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XUNTA DE GALICIA

RAZOR CLAMS biology | aquaculture | fisheries

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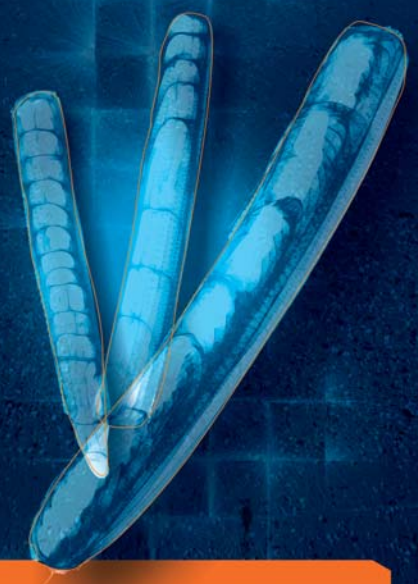
RAZOR CLAMS

biology | aquaculture | fisheries



XUNTA DE GALICIA

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Biology, Aquaculture and Fisheries

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Biology, Aquaculture and Fisheries

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This English edition of the first issue, entitled “Razor shells and razor clams: biology, fishery and culture”, published in 2008 by the Autonomous Government of Galicia, has been extended and modified. It has been published due to the interest displayed in these species in a large number of countries, and also due to the social recognition and protection provided for artisanal and small-scale fishing, carried out by traditional methods and systems, which are highly selective and environmentally friendly.

Numerous international organisations, among them the UN Food and Agriculture Organization (FAO), byway of its Code of Conduct for Responsible Fisheries (CCRF), have stressed the importance of paying special attention to these fishing sectors, favouring the protection and rights of fishers and other workers, and providing preferential access, where warranted, to those fishing resources traditionally exploited in the territorial waters of fishing workers inhabiting coastal areas.

The management and exploitation of razor shells and razor clams in Galicia are a clear example of all of this. Galicia is the place where this book initially found its inspiration and is the subject of a large part of its contents. Later on, for this new edition, several other countries and research groups were incorporated, all of which have provided information on their particular situation as to fisheries and their management. In Section 3, this situation is reflected for a wide range of countries where these fishing resources are relevant: Spain, the UK, Ireland, Portugal, Italy, Greece, the USA, Peru, Chile, Argentina and Thailand.

The information contained in this book will undoubtedly contribute to providing the best possible conditions to guarantee the sustainable management of fisheries and food safety requirements (with regard to quantity, access, use and supply), which is essential for the responsible management and governance of the same.

Rosa Mª Quintana Carballo

Conselleira do Mar, Xunta de Galicia

PREFACE

Several reasons have driven us to publish a second edition of "Razor clams: biology, aquaculture and fisheries" published in Spanish in 2008. In the first place, we felt the need to incorporate newly-acquired knowledge about razor clam species (Pharidae and Solenidae families), together with information contributed from countries with fisheries for these species, and secondly, we felt it was necessary to publish in English. The result is a more mature and more universal work than the first.

We must stress the composite nature of this book, with contributions based on scientific publications, doctoral theses and the reports of public and private agencies; the latter are mainly from administrative organisations and researchers from several countries involved in this subject, where razor clams are relevant or have commercial interest. Several experts on the subjects discussed in this book have reviewed the information provided here. We believe that this last decision, with the editors endorsing the information given, is a valid strategy, more than a limiting factor, in order to continue investigation into this group of bivalves, which so far have not received the attention they deserve, given their ecological and commercial importance.

Commercially exploited species of razor clams represent a substantial food resource, especially in several countries of Europe and South America; in the former, they are distributed along the coasts of the Atlantic Ocean and the Mediterranean Sea, in the latter, along the Atlantic and Pacific coasts of America, and they are also present in Asia. They settle in very different habitats, from sheltered estuaries to coasts highly exposed to the sea. Depending on the species, these gonochoric bivalve molluscs, most of them with K strategy, are distributed from intertidal to subtidal areas, reaching depths of 70 m. They live buried in benthonic substrate, burrowing to a depth of 50-70 cm as soon as they receive some signal that disturbs their environment. This behaviour makes it more difficult to research and record biological parameters in their habitat.

Different harvesting techniques are used for catching razor clams, ranging from extraction by hand and the use of rudimentary tools derived from agricultural activities, mainly at intertidal areas, to apnea diving, or its counterpart with autonomous diving suits, and the use of mechanical and hydraulic dredgers in subtidal areas. In general, each method represents

the need for fishermen or gatherers to harvest this natural resource in shallow sandy areas or a greater depth, respectively, in a rational way.

The book is divided into 3 main sections. The first describes various aspects of the biology of the species in the following chapters: taxonomy and distribution, anatomy, physiology, reproduction, genetics and pathology, with preferential information about the three species that appear on the Spanish coasts and particularly in Galicia (sword razor shell, *Ensis arcuatus*; pod razor shell, *Ensis siliqua*; and grooved razor shell, *Solen marginatus*). The second section of the book focuses on the fishing practices in different habitats and countries and the impact they have on the environment, as well as the latest advances made in the aquaculture of these species. The third section gathers together information concerning the current status of the resource worldwide, mainly from European countries (Spain, Portugal, Italy, Ireland, United Kingdom and Greece), but without forgetting others where razor clam fishing has been and still is important (Chile, Peru, Argentina, Canada, USA, Thailand, Tunisia, etc.).

Contributions in the first section come mainly from research theses and projects carried out during recent years, and from scientific publications arising from them. In this investigation, which has been carried out from the 1990's on, Spanish research centres have been involved, individually or in a coordinated way, especially from Galicia, the "Centro de Investigacións Mariñas" (CIMA) funded by the Fisheries Department of the "Xunta de Galicia" (regional authority) and the Genetics Laboratory of the Cellular and Molecular Department of the University of A Coruña. Part of the money for financing these projects was obtained from funds for marine investigation from the Xunta de Galicia, and especially from National Plan of Aquaculture (JACUMAR) and from two Interreg IIIB European projects: "Sustainable Harvesting of *Ensis*" (SHARE-90) and "Towards Integrated Management of *Ensis* Stocks" (TIMES). The results of these research projects, developed by groups from the above-mentioned institutions, form the backbone of this book and will undoubtedly be useful as a point of reference for future studies on the topic of bivalve molluscs.

The second section, regarding fishing practices and their impact on the environment, comprises original collections and information gathered together for the first time in this publication, since there are no references available regarding the effects produced by different fishing practices. The most frequently-used practices, their role and the environmental impact

of the different techniques on the habitat exploited are here described. Analyses based on multiple criteria are also carried out, in order to suggest the most suitable techniques, depending on the performance and sustainability of the resource in fishing management.

The other parts of this section are made up of contributions regarding the acquisition of seed in hatcheries. Although the bulk of the information is based on Europe, mainly on Spain with regard to traditional species (*Ensis arcuatus*, *Ensis siliqua* and *Solen marginatus*), advances made in Chile with *Ensis macha* are also described. These investigations show that they are organisms with different larval stages, which are sensitive to the effects of manipulation, parasite infections and other kinds of stress related to ongrowing in beds, just like all aquatic organisms in aquaculture activities. Nevertheless, the greatest obstacles identified in farming are the stabling and maintenance of juveniles before transfer for on-growing outside.

In the third section, the situation of the resource in countries where razor clams are commercially important is described and analysed. The book presents information provided by researchers, companies and the extraction area itself, showing a specific view of each producer country, leading to a global analysis of the situation and future possibilities for the resource worldwide. In this sense, as general rule, the decline of natural beds and the lack of regulations lead to the need to implement plans for managing the resource, in order to avoid overexploitation in most of the natural beds and in many cases in order to recover them. In any case, we conclude that fishing these species is sustainable and profitable if it is managed with criteria of rational exploitation.

The editors wish to thank and value the effort made by the authors of the different chapters of the book, mostly enthusiastic students and experts in these species, who initially encouraged us to gather together in one book information about razor clams, which to date has been limited and diffuse. Their enthusiasm in the investigation drove us to complete this work, in order to allow any scientist, teacher, employee in the sector or any person simply interested in knowing about razor clams to have a tool with which to work in this rewarding subject. So, fulfilling its role as a propagator of investigation, the Consellería do Mar (Xunta de Galicia) and Fisheries Department, through JACUMAR, provided the corresponding financial support that has made publication possible. Therefore, we would

like to sincerely thank the institutions to which the people participating in this publication belong. Last but not least, we cannot forget those who do not appear by name, but who have been indispensable: the laboratory technicians and assistants who made it possible for the investigations to be successfully culminated, and also the gatherers of these fruits of the sea, who helped many specialists working on this book, becoming in turn professionals in the sustainable and rational exploitation of this resource, precisely due to the characteristic of the habitats and the wide distribution of the species studied.The logistics of the projects that were the basis of the information summarized in this book would have been impossible had it not been for the help of all parties involved in individual sampling, research in natural environments, monitoring of farming and the insights obtained from daily experience in the field.

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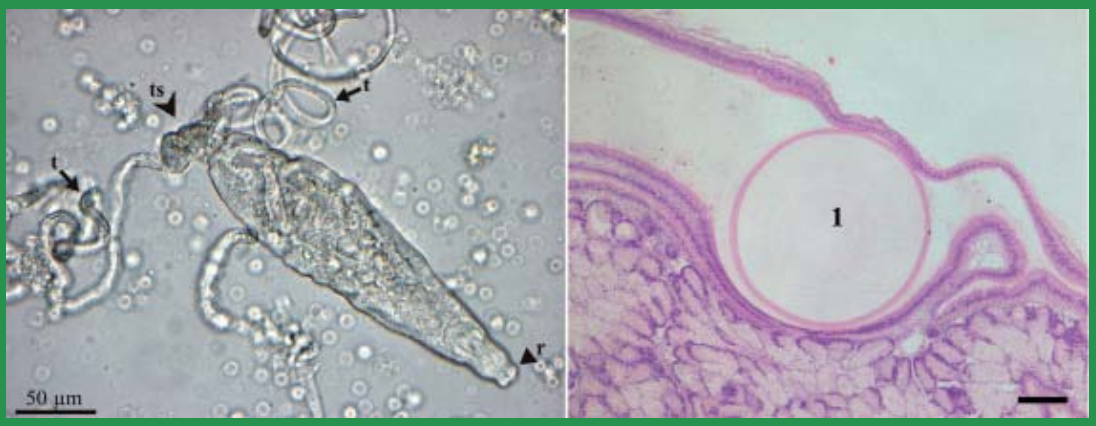

Chapter 1: Systematics and distribution

Susana Darriba Couñago and Juan Fernández Tajes

Abstract

Solenidae are a group of highly specialized infaunal bivalves living in soft sea beds, with a compressed, elongated shell that allows easy penetration into sediments. Species commercially important in Galicia (NW Spain) are the sword razor (*Ensis arcuatus*), the pod razor shell (*Ensis siliqua*) and the grooved razor shell (*Solen marginatus*). The scientific denominations of the razor clam which inhabits Galician coasts show discrepancies in both official documentation and in the few publications where they appeared. In this chapter we show the results of the study, which concluded that the right denomination for this species in Galicia is *E. arcuatus*. Species pertaining to the *Ensis* genus have two cardinal teeth and two lateral ones on the left valve, while species of the *Solen* genus have just one cardinal tooth on each valve. The inclusion of two genera in the same family (Solenidae) or in different families pertaining to the same superfamily (Solenoidea), varies depending on authors. There is a shortage of scientific publications dealing with the geographical distribution of this Superfamily worldwide, and, besides, there is no solid research about their abundance, studies about these species being very limited in nature. Decisive factors in the lack of research into this group are difficulties in sampling, due to its fast burrowing, and the reduced volume of worldwide production compared with other bivalves. In this chapter we publish worldwide distribution maps of *Ensis* and *Solen* genera, stating their different distribution patterns. As for factors determining their location on coasts, the importance of the grain size and nature of substrate, and the level of exposition to swell are all analysed. The *E. directus* (*americanus*) species is mentioned as an example of their potential capacity of movement and colonisation of other areas. This latter species was introduced into Europe at the end of the 1970's, proceeding from the North American Atlantic coast, and spread all over the North of Europe in a few decades.

1. Introduction

Systematics is the part of Biology aimed to create classification systems expressing, in the best possible way, several levels of similarity between living

organisms. Taxonomy is the part of Systematics that provides principles (rules) and procedures for making a classification. The increase in knowledge about organisms and the introduction of new characters for classifying them, have undergone several changes throughout history.

"Solenidae" are a group of infaunal bivalves inhabiting soft sea bottoms and considered by Yongue (1952) as the most highly specialized ones. The particular shape of its shell and its way of living attracted the attention of malacologists and naturalists from the nineteenth century, such as Deshayes (1839), Forbes and Hanley (1848), Jeffreys (1865) and Fischer (1887). These authors mention Aristotle as the first to point out this mollusc's life habits, using the term "*Solen*" in reference to them (Von Cosel, 1990).

In this chapter the historical evolution of systematics of this group of bivalves is summarised and its morphological characteristics are described, which differentiate both genera (*Solen* and *Ensis*), including the commercial species inhabiting Galician coasts, colloquially called razor clams.

The geographical distribution of Solenoidea superfamily around the world is not documented in any kind of scientific or investigative publication, neither has there been solid research about their abundance, while research about ecological aspects of these species is very sparse.

Difficulties in sampling, due to their fast burrowing and the limited volume of worldwide production, compared with that of other bivalves, are determining factors in the lack of research about this group.

In this chapter worldwide distribution maps of *Ensis* and *Solen* genera are published, produced by collecting together the information acquired in the course of various studies concerning their presence and distribution.

2. Systematics

The systematics of this group has suffered several modifications since Linnaeus established the *Solen* genus in 1758. Lamarck, in 1809, proposed the term Solenidae for the family. Later, Schumacher (1817) established *Cultellus* and *Ensis* genus for some of the species assigned to the *Solen* genus in Linnaeus nomenclature.

In the middle of 19th century the Solenidae family was subdivided into the Solenidae and Pharinae families (Adams and Adams, 1858). After this first division,

several taxonomists varied the name and number of subfamilies: Solenidae and Pharellinae (Tryon, 1884); Soleninae, Solecurtinae and Novaculininae (Ghosh, 1920); Solenidae and Glaucomyidae (Thiele, 1935), keeping the *Solen* and *Ensis* genera always within Solenidae.

In Vokes (1967), for the first time, the *Ensis* and *Solen* genera were located in separated families, and the former becomes part of the Pharellidae family, although they were included in the Solenoidea superfamily. Keen (1969) transferred *Ensis* genus to the Cultellidae family, maintaining both within Solenoidea (Von Cosel, 1990).

Von Cosel (1990) considered Cultellidae, where the *Ensis* genus is placed, to be included in the Pharidae family, recognizing the Pharinae and Cultellinae subfamilies. The superior level of the Solenoidea superfamily is maintained.

Currently, the database of European marine mollusca, CLEMAM, in the National Museum of Natural History in Paris, includes razor clams of the Bivalvia class, Heterodonta subclass, Veneroida order and within it two families: the Solenidae family (with the *Solen* genus) and the Pharidae family (with *Ensis, Phaxas, Pharus* and *Sinupharus* genera) (CLEMAM; Checklist of European Marine Mollusca: http://www.somali.asso.fr/clemam/biotaxis.php). Though in this list the superfamily is not indicated, it is still Solenoidea.

On the other hand, Hayward and Ryland (1998) in their manual of marine fauna from Northwest Europe include *Ensis* and *Solen* genera within the Solenidae family, together with the *Phaxas* genus. Recently, in the same line as CLEMAM, Bieler and Mikkelsen (2006) consider the Solenoidea superfamily divided in two families: Solenidae and Pharidae.

Von Cosel (2009) has recently published a long study about the genus *Ensis* based on an inspection of razor shells from the Eastern Atlantic coast. He concludes that the well-established name *E. arcuatus* (Jeffreys, 1865) falls into the synonymy of *E. magnus* (Schumacher, 1817), cited only from the Faroe Islands and from Norway. Nevertheless, we maintain the scientific name *E. arcuatus*.

Genetic analysis, focused on the search for chromosome and molecular markers for studying phylogenetic relations between different species of Solenidae, may make it easier to characterise and differentiate them, helping to solve the problem existing in the taxonomy of this group. Moreover, this research might act as a complement to the morphological analysis carried out when species are characterised. In the chapter about genetics, the importance of this field in developing proper markers for researching taxonomic relations is tackled from a genetic point of view.

3. Morphological characters and distribution

Solenidae shells are elongated, equivalve and non-equilateral. They are open at the anterior and posterior edges and the ligament is external. They have one or two teeth on each valve and may also have one lateral tooth. The anterior adductor muscle is elongated, while the posterior one is small. They possess a pallial sinus.

Commercially important species in Galicia are *Ensis arcuatus*, *Ensis siliqua* and *Solen marginatus* (Fig. 1).

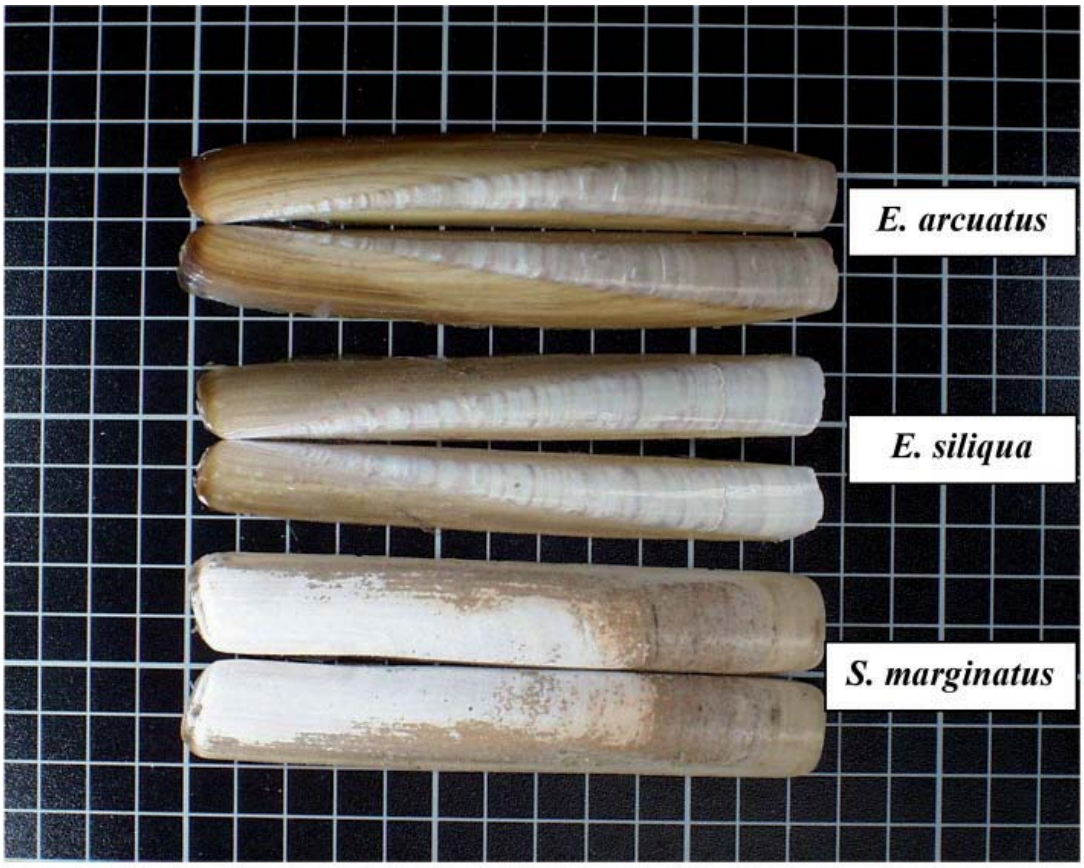

Figure 1. Commercially important species of the Solenoidea superfamily in Galicia: *E. arcuatus, E. siliqua* and *S. marginatus.* Pictures by S. Darriba.

3.1. *Ensis* genus

Species pertaining to this genus have two cardinal teeth and two lateral ones on the left valve; the latter are joined along almost the whole of their length, leaving the ends free and curved upwards. On the right valve there is only one cardinal tooth, which fits between the two teeth of the opposite valve, and one lateral tooth.

The ligament is external and is situated in a surface fissure (Van Urk, 1964).

3.1.1. Worldwide distribution

Figure 2 shows a distribution map of *Ensis* based on information about this genus published in scientific documents and data available on the Internet (OBIS -Ocean Biogeographic Information System, ITIS - Integrated Taxonomic Information System, CLEMAM - Check List of European Marine Mollusca). As is illustrated in figure 3, species from the *Ensis* genus are diversified, several doubts existing about the geographical disposition of its populations. The distribution centre of all of them is located on the European coasts, extending down to the tropical area of West Africa and both coasts of North America. The isolated presence of *Ensis macha* (Molina, 1782) at the northern end of South America must be considered, according to Von Cosel (1990), a remnant population. There are no data about species inhabiting the Indo-Pacific region. In Europe, native species from the *Ensis* genus are exclusively *E. arcuatus*, *E. ensis, E. siliqua* and *Ensis minor* (Fig. 3). Except for *E. minor*, which is located exclusively in the Mediterranean Sea basin, the rest of the species of the *Ensis* genus are distributed from the Portuguese coasts to the Scandinavian Peninsula, also colonising Ireland and the United Kingdom. We must also point out the presence of *Ensis directus,* a non native species, accidentally introduced in 1978.

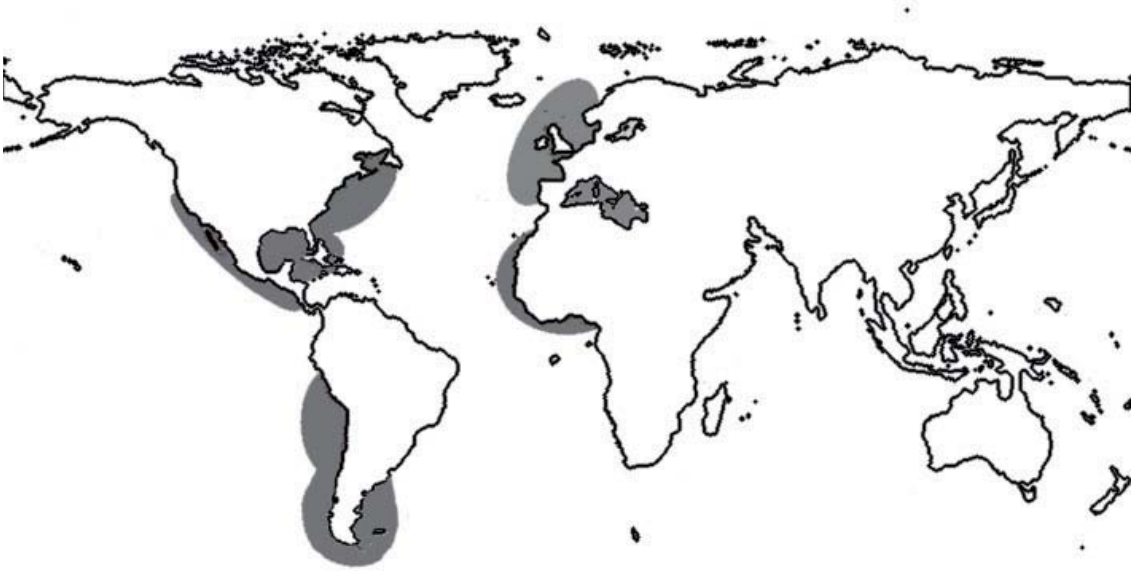

Figure 2. Distribution map of the *Ensis* genus.

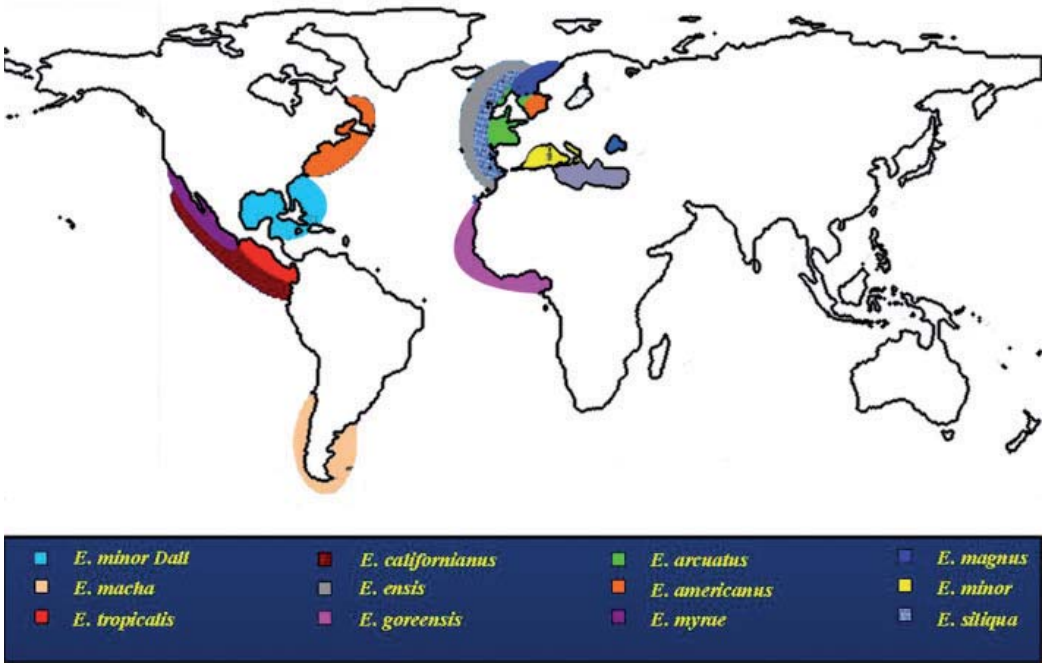

Figure 3. Distribution of *Ensis* genus species.

3.1.2. Location

Regarding ecological factors influencing the location of species, Holme (1954) considers that the size of the substrate grain they inhabit, as well as the level of exposition to swell, are fundamental in the distribution of the three species of the *Ensis* genus (*E. ensis*, *E. arcuatus* and *E. siliqua*) on British coasts. The fact that the distribution is related to grain size is also indirectly determined by the slope and stability of the beach, related at the same time to the effect of swell. *E. siliqua* tend to inhabit beaches more exposed to swell than *E. arcuatus*, which occupy substrate with thicker grains. These observations concur with the results obtained by Holme (1954), where it is concluded that *E. arcuatus* in Great Britain occupy beaches with thicker grains (0.2 a 1.5 mm) than *E. siliqua* or *E. ensis* (0.21 a 0.0313 mm). Moreover, *E. arcuatus* tolerates 35% of thick grain (0.5 mm) and a low percentage of fine sand (<0.0313), while *E. siliqua* does not tolerate more than 5% of thick grain but does tolerate a percentage of even 1% of fine grain. This can be related to the presence of a foot with a stronger muscle in *E. siliqua* than in *E. arcuatus*, as indicated by Henderson and Richardson (1994), comparing *E. siliqua* with *E. ensis.*

Another determining factor in the presence of *Ensis* genus species in a specific substrate is the beaches characteristics: those they inhabit have fundamentally quartz particles, though with a high percentage of lime material. There is no evidence that they are restricted to beaches with sand with a particular mineralogical characteristic, degree of roundness or surface texture (Holme, 1954).

Regarding exposition to swell, according to the results of Holme (1954), on very exposed beaches where the sand is constantly stirred, these species do not appear; *E. siliqua* only appear on beaches slightly protected from the waves and *E. arcuatus* rarely appear on open beaches in the SW of England, confining themselves to protected harbours and estuaries where coarse material is accumulated. None of the three species tolerate reducing areas.

Among the most important species in the Northwest of Europe are:

3.1.3. *E. arcuatus* (Jeffreys, 1865)

The dorsal margin of the shell is almost straight, the ventral margin slightly more curved. Anterior and posterior margins are diagonally truncated. The right valve has one cardinal tooth and one lateral tooth, subsequently elongated, the left valve having two lateral teeth and two cardinal teeth. The lateral tooth reaches half of the length of the external ligament; also the anterior adductor muscle is long, being almost twice as long as the ligament.

3.1.3.1. Habitat

They burrow into sand and gravel, at low intertidal area and at the infralittoral, reaching 36.6 m in depth, according to Tebble (1966).

3.1.3.2. Distribution

They are distributed from Norway to the South of Spain (Alborán Sea), appearing all along the British coastline (Tebble, 1966). In the Ría of Vigo, according to Rolán *et al*. (1989), they appear all along the external area.

3.1.4. *E. siliqua* (Linnaeus, 1758)

Dorsal and ventral margins are parallel and almost straight. The anterior and posterior margins are diagonally truncated, with rounded ends, although less than *E. arcuatus*. The left valve has two cardinal teeth and two lateral ones and the right valve one cardinal tooth and another elongated lateral one towards the posterior end. *E. siliqua* lateral teeth only reach one third of the ligament length. The anterior muscular impression is shorter than that of *E. arcuatus*, although larger than that of *E. ensis*.

3.1.4.1. Habitat

They live buried in the sand in low intertidal and infralittoral areas.

3.1.4.2. Distribution

They are distributed from Norway to the British coast and western coast of Europe, their Southern limit being the Mediterranean Sea and North Africa (as yet unconfirmed, since there have been confusions with *E. minor*). According to Rolán *et al*. (1989), they are found in the Ría of Vigo, along the coast in the central and external zones.

3.1.5. *E. ensis* (Linnaeus, 1758)

Dorsal and ventral margins of the shell are parallel and curved, the anterior edge being rounded. The right valve has a short cardinal tooth and a lateral one elongated rearwards while the left valve has two cardinal teeth and two lateral ones elongated rearwards. Lateral teeth in this species only reach one third of the ligament length.

3.1.5.1. Habitat

They burrow into the sand in low intertidal and infralittoral areas.

3.1.5.2. Distribution

They are distributed from Norway to the Mediterranean Sea and the South of Morocco, appearing all along the English coast.

In Galicia, razor clam species extracted on our coasts used to be called *E. ensis.* Rolán *et al*. (1989) mention the existence of *E. arcuatus* in the Ría of Vigo for the first time while *E. ensis* appears as the least frequent species, as regards to valves, identified as such, in isolated areas of the Ría. The field guide published by Rolán and Otero-Schmitt (1996) calls *E. arcuatus* a razor clam species that appears in the Ría of Vigo, while there is no record of *E. ensis*.

In the 1990's there was disagreement in the scientific name for the Galician razor clam between official organisms and the few scientific documents published (Rolán *et al*., 1989; Rolán and Otero-Schmitt, 1996). Darriba (2001) studied biometric parameters, valve morphology and karyotype of the species. For a morphological study of valves, the study was carried out with the collection of Solenidae from the Natural History Museum in London, where valves of *E. ensis* and *E. arcuatus* species can be found, thus assisting comparative research. Results obtained, and their comparison to research performed by other authors, contribute important information to confirm that the species found on Galician coasts are *E. arcuatus* and not *E. ensis*.

Maximum size is one of the distinguishing characteristics of *E. ensis* and *E.*

arcuatus species. *E. arcuatus* reaches a greater length, between 12 and 17.5 cm, while *E. ensis* reaches between 8 and 13 cm (Holme, 1951; Tebble, 1966; Rolán *et al*., 1989; Poppe and Goto, 1993; Henderson and Richarson, 1994; Hayward and Ryland, 1998). Galician species have sizes even greater than 15 cm long, coinciding with the values mentioned by several authors for *E. arcuatus*.

Another parameter used for distinguishing species from the *Ensis* genus is the ratio between length and width, measured at the middle part of the shell (L/Wm) (De Boer, 1984; Van Urk, 1987; Luczak and Dewarumez, 1992). Holme (1951), researching *E. ensis*, *E. arcuatus* and *E. siliqua*, shows differences for L/Wm ratio, with average values of 7.80, 7.18 and 6.80 respectively for the three species. Darriba (2001) results of L/W ratio are between 8.36 and 6.06 closer in range to the one described by Holme (1951) for *E. arcuatus* (8.29 and 6.20) than for *E. ensis* (9.07 and 6.83). *E. ensis* show higher values because they are thinner, a characteristic mentioned by Van Urk (1964) in a study about the *Ensis* genus in Europe.

Likewise, measurement of the dorsal curve allowed Darriba (2001) to confirm that Galician species coincide with the values provided by Holme (1951) in order to distinguish *E. arcuatus* from *E. ensis* and *E. siliqua*. The dorsal curve of the species that she was attempting to identify coincided with the results of the same author for *E. arcuatus* and were clearly distinguished from those of *E. ensis*, which show a longer curve (Fig. 4).

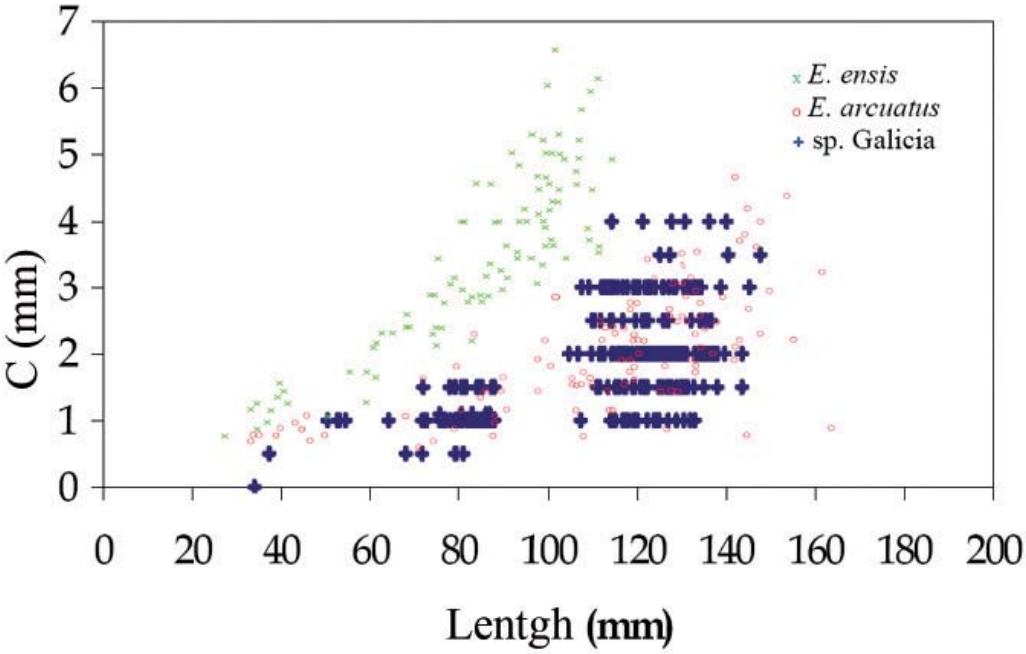

Figure 4. Curvature of the dorsal edge of the shell (C) against shell length for the subject species of this research (curved razor clam-Galicia) and for *E. ensis* and *E. arcuatus.* Taken from Darriba (2001).

Figure 5 summarises the main morphological differences between *E. ensis* and *E. arcuatus* valves and their comparison to the Galician razor clam. The main differences are the roundness of the anterior edge, the pronounced narrowing of the posterior edge and the higher level of curvature observed in *E. ensis*.

	E. ensis **(Linnaeus,1758)**	***E. arcuatus*** **(Jeffreys,1865)**	**Galician razor clam**
Anterior edge			
			
Posterior edge			
			
Dorsal and ventral margins			

Figure 5. Detailed comparative morphological chart of *E. ensis* and *E. arcuatus* valves; part of the Solenidae collection from the N.H.M. of London, and razor clam species from the Cíes Islands. Taken from Darriba (2001).

3.2. *Solen* genus

Species from this genus have a cardinal tooth on each valve. The ligament is similar to that appearing on *Ensis* genus (van Urk, 1964). The most common species on Galician coasts is *S. marginatus* (Pennant, 1777).

3.2.1. Worldwide distribution

Specimens from the Solenidae family are mainly tropical and subtropical, mostly distributed throughout the Indo-Pacific region (Fig. 6), unlike the *Ensis* genus. There are data about species along European Atlantic coasts, the East and Northwest of the Pacific, on North American coasts and in areas of the Indian Ocean, among others. In Europe, *S. marginatus* is the only native species of this genus (Fig. 6).

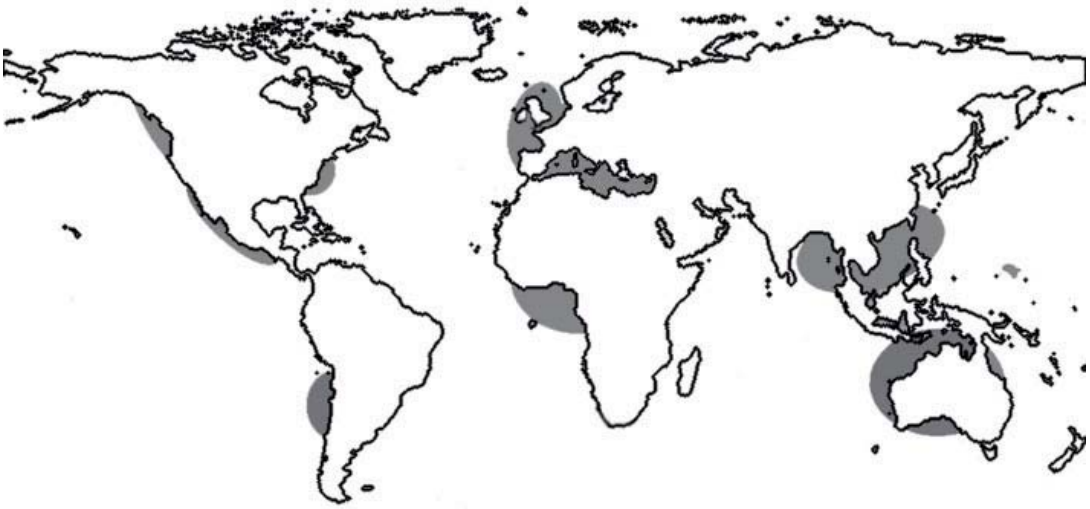

Figure 6. *Solen* genus distribution.

3.2.2. Location

In commercial species it is observed that *S. marginatus* prefers substrates with very fine grain, internal areas and those protected from swell, with medium-low salinities. Among these species are those inhabiting Galician coasts.

The most relevant morphological characteristics of the species are: valves diagonally truncated at the anterior part, straight and parallel dorsal and ventral margins. They also have a prominent dorso-ventral groove at the anterior end of the shell. They do not have lateral teeth, a cardinal tooth appearing on each valve. The

pallial muscles are strongly developed and they do not have a ventral hole.

3.2.3. Habitat

They burrow into the sand, in low intertidal and infralittoral areas.

3.2.4. Distribution

They are distributed from Norway to the Mediterranean Sea and North Africa, appearing in England only on the Southeast and West coasts. In the Ría of Vigo they appear all along the coast, from the interior (Ensenada of San Simón) to the external part (Rolán *et al*., 1989).

4. Mobility

Regarding the distribution of individuals within natural beds, there are references to adult individuals in the deepest areas. Henderson and Richardson (1994) suggest a migration of juveniles towards deeper coasts as they get older and more resistant to currents. Unpublished data of samplings carried out in Galician natural beds also indicate the existence of older specimens in the deepest areas of beds such as A Lanzada (Ría of Arousa) or Cíes Islands (Ría of Vigo) (SW Galicia).

The capacity of mobility of these species is not well known. Drew (1907) and McMahon and McMahon (1983) analysed several possibilities of individual movements, aware that these are very active animals that bury themselves very fast and can also jump and swim, even though these are very limited movements and the capacity to swim is lost in some adult individuals.

Regarding the mobility of this group, it is important to bear in mind that the *E. directus* (*americanus*) species was introduced into Europe at the end of the 1970's, coming from the North American Atlantic coast (between Labrador and the Florida peninsula), due to the transportation of larvae in ballast water of ships arriving in Germany (von Cosel *et al*., 1982; Essink, 1985, 1986). The first record of this species in Germany was in June 1978 in the German Bight (von Cosel *et al*., 1982); since then it progressively spread towards North and South. In a few years dense populations had formed along the whole coast of Germany; in 1982 it was registered in the North and South of Denmark, in 1986 it reached the coast of Belgium and in 1991 the French coast (Luczak *et al*., 1993) (Fig. 7).

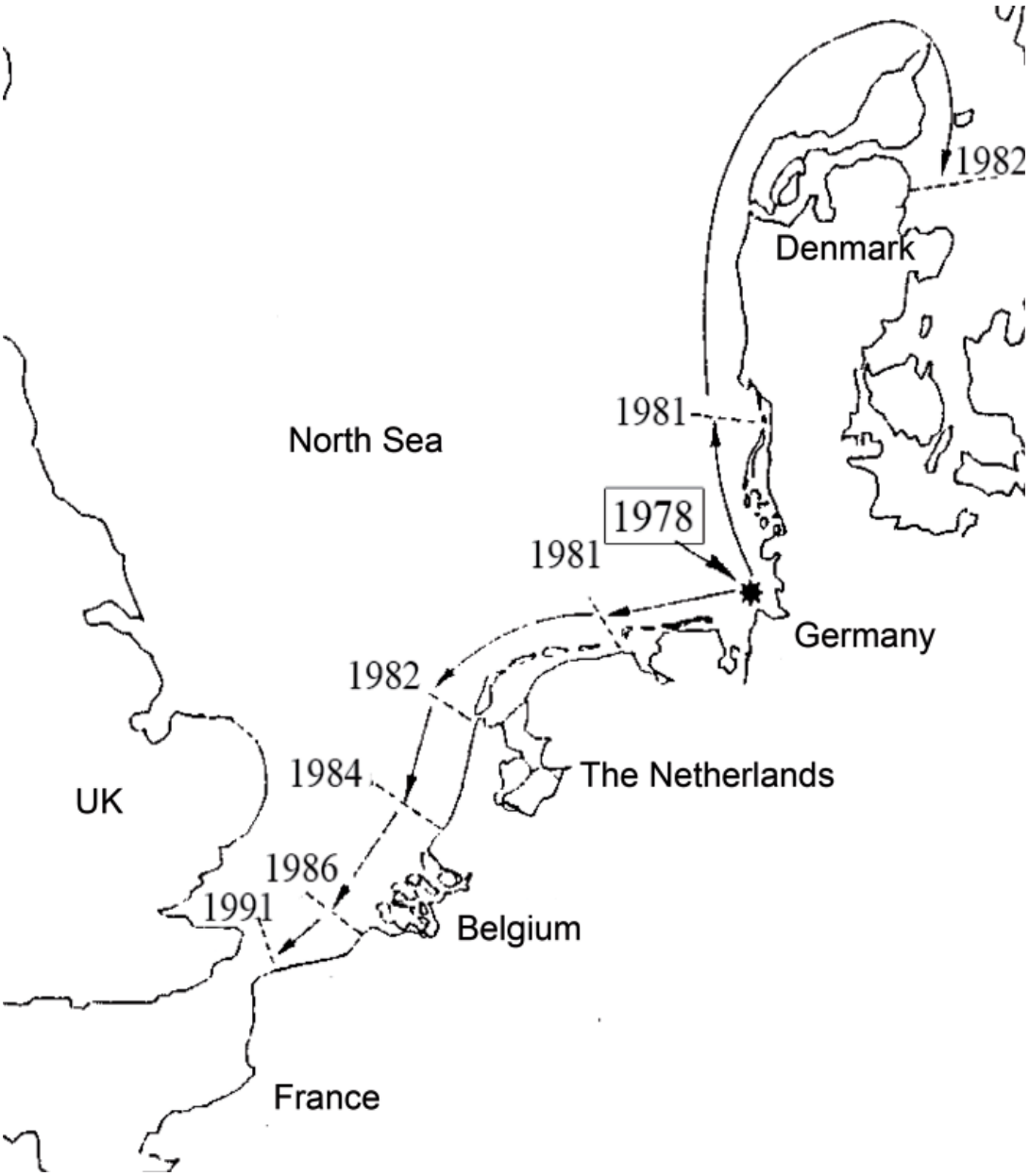

Figure 7. *E. directus* expansion through the Northwest of Europe since their arrival in 1978. Taken and modified from Luczak *et al*. (1993).

Movement from the initial colonisation area towards the North was easily explained by the existence of residual currents at the East area of the German Bight moving in that direction; but the fast dispersion towards the Southwest was more difficult to explain, since larvae had to swim upstream. Essink (1985) and Belgrano *et al*. (1990) explain the spread towards the East as a movement of larvae and post-larvae due to counter-currents which took place at specific moments when North winds were dominant.

Armonies (2001) mentions this case as a rare exception within marine

invertebrates ability to move, calculating the average capacity of dispersion towards the North at 125 Km/year, assisted by the residual current, and at 75 Km/year towards the East. Nevertheless, he does not exclude multiple importations rather than a single insertion in 1978.

Swennen *et al*. (1985) explain the success of *E. directus* in their colonisation of the North Sea area by the fact that they found a gap readily available, as *Mya arenaria* is the only species that lives burrowed in sediment like *E. directus*. But while *M. arenaria* hardly moves and feeds through a long siphon, *E. directus* is a very fast burrower, with short siphons located closer to the surface and it buries quickly when it feels threatened, so *M. arenaria* inhabits more stable sediments, usually with a mixture of mud, while *E. directus* can live in less stable sand.

Armonies and Reise (1999) researched the effect of the invading species on resident communities of Sylt Island (North Sea) and concluded that there was a clear competition with *Cerastoderma edule* (negative correlation); but positive correlations with other infaunal species. The rivalry existing between *C. edule* and *E. directus* had already been registered by Flach (1996).

Predators are another aspect to consider when dealing with the ecology of a species. For Solenoidea, birds, such as gulls, have been mentioned as natural predators (Swennen *et al*., 1985). *S. marginatus* species are able to section part of their own siphons (autotomy) when they feel threatened, in order to confuse predators and escape by burrowing quickly.

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Chapter 2: Anatomy

Susana Darriba Couñago and Carmen López Gómez

Abstract

In this chapter we describe the anatomy of Solenidae in general. It was felt to be interesting to present this in book form, since it is hard to find information about these species in sections about mollusc anatomy in zoology manuals and publications, or in other kinds of documents that can be easily located. These bivalves have specialized in burrowing in soft substrata and living in subterranean galleries, which is why they have a long shell and a big muscular foot, being different from the archetypal bivalve mollusc. The description given in this section is supported by diagrams and micrographs of the different tissues, providing new information about these species.

1. Introduction

1.1. Anatomy

Anatomy is a branch of biology focusing on the structural organisation of living organisms. Knowledge of organism structures is closely related to an understanding of their function, acquired by means of anatomy and physiology, which are almost inseparable, usually being called together “functional anatomy”.

The general characteristics of bivalve mollusc anatomy are collected together in zoology manuals, books and other kinds of documents of easy access (Morse and Zardus, 1997; Larson *et al.*, 2001; Gosling, 2002; Auffret *et al.*, 2003). There are also specific books about the biology of some species or families (Galstoff, 1964; Kraeuter and Castagna, 2001; Shumway and Parsons, 2006). However, studies about anatomy of Solenidae are scarce, and are limited to old scientific publications which are not easily available (Bloomer, 1901a-b, 1903; Drew, 1907; Graham, 1931; Yonge, 1952).

In this chapter, we will carry out a histological review and make a description of the anatomy of the Solenoidea Superfamily.

2. Anatomical description

2.1. Shell

Solenidae are elongated, laterally-compressed organisms. As with other bivalves, the shell consists of 3 layers, one on top of the other, mainly composed of calcite and segregated by the mantle:

- The outermost layer or “periostracum”, of variable colour, where a diagonal line can be distinguished dividing two different areas: dorsal, with horizontal lines, and ventral, with vertical lines.
- The “prismatic layer” calcified with a matt color.
- The “nacreous layer” equally calcified, but shiny.

The ligament, which is external and elongated, is located in the anterior dorsal area (Fig. 1). Right below that is the hinge, where the cardinal and lateral teeth are located (if they have them), matching their counterparts on the other valve and they are an important taxonomic characteristic (Fig. 2). *Ensis arcuatus* and *Ensis siliqua* have two cardinal and two lateral teeth on the left valve and one of each on the right one, while *Solen marginatus* have just one cardinal tooth on each valve.

Muscle scars can be seen on the inner surface of valves. The anterior adductor muscle leaves an elongated and narrow scar along almost the whole valve. The posterior adductor muscle is very small compared to the one previously mentioned and is more rounded. The anterior retractor muscle of the foot leaves two scars close to each other in the distal area of the anterior adductor muscle. On the other hand, very closely linked to the scar of the posterior adductor muscle is located the insertion of the posterior retractor muscle of the foot, rounded and larger than the scars left by its counterpart in the anterior part (Bloomer, 1901a) (Fig. 1).

Alongside the shell, without reaching its ends, is located the pallial scar (dorsal, ventral, anterior and posterior), which outlines the area where the mantle is fixed to the valves. At the posterior end, this pallial scar is C-shaped, with the open part towards the end of the shell, called the pallial sinus (Fig. 1).

2.2. Mantle

The Solenidae mantle has two semitransparent lamellae covering the internal surface of the valves, which fuse in the dorsal area. Each lamella is formed by two epithelia, with connective tissue between them. The internal epithelium is in contact with the pallial cavity and the external one is the secretory epithelium of the shell.

The connective tissue is crossed by several blood sinuses (where many phagocytes circulate), nerves and muscles that act in the retraction and contraction of the mantle edges. The external epithelium is composed of prismatic cells without cilia and with the nucleus located in the basal area. The internal epithelium has ciliated cubic cells with the nucleus in central position (Fig. 3).

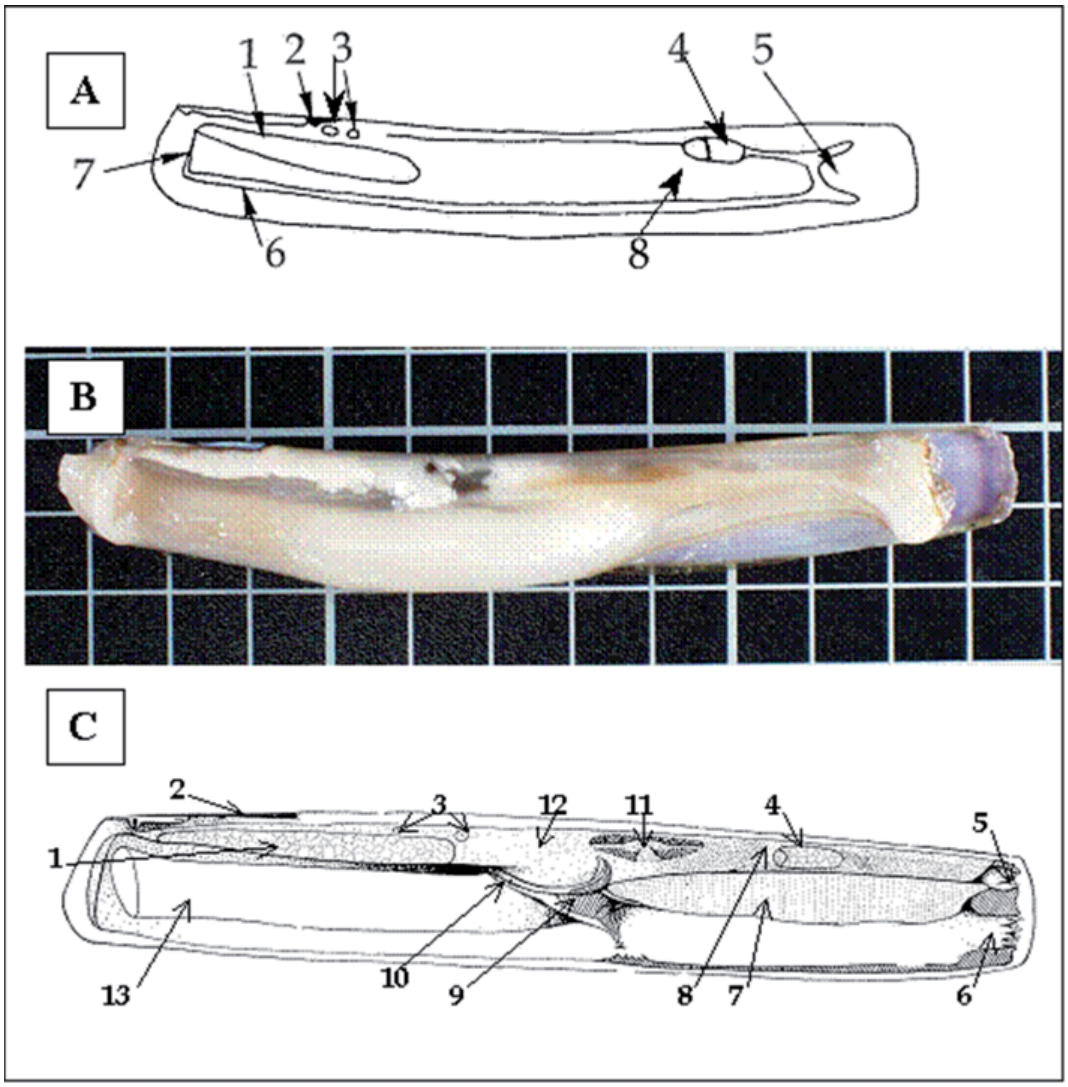

Figure 1. (A) Muscle scars on the internal surface of *E. arcuatus* bivalves.1: Scar of the anterior adductor muscle; 2: Distal area of the ligament insertion; 3: Scar of anterior retractor muscle of the foot; 4: Scar of posterior adductor muscle; 5: Pallial sinus; 6: Ventral pallial scar; 7: Anterior pallial image and 8: Scar of posterior retractor muscle of the foot. **(B)** Illustration of *E. arcuatus* flesh, in mature stage, where gonad is emphasized. **(C)** Diagram of organ distribution in a specimen of *E. siliqua.* 1: Anterior adductor muscle; 2: Ligament; 3: Anterior retractor muscle of the foot; 4: Posterior adductor muscle; 5: Exhalant siphon; 6: Inhalant siphon; 7: Gills; 8: Posterior retractor muscle of the foot; 9: Labial palps; 10: Mouth; 11: Heart; 12: Digestive gland; 13: Foot. Taken from Darriba (2001).

Usually, in bivalves, the mantle edges are divided into three folds: the outer fold secretes the two outer layers of the shell, the middle fold is sensory and the inner fold is muscular and controls water movements. However, the *Solen* and *Ensis* genus lack these three folds, and *Siliqua patula* is one of the few species of the Solenoidea Superfamily where folds are found at the mantle edge (Yonge, 1952).

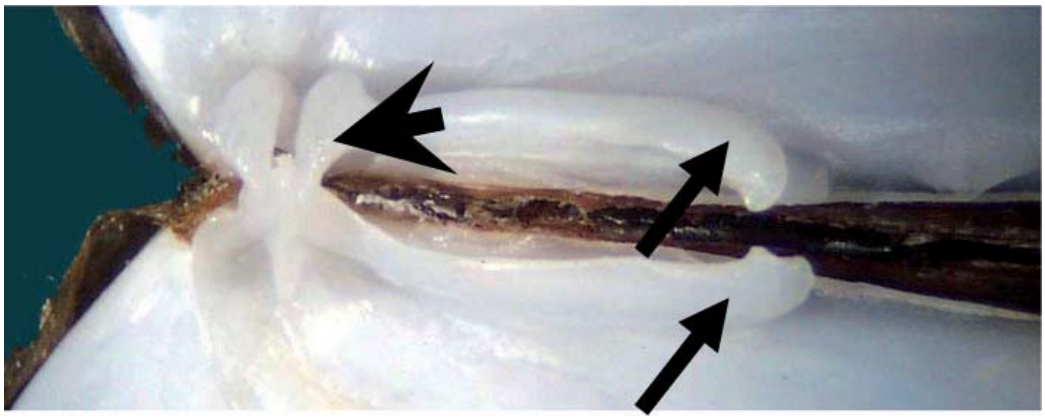

Figure 2. Cardinal (arrowhead) and lateral (arrow) teeth in the hinge of *E. arcuatus* valves.

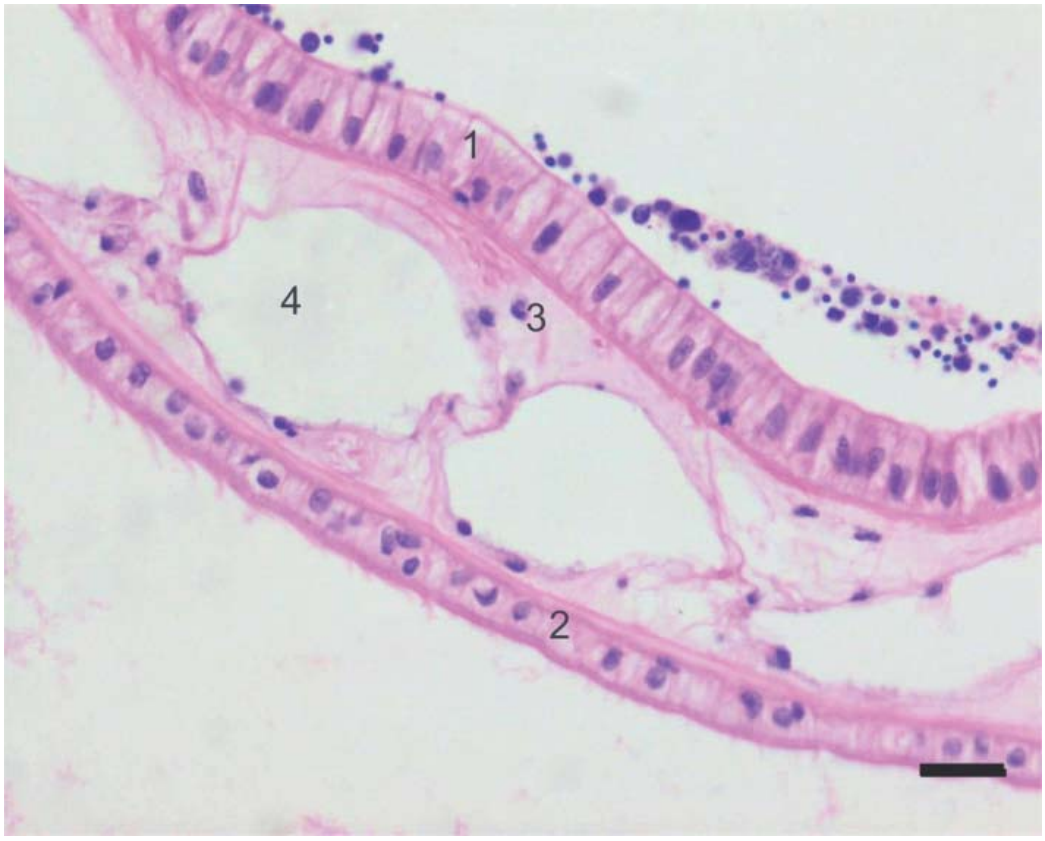

Figure 3. Micrograph of *E. arcuatus* mantle, where two epithelia are observed: the outer epithelium **(1)** with columnar cells, and the inner epithelium **(2)** with ciliated cells separated by connective tissue **(3)** crossed by the blood sinus **(4)** (dyed with Harris-hematoxylin eosin, HHE) (Scale=25 µm).

The mantle edges of Solenidae are sealed, leaving only three apertures which communicate with the outer area: the inhalant siphon, exhalant siphon and anterior aperture for the foot (Fig. 1). *E. arcuatus* and *E. siliqua* have a fourth aperture in the middle area of the ventral part, elliptical in shape and surrounded by small tentacles, which are complemented by the ones on the opposite side (Fig. 4B). Bloomer (1903) concluded that it is a portion of the pedal aperture that developed several tentacles and moved away from the pedal aperture when the walls were fused, migrating progressively towards an anterior position. So, the absence of the fourth aperture might be indicative of more primitive species, such as *S. marginatus* (Fig. 4A). Experiments performed by Bloomer (*op.cit.*) in *E. siliqua* suggested that this aperture has a double function, that is, as a way for obtaining food and as an aperture for exhaling water and foreign matter that reaches the mouth area; this latter seems to be a secondary function.

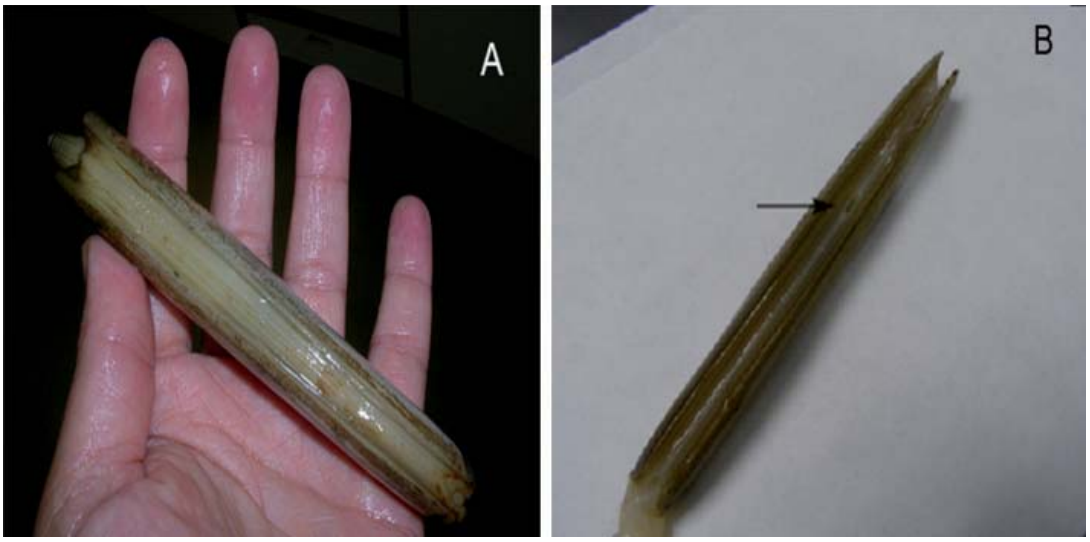

Figure 4. (A) *S. marginatus* ventral area with the whole edge of the mantle sealed. **(B)** *E. arcuatus* ventral area with the fourth aperture (→).

2.3. Foot

The foot (Fig. 1) is large, extended and adapted for burrowing, which allows Solenidae to bury themselves when they feel threatened. It has nerves coming from the pedal ganglion and muscles, distributed in all directions and arranged in layers. Graham (1931) lists them, starting from the most superficial one and continuing with the deeper ones: (1) circular layer of muscle, immediately underneath the epithelium covering the foot, (2) outer layer of longitudinal muscle, (3) layer of diagonal muscles, (4) layer of dorsoventral muscles, (5) inner layer of longitudinal muscles and (6) layer of dorsoventral muscles running in a diagonal direction (Figs. 5 and 6).

The centre of the foot is occupied by the pedal sinus, reached by the hemolymph from the anterior aorta. During maturity, the foot is mainly occupied by gonadal tissue.

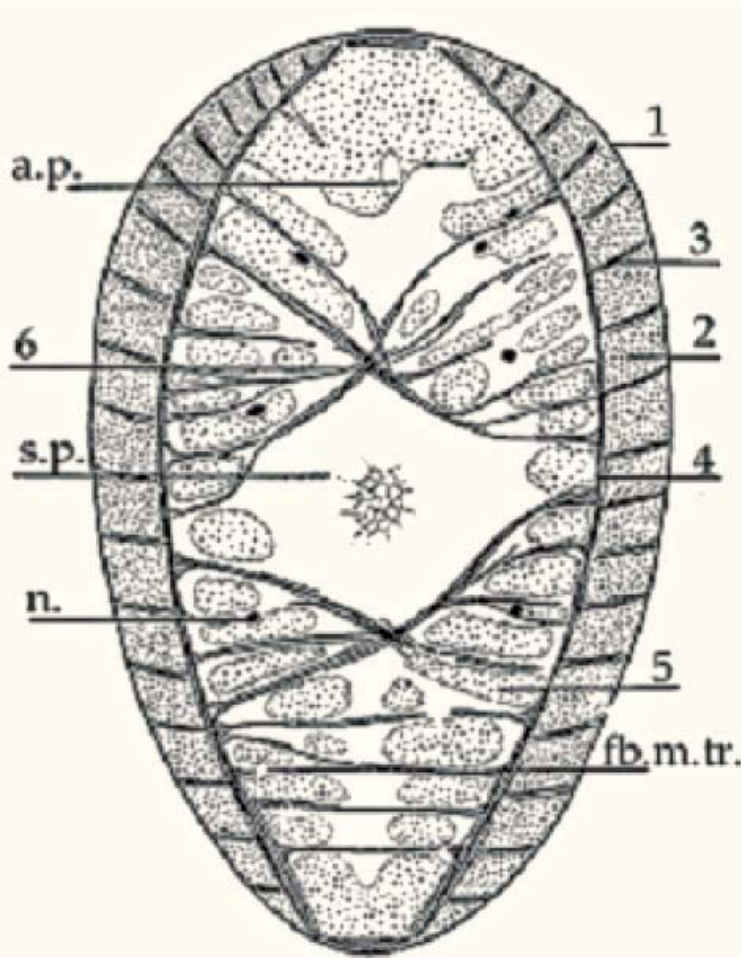

Figure 5. Image of the transversal section of the foot, showing the different muscle layers. **1:** circular muscles; **2:** outer longitudinal muscles; **3:** diagonal muscles; **4:** dorsoventral muscles; **5:** inner longitudinal muscles and **6:** dorsoventral muscles in diagonal direction; transversal muscle fibres, **fb.m.tr**.; nerves **n**.; pedal sinus, **s.p**; pedal artery, **a.p**. Taken from Graham (1931) and modified.

The anatomy of this organ is directly related to the burrowing mechanism, described in detail by Drew (1907). According to this author, Solenidae are very active, can easily bury themselves and can also swim and jump out of the substrate in a movement he calls “leaping”. The foot penetrates into the sediment with dorsal pushing movements until it is totally extended (Fig. 7). During extension the end of the foot is kept small, and the diameter of the protruded part of the foot is smaller than its normal diameter. When the foot is totally extended the distal end swells for firm anchorage, becoming rigid and its diameter increasing when a great amount of hemolymph is incorporated to the pedal sinus. Subsequently, the contraction of retractor muscles provokes the burrowing of the whole body when it reaches the anchorage point. During extension, valves remain open, allowing the siphons and ventral aperture to be open. Just before the rapid contraction of the foot, siphons and ventral aperture close and remain shut until contraction is finished. Thus,

the water inside is released through the anterior area (between the foot and the muscles surrounding it) preventing the entrance of mud and helping descent (Fig. 7). Swimming movements are possible due to the emission of water from the anterior part, allowing Solenidae to advance in the opposite direction.

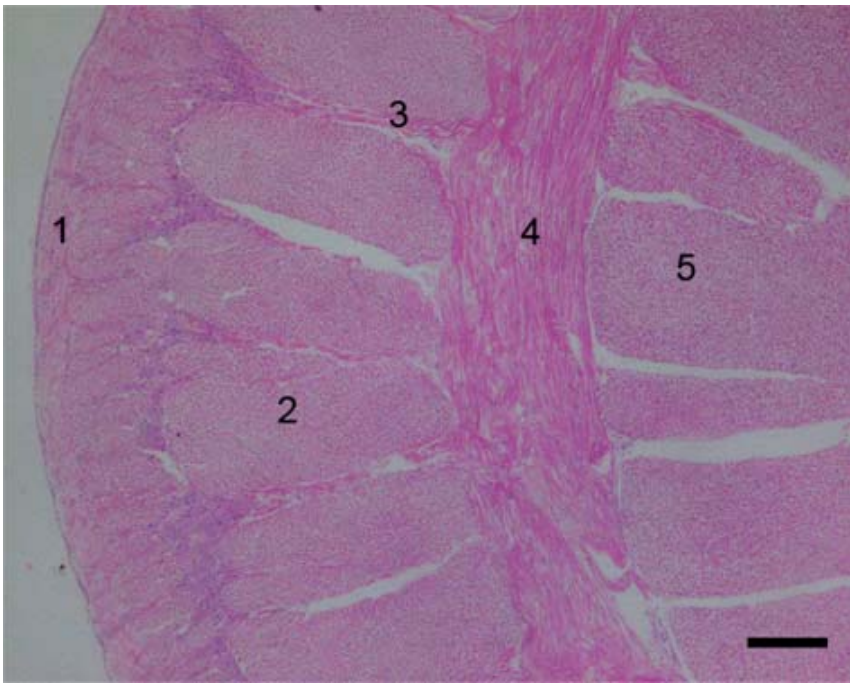

Figure 6. Micrograph of a transversal section of *E. arcuatus* foot and the different muscle layers. **1:** circular muscles; **2:** outer longitudinal muscles; **3:** diagonal muscles; **4:** dorsoventral muscles; **5:** inner longitudinal muscles (HHE, Scale=150 µm).

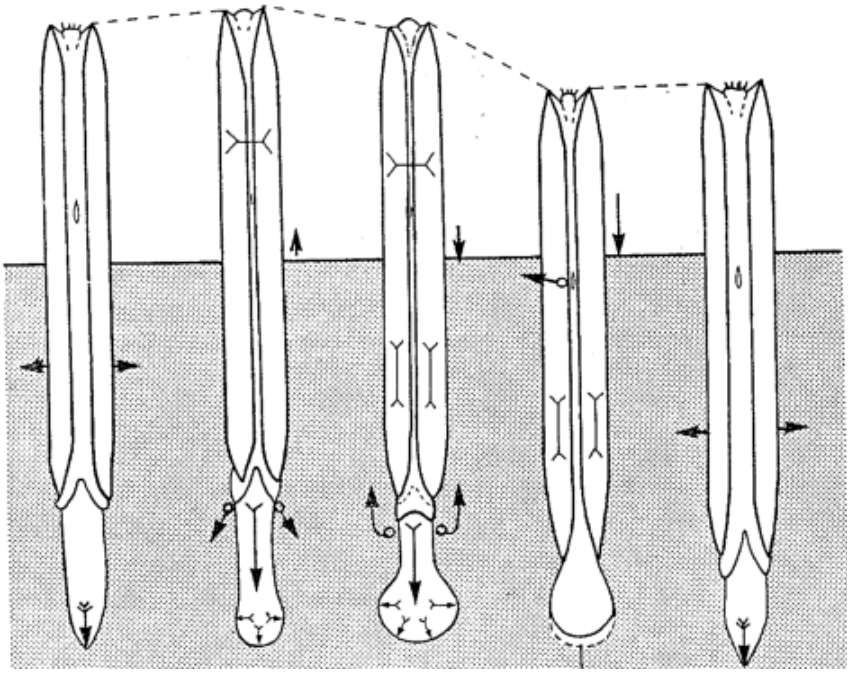

Figure 7. Explanatory diagram of the burrowing system. Taken from Trueman (1967) and modified.

2.4. Circulatory system

The circulatory system is very important for the burrowing mechanism. It is open, as in all bivalves; there are no capillaries bringing hemolymph to the tissues, but it passes through “lakes” and is accumulated in blood sinus. The pedal sinus is a long duct (Fig. 5), homologous to the posterior pedal sinus of other pelecypods, which extends along the foot and muscle fibres surrounding it, allowing the access of hemolymph (Bloomer, 1901a). The heart, included in the pericardial cavity, has a fusiform ventricle, located in the middle area, and two triangular lateral auricles. The pericardial cavity is located in the dorsal area of the soft parts, close to the proximal portion of the foot (Fig. 1C). The ventricle wall has prominent muscle bands composed of smooth muscle fibres and surrounds the final stretch of the intestine (Fig. 8). The wall of the auricles has bands of smooth muscle fibres (Fig. 9).

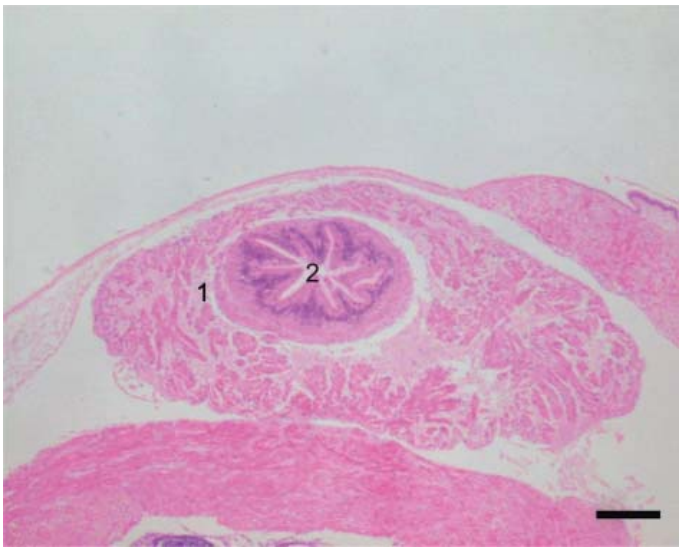

Figure 8. Histological section of *E. arcuatus* pericardial cavity with the rectum **(2)** passing through the fusiform ventricle **(1)** in central position (HHE, Scale=100 µm).

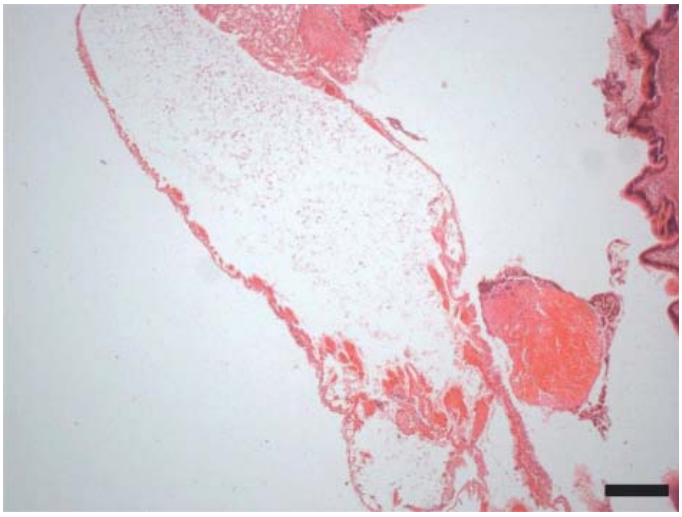

Figure 9. Histological section of *E. arcuatus* auricle (HHE, Scale=100 µm).

2.5. Gills

The pallial cavity is the space located at both sides of the soft parts. It is delimited by the internal epithelium of the mantle and communication with the outside is made through the siphons. The gill chamber is the part of the pallial cavity where the gills are located. Solenidae have two gill lamellae (inner and outer) at each side of the gill chamber, composed of an ascending and a descending lamella; between the two lamellae there are connective joints (interlamellar bridges), which confer on them the characteristics that define eulamellibranchia. Lamellae are pleated and each pleat (or plica) is composed of a variable number of gill filaments, these are ordinary and primary filaments. Ordinary filaments are those found at the crest of the fold and have ciliated cells in the distal area; primary filaments lack cilia and are joined by interfilamentous bridges (Fig. 10).

Gills are in charge of selecting particles that enter through the inhalant siphon and are carried to labial palps, where the selection and transfer of nutrients towards the mouth continues.

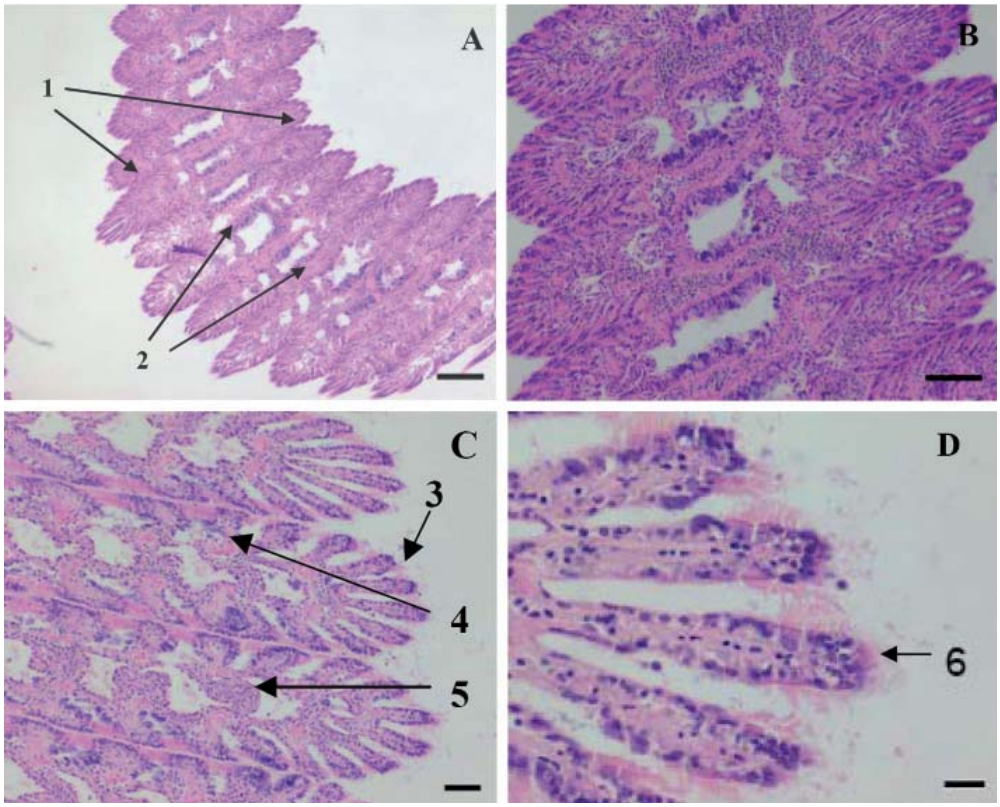

Figure 10. Histological sections of *E. arcuatus* gills (HHE): **(A)** Pleated lamellae (1) joined by interlamellar tissue bridges (2) (Scale=150 µm). **(B)** Detail of lamellar folds consisting of gill filaments (Scale=50 µm). **(C)** Lamellar folds consisting of ordinary filaments (3) in the main filaments and crests (4) joined by interfilamellar bridges (5) (Scale=50 µm). **(D)** Detail of ordinary filaments with ciliated cells (6) distal part (Scale=25 µm).

Labial palps are located behind the gills, consisting of two pairs, one at each side of the foot base. They are triangular and have one smooth surface and one rough (Fig. 11). The rough surfaces of each pair are opposite each other, while the smooth sides face away from each other. The base of the labial palps makes an oral groove and selected particles in suspension reach the mouth through it.

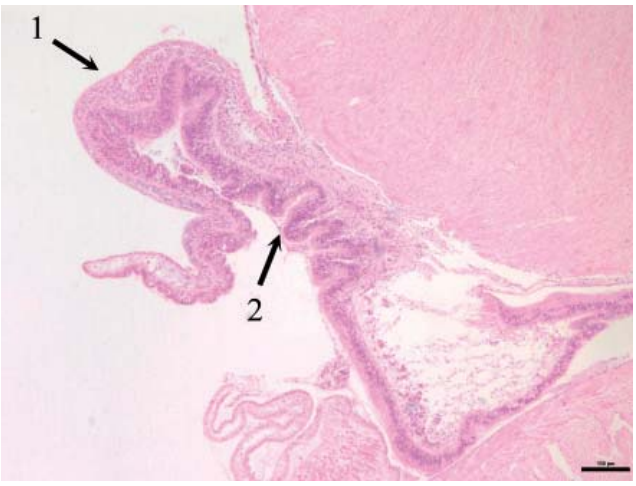

Figure 11. Transversal histological section of a *E. arcuatus* labial palp where we can see the smooth surface **(1)** and the rough one **(2)** (HHE, Scale=150 µm).

2.6. Digestive system

The bivalve digestive system consists of mouth, esophagus, stomach, digestive gland, intestine and anus. It begins in the mouth, which opens externally, anterior to the foot base and posterior to the anterior adductor muscle (Figs. 1 and 21). At each side there is a pair of palps where the material selected by gills arrives, dragged there by the ciliary current. Particles go through a duct that communicates palps with the mouth, forming a U that goes along the foot base.

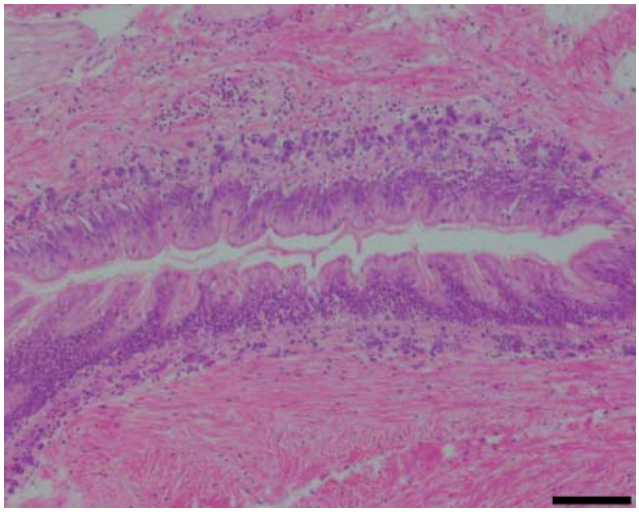

Figure 12. Histological section of the *E. arcuatus* esophagus (HHE, Scale=50 µm).

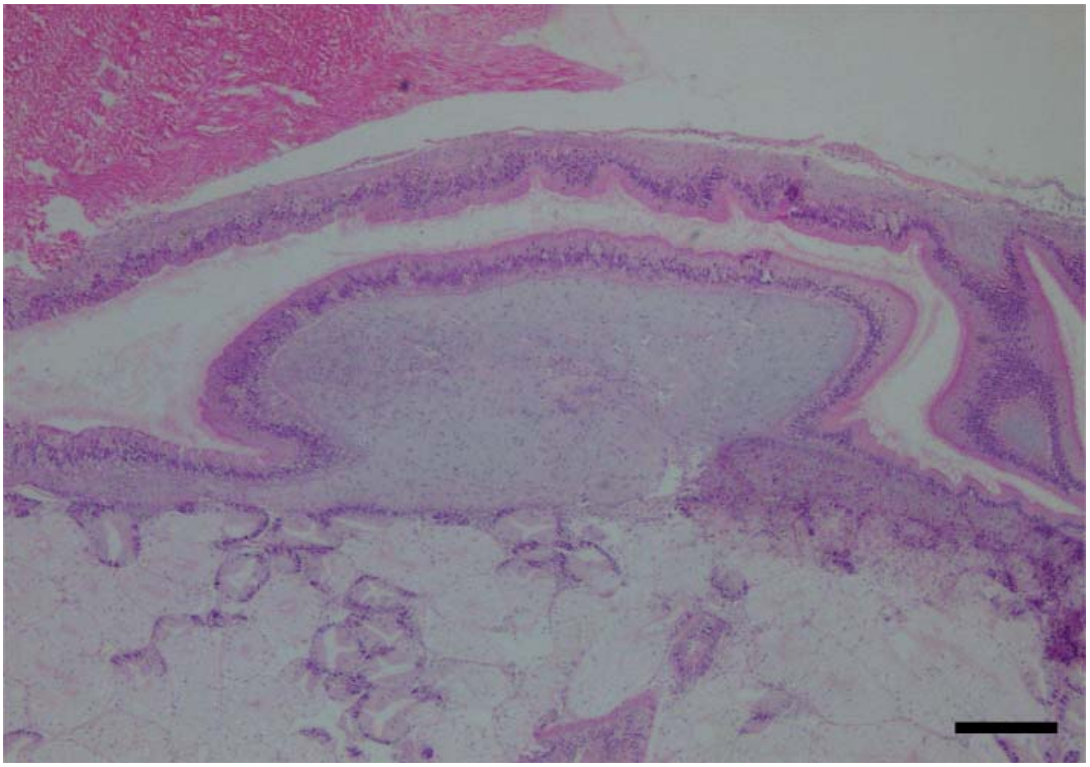

Figure 13. Histological section of the *E. arcuatus* stomach at the anterior cavity (HHE, Scale=100 µm).

The mouth is followed by a short slightly-curved esophagus (Fig. 12) communicating with the stomach. Both mouth and esophagus have a ciliated epithelium with cells secreting mucus, whose function is to lead the particles towards the stomach. The stomach is an irregularly-shaped sac (immersed in the mass of digestive diverticula) divided into three compartments by a muscular wall arising from the anterior wall and expanding into a prominent muscular papilla in the middle area (Fig. 13). The anterior area has two cavities, dorsal and ventral, both covered by a thick cuticular layer forming the gastric shield. The posterior part is not divided by walls, but has several folds in a dorso-central direction (Bloomer, 1901a; Graham, 1931).

Bloomer (1901a) suggests these terms to name the stomach compartments: esophagus portion (anterior-ventral cavity), cardiac portion (anterior dorsal cavity) and pyloric portion (posterior cavity). The pyloric portion (Fig. 14), in its posterior part, contracts in order to form the style sac, which is very long and extends in the interior of the foot base. The crystalline style is a long rod, protein in nature, which crosses the style sac and the centre of the stomach, reaching the cardiac portion, with a hook-shaped ending. Its function is the mixing of food, and it participates in extracellular digestion in the stomach by rotation and the secretion of enzymes. *S. marginatus* has a crystalline style longer than that of *E. arcuatus* and *E. siliqua.*

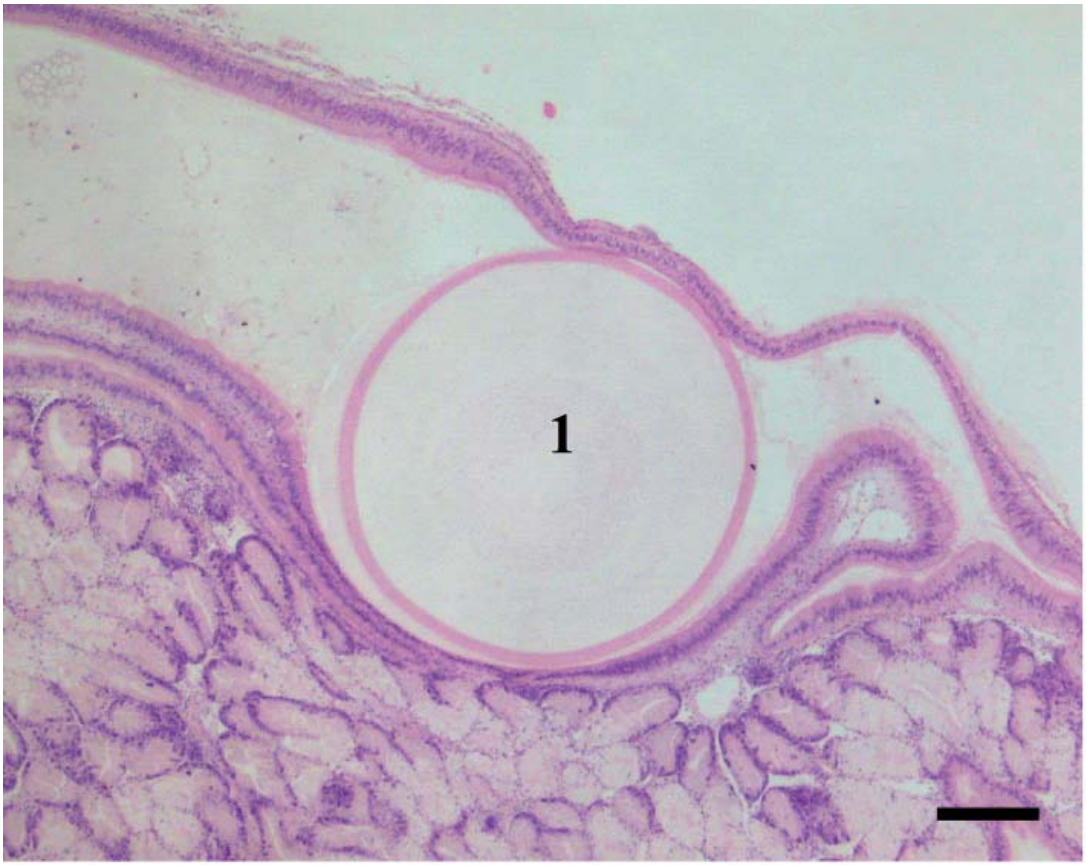

Figure 14. Histological section of an *E. arcuatus* stomach at the posterior cavity or pyloric portion, where the crystalline style is located **(1)** (HHE, Scale=100 µm).

The stomach communicates with the esophagus in the anterior-ventral part (esophagus portion), with the gut in the posterior-ventral part with the style sac in the posterior-dorsal area and with the digestive gland going through the ducts. The digestive gland is a voluminous organ located around the stomach and covering most of it. It is brownish-green and has two lobes which are indistinguishable at first sight, the right lobe spreads on the anterior and right side, while the left lobe covers the left side and is ventrally extended towards the posterior part, under the style sac. The digestive gland of *S. marginatus* is almost totally immersed in the base of the foot, while in *E. arcuatus* and *E. siliqua* only a small part of this organ is in this position.

Intracellular digestion takes place in the digestive gland, which is composed of ducts, starting in the stomach and progressively ramifying, ending in blind tubules (Fig. 15). Primary or main ducts, arising from the stomach wall, have a ciliated part and a smooth part, while secondary ones are not ciliated and have a shorter diameter. Finally, digestive tubules have two differentiated parts, one part consisting of several digestive cells full of vacuoles and the other consisting mainly of secretory cells and flagellated cells (Fig. 16). In ducts, substances flow in two directions, incoming

material goes to the gland for intracellular digestion and absorption, while waste material returns to the stomach and intestine.

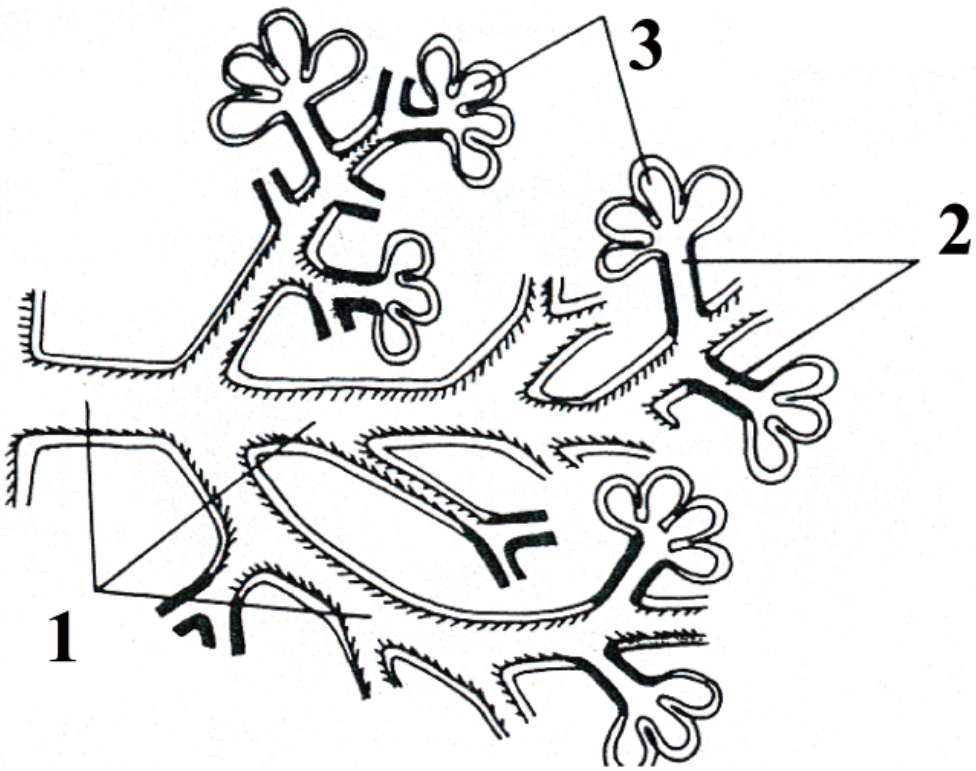

Figure 15. Diagram of digestive glands: **(1)** primary ducts; **(2)** secondary ducts **(3)** digestive tubules.Taken from Auffret *et al.* (2003) and modified.

The mid-gut arises from the posterior-ventral part of the stomach (Fig. 17) and passes into the base of the foot surrounding the style sac, rising towards the dorsal part and continuing with the rectum (Fig. 18). It crosses the pericardium and reaches the anus, located in the branchial chamber, immediately to the rear of the posterior adductor muscle. The epithelium of the rectum has many mucocyte cells (Fig. 19).

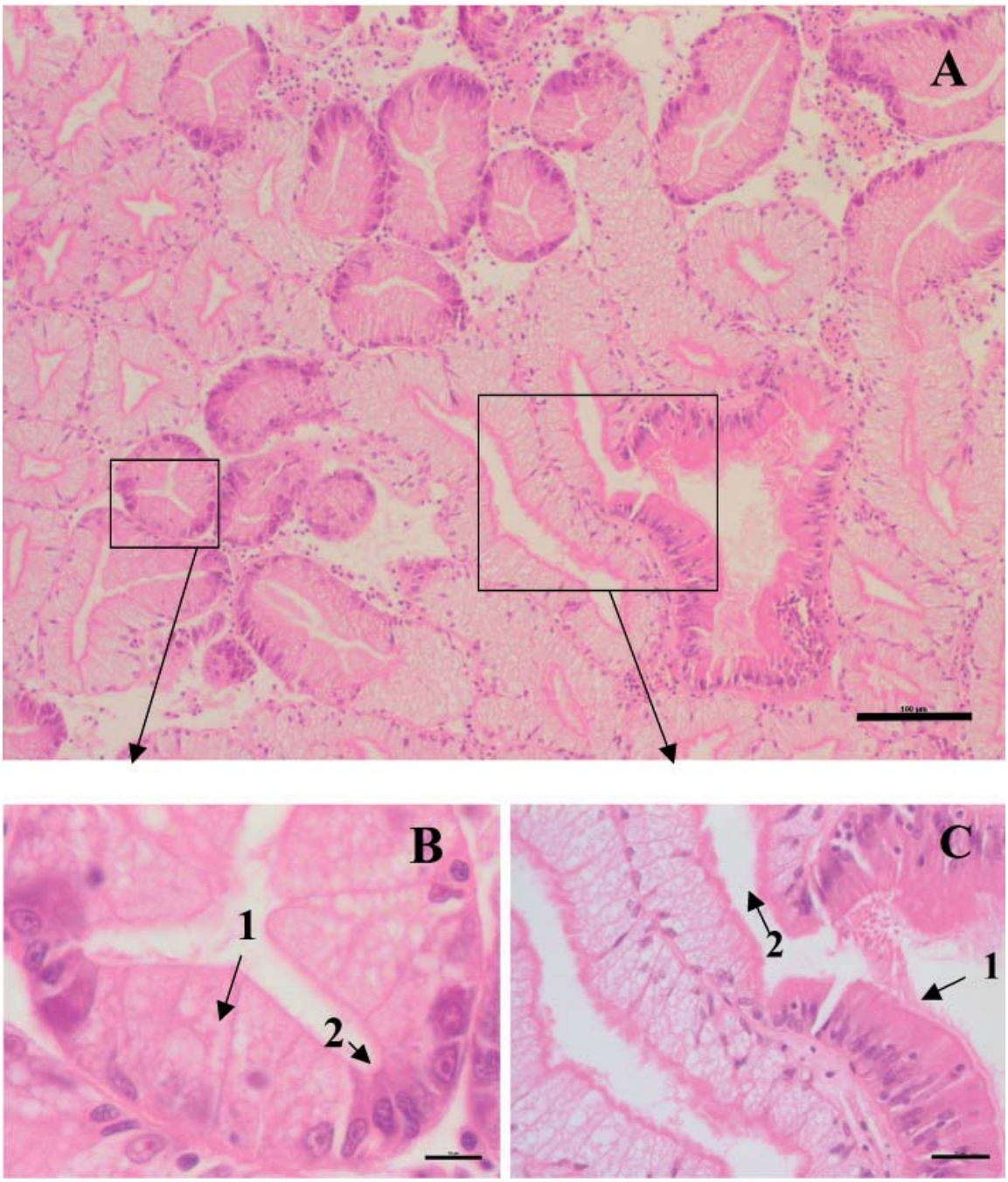

Figure 16. Histological section of *E. arcuatus* digestive gland (HHE). **(A)** General view with primary and secondary ducts, and digestive diverticula (Scale=100 µm). **(B)** Details of a digestive tubule with digestive cells (1) and non-digestive cells (2) (Scale=10 µm). **(C)** Details of an area of the digestive gland with a primary duct with ciliated epithelium (1) and a non-ciliated secondary duct (2) (Scale=25 µm).

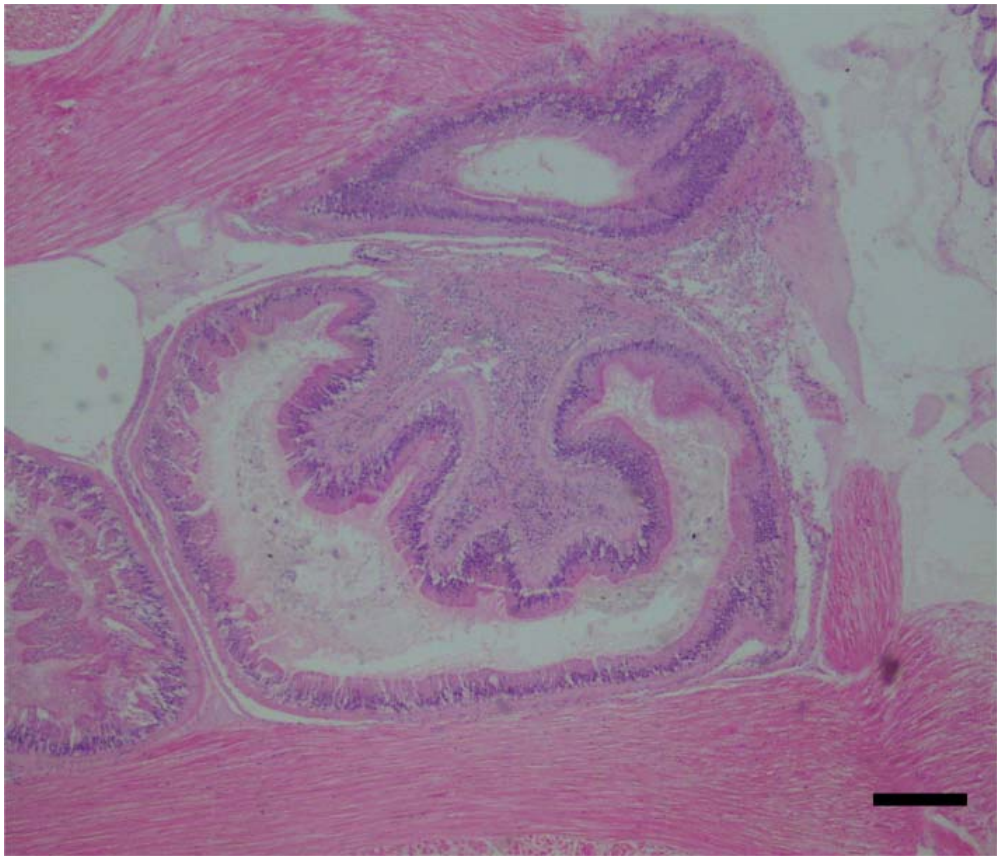

Figure 17. Histological section of *E. arcuatus* midgut at the base of the foot (HHE, Scale=100 µm).

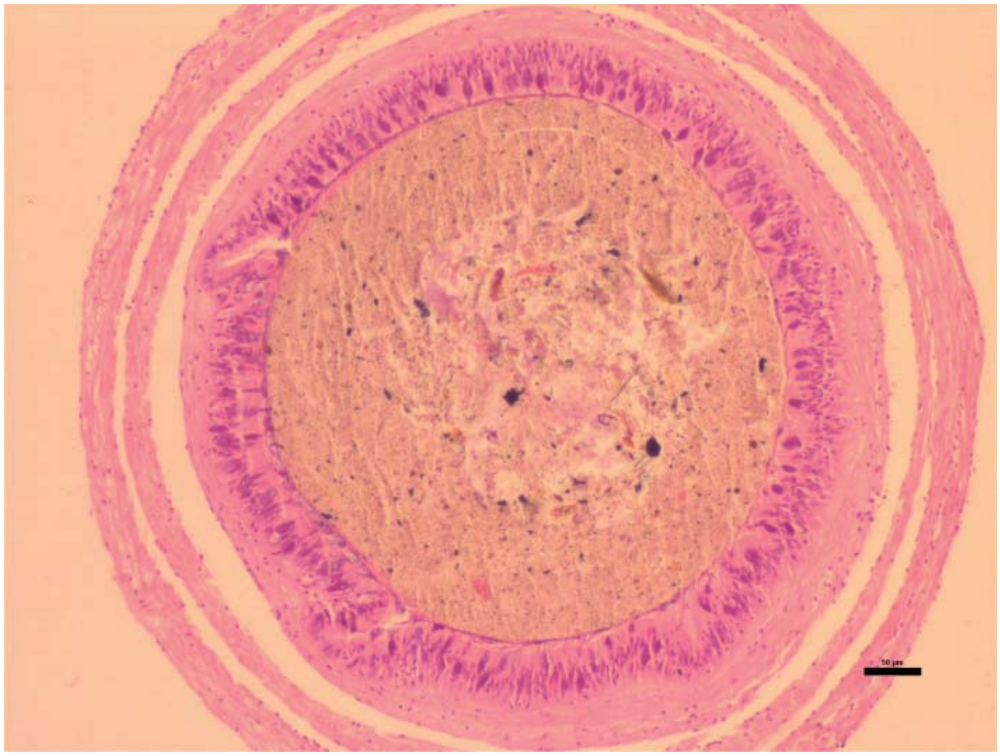

Figure 18. Transversal histological section of *E. arcuatus* rectum, in its final section (HHE, Scale=50 µm).

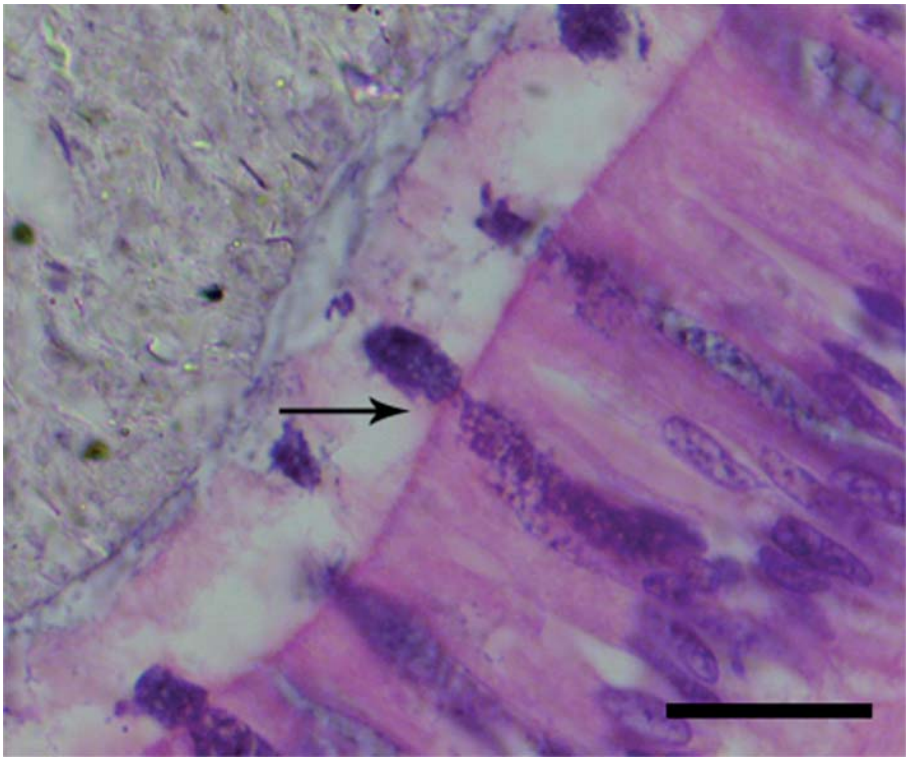

Figure 19. Histological section of *E. arcuatus* epithelium rectum, with mucocyte cells (→) in secretion (HHE) (Scale=10 µm).

2.7. Nervous system

The nervous system of Solenidae is similar to the majority of lamellibranchs. It is composed of pairs of ganglia emitting nerves that ramify and reach different parts of the body (Fig. 20) (Graham, 1931).

The pair of cerebro-pleural ganglia is located in an anterior position and on both sides of the mouth (Figs. 21 and 22A). Macroscopically, it can be seen as two triangular yellowish stains (Fig. 21). Each cerebro-pleural ganglion is formed by the fusion of the cerebral and pleural ganglia (and possibly also the oral ganglion).

The pair of visceral ganglia is located immediately beneath the integument of the ventral surface of the body, very close to the point where the posterior retractor muscle of the foot diverges. Both visceral ganglia are very close and seem to be just one ganglion, although they are not joined.

The pair of pedal ganglia is located in the dorsal part of the base of the foot. Both ganglia are fused into an egg-shaped structure which shows no trace of the double nature (Fig. 22B). At one small point they receive the connectives coming from the cerebro-pleural ganglia, and on the distal side five thick nerves emerge, which reach the foot muscles.

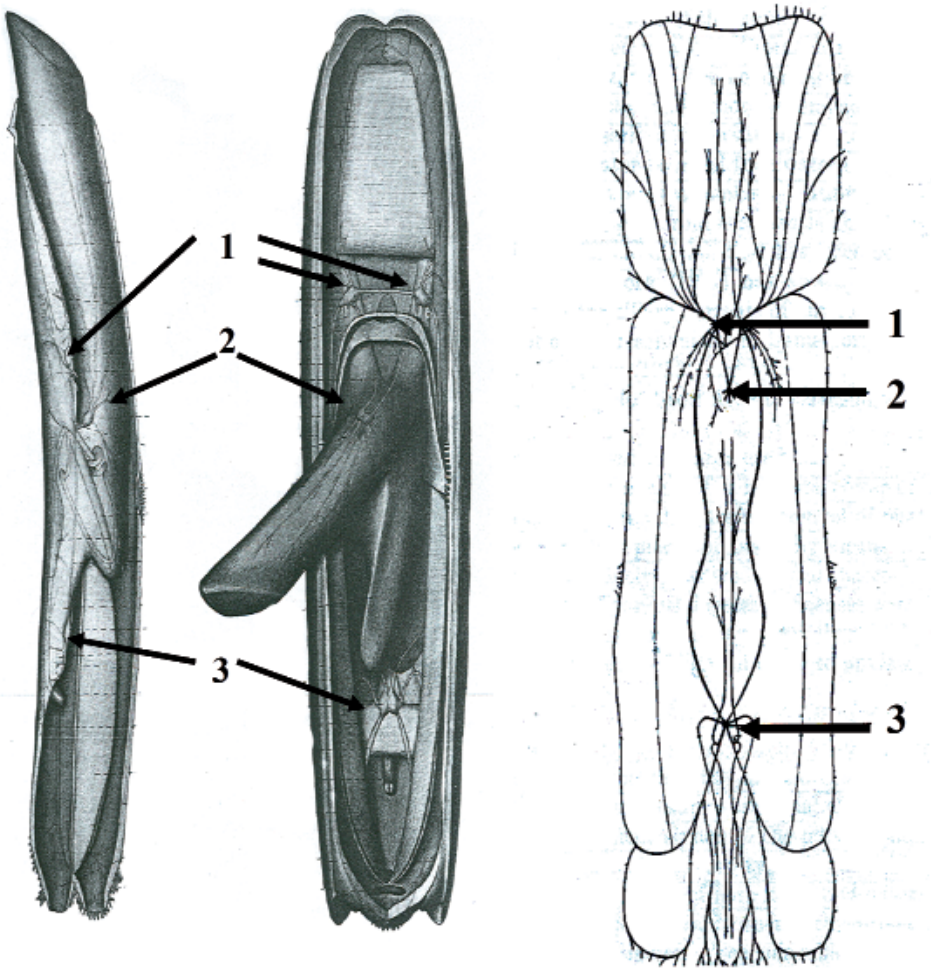

Figure 20. Diagram of the Solenidae nervous system, where pairs of ganglia are highlighted: **(1**) cerebro-pleural ganglia; **(2)** pedal ganglia and **(3)** visceral ganglia. Taken from Drew (1907) and Graham (1931) and modified.

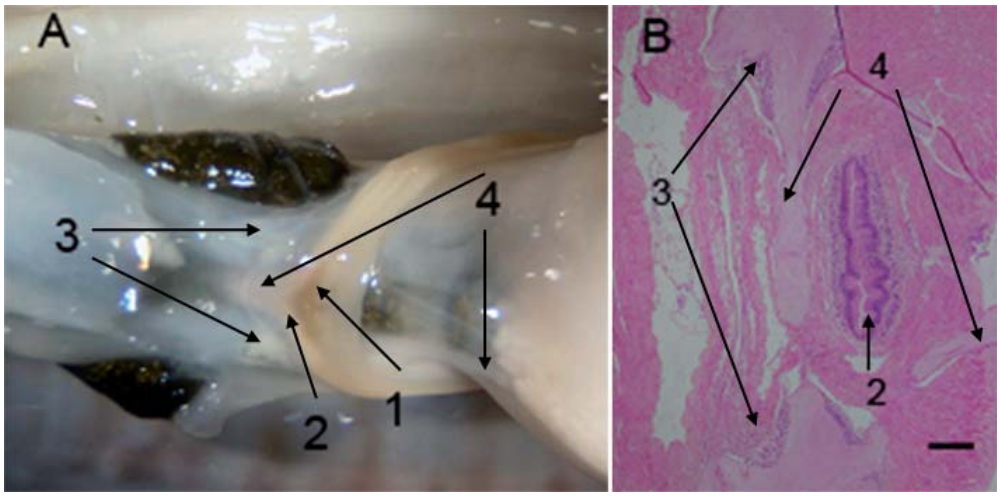

Figure 21. (A) Macroscopic view of anterior area of *E. arcuatus* foot base: mouth (1), esophagus (2), cerebro-pleural ganglia (3) and nerves (4). **(B)** Histological section showing: esophagus (2), brain pleural ganglia (3) and nerves (4) (HHE, Scale=100 µm).

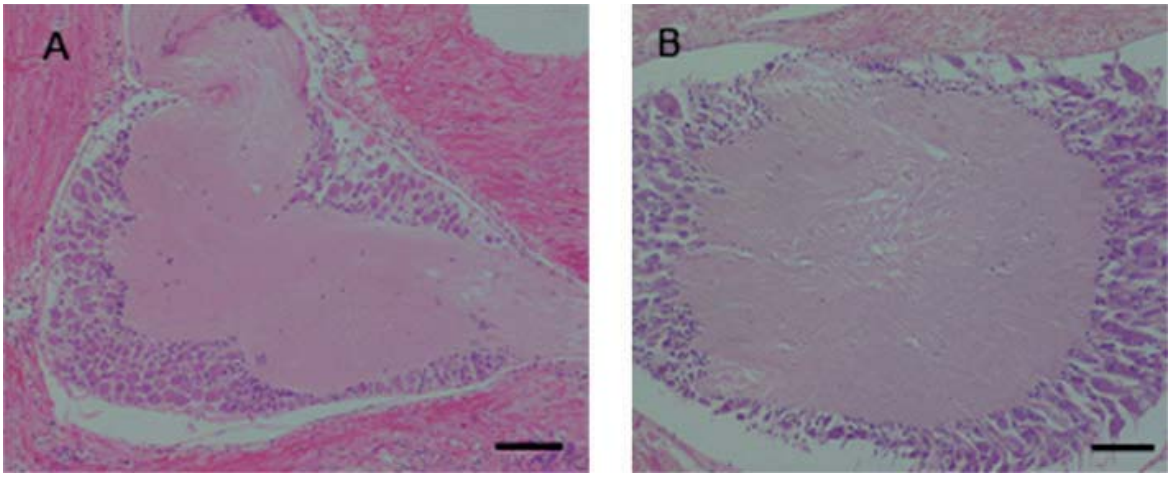

Figure 22. Histological sections of *E. arcuatus* ganglia. **(A)** Cerebro-pleural ganglia. **(B)** Pedal ganglion (HHE, Scale=100 µm).

2.8. Excretory system

The excretory system in bivalves is formed by a pair of kidneys and pericardial glands, though the body surface, and mainly gills, may also emit excretion products (Bayne *et al.,* 1976). The pericardial gland consists of a tubular gland immersed in a large hemolymphatic sinus (Fig. 23A).

The kidney consists of a network of nephridial tubules with an epithelium of columnar cells (Fig. 23B). Kidneys and pericardial glands open onto the pericardial cavity, where the heart and arterial bulb surrounding the rectum are located. Residual products are accumulated in specific cells of the pericardial glands, and periodically they will be discharged to the pericardial cavity, which in turn will transfer them to the kidneys through the pericardial ducts. Other cells of the pericardial glands are probably involved in hemolymphatic filtration as a first step towards forming the urine. Later, the products of filtration flow through the pericardial ducts towards kidneys, where the reabsorption process occurs, obtaining the urine that will be excreted, with a high concentration of ammonium and small amounts of amino-acids and creatinine (Bayne *et al.,* 1976). In Solenidae, the kidneys are easily identified by their brown colour and because they are located dorsal to the base of the foot, under the heart.

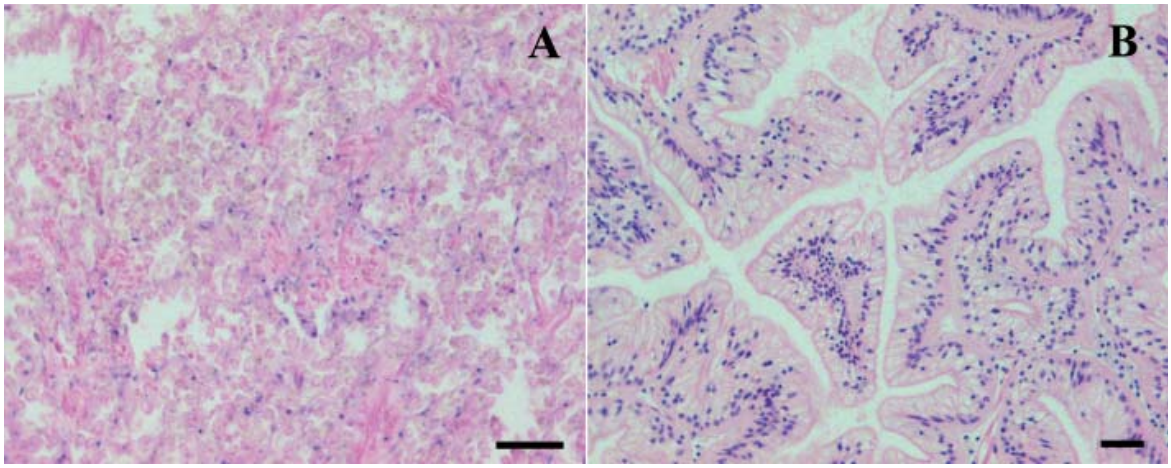

Figure 23. Histological section of the *E. arcuatus* excretory system. **(A)** Pericardial gland. **(B)** Kidney, composed of nephridial tubules of columnar epithelium (HHE, Scale=50 µm).

2.9. Reproductive system

The reproductive system of bivalves has pairs of gonoducts, main genital ducts and several minor channels ending in a network of follicles or alveoli. There is no sexual dimorphism and most of them are gonochoric (separate sexes). Often, the gonad forms a mass that surrounds the intestinal loop in the visceral region (Oysters and Venerids), also invading the mantle in the case of Mytilidae, while in Pectinidae it forms an independent organ protruding from the visceral mass. The formation of gametes in males (spermatogenesis) and females (ovogenesis) takes place in gonadal follicles with a series of cells, which are typical of each phase of the process, leading to the production of spermatozoids and mature oocytes, which are emitted. In some species, sexual products are expelled by renal ducts (Pectinids), although frequently they are released by way of orifices that do not depend on the renal system (Mytilidae). In Solenidae, the gonad forms an independent organ on top of the anterior adductor muscle covering the digestive gland, surrounding the intestinal loops and invading the inner part of the foot in the period of maturity.

Throughout the reproductive cycle different stages of gametogenic development take place, changing the gonad appearance (macroscopically and microscopically). These topics are dealt with in the chapter on reproduction, where a comparative study is made of the three species of commercial Solenidae in Galicia.

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Chapter 3: Reproduction

Susana Darriba Couñago and Dorotea Martínez Patiño

Abstract

Reproduction of several species of bivalves has been studied extensively during the last decades, mainly in commercial species, due to the fact that reproduction research is essential for culture development and fishery management. However, investigation into Solenidae reproduction is more recent. Solenidae species existing in Galicia (NW Spain) (*Ensis arcuatus, Ensis siliqua* and *Solen marginatus*), have been exploited commercially without considering scientific criteria, due to the lack of information about its reproductive strategies. During the last few decades, a great effort has been made to study reproductive patterns and other biological aspects of commercial species on Galician coasts in order to improve management and make progress in aquaculture development. The results obtained have shown that the three species are gonochoric. They have an annual reproductive cycle, which is synchronic, with a varying sexual rest phase depending on the species. It is very long in *E. siliqua* (summer-autumn) and very short in *S. marginatus* (August-September) while in *E. arcuatus* it is medium-length (summer). *E. arcuatus* is characterized by the duration of the spawning period, with spawning during almost the whole of the winter and spring seasons, while *E. siliqua* and *S. marginatus* focus the emission of gametes in a few weeks during the spring. Among the differences between the genera, we would point out the different location of gonadal tissue in the mature stage, appearing in the inner part of the foot and covering the digestive gland and anterior adductor muscle in *E. arcuatus* and *E. siliqua*; while in *S. marginatus* it only develops inside the foot. Another important difference is the absence of reserve cells in *E. arcuatus* and *E. siliqua* gonads, while in *S. marginatus* vesicular cells can be observed. The colour of the gonad is also different in *Solen* species (white in males and brown in females); while *Ensis* genus species are whitish in both sexes, with slight changes only in the mature phase.

1. Introduction

It is essential to have a good knowledge of the reproductive cycle to ensure

the correct management of harvesting practices and the rational exploitation of this species. The basic principle in fishery management is that any captured individual must have reproduced at least once. This aim is usually achieved with a dual approach: determining the minimal size during the first stage of maturity, which would be the minimal size for capture, and defining maturity and spawning stages in order to establish the “close season”. Also, culture development of new species for aquaculture depends on obtaining juveniles from adult breeding stocks; so it is essential to know the reproduction strategies of the species to be cultured.

Regarding Solenidae, there was a lack of information about reproduction, so its exploitation did not follow scientific criteria. An important effort had been made in order to finish with this situation and to investigate this and other biological aspects of the commercial Solenidae species on our coasts. In this chapter we present the main results obtained in research studies about this species during the last decade.

The reproductive cycle is defined by Seed (1976) as “the entire cycle of events from activation of the gonad, through gametogenesis to spawning and subsequent recession of the gonad”, differentiating the reproductive period from a vegetative or rest period. The stages of the cycle among individuals may be synchronic or asynchronic, in last case spawning may occur at different times of the year, such as is the case in tropical species.

Reproduction is a cyclical process that can be annual, semi-annual or continuous. It is determined by the interaction between endogenous and exogenous factors, since the reproductive cycle is a genetically controlled response to the environment (Sastry, 1979). The effect of environmental parameters on the reproductive process has been the subject of research for decades in numerous studies. The most important parameters are the temperature and quality of food available.

Parasites are another external factor that may affect the reproductive cycle of bivalves. Parasites of different taxonomic groups have been described as influencing reproduction. Among them we find trematode (Phylum Platyhelminthes), since they use bivalve molluscs as intermediary hosts in their complex life cycles. Sporocyst and cercaria larval phases are common in bivalves gonads, which act as primary intermediary hosts (see Chapters 6 and 7: Pathology) and appear as a frequent cause of castration in hosts when the normal development of the gonad is interrupted, due to a mechanical effect of compression and tearing of gonadal tissue or due to the depletion of the host energy reserves (Lauckner, 1983; Ponurovsky and Yakovlev, 1992; Coustau *et al*., 1993; Hooker and Creese, 1995; Zhokhov and Pugacheva, 1995; Calvo-Ugarteburu and McQuaid, 1998; Ramón *et al*., 1999).

Research methods regarding marine invertebrates reproductive cycle are numerous and we may differentiate, in a generic way, between direct and indirect methods. Indirect methods are based on detecting larvae in plankton or on settling seeds in collectors, allowing us to estimate the spawning period. Direct methods are based on gonadal tissue analysis.

A direct method for investigating the reproductive cycle is by monitoring the seasonal variation of the volume occupied by the gonad, observing it macroscopically and elaborating a gonadal condition index (GCI). Gonadal condition indexes are mathematical relations, usually simple ones, established between biometric variables easy to determine (such as gonad weight / valve weight). They are frequently used in reproductive research into marine invertebrates (Grant and Tyler, 1983), since the methodology is simple and economical, providing information about the sexual development of individuals. The analysis of temporal variations of these indexes provides useful information for monitoring the reproductive cycle of bivalve populations, however this is not enough to determine the anatomical and physiological changes that take place in the gonad. This restriction may be settled by using another complementary direct method, consisting of a microscopic analysis of the histological sections of the gonadal tissue of bivalves. It is a time-consuming and more expensive technique, but allows us to obtain detailed information about gametogenic development at structural level.

The analysis of histological preparations can be tackled with a qualitative or quantitative approach. The former is based on the elaboration of gametogenic scales, including several discrete stages that classify the different phases the gonad passes through during its maturity cycle. Definition of these scales is based on histological observations of a piece of gonadal tissue, and scales are created based on qualitative details of the descriptive type. Quantitative analysis is based on the calculation of the volume of the the different cellular types occupying the gonad, which are the stereological techniques that have been used since the end of the 1970´s.

In order to devise a gametogenic scale we must consider the germ cells present in the gonad at all times, the size of follicles and the presence or absence of connective tissue, among other factors. To achieve this, it is necessary not only to have specific training in recognizing different types of cells under the microscope but also knowledge about molluscs gametogenic cycle: both aspects vary depending on the species.

Gametes formation process (gametogenesis) begins from precursor cells which give rise to gonias. These can be named as: undifferentiated germ cells, gonia stem

cells, primordial germ cells, primordial gonocytes and protogonias. They are found in periferal areas of gonadal tubules and by active multiplication they create other type of cells known as primary gonias (spermatogonias in males and ovogonias in females). These cells are smaller and have limited cytoplasm, their multiplication allowing the gonad to grow or to be restored.

In males, primary spermatogonias multiply, giving rise to definitive spermatogonias, which are distinguished in primary spermatocytes that become detached from the wall of the follicle, remaining in a continuous layer. They undergo meiosis, giving rise to secondary spermatocytes, which stay within an inner layer, and later to spermatids. Spermatids differentiate in order to become mature spermatozoids, which are located in the centre of the follicle, with the flagellum pointed to the centre.

In the female, primary ovogonias are attached to the follicular wall, some of them remaining in rest stage, while others are immediately divided, giving rise to secondary ovogonias: some of them undergo meiosis and others remain at rest. Oocytes, once formed, begin to grow, and continue to do so until the end of ovogenesis. The ovocyte growth phase is divided into two stages: previtellogenesis and vitellogenesis. During previtellogenesis growth is slower, the nucleus reappears and the cytoplasm increases its volume. During vitellogenesis the chromatin becomes blurred, the nucleus increases its size and reserves are accumulated in the cytoplasm. During the whole process, when oocytes are small they are attached to the wall and when they increase in size they are joined by a peduncle and finally when they are mature, they appear floating in the lumen (free oocytes).

“Atresia” or “oocyte lysis”, that is, the disintegration of oocytes, takes place during ovogenesis. This process is particularly obvious at the beginning of the gametogenic activity (lysis of the first oocytes formed), after partial spawning, and at the end of the reproduction period (lysis of non-emitted oocytes). In certain species (*Ostrea edulis* and *Pecten maximus*) oocyte lysis may affect the gonads during certain periods of the year (Lubet *et al*., 1987a and b). On the other hand, mainly at the end of the sexual cycle, hemocytes may resorb the remains of oocytes or spermatozoids lysis, thus self-cleaning the gonad (Lubet *et al*., 1987a and b).

Below are described the reproductive cycles of each species of commercial Solenidae existing in Galicia (NW of Spain).

2. Razor clam reproductive cycle (*Ensis arcuatus*)

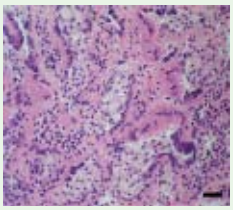
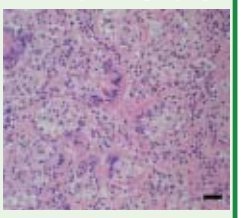
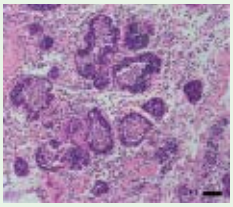
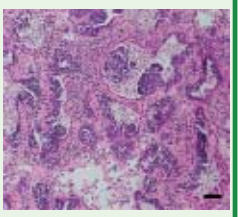
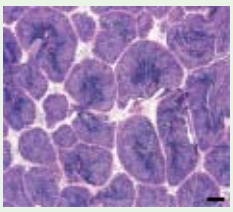
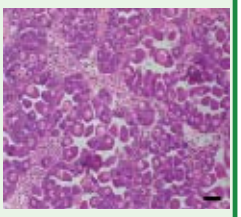
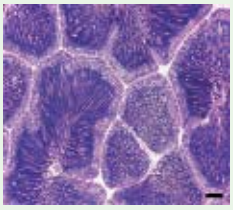
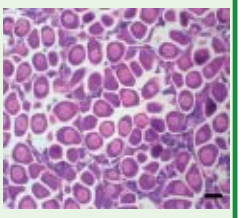
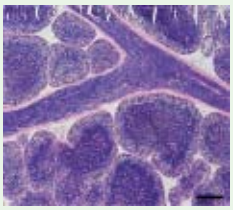
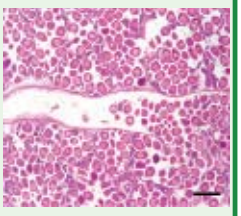
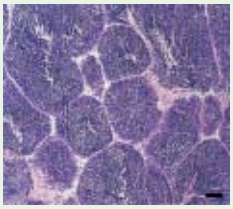
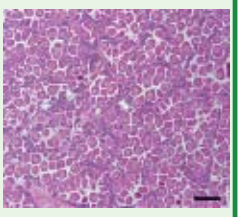
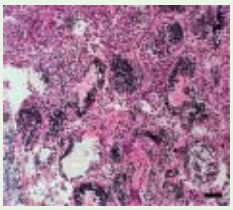
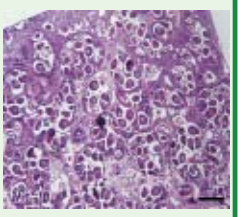
Ensis arcuatus reproductive cycle was studied in detail for the first time on Rodas beach in the Cíes Islands at the mouth of the Ría of Vigo (SW of Galicia) (Fig. 6) (Darriba *et al*., 2004). A gonadal condition index (GCI) was established as GCI=gonad weight / valve weight, and the gonad was monitored by observation of histological sections of the gonads (stained with hematoxylin-eosin) under the microscope. Table I gives a summary with the stages of gametogenic development and its macro- and micro-scopic characteristics, including microphotographs of each one of them for both sexes.

E. arcuatus presents a sexual rest phase (S 0) macroscopically characterized by the absence of gonadal tissue in the inner part of the foot and by the presence of a thin, transparent lamina on the anterior adductor muscle and covering the digestive gland. A few, small isolated follicles were present, consisting of groups of protogonia, and the multiplication of gonias intensifies at the same time as the cycle advances. Abundant hemocytes are present during this stage.

The sexual rest phase takes place during summer and ends in autumn when the gametogenic cycle begins (S I: Start of gametogenesis). Although macroscopically the appearance of gonadal tissue was similar to the description given in the previous stage, microscopically the number and size of follicles increases, with continuing intense gonial mitosis. The first spermatocytes were observed in males and the first previtellogenic oocytes appeared on the follicular wall in females in addition to spermatogonia and oogonia, respectively. The presence of abundant hemocytes is still obvious. This stage is very short, and immediately the number and size of follicles increases, as well as the type of germinal cells that are observed progressing to the stage of advanced gametogenesis (S II).

In stage II (advanced gametogenesis) follicle size increased and occupied the entire tissue, having germinal cells in all phases of spermatogenesis (protogonias, spermatogonias, spermatocytes, spermatids and spermatozoids) or of ovogenesis (protogonias, ovogonias, previtellogenic and vitellogenic oocytes as well as mature oocytes free in lumen). At the same time as this development stage progresses, the percentage of mature gametes increases with regard to the other cells of the germinal line. In females the existence of oocyte lysis during this stage and the presence of hemocytes between follicles are also noted. Macroscopically, gonadal tissue was beige in colour in males, and whitish in females, growing in size on the anterior adductor muscle, partly covering the digestive gland and partly developing inside the foot. Stage II takes place in *E. arcuatus* during autumn and part of winter.

Table I. Gametogenic scale applied to *E. arcuatus*, according to Darriba *et al*. (2004).

Stage	Macroscopic characteristics	Microscopic characteristics	Male microphotography	Female microphotography
S 0: Sexual rest	Thin, transparent lamina on anterior adductor muscle and digestive gland.	A few, small isolated follicles were present, consisting of groups of protogonia and gonia in mitosis. Hemocytes are present (Scale=25 µm).		
S I: Start of gametogenesis	Similar to the previous one.	The number and size of follicles increased, continuing intense gonial mitosis. The first spermatocytes were observed in males and the first previtellogenic oocytes appeared on the follicular wall in females. Hemocytes are still present (Scale=50 µm).		
S II: Advanced gametogenesis	Whitish lamina on top of anterior adductor muscle and whitish stains in lamina covering digestive gland.	Follicle size increased and occupied the entire tissue. Germinal cells were present in all phases of gametogenesis; mature gametes represents only minor percentage (Scale=50 µm).		
S IIIA: Ripe	The gonad grew by completely covering the digestive gland and invading the interior of the foot.	Large number of sizeable follicles and polygonal outline mainly full of mature spermatozoids. In females, follicles are also large in size, with thin walls and mainly formed by mature oocytes with polygonal outlines (Scale=50 µm).		
S IIIB: Start of Spawning	Similar to the previous one.	Some male follicles lost the radial arrangement of the spermatozoa. In females, empty spaces were observed in the follicular lumen, mature oocytes had a rounded profile as the packing level decreased and the gonoducts appeared to be full of gametes (Scale=50 µm).		
S IIIC: Restoration	Decrease of gonadal biomass regarding stages S IIIA and S IIIB.	Smaller follicles with a larger amount of germinal cells prior to mature gametes and a low proportion of them, follicular walls are thicker due to the presence of gonias in division and differentiation of gametocytes (Scale=50 µm).		
S IV: Exhaustion	Digestive gland and anterior adductor muscle are partially covered with whitish stains.	Small follicles, some of them with residual mature gametes, others empty and in all of them an invasion of hemocytes swallowing up the non-emitted ones. In females a general lysis of oocytes prevails (Scale=50 µm).		

This species reaches ripe stage (S IIIA) during the beginning of winter, with slight variations depending on the year. Macroscopically, the volume of gonadal tissue is significantly larger than in previous stages. A thick layer of gonadal tissue is observed on top of the anterior adductor muscle, as well as covering the digestive gland, and the foot is completely invaded, losing its ability for fast digging. In this stage, the gonad is beige in colour and of granular texture in males, while in females it is white and of milky texture.

Microscopically, in males the main feature of ripe stage is the large number of sizeable follicles and a polygonal outline mainly full of mature spermatozoids, with the typical radial disposition and fewer germinal cells at the follicle wall, than in previous phases. In females, follicles are also large in size, with thin walls, and mainly formed by mature oocytes with polygonal outlines, free in the lumen and fewer oocytes in maturation attached to the walls, just like oogonia in mitosis.

Once the ripe stage is reached (S IIIA), some male follicles lose the radial disposition of spermatozoids and a lack of organization is observed, indicating the beginning of its emission towards gonoducts. In females, empty spaces were observed in the follicular lumen, and a lower density of free mature oocytes was observed. Mature oocytes developed a rounded profile as the packing level decreased. At the same time, the gonoducts appeared to be full of gametes. Darriba *et al*. (2004) define this stage as “start of spawning” (S IIIB).

During winter and spring, consecutive spawning occurs, interspersed by gonadal restorations (S IIIC). Macroscopically, the decrease in gonadal tissue volume was notable in relation to previous weeks. Microscopically, in males, smaller follicles can be observed with a larger amount of spermatogonia, spermatocytes and spermatids, as well as a low proportion of spermatozoids and sometimes empty spaces in the centre of lumen, shown by an increase in the thickness of the peripheral layer. In females, restoration after spawning (S IIIC), is also characterized by the smaller size of follicles and the thickness of the walls due to the presence of gonia in division, oocyte proliferation in the first stages of maturation and the small number of mature oocytes. Ovocytary lysis is found in some follicles.

In June the gonad is totally emptied and enters the last stage, called “exhaustion” (S IV). Its characteristic is the existence of few and very small follicles, some of them with residual mature gametes, others being empty, and in all of them an invasion of hemocytes swallowing up the non-emitted ones. In females a general lysis of oocytes prevails. Macroscopically, the gonad is very small.

The succession of the stages of gonadal development is reflected in gonadal

biomass variation during the year. Figure 1 shows *E. arcuatus* gonadal condition index (GCI) during 1998/1999 and 1999/2000 cycles at the bank on Rodas beach in the Cíes Islands (Ría of Vigo – SW Galicia; Fig. 6). In each annual cycle a period with minimal values is repeated during summer, GCI increases during autumn, and a period of consecutive maxima (consecutive spawning) during winter and spring. The annual pattern may suffer slight variations according to the year: in January 2000 a first spawning was observed, while in January 1999 the mature stage had not yet been reached.

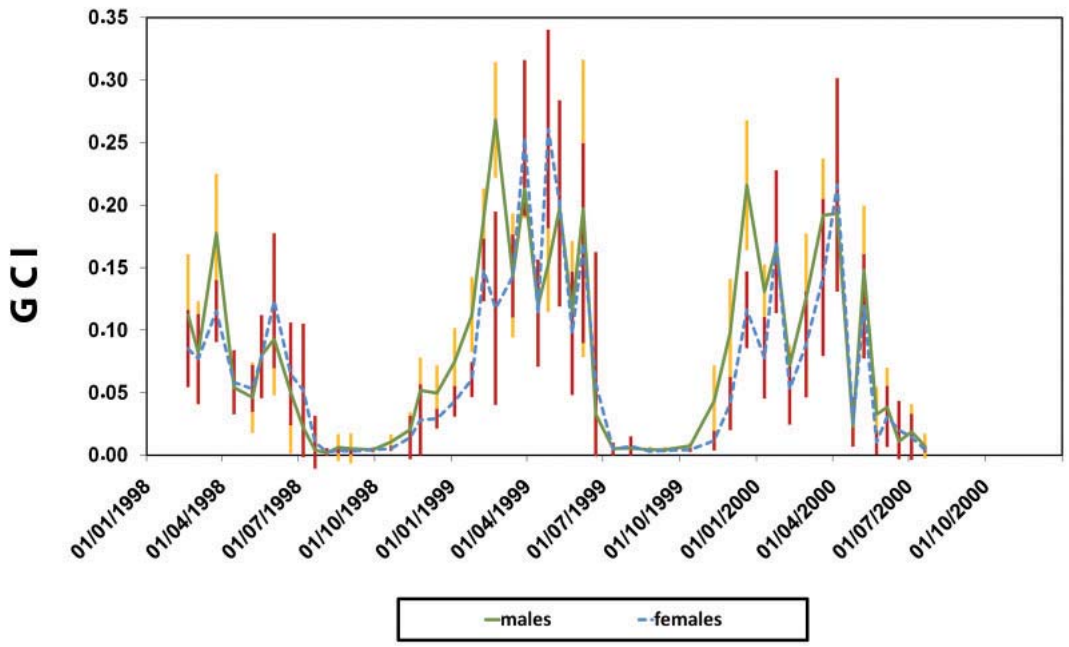


Figure 1. Evolution of *Ensis arcuatus* gonadal condition index (GCI) from February 1998 to July 2000 in males and females at the bank on Rodas beach (Cíes Islands in the Ría of Vigo – SW Galicia). Taken from Darriba *et al*. (2004).

The lengthy spawning period observed in *E. arcuatus* in the Ría of Vigo (SW of Galicia) was not detected in other countries in reproductive studies for other species of the same genus. *Ensis minor* in different regions of Italian coast have a localized spawning between April and May (Del Piero *et al*., 1980; Valli and Giglio, 1980; Casavola *et al*., 1985; Costa *et al*., 1987), just like *E. siliqua* in the South of Portugal (Gaspar and Monteiro, 1998) and in Ireland (Fahy and Gaffney, 2001), although the months vary. There are studies reporting that *Ensis macha* spawns during summer in Chile (Urban, 1996; Aracena *et al.,* 2003). Two spawning periods have been described in studies about *E. macha* in other areas of Chile and Argentina (Avellanal *et al.*, 2002; Barón *et al.*, 2004) and in *Ensis americanus* in the Dutch Wadden Sea (Cardoso *et al.*, 2009).

Differences in reproductive cycle of species of the same genus (inter-specific

differences) or of the same species in different geographical locations (intra-specific differences) have been widely studied in molluscs. Investigations of environmental conditions for evaluating its influence in reproductive cycle development have been widely analysed in several papers, as described in the introduction of this chapter. Temperature and food availability, influencing the reproduction variations between species and locations, are the parameters most frequently studied.

Darriba *et al*. (2004) analysed the relation between temperature and food availability with the *E. arcuatus* reproductive cycle in the Ría of Vigo (SW Galicia), highlighting a clear relationship between oceanographic phenomena and gametogenic cycle (Fig. 2). These authors observed that gametogenesis ceased during summer, coinciding with high temperatures, high surface water temperature, upwelling of cold water, longer daylight periods and phytoplankton blooms, which increase food availability. Gonad development and gametes formation take place during autumn, when upwelling stops, water gets colder and food availability decreases. Successive spawnings, which take place between winter and the beginning of spring, end when water temperature increases and with the first upwelling of the year, followed by the phytoplankton bloom. After comparing results of different species of *Ensis* genus in different locations, Darriba (2001) suggested the existence of a strategy contrasting with the rest of molluscs, concluding that gonadal development of *E. arcuatus* seems to be activated when temperature decreases to a specific value, and inhibits when it increases, which happens during summer, a sexual resting period ensuing.

Salinity is another oceanographic parameter having an important influence on the development of the *E. arcuatus* reproductive cycle. Darriba and Miranda (2005) achieved very important results from the research of *E. arcuatus* reproductive cycle in the Ría of Vigo from 1998 to 2004 (Fig. 3). The heavy rain that fell during the autumn of 2000 and winter of 2001 caused the decrease of salinity to a lower level than usual, close to 24‰, while temperature and food availability were similar to other years (data recorded by Intecmar and provided for this research). During that period, the gonadal condition index showed unusually low values until salinity recovered the usual values in the spring of 2001. Authors concluded that salinity decrease interrupted gonadal development during months when in normal years successive spawning and restorations would take place.

The fact that the gametogenic process needs a large amount of energy and that, for *E. arcuatus*, it takes place in winter when food availability is low, was studied in detail by Darriba *et al*. (2005b). This study analysed cycle reserves by quantifying the levels of glycogen, glucose, proteins, total lipids and triglycerides in different tissues (gonad, digestive gland, foot and anterior adductor muscle) together with the reproductive cycle.

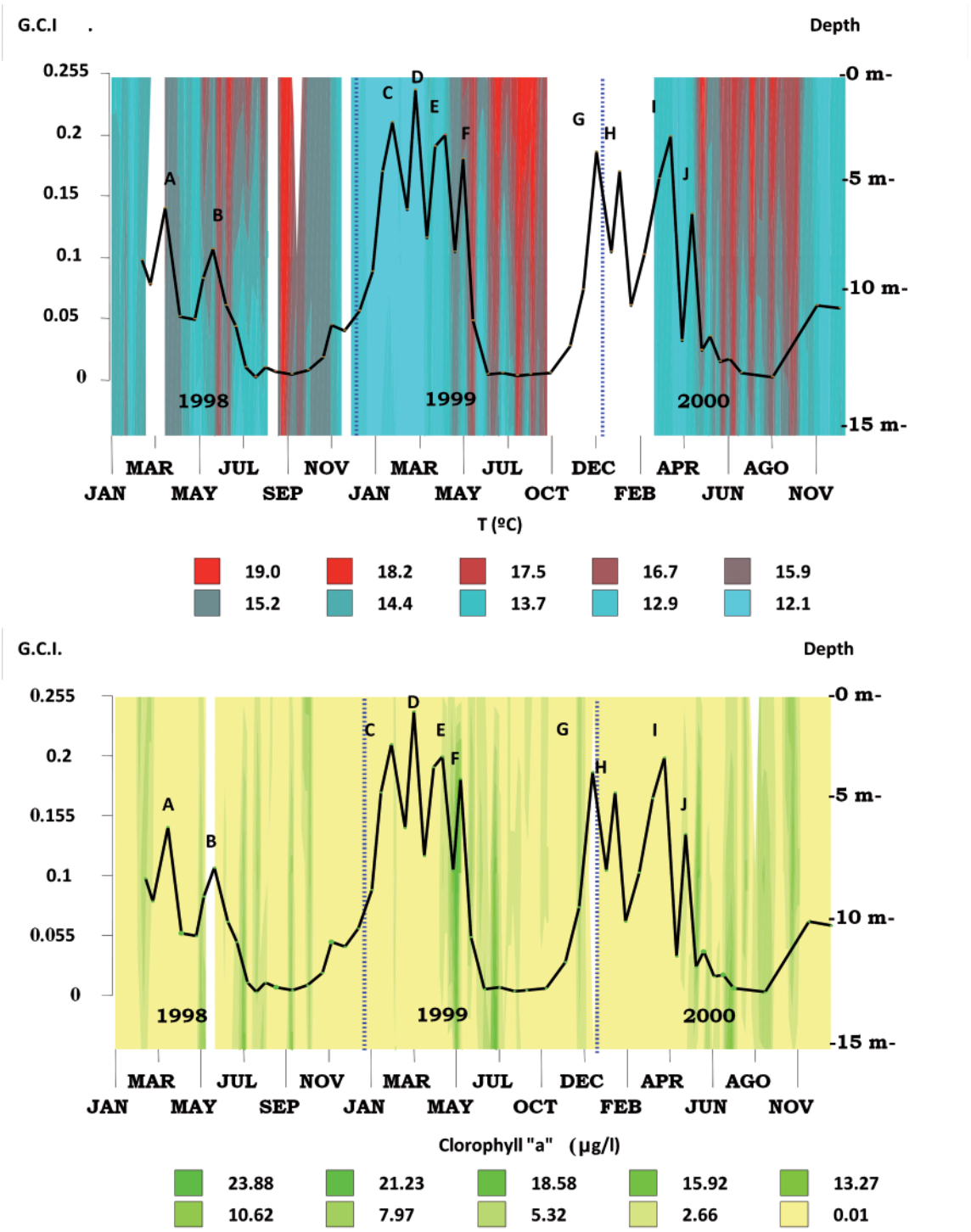


Figure 2. Relation between *E. arcuatus* gonadal condition index (GCI) and temperature in the water column (from 0 to 15 meters) during 1998, 1999 and 2000 (superior). The relation between GCI and the concentration of chlorophyll "a" in the water column during the same period (inferior). Source of environmental data: INTECMAR. Taken from Darriba (2001).

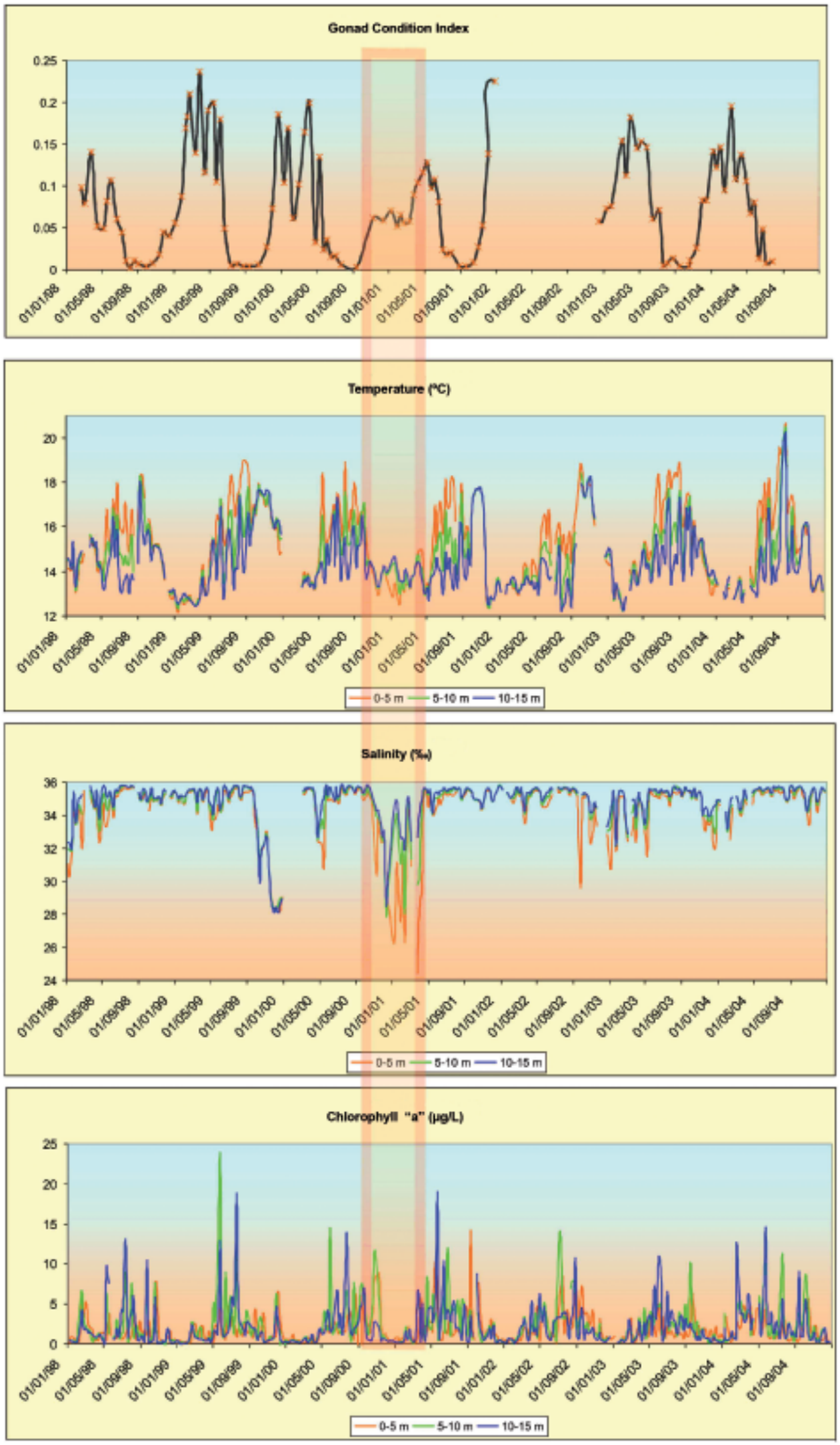


Figure 3. Evolution of *E. arcuatus* gonadal condition index (GCI) on the Cíes Islands (SW Galicia) and evolution of temperature, salinity and chlorophyll "a". Source of environmental data: INTECMAR. Taken from Darriba and Miranda (2005).

For decades we have known that the gametogenic process requires a large amount of energy, so there is an important relationship between the reproductive cycle and the availability of energy for growing (Bayne, 1976; MacDonald and Thompson, 1986). It is also known that bivalve larvae need an important lipid reserve for their metabolism, while adults use glycogen as an important reserve (Holland, 1978). Gametogenesis may take place depending on the food just ingested and/ or the energy stored in several tissues, creating an important store of lipids in the vitelline reserves that larvae will benefit from.

According to Lubet (1996) the annual delay and blocking of gametogenesis (sexual rest) observed in many coastal species coincide with a period for storing energy reserves in order to cope with the effort of reproduction. Darriba *et al*. (2004) highlighted the lack of reserve cells and the existence of a large number of hemocytes in *E. arcuatus* gonad from the beginning of the cycle, suggesting that hemocytes act as a transmission channel of nutrients for the gametes that are being formed, and as a way for obtaining reserves from degraded gametes, complementing the contribution of external nutrients and the degradation of reserves accumulated in other tissues.

E. arcuatus, in the Ría of Vigo during summer, stores reserves since there is great availability of food due to phytoplanktonic blooms. Reserves are accumulated as lipids, mainly triglycerides, in the digestive gland, and glycogen in the anterior adductor muscle and foot. In autumn gametogenesis begins, and the amount of food available in the environment decreases, so gonadal development take place at the expense of reserves accumulated in somatic tissues which decreases its condition index at the same time as GCI increases (Darriba *et al*., 2005b) (Fig. 4).

Variations of biochemical components regarding the reproductive cycle and environmental conditions define the reproductive strategy of the species. Bayne (1976) divided bivalves into two groups, depending on the strategy followed. The group of “conservative” species performs gametogenesis in autumn-winter at the expense of the nutrient reserves accumulated during the previous summer. The “opportunist” species are in gonadal resting phase during autumn-winter and gametogenesis takes place in spring when the amount of food available in the environment is enough to supply the process’s demand for energy. *E. arcuatus* shows a reproductive strategy closer to that of the conservative species.

The most important beds for *E. arcuatus* in Galicia (NW Spain) are in the Rías Bajas (Fig. 6). Darriba *et al*. (2005a) studied the reproductive cycle of this species, simultaneously in time, in a subtidal bed in the mouth of the Ría of Vigo (Cíes Islands) and in a low intertidal bed of the Ría of Arousa (Means-Cambados) proving

the non-existence of intraspecific differences in the reproduction of species, with occasional exceptions.

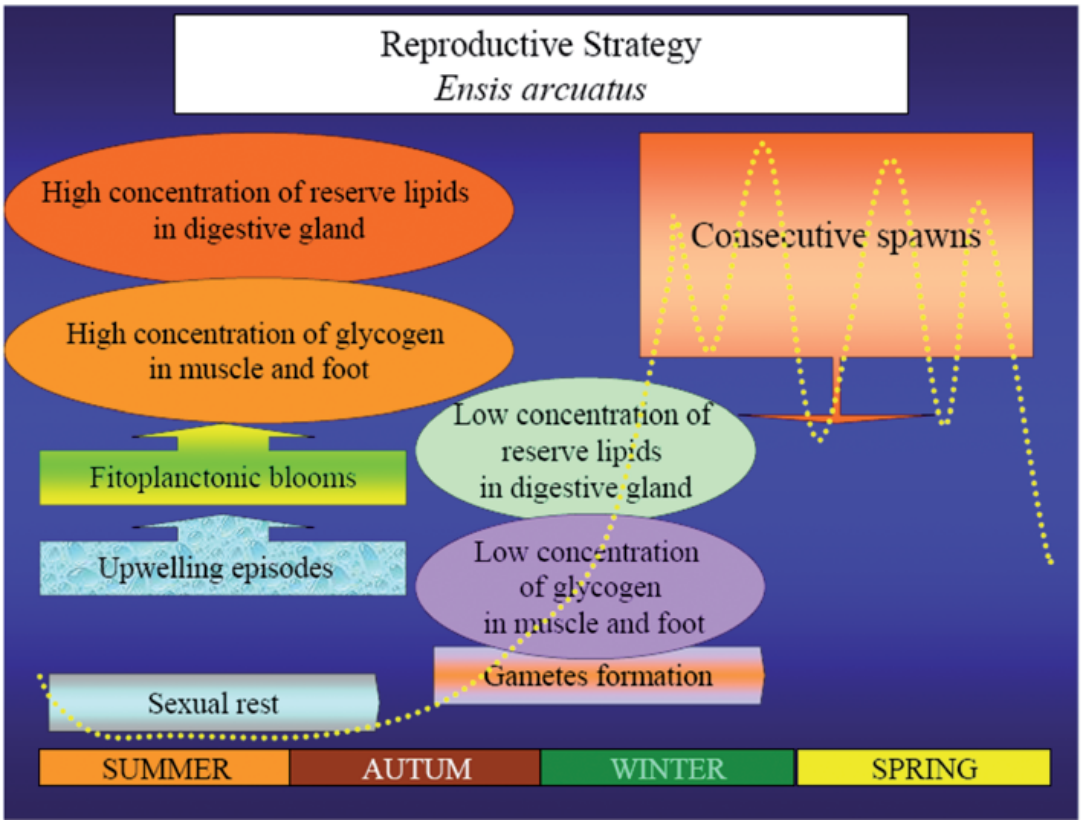


Figure 4. Annual pattern of the gonadal condition index (dotted line) of *E. arcuatus* in the Ría of Vigo, regarding the concentration of reserved substances in different tissues and the oceanographic situation of the Ría.

3. Pod razor clam reproductive cycle (*Ensis siliqua*)

The reproductive cycle of *Ensis siliqua* species was researched for the first time by Gaspar and Monteiro (1998) in the south of Portugal. Subsequently, in Galicia (NW Spain) researchers studied in beds of the Ría of O Barqueiro (N Galicia) (Martínez-Patiño, 2002) and in the Ría of Corcubión (NW Galicia) (Darriba *et al*., 2005c) (see locations in figure 6).

The location of gonadal tissue in *E. siliqua* is similar to that of *E. arcuatus.* For histological studies of the reproductive cycle, these authors used scales based in 6 stages, similar to that described earlier for *E. arcuatus* (Table I). None of these studies mentions the existence of a gonadal restoration stage (S IIIC), observed by Darriba *et al*. (2004) for *E. arcuatus.* This species, like *E. arcuatus* and *S. marginatus*, is dioica, gonochoric or unisexual (separate sexes). Nevertheless, as was pointed

out by Sastry (1979), it is not unusual to occasionally find hermaphrodite samples in strictly gonochoric species. Darriba *et al*. (2005c) found 0.5% of hermaphrodite specimens in *E. siliqua* in the Ría of Corcubion (NW Galicia) and Valli and Giglio (1980) in *E. minor* (0.4%) in Italy.

E. siliqua reproductive cycle is annual, the sexual rest period is longer than in *E. arcuatus*, maintaining this condition during almost the whole summer and autumn period (Fig. 5). The beginning of the cycle takes place at the end of autumn-beginning of winter, with slight variations. Gonadal development takes place during winter, and when the spring begins, maturation is reached. Gamete release is concentrated between the end of April and the beginning of June in Galicia in the Ría of O Barqueiro (1994) (Martínez-Patiño, 2002) and in the Ría of Corcubión (2000-2001) (Darriba *et al*., 2005b). The same species spawns in April-May in the South of Portugal (Gaspar and Monteiro, 1998) and in May-July it does so in Ireland (Fahy and Gaffney, 2001).

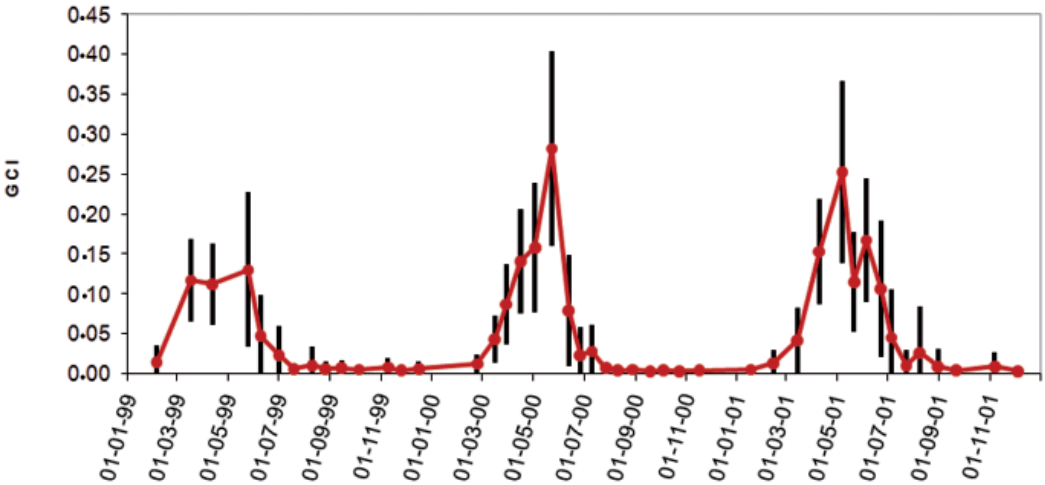


Figure 5. Evolution of *E. siliqua* gonadal condition index from February 1999 until December 2001 in Fisterra (Ría of Corcubión). Taken from Darriba *et al.* (2005c).

Darriba *et al*. (2005c) applied the gonadal condition index (GCI = gonad weight / valves weight) defined for *E. arcuatus* in Darriba *et al*. (2004) and propose its use as a routine and economical technique for managing the resource and for establishing the annual close season just when the reproduction season of the species take place.

Martínez-Patiño (2002) completed the gametogenic cycle study of this species, analysing the water temperature and concentration of chlorophyll “a” (food availability). It is concluded that spawning occurs when the highest temperature is reached, as well as food availability, helping larvae to develop in the environment.

Differences observed between *E. arcuatus* and *E. siliqua* in Galicia (NW Spain), seem to be caused by the different environmental conditions between the Galician Rías Altas and Rías Bajas. From the first study it was concluded that *E. arcuatus* in the Ría of Vigo (Darriba *et al*., 2004) had a shorter sexual rest period and a longer spawning period, while *E. siliqua* in the Rías of O Barqueiro (Martínez-Patiño, 2002) and Corcubión (Darriba *et al*., 2005c) has a longer sexual rest and a shorter spawning period.

In order to analyse the possible differences between species (interspecific) and in the same species in different locations (intraspecific), between 2003 and 2004, the cycles of both species were simultaneously monitored in locations with different environmental conditions (Darriba *et al*., 2005a). *E. arcuatus* was studied in the subtidal bed of the Cíes Islands (North mouth of the Ría of Vigo, SW of Galicia, Fig. 6) and in the intertidal bed of Means beach (Cambados-interior of the Ría of Arousa, SW of Galicia). *E. siliqua* was monitored in two subtidal beds in different coastal areas with different environmental conditions (Barra - Ría of Vigo and Fisterra - Ría of Corcubión, N of Galicia), both in open areas. The results obtained with *E. arcuatus* in the Cíes Islands and *E. siliqua* in Barra beach (both in the northern mouth of the Ría of Vigo, SW Galicia) confirm the existence of interspecific differences detected in the first study. This research also concludes that there are no intraspecific differences for any species in the different locations that were analysed.

4. Reproductive cycle of grooved razor shell (*Solen marginatus*)

In Galicia, the reproductive cycle of this species has been studied by Rodríguez Moscoso *et al*. (1996) and Martínez-Patiño (2002) in a natural bed of the Ría of Ortigueira (NW Galicia, Fig. 6). Similar studies have been carried out in Asturias (Northern Spain) for the same species in different natural beds (López *et al*., 2005; Remacha-Triviño and Anadón, 2006).

Like the others, it is considered a gonochoric or unisexual species. The gonad is not an anatomically differentiated organ; it invades the visceral mass and is inserted along the length of the foot as happens with *Mercenaria stimpsoni* and *Spisula sachlinensis* (Kasyanov, 1989), though it does not cover the digestive gland or the anterior adductor muscle, as occurs with *Ensis* species. Macroscopically, the sex can be clearly determined, since the gonad is white in males and brown in females. Throughout the cycle we can observe a similar evolution in both sexes, although it is slower in males. To describe the gametogenic cycle, Martínez-Patiño (2002) used the qualitative scale shown in Table II.

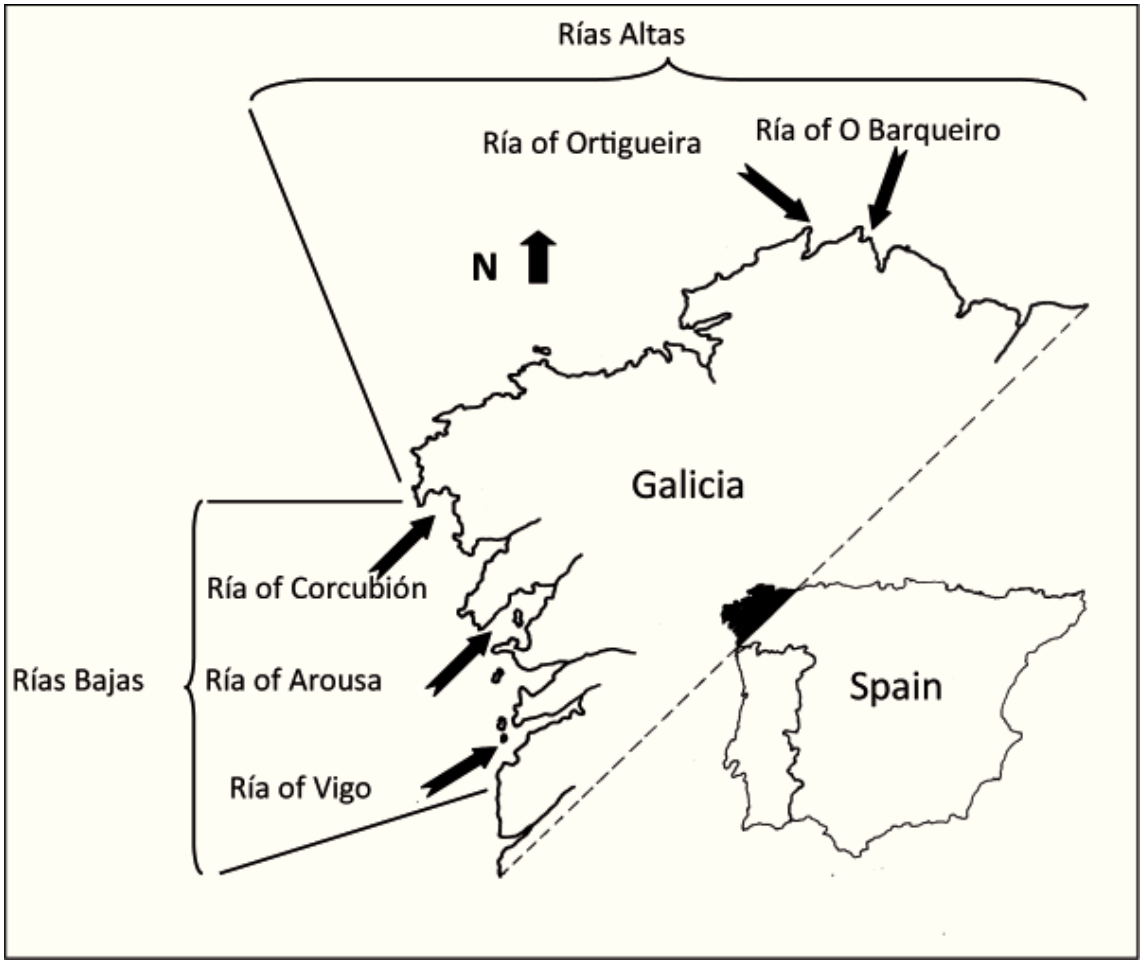
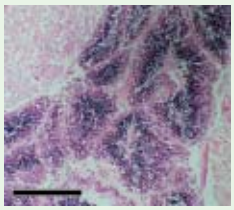
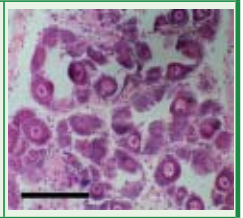
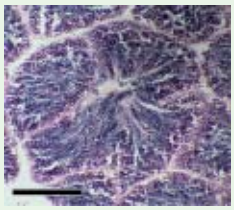
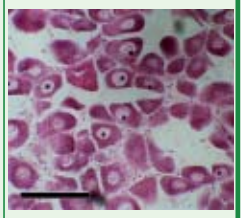
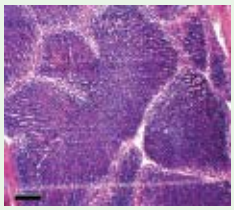
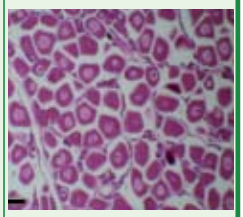
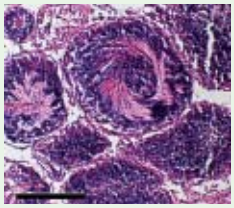
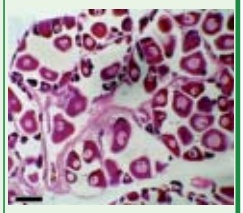
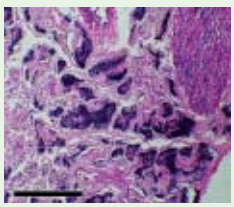
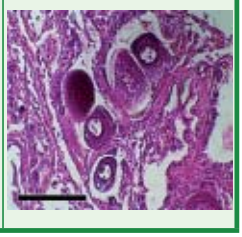


Figure 6. Location of Galician natural beds described in this study.

The results obtained by Martínez-Patiño (2002) show that *S. marginatus* has a short period of sexual rest (S 0) going from August to September, which extends until October in part of the population (Fig. 7). The characteristic of this phase is a decrease in its gametogenic activity which makes it difficult to ascertain the sex, due to the presence of vesicular cells (reserve cells) and due to a major development of the muscular tissue.

Table II. Gametogenic scale applied to *S. marginatus* by Martínez-Patiño (2002). Scale=100 µm.

Stage	Macroscopic characteristics	Microscopic characteristics	Male microphotography	Female microphotography
S 0: Sexual rest	Whitish plain visceral mass is observed.	Sexes are not distinguished due to the absence of follicles and gametes. It is characterized by a large amount of connective tissue.		
S I: Start of gametogenesis	This stage is not distinguished macroscopically.	There is still a large amount of connective tissue, follicles are small. Females have oocytes in the initial stages of development, encrusted in the walls of follicles. In males spermatogonia are observed in the walls of follicles.		
S II: Advanced gametogenesis	Gonad occupies most of the mantle and visceral mass, with different colours, depending on the sex. Brown in females and white in males.	Lack of interfollicular connective tissue. The size of follicles increases. In females most oocytes are free in the lumen, though there are some stuck to the walls by a thin peduncle, with polygonal outlines, since ovogenesis has not yet finished. Most males spermatozoids are placed in radial columns oriented towards the centre.		
S III: Ripe	Gonad reaches its maximum development. Difference of colour is clearly observed, depending on the sex.	Connective tissue has been replaced by follicles full of gametes. In females, oocytes are spherical or polygonal in shape. Male spermatozoids are disorganized and free in lumen. In both sexes sexual cells are observed in different development stages.		
S IV: Spawning	The wall of the visceral mass becomes more flaccid. Colouring still depends on sex.	There are completely empty follicles, and others still have mature sexual cells. As spawning progresses, connective tissue is restored. The latter was limited and disperse until now, as a consequence of follicle diameter decrease. Several amebocytes appear.		
S V: Post-Spawning	In this stage non-emitted gametes give the characteristic colour for males and females. Gonad becomes totally flaccid.	Large amounts of connective tissue and presence of some residual and isolated spermatozoids and oocytes.		

Gametogenic activity (S I) begins during October and will last until December in some individuals. Although in November a small part of the population is already in an advanced gametogenesis stage (S II), the whole population reaches this stage in January and it will extend until April in almost half of the individuals analysed, while the others are already mature (S III). In May the whole population reaches maximum maturity and some start gamete release. Massive spawning (S IV) takes place at the end of May and during June. This period of massive spawning in the natural environment coincides with spawning obtained in aquaculture facilities (Martínez-Patiño *et al.,* 2000). At the end of June-July males and females undergo a weak gonad restoration, leading to spermatozoid phagocytosis in males and ovocytary lysis in females (S V) before the rest period starts. From November to April cytoplasmatic vacuolization and oocytary lysis is observed in some oocytes in previtellogenesis and vitellogenesis, with resorption of non-emitted gametes. Restoration of the gonad is possible thanks to the energy contributed by its own gametes.

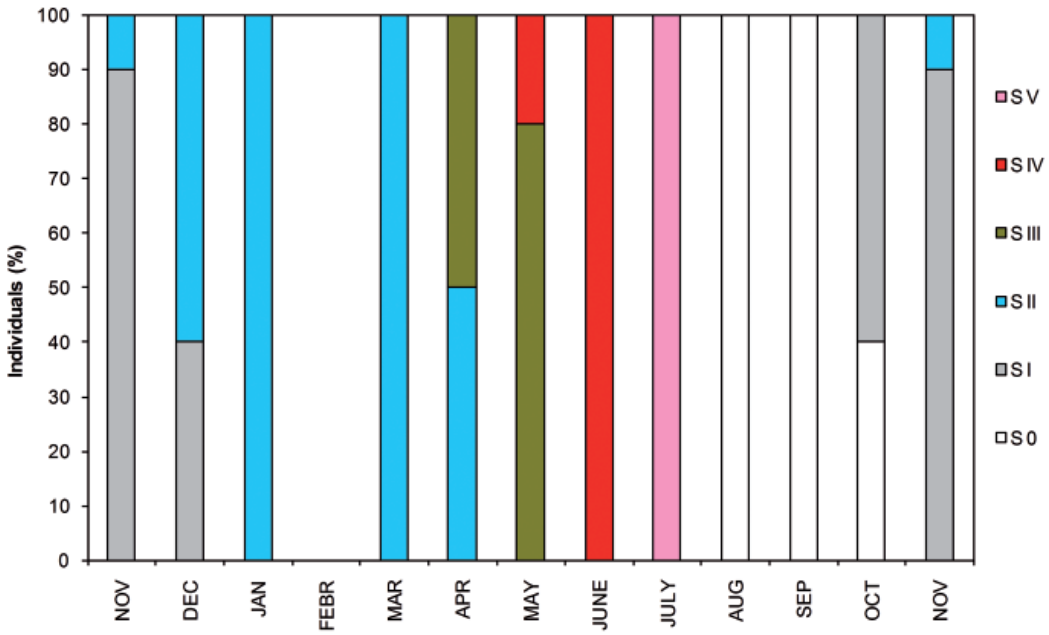


Figure 7. Representation of percentage of individuals in different stages of the *S. marginatus* gametogenic cycle.

The existence of a short spawning period once a year in *S. marginatus* coincides with what was found in other Solenidae species. In *E. minor* (Casavola *et al.,* 1985) in the Gulf of Manfredonia (Southern Adriatic Sea), the spawning period takes place in March-April coinciding with the increase of water temperature. In a study with *E. directus* about recruitment carried out on French North Sea coasts, an important recruitment is found in June, indicating a spawning period probably between April-May (Luczak *et al.,* 1993). In the South of Portugal (Gaspar and Monteiro, 1998) *E. siliqua*

have their spawning period in April and May, while the same species, on Irish coasts, have their spawning from mid-May to the end of July or beginning of August (Fahy and Gaffney, 2001) and in Galicia between the end of April and the beginning of June (Martínez-Patiño, 2002; Darriba *et al*., 2005c). Breese and Robinson (1981) in Newport (Oregon), studying *Siliqua patula* species indicate that the spawning period is from May to July. On the Chilean coasts (Aracena *et al*., 1998) *E. macha* species have just one spawning period, in November and December.

Regarding the sexual rest period, this species strategy is different from that of *E. siliqua*, since *S. marginatus* have a short rest period, in August-September, while *E. siliqua* have a longer rest period (summer and autumn in the Iberian Peninsula) (Gaspar and Monteiro, 1998; Martínez-Patiño, 2002; Darriba *et al*., 2005c), coinciding, though not in the same season with the extended rest period of *E. minor* in Italy, in Manfredonia Gulf (Casavola *et al.,* 1985) and on the coast of Tuscany (Costa *et al*., 1987), which is from May to November. Also on the Italian coasts, but in the Gulf of Trieste, Valli and Giglio (1980) and Valli *et al*. (1985) report the sexual rest period is between June and September.

The results obtained by Martínez-Patiño (2002) in the North of Galicia for *S. marginatus* and *E. siliqua*, in natural beds located in intertidal areas in two rias close to each other with similar environmental conditions (Ría of Ortigueira and Ría of O Barqueiro) suggest the existence of differences in the gametogenic cycle due to the differences between species (interspecifics). The duration of the different periods of the gametogenic cycle are different in the two species researched, with the exception of the short spawning period, which is the same in both species. At the same time, *E. siliqua* shares the natural bed with other species of bivalves, *Donax trunculus,* which have an extensive spawning period (March-April) and a very short sexual rest period (Martínez *et al*., 1993).

Martínez-Patiño (2002) studied the biochemical composition of *S. marginatus* in different tissues throughout the reproductive cycle. From the results obtained he emphasizes that proteins are the main biochemical component in the organism (43 to 67%), followed by lipids (10%) and glycogen (1 to 14%). Gills have the highest concentration of lipids in dry weight, with a low mobility rate during the cycle; similar results were found by Rodríguez-Moscoso (2000) in *Ruditapes decussatus*, concluding that stored lipids have a main role as structural lipids (i.e. membrane components) and thus lipids are not used as storing reserves. Visceral mass with the gonad is second highest in lipid concentration and it is the one with most variations in total lipids during the cycle, following the evolution of the gametogenic cycle, with positive values during maturity and negative values during and after spawning. The highest concentration of proteins was found in the foot. There is most glycogen in

the muscular ridge of the mantle, then in the foot, adductor muscles and visceral mass. The muscular ridge, foot and adductor muscles have their lowest values during gametogenesis, while visceral mass, where the gonad is included, has the highest value in May, coinciding with maturity, and decreasing as spawning occurs. This seems to indicate that there is a glycogen mobilization of these organs towards the gonad for the formation of gametes, which is expressed as an increase of all biochemical components in maturity. All biochemical components researched in different tissues show highest values in August and at the beginning of autumn, coinciding with an optimal physiological state of the animal. This species accumulates glycogen during the sexual rest period, above all in the aforementioned parts.

S. marginatus reproductive strategy is adapted to the conservative model, storing energy reserves to be employed later in reproduction. It accumulates glycogen and transforms it into lipid reserves that are stored mainly in the visceral mass, where gonads are included. Glycogen is a good indicator of the physiological state of individuals. Spawning mainly produces a loss of lipids and proteins (Martínez-Patiño, 2002).

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Chapter 4: Selection and retention of particles in the diet of Solenidae

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Abstract

Ensis arcuatus, *Ensis siliqua* and *Solen marginatus* are three native species found in the Northwest area of Europe, while *Ensis directus* is an invasive species. Its exploitation, distribution and in most cases its biological cycle have been researched, but information regarding its physiology is limited. Only certain aspects of the food acquisition process have been researched, such as retention efficiency, clearance rate and pre-ingestive selection.

For *E. siliqua*, it has been determined that retention is 100% for 8 µm particles while for smaller sizes efficiency declines. The clearance rate was also measured, with an average value at 16±0.5ºC, of 1.11±0.35 $L \cdot h^{-1}$. A clearance rate of 0.084±0.053 $L \cdot h^{-1}$ was obtained for *E. directus*. It was observed that *E. directus* is capable of selecting food before ingesting it. For instance, when given dinoflagellate *Prorocentrum minimum* and diatom *Phaeodactylum tricornutum* (a species of similar size) *E. directus* usually selects the dinoflagellate.

Solen cylindraceus, a species with austral distribution, is the Solenidae where the highest number of physiological parameters has been determined. The retention efficiency and clearance rates obtained (1.16 $L \cdot h^{-1}$ to 25ºC and 1.09 $L \cdot h^{-1}$ to 15ºC) were similar to those for *E. siliqua.* The filtration rate (an average level of 1.14-1.38 $L.h^{-1}$) was measured in relation to several factors that may affect it, such as temperature, salinity and seston concentration. *S. cylindraceus* was fed 90-95% of the time within a range of 5-25 $mg \cdot L^{-1}$ at seston concentrations, at 50 $mg \cdot L^{-1}$ concentrations of food activity decreased to 87% and the decrease was greater, to 68, 50 and 32% of the time when the concentrations were increased to 100, 250 and 500 $mg \cdot L^{-1}$, respectively.

1. Introduction

Growth in bivalves may be directly measured or indirectly estimated by determining the energy balance of the organism: the energy coming from the food

consumed is calculated and the energy consumption of the different physiological processes is deducted. Energy balance is the basis of physiological energy, a discipline which began in the sixties in fish farming (Navarro, 1997). A specific methodology was developed when this research was transferred to the field of bivalves (Bayne and Newell, 1983).

All components of the energy balance equation are sensitive to variations as a response to changes in the environment (Bayne and Newell, 1983). Bivalves must be able to achieve more gain than loss in order to have a remainder of energy necessary for growth and reproduction. Finally, there is a physiological adaptation of the organism to the environment in which it lives. This is why it is necessary to validate the results about energy consumption of the physiological processes obtained in the lab in natural conditions. The data obtained in different conditions, along with the monitoring of environmental features, will offer us a theoretical growth model.

2. Physiological processes

When estimating the food consumed by molluscs it is necessary to know which physiological processes are involved and what environmental factors may affect them. Water pumping, selection at gills, pre-ingestive selection at labial palps, post-ingestive selection in the intestine and assimilation are the physiological processes involved in the acquisition of food.

Environmental factors which may affect them are above all temperature, salinity, phytoplankton concentration and composition, so it is important to know what fluctuations occur throughout the year and in different locations.

The amount of food available for bivalves depends on the concentration of particles in suspension, volume of water transported through gills and the efficiency with which particles are retained (Palmer and Wiliams, 1980). Most bivalves retain particles larger than 4-5 µm with an efficiency of 100%. When sizes of particles are not close to these numbers the retention efficiency decreases (Sobral and Widdows, 2000). Food concentration has a negative effect on retention. At high food concentrations gills are full, causing retention to decrease (Barillé *et al.*, 1993). Food shape and flexibility may also be key factors when selecting: diatoms have a silica frustule and sometimes spicules, so when passing through gills these structures can make retention easier. Flagellates, on the other hand, have a flexible membrane: their shape can be distorted and they pass through gills with greater ease (Bougrier *et al.*, 1997).

In order to determine the efficiency of retention in *S. cylindraceus*, De Villiers

and Allanson (1988), studied the influence of temperature (25 and 15ºC) and salinity (35 and 15 UPS) in this parameter using *Tetraselmis suecica* monoalgal cultures. Results showed that particles of 1.5-2 µm were retained with 15-35% efficiency, those within a range of 2-2.5 µm with 40-60% efficiency and those in a range of 2.5-3 µm with 70-90% efficiency. Maximum efficiency (100%) was achieved with medium-size *T. suecica* cultures, using 7-8 µm particles. Temperature and salinity had hardly any noticeable influence.

Miranda and Blanco (2002) investigated the retention efficiency of *E. siliqua* using a microalgal mixture, with *T. suecica* and *Monochrysis lutheri* species, which covered a range from 3 to 15 µm of equivalent spherical diameter, with average sizes of 4 µm and 8 µm. Cultures were used in different proportions or diets: the first had the same amount of *Tetraselmis* cells as *Monochrysis,* while in the second the ratio was 1 to 8 and in the third it was 1 to 4. Diets were designed in this way in order to check whether the selection capacity in gills was affected by the relative proportions between the types of phytoplankton particles. In order to calculate which species was the one most retained, the final concentration was subtracted from the initial concentration and the result of that operation was divided by the final concentration. Results showed that *T. suecica* was retained with an efficiency of 0.262±0.096 and *M. lutheri* with an efficiency of 0.168±0.076. *T. suecica* was retained more than *M. lutheri* regardless of the relations existing between them. It was assumed that if for *T. suecica* the retention efficiency was 100%, for *M. lutheri* it was 64%. *E. siliqua* behaved like most bivalves. These results were obtained with artificial diets; in a natural environment the highest retention may fluctuate between a range of sizes, just as happens in other species.

Lucas *et al.* (1987) determined the relative retention efficiency of natural seston particles using two samples of *Mytilus edulis* from two different locations in the same seaside area. The results showed that mussels from an estuary retained particles of 16 µm more efficiently, while those from an exposed area retained particles of 10 µm. Differences in retention were not statistically significant.

In *Venerupis corrugatus* relative retention in particles between 5 and 9 µm diameter had an efficiency of 70-100% at low tide (Stenton-Dozey and Brown, 1992). At high tide the same particles were retained with an efficiency of 50-80%. *V. corrugatus* provides maximum retention efficiency to particles between 9 and 13 µm at high tide. These changes were related to the increase of size range in particles at high tide. In other molluscs such as *M. edulis* and *Ostrea edulis* (Vahl, 1972) very low retentions have been registered for the smallest particles.

There are two ways of determining the water volume that can be processed by bivalves for obtaining food, clearance rate and filtration rate. Clearance rate is

defined as the volume of water completely cleared of particles per unit of time. It can be determined with microalgae cells (from culture or natural environment), organic or inorganic particles. There are several methods for measuring it (Riisgård, 2001).

Clearance rates are characteristic to each species, being the result of interaction between different morphological characteristics of the organisms: size and structure of gills, disposition of mantle and siphons (Morton, 1983), etc., and also of environmental parameters existing in the different geographical locations they inhabit.

The succession of stages in an organism's life cycle, just like the changeable environmental conditions, as well as the intrinsic characteristics (geno/phenotypic) of each individual, are the reason why there is a great variability in clearance rates within the same species. Part of this variability is a consequence of the changes in physical-chemical environmental factors, such as temperature, salinity, pH, dissolved oxygen or water flow, which may affect clearance rates; pollutants are another factor: hydrocarbons, especially the aromatic ones, inhibit clearance rates, TBT's produces a neurotoxic effect in the ciliary activity of gills (Widdows *et al.*, 1995). Age, period of reproductive cycle (Chaparro and Thomson, 1998), the state of parasitization (Pérez Camacho *et al.*,1997) and genotypic and phenotypic characteristics of each individual are also factors that may alter rates.

Shumway *et al*. (1985) determined the clearance rate in six species of bivalves, *Ensis directus* among them, using mixtures of cell suspensions of the dinoflagellate *Prorocentrum minimum*, diatom *Phaeodactylum tricornutum* and the flagellate *Chroomonas salina.* The method used was the indirect method (Riisgård, 2001), which is based on measuring the decrease in number of particles that takes place in the experiment tank due to the filtration realised by suspension-feeding bivalves. The rate obtained was 0.084±0.053 $L \cdot h^{-1}$ at 12ºC.

Miranda and Blanco (2002) measured the clearance rate in *E. siliqua* also using the indirect method, obtaining a clearance rate of 1.1±0.3 $L \cdot h^{-1}$ at a temperature of 16±0.5ºC. De Villiers and Allanson (1988) and De Villiers and Hodgson (1993) obtained clearance rates of 1.16 $L \cdot h^{-1}$ at 25ºC and 1.09 $L \cdot h^{-1}$ at 15ºC in *S. cylindraceus*.

Other bivalves show higher clearance rates, such as *Tapes decussatus* with 2-2.5 $L \cdot h^{-1}$ (Sobral and Widdows, 1997), and *Mytilus galloprovincialis* with 4.5-5.5 $L \cdot h^{-1}$ (standardised by length) (Labarta *et al.*, 1997).

Filtration rate, defined as water volume flowing through gills per unit of time, may vary for the same species, due to several factors. Phytoplankton species used

for determining the filtration rate must be retained in the gills with 100% efficiency (i.e. algae diameter ≥ 4 µm for mussels and ≥ 7 µm for pectinidae) in order not to achieve a result that underestimates the rate (Møhlenberg and Riisgård, 1978; Jorgensen *et al.*, 1984; Riisgård *et al.*, 1996).

It is also important that the bivalve for which the filtration rate is being determined has valves wide open, since otherwise the rate would be inferior to the real one.

Filtration rate is greatly influenced by temperature (Winter, 1978): if the temperature increases until it exceeds the optimum condition, the filtration rate will decrease dramatically.

Several experiments have been carried out in order to determine the filtration rate of *S. cylindraceus*. Some were made in order to research the effect of temperature and salinity on filtration rate. These studies share a similar structure: in summer (25ºC and 35 UPS) and winter (15ºC and 35 UPS) some individuals are gathered in groups, then in the laboratory they are subdivided in batches to be acclimatised to several combinations in temperature (from 10 to 40ºC) and salinity (from 5 to 45 UPS), then the filtration rate is determined (De Villiers and Allanson, 1988; De Villiers *et al*., 1989; De Villiers and Hodgson,1993). Several conclusions were reached from the results: for example, at salinity level of 15-45 UPS, the filtration rate reached is 1.14-1.38 $L \cdot h^{-1}$ regardless of the temperature to which they were acclimatised. Filtration rate was influenced by salinity outside that ideal level, the rate decreasing at salinities higher or lower than that range. The season when bivalves are captured has an effect on filtration rate, since values obtained with those captured in winter are always lower than those captured in summer, by a difference ranging from 0.06-0.18 $L \cdot h^{-1}$.

On the other hand, those individuals acclimatised at 35 UPS salinity and at 15, 25 and 35ºC, then subjected to other salinities, underwent an initial decrease in filtration rate. The amount of this decrease correlated with the magnitude of change of salinity and, more specifically, the effect of hyper-saline conditions (increase from 35 UPS to 45 UPS) was less marked than those of hypo-saline conditions (decrease from 35 UPS to 25 UPS). However, after 12-24 hours, filtration rate increased to the values reached by control individuals, the increase being faster in hyper-saline than hypo-saline conditions. But bivalves kept at 10ºC suffered a decrease in filtration rate, and at no time exceeded 0.6 $L \cdot h^{-1}$ in all the salinities used. Individuals subject to salinities of 15 UPS suffered a decrease in filtration rate when exposed to higher and lower salinities. Those exposed to a salinity of 10 UPS, regardless of all the acclimatization temperatures, showed the highest reduction in filtration rate, which was around 0.6 $L \cdot h^{-1}$ during the whole experiment (216 hours). By contrast, filtration rates of individuals

exposed to an increase in salinity recovered control rates in 60-90 hours.

Another experiment was to test the effect of simultaneous changes in temperature and salinity on filtration rates. The response of the acclimatised animals, whether collected in summer or winter, to temperature increase and salinity was an initial increase in the filtration rate, followed by a decrease until it reached the values of control individuals. The response of animals caught in summer to temperature decrease, maintaining salinity or decreasing it, was an immediate and rapid decrease in filtration rate. This decrease was maintained during 12-24 hours and after that it gradually recovered. However, in many cases, filtration rates were not stabilised when the experiment ended (216 hours). The response of animals collected in winter and acclimatised at 10ºC-15 UPS and 10ºC-25 UPS was a decrease in filtration rate that persisted during the whole experiment. Exposure to 20ºC and 15 UPS caused an initial decrease of the rate and then 72 h later it climbed back to its original values.

S. cylindraceus samples showed filtration rates with slight variations between those animals caught in summer and in winter. During summer *Venerupis decussata* and *Crassostrea gigas* showed maximum rates (Walne, 1972), while *M. edulis* and *O. edulis* showed an increase during summer but not a maximum. However, in *Chlamys opercularis* the same filtration rates were observed for individuals caught in summer and winter (Valh, 1972).

In general, in bivalves, starting from a low concentration, when seston concentration increases, the filtration rate quickly increases as well (Winter, 1978) and remains constant when the concentration at which they consume the maximum of food is reached. When this maximum is exceeded the filtration rate decreases continuously in order to keep constant the amount of food consumed. This pattern remains unchanged until the concentration is reached at which the production of pseudofaeces starts.

De Villiers and Hodgson (1993) used natural seston filtered through a 64 µm net and concentrated by centrifugation for their investigations with *S. cylindraceus*. Nine kinds of diets of 5, 10, 15, 20, 25, 50, 100, 250 and 500 $mg \cdot L^{-1}$ were prepared with this concentrate. Results showed that there were no important differences (range 1.12±0.11 to 1.25±0.12 $L \cdot h^{-1}$) regarding the values of filtration rates determined in advance for seston concentrations around 5-100 $mg \cdot L^{-1}$. However, an important decrease was observed in filtration rate for seston concentrations between 250 and 500 $mg \cdot L^{-1}$. Similar responses have been found in other bivalves but with less variation in range of seston concentration. In *Crassostrea virginica* and *M. edulis* (Bayne and Newell, 1983), with natural particulate matter, filtration rate decreases to

zero from seston values of approximately 150 mg·L^{-1}.

Food particles in suspension which have already been retained by gills are transported to the internal face of palps that surround a bivalve´s mouth. Palps control the amount of food that enters the mouth directing the remainder to the rejection tracts of the mantle's surface. This happens when the amount of food in suspension is more than the digestive capacity of the bivalve, when the food rejected is covered with mucus and eliminated as pseudofaeces since it is not processed in the digestive system (Widdows *et al*., 1979). Bivalves have a mechanism of particle selection that has not yet been totally decoded, which allows them to reject inorganic material (Newell and Jordan, 1983; Newell *et al.*, 1989) and certain organic material, depending on the particle, its shape or percentage of organic content (Bayne, 1993).

E. directus have been researched in order to determine preingestive selection with techniques of flow cytometry. For this, Shumway *et al.* (1985) used mixtures of cellular suspensions of *P. minimum* dinoflagellate, diatom *P. tricornutum* and *C. salina* flagellate, which are phytoplanktonn species with similar sizes. Once the proportion of these species in pseudofaeces was analysed, it was observed that diatom was the species most rejected. During the same study *Artica islandica*, *O. edulis* and *Placopecten magellanicus* also rejected the diatom.

Regarding this topic, research is limited and sometimes studies show different results. Cucci *et al*. (1985), researching the behaviour of *M. edulis* fed with a mixed diet of *P. tricornutum*, *Prorocentrum* and *C. salina* similar to the one used for *E. directus*, did not find selection. On the other hand, Bougrier *et al*. (1997) did find preingestive selection in *C. gigas* and *M. edulis*. Both species rejected more *Skeletonema costatum* and *Pavlova lutheri* than *T. suecica.* Authors related rejection of the diatom to the composition of microalgae cells walls.

S. cylindraceus, as an infaunal organism, is subject to tidal periods that determine its diet; when the tide comes in, food is supposed to arrive. Some authors have proposed that tidal cycles have a huge influence on the rhythm of diet (Morton, 1970, 1977; Langton, 1977).

De Villiers and Hodgson (1993) wanted to test how different food concentrations (5-500 mg·L^{-1}) influence food activity. *S. cylindraceus* was subject to a cycle where a tidal cycle was simulated in the laboratory, so periods of 6 hours where bivalves were submerged were alternated with 6 hours where they were uncovered. Animals were fed with phytoplankton extracted from their own natural habitat at different concentrations and at 25ºC. Once the results were analysed, the conclusion reached

was that food activity is very great where seston concentrations are within a range of 5-25 mg·L^{-1}, since molluscs were fed 90-95% of the time. At concentrations of 50 mg·L^{-1} they detected a slight drop in time, 87%, as a consequence of more periods without activity. But at seston concentrations of 100, 250 and 500 mg·L^{-1} food activity underwent an important decrease, filtering only 68, 50 and 32% of the time, respectively. Other bivalve species showed similar behaviour, such as *M. edulis*, *Mya arenaria*, *Crassostrea edule*, *Venerupis pullastra* (Foster-Smith, 1976) and *Ostrea virginica* (Loosanof and Engle, 1947).

Research about energy physiology of native species from the European Northwest, such as *Ensis arcuatus*, *Ensis siliqua* and *Solen marginatus*, as well as *Ensis directus,* an introduced species, is just beginning. Only in *E. siliqua* has its retention efficiency and clearance rate been investigated. For *E. directus,* filtration rate and preingestive selection have been researched. No physiological studies have been made about the other species mentioned.

It is necessary to complete the research begun in *E. siliqua* and *E. directus*, studying not only the food acquisition process, but also its consumption and assimilation, in order to complete all the components of the energy equation; and it is also necessary to test how those processes vary depending on environmental factors (temperature, salinity, seston concentration, etc.) in order to achieve an integrated model for growth. Research in this field should begin with *E. arcuatus* and *S. marginatus* with the same objectives proposed for *E. siliqua* and *E. directus*.

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Chapter 5: Molecular and cytogenetic features of razor clams

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Background

Pod razor shell, sword razor shell and grooved razor shell constitute a group of bivalve molluscs well-known for their commercial importance. However, cytogenetic studies performed so far are very scarce, and there is an increasing need for a cytogenecic and molecular characterisation of the species that would permit the correct evaluation of natural stocks and help avoid commercial fraud by mislabelling.

In this chapter, we will present the basics about the chromosomal features of the three species, which are essential in order to differentiate between the razor clam types. This knowledge of chromosomal regions will help to identify the genetic anomalies and chromosomal polymorphisms that will contribute to the development of a physical map and the induction of potentially interesting triploids.

On the other hand, the characterisation of different genomic regions, both nuclear and mitochondrial, facilitates the search for and development of molecular markers, which, in addition to providing information for authentication of the three species, are also useful for carrying out phylogeographical analysis and assessing the genetic variation in natural and/or exploited stocks. All this information is extremely useful for the development of management programs that can contribute to an increase in the production of these organisms, with consequent socio-economic advantages for the extraction sector.

Despite the commercial importance of this group of marine invertebrates, most of the studies published so far focus on aspects of their anatomy, morphology and culture (Gaspar and Monteiro, 1998; Darriba *et al.*, 2004; da Costa *et al.*, 2010). It is for this reason that the genetic information available on these species is very scarce. The only genetic studies carried out on this group of bivalves are those conducted by researchers from the XENOMAR group at the University of A Coruña in Spain (Fernández-Tajes *et al.*, 2003; Fernández and Mendez, 2007; Fernández-Tajes *et al.*, 2007, 2008; Freire *et al.*, 2009; Fernández-Tajes and Méndez 2009; Arias *et al.*, 2010; Fernández-Tajes *et al.*, 2010). Therefore, this chapter focuses on reporting the research results obtained for these species as a starting point for further studies, which will contribute to an assessment of the global situation of current stocks.

1. Cytogenetic features of razor clams and European razor clams

1.1. An update on the characterisation of bivalve cytogenetics

Cytogenetics is the science that studies chromosomes, as the visible morphological manifestation of a given organism´s genome. The milestone study in bivalve mollusc cytogenetics was the work of Ieyama and Inaba (1974), which incorporated many of the protocols used for karyotype preparation in mammals: colchicine treatment, hypotonic shock and extension of cell suspensions isolated from gonadal or gill tissues. Later, the development of molecular probes and the enhancement of various protocols for chromosome band induction furthered our knowledge about chromosome structure and organisation in these species. This information enabled us to analyse polymorphisms and detect numerical and structural changes (which help understand genomic rearrangements occurring within and between species), as well as permitting hybrid analysis and the understanding of other cytogenetic aspects.

Within the Bivalvia, most of the published work has been done on the species belonging to the Pteriomorphia and Heterodonta sub-classes. The former includes many species of great economic value, such as mussels, oysters and scallops. As for the latter, the number of species analysed is not as large, but it also contains relevant species, such as pod razor, sword razor shell and grooved razor shell.

At present, about 200 bivalve mollusc species have been cytogenetically characterised, with karyotypes available for almost half of them. Among the Bivalvia class, it is generally observed that the most frequent chromosome dotation is 2n=38 (Nakamura, 1985; Thiriot-Quiévreux, 1994). Most of the karyotypes analysed present a large number of metacentric and submetacentric chromosomes; however, this feature must be considered with caution, for most of the species characterised have great commercial interest, which could have biased the results, thus not reflecting the true chromosomal constitution of the class (Thiriot-Quiévreux, 2002).

Amongst the gene families located by cytogenetic means, the ribosomal gene family is one of those most widely studied. The detection of the major ribosomal genes (18S-5.8S-28S) can be performed by indirect methods, such as the visualisation of the Nucleolus Organising Regions (NORs). Nevertheless, direct location by *fluorescent in situ hybridisation* (FISH) is the gold standard technique at the moment. This technique consists of the location of specific DNA or RNA sequences situated in fixated material with the help of a nucleic acid probe that is complementary to the target sequence. The location of these genes, in one or more chromosome pairs, might serve as a species-specific marker and may contribute to a better

understanding of the phylogenetic relations between closely related species.

1.2. Cytogenetic features of razor clams

1.2.1. Karyotypes

The karyotypes of some razor clam species have already been published: *Ensis arcuatus* and *E. siliqua* from the *Ensis* genus, characterised by Fernández-Tajes *et al*. (2008), and *Solen marginatus*, *S. grandis* and *S. constrictus*, described by Fernández-Tajes *et al*. (2003), Zhenxing *et al*. (2003) and Wang *et al*. (1998), respectively.

The number of chromosomes of all 5 species was 2n=38, matching the mean observed amongst bivalve molluscs (Table I).

Table I. Karyotypes of the different razor clam species

Classification Familiy/Species	Chr n (2n)	Karyotype	Location	Author/s
Pharidae				
Ensis arcuatus	38	4m, 1m/sm, 7sm, 7t	Cíes Islands (Galicia, Spain)	Fernández-Tajes *et al*. (2008)
Ensis siliqua	38	3m, 7sm, 9t	Fisterra (Galicia, Spain)	Fernández-Tajes *et al*. (2008)
Solenidae				
Solen grandis	38	13m, 3sm, 1st, 2t	China	Zhenxing *et al*. (2003)
Solen constrictus	38	18 m, 1 st	China	Wang *et al*. (1998)
Solen marginatus	38	9sm, 1sm/st, 1st/sm, 6st, 2t	Boiro (Galicia, Spain)	Fernández-Tajes *et al.* (2003)

Chr n: number of chromosomes; m: metacentric; sm: submetacentric; st: subtelomeric; t: telomeric.

Analysis of the karyotypes of the *Ensis* genus shows clear differences between practically all chromosome groups: *E. arcuatus* presents four pairs of metacentric chromosomes, while *E.siliqua* has three. However, both species have seven pairs of submetacentric chromosomes. Differences can also be noticed in the number of telocentric chromosomal pairs: *E. arcuatus* has seven pairs, whereas *E. siliqua* has nine. The karyotype of *S. marginatus* consists of nine submetacentric pairs, one single pair of submetacentric/subtelocentric chromosomes, another single pair of subtelocentrics/submetacentrics, six pairs of subtelocentrics and two pairs of

telocentric chromosomes, differeing greatly from the karyotypes of the other species analysed from the *Ensis* genus and also from *S. grandis* (13 metacentric, three submetacentric, one subtelocentric and two telocentric) (Wang *et al.*, 1998) and *S. constrictus* (18 metacentric and 1 subtelocentric).

These variations allow us to differentiate between all 5 species. Besides, these differences suggest that the divergence of species belonging to the same genus may have implied several chromosomal rearrangements (Fernández-Tajes *et al*., 2008).

Most of the variations between the *Ensis* and *Solen* genera are mainly due to the fact that the majority of the species analysed have a high number of subtelocentric or telocentric chromosomes, except for *S. marginatus*. According to Surget-Groba *et al*. (2001), those species with a greater presence of chromosomes with both arms would be suggestive of a more apomorphic character. Following this premise, both species from the Pharidae family and also *S. marginatus* would be more plesiomorphic than the members of the Veneridae family studied (Table II).

Two metaphases belonging to *E. arcuatus* (Fig. 1a) and *E. siliqua* (Fig. 1b).

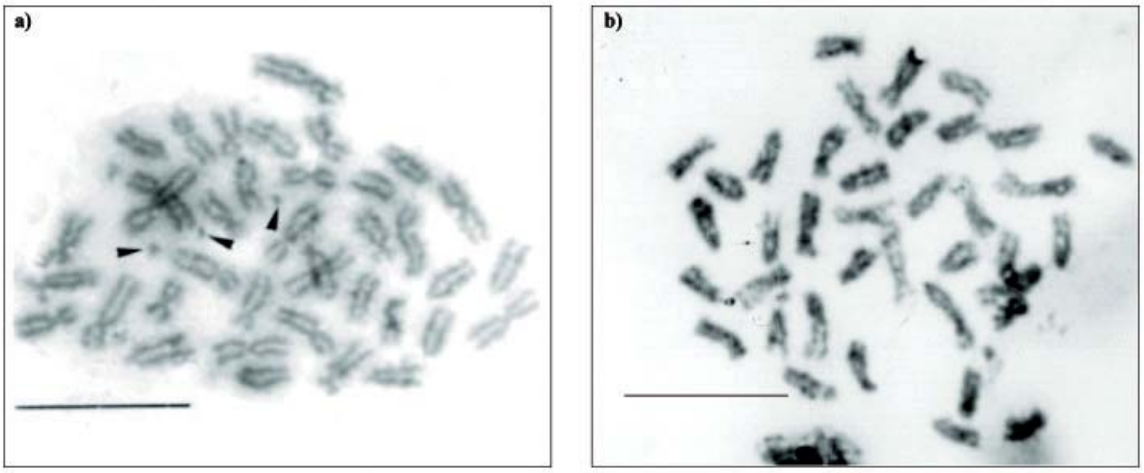

Figure 1. a) Metaphase of *E. arcuatus*; b) Karyotype of *E. siliqua*. Bar: 10 µm. Arrows depict B chromosomes.

The study performed by Fernández-Tajes (2006) showed the presence of B chromosomes, in addition to the standard chromosomal complement of *E. arcuatus* and *E. siliqua*. These B chromosomes can be identified by their small size, and also because they do not follow a Mendelian inheritance pattern, and thus their behaviour in mitosis and meiosis is different from that of regular chromosomes. Their presence has been confirmed in over 1330 vegetal and almost 500 animal species (Jones and Puertas, 1995). In bivalve molluscs, there are reports describing B chromosomes in the cockle *Cerastorerma edule* from a location in Baldaio (A Coruña-Spain) (Insua

and Thiriot-Quiévreux, 1992).

Table II. Karyotypes for the Order Veneroida species.

Classification Family/Species	Chr n (2n)	Karyotype	Location	Author/s
Cardiidae				
Cerastoderma edule	38	1sm/m, 8sm, 3sm/st, 4st, 3t/st	Baldaio (Galicia)	Insua and Thiriot-Quiévreux (1992)
Cerastoderma glaucum	38	3m, 1sm/m, 8sm, 1sm/st, 6st	Baltic Sea	Thiriot-Quiévreux and Wolowicz (1996)
Cerastoderma glaucum		3m, 1m/sm, 9sm, 6st	Sète, Mediterranean Sea	Thiriot-Quiévreux and Wolowicz (1996)
Mactridae				
Mulinia lateralis	38	19 t	Delaware and Virginia (USA)	Wada *et al.* (1990)
Mactra chinensis	38	6sm/m, 3sm/st, 7st/sm, 3st	Japan	Wada and Komaru (1993)
Spisula solidissima	38	4m, 5sm, 10st/t	Magdalen Islands (Canada)	Xiang *et al.* (1993)
Tresus capax	34	10m, 7sm	Vancouver Island (Canada)	González-Tizón *et al.* (2000)
Solenidae				
Solen constrictus	38	15m, 3sm, 1st	Haiyang (China)	Wang *et al.* (1998)
Tellinidae				
Macoma balthica	38	11m, 2m, 6st	Bay of Gdansk (Baltic Sea)	Wolowicz and Thiriot-Quiévreux (1997)
Macoma nasuta	38	8m, 6sm, 5st	Vancouver Island (Canada)	González-Tizón *et al.* (2000)
Psammobiidae				
Nutalia nuttallii	38	7m, 12sm	Vancouver Island (Canada)	González-Tizón *et al.* (2000)
Sinovacula constricta	38	13m, 4sm, 1st, 1t	Qingda (China)	Wang *et al.* (1998)
Donacidae				
Donax trunculus	38	9m, 2sm/m, 4sm, 2sm/st, 3st/sm	Cedeira (Galicia)	Martínez *et al.* (2002)
Veneridae				
Venerupis pullastra	38	3m, 2sm/m, 4 sm, 2sm/st, 3 st/sm, 5st	Baldaio (Galicia)	Insua and Thiriot-Quiévreux (1992)
Venerupis rhomboideus	38	3m, 1 m/sm, 2 sm/m, 6 sm, 2st/sm, 2st, 3t	Arousa Estuary (Galicia)	Insua and Thiriot-Quiévreux (1992)
Venerupis aurea	38	8m, 9sm, 1st, 1t/st	Hiroshima (Japan)	Ieyama (1985)
Ruditapes decussatus	38	6m, 1sm, 2sm/st, 9st, 1st/t	France	Borsa and Thiriot-Quiévreux (1990)
Ruditapes philippinarum	38	6m, 4m/sm, 3st/sm, 6st	Vancouver Island (Canada)	González-Tizón *et al.* (2000)

Chr n: number of chromosomes

1.2.2. Heterochromatic regions

Chromosome banding is one of the most frequently used cytogenetic techniques

for chromosome characterisation. Although it is used routinely in mammal cells, the inability to maintain cell cultures in bivalves presents a handicap for the use of this protocol on molluscs. However, there are certain banding techniques, such as restriction banding or Chromomycin A3 (CA3) staining, that have been carried out in mollusc gill cells (Martinez *et al.*, 2002; Petrovic *et al.*, 2009). The only study to characterise the heterochromatic regions in razor clams so far (Fernández-Tajes *et al.*, 2006) consisted of the use of the Schweizer method (1980), which combines the use of CA3 with DAPI. Results from this study showed the presence of nine CA-positive regions in *E. arcuatus* (Figs. 2a and 4a) and 13 in *E. siliqua* (Figs. 2b and 4b), while for *S. marginatus* the number ranged from two to four (Figs. 3 and 4c).

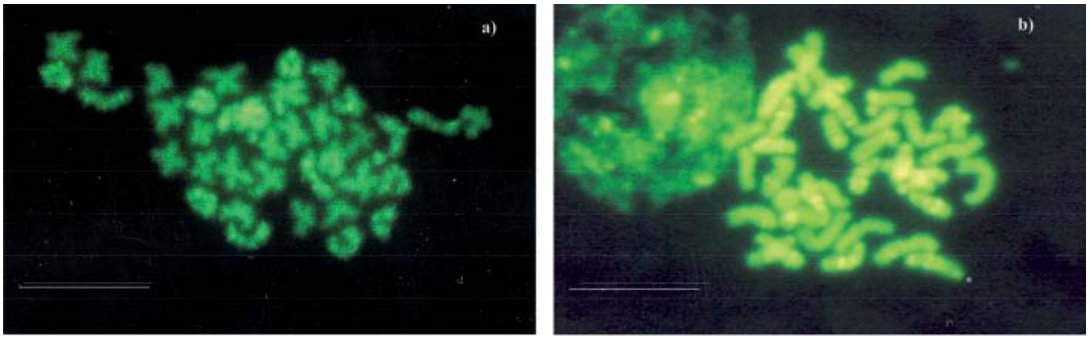

Figure 2. CA3 banding in a) *E. arcuatus*; b) *E. siliqua*. Bar = 10 µm.

DAPI staining showed homogeneity across all chromosomes, with a slight decrease of the signal only in CA3-positive regions.

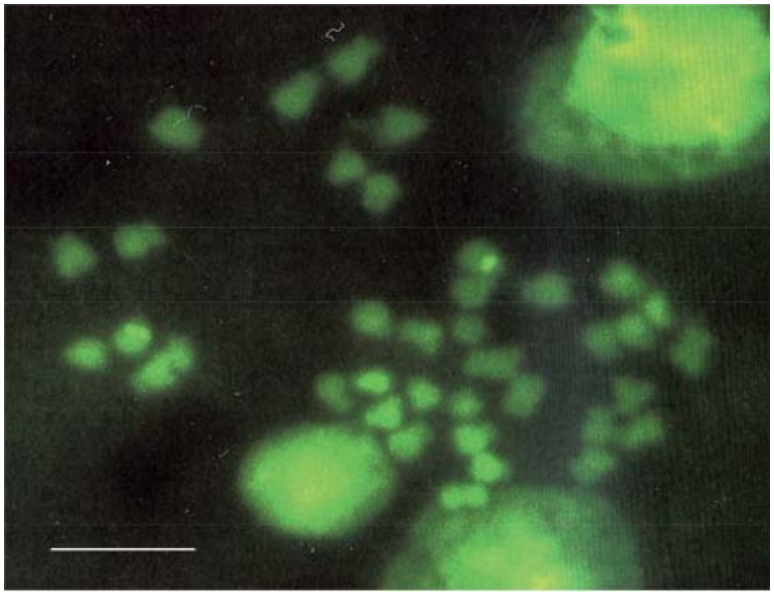

Figure 3. CA3 banding in *S. marginatus*. Bar = 10 µm.

The high number of signals observed in *E. arcuatus* and *E. siliqua* matches

previous results in species from the Order Veneroida, such as *Donax trunculus* (Martínez *et al*., 2002) or *Dreissena polymorpha* (Boron *et al*., 2004). However, CA3-positive regions in *S. marginatus* (1-2) are similar to the number of regions observed in species belonging to the Mytilidae or Ostreidae families, not included in the Order Veneroida.

1.2.3. Location of ribosomal genes by FISH

The location of the ribosomal chromosomal regions has only been studied for *E. arcuatus*, *E. siliqua* (Fernández-Tajes *et al*., 2008) and *S. marginatus* (Fernández-Tajes *et al*., 2003). The results obtained by Fernández-Tajes *et al*. (2008) for *E. arcuatus* showed a hybridisation signal at the subcentromeric level of chromosome 12 (Fig. 4a). Yet for *E. siliqua* the signal appeared at the long arm of chromosome 7 (Fig. 4b). For *S. marginatus* (Fernández-Tajes *et al*., 2003), the location of the major ribosomal genes gave three different signals: two of them in the subcentromeric and subtelocentric regions of chromosome pairs 9 and 12 (Fig. 4c), while the third signal did not appear in any metaphases analysed.

The presence of a ribosomal locus is considered as another plesiomorphic trait (Amemiya and Gold, 1990) and would support the evidence gathered when the number of telocentric chromosomes present in both species of the *Ensis* genus was studied. Nevertheless, the subcentromeric and subtelocentric locations of 18S-5.8S-28S rDNA in *E. arcuatus* and *E. siliqua*, respectively, would be indicative of either a more apomorphic trait or a possible chromosomal rearrangement. Wang and Guo (2007) have suggested the hypothesis that the existence of the two separate ribosomal loci is a consequence of a duplication event in the early genomic evolution of bivalves. In this way, the existence of a single locus in *E. arcuatus* and *E. siliqua* would be due to the loss of one of the ribosomal loci after a chromosomal rearrangement (Fernández-Tajes *et al*., 2008).

Differences in the intensity of the fluorescent signal were observed both within *E. siliqua* and *S. marginatus*. For the former, variation in intensity was observed between homologues, whereas in the latter, two different chromosomal pairs were involved. Something similar has already been described in the *M. californianus* and *M. trossulus* mussels (González-Tizón *et al.*, 2000). These differences in intensities could be attributed to the existence of a low rDNA copy number in these loci, or as a consequence of the condensation degree of metaphasic chromosomes (Fernández-Tajes *et al*., 2003, 2008).

The location of the 5S rDNA in bivalve molluscs has also been carried out on a limited number of species, with a single reference in the Solenidae family

(Fernández-Tajes *et al*., 2003) and none for the Pharidae. The results from this study showed a high number of hybridisation signals in the subtelomeric region of at least ten different chromosomal pairs (Fig. 5).

Figure 4. Idiograms showing the location of the CA3 positive regions and the 18S-5.8S-28S ribosomal loci in a) *E. arcuatus*, b) *E. siliqua* and c) *S. marginatus*. The dark regions are CA3-positive while the circles represent ribosomal loci.

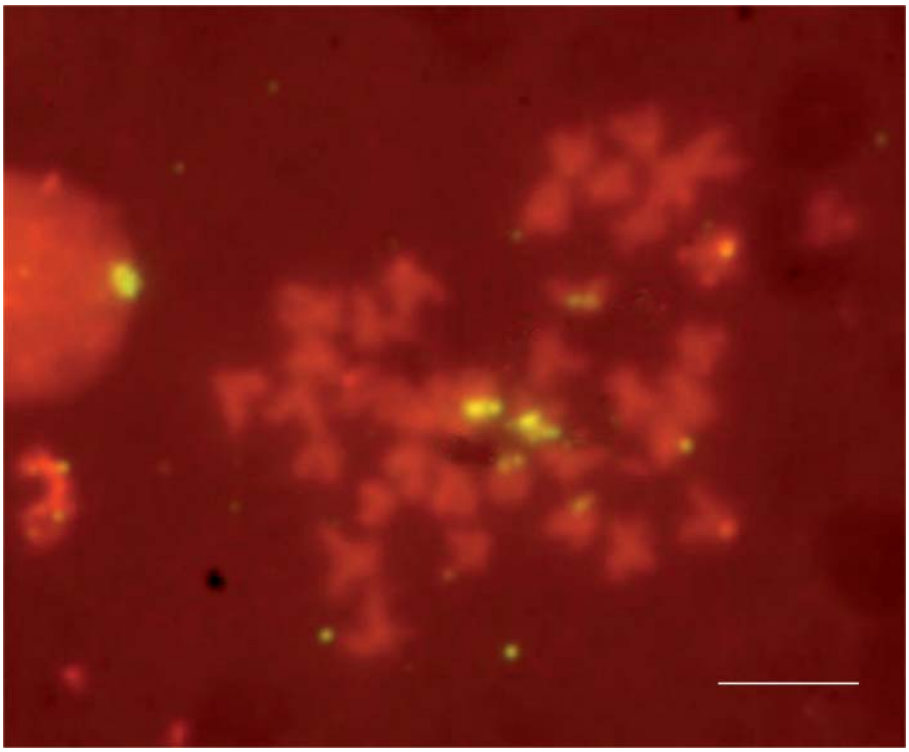

Figure 5. Location of the 5S ribosomal loci in the grooved razor shell (*S. marginatus*). Bar = 10 µm.

The variability in the number of signals observed is likely to be caused by different copy numbers in these ribosomal loci, and contrasts with previous findings in other bivalve species, such as clams (Insua *et al.*, 1999) or mussels (Insua *et al.*, 2001).

The cytogenetic characterisation of the different razor clam species analysed provides evidence that despite their morphological similarities, their chromosomal complements are very different, which suggests they are very diverse species with low inter-species reproductive possibilities.

2. Molecular features of razor clams: development of molecular markers and applications

2.1. Introduction

Knowledge of the nuclear and mitochondrial genomes of the different species is nowadays a fundamental parameter for the sustainable development of the production and management of aquaculture resources.

Advances made in the field of molecular biology over the past few years have meant a revolution in the knowledge and study of the identification of different species, with most of the latest techniques focusing on the detection of genetic variability in certain DNA regions. The differentiation of species in aquaculture is considered of great interest to guarantee the fulfilment of labelling regulations and to ensure consumers dispose of proper information.

In addition, genetic markers are an indispensable device for the measurement of genetic variability, for they are fundamental tools in the evaluation of genetic diversity and the knowledge of the genetic population structure, besides assisting in the comparison of phylogenetic relationships between species.

2.2. Development of molecular markers for the identification of razor clam species

The razor clam species most frequently commercialised are *Ensis arcuatus*, *E. siliqua* and *Solen marginatus*. All three are originally from the European continent, in contrast to *E. directus*, which was introduced from the North American continent. There has also been a recent addition to the species on the market in the form of *E. macha*, a species originally from South America, particularly Chile and Argentina. Its valval morphology and internal anatomy are very simillar to those of the other *Ensis* species, which makes it very difficult to differentiate this species from the European ones if we rely only on morphological features such as muscle insertions or valve curvature. This differentiation becomes even harder in processed samples (cooked or canned), because any distinctive morphological characteristics would be lost.

With the aim of avoiding commercial fraud in the export, import or labelling of

these products, the European Union created the 104/2000 Regulation (EC) for all products related to fishery and aquaculture. In this regulation, it is stated that all products must be properly labelled with their appropriate commercial name together with that of the species, production method and region of capture. However, on many occasions there have been reports of mislabelling, both involuntary and voluntary, which might lead to the selling of some species with prices higher than those permitted. It is because of this that genetic methods for the identification of species have developed so quickly over the past few years. The use of species-specific molecular markers helps assure we can track down the various species.

The recent development of molecular biology has permitted the use of a number of techniques for the identification of commercial bivalve species. DNA-based methods are more reliable than protein-based ones, as DNA is a much more stable molecule and processing conditions do not affect its behaviour and availability. There are a large number of molecular biomarkers for species characterisation reported in the literature. Most of these have been developed by molecular protocols based on the Polymerase-Chain Reaction (PCR). A few examples are the restriction fragment length polymorphisms (PCR-RFLPs) used in the identification of clam (Fernández *et al*., 2001, 2002) and pectinid species (López-Piñón *et al*., 2002), PCR-SSCEP (Single strand conformation polymorphisms) used for clam identification (Fernández *et al*., 2002) or microsatellites, also used for pectinids (Zhan *et al*., 2008).

In the case of razor clam identification, there are a series of methods described in the bibliography that allow the unambiguous identification of the species in the widely commercialised *Solen* and *Ensis* species. The first of these methods was developed by Fernández-Tajes and Méndez (2007). The authors use amplification of the 5S rDNA ribosomal region for the differentiation of five razor clam species: *E. arcuatus*, *E. siliqua*, *E. directus*, *E. macha* and *S. marginatus*. Amplification of this particular DNA region showed length variations between the species from the *Ensis* genus (420 bp) and *S. marginatus* (530 pb), which is easily visualised in an agarose gel (Fig. 6).

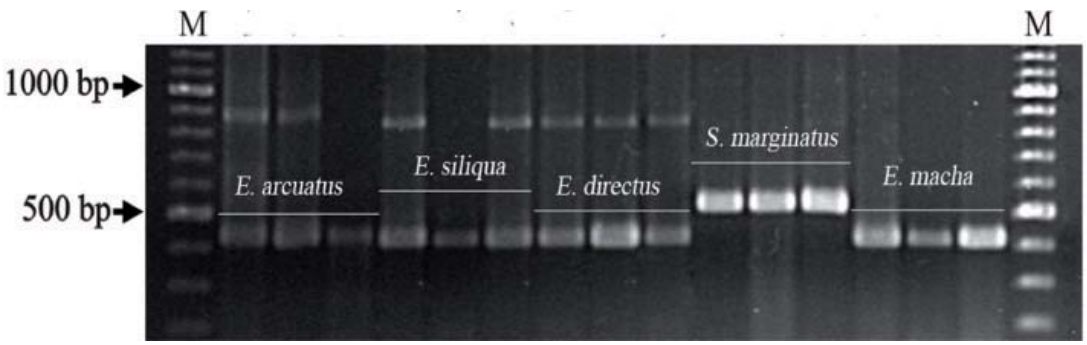

Figure 6. Amplification pattern for the 5S rDNA ribosomal region in *E. arcuatus*, *E. siliqua*, *E. directus, S. marginatus* and *E. macha*. M: 100 bp marker.

The follow-up study of the untranscribed region showed a great similarity between *E. arcuatus* and *E. siliqua*, as well as a great divergence between these two and the species from the *Ensis* genus. This sequential difference made it possible for a search to be made for species-specific restriction sites for discrimination amongst the four species. Digestion with the *Hae*III restriction enzyme produced specific patterns for *E. arcuatus* and *E. directus*, although *E. siliqua* and *E. macha* were still impossible to distinguish (Fig. 7).

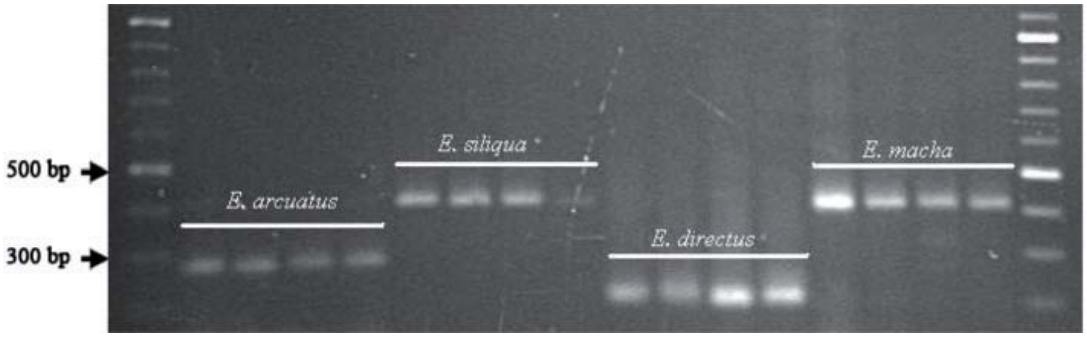

Figure 7. *Hae*III restriction pattern for *E. arcuatus*, *E. siliqua*, *E. directus* and *E. macha*.

Differentiation between *E. macha* and *E. siliqua* was possible after digestion with A*cs*I (Fig. 8). In this way, sequential digestion with both enzymes clearly permits discrimination between the four *Ensis* genus species.

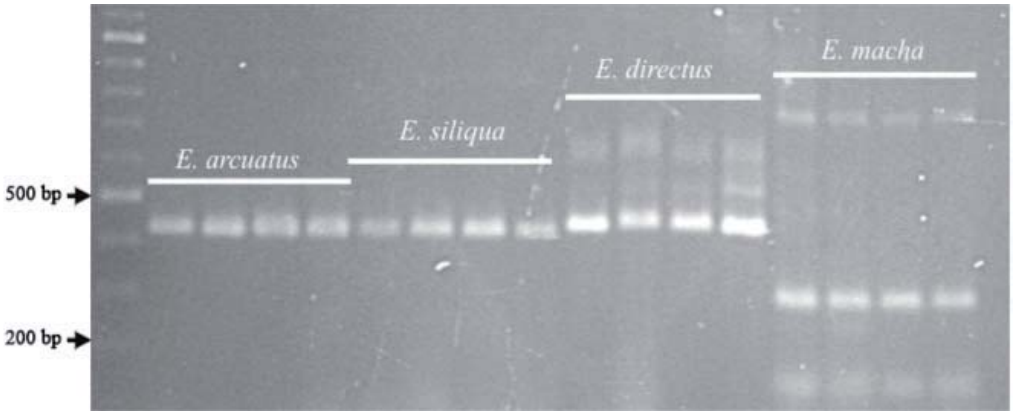

Figure 8. *Acs*I restriction pattern for *E. arcuatus*, *E. siliqua*, *E. directus* and *E. macha*.

The second method consists of differentiation between *E. arcuatus* and *E. siliqua* using RFLPs generated from the ITS1 ribosomal region (Freire *et al*., 2008). The study of the existing nucleotide differences between the two species allowed for the identification of several restriction target sites that, after digestion with the appropriate endonuclease, could discriminate between *E. arcuatus* and *E. siliqua*. Digestion with K*sp*I produced a different restriction pattern for each of the species.

The third method available uses a multiplex PCR for differentiation between *E. arcuatus*/*E. siliqua*, *E. directus* and *E. macha*. This protocol, described by Fernández-Tajes *et al*. (2010) allows the identification of *E. directus* and *E. macha* after a single PCR reaction, while *E. arcuatus* and *E. siliqua* can not yet be distinguished. A further digestion of the amplification products, following the method described by Freire *et al*. (2008) allows for the distinction to be made between the latter two species.

All the protocols described above were developed using both fresh and canned samples, and constitute a quick, effective and reliable methodology for the identification of commercialised razor clam species. Likewise, they may be standardised for their use in food fraud control laboratories.

2.2. Development of molecular markers for the evaluation and characterisation of different stocks of razor clams

Many marine organisms have a high dispersion capability, in at least one part of their life cycle. In the case of bivalve molluscs, the larval phase is, theoretically, responsible for this ability to propagate over long distances. In the absence of oceanographic barriers, this could imply a low or inexistent genetic differentiation. This happens, for example, in bivalves such as mussels (Toro *et al*., 2004) or clams (Vadopalas *et al*., 2004). However, there have recently been an increasing number of studies describing widely differentiated populations, even in species with high dispersal potentials (Reeb and Avise, 1990; Beaumont and Zouros, 1991; Luttikhuizen *et al*., 2003).

Another factor that must be taken into consideration for sustainable resource farming is the knowledge of sources and sinks within a population. Sources are areas that contribute to the conservation of the population, while some groups are maintained by sinks. Knowledge of the population structure and its genetic variation, as well as source-sink organisation, is a key factor to guarantee the conservation and rational exploitation of the species. This information is essential to avoid loss of genetic variation, which is commonly associated with a decrease in biological efficiency and the preservation of the potential for adaptation. Thus, population genetics has become an important tool in the conservation of the natural populations of these species and has contributed to a rational exploitation of the resources, as well as as assisting in the evaluation of the need for reseeding in overexploited populations.

There are only two studies to date that have evaluated the genetic variation in razor clam populations subject to exploitation: these are the works of Fernández-Tajes

et al. (2007) and Arias *et al*. (2010) concerning *E. siliqua*. Both studies examined locations on the eastern shores of the Republic of Ireland and Northern Ireland and the west coast of the Iberian Peninsula with RAPDs. The former used RAPDs on nucleic genomic material, while the second used the same type of molecular markers, but on mitochondrial DNA. In both studies, a high degree of genetic differentiation could be observed between populations.

In the work by Fernández-Tajes *et al*. (2007), genetic differences were assessed for samples obtained from the natural populations of Strangford Lough (Northern Ireland) and from five different locations both in Spain (Celeiro, Fisterra and Barra) and Portugal (Setubal and Olhão). Molecular markers were 61 RAPD loci obtained with 5 different sets of primers. A series of genetic variability measurements, such as Nei´s genetic distance and PhiPT values (Table III) revealed small differences between the Spanish and Portuguese populations, but larger differences between these and samples collected from Strangford Lough. These authors also performed a Mantel test between genetic and geographic distances, obtaining a positive correlation between both variables. This relationship can be explained by an isolation by distance process.

Table III. Genetic distance and PhiPT values in six *E. siliqua* populations.

Population	Fisterra	Celeiro	Barra	Setúbal	Olhão	Strangford Lough
Fisterra	-	0.052	0.069	0.160	0.178	0.184
Celeiro	0.054	-	0.051	0.126	0.196	0.209
Barra	0.065	0.051	-	0.149	0.137	0.225
Setúbal	0.139	0.102	0.123	-	0.190	0.241
Olhão	0.163	0.169	0.118	0.160	-	0.202
Strangford Lough	0.174	0.190	0.214	0.224	0.188	-

Note: PhiPT values above diagonal, Nei´s genetic distance below diagonal (Nei, 1978).

Arias *et al*. (2010) complemented the former study with the analysis of RAPD markers on three new locations: Tyrella (Northern Ireland), Skerries (Republic of Ireland) and Aveiro (Portugal), and also with the use of PCR-RFLPs on a fragment of the mitochondrial 16S gene from all locations. Each of the individuals analysed showed a single RAPD phenotype, and 12 different composite haplotypes were observed when digesting the 16S rDNA with five restriction. Two of the mitochondrial haplotypes appeared in most individuals and, surprisingly, showed a non-overlapping distribution. Furthermore, one of these only appeared in samples collected in Ireland

and the other one only in the Iberian Peninsula samples, with the exception of Aveiro individuals, which shared the Irish haplotype. Genetic differentiation analyses, such as F_{ST} and PhiPT values, molecular variance analysis or grouping algorithms, were performed for RAPDs and mitochondrial PCR-RFLPs, and support the existence of genetic differentiation.

We can see a principal coordinate grouping displayed in figure 9, where the separation between Irish and Iberian locations (except Aveiro) is evident.

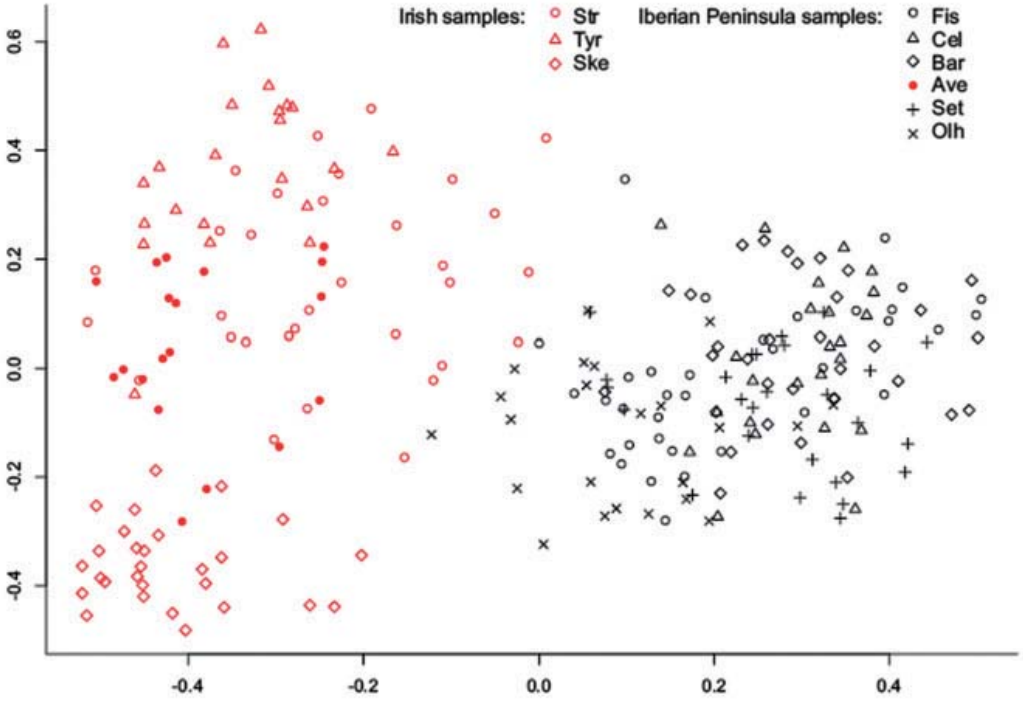

Figure 9. Representation of a principal coordinate graph for the 9 locations studied by Arias *et al.* (2010).

Locations from both Spain and Portugal, except Aveiro, showed low or nonexistent genetic differentiation between them, but high heterogeneity with the other sites of sample collection. Thus, the present or recent genetic flow amongst these locations could be considerable and they would act as a single reproductive population. The Mantel test was significant even when Aveiro was included among these populations, but the low determination rates do not seem to support a process of isolation by distance. This clearly contrasts with the results obtained by Fernández-Tajes *et al.* (2007), but this could be due to the fact that in the latter work a single population from Ireland was used.

Comparisons amongst the Irish populations showed higher differentiation

values, and Aveiro was surprisingly similar to all of these locations. Notwithstanding, the presence in Aveiro of several specific mitochondrial haplotypes is suggestive of a high level of differentiation between this Portuguese location and the Irish ones. The lack of homogeneity when considering Aveiro as against the other populations might mean that this population could in fact be self-recruiting. However, further studies will be needed to verify that the genetic differentiation between this location and nearby ones is constant over time. If this were so, physical and biological factors determining this difference should be thoroughly investigated.

Arias *et al*. (2010) have proposed a series of hypotheses to explain the presence of the same predominant haplotype in Aveiro and the Irish locations, along with the low differentiation obtained for the RAPD markers in these locations. The most acceptable of these would be the undetected anthropogenic transportation of individuals to Aveiro, since this location has a well-known harbour. This could be achieved, for instance, by means of larvae transportation in vessels' water ballast.

The results obtained by Fernández-Tajes *et al*. (2007) and Arias *et al*. (2010) have been confirmed with considerable accuracy by the use of codominant nuclear microsatellite markers (Arias *et al*., manuscript under revision).

One of the main conclusions that can be drawn from the genetic studies performed on *E. siliqua* is the existence of a genetic population substructure in this species. Thus, the elaboration of management plans for the farming of this resource must take into account the genetic results obtained. The high divergence rate between Irish and Iberian locations suggests that by no means should seed from Ireland be used to restock Galician or Portuguese banks or viceversa. Besides, the differences found for Aveiro indicate that this bank could currently be self-recruiting, which suggests that its exploitation should be designed to reduce the impact in the parental generations sustaining the population.

With regard to the rest of the species with commercial interest, no population genetic studies have been performed. Nonetheless, some molecular markers have been developed that may be used for the analysis of genetic variation in exploited populations. This might be the case for *E. arcuatus* and *S. marginatus*, for which some microsatellite markers have already been described (Francisco-Candeira *et al*., 2007; Varela *et al*., 2007). Also, a 5S region containing two sub-unit types with mixed organisation have been characterised for *E. macha* (Fernández-Tajes and Méndez, 2009). The authors have proposed these two sub-units as a useful tool for population genetic analyses.

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Chapter 6: Pathologies in pod razor clam (*Ensis siliqua*)

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Abstract

In Galicia, the extraction of commercially important Solenidae, such as the pod razor clam (*Ensis siliqua*), has increased significantly during the last decade, mainly due to its great commercial success and to the development of plans for its exploitation implemented by several fishermen's associations.

A number of studies have been carried out in order to evaluate different pathologies in razor clam in the Cangas area (Ría of Vigo, Southern coast of Galicia) and in Finisterre (Ría of Corcubión, Northern coast of Galicia). The most noteworthy is the detection of the gregarine *Nematopsis* sp. that usually appears as an ovocyst in the connective tissue of gills, digestive tube and mantle, as well as some cases of coccidia and trematoda. Bacterial proliferations were also detected, whose predominant groups were the genus *Vibrio* and filamentous bacteria.

1. Introduction

Marine mollusc aquaculture is a favourable medium for transmitting and developing pathological problems. This, combined with a high density of farming, in some cases with monoculture, facilitates the propagation of pathogenic agents that may have a limited and localized role or be the origin of epizootics, leading to catastrophic losses.

For some years, numerous diseases have been described, with growth stoppage, various injuries and, in most cases, high mortality being the most obvious symptoms. Although in many cases a connection has been found between high mortality and infectious agents, in other cases it has not been possible to find a real explanation. Probably, constant mortalities have been caused by unfavourable environmental variations, inappropriate breeding conditions and other factors yet to be determined.

The most important epizootics have been caused by protozoa, although several

problems are attributed to fungi, bacteria and viruses. Bacteria from the Rickettsiae group have also been involved in mortalities.

Among diseases caused by protozoa, the most important are those caused by parasites like Haplosporidia and parasites of the genus *Marteilia* (Grizel *et al*., 1974), *Bonamia* (Pichot *et al*., 1980) and *Perkinsus* (Mackin *et al*., 1950; Levine, 1978; Azevedo, 1989). Among the diseases produced by Haplosporidia, causal agents are essentially *Haplosporidium costalis* (Andrews *et al*., 1962) and *Haplosporidium nelsoni* (Haskin and Ford, 1982), which affected the production of *Crassostrea virginica* in the United States.

Other Haplosporidia detected in marine bivalves are *Haplosporidium armoricanum* in *Ostrea edulis* (Van Bannining, 1977; Azevedo *et al*., 1999); *Haplosporidium* sp. in *Crassostrea gigas* (Katkansky and Warner, 1970; Kern, 1976); *Haplosporidium tumefacientis* in *Mytilus californianus* (Taylor, 1966); *Minchinia tapetis* in *Ruditapes decussatus* (Vilela, 1951; Azevedo, 2001); *Haplosporidium edule* in *Cerastoderma edule* (Azevedo *et al*., 2003) and *Haplosporidium montforti* in *Haliotis tuberculata* (Azevedo *et al*.,2006a and b).

The genus *Marteilia* comprises several species, the most important being *Marteilia refringens* (Grizel *et al*., 1974), which caused huge losses in *Ostrea edulis* cultivation on the French Atlantic coast (Grizel, 1985). This parasite has also been detected in other areas, such as Galicia, in flat oysters (Massó, 1978; Alderman, 1979), as well as in mussels (*Mytilus galloprovincialis*) (Gutierrez, 1977; Figueras and Montes, 1987) and in Japanese oysters (*C. gigas*) (Cahour, 1979; Montes *et al*., 1998).

Within the genus *Bonamia*, the species *Bonamia ostreae* (Pichot *et al*., 1980) cause the disease known as Bonamiosis, affecting flat oyster farming (*O. edulis*). This disease was detected in France in 1979, and spread to other European countries such as the Netherlands, Spain, the United Kingdom and Ireland (Van Banning, 1982; Polanco *et al*., 1984; Culloty and Mulcahy, 2001). This species was also detected in the United States (Elston *et al.*, 1986).

Other species of *Bonamia* are *Bonamia exitiosa*, which causes high mortality in *Ostrea chilensis* in New Zealand and Chile (Hine *et al*., 2001; Berthe and Hine, 2003), and *Bonamia* sp., also unresolved as a new species in *Ostrea puelchana* in Argentina (Kroeck and Montes, 2005). Recently *Bonamia perspora* n. sp. was described in *Ostreola equestris* (Carnegie *et al*., 2006), this being the first species of the *Bonamia* type to produce spores.

The genus *Perkinsus* comprises several species, the best known of which is

Perkinsus marinus (Mackin *et al.*, 1950; Levine, 1978), which was detected in the 1950's in *Crassostrea virginica* in the United States and has been the subject of many studies (Ray, 1954; Perkins and Menzel, 1966; Perkins, 1996).

On the European Atlantic coast, Azevedo *et al*. (1989) describe the new species *Perkinsus atlanticus* in the *Ruditapes decussatus* clam in Portugal. Subsequently, fresh studies appeared about this species (Azevedo *et al*., 2000; Martínez, 2000; Hernández, 2000). Other species of the genus *Perkinsus* are *Perkinsus olseni* (Lester and Davis, 1981), infecting the Australian abalone, *Haliotis ruber*, and *Perkinsus qugwadi* (Blackbourn *et al*., 1998), which infects the Japanese scallop *Patinopecten yessoensis*.

Also within the protozoa, we have the genus *Nematopsis*, which uses marine bivalves as immediate hosts (Lauckner, 1983). This genus is described in several molluscs, such as *C. virginica* (Prytherch, 1938, 1940), *Arculata arculata*, *Anadara granosa*, *Perna viridis* and *Paphia undulata* in Thailand (Tuntiwaranuruk *et al*., 2004), *C. edule* and *R. decussatus* in Portugal (Azevedo and Cachola, 1992), *Ruditapes decussatus* in Spain (Navas *et al*., 1992), *Ensis arcuatus* in Ireland (Fahy *et al*., 2002), *E. arcuatus* and *Ensis siliqua* in Spain (Conchas *et al*., 2005).

As for organisms of bacterial origin, these mainly affect molluscs' larval culture. Thus, the main pathological problems are caused by heterotrophic bacteria of the genus *Vibrio* and *Pseudomonas* which are able to proliferate by taking advantage of the favourable conditions found in larval cultivation tanks. Different studies have confirmed the relationship between bacteria and diseases in several mollusc species (Tubiash *et al*., 1965; Elston and Levobitz, 1980; Brown, 1981; Guillard, 1959; Bolinches *et al*., 1986; Riquelme *et al*., 1995; Nicolas *et al*., 1996; Elston *et al*., 1999; Anguiano-Beltran *et al*., 2004; Prado *et al*., 2005).

Regarding mollusc farming, it is worth highlighting a bacterium of the genus *Vibrio*, the *Vibrio P1* (Paillard and Maes, 1989), later classified as *Vibrio tapetis* (Borrego *et al*., 1996), which causes the brown ring disease that affects several species of clams (Paillard, 2004).

Regarding rickettsiae-like organisms, the first description in marine invertebrates was made by Harshbaryer *et al*. (1977) in *Mercenaria mercenaria* from Chesapeake and Chincoteage bays (U.S.A.), and these same researchers also mentioned a rickettsia in *Mya arenaria,* from the same areas. Similarly, rickettsial infections have been found in *C. gigas* and *O. edulis* oysters (Comps *et al*., 1977). Later, new infections were detected in molluscs, such as in *Crassostrea angulata* (Comps and Deltreil, 1979), *M. edulis and M. galloprovincialis* (Yevich and Barszcz, 1980), *R. decussatus*

(Joly and Comps, 1980), *Tapes japonica* and *Patinopecten jessoensis* (Elston, 1986), *Pecten maximus* (Gall *et al*., 1990), *Crassostrea rizophorae* (Azevedo *et al*., 2005) and *Haliotis tuberculata* (Azevedo *et al*., 2006a and b). During recent years, a lethal disease known as "Withering Syndrome of Abalone" has been detected, characterized by atrophy of the foot muscle, which has been related to massive mortality in cultivated and natural populations of *Haliotis* sp. in different geographical areas (Antonio *et al*., 2000; Friedman *et al*., 2000; Moore *et al*., 2000). Recently, the syndrome has been described as a rickettsial-type disease (Haaker *et al*., 1992; Gardner *et al*., 1995; Antonio *et al*., 2000; Moore *et al*., 2000; Braid *et al*., 2005).

Regarding diseases caused by viruses, "gills disease" has been cited, affecting *Crassostrea angulata*, in the Sado and Tajo estuaries in Portugal (Ferreira and Dias, 1973), and giving rise to high mortality in several farming sites in France (Comps, 1983). After "gills disease" a new viral epizootic caused great losses in *C. angulata,* eliminating this species farmed in French parks (Comps *et al*., 1976). Viral infections also appeared among other molluscs: in *C. gigas* (Comps and Bonami, 1977), *Crassostrea virginica* (Farley *et al*., 1972; Meyers, 1981), *Tellina tenuis* (Hill, 1976) and *M. mercenaria* (Meyers, 1981).

As for viral infections in larvae, Elston (1979) describes viral infection in *C. gigas* when analysing significant mortalities in hatcheries. Likewise, Elston and Wilkinson (1985), in a research study about *C. gigas* larvae mortalities, mentioned that they are caused by a virus called "OVVD" (oyster velar virus disease).

As regards diseases with a fungal origin, we may mention *Ostracoblade implexa,* which caused mortalities in certain natural oyster banks (Korringa, 1948; Marteil, 1954). Isolation and culture of *Ostracoblade implexa* was carried out by Alderman and Jones (1967, 1971), who reproduced the disease experimentally.

2. Pathologies detected in the pod razor clam (*Ensis siliqua*)

Research has been carried out into pathologies that may affect pod razor clam, taking into account the many pathologies described in molluscs worldwide, and the increase in extraction of Solenidae during the last decade, including the pod razor clam (*E. siliqua*), whose capture has increased remarkably, due, above all, to its great commercial success and to the development of plans for its exploitation in several fishermen's associations. Among the pathologies were those caused by *B. ostreae* (Montes, 1995; Montes *et al*., 2003) and *M. refringens* (Figueras and Montes, 1987, 1988), detected in Galicia, both of these parasites being included in EU Council Directive 2006/88.

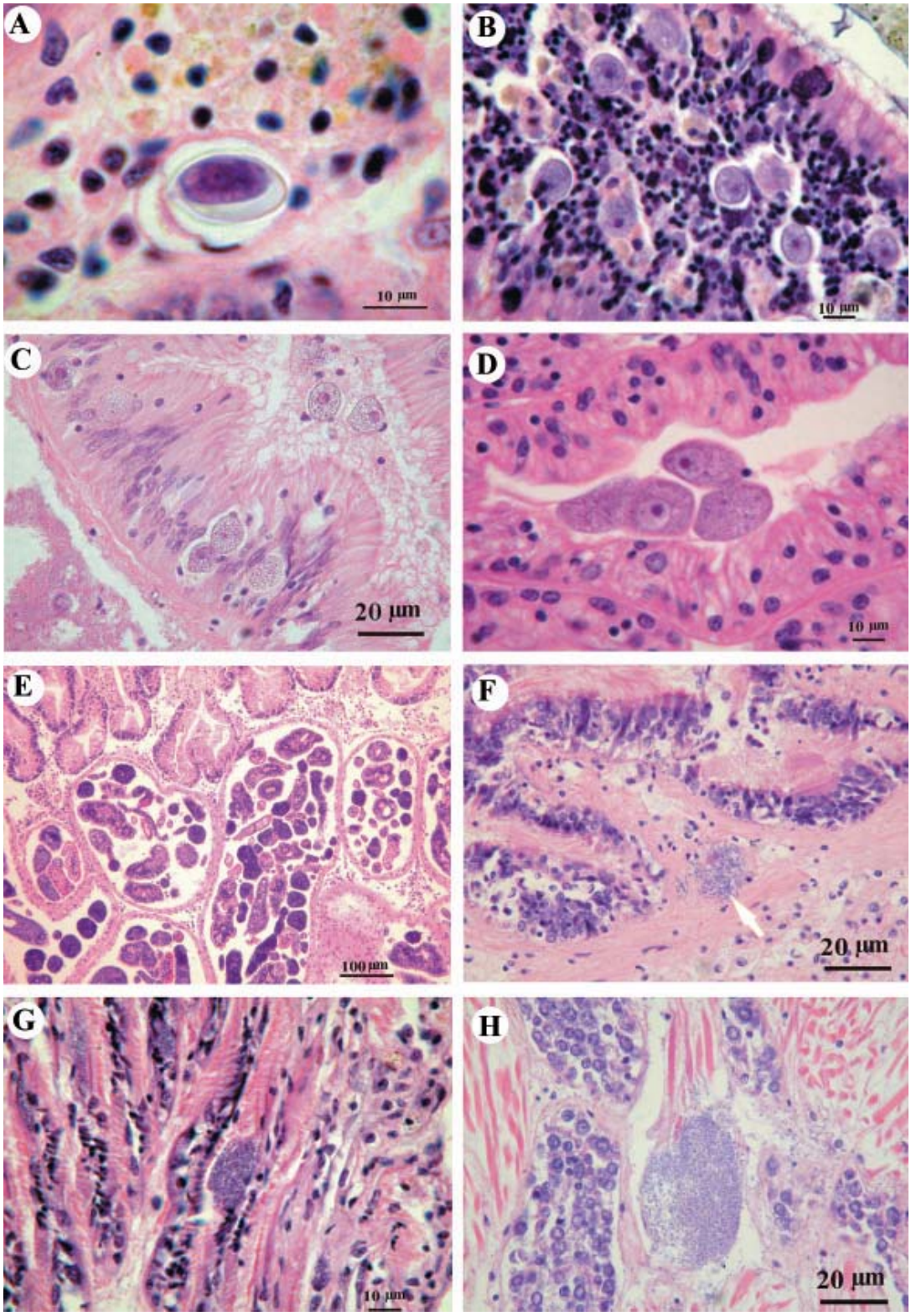

Figure 1. (A) Ovocyst of *Nematopsis* sp. in the connective tissue of the digestive gland. (B) Gregarines in digestive epithelium. (C) Gregarines in epithelium and lumen of digestive ducts. (D) Coccidia in kidney. (E) Trematoda sporocysts containing cercaria in the mantle. (F) Bacterial proliferation in connective tissue of labial palp. (G) Bacterial bag in gill tissue. (H) Bacterial accumulation in gonadal tissue.

Currently, there are few studies available about pathological alterations in Solenidae (Bilei *et al*., 1997; Fahy *et al*., 2002; Conchas *et al*., 2005), this subject being very important when developing mollusc farming or exploiting a resource such as the razor clam.

Research carried out has focused on two very important areas for razor clam extraction in Galicia: Cangas in the Ría of Vigo (Southern coast of Galicia) and Finisterre in the Ría of Corcubión (Northern coast of Galicia). Samplings were planned quarterly during 2003, 2004 and 2005, 30 specimens being collected per sampling.

So far, no pathologies have been described that can be considered a cause for concern. As Conchas *et al*. (2005) point out, the most important step is the detection of gregarines (*Nematopsis* sp.), which usually appear as ovocysts in the connective tissue of gills, digestive tubes and mantle (Fig. 1A), as well as other undetermined gregarines, observed in digestive epithelium (Figs. 1B and 1C). Also, renal coccidiosis was observed during the gamont stage, (Fig. 1D). As for metazoa, trematodes were the parasites that caused most alterations in the host, both during the metarcercaria stage, where they are usually encysted in the foot and mantle, and in the sporocyst stage in gonad, gill, digestive gland and mantle (Fig. 1E), producing a drastic reduction of gonadal tissue and on many occasions total castration of the razor clam.

Research studies about cases of mortality (Conchas *et al*., 2005) have failed to reveal the implication of the above-mentioned parasites, or whether the presence of bacterial proliferations (Figs. 1F, 1G and 1H) may have been simply a *post-mortem* infection. The predominant bacterial groups were *Vibrio* and filamentary bacteria. An analysis of environmental variables associated with these mortalities suggests that certain water temperatures and salinities, together with gonadal development stages, sometimes increase mortality in these bivalves.

Regarding tissue alterations, we would underline the presence of infiltrations in some tissues in certain cases, especially in the gill, digestive gland and gonad. These infiltrations may be due to a response to conditions of “stress” in the razor clam, caused by environmental variables, or to a response to the different pathologies detected.

As a conclusion, we can confirm that the pod razor clam is at present not affected by the main pathologies detected in other bivalve molluscs. Nevertheless, further research will be invaluable in the different areas where this resource is being and will be exploited.

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Chapter 7: Pathology of sword razor shell (*Ensis arcuatus*) and grooved razor shell (*Solen marginatus*)

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Abstract

In this chapter we describe symbiont organisms and pathological alterations affecting bivalve molluscs, *Ensis arcuatus* (sword razor shell) and *Solen marginatus* (grooved razor shell), part of the Solenoidea Superfamily. This superfamily includes species with commercial interest in different countries but, despite that, there are not many references to its pathology. In Galicia, Solenidae are an important marine resource, obtained by the exploitation of natural beds. Moreover, currently, hatchery experiments are taking place in order to establish the basis for future aquaculture activity. Knowledge of all biological aspects, including the pathological one, is fundamental for correct resource management and, in the future, for aquaculture management.

Parasites and pathological alterations found in Solenidae in Galicia may be classified according to their pathogenicity. On the one hand, there are those parasites or alterations that are not a potential danger for the exploitation of these molluscs, due to the low level of prevalence and/or intensity detected and since there is no damage caused to the host (rickettsias, gregarines, ciliates, haplosporidia and coccidia). On the other hand, there are those with a potential danger, such as *Marteilia* sp. and germinoma.

1. Introduction

Solenidae, such as the sword razor shell (*Ensis arcuatus*), pod razor clam (*Ensis siliqua*) and grooved razor shell (*Solen marginatus*) originating from the exploitation of natural beds, are commercialised in Spain.

During recent years, trials have been carried out in hatcheries in an attempt to lay the foundations for developing future aquaculture activity based on these Solenidae (Martínez, 2002). Pathological knowledge concerning these molluscs is scarce, and most research has been carried out during the last decade (Bilei *et al*., 1997; Xu *et al*., 1999; Ceshia *et al*., 2001; Chai *et al*., 2001; López and Darriba, 2006; Darriba *et al*.,

2006, 2010). There are references to cases of mortality in the farming of European razor clams in Galicia, as well as in Asturias (fishermen´s associations, personal communication). Knowledge of symbiont organisms – referring to "symbiont" in its original meaning, which includes mutualist, commensalist and parasitist relationships - and pathological alterations affecting the species included in the Solenidae group, may help to explain the causes of this type of incidents.

There must be three stages in the development of a line of investigation about pathological alterations affecting a group of organisms with commercial interest, and in the search for useful results in the management of the resource exploitation in a region (Villalba, Xunta de Galicia internal report):

- A preliminary approach, allowing the identification of symbiont organisms and pathological alterations affecting the group which is the object of this research.
- Deeper research about potentially harmful pathological alterations detected, in order to acquire a level of knowledge that will allow us to tackle the next stage.
- Development of measures to combat pathological problems so that they will be mitigated or disappear.

In Galicia, pathological research carried out in molluscs to date has focused mainly on **mussels** (*Mytilus galloprovincialis)* cultured on floating marine platforms (rafts) (Gómez-Mourelle, 1993; Villalba *et al.*, 1993a and b; Robledo *et al.*, 1994; Robledo and Figueras, 1995). This research classified in the above-mentioned stages 1 and 2, stage 3 not having been tackled, since there is no real pathological threat for culturing them; **clams** (*Tapes decussatus, Venerupis pullastra, V. rhomboides* and *Tapes philippinarum*), which have been studied mainly in stages 1 and 2 (Figueras *et al.*, 1992; Villalba *et al.* 1993c; López *et al.*, 1998; Villalba *et al.*, 1999; Ordás *et al.*, 2001; Casas *et al.*, 2002a and b; Villalba *et al.*, 2005); **oysters** (*Ostrea edulis*) research of stages 1 and 2 already carried out (Montes *et al.*, 1989; Montes, 1990, 1991; Figueras, 1991; Montes *et al.*, 1991, 1994, 1995) and stage 3 (Montes *et al.*, 2003; da Silva, 2005; da Silva *et al.*, 2005); **cockles** (*Cerastoderma edule*) research on stages 1 and 2 so far (Villalba *et al.*, 2001; Carballal *et al.*, 2001, 2003; Iglesias, 2006; Romalde *et al.*, 2007). Other groups with commercial interest, such as Pectinidae and Solenidae, have scarcely been studied. Since 1998, the authors of this chapter have been undertaking research on stages 1 and 2, previous to developing strategies to combat pathological problems (stage 3) that may affect Solenidae populations (*E. arcuatus, E. siliqua* and *S. marginatus*) in Galicia. In this chapter we present the main results of studies about *E. arcuatus* and *S. marginatus* and include a bibliographic review about pathological problems in Solenidae and other molluscs.

2. Viruses

One frequent problem that appears repeatedly in the description of diseases with presumed viral origin in molluscs is the failure to demonstrate a causal relationship between the virus and the pathology observed. This would imply that the agent was isolated and the disease was reproduced experimentally (Sindermann, 1990). The absence of stable cell lines in molluscs determines to a great extent the development of virological research in this group, and most references to viral infections are based on morphological descriptions at ultrastructural level by using transmission electronic microscopy (revised by Sindermann, 1990; Elston, 1997; Renault and Novoa, 2004). Research about viral diseases in molluscs is far from easy due to the lack of immunoglobulin production, which is a characteristic of the immunological system of these invertebrates, blocking the use of specific serological methods. Other molecular techniques have been used in virus studies in bivalves (Renault and Novoa, 2004; Davison *et al.*, 2005; Romalde *et al.*, 2007; Davison *et al.,* 2009).

There is no information in the literature about the existence of diseases related to viruses in Solenidae, although the development of viral diseases in other marine bivalve molluscs has been documented (reviewed by Farley, 1978; Lauckner, 1983; Johnson, 1984; Elston, 1997; McGladdery, 1999; Bower and McGladdery, 2003; Renault and Novoa, 2004). The main viral agents recorded affecting bivalve molluscs are part of the following families: Papillomaviridae, Polyomaviridae, Malacoherpesviridae, Togaviridae, Retroviridae, Reoviridae, Birnaviridae, Iridoviridae, Picornaviridae and Baculoviridae (Chang *et al*., 2002; Renault and Novoa, 2004; Garcia *et al*., 2006; Cheslett *et al*., 2009; Meyers *et al*., 2009; Davison *et al.,* 2009). Frequently, viruses infecting adult molluscs have been considered as secondary invaders or parasites associated with stressful conditions but not primary pathogens; this happens since usually they are detected in organisms affected by other diseases or by environmental stress conditions (Lauckner, 1983). However, iridovirus (Comps, 1988), herpesvirus (Renault *et al.*, 1994; Elston, 1997) and birnavirus (Suzuki *et al.*, 1998; Kurokawa *et al.*, 1999) have been related to causes of mortality in the absence of primary infections originated by other agents. Also, minor ARN-type viruses have been associated with the mortality of carpet shell clams (*T. decussatus)* in Galicia (Novoa and Figueras, 2000) and of green-lipped mussels *Perna canaliculus* in New Zealand (Jones *et al.*, 1996).

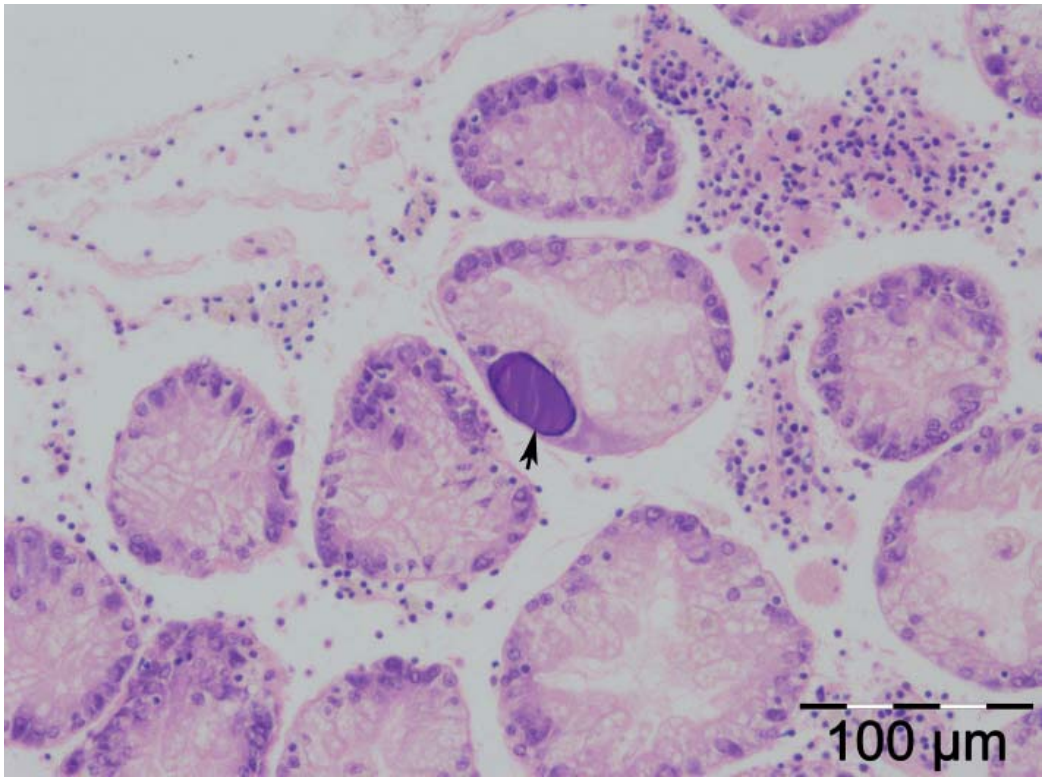

Figure 1. Histological section of digestive gland with basophilic inclusion (arrow) of viral origin in the epithelium of *E. arcuatus* (HHE) digestive gland.

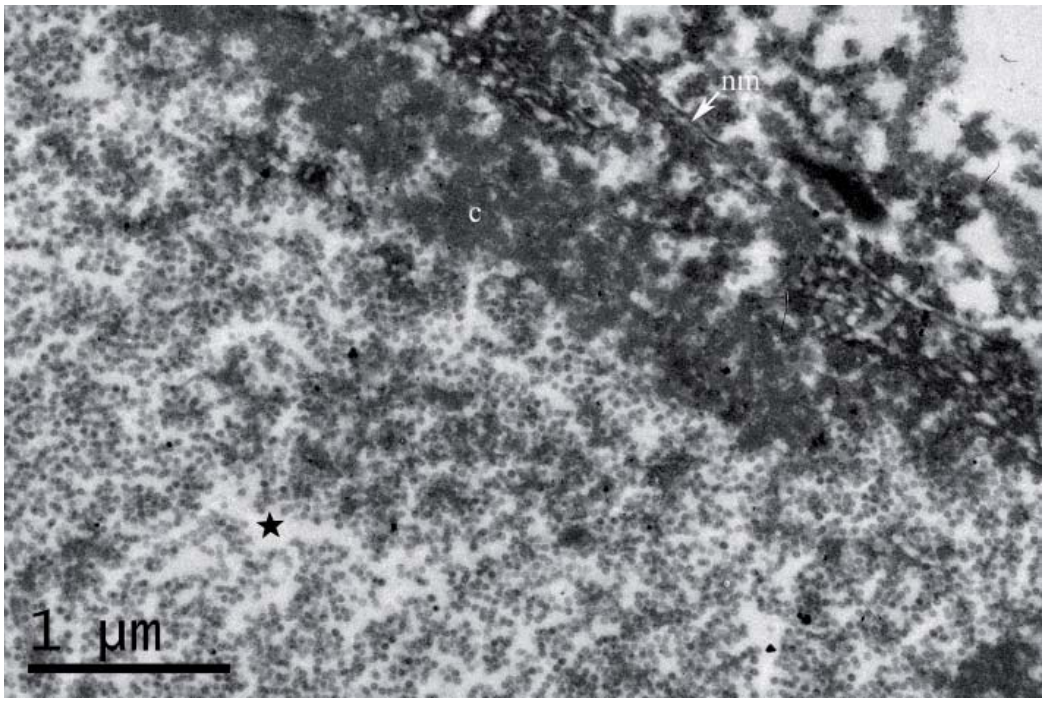

Figure 2. Ultrastructure of viral particles inside basophilic inclusions located in the epithelium of *E. arcuatus* digestive ducts.

Development of neoplastic alterations and the origin of tumours in molluscs

have been related to infections by retrovirus (Oprandy *et al*., 1981; House *et al*., 1998; Renault and Novoa, 2004; Romalde *et al*., 2007). However, in the case of germinoma detected in *E. arcuatus* populations in Galicia (Darriba *et al*., 2006), based on the results obtained so far, it is not related to the presence of a virus (non-published data). Recently, basophilic inclusions have been detected in cells of the epithelium of digestive tubules in *E. arcuatus* (Fig. 1) (Darriba *et al*., 2010) and *E. siliqua* (Ruiz, 2010), similar to those detected in *Crassostrea gigas* and *C. virginica* oysters, originating a viral gametocyte hypertrophy and associated with the presence of viruses, belonging probably to Papillomaviridae and Polyomaviridae families (Farley, 1985; Choi *et al*., 2004; García *et al*., 2006; Cheslett *et al*., 2009; Meyers *et al*., 2009). A later ultrastructural study about *E. arcuatus* inclusions revealed the presence of viral particles (Fig. 2) (Ruiz *et al*., in press).

3. Bacteria

There are no bivalves free of bacteria, they feed by filtration, which is why they have a very high bacterial biota, formed mostly by gram-negative bacteria, and to a lesser extent by gram-positive bacteria (*Bacillus* and *Micrococcus* among others). The most frequent gram-negative bacteria are those pertaining to *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Flavobacterium*, *Pseudomonas* and *Vibrios* genera (Lauckner, 1983; Paillard *et al*., 2004). It has been demonstrated that adult bivalves may tolerate higher concentrations than larvae (Tubiash *et al*., 1965). It is difficult to distinguish between non-pathogen species or strains, for example *Vibrio* and *Pseudomonas* form the bacterial flora of the digestive tract in bivalves (Lauckner, 1983). There are several publications and reviews about bacterial diseases in larvae and juveniles in marine mollusc hatcheries, as well as in adults (Lauckner, 1983; Sindermann, 1990; McGladdery, 1999; Estes *et al*., 2004; Paillard *et al*., 2004; Prado *et al*., 2005; Prado, 2006). Solenidae culture in hatcheries is in an experimental stage (Martínez, 2002), so there is no pathological research about these phases. Regarding adults from Galician natural beds, the presence of bacteria has been detected (unidentified) in different organs (gill, kidney, digestive gland, gonad and blood sinus) of *E. siliqua, E. arcuatus* and *S. marginatus* (Ruiz, 2010; and non-published data) (Fig. 3). In *S. marginatus* from Galicia basophilic and extracellular cysts of prokaryotic organisms have been detected, coated by an eosinophil fibrous cover and located in tubules of water in gills (Fig. 4) (López *et al*., 2008). Similar cysts have been observed in other bivalve molluscs (Villalba *et al*., 1993c; López *et al*., 1998; Carballal *et al*., 2001).

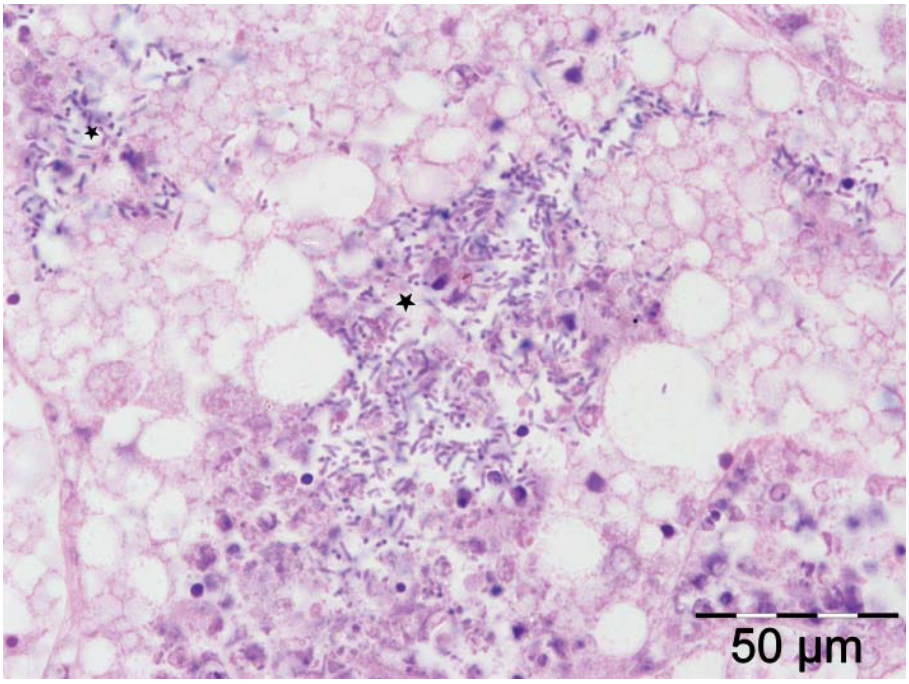

Figure 3. Histological section of the *E. arcuatus* digestive gland with bacterial proliferation (asterisk) in connective tissue (HHE).

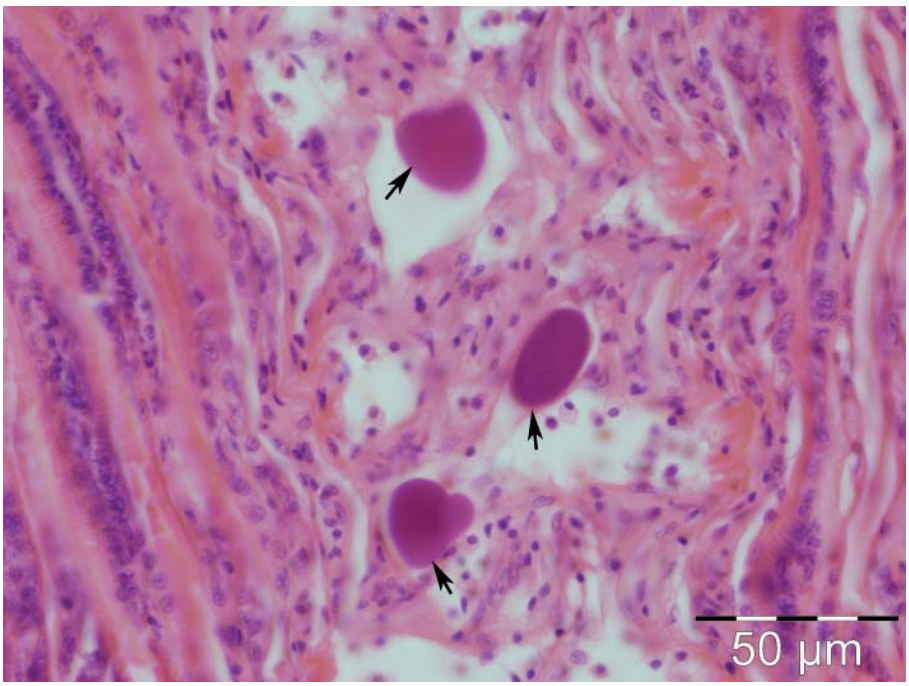

Figure 4. Histological section of *S. marginatus* gill with extracellular basophil cysts (arrows) of prokaryotic organisms located in water tubules (HHE).

Colonies of prokaryotic organisms were detected in the *E. arcuatus* digestive gland and kidney (Darriba *et al*., 2010), in *E. siliqua* and *S. marginatus* digestive gland and gill (Ruiz, 2010; López *et al*., 2008). These are intracytoplasmic basophilic colonies, with rounded-elongated shape (Fig. 5), similar to organisms such as

Rickettsia and *Chlamydia*, mentioned by different authors (Bower *et al*., 1994; Paillard *et al*., 2004). Important tissue damage and host reaction were not observed. However, there are references relating the presence of intense infections by organisms such as *Rickettsia* and *Chlamydia* with high mortality in other molluscs, such as scallops (Le Gall *et al.*, 1988), clams (Villalba *et al*., 1999) and abalones (Friedman *et al*., 2000; Moore *et al.*, 2000). Elston (1986) also relates an incident of massive mortality in Pacific razor clams (*Siliqua patula)* with an infectious process provoked by a prokaryotic intranuclear organism affecting gills, so the pathology was called nuclear inclusion X (NIX). This infectious process was associated with apparently secondary bacterial infections.

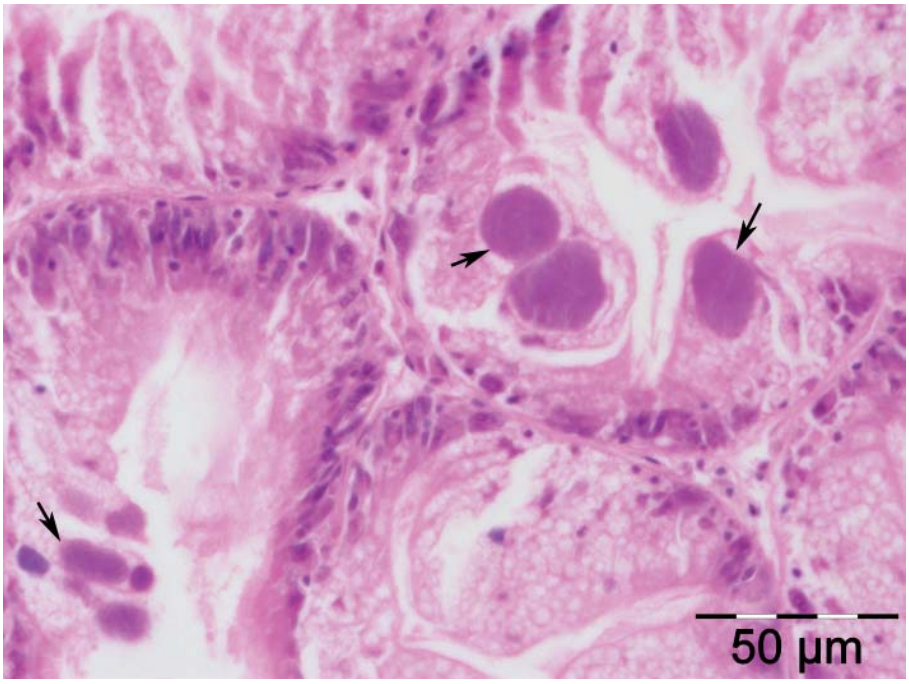

Figure 5. Histological section of *S. marginatus* digestive gland, with intracellular prokaryote colonies of rickettsia type (arrow) in epithelial cells of digestive tubules (HHE).

4. Fungus

Few cases of fungus infections in marine bivalve molluscs have been described. Fungus has a very important role in the biodegradation of calcareus substrate (including shells of molluscs). Several fungus species are known to colonise the shells of dead and living bivalves, and to use the energy existing in the organic matrix of the shell (Lauckner, 1983). The most widely-researched disease caused by fungus was the disease of the shell, foot or hinge, depending on the affected part, which is a mycosis that mainly affects *Ostrea edulis* from Western Europe. Alderman and Jones (1971) described the disease and identified a fungus, *Ostracoblabe*

implexa, as the causal agent. The fungus mycelium develops in the shell, and initially the disease appears as chalky white specks on the inner surface of valves; when the disease proliferates greenish warts are observed, of gummy appearance, formed by conchiolin. The shell becomes thicker and deformed in an advanced state of the disease, which sometimes prevents the valves from closing and usually leads to the mollusc death. *O. implexa* was observed in shells of dead *Solen* sp. molluscs on the French shores of the English Channel (Lauckner, 1983).

5. Protozoa

5.1. Phylum Apicomplexa

Protozoa of phylum Apicomplexa, classes Coccidia and Gregarinia, were described as parasites of marine bivalve molluscs in different countries (Lauckner, 1983; Villalba *et al.*, 1993c; Robledo *et al.*, 1994; Soto *et al.*, 1996; Villalba *et al.*, 1997; López *et al.*, 1998; Carballal *et al.*, 2001). The life cycles of these parasites are not well known and their systematics is continuously being examined. All organisms belonging to Phylum Apicomplexa have gamogonic and esporogonic stages in their life cycles, and some even have a merogonic stage. There are species that complete the life cycle in just one host (monoxenous) while in other cases they experience host alternation (heteroxenous) (Lauckner, 1983; Vivier and Desportes, 1990; Desser and Bower, 1997; Desser *et al.*, 1998). Coccidia are usually detected in kidney tubules, although there are reports of their presence in gonads, intestine, digestive gland and gill (Lauckner, 1983; Desser and Bower, 1997). In recent research carried out in Solenidae natural beds in Galicia, the presence of a non-identified coccidium was observed in *E. arcuatus* kidney (Darriba *et al.*, 2010) and *E. siliqua* (Conchas *et al.*, 2001; Ruiz, 2010). Different stages of the life cycle were detected: merozoite, macrogamont, microgamont, oocyst, sporocyst and sporozoites (Figs. 6, 7 and 8).

Coccidia species mentioned in the literature for marine bivalves belong to the *Pseudoklossia* or *Margolisiella* genera (Desser and Bower, 1997; Carballal *et al.,* 2001). According to Desser and Bower (1997), species belonging to *Margolisiella* have monoxenous cycles and spend the gamogonic, sporogonic and merogonic phases in the same host; also, their sporocyst has 2 to 4 sporozoites. Species pertaining to the *Pseudoklossia* genus have heteroxenous cycles (gamogonial and sporogonial in one host and merogonial in another), and the sporocyst has 2 sporozoites. Based on this and on the fact that the meronte stage has never been observed (although the merozoite has), it is thought that the coccidia detected in *E. arcuatus* and *E. siliqua* might be *Pseudoklossia* sp. That would be a coccidia whose life cycle has 3 phases and whose merogony takes place in another host (heteroxenous). There is no

evidence that this parasitisation damages Solenidae, although there are authors who suggest that a severe infection may cause tissue alterations in the kidney affecting its proper functioning (Morado *et al.,* 1984; Carballal *et al.,* 2001).

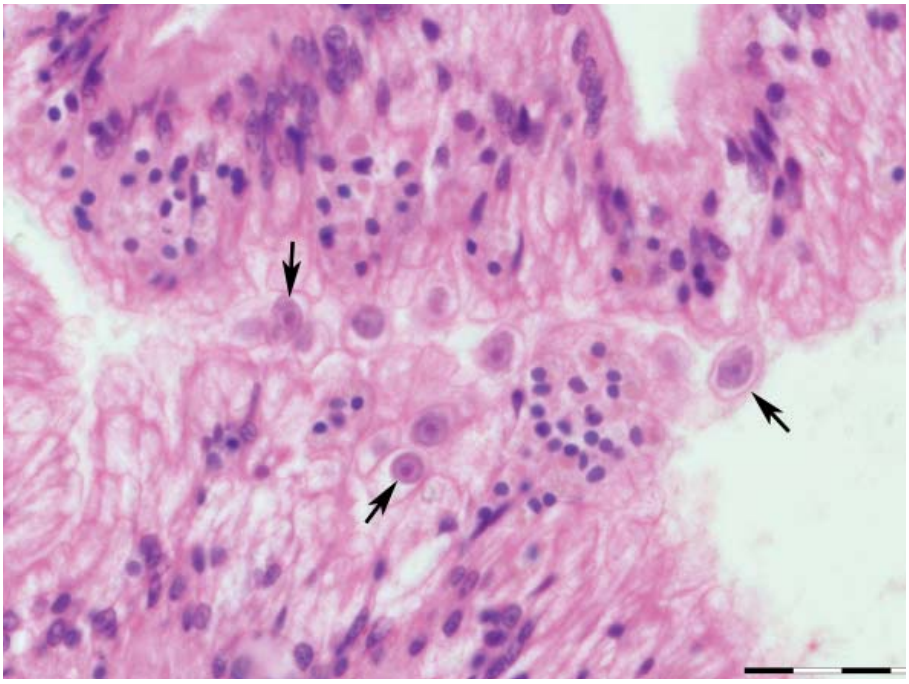

Figure 6. Histological section of *E. arcuatus* kidney with presence of coccidia. Merozoites (arrows) in epithelial cells of kidney tubules (HHE).

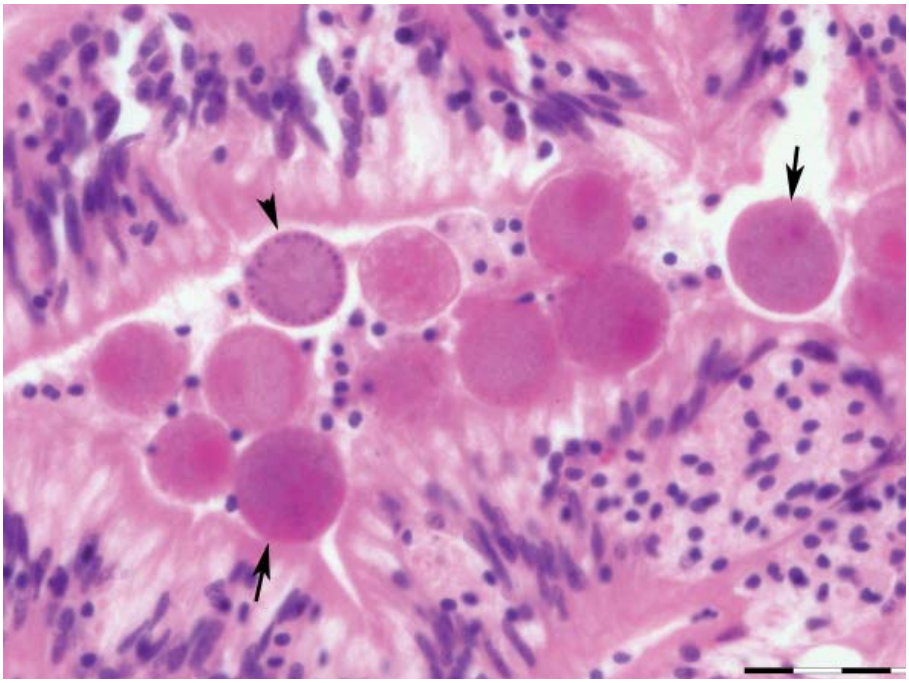

Figure 7. Histological section of *E. arcuatus* kidney with presence of coccidia. Macrogamonts (arrows) and Microgamonts (arrowhead) in the lumen of kidney tubules (HHE).

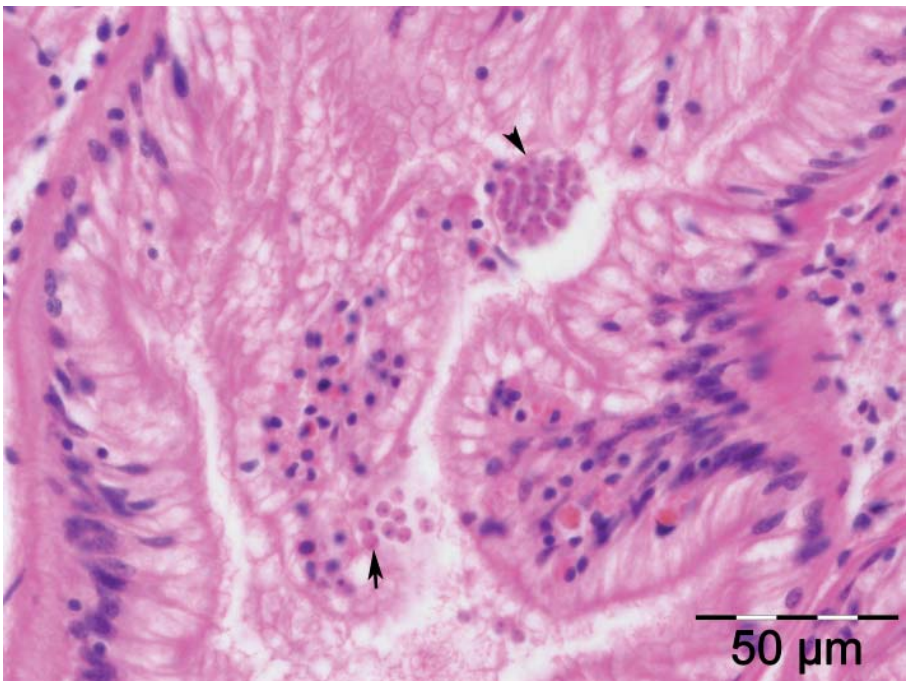

Figure 8. Histological section of *E. arcuatus* kidney with presence of coccidia. Oocyst (arrowhead) with sporocysts inside; and free sporocysts (arrow) (HHE).

In the group of gregarines there are species from the Porosporidae family whose life cycle shows alternations between two hosts: a crustacean and a mollusc (Lauckner, 1983). Several species of the *Nematopsis* genus use a bivalve mollusc as intermediate host (Lauckner, 1983; Azevedo and Cachola, 1992; Bower *et al.,* 1994; Tuntiwaranukuk *et al.,* 2004). The lack of knowledge about the complete life cycle of these species makes it difficult to identify. In Galicia, oocysts of *Nematopsis* sp., were detected in *E. arcuatus* (Darriba *et al.,* 2010), *E. siliqua* (Conchas *et al.,* 2001; Ruiz, 2010) and in *S. vagina* (= *marginatus*) (Soto *et al.,* 1996). Darriba *et al.* (2010) have observed free oocysts and intrahaemocytes in labial palps, gill, mantle, digestive gland, gonad, kidney and auricle in *E. arcuatus*. Each oocyst has just one sporozoite (Fig. 9). These oocysts would be the resistant forms and when they are free they would infect crustaceans. It is important to point out that in the case of *S. vagina* (=*marginatus*) in Galicia, oocysts of *Nematopsis* sp. were detected in the Ría of Vigo (Soto *et al*., 1996) but not in the Ría of Arousa (non-published data). This must be due to the absence of the intermediate host crustacean in the natural beds in Ría of Arousa. There are references to its presence in *S. vagina (=marginatus)* in France (Lauckner, 1983). Usually, it does not exhibit important pathological effects, and its presence was only associated with mortality in cockles in Portugal (Azevedo and Cachola, 1992) and with injuries in cockles branquial filaments in Galicia in high densities (Carballal *et al*., 2001). Another non-identified gregarine was also observed in *E. arcuatus* (non published data) and *E. siliqua* in Galicia (Conchas *et al*., 2001; Ruiz, 2010) located in the epithelium of the intestine and surrounding connective

tissue (Fig. 10). Similar organisms were described as non-identified coccidia in *Ruditapes decussatus* (Joly, 1982; Navas, 2008), and as non identified gregarines in *Ruditapes* spp. (Bower *et al.,* 1992; Villalba *et al.,* 1993c) and in cockles (Carballal *et al.*, 2001). López (1995) recognized the impossibility of distinguishing whether it is a gregarine or a coccidium, since only one phase of its life cycle is observed.

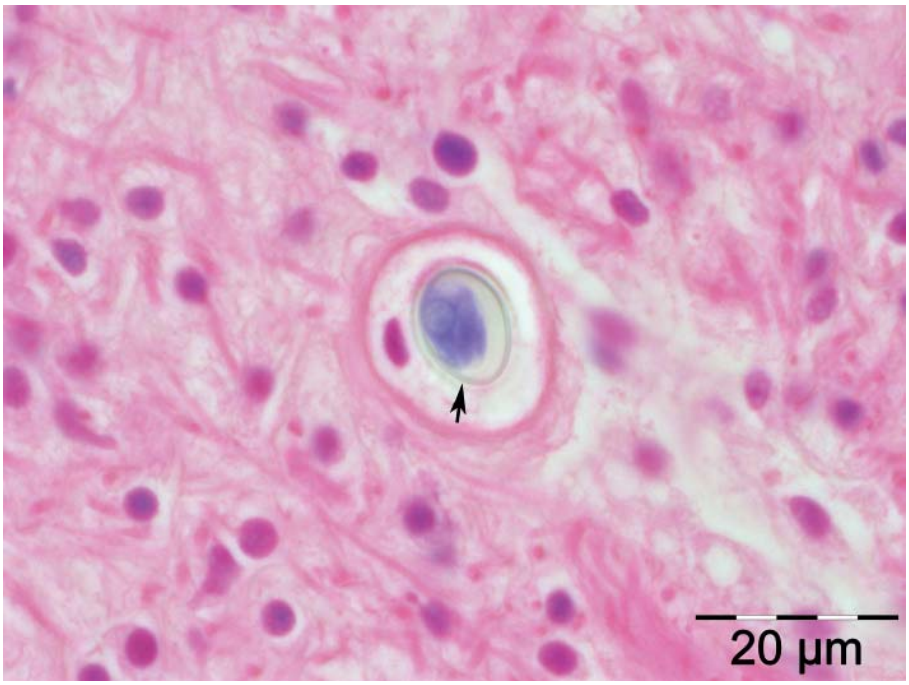

Figure 9. Histological section of *E. arcuatus* digestive gland, with oocysts of *Nematopsis* sp. in connective tissue (arrow) (HHE).

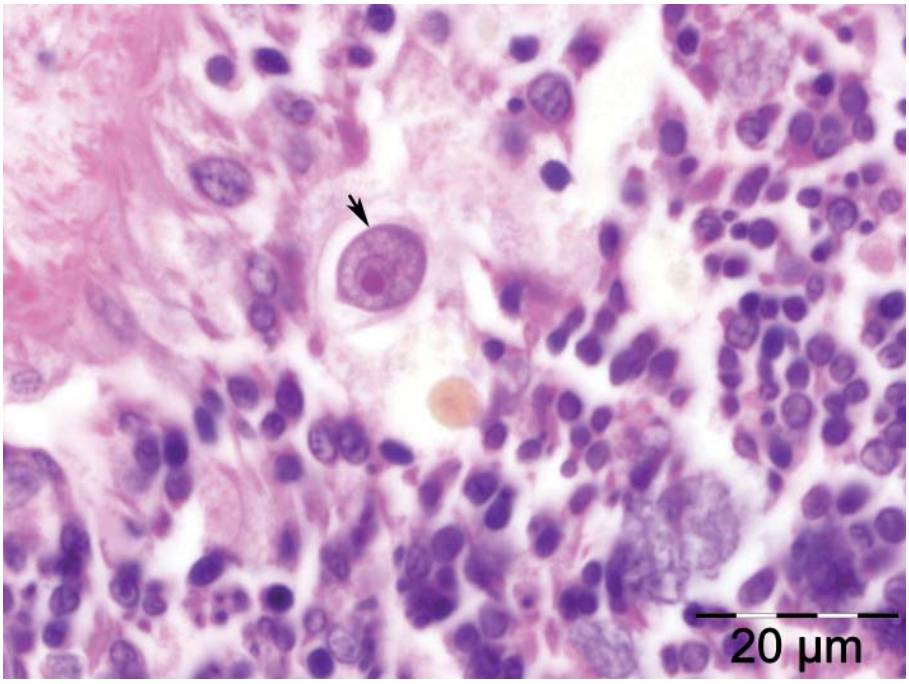

Figure 10. Histological section of *E. arcuatus* digestive gland. Non-identified gregarine (arrow) at the base of the epithelium of a digestive gland (HHE).

5.2. Phylum Myzozoa

The disease of the *Crassostrea virginica* American oyster known as the Dermo was initially considered to be produced by a fungus. Later, morphological research included the etiological agent among protozoa, first in Phylum Labyrinthomorpha (Mackin and Ray, 1966), and later in Phylum Apicomplexa (Perkins, 1976), called *Perkinsus marinus.* Genetic research in the 1990's demonstrated its proximity to dinoflagellates rather than to apicomplexa; Norén *et al*. (1999) proposed the creation of a new Phylum Perkinsozoa, since they considered this species did not fit either dinoflagellates or apicomplexa. Actually, the genus *Perkinsus* is included into the class Perkinsea (infraphylum Protalveolata, subphylum Dinozoa, phylum Myzozoa, subkingdom Alveolata) (Cavalier-Smith, 2004). Different species of this parasite were associated with major mortalities in different marine bivalves around the world, including Spain (Lauckner, 1983; Azevedo, 1989; Santmartí *et al.*, 1995; Burreson and Ragone, 1996; Villalba *et al*., 2004, 2005). Infections for *Perkinsus atlanticus* (=*olseni*) have been described in the following clams: *Tapes deccussatus, T. philippinarum, Venerupis pullastra* and *Venerupis aureus* (=*Paphia aurea)* in different stretches of the Spanish Mediterranean coast (Navas *et al*., 1992; Sagristà *et al*., 1996; Casas *et al*., 2004) and Galicia (Ordás *et al*., 2001; Casas *et al*., 2002a). Despite its presence in Galician coasts affecting clams, this infection has not been detected in research undertaken so far about Solenidae pathology. However, there is a reference to the presence of *P. marinus* in *Ensis minor* in the USA (Lauckner, 1983).

5.3. Phylum Haplosporidia

Phylum Haplosporidia includes parasites known to cause major mortalities in marine bivalves around the world, including Europe, among which are *Haplosporidium* spp., *Bonamia* sp. and *Mikrocitos* sp. (Bachere and Grizel, 1982; Lauckner, 1983; Andrews, 1984; Friedman *et al.*, 1991; Friedman, 1996; Hine, 1996; Carnegie and Barber, 2001; reviewed in Bower and McGladdery, 2003; Hine and Thorne, 2002; Diggles *et al*., 2002; Balseiro *et al*., 2006). Darriba *et al*. (2010) observed plasmodium of the haplosporidia type in the epithelium of digestive channels and surrounding connective tissue of *E. arcuatus,* in Galicia, with high prevalence and low intensity (Fig. 11), and in some cases these plasmodia had as many as 20 nuclei. These authors did not observe tissue damage and/or host reaction. It was not possible to characterize this haplosporidian since its spores were not detected. It must be stressed that the parasite *Haplosporidium edule* detected in *Cerastoderma edule* in Galicia (Azevedo *et al*., 2003), although it was not related to mortalities, might be lethal due to the great intensity and the tissue damage caused. In Galicia haplosporidian-like organisms were also detected in the digestive epithelium of

different kinds of clams (Villalba *et al.*, 1993c; López *et al.*, 1998), but always with very low intensities, and consequently with no tissue damage. Haplosporidian-type organisms were observed in Italy in *Ensis* sp. (Bilei *et al*., 1997) and *Haplosporidum* sp. in *E. minor* (Ceshia *et al*., 2001). Ceshia *et al.* (2001) describe a serious infection where sporulation was observed though they did not identify the species.

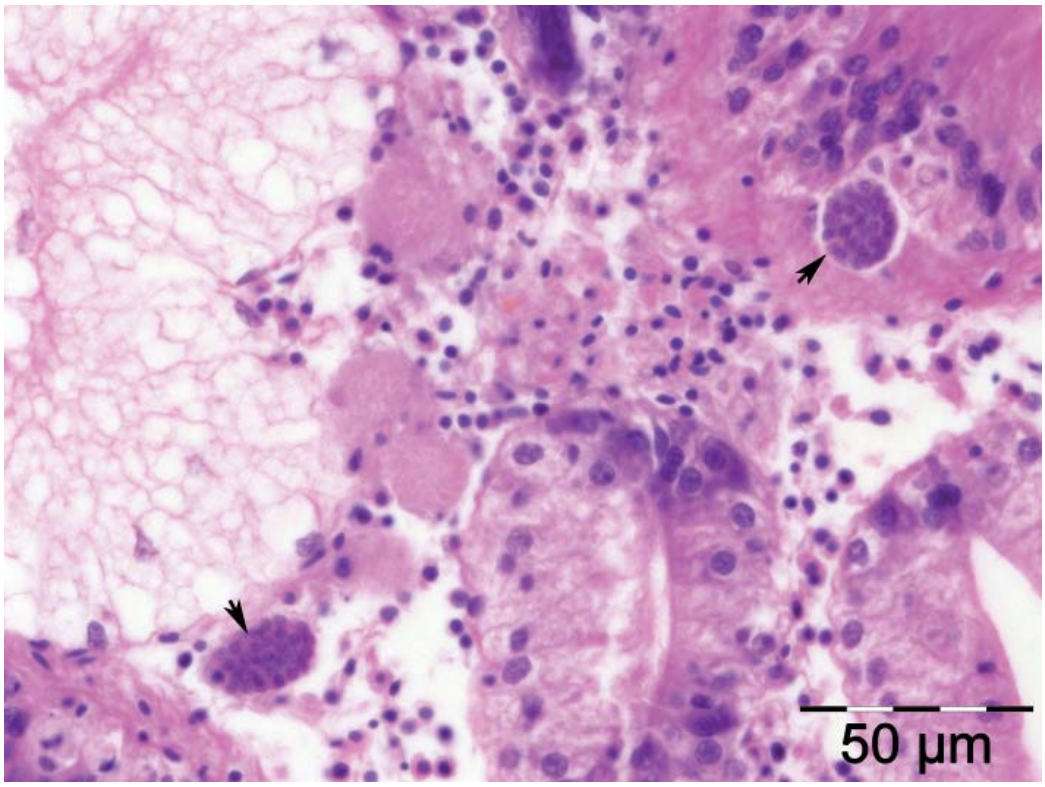

Figure 11. Histological section of *E. arcuatus* digestive gland. Plasmodia (arrows) of an non-identified haplosporidian in the base of epithelium of a digestive duct (HHE).

5.4. Phylum Paramyxea

Marteliosis is a disease of the digestive gland of bivalve molluscs caused by the *Marteilia refringens* protist parasite (Phylum Paramyxea, class Marteiliidea). This disease has caused very high mortality in the European flat oyster *Ostrea edulis* and led to very important economic losses in the French oyster industry. Currently, it is a disease listed as non-exotic, according to European legislation (Directive 2006/88/CE) and of obligatory declaration according to the aquatic code of the World Organisation for Animal Health (OIE, 2009). When studies of the *Marteilia* genus began, it was included in Phylum Ascetospora, together with haplosporidia (Paramyxea class and Stellatosporea class respectively) (reviewed by Desportes, 1981), while in later studies it was necessary to create a new Phylum Paramyxea (reviewed by Desportes and Perkins, 1990; Berthe *et al*., 2000). One characteristic of the species that belong to this new Phylum is internal cleavage to produce cells within cells during sporulation. There are references to the presence of different

Marteilia species in different bivalve molluscs worldwide (Lauckner, 1983, Berthe *et al*., 2004; López-Flores *et al*., 2008a). The presence of *Marteilia refringens* in bivalve molluscs in Galicia has been mentioned by different authors (Figueras and Montes, 1988; Villalba *et al.*, 1993a and b; Figueras *et al*., 1996). In Solenidae, the presence of *Marteilia* sp. is recognised in *S. marginatus* in Galicia (López and Darriba, 2006) and in *E. minor* in Italy (Ceshia *et al*., 2001); as well as *M. refringens* in *S. marginatus* in Andalucía (López-Flores *et al*., 2008b). López and Darriba (2006) detected different stages of *Marteilia* sp. life cycle in *S. marginatus,* initial stages (or primary cell) with elongated or spherical shape, uni- or multi-nucleated, were observed in the epithelium of digestive ducts or tubules (Fig. 12). More advanced stages, at the same location, were detected with greater intensities (Fig. 13). Data presented by López and Darriba (2006) suggest the existence of higher prevalences and greater intensities in summer. Ultrastructural research of *Marteilia* sp. in *S. marginatus* shows the formation of spores from divisions undergone by the primary cell, it being possible to observe pre-sporangia (secondary cells) containing the refractive bodies, spore primordium (tertiary cell) (Fig. 14) and the spore formed by 3 uni-nucleate sporeplasms. López and Darriba (2006) even found 6 spores in a sporangium, while in bibliographies about *Marteilia* spp. the number of spores indicated so far is 4 (Longshaw *et al*., 2001).

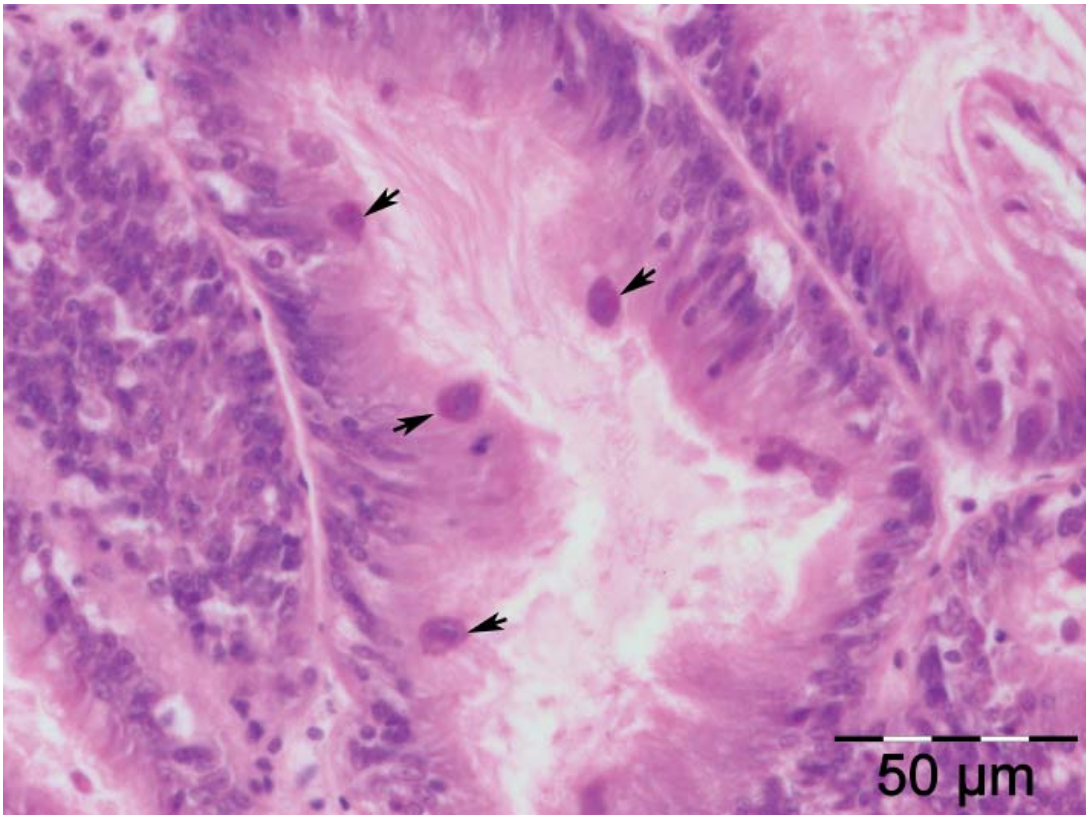

Figure 12. Histological section of *S. marginatus* digestive gland parasitized by *Marteilia* sp. Initial stages of *Marteilia* sp. (arrows) (HHE).

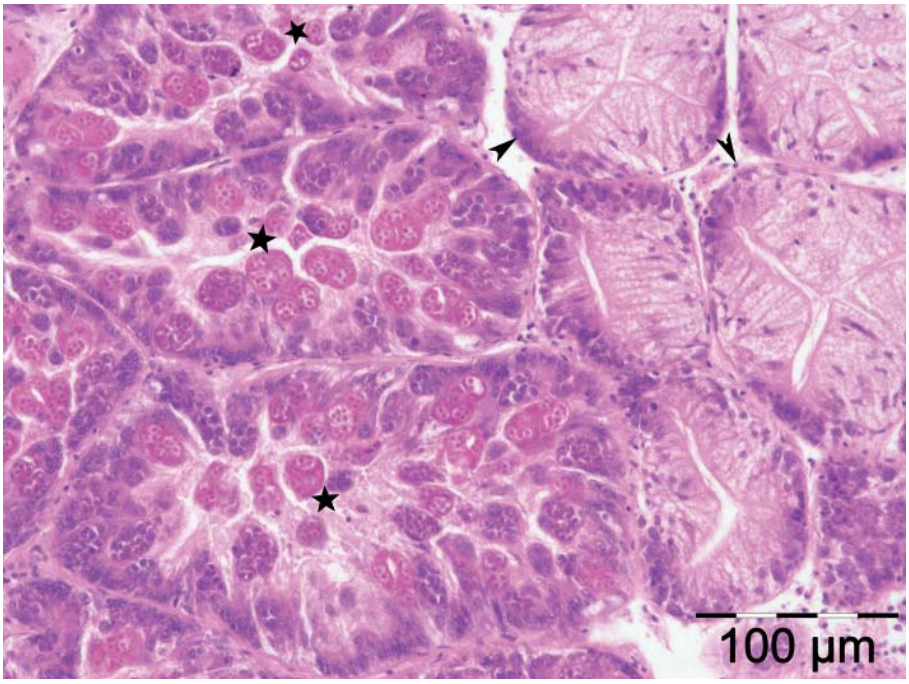

Figure 13. Histological section of *S. marginatus* digestive gland parasitized by *Marteilia* sp. Digestive tubules infected by advanced stages of *Marteilia* sp. (asterisk) (HHE) and non-infected digestive tubules (arrowhead).

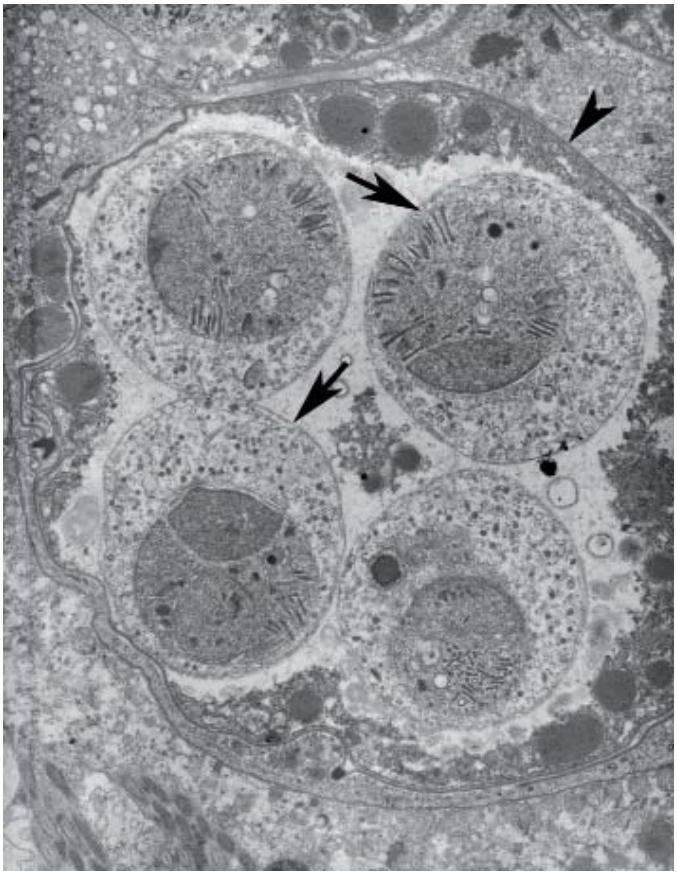

Figure 14. Ultrastructure of *Marteilia* sp. in *S. marginatus*. Sporangium (arrowhead) with 4 spores (arrow) (5000 x).

5.5. Phylum Ciliophora

Ciliates of the *Trichodina* genus (Phylum Ciliophora) were reported in some bivalve molluscs (Carballal *et al*., 2001; Lohrmann *et al*., 2002; Caceres-Martínez and Vasquez-Yeomans, 2003; Xiao *et al*., 2005) without causing damage. However, an inflammatory alteration and reaction was observed in the epithelium of *C. gigas* gill, parasitised by *Trichodina* (Boussaid *et al*., 1999), and mortalities in *C. edule* and *C. angulata* parasitised by this ciliate were reported by Lauckner (1983). Ciliates of the *Trichodina* genus were observed in gills, palps and pallial cavity in *E. arcuatus* (Darriba *et al*., 2010), *E. siliqua* and in *S. marginatus* (non-published data) in Galicia (Fig. 15). For *S. marginatus*, a non-identified ciliate was observed located in gills and pallial cavity (Fig. 16). No hemocytic reaction or tissue damage was detected in Solenidae in Galicia associated with the presence of ciliates, probably due to low intensities, despite observing high prevalence. Xu *et al*. (1999) reported the presence of *Trichodina liana* in *Solen (Plectosolen) gracilis* in China, but he does not give information about prevalences, intensities and damage caused.

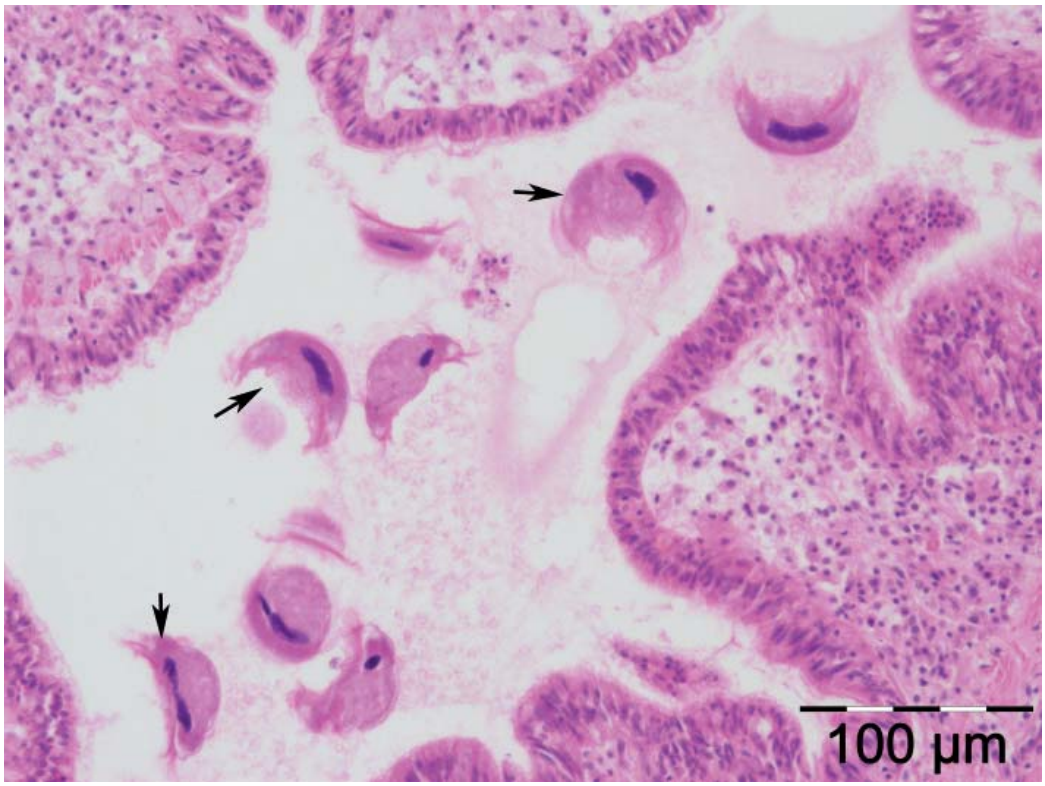

Figure 15. Histological section of *Trichodina* sp. ciliates (arrow) in *S. marginatus* pallial cavity (HHE).

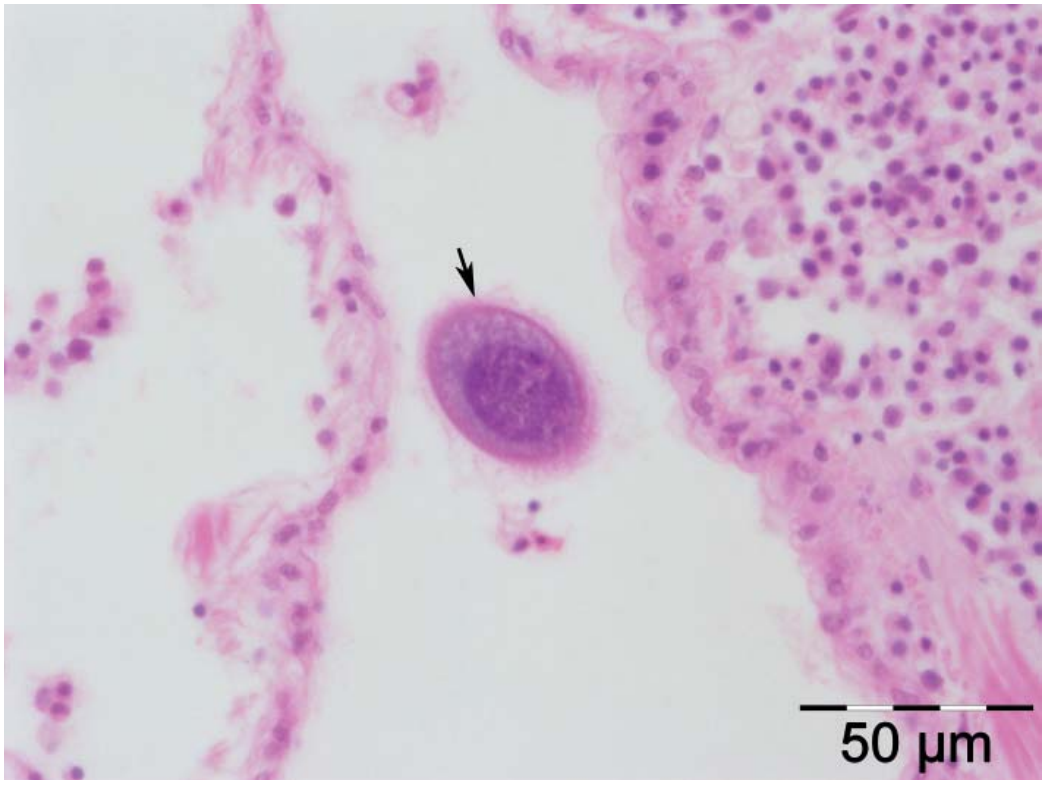

Figure 16. Histological section of an unidentified ciliate (arrow) in *S. marginatus* pallial cavity (HHE).

6. Metazoa

6.1. Phylum Plathelmintha

6.1.1. Turbellaria

Turbellaria are predominantly free-living predators, members of Rhabdocoela and Alloeocoela, and are present in marine molluscs, located in the digestive gland and mantle cavity respectively. In many cases, these species are considered as commensals rather than authentic parasites (Lauckner, 1983). Species belonging to the *Urastoma* and *Paravortex* genus (among others) were observed in bivalves (reviewed by Lauckner, 1983; Carballal *et al*., 2001) located in the cavity of the mantle and gill, and in the digestive gland, respectively. In Solenidae, in Galicia, they were observed with very low prevalence and intensity. In *E. arcuatus* an *Urastoma*-like was observed in the gill (Darriba *et al*., 2010) (Fig. 17), and in *S. marginatus* a *Paravortex*-like in the digestive gland (López *et al.*, 2008) (Fig. 18).

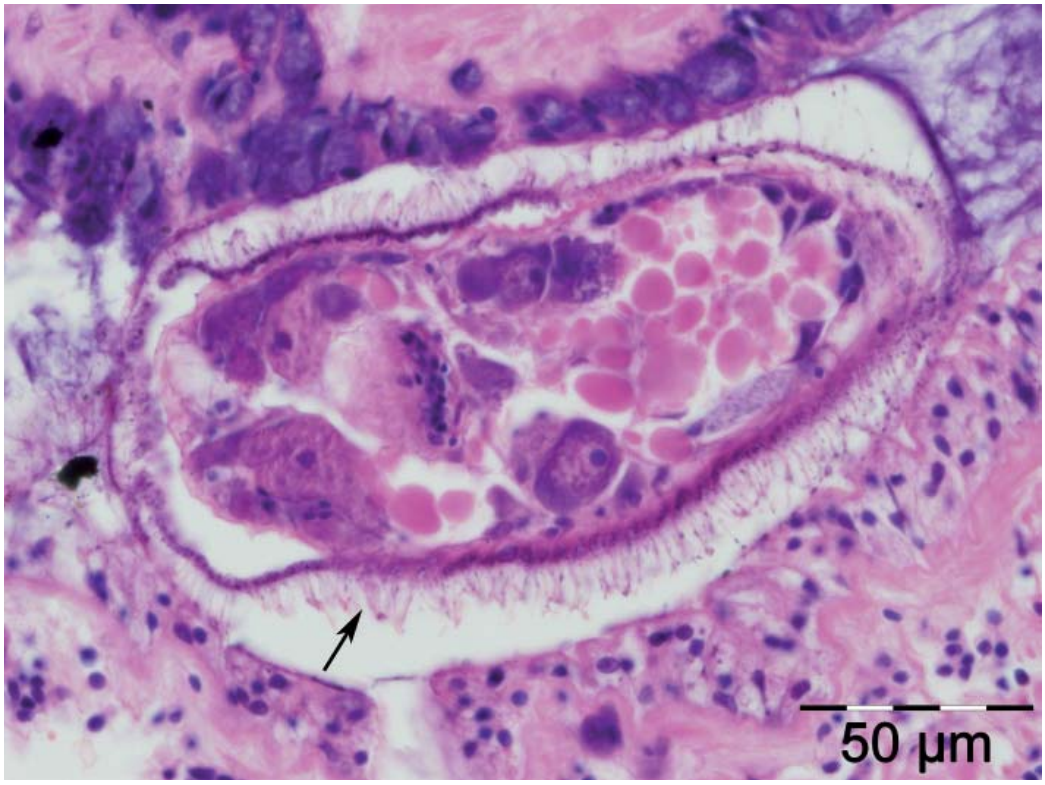

Figure 17. Histological section of *Urastoma*-like turbellaria (arrow) present in *E. arcuatus* gill (HHE).

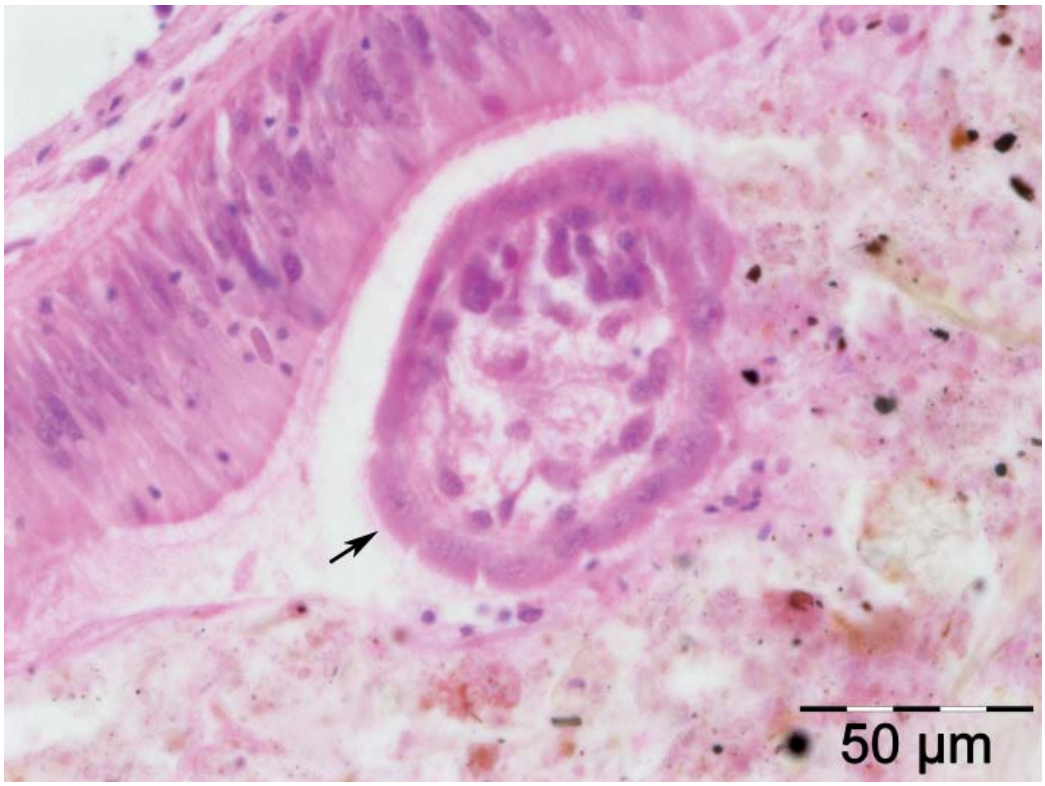

Figure 18. Histological section of *S. marginatus* digestive gland. *Paravortex*-type turbellaria (arrow) in lumen of intestine (HHE).

6.1.2. Trematoda

Digenean trematoda in adult phase are dorso-ventrally flattened worms that usually parasitize birds or fish. Life cycles are very complex, with larval stages that parasitize a minimum of two hosts, one of which is frequently a mollusc. There are different types of life cycles involving bivalves (Table I), which usually act as primary hosts (larval stage of sporocyst and cercariae) or secondary hosts (larval stage of metacercariae) of digenean trematoda, although sometimes they can also be definitive hosts.

Larval stages of digenean trematoda have been described in almost all marine bivalve species, so it is no exaggeration to consider this group as the most important metazoa parasite affecting these molluscs (Lauckner, 1983).

Parasitisation by sporocyst of trematodes of the Bucephalidae family have been detected in the *E. arcuatus* sword razor shell (Darriba *et al*., 2010) in Galicia. Trematodes of the Bucephalidae family belong to the suborder Gasterostomata and have quite uniform life cycles. Their sporocysts and cercariae always occur in bivalves, metacercariae encyst themselves in small teleost fish and adults inhabit the alimentary tract of predator fish. In *S. marginatus* and in *E. siliqua* sporocysts of a non identified digenean trematode have been detected in Galicia (Conchas *et al.,* 2001; Rodríguez *et al*., 2009; Rodríguez, 2010; Ruiz, 2010).

Table I. Example of the life cycles of some digenean trematoda parasites of bivalve molluscs. In brackets the stage of life cycle of the trematode that parasitizes the host is indicated.

Primary host	Secondary host	Final host
Bivalve (Sporocyst + Cercariae)	Fish (Metacercariae)	Fish (Adults)
Bivalve (Sporocyst + Cercariae)	**Bivalve** (Metacercariae)	Fish (Adults)
Bivalve (Sporocyst + Cercariae)	**Bivalve** (Metacercariae)	Bird (Adults)
Gastropod (Sporocyst + Rediae + Cercariae)	**Bivalve** (Metacercariae)	Fish (Adults)
Gastropod (Sporocyst + Rediae + Cercariae)	**Bivalve** (Metacercariae)	Bird (Adults)

Sporocysts observed in *E. arcuatus* in Galicia were pale reddish-yellow, tubular, with strangulated and thickened areas, highly ramified and entangled with

the host's tissue. Thickened sporocyst areas showed cercariae inside, in different developmental states. Mature cercariae were pear-shaped when relaxed, their mouth was in the central part of the body ventral face, and they had a tail at the rear end, formed by a central base and two long very contractile filaments (Figs. 19 and 20A). The average body size of cercariae was 276.5±20.4 µm (media±standard deviation, n=10) long and 56.0±9.1 µm wide in their central area. The anterior end of cercaria was more pointed and it was there where the rhynchus was located, which is the organ of penetration used by the cercaria to pierce the secondary host epidermis and encyst itself in the interior as a metacercaria (Fig. 20B).

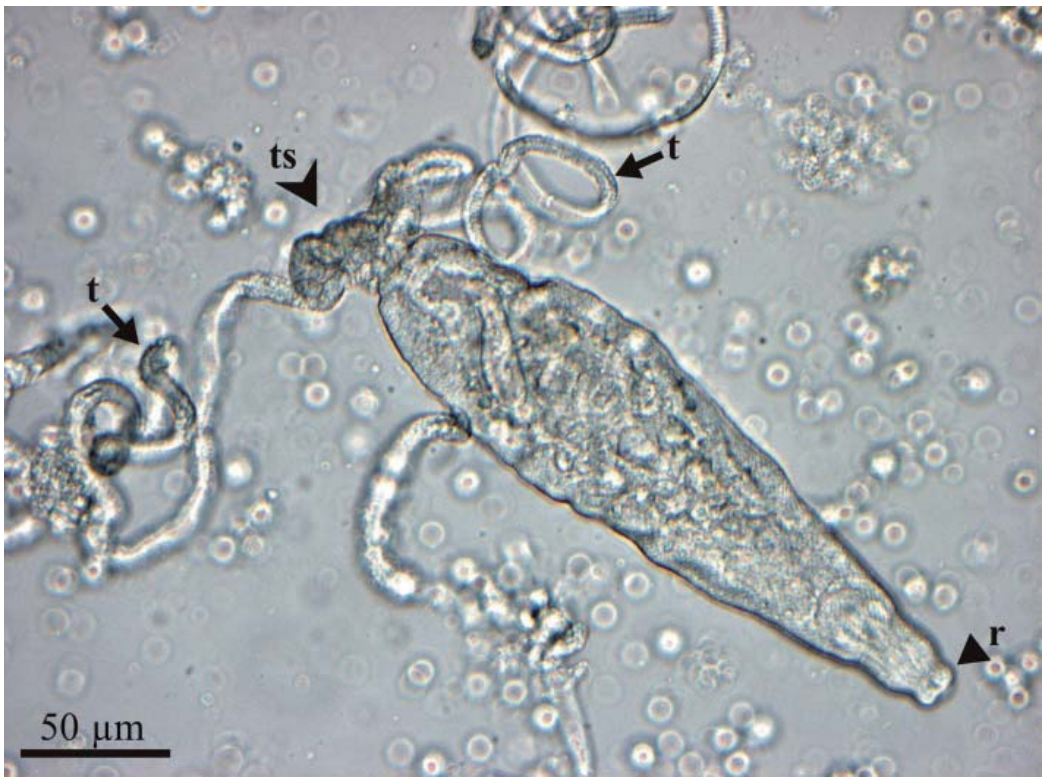

Figure 19. Frotis of trematode cercariae found in *E. arcuatus* (t, tail; ts, tail stem; r, rhynchus).

Examination of histological sections of affected individuals revealed that the sporocyst preferably invaded gonadal tissue (Fig. 21) and, in cases of intense parasitisation, other organs as well, such as the digestive gland, gills and kidney. As a consequence of that infestation, partial or total castration of parasitised individuals was produced. This effect is normal in all bivalves affected by Bucephalidae sporocysts (Cheng, 1967; Lauckner, 1983; Lasiak, 1991, 1993; Coustau *et al.*, 1993; Heasman *et al.*, 1996; Calvo-Ugarteburu and McQuaid, 1998).

The bucephalidae family is one of the most important among digenean trematodes. Its larvae affect several marine invertebrates with commercial economic

importance, such as mussels, cockles and oysters, as well as flat-fish and gadoids.

Bucephalidae larvae are probably the most harmful metazoan parasites affecting marine bivalves, because when they develop their sporocysts, castration and weakening of the host is caused (Lauckner, 1983). For Galician Solenidae, average prevalence was usually less than 7%; therefore this trematode does not seem to cause serious consequences at population level.

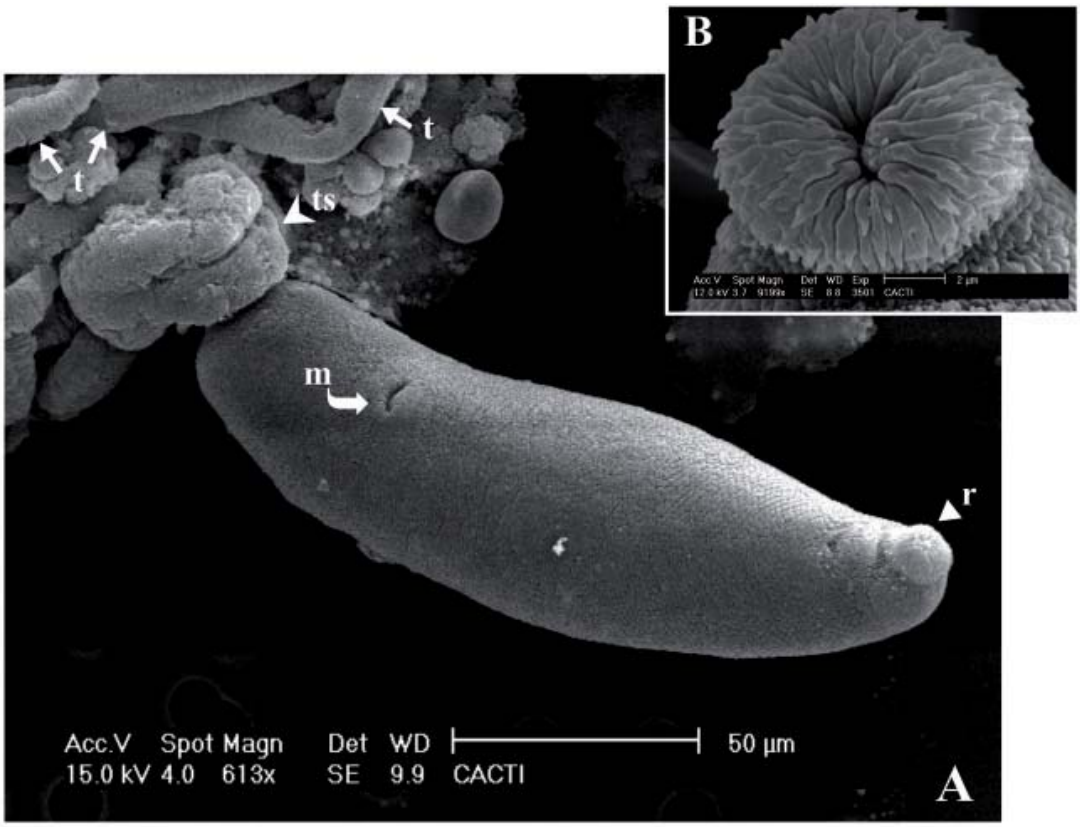

Figure 20. Micrographs of scanning electron microscopy (SEM) of trematode cercariae of Bucephalidae family. **A)** Cercaria (m, mouth; ts, tail stem; t, tail; r, rhynchus). **B)** Rhynchus details.

Regarding this trematode taxonomic position, the only conclusion reached was that it belongs to the Bucephalidae family. In general, cercariae of this family do not have characteristics that can be used for distinguishing their genus, and it is not possible to establish correspondence between a certain cercaria and an adult worm until the life cycle is known by means of the development of experimental infestations (Stunkard, 1974). In Galicia, parasitisation of a bucephalid trematode by sporocysts and cercariae, called *Labratrema minimus*, has been described in numerous banks of the cockle *Cerastoderma edule* (Iglesias *et al*., 2003; Iglesias, 2006). Morphological characteristics of this trematode sporocysts and cercariae – such as the pattern of the infestation of the cockle tissue – were similar to those described for Solenidae.

However, considering that bucephalid trematodes are extremely specific in choosing their primary intermediate host (Lauckner, 1983), it is improbable that they form part of the same species. There are bibliographic references that mention different species of Solenidae as trematodes secondary intermediary hosts.

Metacercariae of the Gymnophallidae family have been described in *Ensis ensis*, *S. marginatus* and *E. siliqua* on the French Mediterranean coast and in South Wales (Lauckner, 1983; Bowers *et al.*, 1996). There has been recent information about parasitisation by metacercariae of *Himasthla elongata, H. continua, H. interrupta* and *Renicola roscovita* in razor clams *Ensis* (*directus*) *americanus* from the Wadden Sea (Krakau *et al*., 2006), as well as *Parvatrema* sp. in *Tagelus plebeius* in Brazil (da Silva *et al*., 2009).

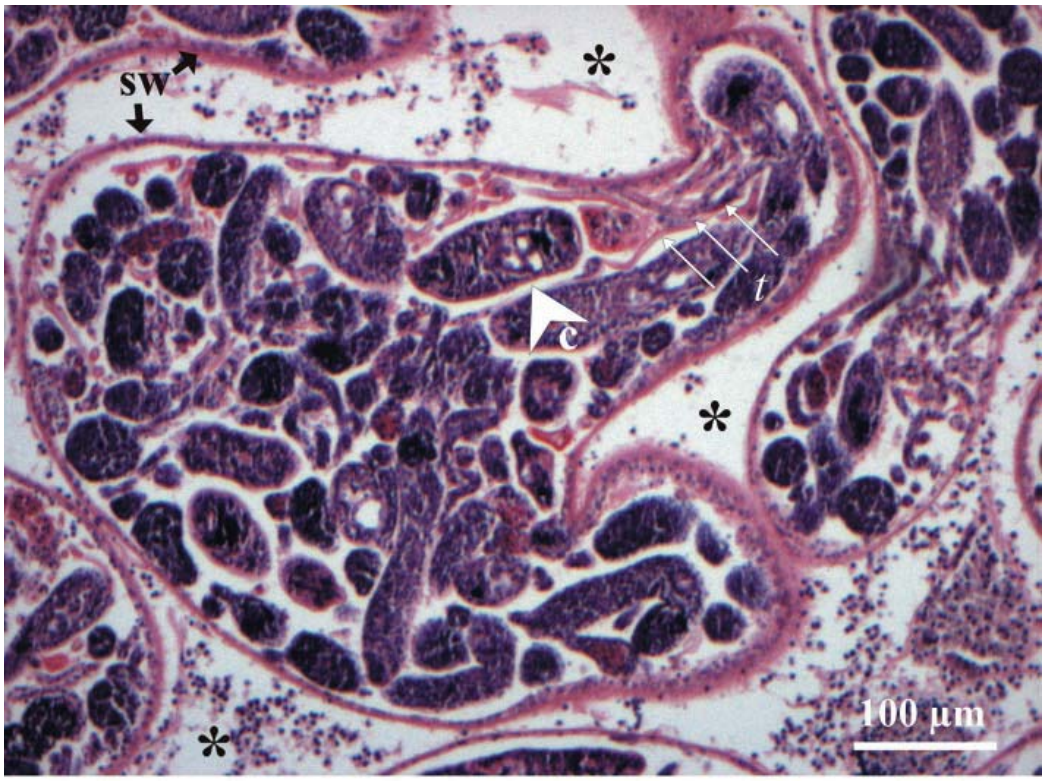

Figure 21. Histological section of *E. arcuatus* gonad. Sporocysts of trematode causing gonad castration (asterisk) (sw, sporocyst wall; c, cercaria; t, tail) (HHE).

In Galicia encysted metacercariae have been detected in palp, mantle edge, digestive gland and gill (Fig. 22), in histological preparations of *E. arcuatus* (Darriba *et al*., 2010) and in *E. siliqua* (Conchas *et al*., 2001; Ruiz, 2010). Based on its size and organs where it has been observed, these metacercariae might be *Renicola roscovita,* although it is not possible to be conclusive basing ourselves on the analysis of histological preparations. In *S. marginatus* encysted metacercariae with high intensities in pericardial glands have been detected in Galicia (Rodríguez

et al., 2009); isolation and research *in vivo* of these metacercariae allowed them to be identified as part of the *Curtuteria* genus (Echinostomatidae; Himasthlinae) (Kostadinova, 2005; Desclaux *et al.*, 2006; Russell-Pinto *et al*., 2006). Later research (Rodríguez, 2010), where these metacercariae of *S. marginatus* were analysed by scanning electron microscopy, showed that they are probably *Curtuteria arguinae*, a species described by Desclaux *et al*. (2006) affecting *C. edule* cockles from Arcachon Bay in France.

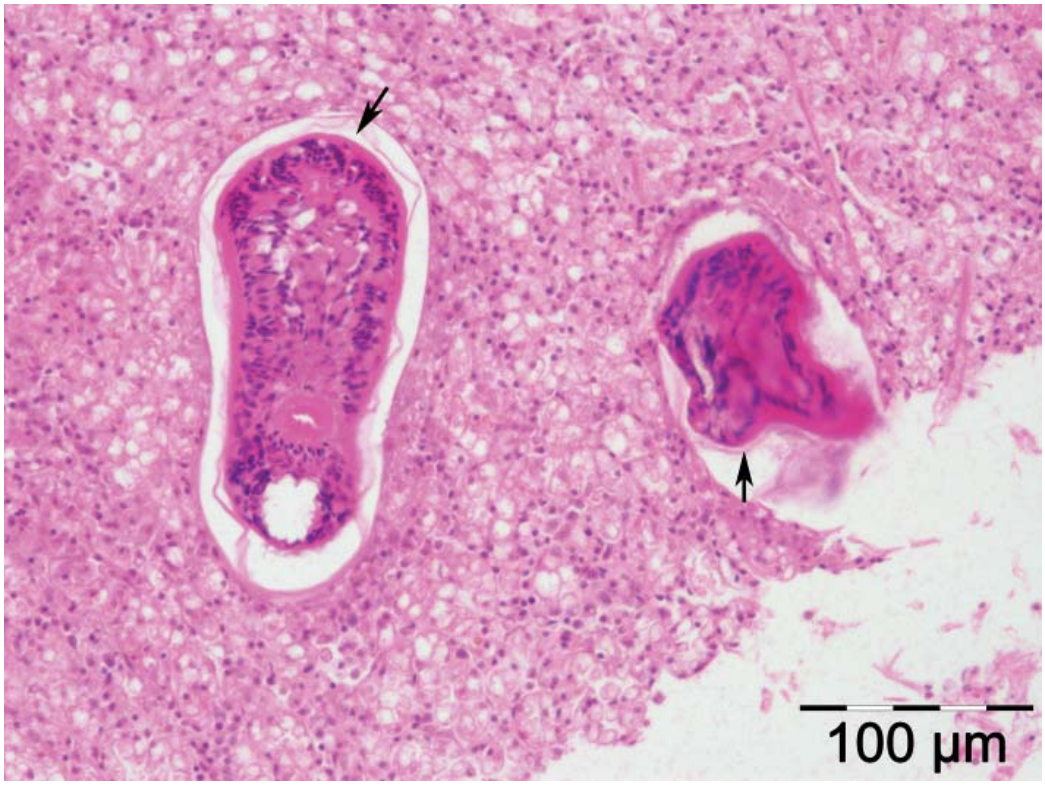

Figure 22. Histological section of *S. marginatus* pericardial gland. Encysted metacercaria (arrow) (HHE).

6.1.3. Cestoda

Organisms belonging to the cestoda class use bivalves as intermediary hosts. The presence of cestoda in bivalves was reviewed by Laukner (1983). *Echinobothrium* sp. was detected in different tissues (mainly in the foot) of *Solen vagina* (=*marginatus*) in France, and *Scolex pleuronectis* in the intestine and digestive diverticula in *Ensis minor* in Italy, as well as cestoda similar to *Scolex pleuronctis* in *S. vagina* (=*marginatus*) in France, *Acanthobothrium* sp. in *E. minor* of the Gulf of Mexico and *Rhinebothrium* sp. in digestive diverticula of *E. minor* (Lauckner, 1983).

Non-identified metazoa similar to the cestoda larvae described by Cremonte *et al.* (2005) were detected in Solenidae (Darriba *et al*., 2010) and in *S. marginatus* in Galicia (non-published data) (Fig. 23).

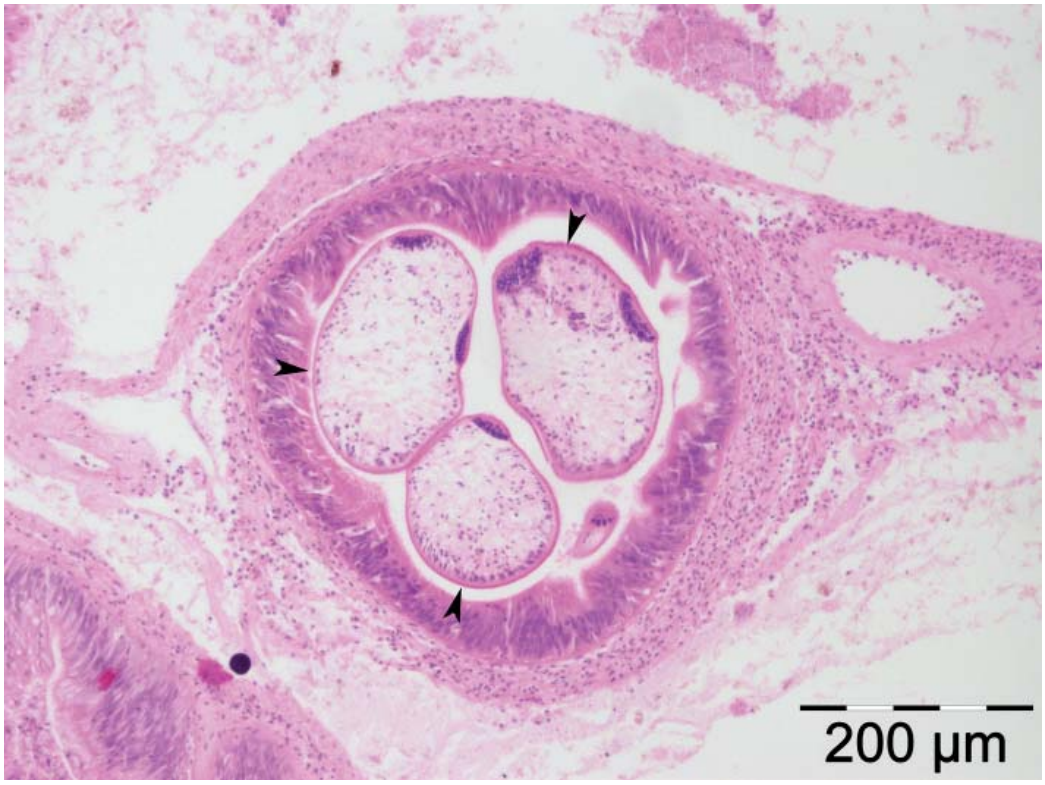

Figure 23. Histological section of *S. marginatus* digestive gland. Unidentified cestode-like metazoa (arrowhead) in the lumen of a digestive duct (HHE).

6.2. Phylum Arthropoda

6.2.1. Copepoda

Different species of copepoda (Crustacean class) have been identified as parasites of marine bivalve molluscs. Some, such as *Mytilicola* sp., modify their bodies and live as endoparasites in the intestine; however there are studies that consider this as a commensal rather than a parasite (Davey, 1989). Other copepods, such as *Modiolicola* sp., *Ostricola* sp. and *Myicola* sp., live in the mantle cavity, where they grip onto the gills (Lauckner, 1983). In Solenidae, in Galicia, non-identified copepods were observed in digestive glands and gill in *S. marginatus* (Fig. 24). Copepod prevalences and intensities were low, and consequently no important damage or reactions from the host were observed. There are references to the presence of copepods in Solenidae around the world, such as *Myocheres major* in the mantle cavity in *Ensis directus* on the Atlantic coast of North America and very abundant *Conchyliurus solenis* in the mantle cavity in *S. marginatus* in France (Lauckner, 1983).

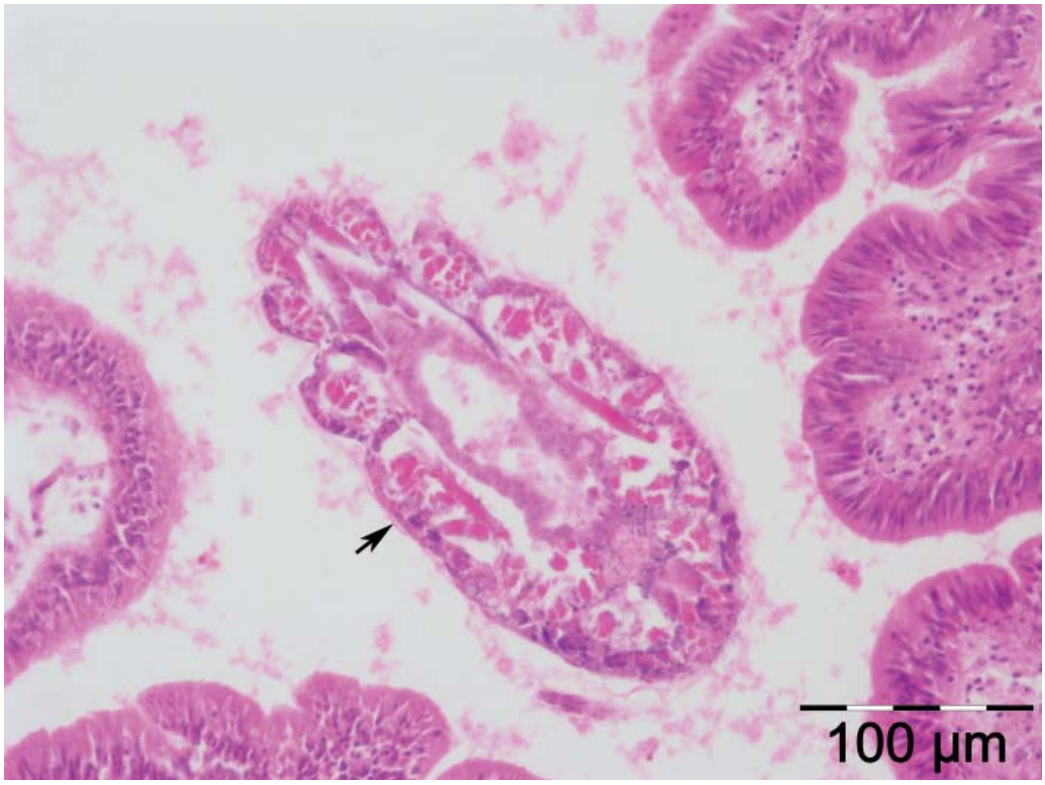

Figure 24. Histological section of an *S. marginatus* digestive gland. Copepod (arrow) in stomach lumen (HHE).

7. Other pathological alterations

There are references to different kinds of deformities affecting soft tissues as well as bivalve shells; some of these are the result of external mechanical impact, infections by microbial agents and the presence of pollutants (heavy metals, petroleum and other chemical agents). Deformities of the shell were sometimes related to the presence of trematoda and other agents (mechanical and/or chemical) affecting the edge of the mantle, whose main function is to secrete the material to produce the shell. In the revision of Lauckner (1983) about this kind of alteration, he mentions a deformity of the valves in *S. marginatus*.

Lauckner (1983) defines nomenclature normally used to refer to tissue deformities, such as hypertrophy, hyperplasia, metaplasm and neoplasia (the latter covers benign and malignant tumours).

Regarding neoplasia, there are references to its presence affecting different organs in bivalve molluscs, disseminated neoplasia being the one with most incidence (possibly, in some cases, of hemocytic origin) and in second place come those affecting the gonad (or gonadal neoplasia) (reviewed by Peters *et al*., 1994; Barber,

2004). Disseminated neoplasia consist of an excessive proliferation, in the sinus of hemolymph and in spaces of connective tissue, of abnormal circulating cells with a relatively large nucleus, an elevated nucleus/cytoplasm rate and a high frequency of mitotic figures (Peters, 1988; Elston *et al*., 1992). Within gonadal neoplasia there are three different histotypes: a) germinoma, which is a follicular proliferation of immature germinal cells that may spread and penetrate the interstitial tissue; b) stromal gonadal neoplasia, formed by spindle-shaped cells; and c) gonadoblastoma, a mixture of the others.

In Spain there are references to disseminated neoplasia in *M. galloprovincialis* (Gutierrez and Sarasquete, 1986; Villalba *et al.,* 1997), *R. decussatus* (Villalba *et al*., 1995), and *C. edule* (Villalbal *et al*., 2001). In Solenidae, it was detected in *S. marginatus* (López *et al.*, 2008), mainly in the gill (Fig. 25), and less frequently in digestive gland and heart; and one case in *Ensis siliqua*, also affecting mainly the gill (Ruiz, 2010). In the case of gonadal neoplasia, Alonso *et al.* (2001) reported a possible case of gonadal neoplasia affecting *M. galloprovincialis* mussels from the Ría of Vigo. Darriba *et al*. (2006) detected the presence of germinoma in *E. arcuatus* males (Fig. 26) and females (Fig. 27). Histological preparations corresponding to *E. arcuatus* germinoma were registered at the Registry of Tumours in Lower Animals with codes 7715 and 7716, respectively. The same type of germinoma was detected equally in males and females in *E. siliqua* (Ruiz *et al*., 2009; Ruiz, 2010). The characteristic of the germinoma observed by these authors is an uncontrolled proliferation of non-differentiated germinal cells located at the wall and lumen of gonadal follicles, and never observed outside the follicles invading the interstitial space. Cells are eosinophilic, with a high nucleus-cytoplasm ratio, mitotic figures being observed at times, indicating the proliferation speed.

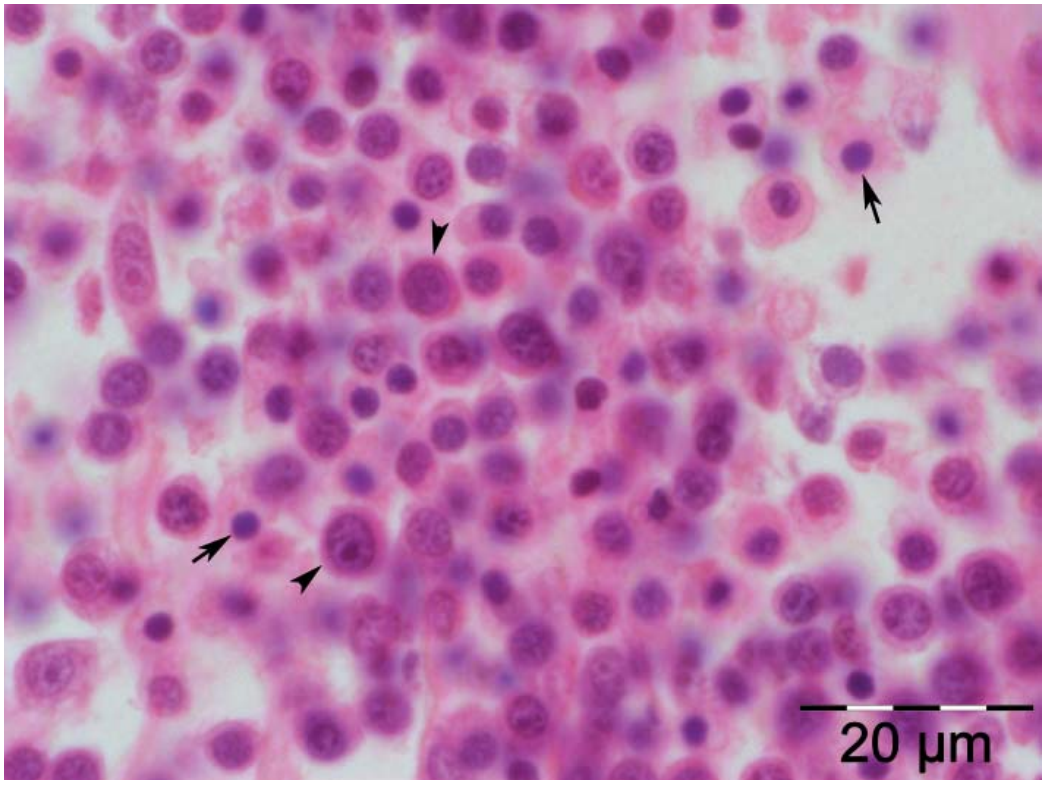

Figure 25. Histological section of *S. marginatus* gills with neoplastic cells (arrowhead) and normal cells (arrow) (HHE).

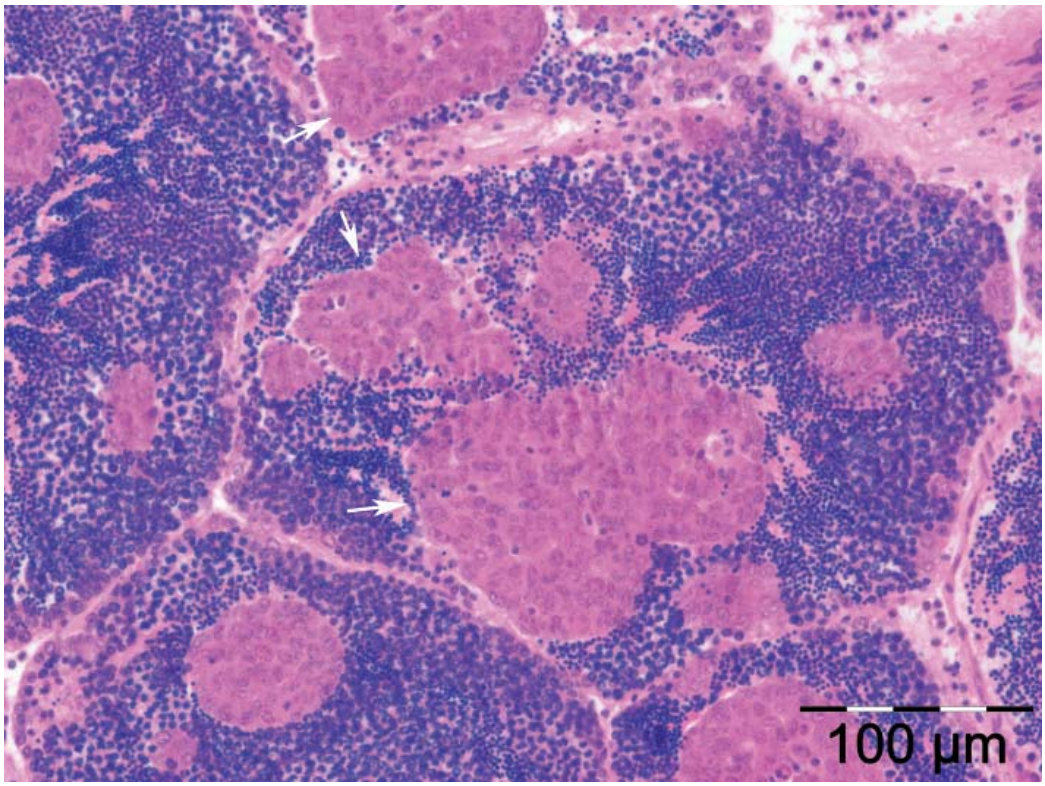

Figure 26. Histological section of *E. arcuatus* gonad. Presence of germinoma (arrow) in male (HHE).

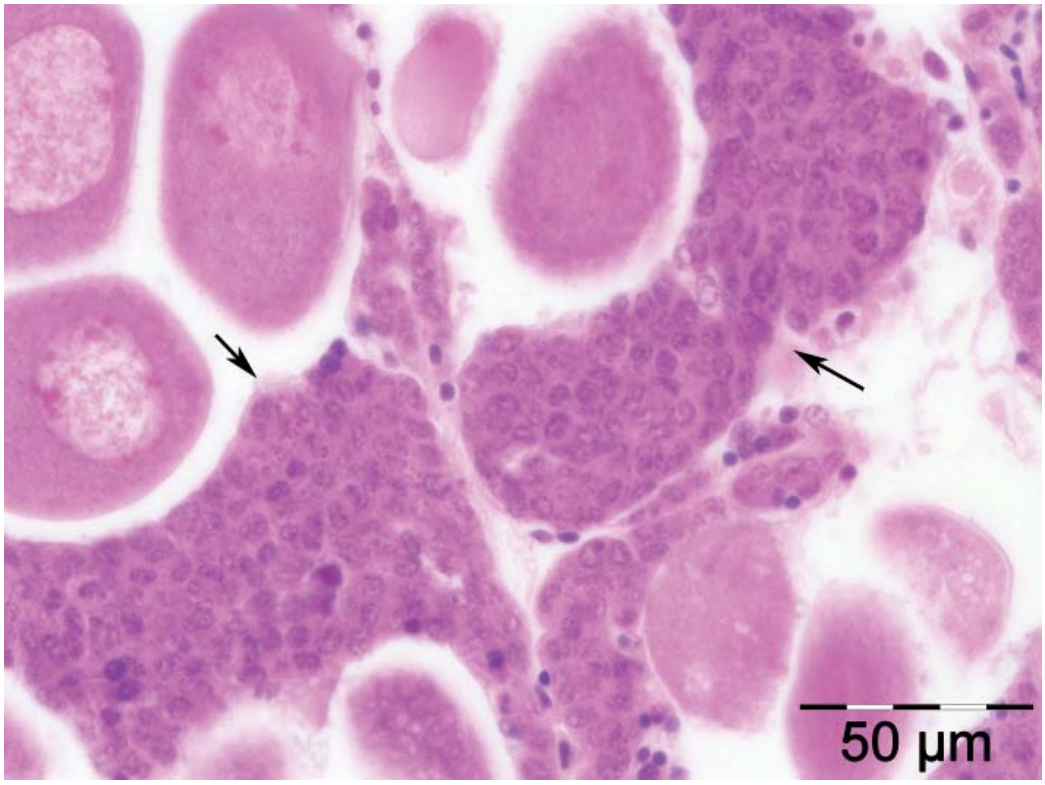

Figure 27. Histological section of *E. arcuatus* gonad. Presence of germinoma (arrow) in female (HHE).

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Chapter 8: Advances in our knowledge of microbiota of hatchery-cultured Solenidae

Susana Prado, Javier Dubert, Jesús L. Romalde and Juan L. Barja

Abstract

Razor clams, mainly *Solen marginatus,* are emerging bivalve species in the aquaculture of Galicia (NW Spain). As with other molluscs, outbreaks of diseases affecting larval and post-larval stages in hatcheries constitute one of the main problems for their culture, seriously reducing their production. In this chapter, we present results obtained in some recent studies developed in our laboratory on the bacteria associated with *S. marginatus*, both at broodstock conditioning and hatchery stages, these studies constituting a step forward in our knowledge of the microbiota of this bivalve mollusc as well as of the potential bacterial pathogens for razor clams.

Representatives of the genus *Vibrio,* specifically within the Splendidus clade, which includes well-known pathogens for other bivalves, are the main bacterial group found. Therefore, it is to be expected that species closely related to *V. splendidus* constitute a key limiting factor for the culture of these clams at hatchery level.

Another remarkable finding is that certain routine procedures in hatcheries, such as the use of antimicrobial substances, may increase the risk of selection of undesirable populations of bacteria, including these opportunist pathogens.

1. Introduction

The aquaculture of molluscs has been developed extraordinarily in the last decade. However, it is still necessary to increase both the production and the number of species exploited. The main species used in marine aquaculture belong to the Mytilidae, Ostreidae, Veneridae and Pectinidae families, the Pacific oyster (*Crassostrea gigas*) and the Manila clam (*Ruditapes philippinarum*) being the dominant species, apart from the mussel species. The introduction of new species of bivalve molluscs of the Solenidae family (*Ensis ensis*, *E. siliqua*, *E. arcuatus* and *Solen marginatus*), commonly named as razor clams, would cover the requirements of some markets, including the delicatessen sector. In fact, razor clams, mainly *Solen marginatus,* are emerging bivalve species in

the aquaculture of Galicia (NW Spain) (da Costa and Martínez-Patiño, 2009).

One of the main problems in the aquaculture of molluscs is the repeated episodes of mortality, which seriously reduce the production. These outbreaks of disease affect larval and post-larval stages in hatcheries, as well as juveniles and adults cultured in natural environments. In the case of hatcheries, these massive mortalities usually involve the complete loss of the stocks of production, with serious economic consequences. Despite these events, the agents responsible are usually not determined (Tubiash, 1975; Brown and Losee, 1978; Di Salvo *et al*., 1978; Prieur and Carval, 1979; Elston and Leibovitz, 1980; Brown, 1981; Garland *et al.,* 1983; Elston, 1984).

For other bivalve species, studies have demonstrated, in most cases, that the problems are caused by bacterial pathologies, members of the genus *Vibrio* being the main etiological agents (Nicolas *et al*., 1996; Sugumar *et al*., 1998; Paillard *et al*., 2004; Prado *et al*., 2005; Beaz-Hidalgo *et al*., 2010a). In the case of razor clams, due to their character as emerging aquaculture species, little is known about the potential pathogens affecting these early stages of development, the majority of works focusing on descriptive pathology of juvenile and adult individuals obtained from natural beds (see reviews by Montes and by Lopez *et al*., in this book).

The lack of systematic and rigorous studies of the bacterial populations associated with mollusc culture and, hence, the scarce knowledge obtained on the subject, has led to a search for solutions focusing on the complete elimination of the microbiota in the culture water during hatchery stages. The different methods employed, from water treatments to chemotherapy, have proved to be inadequate to avoid episodes of mortality.

In this chapter, current knowledge about this subject is reviewed, describing some recent studies developed in our laboratory on the main bacterial pathogens affecting larval and post-larval stages cultured in hatchery.

2. The hatchery environment

The decline of natural beds and hence the reduction of the recruitment of juveniles, makes it necessary to obtain spat under controlled conditions in hatcheries. Hatcheries are nowadays the main source of seed for the aquaculture of many bivalve molluscs of a high economic value, such as oysters, clams or scallops. Seed obtention by induced spawning has been studied from a technical point of view in order to establish the optimum conditions for its performance.

Unfortunately, the optimum conditions for bivalve culture also favour the growth of bacteria and the accumulation of their metabolites (Prieur and Carval, 1979; Murchelano *et al.*, 1975; Brown and Tettelbach, 1985). The disease process is favoured on many occasions by increased larval susceptibility due to external stress factors, including the poor quality of food or water, organic contamination, etc., which also facilitate the growth of potential pathogenic bacteria or opportunist pathogens (Di Salvo *et al.,* 1978; Tubiash and Otto, 1986). This highlights the importance of maintaining optimum water quality and culture density in a hatchery. The use of antibiotics may be beneficial, although their frequent use may lead to the appearance of resistant bacterial strains (Karunasagar *et al*., 1994), and therefore their use is forbidden within the European Union.

Hatcheries vary in design, depending on local conditions and species to be cultured. However, the basic procedures and sections in which they are organised are commonly present: i) conditioning adults for spawning; ii) rearing the larvae; iii) production of microalgae for food; and iv) the system to supply high-quality water for all operations (Fig. 1).

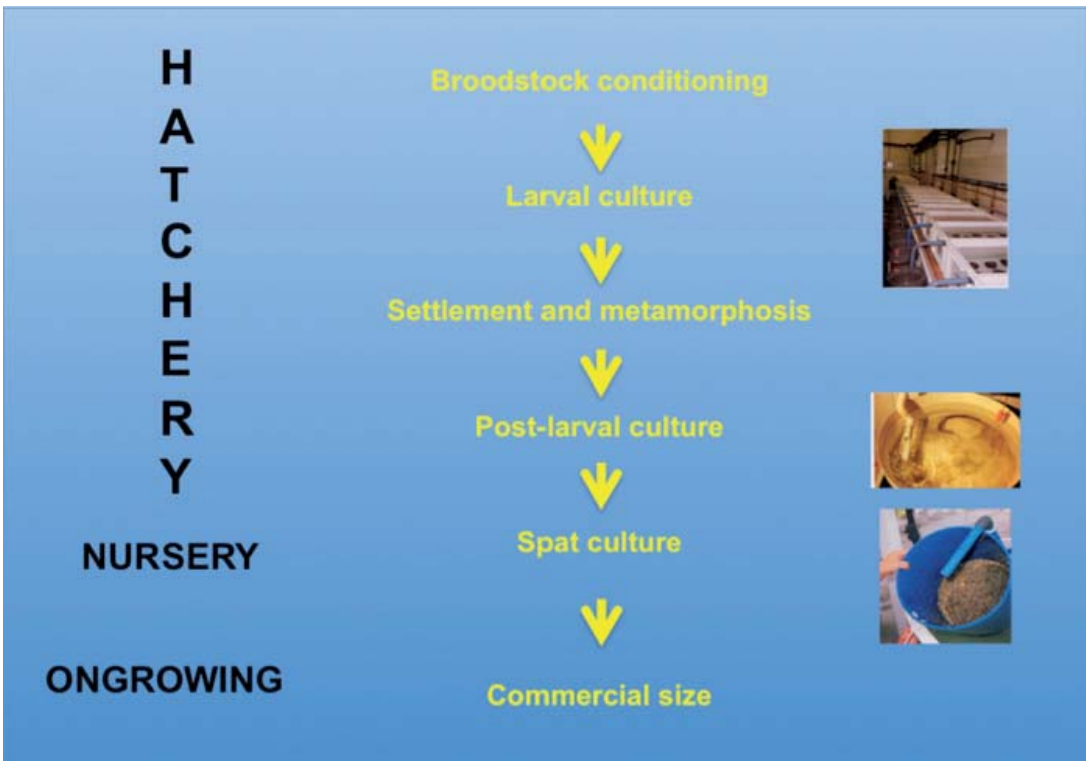

Figure 1. Diagram of a hatchery with the different stages and critical points.

Any of these procedures or sections can be a way for opportunist pathogens to enter the system, and should therefore be considered as critical points for microbiological control of hatcheries. Thus, the broodstock used in hatchery have a natural bacterial

load that could be transmitted to larvae. In addition, several factors can influence the dynamics of the bacteria present. On the one hand, the process of conditioning could enhance bacterial growth (regular supply of food, high temperature, etc.), while on the other hand, the filtered water used for the conditioning of the animals has low levels of bacteria, in order to reduce and control the associated microbiota.

The microalgae added as food for broodstock and larvae are obtained from non-axenic cultures, also possessing an important bacterial load. Finally, the efficacy of the water treatments to control the microbial loads is extremely variable, and any attempt to achieve their complete elimination would be unreasonable, since the presence of beneficial microorganisms in the hatchery environment has been documented (Prado *et al*., 2010).

3. Microbiological studies

The "Centro de Cultivos Mariños de Ribadeo" (Galicia, NW Spain) is a pioneer in the hatchery culture of the razor clam *Solen marginatus* (da Costa and Martínez-Patiño, 2009). Episodes of mortality during larval and post-larval development were observed, showing similar signs to those described for *Vibrio* infections in other bivalve species (Guillard, 1959; Tubiash *et al*., 1965; Di Salvo *et al*., 1978; Prado *et al*., 2005). On this basis, we have carried out a study focusing on the bacterial populations associated with different stocks of *S. marginatus* and their development during their stay in hatchery, with special attention being paid to possible members of the genus *Vibrio*, the best-known pathogens of larval cultures of bivalves. The bacteria associated with larval mortalities, together with those obtained from the broodstock conditioning phase, were characterized in order to identify them and to increase our knowledge of the microbiota of this species.

With this aim, samples were taken periodically for microbiological analysis and processed immediately. Two media were used to isolate bacteria: Marine Agar (MA), for total marine heterotrophic bacteria, and Thiosulphate-Citrate-Bile-Sucrose (TCBS), selective for vibrios. The plates were incubated at 22ºC and selected colonies were isolated after 24 h (TCBS) or 5-7 days (MA). Pure cultures of the strains were preserved frozen at -80ºC in Marine Broth with 15% v/v of glycerol.

The phenotypic characterization of those strains was carried out by classical methods (Romalde *et al*., 1990; Noguerola and Blanch, 2008) studying their morphological, physiological and biochemical properties to establish similarity groups. Furthermore, in order to achieve a better characterization of some of these strains, a selection of them were subjected to molecular studies by 16S rRNA gene

sequencing, as described by Prado *et al*. (2005).

3.1. Broodstock conditioning

Two broodstocks obtained from a natural bed (Redondela, NW Spain) were studied. They were maintained in the hatchery for 3 months and sampled at regular intervals between days 0 and 90. Individual razor clams were processed following Prado *et al*. (2005). Briefly, each individual was opened carefully after being washed with sterile seawater (SSW), the tissues dissected and homogenized separately in SSW. Appropriate dilutions were prepared before being inoculated (100 µL) on plates of MA and TCBS. After the incubation, isolates were characterized phenotypically and genetically as described above.

The initial bacterial loads in broodstock 1 were lower than in broodstock 2, at least in 1 log-unit in TCBS and 2 log-units in MA (Fig. 2). During conditioning there was no reduction in bacterial numbers in any stock; even though a decrease in TCBS count was observed in broodstock 1 after the first month, it was followed by a recovery of initial values.

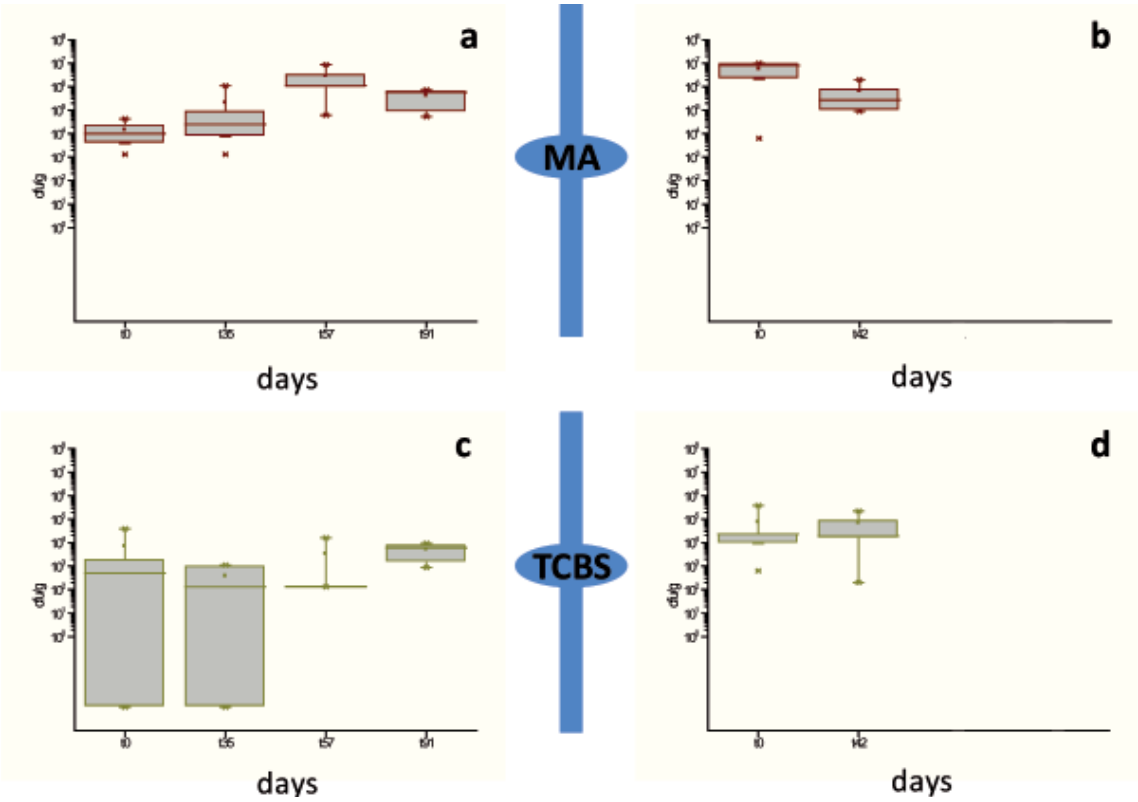

Figure 2. Bacterial loads in broodstock 1 (a, c) at 0, 35, 57 and 91 days and broodstock 2 (b, d) at 0 and 42 days estimated in MA (a, b) and TCBS (c, d). Results are expressed in colony-forming units per gram of gonad (cfu/g) in logarithmic scale.

A total of 32 strains were selected from the different samples on TCBS plates. Nineteen strains were isolated from broodstock 1 and thirteen from broodstock 2. The characterization of these strains showed that 31 of them were fermentative bacteria, presumptively identified as belonging to the genus *Vibrio*. Eight isolates were selected on the basis of their phenotypic profiles and subjected to sequencing of their 16S rRNA genes. Seven out of eight isolates sequenced belonged to the *Vibrio splendidus* group (Fig. 3), which includes pathogens for bivalve larvae (Nicolas *et al.*, 1996; Sugumar *et al.*, 1998; Gómez-León *et al.*, 2005; Beaz-Hidalgo *et al.*, 2010b).

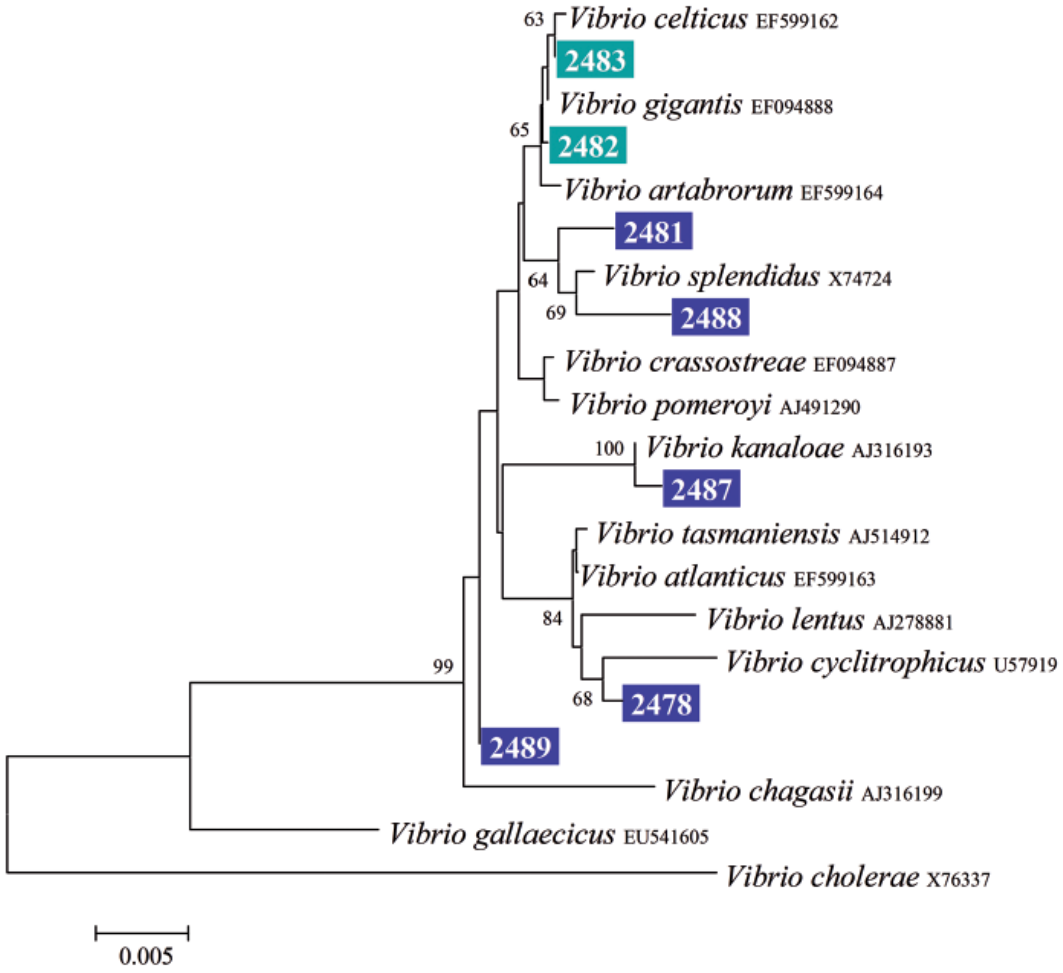

Figure 3. Phylogenetic tree based on partial 16S rRNA gene sequences of selected isolates located in the Splendidus clade using Neighbor-joining algorithm. *Vibrio cholerae* was used as outgroup. The stability of the groupings was estimated by bootstrap percentages from 1000 replicates. Bar, 0.005 substitutions per nucleotide position. Blue: strains isolated from BS-1 / Green: strains isolated from BS-2.

3.2. Larval cultures

In a hatchery, although all larval stages are vulnerable, during settlement and

metamorphosis they are exposed to high concentrations of potential pathogenic bacteria associated with the tank surface, moribund larvae or organic detritus (Sutton and Garrik, 1993). Guillard (1959) was the first to report evidence of the involvement of a *Vibrio* sp. in the disruption of the velum and internal tissues of the clam (*Mercenaria mercenaria*) larvae, which produced 70% mortality in the population. The disease was characterized by bacterial colonization of the mantle, velum disruption, abnormal swimming, visceral atrophy, and lesions in the organs, among other signs. Another characteristic sign of larval vibriosis in hatcheries is the appearance of the phenomenon called "spotting", defined as an accumulation of moribund larvae agglutinated at the bottom of the tanks (Di Salvo *et al.*, 1978).

In our studies, a number of batches of razor clam larvae cultures were monitored during the season, and mortalities were recorded in six of them at different times, ranging from day 15 until day 30 of culture. In the mortality episodes which occurred during larval and post-larval development, similar signs to those described for *Vibrio* infections in other bivalve species were observed. In those cases, samples of dead or moribund larvae or post-larvae were taken by using a sterile inoculation loop and directly spread in MA and TCBS media.

Moreover, since those cultures were experimental, in most of these cases, antibiotic (2.6 mg/L chloramphenicol) was added in the periodic changes of seawater to evaluate its effect on the survival of razor clam in hatchery.

A total of 24 isolates were selected from MA and from TCBS media and examined for their phenotypic properties. Twenty-one of them presented fermentative metabolism, being isolated from the TCBS and presumptively assigned to the genus *Vibrio*. Classical taxonomic studies allowed these fermentative isolates to be grouped on the basis of their phenotypic profiles and to select representative strains for sequencing of their 16S rRNA genes.

It was possible to establish two major groups of *Vibrio* strains. A major group (Cluster 1), comprising 10 strains, included isolates obtained from samples corresponding to all the episodes of mortality, except the only one without antibiotic treatment. These were the dominant type of colony on all TCBS plates, and in MA samples from some episodes. Their phenotypic profiles were very similar. Variability for the feature of growth in TCBS medium is remarkable, forming yellow, green or green-whitish colonies. Genotypic studies showed that they were a group close to *Vibrio splendidus*, with the greatest similarities (99.77-99.70%) with this species, but located in a different branch in the phylogenetic tree (Fig. 4).

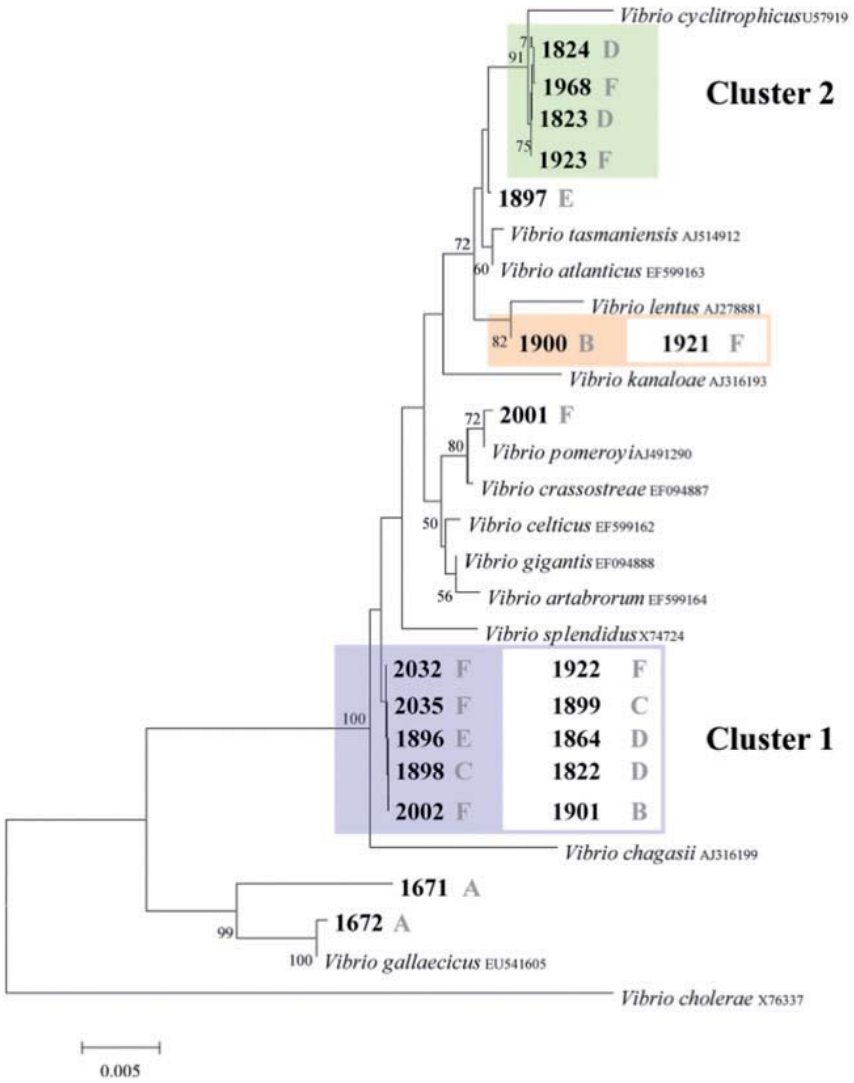

Figure 4. Phylogenetic tree based on partial 16S rRNA sequences of isolates related to the Splendidus clade, constructed by the Neighbor-joining method. *Vibrio cholerae* was used as outgroup. GenBank sequence accession numbers are given with each type species. Bootstrap values higher than 50 are near the corresponding branch. The bar represents substitutions per nucleotide position.

A second group (Cluster 2) was constituted by 4 strains close to the *V. tasmaniensis*-*V. cyclitrophicus* species, showing the highest similarity values with *V. tasmaniensis* (99.57-99.66%). All of them shared most of the phenotypic characteristics, with variable results only for growth at 8% NaCl and 37ºC.

4. Final remarks

Broodstocks captured in the wild, although presenting important differences

(1-2 log-units) in initial bacterial loads, did not reduce the number of associated bacteria during conditioning in the hatchery. The majority of the fermentative strains isolated belonged to the *Vibrio splendidus* group, which includes important aquaculture pathogens. Control of broodstocks constitutes, therefore, a critical point in order to avoid the entry of pathogens in a Solenidae hatchery.

Regarding the microbiota of larval and post-larval stages of *S. marginatus*, again most of the isolates obtained from mortalities were fermentative bacteria, which could be assigned, as in the case of broodstocks, to the *Vibrio splendidus* group. It can be hypothesized that some of those strains could have their origin in the broodstocks being vertically transmitted to the larvae.

The presence of bacteria from the Splendidus clade in the majority of the samples, except those from the only culture without antibiotic treatment, points to the risk of selection of undesirable populations of bacteria with the use of these substances. The use of probiotic bacteria for the hatchery culture of solenids should be considered a promising alternative to the use of antimicrobials, as has been demonstrated for other bivalve cultures.

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Section II: Aquaculture and harvesting methods

Chapter 9: Razor clam culture in Galicia (NW Spain)

Fiz da Costa González, Susana Nóvoa Vázquez, Justa Ojea Martínez and Dorotea Martínez Patiño

Abstract

In this chapter, the culture possibilities of razor clams *Ensis arcuatus*, *Ensis siliqua* and *Solen marginatus* are described. The first step in the culture of these species is broodstock conditioning. When conditioning trials were started from advanced gametogenesis stage successful results were achieved, ripeness being attained two months before it occurred in wild populations. Spawning methods have been developed for these three species; however, *S. marginatus* induction should be improved in order to control egg release better. These razor clam species differed in the time needed to surpass larval development. Metamorphosis in *S. marginatus*, *E. siliqua* and *E. arcuatus* took place after 8, 14 and 20 days of culture, respectively. Best results, from settlement until 1 mm long (one-month-old seed approximately), were achieved rearing the seed without substrate. *S. marginatus* exhibited good survival rates during this period (63.5%), whilst in *Ensis* species they were quite low (5-10%). From 1 mm long upwards, nursery culture must be carried out without substrate in *E. siliqua* and *S. marginatus*, while seed must be held with substrate in *E. arcuatus*. Razor clam seed produced within a hatchery has to be transferred to natural beds when they reach a certain length (namely, from 10-15 mm long upwards). Razor clam seed displayed good adaptation to wild beds where these species occur. *E. arcuatus* and *S. marginatus* on-growing showed satisfactory survival rates and they took three years to reach commercial size. The life cycle of *S. marginatus* was closed, obtaining larvae from the individuals reared in the on-growing experiment.

1.Introduction

Razor clam species in Galicia (NW Spain) are exploited by shellfish gatherers. Their captures are regulated by management plans prepared by the government, together with shellfish gatherers' associations, based on the knowledge of the biology and resource status of these species. High fishing pressure could start to produce signs of overexploitation in natural beds. In addition, a failure in recruitment could lead to a collapse of wild populations. In order to solve this problem, seed

must be transferred to natural beds, either from other wild populations or using hatchery-produced seed. Hence, these species are of interest for aquaculture to improve natural stocks and consequently to produce food.

Intensive bivalve culture has been developed in suitable facilities. Investigations dealing with hatcheries and nurseries started in the 1950´s and industrial scale production expanded during the 1970´s. The Galician Regional Authority set up bivalve cultivation facilities in the late 1970´s in Ribadeo. Hatchery and nursery production of bivalve seed ready to be transferred to natural beds has been undertaken in these facilities since 1978 (Guerra, 1979). Early investigations dealt with European oyster (*Ostrea edulis*) and grooved carpet shell (*Ruditapes decussatus*), and they were based on techniques developed by Loosanoff and Davis (1963) and Walne (1966). After this first step, cultivation technology was adapted to other bivalve species, such as the pullet carpet shell (*Venerupis pullastra*), manila clam (*Ruditapes philippinarum*) and wedge shell clam (*Donax trunculus*). From 1990 cultivation of three razor clam species native to Galicia has been studied: *Solen marginatus*, *Ensis siliqua* and *Ensis arcuatus*. Nowadays, there is no demand for the production of these species in hatcheries; however, research has been undertaken in order to get to know their biological make-up and to study the viability of replenishing wild populations, thus, guaranteeing resource sustainability. During the late 1990´s the depletion of *S. marginatus* populations was observed, mainly in the Northern Galician Rías, due to an abnormally high natural mortality (Martínez-Patiño, 2002).

Some notable technological advances have been achieved in the production of razor clams using hatchery and semi-intensive aquaculture techniques in other areas of the world. The first studies in embryo and larval development of *Ensis directus* were undertaken a long time ago in North America (Sullivan, 1948; Loosanoff and Davis, 1963; Costello and Henley, 1971), and currently attempts are being made to transfer them to industrial scale (Leavitt *et al*., 2002, 2005). Furthermore, *Siliqua patula* cultivation was first studied in the late 1970´s as well as in North America (Breese and Robinson, 1981). *Ensis macha* culture has been studied since the 1990's in order to respond to high fishing pressure on this species (Lépez, 2006). Two collaborative research projects of the European initiative Interreg IIIB, carried out by C-Mar (Queen´s University of Belfast), IPIMAR, BIM, University of A Coruña and CIMA-Xunta de Galicia, called SHARE-90 (Sustainable Harvesting of *Ensis*) and TIMES (Towards Integrated Management of *Ensis* Stocks) allowed the experimental cultivation of *E. arcuatus* and *E. siliqua* in Northern Ireland (United Kingdom).

The aim of this study is to analyse different razor clam hatchery and nursery systems and the on-growing of these species in natural beds, based on the results obtained at the research facilities of the Marine Cultivation Centre (Centro de Cultivos

Marinos) in Ribadeo, Galicia.

2. Hatchery and nursery culture

2.1. Conditioning

Conditioning broodstock is essential for the provision of larvae for hatchery culture. This is the procedure in which hatcheries are able to extend their production season, removing reliance on the relatively brief period in the year when adults of the desired species are bearing mature gametes in the sea. In the case of hatcheries in marginal climates, there are different advantages in producing seed early in the year – often months before stock have developed and matured in nature. The main aim of the process of broodstock conditioning is to achieve maximum fecundity of adults whilst preserving the quality and viability of the resulting fertilised eggs (Devauchelle and Mingant, 1991; Utting and Millican, 1997; Madrones-Ladja *et al.*, 2002; Martínez and Perez, 2003). Broodstock conditioning involves the manipulation of both physical and biological parameters (temperature, photoperiod and diet). European razor clam species exhibit annual reproduction cycles with short ripe and spawning stages, which ranged from 1 to 4 months depending on the species (Table I).

Table I. Spawning season in three razor clam species located in Galicia (NW Spain).

	Spawning season	Location	Reference
E. arcuatus	January-April	Ría of Vigo	Darriba *et al.* (2004)
E. siliqua	May-June	Fisterra	Darriba *et al.* (2005)
E. siliqua	May-June	Ría of Barquero	Martínez-Patiño (2002)
S. marginatus	May-June	Ría of Ortigueira	Martínez-Patiño (2002)

2.1.1. Broodstock conditioning system

Harvesting broodstocks surpassing commercial size is the first stage of broodstock conditioning. An adequate transfer of broodstocks to hatchery facilities allows them to arrive in good condition, thus guaranteeing a high quality of the gametes released. These species are sensitive to manipulation due to their biological features. They live buried deep in the substrate and exhibit large shells which are opened along the extent of their valves. Consequently, individuals must be banded together in groups and wrapped in wet cloth before being transferred to cultivation facilities.

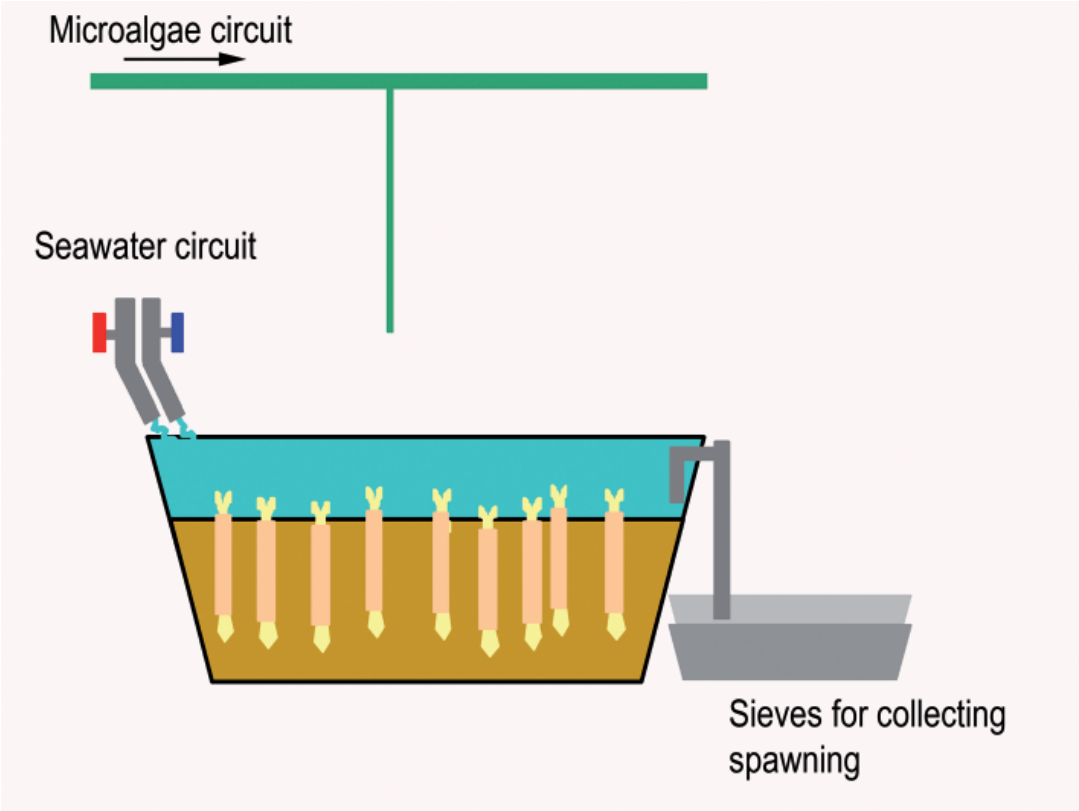


Figure 1. Diagram of a broodstock conditioning system.

Razor clams are allowed to burrow 30-40 cm deep in the sand in tanks, in open circuit with mild aeration, as illustrated in figure 1. Density in holding tanks must be kept under 140-160 individuals m^{-2}. Higher densities may hinder individuals from burrowing.

Razor clams, which are usually buried in the substrate in nature, feed more efficiently if they are kept in a suitable substrate, and also shell gaping is prevented. Broodstock survival in this system was as high as 90%, indicating that this holding system is suitable for these species. However, the use of substrate may also have some disadvantages, such as the problems of managing large volumes of sand, the loss of gametes released during spontaneous spawning because they are prone to lie on sand surfaces, and also that gametes released are collected together with dirt.

Due to the aforementioned disadvantages of the use of substrate, an alternative method for broodstock induction has been developed, holding individuals in row bars tied individually with rubber bands, thus avoiding shell gaping and allowing suitable mobility of razor clams to avoid stress (Fig. 2). In spite of being an adequate system for spawning induction, conditioning trials without substrate had shown high mortality of adults, mainly after 10 to 15 days from the start of the experiment, thus this system is not suitable for maintaining broodstock for long periods of time.

Figure 2. Broodstock buried in sand in conditioning tanks.

2.1.2. Temperature effects

The increase of the seawater temperature during broodstock conditioning, helps vitelogenesis processes (Robinson, 1992). Both *E. siliqua* and *S. marginatus* undergo reproductive development and mature gametes at temperatures ranging from 18 to 20ºC. *E. siliqua* display faster gonad development and higher gonadal condition index at a constant temperature of 20ºC than those held in seawater heated to 20ºC, at a rate of 1ºC per week from environmental temperature (gradual increase in temperature). However, *S. marginatus* displayed the opposite pattern, a higher gonad development being reached in the group subject to a gradual increase in temperature at the end of the conditioning trial. Moreno *et al.* (2007), studied the effect of temperature in gonadal development of *S. marginatus*, and observed that temperature influences gonadal maturation. They found a long delay in the emission of gametes and the reorganisation of the gonad at 17ºC whereas a reduction in spawning period and acceleration of gametes emission was observed at 23ºC (Moreno *et al.*, 2007). On the other hand, gonads in *E. arcuatus* held at environmental seawater temperature (12-15ºC) became ripe and spawned in a shorter time than the ones held in heated flowing seawater at 20ºC (da Costa *et al.*, 2005). These results can be related to the behaviour of adults in the wild, because gametogenesis begins when seawater temperature decreases to a certain level in the environment

(Darriba *et al.*, 2004). The usefulness of these results is that the cost of heating seawater during winter can be reduced in hatchery facilities, which, taking into account the increasing price of oil, could be an important factor when conditioning is undertaken. Similarly results were observed for the scallop *Argopecten purpuratus*, which showed better results when conditioning was undertaken at 16ºC than at 20ºC (Martínez *et al.*, 2000).

2.1.3. Feeding broodstocks

A mixture of marine microalgal species, on a proportional basis, is more beneficial than a diet of a single species. In our experiments we used a mixed diet consisting of *Tetraselmis suecica*, *Isochrysis galbana*, *Pavlova lutheri*, *Chaetoceros calcitrans*, *Phaeodactylum tricornutum* and *Skeletonema costatum*. A 3% ration is not enough for *E. siliqua* conditioned at 20ºC to achieve the best results in gonadal condition index (G.C.I.) and gametogenic development. The best diet is the 6% ration, reaching a G.C.I. higher than the 9% ration, even though, analysing the gametogenic scale, both have a similar gametogenic development. This shows that the 6% ration produces more gametes than a 9% ration, as an excess in feeding ration can lead to a reduction in G.C.I. A 9% ration was excessive, promoting shell growth and resulting in delayed and decreased brood production associated with loss of larval viability (Stephenson, 1973). Utting and Millican (1997) pointed out that a suitable ration for bivalve broodstocks is 6% of dry meat weight in dry weight of algae per day for most species reared at 20ºC. For species reared at lower temperatures 3% may be enough.

2.1.4. Effect of gonad developmental stage at the beginning of conditioning

Experiments were performed at different times of the year in order to investigate the effect of gonad developmental stage when conditioning is started on broodstock of *E. arcuatus*, *E. siliqua* and *S. marginatus*. Broodstock were held in 200-L rectangular tanks in open circuit and were fed on a ration of 4% mean dry meat weight of adults per day. Conditioning was initiated when individuals were in sexual rest, at onset of gametogenesis and at advanced gametogenesis stages. At sexual rest stage conditioned broodstock only reached the ripe stage at the end of the experiment. Similar results were observed when conditioning started at the onset of the gametogenesis stage. When conditioning trials were started from advanced gametogenesis stage successful results were achieved, attaining ripeness two months before it occurred in wild populations.

2.2. Spawning induction

The lack of efficient artificial stimuli to induce spawning still hinders the controlled development of several bivalve species (Mouëza *et al.*, 1999). Spawning induction allows the planning of larval cultivation and also allows controlled fertilisation to be performed. Several spawning methods have been used in bivalves: thermal shock adding microalgae and gametes (Madrones-Ladja, 1997) or hydrogen peroxide (Morse *et al.*, 1977); photochemical methods such as UV-treated seawater flow without adding any feed (Le Pennec, 1981); or high microalgal concentrations as tested by Breese and Robinson (1981) in *Siliqua patula*.

Individuals were placed in 200-L rectangular tanks without any substratum and held in row bars tied individually with rubber bands in open circuit in order to reduce stress (Fig. 3a). Razor clams were subjected to different stimuli:

1. Thermal shock, with temperatures up to 25-27ºC maintained during 1 hour, decreasing to 10-12ºC for 30 minutes. A total of 3-4 cycles were performed. Additional stimulation was provided by adding gametes stripped from one of the conditioned bivalves and microalgae.
2. Changing water level by simulating tides, with brief dry periods as described in da Costa *et al.* (2008). The method consisted of leaving individuals immersed for 30 minutes, and then for one hour in seawater at environmental temperature (15±1ºC). Thereafter, 3-4 cycles of each treatment were repeated.
3. Stripping gonads of the conditioned razor clams.

An effective spawning method was developed to induce spawning in *E. arcuatus* (da Costa *et al.*, 2008). Changing water levels by simulating tides, with brief dry periods, caused spawning in this razor clam (Fig. 3b). Thermal shock was not an effective stimulus to spawn *E. arcuatus*, as observed in the wild population. Gamete release occurs between January and April, when the temperature is low in natural beds. Adult individuals reach resting stage when seawater temperature begins to rise (Darriba *et al.*, 2004).

However, our results showed that *E. siliqua* can be successfully induced to spawn with thermal shock stimulation, increasing seawater temperature from 10ºC to 25ºC (da Costa *et al.*, 2010b). This could be related to the fact that gamete release in wild populations occurs in late spring, when seawater temperature is rising (Darriba *et al.*, 2005).

Although there are several effective spawning methods in different razor clam species, in the spawning experiments conducted, broodstock of *S. marginatus* failed to spawn when subjected to different stimuli. However the following day, after

thermal shock, when they were returned to tanks, spawning occurred (da Costa and Martínez-Patiño, 2009).

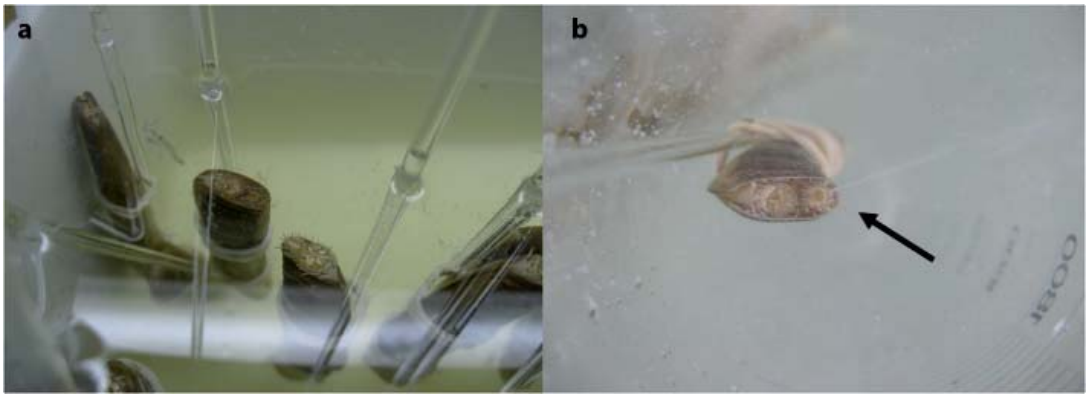


Figure 3. a. Broodstock holding system consisting of individuals tied individually with rubber bands to glass rods. b. *E. arcuatus* female releasing eggs after spawning induction.

Breese and Robinson (1981) generated normal larvae, stripping gonads and with a high microalgal concentration of *Pseudoisochrysis paradoxa*. Loosanoff and Davies (1963) in *E. directus* found that by increasing the temperature from 13 to 25ºC males would spawn and continue to release sperm even after they were cooled to 13ºC. The success of fertilising eggs stripped from conditioned females depends on their degree of ripeness (Loosanoff and Davis, 1963). Early in the season, when the largest egg was only 66 µm, none were fertilisable. Later, when razor clams were riper, a large percentage of stripped eggs underwent cleavage, but most of the larvae were abnormal.

2.3. Embryonic and larval development

2.3.1. Embryonic development

There are two key stages affecting larval development success: embryogenesis and metamorphosis. Here we describe the embryonic development of these razor clam species. The specimens responding to the stimulus were separated into individual 1-L test tubes for the release of sperm or eggs, thus, avoiding polyspermy. A mixture of sperm from 3-4 males was used for fertilisation in a ratio of 2 mL of sperm per litre of egg suspension, with fertilisation being conducted in a 5-L flask. After fertilisation, the eggs were sieved through a 45 µm screen to eliminate excess sperm. Embryos were transferred to graduated beakers for counting, and thereafter they were placed in larval rearing tanks with mild aeration and without feeding supply until D-shaped veliger stage was reached.

The oocyte of *S. marginatus* is surrounded by a 30-µm-thick and 150-µm-diameter egg capsule (Martínez-Patiño, 2002; da Costa and Martínez-Patiño, 2009) (Table II). In *S. marginatus* the thick chorion must have a fluid membrane to allow trochophores to swim inside, and also a lipid droplet to provide energy for embryonic development (Fig. 4). Carriker (1967) suggested that the presence of this jelly coat is an adaptation to fluctuating estuarine environments. This brood protection is an adaptation to osmotic pressure changes and pollution in the environment in *Scrobicularia plana* (Frenkiel and Mouëza, 1979). The size of this oocyte is very large compared to other razor clam eggs. *E. siliqua* eggs ranged from 90 to 100 µm in diameter (Martínez-Patiño, 2002; da Costa *et al.*, 2010b). The oocyte size in *E. arcuatus* is approximately 75 µm in diameter, being smaller than the other razor clam species located in Galicia (da Costa *et al.*, 2008), and similar to that found for other razor clam species, such as *E. directus* (Loosanoff and Davies, 1963; Loosanoff *et al.*, 1966).

Table II. Comparison of embryonic development in razor clam species. Hpf: hours-post-fertilisation.

	Egg size	2-cell stage	Trochophore	D-shaped veliger
E. arcuatus	75 µm	1 hpf	19 hpf	30 hpf
E. siliqua	90-100 µm	1 hpf	12 hpf	24 hpf
S. marginatus	150 µm	-	-	12-24 hpf

Embryonic development of *Ensis directus* at 27-30ºC is quite similar to that of *E. siliqua* at 19ºC, reaching the trochophore stage in 12 hours and D-shape larvae in 27 hours-post-fertilisation (hpf) (Costello and Henley, 1971). *E. siliqua*'s embryonic development is faster than that of *E. arcuatus* (Figs. 4 and 5) (Table II), which reach trochophore and D-shaped larvae stage in 19 hpf and 30 hpf, respectively (da Costa *et al.*, 2008). D-shaped veliger appeared in *S. marginatus* in 12-24 hpf (da Costa, 2009). This species undergoes embryonic development inside the chorionic envelope, which is retained at trochophore stage until the beginning of the development of straight-hinged larvae, as in *Scrobicularia plana* (Frenkiel and Mouëza, 1979). A relationship is suggested between egg size and duration of embryonic development in the 3 razor clam species studied, in which the smallest egg size is associated with the longest embryonic development.

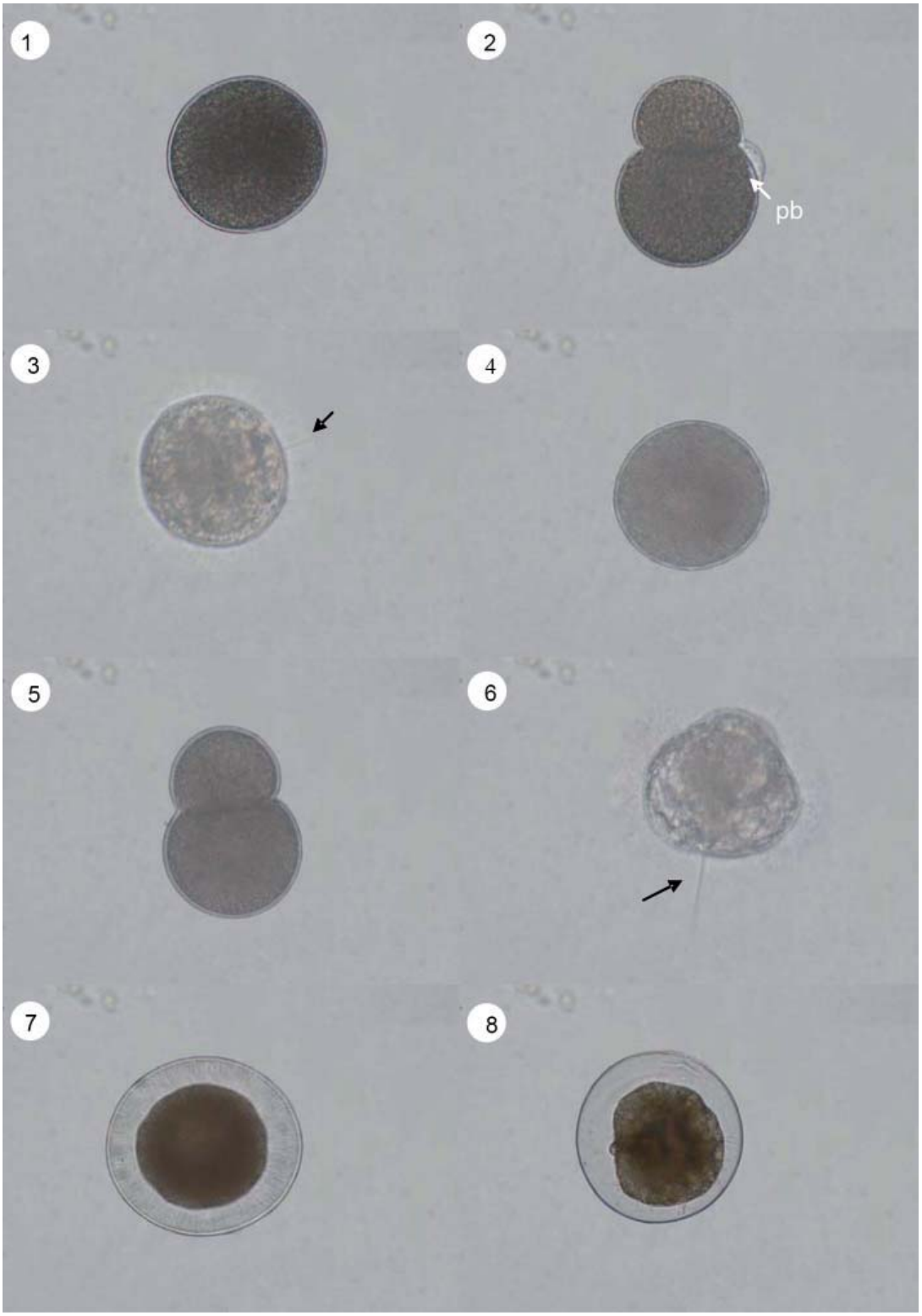


Figure 4. 1. *E. arcuatus* unfertilised egg. 2. 2-cell stage of *E. arcuatus.* 3. *E. arcuatus* trochophore. 4. *E. siliqua* unfertilised egg.5. 2-cell stage of *E. siliqua.* 6. *E. siliqua* trochophore. 7. *S. marginatus* unfertilised egg. 8. *S. marginatus* trochophore. Arrow: apical tuft. pb: polar body.

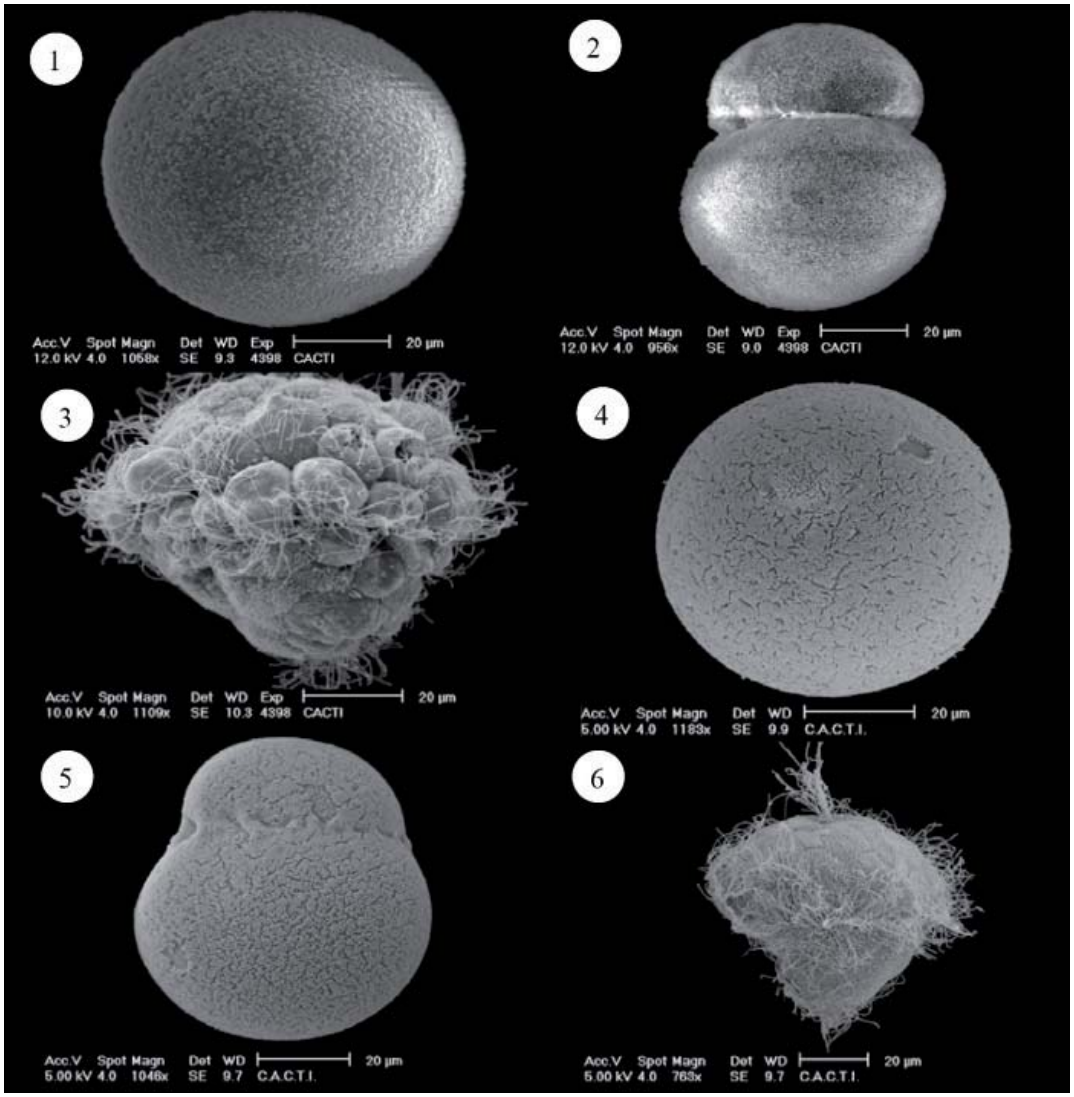


Figure 5. Scanning electron micrographs of different stages during embryonic development of *Ensis.* 1. *E. arcuatus* unfertilised egg. 2. 2-cell stage of *E. arcuatus*. 3. *E. arcuatus* trochophore. 4. *E. siliqua* unfertilised egg. 5. 2-cell stage of *E. siliqua*. 6. *E. siliqua* trochophore.

2.3.2. Larval development

Afterwards, embryos were transferred to 150 or 500-L larval culture tanks with aerated water at 19±1ºC. Water was changed every two days using 1 µm sand-filtered, UV-sterilised seawater. Egg density in each container at the beginning of the experiment was 5 eggs mL^{-1}, decreasing to 1-2 postlarvae mL^{-1} at settlement. Feeding was initiated at D-stage veliger phase. The larvae were fed daily with *T. suecica*, *I. galbana*, *P. lutheri* and *C. calcitrans* in equal proportions at 40 cells μL^{-1} as initial ration. Larvae were collected from each tank through Nytex screens, and shell length was measured for 100 individuals using a microscope connected to an image analyser.

D-shaped veliger of *E. arcuatus* measured 120 µm in length, showing a well developed vellum (Fig. 6) (Table III; Fig. 7; da Costa *et al.*, 2008). At this time "D" larvae have a developed digestive system, consisting of a mouth, a foregut, a digestive gland followed by an intestine and an anus. The post-anal tuft of a few simple cilia was dorsal to the anus. Seven-day-old larvae, which become oval shaped, were slightly umbonate, with a length of 170-180 µm. Fourteen days after fertilisation, they reached the pediveliger stage, with a size of 280 µm, crawling and swimming for short intervals, with the vellum still functional. During metamorphosis the vellum can be shed completely as one unit or can be deciliated gradually before being histolyzed, and gill development begins.

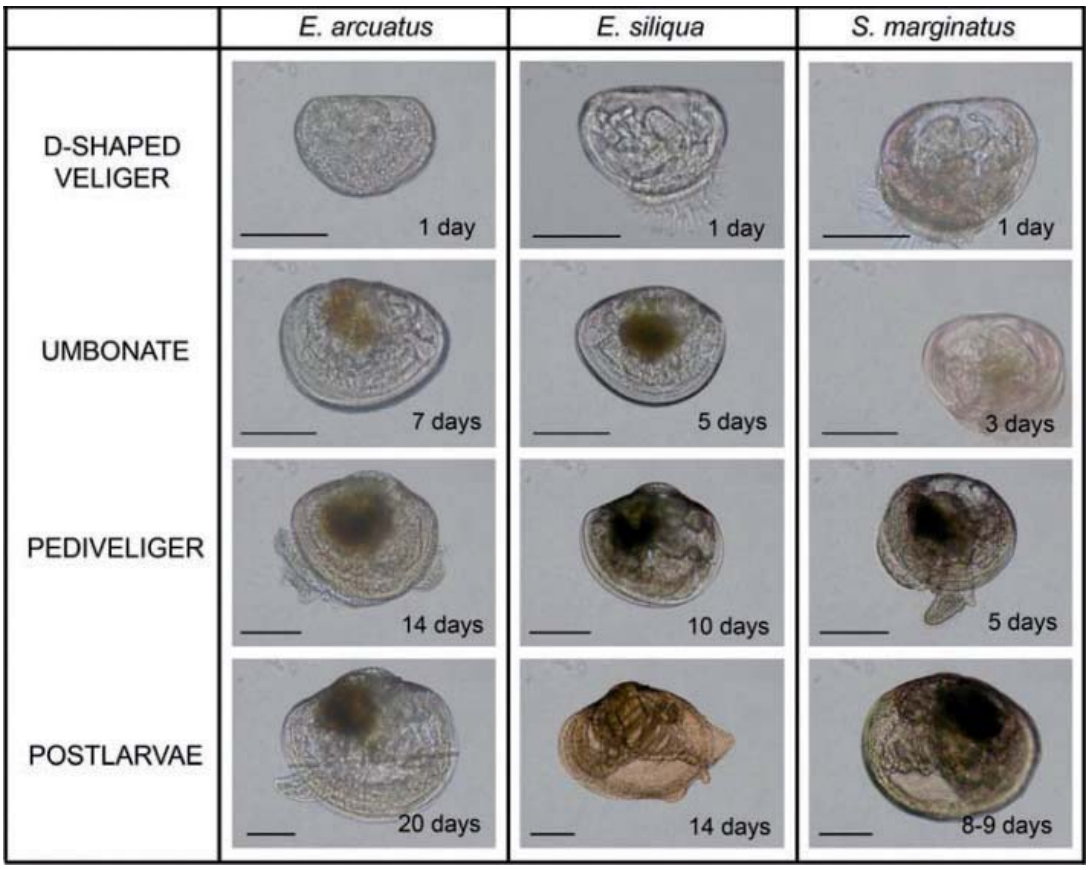


Figure 6. Larval development of *E. arcuatus*, *E. siliqua* and *S. marginatus* reared under the same experimental conditions. Scale bar: 100 µm.

E. siliqua displayed a similar size of D-shaped veliger, ranging from 120 to 125 µm (da Costa *et al.*, 2010b; Fig. 7;). Five-day-old larvae were slightly umbonate, with a length of 210 µm, and the digestive gland became highly coloured. Ten days after fertilisation, they reached the pediveliger stage, with a size of 340 µm, crawling and swimming for short intervals, with the vellum still functional.

Typical straight-hinged D-shaped larvae of *S. marginatus* ranged from 150

to 160 µm long (da Costa and Martínez-Patiño, 2009; Fig. 7). Umbonate larvae appeared after three days of cultivation, with a length of 200 µm. Six days after fertilisation, they reached the pediveliger stage, with a size of 270 µm.

Razor clam species differed in the time needed to surpass larval development. Metamorphosis in *S. marginatus* took place after 8-9 days of cultivation, with a length of 350 µm. In *E. arcuatus* metamorphosis took place at day 20, with a length of 400 µm, while in *E. siliqua* it occurred on day 14 with a similar size. Postlarvae of *S. marginatus* exhibited a more rounded shape than the two species of *Ensis*, and this fact may explain the smaller size observed in *S. marginatus* postlarvae.

Table III. Comparison of larval development in three species of razor clam reared under the same experimental conditions.

	D-shaped veliger	Umbonate	Pediveliger	Postlarvae	Settlement
E. arcuatus	120 µm	180 µm	280 µm	400 µm	20 days
E. siliqua	125 µm	210 µm	340 µm	400 µm	14 days
S. marginatus	160 µm	200 µm	270 µm	350 µm	8-9 days

The different sizes at settlement and length of larval development influence growth rates. *S. marginatus* displayed the highest growth rate, 16-18 µm day^{-1}, whilst the lowest was observed in *E. arcuatus*, 13-14 µm day^{-1} (Fig. 7). Figures 8 and 9 show the length-height and length-weight relationships, fitting both to an exponential curve.

Average larval survival from egg to newly-settled postlarvae for *E. arcuatus* was 15.6% (da Costa *et al.*, 2010a), being lower than that for other razor clam species, such as *E. siliqua* (42.3%) (da Costa *et al.*, 2010b). *S. marginatus* razor clam showed a high larval survival of 53.2% (da Costa and Martínez-Patiño, 2009). López *et al.* (2005) reported larval survival ranging from 25 to 50% in *E. macha* from D-shaped veliger to pre-metamorphic larvae.

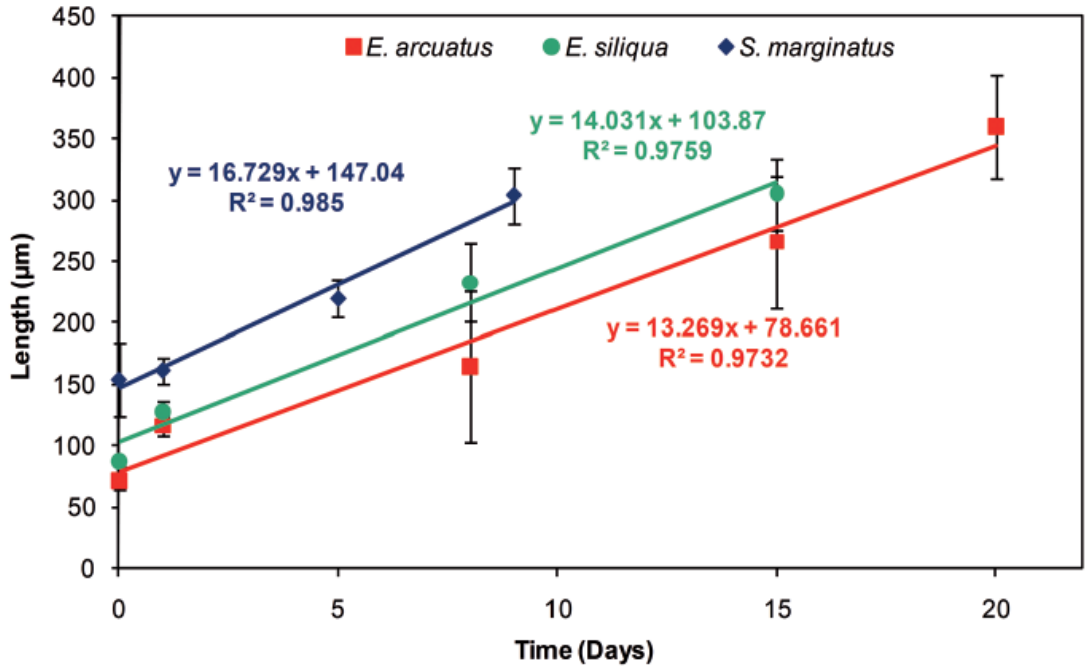


Figure 7. Growth in length of *E. arcuatus*, *E. siliqua* and *S. marginatus* larvae.

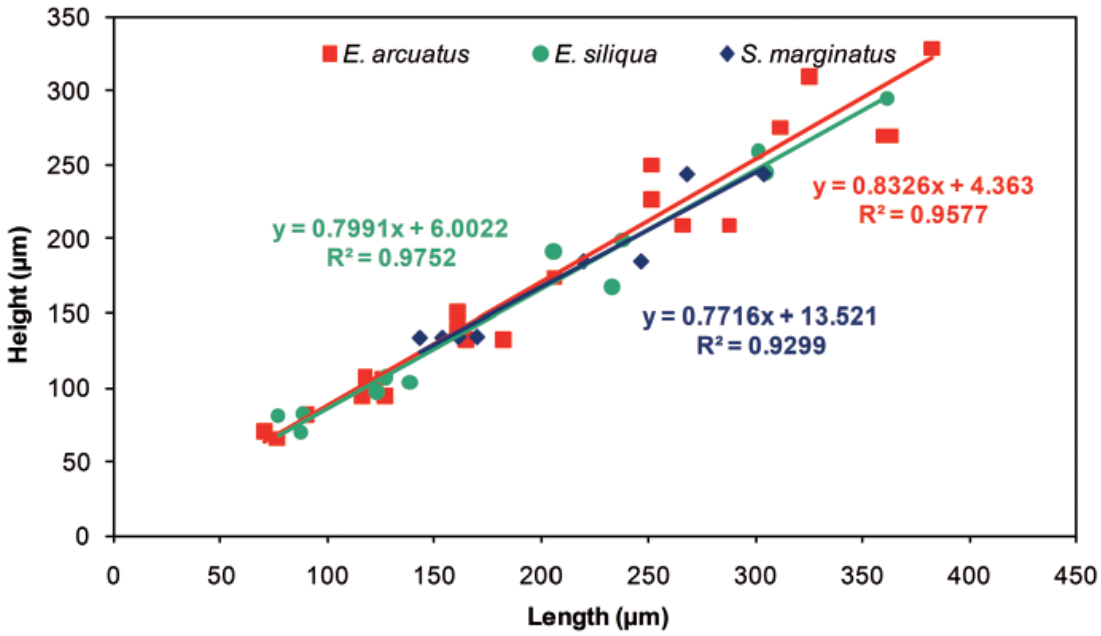


Figure 8. Relationship between length and height of *E. arcuatus*, *E. siliqua* and *S. marginatus* larvae.

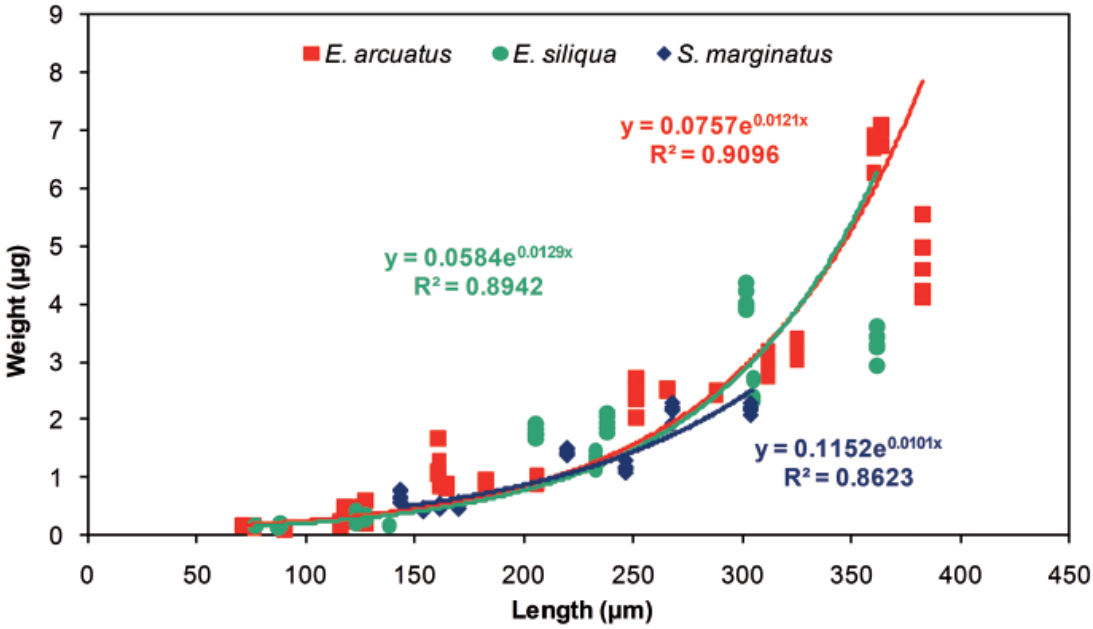


Figure 9. Relationship between length and dry weight of *E. arcuatus*, *E. siliqua* and *S. marginatus* larvae.

2.3.2.1. Effects of temperature upon larval culture in *E. arcuatus*

It is well known that increases in culture temperature shorten the larval period of many bivalves (for example *Mercenaria mercenaria* in Loosanoff and Davis, 1963). However, every species has an optimal temperature of larval culture, maximising growth and survival. This study presents data about the optimum temperature for *E. arcuatus* larval culture, and its relationship with growth and survival. Three different temperatures per duplicate (14, 18 and 24ºC) were tested with *E. arcuatus* D-shaped larvae from the same batch. Larvae were held in 150-L larval rearing tanks, using sand-filtered and UV-sterilised seawater. Water was changed 3 times a week. Larvae were fed every day with *T. suecica*, *I. galbana*, *P. lutheri* and *C. calcitrans* at the same proportion with 40 cells μL^{-1}. Larvae were sampled weekly to measure length and to determine survival.

Larvae reared at 14ºC reached settlement at day 26, whilst larvae reared at 18ºC did so at day 20. Better results in growth in length were achieved with 18ºC (10.72 µm day^{-1}), with similar results for 14 and 24ºC. Survival was higher at 14ºC (23.2%), followed by 18ºC. Larvae held at 24ºC died after 19 days of culture. Rearing larvae at 14ºC is worthwhile due to the higher survival rate observed, even though they take longer to reach settlement.

2.3.2.1. Effects of diet upon larval culture in *E. arcuatus* and *S. marginatus*

Food quality has been identified as an important factor to control, in order to maximise the growth and survival of bivalve larvae (Bayne and Newel, 1983). Research has shown that bivalve larvae should be fed a diet of at least two algal species (Nell and O´Connor, 1991) since a mixed diet is thought to provide a better balance of nutrients. In order to optimise diets in *E. arcuatus,* different microalgal mixtures were assayed. Four different diets per duplicate were tested with *E. arcuatus* D-shaped larvae from the same batch: diet 1: *I. galbana*+*P. lutheri* (Ig+Pl); diet 2: *I. galbana*+*P. lutheri*+*C. calcitrans* (Ig+Pl+Cc) and diet 3: *I. galbana*+*C. calcitrans*+*T. suecica* (Ig+Cc+Ts) and 4: (*T. suecica*+*I. galbana*+*P. lutheri*+*C. calcitrans*) (Ig+Pl+Cc+Ts). Larvae were held in 150-L larval rearing tanks, using sand-filtered and UV sterilised seawater. Water was changed 3 times a week and the temperature was set at 18±1ºC. Larvae were fed daily with 30 cells μL^{-1}. Diet 4 (Ig+Pl+Cc+Ts) displayed a 25% survival rate, a better survival rate than the others, which ranged between 5 and 10%.

A feeding trial with *S. marginatus* larvae was carried out in order to investigate the need for external feeding of its larvae, using different experimental diets than the ones used for feeding *E. arcuatus* larvae. Larvae were fed on three different diets: diet 1: 10 equivalent cells (*I. galbana*, *P. lutheri* and *C. calcitrans*) plus 20 equivalent cells of *T. suecica*; diet 2: 80 equivalent cells of *I. galbana*; and diet 3: starvation during eight days until settlement, and from this point onwards individuals were fed on diet 1. Best results for growth were observed in larvae fed on diet 1. Starved larvae reached the best rate of survival on day 8 (66%). However, they all died after three more days of culture once they were fed again. Previous studies on starvation of *S. marginatus* larvae pointed out that larvae were able to settle at day 10 post-fertilisation after 5 days of starvation when feeding was initiated (da Costa, unpublished data). This suggested that after 8 days of starvation *S. marginatus* had lost too much of its energy reserves to surpass metamorphosis after feeding was initiated. In spite of the large size of *S. marginatus* eggs and the great amount of stored reserves their larvae need to feed on microalgae to undergo metamorphosis.

2.3.2.1. Biochemical and fatty acid composition during larval development in razor clams *E. arcuatus*, *E. siliqua* and *S. marginatus*

Survival of cultured bivalves in hatcheries depends largely on the levels of stored energy reserves which are metabolised to sustain developmental changes during embryogenesis, metamorphosis or starvation. Embryogenesis, the initial anatomical change from egg to veliger, relies solely on endogenous energy reserves supplied to the egg by broodstock (Bayne, 1973), which corresponds to an endotrophic

phase (Lucas *et al.*, 1986). The second stage, in which stored reserves are often essential, is a process that takes place before metamorphosis and depends on food quality and quantity (Whyte *et al.*, 1989, 1990). Several studies have highlighted the importance of fatty acids, especially polyunsaturated fatty acids (PUFAs), to bivalve nutrition and welfare. Napolitano *et al.* (1988) reported that neutral lipids can either store large amounts of saturated fatty acids (SAFAs) as energy reserves or they can be provisionally reserved for PUFA synthesis.

Research was carried out into gross biochemical and fatty acid composition from both neutral and polar lipid compartments of *E. arcuatus*, *E. siliqua* and *S. marginatus* razor clams throughout embryonic and larval development (da Costa, 2009). Proteins are the main stored reserves in the eggs of the three species, followed by lipidic reserves. Of the three species, *S. marginatus* is the one that stores the highest amount of reserves in its eggs (Table IV). Its eggs are rich in stored reserves, given their large size, the chorionic envelope and the lipid droplet providing energy for embryonic development (da Costa and Martinez-Patiño, 2009). These large amounts of stored reserves in *S. marginatus* are responsible for the rapid larval development, which lasts only 8 days. Hence, the time spent in the plankton is minimised, which reduces the risk of predation. *E. arcuatus* and *E. siliqua* showed similar amounts of lipids, proteins and carbohydrates in their eggs.

Several investigators have shown that lipids are the main energy source during embryonic development in marine invertebrates. Although there are several works on the biochemical composition of other bivalve species during larval development, there are no previous studies on razor clams. The energy required for embryogenesis was obtained from stored proteins in all of these razor clam species, these being the major biochemical constituent in eggs (da Costa, 2009). Lipids and carbohydrates remained unchanged during the embryonic development in the three species. In contrast to this, Whyte *et al.* (1990, 1991) found that *Crassadoma gigantea* and *Patinopecten yessoensis* embryogenesis showed a decrease in lipids and proteins in the same proportion, and to a lesser extent in carbohydrates.

Lipids, proteins and carbohydrates are stored in *E. arcuatus*, *E. siliqua* and *S. marginatus* when feeding is initiated at D-shaped veliger stage. No mixotrophic stage was identified by the study of gross biochemical composition: thus, it can be proposed that assimilation of nutrients begins soon after feeding is started. From D-shaped veliger until settlement all biochemical substrates are stored indistinctly to facilitate metamorphosis.

Oocyte diameter may be related to total neutral fatty acids stored. Bivalve species with egg diameter ranging from 65-75 µm, such as *E. arcuatus* and *E. siliqua*

(5 ng egg^{-1}) (da Costa, 2009), the clam *Venerupis pullastra* (1.8-2.8 ng egg^{-1}) (Nóvoa, 2007) or the scallop *Pecten maximus* (4.6 ng egg^{-1}) (Soudant, 1995), stored lower amounts of neutral fatty acids. *S. marginatus* egg diameter was 143-154 µm, and thus they stored a high amount of neutral fatty acids (29.73 ng egg^{-1}) (da Costa, 2009). This could sustain the fast larval development displayed in this species. Part of these fatty acid reserves could be stored in the lipid droplet inside the chorionic envelope present in the oocyte, which is entirely depleted during embryonic development at the gastrula stage (da Costa and Martinez-Patiño, 2009).

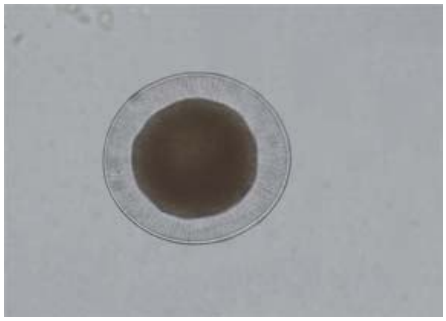
During embryonic development of *E. arcuatus*, a gain in fatty acids of neutral lipids was observed. This behaviour was also observed in the *V. pullastra* clam (Nóvoa, 2007). This author pointed out that it could be the result of the synthesising ability of SAFAs 16:0 and 18:0 and MUFAs 18:1n9, which increased during this developmental stage. It was suggested that fatty acids could be synthesised from simple non lipidic precursors, such as acetyl-CoA. As mentioned above, *E. arcuatus* embryonic development is mainly sustained by the supply of energy from proteins. However, total fatty acids in neutral lipids decreased during the embryonic development of razor clam *E. siliqua*. This pattern has been previously described in the scallop *P. maximus* (Soudant, 1995). A depletion of fatty acids in neutral lipids was observed from day 1 to day 8 in *E. arcuatus* larvae. After day 1 in *E. siliqua* and day 8 in *E. arcuatus* total fatty acids increased in both neutral and polar lipid compartments.

S. marginatus displayed a different pattern in fatty acid changes during embryonic and larval development. Fatty acids in both neutral and polar lipids decreased during embryonic development of *S. marginatus*. In the larval development of *S. marginatus*, from day 1 until day 5, depletion of total fatty acids was observed in both neutral and polar fatty acids. This latter species displayed a different strategy in the use of fatty acids during larval culture compared with other bivalves, due to the large amounts of stored fatty acid reserves in the oocyte. Depositories allow a short larval development, in which settlement is reached with lower amounts of stored neutral and polar lipids than with the content found in oocytes.

Mollusc lipids contain, in addition to or in replacement of the typical marine animal fatty acids, Non-Methylene-Interrupted Dienoic (NMID) fatty acids (Joseph, 1989) which have unusual unsaturation features, as they possess double bonds with more than one methylene group between ethylene bonds. These fatty acids, more precisely 20:2NMID, were demonstrated to be *de novo* synthesised in bivalves by elongation and Δ5 desaturation of 18:1n9 and 16:1n7, which are very abundant in phytoplankton, especially in diatoms (Zhukova, 1991). Preferential incorporation in polar lipids, sometimes in competition with PUFA n3, and selective distribution among

different tissues and organs which favours those exposed to external environment, suggest a structural and functional role in biological membranes (Fang *et al.*, 1993). NMID fatty acid levels were similar to those of some polyunsaturated fatty acids from polar lipid compartment in *E. arcuatus* and *E. siliqua* and from both neutral and the polar lipid compartments in *S. marginatus*, with increasing percentages at the onset of metamorphosis in these three razor clam species. This pattern observed in these three razor clam species highlights the importance of NMID fatty acids in these species.

Table IV. Comparison of main characteristics of *S. marginatus* and *Ensis* eggs. Data from da Costa (2009).

	***Ensis* eggs**
	Egg diameter: 75-90 µm
	Egg dry weight: 0.14-0.16 µg ind.$^{-1}$, 3-4 times less than in *Solen*
	High larval survival in *E. siliqua* (42%) and low survival in *E. arcuatus* (16%)
	Settlement: *E. siliqua* (14 days) and *E. arcuatus* (20 days)
	Total lipids: 11-13 ng ind.$^{-1}$
	Total proteins: 36-44 ng ind.$^{-1}$
	Fatty acids in neutral lipids: 5 ng ind.$^{-1}$
	Fatty acids in polar lipids: 1.2-2.0 ng ind.$^{-1}$
	***Solen* eggs**
	*Solen* eggs have a chorionic envelope
	Egg diameter: 140-150 µm
	Egg dry weight: 0.55 µg ind.$^{-1}$
	High larval survival: > 50%
	Settlement: 8-9 days
	Total lipids: 37 ng ind.$^{-1}$
	Total proteins: 78 ng ind.$^{-1}$
	Fatty acids in neutral lipids: 30 ng ind.$^{-1}$
	Fatty acids in polar lipids: 7.9 ng ind.$^{-1}$

2.4. Nursery culture

2.4.1. Postlarval and spat culture

Postlarvae were reared in sieves with inverted flow, held in 150-L or 500-L larval rearing tanks. Water was fully renewed every two days and the temperature set at 18±1ºC. A mixed diet was provided on a daily basis, consisting of a mixture of *P. lutheri*, *I. galbana*, *T. suecica* and *C. calcitrans* at an initial ration of 80 cells μL^{-1}. When seed reached 1 mm in length, postlarvae were reared in 150 µm mesh sieves in a down-welling system in 1000-L rectangular tanks in closed circuit (Fig. 10). When spats reached 10 mm in length, they were transferred to 200-L tanks in open circuit with a 10 cm layer of sand at the bottom. Temperature was maintained at 18±1ºC. The seed was fed with a mixed diet of *T. suecica*, *I. galbana*, *P. lutheri*, *C. calcitrans* and *S. costatum* in the same proportion.

After one month of culture *S. marginatus* reached 2.1 mm, doubling the size of *E. arcuatus* and *E. siliqua* seeds (da Costa and Martínez-Patiño, 2009; da Costa *et al*., 2010a and b) (Table V) (Fig. 11). Four-month-old *S. marginatus* juveniles reached 19 mm in length, while *E. arcuatus* and *E. siliqua* reached around 30 mm (Darriba, 2001; da Costa *et al*., 2010a and b) (Fig. 12).

Table V. Length and weight of seed during nursery culture. Pf: post-fertilisation.

	Length at 1 month pf	Length at 4 months pf	Weight at 4 months pf
E. arcuatus	1 mm	30 mm	0.4-0.45 g
E. siliqua	1 mm	30 mm	0.4-0.45 g
S. marginatus	1.5-2 mm	20 mm	0.35-0.4 g

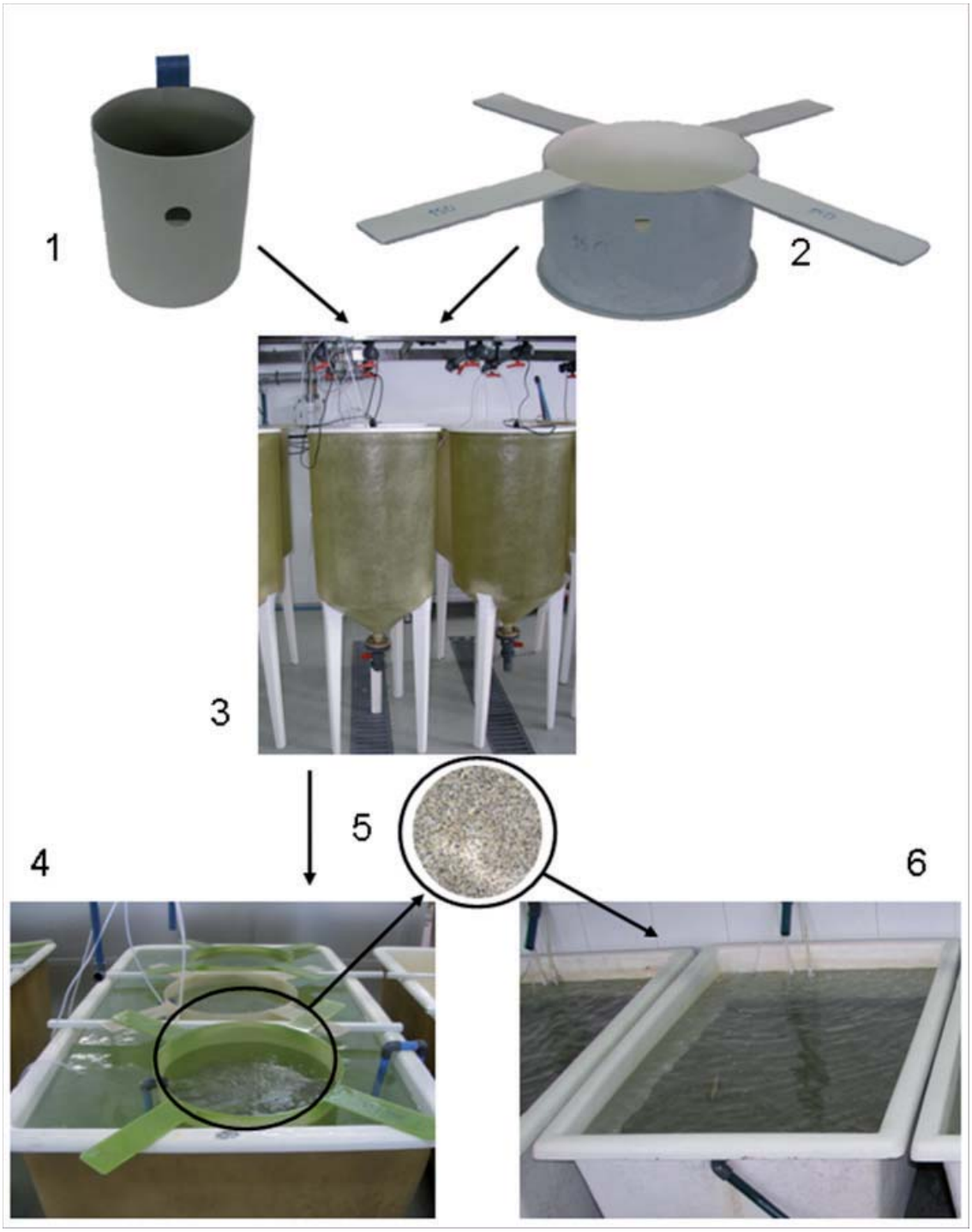


Figure 10. Nursery culture system. 1. Container with an area of 0.125 m^2. 2. Container with an area of 0.5 m^2. 3. Tank with 150-L capacity for postlarval culture. 4. Tank of 1000-L capacity for seed culture. Seed held in container. 5. Detail of the seed held in this system. 6. Tank with 200-L capacity with a 30 cm layer of sand.

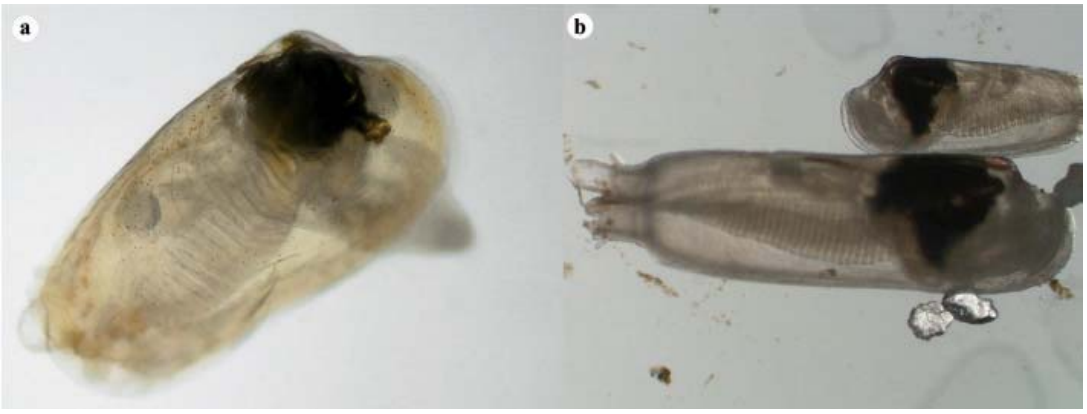


Figure 11. a. One-month-old postlarvae of *Ensis*. b. One-month-old postlarvae of *S. marginatus*.

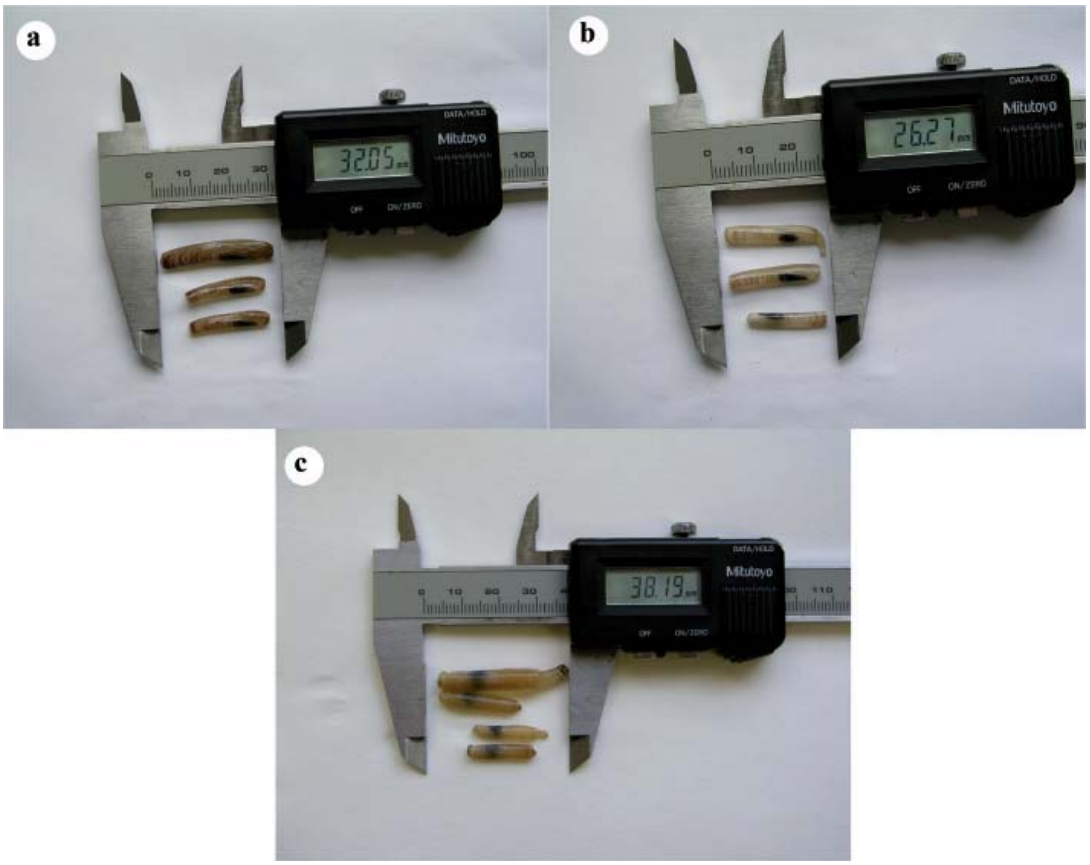


Figure 12. a. *E. arcuatus* seed. b. *E. siliqua* seed. c. *S. marginatus* seed.

Growth in length and length-weight relationship were fitted to exponential curves in these three razor clam species (Figs. 13 and 15). *S. marginatus* displayed a greater weight for the same length, from 15 mm in length onwards. Growth in height was similar between all three species for the whole of the experimental period (Fig. 14) and is fitted to a potential curve in all of them. However, weight during the first 120 days post-fertilisation is similar in the three razor clam species studied, after

which time *E. arcuatus* reached a greater fresh weight due to the greater increase in length.

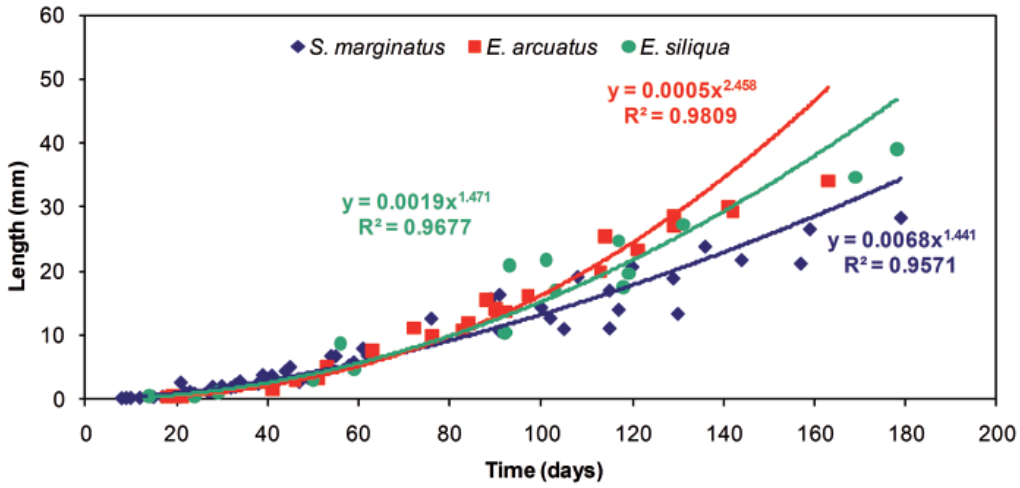


Figure 13. Postlarval and seed growth in length.

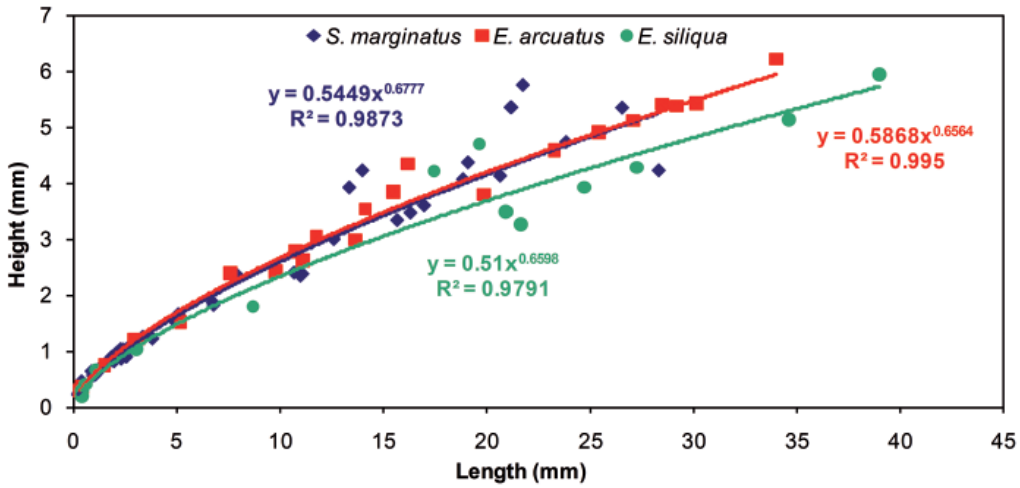


Figure 14. Length-Height relationship in postlarvae and seed.

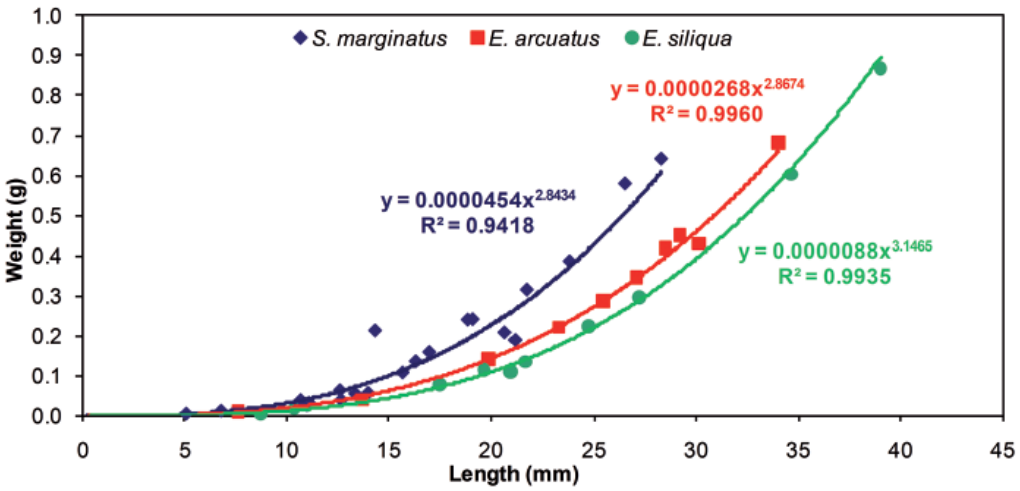


Figure 15. Length-Weight relationship in postlarvae and seed.

2.4.2. Effect of substrate on seed culture

Razor clam aquaculture is constrained by the fact that razor clam need to be buried and that they are sensitive to manipulation, making seed handling and cleaning difficult. Hence, for large-scale seed production, it is important to reduce the volume of the sand needed or even to avoid the use of substratum. Some trials were carried out in order to improve low yields observed in this culture phase, such as testing different sizes of containers for holding the postlarvae and determining what size should be reached for culture without substrate.

Containers with two different areas were tested (0.125 and 0.5 m^2), and also different times for seed transfer into substrate: at settlement, at one month post-fertilisation (approximately 1 mm long) and at sowing time. Best results, from settlement until 1 mm long (one-month-old seed approximately), were achieved rearing the seed without substrate, showing highest survival in the smaller holding containers (0.125 m^2).

One experiment was carried out in each species to investigate the effect of substrate on the survival and growth of spat. Initial seed length was between 1.5 and 4 mm, depending on the species. Three different treatments per duplicate were tested: (1) 0.125 m^2 containers without substratum for rearing seed; (2) 0.5 m^2 containers using fine grain sand (150-600 µm grain diameter); and (3) 0.5 m^2 containers using coarse sand (300-1200 µm grain diameter). Each experiment was performed for 1 month and samples were collected at days 0, 15 and 30.

The rearing of *E. arcuatus* spat of 4 mm long showed a 50% survival rate without substratum, whereas animals grown in two different types of sand displayed survival ranging from 80 to 90% (da Costa *et al.*, 2010a). Growth of *E. arcuatus* spat was similar under the different conditions studied. In contrast, *S. marginatus* seed reared under the same experimental conditions displayed a higher survival rate when farmed without substrate (81.9%), compared with 36.1% for fine and 53.3% for coarse sand, respectively (da Costa and Martinez-Patiño, 2009). Similar results were observed for *E. siliqua*, which revealed better survival in seed grown without substratum (70%) than when grown in sand (33%) (da Costa *et al.*, 2010b). One explanation for these results could be that high stocking density may reduce the energy necessary to keep shells closed due to the weight of neighbours pushing one another. High stocking density during nursery culture may be effective for individuals measuring less than 10 mm. Over this size, stocking density should be reduced to ensure optimal feeding supply. This could lead to increasing energy losses preventing shell gaping, and thus reducing growth rate. Furthermore, it was observed that, after a few months of seed rearing without substrate, individuals showed deformities in their shells (da Costa,

unpublished results). Substrate has to be provided progressively to acclimatise seed for on-growing conditions in natural beds.

2.4.3. Effect of diet on seed culture

Seed culture has been identified as one of the major constraints in razor clam culture (da Costa and Martínez-Patiño, 2009). Observed mortalities may be a result of nutritional deficiencies, as little is known about dietary needs in these developmental stages. Diets used in razor clam hatcheries have generally been adopted from those used for other bivalves with more established culture practices, yet the performance of algal diets is often highly species- and stage-specific. Therefore, investigation should focus on identifying algal diets which maximise survival and growth of *E. arcuatus* and *S. marginatus* spat.

One-month-old *E. arcuatus* seeds were held in 150-L tanks similar to those used for larval culture and were reared in sieves with inverted flow produced by air-lifts. Water was changed 3 times a week and temperature was set at 19±1ºC. Initial length was 1.31±0.29 mm. Three different diets were evaluated per duplicate: diet 1: *T. suecica* and *S. costatum*; 2: *I. galbana*, *P. lutheri* and *T. suecica*; 3: *I. galbana*, *P. lutheri*, *S. costatum* and *T. suecica*. Spat was fed on a daily basis using the same initial ration in all diets, 120 equivalent cells per µL. The experiment was maintained for 2 weeks. Results showed that at the end of diet 2 (*I. galbana*, *P. lutheri* and *T. suecica*) and diet 3, trials showed similar length, with an important difference from diet 1. The presence of *I. galbana* and *P. lutheri* in diet seems to promote better growth compared with diets lacking these algae; however, no effect was observed in survival, which was similar in all diets, with 100% survival at the end of the experiment.

A similar experimental design was used for *S. marginatus*. Initial seed length was 1.17±0.29 mm. Three different diets were assayed per duplicate: diet 1: 25 equivalent cells per µL (*I. galbana*, *P. lutheri* and *C. calcitrans*) and 50 equivalent cells of *T. suecica* per µL; 2: 25 equivalent cells per µL (*I. galbana*, *P. lutheri* and *S. costatum*) and 50 equivalent cells of *T. suecica* per µL; 3: 40 equivalent cells per µL (*I. galbana* and *P. lutheri*) and 50 equivalent cells of *T. suecica* per µL. Spat fed on diet 1 (*I. galbana*, *P. lutheri*, *C. calcitrans* and *T. suecica*) displayed the greatest growth in length. Survival at the end of the experiment was similar between diets, ranging from 97 to 100% in all treatments.

An understanding of the effect of the ration on seed growth and survival is important for the development of optimal and cost-effective feeding regimes in bivalve cultivation. Three ration diets were tested per duplicate: 1, 2 and 3% of mean

dry meat weight of the seed per day in forty-five-day-old *E. arcuatus* seed. Spat were fed a mixed diet of *T. suecica*, *I. galbana*, *P. lutheri* and *C. calcitrans*. Initial length was 1.55±0.65 mm. After one week, growth was faster in seed fed a 3% ration than in those fed the other two rations. Growth of seed on 1% and 2% rations was similar. At the end of the experiment the 3% ration seed reached 4.44±1.49 mm in length. Growth in dietary rations of 1% and 2% was similar, attaining a final length of 4.20 and 4.22 mm, respectively. Seed fed a ration of 1% mean dry meat weight of the seed per day showed lower both growth and survival (60% survival after 21 days) than those fed the other two rations (90% survival at the same time). An adequate quantity of food is essential for good growth and survival rates. This demonstrates the importance of the quantity of food supplied to young juveniles in bivalve nursery cultivation.

2.4.4. Postlarval and seed survival

Postlarval and seed survival was quite low in *E. arcuatus*, *E. siliqua* and *S. marginatus*, being around 5-10% after 30 days and 2-5% after 90 days from settlement. High mortality was observed until reaching 1 mm long but from this size onwards survival was constant. Different modifications were introduced in postlarval culture protocols during razor clam cultivation in our facilities. Rearing without substrate *S. marginatus* postlarvae from settlement until 1 mm long (one-month-old seed approximately), as mentioned above, increased the survival in this phase. As a result of this, survival increased from 5-10% on average up to 63.5% on average (ranging from 12.9 to 100% in 2009) (da Costa, unpublished results). However, postlarval survival still remained low in *E. arcuatus* and *E. siliqua*. Moreover, from 1 mm long until seed was transferred to wild beds, survival was low (5-10%) in all three species. Hence, more research is needed to ascertain the specific dietary and environmental requirements (in terms of rearing temperature) in this important phase of culture process in order to improve yields.

3. Intermediate culture

Bivalve juveniles (seed) produced within a hatchery have to be transferred to natural beds when they reach a certain length, because the cost of maintaining them in a hatchery is too high. If juveniles are introduced into natural beds when they are too small they are subject to very high levels of mortality, mainly as a result of predation. Commercial production of most bivalve species involves a phase of intermediate cultivation during which juveniles are grown under protected conditions, using natural phytoplankton as food, until they are large enough to be less prone to predation and can be transferred to the final grow-out location. Consequently,

different types of containers have to be designed to study growth and survival of razor clams.

An experiment was conducted to investigate the performance of *E. arcuatus* in intermediate cultivation in 5-L plastic bottles (da Costa *et al.*, 2010a). The bottles were perforated and covered with 1 mm mesh to prevent razor clam escape. A plastic screw-top was used to close the container (Fig. 16a). Juveniles with an initial length of 11.17±3.51 mm were kept in the containers for 30 days. The experiment involved comparing growth and survival of juveniles in fine (150-600 µm grain diameter; FS) and coarse grained sand (300-1200 µm grain diameter; CS) and at low (18 g per bottle; LD) and high (36 g per bottle; HD) stocking densities. Significant growth in length was observed in all cases in *E. arcuatus* seed reared in intermediate cultivation under different substrate and density conditions. The increase in mean length of juveniles was higher at low densities and highest for those grown in coarse sand at low density; the increase in mean length of juveniles was lowest in those grown in fine sand at high density. Lowest survival was recorded in coarse sand at high density (86%); other treatments showed similar survival rates (94-95%). Individuals reared at low densities have more space and a higher amount of phytoplankton per individual, which is likely to result in higher growth and survival rates. Enhanced growth in coarse sand may be explained because less detritus is retained in this type of substrate and consequently there may be fewer bacterial infections in individuals grown under such conditions. This could also be related to the substrate preferences of *E. arcuatus*, which normally inhabits sand and gravel bottoms. The problem with this type of container is that it is time-consuming to fill it with juveniles and to sample them subsequently; this would preclude their large-scale use in a commercial situation.

A different type of seed-holding container was designed in order to improve the intermediate cultivation of razor clams using readily-available cheap equipment and protocols that could be rapidly adopted by commercial producers. Hence, PVC cylinders were perforated with 6 cm holes in the sides and 1 mm mesh was used to cover the structure to prevent razor clam escape (Fig. 16b-d). Each structure consisted of two cylinders, each one having a 150 µm mesh at the bottom to retain the sand yet allow its aeration. Growth and survival were tested in fine and coarse sand and at low (20 g per container) and high (40 g per container) stocking densities. The trial began in September 2007 and sampling was performed at monthly intervals until May 2008. There was a marked increase in mean juvenile shell length during the first 30 days of the experiment, with a small increment in the following 30 days (until November) (Fig. 17a). Between November and the end of March no significant growth was observed. During spring, growth began to increase again in all treatments. During the first month of the experiment, highest survival was observed for juveniles

held in coarse sand at low density (74.5%), followed by those held in fine sand at low density (60.1%). After November this trend was reversed, with fine sand and low density showing higher survival, with 26.6% up to May 2008 (Fig. 17b).

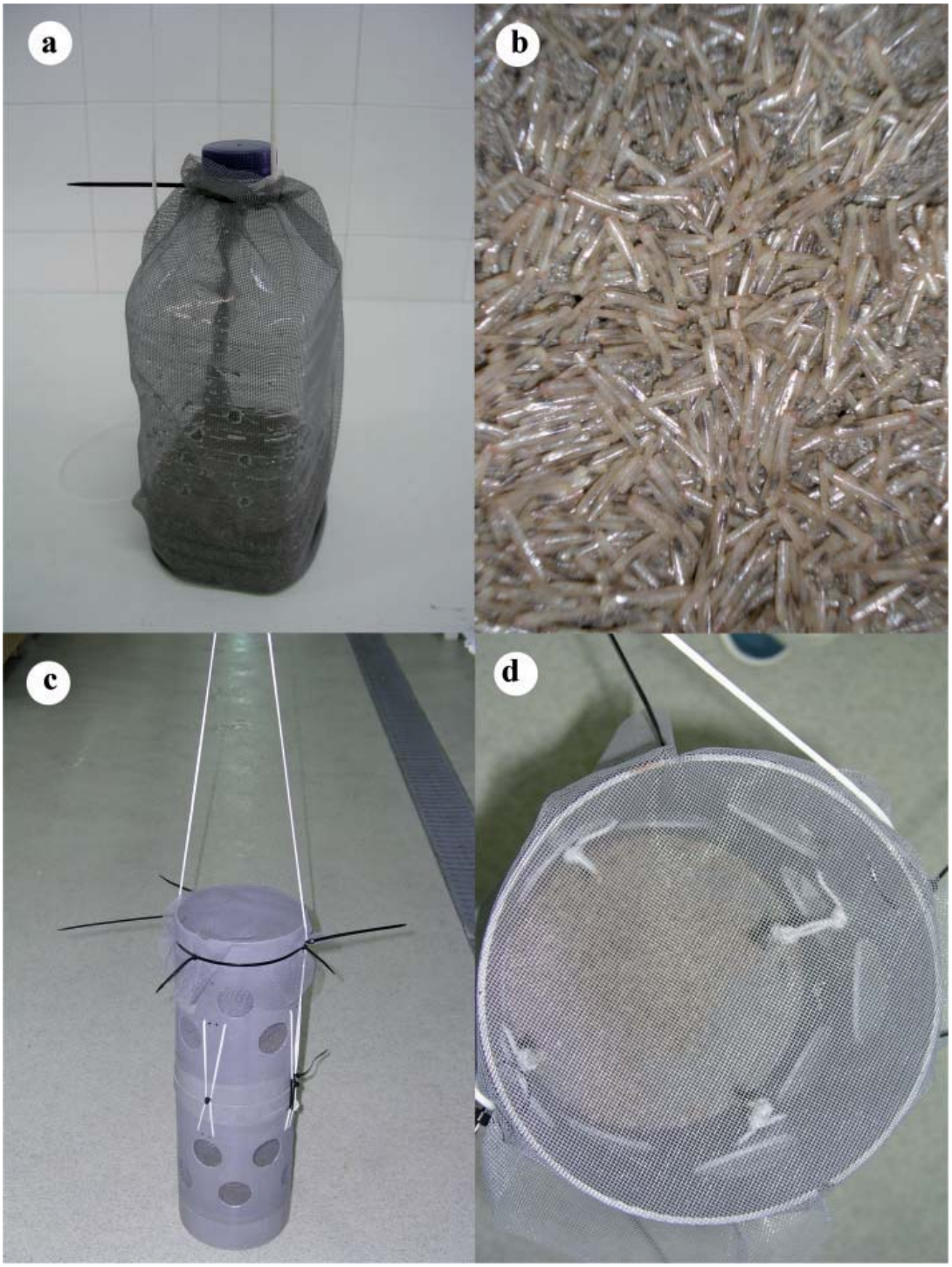


Figure 16. a. Plastic bottle used for on-growing of *E. arcuatus* juveniles. b. *E. siliqua* seed for on-growing experiments. c. Container to hold razor clam seed. d. Upper view of the container, with 1 mm mesh to prevent razor clam escape.

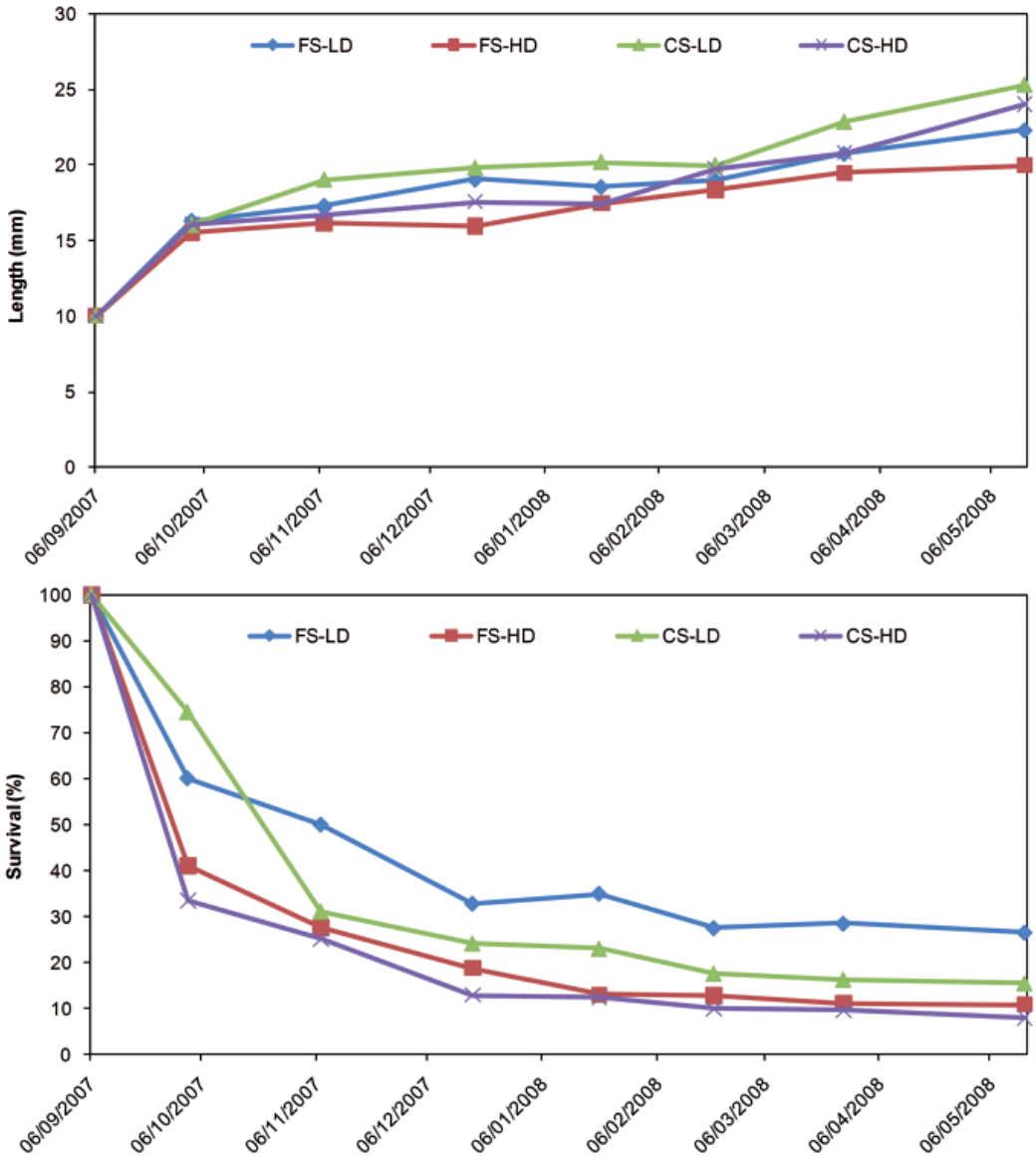


Figure 17. Growth and survival at monthly intervals of *E. siliqua* seed, grown in plastic cylinders under different treatments. a. Increase in length. b. Survival (%). FS: fine sand; CS: coarse sand; LD: low density; HD: high density.

An intermediate cultivation experiment was also performed with *S. marginatus* seed. Initial length of individuals was 5.2±0.7 mm. Two types of substrate were used: fine (FS) and coarse sand (CS) and two holding densities: 8.5 g per container (Low density, LD) and 17 g per container (High density, HD). Lower growth in length was exhibited in coarse sand and high density at the end of the experiment (four months of cultivation). Survival after one month ranged between 50 and 59% in all treatments. Best results observed in survival were for fine sand, irrespective of density, which ranged from 43% to 45%; however, growth in fine sand was not as great as in coarse sand. Lowest survival was observed in coarse sand and low density (19%).

Just as in previous studies, the present one indicates that it is feasible to conduct intermediate cultivation in suspended culture systems using cheap and readily-available materials. However, such studies have yielded very diverse results. In addition, most protocols are labour-intensive and may not be realistically adopted for commercial purposes. In the future, other methods should be tested, such as the use of plastic containers filled with sediment in low intertidal areas and seeded with juveniles, which has been successfully used for intermediate grow out of clams in other parts of the world.

4. On-growing

Bivalve juveniles (seed) produced within a hatchery have to be transferred to natural beds when they reach a certain length (namely, from 10-15 mm long onwards), because the cost of maintaining them in a hatchery is too high. If juveniles are introduced into natural beds when they are too small they are subject to high mortality rates, mainly as a result of predation. It is difficult to study the growth of juvenile razor clams in natural beds because they move around and burrow quite deeply into the sand.

Holding containers for burrowing in the substrate were developed in order to monitor seed on-growing. Individuals were transferred to 50 cm x 50 cm x 50 cm mesh cages with a stainless steel frame, each of which was fitted with a top cover with a mesh net to prevent razor clam migration (Fig. 18). These cages were buried in the sand, leaving approximately 5 cm of each cage protruding above the surface of the sediments. Consecutively, with monthly low tides, one buried cage was sampled to empty out the substratum and to recover all individuals. The rest of the cages were cleaned and predators eliminated. The following month another cage was sampled, repeating the procedure. Every month the top covers of the cages were replaced due to fouling. At this time all razor clams collected in each cage were counted, measured and weighed so that estimates of growth and survival could be made. At the end of each sampling session, the individual razor clams were returned to their corresponding cages.

Initial length of *S. marginatus* seed was 19.10±1.93 mm (4-month-old juveniles), corresponding to 0.24±0.08 g of fresh weight. One-year-old juveniles reached 38.53±3.89 mm, corresponding to a weight of 1.56±0.46 g (Fig. 19; Table VI). Survival during the first year of on-growing was up to 80%. Two-year-old juveniles reached 67.13±5.14 mm in length, with a survival of 62%. Commercial size was reached with three-year-old individuals (80.06±5.17 mm) (da Costa and Martínez-Patiño, 2009). The influence of environmental conditions on bivalve growth

has been mentioned by several authors, where temperature and food are the most important parameters (reviewed in Bayne and Newell, 1983). Elevated growth was observed until November, coinciding with high seawater temperatures and algal bloom in October-November. From November onwards, no growth was recorded but from April onwards, significant growth was observed. The growth period lasted from March-April until September-October. From the end of autumn until late winter no growth was observed. Temperature and chlorophyll a (amount of food) had a combined effect on growth (da Costa and Martínez-Patiño, 2009). Survival in the on-growing experiment ranged between 50 and 83%.

Figure 18. Holding containers for on-growing seed of the razor clams buried in natural beds.

Table VI. Comparison of razor clam on-growing in wild beds. Pf: post-fertilisation.

	Length at 1 year pf	Weight at 1 year pf	Age at commercial size
Ensis arcuatus	64.1±3.0 mm (hatchery)	5.3±1.0 g	> 2.5 years (100 mm)
Solen marginatus	38.5±3.9 mm (wild beds)	1.6±0.5 g	> 2.5 years (80 mm)

The life cycle of this species was closed, obtaining larvae from the individuals reared in the on-growing experiment (da Costa and Martínez-Patiño, 2009). Once individuals reached commercial size (80 mm), they were used as broodstocks in the hatchery facilities to obtain spawning, as described above, to close the cultivation cycle. Seven spawnings were obtained from broodstock reared in the hatchery and four of these were studied. The highest survival rate in the settlement was 83%, with the average being 48%. From settlement to 30 days of culture, the highest survival was 46%, with an average survival in this phase of 35%. Closing the life cycle allowed the inclusion of this species as a potential aquaculture species. Length-height relationship of hatchery-produced seed on-grown in a natural bed of *S. marginatus* was fitted to an exponential curve, which had a exponent of 0.868

(Fig.20a). Gaspar (1996) stated that this adjustment represented a negative allometric relationship, because the exponent was less than the unit, as in *E. siliqua.*

Length-weight relationship was obtained by adjusting data to an exponential curve $W=0.00003L^{2.956}$, where W is weight in g and L is length in mm, which corresponds to an isometric relationship (Fig. 20b).

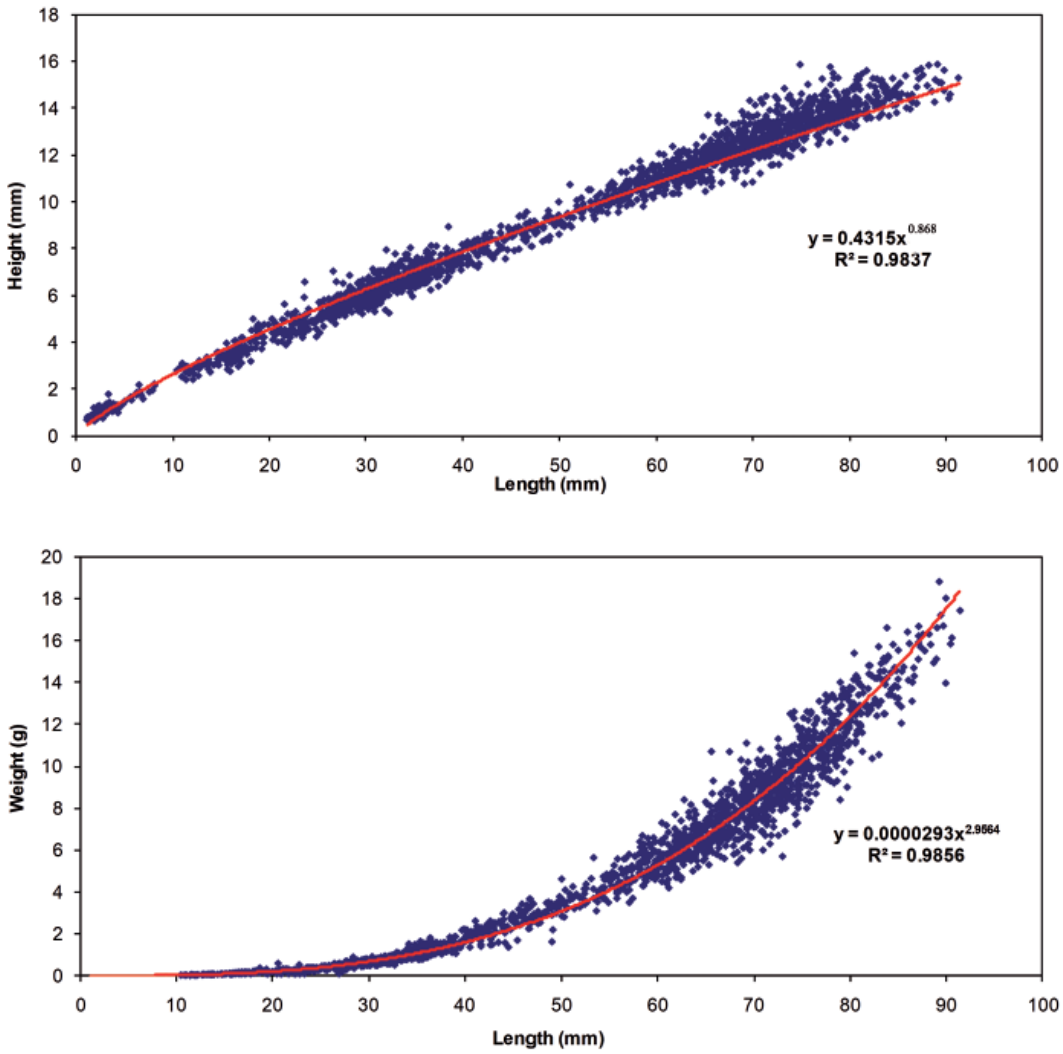


Figure 20. Length-Height and Length-Weight relationships of the juveniles of *S. marginatus* during on-growing experiment.

On-growing experiments were carried out not only with one-year-old hatchery-produced *E. arcuatus* juveniles but also with juveniles captured in a natural bed (Darriba, 2001; da Costa *et al.*, 2010a). One-year-old hatchery-produced juveniles measured 64.1±3.0 mm long, corresponding to 5.3±1.0 g of weight. Mortality of all specimens was recorded after nine months from the beginning of the experiment due to a decrease in salinity caused by heavy rains, with a survival, until then, of 60%. This research allowed description of the mathematical model of growth

during 600 days of life of *E. arcuatus*, which fitted to an exponential curve with a higher dispersion of the data (length) as individuals increased their size (Fig. 21). Length-weight relationship was determined by adjustment of an exponential curve (Fig. 22).

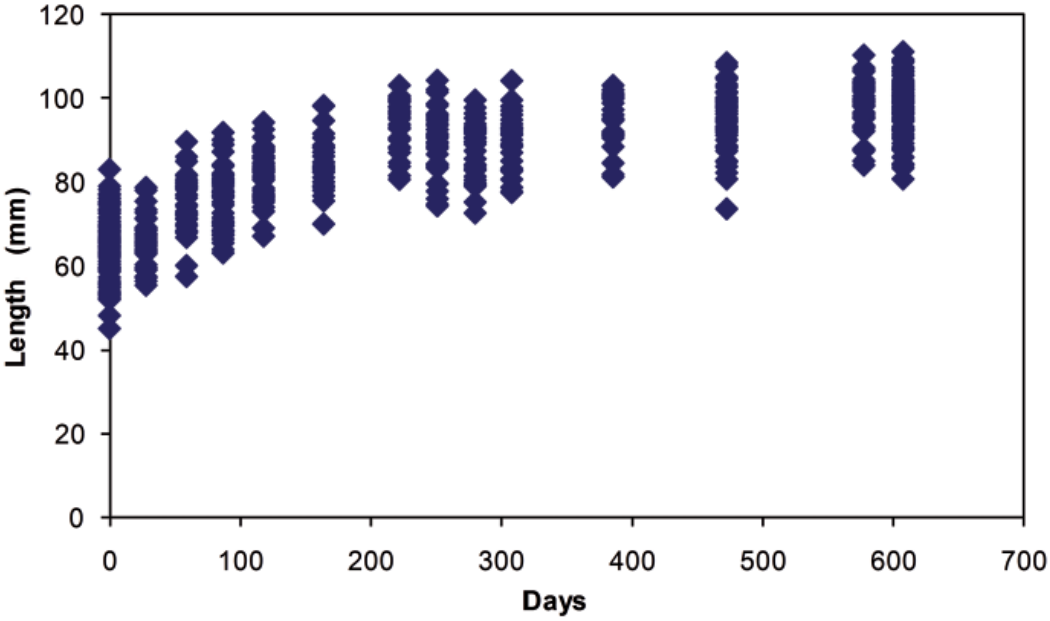


Figure 21. Growth in length of *E. arcuatus* reared in the hatchery and on-grown in cages.

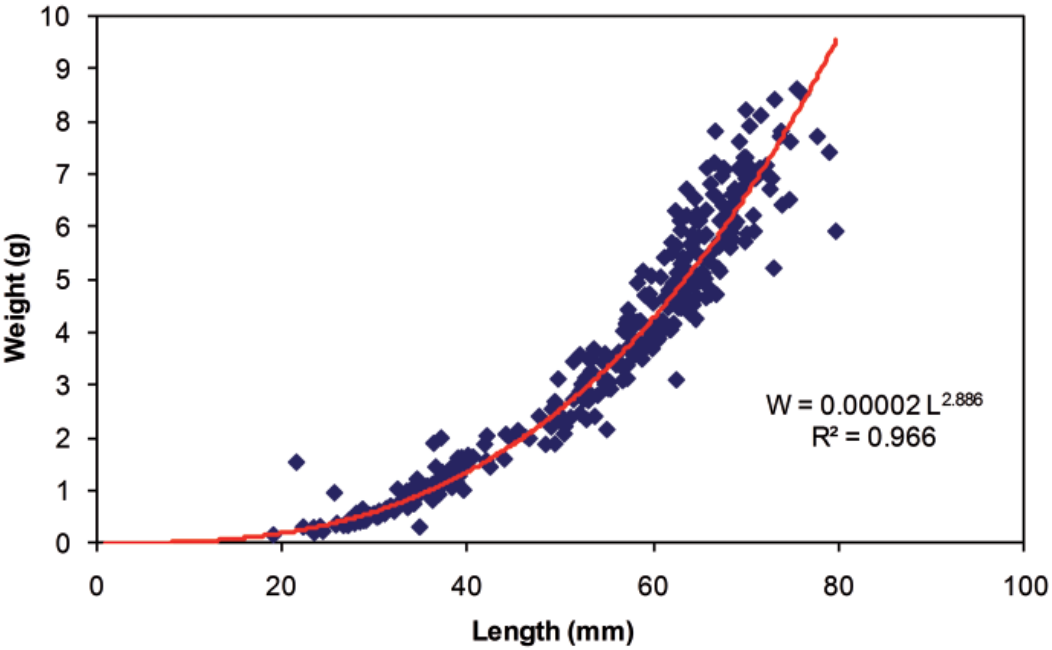


Figure 22. Length-Weight relationship of *E. arcuatus* reared in the hatchery and on-grown in cages.

The determination of growth curve and age of commercial size individuals was

carried out with juveniles captured in a natural bed with 65.0±7.7 mm of length and 3.5±1.2 g of fresh weight (one year old approximately) (Fig. 23). Individuals were held in buried cages for 18 months approximately. One year after the beginning of the experiment length was 94.3±6.3 mm and fresh weight reached 13.2±2.1 g. Individuals reached 98.0±6.2 mm in length, which corresponded to a mean fresh weight of 14.7±2.2 g, which is close to the commercial size of this species (100 mm). During spring and summer significant growth was observed. Survival was 68.5% and 56.8% after six and twelve months of on-growing in cages, respectively. After a year and a half running this experiment all specimens died due to the same salinity decrease as in the experiment of hatchery-produced juveniles.

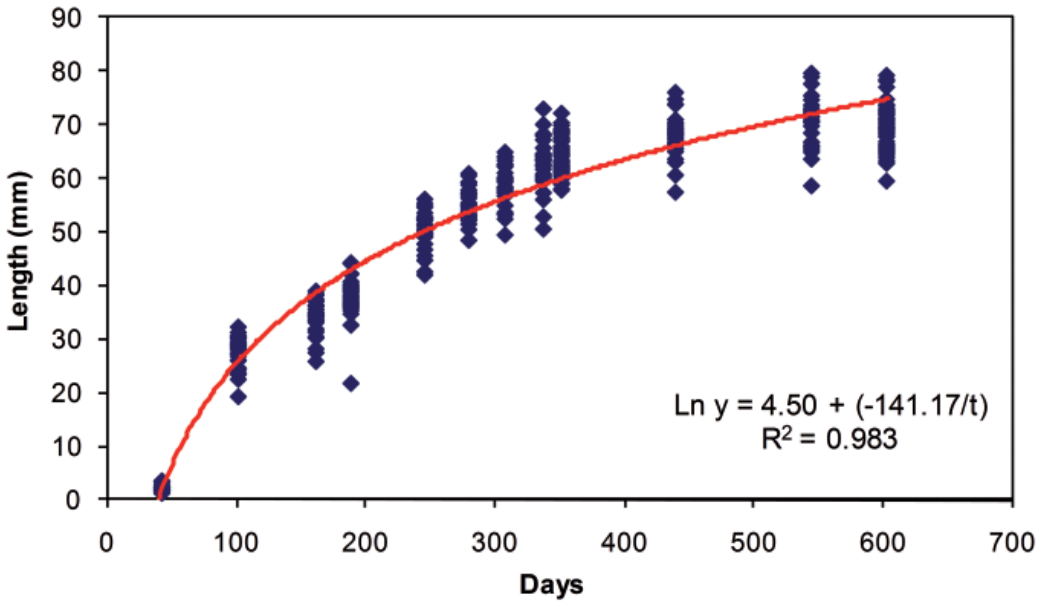


Figure 23. Growth in length of *E. arcuatus* juveniles captured in a natural bed on-grown in cages.

5. Aquaculture perspectives

Hatchery and on-growing culture of *E. arcuatus*, *E. siliqua* and *S. marginatus* has both strengths and weaknesses.

Broodstock conditioning was achieved; however, with the present state of the art, spawning can be obtained only during a limited period in the year. Further aspects of broodstock conditioning in *Ensis* and *Solen* species need to be investigated, such as the combined effects of diet and temperature, and the effect of photoperiod on gonadal development in these species.

Spawning methods are developed for these three species; however, *S. marginatus* induction should be improved in order to better control the acquisition of eggs.

Larval culture in the three species does not display significant problems. Even so, *E. arcuatus* survival still needs to be improved.

One of the major issues impeding the development of hatchery culture of razor clams is postlarval and seed culture. Survival from settlement until 1 mm long is low in *E. arcuatus* and *E. siliqua*, thus reducing the yield of the culture. On the other hand, *S. marginatus* survival from settlement until 1 mm long has been improved, reaching 63.5% on average. Furthermore, culture of seed is constrained by the fact that they need to be buried. Therefore, when rearing razor clams at nursery, a trade off must be made between husbandry (in term of man hours spent maintaining the sand) and yield. Even though it is cleaner and easier to work with systems devoid of sand, better survival rates were achieved for *E. siliqua* and *S. marginatus*, as against those found for *E. arcuatus*.

These studies indicate that it is feasible to conduct intermediate cultivation in suspended cultivation systems using cheap and readily-available materials although the present state of the art precludes large scale razor clam production. Moreover, survival should be improved in order to make intermediate rearing profitable.

On-growing showed good growth and survival rates; however, the negative effects of the no-growth season must be reduced by investigating the best season of the year to initiate razor clam on-growing in natural beds.

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Chapter 10: Razor clam (*Ensis macha*) culture in Chile

María Irene Lépez García, Flor Elizabeth Véjar Durán and Daniel Andrés Arriagada Obregón

Abstract

Razor clam (*Ensis macha*) culture started in 1997, due to the interest from business people elaborating and exporting canned products, which motivated several research projects[1]. This allowed us to discover aspects of the biology, ecology and physiology of the species, leading to the development of technology for producing seeds in controlled environments (Olave *et al.*, 1999; Lepez *et al.*, 2004; Arriagada, 2005) and for on-growing culture (Santos *et al.*, 1999; Lepez, 2005).

Razor clam culture starts with the selection and preparation of broodstock extracted from natural beds. After preparation, gamete release is induced, beginning larval culture, which is performed in controlled environmental conditions, lasting about 20 days. Postlarvae obtained are reared in systems containing sand during 50 days, after which the samplings are 1 cm in valve length. Several on-growing culture systems have been tested, suspended culture in BDC[2] devices being the most successful one.

Despite the efforts made and the time spent, no technology exists that would allow the industrial production of razor clam in Chile.

1. Razor clams seed production (*Ensis macha)* in controlled system

1.1. Selection and conditioning of broodstock

Razor clam broodstock are collected in natural beds and samplings are selected that are longer than 10 cm, with valves healthy and free of epibionts, with active foot and valvar movement, a swollen mantle and undamaged siphons.

[1] FONDEF D96I1095 project. Culture of razor clam (*E. macha*) in VIII Region. FONDEF D99I1073 project. Culture of razor clam: a viable alternative for national productive sector. FONDEF HUAM AQ04I1022 project. Optimization of burrowing bivalve culture: macha, jackknife clam, Chilean razor clam in post-larval and on-growing stages.

[2] The BDC culture device is associated with invention patent Nº 27182003.

Broodstock conditioning is carried out in tanks with water recirculation systems and using a sand substrate. A periodical diet of *Isocrhysis galbana* and *Chaetoceros muelleri* microalgae (300,000 cells mL^{-1}) is given, and stable conditions of temperature (17ºC), salinity (33-35‰) and dissolved oxygen (5-6 mg L^{-1}) are maintained for a maximum period of 90 days (Fig. 1).

Figure 1. Selection and conditioning system of razor clam broodstock.

1.2. Spawning induction

Spawning induction is carried out through consecutive changes of sea water. Induction begins at 11ºC and increases by 6 or 7ºC during 2 to 3 cycles and for about a 30-minute period at each temperature. When the routine of changing temperatures has concluded and after 2 to 4 hours of maintaining broodstock in tanks with filtered and sterilised sea water, the release of gametes begins, and then a mass fertilisation in the same tank (Fig. 2).

1.3. Fertilisation

Fertilisation is controlled by the appearance of the fertilisation membrane and is confirmed with the number of D larvae counted in the first culture control. With the egg just fertilised, embryonic development begins, lasting 48 hours. Trochophore and straight hinge veliger larvae are obtained between 24 and 48 hours after fertilisation, respectively.

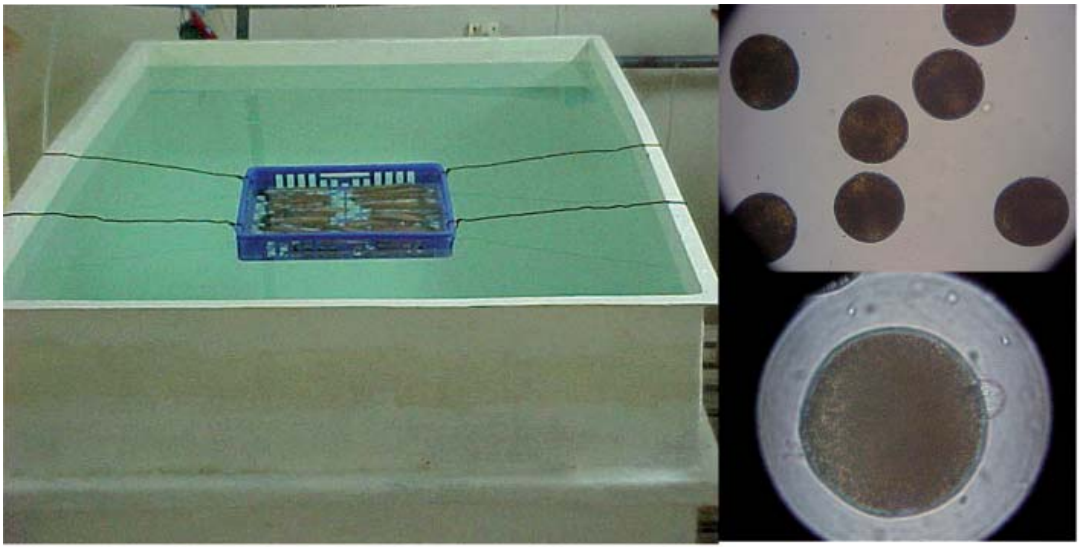

Figure 2. Spawning induction and onset of razor clam embryo development.

1.4. Larval culture

The formation of D larva ends the embryonic development process and starts the stage of larva with shell, which originates larval culture.

Razor clam larval culture lasts 17 to 20 days, from D larva until the premetamorphic stage, at an average temperature of 17ºC (Fig. 3). Rearing is carried out in conical 2000 L tanks, with sea water filtered at 1 µm and treated with UV, with a daily change of water and permanent aeration. Diet is based on *Isochrysis galbana* and *Chaetoceros muelleri* microalgae at a concentration of 20,000 to 50,000 cells mL^{-1}, depending on the number of culture days (Table I).

Table I. Diet and density programme during larval culture of razor clam in controlled system.

Culture days	Development state	Density (larvae/ ml)	Microalgae (proportion)	Concentration (cells/ mL)
1	Trochophore	20	*I. galbana* (100%)	20,000
2 - 7	D Larvae	10	*I. galbana* (100%)	30,000
8 - 10	Umbonate	5	*I. galbana*: *C. muelleri* (1:1)	30,000
10 - 14	Pediveliger	1	*I. galbana*: *C. muelleri* (1:1)	40,000
15 - 20	Premetamorphic	1	*I. galbana*: *C. muelleri* (1:1)	50,000

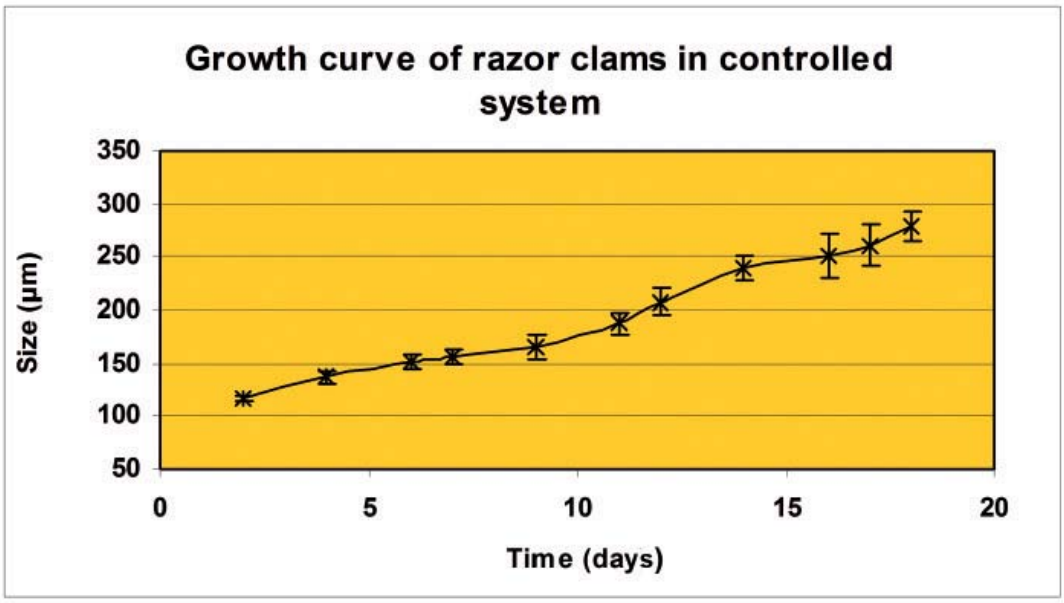

Figure 3. Razor clam larval growth curve in 2000 L culture tanks (Lépez *et al.,* 2004).

1.5. Settlement and metamorphosis

Metamorphosis is induced when pediveliger larvae reach an average size of 290 µm in valve length. Induction to metamorphosis is performed in trays containing substrate sand, in a descending water circulation system, for three days at an average temperature of 15ºC. During this process, diet is kept at low concentrations (20,000 cells mL^{-1}) (Fig. 4).

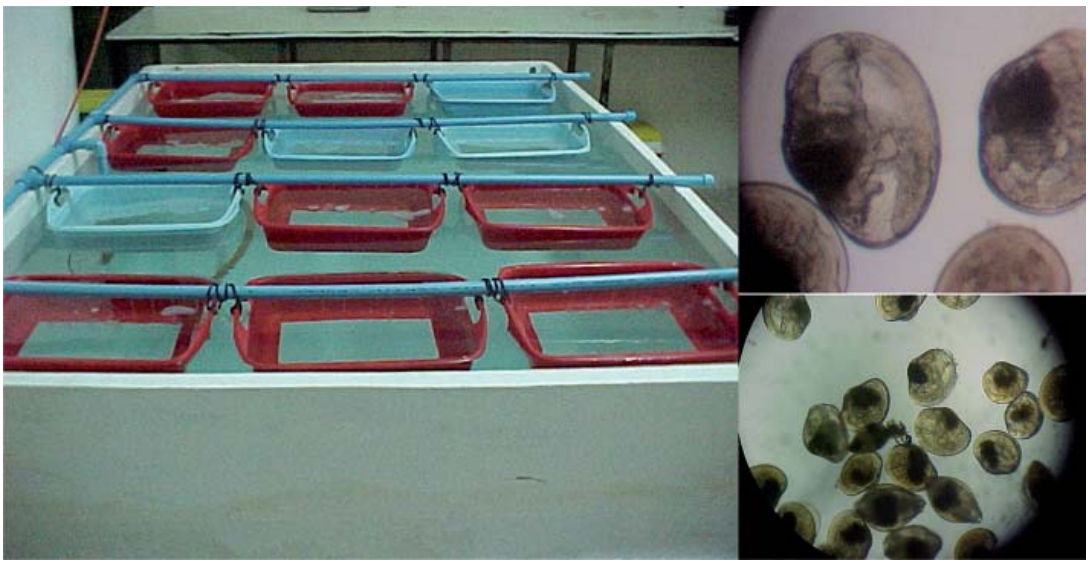

Figure 4. Induction to metamorphosis and pre-metamorphic razor clam larvae.

1.6. Juveniles or postlarval culture

Razor clam postlarval culture is carried out in trays with compacted sand, in a closed recirculation system, until juveniles reach an average size of 1 cm in valve length (Figs. 5 and 6). This phase lasts 50 days after settlement and, during the first days, sea water temperature is gradually brought to the level of environmental temperature. Sea water is filtered at 1 µm when culture starts and at 50 µm when it finishes, while it is only sterilised with UV light at the beginning of culture. A mixture of microalgae at a concentration between 50,000 to 100,000 cells mL^{-1} is used as food.

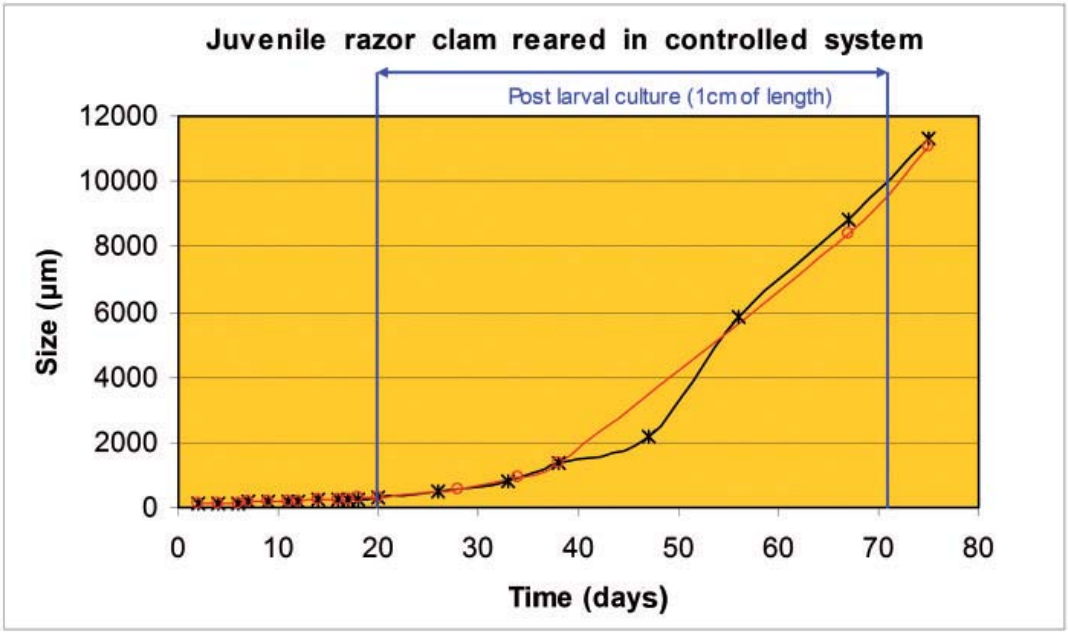

Figure 5. Growth curve of razor clam juveniles in controlled system (Lépez *et al*., 2004).

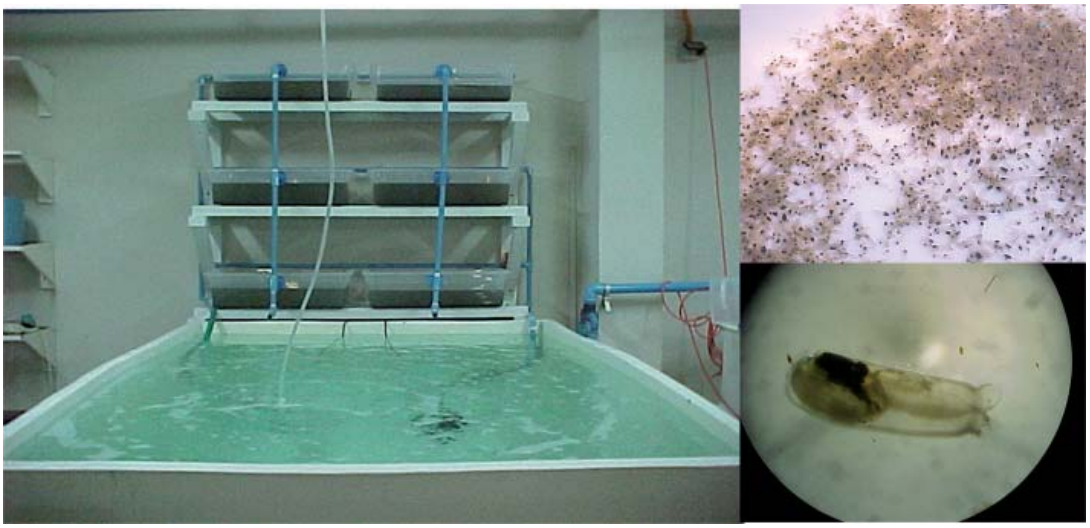

Figure 6. System of culture of razor clam: postlarvae and juveniles of 2.15 mm in valve length (cultured for 20 days).

2. Razor clam on-growing culture

For razor clam on-growing culture, three culture systems have been tested: long-lines, in ponds and by in- and on- bottom husbandry.

2.1. Long-line culture

For razor clam long-line culture a technology has been developed that includes the use of a growing device called “BDC”. Each device has 2 culture units or levels with holes that allow water circulation. “BDC” devices have a 12 cm sand column and must be covered with a plastic mesh in order to prevent individuals from escaping. Juveniles that have been “BDC-farmed” with 1 cm in valvar length are suspended from a floating line or long-line (Fig. 7).

The use of this culture device allows a better use of the space and food, at different depths. It is easy to manage and maintain, and allows work with high seed densities at when the culture is starting. However, this culture system needs large amounts of sand, affecting line weight and flotation costs. Moreover, it requires a large amount of grading, with a low number of individuals per unit when harvesting, which implies a large number of floating lines, “BDC” devices and man-hours used. Table II shows a diagram of long-line culture management.

Figure 7. Razor clam long-line culture: “BDC” devices and 1 cm long razor clam culture.

Table II. Diagram of grading during razor clam on-growing culture with long-line system.

On-growing time (month)	Density (individuals/m²)	Density in "BDC" (indiv./level)	Protective mesh opening (mm)	Starting phase average size (cm)
0 - 7	24,045	1,700	2x4	1
7 - 13	11,315	800	4.5x4.5	3.75
13 - 19	2,829	200	9x9	5
19 - 24	1,414	100	9x9	7

2.2. Pond culture

This system involves keeping ponds on the ground, which may be of an inert material suitable for culture. In order to use this system there must be a continuous flow of sea water and a roof and protective mesh in order to avoid an excess of light and the entry of foreign particles. Ponds must have a 15 cm high sediment column and an aeration system that allows microalgae to be kept in suspension until they are consumed (Fig. 8). Pond culture can be carried out with individuals with an average size of 1 cm, and diet is based on traditional microalgae.

The advantages of this system are related to the protection of individuals against predators. Moreover, it does not require specialized staff for cleaning and maintenance, allows daily control of the state of the system and does not depend on the environmental dynamic. The difficulties associated with pond culture are related to temperature fluctuations, the high renovation rate of sea water required, the large amount of food needed and frequent cleaning of ponds, meaning intensive labour.

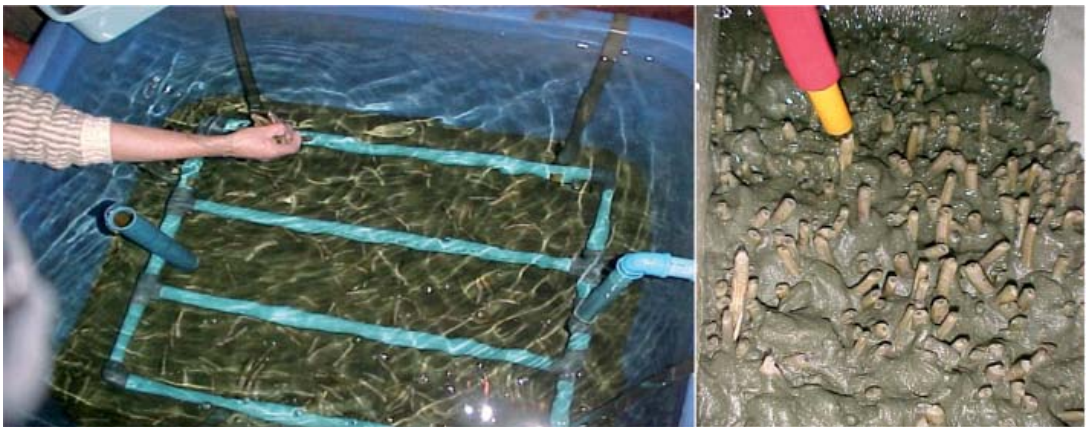

Figure 8. Razor clam pond culture.

2.3. In- and on- bottom husbandry in pens

This culture system is a variation on the one most widely used in clam culture around the world and consists of using an enclosure or protected plot or area of the sea bottom where individuals are left to grow. In Chile, experimental culture has only been carried out using 2 m^2 pens at a depth no greater than 5 m and at low culture densities. In this kind of culture, there are diverse culture samplings, and these are homogenously distributed inside pens, which have to be protected with a mesh to prevent the entry of predators.

The management of this system consists of a frequent inspection of pens (state of mesh, elimination of predators, mortality controls) and quarterly control of growth. Harvest size depends on market requirements. The difficulties of culture in pens include limited availability of suitable areas for culture, the need for qualified divers in order to maintain systems, limitations in culture density and the minimal size required for culture.

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Chapter 11: Environmental impacts of razor clam fisheries

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Abstract

Razor clams are exploited worldwide using several types of fishing techniques or gears, depending on target species and/or the location of fishing beds. Several environmental impacts are associated with this fishery, ranging from changes in the physical characteristics of sediment and the water column, to associated biological communities, as well as target populations. However, few studies have been undertaken concerning the impacts caused by razor clam fishing. Based on these studies, and also on the information available for other bivalve species, the present chapter describes the main environmental impacts of the various fishing methods used for razor clam fishing, and discusses their use according to the habitat exploited. A multi-criteria analysis undertaken suggests that different techniques can be considered the most suitable, depending on the habitat exploited. Hand harvesting has the lowest environmental impact, both in intertidal and subtidal areas. Although scuba-diving is usually forbidden by law, this prohibition should be reviewed as, provided strict restrictions are imposed (e.g. maximum catch per diver / per day) and if it is controlled sufficiently, this fishing method can be environmentally friendly. Several hand tools analysed here were considered only moderately disruptive, and it seems that they do not alter the marine or estuarine ecosystems irreversibly. Within deeper subtidal areas, where diving is not possible, the use of more highly-mechanized techniques or gears should take into consideration the balance between the economic value of the fishery and local environmental sustainability. However, these more mechanized methods appear to be suitable for clean highly-hydrodynamic sandy areas, as communities from these areas are very resilient to physical disturbance and recover within a short period of time. Nevertheless, within some habitats known to be very diverse and sensitive to disturbance, high-impact fishing gear should be avoided.

1. Introduction

Several species included in this group are exploited worldwide and belong

to the Solenidae, Pharidae and Psammobidae families. Disregarding recreational harvesting for razor clams, the exploitation of this resource is particularly relevant in several European countries, along the Atlantic and Mediterranean coasts, but also along both Pacific and Atlantic coasts of North America, as well as in South America. This group of species can be observed in different habitats, ranging from more sheltered systems (e.g. lagoons and estuaries) to open coasts. Moreover, depending on the species, they can also be found in intertidal and subtidal areas at water depths up to 70 m, such is the case of *Ensis siliqua* (Poppe and Goto, 1993), and in sandy or muddy bottoms. These animals are suspension feeders and are traditionally buried vertically in sediment down to depths greater than 60 cm.

Different fishing techniques are used worldwide, depending on the target species and/or the location of fishing beds. These techniques include hand harvesting and the use of rudimentary tools, especially in intertidal areas, scuba diving, and mechanical and hydraulic dredges in subtidal areas. Several environmental impacts are associated with this fishery, ranging from changes in the physical characteristics of sediment and water column (water turbidity, nutrient and metal concentrations, seabed topography, biogenic structures such as corals, shells, etc.), to effects on associated benthic communities, as well as on target populations (e.g. Hall *et al.*, 1990; Currie and Parry, 1996; Pravoni *et al.*, 1998; Coffen-Smout and Rees, 1999; Gaspar and Monteiro, 1999; Gilkinson *et al.*, 2003; Bishop *et al.*, 2005). The nature and severity of the impact depend on the habitat, species and harvesting technique (de Groot, 1984; Churchill, 1989; Mayer *et al.*, 1991).

The objectives of this work centre on these themes, and, based on already available information, several fishing techniques for razor clams will be analysed and discussed in terms of their environmental impact within the different habitats exploited.

2. Environmental impacts of bivalve fishing

Fishing has long been considered one of the major threats to marine biodiversity and ecosystem function (Dayton *et al.*, 1995; Chiappone *et al.*, 2002). However, fishery management has traditionally dealt with the conservation of sustainable stocks of commercial species. More recently, concern about secondary environmental effects of sublittoral fishing activities on the marine environment has increased considerably (de Groot, 1984; Messieh *et al.*, 1991; Jones, 1992; Dayton *et al.*, 1995; Jennings and Kaiser, 1998).

Ecosystem changes caused by fishing are especially associated with mobile

seabed gears, designed to capture seabed- or near-seabed-dwelling species (Veale *et al.*, 2000). In order to catch target species, fishing gear is dragged over the seafloor, inflicting damage either on sediment, the water column or on associated species (e.g. Hall and Harding, 1997; Pravoni *et al.*, 1998; Hiddink, 2003; Masero *et al.*, 2005). The magnitude of the impact depends on several factors related either to fishing activity (towing speed, gear type, gear penetration into the sediment) or to local environmental conditions (season, water depth, tidal strength, currents, nature of substratum and structure of benthic communities) (de Groot, 1984; Churchill, 1989; Mayer *et al.*, 1991). The severity of fishing impact is also related to the scale and intensity of fishing activity and local environmental conditions (Gaspar *et al.*, 2009). In recent decades, the environmental effects of bivalve fishing have been studied worldwide (e.g. Caddy, 1973; McLoughlin *et al.*, 1991; Eleftheriou and Robertson, 1992; Dare *et al.*, 1993; Hall *et al.*, 1993; Jennings and Kaiser, 1998; Hall-Spencer and Moore, 2000; Gaspar *et al.*, 2009). The potential impacts resulting from bivalve fishing, particularly that made on sediment, the water column and biological communities, will be described and summarised in figure 1 (below).

2.1. Sediment and water column levels

Changes in seabed topography are an immediate effect of the passage of fishing gear through the sediment. However, for many years, fishery biologists have disregarded the importance of small seabed structures (e.g. cobble- and pebble-sized rocks, sand ripples, seagrass blades, sponges, bryozoan colonies, dead shells and polychaete tubes) and the consequences of their destruction (Norse and Watling, 1999). The existence of these structures confers complexity to seabeds that otherwise would essentially consist of unconsolidated, fine, muddy or sandy sediments. Habitat complexity is of utmost importance as seafloor structures provide refuge for both predators and prey (Tuck *et al.*, 2000), and are therefore essential for maintaining predator-prey interactions (Persson and Eklov, 1995; Rooker *et al.*, 1998). As structural complexity is vital in benthic environments, its destruction will have harmful effects not only on benthic biological diversity but also on fisheries, as several commercial species feed on benthos (Boehlert, 1996; Norse and Watling, 1999). Some animals, such as scallop species, also depend on hard substrates for juvenile development (Castagna, 1975) and in some cases leaves of seagrass species may be the only hard structure available. One example of this is a bay located in western Bogue Sound (North Carolina), where Bishop *et al.* (2005) carried out a study on the effects of harvesting on the sustainability of local scallop fishery. These authors found that while hand harvesting did not cause uprooting of seagrass or the displacement of juvenile scallops, significant damage was caused to the seagrass bed by dredging. Nevertheless, one month later, while there was no longer any detectable impact on seagrass biomass, juvenile scallops were still observed to

suffer a negative impact. Indeed, abundance of these juvenile specimens increased in control and hand harvested plots, which was attributed to a migration from the dredging plots. After emigrating from disturbed areas, juvenile scallops need to reattach, but finding undisturbed seagrass habitats over extensive fishing areas is unlikely, enhancing the risk of predation, increasing mortality (Bishop *et al.*, 2005) and ultimately compromising fisheries' sustainability.

One of the direct visible effects resulting from seabed fisheries is the creation of several trenches in the sediment, with dimensions that vary depending on the sediment type and the specifications of the gear being used (e.g. gear width, tooth length, cutting depth of the blade and/or water-jet pressure). The depth of the furrows can exceed 50 cm, and their width can vary between 1 and 5 m (Meyer *et al.*, 1981; Hall *et al.*, 1990). The water depth of the fishing habitat, together with the sediment type, which depends on the hydrodynamic conditions (i.e. strength of tidal and bottom currents, and frequency of storms) are determining factors in the recuperation of the seabed after the sediment has been disturbed. Whereas Gaspar *et al.* (2003) and Rosenberg *et al.* (2003) found that the sides of the tracks started to erode from the moment of their creation, Meyer *et al.* (1981) reported a 2-hour period until the trench wall began to collapse. Deeper and longer-lasting trenches are also reported for sandy-muddy areas when compared to sandy sediments (Gaspar *et al.*, 2003). Similarly, the recovery of the seabed structure is faster within shallow water habitats than in deeper areas, where marks can persist for a period longer than 60 days (DeAlteris *et al.*, 1999). Hydraulic dredge trenches may, however, remain visible for 3 years, as described by Gilkinson *et al.* (2003) during an experiment performed using a hydraulic clam dredge on a 70-80 m deep sandy bank off the Scottish continental shelf. The persistence of surface sediment scars is a function of both the cohesive characteristics of the sediments and the local hydrodynamic regime (Dernie *et al.*, 2003).

With the passage of the fishing gear, sediment is re-suspended and large sediment plumes are produced in the water column, the time they remain depending on the local hydrodynamics, though they usually disappear within a short period of time (Medcof and Caddy, 1971; Caddy, 1973; Butcher *et al.*, 1981; Meyer *et al.*, 1981; Mayer *et al.*, 1991; Black and Parry, 1994; Gaspar, 1996; Gaspar *et al.*, 2003; Pranovi *et al.*, 2004). Although turbidity is very common in shallow water areas due to natural disturbances, in low-energy systems, the prevalence of turbidity for long periods may result in increased mortality of invertebrates, particularly suspension-feeders (Currie and Parry, 1996). The dispersal of sediment particles after fishing will be intensified if currents are above the critical threshold for deposition (Falcão *et al.*, 2003). Loss of the fine sediment fraction from the upper sediment layers is common, since these particles, once in suspension, will travel further than other coarser particles and will

leave the area fished (Mayer *et al.*, 1991; Tuck *et al.*, 2000; Watling *et al.*, 2001). Restoration of the sediment composition may vary between a few days (Tuck *et al.*, 2000) and up to six months (Watling *et al.*, 2001). Nevertheless, a permanent change in the sediment's composition may occur when repeated fishing events are undertaken within a certain area (Langton and Robinson, 1990; Pravoni and Giovanardi, 1994; Schwinghamer *et al.*, 1996, 1998; Watling *et al.*, 2001). All of these changes will have repercussions on benthic communities, as will be described later on in this chapter.

Sediments have long been recognized as a sink for nutrients (Henriksen *et al.*, 1983; Sundby *et al.*, 1992; Forja *et al.*, 1994) and contaminants discharged into surface water bodies (Chapman and Mann, 1999). Therefore, sediment re-suspension may lead to an alteration of metal function, of bioavailability for marine organisms (Howell, 1985) and of nutrient fluxes (Falcão *et al.*, 2006). With sediment reworking while the gear is dragged along the seafloor, oxygen penetration into lower sediment layers is boosted. When in contact with oxygen, the functioning of redox-sensitive metals may be changed and substances previously "locked" into subsurface layers may be transported to the water column and afterwards bio-accumulated by some marine organisms. As for nutrients, after fishing has taken place an increase of nutrients in the water column is expected. In a study undertaken on the south coast of Portugal, Falcão *et al.* (2003) found a decrease in ammonium, nitrates, organic nitrogen, phosphates and silicates in the sediment pore-water within fishing tracks immediately after the passage of the gear, indicating a flux into the water column. Findings of Riemann and Hoffmann (1991) for organic and inorganic nitrogen support this evidence. These changes in nutrient cycling in coastal ecosystems may seriously alter phytoplankton primary production. Moreover, primary productivity may also be constrained when high turbidity levels in the water column are present, as phytoplankton growth is limited by light intensity (Barnes *et al.*, 1991).

As is clearly seen, most studies on the environmental impact of bivalve fishing have concerned dredging methods. However, artisanal collection of bivalves by hand or using traditional devices (e.g. hand raking) takes place low in the shore in several countries, both in Europe (e.g. Ireland, Italy, Portugal, Spain, UK) and America (Chile, Argentina, USA, Canada) (see Table I). Kaiser *et al.* (2001), studying the effect of hand raking for cockles (*Cerastoderma edule*) on the Dee Estuary showed that this method implied a low penetration in sediment (between 5 and 10 cm). This apparently low disturbance was probably responsible for an increase in the silt/clay fraction after disturbance. Nevertheless, 14 days later, significant differences were no longer detectable. Although, in contrast to dredging, sediment is left *in situ* (Kaiser *et al.*, 2001; Hiddink, 2003) it can be stirred up, and its cohesive nature can be disrupted, leading to finer particles being washed out during flooding. Falcão *et*

al. (2006) also showed that sediment reworking through harvesting induced changes in HPO_4^{-2} pore-water concentration during tidal inundation. These authors found that phosphate released into the water column in reworked sediments decreased up to two orders of magnitude in muddy flats, but no major differences were obtained for sandy areas. Once more, it seems that even hand-dredging using artisanal fishing gears can have a negative impact on primary production processes, as was previously indicated for subtidal areas.

2.2. Benthic communities

Fishing activities not only directly affect target species, but also benthic communities as a whole (Pravoni *et al.*, 1998), and potentially all the other species in the system with which they interact (Dayton *et al.*, 1995). Moreover, they can also affect non-benthic species, as they are food items for several fish and coastal bird species (Norse and Watling, 1999; Masero *et al.*, 2005). For example, concerning the latter, Masero *et al.* (2005) found that hand digging may have a negative impact on shorebird populations in the Bay of Cádiz, due to the reduction in the abundance of the mudsnail *Hydrobia ulvae* available for migrating and wintering birds. In fact, this gastropod lives at or just below the sediment surface, so it is more easily damaged, killed or buried in disturbed areas, decreasing its availability for predators, such as shorebirds (Masero *et al.*, 2005).

The impact of fishing depends on the size of benthic animals (meiofauna and macrofauna), their life stage and phase in the reproductive cycle, position of individuals in the sediment (infauna or epifauna), as well as the nature of the sediment (soft or hard), fishing effort, resilience, the ecosystem's recovery potential and other local environmental conditions (Gaspar and Chícharo, 2007). Therefore, it is not surprising that fishing disturbance can give rise to different responses of taxa with different life-histories, as Jennings *et al.* (1998, 1999), Frid *et al.* (1999) and Bradshaw *et al.* (2000) demonstrated. Benthic macrofauna shows strong, but narrow, affinities to certain biotic and abiotic conditions, and the disruption of the existing characteristics may lead to changes in the distribution and abundance of benthic species (Dayton, 1971; Dayton and Hessler, 1972; Thistle, 1981; Lissner *et al.*, 1991). Usually, the level of disturbance affecting benthic communities is related to the extent of the fishing impact. Hence, it was to be expected that a cumulative impact would cause greater and longer-lasting changes in the benthic environment. However, the results obtained in several studies seem to indicate that fishing impacts on benthos tend to be higher when a previously unfished area is exploited for the first time (Gaspar and Chícharo, 2007).

The short-term environmental impacts of fishing have received increasing

attention in recent years (e.g. Hall *et al.,*1990; Kaiser and Spencer, 1996; Bergman *et al.*, 1998; Tuck *et al.*, 2000; Hauton *et al.*, 2003b; Gaspar *et al.*, 2005; Constantino *et al.*, 2009a, b). Several studies have shown that fishing activities can reduce benthic biomass and diversity, with negative consequences for secondary production at large spatial scale (Kaiser, 1998; Collie *et al.*, 2000; Kaiser *et al.*, 2000; Jennings *et al.*, 2001). The major impact tends to occur on non-target species (Ardizzone *et al.*, 2000; Ramsay *et al.*, 2000), as the design of the fishing gear is directed towards commercial species. The reduction in density of non-target fauna in disturbed areas may result from mortality caused by fishing, redistribution from disturbed to undisturbed areas, or both of these (Hiddink, 2003). Although high mortality levels can be caused in non-target benthic fauna, after this short-term impact there is a high recovery potential of the benthic community to pristine conditions supported by recruitment events, and the overall effect on populations is probably low (Hall and Harding, 1997).

Another source of disturbance that must be considered is the discarding of by-catch specimens, which are dredged, exposed to air on the deck, sorted and then returned to the sea. During any of these procedures, animals may suffer physical damage that may impair their survival potential (Hauton *et al.*, 2003a; Leitão *et al.*, 2009). High rates of mortality due to desiccation are expected to occur, particularly during the summer, when air temperatures are higher. The inverse relationship between the duration of air exposure and by-catch survival rates was demonstrated by Gaspar and Monteiro (1999) for *Spisula solida* juveniles. These authors found that all individuals transplanted to seawater tanks immediately after being caught survived, while the survival rate of individuals that were exposed to air decreased dramatically with time (84%, 69%, 56% and 46% for deck exposure times of 1, 2, 3 and 4 hours, respectively). However, it is worth mentioning that the survival of discarded individuals seems to be species-specific. For example, juveniles from the species *Donax trunculus* could sustain long periods of air exposure with high survival rates (Gaspar and Monteiro, 1998). This species is characteristic of high-energy areas, such as exposed sandy beaches, and therefore has a natural resistance to high temperatures (Gaspar and Monteiro, 1998). The survival of discards is, therefore, related to their ecology and life histories (Gaspar and Monteiro, 1998) but also to their ability to rebury into the sediment and escape from the scavengers and predators which move into the fished area (e.g. Fonds, 1994; Ramsay *et al.*, 1996; Fonds *et al.*, 1998; Ramsay and Kaiser, 1998; Coffen-Smout and Rees, 1999; Jenkins and Brand, 2001). For example, Coffen-Smout and Rees (1999) observed a delay in the re-burrowing response of the cockle *Cerastoderma edule* after fishing disturbance; Ramsay and Kaiser (1998) also found a reduction in the escape performance of the whelk *Buccinum undatum*, while Jenkins and Brand (2001) demonstrated a similar response in captured undersized great scallop, *Pecten maximus*. Some species,

however, present some biological features that enhance their survival potential. Such is the case of starfish and brittlestars, whose regeneration capability enables them to autotomise their limbs while escaping from predators, which seems to be the reason for their high survival rate (Bergman *et al.*, 1990; Kaiser and Spencer, 1995).

On the other hand, after long-term impacts, both biotic and abiotic characteristics of the ecosystem can be permanently affected. One of the main limitations in studying long-term changes in benthic ecosystems is the interaction between fishing and natural disturbance (Currie and Parry, 1996; Kaiser *et al.*, 1998). Therefore, assessment of such changes is more difficult and is usually based on comparisons between fished and unfished areas. Natural physical differences between both treatments add another source of misinterpretation for evaluating long-term environmental impacts caused by fishing activities (Gaspar and Chícharo, 2007). One alternative is the analysis of time-series data from before and after the beginning of large-scale commercial fishing (e.g. Reise and Schubert, 1987; Greenstreet and Hall, 1996; De Vooys and Van der Meer, 1998; Hill *et al.*, 1999), although the interpretation of the results may be biased by factors such as climate changes (Southward *et al.*, 1995). Benthic communities typically present year-to-year fluctuations due to changes in local conditions such as temperature (Beaumont and Budd, 1982; Southward, 1995; Kroncke *et al.*, 1998; Lindley, 1998), currents (Pingree and Griffiths, 1978; Hill *et al.*, 1995, 1997), frequency and intensity of storms (Hall, 1994; Kaiser *et al.*, 1998) and phytoplankton abundance (Frid *et al.*, 1996). These changes can affect species' reproduction cycles, recruitment or survival, it being important to take natural variability into account when assessing fishing impacts. According to studies already undertaken, changes in sediment characteristics may result in an overall decrease in benthic diversity, abundance and biomass (e.g. Norse and Watling, 1999; Veale *et al.*, 2000; Schratzberger and Jennings, 2002) and changes in dominant trophic groups (Veale *et al.*, 2000), that is, through the selective removal of sensitive taxa (Eleftheriou and Robertson, 1992), and the increase of scavengers (Britton and Morton, 1994; Kaiser and Spencer, 1994, 1996; Evans *et al.*, 1996; Collie *et al.*, 1997). Changes in community structure were reported by Lindeboom and de Groot (1998) for Loch Gareloch on the Irish Sea, where trawling significantly reduced populations of some infauna (e.g. the nut clam *Nucula nitidosa*), whereas opportunistic cirratulid and capitellid polychaetes became more abundant. Fishing impacts tend to select the survival of small-sized animals, as shown by Chícharo *et al.* (2002), who reported that several species such as sea-urchins, sea stars, gastropods and larger bivalves (e.g. *Acanthocardia tuberculata*) become rare or absent under continuous fishing pressure. Fishing can also give rise to the destruction of algal mats, with consequences for the abundance of herbivores and also for other species, such as fish, that use these areas for spawning (Chícharo *et al.*, 2002).

As far as recovery processes are concerned, the overall recovery time of macrobenthic communities depends on the intensity of disturbance, hydrodynamic conditions, sediment particle size and also on the structure of the communities affected (Kaiser and Spencer, 1996; Spencer *et al.*, 1998). It is generally accepted that recovery times of infaunal communities are lower in dynamic sandy habitats than in muddy areas (Schratzberger and Warwick, 1999; Collie *et al.*, 2000; Ferns *et al.*, 2000; Gaspar *et al.*, 2009). In fact, communities undergoing regular physical disturbance events (e.g. exposure to wave action) tend to recover quickly from disturbances, as these communities are usually dominated by mobile species that are adapted to a highly dynamic environment (Gorzelany and Nelson, 1987). On the other hand, communities inhabiting muddy sediments are not exposed to regular physical perturbations and are therefore more specially sensitive to fishing (Moore, 1991). According to several authors (e.g. Commito, 1982; Bemvenuti, 1994; Mortimer *et al.*, 1999; Christensen *et al.*, 2000; Chícharo *et al.*, 2002) long-term fishing impacts on benthic ecosystems may be reflected in a change from resilient benthic communities dominated by long-living *k*-selected species to impacted communities where *k*-selected species are replaced by short-lived *r*-selected species, such as some opportunistic polychaetes. However, in other studies, recolonisation was mainly performed by species with high abundance in the surrounding area, rather than specifically short-lived opportunistic species (Boesch and Rosenberg, 1981; Jones, 1992). Pravoni *et al.* (1998) in the Northern Adriatic Sea, found an intermediate situation, in which, during the recolonisation process of disturbed areas, characteristic species were not the only ones known to be “opportunistic”, such as *Corbula gibba* (Pearson and Rosenberg, 1978), but also others lacking such characteristics, such as *Tapes philippinarum* and *Paphia aurea*. Some of these differences may be related to the size of the disturbed areas, which is known to influence the colonisation mechanisms in soft-bottom habitats (Smith and Brumsickle, 1989; Levin *et al.*, 1996; Thrush *et al.*, 1996). Whitlatch *et al.* (1998) have developed a patch-based, soft-sediment recolonisation model, which has indicated that smaller patches with higher edge/surface ratios should be more influenced by the post-settlement colonist pool than larger patches with smaller edge/surface ratios. Therefore, when a larger proportion of a fishing area is affected it is quite conceivable that the contribution of the associated benthic species to the recolonisation process would be reduced, and recovery would take longer (Hall, 1994; Thrush *et al.*, 1995). The ecological features of some species, as well as their associations with sediment, may also be a determining factor in community recovery. Kaiser *et al.* (1996) found that within a harvested area in South East England, tube-dwelling polychaetes, such as *Lanice conchilega* and *Euclymene lumbricoides*, were less negatively affected than more mobile species such as *Macoma balthica* and *Scoloplos armiger*. While the polychaetes escaped from the fishing gear by burrowing down into the deeper mud/

clay fraction, the other species living within the coarser overlying sediment were more exposed to fishing. Regarding the association of these species with the coarser sediment fraction, it is expected that their colonisation process will be dependent on the re-establishment of this sediment fraction (Spencer *et al.*, 1998). In brief, the rates of recovery for benthic communities following fishing disturbance may range from weeks to years, with recovery times depending on rates of immigration, recruitment and growth (Schratzberger and Jennings, 2002).

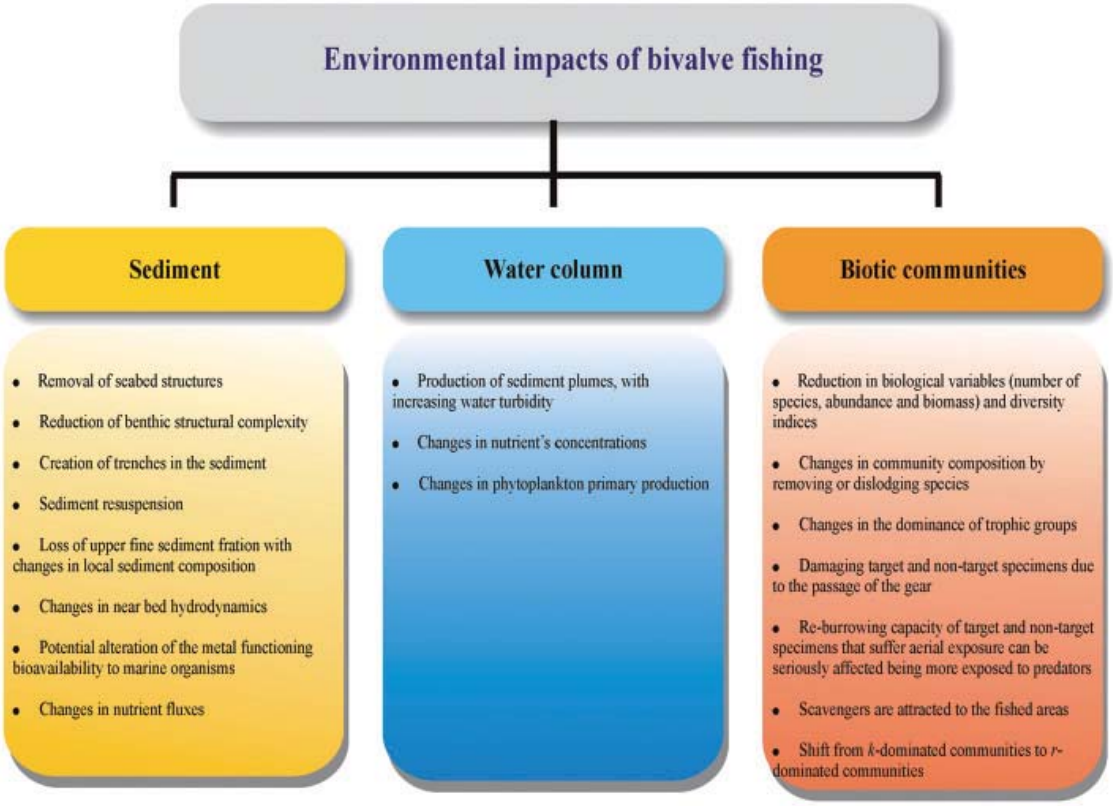

Figure 1. Main potential environmental effects of bivalve fishing in sediment, water column and biotic community levels.

3. Harvesting techniques for razor clams

According to the habitat (intertidal and subtidal areas) and razor clam species exploited, several mechanical and hand harvesting techniques are employed. Fishing gears and techniques used worldwide are summarised in Table I and described below.

Table I. Fishing gears and techniques used to harvest razor clam worldwide, according to habitat (PT - Portuguese; ES - Spanish).

	Fishing gear or technique	Target species	Location	Reference
Intertidal areas	Salting PT - Apanha com sal ES - Extración con sal	*Ensis* spp. *Solen marginatus* *Solen marginatus* *Ensis* spp.	Ireland Portugal Spain UK	Fahy and Gaffney (2001) Gaspar and Constantino (2006) Martínez-Patiño (2002) Pyke (2002)
	Quick lime PT- Apanha com cal viva ES- Extración con cal viva	*Solen corneus* *Solen strictus* *Solen regularis*	Thailand	Veeravaitaya (2007)
	Metallic rod PT - Adriça ES - Fisga	*Solen marginatus* *Solen marginatus*	Portugal Spain	Gaspar and Constantino (2006) Sebe and Guerra (1997) Martínez-Patiño (2002)
	Shovel and grubber hoe PT - Pá e sacho ES - Pala y azada	*Solen marginatus* *Ensis arcuatus* *Siliqua patula* *Siliqua patula, Solen rosaceus* and *Tagelus californianus* *Ensis directus* *Ensis* spp.	Portugal Spain Pacific Northwest, USA NW Pacific (USA, Canada) NE Atlantic (USA, Canada) Ireland	Gaspar (Pers. obser.) Sebe and Guerra (1997) Lassuy and Simons (1989) MacDonald (Pers. com.) MacDonald (Pers. com.) Fahy (Pers. com.)
	Razor clam rake PT - Forquilha ES - Horquilla y rastrilho	*Ensis arcuatus* *Ensis* spp.	Spain Ireland	Sebe and Guerra (1997) Fahy (Pers. com.)
	Clam gun PT - Bomba ES - Tubo	*Siliqua patula, Solen rosaceus* and *Tagelus californianus* *Ensis directus*	NW Pacific (USA, Canada) NE Atlantic (USA Canada)	MacDonald (Pers. com.) MacDonald (Pers. com.)
	Picking PT - Apanha manual ES - Extracción a mano	*Ensis macha* *Ensis arcuatus and Ensis siliqua* *Ensis* spp.	Argentina Spain UK	Barón *et al.* (2004) Sebe and Guerra (1997); Couñago (2001) Pyke (2002)
Subtidal areas	Salting PT - Apanha com sal ES - Extración con sal	*Solen marginatus* *Ensis* spp.	Portugal UK	Gaspar and Constantino (2006) Pyke (2002)
	Double hook PT - Duplo gancho ES - Gancho	*Solen gaudichaudi* and *Ensis macha*	Chile	Olguín and Jerez (2003)
	Long pincher tongs PT - Tenaz ES - Tenazas	*Ensis macha* *Siliqua patula, Solen rosaceus* and *Tagelus californianus* *Ensis directus*	Argentina NW Pacific (USA, Canada) NE Atlantic (USA, Canada)	Barón *et al.* (2004) MacDonald (Pers. com.) MacDonald (Pers. Com.)
	Metallic rod PT - Adriça ES - Fisga	*Ensis macha* *Siliqua patula, Solen rosaceus* and *Tagelus californianus* *Ensis directus* *Solen marginatus*	Argentina NW Pacific (USA, Canada) NE Atlantic (USA, Canada) Portugal	Barón *et al.* (2004) MacDonald (Pers. com.) MacDonald (Pers. com.) Gaspar (Pers. obser.)
	Clam "kicking"	*Ensis macha*	Perú	Mendo (Pers. com.)
	Dredge PT - Ganchorra ES - Rastro remolcado	*Ensis siliqua*	Portugal	Gaspar *et al.* (1998)
	Hydraulic dredge PT - Ganchorra hidraúlica ES - Draga hidráulica	*Ensis siliqua* and *Ensis arcuatus* *Ensis minor* *Solen marginatus* *Ensis arcuatus* *Ensis siliqua* and *Ensis arcuatus* *Ensis directus*	Ireland Italy Italy Scotland Scotland Atlantic Northeast, Canada	Fahy and Gaffney (2001) Del Piero and Dacaprile (1998) Del Piero (Pers. com.) Robinson and Richardson (1998) Hall *et al.* (1990); Tuck *et al.* (2000); Hauton *et al.* (2003a,b) Kenchington *et al.* (1998)

3.1. Intertidal areas

Razor clams live burrowed in the sediment, forming galleries up to 60 cm deep. These galleries are open at the surface of sediment, forming a keyhole named an “eye” (“show” or “dimple”). However, these “eyes” are not always visible, because they are covered with a fine pellicle of sand. In this case, an “eye” can be easily revealed if the harvester walks backwards. Indeed, the pressure of a person’s foot can result in a water-spout from a hole, revealing the animal’s location. When an “eye” is found, the razor clam is harvested by hand or by using rudimentary hand tools, as described below.

3.1.1. Salting and lime and stick

Salt is spread on the beach and in the area of the burrow entrances (Fig. 2). Animals then leave the burrows and are easily picked up from the sand. A similar technique is used in Thailand; however instead of using salt they use quick-lime.

Figure 2. Harvesting of razor clams with salt in intertidal areas (Photo credit: Susana Darriba Couñago).

3.1.2. Metallic rod

This gear consists of a long wire that ends in a cone (Fig. 3). In Ireland, this hand tool is slightly different, as instead of having a cone, the wire has a curved end forming a kind of hook (Fahy, personal communication). The technique consists of introducing the metallic rod gently in the “eye” until the cone penetrates the razor clam. The gear is then pulled back to the surface.

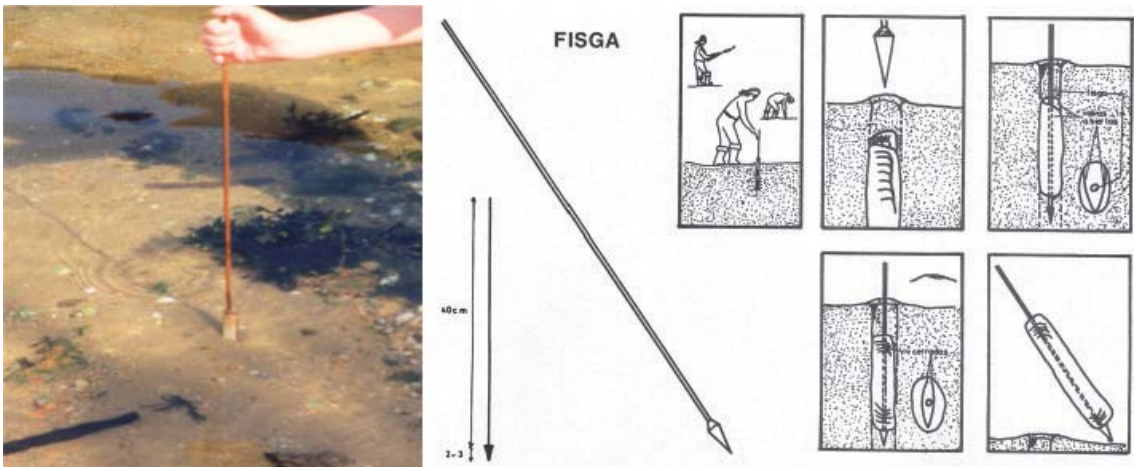

Figure 3. Harvesting razor clam with a metallic rod (left). Diagram explaining the operation of this hand tool (Arnaiz and de Coo, 1977).

3.1.3. Shovel and grubber hoe

Razor clams can be harvested using a shovel or a grubber hoe (Fig. 4.) When the “eye” is found, the shovel blade is placed approximately seaward of the razor clam “show”. The blade should be kept as vertical as possible to avoid breaking the specimen’s shell. Then, a scoop or two of sand is dug away beside the eye and the clam is found by reaching into the sand at the side of the hole.

Figure 4. Harvesters digging razor clams with a shovel (Photo left credit: Quinault Indian Nation) and with a grubber hoe (Photo right credit: Alejandro Guerra Díaz).

3.1.4. Razor clam rake

The razor clam rake (Fig. 5) has about 22 long, sharp and slightly curved tines, and two wooden handles (up to 1.8 m long) forming an angle of around 75-80º. The rake head is forged to these handles. One of the poles is used by the fisherman to drive the rake in, while the other is used to “wash” the catch. This hand tool is used during

low tide in very shallow areas (up to 1.2 m deep) usually characterised by relatively strong currents. In order to find the “eye”, fishermen use a glass-bottomed wooden box. When the “eye” is located, the rake is placed nearby, and then the fisherman pushes the tool with his foot, driving it into the sediment. Then, the sediment is turned over by tilting the rake head towards the sediment surface, using both poles.

Figure 5. Razor clam rake (left) and fisherman operating it (right) (Photo credit: Alejandro Guerra Díaz).

Another type of hand rake can be used in razor clam fishery (Fig. 6). This tool, although very similar to the rake described above, is smaller and has 11 straight 25 cm-long tines. The rake head is forged together with a wooden handle. This gear is used in intertidal areas and is operated in a similar way to the bigger rake.

Figure 6. Harvester digging razor clams with a rake (Photo credit: Alejandro Guerra Díaz).

3.1.5. Clam gun or tube

The clam gun consists of a 10 cm-diameter pipe or tube with a handle and a small air vent at the closed upper end (Fig. 7). The technique consists of placing the tube over the “eye” and then pushing the tube down - with an up-and-down, rocking or twisting motion - until the razor clam is enclosed. The air vent is then blocked with a finger and the core of sand pulled up and dropped on the beach.

Figure 7. Harvesting razor clams with a clam gun (Photo credit: Miguel B. Gaspar).

3.2. Subtidal areas

The exploitation of subtidal razor clam beds can be carried out by divers with or without hand tools or by using dredges, either mechanical or hydraulic.

3.2.1. Diving

Diving to harvest razor clams can be carried out in apnea, using scuba or “hookah” equipment in areas from as shallow as 1 m to as deep as 35-40 m. Divers can remain underwater for stretches of up to six hours at a time. “Hookah” fishing involves diving from a boat using a compressor that supplies air for divers through hoses. In some countries, divers rely on their bare hands, rudimentary fishing gears such as hooks, long pincher tongs and metallic rods as well as net bags attached to a tyre rim to secure their catch.

3.2.1.1. Picking

Razor clams can be picked out of the sediment by divers using only their hands (Fig. 8). Having found the “eye” in the seabed or seeing the siphons projecting above the surface, the thumb and forefinger are quickly pushed into the silt/sand and the animals are grasped and pulled from the seabed with a gently twisting action (around 180º) in order to ensure the foot is not left behind.

Figure 8. Divers harvesting razor clams by hand (Photo credit: José Luis Catoira).

3.2.1.2. Salting

Divers spread salt from a bottle or watering can, onto a previously identified bed of razor clams (Fig. 9). The brine solution, being denser than seawater, settles into the burrows. The increase of the salinity inside the gallery forces the razor clam to rise to the sediment surface, where it is picked up by the diver.

Figure 9. Harvesting of razor clams with salt in subtidal areas (Photo credit: Miguel B. Gaspar).

3.2.2. Using fishing gears

Harvesting by scuba divers can also be accomplished using manually operated fishing gears such as long pincher tongs that are quickly deployed, taking the animal out of the sand, or by using a thin metal rod that is strategically placed inside the feeding clam's siphon. The clam reacts by contracting its siphon, which anchors the metal rod inside the shell, and the diver is easily able to pull the clam out of the sand. The "gancho", which is a metallic fishing gear comprising two long hooks welded to a small metal handle (Fig. 10), may also be used by scuba divers. This gear is used to remove deeply burrowed razor clams from the sediment.

Figure 10. Double hook used in Chile for harvesting razor clams (Photo credit: Irene Lépez G., Daniel Arriagada O. and Flor Véjar D.).

3.2.3. Clam "kicking" (water jet)

This technique consists of using a long hose through which high-pressure water is pumped from the ship (Fig. 11) into the seabed to fluidise it. Razor clams that rise above the seabed are then collected by divers.

Figure 11. Artisanal fishing boat used in razor clam fishery in Peru, showing the deck equipment used to pump water at high pressure through a hose (Photo credit: Jaime Mendo and Roberto Espinoza).

3.2.4. Dredging

3.2.4.1. Razor clam dredges

The razor clam dredge (Fig. 12) is composed of a metallic frame, a toothed lower bar and a mesh bag to retain the catch. The length of the teeth used in this type of dredge takes into account the maximum burrowing depth of the species, so tooth length may reach 60 cm. Dredges are towed with a cable, normally at 3:1 warp depth ratio; the cable is attached to a winch located at the stern of the fishing boat. In order to prevent a high number of damaged individuals, the duration of each tow is very short, usually not exceeding 1 minute (Gaspar *et al.*, 1998). The tow is performed at a speed of 1-3 knots.

Figure 12. Portuguese razor clam dredge (Photo credit: Miguel B. Gaspar).

3.2.4.2. Hydraulic dredges

A hydraulic dredge consists of a rectangular cage made of steel bars to retain the catch, with a cutting blade and a system for delivering pressurised water through jets (Fig. 13). The movement of the dredge is assisted by two skids located along both sides of the collecting cage. As it is towed over the seabed, the sediment is dug by a cutting blade located in front of the dredge mouth that protrudes almost 0.3 m into the sediment. High-pressure water is pumped from the ship through a hose and delivered as a series of pressure jets at the mouth of the dredge and inside the dredge cage, and to the dredge blade. The water injected from the jets located in front of the cage and through the blade fluidises the sand, allowing the blade to cut into the sediment easily, extracting razor clams from it. Hydraulic dredges may be hauled from the bow or the stern of the vessel. In the former case the gear is towed astern either by warping on a big anchor using a winch or by moving backwards by means of the propeller. Each fishing operation lasts between 10 and 20 minutes, depending on the density of the target species, the type of sediment and the amount of debris in the area. Towing speed may reach 3 knots but is usually lower, as the dredges accumulate clams.

Figure 13. Hydraulic dredge for razor clam.

Hydraulic suction dredges may also be used in razor clam fishery. In this case, the catch, instead of being retained in a metallic cage, is continuously brought up through a suction pipe onto the deck of the boat, where bivalves are screened out and the spoil discharged back to sea.

4. Environmental impacts of razor clam fishery

The analysis of literature addressing bivalve fishing impacts shows that there is only a limited number of studies concerning razor clams. The main results of these studies are summarised in tables II to IV and discussed below. It is worth mentioning that results were separated into 3 parts: (1) physical disturbance (sediment and water column); (2) benthic communities; and (3) target species.

4.1. Sediment and water column

As far as harvesting methods used in intertidal areas are concerned, little information is available on the impacts in sediment and water column. Regarding the use of hand-harvesting tools, the occurrence of slight morphological changes, such as the formation of small monticules and depressions, were described by Sebe and Guerra (1997). However, according to these authors, changes disappear within a few hours. The hand-harvesting tools, particularly the grubber hoe and rakes, boost sediment aeration and oxygenation, which may lead to the release of nutrients and

contaminants for the water column, as previously explained. To our knowledge, the first attempt to assess the impacts of salting in the intertidal area was performed recently in the Ria Formosa lagoon (Southern Portugal) (Constantino *et al.*, 2006, 2009b). The main consequence of this method is the immediate increase of salinity in sediment pore water. Nevertheless, with flooding, the amount of salt added during harvesting is diluted and it is expected that salinity will return to pre-fishing levels within the first hours of flooding.

With regards to subtidal areas, most studies concern dredges. As indicated previously, diving is also used in razor clam fisheries, using either salt or rudimentary tools. Once more, salting an area in order to gather specimens emerging from sediment does not seem to have a major impact on the environment, as this technique only implies a short-term and local increase in salinity values. However, the use of harvesting gears such as the "gancho" that involves stirring up sediment might lead to sediment re-suspension and also to the release of nutrients and, eventually, contaminants. Nevertheless, when compared to dredges, these techniques seem to have a low impact in sediment and the seawater column. As described for most bivalve fisheries, the main impact resulting from razor clam dredging is the creation of several trenches and holes within the fishing areas (Hall *et al.*, 1990). These trenches can reach up to 2 m wide and usually decrease in size during the days following fishing (Tuck *et al.*, 2000), but signs of physical disturbance can remain for 40 days (Hall *et al.*, 1990) or even 11 weeks (Tuck *et al.*, 2000), depending, for example, on site exposure. Hall *et al.* (1990), studying the impact of hydraulic suction dredging for *Ensis* sp. in Scotland, observed that samples from dredged areas showed higher median particle size. The authors suggested that this could result from the removal of the fine sediment fraction, as these particles are easily re-suspended and can be transported and deposited elsewhere. However, as samplings were taken within the trenches, a possible increase of this parameter with sediment depth was also suggested. The first hypothesis is very plausible, as the reduction of the silt percentage in fished areas immediately after dredging was also observed by Tuck *et al.* (2000), even though after 5 days differences were no longer significant. A similar result was obtained by Hauton *et al.* (2003b) for a maerl area in the Clyde Sea (Scotland). In this study, although only a small proportion of maerl was dredged up, the number and total dry weight of dead maerl were significantly lower after dredging. These authors indicated that dredging also encouraged the suspension of sediment, reducing the local visibility from 5 m before dredging to only a few centimetres afterwards. Most studies also noted sediment fluidisation in disturbed areas as a major impact of dredging (Hall *et al.*, 1990; Tuck *et al.*, 2000; Hauton *et al.*, 2003a). This effect can remain for more than 11 weeks after fishing (Tuck *et al.*, 2000), suggesting that dredging may break natural cohesive and biological bonds in the sediment (Black and Parry, 1994).

4.2. Benthic communities

The analysis of the information available concerning the effects of razor clam fisheries on benthic communities showed that mechanised fishing methods generally cause more harmful effects than non-mechanised ones. To our knowledge, only two studies addressed the impact of non-mechanised gears on benthic communities, both undertaken in the Iberian Peninsula (Sebe and Guerra, 1997; Constantino *et al.*, 2006, 2009b). Concerning the latter methods, impacts at this ecosystem level are low and can almost be disregarded. Concerning the salt fishery method, no effects were registered in benthic communities, since the fluctuation patterns were observed in both control and experimental areas. Thus, the differences observed seem to result from natural variability of benthic populations within study areas. With regard to hand tools, the razor clam rake seems to be the technique affecting a higher percentage of non-target species, as this technique involves stirring up large quantities of sediment (Sebe and Guerra, 1997). Nevertheless, no damage was observed in the dislodged individuals. It is worth to note that the metallic rod and diving were the most selective harvesting methods (Sebe and Guerra, 1997).

In relation to mechanised techniques, the main impacts on benthic communities concern the reduction in the mean number of species, abundance, biomass and diversity indices after fishing, although sometimes the differences are not significant (Hall *et al.*, 1990; Tuck *et al.*, 2000). For example, Hall *et al.* (1990) found that, one day after dredging, abundance was significantly lower in fished plots than in control plots, whereas Tuck *et al.* (2000) did not observe any effect on diversity measures. During the latter work, after 5 days, differences in number of species, abundance and biomass were no longer detectable. Changes in benthic communities' composition can also be observed within a fished area. However, according to the studies already undertaken, recovery processes occur within a few days, as demonstrated by Tuck *et al.* (2000) in Scotland. In that study, two major taxonomic groups presented opposite behaviours, with polychaetes being affected by dredging, while amphipods increased their abundance. Another group that can be damaged by razor clam fishing are sponges. The plume of sediment that is produced after the passage of the gear through the sediment will be deposited in the nearby areas and may cover these animals, affecting their filter-feeding behaviour (Hauton *et al.*, 2003b). Some mobile animals (e.g. gobbies -*Pomatochistus minutes*- or crustaceans -*Galathea intermedia*) can disappear from the dredged area immediately after dredging, probably due to their ability to escape (Hauton *et al.*, 2003b). Hall *et al.* (1990) found that abundance of the top ten species decreased in fished plots immediately after dredging, but no significant differences were detected between treatments either 1 or 40 days later. On the other hand, the attraction of scavengers (e.g. crabs, whelks, and fish) to the fished area, feeding on the exposed and/or damaged specimens, is

one of the major effects observed on the local epifauna, attested expressly by Tuck *et al.* (2000) and Hauton *et al.* (2003b). The effect of fishing gears is also observed either by the damage caused to individuals on the sediment during the passage of the gear or by removing the animals, transporting them to the deck. Gaspar *et al.* (1998) in an experimental study using a razor clam dredge, observed that 10-15% of the total abundance of the catch was comprised by non-target bivalves, such as *Acanthocardia tuberculata*, *Ensis ensis*, *Mactra corallina*, *Mactra glauca*, *Pharus legumen*, *Tellina crassa* and *Venus striatula*. The removal of the longer-living razor clams from Gormanstown (Ireland) resulted in their replacement by a number of other opportunistic bivalve species (suspension feeders) with shorter life cycles, such as *Pharus legumen* and *Lutraria lutraria* (Anonymous, 2006). Other groups, such as polychaetes, brittlestars, crustaceans, other echinoderms and anemones are also frequently damaged by dredging (Gaspar *et al.*, 1998; Hauton *et al.*, 2003a and b). The by-catch composition depends on the affected benthic communities. In the Clyde Sea area, Hauton *et al.* (2003a) observed that the heart urchin *Echinocardium cordatum* comprised approximately 60% of the total discard biomass, being also one of the main species dislodged by hydraulic dredge. Despite being a fragile species, on each occasion, 30-40% of discarded animals were damaged, while for those dislodged, the percentage of damage was 20-30% (Hauton *et al.*, 2003a). The impact on the *E. cordatum* population can, however, be more severe, as specimens suffering air exposure were unable to rebury successfully within three hours after being returned to the sea (Hauton *et al.*, 2003a).

Recovery to pre-fishing levels is based on the migration and passive translocation of organisms from nearby areas. Recovery time will be longer with the increase of the disturbance area and decrease of hydrodynamic conditions. By contrast, negative impacts will be attenuated in areas strongly influenced by wind and tide-induced currents (Hall *et al.*, 1990).

4.3. Target species

Salting, both in intertidal and subtidal areas, does not seem to have negative consequences for target populations, as individuals are picked up by hand and animals' integrity is not known to be damaged. Overexploitation of resources is the major potential impact that can result from this method. Nevertheless, some laboratory experiments seem to indicate that this technique can affect the species' physiological condition (ingestion rate, excretion rate) and re-burrowing capacity (Diniz, 2004). The remaining techniques appear to be potentially more destructive, though. Both rudimentary tools and dredges can damage individuals by cutting their siphons, ripping their pedal muscles and/or breaking their shells (Gaspar *et al.*, 1994;

Robinson and Richardson, 1998; Hauton *et al.*, 2003a).

Several authors have attested breaks in shell margins, promoting the incorporation of sand grains in the matrix shell, which will affect its growth (Gaspar *et al.*, 1994; Robinson and Richardson, 1998). This result was associated with repeated disturbance events and the frequency of damage was higher in larger and older specimens (Gaspar *et al.*, 1994). During an experimental study in southern Portugal, Gaspar *et al.* (1998) found that the increase in tow duration was found with have higher negative impacts on the total number of damaged specimens. On the other hand, these authors observed a decrease (2 to 3 times) in the number of damaged specimens with the increase of tooth length.

Density and population structure of *Ensis* spp. can also be affected by dredging operations. A decrease in the number of individuals and in the length of the specimens exploited by suction dredging, caused by the selective removal of larger animals, was described by Robinson and Richardson (1998). Hauton *et al.* (2002) also reported a 90% decrease in razor clam biomass from a 0.4 m wide dredge path. After razor clam dredging, a large number of predatory crabs were attracted to the disturbed area (Robinson and Richardson, 1998), which can have a negative impact on dislodged or damaged individuals. In fact, Hauton *et al.* (2003a) found that undersized individuals contributed considerably to the biomass of the dislodged megafauna. *E. siliqua* specimens seem to be more resistant than the ones from the *E. arcuatus* species. Despite 35 g m^{-2} of *E. siliqua* being dislodged by the dredge, 52% of the dislodged specimens were intact, whereas for *E. arcuatus*, dredging dislodged 18 g m^{-2}, of which 60% were severely damaged. In that experimental study, dislodged individuals were found to have reburied successfully. However, undersized razor clams (*E. siliqua* and *E. arcuatus*) were also included in the six most discarded species, and in these circumstances Robinson and Richardson (1998) observed that the re-burrowing capacity of *E. arcuatus* returned to the sea decreased, making specimens more vulnerable to predation.

Table II. Impacts on sediment and water column.

Target species	Fishing method	Effects	Location and habitat	Reference
Ensis sp.	Hydraulic suction dredging	After dredging several trenches and holes were observed in approximately 20 to 30% of the fished area. Samples from dredged areas presented significantly higher median particle diameter, which was suggested to be related either to the removal of the sediment fine fraction or to the increase of this parameter with depth, as samples from fished areas were taken in trenches. Sediment in holes and trenches were almost fluidized. Signs of fishing disturbance disappeared 40 days after dredging.	Loch Gairloch, Scotland; Fine sandy bottoms; 7 m depth	Hall *et al.* (1990)
E. ensis, *E. siliqua*, *S. marginatus*	Hand-tools	Slight morphological changes may be observed, such as the formation of small monticules and depressions that disappear in few hours. The grubber hoe and rakes, but particularly razor clam rake promotes sediment aeration and oxygenation.	Galicia (NW Spain); Intertidal and shallow subtidal areas	Sebe and Guerra (1997)
E. siliqua	Hydraulic dredge	The effect of the dredge passage was noticed by the resuspension of sediment with consequent reduction in visibility and the creation of a trench (approx. 35 cm width). The walls of the trench quickly collapsed partially filling the trench. Sediments become more fluidised.	Gormanstown, Co. Meath, Ireland	Anonymous (nd)
E. ensis	Hydraulic dredge	Sediment was fluidised by the dredge within the track. The sides of the trench left by the dredge quickly fell in and within 10 minutes, the track quickly firmed up and returned to the consistency observed before fishing.	Clifden Bay, Ireland; clean fine/medium sand with low silt percentage	Anonymous (nd)
Ensis spp.	Water jet dredging	The impact of water jet dredging was observed by the trenches created along the fished track. In some places, trenches reached up to 2 m wide that were slightly reduced after five days and no longer detectable after 11 weeks. Sediment within fished tracks also become more fluidised and 11 weeks later, sediments within fished areas were still more fluidised than within control areas. Water jet dredging resulted in a reduction of silt percentage in fished areas immediately after dredging but differences were no longer significant five days after dredging.	Western Isles, Scotland	Tuck *et al.* (2000)
E. siliqua and *E. arcuatus*	Hydraulic blade dredge	Dredge tracks caused by the fishing gear presented more fluidised sand.	Clyde area, Scotland	Hauton *et al.* (2003a)
Ensis spp.	Hydraulic blade dredge	Number and total dry weight of dead maerl were significantly lower after dredging. However, only a small proportion was dredged up, as most of it was dragged and then buried. Dredging tend to increase the percentage of sand fraction in surface sediments. Dredging also promoted the suspension of sediment and local visibility was reduced from 5 m before dredging to only a few centimetres after. The dredge track had an average depth of 10.3 cm and 103.6 cm width and one month after dredging, depth had been reduced to 6.5 cm and width to 99.0 cm.	Stravanan Bay, in the Clyde Sea; Maerl area; 10 m depth; megarippled with coarse maerl-gravel	Hauton *et al.* (2003b)
Solen marginatus	Salting	The salinity in sediment pore water increased substantially to 68 ppm immediately after covering the area with salt. One hour and two hours after, values starts to decrease with flood tide, but were still higher than 37 ppm (55 and 50 ppm, respectively). Values stabilized 24 hours after salting.	Ria Formosa lagoon, southern Portugal; intertidal area	Constantino *et al.* (2006, 2009b)

Table III. Impacts in benthic communities.

Target species	Fishing gear or technique	Effects	Location and habitat	Reference
Ensis sp.	Hydraulic dredging	There were differences in mean number of species, number of individuals and diversity indices for control and fished areas 1 and 40 days after dredging but only total abundance was significantly lower. Differences were no longer detectable 40 days after. Although abundance of the 10 most abundance species was lower in fished plots immediately after dredging. No significant differences between treatments after either 1 or 40 days were detected.	Loch Gairloch, Scotland; Fine sandy bottom; 7m depth	Hall *et al.* (1990).
E. ensis, *E. siliqua*, *Solen marginatus*	Hand tools	The razor clam rake is the technique that affects a higher percentage of non-target species (59.9% of total catch), due to the greater removal of sediment, followed by the small hand rake (45%) and the grubber hoe (30.6%). *Scrobicularia plana* was the most affected species by the grubber hoe, while *Dosinia exoleta* was the most affected using the razor clam rake. Nevertheless, no damages were observed in non-target species. The metallic rod was the most selective, as non-associated fauna was collected.	Intertidal and shallow subtidal area	Sebe and Guerra (1997)
E. ensis, *E. siliqua*, *S. marginatus*	Diving	No associated fauna was collected.	Subtidal	Sebe and Guerra (1997)
E. siliqua	Dredge	Several non-target species, such as *Acanthocardia tuberculata*, *Ensis ensis*, *Mactra coralina*, *Mactra glauca*, *Pharus legumen*, *Tellina crassa*, *Venus striatula*, some crabs, hermit crabs, brittle stars and polychaetes, are caught during fishing. Non-target bivalves roughly account for 10-15% of total abundance.	Lagos, southern Portugal; 10 m depth	Gaspar *et al.* (1998)
E. siliqua	Hydraulic dredge	Several crab specimens (*Corystes cassivelaunus*, *Liocarcinus depurator* and *Carcinus maenas*) and fish were attracted to the dredge track scavenging on the fauna uncovered by the passage of the dredge.	Gormanstown, Co.Meath, Ireland	Anonymous (nd)
Ensis spp.	Water jet dredge	Reduction of number of species and abundance in the fished track one day after fishing, but no differences were observed after five days. Biomass generally decreased in fished tracks until 5 days after dredging. Diversity measures showed no effects. While polychaetes decreased after fishing, amphipods increased in fished areas. The major effects on the scarce epifauna was the attraction of crab species (especially *Carcinus maenas*) known to have a scavenger behaviour, to the fished area.	Western Isles, Scotland	Tuck *et al.* (2000)
Ensis spp.	Hydraulic blade dredge	The heart urchin *Echinocardium cordatum* comprised approximately 60% of the total discard's biomass. Despite being a fragile species, in each occasion, between 60-70% of the specimens were intact. Polychaetes, brittlestars *Amphiura brachiata* and anemone *Cerianthus loydi* were severely affected by dredging. *Echinocardium cordatum* also dominated the dislodged megafauna by the hydraulic dredge and 20-30% suffered severe damage by the dredge. *E. cordatum* specimens that suffered aerial exposure were unable to rebury successfully within three hours after being returned to the sea.	Clyde Sea, Scotland	Hauton *et al.* (2003a)

Target species	Fishing gear or technique	Effects	Location and habitat	Reference
Ensis spp.	Hydraulic blade dredge	After dredging, filter-feeding sponges were covered by silt due to the deposition of the sediment plume resulting from the passage of the dredge. Some gobies (*Pomatoschistus minutus*) and crustaceans (*Galathea intermedia*) were absent from the dredged area and may probably have dispersed as the gear approached. Individuals from several species were damaged by the dredge (e.g. *Cerianthus loydi*, *Cancer pagurus*, *Echinocardium pennatifidum*, *Lutraria angustior*, *Mya truncata*). Two hours after fishing, crabs, whelks and fish were observed within the track feeding on the exposed damaged or dead animals. Some species also fed on undamaged individuals, such as found for an *E. arcuatus* specimen while attempting to re-burrow.	Clyde Sea, Scotland; maerl bed; 10 m depth	Hauton *et al.* (2003b)
Ensis spp.	Hydraulic dredge	The removal of the longer lived bivalves will cause faunal changes, such as their replacement by other opportunistic bivalve species (suspension feeders) with shorter life cycles. Carnivores and scavengers also increased for a period after removal of the razors.	Gormanstown, Co. Meath, Ireland	Anonymous (2006)
E. siliqua	Hydraulic dredge	The target species of the fishery, *E. siliqua*, is being displaced by another suspension feeding bivalve, *Lutraria lutraria.* Although this species is also considered vulnerable to hydraulic dredging its shell is more robust than that of *Ensis*, increasing its survival potential. As a consequence, the ratio of *Lutraria* to *Ensis* biomass at Gormanstown has been altered. No age class older than age eleven has accounted for 10% of the *E. siliqua* population since 2001, although that was usually the case in the four preceding years.	Gormanstown, North-West Ireland	Fahy and Carroll (2007)
E. siliqua	Dredge	In terms of abundance, the by-catch represented 22% of total catches, while in biomass the percentage was 12%. The mortality of discards corresponded to 13%. Within the most abundant by-catch species, the most affected were *Acanthocardia tuberculata*, *Echinocardium cordatum* and *Polybius henslowi*. Within the *Ensis* catches, an average of 3% corresponded to undersized razor clams. Of these, 83% were undamaged or only slightly damaged.	Setúbal (western Portugal); 8-10m depth; sandy bottoms	Constantino *et al.* (2006)
Solen marginatus	Salting	Concerning abundance values, both control and experimental areas showed a similar pattern of variation, with a decrease 1 and 2 days after simulating salt fishery. After 7 days the abundance presented a tendency to increase. The number of species decreased 1 day after the start of the experiment, in both areas. Higher values of abundance and number of species were recorded 90 days after the start of experiment. No significant differences were detected between control and treatment plots during the study period.	Ria Formosa lagoon, southern Portugal; intertidal area	Constantino *et al.* (2006, 2009)

Table IV. Impacts in target species.

Target species	Fishing gear or technique	Effects	Location and habitat	Reference
E. siliqua	Dredge	The authors found that target species had several shell margin breaks consisting of deep clefts in the outer shell layer in which sand grains were embedded. This is possibly related to repeated disturbance events. In higher and older specimens the frequency of clefts tended to increase.	Barrinha, southern Portugal; Fine sand; 3-7m depth	Gaspar *et al.* (1994)
E. siliqua	Hydraulic dredge	Undamaged razor clams that have escaped from the dredge were observed re-burrowing successfully. Broken specimens were recorded both in the catches (approx. 30% of the catch) and along the dredge trench. Damage clams were not able to extrude the sediment from their shells.	Gormanstown, Co Meath, Ireland	Anonymous (nd)
E.ensis	Hydraulic dredge	Few broken clams were observed both on the bottom and in the dredge, although numerous foot muscles were recorded within the dredged area, contributing to the attraction of scavengers.	Clifden Bay, Ireland; clean fine/medium sand with low silt percentage	Anonymous (nd)
E. siliqua	Dredge	The tow duration and dredge tooth length was shown to influence the number of damaged target species. An increase in tow duration increased not only the total number of clams but also the number of damaged specimens. On the other hand, considering the same tow duration, greater tooth length led to a lower number of damaged clams (2 to 3 times).	Lagos, southern Portugal; 10 m depth	Gaspar *et al.* (1998)
E. ensis, *E. siliqua*, *S. marginatus*	Hand tools	Several rudimentary tools were tested, namely small razor clam rake, grubber hoe, razor clam rake and metallic rod. The results obtained showed that the highest percentage of damaged specimens was obtained using the small razor clam rake, followed by the metallic rod. By contrast using the razor clam rake this percentage was lower than 8%. In terms of catches (ind. per hour), the small razor clam and the metallic rod showed the best results, while concerning the fishing yield (kg per hour), the metallic rod presented values considerably higher.	Galicia (NW Spain)	Sebe and Guerra (1997)
E. ensis, *E. siliqua*, *S. marginatus*	Hand picking	No damage in harvested specimens was observed. This technique presented the lowest fishing yield (catch in number) when compared to the hand tools and diving, while in terms of biomass, fishing yield was higher than the observed for all hand tools, except for the metallic rod.	Galicia (NW Spain)	Sebe and Guerra (1997)
E. ensis, *E. siliqua*, *S. marginatus*	Diving	Both by apnea and scuba diving all the individuals collected were undamaged. A higher number of individuals per hour was obtained in apnea, the fishing yield (in biomass) was higher in scuba diving.	8 to 10 m depth	Sebe and Guerra (1997)

Target species	Fishing gear or technique	Effects	Location and habitat	Reference
E. arcuatus	Suction dredging	After suction dredging, local populations of *E. arcuatus* from the dredged site showed a decrease in density and in the length of the razor clams. Size-class structure of these populations suggested a direct effect of suction dredging by a selective removal of larger specimens and a reduction in their density. The results of the present study suggested that repeated suction dredging operations increased the occurrence of disturbances in the shell growth, particularly the incorporation of sand grains in the shell matrix. *In situ* experiments on the re-burrowing capacity of *E. arcuatus* returned back to the sea showed that these specimens become more vulnerable to predators due to a low re-burrowing capacity. A large number of predatory crabs were attracted to the disturbed area.	Orkney Islands, UK; Fine sand and broken shell; 3-6m depth	Robinson and Richardson (1998)
Ensis spp.	Hydraulic blade dredge	Dredging removed approximately 90% of the razor clam biomass from a central 0.4 m swath of sediment		Hauton *et al.* (2002)
E. siliqua and *E. arcuatus*	Hydraulic blade dredge	Undersized razor clams (*Ensis siliqua* and *Ensis arcuatus*) were included in the six most represented species in the discards' content. These individuals also contributed considerably to the biomass of the dislodged megafauna. The dredge inflicted severe damage to both species of razor clams, either ripping their pedal muscles or breakig their shells. 35 gm^{-2} of *E. siliqua* were dislodged by the dredge, 52% of which were intact. For *E. arcuatus*, dredging dislodged 18 gm^{-2}, of which 60% were severely damaged. Disregarding the condition of the seabed, dislodged *E. arcuatus* and *E. siliqua* have reburied successfully.	Clyde Sea, Scotland	Hauton *et al.* (2003a)
Ensis spp.	Hydraulic dredge	Target species were caught in low density, reflecting their low abundance within the maerl habitat.	Clyde Sea, Scotland	Hauton *et al.* (2003b)
S. marginatus	Salting	90% of the individuals submerged during 1 or 2 minutes in a hyper-saline solution were unable to rebury while in control treatment this percentage decreased to 45%. Ingestion and excretion rates decrease in the individuals from treatments, but differences were not significant.	Laboratory experiments	Diniz (2004)
E. siliqua	Hydraulic dredge	Razor clam populations at Gormanstown make up most of the biomass of the bed. Due to exploitation, their population has, however, been altered. In recent years the size range of clams caught has narrowed and larger animals are becoming less abundant. The authors suggest that older age classes, which were removed by dredging are not being replaced.	Ireland	Anonymous (2006)
E. siliqua	Dredge	*Ensis siliqua* was the most abundant species in the catch corresponded to 89% and 88% in terms of biomass and abundance, respectively. Of the total *E. siliqua* caught during the experiment approximately 15% were heavily damaged or dead.	Setúbal (western Portugal); 8-10 m depth; sandy bottoms	Constantino *et al.* (2006)

5. Discussion

The main objective of the present work was to present a review of the literature concerning the environmental impacts of bivalve fishing, with particular relevance to techniques and gears used for razor clam fishing. Bearing this in mind, the main methods used worldwide were described, as well as the resulting impacts on sediment, water column, associated biological communities and on target species. The most striking observation was the extremely small number of studies undertaken regarding razor clam fishing. Moreover, scientific understanding of adverse environmental effects varies considerably among different fishing gears and techniques. For example, dredges and hydraulic dredges are relatively well studied, whereas no information is available for clam “kicking” and the “gancho”.

In order to summarise the main effects caused by the different techniques and gears, a Delphi Survey technique and multi-criteria analysis was carried out, based both on the results provided by the studies already undertaken and on the knowledge of experts. For this purpose, five researchers were convened to rate habitat impact, by-catch and target species damage for each of the thirteen fishing techniques used in razor clam fisheries. Habitat impacts were categorized into two types: (1) effects on physical structures (e.g. boulders, cobbles, gravel, mud or sand seafloors), and (2) effects on associated biotic communities; by-catch was considered as a whole, that is, it was not divided into different categories; impacts on target species were categorized into two groups, those above and below the MLS. It is worth noting that, in this analysis, impacts due to recreational fishing were not taken into account. Based on the information gathered from our review of the literature, the expert judgment of the five researchers and discussions between them, the effects of each fishing technique were rated. Each category was analysed separately and a qualitative classification was given to each one of the techniques or gears, with impacts rated as *high*, *moderate*, *low* or *negligible*. By-catch classification is similar, but without the *negligible* class. Consensus ratings of habitat impacts, by-catch and target species impacts are presented in figure 15.

According to the results obtained for both physical and biological impacts on associated communities, most of the methods were considered to have negligible or low impacts on the environment (Fig. 15). In terms of biotic communities, only clam “kicking”, razor clam dredges and hydraulic dredges (the only two mechanised methods) were rated as being moderately or highly disturbing methods (Fig. 15). Regarding physical impacts, two rudimentary tools (i.e. shovel and grubber hoe and clam gun) were also considered moderately disturbing, as they involve the turning-over or removal of sediment. This may have consequences, namely in the

release of nutrients for the water column with flooding, as previously explained. Concerning by-catch, the classification of each fishing method was based on the following criteria: highly selective methods affecting only target species were considered to have a low impact; methods where target specimens were located by the “eye” but where sediment is turned over or removed were considered to have moderate impact; less rudimentary methods not exclusively directed to the target species were considered to have high impacts. As a consequence of these criteria, within a single category, there are some methods with more impact than others, such as the case of clam gun and double hook when compared to razor clam rake, shovel and grubber hoe (Fig. 15).

Figure 15. Classification of habitat, by-catch and target species impacts for each fishing method, as determined by five researchers. N, negligible; L, low; M, moderate; H, high.

Finally, regarding target species, both damage to and death of specimens were considered when rating the severity of impact of each fishing method. Salting (intertidal and subtidal), clam gun and hand-picking were the techniques with potentially negligible impacts concerning specimens above MLS. In intertidal areas, regarding animals below MLS, salting and the clam gun were considered to have low impact, because when clams are removed from the sediment, they are left exposed to predation and desiccation, particularly during summer (Fig. 15). In contrast with the results obtained for the previous components, metallic rod and hydraulic clam dredging seem to be the most damaging fishing methods (Fig. 15). In the case

of the metallic rod, this result is obvious, as this gear pierces the animals' body, causing their death. On the other hand, this gear is usually used in class B areas, which implies that harvested bivalves must undergo depuration before consumption, but depuration is not possible when animals are dead. Regarding hydraulic clam dredging, the water jet, used together with the subsequent passage of the dredge, often results in serious damage both to adult and juvenile razor clams. Some of them are simply dislodged and are not collected but exposed to predators that are attracted to the fishing areas. When they are collected, transported to the deck and afterwards discarded to the sea, their burrowing capacity is usually affected and they are, once more, easy prey for scavengers.

Evaluation of the total environmental impacts of each technique or gear was undertaken by attributing a score to each one of the components analysed: 3, 2, 1 and 0 points to *high*, *moderate*, *low* and *negligible* respectively, and then an average value was determined (Fig. 16). In general, according to their impact scores, three different groups are distinguishable, namely: (1) low-impact techniques that include hand-harvesting methods (salting and hand-picking); (2) medium impact gears, encompassing rudimentary fishing gears (pincher tongs, clam gun or tube, double hook, metallic rod, shovel, grubber hoe and razor clam rake); and (3) high-impact fishing methods that comprise clam "kicking" and mechanised fishing gears (razor clam dredges and hydraulic dredges) (Fig. 16).

According to the information gathered, different techniques can be considered the most suitable, depending on the habitat exploited (Fig. 17). Hand harvesting can be considered an environmentally friendly technique both in intertidal and subtidal areas. Within subtidal areas, hand harvesting is undertaken by diving in apnea, "hookah" or scuba, although the latter is usually prohibited by law. Considering the limited information on scuba diving impacts, we think that there are reasons to support the review of this prohibition. In fact, by imposing relevant restrictions, namely a maximum catch per diver and day and under stringent controls, hand harvesting by scuba diving can be more selective than apnea. What is more, as divers have more time to explore the seabed, they can be more selective regarding marketable specimens. Although presenting a greater impact, the use of rudimentary tools does not seem to have severe consequences for environmental sustainability. In deep subtidal areas, where diving is not possible due to the high risk for divers, the use of more mechanised techniques or gears is unavoidable (Fig. 17). Decisions concerning the use of these methods should take into consideration the balance between the economic value of the fishery and local environmental sustainability. These more mechanised methods also appear to be suitable for highly hydrodynamic clean sandy areas, as communities from these areas are very resilient to physical disturbance and recover within a short period of time. On the other hand, some

habitats known to be very diverse and sensitive to disturbance, such as macroalgae and seagrass bottoms and “maerl” areas need to be preserved and highly impacting fishing gears avoided or even prohibited within these habitats. The same suggestion is valid for estuarine and coastal lagoon systems that usually support communities with high diversity and are often used as nursery areas by several commercial species. Moreover, because coastal areas attract people and develop to become urban areas, sediments from these areas can also be contaminated or present high nutrient levels. Mechanised methods have a higher impact on sediment disturbance and ultimately, contaminants can be bio-incorporated by several animals, some of them with commercial interest, with potential consequences for human health.

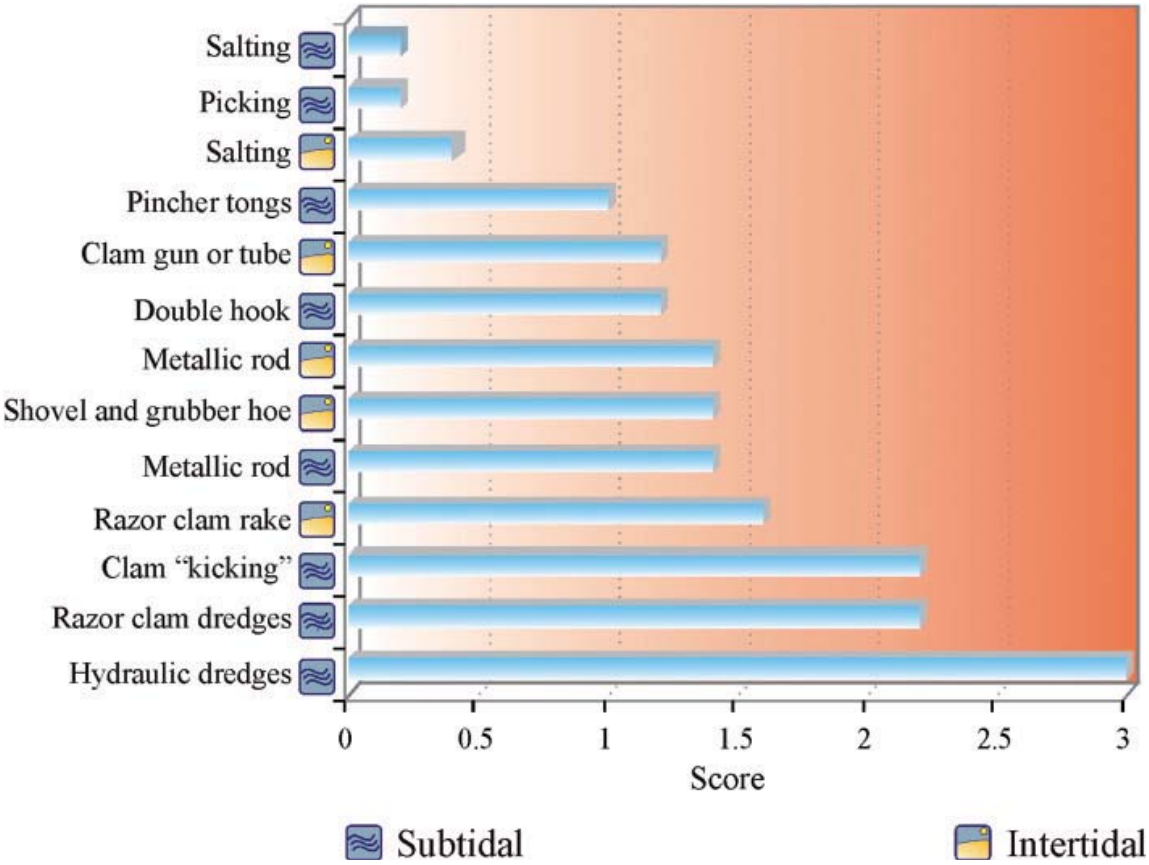

Figure 16. Severity ranking of ecological impacts for all fishing techniques.

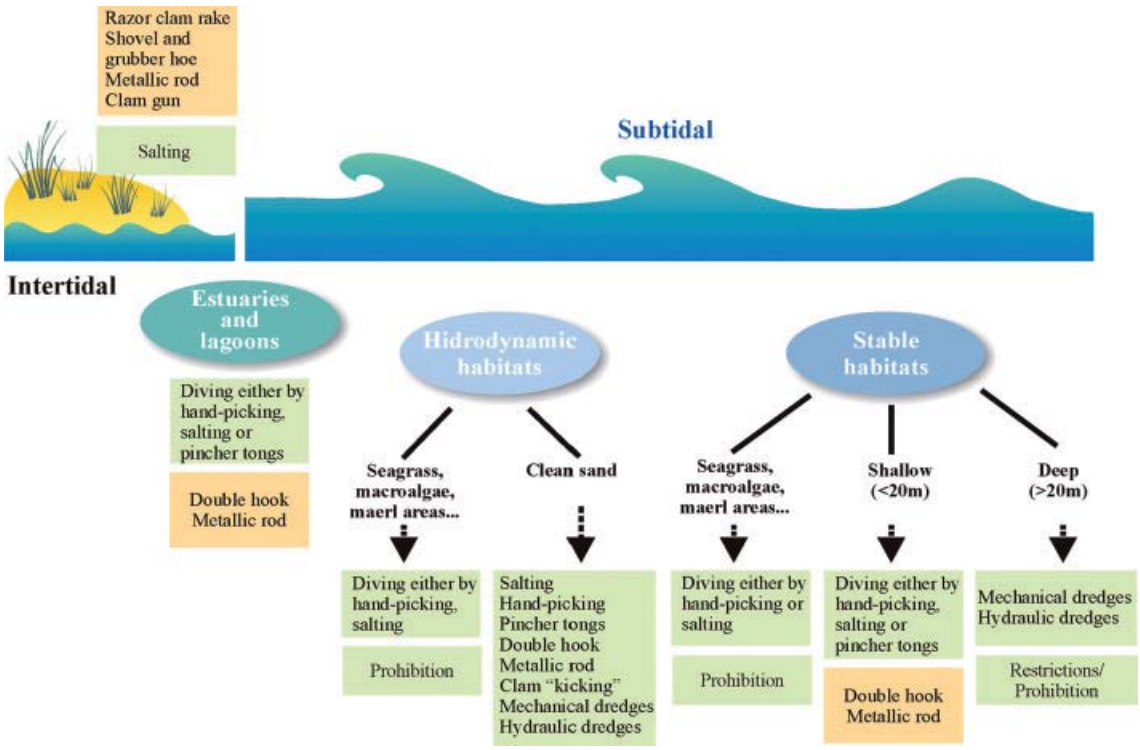

Figure 17. Proposed fishing techniques for razor clam in different habitats.

It is worth stressing that the environmental impact caused by the different fishing gears and techniques may vary with the scale of their use. Even a gear type that has relatively low habitat impact per ton of target catch may have a very large cumulative impact on ecosystems if the fishing effort is very high. Nevertheless, the results obtained with the multi-criteria analysis may constitute a basis to be used by public administrations in the management of razor clam fisheries. The introduction or prohibition of certain fishing techniques in some areas should take into consideration the vulnerability of the habitat to disturbance. Therefore, in these systems, fishing methods that produce severe environmental impacts should be forbidden.

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Section III: Razor clam fisheries

Chapter 12: Razor clam fisheries in the United Kingdom of Great-Britain and Northern Ireland

Chris Hauton, Trevor Howell and Adele Boyd

Abstract

This chapter summarizes the recent development of the razor clam fishery in Great Britain and Northern Ireland. Two species are landed in the UK, mainly for export to the Far East and southern Europe. The pod razor *Ensis siliqua* is generally exported as a live product to the Far East. *E. siliqua* and the curved razor *Ensis arcuatus* are also landed in the Western Isles in northern Scotland and are generally exported to southern Europe for inclusion in mixed seafood dishes. The fishery for razor clams in the UK has always been dominated by hand gatherer fishers working in Scotland, although there has been some interest in razor clam fishing throughout England, Wales and Northern Ireland. A very small number of fishers in Scotland have, since the mid 1980's, fished for razor clams using a variety of different hydraulic dredges. However, the development of a hydraulic dredge fishery remains limited, in part because of fishery legislation and, in part, because of the challenges of using hydraulic gear to catch these relatively-large but thin-shelled and deep-burrowing bivalves. Razor clam landings in the UK are regulated in accordance with pan-European standards for minimum landing size (MLS), contamination with microalgal toxins and microbiological contamination. There are, however, no national or local programmes for monitoring clam stocks and no quotas or TACs have been established. The fishery in the UK has been subject to very little commercialization although a summary is provided in this chapter of restocking studies carried out in Northern Ireland as part of the INTERREG-funded SHARE and TIMES projects (2003-2008).

1. Introduction

Six species of razor clams (*Ensis* spp. and *Solen marginatus*) have been reported from UK waters, five widely distributed and a fifth species that has been reported on two single occasions. The UK fishery for razor clams is dominated by the intertidal and subtidal collection by hand of the pod razor *Ensis siliqua* and exists almost exclusively in Scottish waters.

A small fishery for razor clams has existed in Scotland since at least 1990. However, recent improvements in handling, depuration and transportation combined with an increase in demand from southern Europe, has led to a significant growth in this fishery, especially since 2004. In the late 1990´s to early 2003 there was a growing interest in the use of mechanical fishing gear to harvest clams and a number of independent studies in Scotland, Northern Ireland and Wales investigated the potential, and potential impact, that this development might have. The key findings of these reports have, to some extent, shaped the development of the fishery since then. The widespread development of a mechanised fishery has not materialized; in part because of: 1) persistent concerns identified in those studies as to the impact of hydraulic gear on benthic communities including the target species as well as the sedimentary environment, 2) questions regarding the sustainability of a fishery for this slow-growing and intermittently-recruiting species (*E. siliqua*), and 3) challenges associated with fishing these deep-burrowing but fragile species using dredge gear. Subsequently, commercial landings of razor clams continue to be dominated by hand gatherers working on the intertidal, or by commercial scuba divers working subtidally.

The fishery is still at a relatively small scale and subject to very little monitoring or overt commercialization or restocking programmes. To an extent, the fishing pressure imposed by the hand collecters is self regulated, ensuring sustainability. Some limited, and possibly inappropriate, EC regulation is imposed on the UK razor clam fishery, as discussed below.

2. Target species and their distribution

Four species of razor clam (*Ensis* spp.) are commonly recorded in UK coastal waters, although estimates of densities and distribution have been reported as largely ‘unknown’ (Aqua-Fact, 1998). The two most cosmopolitan species include the pod razor *Ensis siliqua* and the curved razor *Ensis arcuatus.* Both species form large but sparsely-populated infaunal beds from the intertidal to depths of 10-20 m (Hall *et al.*, 1990); however, some reports have recorded razor clams in water depths of approximately 60 m (Holme, 1953). Razor clams, classified as large benthic infauna, have been recorded burrowing into sediments to depths as great as 75 cm or 1 m (Howard *et al.*, 1998; Hauton *et al.*, 2002; Muir, 2003).

E. siliqua and *E. arcuatus* have a circum-UK distribution with *E. siliqua* generally found inhabiting fine sands and muddy sediments and *E. arcuatus* in more coarse-grained sediments (Henderson and Richardson, 1994; Hauton *et al.*, 2002).

The invasive American Jacknife clam *Ensis directus* was first reported on the south coast of England in 1990 (Howlett, 1990). Thereafter populations have been reported in the Thames Estuary, along the Kent coast and the Wash in East Anglia (Fig. 4; Palmer, 2003) as well as isolated records from the Firth of Forth and north-west coast of mainland Scotland. *E. directus* had first been reported in the North Sea in 1979 and it is widely accepted to have been transported to Europe in the ballast water of commercial ships arriving from the United States (Von Cosel *et al.*, 1982). This species quickly spread along the European coast, being reported in the Elbe estuary and Skaggerak in 1982 and in the Bay of Seine from 1998.

The 'common razor clam' *Ensis ensis*, paradoxically, has a more restricted distribution, predominantly being recorded along the Channel coast of England and on the north and west coasts of Wales. A fifth species, *Ensis minor,* has been recorded in UK waters - specifically in Carmarthen Bay in Wales (Dansey, 1999) and in the Firth of Forth on the east coast of Scotland (Smith, 1974). However, these two isolated records have not been corroborated by more studies. A final species, the 'grooved razor clam' *Solen marginatus*, has been widely recorded on the south coast of England, through Wales and north towards Blackpool on the west coast and to the Wash on the east coast. Isolated records have also identified *S. marginatus* in Northumberland (in 1994) and on the Shetland Islands (National Biodiversity Network's Gateway).

Of the six species reported in UK waters only the pod razor *E. siliqua* and the curved razor *E. arcuatus* are routinely harvested for domestic and export markets, although interests in fisheries for *E. directus* have been expressed by the industry since 2000.

Reports of typical razor clam density around the UK are rare in the literature. Estimates from the Wash indicate that *E. directus* can dominate the infaunal biomass (constituting ~ 95% of recorded macrofaunal biomass). Densities of up to 200 adults per square metre, and in excess of 2000 individuals per square metre in February, immediately post larval settlement, have been reported (Palmer, 2003, 2004). Species of the genus *Ensis* in Carmarthen Bay in Wales have been recorded at densities of three to six individuals per square metre (SWSFC). Perhaps the most systematic snap-shot survey of razor clam abundance has been produced in Scotland. In 1992, Fisheries Research Services, Aberdeen (now Marine Scotland-Science) conducted a suction dredge census of infaunal bivalves throughout Scotland (McKay, 1992). The data from this census are summarised in figure 1. Population abundances were estimated from clam catch rates and expressed in terms of kilogrammes of clams collected per hour of fishing. Estimates should be regarded as indicative only as the efficiency of the suction dredge was not determined in this study. A more recent

Scottish survey, based on visual assessments made by divers of razor clam siphons has reported abundances in the range of 0.3 - 40 clams / 2.5 m^2 for three sites in Scotland (defined as 'The Clyde', 'The Highlands' and 'Western Isles'; Muir, 2003). In this study, absolute abundance was found to vary as a function of past fishing pressure, depth, location and season. A major factor affecting visual estimates of razor clam abundance, however, was later shown to be wind-driven ground swell in shallow waters. This ground swell forced the clams to burrow deeper into the sediment for protection, reducing counts based on clams' siphons at the surface.

All studied populations of razor clams in UK waters appear to have a multi-modal size-frequency distribution but with sporadic recruitment and a population structure that is skewed towards larger size classes (e.g. Henderson and Richardson, 1994; Muir, 2003). In a review of razor clam populations in Scotland in 2002 it was reported that over half of the sampled *E. siliqua* were over 14 years of age (Hauton *et al.*, 2002). The beds of *E. directus* surveyed in the Wash region similarly demonstrated sporadic recruitment events. The survey conducted by Palmer (2003) identified that populations were dominated by the 1994 year class, although there was evidence of individuals from 1993 and 1995 and also some survivors from a 1998 settlement. It has been argued that large patches of adult clams may hinder settlement, either because the young razor clams cannot successfully settle and compete with the adults for food and space, or the adults themselves may be key predators of their own eggs and larvae.

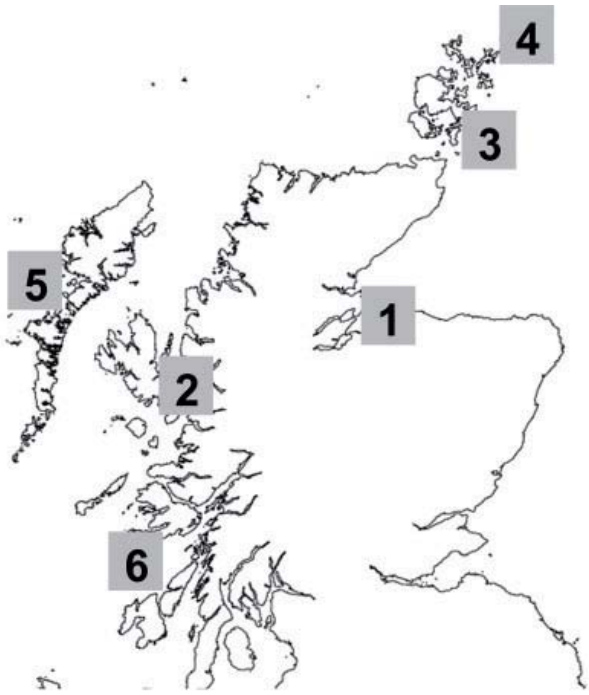
3. Production

3.1. Fishing gears

The UK fishery for razor clams is dominated by hand collection of *Ensis siliqua* from the intertidal and from the subtidal by commercial divers. A very small fishing fleet has fished using mechanical dredge gear since at least the early 1990's in Scotland (Allen, 1990). In part, this limited fishery is a function of current regulation (see section 3.4), a limited market and the challenges associated with the successful harvest of large clams, undamaged, from depths as great as 1 m in the sea bed.

There is no single design of fishing gear established in the UK to harvest *Ensis siliqua*. A number of different designs have been operated by individual boats, with the vast majority being classified as hydraulic batch (i.e. non continuous) dredges. The hydraulic dredges are supplied by seawater pumped down to the dredge from the surface from vessel-mounted pumps. This seawater supply is used to: 1) first prevent the bivalves from burrowing deeper by fluidising the substrate around each

clam; and 2) wash the catch in the collecting cage and prevent it from becoming blocked with sediment. Dredge designs employed in the UK have varied in design, with the degree of gear sophistication reflecting the inspiration of individual fishers, but are based on the principles described above.



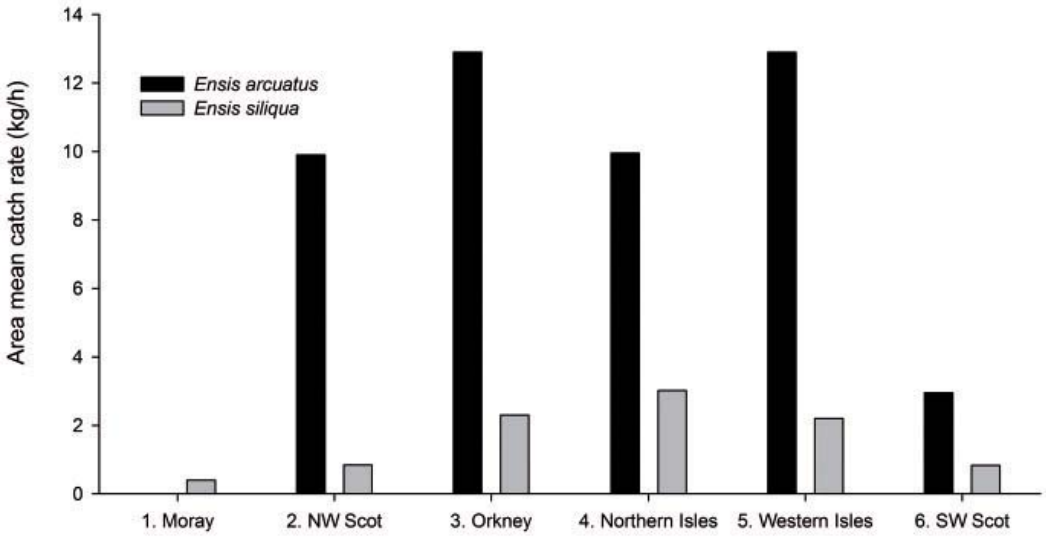


Figure 1. Suction dredge census of razor clam (*Ensis siliqua* and *E. arcuatus*) distribution throughout Scotland in 1992 based on a survey carried out by Fisheries Research Services, Aberdeen (now Marine Scotland – Science). Figure based on data from McKay (1992).

Detailed studies have been made of three blade dredges, of similar style, in 1998 and 2002 (Fig. 2 and Plate 1; Anon., 1998; Hauton *et al.*, 2002). At the time, these dredges were typical of those being used in the UK. Whilst it is true that more effective designs were being developed the fishing principles have tended to remain. The dredges studied in 1998 and 2002 comprised a box-section blade or 'tooth' (minimum 39 cm wide) that penetrated the sea bed to a depth of ~ 34 cm (Fig. 2). The blade was tapered along its length to produce a ~ 10 mm gap at the leading edge through which water was delivered into the sediment. On some dredges additional holes were drilled into the front face of the blade to allow water to be jetted forwards (Plate 2). Extra, backward-pointing, holes created at the top of the blade directed jets of water to wash the catch into the collecting cage. The collecting cages (typically 185 x 80 x 55 cm, but some much larger) were a simple box structure extending behind the dredge mouth to retain the catch. The collecting cage was fitted with a hinged door at one end to allow the retrieval of catch on deck after hauling. Seawater was delivered to the dredge from the vessel-mounted pump (~ 3200 litres per minute and a pressure of ~ 2 bar in some dredges) via 10 or 15 cm diameter layflat hose. On reaching the dredge the water was split between the hollow blade and the collecting cage. The water from the blade was used to fluidise the sand ahead of the dredge. The angled blade and additional water jets then washed the stranded clams and other fauna into the collecting cage. This basic design of dredge has existed for a number of years within Scotland, especially around the Western Isles on the north west of Scotland (Anon., 1998; Tuck *et al*., 2000). Variations on this design were in use at the time of these early studies and included a larger dredge, part of which is shown in Plates 3 and 4. In this case the hollow box-section blade did not have any holes in its leading face. In addition, the majority of the water supplied to the dredge was directed forward and used to produce a series of jets acting from in front of and above the blade as shown in Plates 3 and 4.

A second type of gear, the 'jet dredge' was also in use in Scotland during the period 2000-2002 and was based on dredge designs available in Ireland at the time (Plate 5). This gear did not use a blade or tooth to inject water in to the sea bed. Instead water was injected solely from a row of large bore nozzles fitted to the leading edge of the dredge.

Dredges are deployed in depths of 4-10 m and fished for approximately 10 minutes. Towing speed is variable but essentially very slow. Catch damage rates are minimal when the dredge is not towed as such and the boat is instead allowed to drift with the wind or tide. Even in unconsolidated sediments towing speeds of less than 10 m per minute are most successful. At the end of each tow the gear is hauled and emptied on deck for sorting. It is usual practice to immediately reshoot the dredge so that fishing continues whilst the catch and discards are sorted.

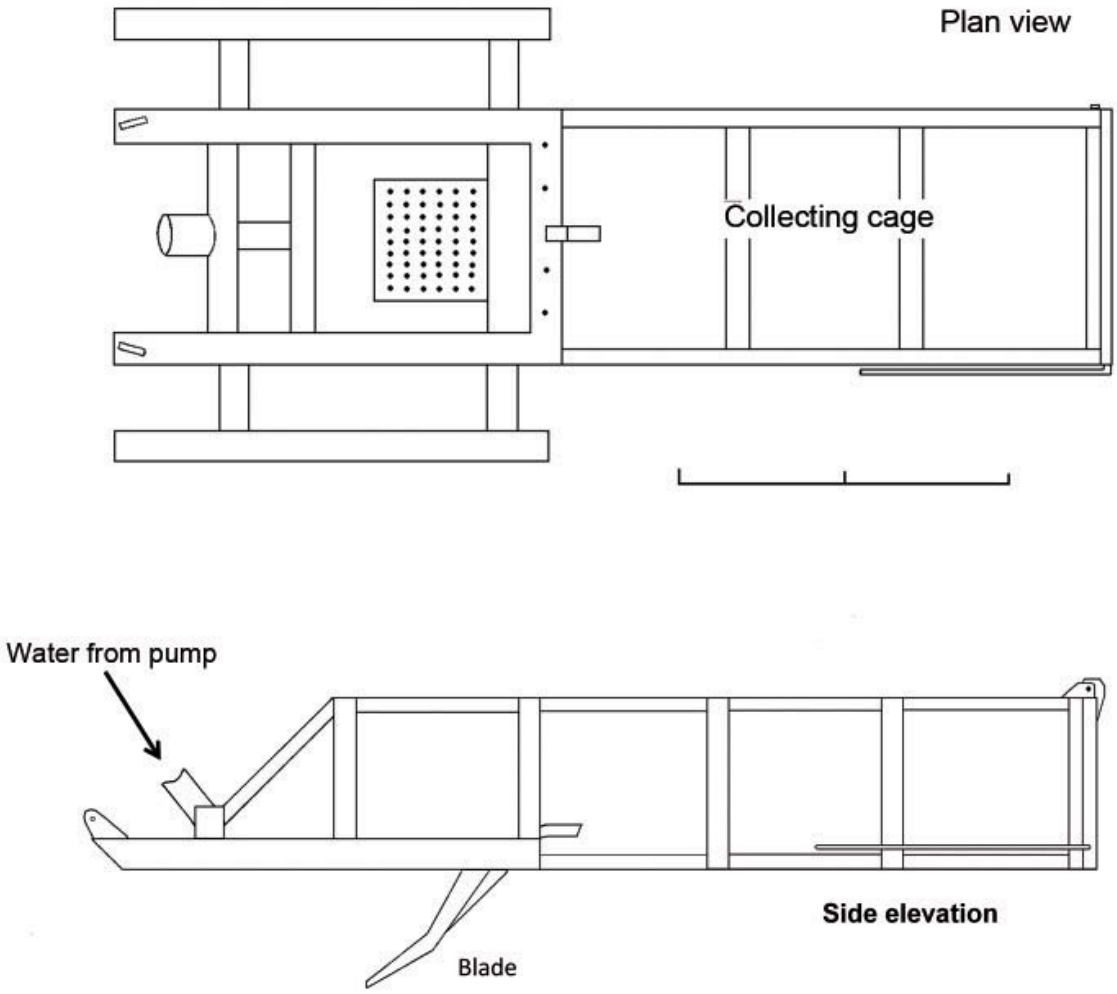


Figure 2. Plan view and side elevation of an hydraulic blade or 'tooth' dredge typically in use in the late 1990's and into the early 2000's. Scale bar represents 1 m. Note, mesh on collecting cage has been omitted for clarity.

Plate 1. View of a typical hydraulic blade dredge used in parts of Scotland in the period 1988-2003. View is from the front of the dredge and shows the layflat hose and manifold used to deliver water from the deck pump. Image also shows stabilising runners on the sides of the dredge and the mesh of the collecting cage. The hollow blade or 'tooth' can just be seen on the underside of the dredge (arrowed).

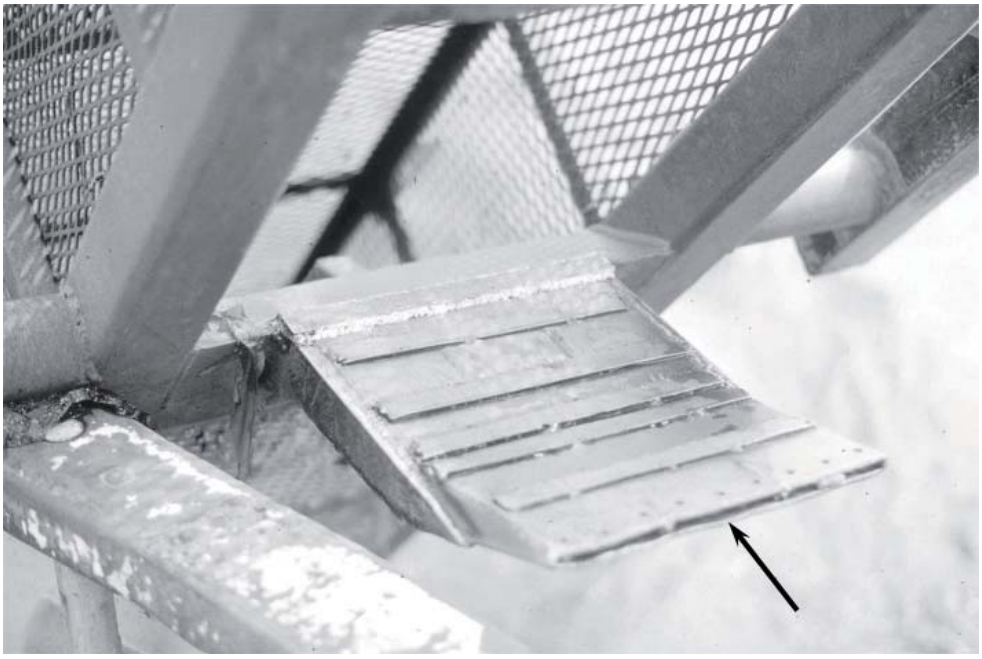


Plate 2. View of the box section blade or 'tooth' of the hydraulic dredge used in parts of Scotland in the period 1988-2003 (as in Plate 1). Image shows the longitudinal taper and the 10 mm gap along the leading edge (arrowed). Image is taken of the underside of the dredge viewing the front face of the blade at an oblique angle.

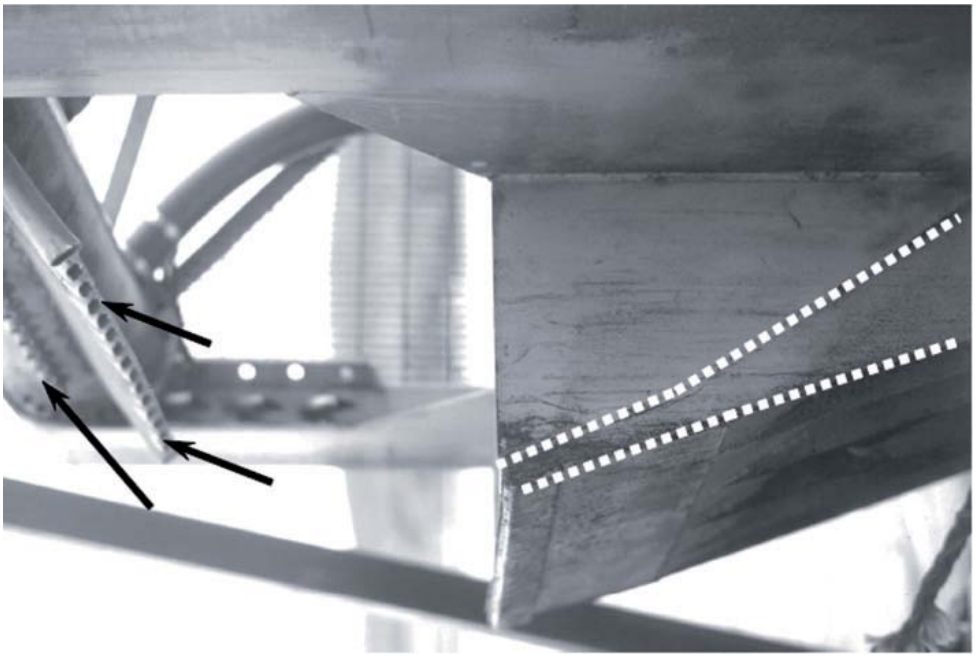


Plate 3. View of the blade arrangement of alternative hydraulic blade dredge used in Scotland during the period 2000-2002, showing the additional water jets positioned in front of and above the blade (arrows) used to fluidise the sand. Picture is of underside of the dredge perpendicular to the leading edge of the blade. White dotted lines outline the box-section blade with modified side walls to improve dredge catch efficiency.

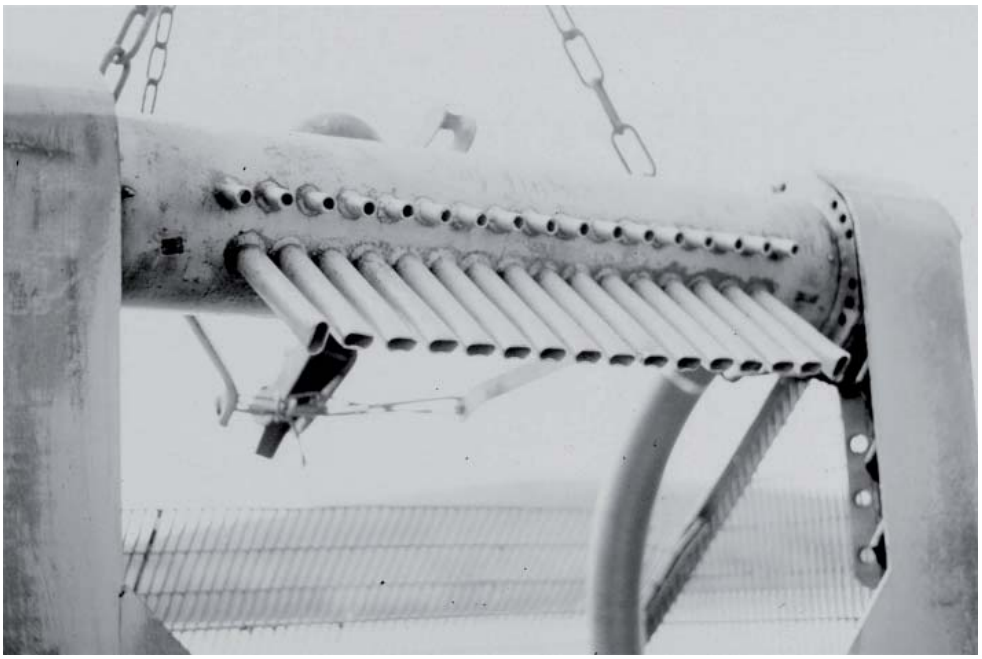


Plate 4. View of the water jet arrangement of an alternative hydraulic blade dredge used in Scotland during the period 2000-2002 (as in Plate 3). Image is of the underside of the dredge showing nozzles that produce water jets to fluidise the sediment in front of the blade.

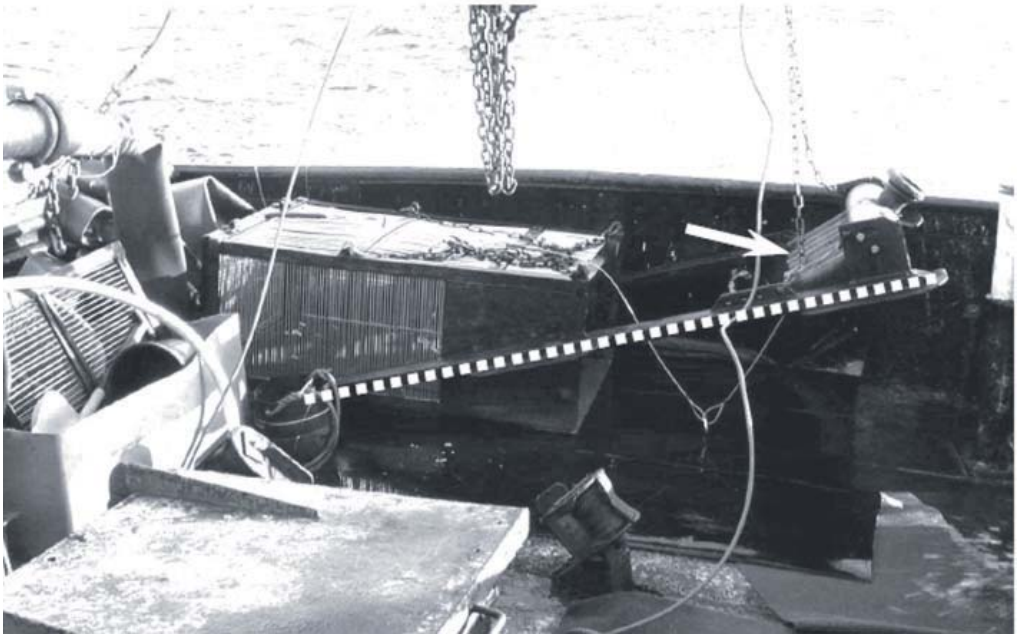


Plate 5. Alternative dredge design used in Scotland in the period 2000-2002 and based on design used in Ireland at the same time (the 'jet dredge'). White dotted line indicates dredge runner that, when fishing, runs horizontally over the sea bed such that the collecting cage is angled into the seabed to collect clams. In this design all of the water delivered to the dredge manifold is injected into the sea bed from the leading nozzles (just visible, arrowed).

3.2. Fishing fleet characterization

The shallow depth at which razor clams are found in the UK governs the nature of the small fleet. All vessels working in razor clam beds must operate according to local regulations, which in England have been established by Sea Fisheries Committees (described in section 3.4.3). Whilst each Committee's byelaws vary, the small number of vessels operating dredges tend to be less than 18 m in length and operate a single dredge per vessel. Shallow draft vessels are more versatile in the inshore waters in which they operate. The typical vessel engine power developed by these vessels is ~ 110 kW (Fig. 3). There is currently no restriction on the size, power output, or pump pressure of the deck mounted water pump used to supply the dredge.

3.3. Number of professional scuba divers or intertidal fishermen involved in the industry

Currently, no statistics on the number of divers or intertidal fishermen are centrally recorded in the UK.

Razor clam landings in the UK are dominated by hand gatherers in Scottish Waters. Clams are harvested from the intertidal at low water or from the shallow subtidal by commercial scuba diving. This fishery has expanded in the last decade as diver fishers have sought alternatives to the scallop (*Pecten maximus*) that has been subject to fishery closures arising from instances of algal biotoxins. 'Salting' is commonly used to extract the clams from their burrows. Conventional table salt or, more commonly, rock salt is used to produce a concentrated brine solution. This is deposited on the siphons of the razor clams at the surface. The concentrated brine solution has been shown to raise the salinity of the interstitial waters up to as much as 85‰ for at least an hour after salting (Muir, 2003). This produces an osmotic shock in the clams and they evacuate their burrow to escape, whereupon they can be collected and banded for market. An alternative method that does not use salt is employed by fishers in the Highlands and Western Isles where problems of availability and cost restrict the use of salting. In this technique the clam is caught at the surface by hand and pinned to the side of its burrow to prevent escape digging; the clam is then pulled from the sediment with one continuous twisting action to ensure that the foot is not ripped off as the clam is extracted.

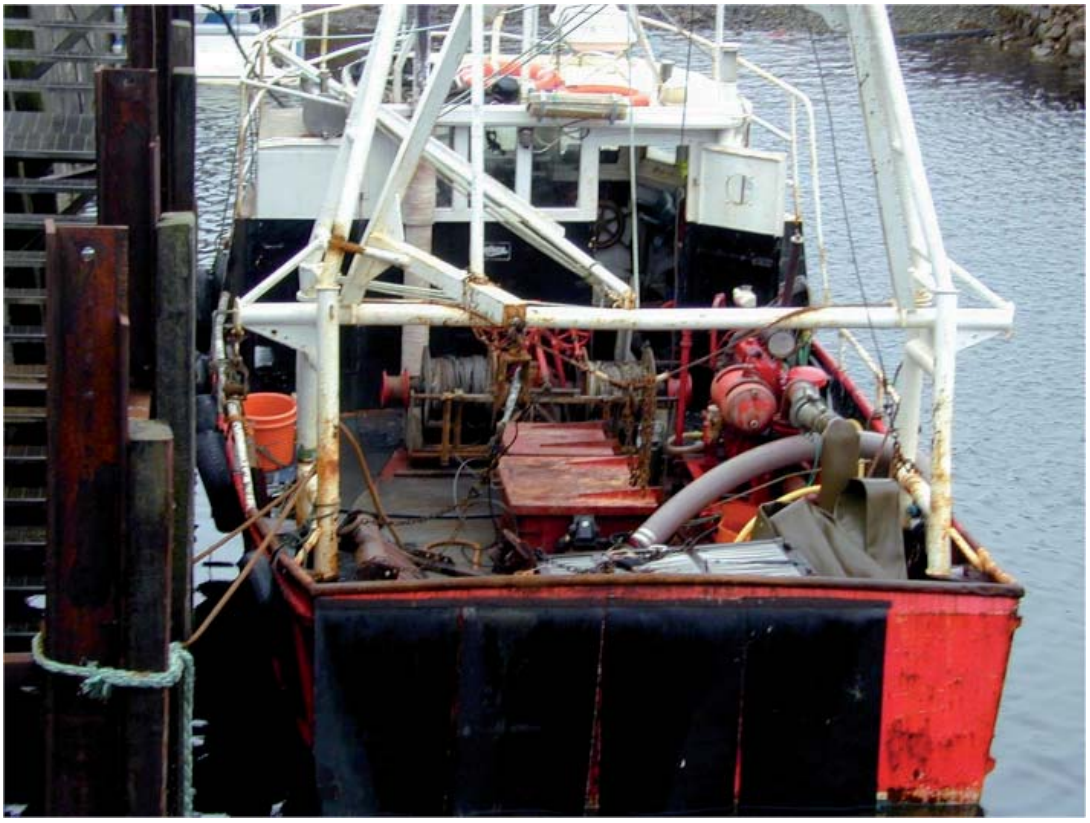


Figure 3. Image over the stern of a typical hydraulic dredge boat operating in Scottish waters during the period 2000-2002.

The selective nature of this fishery has been reported to result in minimal discards (1-8%) and by-catch (Muir, 2003). To an extent the fishery is self regulating, with the fishers concentrating efforts on the largest, and therefore most mature, specimens of *E. siliqua*. When an individual bed of clams is depleted of its largest clams the fishers tend to move to alternative sites, rather than collecting smaller clams (Muir, 2003). Modal sizes of diver-collected clams vary with season and location. In the Clyde Sea the modal length of landed clams has been reported as 201-210 mm during the spring and summer but dropped to 181-190 mm in the winter, whilst in the Highlands the modal size was recorded as 181-190 mm in summer, dropping to 171-180 mm in the autumn and down to 161-170 in the winter. No clams were landed by divers that were smaller than 139 mm in length (Muir, 2003). Market forces also drive the selection of larger clams, with customers in the Far East favouring clams in excess of 140 mm in length.

In recent years there has been interest in the application of electrofishing for razor clams in the UK, particularly in South Wales and in Scotland. Since 1998 the use of electricity to catch fish has been banned throughout Europe under EU Council Regulation (EC) No 850/98, article 31. In order for electrofishing to be permitted, the UK would be required to seek a derogation under that Council Regulation. This derogation would require appropriate scientific support to prove that the technique was not detrimental to stocks of the target species, to non target species, or the wider marine environment. That evidence does not yet exist, although research has started and more is planned (mainly by Marine-Science Scotland) in support of this issue. In the interim, fishing licenses for razor clams in Scotland have been revised to specifically state that: “The vessel shall not carry on board nor deploy into the sea any electrical equipment, including electrical generators, cables, probes, grids or any other equipment capable of transmitting electrical currents to the seabed.” In addition to the potential harm caused to organisms within the marine environment there are concerns regarding dangers posed to human health in operating such systems at sea from small vessels.

3.4. Legislation in force

In the UK there is currently no established quota or TAC for razor clams. The fishery across the UK is still considered of a sufficiently small scale that such legislation has not been considered.

3.4.1. Regulation of minimum landing size

The minimum landing size (MLS) for all species of razor clams in the UK is in

accordance with that set by EC Council Regulation Number 850/98 which sets the MLS for all *Ensis* species of 100 mm in length.

However, work conducted in Scotland (Muir, 2003; Muir and Moore; 2003) has shown that 100% sexual maturity is not reached in *Ensis arcuatus* until a length of 121-130 mm (minimum size of sexual maturity: 81-90 mm) and until 131-140 mm for *E. siliqua* (minimum size of sexual maturity: 118 mm). Estimates based on laboratory-reared *Ensis* spp. collected as juveniles from the field indicate that the minimum age to sexual maturity in Scottish waters is 4-5 years. This indicates a slower growth rate in northerly latitudes when compared with populations in Wales and Portugal. It has been reported that Welsh populations of *E. siliqua* reach the MLS after approximately two years but that they do not reach sexual maturity until at least three years old and 'possibly older' (Henderson and Richardson, 1994). In light of these data it has been argued that the EC MLS for razor clams should be increased throughout Europe (Hauton *et al.*, 2002; Muir, 2003) or that species-specific MLSs should be established (Muir, 2003; Palmer, 2004).

3.4.2. Regulation of microbiological quality and algal biotoxin contamination

Within the UK there are currently (2010) a total of 9 Production Areas classified for razor clams (*Ensis* spp.). This classification includes five Class A areas and 4 Class B areas. These include beds in Loch Fyne, the Forth Estuary, Arisaig, Rabbit Islands, Arran and North Bay (Ayreshire) in Scotland and beds in Poole Bay in England. At present (2010) there are no Production Areas classified for razor clams within Northern Ireland.

Clams from Class A production areas can be sold on the domestic market without further depuration. Clams harvested from Class B waters must first undergo depuration, details of which are summarised in section 4.

In addition to the above testing for microbiological quality of landed razor clams, European legislation regulates against the contamination of shellfish flesh with microalgal biotoxins. The Food Standards Agency is the Central Competent Authority to conduct an Official Control Monitoring Programme for biotoxins in Classified Shellfish Production Areas under the Regulatory requirements of EC Council Directive EC854/2004. This legislation requires all shellfish production areas to be monitored on a basis of risk (default weekly) through Representative Monitoring Points (RMPs). EC regulation 853/2004 prescribes the statutory maximum permitted levels (MPL) of biotoxins in live bivalve molluscs (Table I).

Table I. Maximum permitted levels of algal biotoxins in shellfish (established under EC Council Directive EC 853/2004).

Toxin	MPL
OA (okadaic acid), DTX (dinophysistoxins) and PTX (pectenotoxins) together	160 µg OA equivalents/kg shellfish flesh
YTX (yessotoxins)	1 mg YTX equivalent/kg shellfish flesh
ASP (domoic acid toxins)	20 mg domoic/epi-domoic acid/kg shellfish

In England and Wales samples are collected on a monthly basis, except in areas with historical occurrence of PSP or *Alexandrium* species in phytoplankton samples. If toxins are detected in any one area then the sampling interval is reduced to weekly. In 2009 the sampling programme in England and Wales was dominated by tissue samples from mussels (52%), native oysters (21.5%), pacific oysters (11%) and cockles (15%).

In Scotland, shellfish beds are monitored on a weekly basis for PSP according to seasons and on a variable timescale for DSP (weekly from April to November, fortnightly in December, monthly from January to March) and ASP (weekly from July to November, fortnightly from April to June, monthly from December to March). In the period 1st April 2008 to 31st March 2009 sampling was dominated by mussels (84%), Pacific oysters (9%), common cockles (3%) and razor clams (3%). In the 2008-2009 sampling season one toxic episode was reported for DSP in razor clams in Scotland. In addition, PSP levels in razor clams from the Forth Estuary exceeded action limits on two occasions.

In Northern Ireland the shellfish beds within Belfast Lough are monitored for biotoxins on a fortnightly basis, as is the wild mussel bed within Donaghadee Sound (when open). Biotoxin monitoring within the other shellfish beds in Northern Ireland is undertaken on a monthly basis when harvesting is taking place.

3.4.3. Regulation of fishing gear

In England and Wales fishery byelaws have been established and enforced by local Sea Fisheries Committees (SFCs). In England and Wales there are there are 12 Inshore Sea Fisheries Committees (Fig. 4). In England, dredging, including hydraulic dredging, is currently specifically prohibited by the following Sea Fisheries Committees:

Cornwall, Eastern District (partial) and Sussex (suction dredging). A number of other Committees in England restrict dredging to particular sea areas within their jurisdiction or do not make any specific restriction on dredging within their byelaws.

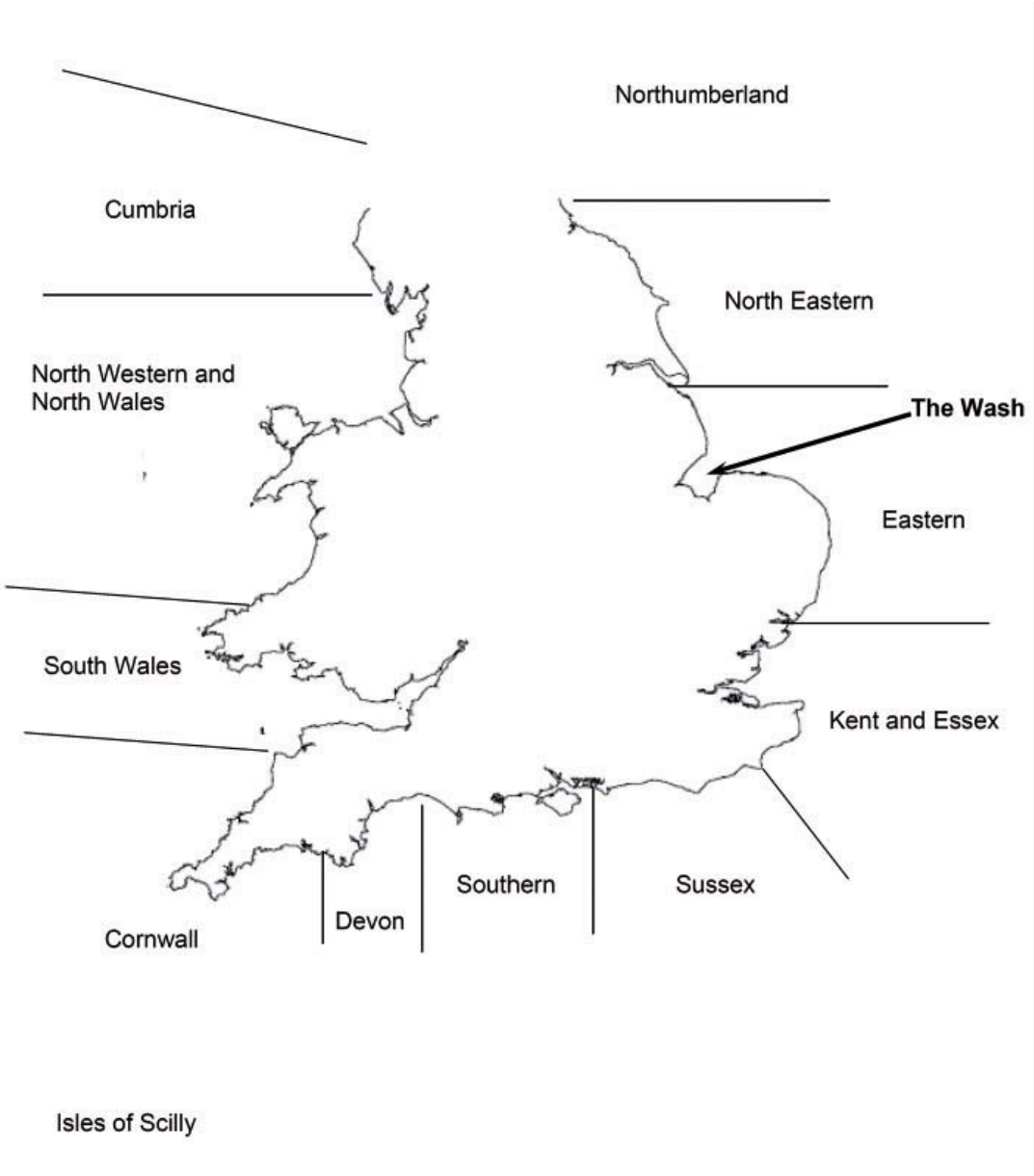


Figure 4. The twelve Sea Fisheries Committees in England and Wales as at the end of 2009. Note that the zones indicated are only illustrative. Figure also indicates the position of 'The Wash' where populations of *Ensis directus* were first reported; see text for further explanation.

Committees in Wales have adopted a precautionary approach to fishing razor clams using mechanical gear. Suction dredging, continuous hydraulic dredging and hydraulic batch dredging are banned within the 6 mile limit by the South Wales and the North Western and North Wales Seafisheries Committees. Within the respective

byelaws, however, it has been established that limited hydraulic dredging can be granted under special derogation from the respective Committee. These derogations are designed to support limited 'fishery-scale' trials of the repeated hydraulic dredging of clam beds in order to develop knowledge of the long term sustainability and effects of a fishery.

It should be mentioned, however, that the status of the Sea Fisheries Committees within England and Wales is currently (2010) under review as part of the Marine and Coastal Access Act. As of 1st April 2010 the two committees in Wales were modernised to become IFCAs (Inshore Fisheries and Conservation Authorities) and have been subsumed into the Welsh Assembly. In time the separate byelaws within all twelve SFCs may be subject to rationalization or revision.

Throughout a large part of Scotland the restriction of hydraulic dredging is established under an amendment to the Inshore Fishing (Scotland) Act 1984 (Inshore Fishing (Prohibition of Fishing and Fishing Methods) (Scotland) Order 1989) which came into effect on 1st January 1990. Under section 2(1) of this Order suction dredging is defined as follows: "the raising from the sea bottom of material, fish and shellfish with gear involving the use of a solids pump or air lift, or water jets to dig into the sea bottom" and includes the use of any type of hydraulic gear. The inclusion of hydraulic dredging within this definition was reviewed in 1998 by Fisheries Research Services (now: Marine Scotland-Science) who concluded that suction dredging and hydraulic dredging produced very similar physical impacts on the sea bed and therefore that the inclusion of water jet dredges within the definition of suction dredging should remain (Anon., 1998; Tuck *et al*., 2000). However it was also concluded that, within the restriction of the Order, a limited hydraulic dredge fishery should be possible in some areas and that this could be pursued through the establishment of a Regulating Order, similar to the approach that has been taken in Wales. A Regulating Order bestows on the beneficiary the right to manage and regulate a fishery or a number of fisheries within a defined area. Furthermore the beneficiary, such as a group of fishers or a Sea Fisheries Committee, can charge tolls and royalties in order to improve the fishery (Clarke, 2001). After some initial interest in an application for hydraulic dredging under a Regulating Order for the Highlands (Fishing News, 2000), interest in this method of fishing has declined.

In Northern Ireland regulation of shellfish harvesting is the responsibility of the Department of Agriculture and Rural Development (DARD). In Northern Ireland the Razor Shells (Prohibition of Fishing) Regulations (Northern Ireland) 1998 banned the use of dredges and suction dredges to harvest razor clams in Dundrum Bay. This legislation came in to force on 1st January 1999. The Inshore Fishing (Prohibition of Fishing and Fishing Methods) (Amendment) Regulations (Northern Ireland) 2008

which came into effect on the 11th of July 2008, extended this ban by prohibiting the use of suction dredges for the capture of sea fish within Northern Ireland Inshore waters.

Additional local regulations have been established throughout the UK to offer protection to seabed areas of exceptional conservation status under the European Union's Habitat Directive (Council Directive 92/43/EEC of 21 May 1992). For example, on the east coast of England in the Wash candidate Special Area of Conservation (cSAC) hydraulic dredging is banned (Prohibition of Fishing Order SI 1276, 1998).

3.5. Evolution of landings per razor clam species

Recorded landings of razor clams in the UK are summarized in figure 5 (data from FAO Fishstat Database). As mentioned above, landings in the UK are dominated by the pod razor *Ensis siliqua* with *E. arcuatus* also contributing to landings on a regional basis. The majority of landings are for export, with live product being shipped by air to Spain and Portugal as an ingredient for paella and also to Hong Kong and Korea where the clams are cooked and served whole and 'in shell' (Kelso and Service, 2000). Smaller markets for UK razor clams have existed in Holland, Denmark and France and southern Europe. Prices of razor clams are subject to market forces with quoted values ranging from ~ £ 1,600 per tonne (Allen, 1990) to ~ £ 4,000 per tonne in 1998 and with an average of £ 2,500 per tonne (Fishing News, 1998). In 1998 the UK razor clam fishery was valued at £ 343,252 (Kelso and Service, 2000). By 2004 UK export values for razor clams ranged from approximately € 1,300 per tonne (exported to Ireland) to approximately € 5,700 per tonne (exported to South Korea) (Anon., 2005).

Table II. Summary of landed razor clams (live weight and value) as a function of vessel size for Scotland in 2008. Note this table does not distinguish between vessels operating hydraulic dredges and vessels supporting diver fishers. Data taken from National Statistics (2008).

Overall vessel length	Live weight (tonnes)	Value (£ '000s)
< 8 m	0	0
8 – 10 m	254	720
10 – 12 m	108	272
12 – 15 m	39	116
15 – 18 m	125	388
TOTAL	526	1,496

Latest available statistics for Scotland indicate that a total of 161 tonnes of razor clams were landed by boats using mechanical dredges in 2008, with a landed value of £ 404,000 (National Statistics, 2008). Statistics for Scotland indicate that a total of 360 tonnes of razor clams were landed by boats employing 'hand shellfishing' with a value of £ 1,079,000 (National Statistics, 2008). Table II summarizes the distribution of live landed weight and value of razor clams based on overall vessel length.

Landing statistics by district available for Scotland have been summarized in table III, which identifies the value and live weight of razor clams landed by landing district for 2008. Comparison of the 2008 total live weight (Table III) with the data shown in figure 4 emphasizes the complete dominance of the Scottish fishery in landings of razor clams.

Table III. Summary of total landings of razor clams in Scotland in 2008 (National Statistics, 2008) reported by landing district. Data do not distinguish between hand collected and dredged razor clams.

Landing district	Live weight (tonnes)	Value (£ '000s)
Pittenweem	98	230
Buckie	26	71
Mallaig	20	61
Oban	35	103
Campbeltown	281	851
Ayr	65	178
TOTAL	526	1,496

4. Commercialization and depuration

The hand collection fishery focuses on producing a high quality intact product. Clams are sold live, banded in bundles, through a network of shellfish dealers. Diver landings of clams on mainland Scotland are comprised of up to 93% *E. siliqua,* which are then quickly exported live and by air to the Far East (Muir, 2003). In the Highlands and Western Isles, which do not have such quick links to export markets, the landed clams can be a more equal mixture of *E. siliqua* and *E. arcuatus*. During the winter months *E. siliqua* might only comprise 49% of the landed catch in the Highlands and Western Isles (Muir, 2003). Conventionally, these clams are shipped by road to

Europe and sold as a processed product and so are not dependent on supplying a large and intact clam to secure a high-price. Export markets for dredged clams are the same as for hand collected clams. The only constraint on the export of large dredged *E. siliqua* to the Far East is the requirement for an intact product; shellfish dealers tend not to accept *E. siliqua* for Far East markets that are excessively chipped or otherwise damaged (for example: split or cracked shells, foot muscle displaced).

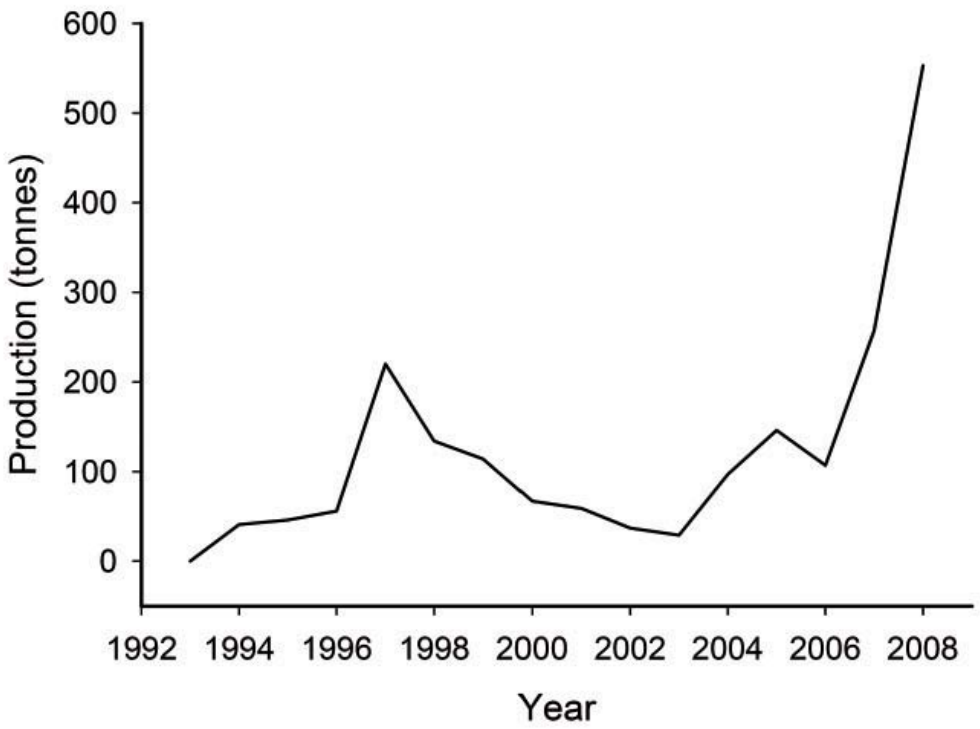


Figure 5. UK capture production of razor clams (all species) in tonnes from 1993-2008; data from the FAO Fishstat database. No landings were recorded prior to 1992. Data from FAO Fishstat database.

In the UK the Competent Authority for monitoring shellfish for faecal contamination, according to regulations laid out in the EC Shellfish Directive 91/492/EEC, is the Food Standards Agency (FSA). In England and Wales, testing is coordinated on behalf of the FSA by the Centre for Environment Fisheries and Aquaculture Sciences (CEFAS) and is carried out by Local Enforcement Agencies (LEAs). In Scotland, monitoring is carried out by Marine Scotland - Science (formerly Fisheries Research Services) on behalf of the Food Standards Agency Scotland (FSAS), whilst in Northern Ireland biotoxin analysis is carried out by the Agri-Food and Bioscience Institute (AFBI) on behalf of the Food Standards Agency Northern Ireland (FSANI).

As described in section 3.4.2., bivalve mollusc production areas are classified

according to contamination with *E. coli*. Table IV summarises the contamination limits and requirements for bivalve shellfish sold within the UK.

Table IV. Summary of the permitted levels for shellfish waters classifications within the UK.

Classification		Permitted level	Outcome
A	< 230	Less than 230 *E. coli* / 100 g flesh	May go direct for human consumption if end product standard is met
B	< 4600	Less than 4600 *E. coli* / 100 g flesh (90% of samples must be less than or equal to 4600 *E. coli* / 100 g flesh	Must be subject to purification, relaying in Class A area (to meet Category A requirements) or cooked by an approved method
C	< 46000	Less than 46000 *E. coli* / 100 g flesh	Must be relayed for at least two months in an approved relaying area followed, where necessary, by treatment in a purification centre, or after an EC approved heat treatment process
Above 46000 *E. coli* / 100 g flesh			Prohibited areas – molluscs must not be subject to production or be collected

In 2000 the results of a joint study, run by CEFAS and the SeaFish Industry Authority, investigated the most appropriate conditions for the depuration of razor clams collected from class B waters (Younger *et al.*, 2000). Optimal depuration took place when clams were held horizontally in depuration tanks in bundles of 10-12 animals restrained using elastic bands. Razor clam activity was very much reduced at salinities below 35‰, temperatures below 5ºC and oxygen saturations of less that 5 p.p.m., and this reduced activity delayed the depuration process. Clam activity was also reduced when the volume ratio of shellfish:water was less than 1:2.2. More recently it has been confirmed that depuration is most successful when clams reach the depuration tanks within 6 hours of harvest and also that the siphons of clams that have been collected by suction or hydraulic dredging can become blocked with sediment or 'grit', which impacts on depuration. For this reason no recommendations have yet been established on the optimal conditions for the depuration of dredged clams in the UK.

5. Razor clam bed monitoring

Currently there is no national sustained monitoring of razor clam beds within UK waters. The fishery is dominated by selective hand collection from the intertidal or subtidal using divers and no scheme to monitor beds or establish TACs has been established.

6. Razor clam restocking or stock enhancement

Within the UK there is also no established programme for restocking of razor clam beds for fishery enhancement. The INTERREG-funded Sustainable Harvesting of *Ensis* (Razor shellfish) (SHARE) project (2003-2007) sought to address the issues required to establish a basis for sustainable cost-effective production or razor clams in the Atlantic Area (Constantino *et al.,* 2007). This project involved a collaboration of institutes throughout Europe (Queens University Belfast Centre for Marine Resources and Mariculture (C-Mar) in Northern Ireland, the University of A Coruña and CIMA in Spain, IPIMAR in Portugal, and Bord Iascaigh Mhara (BIM) in Ireland). Studies in C-Mar investigated broodstock holding systems (Cromie and McDonough, 2006), the potential for larval culture, and *E. siliqua* seed culture trials (Constantino *et al.*, 2007).

Initial findings from the C-Mar studies reported that maintaining broodstock in sand was preferable over simple banding with elastic bands (85% survival after 33 days in sand compared to 33% survival when banded). After larval settlement and metamorphosis razor clams were successfully reared, on a microalgal diet, to a size of 7 mm in captivity - a size at which the juveniles were suitable for transplanting out onto beds. *E. siliqua* seed was successfully reared in the hatchery to a size of over 20 mm by 168 days post fertilization.

The SHARE project was followed by the INTERREG-funded 'Towards Integrated Management of *Ensis* Stocks' (TIMES) project (2007-2008). The TIMES project involved partners from Queens University Belfast (C-Mar), the University A Coruña, CIMA and IPIMAR. This project focused on the keys areas identified for further investigation through the SHARE project. C-Mar in Northern Ireland focused on investigating the effect of photoperiod on gametogenesis of *E. siliqua* broodstock under hatchery conditions, and investigating systems for the hatchery culture of *E. siliqua* larvae, specifically investigating the effect of diet, stocking density and light intensity/photoperiod (Cromie *et al.*, 2008).

Findings showed that by increasing the photoperiod in the hatchery to 16 hours light:8 hours darkness during the winter months (November to January) *E. siliqua* broodstock could be conditioned to start spawning at the start of January, two months earlier than observed in wild populations (Cromie *et al.*, 2008). Hatchery culture trials indicated that microalgal pastes were not an effective substitute for a diet of live microalgae in the hatchery cultivation of *E. siliqua* larvae. Low survival rates observed during the stocking density experiments and light intensity/photoperiod experiments may have influenced the results obtained (Cromie *et al.*, 2008).

Experimental investigations undertaken at C-Mar during both the SHARE and TIMES projects demonstrated successful spawning induction, larval rearing to metamorphosis and settlement, and postlarval rearing of *E. siliqua* under hatchery conditions. However, the technology and methods developed within these projects have not been expanded to a larger scale in the UK, in part due to a lack of continued funding to support this work. However, another acknowledged problem of razor clam ranching is that clam stocks are mobile (Swennen *et al.*, 1985; M. Pyke and C. Hauton, pers. obs., but see also Muir, 2003) and this may limit the financial success of any project designed to seed natural beds with juvenile clams to support subsequent fishery activity.

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Chapter 13: Biology and commercial exploitation of razor clams in Ireland

Edward Fahy

Abstract

Three species of the genus *Ensis* occur in Irish coastal waters. *E. siliqua,* contributes most to commercial landings; its principal fisheries are in the North West Irish Sea. *E. arcuatus* is found in sheltered areas off the western coast; *E. ensis* is rare. All three are K-selected species: they are slow growing, reproduce at a low rate and most of the biomass resides in the older year classes.

Traditionally razor clams were extracted by digging or by sprinkling salt on their burrows at low tide. Some of the harvest was used for human consumption, a proportion as bait.

The impact of hydraulic dredging was analysed at Gormanstown off the North East coast. This kind of extraction causes a high direct and by-catch mortality in older animals and juveniles do not replace them with sufficient speed to maintain the population. This effect may explain the patterns of landings of razor clams shown in FAO statistics. The consumption of razor clams in Ireland is low and most are exported to continental Europe, mainly to Spain, a small proportion going to the Far East.

1. Introduction

Casual observation of shells washed onto beaches suggests that razor clams are widespread in Irish coastal waters. Traditionally they were locally harvested at low spring tide by individuals who dug them out or who sprinkled salt on their burrows, which the razors rapidly vacated. Some harvesting was undertaken for human consumption but a proportion of razor clams caught by these methods was for use as bait.

Three species of the genus *Ensis* occur in Irish coastal waters. *E. siliqua* (L.), the pod razor, contributed the majority of the landings since 1997 (probably more than 95%), principally from the North West Irish Sea and very largely from the Gormanstown bed adjoining County Meath (Fig. 1); a more comprehensive account of the North

West Irish Sea fishery is provided in Fahy and Carroll (2009). *E. arcuatus* (Jeffreys) is best represented by small patches enjoying shelter from the Atlantic swell along the western seaboard. *E. siliqua* also occurs in small patches on more exposed parts of the West coast (Fahy *et al*., 2001). Occasionally very large specimens of *E. siliqua* (>20 cm) have been harvested among *E. arcuatus*. Both species occur along the Southern Irish coast. *E. ensis* (L), the common razor shell is, in fact, quite rare. Evidence suggesting the occurrence of a larger population of this species was encountered only in Blacksod Bay, County Mayo, during exploratory fishing for razor clams (Fig. 1).

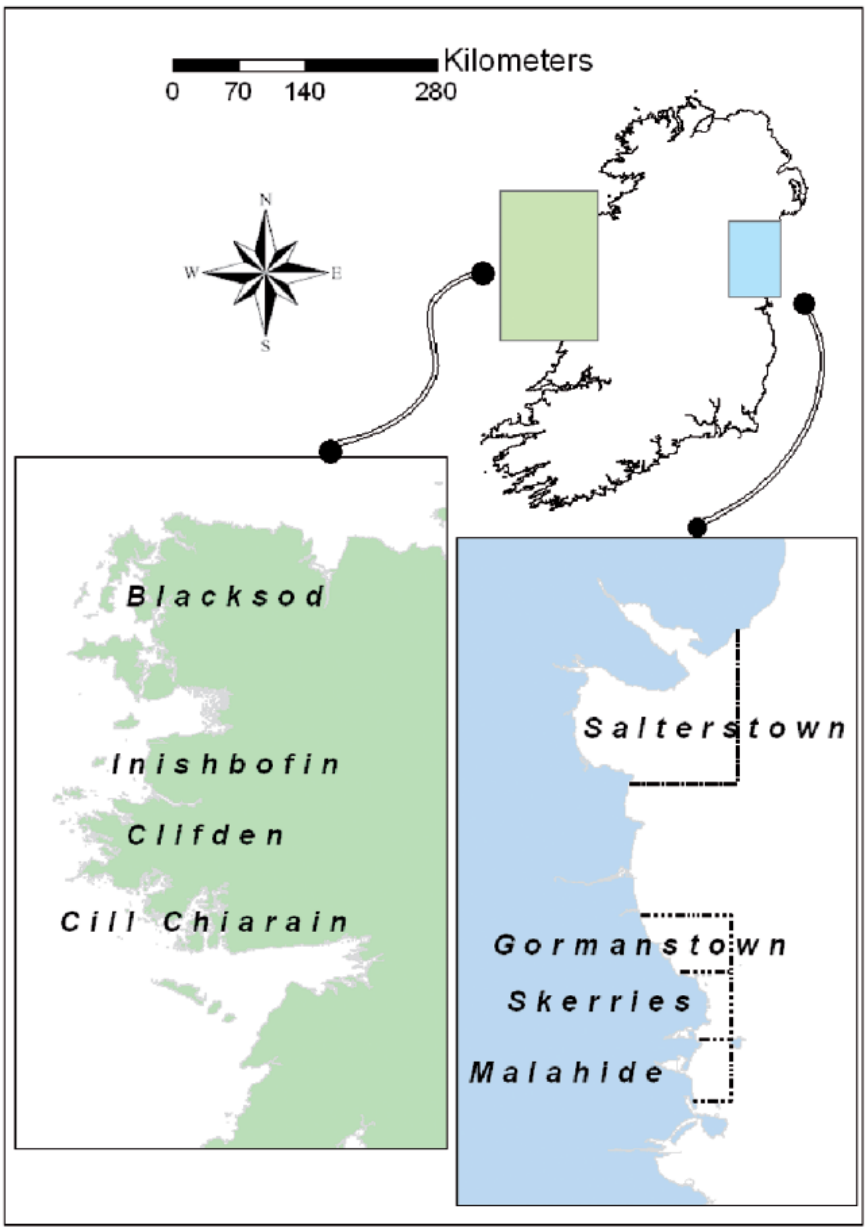

Figure 1. Locations in Ireland at which razor clams were sampled. The administrative "boxes" in the North West Irish Sea in which exploitation is administered are identified.

Razor clams are very mobile. Among bivalves they are second only to scallops in the waters around Ireland and when they are fished they behave in a way that exaggerates their abundance: once a proportion of the biomass has been removed from an area in which the animals occur at higher density (these are areas on which fishers concentrate effort and in which razor clams cluster, presumably to benefit from shelter or a greater supply or food), individuals on the outer fringes of the bed displace and occupy the depleted area. Fishers interpret this as indicating a greater abundance than actually exists and they respond by intensifying harvesting. Once fished down, stocks are very slow to recover. Landings statistics reported by FAO demonstrate the consequences of harvesting razor clams in Western Europe (Fig. 2). For a time a single nation supplied the majority of landings, Spain up to 1987, Portugal for the next ten years and, from 1999, for three years Ireland provided the bulk of the razor clam harvest in Western Europe.

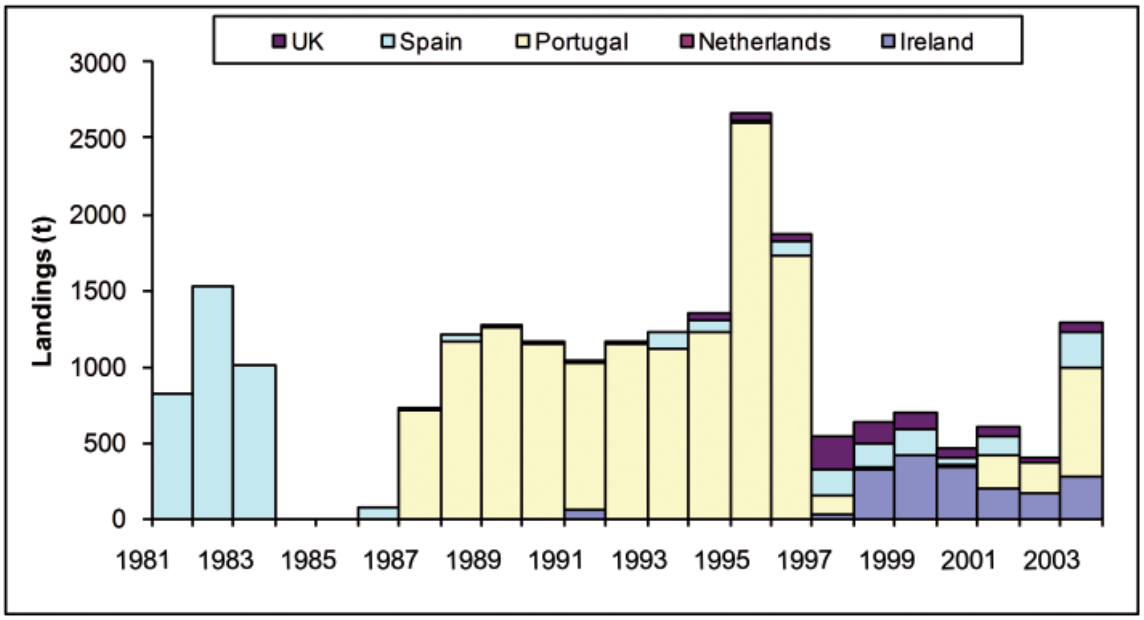

Figure 2. Landings of razor clams by north-east Atlantic nations, 1982–2004 (Source: FAO landings statistics for zone 27, *Solen* spp, code RAZ). Figure from Fahy and Carroll (2007).

2. Habitat and distribution

Before 1997, when commercial harvesting commenced in the Irish Sea, relatively little was known of the biology and habitat requirements of razor clams. Being interstitial species, the sediment characteristics of the substratum supporting them is important. Some examples are shown in Fahy *et al.* (2001) and Fahy and Carroll (2007, 2009). *E. ensis* occupies the smallest mean grade size which consists almost entirely of fines. *E. arcuatus* has a preference for much coarser

sediments in which the fines are reduced to <12%. *E. siliqua* is intermediate, tending towards *E. ensis* in its sediment preference. These preferences go some way to explaining the distribution of the species. The three species have not, to date, been discovered sharing the same sediment type at comparable densities.

3. Commercial exploitation and its consequences

Razor clams are not consumed to any extent in Ireland and the yield from commercial exploitation is almost entirely exported to continental Europe, largely to Spain, a small quantity going to the Far East. The pod razor, *E. siliqua*, is suitable for canning which has exerted much of the demand. In the aftermath of spawning condition falls and the animal may lose 10% of its weight (Fahy and Gaffney, 2001) at which stage the canning market loses interest in it. The western seaboard has very limited resources of razor clams. Although some landings were made up into the early years of the new century, there are insufficient resources to keep one inshore boat (10-12 m) gainfully employed all the year round.

3.1. The North West Irish Sea fishery

Landings of razor clams to Ireland, their value and the quantity from the Gormanstown clam bed in Co. Meath, are shown in figure 3. In 1997 an individual fisher, financed by an entrepreneur, had his boat fitted out in the Isle of Man to dredge hydraulically. The development was meant to be secret but, the story goes, the fisher was proud of his new gear which rapidly became common knowledge (Fig. 4). Whatever the truth of that story, a number of vessels (perhaps as many as 50) quickly obtained similar equipment and converged on the Gormanstown bed in Co. Meath. The boundaries of the Gormanstown bed were established for reasons of water quality. Other than that there was no attempt to regulate the fishery. As many as 25 boats were to be seen fishing simultaneously there on occasion.

Demand for razor clams was not large. Seven buyers purchased them in the first year and the most powerful of them sought control of the market. The majority of boats fishing Gormanstown were independently owned. The market was rapidly saturated and excess landings were inevitably dumped. Reflecting a decline in demand, the first sale price, over a period of less than 12 months, fell by 32%. More recently other beds have opened within the regulatory “boxes” shown in figure1. Water quality is still the factor deciding whether or not fishing is permitted. The fishery remains an open access one although the boats now only fish to fill specific orders.

Up to the end of 2005, Gormanstown had yielded a minimum of 1,600 t of razor clams. Exactly what biomass the 1,600 t came from is difficult to estimate. The razor clam bed at Gormanstown was reported by divers to be densely occupied by the animals (as many as 100 per m^2). The shellfish bed was disturbed by a variety of custom-built hydraulic dredges, penetrating the substratum to a depth of 30 cm and fluidising the sea-bed at a range of pressures. The behaviour of the animals further complicated interpretation of results. In winter and spring razor clams may reside deeper in the substratum to protect against shell fracture in turbulent weather. Breakage rates resulting from harvesting in the early fishery (1998 and 1999) accounted for 30% of the landings in quarters 1 and 4 and 18% in quarters 2 and 3 (Fahy and Gaffney, 2001). However, these rates were calculated from the contents of the dredge basket and did not take into account what was left behind on the sea bed. Razor clams which appeared undamaged were purchased but a proportion of these were later rejected because the flesh was bruised. Collateral dredging damage might well have accounted for an additional 50% of the landed weight but this percentage is surmise.

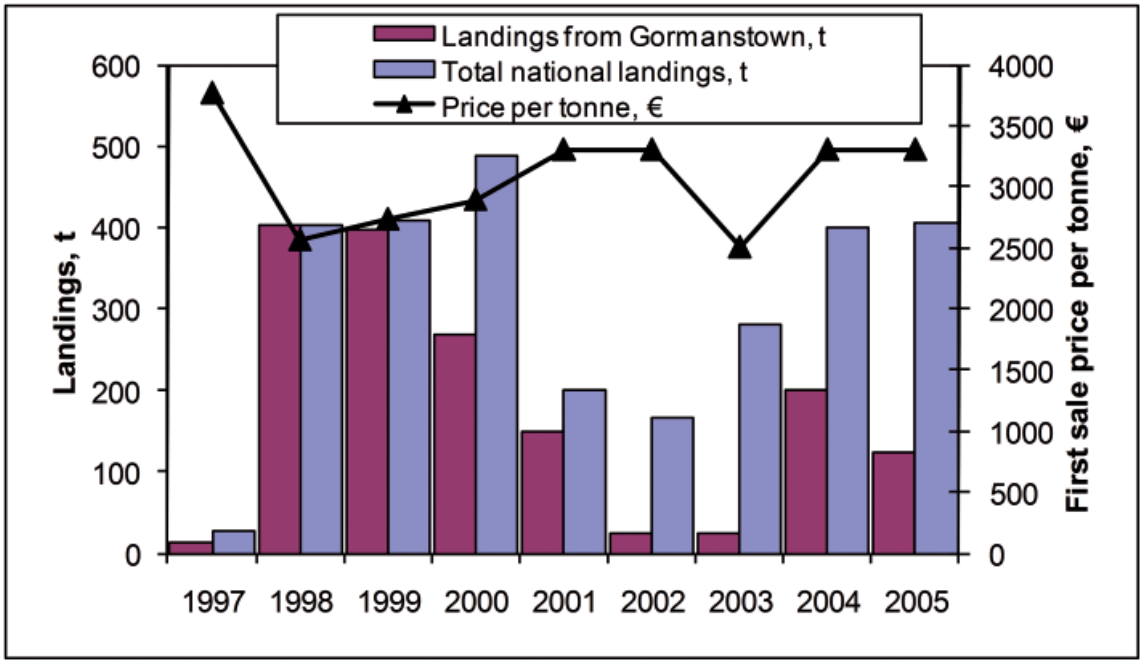

Figure 3. Landings of razor clams to Ireland and landings from the Gormanstown bed in Co. Meath, 1997 to 2005. First sale price is given in € and € equivalent. Source of data: landings statistics from the government department responsible for fisheries supplemented and varied by information from industry.

The consequences of hydraulic dredging for the Gormanstown bed and its benthos were monitored over a period of seven years (Fahy and Carroll, 2007) (Fig. 5). The effects of hydraulic dredging on interstitial fauna depend largely on the type of sediment and the nature of the fauna frequenting it. *E. siliqua* favours the

sediment grade composition making up the clam bed at Gormanstown and there is a possibility that repetitive fluidising could make the substratum inhospitable. The tidal regime in the North West Irish Sea is weak so that suspended fines are likely to settle back onto the disturbed area but that fact is not established. At Gormanstown the quantity of broken shell increased and the sorting coefficient of the sediments rose but there is, as yet no evidence that fines have been washed away.

Figure 4. Boats equipped for hydraulic dredging.

Studies suggest that fine sand and mud communities are more vulnerable to dredging than those in high energy environments such as coarse sand. But long lived bivalves in either are likely to be depleted and will require long periods to restore their numbers.

Various studies are available showing the effects of environmentally damaging fishing techniques such as scallop dredging and trawling. They invariably have the effect of reducing species diversity. Hydraulic dredging in Gormanstown on the other hand, increased diversity by challenging the *Ensis* monoculture and providing opportunities for other species which were present throughout, but only in small numbers. The effect was clearly demonstrated through the Shannon-Wiener index of diversity (Fahy and Carroll, 2007).

A glimpse of what may occur on a more widespread scale once harvesting of razor clams commences is available from the Gormanstown case study. Some

spat settle every year but probably not very many. The older animals are selectively removed or killed in the course of hydraulic dredging and juveniles do not replace them sufficiently fast to maintain the population. It is the mechanism which is most likely to explain the pattern of landings in the FAO statistics (Fig. 2).

Figure 5. Razor clams (*E. siliqua*) washed ashore in the vicinity of hydraulic dredging.

Dayton *et al.* (1995) observed that a long lived suspension feeding community is likely to be replaced by a community of opportunistic deposit feeders and mobile epifauna after large scale and intense fishing disturbance. Certainly, numbers and biomass of scavengers and predators increased as exploitation at Gormanstown proceeded. *Pharus legumen*, whose population expanded considerably, is indeed a deposit feeder and an opportunist. But the largest effect to date has been the replacement of one suspension feeder, *E. siliqua*, with another, *Lutraria lutraria*, and that is likely to be due in some measure to the continuation of hydraulic dredging on this clam bed.

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Chapter 14: Commercial razor clam fisheries in Spain

Alejandro Guerra Díaz and Carlos Gabín Sánchez

Abstract

In Spain, three species fundamentally formed the group of commercial razor clams: *Ensis arcuatus, Ensis siliqua* and *Solen marginatus.* The most important production comes from the *Ensis* spp. species, whose capture is carried out mainly from boats by fishermen-divers expert in breath-hold diving systems. This shellfish-gathering system is very selective and its use is limited to areas lower than 20 m deep. For catching the species in the intertidal area, especially *S. marginatus,* extraction gear is used derived from tools used by farmers in coastal areas bordering on catching areas.

On the Spanish coasts, both species of *Ensis* genus are mainly found on the Galician coasts (not the whole length of the Spanish coastline), while *S. marginatus* appear along Atlantic and Mediterranean coasts, in intertidal and subtidal areas of protected inlets and the waters of rias, canals and streams. The commercial value of both species of *Ensis* genus is higher than that of *S. marginatus.*

Spain is the biggest importer of Solenidae, which come from European (Ireland, Scotland, Italy, France, etc.) and South American countries (Argentina, Chile, Peru). National production is mainly for fresh consumption, while imported production is for the canning industry, where Spain is the main European exporter.

The biological and anatomical characteristics of Solenidae determine that depuration, which is an obligation when extractions are not from areas classified A (direct consumption), processing, transport and sale need specific methods to extend the lifespan of the specimens and their organoleptic characteristics.

Several adverse circumstances surround the exploitation of these species, such as: confused commercial denominations, large and disperse space distribution and the fact that most catches are not legally commercialised, meaning that official registers do not reflect faithfully the amounts extracted nor the real situation of fisheries.

1. Introduction

Solenidae are infaunal bivalve molluscs (Fig. 1) found along almost the whole of the Iberian Peninsula coast, mainly in protected areas with sandy substrates, which assists the recruitment of larvae. Razor clam populations are found in the area stretching from the lower part of the intertidal zone to the 20 m infralittoral; this wide distribution area, together with the type of habitat and the characteristics of the substratum where they are buried, have determined that through the years shellfish gatherers have designed specific catching gear and methods adapted to the characteristics of the area and species in question.

There are registers mentioning that in prehistory, during the Palaeolithic age, camps were established at the mouth of large rivers. The presence of these fossils, coming from parts of marine molluscs, is a clear sign of their consumption in their diets as well as the use of their valves as tools. Martínez *et al*. (1998) mentions the presence of pod razor remains in the most important prehistoric towns during the Bronze Age, in Galicia in Celtic hillforts[1]. In the same way, the existence of dungheaps or places with piles of shells in other Spanish regions is reported in some records.

A wide distribution area for pod razors along the coast, together with the limited commercial importance and the poor reliability of identification in dispatch centres and fish markets are the reason why there are barely any reliable official registers about production and sale. Aside from this general situation, there are specific areas of Galician coasts where Solenidae exploitation has had special importance, mainly shellfish-gathering in natural banks located in the Finisterre area (Northwest of Galicia), where trawl fishing was practised in the 1970's (Catoira and Traba, 1997).

Razor clam production data, regarding Spain, mentioned in FAO registers from 1994, are catches made in Galicia that represented 25% of European production in 1998. The Irish Sea Fisheries Board (B.I.M., 2005) mentions that in 2004 the Spanish market was the most important in the EU, importing razors clams from more than 40 countries; mainly, and in order of importance, Argentina, Italy, Peru, Chile and France, which constitutes about 43% of the Solenidae imported by the EU.

[1] Hillforts are the most primitive prehistoric settlements and village nuclei known in Galicia (6,000 to 2,400 a. C.). The term "pod razor", means pertaining to the Solenidae group, whatever the species.

2. Exploited species: razor clams

Common name: Sword razor
Genus: *Ensis*
Species: *arcuatus*
Commercial code FAO: EQE

Common name: Pod razor shell
Genus: *Ensis*
Species: *siliqua*
Commercial code FAO: EQI

Common name: Grooved razor shell
Genus: *Solen*
Species: *marginatus*
Commercial code FAO: RAE

Figure 1. Razor clam commercial species present on Spanish coasts.

3. Distribution

As we know, it is not easy to determine the amount and production of each species on Spanish coasts and it is also challenging to identify them when trading and consuming them[2] (Table I). Moreover, we have to add the reduced global value of fisheries and the dispersion of catches along the coast.

Commercial denominations of Solenidae have several local meanings, which are adapted to the real name through lists of commercial denominations of fishing products, reviewed each year by the General Secretariat of the Sea, part of the Ministry of Environmental, Rural and Marine Affairs and it is published in the BOE[3]. However, confusion in local denominations is common, so in fish markets as well as in dispatch centres the two species of *Ensis* genus present on Spanish coasts are confused, namely *Ensis arcuatus* and *Ensis siliqua*.

Table I. Commercial names for each species in different Spanish regions

Commercial name	Scientific name	Commercial name by region			
		Galicia	Asturias	Baleares	Cantabria
Navaja (Sword razor)	*Ensis arcuatus*	Navalla			
Navaja (Sword razor)	*Solen spp.*	Longueiróns	Muergo /Longueirón	Manecs	
Navaja (Sword razor) or muergo (Grooved razor shell)	*Ensis spp.*	Navallas pequenas			
Longueirón (Grooved razor shell)	*Solen marginatus*	Longueirón vello	Muergo /Longueirón		Muergo
Longueirón (Grooved razor shell)	*Solen vagina*				

[2]In the report "Repertorio en torno a las especies alimentarias más utilizadas en España", by Dr. Felicísimo Ramos Fernández (http://www.historiacocina.com/especiales/diccionario/N.pdf), for pod razors, these denominations and synonyms are mentioned as the official Spanish name of this mollusc: muergo, navaja arqueada, navaja ruda, tagelo berberecho, tagelo pico de pato, anguillolo negro; anguillolo; avero; cadela, caiño; canyut; carabela, caballete; datil indoduna; espadín; ganivet; langueiró; lingueirón; longueirón; longueirón vello; manec de ganivet; meisán; morguera; navalla; pisadote.

[3]In BOE nº 70 dated 03.22.07, appears the Resolution, dated 02.27.07, from the General Secretariat of Marine Fishing where the list of commercial denominations of fishing species admitted in Spain is indicated. Based on Article 4, Section 2 of Regulations (CE) nº 104/2000 from the European Council dated 12.17.09.

These species' distribution areas lie all along the coast (Table II), although there are no data about their production and harvesting to validate this, except for the Northwest Atlantic.

Table II. Areas from Spanish coasts where Solenidae are registered in the BOE[4] database. The water classification of the extraction areas is indicated.

	Area	Classification area	Species or group of species
País Vasco	- Ría de Hondarribia - Ría de Mundana - Ría de Plancia	B B B	Navaja (*Pharus legumen, Solen margintus, Ensis ensis* and *Ensis siliqua*)
Cantabria	- Bahía de Santoña - Bahía de Santander - Ría de Mogro - Ría S. V. de la Barquera - Ría de Tina Menor	B B B A B	Muergo (*Solen* spp.) and navaja (*Ensis* sp)
Asturias	- Ría del Eo - Ría de Villaviciosa	B B	Muergo (*S. marginatus*) and *E. arcuatus.*
Galicia[5]			Not divided by species
Andalucía	- Río Guadiana (estuary) - Marismas (mud flats) del Guadiana - Río Carreras (estuary) - Isla Canela - Barra del Terrón - Marismas (mud flats) del Río Piedras - Río Piedras (estuary) - Estuary of Río Guadalquivir - Río San Pedro and mud flats - Saco de la Bahía de Cádiz - Sancti Petri - La Atunera	B B B A A B A B B B B A	Longueirón, navaja (*Solen* spp,, *Ensis* spp.) Longueirón (*Solen* spp.)
Cataluña	- Alcanar- S. C. de la Rápita - Shores of Río Ebro delta	A A	Longueirón (*S. marginatus*)
Islas Baleares	- South Coast of Majorca	A	Navaja (*Solen* spp.)

[4] In the appendix of Order APA/3228/2005, dated September 22th (BOE, nº 249), a list of areas producing molluscs and other marine invertebrates from the Spanish coasts is shown, and with it the presence of Solenidae in the areas mentioned.

4. Production

In figure 2 are shown razor clam catches in Europe during recent years. During 2003 they reach their highest values, this recovery being due to the catches declared from the Netherlands, which have not appeared in FAO fishing statistics so far.

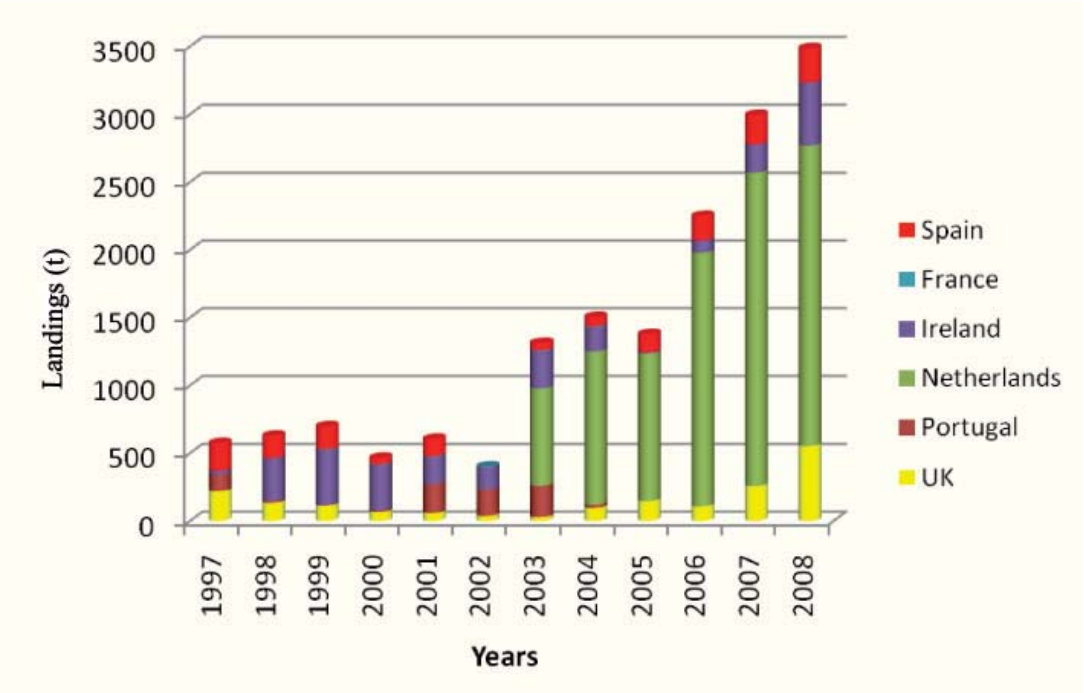

Figure 2. Evolution of Solenidae catches in Spain and other European countries.

During the last years, more than 85% of Solenidae catches are concentrated on the East Atlantic coast. Ireland is the European country with the most stable production, while the United Kingdom shows a downward tendency, being in 2003 less than 10% of the values declared in 1997. Other European countries (France, Portugal and Spain) show irregular data, due to the collapse caused by overfishing in some production areas, combined with the poor specific data of actual catches.

4.1. Solenidae production areas on Spanish coasts

Solenidae production areas on Spanish coasts which are important for commerce are:

4.1.1. The Southern Atlantic coast

In the South Atlantic region *S. marginatus* is the predominant species, known as longueirón in the province of Huelva and muergo in Cadiz. Some statistics mention

E. siliqua species mainly due to unloads in Spanish harbours of individuals caught on Portuguese coasts. On just a few occasions, some specimens of *Ensis ensis* have been obtained, together with other species such as *Venerupis rhomboides.*

S. marginatus natural banks go along the coast from Huelva province, from the mouth of Guadiana river, to the mouth of the Ría of Huelva (Punta Umbría), and they are more abundant, sometimes amounting to more than 2500 units/m^2, in estuaries of Río de Piedras and Rio Carreras stream. In Cadiz province they go from the mouth of the Guadalquivir River to Atunara (on the Mediterranean Sea) with areas of extraction in San Pedro, Saco de la Bahía and Sancti Petri Rivers.

In these areas, razor clams settle in substrates of very fine sand, poorly calibrated, with a sparse presence of bioclasts and a percentage of organic matter higher than 10%. The reproductive period of these populations is from April to July (Del Castillo, 2001).

There are only historical data of pod razors and sword razor shells at the fish market of Cristina Island from 1985 to 1997, all of them under the title of pod razors, so it is supposed they come from Portuguese coasts and were caught with dredges. From 1997 on, extraction by apnea diving was started but since it was not officially authorized, sales were not made at the fish market.

In Huelva province, harvesting has been established between 30-40 kg/collection/day, while in an area with average abundance they are obtained diving for 4 hours. Taking into account that one kg is equivalent to 3 dozen individuals larger than 80 mm, the catch per day is supposed to be about 1,000-1,400 razor clams per diver.

Estimates of the number of shellfish gatherers vary depending on the source, with an average of 30-40 persons dedicated only to this work during the year. This number may be higher, reaching 90-100 in seasons with higher demand (summer and Christmas holidays). A diver works together with the owner of a boat and a packer, who are usually members of the same family. In this sense it is estimated that 10 families live from this activity.

On the coast, the fishing area is from about 20 m to the coast, in periods of 2.5 -3 hours, depending on the depth.

In 2007, the price per kilo at distribution centres or fish markets, is lower than the first years of the present millenium, due to the increase in supply, costing 5-6 €/kg (Royo, pers. com.).

4.1.2. Cantabrian coast

In Asturias, there are natural banks of grooved razor shell (*S. marginatus)*, in the Ría del Eo and Ría de Villaviciosa, and their exploitation is regulated. In the Ría del Eo they appear in the intertidal strip emerging in 0.8 m of Mean Low Water Springs (MLWS), related to the *Zoostera marina* plant. There is also presence of *E. arcuatus* populations at densities of 0.55 units/m^2 (López *et al*., 2006a). Data of *S. marginatus and E. arcuatus* catches in production areas from the Asturian coasts (Rías del Eo and Villaviciosa) are shown in Table III (Rodríguez, pers. com.; web: http://tematico.asturias.es/dgpesca/din/estalonj.php).

Table III. Solenidae catches on Asturian coasts.

Year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
kg	**172**	**650**	**1,440**	**1,285**	**2,280**	**800**	**1,630**	**6,900**	**8,700**	**1,164**

In Cantabria, although there are no records of catches, the situation and state of this resource is mentioned (Mediavilla, 2005), and the cartography of solanaceous populations has been made (A.C.E.M., 2003). Nevertheless, along the coasts of both autonomous communities, the presence and consumption of grooved razor shell is important for retail markets.

4.1.3. Northwest Atlantic coast

In Galicia more than 95% of pod razor and sword razor shell production of Spanish coasts is extracted. The background of pod razor shell (*E. siliqua*) exploitation is collected in a report by Catoira and Traba (1997), showing extraction data (Table IV) during 1966-1976 registered in the Finisterre area (Costa da Morte in A Coruña province).

Table IV. Catches in the Finisterre area years prior to the existence of Exploitation Plans.

Year	1966	1967	1968	1969	1977
kg	**24,750**	**3,800**	**308**	**43,000**	**4,800**

Shellfish-gathering activity in this area during these years was carried out from vessels (each craft has between 7 and 9 crew members); and they used the

trawl-fishing method from these craft, called “pod razor trail”. Extraction activity lasted from one to three months, between October and March. Fishing production during this period was between 150.7 kg/boat/day in 1966 and 66.3 kg/boat/day in 1978. From this year it was suspended until 1983 due to the low profitability of exploitation. That year, sword razor shell catches with the apnea diving system started again, however the activity was not sustainable due to poachers. Until Exploitation Plans[5] came into force in 1993, monitoring and contribution of catching data and sales in fish markets were poor, especially for secondary species, such as razor clams and sword razor shells. In 1993 an Exploitation Plan called that of “specific resources” was created for these species in the Finisterre area. Catches in this area, although important, decreased during the last years, from 64,000 kg in 1993 to 21,700 Kg in 1996, meaning respectively 95% and 32.7% of pod razor shell catches in Galicia (Catoira and Traba, *op.cit.*).

Darriba (2001), reporting data from the Consellería de Pesca, Xunta of Galicia, points out that between 1993 and 1999 Solenidae catches increased from 68 to 172 t/year, decreasing to 140 t in 2000. This author also says that razor clam catches (*E. arcuatus*) were the most important during the last years, reaching 72% of the total production of Solenidae extracted in 2000.

4.2. Evolution of Solenidae

4.2.1. Evolution and value of catches

In figure 3 the evolution of catches during recent years is indicated, according to data of sales at fish markets, provided by the Consellería do Mar, Xunta of Galicia. The minimum production during 2002 and 2003 coincides with the sinking of the oil tanker the *Prestige* opposite the Galician coasts. This fact determined the prohibition of fishing for the following six months, affecting especially Solenidae extractions in banks along the Atlantic coasts, the most important in Galicia. The figure also shows the progressive importance of *E. arcuatus* catches during the last years, occurring alongside the slow decrease of the *S. marginatus* catches, which barely exist in 2006.

E. siliqua production levels, inferior to those of razor clams (*E. arcuatus*), are

[5] Decree 59/1992 of the Xunta de Galicia creates Exploitation Plans, which force shellfish gatherers to practise the activity by a sustainable exploitation model of the resource. So, from 1993 in the Autonomous Community of Galicia, official data of catches start to be registered, indicating the evolution of production in areas where management of the resource is undertaken through Exploitation Plans. There are established ways of management and annual production objectives, for the extraction areas -authorisations- of each fishermen´s association. Regulation (CE) nº 850/98 of the Council, dated 03/30/98 points out the minimum size for catching these species: 100 mm for pod razors (*Ensis* spp., *Pharus legumen*) and 80 mm for the grooved razor shell (*Solen marginatus*).

maintained throughout the years, with average increases of 5% (Fig. 3).

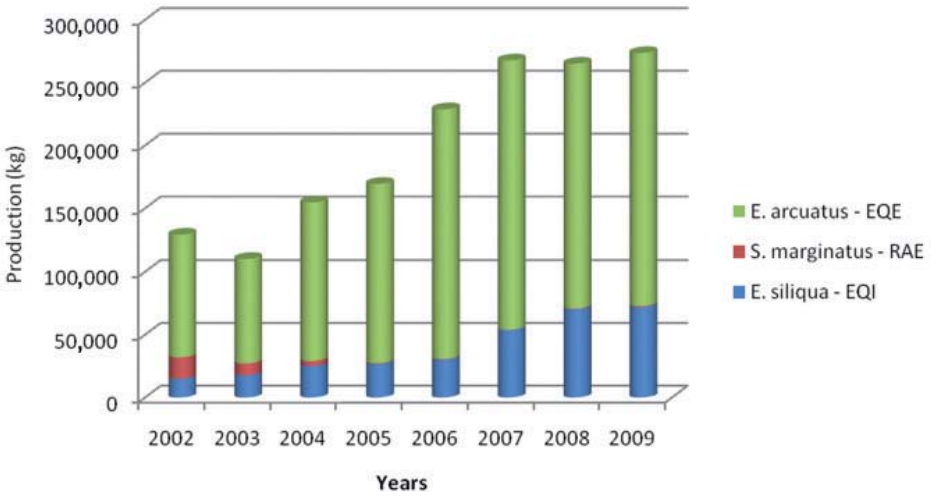

Figure 3. Evolution of Solenidae catches year on year.

In the evolution of average sale prices in fish markets, we do not appreciate any great increase in the last years (Fig. 4). We wish to point out the increases in 2002 and 2003, motivated by the poor harvest of these species, since fisheries of these species, mainly established in the area affected by the petroleum spilled by the *Prestidge*, were closed during six months from November 2002 on. Prices stabilised again during 2004 and 2005, varying between 10.5 € and 12.5 €/kg; slightly higher for sword razor shell than for pod razor. Temporary closure of the most important fisheries during six months produced an increase in prices due to the shortage of supply.

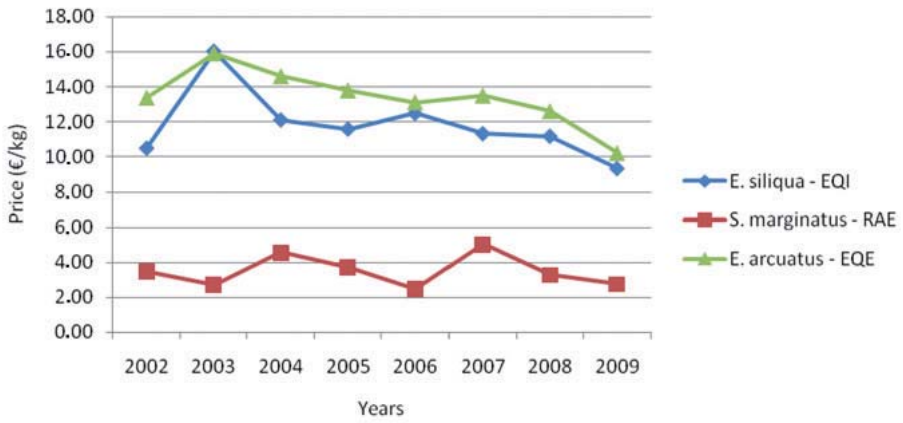

Figure 4. Evolution of average sale prices (€/kg) at Galician fish markets during recent years.

The grooved razor shell kept a stable price throughout the years, although lower than both *Ensis* species. This species has the least demand, due to its external aspect: it is less attractive, has worse quality meat, and the methods used for

catching them damage several individuals. All of this determines the low commercial value of this species.

4.2.2. Production areas and value of catches in Galicia

On table V the origin of the production is shown, by species and catching areas, with data from 2005. In the Rías Bajas (Rías de Vigo and Pontevedra and slightly less in Ría de Arousa), is where most pod razor and sword razor shell catches are concentrated. The evolution in the last years shows that most important fisheries of pod razor shell in the 1980's, held the coast between Carnota and Finisterre as a referent (North coast of Galicia), which decreased due to overfishing. Currently, the Ría de Vigo is the main production area, especially around the Cíes Islands where sustainable and stable management of catching is carried out. Production and sales data of grooved razor shell are available only in the Rías Bajas. Low catches in other areas, often are not recorded in official statistics although their presence is constant along Galician coasts.

Table V. Amounts of razor clams (kg) sold at fish markets in each area. Data provided by the Analysis and Registers Service of the Consellería do Mar (Xunta of Galicia)[6].

	E. siliqua EQI		*S. marginatus* RAE		*E. arcuatus* EQE		Total	
	(€)	(kg)	(€)	(kg)	(€)	(kg)	(€)	(kg)
Zone I-Vigo	4,820	54,690	15	90	71,574	769,259	76,408	824,039
Zone II-Pontevedra	395	4,491			53,296	540,897	53,691	545,388
Zone III-Arousa	42,073	392,038	318	500	63,149	638,569	105,541	1,031,107
Zone IV-Muros	1,038	7,719			12,510	100,873	13,548	108,592
Zone V-Fisterra	19,709	184,404			19	175	19,728	184,579
Zone VI-Costa da Morte	2,644	19,828	260	779	300	1,710	3,203	22,317
Zone VII-Coruña-Ferrol					8	54	8	54
Zone VIII-Cedeira			75	463			75	463
Zone IX-Mariña	1,322	7,478					1,322	7,478
	72,001	**670,648**	**667**	**1,831**	**200,854**	**2,051,536**	**273,522**	**2,724,015**

In table VI are indicated the vessels forming the fleet (usually with two crew members per craft) dedicated to catching pod razor and sword razor clam, according to the Fishing Boat Register in the Autonomous Community of Galicia, Consellería

[6] Data obtained from Plataforma Tecnolóxica da Pesca; www.pescadegalicia.com. On this web page are detailed the catches by fishermen´s associations of each zone.

do Mar, Xunta of Galicia.

Table VI. Distribution of ships dedicated to fishing pod razors and sword razor clams from vessels by the apnea diving system in Galicia.

Area	Nº of vessels
- Ría de Ferrol	**2**
- North West Coast	**31**
- Ría de Muros-Noia	**10**
- Ría de Arousa	**81**
- Ría de Pontevedra	**36**
- Ría de Vigo	**40**

4.2.3. A management model: the group of divers/shellfish gatherers from Cangas fishermen´s association

Extraction areas and sales of pod razors and sword razor shells at fish markets in Galicia (Fig. 5) have two main references: Finisterre, on the Costa da Morte (A Coruña province) and Cangas (Pontevedra province). Earlier the evolution, decrease and situation of fisheries in the Finisterre[7] area was described. Simultaneous to its decrease, from 1993 on the most important fishery of pod razors and sword razor clams (*Ensis* spp.) currently exploited in Galicia was initiated. Located around the Cíes Islands, catches are oriented mainly for fresh consumption and are known and appreciated for their quality (B.I.M., 2005). In this area, for more than ten years, exploitation of these species has been managed by the “Agrupación de Navalleiros de Cangas”, expert divers known locally as “os navalleiros”.

Fishing is around the Cíes Islands at Barra beach in Cangas (42º15´ N - 8º54´ O). The fleet consists of 18 craft with 25 professional divers/shellfish gatherers performing this apnea-diving activity from the craft with two or three “navalleiros” each one. These shellfish gatherers fish following an exploitation plan that controls the catch limit, close season periods and the extraction system. This “management plan” validated by the fishing administration of Galicia, also allows the extractions to be stopped or controlled when prices decrease, rotation of areas, etc. The activity is carried out during two or three weeks, and during this time between 150 to 200 dives are made at 6-10 m deep. With each dive (lasting between 1 and 15 minutes), most experienced divers may collect between 15 and 20 individuals, until they reach the maximum catch agreed with the Exploitation Plan (20 kg/crew member); which may be less, depending on the

[7] These areas (Carnota, Muxía, Corcubión, Cee and Finisterre) were the referent of pod razor shell catches, a position which was lost between 1999-2005, and it is supposed that this was recovered by 2006 (Fig. 6). Also, we must emphasise the quality, from a commercial point of view, that this product has, fished by apnea diving; and it is recognized (July 2007) with label and brand-name to identify them “Galicia Calidade”. These are the first shellfish from Galicia to achieve this sign, which implies it is a quality product as well as being a system for managing the resource.

demand (annual catch is around 13 kg per day, on days when it is possible to fish (from Monday to Thursday). In figure 6 we indicate the evolution of sales of razor clams and sword razor shells at fish markets from both fishermen´s associations with the highest amount of catches: Finisterra and Cangas.

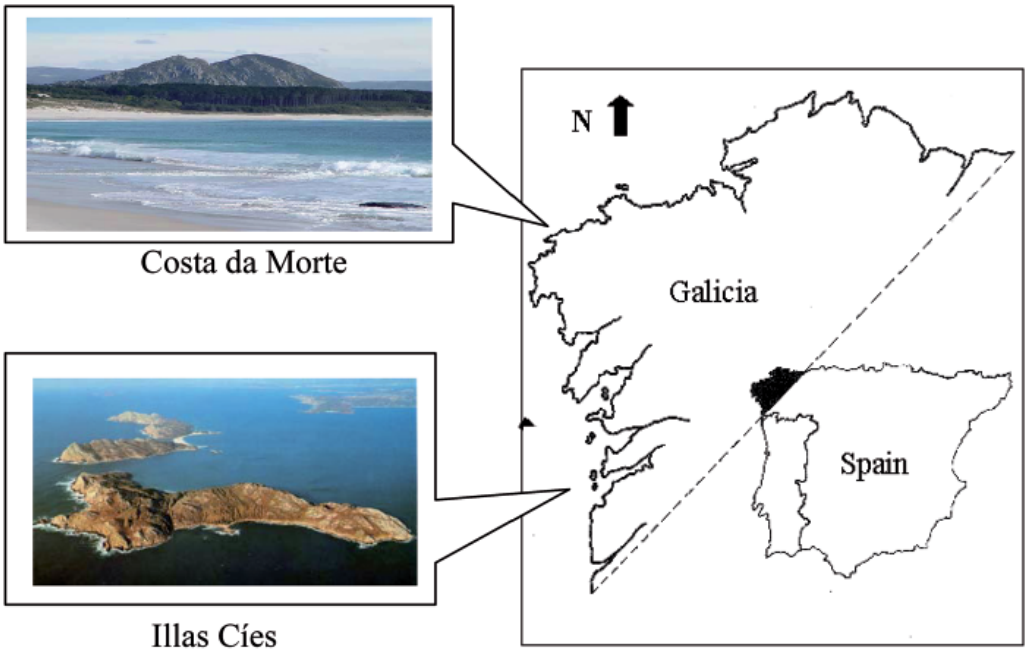

Figure 5. Fishing pod razors and sword razor shells in Galicia has two main references: Finisterre on the Costa da Morte, and the Cíes Islands in the Ría de Vigo. From here more than 95% of *Ensis* spp. species from the Spanish coasts are caught.

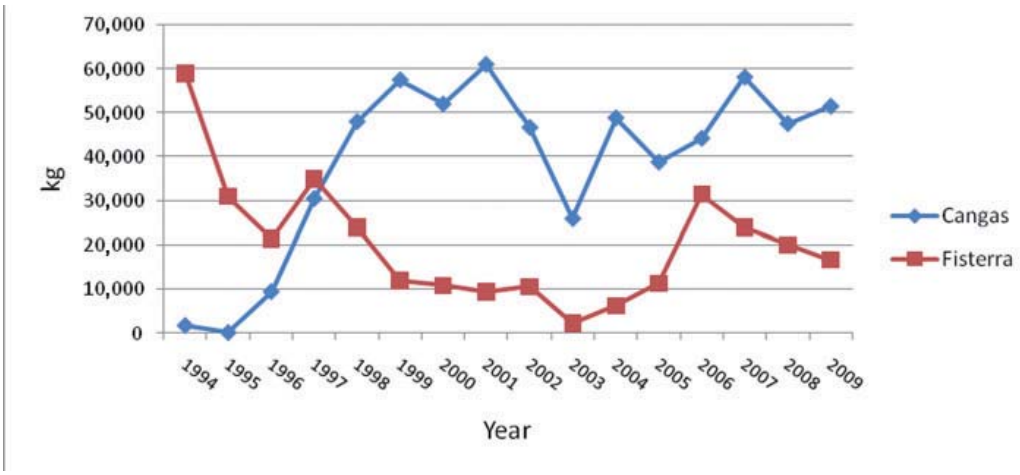

Figure 6. Evolution of *Ensis* spp. sales at fish markets recorded by fishermen's associations with the longest tradition in the exploitation of this resource: Finisterre and Cangas.

Having 2005 as a reference, at the fish market of the Pescadores de Cangas (Fig. 7) fishermen's association, sword razor sales were close to 38 t, which is 2,100

kg/craft/year; and a total sale value of 571,966 €; that is, 31,776 kg/craft/year. Pod razor shell catches were only 2.5% of the total of kg of Solenidae extracted. In this case, sword razor shell has a higher average price: 18.00 €/kg against 15.00 €/kg in pod razor shell.

4.2.4. Production potential in Galicia

Arnaiz (2005), in a study about the state of shellfish gathering in Galicia, mentions the productive potential of commercial molluscs exploited on natural banks of Galician coasts. This author, basing his research on several actual assumptions (sale at fish markets, bank surface area, year of best production, etc.) estimates the potential of the production and the biomass "wasted" by ships. According to these calculations, and in relation to both species (pod razor shell and grooved razor shell), current production, according to the author, amounts to 1.7% and 3.1% of the productive potential. He also mentions, regarding the presuppositions of the research, that the (theoretical) production of both species might be of 2,283 t/year and 1,114 t/year respectively (Table VII).

Table VII. Sales data at fish markets corresponding to the best production year, at the most productive natural banks of these species (adapted from Arnaiz, 2005).[8]

Species	Surface of the bank (m^2)	Fish market sales (t)	Best production year	Average density (ind./m^2)	Expected potential	Wasted potential	% of extracted potential [2]
Sword razor	5,794,455	124	2001	21.37	-	-	-
Pod razor shell	6,431,214	40	1998	6.17	2,283	2,243	1.7
Grooved razor shell	6,384,665	31	1997	4.92	1,014	983	3.1

4.2.5. Fishing gear and systems for extractions and regulations

In Galicia there are several extraction methods and systems, most of the gear coming from the devices used in agricultural activities, which were adapted by shellfish gatherers over the years for extracting infaunal specimens. Arnaiz and de Coo (1977) describe and analyse the evolution and adaptation of this gear to the working area. Sebe and Guerra (1997), value the impact level in substrate, in species to be caught and in related species which are caught using the different gear (sacho, rake, fish

[8] The author compares wasted potential biomass estimated and sales -actual- at fish markets. He also mentions that the wasted potential is the difference between actual sales and the expected potential. There is no reference to an existing production at the bank, but to the one that is not produced due to low densities existing at the banks.

spear, fork, apnea diving, etc.). These authors conclude that “apnea”, the system most used by professional shellfish gatherers, is the most selective one and allows the best regulation of the resource[9] (these aspects are described in detail in Chapter 11).

Figure 7. In the subtidal area of the Cíes Islands (Ría de Vigo), is located the most important natural bank of *E. arcuatus*, which has been exploited for years under sustainable management criteria. Extraction is done by the apnea-diving system. Pictures by J.L. Lorenzo, provided by the San José fishermen´s association in Cangas (Pontevedra).

[9] CA regulation of Galicia, Decree 424/93 (DOG nº 13 dated 01/ 20/94) defines bivalve mollusc fishing gear, among them the specific gear for pod razors and sword razor shells.

5. Marketing and purification

A report by the Irish Sea Fisheries Board (BIM, 2005), gathers information from the "Gloge Trade Information Services database (GTIS)" and indicates that the value of Solenidae (of the *Ensis* genus), imported by the EU, was 550 million euros; Spain represents 43% of this total value of imports, followed by Italy with 8%. Imports from countries outside the Union are becoming more and more important, making up more than 65% of the total in 2004. On the other hand, Spain is one of the main importers of *Ensis* spp as a processed product (mainly canned). 63% of these exports are sent to Italy and Portugal, up to 429 million € in 2004. The origin of imports has been changing during recent years, since the price from several non-European countries may be 50% lower.

The price of the fresh product in the markets fluctuates throughout the year, with increases associated with vacation periods, especially from November to December and falls during summer. This is shown in figure 8, which records the evolution of average prices of pod razors and sword razor shells at fish markets of the Rías Bajas (Rías of Vigo Pontevedra and Arousa).

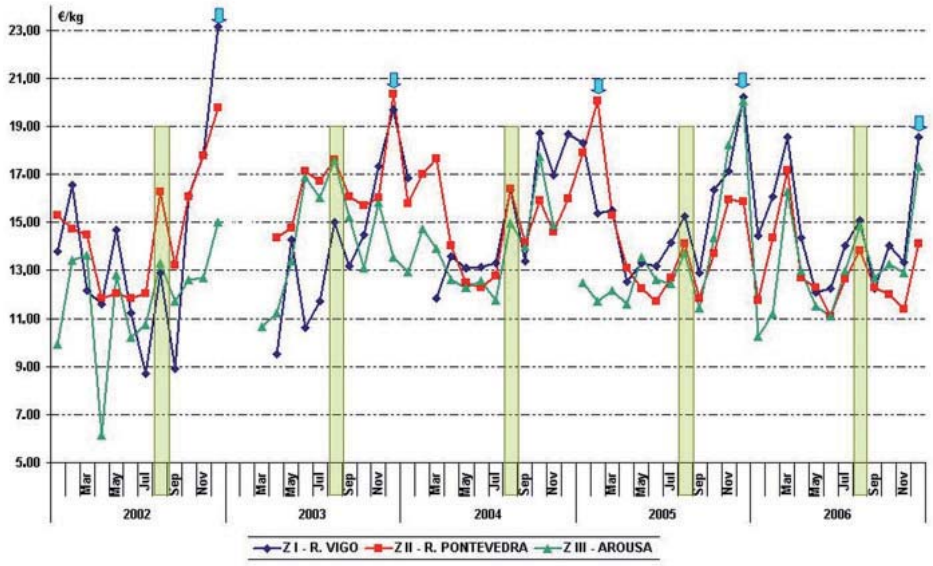

Figure 8. Evolution of average prices (€) at the most important fish markets in the Rías Bajas (Galicia), between 2000 and 2006. The arrows and vertical bars show the increase in prices each year.

The domestic market is mainly oriented to direct consumption of the fresh product and a little less of the processed product (canned and vacuum-packed), imports mainly from Portugal and Netherlands arriving at this market. The origin, presentation and quality of the product determine the fluctuation of prices within wide ranges (B.I.M., *op. cit.*).

In Spain most of the coast where these species are caught is classified as a class B zone (Table II) which means forced purification before consumption. The regular system used for purification of bivalve molluscs at commercial facilities has several problems when applied to Solenidae. The weakness of its adductor muscle means the valves open with ease when they are not buried for a while, accelerating the loss of humidity and death. Several authors (Pyke, 2002; López *et al*., 2005, 2006b) have analysed the systems and methods for achieving more effective purification with less humidity loss, and have proposed good practices for managing these species while they are transported and traded.

López *et al*. (2005) confirmed that keeping the individuals tied in packages of 15-20 specimens (firstly, non-damaged specimens were selected) (Fig. 9) and placed horizontally, makes it possible for them to pass through the purification process with 100% survival and in a good condition for marketing. Several experiences have demonstrated that exact purification periods cannot be established for each species, since there is a great variability, depending on the bacterial load of the specimens, the load of the purification tower and also stabling conditions.

Figure 9. Illustrations. (Above): table with pod razors prepared to be sold at fish market. (Below): purification process and tighter procedures for management and conservation of razor clams.

Packaging systems and transport to points of sale are similar to those in Ireland, Spain and the UK, using boxes of 10-12 kg/box. Keeping them cold allows the specimens to stay alive and ready for consumption within 5 or 6 days.

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Chapter 15: Razor clam fisheries (*Ensis siliqua* and *Solen marginatus*) in Portugal

Miguel B. Gaspar, Rita Constantino and Carlos C. Monteiro

Abstract

Three species of razor clams may be identified off the Portuguese coasts: *Ensis siliqua*, *Ensis arcuatus* and *Solen marginatus*. Among these, only *E. siliqua* and *S. marginatus* are exploited commercially. *E. siliqua* is caught along the coast of mainland Portugal using a dredge, while *S. marginatus* is caught in estuaries and coastal lagoon systems, by fishermen or divers using a metallic rod or salt. In Portugal bivalve dredging is a fairly new activity, having been started by the Spanish dredge fleet at the end of the nineteen-sixties. The great demand for pod razor shell in Spanish markets between 1995-1996 was the reason why the dredge fleet moved a large part of its fishing effort towards *E. siliqua*, impoverishing beds of this species in 1997. As a consequence, the exploitation of this species was prohibited. In the middle of 2001, the fishery was restarted, but under regulations that limited fishing effort. Currently, the dredge fishery is managed by daily quotas, number of fishing days per week, fishing hours per day and a close season. Catching quotas are adjusted every year depending on the conservation status of exploited beds, taking into account the monitoring surveys undertaken by the Portuguese Institute for Fisheries and Sea Research (IPIMAR). Regarding *S. marginatus* beds, their conservation status is indirectly monitored, through small surveys conducted on fishermen dedicated to this activity. Marketing of bivalves is done through authorized dispatch centres, but they may be either directly traded, or traded after a depuration period, depending on the area of origin. Most pod razor shells and grooved razor shell caught in Portugal are exported live to Spain.

In this chapter razor clam fisheries that occur along the mainland in Portugal are described, dealing with subjects such as fishing gear used, catching methods, number of vessels and fishermen/harvesters involved, fisheries management, evolution of landings and marketing. The possibility of implementing a restocking program of pod razor shell and grooved razor shell beds is discussed.

1. Introduction

Catching bivalves in natural beds is a traditional activity whose origins are lost in the mists of time. During the eighteenth, nineteenth and twentieth centuries, down to the end of the nineteen-sixties, bivalve extraction was performed mainly in estuaries (Tajo, Sado and Mira rivers) and coastal lagoon systems (Ría de Aveiro, Óbidos lagoon and Ría Formosa). Exploitation of bivalve beds that occur along the coast, as an industrial activity, is something fairly new. It was started in 1969 by the Spanish fleet, which used to fish off the Portuguese South coast between the mouth of the Guadiana River and Torres Aires, under a border agreement (Sobral *et al*., 1989). This activity, developed by fishermen and initially aimed at pod razor shell (*Ensis siliqua*), did not take long to attract the attention of Portuguese fishermen, first in the Algarve and later on the West coast, where catches with dredges have been reported since 1982 in areas to the North of Aveiro and from 1984 in the area of Setúbal. The relative ease of fishing on the coast and the high productivity of this activity, during a period when pod razor shell demand was rising, led to a movement among the fishing community in 1982 applying pressure for the granting of licenses to catch these species. With no information about the distribution of natural beds and about the abundance of the commercial species, the administration attributed, temporarily (1 year), a specific number of fishing licenses (about 50). Knowing the preferences of consumers and with no perspectives of introducing other species in the Spanish market, the dredge fleet directed the fishing effort to both the surf clam (*Spisula solida*) and pod razor shell (*E. siliqua*). Recently, this fleet has also aimed its fishing efforts at other commercially important species, such as, the wedge (*Donax trunculus*), the stripped venus (*Chamelea gallina*), and the smooth clam (*Callista chione*). It is important to mention that the interest demonstrated by the Spanish market in razor clams, encouraged fishermen, who used to fish in estuaries, lagoons and other coastal lagoons systems to exploit *Solen marginatus* beds in the 1980's. Due to the increasing importance of the bivalve fishery, in 1983 the Portuguese Institute for Fisheries and Sea Research (IPIMAR) started a plan to monitor bivalve beds along the coasts.

Initially, samplings were aimed at gathering information about the areas where the commercial species were distributed, their abundance and about the structure of exploited resources. These samplings have been very important, since they have allowed the establishment of measures for the management and control of fishing, which are fundamental to ensure the sustainable exploitation of resources.

This chapter describes *E. siliqua* and *S. marginatus* fisheries existing in Portugal. It describes catching areas, catching methods, number of vessels and of fishermen/

harvesters involved in this activity, the evolution of landings, marketing rules and legislation in force. The possibility of restocking pod razor shell and grooved razor shell beds is discussed at the end of the chapter.

2. Exploited species and their distribution

Three species of razor clams may be identified off the Portuguese coasts: *Ensis siliqua*, *Ensis arcuatus* and *Solen marginatus*. Only *E. siliqua* and *S. marginatus* are the aim of catches, since the extremely low abundance of *E. arcuatus* does not allow us to speak about the specific exploitation of this species.

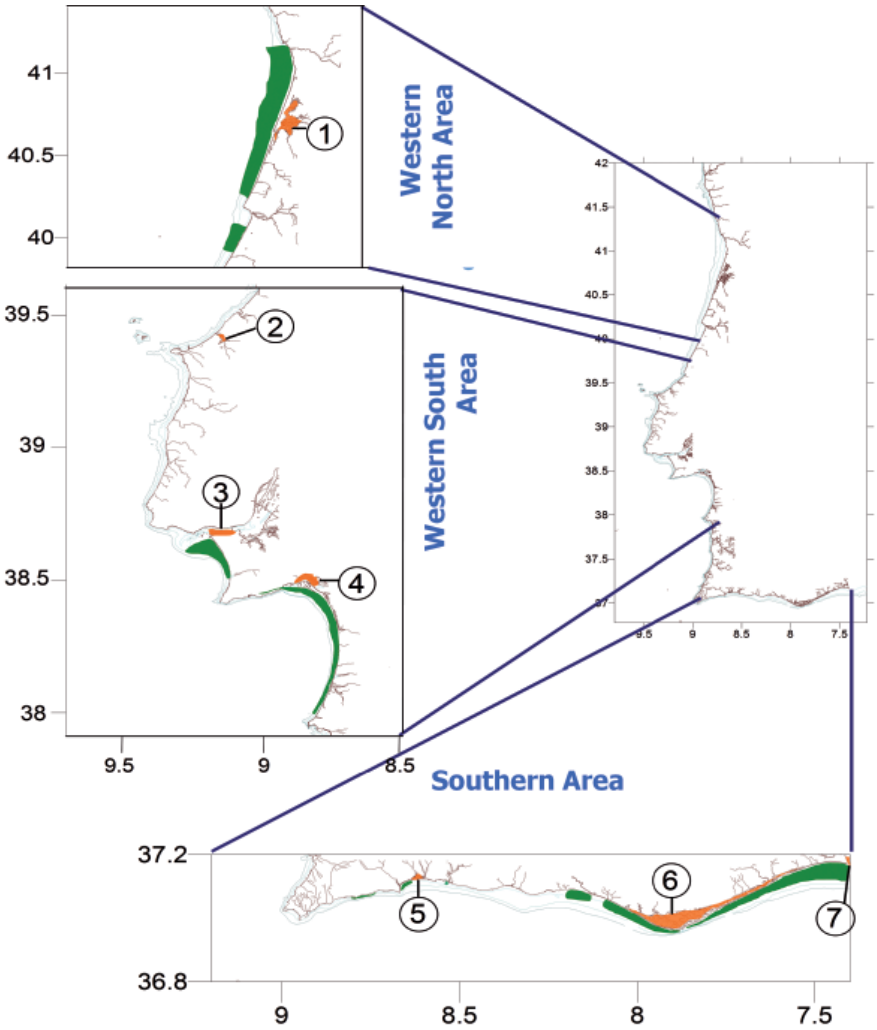


Figure 1. Location of main *Ensis siliqua* (in green) and *Solen marginatus* (in orange) beds on the Portuguese coast. 1: Ría de Aveiro; 2: Óbidos lagoon; 3: Tajo estuary; 4: Sado estuary; 5: Alvôr coastal lagoon; 6: Ría Formosa coastal lagoon; 7: Guadiana estuary.

The distribution of the two species exploited is clearly different, whether bathymetric or by location areas (Fig. 1). While *E. siliqua* may be caught along almost the whole length of the Portuguese coast, at a depth of between 5 and 25 meters, *S. marginatus* is only found in abundance in estuaries and coastal lagoon systems, in intertidal areas as well as in subtidal areas up to 10 m depth. Currently, the main beds of *E. siliqua* are located between the mouth of Douro and Figueira da Foz rivers; between Costa da Caparica and Fonte da Telha; between Comporta and Sines; between Zavial and Salema; between Lagos and Alvôr; between Vilamoura and Olhos d'Água; and between Quarteira and Vila Real de Santo António (Fig. 1). The difference in spatial distribution of both species is related to the type of sediment required by each one. *E. siliqua* is only found on clean sandy beds, while *S. marginatus* occurs in muddy sands, sandy muds or mud bottoms.

E. arcuatus may be caught together with *E. siliqua*, since both coexist in the same natural beds. Nevertheless, its abundance on the coast is very different whether it is to the North or South of Pedrogão's parallel. Recent monitoring samplings, carried out by IPIMAR, demonstrated that *E. arcuatus* is more abundant to the North than to the South of that parallel and catches to the South are just occasional.

3. Catches

As mentioned earlier, exploitation of pod razor shell is carried out in beds always submerged along the coast, whereas grooved razor shells are fished in estuaries, coastal lagoons and other coastal lagoons systems. It is necessary to know the number of vessels and fishermen involved in these fisheries, in order to understand their socio-economic importance in the different areas where this activity is developed.

3.1. Extraction methods

Fishing methods vary depending on the target species and the area in which fishing is carried out (coastal areas or estuaries and coastal lagoon systems). *Ensis siliqua* exploitation is performed with a dredge, a fishing gear that is towed by a vessel, as beds of this species are always submerged. Regarding *Solen marginatus*, the catching method varies, depending whether the beds are intertidal or subtidal. In intertidal areas, fishermen and harvesters use salt or a metallic rod for carrying out their catches, while divers develop this activity in subtidal areas using salt. This kind of activity, called "apanha" (harvesting), are defined as "any fishing method, characterized by being an individual activity, where in general no device made for

this purpose is used, but hands or feet, or maybe an animal, but without causing serious wounds when catching them", according to legislation nº 1102-B/2000, dated 11-22-2000. The fishing gear and methods mentioned has been described in detail in Chapter 11, which deals with the impact of different exploitation techniques on the ecosystem.

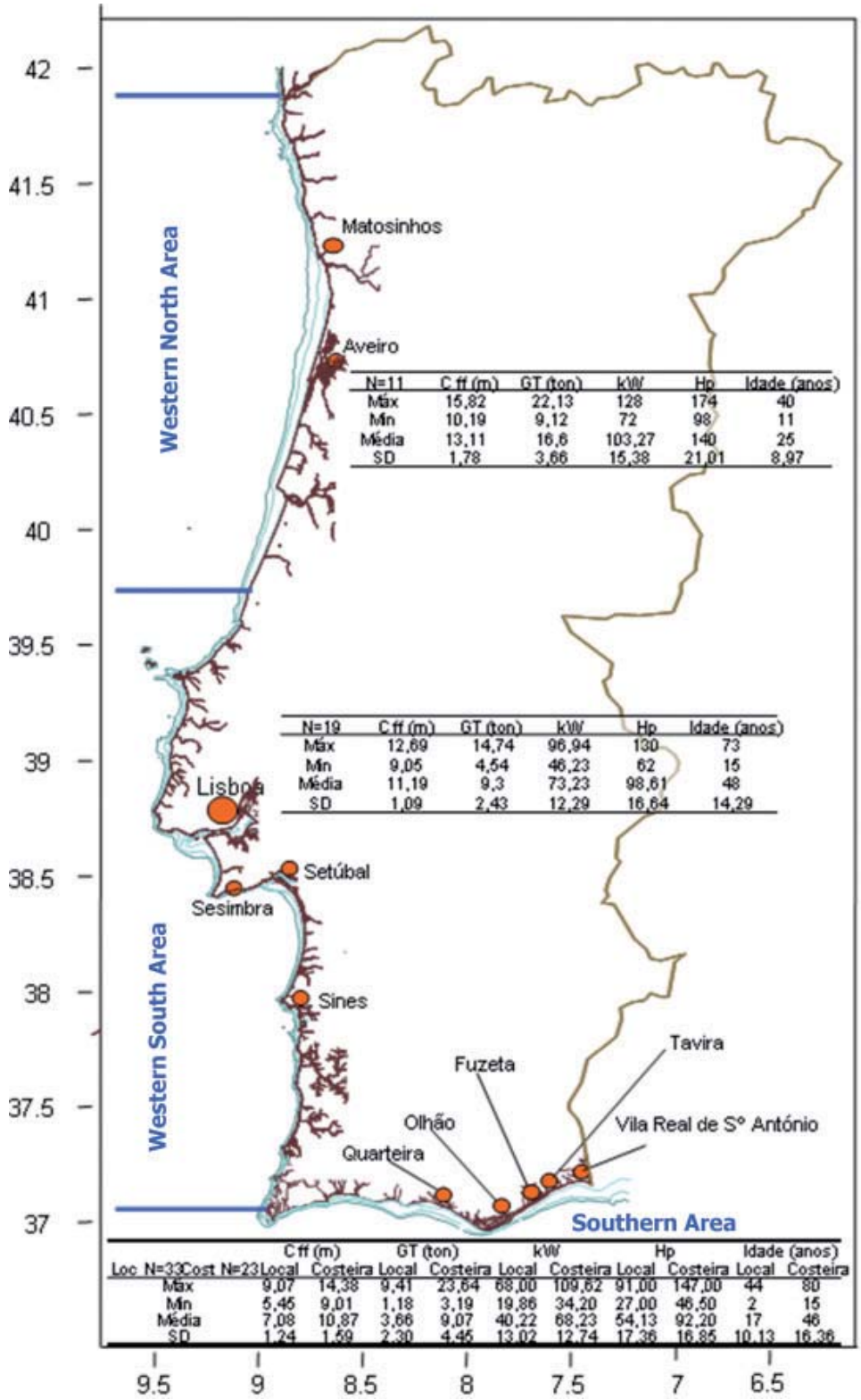
3.2. Catching areas, fish-landing ports and dredge fleet

According to current legislation, the Portuguese mainland coast is divided into three major areas, regarding bivalve dredge fishery (Fig. 3): the North Western Zone (NWZ), from Caminha to the Pedrogão parallel (39º55'06'' N); South Western Zone (SWZ), bounded in the North by Pedrogão and in the South by the parallel crossing through the lighthouse of Cabo de São Vicente (37º01'15'' N); and the South Zone (SZ), bounded on the North by the São Vicente parallel mentioned above and on the West and East by the Portuguese marine territory. These three catching areas have been defined taking into account the coastal morphology, the location of main fish-landing ports and the location of the main bivalve beds.

The dredge fleet is distributed in several fishing ports along the coast. The main bivalve landing ports are: Matosinhos and Aveiro in NWZ; Setúbal, Sesimbra and Sines in SWZ; and Quarteira, Olhão, Fuzeta, Tavira and Vila Real de Santo António in SZ (Fig. 2).

Fishing vessels, depending on their characteristics, may be considered local (EPL) or coastal (EPC). EPL (Fig. 3A) are characterised by having low-powered engines and a maximum overall length (Cff) equal or less than 9 m and/or with an engine power lower than 75 kW (=100 Hp) or 45 kW (=60 Hp), depending if the vessel has a cabin or not. EPC (Fig. 3B) have an overall length higher than 9 m, a gross tonnage (GT) lower than 180 t and an engine power greater than 25 kW (=35Hp).

Regarding the dredge fleet, when comparing the characteristics of the fishing vessels in the three areas, a clear tendency was observed. Indeed, overall length, gross tonnage and engine power of the vessels decreases from the North to the South (Fig. 3), which is related to the harsh hydrodynamic conditions in NWZ, such as strong swell, compared to SWZ and SZ areas. This difference on environmental conditions is reflected in the mean number of working days per year. On 2009, dredge vessels in NWZ fished an average of 81 days, while in SWZ they fished during 108 days and at SZ during 122 days. Other factors, such as bathymetric distribution of bivalve populations and the distance of landing fishing ports with respect to fishing grounds, are also important in explaining the difference of dimensions and of vessels' power in the different areas.



N=11	C ff (m)	GT (ton)	kW	Hp	Idade (anos)
Máx	15,82	22,13	128	174	40
Min	10,19	9,12	72	98	11
Média	13,11	16,6	103,27	140	25
SD	1,78	3,66	15,38	21,01	8,97

N=19	C ff (m)	GT (ton)	kW	Hp	Idade (anos)
Máx	12,69	14,74	96,94	130	73
Min	9,05	4,54	46,23	62	15
Média	11,19	9,3	73,23	98,61	48
SD	1,09	2,43	12,29	16,64	14,29

	C ff (m)		GT (ton)		kW		Hp		Idade (anos)	
Loc N=33 / Cost N=23	Local	Costeira	Local	Costeira	Local	Costeira	Local	Costeira	Local	Costeira
Máx	9,07	14,38	9,41	23,64	68,00	109,62	91,00	147,00	44	80
Min	5,45	9,01	1,18	3,19	19,86	34,20	27,00	46,50	2	15
Média	7,08	10,87	3,66	9,07	40,22	68,23	54,13	92,20	17	46
SD	1,24	1,59	2,30	4,45	13,02	12,74	17,36	16,85	10,13	16,36

Figure 2. Catching areas, main fish-landing ports and characteristics of the dredge fishing fleet by area. Cff: Vessel overall length; GT: Gross tonnage; kW: power of engine (in Kilowatts); Hp: power of engine (in horse power).

Figure 3. Dredge fishing vessels. (A) Local fishing vessels (EPL). (B) Coastal fishing vessels (EPC).

In 2009, the dredge fleet consisted of 86 vessels, where 11 of them perform their activity in NWZ, 21 in SWZ and 54 in SZ. It is worth noting that the dredge fleet in SWZ and NWZ only consist of coastal vessels, while the dredge fleet in SZ comprised 32 and 22 local and coastal vessels, respectively (Fig. 3). All vessels composing the NWZ, SWZ and SZ coastal fleet have a wooden hull and flush deck. In turn, most local vessels have a hull made of fibreglass and are usually open deck. The number of crew members varies depending on the size of the vessel: it could be 3-4 men at EPC and 1-4 at EPL.

It is important to underline that during recent years, the dredge fleet has considerably decreased on the North and South Western coasts, due to the deplection of the bivalve stocks. One example of this, is the drastic decrease in the number of dredge fishing fleet in NWZ, which in 1995 decreased from 90 to 11 vessels.

3.3. Number of fishermen/harvesters and divers

The number of fishermen/harvesters and divers dedicated to catching *S. marginatus* is not known, since there is no specific license for catching this or other species. Currently, in Portugal there is only one type of license aimed at authorizing the hand-picking ("apanha") of a diverse group of species, from polychaetes and gastropods to bivalves such as grooved razor shells. It is estimated that there are around 250 fishermen that, regularly, dedicate their activity to harvest razor clams in estuaries, coastal lagoons and other coastal lagoons systems. However, during summer time, the period when the demand for this species increases, the number of

harvesters may increase significantly.

3.4. Fisheries management

Generally, artisanal fisheries along the Portuguese coast are under national or regional management, not being common resources of the European Union. In this case, they are considered as different population units between countries of the EU, which justifies the inexistence of common regulations for these fisheries. The only guideline for all countries involved relates to the minimum landing sizes.

The legislation that regulates the dredge fishery has been significantly improved in recent years, thanks to research studies that were undertaken concerning biology, ecology, selectivity and fishing impacts on ecosystems (Gaspar and Monteiro, 1998; Gaspar *et al.*, 1994, 1998, 1999; Chícharo *et al.*, 2002; Falcão *et al.*, 2003). These studies were very important, since they allowed the implementation of several technical measures based on scientific information.

In general, bivalve catches are managed through daily fishing quotas per vessel and per species, as well as a close season. Fishing is also limited by the maximum number of licenses issued, by the number of fishing days per week and by the number of fishing hours per day. On the other hand, technical characteristics of the fishing gear have been modified during the last years, adapting them to the bio-ecological characteristics of the target species. In table I we summarised the information related to the legislation in force that regulates the pod razor shell dredge fishery. The exploitation of *S. marginatus* beds by harvesters or by apnea divers is not as regulated as the dredge fishery. Indeed, the catches are not limited by either a daily quota or effort limitations, and there is no closed season in the fishing activity. The only fishing artefact that could be used in the fishery is a metallic rod.

Table I. Razor clam dredge fishery. Legislation in force.

Management measures	Dredge fishery
Characteristics of the fishing gear used:	**Dredge mouth:** Maximum length: 1 m. Maximum tooth length: 550 mm for razor clams. Minimum tooth spacing: 15 mm. **Grid dredge:** Space between the metallic bars: 9 mm for razor clams. **Traditional dredge:** Minimum mesh size: 35 mm (measurement from knot to knot with the mesh straight) for razor clams.
Fishing vessels:	Maximum engine power: a) North Western Zone – 110.3 kW. b) South Western Zone – 95.6 kW. c) South Zone – 73.5 kW. The maximum number of vessels allowed is established every year for each production area, depending on the status of the resources.
Authorized number of fishing days:	**North Western Zone**: a) Five days a week (Monday to Friday). **South Western Zone**: a) Between 5 am and 5 pm. b) Fishing is allowed one tide per day and 6 days a week, between Sunday and Friday. **South Zone**: a) Six days a week (Monday to Saturday). b) Fishing allowed one tide per day between 6 am and 3 pm.
Minimum landing size:	For *Ensis siliqua* and *Solen marginatus* it is 100 mm, measured along the antero-posterior axis.
Daily catch quotas:	Daily catch limits are established every year depending on the status of the stocks. Daily catch quotas are set per species and vessel. The higher the GT, the higher the daily quota.
Restrictions:	It is forbidden to replace the tooth bar by a blade and to use running sledges in traditional dredges. Sorting of the catch and discarding must be carried out immediately after hauling the gear onboard and it is prohibited to discard bycatch into inner marine waters or at fishing ports. Dredge vessels cannot operate at depths less than 2.5 m and less than 300 m from the coastline in concession areas during summer.
Control of the catches:	The dredge fleet that operates in the North Western Area are obliged to land their catches in the Aveiro and/or Matosinhos fishing ports. The dredge fleet that operates in the South Western Area are obliged to land their catches in the fishing ports that exist in the area.
Trading:	All catches must be sold through the wholesale fish market.
Close Season:	Due to biological reasons, a close season is established every year between May 1st and June 15th, for all bivalve mollusc species.

3.5. Production

Pod razor shell and grooved razor shell are caught throughout the year, except during the close season (for pod razor shell) and in periods of contamination by biotoxins (resulting from filtration of toxic microalgae). If that is the case, the exploitation of these species is temporarily suspended.

Figure 4 shows the evolution of pod razor shell and grooved razor shell landings between 1993 and 2009 (Direcção Geral das Pescas e Aquicultura - DGPA), respectively. Between 1994 and 1995, a significant increase was observed in pod razor shell landings, from 684 t to 1412 t. The reason for this increase was the great demand for pod razor shell in Spanish markets in 1995, causing the SWZ dredge fleet to direct fishing effort almost exclusively towards *Ensis siliqua*. This excessive increase in fishing had a dramatic impact on the populations of this species, leading to the depletion of *E. siliqua* stock in 1996. The significant decrease of pod razor shell landings in the South Western Zone, together with the great demand for this species in the Spanish market, led the dredge fleet of the South Zone to increase fishing effort to *E. siliqua* in 1996. Just as happened in the South Western Zone, the pod razor shells beds soon became exhausted (in 1997), due to an uncontrolled exploitation. This is clearly reflected in landings observed during that year, decreasing from 992 t in 1996, to 87 t in 1997 (Fig. 4). Overexploitation of *E. siliqua* beds lead to the need of implementing new management measures (June 1997) in order to promote the recovery of the razor clam beds. Therefore, the fishing of *E. siliqua* was forbidden. So, until 2001 when the dredge fishery for *E. siliqua* was reopened, *S. marginatus* was the only razor clam species landed. In 2001 commercial exploitation was resumed but under regulations that limited the daily catch per boat and species. Since then, pod razor shell landings have been relatively constant, although there has been a slight decrease during the last two years (2008 and 2009) with an average of 106 t/year during the last 5 years (Fig. 4). Landings of *S. marginatus* clams followed the same tendency observed for *E. siliqua* with an increase during 1995 and 1996 followed by a decrease in the next two years. Since 1998, landings of this species have been constant attaining around 24 t/year (Fig. 4). However, it has to be emphasized that a large proportion of catches of this species is not declared.

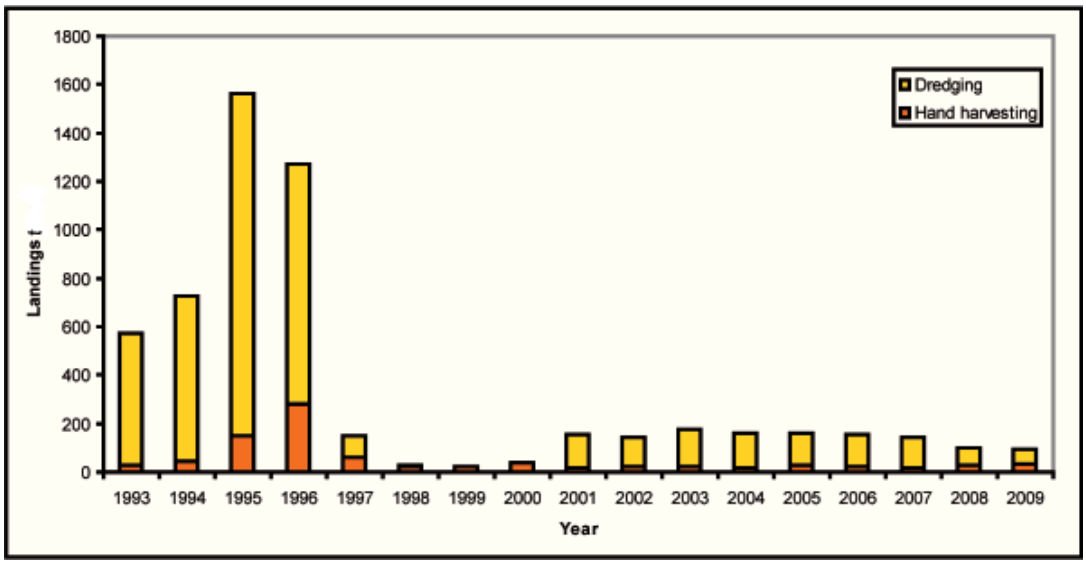


Figure 4. Evolution of pod razor shell (*Ensis siliqua*) and grooved razor shell (*Solen marginatus*) landings between 1993 and 2009 (DGPA), caught by the dredge fleet and by harvesters, respectively.

4. Health regulations for trading

As was mentioned earlier, in mainland Portugal the razor clams landings during the last years have reached an average of 163 t/year. Part of these catches is consumed locally, but most is exported to Spain. Trading these species, just like other bivalves, is carried out through authorized dispatch centres. However, selling them directly in the market of live bivalve molluscs depends on the area in which they were caught. Since razor clams are filter feeders they concentrate contaminants to a much higher level than that of the surrounding seawater where they grow. Therefore, it is extremely important to know microbiological contamination of the water in the areas where bivalve molluscs are caught, in order to minimize possible risks to public health.

Harvested areas where bivalves are caught are classified as A, B, C or D (Table II) (Regulations (EC) Nº 854/2004, dated April 29th 2004; Regulations (EC) Nº 2073/2005 November 15th; Regulations (EC) Nº 1441/2007 5th December and Regulations (EC) Nº 1021/2008 October 17th) based on the extent of contamination shown by analysis of faecal indicator bacteria. In the specific case of pod razor shells and grooved razor shell, dredging and harvesting is only allowed in areas classified as A or B. Bivalves caught in areas classified as A may be marketed directly, since these are areas with low contamination levels. Oceanic beds of *E. siliqua,* which are always submerged, are an example of these areas. If bivalves come from B areas, where the levels of *Escherichia coli* is higher than the minimum allowed for direct

consumption, they will have to be depurated (in order to comply with national and EU rules), and only after that will they be ready for trading. In general, the areas where *Solen marginatus* come from are classified as B, and depurating them is compulsory. However, despite the legal obligations laid down, public health is still at risk, since it is widely recognised that a large number of catches go straight to Portuguese and Spanish restaurants without being depurated.

Table II. EU shellfish harvesting area classification criteria.

Classificatión	Criteria	Trading
Area A	Up to 230 *Escherichia coli*	Direct trading
Area B	Between 230 and 4600 *Escherichia coli*	Depuration for 24 h
Area C	Between 4600 and 46000 *Escherichia coli*	Industrial processing
Area D	More than 46000 *Escherichia coli*	Harvesting not permitted

5. Monitoring bivalve resources

Commercial bivalve stocks are characterized by a great spatial-temporal variability determined both by the success or lack of success of the recruitment and by the fishing effort exerted. Natural repopulation of these resources is important, since these bivalve species are characterized by fast growth and a short lifespan (4-5 years). Thus, in years when the recruitment fails, it is necessary to diminish fishing effort in order to conserve the biomass of the breeding stocks at levels that will allow a swift recovery of the populations in the following years ensuring the sustainability of the fishery on a year-by-year basis. For this reason, every year IPIMAR carries out bivalve surveys along the Portuguese coast in order to assess recruitment and the status of the stocks. With this information, IPIMAR proposes the Administration, the daily quotas that must be assigned for each species and for each fishing area. These surveys are not directed to assess the stocks of *S. marginatus,* since its beds are located in areas with sensitive ecosystems where dredging for bivalves is not allowed. Therefore, other sampling strategies would have to be adopted, which would necessarily involve unduly expensive human resources. Our knowledge of the status of *S. marginatius* beds is indirectly assessed, through confidential enquiries conducted every month among fishermen dedicated to the extraction of this species. With this information it is expected to find out the areas where harvesting has taken place, the number of daily working hours and the total amount caught. However, it is important to note that this survey is not being undertaken on a yearly basis

6. Restocking and/or stock enhancement

Farming bivalve molluscs is a traditional activity in Portugal, but has had an artisanal nature so far (DGPA, 2003). The development of mollusc farming has a strategic importance for the national production sector. The Portuguese coast, and especially the Algarve coast, display the right features for developing this activity that have an enormous growing potential. In this sense, IPIMAR started a group of research projects aiming at: 1) diversifying production; 2) improving seed production techniques (nutrition, health, quality, etc); and 3) developing new production technologies.

Aquaculture, using the artificial reproduction technologies usually applied to bivalves, might have a fundamental role in obtaining the seed for restocking overexploited bivalve beds, as is the case of the razor clam beds. The implementation of restocking and/or stock enhancement programmes are especially important for species that need a long period of time to recover their populations. Experience has taught us that the recovery of bivalve beds, especially of pod razor shell beds, is very slow, and can take between 4 and 12 years. Therefore, restocking programmes may contribute to reduce recovery time. To achieve this, firstly it is necessary to know and to control the life cycle of the species in order to be able to produce large amounts of seed; and secondly it is necessary to define the best restocking strategy. In this context, IPIMAR initiated a project aimed at improving the methodology for the artificial production of bivalve juveniles with economic interest, such as razor clams (*Ensis siliqua* and *Solen marginatus*). This project has the following specific objectives:

- To define the ideal reproductive period of each species, in order to determine the best season for inducing spawning;
- To identify the conditioning methodologies for larval and post-larval farming;
- To develop and/or improve fertilization and incubation;
- To evaluate and compare the larval quality obtained from wild and conditioned razor clam broodstock;
- To determine the effect of different diets on the storage and use of the different biochemical compounds, during larval and post-larval rearing;
- To produce high quality larvae, post-larvae and seeds, in order to guarantee the success of restocking;
- To define the best restocking strategy.

However, we consider that in order to achieve success in introducing a National Program for restocking bivalve beds, it is necessary to involve the sector, not only

by actively participating in restocking actions but also by financing the program. This last point is of utmost importance, since it will enable fishermen to be aware of the co-management they are involved in, and the need to respect the daily quotas limits, which means that they will have to supervise their own activity.

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Chapter 16: Razor clams in Italy

Donatella Del Piero

Abstract

In the past, razor clams *Ensis minor* (Chenu, 1843) and *Solen marginatus* (Pulteney, 1799) represented an important source for clam fishing in Italy. Marked oscillations observed in recruitment as well as in growth rate are currently limiting the importance of *E. minor*, whereas *S. marginatus*, a less valuable species, seems to comprise the bulk of catches, as suggested by the results of surveys carried out in North-Eastern Italy and by the information collected either from the Maritime District Authorities where this activity is carried out, or from market operators. Exploitation by hydraulic dredge faces a decline but, on the other hand, the increase in fishing activity by professional divers entails serious problems in the management of resources.

1. Introduction

Razor clams are widely distributed along the Italian coasts (Bruschi *et al.*, 1985; Poutiers, 1987) and the target species, *Ensis minor* (Chenu, 1843) and *Solen marginatus* (Pulteney, 1799), harvested with hydraulic dredges, are especially appreciated. The trend in the last years has unfortunately been a negative one, because razor clams are not so abundant as before. Until the end of the 1950's both species were harvested by hand at low tide using a special hook (Ghirardelli, 1981). The invention of the hydraulic dredge, a development of the Nantucket dredge (e.g. Froglia and Bolognini, 1987; Froglia, 1989), designed by Mr. Raugna, an Italian engineer, brought new opportunities for the harvesting, not only of *Ensis* and *Solen* razor clams but especially of the *Chamelea gallina* baby clam. *Ensis* and *Solen* have the same common name in the Italian language (i.e. "cannelli", "cannolicchi", "capelonghe" and sometimes "cannolicchio giallo" or "yellow razor clam") but different vernacular names exist in various Italian regions. In North-Eastern Italy, for example in Friuli, *E. minor* is known simply as "capalonga", but *S. marginatus* is named "tabachina" (from the tobacco thrown into sand holes occupied by clams, which is irritating for the molluscs and triggers their rapid emergence). Other vernacular names are: "capa de deo" (*Ensis*, "finger clam") and "capa de fero" (*Solen*, "hook

clam") related to the method of catching, in the tidal area, before the development of mechanical harvesting. Since both species have the same common name, they may be confused in the marketplace, where it is considered that *S. marginatus* is less valuable than *E. minor*.

The scientific names adopted come from indications published, for example in CLEMAM, Bedulli *et al.* (1995), Schiaparelli (2006) and from "Fauna d'Italia" faunaitalia.it, an online site. Other names and authors are found in scientific literature (e.g. Turgeon *et al.*, 1998).

2. Harvested species and distribution

S. marginatus (Pulteney, 1799) -Solenidae- and *E. minor* (Chenu, 1843) -Pharellidae- (Bedulli *et al.*, 1995; Faunaitalia, 2003), Solenidae and Pharidae (Gofas *et al.*, 2001; CLEMAM) are widely distributed along the sandy shores of Italy (Figs. 1 and 2) (Poutiers, 1987; Cesari, 1994; Cesari and Mizzan,1994, and others), with a slight preference for finer sediments observed in *Solen*. Both species have also been reported in lagoon areas (Cesari and Mizzan, *op.cit.*).

Figure 1. Schematic representation of Italy. Source: http://www.mais.sinanet.apat.it/cartanetiffi/ (modified).

Figure 2. Schematic representation of the Friuli coastal area. Source: http://www.irdat.regione.fvg.it/ (modified).

3. Fishing

Since 1986 the minimum legal size has been fixed at 8 cm in length, up from the 6 cm established before. Now the Mediterranean Regulations (European Council Regulation No 1967/2006 of December 21st 2006), in force from June 2010, have created great concern among fishermen owing to the limit set for the distance from hydraulic dredges to the coast at 0.3 nautical miles, so that currently the fleet is banned from the most productive *E. minor* beds, with only professional scuba divers or artisanal operators allowed. Only the adoption of a national plan, approved by the E.C., could modify the present situation.

Due to devolution of powers to fishermen's consortia, clam assessment is a matter for these organizations, so almost every Maritime District has its own regulations, within the framework of national law, depending on local factors. For example, in autumn 2009 and spring 2010 the *C. gallina* fishing was suspended in the Maritime District of Monfalcone due to shortage of the product and poor demographic data found after surveys made in 2009 (Del Piero, unpublished data reported to CO.GE.MO. and the FVG (Friuli/Venezia/Giulia autonomous region)). This means the exact number of dredges exploiting razor clams is almost impossible to calculate. The number of hydraulic dredges counted in September 2009 was 482, GT 6579, Kw 52388 23 (MIPAF). The number of professional scuba divers presently fishing razor clams is also difficult to verify, because a single licence is issued for many species. Nevertheless, some information was obtained from Latium, Civitavecchia. Here, 9 authorizations were granted but none was reported for razor clam; the same took place in Fiumicino, with 3 licences; in Campania, 21 licences were issued by the Naples Maritime District; there were no authorizations in Salerno; in the Marche

region, no scuba divers were authorised (Prof. Piccinetti, personal comment); the region of Veneto has seven licences in the Venetian Maritime District and twelve in Chioggia. In the Friuli region, the Trieste Maritime District granted 4 authorizations in 2008 and three in 2009, but none was reported for razor clam. It is worth noting that a fisherman can obtain a licence in more than one Maritime District.

Fishing, organised on a District basis, is carried out from boats equipped with hydraulic dredges derived from the Nantucket dredge (Froglia, 1989) (Fig. 3), but independently developed in Italy by Mr. Raugna, an engineer from Grado (Italy), in the late 1950's, and soon adopted by regional and national fleets. The most important development occurred at the beginning of the 1980's, when the two pipes conveying the sea water from the pump to dredge nozzles were substituted by a single hose, and the dredge grid was replaced by bars properly spaced (7 mm). This was an extraordinary development because from that time onwards, the bar-equipped dredges performed better in areas where it was previously impossible to fish, due to the muddy sand, opening *de facto* new areas for exploitation. Older banks were in fact nearly exhausted, as indicated by the lower mean size or the rarefaction of clams reported by several papers (e.g. Del Piero *et al.*, 1980, 1989).

Figure 3. Hydraulic dredge. Photo credit: Dr. Francesco Biancuzzi.

Another important point is the damage that these dredges, digging down at least 20 cm in sediment, could cause to the seabed and to the non-target benthic population. Studies realised by Vaccarella *et al.* (1994, 1998) along the Apulian

coast, estimated from thirty to sixty days for recovery, but the debate on this or other seabed gear is far from over (Hall-Spencer *et al.,* 1999). The real size of catches is difficult to estimate: Poutiers (1987) reports several hundreds of metric t in the Adriatic Sea. For the northern Adriatic Sea, data from scientific surveys since 1979 reveal marked oscillations from year to year, with a negative trend (Del Piero *et al.*, 1989; Del Piero, 1994; Goriup *et al.*, 1995).

Valli *et al.* (1982), working on fishing data from 1974 to 1981, reported a maximum of 281,900 kg for *E. minor* in the Friuli region in 1976. Data were based on 88% of the fleet (Marano and Grado), due to the impossibility of obtaining the quantities for the Lignano fleet. It has to be stressed that the data are computed every year from January to December, not for the period of the fishing season (from October 20th to March 15th). The difficulty of obtaining reliable data from commercial fishing (both in terms of quantity and fishing effort) brought about an abandonment of such data collection, and only strict collaboration with fishermen has allowed us to obtain more information about fishing results during recent years. Recent commercial data provided for this research by a fishermen´s association (courtesy of Dr. Anaclerio) is presented in Table I. For locations mentioned see figure 2. It is worth recalling that both *E. minor* and *S. marginatus* are registered under the same common name and that probably the *Ensis* portion is even lower, not so far from data provided by Valli *et al.* (*op.cit.*), when only *E. minor* was present in commercial catches. From 2002 until 2007 there was no hydraulic fishing in the FVG region, though some were collected by hand, which is more difficult to count. The term “by hand” does not mean it is only performed by professional scuba fishermen, but also people fishing by hand from fishing vessels with authorization for other activities, such as trawling. Only from 2008 on (after a brief survey made of fishing beds in October) were some hydraulic dredges authorized to fish razor clams in order to mitigate the crisis in other sectors (*Callista chione* or *Chamelea gallina*). The results of the campaign revealed a fairly good situation both in terms of size and quantity, with maximum density 4.9 ind/m^2 and 55.27 g/m^2 fresh weight, overall mean density 1.7 ind/m^2 (s.d.=1.10) and 18.13 g/m^2 (s.d.=12.80). *E. minor* was the species most heavily fished and individuals’ size ranged from 6.6 cm (young-of-the-year) to 11.9 cm (probably 4+ years) (Fig. 4). Recovery was really impressive and the size spectrum satisfactory, but it is hard to tell whether it was the fishing stoppage or the disappearance of *S. marginatus* (first observed in good numbers in 1994) that was the origin of the recovery, or whether (most probably) both played a role.

Consumers want live clams and there is no market for processed or canned ones. Both species seem to undergo depuration processes, so fishing is allowed only in areas classified by regional agencies, after the national law that came into effect in 1999. Nevertheless, many experiments were conducted to improve

depuration (Dr. Franci, personal comment). The trend in the Italian markets from 2006 to 2008 is slightly positive (Table II) while in 2009 quantities actually doubled. It must be emphasized that market data may not be objective, but the increase is really impressive.

Quantities are given as in the original file, without transformation, and imported clams are reported only for 2009 and were computed in the total. Results from different market typology were pooled. It is still unclear where imported clams come from and, more importantly, which species they belong to. Minimum and maximum prices are reported in ISMEA data. Overall maximum price was 18.02 €/kg observed in 2009 in local fishing.

Table I. Razor clam unloads (kg, hydraulic dredges) at Friuli, Venezia, Giulia (Dr. Anaclerio, Federcoopesca, modified). 2010 quantity refers to the January-March period.

Year	Kg
2008	4,578
2009	3,906
2010	7,330

Table II. Quantities from Italian markets as reported by ISMEA, imported clams in italics, computed in the total (courtesy of Dr. F. Carbonari, modified).

Year	Kg	€/Kg min	€/Kg max
2006	14,169	6.42	16.51
2007	14,447	3.83	15.21
2008	15,597	5.66	16.67
2009	29,851	4.34	18.02
	2,055		

As mentioned before, it is not possible to obtain information about the species actually caught and there is no scientific literature on the topic.

As stated above, fishermen are concerned about fishing by professional scuba divers and they perceive this as unfair competition, since it is more difficult to control. Licences are granted by maritime offices on a local basis, and fishing must

be performed according to the laws other fishermen are subjected to. Individual quantities are not fixed, but 40 kg/day, at least, seems reasonable, as many Maritime Districts officers confirmed. The number of licences may vary from year to year and the target species, at least in North Eastern Italy, is *S. marginatus*, whilst *E. minor* is very rare. Since both species are reported as "cannolicchi" there is no quantification of the amount of each one. The prevalence of *S. marginatus* ("cannolicchio giallo" / yellow razor clam) was confirmed by the officers interviewed in Tuscany, Latium and Campania.

Table III. Regional razor clams catches (Kg), as reported by Dr. Labanchi (IREPA) from 2006 to 2009 (from the original table in Italian, modified) (Source: Osservatorio Mipaaf-Irepa).

Market	2006 Kg	2007 Kg	2008 Kg	2009 Kg
Latium	90,321	33,202	36,214	106,079
Campania	36,137	148,271	94,347	124,748
North Apulia	54,429			39,547
Molise			14,544	12,451
Veneto	95,348		64,902	79,997
F.V.Giulia	15,410			894
Italy	291,645	181,473	210,008	363,716

4. Italy: An overview

Recovery among *E. minor* (at least in the North Adriatic Sea) was observed since a survey done in 2008, which apparently started in 2007, because in July 2006 an *ad hoc* survey done by fishermen brought very poor results. So the *S. marginatus* cycle, which started in 1993 and was first described by Gorgato (1998-1999) and afterwards by Cociancich (2000-2001) for the Friuli and Veneto regions, respectively, seems to be closed for now.

Reports from other regions (partly from Relini *et al.*, 1999). Regions indicated in figure 1.

4.1. Latium

Regarding the ICRAM report released in 2002, the presence of *Ensis* and *Solen*

is most noteworthy between 2 and 4 m deep and a dramatic drop in commercial fishing is noted, while previous papers (A. Ge. I.,1995) reported a yield of about 15.3 kg/h in the southern part of the region and (Mariani, 1999) a density between 0.97 and 1.43 ind/m^2 for *E. minor*.

4.2. Campania

Catches increased in 2005, mainly because fishermen from the Naples area were fishing razor clams: biology, aquaculture and fisheries-authorized fishing in the Salerno maritime district (IREPA, *op. cit*.).

4.3. Sicily

Rinelli *et al.* (2001) collected samples along the Southern Coasts of Sicily, but estimates were not very encouraging for commercial use, even though data reported from Costa *et al.* (1987) indicated that *Ensis* could be exploited (due to the high market value) at fairly low density (e.g. 10 g/m^2).

4.4. Apulia

Vaccarella *et al.* (1996) reported a maximum above 100 kg/1000 m^2 in the maritime districts where the species are found (Termoli and Manfredonia).

4.5. Molise

Fishing started in 2008 with positive results.

4.6. Marche

In the past there was a flourishing fishing industry, but currently neither fishing nor specific scientific research are realised (Froglia, inf. pers), and razor clams (*Ensis* spp. and *S. marginatus*) are only mentioned as bycatch and discards (Morello *et al*., 2005).

4.7. Veneto

The stock was examined, with annual sampling from 1997 to 2002. Fishing was suspended for many years, due to fishing of the more attractive *Chamelea*. Surveys

reveal an important recruitment for both species and a progressive decrease of *E. minor*, replaced by *S. marginatus*, particularly in the northern part of the Venice Maritime District. The process seems to be the same in the region of Friuli. The highest density sampled (1.56 ind/m^2) was recorded in the central-northern part of the district for *S. marginatus* during the 2002 campaign. There is no official news about fishing activity in the Venice lagoon, where both species are reported, and it is important to point out that Manila clam activity is the focus of business in this lagoon, legal or otherwise.

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Chapter 17: Razor clams in the Hellenic Seas

Sofia Galinou-Mitsoudi

Abstract

The presence of shellfish has been reported since the time of the ancient Greeks, among them razor clams. Currently, six species are recorded, namely: *Solen marginatus* (Pulteney, 1799), *Ensis arcuatus* (Jeffreys, 1865), *E. ensis* (Linnaeus, 1758), *E. minor* (Chenu, 1843), *Pharus legumen* (Linnaeus, 1758) and *Phaxas pellucidus* (Pennant, 1777). We will present the distribution and the state of commercialisation of these species in the Hellenic seas. Two of the six razor clam species (*S. marginatus* and *E. minor)* are officially commercialised as bait, and their fishing is regulated by national legislation. Currently, the profits made from harvesting razor clams are very small, due to the limited interest of the Hellenic market and, consequently, the low price of the product. Given these circumstances, razor clam exploitation is still a secondary activity for stakeholders.

Future research and management plans for the development of razor shell populations could be a challenge for the development of sustainable exploitation of razor clams.

1. Introduction

In Greece, razor clams are among the best-known bivalve species. These shellfish were recorded and described under the name *sōlēn* (=σωλήν) for the first time in zoological works by Aristotle in the 4th century B.C. (Voultsiadou and Vafidis, 2007; Voultsiadou *et al*., 2010). Today, they are known as “solenes” (=σωλήνες = tubes) or “ammosolenes” (=αμμοσωλήνες = tubes of the sand). Currently, razor clams are used mostly as bait and not exploited as edible species.

Five razor clam species have been recorded in the Hellenic seas (Zenetos *et al*., 2005; Manousis *et al*., 2010; CLEMAM - June 2010): *Solen marginatus* (Pulteney, 1799), *Ensis arcuatus* (Jeffreys, 1865), *E. ensis* (Linnaeus, 1758), *E. minor* (Chenu, 1843), *Phaxas pellucidus* (Pennant, 1777) and also *Pharus legumen* (Linnaeus,1758)

(Delamotte and Vardala–Theodorou, 1994; pers. obs.). Although the market supplies razor clam species without stating their scientific names, there are only two official species of commercial bait: *Solen marginatus* and *Ensis minor*.

This chapter is the first to focus on an individual commercial Hellenic shellfish, the razor clam, and its purpose is to gather together and review the available scientific information, and also to provide recent personal observations on shellfish fishing and distribution in the Hellenic Seas, for scientific fishery management purposes as well as for the protection of razor clam populations.

2. Species harvested and their distribution

On either side of continental Greece lie the Aegean Sea (to the East) and the Ionian Sea (to the West). The Greek coastline is very extensive, due to the many gulfs and over 3,000 islands, and it is in the main gulfs where the deltas of small or large rivers and/or wetlands are located. This kind of coastal environment is the habitat for razor shells, as indicated in figure 1. Most of these coastal areas of Alexandroupolis (Evros river estuaries), Porto-Lagos, Kavala (Nestos river estuaries), Thessaloniki and Thermaikos Gulfs (Axios, Loudias, and Aliakmon river deltas), N Patraikos Gulf (Mesologi lagoon) and Amvrakikos are wetlands of international importance and protected by the Ramsar Convention. Hellenic razor clams and their geographical distribution are listed in the following section.

2.1. *Solen marginatus*

The presence of this species has been reported on the coasts of the Peloponnesus (both in the Ionian and Aegean Seas) and in the Amvrakikos, Thermaikos, Pagasitikos and Saronikos Gulfs (Zenetos,1996; Zenetos *et al*., 2005; Katsanevakis *et al*., 2008; Manousis *et al*., 2010). After personal observations *in situ* in March 2010, it was reported that *S. marginatus* seems to appear regularly, and its distribution in Hellenic waters is widespread, as the species was found on most coasts of the NE Aegean (Alexandroupolis, Porto-Lagos, Strymonikos, S Lemnos Island-Moudros) and in other places in the Thessaloniki Gulf, such as NW Thessaloniki Bay (Kalochori), NW Thessaloniki Gulf (Kavoura-Leukoudi), and SW Thessaloniki Gulf (Ajia Triada) (Fig. 2).

2.2. *Ensis ensis*

In the Hellenic Seas, *Ensis ensis* is a relatively rare shellfish. It has been reported

in Saronikos, Patraikos in the SE Ionian Sea (Katakolo), N Evoikos and Pagasitikos Gulfs, as well as in the Thermaikos and Thessaloniki Gulfs and in two islands of the NE Aegean Sea (Sakellariou, 1957; Zenetos, 1996; Zenetos *et al.,* 2005; Manousis *et al*., 2010). This species has also been found close to estuaries and lagoon areas (personal observation): Strymonikos Gulf (Asprovalta), E Thermaikos (Potamos Epanomis), and the NE Ionian Sea (Hgoumenitsa) (Fig. 3).

Figure 1. Hellenic biotopes of razor clams. 1: Alexandroupolis (Evros River Delta), 2: Porto-Lagos (lagoon), 3: Gulf of Kavala (Nestos River Delta and lagoons), 4: Strymonikos Gulf (Strymonikos River estuaries), 5: Thessaloniki Gulf, 6: Thermaikos Gulf (Axios, Loudias, Aliakmon Rivers Deltas and lagoons), 7: Pagasitikos Gulf, 8: N Evoikos Gulf, 9: Saronikos Gulf, 10: Katakolo, 11: Patraikos Gulf (South) and Mesologi lagoon (North),12: Amvrakikos Gulf (lagoons), 13: Hgoumenitsa (Kalamas River Delta), 14: Samothraki Island, 15: Lemnos Island, 16: Kaloni Gulf (Lesvos Island) and 17: Island of Crete.

E. arcuatus (Jeffreys 1865) is another razor clam species similar to the one previously mentioned, but shorter (15 cm) and more banana shaped (both margins

are curved) (www. seawater.no/fauna). The dorsal margin is almost straight while the ventral margin is curved.

In the Hellenic Seas, *E. arcuatus* has been reported by Zenetos (1996) and Zenetos *et al*. (2005) from the Island of Crete, the Gulfs of Saronikos, Pagasitikos, Thermaikos and Thessaloniki as well as the SE Ionian Sea.

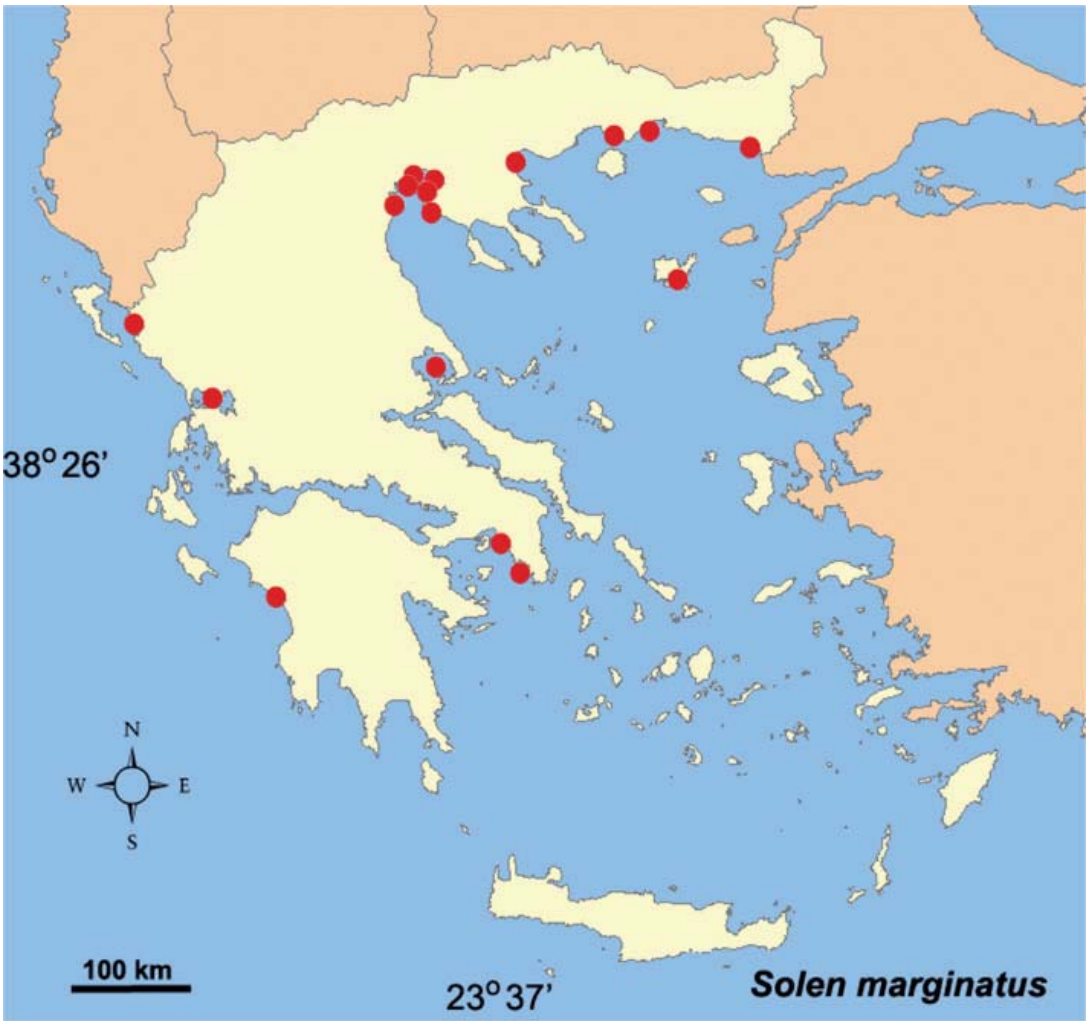

Figure 2. Geographical distribution of *S. marginatus* in the Hellenic Seas.

2.3. *Ensis minor*

In the Hellenic Seas, *Ensis minor* is a fairly common shellfish and has been reported in the Island of Crete, Saronikos and Pagasitikos Gulfs as well as in Thermaikos and Thessaloniki Gulfs (Katsanevakis *et al.,* 2008; Manousis *et al*., 2010). After personal observations *in situ*, this species has been recorded close to estuaries, mainly at the western sites (towards the freshwater and current directions) and/or lagoon areas of Alexandroupolis, Porto-Lagos, Strymonikos Gulf (Asprovalta), SW Gulf of Thessaloniki (Ajia Triada), and NW Thermaikos Gulf (Kitros) (Fig. 4).

3. Fisheries

According to razor clam species' distribution so far recorded in Hellenic waters (Figs. 1 - 4), the main biotopes of the commonest razor clams are coastal zones with fresh water currents, with or without lagoons, and wetlands. We must bear in mind that the main current directions are from E to W. Habitats of this type are situated in the North Aegean Sea, including the Thessaloniki and Thermaikos Gulfs, as well as the Saronikos and Amvrakikos Gulfs, in central Greece and the Ionian Sea, respectively. Consequently, razor clam fishing activities are carried on in these places.

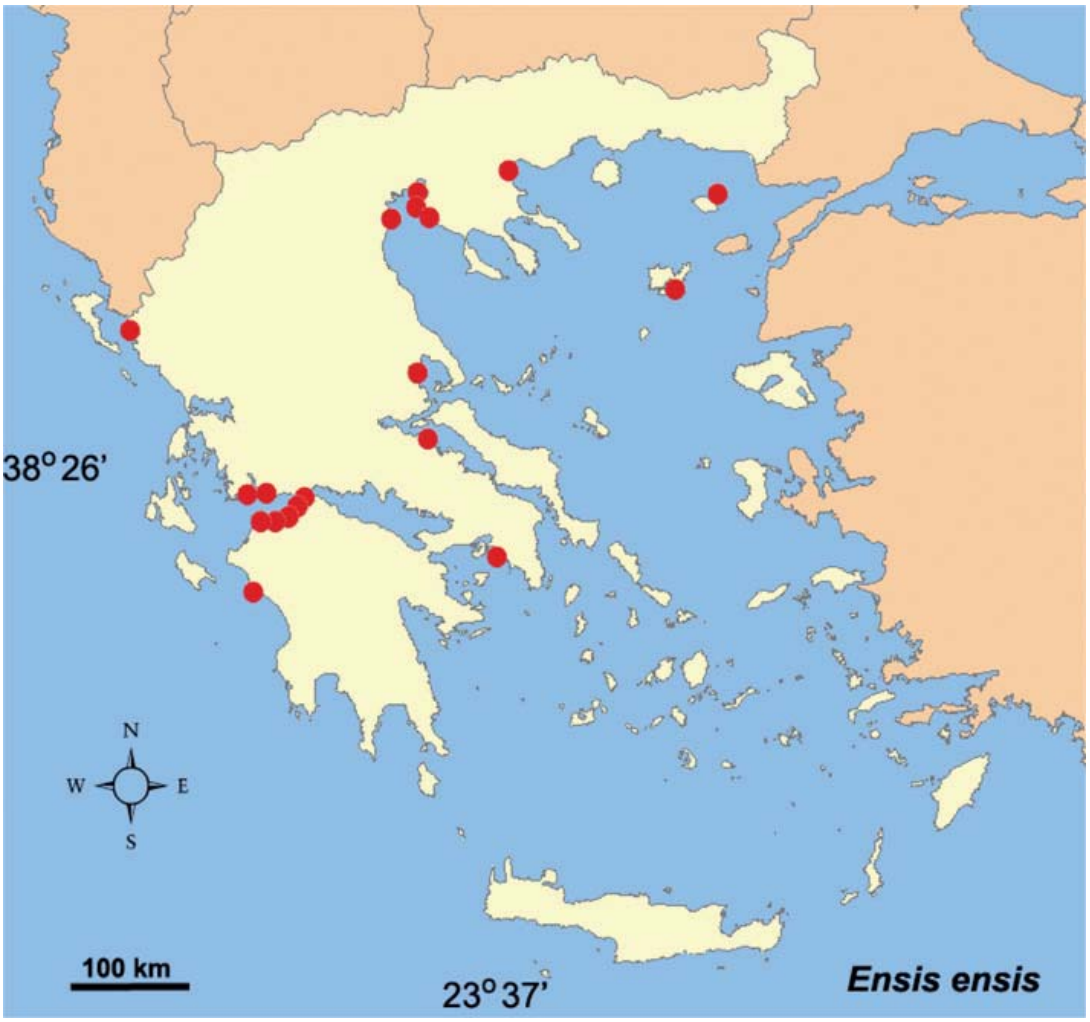

Figure 3. Geographical distribution of *E. ensis* in the Hellenic Seas.

Although the razor clam species are well known, interest in them only emerged in the 1990's and still focuses on the two biggest and commonest species, *Solen marginatus* and *Ensis minor.* Fishing of these species has been regulated by national

legislation since 2002 (PD 109/2002), which concerns *E. siliqua* rather than *E. minor.* Exploitation of both razor shell species (*S. marginatus* and *E. minor*) is for human consumption and (officially) for fishing bait. Professional fishermen may belong to local Alieutic associations for bait fishing, but work independently. After harvesting, both local, national and even international bait markets are supplied through specific bait mongers or fishmongers.

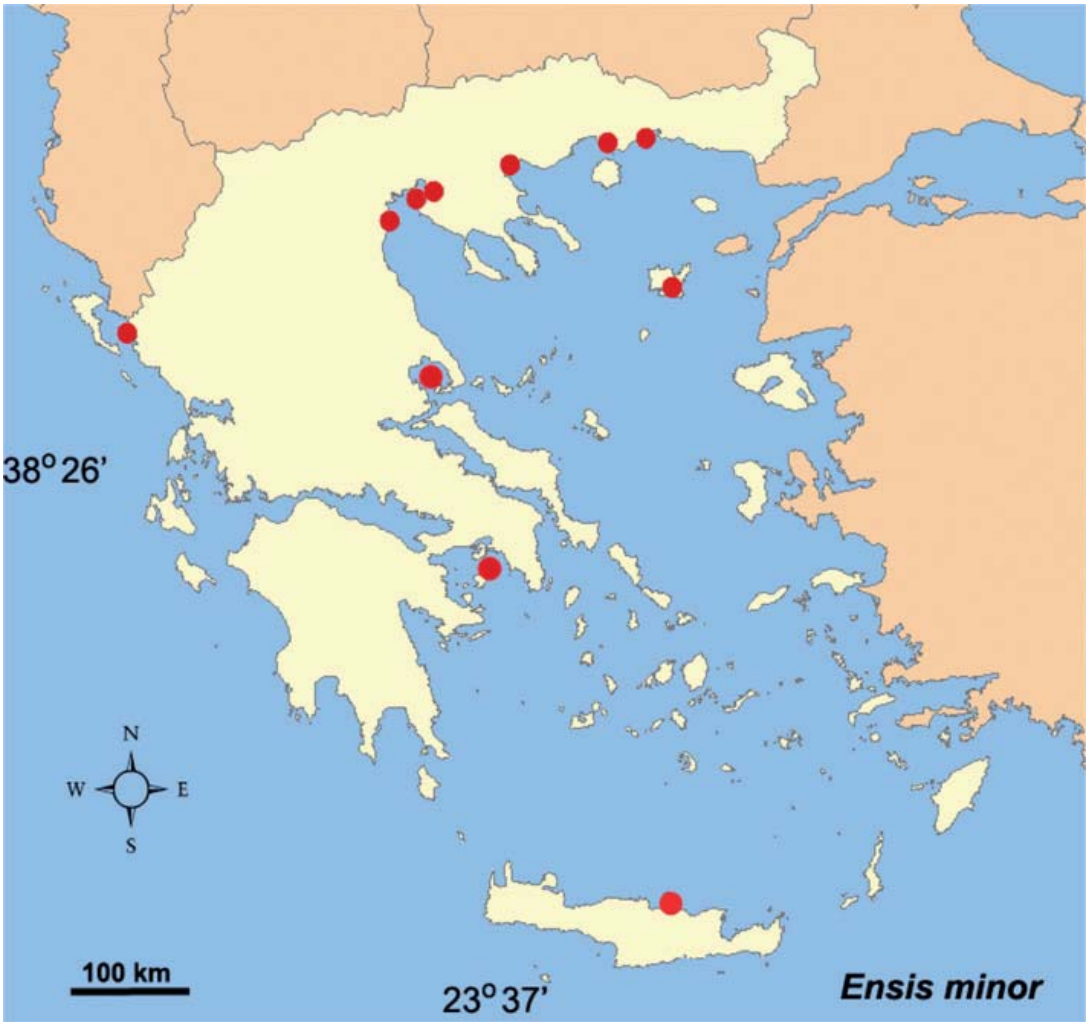

Figure 4. Geographical distribution of *E. minor* in the Hellenic Seas.

3.1. Harvesting methods

Traditionally, fishermen have collected razor clam during ebb tides in midlittoral zones using shovels. According to PD 109/2002, fishermen have to fish using boats, with or without a scuba-diving system with continuous air pumping (Fig. 5), and with the officially permitted gear for professional fishing as in the following description:

- Shovels up to 25 cm long and 12 cm wide in their metallic part and a 110 cm long shaft.

- Forks with up to 7 metallic teeth, up to 20 cm long and 16 cm wide, and a 110 cm long shaft.
- Spatulas with metallic parts up to 15 cm long and 10 cm wide and a 15 cm long shaft.

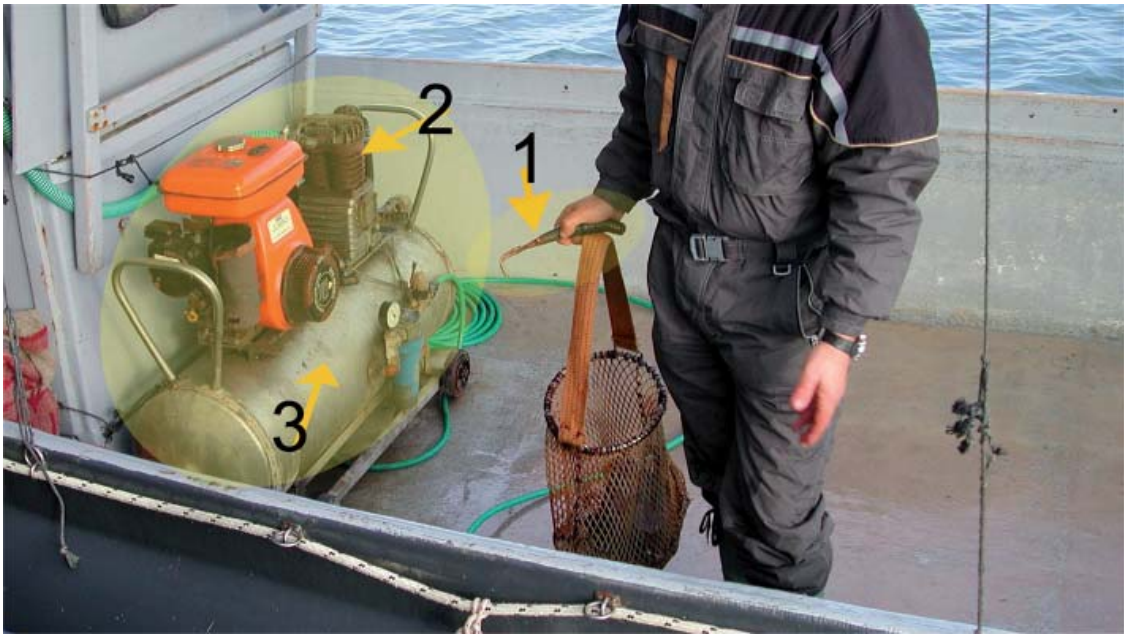

Figure 5. A professional fisherman's continuous air pumping system. 1: fork-type handmade fishing gear, 2: air compressor, 3: air chamber.

The gear mentioned is rather ineffective, due to razor shells' fast and deep penetration into the substrate and so, alternatively, fishermen prepare handmade gear like that in figure 6. Fishermen look for razor clam siphon holes on the sea bottom (Fig. 7a), sink the gear into the substrate (Fig. 7b), and the shellfish, irritated by the metallic "invader" close their valves, "trapping" the gear; fishermen can then remove the shellfish from the substrate (Figs. 7c-e).

Figure 6. Handmade fishing gear specific for razor clam collection (made by diver Manolis Kazlaris).

Figure 7. Razor clam fishing in shallow waters: a) looking for siphon holes, b) sinking the gear into the hole, c-e) a trapped razor clam.

3.2. Production data: evolution

To date, there are no official data available for razor clam production, since bait fishermen are very few, and their production is scarce, cheap and supplementary. Currently, the priority for bait fishing is the harvesting of polychaete worms, sipunculids and the *Solecurtus strigilatus* species of bivalve baits.

Main distribution periods of bait to markets are holidays and/or weekends spent doing recreational fishing. Nevertheless, the current economic crisis affects the bait market, and it is believed that the crisis will produce a reduction in recreational activities, and consequently bait disposable in the marketplace for fishing.

3.3. Exploitation management

Exploitation management of razor clams is regulated by some rules (spatial, temporal, biological, social, etc.), and according to PD 109/2002 for bait fishery, the following are included:

- Fishing with scuba diving and continuous air pumping is forbidden from July 1st to October 31st.

- The schedule for daily fishing is from one hour after sunrise to one hour before sunset.
- The fishing of razor shell species is restricted to professional fishermen.
- Minimum commercial length of the species is 8 cm.
- Maximum number of shellfish/day to be collected is 200 individuals/species.
- Fishing of shellfish is forbidden in ports and harbours, at a distance <150 m from aquacultures, <500 m from semi-permanent fishing gear (i.e. pound nets), lagoon mouths, or extensive/semi-intensive aquaculture.

The General Fishery Department of the Ministry of Rural Development and Food, together with the peripheral departments in each municipality, prepare the exploitation management plans to be established as legislation, while the local port authorities are responsible for legal fishing.

3.4. Production economy

The few collectors of razor clams work mainly in NE Greece (Alexandroupolis, Porto-Lagos, Kavala) and the Ionian Sea in the Amvrakikos Gulf. Most of these fishermen seem to prefer the collection of razor shells to be as cheap as possible; hence they fish in the midlittoral zone up to a depth of one fathom without expensive equipment, in order to diminish production costs.

What is harvested is merchandised in trade centres for selling raw or frozen. The product is very cheap, as already mentioned (~1.5 €/20 individuals), and consequently interest is very small.

4. Sanitary control regulations

Bivalves, due to their bioaccumulation ability, could be bioindicators for environmental quality, the existence of biotoxins, and the hygiene and safety of seafood for human consumption. Since 1980, mussels are among the organisms most used as bioindicators of marine pollution, such as *Mytilus californianus* (Gordon *et al*., 1980), *M. edulis* (Bourgoin, 1990), *M. galloprovincialis* (Regoli and Orlando, 1993; Catsiki and Florou, 2006). Later, additional bivalve species were proposed as bioindicators such as the razor clam *S. marginatus* (Thompson *et al.,* 1999).

Most densely-populated biotopes of Hellenic razor clams are affected mainly by agricultural, industrial and urban activities, and consequently different types of

pollutants bio-accumulate in bivalves' body. Since in Greece the razor clam species are only for use as bait, hygiene control is a secondary priority. Nevertheless, in some traditional local taverns they are occasionally served as relishes.

5. Monitoring wild beds

Assessment of the razor clam population has not been conducted in the Hellenic Seas, since published information about commercial or protected bivalve species populations is rather limited (Katsanevakis *et al*., 2008).

Currently, the low commercial interest in razor clams, low market prices and the non-existent demand for these species, whether cooked or not, in any kind of outlet, are discouraging both the authorities responsible and relevant researchers from investigating the dynamics of the natural razor clam population.

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Chapter 18: Overview of the state of the razor clam (*Solen marginatus* and *Ensis minor*) in Tunisia

Nadia Ayache, Leila Hmida, Chiheb Fassatoui and Mohamed Salah Romdhane

Abstract

During the last decade, there has been a significant increase in the interest of countries such as Spain and Portugal in razor clam production. In Tunisia, such interest is manifested in razor clam research projects carried out in order to study the biology, ecophysiology and life cycle control of these bivalve molluscs, projects which can lead to rational exploitation and aquaculture development. A few studies have reported the presence of two species on our coasts: *Solen marginatus* and *Ensis minor*.

Recently, *S. marginatus* has figured in the list of edible bivalves with interest for potential exploitation. Nowadays, some field prospection is being done alongside research into the reproductive cycle, geographical distribution and relative density of *S. marginatus* along the Tunisian coasts. These preliminary studies show a continuous reproductive cycle with a short resting period and a long reproductive period (gametogenesis and spawning). High density was recorded in the Gulf of Tunis, reaching a maximum of 25 specimens/m². Reparation of the relative density of *S. marginatus*, in the Gulf of Gabes, are in progress, where the highest density on Tunisian coasts has been recorded, with more than 30 specimens/m².

1. Introduction

Razor clams are a species with an extensive geographical distribution (Fischer *et al.,* 1987). In Tunisia, only two species, *Solen marginatus* and *Ensis minor*, have been widely reported as being present.

Razor clams are usually present with relatively high density in the areas of the Bizerte Lagoon, Gulf of Tunis (Northern Tunisia) and Gulf of Gabes (Southern Tunisia) for *S. marginatus* (Marzouki, 2006), and in the Gulf of Tunis and Bizerte Lagoon for *E. minor* (Ayari and Afli, 2005).

In Tunisia only three species of bivalves are exploited: *Ruditapes decussatus*,

Mytilus galloprovincialis and *Crassostrea gigas*. Recently, some others (*Tellina planate, Mactra glauca* and the razor clam *S. marginatus*), have appeared among edible bivalves of great economic interest. A few studies have been performed to identify the growth and reproductive cycle of *S. marginatus* and its stock assessment in the Gulf of Tunis (Charef, 2006) and Gulf of Gabes (Ayache, 2008; Hmida *et al.*, 2010). Other ongoing studies will report on the distribution and density of other species of razor clam.

2. Distribution of species

Research studies have reported the presence of *Ensis minor* in the area of the Bay of Tunis and Bizerte Lagoon (Northern Tunisia) (Ayari and Afli, 2005, Fig. 1). According to some fishermen, density was approximately 2 to 7 individuals/m²; *S. marginatus* is more common in the areas of the Gulf of Tunis (Northern Tunisia) and Gulf of Gabes (Southern Tunisia) with a density of 27 and 15 specimens/m² respectively (Marzouki, 2006) (Table. I).

Table I. Main density of *S. marginatus* in the Gulf of Gabes.

Location	Geographical coordinates	Characteristics	Density
Port of Zaboussa	N 34.21.000 E 10.12.841	Sand/muddy sediment; prohibited zone, great diversity of fauna	≈30 specimens/m²
Oued Maltine	N 34.25.12 E 10.20.36	Muddy sediment, Fragments of shells, (clams and cardium), high diversity of fauna, (gastropods and bivalves)	≈20-25 specimens/m²
Sidi Hmed	N 34.28.130 E 10.24.941	Sand/muddy sediment densely overlaid by macroalgae	≈1-10 specimens/m²

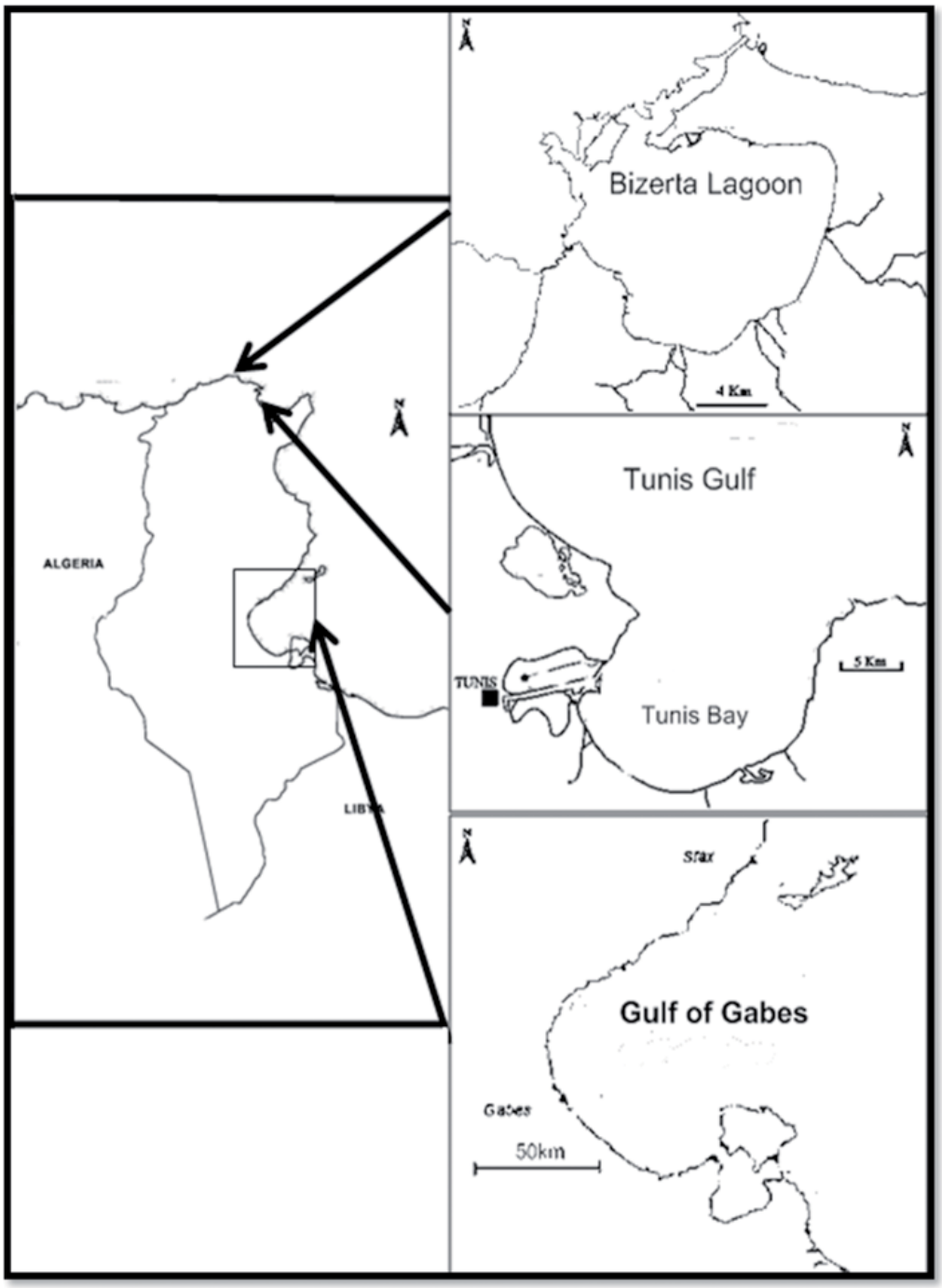

Figure 1. Common distribution of *S. marginatus* and *E. minor* on Tunisian coasts.

The density of *S. marginatus* reaches 22 to 26 specimens/m^2 (125-200 g/m^2) in the southern part of the Gulf of Tunis (Fig. 2) and a density of between 7 and 30 (25-125 g/m^2) specimens in the northern part of the Gulf of Gabès (Fig. 3). The highest density is observed in river estuaries (Mejerdah, Meliane and Abid) (Charef, 2006). The presence of razor clam on the East coast has been reported (Monastir and Hergla), but with a lower density.

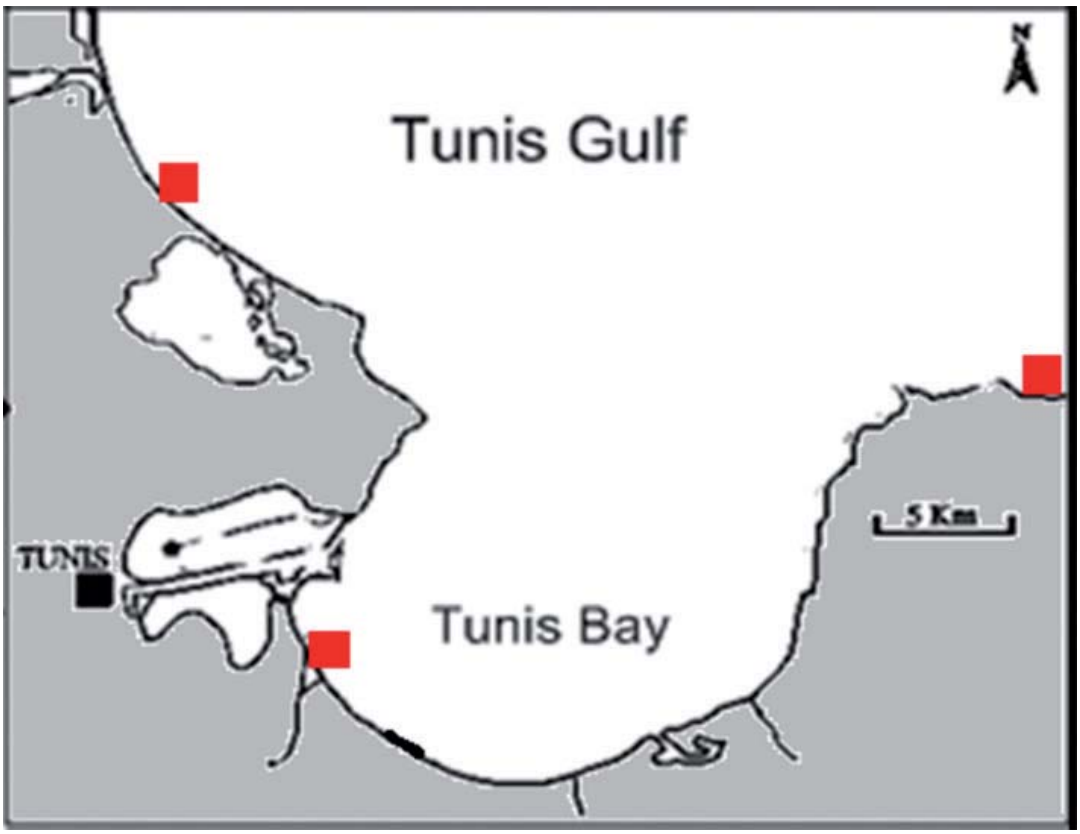

Figure 2. Main density distribution of *S. marginatus* in the Gulf of Gabes. Tunis (Charef, 2006).

These studies will be completed and enhanced by further research in order to evaluate the stock of *S. marginatus* on our coasts and provide information about its distribution.

Stock evaluation of *S. marginatus* (Bivalve, Solenidae) (Pennant, 1777) is in progress in the Golf of Gabes, Southern Tunisia. During this study, a sampling of more than 2000 samples has been performed along 45 km of coast, from El Mahres to Zaboussa (Gulf of Gabes, South of Tunisia), using a 10 x 10 meter Quadra and a 300 meter interval between radials in order to establish a map of the distribution of razor clam in the area, with their relative density. An average length and weight of 85 mm and 9 grams respectively have been reported, with a maximum length of 110 mm and maximum weight of 21 grams. This study has reported a huge variation in *S. marginatus* density, ranging from low density (less than 10 specimens/m^2) to compact population groups (more than 20 specimens/m^2) (Fig. 3). The stock will be estimated and a map of distribution and relative density drawn as follows.

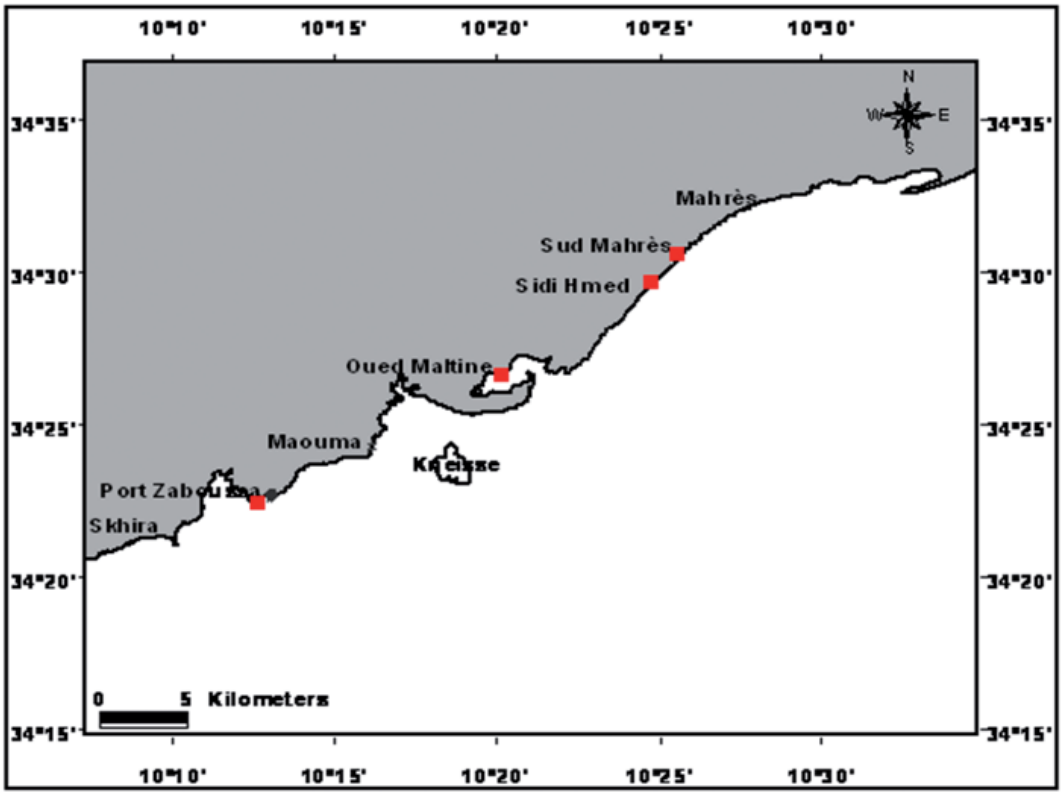

Figure 3. Main density distribution of *S. marginatus* in the Gulf of Gabès.

3. Exploitation and harvesting

3.1. Harvested species

In Tunisia, only the razor clam *S. marginatus* is usually harvested, as bait for sport fishing and occasionally for human consumption, and can be found in the fishmarkets of Tunis and Bizerte. This activity depends greatly on climatic conditions, and is practised more frequently in spring and summer.

3.2. Harvesting methods

In the Gulf of Gabes, with semidiurnal tides, the razor clam *S. marginatus* was traditionally collected using “salt-excitation” techniques (Ayache, 2008) (Fig. 4). In the Bay of Tunis, Bizerte Lagoon and coast, where harvesting takes place, the collecting of *E. minor* was performed by Van Veen Grab, together with that of other benthic communities (Afli *et al.*, 2008), or by “salt excitation”. No mechanized methods are used on Tunisian coasts, a fact which can be mainly explained by the scant commercial interest in razor clam species.

Figure 4. Salting method used in Tunisia.

3.3. Production data: evolution

No production data have been recorded to date. This can be explained by the fact that razor clams do not figure among bivalve species officially exploited on Tunisian coasts. We only know that harvesting activity takes place, especially in summer in Bizerte Bay and in spring in the Gulf of Gabes, reaching a total of 200-300 specimens/day and 200 specimens/day, respectively.

3.4. Exploitation management

Razor clam, and particularly *S. marginatus*, are among the edible bivalves not yet exploited on our coasts but which, however, have potential economic interest. Some research has been carried out to establish the reproductive cycle of *S. marginatus* and its density in Tunisia (INSTM, 2006), and especially in the Gulf of Gabes (Ayache, 2008, Hmida *et al.*, 2010). According to the INSTM, the stock in the Gulf of Gabes was estimated at 180 t/year. Detailed studies will be performed in order to establish the density and distribution of razor clam species by area, and then it will be easier to judge exploitation possibilities.

Current findings encourage us to believe rational exploitation of razor clam

species can be achieved, especially of *S. marginatus.*

3.5. Production economy

For example, *S. marginatus* is only harvested in the Bizerte, Tunis, Sfax and Gabes areas. Razor clam are mainly used as bait by fishermen, but rarely consumed by humans. They are sold in the ports of Bizerte and Tunis (1 €/6 or 8 specimens, according to their length, in Bizerte, and 0.5 € each in Tunis). In the Gulf of Gabes some specimens of *S. marginatus* are harvested during the traditional harvesting of the *Ruditapes decussatus* clam and sold to fishermen (less than 5 €/ 30 specimens).

4. Sanitary control regulations

Before any exploitation, we have to be sure about the safety of the razor clams harvested on our coasts, just as with other clams exploited. There are no data about sanitary control regulations for razor clams.

In Tunisia we have 18 clam purification and exportation facilities. In 1990, a national epidemio-surveillance network was set up in every zone of clam production to control bacteriological, biological and chemical contaminants. Veterinary Services under the direction of the Ministry of Agriculture and Hydraulic Resources are responsible for managing this network.

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Chapter 19: North American Jackknife and Razor Clam Fisheries

Shawn Roach, Barry MacDonald and Ellen Kenchington

Abstract

The commercial jackknife and razor clam fisheries in North America have existed for well over a hundred years. A contributing factor to their longevity has been a high market value for the product. Two species, *Ensis directus* on the east coast and *Siliqua patula* on the west coast are the major species harvested for food markets. Other species are harvested for bait. North American data complied over a 60-year period show that commercial landings have decreased, while those for the recreational fishery have increased. The increase in recreational diggers has prompted concerns over diminishing clam stocks and a lack of regulation. Federal and local governments and agencies have in many areas introduced management plans for both fisheries which have shown some success in preventing overfishing of beds. These timely efforts, and an interest in these species for aquaculture, suggest that both recreational and commercial fisheries are likely to continue into the future.

1. Harvested Species and Distribution

The North American jackknife and razor clam fisheries principally harvest four species of veneroid clams (Fig. 1). Two of these, *Ensis directus* (Conrad, 1843) and *Siliqua patula* (Dixon, 1788) commonly called the Atlantic Jackknife Clam and Pacific Razor Clam repectively, are in the Family Pharidae (H. Adams and A. Adams, 1858). *Solen rostriformis* (Dunker, 1862) (syn. *S. rosaceus* Carpenter, 1864), the rosy jackknife, also belongs to the Superfamily Solenoidea (Lamarck, 1809), but is placed in the Family Solenidae (Lamarck, 1809). The California tagelus, *Tagelus californianus* (Conrad, 1837), belongs to the Superfamily Tellinoidea (Blainville, 1814) and is in the Family Solecurtidae (Orbigny, 1846). These taxonomic designations follow the Integrated Taxonomic Information System database, ITIS, (http://www.itis.gov) which adheres to a set of validation standards consistent with the International Code of Zoological Nomenclature.

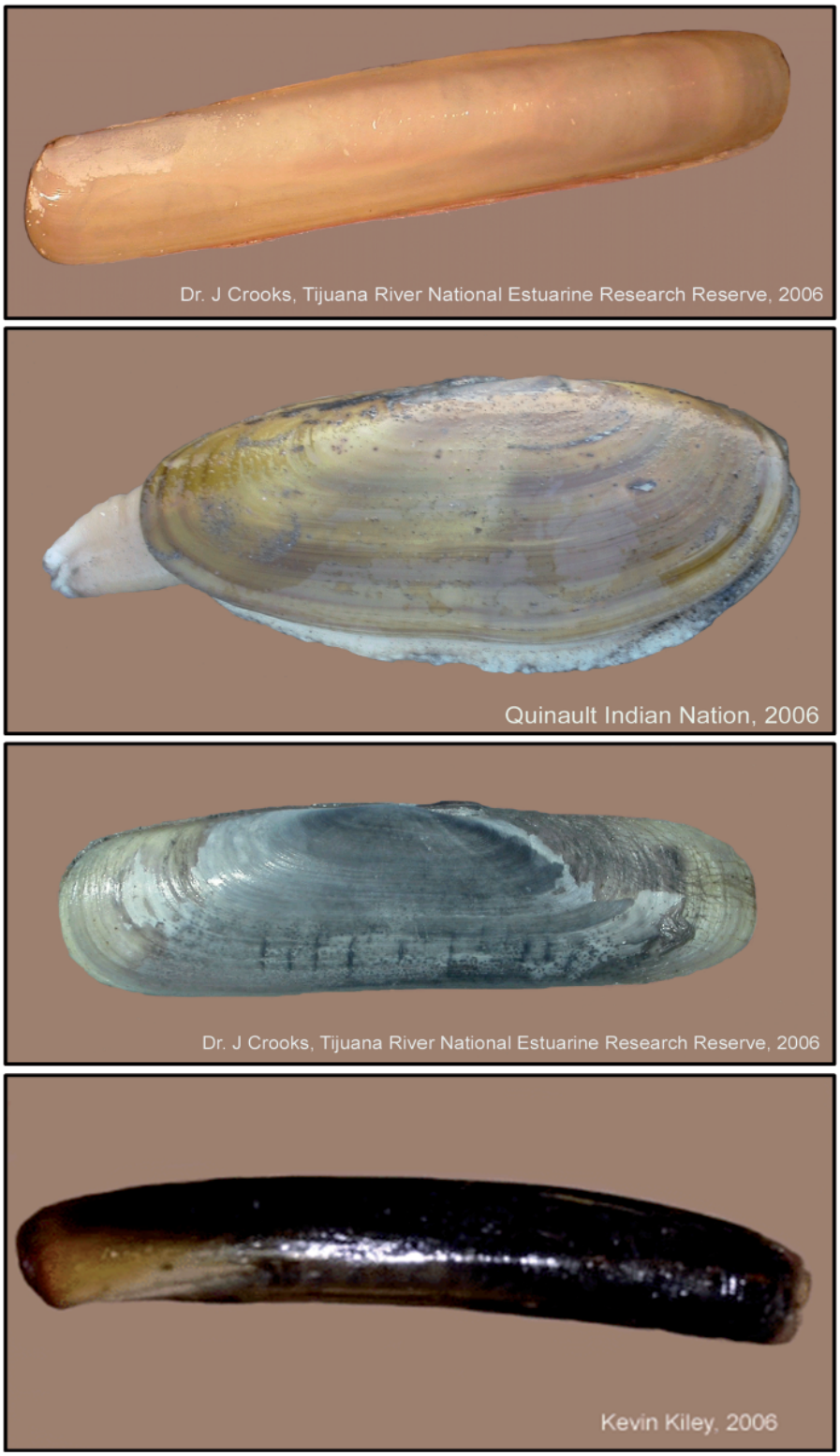

Figure 1. From top to bottom: rosy jackknife (*Solen rostriformis*), Pacific razor clam (*Siliqua patula*), California tagelus (*Tagelus californianus*), and Atlantic jackknife clam (*Ensis directus*). Photos used with permission.

Jackknife and razor clams are long, sleek burrowing bivalves found primarily in shallow coastal waters, including intertidal beaches. Relatively easy access has enabled both commercial and recreational fisheries to develop. They are generally found on flat or gently sloping, exposed sandy beaches with moderate to high surf, but some species prefer calm, enclosed bays. They can be found buried in the muddy sands within the intertidal and subtidal areas at water depths up to 55 m. All species of razor and jackknife clams have the ability to burrow very quickly and can be found just below the substrate surface to depths greater than 60 cm.

The four species commercially fished in North America differ in distribution, with three species found on the west coast and one on the east coast (Fig. 2). The west coast species, *S. patula* is widely distributed from Prismo Beach, California to the Aleutian Islands in Alaska, while the east coast species, *E. directus*, is found from Labrador to as far south as Florida (Savard, 1994; Leet *et al.*, 2001). *S. rostriformis* and *T. californianus* have much more restricted distributions on the west coast where they overlap with each other and *S. patula*. Many other species in these genera are also harvested in low numbers by recreational diggers for personal use.

Figure 2. Geographical range of commercial jackknife and razor clam species in North America. Overlapping distributions have been displayed separately, extending from the coast and parallel to their actual coastal distribution, for clarity.

2. Fisheries

Jackknife and razor clams are marketed for both human consumption and bait. *S. patula* and *E. directus* are considered valuable food items, while *S. rostriformis* and *T. californianus* are primarily sold as bait for crab fisheries (Emmett *et al.*, 1991). Catches are typically small but moderate to high prices sustain these fisheries (Barón *et al.*, 2004).

In many States the fishery has transformed from a commercial fishery to a recreational fishery. For example, in Oregon State the commercial fishery accounted for ~90% of the harvest in the 1940´s declining to only 13% in the 1990´s (Oregon Department of Fish and Wildlife, 2001). This decline has been attributed to overfishing, low recruitment, inefficient harvesting methods, market competition with cheap, dredged clams from the east coast and competition with recreational fishers. The commercial fishery is constrained by the lack of allowable efficient and effective harvesting tools. The hydraulic dredge, which is both efficient and effective, is banned in many states and provinces. Concomitantly, new road construction along the coast has eased access for recreational diggers (Link, 2000). With more areas to fish and discovery of virgin populations, the recreational fishery expanded during the 1970´s through to the 1980´s. However, by the early 1980´s, catches started dropping while effort stayed high sparking action from government agencies to better regulate and manage their clam resources. Throughout this transition period, the fishery saw numerous closures due to high biotoxin levels and seasons were drastically shortened. This led to decreases in landings and economic loss.

Overfishing by recreational diggers, who are difficult to monitor, can be a serious problem. In Washington State, prior to 1980, ~750,000 digger trips were documented, repeatedly harvesting more than 13 million clams in one season. This number has steadily decreased to 250,000 digger trips with a harvest of less than 3 million clams per season (Washington Department of Fish and Wildlife, 2003). High fishing pressure has been blamed for recent recruitment problems in this area.

There is no commercial razor clam fishery in California and sport / recreational fishing is limited to beaches on the northern coast.

Although the emphasis in some states has shifted to the recreational fishery, the commercial fishery continues at a smaller scale, with fisheries conducted on both coasts of North America. On the west coast, eight major concentrations of jackknife and razor clams support both the commercial and recreational fisheries (Babineau, 2000).

Like Canada, the bulk of the US commercial fishery occurs on the west coast, with fisheries in Oregon, Washington and Alaska. Catch statistics for the US are much harder to compile and often state and federal statistics do not align. According to data gathered from the west coast of the U.S., the commercial harvest has decreased significantly from the late 1950's and into the early 1960's (Fig. 3). Landings of *S. patula* peaked in 1951 at 1455 t and are currently averaging 355 t/year for the last 10 years. Most of the *S. patula* (U.S.A.) data used for figure 3 was compiled from individual state data and can be considered accurate.

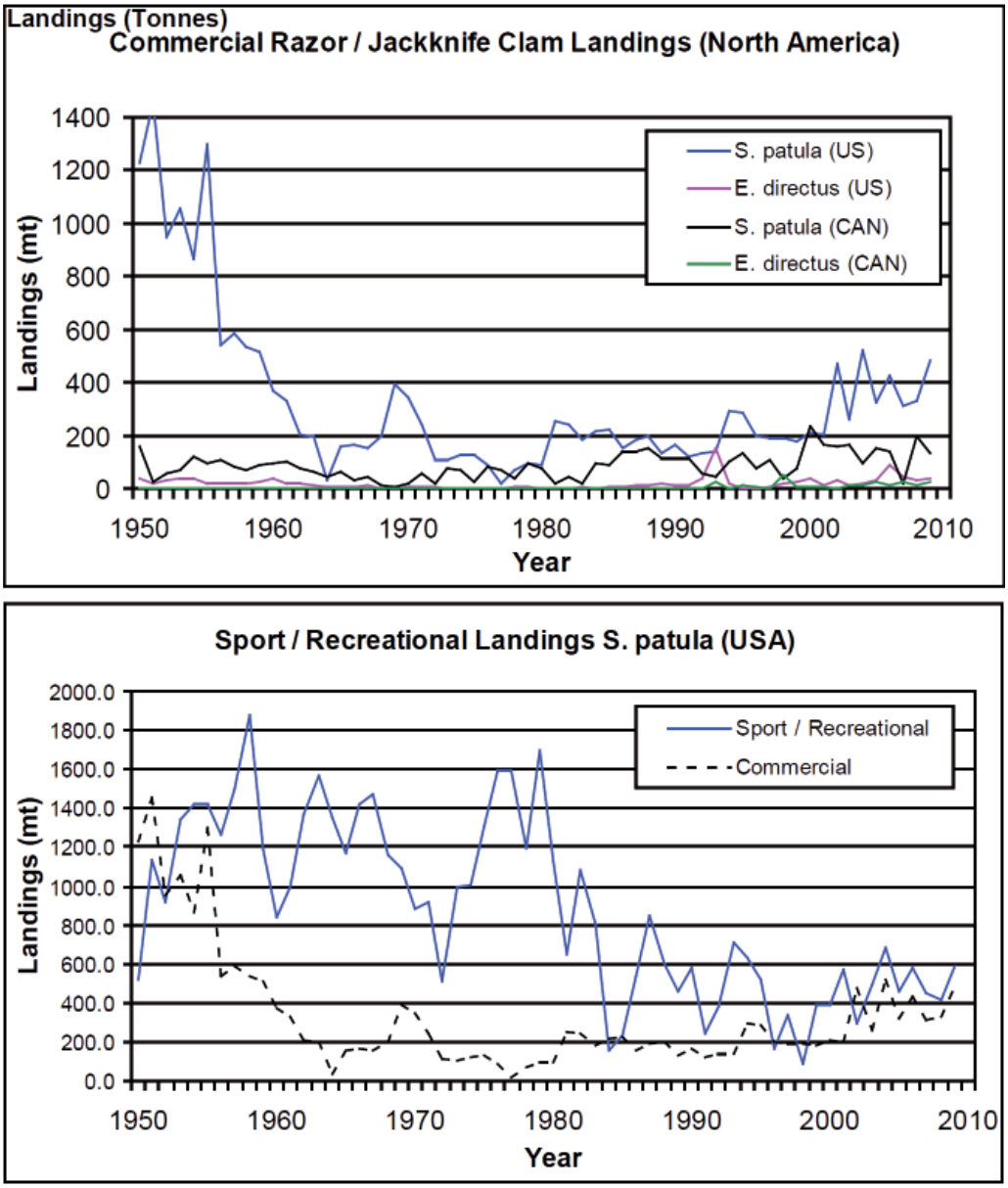

Figure 3. Commercial / Recreational landings statistics for the USA and Canada 1950-2009. (NMFS and Washington, Oregon, and Alaska Department of Fish and Game (USA); Jones and Davies, Invertebrate Post season Review (2005), Haida Fisheries Program and DFO (Quebec Region).

The smaller east coast commercial fishery for *E. directus* takes place in the mid-eastern United States with small and/or sporadic fisheries also occurring in Quebec, Canada where an average of 14 t have been harvested from the Gulf of St. Lawrence from 1993 - 2009 (DFO, 2010, pers. comm.; Kenchington *et al*., 1998; DFO, 2004). The National Marine Fisheries Service data, which is the source for figure 3, *E. directus* U.S.A., is an underestimate based on the limited state / town landings that were obtained.

Where landings data prior to 1950 was available (commercial and recreational) it generally indicated high initial landings that quickly dropped to approximately 1950 levels. The year 1950 was chosen for figure 3 as it represented the fullest available data set.

3. Method of extraction

The traditional method of harvesting razor and jackknife clams utilises hand tools such as picks, spades and clam hoes, although some use home-made pieces of equipment like “clam guns” or “clam tubes” which essentially do the same thing as a spade but are less destructive to the bottom environment (Fig. 4). This method is slow and labour intensive.

Figure 4. Harvesting *S. patula* in Washington State U.S.A. (Photos courtesy of Washington State Department of Fish and Wildlife).

Harvesting by SCUBA divers has been practised with moderate success (Kenchington *et al.*, 1998; Barón *et al.*, 2004). Exposed clams respond to vibrations and burrow quickly upon approach, while divers can move in while they have their siphons extended and are more vulnerable. Typically, long pincher tongs are quickly deployed - lifting the animal out of the sand, or else a thin metal rod is strategically placed inside the feeding clam's siphon. The clam reacts by contracting its siphon, which anchors the metal rod inside the shell, and the diver is able to pull the clam out of the sand.

Mechanical hydraulic harvesters are more efficient than hand tools, but have been banned or heavily regulated due to their detrimental impact on the environment (Coen, 1995; Kenchington *et al.*, 1998; DFO Gulf, 2001; Canada, 2003; Hauton *et al.*, 2003). These harvesters are usually floating boats or barges that use a jet of water to force the clams to the surface of the sediment where they are collected by a rake or suction and placed on a conveyor belt that places the clams aboard the boat (Fig. 5).

Figure 5. Hydraulic harvesting of *Ensis directus* in Quebec, Canada (Photo courtesy of H.F. Ellefsen, Department of Fisheries and Oceans).

4. Management

The lack of regulatory mechanisms during the height of the recreational fishery in the early 1970´s caused widespread stock declines in the 1990´s. In attempts to conserve these fisheries, governmental agencies throughout the US and Canada reviewed regulations and adopted harvest management plans to help curb the dwindling stock problems.

Most of these plans included increased stock monitoring, the gathering of more timely and accurate data, optimizing fishing effort and developing appropriate management measures (DFO Gulf, 2001; Canada, 2003). Through these actions and public awareness, the overexploitation of the stocks has been somewhat reduced.

In many states the recreational fisheries are governed by daily bag limits, minimum size limits and controlled access to fishing areas. Bag limits range from 10 to 15 clams per day in Washington and Oregon States to 300 per day in Atlantic Canada (Link, 2000; DFO Gulf, 2001; Canada, 2003) and are adjusted according to stock status. Canada does not require recreational diggers to hold a licence so no records of landings are recorded.

In Canada there are size limits restricting harvest of clams that are less than 100 mm in length on the East coast and 90 mm on the West coast. The Canadian Government hopes that by setting a minimum size limit, the fishery will be able to sustain a healthy cohort of sexually mature individuals (Canada, 2003). However, the US does not implement this regulation anymore because they found high incidences of undersized clam wastage.

On Canada's west coast successful co-management by the Canadian DFO and the Council of the Haida Nation has resulted in a very successful and stable fishery (DFO, 2001). Commercial landings at North beach (McIntyre Bay, British Columbia) have been relatively stable since 1923. The landings in 2000 of 237 t were a record going back to 1943 and the last ten year average of 146 t is greater than any consecutive 2 year average since 1950.

In the US, rotational fisheries have been established. Beaches are zoned using readily visible boundary markers and typically one half of the beach is open to fishing one year and the other half in the following year, allowing a fallow year for recovery and recruitment.

5. Economic value

These fisheries function under a supply and demand economy which is influenced by factors such as the state of the stock, the environment and local and global economies. Due to the labour intensive nature of the fishery, demand consistently exceeds supply, maintaining a market with relatively high and stable prices. Currently, there are many local and overseas markets (Europe and Japan) demanding fresh and frozen clam products.

The transformation from commercial enterprises to recreational fisheries has meant a loss of jobs in one sector and an increase of economic benefits in the other. Upwards of 30,000 diggers visit California, Oregon and Washington State beaches on the weekends, injecting more than $12 million per year into the local economy (Puget Sound Action Team, 2003; Fish and Wildlife Science, 2004).

Conversely, the total North American commercial catch from 1950 to 2005 for *S. patula* and *E. directus* was estimated at approximately $14 million US funds (DFO, 2001; NMFS and Juanita Rogers DFO, pers. comm.). This averages out to approximately $255,000 per year over the 55 year period.

6. Aquaculture

Due to diminishing natural stocks and pressure from buyers for more product aquaculture may be one venue to supply increasing demand (Kenchington *et al.*, 1998; Leavitt and Burt, 2001; Leavitt *et al.*, 2005).

Both *S. patula* and *E. directus* have already been cultured successfully in small numbers at research facilities. A larval phase upwards of 10 weeks makes them susceptible to the effects of handling, parasitic infections and other stresses associated with hatcheries. However, the greatest obstacle lies in the containment of juveniles. Specialized caging systems must be developed to keep juvenile animals from escaping as they are highly motile. Grow out cages / netting developed for other clam species, such as quahogs, are unsuitable. Currently, there are few commercial hatcheries in North America producing razor or jackknife clam spat for commercial grow out, but there are a handful of government run facilities that produce spat to reseed wild clam beds for the recreational fishery.

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Chapter 20: Fishery and some biological aspects of razor clam (*Ensis macha*) in Peru

Jaime Mendo and Roberto Espinoza

Abstract

This research study presents information related to fishing and some parameters of the *Ensis macha* population on the Peruvian coasts, the fishing of which started in 2002, induced by the opening of international markets, which increased its value. Only a few studies have been undertaken about the biology and population dynamics of this species, but even so these can be used as a basis for optimal management and sustainable exploitation of this resource. Currently, many exploited natural beds are exhausted, due to the lack of strategies and control measures. The use of water pumps for extraction and their impact in productivity are discussed. Last but not least, some management measurements are suggested for the proper exploitation of *E. macha* beds.

1. Introduction

Landing levels of the razor clam *Ensis macha* (Molina, 1782) from artisanal fishing along the Peruvian coasts have increased during the last four years, making it one of the most interesting bivalves for artisanal fishery. This is due to the opening of international markets, which has led to a significant increase in its commercial value when compared with previous years.

This razor clam “boom” on Peruvian coasts started in the area of Pisco in Bahía Independencia, where registered landings were less than 50 t until 2002, but which grew from 1,241 t in 2002 to 2,521 t in 2004. Landings of this resource are between 25 and 50% of the total landings realised here. Until 2005, landings in Bahía Independencia were almost 100% of total razor clam unloads on the Peruvian coasts, decreasing to 50% as from 2006. It is clear that there has been a drastic reduction in output from the most productive banks in Bahía Independencia (Morro Quemado). However, the incipient nature of knowledge about the biology and population dynamics of this species has prevented basic guidelines from being established for the creation of a management plan which would lead to a sustainable

use of this species. On the other hand, it is known that this fishing, carried out by divers with compressors, has gone from extraction by hand to water pump extraction, which has increased the fishing effort to a dangerous extent.

Currently, artisanal fishermen working with different species on the coasts have oriented their activity towards looking for banks, prompted by exporters who supply water pumps for extraction. It has not been scientifically proved so far whether the pump used affects the recruitment of this species or the product quality. However it is logical to think that stirring up large amounts of sand might have an impact on habitats and resources. This kind of massive extraction caused the collapse of one of the largest banks in Bahía Independencia, and unless some measures are taken, different banks along the Peruvian coasts might go the same way.

The present chapter presents published and unpublished information about *Ensis macha* fishery in Peru, especially in Bahía Independencia, where the first population studies regarding this resource have been initiated.

2. Target species and distribution

Ensis macha is distributed along the American coast of the Pacific Ocean from Caldera (27˚S) to Magallanes, Chile (55˚S); and on the Atlantic coast as far as Golfo de San Matías, Argentina (40˚S) (Gallardo, 1978; Osorio *et al*., 1979; Lasta *et al*., 1998). In Peru they have been reported as *Ensis* sp. at Pisco, 14˚S (Paredes *et al*., 1988), at Las Lomas, 16˚S (Guzmán *et al*., 1998); and recently in Bahía de Ancón, 12˚S (Indacochea, com. pers., 2004), San Juan de Marcota, Huacho, Calma and Chimbote (Berrú *et al.,* 2005 a, b and c) (Fig. 1).

Along the Peruvian coast, razor clams live in sandy sediments of fine and medium-fine grains and can be found forming patches from superficial subtidal areas to depths close to 26 metres (Guevara, 2006).

There is little information about the density and biomass of *E. macha* in Peru. For Bahía Independencia, Espinoza (2006) reports density values of between 25.1 and 147.5 ind. m^{-2} from 2002 to 2004, with a tendency to decrease. Average density and biomass were 78.1 ind. m^{-2} and 2.8 kg m^{-2} respectively during the same period. These density values were observed between 4 and 12 m deep, although at greater depth higher densities of this resource were observed. In other places along the Peruvian coast densities lower than 31 ind. m^{-2} have been reported, as shown on Table I. *E. macha* density values observed at Bahía Independencia are higher than those observed by Ciocco (unpublished data) for Argentina, with average densities

of 51.6 ind. m^{-2} and average biomass between 1 and 1.5 kg m^{-2} , and for Chile, with average densities lower than 1 ind.m^{-2} (reported by Aracena *et al*., 1998) and lower than 3.2 ind. m^{-2} (reported by Jaramillo, 1998).

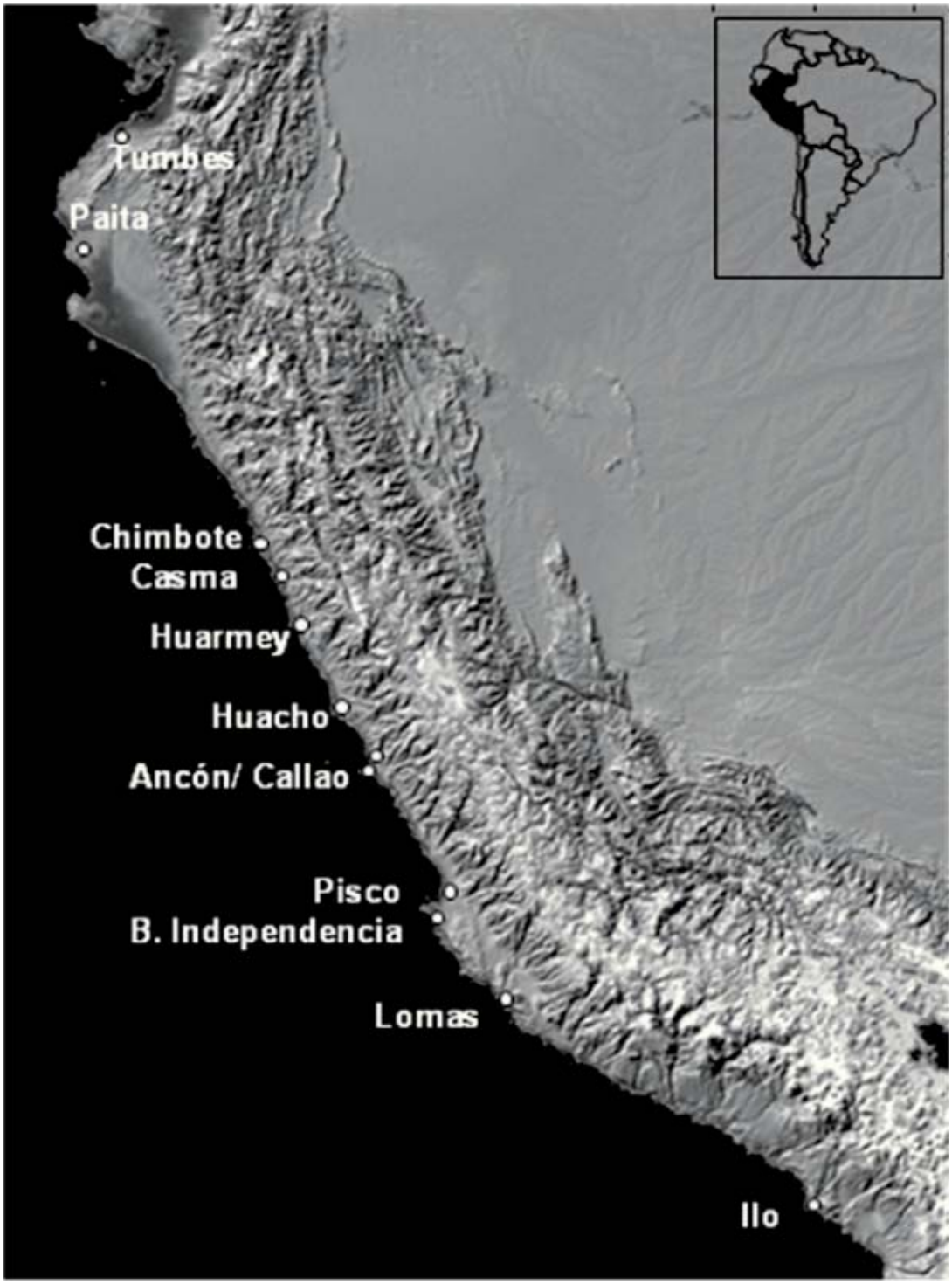

Figure 1. Major distribution areas of *E. macha* along the Peruvian coast.

Table I. *Ensis macha* density (ind. m^{-2}) and sizes (mm) observed in different banks along the Peruvian coast.

Area	Depth (m)	Density (ind.m^{-2})	Size (mm) min - max	Author
Casma	12 - 26	1.0 - 3.4	15 - 42	Berru *et al*. (2005a)
Huarmey	12 - 23	0.5 - 10.9	04 - 75	Berru *et al*. (2005b)
Chimbote	> 7	0.3 - 31.0	57 - 80	Berru *et al*. (2005c)
Lima (Ancon)	8 - 18	1 - 2	-	Arguelles *et al*. (2005)
Pisco (B. Indep.)	4 - 12	25.1 - 147.5	90 - 175	Espinoza (2006)

3. Fishing

3.1. Landing sites

Historically, the most important landing port for *Ensis macha* has been Pisco; however, small volumes of this resource have also been landed at other places in the area of Chimbote, Casma, Huarmey and Callao, aimed at local consumption. From 2002, with the opening of the international market, unloads of this resource in the area of Pisco increased considerably. Progressive deterioration of the resource during the following years caused the use of new unloading sites, such as Ancón and Huacho. Fishermen are currently exploiting new banks and other fishing areas along the Peruvian coast in order to maintain the extraction and exportation of this resource. The areas of Huacho and San Juan de Marcona have currently the largest unloads for export.

3.2. Fleet

The fleet consists of wooden vessels of up to 8 m long, with inboard or outboard motors, with a compressor supplying air to divers (Fig. 2). This fleet moves from one zone to another depending on resource availability, and it is exclusively aimed at extracting benthic invertebrate (octopus, scallop, razor clam, mussels, winkles, clams, fish, crabs, sea urchins, etc.). Traditionally, most of the fleet is concentrated at Pisco, with around 270 vessels (Mendo *et al*., 2005), which rose to 1000 vessels during the strong “El Niño” phenomenon in 1982/83 and 1997/98, due to the massive increase of the Peruvian scallop population (*Argopecten purpuratus*).

Figure 2. Shellfish vessel with motor pump for extracting *Ensis macha.*

Shellfish vessels oriented to catching razor clams have water motor pumps with three outlets, installed on the deck and managed by two crew members (Fig. 5). These pumps emit a jet of water that allows the sandy sediment to be stirred up, making it easy for this resource to be extracted.

3.3. Landings and CPUE

E. macha statistics show landings of less than 50 t along the Peruvian coasts during 2000 and 2001 (Fig. 3). Before 2000 no unloads were registered, although it is known that this resource has been consumed by coastal communities for many years. From 2002 on, an increase in unloads was registered, due to the demand from the external market, reaching maximum values of around 300 t per month during the second half of 2004 and the first months of 2005.

As can be seen from figure 3, almost all unloads come from Laguna Grande at Bahía Independencia (Pisco); however, from 2006 unloads in Laguna Grande decreased due to the exhaustion of the bank of razor clams at Morro Quemado, the most productive one at Bahía Independencia. The dramatic decline of this bank is not shown in Laguna Grande unloads since fishermen, after its exhaustion, evaluated and exploited other less productive banks within Bahía Independencia. Fishermen looked for new banks in other areas of the Peruvian coasts, which have maintained total unloading volumes of approximately 150 t per month. In 2006 other areas, such as Huacho and San Juan de Marcona, have contributed with important unloads, to the total landings in Peru.

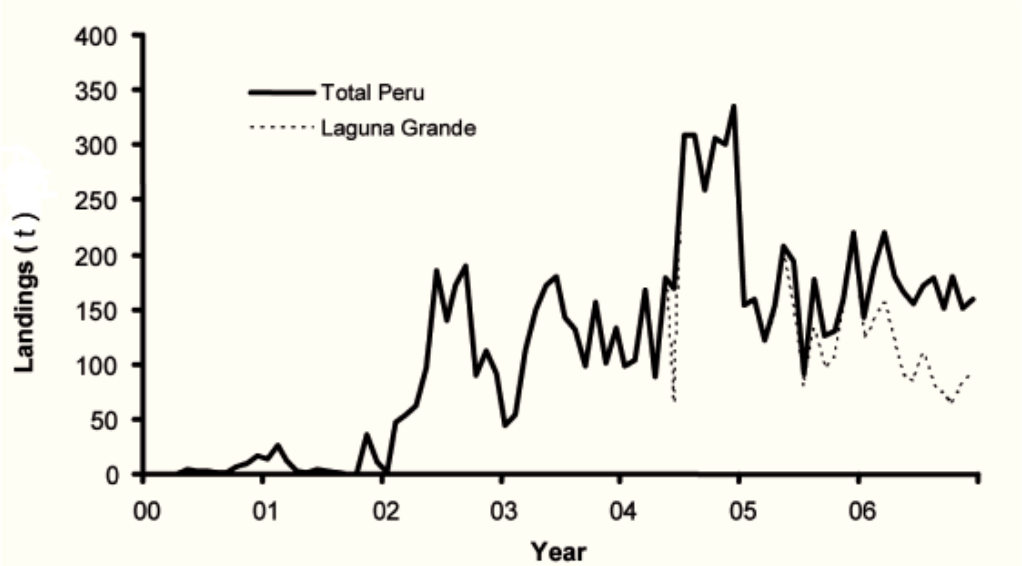

Figure 3. *E. macha* unloads (t) on the Peruvian coast and Laguna Grande (Bahía Independencia, Pisco), 1996-2006.

Regarding catch per unit effort, data registered by Espinoza (2006) display values between 60 and 990 kg/vessel/day and between 30 and 330 kg/diver/day (Table II).

Figure 4 shows the CPUE data registered at Bahia Independencia from 2000 to 2008 showing a strong decline of razor clam relative abundance.

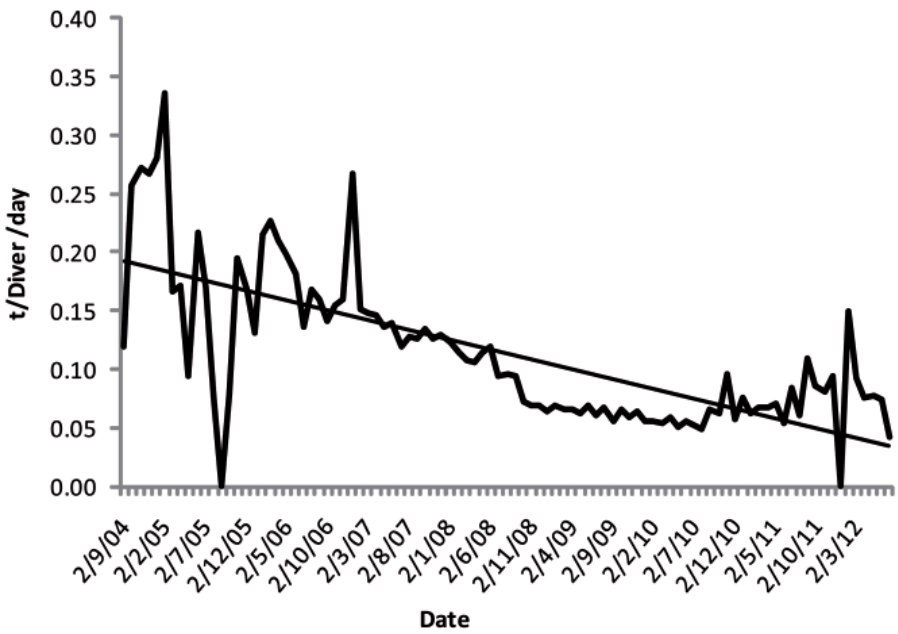

Figure 4. Monthly catch per unit effort (t/diver/day) in Bahia Independencia from 2000 to 2008.

Table II. Catch per unit effort of the extracting fleet at Bahía Independencia registered in July 2004.

	Nº vessel/ day	Nº divers/ day	Daily catch (kg)	Kg/vess/day	Kg/diver/day
Average	26	70	11,378	447	167
Min	1	2	60	60	30
Max	54	149	24,740	990	330
sd	15	42	6,905	145	52

3.4. Composition of catches

E. macha individuals obtained from unloads and processed by exporting companies have sizes ranging from 9 to 20 cm, with an average of 14.4 cm (Fig. 5). Two cohorts can be clearly identified and 10 % of the individual are smaller than the minimum legal size (12 cm).

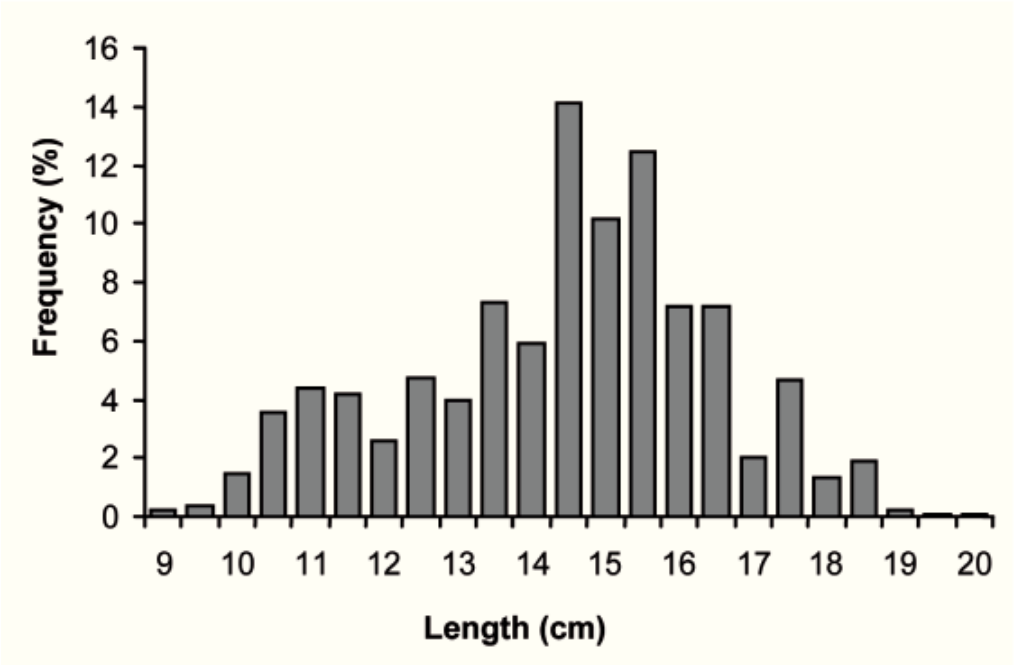

Figure 5. *Ensis macha* composition by size (%) in unloads in the Huacho area registered by the GAM CORP company, June 2006.

3.5. Extraction methods

Traditionally, and until 2003, razor clam extraction was performed with the apnea diving system and with semiautonomous diving using an air compressor. From 2004 on, artisanal vessels implemented motor pumps, making the job of removing the

substrate and extracting razor clams easier for shellfish divers (Fig. 2). Zeballos and Zavala (2004b) carried out an experiment *in situ* and estimated that catches using a motor pump are 4 times greater than those obtained extracting by hand. Moreover, the volume of sand stirred up by using the motor pump is 7 times higher, altering significantly the natural substrate where species inhabit.

It is known that in other places there are also alternative ways for extracting, such as dredges, for *E. arcuatus* sword razor (Robinson and Richardson, 1998), or hydraulic dredges, for the *Chamelea gallina* bivalve (Moschino *et al*., 2003), which also alter the natural substratum. Post-fishing mortality produced by the damage done by the pump, might be many times greater than species' natural mortality rates. Damaged specimens and juveniles thrown back into the sea are usually unable to burrow, so they would be more vulnerable to the attacks of predators; also they may be damaged or suffer stress and eventually die (Gaspar *et al.*, 1994).

4. Production data

4.1. Processing

Razor clam processing starts on the beach after extraction, when damaged specimens and those of non-commercial size are eliminated. Selected individuals are transported to the depuration plant, where they go through a depuration and de-sanding process during 10 to 12 hours. After that, the product is selected by size (10-13, 13-15, 15-up) and then washed with chlorinated water, put in trays, IQF frozen, packed and finally cold stored.

4.2. Export data

Data available concerning razor clam exports are grouped together with mussels and sea cucumbers, among other shellfish, and their volumes are relatively high. According to PROMPEX (www.prompex.gob.pe/PROMPEX/Portal/Sector/) during 2004 and 2005, 1,496 t and 3,273 t respectively were exported in this area, representing a value of 3.91 and 7.86 million dollars, respectively. The price of razor clam on the beach is between 3.50 and 3.75 Peruvian soles per kilo (approximately between US$1 and 1.2). This price does not include the payment of transportation to the plant. The export price is around US$3.10 (prices from June/July 2006).

4.3. Destination

According to PROMPEX, the main destination of the Peruvian razor clam is Spain, with around 70% of exports. Hong Kong imports 7%, the United States and

Korea 4% each. Chile also imports razor clams from Peru, but it does not appear in statistics.

5. Exploitation management. Fishing gears and possible regulations

E. macha extraction is not regulated. This is due to the incipient knowledge of this resource's population dynamics and the lack of direct evaluation of banks for adaptive management. A collapse of the banks exploited by the shellfish fleet is imminent unless a management plan is applied and unless specific measures are taken to enable these banks' productivity to be sustained.

So far, the only step taken by the Production Ministry has been to issue a regulation banning the extraction of razor clam with "hydraulic pumps" (RM-025-2005 dated February 2nd 2005). However, this regulation has been disobeyed by fishermen, who allege that they do not use hydraulic pumps but water motor pumps. Moreover, the regulation forbids the use of motor pumps until the Instituto del Mar del Perú –IMARPE determines the impact of extracting *Ensis macha* razor clams and other benthic resources and until IMARPE estimates the impact of razor clam extraction on a trophic level within the community and on larval settlement of other species. Efforts made by IMARPE, transposed to internal reports (Zeballos and Zavala, 2004a; Zeballos *et al*., 2004), demonstrate the impact of motor pumps on substrate (sand disturbance) although no publication has so far been issued concerning the impact at community trophic level or on larval settlement.

Currently, banks are being exploited without control either of extraction or trading. Some fishermen have decided to set catching quotas without technical support, which could extend a little longer the exploitation of banks.

It is known that recruits of this species share their habitat with adult individuals (Jaramillo, 1998), living very close to the sediment surface, due to their limited burrowing ability and since they are submitted to a huge mortality due to predators or catastrophic physical events, which re-suspend sediment in large volumes. In this context, the use of a motor pump would have a similar effect and would destroy the sedimentary microhabitat when disturbing down to 10 cm deep in razor clam banks (Guevara, 2006). Water pressure could not only kill juvenile individuals directly, destroying their shells, but also those who survive would be exposed to predation.

Well-organised exporters move along the Peruvian coast offering diving systems and motor pumps to fishermen from small fishing coves, and controlling prices. This system, just like any other clandestine trafficking process, only contributes to

a drop in prices and subjugates extractors by granting them a certain degree of temporary welfare. The increase of catch per unit effort obtained with this equipment is compensated for by the low prices paid by exporters. In the case of exports to Chile, for example, exports are made with no added value, which would cause loss of jobs.

On the other hand, there is not a monitoring and control system of fishing effort and sanitary regulations, putting at risk the sustainability of banks' production and the opening of markets, respectively. Currently, razor clam are exploited with motor pumps in fishing coves at La Gramita (South of Casma), Culebras (North of Huarmey) and Tamborero (South of Huarmey). Probably, once these banks are exhausted they will look for others, just as happened with banks of razor clam at Bahía Independencia, Ancón and Huacho.

The arrival of these products at the market in the above-mentioned conditions, breaks the rules about safety conditions that we are obliged to follow as a nation, and so we are open to being penalised, by measures such as the seizure of our exports, since we are processing and exporting bivalve molluscs from non-classified and non-authorised areas, failing to observe the protocol controls we agreed on.

The impressive current boom of the razor clam resource and, in general, of other infaunal bivalves (mussels, different types of clams, razor clams, etc.) confirms our paradoxical role as producers of large amounts of raw material accompanied by hardly any redistribution of the wealth it represents. This, added to the context of an extraction activity with methods far from the minimal criteria of sustainability, may mean throwing away Peru's very valuable position within the exclusive group of countries authorised to export to the European Union.

As has been set out by IMARPE in several documents, there is a need for research leading to an assessment of the impact caused by motor pumps on banks' structure, recruitment/settlement and the quality of the resource. However, from information obtained in other places worldwide that demonstrate the impact on razor clam banks' productivity, we believe the use of motor pumps should be reconsidered. This measure must be accompanied by a biological and socio-economical evaluation that will allow the damage to the resource as well as to artisanal fishermen themselves to be demonstrated.

Another aspect that should be taken into account is the productivity of banks; for this it is necessary to carry out constant evaluations in order to set catching quotas that would avoid overexploitation. The geographical distribution of banks, as well as connections between them through the dispersion of larvae, are important aspects

when implementing an adaptive management plan with an ecosystem approach. The setting of minimum population levels for banks (points of reference), should be done on the basis of a thorough knowledge of the structure, productivity and larval drift of razor clam metapopulations.

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Chapter 21: The state of the razor clam (*Ensis macha*) in Chile

Irene Lépez García, Daniel Arriagada Obregón and Flor Véjar Durán

Abstract

In Chile two species of razor clam, also known as the "huepo" or "machuelo", are registered. Neither *Solen gaudichaudi* nor its biological background are very well-known, since it is confused with *Ensis macha*, a species that occupies most of the fishing activity regarding this kind of resource. *E. macha* is distributed from the North to the South of the country, reaching most of the South Argentinean coast. Its reproductive cycle is characterized by a spawning period starting in October and ending in December every year. The species displays slow growth, with no seasonal changes and with an asymptotic size going from 189 to 220 mm of LV.

The resource is mainly exploited by artisanal fishermen diving with hand-held gear (hooks or pincers) from vessels not longer than 15 metres, the extraction activity being concentrated in the Eighth Region (Golfo de Arauco) and Tenth Region (Corral). The development of fishery is disturbed by the high variability in historical and monthly unloads, since artisanal fishing is affected by restrictions and adverse weather conditions.

The economy is centred mainly on the preparation of the canned product (70.2%), followed by frozen products (25.2%) and fresh-cooled products (4.6%). Resources generated by the industry were worth over US$15 million (FOB) during 2005. The main countries receiving this kind of products are Spain (80.1%), Malaysia (9.1%) and Singapore (8.2%). The fishing of this resource is currently controlled under an Exploitation Plan, whereby new craft and users are not allowed to join this fishing activity. No minimum extraction size has been established, but there is a reproductive close season between October and November every year.

1. Introduction

Chile is a country where fishing and aquaculture play an important role, and whose main strengths and comparative advantages lie in the high productivity of the marine ecosystem existing on its coasts. Upwellings in the North-Central area of the country, as well as the cold water of the South-Austral area originate many

fishing resources available for developing extraction activities, both artisanal and industrial. Furthermore, the quality of the water, the ecosystems and geography allow fish-farming to use a plethora of resources.

During 2005, fishing unloads in addition to harvesting from farming facilities amounted to a total of over 4.9 million t. Fishing exports during that year reached US $3,080 million, corresponding to 1.6 million t of exported volume (Subpesca, 2005); commercial fishing represented 39% of export value (US$ 1,201 million), while aquaculture represented 61% (US$1,879 millions).

Aquaculture has flourished since 1988, with an average rate of increase of around 20% per year. In 2005, 716 t were harvested from farming centres, 80% of them made up of salmonids, while molluscs represent 12.4% (109 thousand t) and algae 8%.

One of the most important resources for artisanal fishing in the South-Central area of the country is the “huepo” or razor shell (*Ensis macha*), a species that is the basis of canned fish exports to Spain and to several Asian countries. A statistical history of this fishing shows the effect of over-exploitation of its natural banks, causing extraction in the South of Chile to fall to near zero levels (10th Region). Over-exploitation is the reason why this resource has been researched from biological, population and social-economic points of view, and why farming started to develop.

2. Target species and their distribution

In Chile two species of razor clams are extracted, known as “machuelo”, “huepo”, “espárrago de mar” (sea asparagus) or “navaja de mar” (razor shell): *Ensis macha* (Molina, 1782) and *Solen gaudichaudi* (Chenu, 1843), the former of these being the most important for fishing.

Solen gaudichaudi (Fig. 1) belongs to the Solenidae family, and its geographical distribution runs from Coquimbo (4th Region) to Golfo de Arauco (8th Region) (Fig. 2), where it inhabits sandy beds at the sandbanks of some rivers in the Central and Southern areas of Chile. The shape of its shell is elongated and slightly curved and its valves are convex and half-open at the ends. The outer part of the shell is smooth, with concentric growth lines, and faint thin radial lines. The purplish-white valve is covered with a shiny, strong, greenish / dark-brown or light-brown periostracum, and reaches a maximum size of 12 cm. Not much is known about its biology, since it is mistaken for *Ensis macha* (Osorio, 2002). *Ensis macha* (Fig. 1) belongs to the

Pharidae family and is distributed from Caldera (3rd Region) to the Strait of Magellan (12th Region) on the Chilean coasts (Fig. 2), reaching the Golfo de San Matías on the Argentinian coast. The shell is bivalve and elongated, with parallel edges and a smoothly curved surface. Valves are convex and half-open at the ends, with a rounded anterior edge and a truncated posterior one; they are covered by a strong, dark-brown periostracum, and the ligament is external. The surface is smooth, with concentric growth lines. The umbo, close to the anterior edge, can barely be seen, and the hinge has three teeth (Osorio, 2002).

Figure 1. *Solen gaudichaudi* and *Ensis macha* specimens from Chilean coasts.

Wherever it is found, *E. macha* inhabits superficial sandy bottoms, from the intertidal to about 20 m deep, forming groups or banks (Osorio and Bahamonde, 1968). The habitat of the razor clam is characterised by a mixture of grain sizes, where fine and very fine sand with a low level of organic matter predominate (Aracena *et al.,* 1998; Jaramillo, 1998).

Oceanographic conditions predominating in the areas where the species inhabits are variable and depend on their geographic distribution. In the main fishing area of these species (Golfo de Arauco, Fig. 2), the vertical structure of the water column is marked by two periods, the first with a stratified water column with an area of marked slopes, characteristic of the spring-summer season and the second one by a homogenous water column without stratification, in the autumn-winter season (Aracena *et al*., 1998; Jaramillo, 1998). On the other hand, in more Austral areas, temperature and salinity show only slight temporal variability or seasonal variations (Jaramillo, 1998).

The density of razor clams displays important seasonal variations. Adults vary between 0.005 and 0.17 individuals/m^2 in summer and between 0.11 and 0.72 individuals/m^2 in winter, while juveniles show densities of between 0.66 and 10 individuals/m^2 in summer and between 0.11 and 8.87 individuals/m^2 in winter. Adults and juveniles live together in all the researched areas, without any sign of negative influence of adults being found in juveniles' settlements (Aracena *et al*., 1998). In

Magallanes (Gorny *et al*., 2002) very high density values are found compared with other regions, fluctuating between 19.69 and 45.20 individuals/m^2, since this area has not been exploited.

Figure 2. Geographical location of Chile, its regions and distribution of *Solen gaudichaudi* and *Ensis macha*.

Regarding incidental catches, the presence of 17 species of polychaetes has been determined, where *Diopatra chilensis*, *Nassarius dentifer* and *Nassarius gayi* winkles might be potential predators of razor clam juveniles. Other members of the epifauna on razor clam banks are *Hepatus chilensis* and *Pseudocorystes psicarius* crabs, *Xanthochorus cassidiformis* winkles and the small isopods *Serolis gaudichaudi* (Aracena *et al*., 1998).

3. Extraction methods

The fleet exploiting the resource consists of boats and motorboats less than 15 m long totalling around 1,200 vessels, which may operate by an inboard or outboard motor (Olguín and Jerez, 2003). The fishing system used is semi-autonomous diving, so vessels are equipped with a compressor, and for collecting molluscs the diver uses two tools: hooks and pincers (Olguín and Jerez, 2003). Extraction activity starts at dawn, when vessels go to banks already known by fishermen, and it finishes when they go back during the afternoon of the same day. Bivalves are kept in plastic mesh bags until they are unloaded on the beach, where buyers weigh and stock up razor clams in trays, which are transported in refrigerator lorries towards food preservation plants (Fig. 3). The product that reaches the plant displays an important percentage of sand and damaged valves due to the inappropriate handling of the mollusc by fishermen during the transfer in vessels.

Figure 3. Vessels' arrival on the beach and the weighing of razor clams for sale.

There is great uncertainty regarding the fishing effort exerted on razor clam banks (Sánchez *et al*., 2003). The information collected by Sánchez *et al*. (2003) and Lépez *et al*. (1997) indicates that there are more than 500 vessels and about 1,500 divers/collectors working on this activity. Knowing the actual number of vessels and divers/collectors participating in this activity is a task that requires a huge logistical effort by the competent services and is one of the most important activities in establishing a management plan for this fishing (Sánchez *et al*., 2003).

The characteristics of vessels that worked with this resource in 2003 show that the fleet consisted mainly of wooden craft (81%), with an outboard motor and between 4.5 and 9.5 meters long. The age of the fleet varies: there were boats less than a year old, and others 30 years old (Sánchez *et al*., 2003). Working equipment on the vessels consists of 3 to 4 fishermen (1 assistant and 2 to 3 divers per boat) and seasonal fishing performance ranges from 20 to 55 kg/hour. The price of the resource at the beach does not show seasonal variation, and the distribution of incomes varies, depending on the extraction method and the activity performed (Sánchez *et al*., 2003).

Other antecedents show that in razor clam fishing, the “good” months are in summer (January-March; October-December). This is due to the weather conditions, which allow a higher number of fishing trips (Sánchez *et al*., 2003), these being about 10 and 25 per month. But during the bad months it is only possible to make from 1 to 10 trips. During the good months, between 200-550 kg/day are unloaded and during the bad months, unloads are around 50 and 250 kg/day per vessel (Sánchez *et al*., 2003).

4. Fishing and the development of production

Razor clam fishing officially started, according to Chilean fishing statistics, in 1988. In that year 1,741 t were unloaded. After that, and as a consequence of the increasing interest in exporting this product, a huge increase was produced in volumes extracted, which reached more than 8 thousand t during 1991 (SERNAPESCA, 2000).

During the last 11 years (1998-2008), extraction of the resource from natural banks has been constant, with an average of close to 4,800 t per year (Fig. 4). In 2001, an important increase of unloads was observed, reaching 7,000 t, and then during 2002 and 2003 there was a sharp fall, registering only 4,000 t. The following year (2004) a slight recovery was observed; however, this situation changed in 2005, when there was a fresh decrease in unloading statistics: 3,559 t in 2008. The

variability of unloads highlights the instability of fishing and, hence, the availability of raw material for processing plants supplied from this resource.

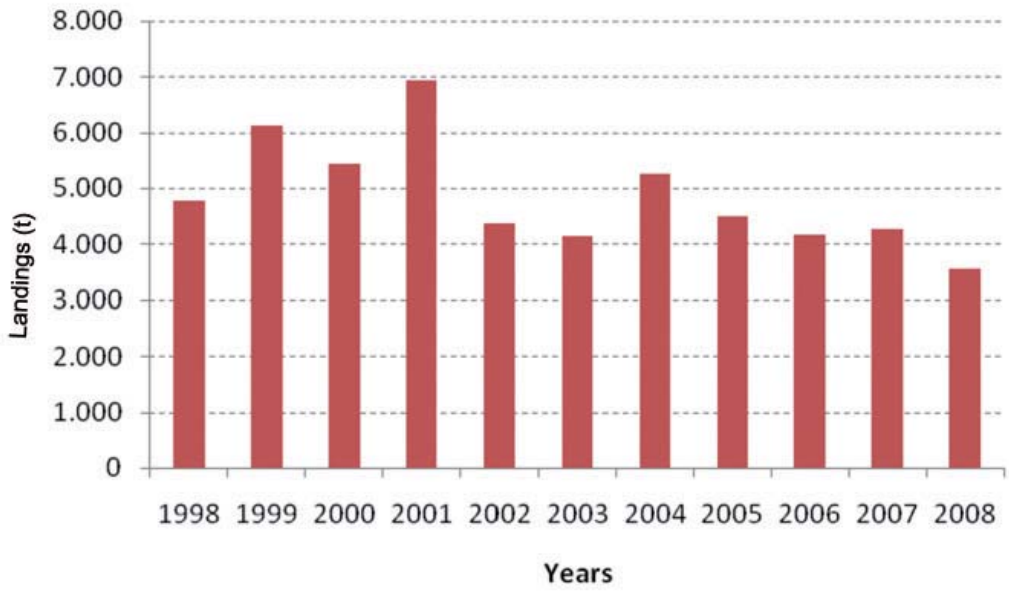

Figure 4. National unloads of razor clams between 1998 and 2008 (SERNAPESCA, 2008).

The areas of the country with the largest participations in razor clam unloads are Golfo de Arauco (8th Región) and Corral (10th Región), with 82% and 14% respectively of the national total in 2005. These regions are the most important ones in the extraction of this resource; however, during recent years, unloads in Magallanes region (3%) and Maule region (1%) (SERNAPESCA, 2005) have been added to the official statistics, areas where this resource might be an important source of income for artisanal fishermen.

Another characteristic of razor clam unloads is their exceptionally seasonal nature, since higher volumes are extracted during summer time annually, while during autumn and winter (June to September) unloads are lower (<400 t). In 2008, about 77% of unloads were performed in summer (December to April), while only 12% were carried out in winter (Fig. 5). This seasonality is due to poor weather conditions affecting extracting areas during winter time, limiting the number of fishing trips made by artisanal fishermen. This directly affects the extracted volumes, which are considerably reduced. Moreover, during most of November, there is a reproductive close season for the species, so unloads registered during that month are lower or non-existent.

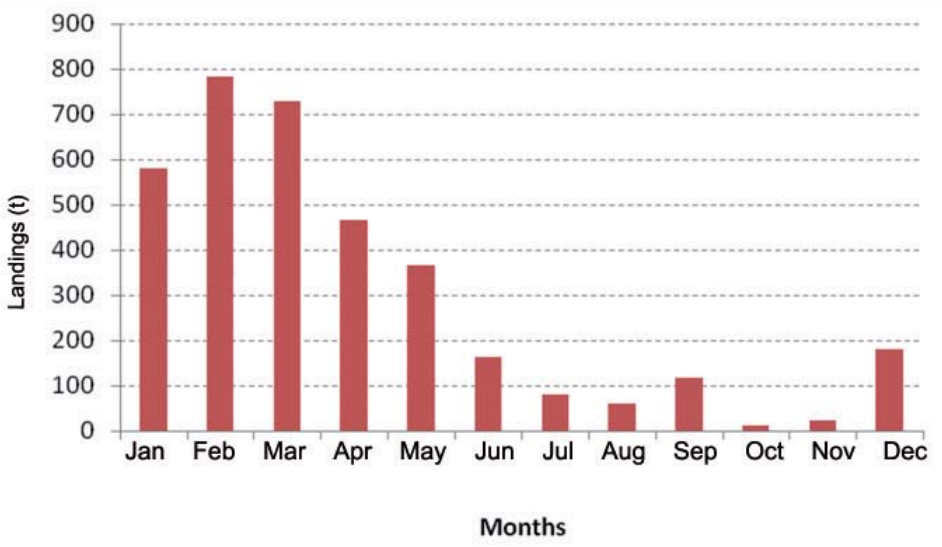

Figure 5. Razor clam unloads during 2008 (SERNAPESCA, 2008).

5. Production economy

During 1998 and 2008, unloads were aimed at raw material for canned, frozen products and a small amount for the fresh-cooled product (Fig. 6). In 2008, the canned product represented 70.2% of the sum total of raw material generated by this resource, and the frozen product 25.2%, while the fresh-cooled total (4.6%) was marginal (SERNAPESCA, 2008).

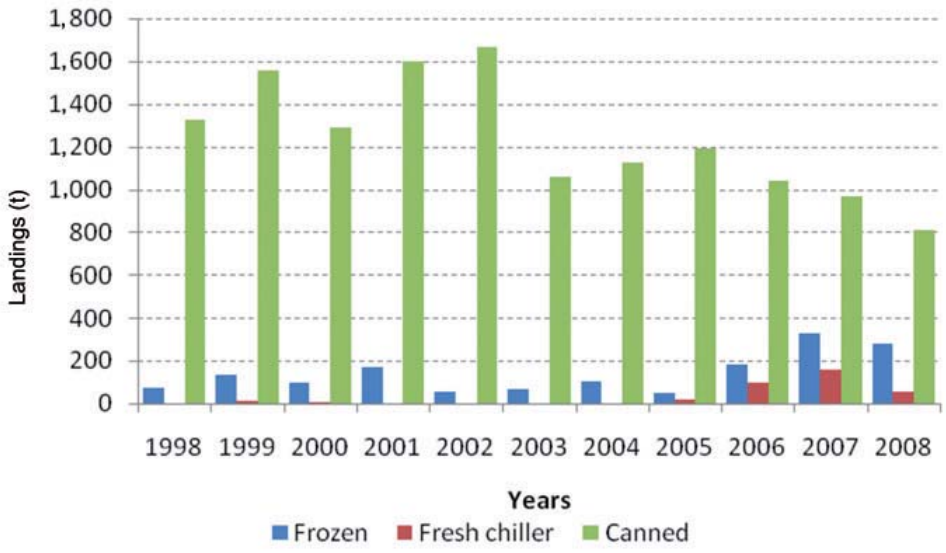

Figure 6. Statistics of razor clam production in Chile between 1998 and 2008 (SERNAPESCA, 2008).

During the last 11 years, production levels have been changing, mainly due to the high dependence of processing plants, unloads carried out by artisanal fishermen, and regulations established by a government authority.

Price variation of products derived from razor clams has meant a slow increase in producers´ incomes. The price per t of razor clams fluctuated until 2001, when it reached a minimal value (US$ 6,200). From 2002 an increase in the price per t was observed, reaching a maximum of US$ 9,861 FOB during 2005 (Fig. 7).

Incomes for razor clam exports also underwent important fluctuations until 2001, when they reached US$ 11,800 but just as happened with the price of razor clam per t, from 2002, a slow but significant increase of incomes was observed for the industry processing this resource (Fig. 7).

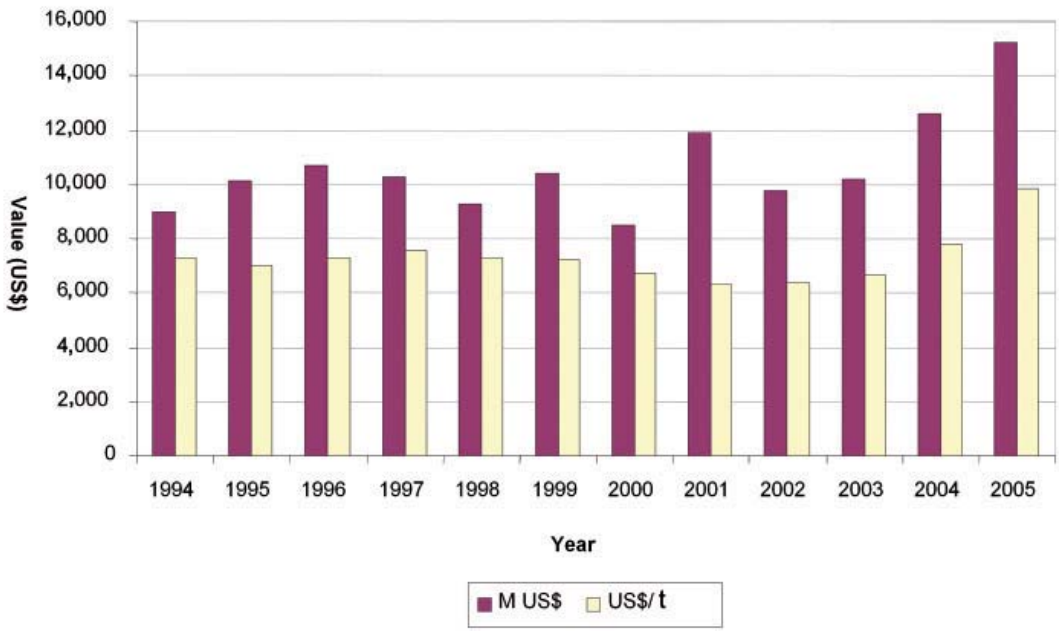

Figure 7. Price variations per ton, and total incomes for Chilean razor clam between 1994 and 2005 (Source: IFOP, Instituto de Fomento Pesquero, information given by Customs).

Since the destination of razor clam unloads is mainly for canned products (99.5% of production), the main price variations have been observed in this product. The average price of a t of canned razor clam, between 1995 and 2005, was US$ 7,290 FOB. In 2005 the price of a t of canned product reached US$ 9,900 FOB, while frozen production reached US$ 2,840 FOB (Source: Instituto de Fomento Pesquero, information given by Customs). During 2005 exports of canned product reached 1,543 t, so the industry that year invoiced for about US$15.2 million worth of this product (Fig. 7).

Razor clam exports are mainly aimed at Asian countries; however, Spain is the main importer of this product. In that sense, Spain registered a large increase in import volumes from 2002, beating the 1,200 t of the canned product during 2005 (Fig. 8). Malaysia was the second country importing canned razor clam during 2004 and 2005. However, volumes imported by this country do not outweigh those imported by Singapore, predominant in statistics until 2003.

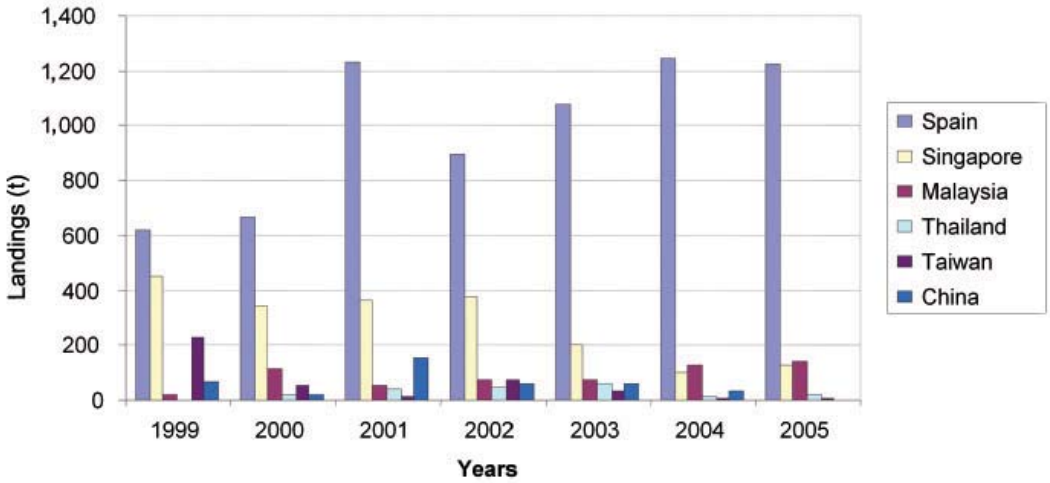

Figure 8. Main destinations of Chilean razor clam exports between 1999 and 2005 (Source: IFOP, Instituto de Fomento Pesquero, from information given by Customs).

During 2005, the historical tendency was repeated, Spain (80.1%), Malaysia (9.1%) and Singapore (8.2%) being the main countries importing razor clam.

6. Exploitation management. Fishing arts and regulations

The General Law of Fishing and Aquaculture (LGPA) is the regulatory framework for the sustainable management of hydrobiological resources and their environment in Chile. Fishing this resource has been carried out under an exploitation regime from January 1st 2003 to date. The state of full exploitation refers to that situation where fishing reaches such a level of exploitation that with the catching of authorised commercial units, there is no longer an excess in productive surplus of the hydrobiological species (Article 2º of LGPA). Before that, it was under the "General Access Regime", which was in force from January 1st 1998 to December 31st 2002. All national fisheries are submitted to this kind of regime by default, unless the opposite is indicated. "General Access Regime" implies that the fishing effort is not directly under control (there are no direct restrictions for new users to join), but it may imply setting quotas or other conservation measures.

Since the resource has been under strong pressure due to extraction, the fishing authorities have ordered a Regime of Full Exploitation for fishing, with restrictions like close seasons and the suspension of access to the fishing areas.

6.1. Biological and reproductive close seasons

1. Close season between October 1st and November 30th every year for the coastal area between 4th and 11th regions (year of publication, 1998).

2. Close season between August 1st and November 30th every year for 12th region coastal area (year of publication, 2003).

3. An additional close season is established between May 1st and July 31st every year for the 10th region (year of publication, 2005).

6.2. Suspension of access to fishing areas

1. From January 1st 2003 until June 30th 2008, inscription in the Artisanal Register of the 8th and 10th Regions was suspended in every category of fishing sections for the “huepo” species.

2. Inscription in the Artisanal Fishing Register of the 12th Region is suspended for 3 years in the fishing section for the “huepo” species (publication date, March 2006).

Despite measures taken by government authorities, no restrictions have been established regarding a minimum size for extraction or an extraction quota for artisanal fishermen.

Overexploitation symptoms in natural banks have been seen every year, so from 1996 on, biological and technological research was initiated for farming *Ensis macha* species.

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Chapter 22: Razor clam fishing in Patagonia, Argentina

Enrique Morsan and Néstor F. Ciocco

Abstract

Shellfisheries in North Patagonia constitute a traditional subsistence activity focused on scallops, mussels and some clam species. Among the latter, the razor clam (*Ensis macha*) represents an alternative to diversify traditional shellfish captures. Their beds are distributed along the coast structured as metapopulations consisting of discontinuous population subunits with different degree of connectivity. In these population subunits, densities often reach 60 individuals per m^2 (biomass: 1-1.5 kg. m^{-2}). In Argentina *E. macha* is mainly exploited at two sites from San Matías Gulf (42º S; North Patagonia): El Sótano (3-5 vessels) at the NW and Puerto Lobos (2 vessels) at the Centre-South of the gulf. The scale of the fishery is artisanal, with out-board motor vessels equipped with an air compressor that enables the diver to work with an "hydro-jet" system to remove the clam from the substrate. The average CPUE at El Sótano ranged from 4.8 to 19.6 Kg $diver^{-1}$ hr^{-1} between 2005 and 2009. Razor clam population dynamics is under study and fishery data are regularly collected by the Fishery Administration but with no biological reference points yet to regulate harvesting. Despite the fact that razor clam captures represent less than 1% of total shellfish landings of the artisanal fleet, there are favourable expectations based on the high price of first sale (around 3.56€ . kg^{-1}), and on the fact that the main beds remain unexploited.

1. Introduction

During the last decades bivalve fishing has become a traditional subsistence activity for many families in North Patagonia, whether by the local sale of catches or by domestic or semi-industrial processing of canned products. Bivalve harvesting on the Patagonian coasts of Argentina dates back to around 5500 years ago, when aboriginal gatherer hunters moved along these shores. After the European immigration in 1890-1920, bivalves began being harvested, not only by hand from the shore, but also using rowboats or small motorboats. These capture methods continued until the 1970's, when the activity quickly expanded in North Patagonian gulfs, later developing into small-scale coastal fishing using inboard motor vessels,

equipped with dredges. This was multispecies fishing, mainly targeting the tehuelche scallop *Aequipecten tehuelchus* and the mussel *Mytilus platensis* (Orensanz *et al.*, 1991; Ciocco, 1995). The availability of resources, together with the great demand from the market, led either to the expansion and later collapse of this traditional fishing or to the cyclic alternation of good and bad catching periods (Morsan and Zaidman, 2008). Consequently, this coastal fishing started gradually to give way to its artisanal counterpart, aimed at the small remaining beds of traditional bivalve species, and to the search for new species with possibilities as yet unknown to the market, such as several infaunal bivalves, crabs and snails.

Infaunal species (clams and razor clams) are important components of the coastal communities of North Patagonia. Until 2000, exploitation of these resources was almost nonexistent in North Patagonian gulfs, except for artisanal fishing of the purple clam (*Amiantis purpurata*), which developed from 1996 on, and the harvesting of the striped clam (*Ameghinomya antiqua*) at San José Gulf. From the beginning of 2000, the exploitation of infaunal species was aimed at the southern geoduck (*Panopea abbreviata*) and the razor clam (*Ensis macha*), although it never became established as a regular fishing activity.

Towards the end of the 1990's, small samples and pilot catches of *E. macha* from the shellfishery by diving of San José Gulf began to be distributed in the internal market. Unlike in Chile, where *E. macha* is one of the most important infaunal resources (which has generated populational studies; Urban, 1996; Chong *et al.*, 2001), in Argentina this species has only in recent years begun to awaken interest as an alternative to diversify traditional shellfisheries. In fact, the Centro Nacional Patagónico (Chubut Province) and the Instituto de Biología Marina y Pesquera "Almirante Storni" (Río Negro Province) have begun joint investigations in recent years with the aim of improving the knowledge of biological and populational aspects of alternative infaunal resources for artisanal fishing. In this sense, bed surveys, as well as studies of the age, growth and reproduction of razor clam, purple clam and striped clam were carried out, including descriptions of artisanal extractive activity.

This chapter focuses on the evolution of *Ensis macha* catches, methods and harvest areas, number of vessels and fishermen, trading rules and the economic impact of this activity on coastal communities from North Patagonia.

2. Exploited species and their distribution

Two species of razor clams occur along Argentinian coasts: *Solen tehuelchus* (d'Orbigny, 1843) and *Ensis macha* (Molina, 1782) (Fig. 1). *S. tehuelchus*, the

smallest razor clam species, is endemic to the Southwest Atlantic where inhabits sandy bottoms of both intertidal and subtidal areas from Rio de Janeiro (22º 27' S, Brazil) to San Matías Gulf (41º S, Argentina) (Lasta *et al.*, 1998). Their shells are very fragile and elongated with a yellow-greenish periostracum. In Argentina *S. tehuelchus* seldom reaches the market, and their sporadic catches are not included in the landing statistics (Penchaszadeh *et al.*, 2007).

Figure 1. Razor clam species inhabiting Argentine waters. *Ensis macha* is the only species exploited.

Ensis macha is the largest razor clam species and the one of greatest commercial interest in Argentina. They have a flattened cylindrical shell which is more elongated than that of *S. tehuelchus,* with slightly curved parallel edges, truncated and half-open ends and periostracum olive-brown in colour; the umbo is located at the front end. This species is found in both the Pacific and Atlantic Oceans. *E. macha* inhabits muddy and sandy beds ranging from shallow waters down to 55 m deep, from Caldera (Peru, 27º S) to Magallanes (Chile, 55º S) along the Pacific, and along the Argentinian Atlantic coast to San Matías Gulf (Argentina, 41º S) (Lasta *et al.*, 1998; Scarabino, 1977). Just like other sedentary species, they are structured as metapopulations consisting of discontinuous population subunits with different levels of connection between them by pelagic larvae. These population subunits frequently

show aggregate distribution, reaching densities of up to 60 individuals per m^2 (biomass: 1-1.5 kg m^{-2}) as in El Sótano, Punta Pozos and Puerto Lobos (Fig. 2).

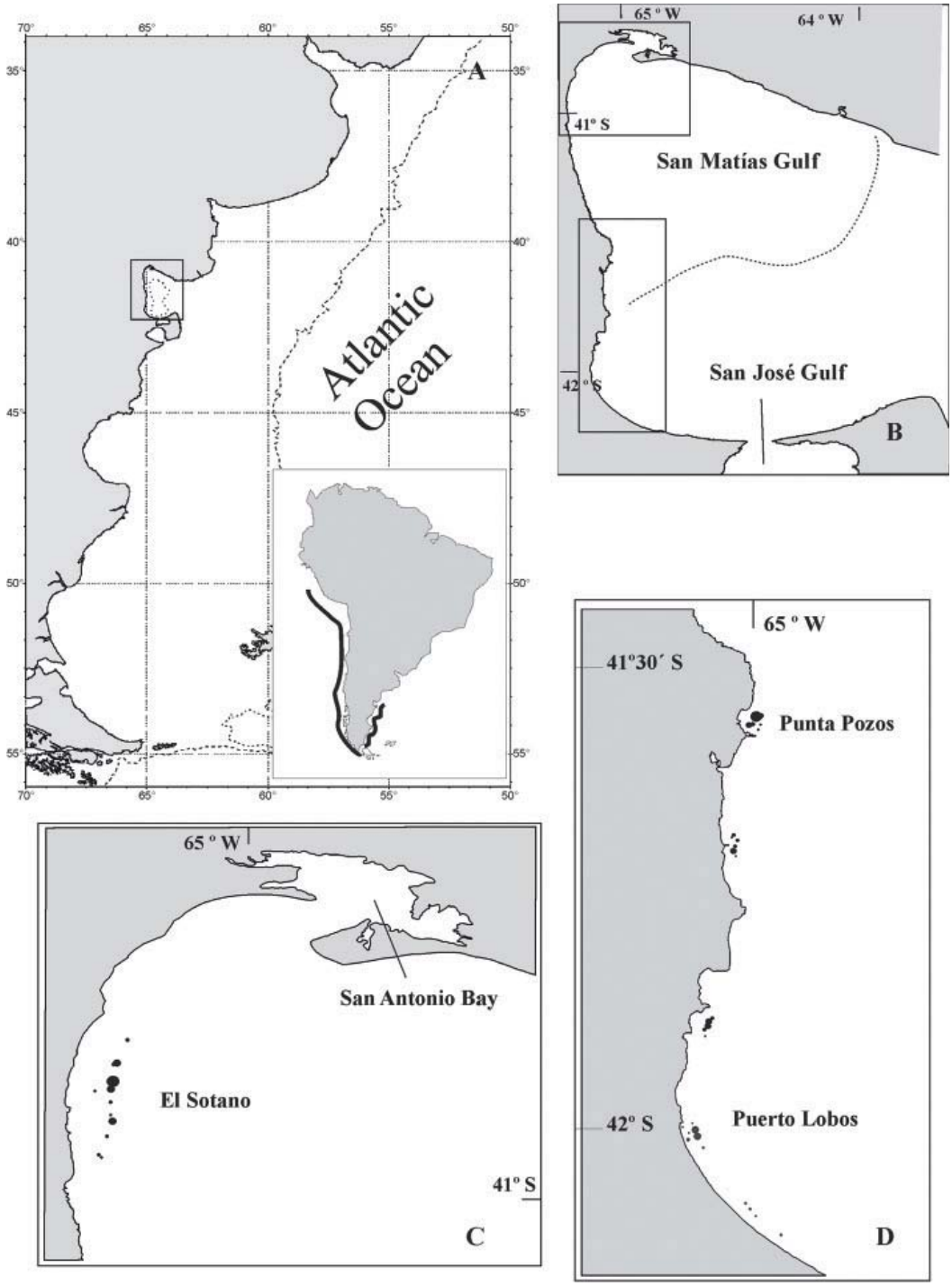

Figure 2. Spatial pattern of razor clam in Patagonia on different scales. A: Argentine coast with the geographical distribution of *E. macha*. B: San Matías Gulf map indicating the only two places where razor clam is exploited in Argentina. C and D: Small-scale spatial distribution of abundance in the North (C) and South (D). Large black circles represent 60 clams m^{-2}.

3. Catches

3.1. Harvesting methods

Razor clam harvesting in Patagonia has always been undertaken by divers with "hookah" systems operated from artisanal vessels (<9.9 m long), equipped with an air compressor that enables up to 3 divers simultaneously, and a crew composed of a skipper and a fisherman. The harvesting process starts with a diver exploring the seabed until he finds densely populated razor clam beds, which are recognised by the burrowing marks left by siphons on the sediment surface. Razor clam extraction is carried out using a "hydro-jet" system as illustrated in figure 3. It consists of an on-board water pump that takes in seawater through a hose with a filter and expels it by pressure into the end of another hose with a thinner section (Ø 55 mm). At the end of this hose there is a rigid metal structure, which facilitates handling by divers and ends in a small section with 5 holes: 1 central and 4 peripheral holes at a 45º angle to the central one (see Fig. 3).

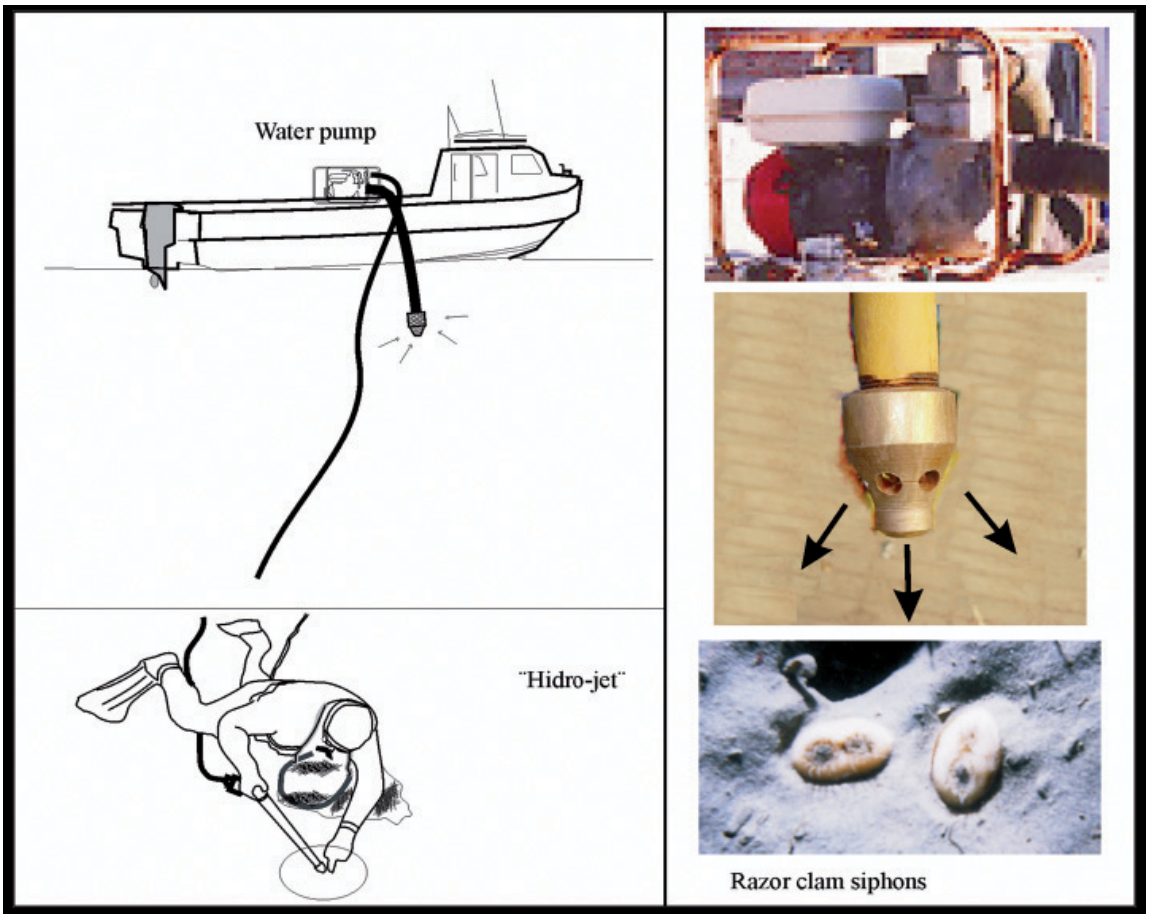

Figure 3. Razor clam harvesting system. On-board water pump (top), end of stinger with several holes to expel water jet (centre) and razor clam siphon tips (bottom).

The expelled water removes the surrounding sediment, making it easy to pull out individual clams. In some cases the pump is modified to maximise either water volume or pressure; in others, the number of holes is increased in order to widen the sediment removal area. Fishermen decide on these options depending on convenience and effectiveness. Razor catched clams are placed in a net bag with a rigid mouth, which, once full, is hauled on board the vessel and replaced by an empty one. The normal depth at which divers work is about 12-25 m; each working day is approximately 4 hours. The operation area of the divers' team (2 or 3) has a radius of approximately 30 m around the vessel. The variation in the daily position is around 100 to 150 m. The average Catch Per Unit Effort (CPUE) per year, estimated from the fishing records of vessels at El Sótano (overall data with razor clam as main target) ranged from 4.8 to 19.6 Kg diver^{-1} hr^{-1} between 2005 and 2009. Ciocco (1999) performed catching efficiency tests with other fishing gear at Puerto Lobos obtaining the following CPUE estimates (Kg diver^{-1} hr^{-1}): shovel = 3.1; pliers = 3.9; balls = 3.5; suction =1.8; air/pressure = 4.1.

3.2. Catching areas

E. macha is only exploited at two sites in San Matías Gulf (Fig. 2). The most important one is El Sótano, in the NW angle of this gulf, next to Bahía San Antonio. The fishing beds are a few miles away from the landing site (Fig. 4), which is an appropriate sector of the beach also used by fishermen to sort out individuals. Catches are then transported overland to the artisanal fishing terminal of San Antonio Oeste (Río Negro province) where they are washed and stored until their shipment to centres of consumption.

Figure 4. Artisanal fishing vessel and view of a fisherman's camp on the coast of San Matías Gulf (Río Negro, Argentina).

Exceptionally, shellfish harvesters from Puerto Madryn (Chubut province) catch reduced amounts of *E. macha* at San José Gulf (Península Valdés; 42º 20' S), where the resource is abundant, especially along the West and South coasts of that area

(Ciocco, 2000).

3.3. Number of vessels and fishermen

In San Antonio Oeste (North of San Matías Gulf) there are permanently 74 artisanal vessels, of which 30 operate regularly, making about 1000 fishing trips per year. Due to the nature of the equipment required for razor clam extraction, only 3 to 5 vessels work at this place. At Puerto Lobos (Centre-South of San Matías Gulf) only two vessels catch razor clams on the border between Rio Negro and Chubut provinces.

3.4. Fishing regulations

Razor clam fishing regulations only refer to their commercialisation; there are no specific rules based on biological reference points derived from *E. macha* population studies or stock assessment. *E. macha* fishing in the provinces of Rio Negro and Chubut follows the general rules established for artisanal fishing, meaning that the skipper of the vessel is required to submit a Fishing Report to the provincial fishing authorities, indicating data on the vessel, its positions, catch by species, fishing effort in hours and number of divers for each fishing trip. The report must be forwarded to the fishing police on arrival at the beach used as a fishing port. From that point on, it is compulsory to have all catch documents in order to ensure traceability of the products transported overland in refrigerated trucks to consumption destinations. Prior to their commercialisation, all molluscs require a laboratory analysis indicating the absence of contamination by biotoxins in order to guarantee the sanitary safety of the product. However, in practice, collecting these data is not easy, and both weak controls and great informality in trading practices have been detected.

3.5. Production and commercialisation

As expectable, the main motivation of bivalve fishermen is to maximise their daily catch, which is linked to expectations for the best economic performance in the least time. This factor determines the spatial allocation of fishing efforts, leading to a progressive exhaustion of the densest beds, which are abandoned when profitability drops below break-even point (Orensanz, 1986; Caddy, 1989). Locating denser beds requires a higher investment in search time. Once this stage is finished, divers devote their time exclusively to harvesting. Sometimes it is impossible to distinguish between these two stages, but presumably the time spent searching will increase as beds are progressively exhausted (Caddy, 1979). Theoretically, this recurrent exploitation and harvesting process occurs on a small spatial-temporal scale, and determines that a fisherman‘s permanence in a specific place is conditioned by the dimension and density of beds and by the daily catch, that is, by spatial distribution

of the resource and also by the CPUE (Orensanz and Jamieson, 1998).

Razor clam fishing does not follow this pattern strictly, since fishermen share their interest both in this resource and in other species and they assume previous sale commitments for different shellfish, which means that on each fishing trip the effort must be allocated between 2 or 3 beds of different species. Market demand is not constant but erratic, and is low compared to other resources. Although it seems the global trend has been towards an increase over the last 5 years, annual razor clam catches reached just 16.3 t in 2008 and 10.5 t in 2009 (Fig. 5A), which represents less than 1% of fish landings of the artisanal fleet working in San Antonio Oeste harbour (Fig. 6).

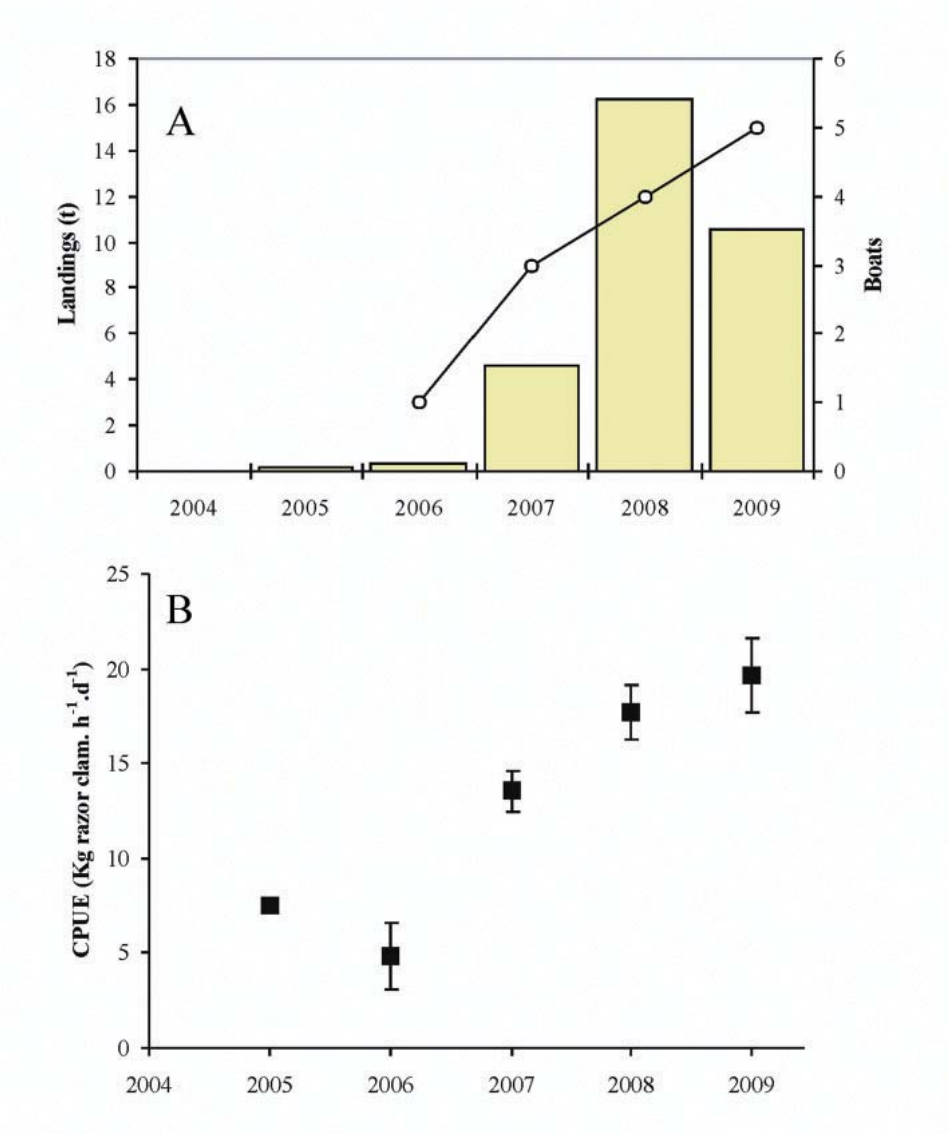

Figure 5. A: Annual *Ensis macha* catches landed by the artisanal fleet of San Antonio Oeste (bars) and number of fishing vessels. B: Average annual catch per unit effort (in kg of razor clam per hour and per diver).

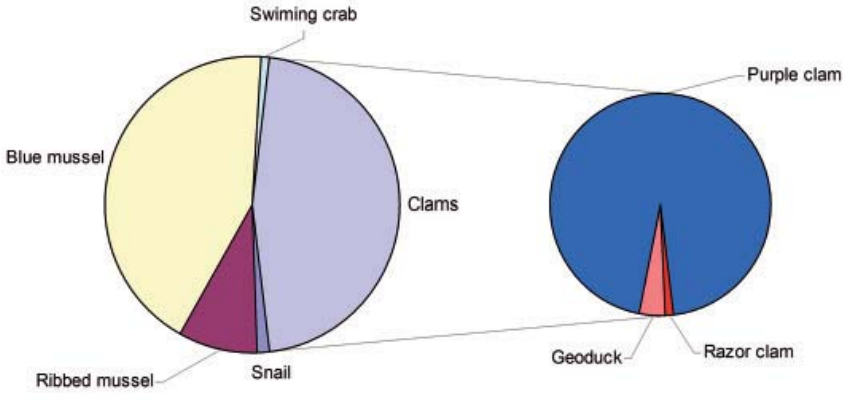

Figure 6. Proportion of razor clam landings (average 2005-2009) with respect to total shellfish landings from the artisanal fleet in San Antonio Oeste. Total annual landing is 1370 t, where clams represent 46%, and razor clams 0.6%.

Average annual CPUE shows a tendency to increase (Fig. 5B). This aspect must be analysed in the context mentioned above, where in one trip the effort is divided between several target species. This trend has been interpreted as the result of factors such as: i) a greater importance of razor clams in the total of exploited resources, due to the greater interest of fishermen and ii) the training of divers in the harvesting of the resource.

These data may be reorganised in order to explore the repercussions that catches of each species have on the community of artisanal fishermen. Figure 7 shows mollusc species which are fishing targets, on two axes and with four parameters. The horizontal axis represents the economic aspects specific to each species; on the right is the price paid to the fisherman on first sale, and on the left the total income in terms of this price and the average annual catches (2005-2009). The vertical axis describes the level of distribution of the income generated, representing the number of fishermen involved as well as the number of vessels. From the point of view of optimising social income, an ideal situation can be defined as one where all values tend towards the maximum. It is evident that catches of mussels (*Mytilus platensis*) or purple clams (*Amiantis purpurata*) dominate the economic flow despite their low price on first sale. Southern geoduck clam (*Panopea abbreviata*) and razor clam are, at different levels, products with less participation in artisanal fishing, with smaller landings and catches depending on the ups and downs of demand. Moreover, razor clam has the highest price among target resources of regional artisanal fishing (around 3.56 € kg^{-1}).

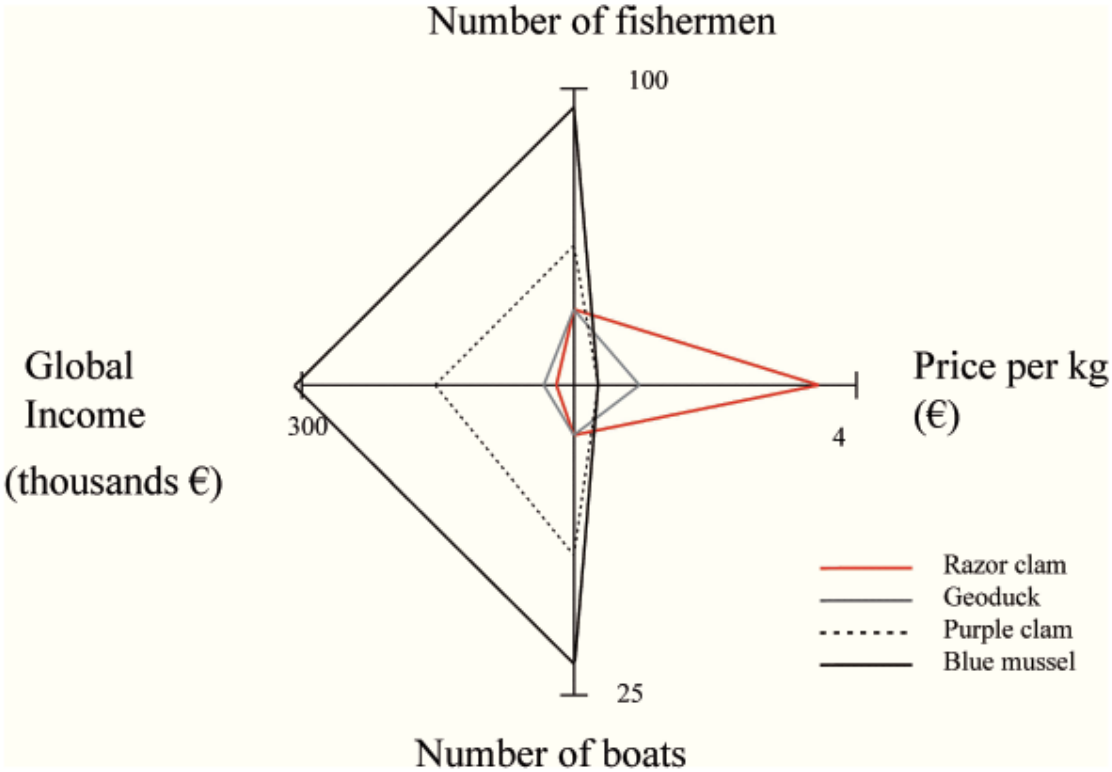

Figure 7. Economic income axis (horizontal) and participant or distributional axis (vertical) to illustrate the contrast between social repercussions of three clam species. All values are averaged annually, taking into account the last 5 years (details in text).

4. Monitoring of populations

The research institutions mentioned in the introduction conduct investigations about, among other things, how coastal marine ecosystems function, including the most interesting species from the fishing perspective. The goals of this research are to understand distribution patterns of benthic resources on different scales, to study their population dynamics, to examine interspecific interactions and to develop sound strategies for sustainable management of the resources to be exploited.

One of the limitations for the management of artisanal benthic fishing is obtaining the information needed for management at different spatial scale. This is only possible with the active collaboration of fishermen. In both institutions several research projects are in progress to explore participatory modes to monitor shellfish resources, analyse available information and joint decision-making among the enforcement authority, fishermen and scientists, in the framework of an ad-hoc technical team.

Administration of resources is the responsibility of the authorities with jurisdiction over the coast involved. These provincial authorities and regional research centres coordinate tasks as assessment of resources and submitting of financial proposals in the form of research projects to national authorities, as well as discussing the options for regulation within a co-management framework.

Razor clam populations are not specifically monitored in the provinces of Rio Negro or Chubut, but they are included in general prospecting programmes for benthic resources.

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Chapter 23: Razor Clam Fisheries in Thailand

Narong Veeravaitaya and Sansanee Choowaew

Abstract

Most razor clams found in Thailand are *Solen* spp. They can be found in several provinces along the coast, both in the Andaman Sea and the Gulf of Thailand. The largest razor clam population is located at Don Hoi Lot Tidal Flat in Samut Songkhram Province and the dominant species is *Solen corneus* (Lamarck, 1818). According to statistics, the production of razor clams has declined in recent years due to high demand and overharvesting by local fishers. Threats found in the area, namely polluted water, mud deposits, high density of horse mussel and improper harvesting practices for catching razor clam, may also have contributed to the decline of razor clam populations. Several strategies have been implemented by local administrative agencies aiming to increase razor clam populations. With this purpose, a conservation program has been designed in collaboration with local stakeholders.

1. Introduction

Thailand is a Southeast Asian country located between latitude 5º 45’ and 20º 30’ North and longitude 97º 30’ and 105º 45’ East, covering a total area of approximately 513,115 square kilometres. Coastal areas are divided into three main parts: Eastern, Central and Southern. The Central region is characterized by low plains, the lower half of the region stretching toward the Gulf of Thailand. There are four major rivers of the region discharging into the Gulf of Thailand: Bang Pakong, Chaopraya, Tachin and Mae Klong Rivers. The topography of the Eastern region is dominated by coastal mountains and hills, from which a number of minor rivers discharge into the Gulf of Thailand. The Southern region is a peninsula with the Gulf of Thailand on its East Coast and Andaman Sea on the West Coast (UNEP, 2004). The major fishing activities in the coastal area are brackish-water aquaculture and the main cultured species are giant tiger prawns (*Penaeus momodon* Fabricius, 1798), whiteleg shrimps (*Litopenaeus vannamei* (Boone, 1931), green mussels (*Perna viridis* Linnaeus,1758), blood cockles (*Anadara* spp.) and oysters (*Crassostrea commercialis*) (FAO, 2010). However, there is no relevant aquaculture activity for

razor clams, most of which are caught from intertidal areas. Since 1970, bivalve fishery has deteriorated due to organic pollutants discharged directly from the river to the sea as well as overfishing in the Gulf of Thailand (Suvapepun, 1991).

2. Target species distribution

In Asia, most individuals of the *Solen* genus are found in the lower part of the intertidal zone and are distributed between Indonesia, the Philippines, China and Japan (Tuaycharoen and Matsukuma, 2001).

Figure 1. Photograph of razor clam (*Solen* spp.). Source: Veeravaitaya (2010).

In Thailand, razor clams are found both on the Andaman Sea (in Phuket province) and in the Gulf of Thailand (Fig. 1), in Songkla, Prachuap Khirikhan, Phetchaburi, Samut Songkhram and Samut Prakarn provinces. Tuaycharoen (1999) reported three species of razor clams in Samut Songkhram, *S. strictus* (Gould, 1861), *S. corneus* and *Solen* sp. *S. corneus* is also found in Samut Prakarn Province and *Solen* sp. in Phetchaburi province. Tuaycharoen and Matsukuma (2001) reported three species of razor clams: *S. brevis* (Gray, 1832), *S. grandis* (Dunker, 1861) and *S. exiguous* (Dunker, 1862) along the coast of the Andaman Sea, namely in Trang and Satun provinces, and six species: *S. corneus*, *S. regularis* (Dunker, 1861), *S. strictus*, *S. malaccensis* (Dunker, 1862), *Solen* sp.(1) and *Solen* sp.(2) in the Gulf of Thailand and in the provinces of Chantaburi, Samut Prakarn, Samut Songkhram and Phetchaburi. At Don Hoi Lot, Tuaycharoen and Kongrabeab (2005) 4 razor clam species were identified: *S. strictus*, *S. thailandicus* (Cosel, 2002) (formerly known as *Solen* sp.(1), *S. corneus* and *S. regularis*). In 2008-2009, Veeravaitaya (2010) observed that the dominant species in Don Hoi Lot was *S. corneus*, followed by *S. strictus* and *S. regularis* (Fig. 2). Razor clams can also be found in 2 other provinces: Chumporn and Trat (Fig. 3).

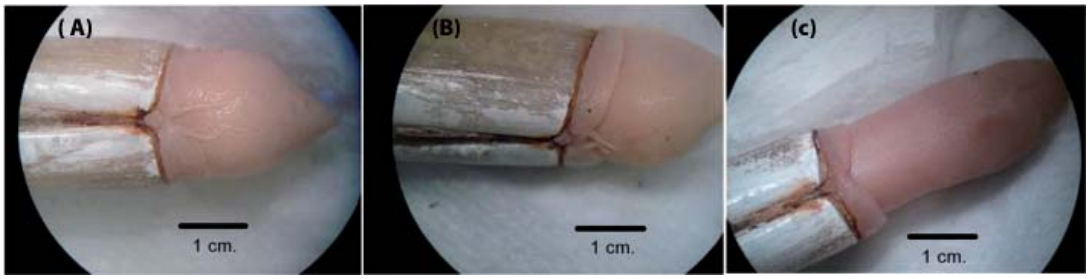

Figure 2. Tentacle characteristic of razor clams in Don Hoi Lot: *S. corneus* with long tentacles (A) *S. strictus* with short tentacles (B) and *S. regularis* with no tentacle (C). Source: Veeravaitaya (2010).

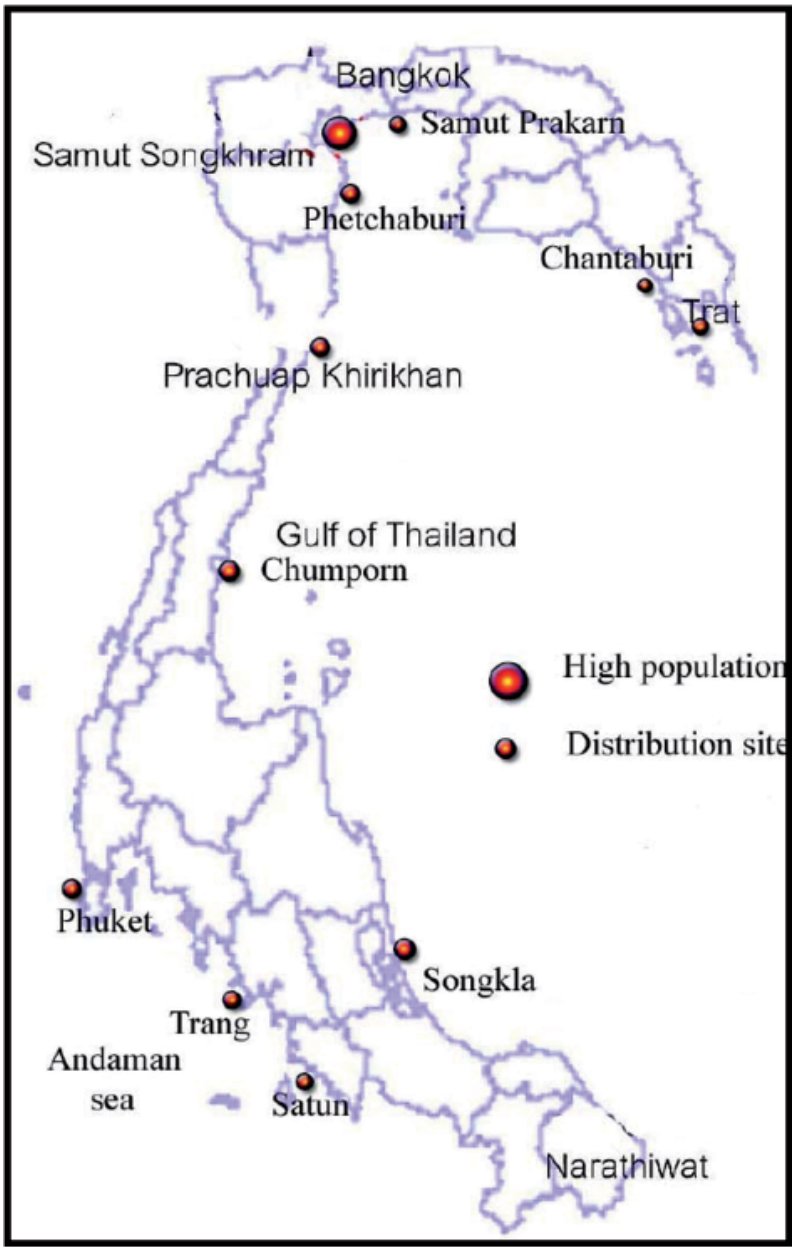

Figure 3. Distribution of razor clams in Thai coastal areas.

3. Production

3.1. Razor clam harvesting methods

There are 5 traditional methods used by local fishers to harvest razor clams during low tide when sand banks are exposed (Department of Fisheries, 1995; Worrapimphong, 2005), such as:

- Method I: Lime dipping. This method is the original and traditional method, whereby local fishers search for razor clam holes using their fingers to knock on the sandy surface. If razor clams are accessible, they eject water from the siphon through the hole, and subsequently, a small bamboo stick dipped in lime is poked into the holes. The razor clams react, emerge from their holes and are caught by the fishermen.

- Method II: Applying lime. Local fishers apply lime directly on the seabed where razor clams live. The razor clams escape from their holes and are caught by fishers.

- Method III: Applying a lime solution. Local fishers dissolve 1-2 kg of lime in 5 litres of water and apply the solution to the seabed. This method is similar to Method II.

- Method IV: Applying an acetylene solution. Local fishers apply an acetylene solution to the seabed, and subsequently all the razor clams react, jumping from their holes. This method is similar to Method II and III but far more effective. However, the acetylene solution has more impact on other species than methods using lime.

- Method V: Digging. This method is the best for collecting razor clams because no chemical is involved. However, the digging method is inefficient because more labour is used and production is lower than with other methods.

Kanthom and Sukawong (1991) reported that the traditional method for catching razor clams in Don Hoi Lot by using small sticks with lime allowed local harvesters to select the size of razor clams, whereas a dispersion method, scattering the lime solution, would kill all the razor clams. The study also showed the effect of a concentration of quicklime on razor clams' mortality rate. The lethal concentration (LC50) value for 48 hours of small size clam with a shell length of 1.5-2.9 cm was 321.50 mg/L, whereas LC 50 value for 48 hours of medium size (3.1-4.4 cm) and

large size (4.2-7.0 cm) was 376.21 mg/L and 338.32 mg/L, respectively. Safest concentrations of small, medium and large sizes were 32.15 mg/L, 37.62 mg/L and 33.83 mg/L, respectively.

Currently, only razor clam harvesting methods I (Fig. 4) and V are allowed, since other methods have a hugely negative impact on both target and non-target species.

Figure 4. Local fishers harvesting razor clams by lime-dipping during daytime and night-time.

3.2. Fishing areas

Don Hoi Lot is the most important razor clam fishing ground and is described mainly as a tidal flat consisting of muddy sand. It is a unique coastal area created by river sedimentation at the estuary of the Mae Klong River. The tidal flat extends approximately 8 km into the sea and is 4 km wide during low tide, the area consisting of fine silt and characterized by being rich in nutrients. Tides occur in the area twice a day, with water rising North during high tide and receding South during low tide. A report by Oiamsomboon (2000) indicates that Don Hoi Lot covers an area of 24.09 km^2. This site can be divided into 5 sections of elevated sediment deposits or islets, covering approximately 0.69, 1.51, 4.64, 8.45 and 8.80 km^2, respectively. Don Klang is the largest islet, occupying 8.80 km^2 and located in the centre of Don Hoi Lot (Fig. 5).

3.3. Number and social characterization of razor clam harvesters

In 2005, Worrapimphong reported that 82 persons/day were harvesting razor clams in Don Hoi Lot, the number varying each month according to razor clam abundance, season of the year and the opportunities for obtaining other work. In 2008-2009, Veeravaitaya (2010) carried out a field survey in Don Hoi Lot and

observed that the number of razor clam harvesters had decreased to 20, reflecting the reduction in the razor clam population in the area. The ratio of male to female harvesters was 1:1 with an average age of 35 (ranging from 17 to 53) and 47 (ranging between 37 and 60) for male and female harvesters, respectively. Most of them are local inhabitants and have lived in the area for more than 38 years. In terms of educational skills, 90% have secondary school level and the size of the average family was 4.5 individuals. Most hand gatherers had experience in razor clam harvesting from 5-40 years, with an average of 22.5 years. Harvesters' income comes from several activities, such as razor clam harvesting, the harvesting of other molluscs (Ark shell and Tiger moon snail) and other non-fishing activities.

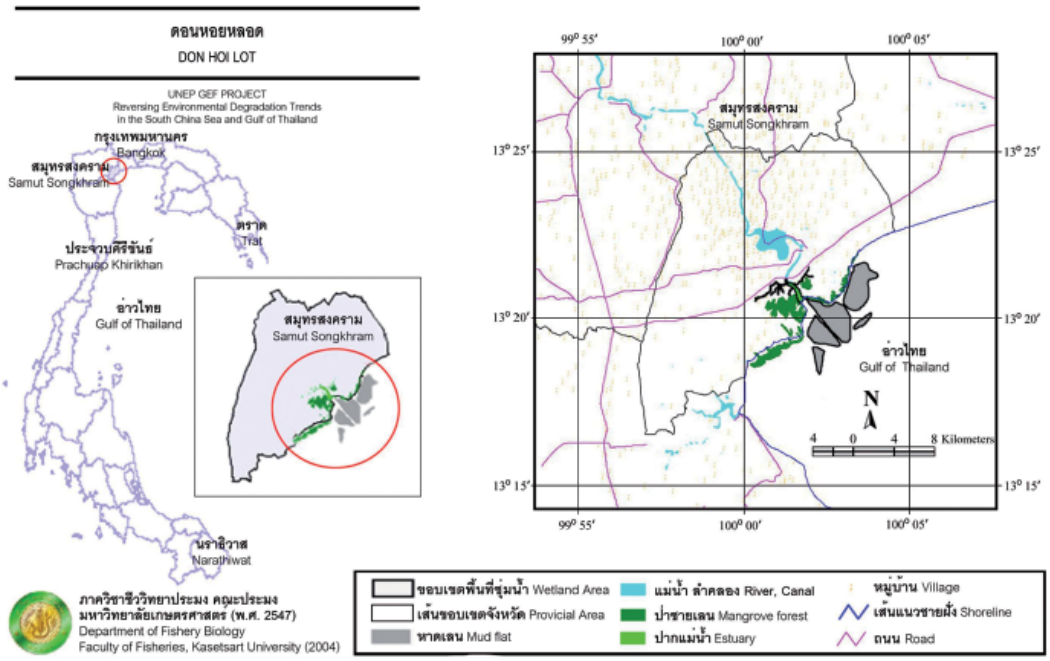

Figure 5. Location of Don Hoi Lot; Source: UNEP (2004).

3.4. Legislation in force

In 1993, the Samut Songkhram Provincial Forest Office declared an area of 48 ha (300 rai) of newly-formed mudflat in Bang Cha Kreng, Bang Bo and Bang Kaew Sub-Districts as a Restricted Preservation Area (Chulalongkorn University, 1996). Later, Chulalongkorn University prepared the Nature and Environment Conservation Plan for Don Hoi Lot which was submitted to the Office of Environmental Policy and Planning in August 1996. Three conservation management zones were proposed: a Preservation Zone (including mangroves, newly formed land with mudflats, and 5 areas of mudflats); a Conservation Zone; and a Development Zone. At least 560

ha (5.6 km^2 or 3,500 rai) were declared the *Anadara* sp. Conservation Area. In May 2000, the National Environment Board approved the conservation and sustainable use of Don Hoi Lot, a plan of action drawn up by several governmental and non-governmental agencies.

Samut Songkhram Province, Muang District, Bang Cha Kreng, Bang Kaew, Laem Yai and Klong Kone sub-districts are responsible for regulating land use and land encroachment whereas the Department of Fisheries is responsible for regulating fishing activities in the area.

3.5. Production

According to Department of Fisheries statistics (1989), the total catch of razor clam from 1983 to 1989 was 1,275, 299, 395, 299, 414, 267 and 795 t/year, respectively, while the total catch of razor clams from 1998 to 2004 was 74, 318, 298, 54, 21, 206 and 95 t/year respectively (Department of Fisheries, 2000, 2001, 2002, 2003, 2004, 2005 and 2006). In 2008-2009, the total catch of razor clams in Don Hoi Lot was about 11.2 t/year (Veeravaitaya, 2010). From the above-mentioned figures, it is shown that the total catch of razor clams tended to decrease steadily from 1983 to 2009 (Fig. 6) due to high demand, consumption, a high commercial price compared to other molluscs, and degradation of the environment. The economic role of razor clams at Don Hoi Lot is related to ecotourism, as each year this flagship species is used as a symbol of the area. According to Jarinrattanakorn (2001), the production values of razor clams harvested by local fishermen were more than a million baht/ year since 1983.

In seasonal terms, fishing intensity is greatest between June and September and least during February. The time consumed in harvesting razor clam was about 2,846 hours/year, with a maximum harvesting time in July of 388 hours and a minimum in February of 146 hours. As for CPUE, a maximum was observed in October (0.30 kg/ fisher/hour) and a minimum in May (0.16 kg/fisher/ hour) with an average of 0.23 kg/ fisher/hour (Veeravaitaya, 2010).

Total catch is directly related to the density of razor clam populations. Several studies aiming to estimate razor clam densities have been carried out in Don Hoi Lot. In 1981, Pradatsundarasar (1982) estimated a density of 10.00±1.23 individuals/m^2 while in 1982 Jongpepian *et al*. (1985) reported that razor clam density ranged from 0 to 72 individuals/m^2. In 1986 and 1988, their densities were 26.80±11.59 individuals/ m^2 and 65.51±57.60 individuals/m^2, respectively (Pradatsundarasar *et al*., 1989). In 1991, Khumsupha *et al*. (1991) found densities of between 0 and 175 individuals/m^2. Between 1994 and 1997 a dramatic decrease of razor clam density was observed,

from 49.86±37.38 individuals/m^2 (1994-1995; Sriburi and Gajaseni, 1996) to 4.6±3.7 individuals/m^2 (1996-1997; Boutong, 1997). In the 2008-2009 period, Veeravaitaya (2010) also reported that razor clam density was less than 1 individual/m^2.

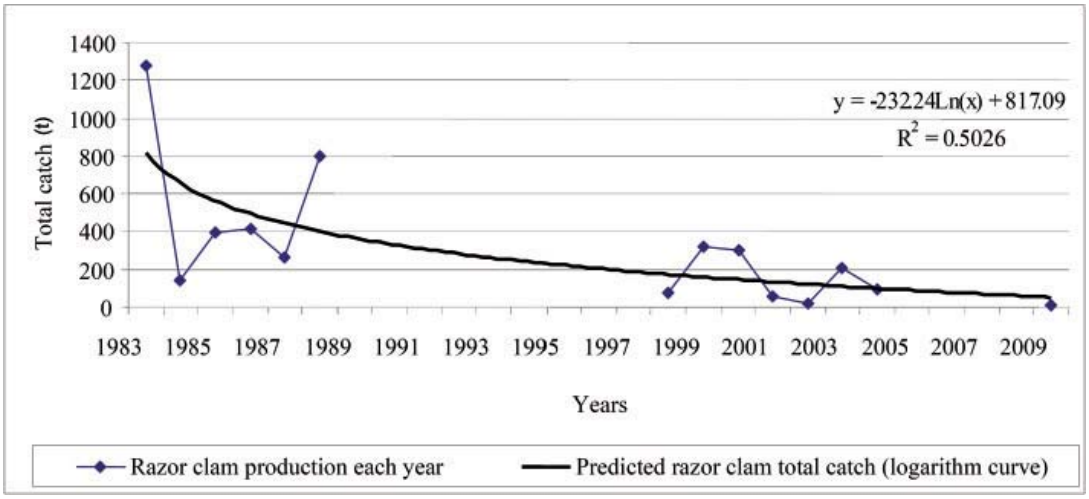

Figure 6. Predicted equation of total catch of razor clams from 1983 to 2009. Source: Veeravaitaya (2010).

4. Commercialization and depuration

There are two ways of selling razor clam in local markets: fresh and dried mixed with salt or sugar. However, they can also be sold by local fishers as raw material for food processing, to increase their value. Most razor clam products are sold only in domestic markets in several provinces such as Prachuap Khirikhan, Phetchaburi, Samut Sakorn, Chonburi, Samut Prakarn, Rayong and Samut Songkhram (the biggest market in Thailand) (Department of Fisheries, 1995). In Don Hoi Lot, most collectors harvested not only razor clam but also Ark shell and Tiger moon snail throughout the year, and the razor clam harvesting period lasted 18-31 days, an average of 23 days per month. Intensive razor clam harvesting took place at Don Na San (in front of the Prince Chumporn Khedudomsak Memorial), Don Laem and others at 60, 20 and 20%, respectively (Veeravaitaya, 2010).

The selling price for fresh *Solen* spp. ranged from 60-220 baht/kg, an average of 140 baht/kg, while the selling price for Ark shell and Tiger moon snail were 13 and 32.5 baht/kg, respectively. Fishermen transport razor clams to be sold directly to the market, to middle-men and for household consumption at 15, 75 and 10%, respectively (Veeravaitaya, 2010). The selling price of fresh razor clams, including their shells, varies every year depending on supply and demand (Table I).

Table I. Selling price for razor clams from 1982 to 2009 at Don Hoi Lot, Samut Songkhram Province.

Year	Fresh seafood with shell (baht/kg)	Dried seafood without shell (baht/kg)
1982-1983	No information	20
1984-1985	No information	20
1986-1987	6-8	30-40
1988-1989	8-15	40-60
1990-1991	15-20	60-80
1992-1993	20-45	100-120
1994-1995	45-60	150-300
1996-1997	60-80	400-500
1998-1999	80-120	No data
2003-2004	70-120	400-500
2008-2009	60-220	No data

Source: Department of Fisheries (1995); Papawasit *et al*. (2004); Veeravaitaya (2010).

5. Razor clam bed monitoring

Currently, there are no monitoring programmes to evaluate the state of razor clam beds. Nevertheless, it must be stressed that several studies have been carried out in order to investigate the influence of environmental factors such as grain-size composition (Pradatsundarasar, 1982; Tumnoi, 1996; Tuaycharoen and Charoenpornthip, 1997; Tuaycharoen *et al*., 2002; Veeravaitaya, 2010), hydrodynamics (Purchon, 1968), physical/chemical parameters of water (Tumnoi, 1996; Mongkulmann, 2001; Phuwapanich *et al*., 2003; Veeravaitaya, 2010) and heavy metals (Tuaycharoen *et al*., 2000) on the distribution of razor clam populations.

A recent study carried out by Veeravataya (2010) allowed the identification of the greatest threats for razor clam beds in Don Hoi Lot, such as: the impact of local communities; pollution; mud deposits; the spread of invasive exotic fauna (horse mussel); the harvesting of venus shellfish; limited water circulation; the use of sodium hydroxide for harvesting razor clam. Local fishers stated that, if these threats continue, some mollusc species, especially razor clams and the wedge shell (*Donax faba*), will disappear or have low population densities within the next 5-10 years (Veeravaitaya, 2010).

6. Razor clam restocking and stock enhancement

Nowadays, there are no programmes for restocking razor clam beds in Thailand. Nevertheless, there are several studies aimed at understanding the razor clam's life cycle, reproductive cycle and spawning season (Tuaycharoen and Voraingtara, 1991; Sriprathumwong *et al*., 2002; Veeravaitaya, 2010) as well as the influence of phytoplankton abundance (Boutong, 1997; Veeravaitaya, 2010) and environmental factors (Khumsupha *et al*., 1991; Veeravaitaya, 2010) on reproduction and growth. This kind of information is important for ensuring the success of enhancement of future razor clam beds or restocking programmes.

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