# CULTIVATION OF AMERICAN RAZOR CLAM

Screening of potential for commercial cultivation



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Dansk Skaldyrcenter

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# 2.0 Preface

This report summarizes the findings of the pilot project 'Growing American razor clam -Screening of potential for commercial cultivation." The report is the result of collaboration between University of Aarhus (AU) and the Danish Seafood Center (DSC). The project was implemented with financial support from EU Fisheries Guidance (FIFG) and the Directorate for Food (DFFE) Ministry of Food, Agriculture and Fisheries. In connection with the liquidation of the project has been established an advisory group to support and guidance during the project. The reference group consisted of the following members: Arne Bækgaard (Chairman, Breeders' Association), Per Dolmer (Senior Researcher, DFU), Kurt Thomas Jensen (Associate Professor, AU), Tomas Jensen (Ranger / Biologist, Wadden Sea Centre, Ribe), Michael Victory (Scientist, NERI), Ditte Drying (Manager, DSC), Maj-Britt Hedegaard (DSC) are thanked for a great and solid performance during the generation of the samples. Thomas Jensen (AU), Ditte Drying and Christian Skaaning Laursen (DSC) are thanked for inspiring scientific discussions underway. Frans Francis O. Høyer (DSC) are thanked for preparation of market analysis. Finally, it sounds a thanks to the people who unperturbed cold water gave a hand with the collection of razor clams.

The report is available on the Danish Shellfish Center's website. Nykøbing Mors, 30 September 2005

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# 3.0 Introduction

This project has aimed to explore the potential for cultivation American razor clam (*Ensis americanus*). American razor clam is an excellent edible mussel, which represents an untapped resource in Danish waters. As food is marked, razor clams especially known in the southern European and Asian markets. Isolation of razor clams will expand the Danish aquaculture sector with a new high value products, which represents a potential export product. Commercial fishing on razor clams using bundskrabere take place several locations in Europe. During the capture process damaged a portion of the collected razor clams, and fisheries can also have negative implications for other benthic fauna. The adverse effects of fishing on natural populations supports the logic of developing aquaculture of razor clams, especially in shallow areas Limfjord.

The project carried out a market study, and biological studies. Documentation of the potential for implementing rearing from fry for sale ready mussel consumption. During the project were induced spawns and produced broods that survived one year under controlled conditions, and at

this writing is still alive. Project also deals with the possibility that growth of razor clams in place water phase, similar to the methods of mussels and oysters. There is in this context for conducted preliminary studies of alternatives to sand as a substrate for razor clams or other species of burrowing bivalves.

There is the project established international contacts with researchers involved, cultivation of burrowing bivalves, including different knife clam species through an exchange of new knowledge and general experience.

The project results show that the American razor clam has great potential as breeding organism. The results obtained provide the basis for further studies and refining breeding processes, and it is estimated that actual experimental productions may be started within a foreseeable future.

# Introduction to American razor clam

American razor clam known in Europe under the Latin name *Ensis americanus* (Conrad (Binney 1870), while in other parts of the world known as *Ensis directus* (Conrad 1843). The species originates from the North American east coast, but was in the late 1970s observed in the German Wadden Sea. It is believed that the introduction was done through ballast water. In Denmark, the species first found by Romo in 1981; have since spread the Limfjord and the inner Danish waters. American razor clam had no visible negative consequences for other benthic fauna.

Besides American razor clam, there are four other razor clam species in Danish waters: Small razor clam (*Phaxas pellucidus*), curved, razor clam (*Ensis arcuatus*), straight razor clam, also known scabbard clam (*Ensis siliqua*) and ordinary razor clam (*Ensis ensis*). These occur primarily in deep water, while the American razor clam is the only one occurring in the Limfjord and other shallow areas in greater numbers. The latter three are like the American razor clam delicious eating clams, and research on international plan of the culture of curved and straight razor clam.

American razor clam live underground and can be found in a wide spectrum of bottom materials from coarse sand to packed mud, and preferably where there is power. As filtrator lives of algae, bacteria and other organic material in seawater. Since ånderørere are short, sits right in the shell surface when the filter. Will the disturbed, it can pull down an up to 30 cm deep, almost vertical again. If a razor clam, for one reason or another released from scratch, it using a sharp twitch of the foot move up to half a meter. The mussel is thus possession of an effective mechanism to escape predators. Adults razor clams do not have many enemies, however they are in certain areas prayed on byeiders and oystercatchers.

American razor clam is særkønnet and maturing as a one or two. When mussels spawn in late spring, sending the eggs or sperm into the water, where fertilization takes place. The fertilized egg develops into a larva that lives free swimming in the water for approx. 16 days before mowing bottom (settling) occurs. In settling at have connection with settling changing shell

shape and appear gradually to have same appearance as an adult razor clam. From this stage liver mussel buried.

Length growth in razor clams are often described by a growth equation, which taken into account that the annual growth rate declines with age. Razor clams from Sundsøre in Limfjord gain during the second year of life a size of just over 90 mm. During the next season they grow to approx. 113 mm. Maximum length for American razor clam in home waters is approx. 18 cm. This length is mussels 7-8 years old. American razor clam is equivalent in size to the other razor clam species caught and sold in Europe.

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PART 1 Result Collection Market Analysis Outcome measures

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### 4.0 Result Collection

### 4.1 Production Phases

Pilot project results all point towards the breeding of American razor clam has great potential. This chapter describes the results of the project brief in for connection with an examination of the expected production process for razor clams.

Stages in the cultivation of American razor clam

Hatchery Production Creating parental broodstock Determination of broodstock maturity Induction of spawning Production of larvae Acclimation of juveniles Ongrowing Suspended Systems Harvesting for consumption

### 4.2 Hatchery Production

In nature, the occurrence of juveniles of the American razor clam is not high and stable enough to that a production can be based on fry collection from wild stocks, as known from such as mussels. It is therefore developing techniques for the production of fry in the hatchery, while growing up until fish size can be usefully done in the wild.

Creating parental broodstock

For production of fry up a stock of adult razor clams, which by maturity can induce spawning. A broodstock was the project successful established under conditions similar to the razor clam's natural environment. Survival was very high and the mussels exhibited normal behavior.

For a broodstock recommended that:

• Use individuals who are older than two years, equivalent to a length of> 10 cm.

• Collect parentaldyr in early spring, shortly before the gonads are mature and leave latter part of maturation to take place in broodstock conditioning system.

- Use coarse sand as a substrate.
- Use coarse filtered seawater with its natural content of food particles.

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Determination of broodstock maturity

Time point at which the mussels are mature and can be induced to spawn, determined of the reproductive tissue development. The development is seasonal and is determined by temperature and food conditions that affect the mussel. Prevalence of gonad over foot and abdominal area of a few individuals from broodstock is one in production contexts adequate method to assess ripeness. There may be supplemented by microscopy of samples of gonad.

In mature mussels gonad seen as distinct bright strings in the foot, a layer that extends across the abdomen. When sex products become ripe, over layer becomes thinner and appear after spawning as a thin, yellowish skin over abdomen.

Reproductive cycle of American razor clam in Limfjord, season 2004-05:

- Mussels spawn from May to July with the prime time in late May
- Mussels have a rest period from August to early winter.
- From early winter build the reproductive tissues again.
- The final maturation takes place during the spring until spawning occurs.

Induction of spawning

Mature razor clams can be stimulated to spawn by temperature influences. By raise the water temperature a few degrees succeeded in seven out of eight attempts to induce spawning on razor clams from broodstock. All induced spawns resulted fertilized eggs developed into larvae. This result is a significant step in towards the cultivation of American razor clam.

The project results showed that:

• Temperature stimulation is a reliable and convenient method for induction

• Based on larval survival and timing of the natural maturation of the gonads, is mid-May, the most favorable time to induce spawns

• A spawning can be determined by the water becomes milky white of egg and sperm

• If microscopy shows egg surrounded by sperm

• Spawning takes place over several hours, after which the adults should be separated from the fertilized eggs

 $\bullet$  There are likely between 2 and 10 million fertilized eggs from a spawning of 20 - 30 adult individuals

Production of larvae

The fertilized egg develops within a day to a shell-bearing larvae that swim around in the water. In approx. 16 days the larvae develop in water phase, after which it is ready to and released to live buried in the bottom.

The project results showed that:

• There should be regularly changed water by the larvae. In the beginning every day, later every other days.

• Food in the form of cultured microalgae to be added within one day after fertilization. The food alone, the first day consist of Isochrysis and after a week also Tetraselmis and Chaetoceros. This was the microalgae, which were available from algal production in the Danish Shellfish Center. i The algae are used worldwide in shellfish farming.

• Relatively low food concentrations is more optimal than high concentrations.

• The larvae perform best at relatively low densities.

• The rate of growth through the larval stage averaged 11 micron/day. Which is comparable with the results obtained from other bivalves.

•Larvae settled at the age of 16 to 19 days

• fry settlede without using special manipulation

• Observation of øjeplet of larvae indicates however that lysforskelle significance in connection with settling.

• As settlings substrat between fine or coarse sand is preferable (250 - 1000 micron)

• Sand thickness should be increased in line with growth of offspring after settling. It can under current farming conditions is expected that less than 1% of the fertilized eggs resulting in settle spat, but optimization will probably bring this ratio up. In 2004 produced approx. 250 settled mussels, while in 2005 approx. 2.400 settled individuals. The highest mortality occurs in the early larval stage, before and immediately after the larvae begin to take nourishment. The period immediately before settling represented a critical time for larval survival.

Acclimation of juveniles

When the fry first Settlers, shows the pilot project, the likelihood that they survive until ongrowing is good. In between settling and the fry are ready to on-growing can fry up by conditions similar to those it will encounter during on-growing.

The project was fry at the age of six months established in the tub with sand and irrigation water directly from the fjord. That enabled fry under controlled against the possibility of physiologically adapt to the open-water natural conditions and the more diversified food spectrum that is likely to respond to fry nutritional needs better than a diet based on cultured algae. In a production situation is expected acclimatization that would last for some months.

# 4.3 Ongrowing

Ongrowing expected in future to be conducted in suspended systems in open water or bottom culture in terrestrial plants. Based on the growth rate of razor clams in their natural environment

of the seabed, the mussels reach an MERCHANTABILITY size during second to third year of life. From the production of mussels is known that the high end food availability in the water phase results in faster growth and higher meat content than in benthic individuals. Similarly, breeding of razor clams in the water column maximum likely cut production time significantly and facilitate the work of the harvest.

Since suspended systems from the existing knowledge is assessed as the most innovative solution to on-growing of razor clams were made during the project preliminary experiment with systems for placing into the water above the bottom.

# Suspended Systems

Under natural conditions live razor clams dug at the bottom that provides support and keeps them in an upright position. To suspended farming systems should therefore is a material that supports the mussels while being flexible enough that they can grow and move.

Sand is so dense that it is in practice uhåndtérbart to use as a substrate. We were performed pretests with alternative materials such as growing medium. Systems with cut upholstery foam and brushes were tested indoors and in open water. In particular configurations of foam showed great potential, since knife mussels could burrow into the cut holes and exhibited normal behavior.

Structures with foam offers great possibilities for the farming of razor clams, since the mussels may move in the material and filter as normal.

Based on pre-tests following criteria can be defined for suspensive systems:

- The material should support razor mussels and keep them upright.
- Knife mussels should be able to move into the structures and filter according to normal behavior. However, precautions should be taken so that the mussels can not escape system.
- Structures must be able to adapt to the mussel growth. Can be created systems with specific targets for specific size classes.
- It is desirable that the system must be able to hang on long lines to complement existing breeding sites for mussels.
- Structures must withstand mechanical and chemical interactions in the open water.
- The system must be practically manageable.
- The system must be cleansed of påvækstorganismer.

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Harvesting for consumption

Suspended systems will be easy to handle in a crop situation, the mussels can land in the systems that further handling can take place. Furthermore, under also exists the possibility that the mussels become knife systems are being cleaning and possible purge of mussels but then to let the system follow razor clams during transportation to distribution centers. In this way they avoided a major handling of the knife mussels until shortly before they reach the consumer.

4.4 Market Analysis

The project's market analysis showed that the European market primarily consists of the Spanish. The market is still somewhat vague, but there exists a rich potential for exports, if a steady supply is guaranteed. Market from other European countries is diffuse and not understood.

Cultivated razor clams is intended primarily for the fresh market and can be produced specifically to a given market with regard to the length of the mussel. On the fresh market exists kilogram prices of 4 to 15 euros for fishing razor clams, depending on quality and origin. Cultivated razor clams are expected to be of higher quality than fished razor clams and likely to compete with the highest quality of fished razor clams from Galicia in the Spanish market fetching up to 30 - 35 euros. kilo. kg.

# 4.5 Future focus areas

There should, in future, further work on optimization and rationalization of a system to parentalmuslinger. If structures without sand proves workable, it may be used both on-growing to the maintenance and conditioning of parental mussels.

It should consider whether it is most favorable to gather mussels to a broodstock in early spring and leave the last part of gonademodningen place in parent stock, or whether it is possible to stimulate gonad development outside the normal season by conditioning. If this approach is suitable, can collection and larval production is independent of the season.

Possible induction methods and the optimal time for induction was detected during this pilot. Alternative induktionsprocedurer, construction of facilities for gydningsinduktion and induction of a large scale should be the focus of future studies.

The reason for the observed larval mortality should be a focus area, as the basis for optimization of rearing conditions in the hatchery. These studies, and larval behavior should be considered for development and adaptation of farming systems, eg Development of a suspension system for the last larval stages and the first settled stages.

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From settling to fry reaches a size where it can be established in on-growing system, must work with the biological mechanisms that have an impact on growth and survival, but more specifically on the design of akklimatiseringssystemer that can provide a good link between conditions in the hatchery and ongrowingsystemet.

Much future research should focus on ongrowing phase. Including development and testing of farming systems and analysis of razor clam's behavior, survival, growth and the quality of the finished product. Moreover, development of methods of transportation and storage of fresh razor clams.

The knowledge gained on the cultivation of razor clams, may in future form foundation for the development of rearing methods for other burrowing clam, which is suitable for human

consumption, such as sand clam (*Mya arenaria*), cockle (*Cerastoderma edule*) and wedge clam (*Donax vittatus*).

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# 5.0 Outcome measures

# Uncovering market for farmed razor clams

There has been a market that has shown that the European market primarily for at a stable production, there exists a rich potential for exports.

Biological knowledge is a prerequisite for the development of cultivation It is possible to produce razor clam spat in a hatchery and keep it acclimation systems until it is ready for ongrowing. There are made with præforsøg on-growingsystemer suspended in open water and experience with the procedures in different rearing stages in the hatchery. There are biologically significant potential for farming American razor clam.

# Knowledge about keeping a broodstock

Broodstock may be kept at densities of 250 individuals  $/m^2$  in a simple irrigation systems in tanks of coarse sand. By the proper water flow is mortality is very low. We recommend using razor clams, which have a 10 cm. the minimum length of 10 cm.

# Reproduction in controlled conditions

The reproductive cycle of razor clams in the Limfjord has been described for 2004 season - 2005. Maturity can with razor clams assessed from the gonads size and by microscopy of tissue samples. In a hatchery can mature mussels successfully brought to spawn at a mild temperature exposure.

# Experience and knowledge of survival and growth of fry

There is the project gained valuable knowledge regarding the handling of larvae and settle fry. It is identified, at what age, there are critical phases in relation to job satisfaction and survival of larvae and juveniles settle. Suitable settling substrater and food conditions for larvae have been resolved. Calculated an estimate of survival from egg to settling and from settling to on-growing be exercisable.

# Foundation for later studies on reproduction

Alternative induction and the opportunity for conditioning parental mussels can be examined. The observed mortality of larvae expected to be reduced significantly under the right conditions, so this should be a focus area for future studies.

# Foundation for later studies of growth and quality of drinking mussels

For results are available from long-term trials of growth in suspension systems that can growth and condition of mussels collected in the wild serve as comparison. Lessons from parental broodstock shows which growth and quality that can be expected in terrestrial plants.

# Recruitment of a hitherto untapped production opportunity in aquaculture

In a further development of methods for growth and a better yield from hatching of razor clams, there is evidence within a few years to begin a production of razor clams. Razor clams could be a valuable supplement for breeders who have already established areas with long lines to production of mussels. The overall potential for the cultivation of razor clams must thus said to be extremely large.

# 6.0 Market Analysis

6.1 Preface

This market analysis prepared by director Francis O. Høyer, Danish Seafood Center and export Fellow Jacob Duus, Denmark's trade office in Barcelona.

The analysis was prepared following the implementation of development project "Growing American razor clam. Screening of potential for commercial cultivation ", which is implemented in collaboration between University of Aarhus and the Danish Seafood Center in 2004 and 2005.

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6.2 Market Analysis for Razor Clams

6.2.1 Introduction

In continuation of the project, there have been several studies and explorations of through this to illuminate the market for razor clams. Analyses were based on a series of questionnaires, telephone interviews, exhibition visits and consultant services, in markets which are prima facie interest in Danish context.

The market analysis was originally scheduled to include a larger number of markets than has become. This is due in part to the European market is concentrated around Spain and Portugal, as well as data for other markets have been inaccessible. Jo more distant markets are located, the more analysis should be made at qualitative data, without being able to substantiate these with quantitative. On the this close markets such as Spain and Portugal have the available data also been subject to some degree of uncertainty.

Compared to the generally large seafood market with oysters, mussels, crabs, snails is razor clams a marginal market, although in relation to a possible Danish production, seem particularly interesting. In contrast to the availability of data for other shellfish are razor clams subject to some uncertainty because of their marginal significance in the larger markets, like major conflicts of interest distribution contributes ambiguous indications of market accessibility and size. Qualitative interviews are, therefore, wherever possible, tried and unsubstantiated data. verified with quantitative data.

Despite the exhibition visits and several interviews seems the U.S. market to be very diffuse. There is no doubt that the market is interesting, and that in future can be a major customer. The market is characterized by random landings without stable supplies, which greatly affect the distribution's interest in this product. On stable supply for the U.S. market make it a target export market for fresh razor clams.

On the Asian market, which in this study is only peripherally concerned would appear supply to be low and prices higher. Several merchants from NL, UK and IRL exporting fresh mussels to the Far East. In contrast to the market in Spain demand for this market the largest clams 20 to 24 cm. Despite high freight rates, it is interesting that price plays a minor role as long as quality, fresh unit and the size is in order.

At the close markets in Europe, Spain is the main objective. Although market statistics seen is diffuse, it seemed to be very systematic in its logistics and marketing.

The remainder of this analysis will highlight immediate opportunities in this market and briefly describe the general situation on quality, prices, volumes and market distribution.

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6.2.2 Market:

Generally, the market may be divided into 2 main categories. The fresh market and industrial.

In the fresh market traded mussels alive and being through wholesalers dedicated to restaurants and retail shops. The larger wholesalers are also importers and again more marketing. First, through markets and auctions in Mercabarna, Mercamadrid and Mercavalencia, and directly to other wholesalers, restaurants and retail stores. The smaller wholesalers buy directly from importers of the mentioned markets. Mussels are here wrapped in bundles of 250 to 300 g. Furthermore, the called "Depudoras" who buy large lots in bulk. Here are the products packaged on and again sold through the above channels.

Finally there is the industrial market, where the mussels are processed in different ways and sold as canned. The processors often buy directly from the individual fishermen and buyers.

Of the total product volume assessed approx. 70 % go to the industrial market, while the remaining 30% is sold on the fresh markets. This has in this study was only possible to obtain statistics for the fresh market.

# 6.2.3 Size and Quality:

It is not so much the nature of the different shells, as is the size, quality and price that sells the product. Quality Parameters freshness and size are critical importance in Spanish markets. The size can be up to 17 to 18 cm and fit one serving with 6 - 7 pcs. on a plate. Mussels are purified and free of sand and moreover, fresh (up to 7 days) described it as high quality.

To ensure quality requires that the mussels come from a Class A area, and to packaging and temperature conditions during transport are optimal. Transportation is on ice, with temperatures between 0 and 3 degrees. Moreover meaty in each mussels.

# 6.2.4 Supply and demand:

As previously mentioned, there are very ambiguous indications of market size and prices. At the 2 markets Mercamadrid and Mercabarna that there is statistics, in 2004 was a total sold approx. 286,000 kg fresh razor clams to a average price of 6 to 7 euros./kg. The fresh market is approx. 1/3 of total market in Spain. This must be added sales from other markets, and it sold directly to importers. A conservative estimate of the total Spanish market becomes 1000 - 1200 tons annually. Since the industrial market will pay less, estimated the average price to be around. 5 euros. kg. In Spain, the estimated total purchase price for wholesalers to be around. 5 to 6 million. euros or just over £ 40 million.

As a precaution if it can be assumed that the market in Portugal is of similar size – and that the overall market for razor clams in southwest Europe in the amount USD 60 - 80 million annually.

The supply of razor clams are spread over a number of countries, NL, UK, IRL, Spain and Portugal. Razor clams fished either by scraping tools or assembled by divers, and it is a general assumption that, especially fisheries are not sustainable. It can therefore assumed that the availability of term is decreasing.

The analysis of respondents expressed any enthusiasm for a possible opportunity farmed razor clams, as it can better ensure quality, and any greater range.

With the reservations that are also drawn from the analysis, it must therefore be concluded that as well as market prospects for farmed razor clams is certainly present.

- 6.3 The market for razor clams in Spain
- 6.3.1 Summary

An estimation of the Spanish market for razor clams is hampered by lack of import statistics for canned, and poor record keeping of catch data in terms fresh razor clams. Origin criteria for selling razor clams indicates not country where they are caught, but may be occupied for an importer or an intermediary, which transmits it to the auction in Barcelona or Madrid, which further complicates the quantification of the market. Some key indicators of the Spanish market, but sales in Spain's two main auction wholesale markets in Barcelona and Madrid.

The study includes the specification of sold species, inflation and the comparison fish market in Madrid and Barcelona. Section on catching razor clams includes also quantity and price for the three species of razor clams in Spain.

# 6.3.2 Purpose of investigation

The premise of this market has been to provide a general description of the market for razor clams in Spain as well as a description of how razor clams fished and possibly import from, and in this context how supply situation is currently. Also trying out how the demand is and the relationship between the fresh and processed markets. In this context, it examined how razor clams are traded and at what prices. To increase the validity of the data acquired, it tried to find more sources to the same data, which largely succeeded.

Similarly, the qualitative information helped to estimate the Spanish market of razor clams. It must be mentioned that there are uncertainties associated with estimation.

6.3.3 Razor Clams

In Spain there are three species of razor clams. The local agricultural and Fisheries Council in Center Galicia has indicated that there exist the following three species, which is confirmed by the Center for Marine Research, Susana Darriba Recursos Marinos have indicated, however, that only caught two species of razor clams. Several statistics provide simultaneous information on three species. Besides the commercial razor clam name is also listed

Scientific name, name, and the FAO classification.

• Navalla (Ensis arcuatus) (FAO / EQE)

• Longueirón Vello (Solen marginatus) - RAE

• Longueirón (Ensis siliquia) (FAO - Eqi)

It has no requirements for maximum size, but the minimum size:

•Longueirón Vello: min. 80 mm

• Longueirón: min.100 mm

As regards the customs classification of razor clams, the following information obtained through the customs service in the Foreign Ministry. Mussels within HS: 0307, with the subdivision divided by the mussel species. Customs-related information available however, the nature, which further specified in Annex 1 and 2 and briefly summarized below:

• 03072100: Scallops and mussels of the genera Pecten, Chlamys or Placopecten. If the scallops of this generation so Customs mussel 03072100XX and the rest depending on the genus.

• 030731: Mussels (Mytilus spp, Perna spp). If the mussels from this generation, as Customs mussel 030731XX/030739XX and the rest after the genus.

If razor clam not under 03072100 and 030731, then choose customs classification of the "clam 2" documen under Knivmuslingen be classified under "Other, including flours, meals and pellets of aquatic invertebrates."

Given the above it is not possible to obtain import figures for razor clams.

See source list

Resursos Marinos, please see source list

# 6.3.4 Structural Spain

Spain's division into 17 autonomies, each with varying degrees of autonomy, often complicates a strict definition of competencies and responsibilities. In fishing, it is JACUMAR under the Ministry of Agriculture and Fishing, the authority responsible for fisheries - and shellfish. During JACUMAR each of the 17 autonomies a local department "Consejería de Agricultura y Pesca", which is the operational regional authority responsible for regional activities

The Spanish market for razor clams can be said to be very little documented when catch in each port is not always recorded, and the vertical value chain from catch for retail is very fragmented, with little integration between the individual joints.

The local branch of JACUMAR in Andalusia, however, explained by telephone contact with them that they are briefed on a relatively large catch of razor clams in Andalusia, but it is still not registered. However, it has plans to launch register ring. Nationally, access to information equivalent unstructured. The only autonomies, which can estimate the catch and provide figures on their catch of razor clams, are Northern Autonomies: Galicia, Asturias and Cantabria.

Another explanation for the unstructured situation may be due to JACUMAR on nationally disseminate its legislation to autonomy controls while autonomy themselves responsible for organizing and structuring their markets and sectors. i.e overall national legislation operationalized not in the same structure in individual autonomies.

# 6.4 Supply Situation

# 6.4.1 Geographical distribution of catch

In Galicia, in north-eastern corner of Spain captured the largest amount razor clams in Spain. You drive typically catch a local "factory" where mussels cleaned of sand and impurities. From there they are distributed on to local wholesale markets in Galicia "Llonjas" and to the 22 wholesale markets that exist in Spain. Of these the two most significant Barcelona and Madrid, with a share of 15% and 30% of the Spanish wholesale market turnover. Furthermore sold razor clams to companies for further processing.

http://www.mapya.es/jacumar/presentacion/presentacion.asp and telephone contact with the local council. See map on the front

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It has not been possible to confirm whether this procedure is the same for in Galicia consumed while a significant amount razor clams locally, so that the received data should be interpreted as estimated.

In 2000 JACUMAR seven plans for fish, molluscs and shellfish, of which one project involves the rearing of razor clams. The project implemented in cooperation between autonomy of Galicia, Andalusia, Asturias and Cantabria. The purpose is to analyze current situation, to what degree one commercially exploit the species opportunities and develop the farming of the species. In light of the plan launched in 2000, Galicia, Asturias and Cantabria initiated a plan for the farming of razor clams from 2004-2006

It has not been possible to obtain the plan or some preliminary performance, since these are still under development. It should also be mentioned that currently also caught razor clams at 11 locations in Andalusia, 1 place Cantabria and seven sites in the Basque Country.

# 6.4.2 Auctions / Grossistled

Table 1 below shows the catch and value of razor clams for three of the main autonomies at auction / wholesale markets in the three autonomies. Data from year 2004. Data from other autonomies are not available.

	Gali	cien	Canta	brien	Astu	rien
	Fangst i kilo	Kilopris	Fangst i kilo	Kilopris	Fangst i kilo	Kilopris
Ensis						
arcuatus						
(Navalla)	125.686	14,60	1185,17	11,15		
Ensis siliqua						
(Longueirón)	25.063	11,90				
Solen						
marginatus						
(Longeirón						
Vello)	3.927	4,54	3,219,4	8,03	2.282,5	3,49
Total	154.676		4,419,17		2,282,5	

Table I, Average price and volume developments for razor clams in three autonomies in 2004. Catch in 2004 Source: The local agricultural and Fisheries Council in Galicia. Anuario the Pesca de Galicia the 2004th. Price indicated in Euro.

JACUMAR and the Regional Agriculture and Fisheries Council in Galicia have both reported above figures, so their validity can be considered good.

Table 1 shows that the species *Ensis arcuatus* is the most fished and the catch of razor clams is geographically concentrated with 96% in Galicia. Therefore be examined price and volume movements in Galicia for the years 2002, 2003 and 2004.

http://www.mapya.es/jacumar/presentacion/presentacion.asp

	2004		20	2003		002
	Kilo	gns./kg	Kilo	Gns/kg.	Kilo	gns./kg
Ensis arcuatus (Navalla)	125.686	14,59	83.312	15,91	97.080	13,37
Ensis siliqua (Longueirón)	25.063	12,09	17.656	16,01	14.899	10,47
Solen marginatus (Longeirón Vello)	3.927	4,53	9.268	2,73	17.036	3,48
Total	154.676		110.236		129.015	

Table II. Average price and volume developments for razor clams for the year 2002, 2003 and 2004 on the auction / wholesale markets in Galicia. Price in euros.

The above Table II illustrates the volume and price difference between the nature sun marginatus and the other two species. In terms of volume, total sales also said to be very volatile.

The following bar charts I and II shows the traded price for razor clams at month at the wholesale markets respectively in Barcelona (Mercabarna) and Madrid



For 2004 there is talk about all year, while for 2005 is the year the first 8 months.

#### Gennemsnitspris pr. måned



Bargraph I and II shows that the average price trend in both wholesale markets are quite regularly over the year but with a smaller increase in August and December. The increased sales in August could be explained by the many Spanish domestic tourists this month, while sales in the month of December shows eating seafood in the Christmas day.

The following bar charts III and IV show sales per kilogrammonth wholesale markets respectively in Barcelona (Mercabarna) and Madrid (Mercamadrid).

For 2004 there is talk about all year, while for 2005 is the first 8 months.



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As seen from the above two tables, provided the Spanish market with razor clams from many different parts of Spain and other European countries. To this must be said that the origin criteria defined in terms of which supplies arriving at Madrid's Wholesale Market. That is, a wholesaler may have imported from Ireland and forwarded them from his premises to Madrid's wholesale market. This almost prevents a clear specification of the origin of razor clams. The same is case

with the city of Huelva in southwestern Spain. So far it has been possible to acquire knowledge, captured no significant quantities of razor clams in Huelva area. Therefore, the increasing amount of razor clams from Huelva be imported from Portugal. It is conceivable that cleans knife mussels in Huelva before forwarded to Madrid's Wholesale Market.

Also mentioned that the local population in Galicia eat significant amounts of razor clams at both restaurants as private, and that there is no data available these catches and consumption.

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6.4.4 Geographical origin of fresh razor clams

Mainly imported fresh razor clams from Ireland, Scotland and Holland. As shown in the tables is also imported from other countries, but significantly less so.

From Ireland and Scotland come the cheapest razor clams. De er meget store og seje i They are very big and tough in meat, and sold by wholesalers to 3-4 euro / kg and sold approx.to 5-6/kg, depending on quality A typical sale of a wholesaler is approx. 25 to 100 kg./ week.

Many wholesalers import much from Holland when the supply and quality are generally very stable. These are among the cheapest mussels on the market because these are relatively large and cooler in the flesh. Average pay merchants 3 to 4 euros per kg and sells those for 5 - 6 euro / kg.

The next best razor clams imported from Portugal. These are very similar mussels Galicia and is almost as fine in the flesh, but often have sand, which is why they are not sold as expensive as mussels from Galicia.

The best and most expensive shells in the Spanish market come from Galicia, which also Spain's largest manufacturer of razor clams. Generally, the Spanish wholesalers that razor clams from Galicia is the best quality, as these are completely drawn, are finer in the flesh, has the right size and is smaller and is therefore also the most expensive.

Wholesalers sell mussels for 12 to 18 euros / kg, but no one will say supplier prices.

It was reported by the head of Madrid's fish wholesale market, a wholesaler typically have a margin of 8 to 12% profit.

# 6.4.5 Distribution of retail

Due to the limited amount of information on retail sales of razor clams have we had contact with Daniel Martinez, director of Mercabarna and Roldan, Director of Mercamadrid and wholesalers dealing in razor clams on these markets.

Through this we have obtained information ang balance between the processed and fresh market. However, we have for canned been in contact with supermarkets Plus Caprabo and El Corte Inglés. Four supermarkets were visited; Plus, Dia, Caprabo and El Corte Inglés. Plus, similar to Aldi in Denmark and offer discount goods. Super Caprabo corresponds to Irma and Super Supermarket in Denmark. This segment offers a wide selection of quality products and have including chambers of fresh fish. Prices are medium to high.

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El Corte Ingles, equivalent to ISO and Magasin in Denmark. Here you are offered a wide product mix, focusing on the very quality-conscious consumers and has a fish section with fresh and frozen fish. Prices are high.

Frosne	knivmuslinger	– Supermarke	der:
Trosne	Kinvinusiniger	- Supermarke	uer.

		- ap ci initi i			
Supermarked	Antal	Pris	Vægt	Oprindelse	Art
Caprabo	7	4,20 - 5,46	0,335 – 0,435 g.	Vides ikke	Longueirón
El Corte inglés	11	9,90	450 g	Irland	Longueirón

As the table above shows, there is no large supply of frozen razor clams. Information can be viewed as complementary to the other. These are only found in the aforementioned supermarkets. It was not possible to find Frozen mussels in the markets of Barcelona, Mercabarna or Mercamadrid.

Ferske knivmuslinger – Supermarkeder:								
Pris Oprindelse Art								
El Corte inglés	17,95 kilo	Galicien	Longueirón					

Fresh razor clams - Markets:

Two fish markets (retail) were visited and they spoke with four outlets. On one of markets was stated that sells only razor clams Friday and Saturday.

Origin: The offered razor clams came from: Galicia, Portugal, Holland and Ireland.

Price: The price is very conditional origin and varies according to the stated amount between 12 - 15 euro / kg for imported razor clams and up to 30 to 35 euros / kg for razor clams from Galicia is smaller, has a darker and a more curved shell. The larger knivmuslingen is, the cheaper because the meat is tougher, and also is razor clams from Holland and Ireland are not well treated as those from Galicia.

Weight: Razor clams are sold in bunches, usually shared a kilo up to 3 to 4 bundles.

Classification: Classification carried out by its size and origin.

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Dåsekonse	erves – Su	ıpermarl	ceder:

	Antal	Pris	Nettovægt	Bruttovægt	Oprindelse	Art
Plus		1,65	138 g	78 g.	Chile	Navalla
Caprabo		2,65	98 g.	56 g.	Chile	Navaja

Dasekonserves - Supermarkeder - Er Corte ingles.									
Mærke	Guau	Escuris	DANI	Grandes Hoteles	Conservas Peña	Portomar	Paco Lafuente	NOLY	
Pris	9,80	4,25	2,75	2,85	4,00	4.20	4.00	2.45 - 3.25	
Vægt	170 g		150 ml	120 ml	120 ml	120 ml			
Antal pr dåse	6-8	6 – 8		7-9	6-8	3 – 5 store	5-6	6-8	
Netto vægt	270 g		138 g	112 g	111 g	111 g	115 g	110 – 138 g	
Brutto vægt	165 g		78 g	63 g	63 g	63 g	65 g	62 – 78 g	
Oprindelse	Pontevedra/ Galicien	La Coruña	Chile	Chile	Pontevedra /Galicien	Pontevedra/ Galicien	Galicien	Peru	
Fabrikant		Perlas de Arosa					Conserveria Gallega		
Form	Naturel	Naturel	Nature	Naturel	Naturel	Naturel	Naturel	Naturel	

Dåsekonserves – Supermarkeder – El Corte Inglés:

The above two tables indicate that the razor clams canned mainly comes from Galicia and Chile.

Knife mussels from Galicia are typically of better quality and therefore also sold to a expensive price. Because of the price it is only possible to buy razor clams from Galicia in El Corte Ingles, which also indicates that the mussels from Galicia are sold to the very quality conscious consumer. The Plus and Caprabo exist only a few razor clams from Chile, which indicates that the discount segment and the middle quality-oriented segment is offered only the cheaper products from Chile.

As seen in the above two tables it is mainly the Spanish company DANI, importing from manufacturers in Chile.

# 6.4.6 Demand

From the above tables can be concluded that the largest market for razor clams are fresh razor clams. It has not been possible to quantify frost or canning market.

For the sale of fresh clams, says the wholesalers do not have a extensive sales of razor clams, this means the wholesalers around sell between 300 to 400 kg. per. weeks, not that demand for razor

clams on the Spanish market is particularly high, since there exists a strong tradition of eating these mussels in Spain, but disclosed that it is greater in Portugal.

Wholesalers who have a more comprehensive sales, ie. 1500 - 4000 kg pr. week believe that this is a viable product that sells well.

Director of Mercabarna, Daniel Martinez, does not believe that razor clams are a highly profitable product in Spain. He believes there are other shells that are more demand. Total sales of razor clams on Mercabarna in the first seven months of 2005 amounted to 154,000 kg. With 21 working days in a month, is the sales 1000 kg over the ten stands, dealing with razor clams on Mercabarna.

Compared to other bivalves are the catch of razor clams are not particularly large, this has nothing to do with supply but with demand, says Daniel Martinez.

# 6.4.7 Structure

Wholesalers from Mercabarna inform all suppliers of razor clams from Galicia buy them on the market in Galicia, then sold to wholesalers Mercabarna. Thence sells wholesale fresh razor clams on to restaurants, large supermarkets and other retail as fish markets in Barcelona's city center.

Ireland, Scotland and Holland buys often from suppliers who themselves fishing mussels and deliver these to wholesalers. For this reason, merchants sell mussels cheaper to particular restaurants, supermarkets and other retail as the markets in Barcelona center.

Wholesalers' margin achieved on sales of razor clams is 2.7, and generally sold with a margin of 10%. However, there are many wholesalers who would not disclose the exact margin. VAT is 7%.

In all canned collector manufacturer companies fresh mussels from the harbor or sewage plants. Then resold the product to a distributor who resells under his own name. This is the same for domestic and imported products

# 6.4.8 Penetration Opportunities

As mentioned above, says the director of Mercabarna and wholesalers who have a weekly sales of between 300 to 400 kg is not that the market for razor clams is profitable in Spain, because there is a strong tradition of razor clams in Spain.

However, believe the wholesalers who have a weekly sale in 1500 - 4000 kg, it is a strong product that is profitable. Even have representatives from Galicia, Asturias and Cantabria signed

a joint development plan for sustainable development production of razor clams in those regions, which reportedly caused a increased commercial value.

Telephone conversation with the port of Vigo and the company DANI

Skipped - Page 33 Skipped - Page 34 Skipped - Page 35

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6.6 Bilag 1

Intershell UK director Will Carrier interview:

# We are working with "Ensis americanus" and would like to know if the market differentiates between the species or is it merely the size and quality that matters?

In general the size and quality is the major selling point of the razor clams; however you will find a great size differential between certain species. For example, the Ensis ensis at maturity is much smaller and thinner than the Ensis siliqua at maturity, this makes it a better seller into the likes of the EU. However, the likes of the Spanish 'end user' prefers a product to be no more than approximately 17 to 18 cm in length, this way they can lay half a dozen side by side on a plate in a restaurant which is appeasing to the consumers eye. But in the likes of the Far East a product of this size would not be marketable as it is just too small. Instead the Far East require a product in excess of 20 cm and as high as 24 cm if possible. For want of a better expression it is "more phallic" to have a larger product in the Far East as it shows greater prowess and power to the onlooker. Looking at the Americanus species it is very similar to the siliqua and I foresee no reason why it would not sell well.

Your estimation of the supply and demand on the European market, and if possible broken down to the individual markets?

Although a significant supply of razors to the EU market is derived from the UK, Ireland and Holland, the big Spanish players have found that countries such as Peru and Chile have a species

called the Ensis macha. This is good enough for them to set up their own canning plants in these countries and export back to themselves in Spain.

Furthermore, the Spanish have their own "prized" razor known locally as the Galician Razor; this they will use over anything else; however the cost is considerably more due to the high meat yield and freshness due to its locality of catch.

# You have three main markets in the EU to consider for the razors...

The live markets such as Mercabarna, Mercamadrid, Mercavalencia and so on. This is where you will see the highest economic return from your sales as you are selling direct to a wholesaler who will then trade to the retailers. Selling the product this way requires that it is packed to EU and customer standards, if you fail to comply with these standards the chances are the product will be confiscated and destroyed by the vets who patrol these markets looking out for non compliant goods.

The depudoras; these are companies that buy in bulk and are happy to accept the product in what ever sort of packing the good arrive in. They will buy the products (as long as they are in good condition) repack and sell to the wholesalers on the live markets. These depudoras will obviously demand a lower price for the products as they need to repack them and they also make a percentage on the sale value.

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The canning industry; there is a vast amount of produce canners operating in the likes of Spain who will buy the produce in bulk; once again the packing is not a requirement as long as the goods are health at time of arrival. As the product is not going to be sold to the consumer in a live / fresh state; instead it will be cooked, de- shelled and placed in cans this will require a cost from the producer, therefore they will offer a lower price for purchase. The one good point in dealing with the canning industry is the great volumes they can purchase at any one time. Furthermore, if you have gained trust from your canners they will invariably accept the razors in a frozen format; this allows for full truck loads to be moved safely. The format for freezing is either blast or IQF with the later being the more preferable.

# How much goes to processing and how much to the fresh market?

A percentile breakdown would be in the region of 70% to processing & 30% to the live market.

# What are the app. prices on producer (fishing), wholesale and retail levels?

Approximate purchase value from a producer / fisherman is in the region of 4.00 euros per kilo Approximate sale price to a wholesaler would be in the region of 5.00 euros to 5.50 euros per kilo. This will be a constant with the only variable being the likes of Easter and Christmas where demand is high and the prices rise to reflect this. You would also find that the attainable price for a 'dived' razor will be slightly higher than a dredged razor.

# How is the different quality levels defined?

The quality is defined from a few points as follows...

A) The catch area, is the water graded A, B or C? A grade A product requires less depuration than a grade B and so on; thus allowing the product to reach market in a shorter time period and demanding a better price.

B) The species itself; as previously mentioned the siliqua will obtain a better price than the ensis due to its size.

C) The depuration method; a product will not sell if it has a high sand content; therefore depuration to remove the sand is of paramount importance in the quality of the product.

Packing and transport temperature will play a large part in how the product also sells, it has to be correct for the live market wholesalers and the transport temp has to be optimum in ensuring the healthy arrival of the goods. A tired product will not sell!!!

# How are the clams transported, and is there any loss (mortality) during this?

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We here at Intershell have gone through the trial and error stages of packing and transportation. The outcome is that the products have to be packed with the correct materials (long lasting self contained ice packs). The transport needs to be efficient, and where possible direct to customer as opposed to Groupage where the goods are moved from one trailer to the next in different locations in order for them to arrive with the customer. The transport has to obviously be refrigerated and we suggest a temperature between 0 and +3 degrees centigrade. This method is obviously not optional for Far East sales and for this reason we utilise air freight services; these are expensive but the cost can be covered within the invoice value that the customer receives.

# Are there any season variations on the supply and demand?

Yes, you will find increases on the lead up towards Christmas and Easter, however after these periods you will notice a slump and the market levels off. The summer months sees a downfall in sales as the high environmental temperatures imposes a great threat to the mortality of the razors.

# How long do the fresh clams stay alive once out of the water?

If handled correctly, packed correctly and transported efficiently at the right temperature you will see a healthy product still in seven days. After this time the product begins to fade and although it will not die until day 9 or 10 the consumer will notice a great difference in the quality and taste of the product.

Do you see any perspectives in growing razor clams in an off bottom system?

Yes, if this can be done and in a grade A environment where the collection of the product does not bring them into contact with sand will mean little or no depuration; thus arriving faster and healthier at market.

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PART 2

**Biological studies** 

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# 7.0 Parentalbestand

As a basis for the biological studies were collected razor clams to establishing a broodstock. The crop would supply adult mussels to show whether it is possible to get razor clams to thrive in a semi-controlled environment as a model for production-like conditions.

# 7.1 Collections

# 7.1.1 Fields

It is merely used manual methods for the collection of razor clams in this project, a mechanical method would cause a great disturbance in the bottom collection areas. There can be different manual methods depending on whether the water is covered or not. The selection of site for the collection was made from accessibility, presence of razor clams, depth and bottom conditions.

An area at Sundsøre (56 ° 42.35N; 9 ° 10.21E), a few hundred meters south of ferry to Hvalpsund ferry proved to meet the stated requirements. In this neighborhood living American razor clam in shallow water and their density was high, patchy up to 100 per. m<sup>2</sup>. Most of the mussels knife that was used to parentalbestanden is collected by Sundsøre, while the remainder is collected by Sillerslev (56 ° 40.87N; 8° 44.15E). In both areas it will only gradually deeper from the shore, and there are areas with eelgrass. There lived razor clams in both open and vegetated areas.

# 7.1.2 Procedure

The shallow areas gave the opportunity to combine digging and snorkeling during collection. In areas with a water depth of up to 1 meter is possible for a standing person to dig for razor clams with grip. Water depth however make it impossible at seeing breathing holes and assemble the dug razor clams up, why it is advantageous to have a person lying in the water. It is easy to realize how razor mussels have their holes. The pigmentation and shape differs knivmuslingers

clearly distinguishable from sandmuslingens which is the only other buried clam which was found on same habitat as knivmuslingen.

The mussels were during collection stored on a network of submerged and then wrapped in damp cloths before repatriation. Visibly damaged individuals were frozen immediately upon arrival at the seafood center for use in dry weight and gonad analyser.

The yield ranged from 50 to 300 per razor clams collection. The result from a collection was highly dependent on wind and weather, and it proved impossible in practice to collecting razor clams in a period in the winter of 2004 - 05

The live razor clams was on arrival to seafood center placed in tanks of 20 cm sand and flowing seawater. During establishment had the mussels themselves burrow. This frasorteredes those individuals who had hidden damage in breakage of capes or another. Mussels that died or had not dug down within 12 hours after installation, were frozen. Mussels with visible and hidden damage amounted to about together.20% of the total number collected (Table 7.1). After the initial period of parentalkarrene, mortality was among knife mussels is very low. The mussels were established at densities of either ca. 160 or 320 individuals per. m<sup>2</sup>. There was no difference in mortality between the two densities, indicating that the mussels to thrive at high densities.

Tabel 7.1 Ov Dato	ersigt over inds Sted	amlinger af knivmusl Antal brugbare	inger. Antal skadede	Antal i alt
10-05-04	Sundsøre	115	25	140
11-05-04	Sundsøre	256	47	303
16-05-04	Sundsøre	310	112	422
07-06-04	Sundsøre	3	4	7
10-06-04	Sundsøre	140	52	192
13-06-04	Sundsøre	175	31	206
22-07-04	Sundsøre	168	42	210
08-08-04	Sundsøre	123	30	153
03-09-04	Sundsøre	68	22	90
05-09-04	Sundsøre	185	60	245
28-02-05	Sillerslev	60	10	70
01-03-05	Sundsøre	53	5	58
31-03-05	Sillerslev	120	30	150
17-05-05	Sundsøre	159	37	196
Total		1935	507	2442

Number of usable

Number injured

Total number

Table 7.1 Overview of the collections of razor clams.

Date Location

As an alternative to digging razor clams may be forced to leave the base by pouring a saturated salt solution in their breathing holes. This method has other proved useful but gave no convincing results in this project. After tentatively salting animals from broodstock there were several that did not dug in again, even after several days. Salting is therefore not immediately as a workable method for harvesting of clams, which will subsequently be used in experiments. Finally, there may also be considered in less manpower-intensive, mechanical methods in individual cases to collect larger amounts to the creation of commercial parentalbestande, or explore alternative, low-impact methods.

# 7.1.3 Runtime Data collected mussels

Figure 7.1 shows the distribution of lengths of the collected mussels from Sundsøre and Sillerslev, grouped by month. In May 2004, two size classes 100-120 mm and 120 to 150 mm dominant, representing respectively two and three year old individuals.

Razor clams, which were older than three years, was limited by found collections. Mussels from class 100 to 120 mm grew approx. 1 cm from May 2004 to early spring 2005. Growth of mussels parentalkarrene followed the growth rates observed in mussels collected. In the shell emerged however in most individuals a minor disturbance call from the management of the collection.



Figure 7.1 The size distribution of collected razor clams (Sundsøre and Sillerslev pooled). Data indicated that in February 2005 also contains data for mussels collected the firstMarch, while data set by March 2005 is on mussels collected on 31 marts. March.

For creating parentalbestanden can be usefully applied mussels that are over 100 mm long, which corresponds to an age of at least two years, since they are likely to have a greater reproductive potential than younger individuals. In gonadeundersøgelsen included individual one-year mussels, all of which contained mature gonadevæv in May. By virtue of that annual mussels actually smaller than older mussels, there are less reproductively tissue present in small clams to large. Moreover, it is likely that small mussels better allocate energy to growth rather than reproductive tissues, while large mussels can put more energy to build reproductive tissues.

# 7.2 Parentalkar

The collected razor clams was established in already existing vascular growth hall the Danish seafood center. There were used two betonkar five times a half meter (Figure 7.2). The vessels were about 50 cm deep. As the razor clams are mobile, were each tank divided into units of 1 meter long with vertical walls of PlastNet who had 8 - 10 mm. holes at 8 to 10 mm. There was water exchange between the devices, but mussels were kept separate. Overall, this led to the creation of a total of 10 units.

There were set nets with mesh size 5 mm for outlet holes in the vasculature.

In the growing pool is running seawater that is taken in from the Limfjord on one side of Ørodde and passed out on the other. The inlet is centered within the vascular bundles, while holes evenly distributed in the vascular wall served as expired. The injected water was distributed throughout length using gutters with holes in the bottom so the water flowed out at a height 20 cm above the sediment. Since the water before discharge only through a coarse filtration contained the food particles. It was sought to have a high water flow in the vessels. This was done to ensure that despite the location of the inlet and outlet at the top of the tanks was a high water movement over the sediment surface and to optimize the food supply to mussels.



Figure 7.2 Distributed by two long tanks were razor mussels established in 10 units with an area of  $1.0 \times 0.5$  m. Between the units that were tagged with networks, there was free water exchange. The water was distributed throughout tank using a gutter with holes.

The tubs were filled with 20 cm of washed gravel sand, grain size 0 to 4 mm, which was flushed with seawater for a week before that was established razor clams in it.

Adults razor clams do well in a wide range of particle sizes and may live in sediments with much higher silt than the chosen. Fine sediment may be compact and hard to dig in, so to make it easy to release the mussels when they were used in experiments were chosen coarse sand. They did not efforts to prevent a anaerobic sediments. Razor Clams and many other burrowing animals over the coming anoxic sediments by drawing seawater into their time by which the surrounding environment kept oxygenated and mussel avoid the influence of noxious reduced compounds.

### 7.2.1 Sediment Analysis

To determine the distribution of particle sizes in the selected sediment samples were taken 10 samples for fractionation. There were also conducted tests on 10 samples from each collection point in the Limfjord. Sampling was conducted for 12 cm depth with a tube with an inner diameter of 15 mm. By fractionation was each sample divided into fraktionen size intervals by means of sieves of 63, 100, 250, 500 and 1000 m. The fraction at below 63 m were collected with rinse water and left in a few hours to settle, then gently water was poured off. For determination of the weight of each fraction, samples were dried at 103 ° C for 48 hours and



burned at 550 ° C for 12 hours. Statistical treatment of results was conducted with SPSS.

Figure 7.3 Sediment Distribution in parentalbestanden and from collection points at Sundsøre and Sillerslev.

Fractionation of the sand showed that the grain size from 100 to 500 m was dominated in parentalkarrene (Figure 7.3). The figure shows also the distribution of particle sizes in sediment at the collection points at, respectively. Sundsøre and Sillerslev. Both of these were also dominated by the grain size from 100 to 250 and 250 to 500 micron. The sediment from parentalbestanden contained significantly more sand than 500 meters beyond the two collection points. This was just intent of election of sediment.

Grain size distribution in parentalkarrene changed during the project due to sedimentation of fine particles introduced with the seawater. This led to put a layer of fine particulate matter in the sediment surface. This high sedimentation gave no visible problems for the mussels. For practical reasons it is recommended once every six years to remove the joiners mud.

The graph shows the flared weight (percent of whole sample weight) for each size fraction. For Sundsøre and Sillerslev are grains of 100 to 500 m dominant, while the sand used in parentalkarrene is coarser and dominated by 250 to 500 m.

The collected razor clams dug himself lightly into the sediment. During Summer med sand worms and clams, brought in as larvae with water. This was a good indication that the sediment in the tanks was appropriate because it also naturally these macro zoo-benthic species present, where knivmuslingen live. However, the incidence of sand worms evidence that sand was relatively coarse. In nature often seen a shift from high prevalence of sand worms of razor clams, where sediment changes from coarse to fine more. The tubs were overgrown with sea squirt in the summer months, but cleaning once a month to keep the fouling down. Gutters were daily cleared of any incoming silt. In a commercial production should be under more controlled conditions, if any, in a recirculating system where the presence of other organisms can be controlled.

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# 7.2.2 Physical parameters

Conditions in the water in parentalkarrene followed the ambient conditions in the Limfjord. Oxygen concentration, temperature and salinity were monitored to ensure that conditions in tanks were satisfactory. Oxygen and salinity were measured manually, while water temperature was measured using a temperature logger (Hobo Water Temp Pro). Oxygen in water was by all measurements above 5 mg / 1. The salinity ranged between 28 and 34 ‰. The temperature in the tanks ranged between 0 and 20  $^{\circ}$  C (Figure 7.4). All measured salinities and temperatures fell within the tolerance limits for adult razor clams (Freudendahl and Nielsen 2005).

Figure 7.4 Water temperature in broodstock tank for the season in May 2004 to June 2005.



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# 8.0 Reproductive Cycle

During the pilot project was the development of reproductive tissues (gonadevæv) in American razor clam in Limfjord investigated for the 2004 season -05. Unless launched the conditioning of adult razor clams to a current production year is very much dependent on the natural reproductive cycle. The timing of maturation is crucial in determining when the collection of maternal and spawning should begin. Furthermore, they wished to develop a method for determining the maturity of American razor clam, for use in a future production.

Production of gonads follow in Danish waters, an annual cycle that is regulated by water temperature and food conditions. Spawning occurs in spring and summer months, after which the shell goes into a resting phase where any remaining gonad reabsorbed. Mussel begins construction of new gonad during winter months. In the spring of mature gonads, and around May is ready for mussels spawn.

American razor clam is særkønnet (monoecious?). Gonad the same location in males and females (Figure 8.1). Woven exist:

A) Around the hinge of the mussel from which it extends above the stomach.

B) In the foot, where the upper portion may be an infiltration of muscle and gonad.

There is also a string of gonad tissue down in the foot.



Figure 8.1 A ripe female opened, so soft tissue is seen. The mussel has been harvested, 11 May 2004 and has a shell length of 115 mm. A) Gonadevæv at the hinge and in the abdominal region. B) Gonadevæv in the foot.

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# 8.1 Investigation of gonadal tissue

The study included mussels from Sillerslev and Sundsøre. The study included mussels fra og Sillerslev Sundsøre. By collecting, parental animals were approx. City Collecting, parental animals kom ca. fifth of the ophentede mussels damaged. fifth av ophentede mussels Damaged. These are used in gonadeundersøgelsen. Det er used in gonadeundersøgelsen. It further included 100 individuals whose feet were sent to B). IT yderligere included 100 enkeltpersoner hvis feet blev sendt til B). Genetic studies in Spain (see appendix B). Genetic studies in Spain (see Appendix B). For collections was observed stressgydninger that otherwise could have affected the image of mussel's developmental status. For collections var observed stressgydninger som kan otherwise have påvirket the image of mussel's developmental status. Monitering af gonadeudviklingen blev Monitoring of gonad stage was limited by the fact that in a period in the winter of 2004 to 05 was not possible to collect razor clams because of poor wind conditions. When used in gonadeundersøgelsen they were frozen mussels thawed at room temperature, after which they were cut up along capes second.

# 81.1 Method for assessing the maturity

To evaluate the developmental stage of the collected razor clams, two index defined for visual inspection of the open clamshell. It was also selected a sample for microscopy from each individual. Apart from this mussel were gender and developmental stage of sperm or eggs recorded. The two index as well as samples from mikropering were combined to give a nuanced picture of the razor clam trends and to assess the method in the future it will use to determine when a mussel is ripe and ready to spawn.

# Ab Index

For the spread of genital tissue at the hinge and abdomen allocated index of 0 to 4

- Index 0 indicated that it was not possible to recognize gonad tissue
- Index 1, 2 and 3 gave respectively 1 / 4, 2 quarters 3 quarters of region was covered by gonad.

• Index 4 indicated that nearly the entire stomach and surrounding regions were covered by gonad tissue

Fodindex

To describe the prevalence and density of gonad tissue allocated index of 1 to 5. The assessment was performed by the same two people throughout the investigation.

• Index 1 indicated that there was no visible gonad tssue foot. Index 1 could both assigned mussels that had just gydt, and mussels in the neutral phase.

•Index 5 indicated a very wide distribution.

• Index 2, 3 and 4 were intermediate stages.

Samples for microscopy

In addition to the visually based gonad index were provided from each individual sampled gonad tissue from the abdomen and foot. Samples were microscopes, and if there was visible gonad tissue were mussel sex and stage of gonadal development noted.

There were photos (Soft Imaging System from Olympus Analysis) of the individual developmental stages in both sexes. For both males and females were gonad tissue development stage determined from the following categories:

• Neutral Gonad tissue not visibly seen only connective tissue. The mussel can not gender determined.

• Under construction Presence of the first developmental stages of eggs and sperm. Eggs and sperm sacs (follicles) only of small size. The eggs are stapled to follicles with a stem.

• Incipient ripening prevalence of connective tissue is reduced. Follicles are larger. The eggs are no longer stapled to the follicles. Males forming sperm distinct columns.

• Ripe In females the follicles full of large eggs, which can become a multi-angular shape, depending on space available. In males the follicles are fully of sperm.
• Partially spawned Gender tissue is more or less empty of eggs or sperm. There is degraded cell walls of the follicles and many empty spaces in germ tissue.

• Spent Connective tissue dominates. There are few, not gydte eggs or areas with sperm present.

## 8.1.2 Conditioning

To determine the accounts production of razor clams, the ratio between length and the dry mass of soft tissue is used. To investigate whether aerobic fitness can demonstrate maturity of razor clams were made analysis on the meat content of all mussels from gonadeundersøgelsen.

Length was measured with digital callipers to the nearest 0.01 mm at the longest point along the shell (Figure 8.2). To determine the ash-free dry weight (AFDW) was soft tissue removed from the husk, dried at 103 ° C for 48 hours and burned at 520 ° C 12 hours. The difference between the weight of the dried and burn soft tissue constituting the ln[AFDW] / ash-free dry weight, measured to the nearest 0.001 g. Stamina calculated as ln [AFDW] / ln [length]. Dry mass was not determined for mussels from December 2004.



Statistical analysis was performed with SPSS 13.0. Test for equal gender distribution was analyzed by Chi-square or binomial tests. Timing differences in the frequency of different developmental stages and distribution of the feet and stomach index was determined the General Loglinear Analysis. Analyses of seasonal variation in lean meat was made with Oneway Variance Analysis.

## 8.2 Results

The development of eggs and sperm travels through the years a pattern reflected in the gonads stage of development as well as abdominal and fodindex. In Figure 8.4 illustrates the distribution of mussels in various stages of the month, which made fundraising.

Statistically differed from the mussels and Sundsøre Sillerslev not from each other in the development of gonad tissue and were therefore pooled and treated together.

Data shows that about. 50 % of the studied mussels were mature in May 2004 and 2005 (Figure 8.3). This image seen in the feet and stomach index, where half of the mussels were high index. Under the microscope prepared samples could be seen that the remainder of mussels were wholly or partially spawned, reflected by the clams, which in May was low index of gonad tissue in feet and abdomen. Statistical analysis of the distribution within respectively fodindex, stomach index and tissue samples between the different months showed that there was no difference between

May 2004 and 2005. For fodindex there was several mussels with a high index in May 2005 compared to 2004.

From June to August, the percentage of clams with no or almost no visible gonad tissue in the foot from 73 to almost 100%. As the mussels had gydt during the summer, performed mostly with stomach index 0 or 1 Comparing this with the scheduled development stage, there is much to suggest that mussels are gydte, yet have something gonad tissue back at the abdomen. Tissue is usually light brown and gritty, and eggs or sperm are reabsorbering.



Figure 8.3 Gonad tissue from U.S. razor clam. Top left: Mature male. Top right: Gydt Bottom left: Mature female. Bottom right: Gydt she said. Scale the image top left are valid for all images.

In September there was more with higher abdominal and fodindex than in previous months and individual mussels were classified as partially gydt. In June and July, each mussels from tissue samples registered in incipient maturation. Although no mussels in August was found with mature or partially gydt tissue, there is evidence that there may exist a secondary spawning period in late summer. However, the number of collected mussels not very big for the summer months and September, so there is a uncertainties related to the finding.

By December, all studied mussels by building gonavevæv. Structure initiated following several months before the gonads final maturation. In December, third of the diameter at maturity, which corresponds to a several-fold smaller volume. In February and March were more than 50% of the collected mussels mature or incipient maturation, reflected in both the index and tissue samples. In late March, there was dominance of mussels with index 3, while 22% had been recruited to index 4th While individual mussels were still in index 1 and had not gonad tissue in the foot. In

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May, the first mussel with a maximum fodindex observed and the tissue samples showed that 18% had gydt.



Figure 8.4 Distribution of fodindex (top), abdominal index (middle) and developmental stages (bottom) for gonadtissue in razor clams from Sundsøre and Sillerslev from May 2004 to May 2005. In fractions included both males and females. Above each column is the number of clams (N) for each month indicated. In month indicated with \* were not collected razor clams. February data includes mussels March, while March data indicates mussels collected in late March.

## Gender distribution

Frequencies of males and females were similar as a function of both time and place (Pearson Chi Square, P > 0.05 and Binomial test, P > 0.05) and did not differ significantly from the relationship 1:1. Testing is performed for mussels collected at Sundsøre or Sillerslev the months, which was collected razor clams (Table 8.1). Developments in males and females followed the same pattern of abdominal and fodindex (General Loglinear Analysis, P > 0.05) and developmental stages.

Måned	Hunner	Hanner	Total
Maj 2004	82 (44%)	106 (56%)	188
Juni	27 (59%)	19 (41%)	46
Juli	3 (27%)	8 (73%)	11
August	2 (50%)	2 (50%)	4
September	15 (34%)	29 (66%)	44
December	5 (38%)	8 (62%)	13
Februar	43 (48%)	46 (52%)	89
Marts	10 (42%)	14 (58%)	24
Maj 2005	26 (52%)	24 (48%)	50
Totalt antal	213	256	469

Table 8.1 Number of females and males collected (Sundsøre and Sillerslev pooled) and percentages broken down two sexes for each month. February data includes mussels from the first March, while March data includes mussels collected in late March.

# Must Length and gonad tissue

Between mussels of different shell lengths, there was no difference in the assigned index (Nominal regression, Chi Square, P > 0.05), and there was thus no tendency to larger mussels were awarded higher index. Since diffusion is therefore the same for different store sizes of razor clams, the actual quantity of gonad tissue be greater in large individuals in relation to small.

# Conditioning

Stamina with razor clams, measured as  $\ln [AFDW] / \ln [length]$  shows the relationship between the weight of soft parts and the length of the shell (Figure 8.5). The relative lean meat from the compiled razor clams was highest in August and September when mussels recovered from the spawning period. The fitness decline during winter when food availability is limited. During the spring months when plankton again flower up, there will be more food available and the relative weight of soft-tissue increases. Data shows that under the prevailing conditions in the Limfjord will be advantageous to harvest razor clams early autumn, where the contents of soft parts is high. August and September differed significantly from the other collection months know that the mussels had a high lean meat (Oneway ANOVA, df 6,386, P <0.05).



Figure 8.5 Boxplot of fitness in mussels collected at Sundsøre and Sillerslev (pooled). The plot shows the median and 25 and 75 percentiles. Number of individuals of each month (N) is specified for each column \* denotes statistical outliers.

### 8.3 Discussion

### Gonad stage

Gonad stage in American razor clam is seasonal and is determined by temperature and food conditions that affect the mussel. Nærværende undersøgelse The present study included a total of 551 individuals, collected by Sundsøre and Sillerslev from May 2004 to May 2005. The results show that the spawning period for the American razor clam Limfjord stretches from May to July in late May as the primary spawning time. Mussels undergoing a rest period from August to early winter. Herefter Then begin construction of the gonads during the winter months until spring when the final maturation takes place.

It is recommended to collect parentalmuslinger before May and induce spawns in May Mussels collected later will most likely have gydt in nature and experimentation with induction also proved unsuccessful at this time.

The observed trend is consistent with observations in other razor clam species at both the northern and southern hemisphere (Aracena et al. 2003; Breese and Robinson 1981; Gaspar and Monteiro 1998; Kenchington et al. 1998; 1998; Mühlenhardt-Siegel et al. 1983).

Prevalence of gonad tissue is a good indicator of mussel's ripeness. At mature mussels gonad tissue seen as distinct strands in the foot, a thick layer extends over the entire abdominal region. When sex products become gydt becomes layer thinner and appear after spawning as a thin, yellowish skin over the abdomen. There was an overlap between the results from the two index and vævssprøverne. An assessment of whether the knife mussels are mature, can in practice be based on a visual assess the distribution of gonad tissue over foot and abdominal area with few mussels instead of microscopy of tissue samples or actual slides, which is both time-and equipment-intensive.

Studies conducted in the Wadden Sea by the occurrence of razor clam larvae in the water phase has suggested multiple spawning periods during the summer at the American razor clam (Armonies 1992). In the present study from the Limfjord, there are also signs that individual mussels ripens later than most, suggesting a secondary spawning period in September. Assigned higher indices in September than in August. In September was partly gydte mussels present in contrast to August, when microscopy samples showed that all studied mussels had gydt.

Aracena et al. 2003) have described that the index based on dry weight is not good at follow gonad stage with razor clams. The same conclusion can be drawn from present data material. There is seasonal variation in gonad stage as well as conditioning, but the meat content could not specifically say anything about the mussel stage of development.

### Gender Ratio

Similar to other studies had knife mussels from Sundsøre and Sillerslev a sex ratio of 1:1, and was observed hermaphrodites. The results are in consistent with studies conducted in *Ensis siliqua* and *Ensis macha* (Baron et al. 2004; Gaspar and Monteiro 1998). In *E. siliqua* were not registered gender-based differences in the gonads color or texture. This is observed in *E. macha*, where the male gonad tissue is gray-white, while the female tissue is white to cream colored. A similar difference in color and texture are seen in U.S. razor clam. Genital tissue in males is golden, while in females are whitish.

Gender Determination may be of relatively high safety without the use of microscope.

## Age of sexual maturation

There have been some ignorance about the age of sexual maturation in U.S. razor clam. Under this project were observed gonad tissue in several perennial mussels. *E. siliqua* were also found in gonad tissue annual mussels (Gaspar and Monteiro 1998). About the development of entirely gonad tissue young mussels probably depend on whether the shell originates from an early or late spawning year earlier.

There were no quantitative studies of gonad tissue since location of gonadtissue around the abdomen makes it impracticable to generate all tissue out. Visual observations of gonad tissue showed that gonad tissue had same extent independent of mussel size. However, there was a tendency for gonad tissue in larger mussels were a little thicker.

Quantitatively, the actual quantity of gonad tissue and thus the reproductive potential is therefore greater in larger mussels (due to their larger volume) compared with younger individuals. From knivmuslingens third and fourth years of life the growth rate is somewhat lower than in young mussels and devoted energy to gonad production over length growth. It is therefore advisable to using larger animals in the second year of life.

9.0 Spawning and fertilization

The purpose of the work package was to investigate whether it is possible to induce and control spawning and fertilization in American razor clam hatchery. Temperature and stripping was tested as spawn inducing factors.

Materials and methods

In spring and summer of 2004 and 2005, it was investigated whether the razor clams can induced to spawn by a slow increase in temperature. Prior to the experiments was 20 - 30 mussels from broodstock established in  $60 \times 60$  cm tank with 12 cm of water phase and a 15 cm deep sand layer with a sediment size of less than 4 mm. The water was filtered down to 2 micron. The mussels were allowed to burrow in baseline. The tubs were aerated with 0.22 m filtered air. Table 9.1 shows the dates on which induction took place during the pilot project. In 2005, various creation modes tested and the establishment of true, location of the mussels on the bottom of a trough and establishment of a lattice structure.



Figure 9.1 The left picture: Compilation of conditioning. The tubs were filled with 15 cm of sand. The aqueous phase was 12 cm. The water was aerated, but there was no running water or food additive. There was established 20 to 30 mussels. The image on the right: Induction of spawning without sand. Mussels ranged bottom of a tub. Note the deferred ånderør. The white material is gydte sex products.

The initial water temperature in spawning table was similar to the temperature, the adults came from the broodstock system. The temperature was increased by  $2 \pm 0.1$  ° C every 20 to 30 minutes until found spawning. Samples were taken from water samples that were microscope for the presence of eggs and sperm. Temperature and oxygen were monitored. The oxygen level was never critical. Besides temperature inducedspawns were in May 2004 in one case observed a spontaneous, natural spawning in broodstock system itself. Finally, fertilization of eggs based on stripping tested. By stripping extracted and mixed gonad tissue from males and females.

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### 9.1.1 Results

In five out of six attempts in the period 31/05 to 24/07 - 2004 succeeded in inducing spawning by temperature stimulation. Spawning was noted when the water was turbid and milky white in color, and by microscopic analysis of water samples showed that there were eggs and sperm into the water. From late May to late June 2004 began spawning after a temperature increase of between 6 and 7 ° C. i.e. from a starting temperature in broodstock system at 15 to 16 ° C temperature was increased to 21 to 23 ° C before the mussels spawned (Table 9.1). Spawning was harder to induce in July. I midten af juli In mid-July

2004 skulle temperaturen hæves 9 °C, mens den sidste induktion i juli mislykkes 2004 would increase the temperature 9 °C, while the last induction in July fail (Table 9.1). It was not possible to induce additional spawns by running multiple temperature cycles in a row at the same parentalmuslinger. In 2005, induction made at an earlier date between 27/04 and 11/05, where the output temperature parentalbestanden was lower, between 9 and 11 °C. It succeeded in all

three cases to induce spawning after a temperature boost of 5 to 6  $^{\circ}$  C compared to the initial temperature (Table 9.1).

Date	% Mature	substrate	spawned	broodstock	spawning	total
				Temp	temp	eggs
Dato	Modne (%)	Etablering	Gydning	Temperatur- forøgelse (°C)	Antal æg (ml <sup>-1</sup> )	Totalt antal (mio).
31-05-04	45	I sand	Ja	6	21 ± 14	1,98
17-06-04	15	I sand	Ja	7,2	$40 \pm 24$	1,89
21-06-04	15	I sand	Ja	6,5	12 ± 1	0,55
12-07-04	6	I sand	Ja	9	$0.7 \pm 0.7$	0,00
24-07-04	6	I sand	Nej	9	-	-
27-04-05	-	I sand	Ja	6,1	55 ± 8	1,76
28-04-05	-	På karbund	Ja	5,8	$160 \pm 87$	7,11
11-05-05	48	Gitter	Ja	5,3	213 ±163	10,48

Table 9.1 Data for induced spawns. The column percentages mature indicates how large a percentage of the induced mussels that can be expected to have gydt in advance. This data is based on microscopy samples during the investigation of the reproductive cycle. The table also indicates how the adults were established during the spawning season, the spawning took place after the temperature influence, where large temperature increase, which was conducted (measured as the difference between the temperature in parentalbestanden and temperature when spawning registries). Number of eggs is the measured concentration, and total number (million) is an estimate of the total number of fertilized eggs that got out of induction, measured within the first day.

Figure 9.2 indicates that there is a correlation between the temperature increase has necessary to induce spawning and respectively temperature broodstock system and date for induction. he figures show that the inducing temperature increase rises, as temperature rises, and that there should a higher temperature stimulation of late in the spawning season. Table 9.1 also shows a tendency for the number of eggs gydte depends induktionstidspunktet. There was gydt most eggs in April and May compared in July.

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Figure 9.2 The inducing temperature increase compared to the temperature of broodstock system for induktion left) and date of induction (right).

Spawning occurred between 4 and 25 minutes after temperature stimulation. In the vast most cases began spawning after 10 minutes. The first water samples contained only sperm, while eggs were observed later. Spawning continued between 1 to 3 hours after induction.

It is known that spawning in one mussel hormonal influence scallops to spawn. This ensures that the mussels spawn at the same time as egg and sperm meet each other in the water. During spawning had mussels siphon distended and vomited sequence shown clouds of sex products. In some cases, it was observed that mussels were placed higher in sediment than normal. No differences were found mortality of mussels associated with the induction.

In 2005, various establishment methods tested. The results show that American razor shell temperature can be induced regardless of whether the mussels are placed at the bottom of vats of lattice structures or in sand at Table 9.1 shows a tendency for induktionsmåden influence ægantallet as this was less for establishment in sand compared with the other methods.

Mussels that were used for induction in 2005, had an average length of 115 mm. There was no significant difference in the size of the used mussels during the three inductions (Oneway Anova, P = 0.832). It was possible to obtain fertilized eggs by stripping, but larval numbers and the subsequent survival was minimal. Induction of spawning by the addition of large quantities of food for the adults was also tested, but rejected again because it was difficult to detect spawning.

During induction in 2004 decreased oxygen levels generally from about 9 to 5 mg / l. The oxygen level decreased but never to a critical level. The oxygen level was high during induction in 2005 (from 90 to 10 mg / l). The salinity was between 26 and 33 %.

### 9.1.2 Conclusion on results

They managed to induce spawning in American razor clam and obtain fertilized eggs both 2004 and 2005. Based on the results can be concluded that mature razor clams at a warming of 5 to 9 degrees above the temperature of the broodstock system can be induced to spawn. Induction using the temperature was a reliable and practically useful method that allows you to set a specific time for fry production.

Spawns obtained by induction led to the fertilized egg went through that embryonic development. The density decreased in all litters during the first days, but usually survived until day 10 - 12. From only one out of seven induced spawns larvae survived to settlings stadiet. Based on this study, expected between 2 and 10 million eggs from 20 to 30 spawning mussels (10 - 15 females).

Spawns were induced between late April and mid July. Based on inductions success, larval survival and gonadeundersøgelser is mid-May the most favorable time to induce spawning in American razor clam during the Limfjord prevailing conditions. Induction during this period reduces the risk of induction on immature or partially gydt tissue.

### 9.2 Discussion

Seasonally Dependent inductions success

Of 8 trials temperature induction managed to stimulate spawning in 7 cases.

Induction of spawning were made relatively late in the razor clam in the gydningsperiode 2004 due to the time of project initiation, and according gonadeundersøgelserne had most of the mussels already gydt in nature, since they were collected.

Induction was therefore carried out at suboptimal material in 2004. Probably mussels have been pressured to spawn the last volume, respectively. sperm and eggs were back in genital tissue. This may explain why in July to high temperature stress to and why they litter, which was obtained by gydningsinduktion in 2004, not fared particularly well.

The high mortality of egg and larval stage may be due to the gametes carried a low content of stored food, or were not fully developed at gydningsinduktionen.

Apart from the high mortality, there was no indication in either the fitness index or growth of larvae that litters the summer months were in worse condition.

In 2005, inductions conducted in late April and early May. There were a small temperature increase at this time than in summer 2004. Based juvenile, mens by induction on 11-05-2005 manages to produce settlede juveniles, while larvae from induction from late April failed settlingsstadiet.

Results indicate that early induction is performed on reproductive tissues that were not quite mature, and that the tendon was carried out on mussels, which were wholly or partially gydte.

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Thus, the proportion of mature or nearly mature individuals somewhat larger in May for both 2004 and 2005 than in the subsequent summer months. Finally, the mortality in larval stage due to several factors other than induktionstidspunktet.

Under this project there have been eight spawns (7 + 1 induced spontaneous), two led to settled mussels. The litter that settled was from May and the results show that May is the best time to induce spawning. The experiments also indicate a correlation between induktionstidspunktet and temperature of the broodstock system.

A slight stimulation was required earlier in the season when the mussels were mature, than late in the season where the mussels were gydte or partially gydte. There is also a weak tendency that it was easier to stimulate the beginning of May, compared in late April. The required temperature stimulus increased during summer as the temperature in broodstock system increased and the likelihood that the mussels already had gydt rose.

#### Processes during induction

It was possible to stimulate spawning both in mussels established in vascular bundles and lattice structures. The largest number of fertilized eggs were obtained when the mussels was established in lattice structures or the vascular bundles. The reason is that by induction without sediment was possible to absorb the entire amount of eggs and sperm that were at the bottom of tank up.

From the same number of parental animals were there at spawning time in 2004 gained between 0.5 and 2 million eggs, while in 2005 reached up to 10 million fertilized eggs.

Ægantallet at the first spawning in 2005 and induction in 2004 was almost identical. This may be due to identical induction (with sand), or the number of mature gametes in gonadevævet were similar at these times.

The advantage of induction of mussels established in sand is that it allows to mussels may gydningsinduceres while they are in broodstock system, which fertilized eggs or larvae can be

collected in specially adapted collectors. By development of the proper design of the vascular system will gydningsinduktion in broodstock system be handy in a future production situation.

Induction without sediment requires mussels dredged and subsequently established in lattice structures or hills. Excavation and restoration of sand or lattice structures has not caused problems during this project, and such an approach can be used in the future. Mussels, which were restored in the sand, dug themselves down quickly, while mussels established in lattice structures, or on the bottom of the tub seemed a little stressed about, that they could not burrow.

Mussels at the bottom was moving around a lot and tried in vain to dig down. The lattice structures were mussels in the normal vertical position but it managed several times to swim out of the structures and then come to lie at the bottom of the vessel. Lattice Structures or possibly trays should be adapted to mussels, since the procedure without sediment else worked well with high gydningsaktivitet. It is easier to separate the adults from their broods, whose induction made without sand as a substrate for the adults.

Alternative methods for inducing spawning

Stripping does not seem to be a workable method. Since several developmental stages can occur simultaneously in the gonads, the limited success of stripping due that it was not possible to separate mature and immature eggs. This may explain the low number of fertilized eggs and larvae were obtained. Another drawback is that parentaldyrene killed by method, while parentaldyrene after temperature induction survive and subsequently be conditioned to again build gonad tissue. Moreover, stripping for gonad products. a labor-intensive method because each animal must be cut open and emptied of gonadtissue.

There are methods for induction of spawning, the mussels given hormone injections, but more simple methods such as in terms of temperature effect is preferable if the effect is the same.

Besides spawning induction have during this project has been a spontaneous spawning. The advantage of spontaneous spawning is that the mussels spawn when sex products are ripe; whereas induction is a risk of induction of mussels that have not mature or already have gydt. The biggest problem with spontaneous spawning is that the exact time of spawning is not determined by the breeder.

Lessons from this project indicates that there is a risk that the adult razor clams can filter out some of their own offspring. There is thus observed only a few shells from fry in the broodstock tanks. This indicates that the current karopbygning not suitable for extensive farming of razor clams. Small modifications can make the system usable. There must therefore be installed collection systems that can separate the fertilized eggs from adults when spawning must take place without moving mussels from the broodstock system Conditioning of mussels could also done in such a system.

### Mass spawning

By mass spawning, as used in this project, it is not possible to verify number of eggs and sperm that are part of fertilization. This can be problematic as several researchers indicate that the later larval survival and development is influenced by the eggs fertilized by multiple sperm (polyspermy). High sperm concentrations increases the risk of polyspermy. It is important to wash the excess sperm away, after fertilization has occurred. Fertilized eggs and the first cell divisions were observed shortly after spawning had begun. The sperm was only removed by sieving after a half to a whole day. Has been observed high concentrations of spermatozoa around the egg cells and polyspermy probably occurred to some extent. It is described that polyspermy example can be minimized in larger water volumes with lower densities of reproductive products. The density of eggs during this project, however not differed from values reported in the literature. Moreover led the last spawning to settled mussels despite high numbers of eggs initially

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### 10.0 Larval Production

To ensure survival and growth of larvae is an important part of production in the hatchery. Experiments with hatching of razor clams is only a few places in the world, and there is large seen unpublished literature. The primary purpose of klækningsforsøgene under this project was to

•Investigate the entire workflow from induction, to free-swimming larvae to settling and fry for ongrowing.

- Investigate whether it is possible to get razor clam larvae to survive and grow
- Gain experience in working practices / processes and develop methods
- Identify any critical factors that require further investigation for the future commercial production

The studies should thus determine the potential for breeding razor clam larvae and provide an estimate of the success that can be expected in the future production.

### 10.1 Materials and methods

#### Larval Care

For the first spawning was used stationary tub with objectives  $60 \times 60 \times 60$  cm the earliest larval stages. After the first filtration was established larvae in tanks of 10 l which was filled with 8 l seawater. For all subsequent litters were used 10 l vats.

Added seawater was filtered through a sand filter and a filter station with filters of 20 to 2 micron, and the effluent was led into the sewer. It was planned to use UV, but technical problems meant that the experiments in both 2004 and 2005 drove without UV. There were connected to air supply of 0.22 m filtered air.

#### Handling of larvae

There was replaced water in the tanks every or every other day. During water exchange larvae were retained on the screens, selected according to larval size. Experience shown that size differences between dead and live larvae may be so small that it is difficult to sort the shells and dying larvae from. Larvae were then washed into cleaned tanks of fresh seawater. In two cases where the larvae showed signs of being in low fitness was a litter divided into several size fractions to examine the sizes that were most viable.

### Monitoring

To follow the survival and growth were regularly sampled  $3 \times 1$  ml samples from water phase. In the earliest larval stages were often sampled multiple times daily, while sampling was done once a day later in the larval stage. Samples were taken integrated through the water column as larvae from all depths were included. The larvae in the samples were counted using stereolup and the total number estimated from water volume in each vat. The total number of larvae from a spawning was calculated as the sum of larvae in all vat.

Larval length and width were measured using a photo program (Digital Image analysis via Soft Imaging Analysis from Olympus) in stereolup. There were photos documentation of the development stages, and larval behavior and condition were registered.

Fitness Values between 1 and 5 were attributed on the basis of larval activity:

- Index 1 No larvae swim. Bottom Landscape larvae are inactive.
- Index 2 Very few larvae swim. Bottom Landscape larvae are inactive.

• Index 3 Partially active larvae, fourth swimmer, while the rest lies on bottom. Get bottom-lying larvae are active.

- Index 4 Active larvae, half swimmer. Bottom Landscape larvae are relatively active.
- Index 5 Very active larvae, 2 / 3 swimmer. Bottom Landscape larvae are active.

To describe the feeding mode was given values between 1 and 4:

- Index 1 Completely transparent larvae
- Index 2 Almost transparent larvae
- Index 3 Mostly dark larvae, not the entire system is filled with feed
- Index 4 Very dark larva, both the esophagus, stomach and intestine is filled with feed

Were also made measurements on temperature, oxygen and salinity in the tanks to ensure that these parameters were within acceptable limits.

Food

As a food source for the larvae were applied three species of microalgae grown in monocultures.

There was no added food to the larvae the first day, where they still live on food from egg. Subsequently, the larvae fed with *Isochrysis galbana* for some days, after which were added *Chaetoceros calcitrans* and *Tetraselmis suecica* in relation shown in Table 10.1. Capture and digestion of the algae was checked under a microscope or stereolup.

Were fed twice a day.

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Dage efter gydning	Isochrysis	Tetraselmi s	Chaetocer os	Total konc.	Forhold
Kuld 21/05-04					
1 til 6	82			82	1:0:0
fra dag 11	105	16		121	7:1:0
Kuld 31/05-04					
2 til 8	217			217	1:0:0
fra dag 9	80	14		94	6:1:0
Kuld 17/06-04					
1 til 11	157	_		157	1:0:0
fra dag 11	. 84	7		91	12:1:0
Kuld 21/06-04					
1 til 7	162			162	1:0:0
fra dag 7	121	10		132	12:1:0
Kuld 42/07-04					
Kuld 12/07-04	105				1-0-0
2 til 16	195				1:0:0
Kuld 27-04-05, 28	3-04-05 og 11-0	05-05			
1 til 5	19			19	1:0:0
7	14		11	25	4:0:3
8 til 11	17	3 3	6 7	25	6:1:2
12 til 15	20			30	6:1:2
16 til 21	23	4	8	35	6:1:2

Table 10.1 Food Additive per. feeding in the larval stage. Were fed twice daily. Table 10 indicates the average concentration in the vessels (in 10 cells/ml) immediately after algal addition. Values from 2005 are based on algal counts, while values from 2004 are calculated from the added volume and censuses from 2005.

The addition of food was in 2004 based on larval removal of micro-algae, which visually, was subjected to water color. In 2005 we made provision of algal concentration using an optical particle counter. Larvekuldene in 2004 had an average algal concentration in the blood vessels just after food additive at 135 cells/ml

Larvekuldene in 2005 were exposed to concentrations of 19 to 35 algal cells/ml depending on larval age.

10.2 Results and discussion

Storage and handling of larvae

During his studies were the larvae from each litter distributed on 10 tub. got all the larvae from the same litter generally the same daily handling and food additive. For the litter that went down, dead larvae in different vessels almost simultaneously. For the litter from the 21-05-04 dawns on settled fry almost without exception from the same vats, while all vessels from the litter from the 11-05-05 gave rise to settling.

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During the first spawning in 2004 were different routines with respect to water turnover and filtration tested. Two tanks were not filtered after 4 days while they others were filtered by a day. This had no effect on the larvae that did themselves equally well regardless of filtration time. This suggests that the removal of excess sperm may not be so important. By successive spawns was water with filtered first half to a full day after fertilization.

In two cases, 30 to 80 m fraction is filtered on days 3 and 5, stored in separate tanks. These larvae survived only in a few days, indicating that with the benefit of an early time can filter out small larvae with low quality.

In both 2004 and 2005 the oxygen level in larval water at 7 to 10 mg/l corresponding to a mætningsprocent at 78 to 111%. The salinity was 26 to 31 % and water temperature 15 - 20  $^{\circ}$  C.

Added seawater was UV illuminated during this project. Whether UV treatment could have prevented some of the mortality in the larval stage by reducing the flow of bacteria and viruses is unclear. Chilean studies of mussel *Mesodesma donacium* showed a higher survival of larvae receiving non-UV-treated, 10 m filtered water compared with larvae that received UV-treated, 1 m filtered water. The cause is believed to be that the bacteria entering the water, with high probability function as food for the larvae.

#### Larval development

The embryonic development and development through the larval stage of American razor shell seen in the following pictures (Figure 10.1 and Figure 10.2). The eggs measure mens between 65 to 80 micron. The smallest recorded larvae had a length of 70 m while the largest free-swimming larvae were up to 340 micron.

After several cell divisions (Figure 10.1 B and C), starting each cell being differentiated, and the larvae are ciliated. After approx. 24 24 hours developed a skalpar that is just above the hinge. D-Larvae are thus shaped like a big D and is called a D- veliger. In this stage the larvae begin to actively take nourishment. By means of a mantle called velum, which is beset with cilia, food particles may be headed to the mouth. Moreover, serves cilia with a flagellum to larval propulsion in the water. D-veligers stage growing larvae around. 11 microns/day.

When the larva is about 5 days old developed Umbo as a small, outward-facing bulge in the shell at the hinge. When settlingstidspunktet approaching shell gradually assumes a more oblong shape, developed a foot and velum reduced. In pediveligers stage is the foot developed, while the velum is still present. The first records of a foot was when the larvae were 13 days old. In the oldest larvae have been observed developing a byssus, which is probably an adaptation to improve lift and transport through the water (Sigurdsson et al. 1976). Larvae were settlingsklare in an age of 16 days.



Figure 10.1 Larval Development at American razor clam. (A) Eggs (65 micron) surrounded by sperm. (B) First cell division, age approx. 20 min. (C) multicellular stage, age 4 hours. (D) Rotating trochophore-larva (70 micron), age 18 hours. (E) First must-bearing stage, D-veliger, age 23 hours. (F) Veliger shell and velum. Larvae of the image is 90 meters long and 5 days old.

Figure 10.2 Larvae of American razor clam. A and B strains from the water phase, while C comes from bottom. Besides the different morphological traits indicated the front (anterior) and



rear (posterior). (A) larva in pediveliger stage where velum not yet been reduced, but the foot is developed, age 13 days. (B) Age 16 days. The larva is a bit more developed, but the velum is not yet fully reduced. The stand is mature and has a distinctive charm. (C) Age 19 days. The mussel is now settle and during metamorphosis, length 720 microns.

Survival

The following results are based on 8 track litter from spawning in 2004 and 2005.

10.2 indicates the total larval number and density of all 8 litters. Most of litters survived between 11 and 13 days, and of two litters, survival was so good that there were mussels that settled. Evolution of larval numbers followed the same pattern all larval brood. The survival of each litter were compared to determine whether mortality occurs at the same time. This serves as a basis for whether there exist critical stages in the larval stage.

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Table 10.2 The number of larvae as a function of time given to all 8 larval brood. Total number of larvae are displayed for the entire litter. Density  $\pm$  SE is the average density (larvae/ml in the tank.)

	21/	21/05-04		/05-04	17/	06-04
Alder	Total antal	Densitet	Total antal	Densitet	Total antal	Densitet
0	-	-	1.981.200	17,0 ± 5,5	1.886.040	40,3 ± 16,7
1	-	-	912.600	9,8 ± 2,9	-	-
2	-	-	683.280	4,9 ± 2,6	-	-
3	3.036.000	6,8 ± 3,8	656.800	9,1 ± 2,1	834.400	52,2 ± 20,4
4	1.409.400	3,1 ± 2,0	-	-	584.000	36,5 ± 15,5
5	118.800	0,3 ± 0,3	753.600	11,7 ± 1,9	584.000	36,5 ± 17,5
6	180.800	$2,3 \pm 1,1$	645.600	-	726.400	22,7 ± 14,3
7	139.200	$1,6 \pm 0,7$	-	-	196.800	6,2 ± 3,7
8	79.200	$1.0 \pm 0.4$	4.000	-	-	-
9	71.200	0,8 ± 0,4	-	-	-	-
10	46.400	0,6 ± 0,2	-	-	-	-
11	26.400	0,3 ± 0,2	-	-	2.400	0,2 ± 0,1
12	13.600	0,2 ± 0,1	-	-	-	-
13	7.200	0,1 ± 0,1	2.667	-	-	-
14	-	-	-	$0.2 \pm 0.1$	-	-

	21/	21/06-04		/07-04	27	27/04-05	
Alder	Total antal	Densitet	Total antal	Densitet	Total antal	Densitet	
0	552.240	11,8 ± 0,8	-	-	-	-	
1	131.040	-	-	-	576.000	$18,0 \pm 6,0$	
2	82.400	5,2 ± 2,7	11.200	0,7 ± 0,6	272.000	8,5 ± 4,5	
3	126.400	7,9 ± 3,3	-	-	205.333	6,4 ± 4,7	
4	-	-	-	-	344.000	10,8 ± 5,6	
5	-	-	4.800	-	368.000	15,3 ± 4,4	
6	-	-	-	-	288.000	12,0 ± 3,3	
7	11.200	0,9 ± 0,5	-	-	384.000	12,0 ± 4,1	
8	-	-	-	-	242.667	7,6 ± 2,8	
9	-	-	-	-	269.333	11,2 ± 1,2	
10	-	-	-	-	242.667	10,1 ± 4,6	
11	-	-	6.000	0.8 ± 0.1	5.333	$0.5 \pm 0.2$	

	28	/04-05	11/	05-05
Alder	Total antal	Densitet	Total antal	Densitet
0	-	-	10.484.000	212,7 ± 66,5
1	7.108.000	159,9 ± 35,7	7.978.667	166,2 ± 36,8
2	4.164.000	86,5 ± 23,5	6.160.000	128,3 ± 33,6
3	4.309.333	82,7 ± 27,7	1.709.333	35,6 ± 10,8
4	4.058.667	92,9 ± 19,1	608.000	12,7 ± 4,9
5	2.910.667	68,1 ± 15,3	1.221.333	$38,2 \pm 20,6$
6	3.402.667	67,4 ± 17,2	-	-
7	2.613.333	54,1 ± 11,5	170.667	5,3 ± 2,1
8	2.538.667	51,8 ± 12,1	136.000	5,7 ± 2,2
9	2.190.667	48,3 ± 7,7	10.667	$1,3 \pm 0,8$
10	2.349.333	41,8 ± 16,4	-	-
11	861.333	18,0 ± 9,2	-	-
12	241.333	10,3 ± 4,5	13.333	$1,7 \pm 0,8$
13	-	-	-	-
14	-	-	-	-
15	-		13.333	0,6 ± 0,6

The same trends in survival could be seen in all litters. Figure 10.3 shows survival expressed as percentage of larvae in relation to the initial egg number. The greatest mortality occurs within the first day. Thus, the percentage of mens den died in relation to the initial number of eggs between 24 and 76% the first day, while the after day 3 were between 56 and 87%. After this event, larval numbers fairly stable between days 3 and 6. From day 7, there are again indications of increased mortality, which coincides with some litter crashes. For these litters was the last recordings of live larvae performed between days 7 and 12, ie before settling would have occurred.

In the litter where settling occurred, was it between days 16 and 19. Given Figure 10.3 can thus be concluded that there are two critical stages during rearing phase, respectively. før settling. within the first day and just before settling. There was no litter from settling. later than May, which survived until settling. For the litter that went down before settling, there was no immediate link between timing of spawning and the number of days the larvae survived.



Figure 10.3 Survival of larval stage. Percentage of larvae in relation to the initial egg number as a function of tiden. • Spawn 31/05-04, • Spawn 17/06-04, • Spawn 21/06-04, • Spawn 11/05-05 (larvae reached forward to settling).

Improvement of survival should be a major focus area for future studies on razor clams, and also has international attention (see appendix B).

Mortality can be caused by many different factors such as parental fitness and maturity and hence the quality of eggs on gydningstidspunktet, morphologically related critical phases (eg transition between egg nutrition and taking nourishment), external shocks in the form of disease from viruses or bacteria, wrong nutrient conditions (lack of symptoms or rapid growth), possible saturation of oxygen and lack of turbulence.

Density

Larvae were attempted kept at a start density of eggs at either <50 or> 200/ ml (Table 10.2 and Figure 10.4). The density of larvae affects the survival and growth and results from studies of other mussel species have shown that a successful result usually seen at low initial densities (Utting and Spencer 1991; Lepez, personal communication).

Litter, 28 April 2005 was the first day densities of 40-280 larvae/ml During the fourth to 8 day decreased the density of 40 to 150/ml Litter, 11 May 2005 had the first day densities of 90 to 500 larvae/ml Between days 4 and 9 were the density in the most tanks 5 to 20/ml, and from day 9 density was 1 to 3/ml.

Evolution of the density and the final result was different for these two litters. Litter from May 2005, settling, whereas larvae from April, which experienced high densities throughout most of the larval stage, went down after 12 days. Litter, 11 May 2005 saw the highest mortality in early larval stage and then average density was less than 30 larvae/ml This indicates that the chances of success at increasing larval cultures with low densities. The outcome may also be due other factors, eg different gydningstidspunkter.



Figure 10.4 The average density of larvae as a function of age. Litter from 11-05-05, which had high start density at settle. Litter with high start density from 28-04-05. Litter from 21-05-04, who had low start density and eventually settle. Litter low start density from 2004 and 2005.

Growth

Must Length measured on larvae from spawning in 2004 and 2005 is depicted in Figure 10.5. There was no evidence of differences in growth either between litters or between years and even

between different densities in the larval stage. By age 12 to 14 days there were several litters in which the larvae ceased to grow (see Figure 10.5) and then died, whereas the litter, which reached settling showed steady growth.

A balanced covariansanalyse comparison of each litter could unfortunately not performed and therefore it was not possible to statistically show whether the growth was similar for litters. By adjusting all the measurements to a linear regression produces a daily increment of 11 micron through the larval stage.

Table 10.3 indicates the daily length growth for each litter.



Figure 10.5 Average Growth (m)  $\pm$  SE as a function of age for all broods from 2004 and 2005. The photo shows the length and width of a D-veliger.

Gydningsdato	Vækst (µm dag¹)	R <sup>2</sup>
21/05 2004	15,2	0,89
31/05 2004	9.6	0,45
17/06 2004	10,5	0,52
21/06 2004	11,2	0,76
12/07 2004	16,1	0,46
27/04 2005	11,8	0,78
28/04 2005	5,5	0,45
11/05 2005	14,4	0,82

Tabel 10.3 Daily length growth (microns/day for larvae from 2004 and 2005  $\,$ 

The relationship between larval length and width can be expressed by the equation y = 0.8072 x + 2.0093 (R <sup>2</sup> = 0.95). Forward to settling larvae may length-width relationship therefore described as linear. The results for length-width ratio is consistent with the few literature values available for the fry of American razor clam (Figure 10.6).



Figure 10.6 Length-width ratio in the larval stage. • larvae of spawns in 2004 and 2005 • Loosanoff et al, 1966, • Sullivan, 1948.

### Larval condition

Larval fitness and feeding mode was monitored at all samplings. Figure 10.7 showing larval fitness as a function of age for two of the litters. Larval activity was high the first 7 to 12 days when more than half of the larvae swam. During this period larvae stayed only for brief intervals on the bottom, and there was a distinct activity in stomach on the bottom-lying individual. Then there could of all litter recorded a decline in activity as the settlingstidspunktet approached, and the larvae were larger and heavier, and the velum was reduced. This was reflected in the index of fitness, which, as illustrated in Figure 10.7, decreased with larval age. Low swimming activity is not necessarily a sign that larvae are about to die. The decrease in fitness is consistent with the increased mortality related to settling. Dying larvae had low activity and showed a erratic swimming pattern.



Figure 10.7 Frequency distribution of fitness index as a function of larval age. Index = 5 (Very active larvae), Index = 4 (Active larvae), Index = 3 (Partly active larvae), Index = 2 (Get active larvae), Index = 1 (No active larvae). (A) Spawning 28/04/2005, (B) Spawning 05/11/2004.

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Figure 10.8 indicates larval feeding mode as a function of age for two of the litters. Based on the amount of food in the gastrointestinal tract were the larvae feed in good condition throughout the larval stage. The larvae begin to take food when they are around a day old. There is no difference in feeding mode from each litter or between larvae from 2004 and 2005.



Figure 10.8 Index of feeding mode as a function of time. Index = 4 (Very dark larvae with large food content), Index = 3 (Mostly dark larvae), Index = 2 (almost transparent larvae), Index = 1 (Completely transparent larvae). (A) Spawning 28/04/2005, (B) Spawning 11/05/2005.

Food

Through the first part of larval development takes the larvae are not food for themselves but live entirely of egg storage of carbohydrates. There was only added to food after a day, where the larvae had developed into the bearing D-veliger. The high, initial mortality were mainly recorded in the period before the larvae began to take food, suggesting that mortality may be because the eggs had a low content of storage of carbohydrates. It was observed that the larvae, since they were one day old, were able to consume Isochrysis. Few days old larvae had to turn hard to digest Tetraselmis. Since Chaetoceros is less than Tetraselmis, it is expected that Chaetoceros can added at an earlier stage than Tetraselmis.

Litter that settled in 2005, was added to a markedly lower amount of food than larvekuldene in 2004. The high amount of food in 2004 resulted in better fitness, diet, condition or faster growth compared with measurements from 2005. In 2004, during the latter part of larval registered dirty larvae, which may be attributable algae sedimentation, especially in the latter part of the larval phase, where larvae are bottom seeker. The fact that differences in food enrichment is not reflected in the growth shows that it not worth adding microalgae at high concentrations.

Critical stages in the larval stage

It was possible to get most of the litters from 2004 and 2005 to survive between 11 and 13 days, and 2 out of 8 litters settled. Mortality was high the first day, but does not differ from observations of other knife clam species. There was razor clam species *Siliqua patula* observed a survival of 30% after 48 hour (Breese and Robinson 1981), while the survival of eggs and D-veliger to the burrowing, Chilean mussel, *Mesodesma donacium* were between 9 and 80% (Romero University Católica del Norte, pers.komm.)

Utting and Spencer (1991) indicates a survival between the embryonic stage and the D-veliger stage at 30 to 50% for several mussel species. The middle part of the larval stage was not critical. During this period, mortality was low, and larvae had a high activity level. Results indicate that, besides the high mortality within the first day, exists a bottleneck just before settling. There could in this period registered an increase mortality and increased occurrence of shells and death larvae in some cases consumed by ciliates. Between about settling can be observed coincidence between reduction in larval numbers and the reduction in larval activity.

Results from 2004 and 2005 shows that under current farming conditions the even for successful litter is expected that less than 1% of spawned eggs survive until settling. If taken strictly into account that 25% of the litters and 0.03% of the eggs survived, it is roughly estimated expected that 0.01% of all spawned eggs survive.

11.0 Settling and subsequent juvenile stage

Razor clams live buried. Methods and systems designed for growth of settle fry will therefore differ from what we know from example. oysters and mussels. Methods are being developed for razor clams are likely to be transferred to other species of burrowing bivalves. During the pilot project focused farming methods, where sand was used as substrate for juveniles. The purpose of experiments were:

- To determine if the fry can thrive in rearing facilities
- To assess whether the settling and juvenile stage is a bottleneck in fry production
- To assess whether juveniles can be grown to a size that is suitable for on-growing

## 11.1 Material and Methods

### 11.1.1 Settlings material

Focus was directed against the settling could take place under controlled conditions with sand as a substrate. The litter from the 21-05-04 was used 5 cm of sand with grain size <1 mm. The litter from the 11-05-05 was used 1 cm of sand with grain sizes <5 mm. For sand filtered to 1 mm fractions were 250 to 500 m and 500 - 1000 m and constituted. 56 % og 32 % of the sand burned weight. Fraction below 250 m accounted for only a few percent. The same situation prevailed in the sand sighted at 5 mm. There was grain exceeding 1 mm but 10% of the total weight.

The depth of sediment was increased as the fry grew, and there was also used sand to growth of fry after settlings fasen. Litter from the 31-05-04 died shortly before settling. For this litter was prepared settling substrate in the form of sand-sighted at 500 meters, weight dominated by grain size from 100 to 250 m. However, to test sediments with the grain size was is to the vessels with an added layer of 1 cm.

## 11.1.2 Monitoring

When larvae were 13 to 15 days old, showed their behavioral and morphological characteristics that they were ready to settle. The following was put sand in the vasculature by next water change. For more accurately record the time of settling were after sediment addition, sampling for monitoring in both aqueous and sediment. When no longer found larvae in the water was changed practice only to take sediment samples to monitor the density, survival and growth. The fry from samples were reestablished in the vasculature after registration.

For fry from 2005 was limited sampling of the project. For fry from 2004 were taken sediment samples between the beginning of June until late July, then in summer and autumn period was conducted monthly total count of all individuals. The total counts were fry sieved sediment. Length measurements were made with graph paper or digital calipers, depending on mussel size. To evaluate the importance of space conditions was fry after total count established by different densities. There was also regularly monitored the oxygen content, temperature and salinity.

In early December was also wet weight recorded and made dry study of 32 individuals. The meat was dried for 48 hours at 103 ° C and calcined at 520 ° C for 12 hours.

### 11.1.3 Hatchery Culture

Hatchery Culture covers the period between settling and until the fry have reached a size where it can be put into open water to on-growing. During this period stood fry in the hatchery for it had an age of six months. Then followed a akklimatiseringsfase where the fry were established by conditions similar to open on-growing. water, physiologically to adapt them to the next ongrowing.

### Hatcheries-phase

Until the fry were three months old, was the water in the tanks changed weekly, with seawater filtered to 10 m. The feeding was carried out twice daily by manual addition of algae. In 2004, food supply regulated based on water color, and there was an average addition of 120,000 cells/ml in the early part of the fry stage and 162,000 cells/ml in later phases. The added amount of algae in 2005 was steadily revised downwards on the basis of daily algal counts. There was added approx. 35,000 cells/ml in the first part and then 50,000 cells/ml. When the fry were 3 months in 2004 was keen continuous supply of 10 m filtered seawater and algae. From the fry was 5 months, it was added only filtered seawater through a sand filter. Inlet and outlet on the continual system was located at the top of each side of the vessel. Food and water flow was adjusted so that the water was slightly tinged with algae, so algal concentration was constantly maintained at an appropriately high level.

Replacing the entire body of water and removal of any sediment out algae were conducted as needed.

## Akklimatiseringsfase

The project was the fry from 2004 at the age of six months established in tanks of irrigation water directly from the fjord. That enabled fry under controlled against the possibility of physiologically adapt to the open sea temperature and salinity and the more diversified food spectrum, probably more than one diet based on a few species of cultured algae would respond to fry food needs and need for specific fatty acids.

Initiation of acclimatization was the water temperature in the fjord fallen so much that there was a temperature difference between water in the hatchery and the fjord at 14 degrees. The fry was over a week refrigerated with 1 to 2  $^{\circ}$  C/day During chilling was daily addition of cultured microalgae and made exchange of water with seawater directly from the fjord. Before the water was discharged to the tanks with fry had nevertheless been allowed to, during aeration, taking the same temperature as the water surrounding the fry. After cooling, the process was set to fry the actual acclimation. It was observed that the fry thrived best at a certain water turbulence, and it is therefore important to create a water flow over the mussels.

In a production situation is expected akklimatiseringsfasen that could last for some months. The exact duration will depend on the age of reproduction and growth and what systems will be developed for mussels of different sizes. There will also be able to delete the last part of the acclimatization place in the very same structures that mussels should be in the on-growing in open water.

# 11.2 Results

## 11.2.1 Morphological development of the fry

Larval morphology changed in the period around settlingstidspunktet through a process called metamorphosis. During the transition phase between the larval and fry stages developed foot and velum is converted into incoming and outgoing siphon (Figure 11.1 A and B). Husk becomes progressively more elongated as the Umbo and hinge moves farther and further down towards the front of the shell (Figure 11.1 B and C). In Figure 11.1A shows a 15 day-old individual who was found in the water phase and not yet metamorphosis. The larva has developed øjeplet at the base of the foot. Figure 11.2 shows photos of the characteristic hinge with a razor clam.







Figure 11.1 Morphological development in broods of American razor clam. Besides morphological characters is the location of the front (anterior) and rear (posterior) indicated. (A) A 15 days old individuals in the premetamorforiske stage. Have been developed øjeplet. Individuals from the water phase. (B) First individuals found in sediment in 2004. 19 days and 722 m. (C) A 23 days old individual. Among the first specimens found in 2005. These had an average size of  $1.3 \pm 0.1$  mm. (D) 47 days old individual. The average size of  $3.1 \pm 0.4$  mm. (E) 82 days old individual. Size 20 mm (F) 133 days old individuals. The average size of  $31.4 \pm 0.4$  mm.



Figure 11.2 The hinge of American razor clam.

### 11.2.2 Settlingstidspunkt

Based on the findings from this project goods larval stage in American razor clam between 16 and 19 days. For litter from 21-05-04 was the last swimming larvae observed day 16 (pediveliger) and the first settled individuals day 19th For the litter from the 11-05-05 were found floating larvae day 15 (premetamorfoseret) and settle fry day 23rd Larvae settled the two coarse sediment types, composed mainly of grain sizes between 250 and 1000 m.

### 11.2.3 Survival of fry stages

On the litter from 2004, the total number of live fry until day 60 calculated on background of sediment samples. Unfortunately fluctuated the resulting produced results as much that it was not statistically possible to conclude on the total number of juveniles in this period. From day 67 was the number based on counts of all mussels (Total counts). Figure 11.3 indicates the data from day 67 of the total number of mussels from spawning, 21 May 2004.



Figure 11.3 The number of surviving mussels as a function of age. Data for juveniles from the spawning 21 May 2004.

After settling, the survival of fry relatively high. Based on counts were survival 55% after the first year of life among the litter from 21-05-04. Survival through under 1 %. larval stage for settling was to compare below 1%.

Due to completion of project monitoring of the fry from 2005 has been limited. Estimates based on sediment samples showed a total count of 2,692 after 23 days. After a total of 2,346 live specimens back, corresponding to a survival rate of 87% from the first settled individuals were observed for an age at  $3\frac{1}{2}$  months.

For total counts were all individuals out of the sediment. Overall, the day after a count greater mortality than normal, especially in cases

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where the mussels were restored at high densities. It must be concluded that handling of the fry must be relatively minimal. In a production situation where work on a larger scale may be taken more and larger subsamples than in the project capable of achieving a better measure of the density and number of offspring.

11.2.4 Growth of fry stages

Size in relation to age at the fry in 2004 and 2005 are shown in Figure 11.4 and Table

11.1. Within a year grew fry from 2004 from 0.7 mm to 40 mm. The sparse amount of data from 2005 show that growth was lower this year, probably because of lower food additive and higher density.



Figure 11.4 Average length (mm) as a function of breeding age (days). • Hatchery from 2004 • Hatchery from 2005

Årgang	Alder (dage)	Længde ± S.E.
Yngel fra 2004	19	0,7
	38	$2,5 \pm 0,2$
	67	$10,5 \pm 0,2$
	96	$22,3 \pm 0,3$
	133	$31,8 \pm 0,5$
	189	$36,7 \pm 0,6$
	364	$40.0 \pm 0.6$
Yngel fra 2005	23	1,3±0,1
	106	$11.2 \pm 0.3$

Table 11.1 The average length (mm)  $\pm$  SE for broods from 2004 and 2005.

Table 11.2 indicates the growth rate of fry from 2004 for various periods through fry culture. In May to July, i.e. during the first month and a half after settling, fry grew on average 0.08 mm/day. The highest rate was observed between July and October. It appears that growth is slowing in the autumn and winter. Growth through the winter to May 2005 averaged 0.02 mm/day

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There was significant difference in brood size in late November and May (Oneway ANOVA, P = 0.000), reflecting that the fry had significant growth through winter and spring, and that it was primarily the small fish, who died in period. This was seen by dead specimens collected from over the winter and spring were significantly less than the length measurements made on the fry during the former total count made on 26-11-04 (Oneway, P = 0.000).

Based on registrations in 2005 was the growth rate for this year's spat between days 23 and 106 of 0.12 mm/day

Tidsperiode	Alder på yngel (dage)	Antal dage	Fase	Vækstrate (mm dag <sup>-1</sup> )	R²
Maj – juli	19 - 63	44	Klækkeri	0,08	0,54
Juli – oktober	67 - 133	66	Klækkeri	0,31	0,98
November - maj	189 - 364	175	Akklimatisering	0,02	1

Table 11.2 Growth rate (mm/day) for juveniles from 2004. Rate specified for various periods through fry stages

Table 11.3 indicates the various weight relationships for 6 months old fry. Correlations between dry weight and length were made to describe of offspring mode before the fry were established during akklimatiseringsfasen.

Ligning	$\mathbf{R}^2$
Vådvægt kød (g) = 0,038 × længde (mm) – 0,7513	0,9064
AFDW kød (g) = $0,003 \times 1$ angde (mm) - $0,0623$	0,8672
AFDW kød (g) = $0,3286 \times \text{skal vægt}$ (g) - $0,0052$ .	0,9003

Table 11.3 Correlation between weight and length for 6-month-old juveniles from 2004.

# 11.2.5 Density

The density was markedly higher in 2005 than in 2004. The density of 3 months old juveniles ranged in 2005 between 20,000 and 50,000 individuals/m<sup>2</sup>, while the density of juveniles of a similar age in 2004 ranged between 4,000 and 15,000 individuals/m<sup>2</sup>. For 6 months old fry was density between 800 and 5000 individuals/m<sup>2</sup> and for one-year juveniles were density between 140 and 4000 individuals /m<sup>2</sup>

Figure 11.5 indicates correlations between breeding length and density. Growth was densitetsafhængig so that when higher densities had lower growth (Oneway, P = 0.000 for all ages).



Figure 11.5 Boxplot of length (mm) as a function of the density (number/m<sup>2</sup>). Top: 3 months old juveniles. Middle: 6 month old juveniles. Bottom: 1-year brood.
#### 11.2.6 Physical parameters

During klækkerifasen temperature ranged between 20 and 23 ° C while the temperature during chilling before akklimatiseringsfasen was lowered to 6 ° C (Figure 11.6). Then followed temperature inlet temperature. The temperature was low, between 0.8 and 7.1 ° C until April, when temperatures began to rise. The salinity ranged between 28 and 31 ‰ throughout fry culture. Oxygen concentration was between 7.7 and 8.6 mg / l, corresponding to an oxygen saturation between 108 and 109



Figure 11.6 The temperature during acclimatization of fry from 2004. Water temperature followed the temperature of the ingested water from the Limfjord.

11.3 Discussion

When the fry first settle is the probability that it survives until on-growing good. During the project, which in 2004 produced approx. 250 settled mussels, while in 2005 was produced approx. 2.400 settled individuals. The project showed that it is possible to keep juveniles from U.S. razor clam in the tub with sand in indoor rearing facilities in over a year. These results are promising for a future production.

## 11.3.1 settling

Were tested at depths of 1 to 5 cm sediment of settling. Based on settling success there are no indications that one sediment depth is more favorable than the other. The depth of the sediment should be increased as the fry grows.

Ordinary beach sand, dominated by particle sizes of 250 to 500 meters, turned out to be a functioning settling substrate and can be used as a substrate in the whole period to on-growing. Particle sizes above 1000 m should for practical purposes sieved off as this complicates the separation of the youngest juvenile classes and sediment by sieving.

Sediment with particle sizes below 500 m was not ideal as settling substrate as it was time consuming and unwieldy for the daily management. It was easy suspended, and the water in the tank remained muddy for a prolonged period after handling. The high resuspension will not bid fry optimum conditions. The results also indicate that the addition of settling substrate after 13 to 15 days appropriate. The duration of the planktonic larval stage and settlingstidspunktet at the American razor clam were consistent with records for other mussel species.

Registering a øjeplet of larvae indicates that lysforskelle significance in connection with settling. Using this photosensitive area larvae can detect between water surface and bottom, allowing them to search from bottom to settle. In contrast to the results from Chinese studies an Asian knivmuslingeart, the project showed that beyond the normal ceiling lights were not necessary to sunlight for the larvae to induce settling. The difference in lysnedtrængningen between water surface and the bottom has probably been enough to fry were looking toward the bottom.

Estimates based on sampling in the water phase and sediment fluctuated part due to less activity and bottom seeking behavior in the last larval stages and of offspring heterogeneous distribution and the relatively low density in the sediment. Although neither results from 2004 or 2005 indicates an increased mortality in settling, it was not possible to show it statistically. U.S. scientists registered an increased rate of death just after settling at a brood size of 0.5 mm (Dale Leavitt, Roger Williams University, USA).

## 11.3.2 Hatchery Behaviour

During the first week after settling was fry very active and showed great defaecation. It is also observed that the fry can swim through the movements of the foot. This behavior may explain the secondary operation, which is observed in nature including in the Wadden Sea. In design of tanks for the fry, must therefore take into account that the fry can located in the water phase, such as by installing filters on the outlet to prevent fry Wash out with the effluent.

The active digging behavior may be an adaptation to the razor clams in the wild often living in the stream-filled areas where the risk of rearrangement of the sediment is high. Later in development was fry less active. Hatchery 1 mm dug in less than 1 minutes while it took longer for larger broods. Fry tolerate some handling and were able to burrow after having been up of sediment in the surveys. There was, however, registered a slightly higher mortality in the period after handling. To avoid increasing mortality due handling, it was decided not to take the mussels up too often to monitored on them.

There is therefore a maximum subsided census and survey once each months. The number of fry from the 2004 limited the ability to take statistically usable aliquots in connection with monitoring rather than low total counts.

## 11.3.3 Hatchery Culture

For fry from 2004, survival was 55% during the first year after settling. At a optimization of conditions and refining farming systems it is expected that even higher survival can be achieved. It was thus possible to cultivate fry razor clams, which is the correct size of a few centimeters, where it is expected to could be released into open water. Launching size and hence the release date will depend on which on-growing system chosen. The exact duration of fry culture will depend on the increment, which may be obtained from fry in fry culture. By optimizing the growth rate, will release into the open water could be made at an earlier stage. Fry in 2004 grew to 4 cm within the first year. If on-growing system usable for less brood sizes can fry exposed during the autumn of that year, as they are gydt and length of fry culture will be shorter. It is expected to be akklimatiseringsfasen take several months and fry during the latter part of this phase can be established in on-growingsystemerne.

Results from the pilot project indicates that there was similar survival during klækkerifasen and akklimatiseringsfasen. However, there is a tendency for survival and growth decreased during the autumn months from the end of klækkerifasen. There were recorded mortalities in the chilling, which was carried between hatchery and akklimatiseringsfasen.

## Food and survival

During klækkerifasen was the added consumption of the produced microalgae, Isochrysis, Chaetoceros and Tetraselmis. Mortality in the latter part of klækkerifasen can because they produced microalgae eventually constituted an inadequate dietary composition for the fry, and therefore got to lack important nærringsstoffer.

In akklimatiseringsfasen diet consisted of particles introduced into the seawater, including plant and animal plankton, bacteria and organic particles. The natural food in seawater probably has a better mix of nutrients than the cultured microalgae, and thus meets the greater nutritional needs of offspring and especially the need for essential fatty acids. Chilean studies at the University of Temuco, Chile showed the importance of adding farmed mussels others food sources other than algae.

Compared with klækkerifasen was the added amount of food the less akklimatiseringsfasen and low algal concentrations over the winter and spring probably led to food restriction. To increase food availability for each individual may in future add extra algae, increasing water flow or reduce yngeldensiteten. An increase of the flow may be preferable because it is observed that the fry grow best at high water flow. This was especially the smallest individuals who turned out to be vulnerable and die, probably due to less energy depots, which could be used during winter. This indicates the importance of that juveniles are in good condition.

# Growth

Earlier descriptions of the growth of razor clams are based on measurements between winter rings on the washed-up shells. Under this project the growth rate is based on length measurements of individuals of known age, why the exact rate can be determined. The size of the fry and the growth rate registered during the first month klækkerifasen was comparable with the growth registered by the razor clams both rearing and from nature (Beukema and Dekker 1995; Breese and Robinson 1981; Kenchington et al. 1998; Wong et al. 1986; Lepez, personal communication).

Growth declined in the autumn period in the late klækkerifasen and was low during akklimatiseringsfasen. Reduction of growth in late klækkerifasen can at due to micro-algae no longer constituted an adequate food source, or that yngeldensiteten led to food restriction, as the fry grew.

Growth during akklimatiseringsfasen is probably seasonal and may attributed to low temperatures and low food content through the winter. Growth is so also in the wild decreased in winter. Building a layer of fine organic material at the bottom of akklimatiseringskarrene may also have had a negative impact on growth by inhibiting filtrationsevne of offspring. Growth may therefore be reduced due to system-specific factors.

The growth rate through the winter and spring was 0.02 mm/day Higher growth rates are required to obtain a human size during an acceptable time frame. It will expected that the growth rate increased during spring and summer due to algal blooms and temperature rise as high growth rates reported in the literature for the second year of life (Beukema and Dekker 1995; Mühlenhardt-Siegel et al. 1983; Swennen et al. 1985).

# Density

Results from the pilot project show that the density influence of offspring growth. The spat, which stood at high densities grew significantly less than juveniles at lower densities. A combination of higher density and lower food additive can probably explain the lower growth in 2005 compared to growth in 2004.

The density of the pilot project were higher than recorded for even very successful litter in the countryside (Armonies 1996; Armonies and Reise 1999; Baron et al. 2004; Beukema and Dekker 1995).

In light of this it can be concluded that it would be more optimal and could under enhance the growth potential to raise the fry at lower density than that practiced in project. There can be usefully used densitetsjustering Having farmed, so the density in the tanks reduced as the fry grows.

## 12.0 On-growing in suspended systems

It is eventually a major goal to find systems that can support a stable production of razor clams in economically profitable systems that are adapted for razor clam biology and lifestyle. Ongrowing expected to be conducted in suspended systems in open water or in bottom culture in terrestrial plants.

Suspended systems assessed from existing knowledge as the most innovative and workable solution to on-growing of razor clams. Based on the growth rate of razor clams in their natural environment of the seabed will razor clams have reached a marketable size during the second or third year of life. From production of mussels is known that the high food availability in the water phase resulting in faster growth and greater meat content of suspended mussels than in benthic. Similarly, growth of razor clams in the water column probably cut production time significantly. Meanwhile, the mussels less susceptible to benthic predators. Compared with bottom culture will husbandry in suspended systems be an advantage in the harvesting of mussels.

In nature, living razor clams buried at the bottom that provides support and keeps the shell in a vertical position. f the razor clams are left lying in seawater, they can survive for several days. But sooner or later they will have trouble holding the shells closed and may then start to crack along the shrouds second. There must therefore suspended systems there is a material which supports the mussels and simultaneously is flexible, so mussels can grow and move into it. It would be tempting to use sand. In Chile has already done experiments with sand in the suspended systemer. However, it soon becomes so heavy that they are bulky and will require strong hardware. There is moreover, a considerable work involved in prepare and shifting sands before systems is ready for establishment of razor clams.

Therefore in this project, conducted experiments with alternative materials like growth medium. Breeding in the water column can help to ensure a uniform product not to the same extent as benthic mussels containing sand etc. purged before the mussels can be sold on the market.

#### Setup with juveniles

It is expected that razor clams from hatcheries are ready on-growing in Limfjord when they have a length in centimeters. In autumn 2004, juveniles with a length of 2.5 cm located in units with, respectively, soft, longhaired brush that was turned on head and silikonerør as a substrate. Silicone tubes, which were chosen with a diameter there were approx. 1 mm wider than the widest point on the mussels, did not properly support. Furthermore, the fry move out of the structures. The bristles were all dug clams down, and survived for several weeks and seemed to thrive. This idea was promising and should be attempted on adult subjects, but was left again because of problems accessing brushes in the right dimensions to adult razor clams. Furthermore, mussels placed in coarse bristles and soft mats. They did not give proper support, and the mussels were not able to dig themselves into them.

Testing the concept for adults

Instead, a small number of adults placed in two different systems, respectively furniture foam and round brushes. Based on indoor experiments in growing hall of Danish Seafood Center, it was shown that both systems were suitable for razor clams, as they dug in and had a normal behavior. However, some individuals tried to dig up in the beginning. These were put back into the structure. In both systems were set mussels of different sizes to see whether they could live with it and test whether materials were flexible enough to adapt to growing razor clams.

After two months of indoor tests, a small set-up designed to test systems in open water in the fjord. Modules with foam and brushes were hung from longlines in Shellfish Centre testing ground for Salling. Success rates were measured at behavior, survival and growth of the established mussels, but also on site configuration in terms of robustness to mechanical stress and manageability.

A total suspended three modules:

Two sets of foam:

a) Blocks of foam filter with two different cell sizes (PPI PPI 10C and 45C, courtesy of Brampton Plastics Industry Inc.) was cut, so they matched in height to two oyster hills facing each other. For each type of foam was three blocks prepared so that the surface area was  $25 \times 25$  cm when the blocks were put side by side. Each block was cut up, then to form cavities for razor clams.

b) Levels of filter foam of two different cell sizes (PPI PPI 10C and 45C, courtesy of Brampton Plastics Industry A / S) with length 1 meters, width 18 cm and a thickness of 1 cm was obtained. In each lane was attached elastic band sewn at each 3 cm. Between each solid sewing was space to establish a razor clam. Each runway was eventually rolled up and put on grabs.

For each type of foam were produced three tracks.

Setting with round bristles:

c) Cylinders presses with a diameter of 5 cm was attached offset on a wooden panel. A similar setup was made with bottles of cleaners with a diameter of 8 cm. Mussels stood vertically between the bristles.

Mussels became established in the substrates just before the establishment of longlines. During transportation on the boat stood ready mounted oyster trays immersed in a vat of water. After 20 days the system was taken down. Status was that the mussels in the foam blocks had tried to escape. Foam blocks were not put strongly enough.

Foam rollers worked fine however. Mussels was satisfactory in the structure, and mortality were minimal. From brushes were some mussels escaped while the rest was satisfactory. It is estimated that a poster with structural foam holds significant opportunities for breeding of razor clams. The system must be further adjusted and should run long-term tests.



Figure 12.1 Images from experiments on structures. Top left: Brushes with razor clams. Top right: Foam blocks, respectively large and small cells. The foam was cut to, so that the mussels could be vertical. In the middle left: Rolls of foam with clams. Mussels held in position by a elastic bands. In the center right: razor clams established in foam blocks, ready to be put into the fjord. Bottom left: The blocks were placed inside two oyster trays. In this way, there are nets above and below structure. Bottom right: Mussels in foam blocks after 20 days in the fjord.

From these experiences, and general considerations should be taken into account the following requirements in the development of a suspended ongrowingsystem to burrowing bivalves:

• The material should support razor mussels and keep them upright.

• Knife mussels should be able to move into the structures and filter according to their normal behavior. However, precautions should be taken so that the mussels do not escape system.

• Structures must be able to adapt to the mussel growth. Can be created systems with specific targets for specific size classes.

• It is desirable that the system must be able to hang on long lines to complement existing breeding sites for mussels.

- Structures must withstand mechanical and chemical interactions in the open water.
- Structures must be appropriate with respect to:

Establishment of juveniles in the system

Cleaning and cleaning påvækstorganismer

Harvesting of the finished product

Handling of boats, known from blåmuslingeopdræt

Harvesting for consumption

Suspended systems will be easy to handle in a crop situation, the mussels can land in the systems that further handling can take place. Furthermore, also exists the possibility that the mussels become knife systems are being cleaning and possible purge of mussels but then to let the system follow razor clams during transportation to distribution centers. In this way they avoided a major handling of the knife mussels until shortly before they reach the consumer.

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PART 3	

Appendix

14.0 Appendix A. Expected operating economy

The pilot project demonstrated that there can be produced fry in the hatchery, and the market value of razor clams are good. Razor clams can complement conventional breeding with a exclusive product. On-growing expected to happen in long lines, so farmers who already has a breeding facility with mussels can put one or more lines appear production of razor clams. Capital costs for plant breeding is not included in the calculations.

The first tests of the methods and structures for ongrowing of razor clams were initiated under the project.

Baltic Hills with foam showed promising results and anticipated operational economy of such a suspension system is estimated under this chapter. There are made calculations for a single production line. Calculation of ongrowing in suspended culture system:

• It is estimated that in a long line can be established 625 razor clam modules consisting of two oyster trays. The price of an oyster tray is about 74 kr, and the total cost of the hills, therefore approx. 92.500 kr. Trays have a lifetime of 10 years giving an annual cost of £ 9,250

• There is annually allocated  $\pounds$  20,000 for the purchase of foam material. When farming system developed, it could be mass produced, giving lower production costs and lower price.

• Recognised wage bill for the establishment of nurseries, rehabilitation of hills, monitoring of breeding farms and harvests. There is allocated 667 hours annually, which corresponds to 13 hours weekly for 52 weeks. The hourly wage is set at 150 DKK expected that the line with razor clams inspected under the supervision of the other breeding sites.

• The estimated retail price for fresh clam knife is 14.59 euro / kg, which corresponds to 109 kr / kg. This was the price of Ensis arcuatus in the Spanish market in 2004. Ensis arcuatus is very similar to the American razor clam with respect to size and meat quality. The same market should be expected for the two razor clam species. Price for razor clams is much higher in Asia. Prices are fished for clams and it is expected that farmed mussels can bring a higher price due to better quality and lower sand content. Prices are dependent on supply and demand. When breeding it is possible to time deliveries for the most favorable prices. The level is such påvirket influenced that the supplies from fisheries is not stable. More customers will likely demand for the product if stable supplies can be ensured eg by breeding. By City recognition of a profit to the wholesaler of 8% obtained a price of 100 kr / kg. There are approximately 53 consumption mussels on a kg fresh mussels, which gives a unit price of DKK 1.90

• It is expected that there may be 150 drinking mussels in each module. It is assumed that mortality from fry stocking until harvest is 20%. It is calculates that the fry can be produced to 0.05 million each. For estimation of the expenditure, the breeder has to acquire brood put a unit price of 0.10 million, the annual cost for a juvenile to a line then becomes a total of 1.2 x 150 human mussels  $\times 0.10 \times \text{kr} - 625$  modules = £ 11,250

Calculation of the hatchery

• An assumed a density of 160 dams/m<sup>2</sup>. It is expected that one person is able to fit a broodstock of 400 m<sup>2</sup>. Based on results from 2005, 20 adults to a number of juveniles settle at 2300th The project results showed a survival of 0.023% from egg to settling. It is expected that the survival by refining the procedures in the hatchery and subsequent acclimation of fry can be increased significantly. For a doubling of survival will 64,000 parentaledyr leads to a juvenile count of 1,472,000 pieces.

• Operating costs (algae production, power, water pumps) and salary costs for a fulltime employment is recognized.

# Ongrowing

	Number	Price	
Ongrowing	Antal	Pris	
Materialeudgift Lønudgift Køb af yngel Samlet udgift	112.500	29.250 100.000 11.250 140.500	
Salgspris for konsummuslinger	90.000	178.394	
Årligt overskud pr. line		37.894	

Klækkeri	Antal	Pris
Lønudgifter		264.375
Driftsomkostninger		500.000 764 375
Antal yngel produceret	14.720.000	101.010
Produktionsomkostninger pr yngel	-	0,05
Indtægt ved salg af yngel Overskud på yngelproduktion		1.472.000 707.625

Materialeudgift - Material Cost Lønudgift - Wage costs Køb af yngel - Purchase of fry Samlet udgift - Total expenditure Salgspris for konsummuslinger - Selling price for market mussels Årligt overskud pr. - Annual profit per. line

Klækkeri - Hatcheries Lønudgifter - Salaries Driftsomkostninger - Operating expenses Antal yngel produceret - Number of hatchery-produced Produktionsomkostninger pr yngel - Cost per fry Indtægt ved salg af yngel - Proceeds from sales of fry Overskud på yngelproduktion - Surplus on fry production 15.0 Appendix B. International Network

Under the project has established international contacts with researchers and institutions working with the cultivation of razor clams. International cooperation makes it possible a professional forum to resolve issues and share experiences. There has been workshops. study trips to Northern Ireland, Portugal and Chile participating in meetings or workshops.

15.1 Meeting held at the Centre for Marine Resources and Mariculture (C-March), Northern Ireland, 26 – 27. - 27 October 2004

The purpose of the visit was to hear about an ongoing, international research project on breeding of razor clams and make experiences and discuss opportunities for future collaboration.

C-Mars under Queens University and is the center for research in aquaculture Northern Ireland. The Centre was established in 1994 when the Northern Ireland fishing was threatened and aquaculture industry was underdeveloped compared to the rest of Ireland. During the visit meetings were held with research manager Niall McDonough and CEO David Roberts. During the meetings was the primary topic of discussion that in Limfjord planned breeding in the water column, while the Irish research team is planning bottom culture. Either in dedicated areas on tidal flats or the release of harvested areas to support the existing wild population. Breeding in water column offers numerous advantages in terms of better food conditions and thus probably a more lean meat, less need for cleaning, and easier harvest.

Due to the risk of overexploitation of wild razor clam stocks launched in March in C Spring 2000 tests on hatching of razor clams. Adult individuals of the species, *Ensis siliqua* were collected in spring, conditioned for some weeks and brought to spawn through temperature stress. Induction of spawning and fertilization success, but the produced fry did not survive the larval stage. Then, there was no work on razor clams C in March, before in 2004 in collaboration with Spanish and Portuguese institutions were granted research funding through the EU Interreg Programme.

The international project aims to develop methods for sustainable production and fisheries for razor clams. C-mars share of the international project is to transfer knowledge and methods from the Spanish institution CIMA, where in several years work on breeding techniques for E. arcuatus, E. siliqua and Solen marginatus.

In addition to academic discussion of our and their results, the possibility of a future cooperation discussed. Danish Seafood Center / University of Aarhus shown on every respect as an equal partner to the C-Mars, and both they and we thought very prepared for future cooperation. Following the meeting were launched a networking list of people who work with the farming of razor clams.

Fishing for razor clams in Ireland

Commercial mussel fishing knife is just a few years old in Great Britain and Northern Ireland although an increasing number of fishermen reschedules for razor clams from traditional fishing. Fished three different species: Ensis Ensis, E. arcuatus and E. siliqua. It primary market in Europe is made by the Spanish, but also the Asian market is a significant decreases. Use both manual and mechanical methods of fishing. Mechanically applied bundskrabere being dragged through the seabed to a depth of up to 30 cm. The first bundskrabere was commissioned in the late 1990s. Often scrapers fitted with nozzles that sends a stream of water into the bottom to soften sediment and release the mussels before they separated and collected via a set of teeth.

If handled properly, the amount of damaged razor clams limited. Despite this is the quality of knife mussels higher at hand fishing. Proper handling of razor clams is essential to ensure the consumer a decent product.

Knife Mussels bundled with rubber bands immediately after capture and kept moist then and cool. If the cold chain is kept intact, the mussels on arrival consumers keep refrigerated for several days.

Razor clams fished within quite small areas, which means that the same stock repeatedly exercised within a short time. They fished areas may to some extent recolonise via brood reduction and eventual migration of adults. Fears that the fishing makes one for hard use of the population has given rise to the establishment of the international project on sustainable production and fisheries for razor clams.

15.2 Meeting with Interreg group, Portugal on 3 - 4. February 2005

The international project "Sustainable Harvesting of Ensis (SHARE) Project 'is financed through the EU 6th rammeprogram, Interreg IIIB Atlantic Area. Projektet har The project has aims to generate knowledge that can ensure that fishing on razor clams made sustainable. Included are a production of fry for stocking, rearing of razor clams in various environments, studies of genetic variation between within species, and studies of the impact of cockle fishing knife on razor clams and other benthic fauna. The project runs from 2004 to 2007 and has five participating institutions:

Irland Irish Sea Fisheries Board (BIM), Ireland

Centre for Marine Resources and Mariculture (C-March), Ireland

Spanien Centro de Cultivos Marinos (CIMA), Spain

Spanien University of La Coruna, Spain

Portugal National Marine and Fisheries Research Institute (IPIMAR), Portugal

Meeting Participants

Rita Pantarra and Miguel Gaspar (IPIMAR, hosted the meeting)

Adele Cromie, David Roberts and Niall McDonough (C-March, coordinator for SHARE)

Dorotea Martinez Patino and Fiz Da Costa (CIMA)

Andres Lage, Josefina Felpeto, Ana Tizon and Merdedes Moreno (La Coruna)

The meeting began with an interesting tour of a center for fisheries surveys, which primarily worked with the farming of snails and oysters. Thereafter there was a tour of IPIMAR's center for fish culture.

The purpose of the meeting between the participants in the SHARE project was to update the other on the surveys that were made since the last meeting in June 2004. The meeting gave participants to discuss performance, future studies and get good advice for solving different problems. Seafood Center / Aarhus University was invited to this meeting house as a result of the visit to Northern Ireland October 2004. Based on each institution's research, various aspects of razor clam farming discussed. Especially research in suspended systems in Danish Seafood Center aroused great interest among the other participants.

Missions under the SHARE project is distributed, then C-Mars and CIMA works with farming methods. There were at CIMA studies on gonad stage in E. siliqua and E. arcuatus. Temperature on the conditioning of adult and through changing food conditions and the addition of gonadevæv were studied.

Different systems were used for different sizes of settlede mussels in all cases with sand as a substrate. On-growingforsøg were made by creating juveniles in 'cages' on the foreshores surfaces where the fry can survive for at least a year.

IPIMAR examining effects of cockle fishing knife. The proportion of damaged razor clams and fishing influence on other benthic fauna was studied via samplings from research trawls and commercial features of Irish and Portuguese waters. There are studies were performed at different water depths. Results showed a high by-catch mortality. Moreover IPIMAR had compiled a database of literary references razor clams.

BOS stood for studies on the processing and transport of razor clams.

University of La Coruna carrying out genetic studies in several razor clam species. Was conducted studies on the genetic variation (Mitochondrial DNA and microsatellites) in geographically diverse populations of the same knivmuslingeart and various species in order to develop reliable methods for speciation.

Since the University of La Coruna were very interested in involving the U.S. razor clam from Danish waters, were to follow up the meeting gathered American razor clam by Sundsøre and Sillerslev and in Juvre Deep in the Wadden Sea. From each site were collected 50 individuals. The foot was dissected out, preserved in ethanol and sent to La Coruna. The results are in this report was finalized yet received but will be available.

Results from genetic studies can be used to

a) Evidence of variability between populations from different locations in Limfjord, and whether American razor clam from different areas in Limfjord can be regarded as being from one population.

Knowledge of any differences between geographically distinct populations of razor clams can serve as a basis for site selection for the collection of clams and the release of juveniles.

c) Analysis of populations from Limfjorden in comparison with data from Wadden Sea contribute with information about the history of American broadcasting razor clam.

15.3 Workshop, Chile, 19 – 20. April 2005

International workshop: "The status of breeding burrowing clams"

In Chile financed research in aquaculture, mainly through the National Research FONDEF. At the University of Concepción, in the years studied rearing knivmuslingen, Ensis Macha. The international workshop was held as introduction to a new project on the optimization of rearing methods of razor clams.

The workshop was divided into four sessions:

I. Hatchery Production

II. On-growing

**III.** Feed Optimization

**IV.** Joint Actions

Participants came from institutions

Spanien Centro de Investigación Marinas (CIMA), Spain

The marine biology department at the University Catolica del Norte, Chile

Chile Laboratorio de Nutricion y Calidad de Agua at University Catolica of Temuco, Chile

Chile Centro de Ciencia y Tecnologia, University Arturo Prat, Chile

The marine biological and oceanographic station at the University of Concepción, Chile

Danmark Danish Seafood Center / University of Aarhus, Denmark

The yield of the workshop

The workshop gave an insight into the research conducted at the farm of burrowing mussels in Chile and gave the opportunity to share with others in the promising results obtained in the DSC. Again aroused the experiments with the cultivation of razor clams in suspension systems with alternative substrates great interest. In particular, a meeting with Irene Lepéz and Eduardo Tarifeño where problems of pelagic on-growing system discussed in more detail, very rewarding. Possibilities for future collaboration with the University of Concepción seems very promising.

The visit has also created a good network that can operate across continents and language barriers between English-and Spanish-speaking research community.

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Razor Clams in Chile

Ensis macha and Tagelus dombeii live along large parts of the Chilean coast and, together with another burrowing clam, Mesodesma donacium an important resource in the Chilean seafood industry. Fishing performed manually from small rowing boats, owned by a fisherman who has 2 to 3 divers hired. The mussels dredged from the bottom with small tretandede rakes. Divers equipped with air from the boat and work up to 20 meters depth in three to five hours daily. Boat owner gets 2/3 of the proceeds of fishing; the remainder shared between divers.

Razor clams sold typically at local markets along with mussels, snails, barnacles, sea squirt, seaweed and various fish. In retail distribution, 25 razor clams purchased for approx. 10 kroner. There is no real control over the amount of fishing razor clams, and researchers were therefore worried that mussel populations could be declining.

Because of this, but also to create a new product that works with various methods for rearing razor clams.



Figure 15.1 Ensis macha, Tagelus dombeii and Mesodesma donacium.

## I. Hatchery Production

For production of fry of Ensis macha, the following procedures. Collected mature mussels brought to spawning after two weeks of acclimation. After spawning conditioned animals by temperature stimulation over three months through gonadtissue rebuilt and mature. Fry survival was generally highest, if females were matured in nature rather than by conditioning. Mother animals have established in large barrels with a layer of sand around. 20 cm. Recirculated seawater with added algae supplied via an irrigation (Figure 15.2). Once daily change of water flow from downward to opaggående through the sand to prevent anoxic conditions.Induction of spawning is done by immersing 30 razor clams, located in the hills outside sediment in a tank of 2,000 liters. Induced by temperature stress or changes in food supply.

Larvae of Ensis macha is in the water phase in 15 to 20 days before metamorphosis. The added amount of food increased as the larvae grow. Trays with a 1 mm sand with a particle size approx. 125micron acts as settling system. The hills fed UV-treated, 1micron filtered, recirculated seawater. During settlingsfasen reduced food concentration. Settled mussels are in the hills with a thin layer of sand and added to filtered seawater, which in the initial stage of UV-treated and filtered at first An A

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high mortality of nysettlede and a large variation in the growth of post larvae were mentioned which aspects to focus further on.



Figure 15.2 Systems respectively parent population and nysettlede Ensis macha.

Besides Ensis macha, there were lectures on the Chilean working with farming razor clam species, Tagelus dombeii and the burrowing clam, Mesodesma donacium, Spanish work farming Solen marginatus, Ensis siliqua and Ensis arcuatus and worked with Ensis americanus in the DSC.

Session II. On-growing

Ongrowingforsøg in the water phase with hanging systems with sand as substrate is made with Ensis macha. The initial experiments were made with perforated cola bottles with a 2 cm sand layer (Figure 15.3). Broods of 1 cm was established in bottles and hung on longlines. During subsequent trials have been developed larger containers after same principle. The weight of the sand is a big problem. The containers are stacked in pairs and wrapped in nets. Nine stacks combined into one unit. Including sand weighs approximately one unit. 700 kg. Sand layer is magnified as the mussel growth and density is reduced to make mussels more space. The results indicate that juveniles of 1 cm within one year 6 cm under the conditions in the waters just north of Concepcion.

Besides suspension systems have also subsided trials on-growing in mind country, and bottom cultures. Tank Culture known as manpower intensive and requires a high algal production. Disadvantages of bottom culture is that there are only a limited number suitable sites, and the need for divers to monitor. Here likelihood that the mussels escapes weighed against the net size of a grid and risk of fouling.

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Figure 15.3 On-growing system

For Tagelus dombeii have also worked with on-growing in suspended systems and bottom culture. The suspended systems are used PVC pipe with a diameter of 10 cm. On longlines were made densitetsforsøg combined with tests of various substrates. Results showed no significant difference in growth or mortality between treatments, although survival was slightly higher at the lowest densities and medium grain sizes (250 – 500micron).

Session III. Feed Optimization

Knowledge of the mussel's natural food choices can contribute to determining the food cultivated mussels to be offered. Studies of stomach contents from Ensis macha showed that the diet consists of micro-algae, other unicellular organisms and body parts from crustaceans. The results show a potential for using alternative diets such as preserved algae, bacteria, artificial diets or dissolved nutrients.

Was conducted studies of the effect of diets with varying composition of proteins, carbohydrates and fat on the quality of eggs and larvae as well as on growth and survival in post-larvae. Moreover, studies were performed on the seasonal variation in contents of lipids and proteins in genital tissue.

## Session IV. Joint Actions

Optimization of system designs, and development of biological knowledge of burrowing mussels are heading for areas that can benefit from cooperative. Creating an information network on the Internet for researchers working with breeding of burrowing clams, was put forward.

Survival of juveniles is an important prerequisite for stable production. There should therefore made to clarify the larval nutritional needs and sources, and clarify which physical conditions, resulting in a high and stable survival. Efforts should be made to develop systems specifically tailored to particular life stages.

Probably, the same design used for various burrowing clams. Design systems should be in interaction with more detailed study of what is naturally affect growth and survival of burrowing clams. Systems have been tested in small scale does not necessarily work on a large scale.

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Therefore it is important to put resources for large scale experiments to simulate a real farming situation.

For ongrowing system, it is problematic that sand is easy handling heavy. There can therefore be usefully explored for alternative materials.

The market exists and has high demands for supplies, but requires a yet to be achieved efficient production of fry. Bottom Culture is a relatively inexpensive form of culture, since it includes a minimum of materials, but it requires a large area and can occur problems with predation and during harvesting.

The need for knowledge about nutritional needs are still great. The ability to exploit alternatives to traditionally produced microalgae should be investigated. There can be usefully developed micro-algae that produce certain fats to a greater degree than normal strains. In this way they can muslingelarvers requirements for certain food conditions met.

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16.0 Appendix C. Media coverage

Printed articles concerning the rearing of American razor clam

Date	Media	Title
2003	Børsen Exchange	Bright future for farming in the Limfjord
08/06-2003	BT	Sea jewels on his way to Danish stomachs
31/07-2003	Fishing Journal	Danish waters with exotic content
21/05-2004	Morso Folkeblad	Full house at the Danish Shellfish Center
22/05-2004	Morso Folkeblad	Limfjord - a strong brand

29/05-2004 Limfjord	Thisted Dagblad	Knowledge Center breeding ground for shellfish from the
09/06-2004	Nordjyske Times	Razor clams is the next right on the menu
11/06-2004	Morso Folkeblad	Seafood Center invites you inside - and outside
15/06-2004	Morso Folkeblad	Limfjord gold shines with renewed force
01/07-2004	Fishing Journal	Mussels and oysters with several legs to stand on
10/08-2004	Morso Folkeblad	Dead and dying mussels knife sticking out of the bottom
11/09-2004	Morso Folkeblad	Experiments with razor clams are good news
16/04-2005	Morso Folkeblad	Promising experiments with cultured razor clams
2005 American raz	ByrumLabflex Catalo	Radio broadcasts dealing with the rearing of

Channel		Date	Title b	roadcast	
DR, Central / V	West	06/09-2004	Promising experiments with razor clams		
DR, Central / V	West	08/09-2004	Razor clams on the way in regional waters		
DR P4 Midt /	Vest	05/04- 2005	Razor clam farming in Danish Seafood Center		
Radio Limfjord 05/04- 2005 Razor clam farming in Danish Seafood Center			ter		
TV shows dealing with the rearing of American razor clam					
Regional channel Date / Time Title Appoint consummation					
TV2 Midt-Vest06-09 2004 Middags- & Dinner & Evening BroadcastBreeding ofrazor clams in the LimfjordFrazor Clams in the LimfjordBreeding of					
Lectures dealing with the rearing of American razor clam					
Date	Works	shop & Meeting	5	Subject	
04/02-2005	Portug	ortugal Meeting, Portugal Breed of Ensis americanus - Preliminary results		nary results	

19/04-2005 Chile Workshop, Chile Hatchery Production Ensis americanus

20/04-2005 Chile Workshop, Chile On-growing of Ensis americanus