Histological and parasitological studies of the blue mussel *Mytilus edulis* L.

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Dissertation

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Avhandling för filosofie doktorsexamen i Marin Zoologi vid Göteborgs Universitet (examinator: Prof. Rutger Rosenberg) som enligt beslut av naturvetenskapliga fakulteten kommer att försvaras offentligt torsdagen den 17 april 2003 kl. 10.00 i föreläsningssalen vid Tjärnö marinbiologiska laboratorium, 452 96 Strömstad.

Fakultetsopponent: Professor Andrey Granovitch, Dept. of Invertebrate Zoology of St. Petersburg State University, St. Petersburg, Russia.

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Abstract: In environmental monitoring programs chemical analysis of marine organisms, water and sediment indicate the concentrations of identified pollutants. However, chemical concentrations do just indirectly reveal health of various organisms. A more direct assessment of organism health is to use histopathology to unveil sublethal effects of contamination on particular organisms. Histopathological studies of the blue mussel, *Mytilus edulis*, may be used to assess biological effects of human contamination of coastal waters. However, to fully reach this goal, better knowledge of the mechanisms that could affect the tissues of mussels must be gained. Moreover, histological studies add complementary information to ecological studies of mussel populations.

Histological changes of mussel tissue occur owing both to internal processes, such as the reproductive cycle, and to external factors, such as, anthropogenic contamination, salinity and parasites. In this thesis I show that interactions between several factors (external and internal) affect the variation in tissue structure during the life of mussels. A focus is also to test whether it is possible to relate the level of the immune defence (i.e. the production of hemocytes) or the prevalence of parasites to variation in external biotic and abiotic factors.

I found that, when using histological methods to assess effects of contaminants, variation in several natural factors may interact with effects of anthropogenic factors, which limits the usefulness of histological changes as a direct indicator of environmental pollution. However, my result indicates that so called granulocytoma (clusters of granular hemocytes) found in mussels from impacted sites, could be a possible indicator of industrial impact.

The larval stage of a bird parasite, the trematode *Renicola roscovita*, has a complicated life cycle and the first intermediate host is a snail. Larvae are released from snails and inhalated into mussels where they encapsulate in the tissue. I found labial palps of mussels to be filled with the metacercariae of this parasite and such a heavy parasite load is likely to interfere with food uptake. The degree of parasite infection decreases rapidly, however, with distance to rocky shore populations of the snail (*Littorina* spp.), and mussel populations (e.g. rope cultured mussels at >50 m from rocky shores) are not likely to be affected seriously by this parasite.

The flesh weight of the mussels varies with the phase of the reproductive cycle. Therefore, it is important for mussel farmers to know the timing of this cycle. When food is available, glycogen is synthezised in special storage cells in the mussel mantle. The glycogen is later used in the build up of gametes (the gametogenesis). I found a strong correlation between glycogen content and different stages in the reproductive cycle of mussels from Swedish populations of *Mytilus edulis* and from a Spanish population of *Mytilus galloprovincialis*. Thus, the glycogen content of mussels might be used as an indicator of the reproductive cycle and indicate optimal time of harvest. Moreover, the glycogen content also indicates spatial and temporal variation in food availability. To study this relationship in more detail requires probing of individual mussels repeatedly over time. I explored different techniques for tissue sampling without killing the mussels and found some that were possible to use for this purpose. These techniques might also be valuable for sampling endangered species of mussels.

Keywords: *anthropogenic, aquaculture, blue mussel, cercaria, contamination, disease, drilling, environment, glycogen, granulocytoma, health condition, hemocyte, histopathology, life cycle*, Littorina *spp., metacercaria, monitoring,* M. edulis, M. galloprovincialis, *parasite,* Renicola roscovita, *reproduction, salinity, season, sporocyst, tissue, toxin, trematode.*

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Till Kit

Började falla som ett löv.
Ångrade sig halvvägs ned,
förvandlades till en gul fjäril
och flög sin kos.

(Eeva Kilpi)

List of papers

This thesis is based on the following papers, referred to by their Roman numbers:

I Svärdh, L. (2003). Tissue sampling from live blue mussels, *Mytilus edulis*. A field study from the Swedish west coast. J. Sea Res., 49:3, 1 – 5. In press.

II Svärdh, L. and Johannesson, K. (2001). Incidence of hemocytes and parasites in coastal populations of blue mussels (*Mytilus edulis*) – - testing correlations with area, season and distance to industrial impacts. J. Invertebr. Pathol., 80, 22 - 28.

III Svärdh, L. (1999). Bacteria, granulocytomas and trematode metacercariae in the digestive gland of *Mytilus edulis*: Seasonal and interpopulation variation. J. Invertebr. Pathol., 74, 275 – 280.

IV Svärdh, L. (submitted manuscript). Is the glycogen content of blue mussels (*Mytilus* spp.) a good indicator of mussel reproductive activities?

V Svärdh, L. and Thulin, J. (1985). The parasite fauna of natural and farmed *Mytilus edulis* from the west coast of Sweden, with special reference to *Renicola roscovita*. Medd. Havsfiskelab. Lysekil, nr 312.

VI Svärdh, L. (submitted manuscript). Prevalence of trematode larvae in wild and cultured blue mussels, *Mytilus edulis* L..

A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarizes the accompanying papers. These have already been published or are manuscripts at various stages (in press, submitted, or in manuscript).

Contents

INTRODUCTION

Histological studies aim to describe and understand the structure of living cells, tissues and organs. When foreign particles affect the structure, a change occurs in the tissues that could affect the health condition of the living organism. Therefore, histological studies are tools to control the physiological condition of an organism at sublethal levels of environmental stress. However, it is not always easy to define the ultimate cause of an observed histological change and, moreover, several extrinsic factors may interact to produce such changes. It has been suggested that the histological status of, for example, mussels can be used to assess contamination of coastal waters (Yevich et al. 1986). In such a case it is important to know how variation in tissue characters relates to natural factors (e.g. period of the life cycle, salinity, temperature and other abiotic factors), and how the variation interacts with factors such as toxins, diseases and parasites.

Along the Swedish west coast*Mytilus edulis* is one of the most dominant species in shallow, hard-bottom communities, particularly where there is a strong water movement. The ecology of this species is thoroughly investigated (see e.g. Loo 1991, Riisgard and Randlöv 1981) but histological studies of Nordic *M. edulis* populations are rare. Mussels filter particles from the surrounding waters, and owing to the exposure to large volumes of particles and water they accumulate toxins in the tissues. For instance, after exposure to silver (50 $\mu g\ l^{-1}$) for 12 weeks, the concentration of this heavy metal in the soft tissue of the mussel *M. edulis* was measured to 9.95 μg /g wet weight compared to 0.03 μg /g wet weight in the control mussels (George and Pirie 1986). Environmental monitoring programs around the world use *M. edulis* as an indicator of various chemical contaminants showing spatial and temporal variation in coastal contamination (Jones et al. 2001). In many countries, marine mussels are cultured in large quantities and histopathological research has here a high priority with many studies originating from these countries (e.g. Mortensen 1993, Fuentes et al. 1998). The main focus of these studies has been parasite infections, since heavy infections interfere with culturing activities causing tissue damage. For instance, Villalba and his colleagues (1997) reported host castration and loss of storage tissue in *M. galloprovincialis* as an effect of sporocyst infection by the trematode *Proctoeces maculatus*. Not only parasites could infect mussels; virus and bacterial infections are also reported (e.g. Jones et al. 1996, Hernroth 2002).

The overall aim of my thesis has been to assess variation in histological characters owing to natural and anthropogenic factors with particular focus on Scandinavian coastal waters, and to evaluate if histological screening can be used to assess water quality. Another focus has been to use histology as a tool for finding indicators of commersial importance, such as the optimal time for harvest.

TAXONOMY OF *MYTILUS*

The taxonomy of *Mytilus* spp. is complex and there is at present no consensus about the taxonomic status of the three taxa *Mytilus edulis, M. trossulus* and *M. galloprovincialis.* Most authors consider *M. edulis* to be distributed along the North Atlantic coast of Europe and N. America, while *M. trossulus* is present in brackish water habitats like the Baltic. *M. galloprovincialis* has subtropic distribution and is in Europe present along the French and Spanish Atlantic coasts and further into the Mediterranean. This species is also found in New Zealand, Australia and South America. These taxa are treated as semispecies by Väinölä and Hvilsom (1991) in a study of genetic variation (allozyme) and they also describe a hybrid zone from the Kattegat through the Öresund between *M. edulis* and *M. trossulus*. However, a study of blue mussels from the north-eastern Baltic Sea uses *M. edulis* through out (Westerbom et al. 2002). In contrast, Koehn (1991) studying allozyme differentiation, consider all three as distinct species and so do Hummel et al. (2001). In my study I follow Koehn (1991) and treat them as species.

EXTERIOR DESCRIPTION OF *MYTILUS EDULIS*

The mussel body is enclosed between two shell halves, and connected with the inner surface of the shell by a weak attachment of the epithelium of the mantle and a more tenacious adherence by muscles. The mussel body is almost completely enveloped by the two mantle lobes (Fig. 1), and if one of the shell halves is taken away, one of these lobes is visible. In the mantle tissue the gonads are developed, and when the gonads are ripe, the mantle becomes pale and whitish in males and more orange in females. The paired gills extend along the body under the mantle lobe and are in close contact with the labial palps, two at each side of the mouth (Fig. 2). Water currents are inhaled through the shell openings, pass through the gills and enclosed particles get caught by the cilia on the gills. The particles are then transported to the labial palps and further into the mouth, the stomach and the digestive gland (Fig. 1). Some particles are rejected at the surface of the labial palps, bound to mucus and transported out as pseudofaeces through the shell opening. The selection depends on the size of the particle, but also if the mussel wants to change the amount of ingested particles.

Blue mussels are capable to move by using the foot and thebyssus threads which are secreted by a gland in the foot (Fig. 2). The threads attach to a hard surface, and the mussels then pull themselves from one position to another.

CULTURING *MYTILUS* SPP.

Mytilus spp. are excellent to culture for several reasons. A natural abundance and settlement of larvae on hard substrates along shorelines have made mussel farming to an industry of great importance in many countries. The methods used are of two main categories; on-bottom cultivation and off-bottom cultivation. In Sweden an off-bottom method, a long-line system, is used. The mussels are grown on textile bands hanging vertically from horisontal headlines and can utilize a great proportion of the water column. The mussel industry in Sweden has potentialities, since the conditions for a fast growth and good quality of the mussels are very positive. The cultures in Sweden have been spared diseases so far, but knowledge about mussel diseases and parasite infections is necessary to prevent attacks in the future. There have been problems, however, with toxic algae, and despite possibilities to move whole farms from one fiord to another, in order to avoid occasional toxic algaes, the results have been of varying success.

METHODS

There are many tools available in studies of cells and tissues of mussels. Various staining techniques can be used to support the distinguishing between different tissues, and with image analyzers, lesions and cells can be quantified by size, volume and area. In a study of effects of PAH and PCB on *Mytilus galloprovincialis* from Spain, histochemical analyses were used to assess enzyme activity (Porte et al. 2001). Immunohistochemistry extends the basic histological techniques to also identify and locate specific amino acids and proteins active within tissue sections. For instance, this method was used to characterize peroxisomes in the digestive gland of *M. galloprovincialis* from Biscay Bay north of Spain (Cancio et al. 2000). In my thesis I used common histological technique: embedding in paraffin, staining with hematoxylin/eosin and using light-microscope to assess changes and pathological conditions.

Live mussels were sampled from wild or cultured populations. When sampled far from the laboratory, mussels were kept alive in a cool-bag and then transferred to an aquarium as soon as possible. The mussels were prepared for histological examination by opening the shells by cutting through the adductor muscle. The mussels were immediately killed by a cut through the pericardium. Transverse sections were placed in a fixative for 24 hours, washed in changes of 70 % alcohol, dehydrated in tetrahydrofuran (THF) and finally embedded in solid paraffin. The paraffin blocks were cut into 7 □m sections using a rotary microtome and a randomly chosen sequence of 4 – 5 cuts was transferred to microscope slides and stained in hematoxylin/eosin.

Sometimes it is desirable to avoid killing the mussels as a consequence of sampling, for example, sampling of rare or endangered species, or resampling of the same individual. Biopsy has successfully been used to take cell suspension from live marine mussels (e.g.

Santarem et al. 1994), but one difficulty is to get sufficiently large pieces of tissue for further analysis. In order to find the optimal conditions for a repeated tissue sampling from live mussels, I compared the effects on growth and survival using different sampling methods (Paper I). In one part of the mussels I took samples through a drilled hole in the shells, in another part I sampled through the shell openings. The instruments used were surgery forceps and injection needles. A part of the drilled mussels had the holes sealed again with cement.

More than 12 % of the mussels died during the two months experiment, most of them from the treatment "drilled and sealed with cement". Growth was also significantly lower in the mussels undergoing this treatment. The results suggest that there is no obvious way to sample live mussels. The aim and the duration of the study should decide what method to use for tissue sampling. For long-time experiments and repeated sampling, opening the mussels by prizing apart the valves is better than drilling the shells. For a short experiment, drilling the shells, leaving the holes open and using surgical forceps, seems to be an acceptable compromise between treatment mortality and sampling efficiency.

INTERNAL DEFENCE OF *MYTILUS EDULIS*

Filtering great volumes of water, the mussels are exposed to challenges of different kinds of pathogens and toxins. However, they have a very effective internal defence, in which several organelles take part. Enzymes are important, and lysosomal enzymes for instance, are able to hydrolyze components of bacterial walls (Cheng 1983). Peroxisomes contain enzymes that could break down or degrade substances and they are known to proliferate in the presence of xenobiotics (foreign compounds). Krishnakumar et al. (1995) found a proliferation increase of peroxisomes in mussels exposed to PAH, which make these organelles very useful as biomarkers. Of great importance at cell level are the hemocytes which are divided into two main groups, called granulocytes and agranulocytes, depending on the presence or the absence of cellular granularity. In the granules, peptides are stored and it seems that these peptides are involved in the internal defence (Roch 1999). In some cases the hemocytes have shown a chemotactic capacity toward pathogens (Schneeweiss and Renwrantz 1993) and according to Bayne (1976), the defence starts with a migration of hemocytes towards the damaged or infected parts, followed by phagocytosis and often digestion of foreign particles. Hemocytes containing engulfed particles can migrate to the digestive gland or other epithelial linings and be discharged to the exterior. Too large particles, e.g. parasites, can be encapsulated by hemocytes forming so called granulocytomas, or be surrounded by a layer of collagen.

HEMOCYTE INTENSITY

Except for taking part in the internal defence, the hemocytes are also involved in digestion, transportation and wound repair. Hemocyte intensity is often positively correlated with various anthropogenic and natural factors of contamination, e.g. industrial wastes (Wedderburn et al. 2000), and season (Santarem et al. 1994), but how hemocyte intensity is affected by variation in more than one factor at the same time has not been studied before. The study presented in Paper II was designed to analyze the relationship between hemocyte intensity and several environmental factors simultaneously. The factors were both natural and anthropogenic, and the blue mussel populations studied were from the eastern coasts of the Skagerrak/Kattegat. The mussels were sampled during two occasions, in two areas and in each area at four localities, two close to and two far from industrial impacts. The hemocyte intensity varied greatly among samples, 7.0 – 33.9 % and the results revealed that some factors interacted significantly with each other. In the northern area, the differences between the sampling seasons were large while there were small or no differences in the southern area. Also in the northern area, distance to industrial impacts seemed to affect hemocyte intensity, while in the southern area distance seemed to be unimportant. Surprisingly, in the northern area the mussels sampled far from industrial impacts, had higher values of hemocyte intensity than those close to impacts. On average, the mussels from the northern area had more hemocytes. An explanation of this unexpected pattern of variation might be that the mussels from the northern area suffered from increased physiological stress by being more infected by parasites, and/or more exhausted by reproduction. Suresh and Mohandas (1990) report that mobilization of hemocytes towards the gonads occurs to remove remains of the gonads after spawning. In a study of populations of *Mytilus galloprovincialis* from Spain, it was suggested that new hemocytes are generated in association with parasite infection (Carballal et al. 1998). Our results revealed a positive correlation between parasite and hemocyte intensities of populations, which support such a relationship. On the other hand, we found no relationship between gonad developmental stage and hemocyte intensity.

These results question the usefulness of hemocyte intensities as indicators of human impact. This is true, at least in the study area where the level of anthropogenic impact is perhaps too low in relation to the variation in hemocyte intensity caused by variation in natural factors, and also that several other factors interacted with impact levels and obscured any patterns present.

GRANULOCYTOMAS

Granulocytomas are clusters of granular hemocytes, sometimes surrounded by a layer of collagen. The origin is unknown, but it has been suggested that granulocytomas are produced when mussels are exposed to contaminants during long time periods (Neff et al. 1987). In two of the studies included in this thesis, granulocytomas were observed, and were found in mussels from populations near industrial impacts. In the mussels from coastal populations along the eastern Skagerrak/Kattegat (Paper II), only a few granulocytomas were found, all in mussels sampled near industrial impacts connected to oil trading. In Denmark (Paper III) there were more granulocytomas in mussels from a site near industrial impacts (e.g. an oil refinery) than in mussels from sites less close to impact areas. This supports the suggestion that granulocytomas are caused by contamination from oil spill (Neff et al. 1987). When Rasmussen (1986) used electron microscopy to study un-encapsulated granulocytomas in mussels from a polluted area in Lilla Bält, Denmark, he discovered picorna-like virus particles inside the granulocytes composing the granulocytoma. Rasmussen suggested that viruses were phagocytosed by granulocytes, when first entering the hemolymph. Within the granulocytes, the virus multiplies and the granulocytes become immobilized and aggregate, forming granulocytomas. In another study, Rasmussen et al. (1985) suggested that granulocytomas induced from toxins are surrounded by a layer of collagen to prevent spreading of toxic material to adjacent tissues. This is partly in accordance with an observation from Villalba et al. (1997) who found parasite remains inside granulocytomas. Producing granulocytomas could then simply be a defence strategy against foreign particles and produced whenever the contamination by any kind of particles is high enough. Anyway, referring to the results from paper II, granulocytomas seem possible indicators of anthropogenic impacts also at low levels of impact such as the ones along the Skagerrak coast of Sweden.

MICROBIAL INFECTIONS

In my study of Danish mussels (Paper III) I found some inclusion bodies of *Chlamydia*-like organisms in the tubuli walls. They seemed, however, not to have harmed the tissue of the host. Heavy infections could possibly be of more harm, since the bacteria replace the tubule cells and in this way disturb the digestion. My conclusion, that these histological findings were similar to bacteria, is based on the description given by Cajaraville and Angulo (1991), who describe *Chlamydia*-like organisms as small spherical bodies found within the epithelium of the digestive tubule. These observations were diagnosed by light microscopy. When using an electron microscope, they found the small spheres to consist of rod-shaped prokaryotic organisms.

It is well known that marine bivalves, being filter-feeders, may accumulate pathogenic bacteria and viruses that could cause harm to humans eating the mussels, but this thesis does not consider secondary consequences of bacterial infections.

THE STORAGE OF GLYCOGEN

Mytilus edulis, like other bivalves, has specific storage tissue in the mantle to store glycogen during the time of the year when the food supply is good. The glycogen is then used for the gametogenesis. Both food supply and the development stage of the gonads are correlated with the glycogen content (see Gosling 1992 for review). Gabbott (1975) suggested that the metabolism of glycogen and the gametogenesis are both controlled by food supply and temperature. This hypothesis has been supported in other studies. When comparing *M. galloprovincialis* from two areas in Ria de Sada in Galicia, Spain, difference in glycogen content was revealed among mussels from two sites. There were higher glycogen values in mussels from the site with higher chlorophyll *a* concentrations in the surrounding water than from the site with lower concentrations (Fernandez-Reiriz et al. 1996). de Zwaan and Zandee (1972) studied blue mussels from the Waddenzee during one year, and found a decreased glycogen content during the period when food supply was low.

As the glycogen content apparently is linked to the reproductive cycle, it could be possible to define the reproductive stage in mussels from a known glycogen level. In Paper IV I described the annual glycogen content in mussel populations from two Swedish areas, and in comparison the cycle was followed in a Spanish population of *M. galloprovincialis* during the same year. I also tested how well the glycogen content correlates with the reproductive cycle. The results confirm that the pattern of glycogen reserves and the reproductive cycle were linked together, regardless of species and locality. Mussels in resting stages had the highest glycogen values and mussels in the stages just before spawning had the lowest values. The glycogen levels, however, differed among localities and seasons. In the Spanish mussels the glycogen content was low in January but at about average during the other seasons. The Swedish populations had their lowest values in April/June and the highest in August/October. This could be valuable knowledge for Swedish mussel farmers, as the level of the glycogen content is dependent of the food availability and the locations of farms might be evaluated by comparing mussel glycogen contents between sites.

REPRODUCTION OF *MYTILUS EDULIS*

A wide variety of stimuli have been suggested to induce spawning, including temperature, mechanical shock and chemicals. Also interaction between several factors, including the physiological status of the mussels has been suggested. The reproductive cycle includes

phases of developing, spawning, spent reproductive products and resting stages (Seed 1969). Gonad developmental stage of a population differs both over spatial and temporal scales and some variation is also present among individuals of a population (Seed 1969, 1976).

From August 2000 until June 2001 populations of*Mytilus edulis* from Sweden and *M. galloprovincialis* from Spain were sampled in order to describe the timing of the reproductive cycle of the Swedish *M. edulis* compared to the cycle of the Spanish *M. galloprovincialis* (Paper IV). Swedish blue mussel populations have been reported to start spawning when the temperature reaches 10 °C (Loo and Rosenberg 1983) that usually occurs in May. In my study some individuals were still in the pre-spawning stage as late as in October. My suggestion is, that because of food competition, different individuals are supplied with different amounts of food particles and therefore have different timing in the glycogen synthesis and the reproductive development.
Spanish *M. galloprovincialis* have two mass spawnings in spring (Cáceres-Martínez and Figueras 1998 Villalba 1995) and my results provide some support of this observation. The results indicate that Swedish and Spanish mussels all started gametogenesis in late summer/early autumn 2000, but in Swedish mussels gametogenesis progressed through a long period, while in the Spanish mussels it ended in January 2001 and started again in April.

PARASITE INFECTION

Marine bivalves may host a wide spectrum of parasites that might cause mass mortality (Calvo et al. 1998, Montes et al. 1991). Although these infections are not always lethal, they could cause impaired food uptake or impairment of gametogenesis as an effect of parasites utilizing the glycogen reserves. Without killing the hosts, a parasite infection could disturb host population dynamics and also be of great harm for mussel farmers. Swedish mussel farmers would therefore benefit from an improved knowledge about parasite dispersal and effects on the mussel hosts.

A common parasite of *Mytilus edulis* in Swedish waters is the trematode *Renicola roscovita* (Granovitch and Johannesson 2000). The adult of *R. roscovita* is endoparasitic in sea gulls and the eggs are released into the surrounding water with the host´s faeces. If they land up in the water, a ciliated, free-swimming larva hatches from the egg. If successful in finding a snail (*Littorina* spp.), the larva penetrates the epithelium of the snail (its first intermediate host) and a second larval stage (the sporocyst) is developed into a stage where each sporocyst includes a number of cercariae. These cercariae are released from the snail and spread into the surrounding water. Cercariae in general have specific host-finding strategies, sensitive to various chemical and physical cues of the second intermediate host (Haas 1992). However, Werding (1969) found *R. roscovita*

cercariae to be bad swimmers, using their tail only for keeping the larvae floating in the water, and nothing, so far, is reported to conflict that. Cercarial contact with the second intermediate host will probably be facilitated via water propulsion and the filtration of the host. An inactive cercaria could either enter the mussel mantle cavity by hazard or be carried away by the expelled water current. In a study of unspecified trematode cercariae, Stunkard (1964) describes a laboratory experiment where different bivalves were exposed to thousands of cercariae, and he observed "a stream of cercariae sucked into the incurrent siphon of *M. edulis*". Once inside the second intermediate host, the cercariae lose their tails and encyst in the tissues forming metacercariae. If the metacercariae are not eliminated by the mussel defence system, they could survive encysted, waiting for the final host, the sea gull, to eat the mussel.

Seasonal differences of larval infections from*R. roscovita* were shown in the study of wild mussel populations in Denmark (Paper III), where interactions were found between the factors population and month. The results showed that the prevalence of metacercariae varied over months but that the pattern of variation was different among populations. In one population there was a large difference between the sampling months and in the other two there were just minor differences. This pattern is slightly supported by the results of Paper II in which populations from the eastern coast of Skagerrak/Kattegat were sampled on two different occasions (summer and autumn). This time we found a tendency to interaction between sampling time and area. Time of sampling may be more or less important depending on which locality is sampled, and it does not seem possible to describe a general seasonal pattern in parasite infection rates at the Skagerrak/Kattegat coasts. It seems from Papers II and III that the metacercariae from *R. roscovita* can be found in mussels any time of the year, if the biotope is favourable to the life cycle. The intensity, however, could vary over seasons due to various reasons, such as the trematode larvae being less resistant to freezing than their molluscan hosts **(**Lauckner 1983) or the emission of cercariae being restricted to a short season. The latter is not known from Swedish waters but according to Lauckner the major attack of cercarial emission (*Himasthla elongata* and *R. roscovita*) from snails at the German North Sea coast occurs in late May to early June and coincides with the settling of young bivalves. In October the cercarial attack has ceased. However, metacercariae, when once encysted, could survive a year or more in the mussel tissues (Pekkarinen 1988), and this might reduce the effect of seasonal variation and winter freezing.

Different microenvironments of the mussels might have different histopathological effects. When I compared the occurrence of trematode larvae in bottom dwelling (natural) and suspended (farmed) blue mussels at the Swedish west coast (Paper V), I found a large difference in prevalence of *R. roscovita* metacercariae. In the two bottom-dwelling populations 96 and 100 %, respectively, of the mussels were infected, but only 4 and 12 % of the suspended ones. Most (> 70 %) of the metacercariae found were encysted in the labial palps. This probably caused a mechanical damage in the tissues which impaired the possibility for the mussels to transfer particles to the mouth. Thus

microenvironment of the mussels may be very important. In this case, the difference could be explained by problems of the parasite in maintaining its life cycle in the suspended biotope where the mussels were at a distance from the first intermediate host (snails of *Littorina*).

To test this hypothesis I assessed the influence of mussel environments at different distances from the shore, on the distribution of *R. roscovita.* I recorded the presence and densities of metacercariae in natural and farmed blue mussels at various distances and depths from rocky shore habitats inhabited by *Littorina* (Paper VI). The results indicated that cercariae of *R. roscovita* infected mainly mussels at short distances from rocky shores which is what one could predict from a short dispersal of cercariae released from rocky shore snails (e.g. *Littorina littorea*). This also explains why natural populations of mussels living intertidally and subtidally close to populations of snails are much more infected than mussels farmed on ropes at greater distances from the shore.

Not only the environmental conditions fortrematode larvae are important, but also the physiological status (e. g. stage of the reproductive cycle) of the host could affect infestation rate of parasites. If, for example, the mussel spends all its energy in developing the gonads, the cercariae will probably be more successful in encysting.

THE AQUACULTURE PERSPECTIVE

Histological changes occur in the mussel tissue owing to natural processes, such as the reproductive cycle. External factors, both natural and anthropogenic, that interact with mussel life are likely to affect the histological changes in the life of the mussel. In this way, histological studies add complementary information to ecological studies of mussel populations. My results support earlier observations that populations of *Mytilus edulis* in Sweden spawn once a year. Unexpected, though, was that the gametogenesis starts already in the autumn with the developing of the gonads and lasts until late spring the next year. Thus the period during which energy is accumulated and stored as glycogen is short, only a few months during the summer. The glycogen content relate strongly to the different reproductive stages and furthermore the pattern of relation is similar between the Swedish *M. edulis* and the Spanish *M. galloprovincialis*. This suggests that there is a fundamental mechanism that relates the reproductive cycle to the glycogen storage reserves that is similar over the two species and the areas studied. The presence of such a mechanism is promising for the possibility to use the glycogen content as a tool to determine the pre-spawning stages (the stages with the highest flesh weight). However, such a tool requires detailed knowledge about the relationship and my present data is not sufficient in that sense. It is, for example, necessary to get results from more than one year, to estimate temporal variation in this relationship.

The drilling technique that I tested might be useful for histological studies, but as the growth was affected by this treatment, the aim of the study together with the

vulnerability of the population should decide what method to use for tissue sampling. I suggest that the drilling technique is useful in repeated sampling, e.g. in order to follow the gonad development in the same mussel individual.

Trematode larvae seem not to be a problem for most Swedish mussel farms as these are rope cultures and located at some distance from the nearest shore. Since dispersal distance of cercariae seems to be the limiting factor to avoid infections of *Renicola roscovita* metacercariae in mussel farms, it is to consider the distance to the nearest *Littorina* spp. population, as these snails are most likely the source of the larvae.

THE ANTHROPOGENIC IMPACT PERSPECTIVE

Intensity and prevalence of parasites, granulocytomas and hemocytes varied among populations of *Mytilus edulis* and were correlated to a number of natural factors including sampling area (which in this study often means various salinities), time of sampling and microenvironment of the mussels. I also found a positive relationship between parasite and hemocyte levels. Low levels of impact from human contamination, such as along the eastern coast of Skagerrak/Kattegat, did not relate to any of the histopathological changes assessed, while heavy impacts, such as at Lyngs odde in Lilla Bält (chemical and cellulose industries, oil refineries and ammonia tanks) did so. My results show that histopathological investigations trying to trace moderate or low levels of human impacts must carefully consider interactions between natural and anthropogenic factors that affects the histopathology of mussels. With the present knowledge, histopathology seems to be a limited tool as a useful indicator of impacts at the levels of contamination found in Scandinavian waters. On the other hand, histology might be a good indicator of the general physiological status of mussels under various natural conditions, but studies must include intense sampling at various temporal and spatial scales to disclose general patterns of variation.

FUTURE PERSPECTIVES

Histological studies are one way to find out more about the fundamental physiological functions of invertebrates, such as the reproductive and the digestive cycles. For example, the vulnerable Swedish mussel populations of *Acesta excavata* and *Margaritifera margaritifera* both seem to fail in their reproduction. An histological study will, for example, unveil problems in the gametogenesis of natural populations.

It seems possible to use glycogen content as an indicator of reproductive stage in Swedish blue mussels, although repeated sampling over successive years is necessary for a more profound result. Moreover, the impact of nutrient enriched waters on variation in the gametogenesis should be investigated.

The ecology of parasites of bivalves and other invertebrate species is often overseen although many parasite species have strong effects on host fitness and performance (e.g. sterilization, tissue damage, changing behaviours) (Bethel and Holmes 1977, Jonsson and André 1992). Upon introduction of new invertebrate species to Swedish waters, both potential hosts and parasites, knowledge of parasite-host interactions with species involved is urgent.

CONCLUDING REMARKS

The overall aim of my thesis was to assess variation in histological characters in blue mussel populations, owing to natural and anthropogenic factors, and from the results of my work some general conclusions can be drawn.

When using histopathological studies to assess effects of impacts of contamination in the water, one must carefully consider the interactions between anthropogenic factors and natural factors, e.g. salinity in the water and the mussel reproductive stage. In mussels from Scandinavian waters, my suggestion is that the presence of granulocytomas may be used as an indicator of water contamination.

Populations of *Mytilus edulis* at the Swedish west coast spawn mainly once a year, but there are individual variations, depending on the level of stored glycogen, which in turn depends on the availability of food. The period with a surplus of glycogen is short, just a few months after spawning. Then the gametogenesis starts and lasts from autumn to late spring the next year.

Parasitic metacercariae were found to be present in the mussels at all seasons, however the prevalence varied over time and space. My results indicated that the spatial variation was an effect of cercarial dispersal while the temporal variation may be an effect of the strength of mussel immune defence.

I found metacercariae to cause mechanical injuries in the labial palps of the mussels, which suggest that the metacercariae might affect the food uptake.

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Fig. 1

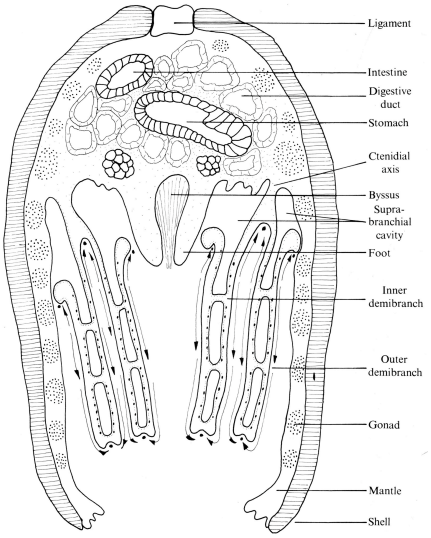


Fig. 2

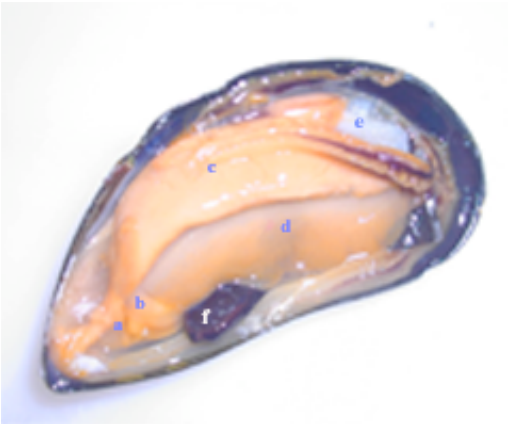


Fig. 1 Cross-section of the mantle, the gills and the digestive gland (Bayne 1976).

Fig. 2 Opened mussel showing a) mouth opening, b) labial palps, c) ripe gonad in mantle, d) gills, e) posterior adductor muscle, f) foot.

Fig. 3

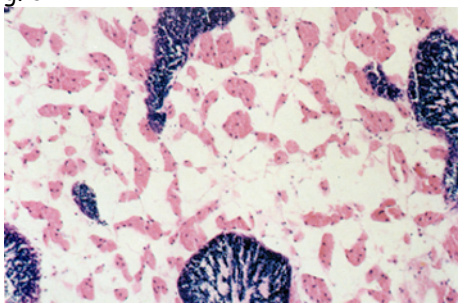
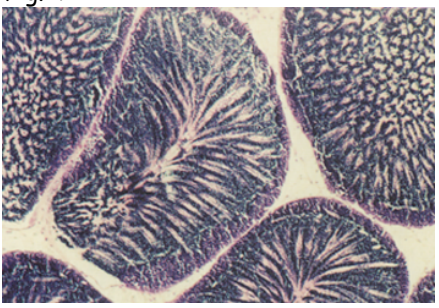


Fig. 4



Two different stages of the reproductive cycle:

Fig. 3 Developing male gonad in the mantle. Red and white storage cells.

Fig. 4 Ripe male gonad in the spawning stage. Dark blue ripe gametes and purple still developing gametes.

REFERENCES

Bayne, B. L. 1976. Marine mussels: their ecology and physiology. Cambridge University Press, Cambridge.

Bethel, W. M. and **Holmes, J. C.** 1977. Increased vulnerability of amphipods to predation owing to altered behaviour induced by larval acanthoccephalans. Can. J. Zool. 55, 110 – 115.

Calvo, L. M., Walker, J. G. and **Burreson, E. M.** 1998. Prevalence and distribution of QPX, Quahog Parasite Unknown, in hard clams *Mercenaria mercenaria* in Virginia, USA. Dis. Aquat. Org. 33, 209 – 219.

Cajaraville, M. P. and **Angulo, E.** 1991. Chlamydia-like organisms in digestive and duct cells of mussels from the Basque coast. J. Invertebr. Pathol. 58, 381 – 386.

Carballal, M. J., Villalba, A. and **López, C.** 1998. Seasonal variation and effects of age, food availability, size, gonadal development, and parasitism on the hemogram of *Mytilus galloprovincialis*. J. Invertebr. Pathol. 72, 304 – 312.

Cáceres-Martínez, J. and **Figueras, A.** 1998. Long-term survey on wild and cultured mussels (*Mytilus galloprovincialis* Lmk) reproductive cycles in the Ria de Vigo (NW Spain). Aquaculture 162, 141 – 156.

Cheng, T. C. 1983. The role of lysosomes in molluscan inflammation. Am. Zool. 23, 129 – 144.

Fernandez-Reiriz, M. J., Labarta, U. and **Babarro, J. M. F.** 1996. Comparative allometries in growth and chemical composition of mussel (*Mytilus galloprovincialis*Lmk) cultured in two zones in the Ria Sada (Galicia, NW Spain). J. Shellfish. Res. 15:2, 349 – 353.

Fuentes, J., Molares, J. and **Villalba, A.** 1998. Growth, mortality and parasitization of mussels cultivated in the Ría de arousa (NW Spain) from two sources of seed: intertidal rocky shore vs. collector ropes. Aquaculture 162, 231 – 240.

Gabbot, P. A. 1975. Storage cycles in marine bivalve molluscs: A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. Proc. 9th Europ. mar. biol. Symp., 191 – 211. Ed. H. Barnes, Aberdeen University Press.

George, S. G. and **Piries, B. J. S.** 1986. Biochemical and ultrastructural observations of long-term silver accumulation in the mussel, *Mytilus edulis*. Marine Environ. Res. 18, 255 – 265.

Gosling, E. 1992. Ed. The mussel *Mytilus edulis*: Ecology, Physiology, Genetics and Culture. Developments in Aquatic and Fisheries Science Vol. 25. p. 204. Elsevier Science Publications, Amsterdam.

Granovitch, A. and **Johannesson, K.** 2000. Digenetic trematodes in four species of *Littorina* from the west coast of Sweden. Ophelia 53 (1), 55 – 65.

Haas, W.1977. Physiological analysis of cercarial behavior. J. Parasitol. 78 (2), 243 – 255.

Hernroth, B. 2002. Uptake and fate of pathogenic microbes in the blue mussel, *Mytilus edulis*. Academic dissertation, Göteborg University. ISBN: 91-628-5200

Hummel, H., Colucci, F., Bogaards, R. H. and Strelkov, P. 2001. Genetic traits in the bivalve *Mytilus* from Europe, with an emphasis on Arctic populations. Polar Biol. 24, 44 – 52.

Jones, J. B., Scotti, P. D., Dearing, S. C. and Wesney, B.1996. Virus-like particles associated with marine mussel mortalities in New Zealand. Dis. Aquat. Org. 25, 143 – 149.

Jones, S. H., Chase, M., Hennigar, P., Landry, N., Wells, P. G., Harding, G. C. H., Krahforst, C. and **Brun, G. L.** 2001. Monitoring for toxic contaminants in *Mytilus edulis* from New Hampshire and the gulf of Maine. J. Shellfish. Res. 20 (3), 1203 – 1214.

Jonsson, P. R. and **André, C.** 1992. Mass mortality of the bivalve *Cerastoderma edule* on the Swedish west coast by infestation with the digenean trematode *Cercaria cerastodermae* I. Ophelia 36, 151 – 157.

Koehn, R. K. 1991. The genetics and taxonomy of species in the genus *Mytilus*. Aquaculture 94, 125 – 145.

Krishnakumar, P. K., Casillas, E. and **Varanasi, U.** 1995. Effects of chemical contaminants on the health of *Mytilus edulis* from Puget Sound, Washington. II. Cytochemical detection of subcellular changes in digestion cells. Mar. Biol. 124, 251 – 259.

Lauckner, G. 1983. In "Diseases of Marine Animals" (O. Kinne, Ed.), Vol. II, Chapter 13. Biologische Anstalt Helgoland, Hamburg, Germany.

Loo, L. O. 1991. Benthic – pelagic coupling in a boreal marine ecosystem. Academic dissertation, Göteborg University.

Loo, L. O. and **Rosenberg, R.** 1983. *Mytilus edulis* culture: growth and production in western Sweden. Aquaculture 35, 137 – 150.

Montes, J., Villalba, A., Lopez, M. C., Carballal, M. J. and **Mourelle, S. G.** 1991. Bonamiasis in native flat oysters (*Ostrea edulis* L.) from two intertidal beds of the Ortigueira estuary (Galicia, N. W. Spain) with different histories of oyster culture. Aquaculture 93, 213 – 224.

Mortensen, S. H. 1993. A health survey of selected stocks of commercially exploited Norwegian bivalve molluscs. Dis. Aquat. Org. 16 (2), 149 – 156.

Neff, J. M., Hillman, R. E., Carr, R. S., Buhl, R. L. and **Lahey, J. I.** 1987. Histopathologic and biochemical responses in Arctic marine bivalve molluscs exposed to experimentally spilled oil. Arctic 40 (Suppl. 1), 220 – 229.

Pekkarinen, M. 1988. Gymnophallid trematode parasites in the Baltic clam *Macoma balthica* (L.) off the southwest coast of Finland. Academic dissertation, University of Helsinki.

Porte, C., Sole, M., Borghi, V., Martinez, M., Chamorro, J., Torreblanca, A., Ortiz, M., Orbea, A., Soto, M. and **Cajaraville, M. P.** 2001. Chemical, biochemical and cellular responses in the digestive gland of the mussel *Mytilus galloprovincialis* from the Spanish Meditterranean coast. Biomarkers 6 (5), 335 – 350.

Rasmussen, L. P. D., Hage, E. and **Karlog, O.** 1985. light and electronmicroscopic studies of the acute and long-term toxic effects of N-nitrosodipropylamine and N-methylnitrosurea on the marine mussel *Mytilus edulis*. Mar. Biol. 85, 55 – 65.

Rasmussen, L. P. D. 1986. Virus-associated granulocytomas in the marine mussel, *Mytilus edulis*, from three sites in Denmark. J. Invertebr. Pathol. 48, 117 – 123.
Riisgård, H. U.and **Randlöv, A.** 1981. Energy budgets, growth and filtration rates in *Mytilus edulis* at different algal concentrations. Mar. Biol. 61, 227 – 234.
Roch, P 1999. Various aspects of mollusc immunity. B. Soc. Zool. Fr. 124 (4), 313 – 324.
Santarem, M. M., Robledo, J. A. F. and **Figueras, A.** 1994. Seasonal changes in hemocytes and serum defense factors in the blue mussel *Mytilus galloprovincialis*. Dis. Aquat. Org. 18, 217 – 222.
Schneeweiss, H. and **Renwrantz, L.** 1993. Analysis of the attraction of haemocytes from *Mytilus edulis* by molecules of bacterial origin. Dev. Comp. Immunol. 17, 377 – 387.
Seed, R. 1969. The ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. I: Breeding and settlement. Oecol. 3, 277 – 316.
Seed, R. 1976. In "Marine mussels" (B L Bayne, Ed.): Their ecology and physiology. Cambridge University Press, 13 – 60.
Stunkard, H. W. 1964. Studies on the trematode genus Renicola: Observations on the life-history, specificity, and systematic position. Biol. Bull. Woods Hole 126, 467 – 489.
Suresh, K and Mohandas, A. 1990. Number and types of hemocytes in *Sunetta scripta* and *Villorita cyprinoides* var. cochinensis (Bivalvia), and leukocytosis subsequent to bacterial challenges. J. Invertebr. Pathol. 55, 312 – 318.
Villalba, A. 1995. Gametogenetic cycle of cultured mussel, *Mytilus galloprovincialis*, in the bays of Galicia (N. W. Spain). Aquaculture 130, 269 – 277.
Villalba, A., Mourelle, S. G., Carballal, M. J. and **Lopez, C.** 1997. Symbionts and diseases of farmed mussels *Mytilus galloprovincialis* throughout the culture process in the Rías of Galicia (NW Spain). Dis. Aquat. Org. 31, 127 – 139.
Väinölä, R. and **Hvilsom, M. M.** 1991. Genetic divergence and a hybrid zone between Baltic and North Sea *Mytilus* populations (Mytilidae: Mollusca). Biol. J. Linnean Soc. 43, 127 – 148.
Wedderburn, J., McFadzen, I., Sanger, R. C., Beesley, A., Heath, C., Hornsby, M. and **Lowe, D.** 2000. The field application of cellular and physiological biomarkers, in the mussel *Mytilus edulis*, in conjunction with early life stage bioassays and adult histopathology. Mar. Poll. Bull. 40 (3), 257 – 267.
Werding, B. 1969. Morphologie, Entwicklung und Ökologie digener Trematoden-Larven der Strandschnecke *Littorina littorea*. Mar. Bil. 3, 306 – 333.
Westerbom, M., Kilpi, M. and **Mustonen, O.** 2002. Blue mussels, *Mytilus edulis*, at the edge of the range: population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea. Mar. Biol. 140, 991 – 999.
Yevich, P. P., Yevich, C. A., Scott, K. J., Redmond, M., Black, D., Schauer, P. S. and **Pesch, C. E.** 1986. Histopathological effects of Black Rock Harbour dredged material on marine organisms. Technical Report D-86-1, U.S. Environmental Protection Agency, USA.

de Zwaan, A. and **Zandee, D. I.** 1972. Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis*. Comp. Biochem. Physiol. 43A, 53 – 58.

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Short communication

Tissue sampling from live blue mussels, *Mytilus edulis*. A field study from the Swedish west coast

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Abstract

Histological techniques are often used to study environmental effects on mussels, but since these techniques include killing of the individuals, rare or endangered populations cannot be studied using conventional tissue sampling. This study is an attempt to find a method that can be used repeatedly with the same mussel individual and which does not affect growth and survival. From 200 mussels, *Mytilus edulis*, tissue was sampled in different ways, such as drilling a hole in the shell or prising apart the shell valves. Two kind of instruments were used, an injection needle and surgery forceps. Some of the drilled mussels had their holes sealed again with cement.

Drilling a hole in the shell, removing tissue sample with surgical forceps and then leaving the holes open did not seriously harm the mussels during the two months the experiment lasted. But if the holes were sealed with cement, both length and weight growth were negatively affected (35% lower length growth and 36% lower weight growth compared to the control mussels). Mortality was highest among the drilled and sealed mussels (80% higher than among the other treatments). The vulnerability of the population, the aim of the study and the duration of the experiment should decide what method to use for tissue sampling. For long-term experiments and repeated sampling, opening the mussels by prizing apart the valves is a better alternative than drilling holes in the shells, but depending on the morphology of the species it could be difficult to sample the anterior part of the mussel body. For a short experiment and to sample anterior parts, drilling the shells, leaving the holes open and using surgical forceps, seems to be an acceptable compromise between the different treatments used.

Keywords: *Mytilus edulis*; Drilling; Growth; Mortality; Sealing; Techniques

1. Introduction

Histological techniques to study environmental effects on mussels, the occurrence of parasites or the health of mussels are widely used and well tested. Studies of parasite infections demand large numbers of mussels. Rare or endangered mussel species (such as the pearl mussel *Margaritifera margaritifera* in Europe) and otherwise vulnerable populations cannot be studied using conventional tissue sampling techniques for which whole individuals are killed. Such populations and species are, however, often the ones most in need of investigation, because environmental and pathological effects on reproduction and growth may be the proximate causes of threat. Methods of sam-

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pling tissue from live animals may be an alternative, important for threatened or rare species, but it may also be desirable in situations where the same individual has to be sampled on several occasions. Live tissue sampling requires methods that do not seriously harm the mussel. Ideally such a method should meet the following demands: (i) no effect on growth, reproduction and behaviour of the mussel, (ii) easy and quick to use, and (iii) allows repeated use on the same individual. One difficulty is to obtain sufficiently large pieces of tissue for further analysis. Berg et al. (1995) sampled 1 cm^2 (approx. 34 mg) mantle pieces from the anterior end of freshwater mussels using biopsy and Naimo et al. (1998) sampled 7.7 mg plugs from the foot tissue of freshwater mussels. In marine mussels biopsy has been used in several studies, but only for cell suspension, not for taking pieces of tissue (e.g. Santarem et al., 1994; Mikhailov et al., 1997). The aim of the present study was to compare the effects on growth and survival using different methods of live tissue sampling of marine mussels *Mytilus edulis*, in order to find optimal conditions for a repeated sampling procedure.

2. Material and methods

Two hundred two-year-old rope-cultured blue mussels (*Mytilus edulis*), 4–5 cm in length, were used in the experiment, which included ten treatments combining drilling and prising apart the valves, and sampling by needle or forceps. Control mussels were weighed and measured and in addition mussels to compensate for the effect of drilling and for the effect of prising apart the shells, so-called procedure controls (Underwood, 1997) were used. Thus the following treatment were used:

1. The shells drilled, the holes sealed, no tissue sampled.
2. The shells drilled, the holes left open, no tissue sampled.
3. The shells drilled, the holes sealed, tissue sampled by surgery forceps.
4. The shells drilled, the holes left open, tissue sampled by surgery forceps.
5. The shells drilled, the holes sealed, tissue sampled by injection needle.
6. The shells drilled, the holes left open, tissue sampled by injection needle.
7. The mussels not drilled or sampled but handled in the same way as drilled ones (PC).
8. The mussels only weighed and measured (C).
9. The shells not drilled but forced to open, tissue sampled by injection needle.
10. The shells not drilled but forced to open, no tissue sampled (PC).

Mussels were placed in a random order in plastic pots (one for each treatment) in the laboratory. The length and wet weight were measured. Seawater was poured into the pots, to avoid the mantle cavity being air filled after the drilling. Since the aim was to test different sampling methods, no pre-determinate tissue or fluid was sampled and no tissue analysis was done. The mussels were supposed to be in the same stage of the gametogenesis, that is the spawning period.

1. Drilling the shells (n = 120): A drilling-machine ('SKIL 2125H') with a speed of 230 r. p. m. was used and the holes were made in the same shell-half in all mussels, near the anterior end. The holes were made with a metal drilling bit (∅ 3 mm). From 80 of the drilled mussels a small piece (approx. 3 mm^3) of tissue was removed through the hole, in 40 mussels with surgical forceps, and in another 40 mussels with an injection needle. The holes of 50% of the drilled mussels (n = 60) were sealed with 'Justi Resin Cement' from Ivoclar, Lichtenstein; a 'non-toxic' cement used by dentists.

2. Prizing apart the shells (n = 40): The shells were opened at the posterior end by inserting a thin piece of cork between the valve-halves when the mussels already had their shells slightly open. This is fairly easy to do if the mussels are placed with the anterior end towards the bottom of a pot and the siphons turned upwards. In 50% of the opened mussels a sample was taken from the posterior end of the mussel mantle using an injection needle (n = 20). The other 50% of the mussels were handled in the same way but no samples were taken (n = 20).

All the mussels were placed in a randomised order in baskets, each mussel surrounded by a marked net-tube. There were five baskets, with 40 mussels each and covered with a net. The baskets were placed in the sea.

Every second week the mussels were checked and fouling algae and barnacles were removed from the baskets with a hard brush. After two months the experiment was terminated, and the baskets were transferred to the laboratory. Growth was estimated from weight and length increase and shells of the mussels were examined.

3. Statistical analysis

As some of the mussels died during the experiment, the data sets became unbalanced. However, the different null hypotheses were first tested using all the observations in an unbalanced data set. If a null hypothesis was rejected, the same hypothesis was tested again with a data set that had been balanced by either random elimination or by addition, reducing data to a proper replicate number. The mortality however, was tested using the result from the original 200 individuals.

The results of the experiment were analysed by a two-factor ANOVA, testing for effects of the fixed factor 'treatment' with ten levels and the random factor 'basket' with five levels, A–E. Post-hoc, the Student-Newman-Keuls (SNK) (Underwood, 1997) tests, were used to assess differences among levels of a significant factor.

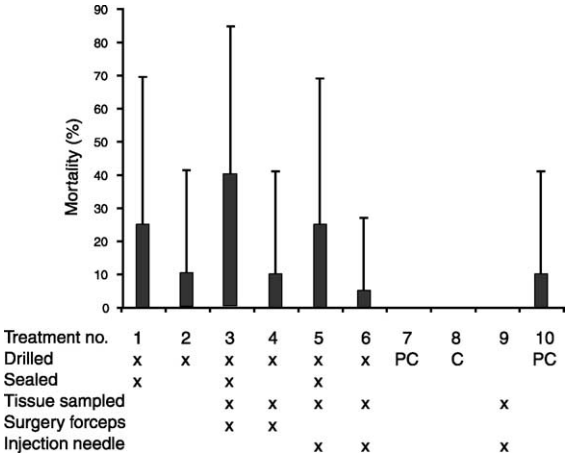

Fig. 1. Mortality prevalence in 200 individuals of *Mytilus edulis* exposed to ten different treatments (20 individuals in each) for a period of two months. C is the control group and PC is the procedure group (see text). Error bars: 95% confidence interval.

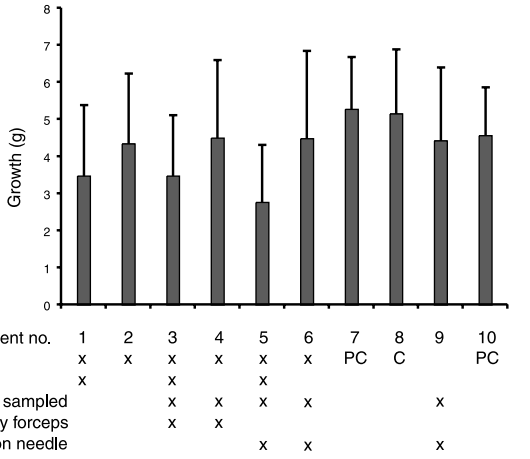

Fig. 2. Effects of ten different treatments on the weight growth of 150 individuals of *Mytilus edulis* (15 individuals in each treatment) after a period of two months. C is the control group and PC is the procedure group (see text). Error bars: 95% confidence interval.

4. Results

Twenty-five individuals (12.5%) died during the experiment. There was no interaction between the factors treatment and basket, on the mortality, weight or length and no effect of basket alone. The factor treatment, however, showed to be highly significant

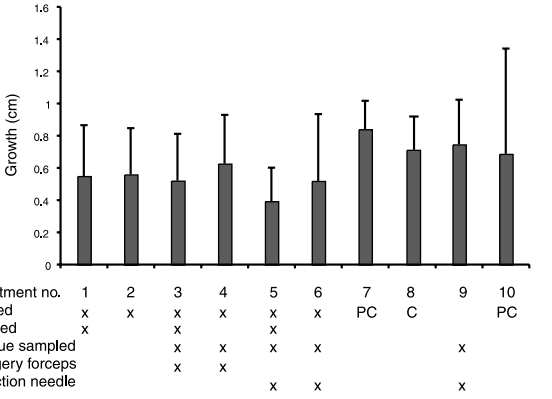

Fig. 3. Effects of ten different treatments on the length growth of 150 individuals of *Mytilus edulis* (15 individuals in each treatment) after a period of two months. C is the control group and PC is the procedure group (see text). Error bars: 95% confidence interval.

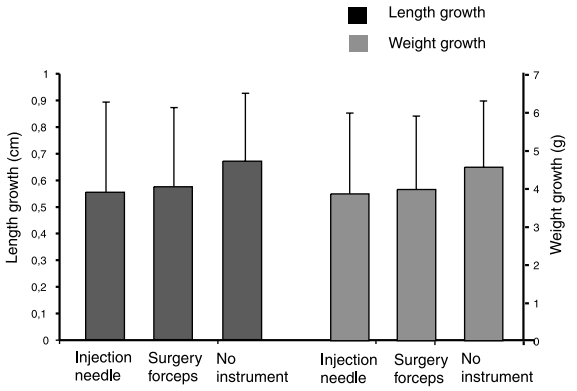

Fig. 4. Effects of instrument used on the growth of 150 individuals of *Mytilus edulis* (15 individuals in each treatment) after a period of two months. Error bars: 95% confidence interval.

and the SNK test revealed a tendency of higher mortality in the mussels with drilled and sealed holes than for mussels from the other treatments and a significantly higher mortality ($p = 0.02$) for drilled and sealed mussels which were sampled with surgery forceps (Fig. 1). The mussels with drilled and sealed holes also had low growth values (Fig. 2, $p_{weight} = 0.01$ and Fig. 3, $p_{length} < 0.001$).

The holes in the shells were repaired in all live mussels and in the shells with sealed holes, the cement string seemed to be unaffected. There was no significant difference between mean growth as effects of the instrument used ($p_{weight} = 0.11$ and $p_{length} = 0.07$), though the mussels in which no instrument was used had the highest growth (Fig. 4). No significant interaction was shown between instrument used and sealed/open holes ($p_{weight} = 0.60$ and $p_{length} = 0.74$).

5. Discussion

Drilled, sealed mussels grew less than the control mussels, and the use of cement decreased both weight and length growth. Kideys (1994) studied the effects of tagging *Buccinum undatum* L. using an electric drilling machine to make a hole (∅ 2 mm) for tagging in the shell. Tagging decreased the growth rate, but the effects of drilling were not separated from other tagging effects in his study. All of the individuals were drilled, and the holes were observed to be repaired and the inner shell surface to be covered with new shell material. Kideys (1994) suggested that a hole in the shell causes the animal to invest extra energy in repair of the shell, which delays growth. My results support his suggestion. Empty shells were found in all baskets, and the fact that mussels from the drill treatments with their holes sealed died at a higher rate than mussels of other treatments (72% of the dead mussels were from this treatment) indicates that the cement was harmful to the mussels after all. Many studies describe the method of using an injection needle to inject a solution into a mussel (e.g. Cancio et al., 1998) or to withdraw blood cells (e.g. Santarem et al., 1994). The methods are widely used and nothing in the literature indicates that they are harmful to the animals. My study supports the use of surgical forceps as an alternative to injection needles; among the treated mussels growth was not affected by the type of instrument used. The advantage of using surgical forceps is the possibility to sample larger tissue pieces.

This study suggests that the vulnerability of the population and the aim of the study should decide what method to use for tissue sampling. Also the duration of the study will be of importance. For long-term experiments and repeated sampling, opening the mussels by prizing apart the valves is better than drilling holes in the shells. For a short experiment drilling, leaving the holes open and using surgical forceps, seems to be an acceptable compromise between the different treatments investigated.

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References

Berg, D.J., Haag, W.R., Guttman, S.I., Sickel, J.B., 1995. Mantle biopsy: a technique for nondestructive tissue-sampling of freshwater mussels. J. N. Am. Benthol. Soc. 14, 577–581.

Cancio, I., Orbea, A., Völkl, A., Dariush Fahimi, H., Cajaraville, M.P., 1998. Induction of peroxisomal oxidases in mussels: Comparison of effects of lubricant oil and benzo(a)pyrene with two typical peroxisome proliferators on peroxisome structure and function in *Mytilus galloprovincialis*. Toxicol. Appl. Pharmacol. 149, 64–72.

Kideys, A.E., 1994. Effect of tagging on the growth rate of the

neogastropod, *Buccinum undatum* in the laboratory. Fish. Res. 20, 283–289.

Mikhailov, A.T., Torrado, M., Korochkin, L.I., Kopantzeva, M.A., Méndez, J., 1997. Male-predominant carboxylesterase expression in the reproductive system of molluscs and insects: Immunochemical and biochemical similarity between *Mytilus* male associated polypeptide (MAP) and *Drosophila* sex-specific esterase S. Comp. Biochem. Physiol. 118 B, 197–208.

Naimo, T.J., Damschen, E.D., Rada, R.G., Monroe, E.M., 1998. Nonlethal evaluation of the physiological health of unionid mussels: methods for biopsy and glycogen analysis. J. N. Am. Benthol. Soc. 17, 121–128.

Santarem, M.M., Robledo, J.A.F., Figueras, A., 1994. Seasonal changes in hemocytes and serum defence factors in the blue mussel *Mytilus galloprovincialis*. Dis. Aquat. Org. 18, 217–222.

Underwood, A.J., 1997. Experiments in Ecology. Cambridge University Press, Cambridge, UK.

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Incidence of hemocytes and parasites in coastal populations of blue mussels (*Mytilus edulis*)—testing correlations with area, season, and distance to industrial plants

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Abstract

Blue mussel hemocytes (cells with immunoresponse activities) are suggested as indicators of anthropogenic contamination. We compared hemocyte numbers, granulocytoma (aggregated hemocytes), and parasites among populations of mussels from different areas of Skagerrak (a north and a south), seasons (summer and autumn), and impact levels (close or far from industrial activities). Seasonal hemocyte numbers were larger in the north compared to the south. Northern unimpacted populations had higher hemocyte numbers than populations close to industries, while no differences were found in the south. More uneven tissue distributions were found in populations far from industries in the north area and in populations close to industries in the south area. Parasites were more common in northern mussels than in southern, but no relationship to impact level was found. Mussels with granulocytoma, however, were found in all populations from the impacted sites while in none of the other populations suggesting granulocytoma as a possible indicator of industrial impact.

Keywords: *Mytilus edulis*; *Renicola roscovita*; *Trematoda*; Hemocyte; Granulocytom; Anthropogens

1. Introduction

The health condition of marine mussels is used worldwide as an indicator of the water quality of coastal areas. The rationale of using mussels is that low or moderate contamination by various substances may be hard to assess by direct measurements, but since sublethal effects tend to affect the mussels, these can be assessed more easily. As the hemocytes in mussels take part in the elimination of foreign particles (through phagocytosis), these cells are considered to be particulary well suited as indicators of health (see e.g., Weeks et al., 1992). There are two main groups of hemocytes: granular and agranular and both are able to take up particles (Cajaraville et al., 1995). As in vertebrates, mussel hemocytes accumulate in infected tissues. An increase of foreign particles in the mussel digestive gland could also alter the proportion of circulating and accumulated hemocytes. Populations of *Mytilus edulis* from NE England, for example, showed increased numbers of granular hemocytes in the digestive gland at impacted compared to less impacted sites in an industrialized region (Wedderburn et al., 2000). Similarly, in Denmark, mussels from a site near industrial plants in Lillebaelt had a higher degree of granulocytoma than mussels from a less industrialized area in Isefjorden (Svärdh, 1999). Granulocytoma are clusters of granular hemocytes, sometimes surrounded by a layer of collagen. The origin is unknown but it has been suggested that granulocytoma are produced when mussels are exposed to contaminants during long time periods (Neff et al., 1987). Also, remains of parasites have been found inside granulocytoma (Villalba, pers. comm.).

Parasites may be treated in the same way as foreign particles by the mussels, and therefore parasite infestations might also stimulate hemocyte formation in the tissues. Parasites surrounded by hemocyte infiltrations have been found in mussels from Mexico (Cáceres-Martínez et al., 1998) and Spain (Figueras et al., 1991).

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Several factors could thus affect the number of hemocytes in the mussels, either as single factors or as interacting factors. The aim of the present study was to look for correlations between single and interacting biotic and abiotic factors to hemocyte occurrences in natural populations of coastal waters. Such correlation, if found, would suggest cause–effect relationships that could be tested further with experiments. An additional aim was to evaluate the importance of natural and human induced factors in combination, as the natural factors (salinity and seasonality) are likely to interact with anthropogenic factors. Such interactions will constrain the use of hemocyte number as an environmental assessment tool.

2. Materials and methods

Mussels were sampled at eight sites along the eastern coast of Skagerrak/Kattegat (part of the North Sea) from Halmstad on the Swedish west coast to Oslo in Norway (450 km). In the north part of the sampling area (sites 1C, 2F, 3F, and 4C) the salinity varies between 20 and 30 ppt and in the south area (sites 5C, 6F, 7F, and 8C) between 5 and 15 ppt. All sites were sampled twice, in September 1998 and in June 1999. Four of the sampled sites were situated close to (< 200 m) oil harbours and other industrial plants, while four were from less affected areas (Fig. 1). Ten to fifteen mussels of 4–8 cm shell length were taken at each site. They were stored alive for 1–2 days and thereafter fixed for morphological sectioning. The digestive gland and part of the mantle were placed in Bouin's fixative, washed and dehydrated through an ascending series of alcohol and tetrahydrofuran (THF) and finally embedded in solid paraffin. Tissues from five mussels per population and sampling occasion were cut into 7 μm sections using a rotary microtome and transferred to microscope slides. The tissue was stained in Ehrlich hematoxylin/eosin.

Stage of gonad development, numbers of granulocytoma, and numbers of parasites were recorded from the whole preparations using a light microscope. Hemocyte area was measured in four photographs covering random parts of each preparation, using an image analyser system. From these measurements mean value of hemocyte occurrence was estimated for each population and season.

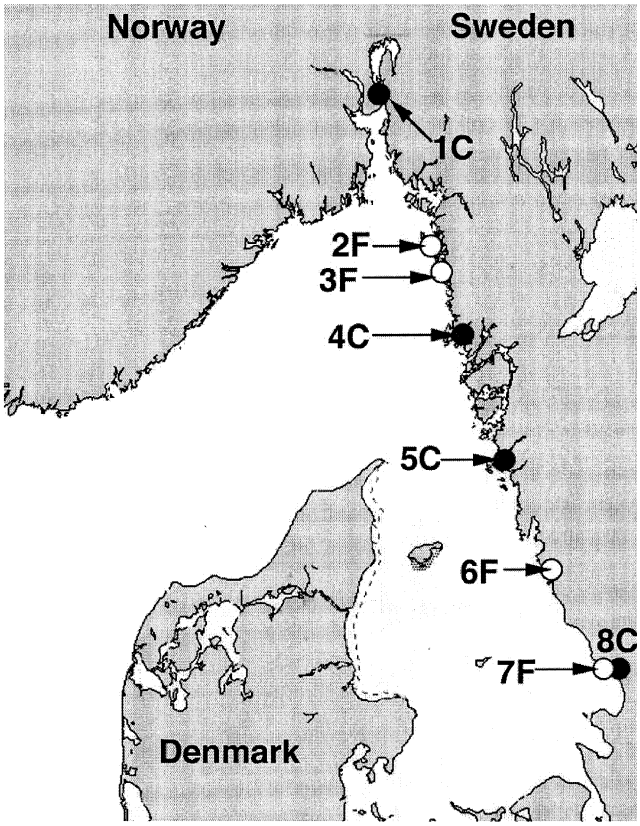

Fig. 1. The sampling sites. "F" and "C" indicate nearness to industrial impacts (see text).

Gonad development was assessed for each mussel using the gonad index of Seed (1969). The stages were grouped into four reproduction periods (Table 1). The effects of locality (a random factor with eight levels), sampling area (random, two levels), nearness to industrial impacts (fix, two levels), and sampling season (random, two levels) on hemocyte number were analysed using an analysis of variance (ANOVA). Hemocyte variation, that is how hemocytes were distributed within

Table 1
Gonad stages according to the index of Seed (1969) and the corresponding gonad developmental stages used in the present study

Index	Description	Gonad development
o	No trace of sexuality observed, resting gonads	Resting
2d	Ripe gametes appear in the centre of the follicles	Pre-spawning
3d	Half of the follicles occupied by ripe gametes	Pre-spawning
4d	Ripe gametes, but still gametogenesis in progress	Pre-spawning
V	Fully ripe condition, spawning starts	Spawning
4s	Spawning	Spawning
3ps	Few early stages of gametogenesis, rounded eggs	Post-spawning
2ps	Follicles less than half full of mature gametes	Post-spawning
1ps	Residual spermatozoa and ova still present	Resting

each mussel, was estimated from coefficient of variance (s/mean) for the four photographs of each mussel. A high coefficient of variance indicated an uneven distribution of the hemocytes, while a low coefficient revealed a more homogeneous distribution. The former expected for infected, and the latter for uninfected mussels. In the linear model (Underwood, 1997):

$$Y_{ijk} = \mu + A_i + I_j + S_k + L(I \times A) + A \times I_{ij} + A \times S_{ik} + I \times S_{jk} + S \times L(I \times A) + A \times I \times S_{ijk},$$

where μ is the grand mean, A and S the effects of sampling area and sampling season, respectively, I is the effect of nearness to industrial impact and $L(I \times A)$ locality nested under the interaction of nearness and sampling area. Cochran's test was used to test for heterogeneity of variances and post hoc (SNK) tests to assess differences among levels of a significant factor.

3. Results

Cochran's test revealed heterogeneity of variances among samples and therefore the data were transformed using an arc-sin transformation. The transformation successfully eliminated the heterogeneity. The number of hemocytes in the mussels varied greatly among samples (7.0–33.9%), and the interactions of season and area, and of impact and area, had significant effects on this variation (Table 2). Thus, in the north area differences in hemocyte numbers between sampling occasions were more pronounced than in the south area (Fig. 2).

Likewise, in the north area distance to industrial plants correlated with hemocyte number, while in the south area distance seemed to be unimportant. Surprisingly, populations far from industrial plants had higher hemocyte numbers than close populations in the north area (Fig. 2). There was also a general effect of area on level of hemocyte infection with mussels of the north area having on average more hemocytes than mussels of the south area (Fig 2). Nearness to industrial

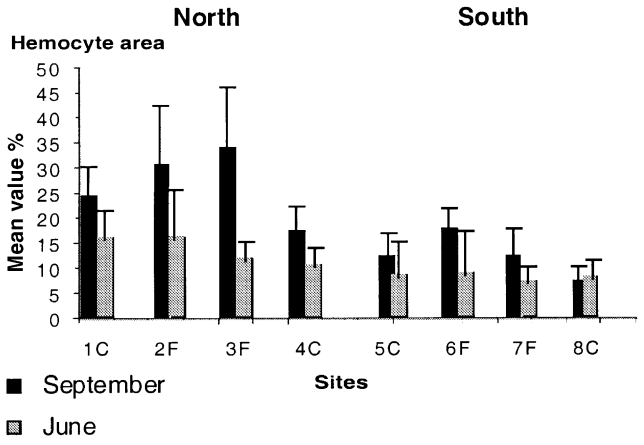

Fig. 2. Hemocyte area in eight populations of *Mytilus edulis*. "F" and "C" indicate nearness to industrial impacts (see text).

impact and area also interacted significantly in their effects on the distribution of hemocytes in the tissues (Table 3, Fig. 3). Eighteen of 80 mussels (23%) were infected by parasites and nearly all infections were metacercariae of trematod species, although one mussel from locality 7H had trematod sporocysts all over the digestive gland. An ANOVA (same model as before) revealed area as the only factor that significantly influenced the number of parasite infected mussels (Table 4). North mussels had on average more parasites than mussels from the south area (Fig. 4). The number of mussels being in different gonad developmental stages differed heavily among populations (Fig. 5) but no clear influence of season, or of area was found (Table 5). Furthermore, no relationship between gonad development and hemocyte number was found (one factor ANOVA, $p > 0.05$). The observed numbers of granulocytoma were extremely low, only six mussels of 80 were infected by granulocytoma. However, all four populations sampled near industrial plants had 1–2 infected mussels each and the difference between far and close populations were indeed statistically significant (four-factor ANOVA, model as before, $p < 0.05$ for the factor

Table 2
Effects of locality, sampling season, nearness to industrial impact (I), and area (A) on the intensity of hemocytes in 80 individuals of *Mytilus edulis* sampled along the eastern coasts of the North Sea

Source	df	SS	MS	F	P
Locality (impact × area)	4	348.35	87.09	3.74	**0.0085**
Area	1	1899.68	1899.68	81.61	**0.0001**
Impact	1	197.43	197.43	8.48	**0.0049**
Season	1	1466.56	1466.56	3.65	0.3069
Season × impact	1	128.95	128.95	0.81	0.5334
Season × area	1	401.40	401.40	8.10	**0.0465**
Impact × area	1	178.63	178.63	7.67	**0.0073**
Area × impact × season	1	159.15	159.15	3.21	0.1475
Season × locality ($I \times A$)	4	198.13	49.53	2.13	0.0875
Residual	64	1489.82	23.28		

The effects of single factors and of interactions among factors are evaluated by a four-factor ANOVA.

Table 3
Effects of locality, sampling season, nearness to industrial impact (I), and area (A) on the coefficient of variance in 80 individuals of *Mytilus edulis* sampled along the eastern coasts of the North Sea

Source	df	SS	MS	F	P
Locality (impact × area)	4	0.12	0.03	1.34	0.2644
Area	1	1.7×10^{-4}	1.7×10^{-4}	0.01	0.9316
Impact	1	0.004	0.004	0.19	0.6618
Season	1	0.05	0.05	31.85	0.1117
Season × impact	1	0.02	0.02	53.76	0.0863
Season × area	1	0.002	0.002	0.07	0.8022
Impact × area	1	0.15	0.15	6.29	**0.0147**
Season × locality ($I \times A$)	4	0.09	0.02	0.95	0.4402
Area × impact × season	1	3.64×10^{-4}	3.64×10^{-4}	0.02	0.9039
Residual	64	1.48	0.02		

The effects of single factors and of interactions among factors are evaluated by a four-factor ANOVA.

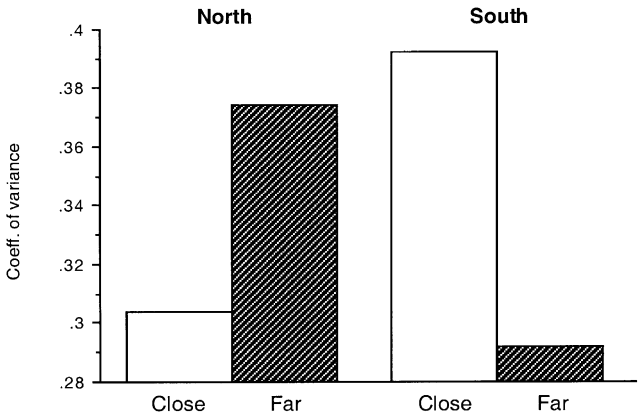

Fig. 3. Distribution of hemocytes in mussel tissue (coefficient of variation) where high variation implies an uneven distribution. "Far" and "Close" indicate nearness to industrial impacts (see text).

impact). No other pathological conditions were observed.

4. Discussion

Hemocyte numbers differed significantly among samples, and these differences were often correlated to interacting factors, for example, season and area, and area and closeness to industrial impact. The most general differences seemed to be due to mussels from the north area having more hemocytes than mussels from the south area, and to mussels sampled in September being more affected than mussels sampled in June. The difference between area correlates with salinity differences, where the north area has a higher salinity than the south area, but it is impossible to say whether this is a cause–effect relationship or not. Nearness to industrial impact have been shown to increase hemocyte numbers in natural populations (Svärdh, 1999; Wedderburn et al., 2000), and during exposure to contaminants under laboratory conditions (Coles et al., 1995). Interestingly, our results revealed no (in the south area) or an opposite relationship (in the north area) between hemocyte numbers and level of industrial contamination, with most hemocytes appearing in populations living in clean and high salinity water. We have no explanation for this, but an important consequence is that hemocyte numbers in blue mussels seem less useful as indicators of industrial impact under the conditions of our study.

Possibly because impact levels were low compared with the effects caused by variation in natural factors such as salinity (area) and season. Not only hemocyte numbers but also hemocyte distribution within mussels

Table 4
Effects of locality, sampling season, nearness to industrial impact (I), and area (A) on *Renicola roscovita* infection in eight populations of *Mytilus edulis* along the eastern coasts of the North Sea

Source	df	SS	MS	F	P
Locality (impact × area)	4	0.75	0.19	0.33	0.8570
Area	1	3.61	3.61	6.35	**0.0142**
Impact	1	0.61	0.61	1.08	0.3033
Season	1	3.61	3.61	1.71	0.4156
Season × impact	1	0.61	0.61	1.00	0.5000
Season × area	1	2.11	2.11	5.45	0.0798
Impact × area	1	0.61	0.61	1.08	0.3033
Area × impact × season	1	0.61	0.61	1.58	0.2771
Season × locality ($I \times A$)	4	1.55	0.39	0.68	0.6075
Residual	64	36.4	0.57		

The effects of single factors and of interactions among factors are evaluated by a four-factor ANOVA.

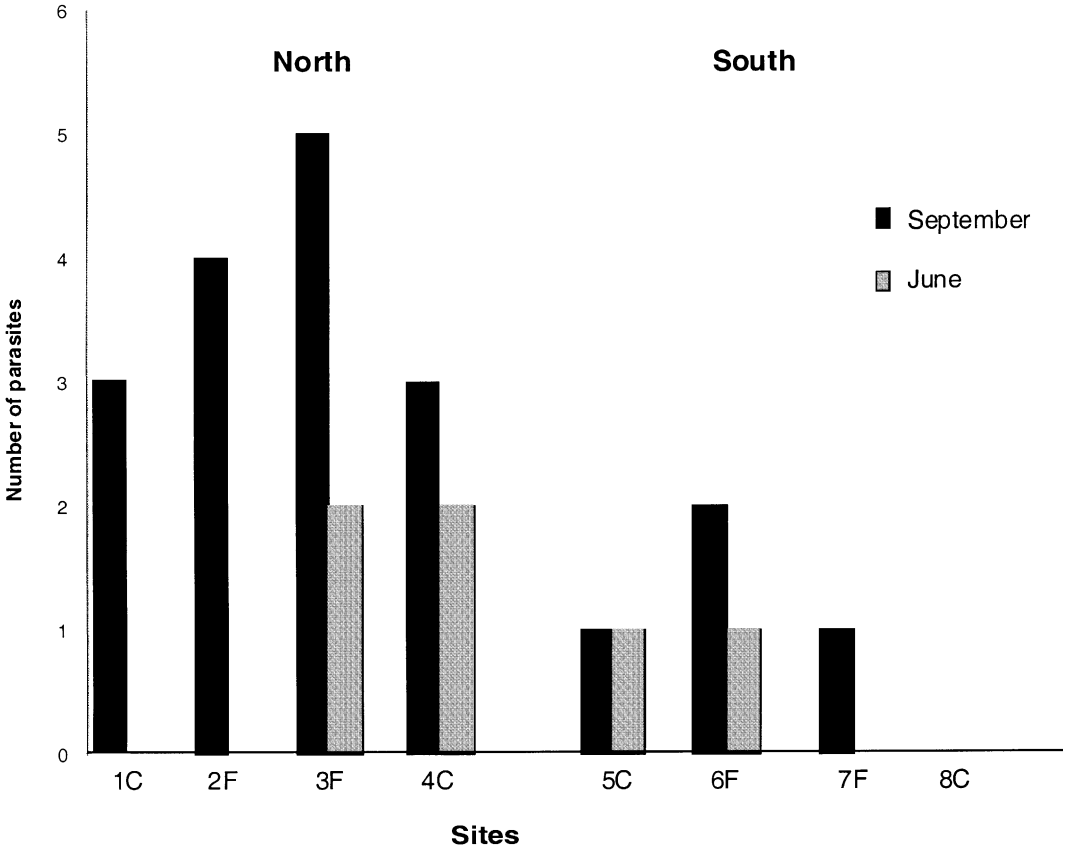

Fig. 4. Total number of parasites in *Mytilus edulis* ($n = 10$) from eight populations. "F" and "C" indicate nearness to industrial impacts (see text).

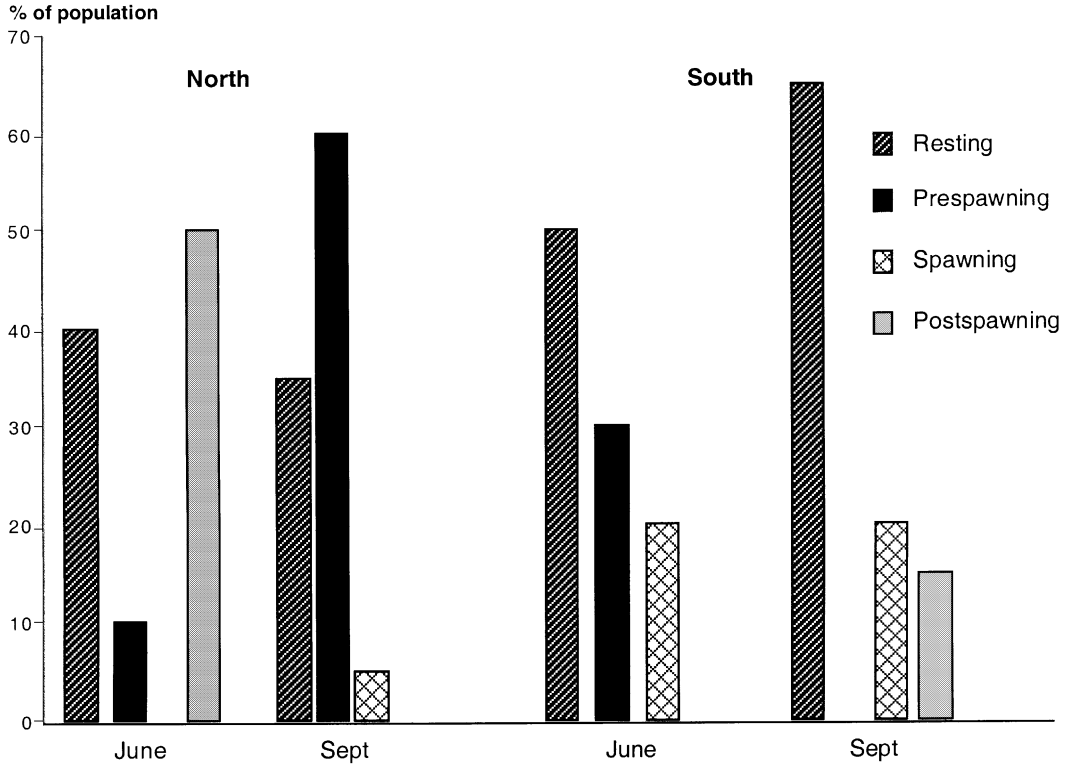

Fig. 5. The distribution of mussels over different gonad developmental stages (see Table 1). Samples were pooled into one north and one south group.

Table 5
Chi-square tests of differences in gonad development among populations (see Fig. 5)

	North/ September	South/ June	South/ September
North/June	18.2*	16.2*	11.0*
North/September		4.3 n.s.	18.6*
South/June			9.4**

* $p < 0.001$.
** $p < 0.05$.

follow this pattern with most uneven tissue distributions in mussels of high-salinity water far from industries.

However, no granulocytoma were found in the mussels from populations far from industrial plants while a few were found in each of the impacted populations (in 15% of the mussels). This supports the suggestion from Neff et al. (1987) that granulocytoma only develop in mussels exposed to contaminants. It is also in accordance with an earlier study of Danish populations of blue mussels, where a heavily polluted site had granulocytoma in 18% of the mussels, compared to 0.5% of the mussels from a non-polluted site (Svärdh, 1999).

Parasite infection is a factor that might induce hemocyte formation. In the present study parasite infection rate covaried with hemocyte numbers as the samples from the north area had higher parasite infection rates. Possible hemocyte production is stimulated by parasite infections (Carballal et al., 1998). However, the covariation of parasite and hemocyte numbers might simply be that they both increase with salinity.

Gonad development has been suggested to be important to hemocyte production. Santarem et al. (1994) found that the number of circulating hemocytes in *Mytilus galloprovinciales* was lower among post-spawning individuals than among others. Possibly hemocytes take care of spawning remains (Suresh and Mohandas, 1990). Mostly gonad development varies over season and over sites (Seed, 1976) and if hemocyte production is affected, it is critical to record the developmental stage of sampled populations as well. However, our result did not suggest any relationship between gonad developmental stage and hemocyte number. This supports the results of Carballal et al. (1998) who did not find any change in circulating hemocyte concentration during the post-spawning period.

Using hemocyte number as a tool for assessing levels of anthropogenic impacts seems not at all straightforward. Indeed, spatial and temporal variation is substantial and several factors might interact to set the intensities of various infections at a certain instance, and the relationships seem often to be complex. Mussel granulocytoma, on the other hand, seem possible indicators of anthropogenic impact also at low levels of impact such as the ones along the Skagerrak coast of Sweden and Norway. However, levels of hemocyte numbers and distribution within the mussel tissue, as well as parasite infection rates, seemed not at all indicating release of industrial wastes. Thus, careful control of variation caused by natural factors must accompany the use of any histopathological technique in each particular area.

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References

Cáceres-Martínez, J., Vásquez-Yeomans, R., Sluys, R., 1998. The turbellarian *Urastoma cyprinae* from edible mussels *Mytilus galloprovincialis* and *Mytilus californianus* in Baja California, NW Mexico. J. Invertebr. Pathol. 72, 214–219.

Cajaraville, M.P., Pal, S.G., Robledo, Y., 1995. Light and electron microscopical localization of lysosomal acid hydrolases in bivalve haemocytes by enzyme cytochemistry. Acta Histochem. 28 (5), 409–416.

Carballal, M.J., Villalba, A., López, C., 1998. Seasonal variation and effects of age, food availability, size, gonadal development, and parasitism on the hemogram of *Mytilus galloprovincialis*. J. Invertebr. Pathol. 72, 304–312.

Coles, J.A., Farley, S.R., Pipe, R.K., 1995. Alteration of the immune response of the common marine mussel *Mytilus edulis* resulting from exposure to cadmium. Dis. Aquat. Org. 22, 59–65.

Figueras, A.J., Jardon, C.F., Caldas, J.R., 1991. Diseases and parasites of rafted mussels (*Mytilus galloprovincialis* Lmk): preliminary results. Aquaculture 99, 17–33.

Neff, J.M., Hillman, R.E., Scott Carr, R., Buhl, R.L., Lahey, J.I., 1987. Histopathologic and biochemical responses in arctic marine bivalve molluscs exposed to experimentally spilled oil. Arctic 40 (Suppl. 1), 220–229.

Santarem, M.M., Robledo, J.A.F., Figueras, A., 1994. Seasonal changes in hemocytes and serum defense factors in the blue mussel *Mytilus galloprovincialis*. Dis. Aquat. Org. 18, 217–222.

Seed, R., 1969. The ecology *of Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. I. Breeding and settlement. Oecol. 3, 277–316.

Seed, R., 1976. The annual cycle and geographical variations. In: Bayne, B.L. (Ed.), Marine mussels: Their ecology and physiology. Cambridge University Press, Cambridge, pp. 22–29.

Svärdh, L., 1999. Bacteria, granulocytoma and trematode metacercariae in the digestive gland of *Mytilus edulis:* Seasonal and interpopulation variation. J. Invertebr. Pathol. 74, 275–280.

Suresh, K., Mohandas, A., 1990. Number and types of hemocytes in *Sunetta scripta* and *Villorita cyprinoides* var. *cochinensis* (Bivalvia), and leucocytosis subsequent to bacterial challenge. J. Invertebr. Pathol. 55, 312–318.

Underwood, A.J., 1997. Experiments in ecology, their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge.

Wedderburn, J., McFadzen, I., Sanger, R.C., Beesley, A., Heath, C., Hornsby, M., Lowe, D., 2000. The field application of cellular and physiological biomarkers, in the mussel *Mytilus edulis*, in conjunction with early life stage bioassays and adult histopathology. Mar. Poll. Bull. 40 (3), 257–267.

Weeks, B.A., Anderson, D.P., DuFour, A.P., Fairbrother, A., Goven, A.J., Lahvis, G.P., Peters, G., 1992. Immunological biomarkers to assess stress. In: Huggert, R.J., Kimerle, R.A., Mehrle Jr., P.M., Bergman, H.L. (Eds.), Biomarkers: Biological, Physiological, and Histological markers. Lewis Publishers, Chelsea, pp. 211–234.

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Bacteria, Granulocytomas, and Trematode Metacercariae in the Digestive Gland of *Mytilus edulis:* Seasonal and Interpopulation Variation

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During the period October 1983–September 1984, mussels were sampled at monthly intervals from three populations in Denmark. The mussels were prepared for histopathological studies and 20 individuals were randomly chosen from each month and each population for a study of the prevalence and variation of the histological changes. The aim of the study was to test the hypothesis that the prevalence of parasites, bacteria, and granulocytoma differs among blue mussel populations during a year, due to the reproductive cycle and the environment. The only bacterial infection found was *Chlamydia* sp. and this was rare. Neither sex, month, nor population was an important determinant of the prevalence of bacteria. Metacercariae of the trematode *Renicola roscovita* had a prevalence of almost 54% in a population from Lilla Baelt but only 4% in a population from Isefjorden. A statistically significant interaction between month and population indicated that the prevalence varied over months but the pattern of variation was different among populations. No other parasites were found. The prevalence of granulocytomas within populations was positively correlated with the degree of anthropogenic contamination of the localities, which might suggest a functional connection. Neither month nor sex had a significant effect. 1999 Academic Press

***Key Words: Mytilus edulis; Chlamydia* sp.; *Renicola roscovita; Littorina littorea; Larus argentatus;* parasitic; granulocytomas; anthropogenic contaminants; interactions; histopathology.**

INTRODUCTION

Histopathological studies are useful in the early diagnosis of bad living conditions for organisms because the effects of environmental stress, for example, first appear in cells and tissues. Knowledge about histopathological changes caused by microorganisms, parasites, and tumors is essential in understanding the role of sublethal effects in mussel populations living in stressed environments.

Bacteria are commonly found in invertebrate species, including blue mussels (Lauckner, 1983). Cajaraville and Angulo (1991) studied *Mytilus galloprovincialis* from the Basque coast of Spain and reported "*Chlamydia*-like organisms" in the digestive cells. Gulka and Chang (1984) found host response to ricketsial infection in blue mussels from Rhode Island, United States. Bacteria belonging to the *Ricketsia* group are known to cause diseases which can be transferred from animals to man, and viruses have been observed associated with the bacteria (Bell, 1971). Also, viral infections have been observed in mussels. Rasmussen (1986), for example, found a picorna-like virus in blue mussels from Lilla Baelt in Denmark, and a disease in oysters that causes great harm for the oyster industry is caused by an iridovirus (Comps and Duthoit, 1976).

In mussels, parasitic copepods, trematodes, and ciliates may, or may not, cause damage to the host (Villalba *et al.,* 1997). The trematode *Renicola roscovita* uses *Mytilus edulis* as second intermediate host and *Littorina littorea* as the first intermediate host. The final host is the gull *Larus argentatus* (Lauckner, 1980). In the snails, cercariae are released from sporocysts and emerge into the water. Those which are lucky enough to stream into the mantle cavity of a blue mussel can penetrate the tissues and encyst, forming the new larva stage, the metacercaria.

Phagocytic leucocytes play an important role in the immunological system of mussels (Cheng, 1983). If parasites and particles like viruses and bacteria irritate the mussel, the blood cells will probably react in some way or another. Santarem *et al.* (1994) showed a difference in the frequencies of agranular and granular bloodcells between individuals of *M. galloprovincialis* infected by the copepod *Mytilicola intestinalis* and noninfected individuals. Cáceres-Martínez and Vásquez-Yeomans (1997) found heavy hemocytic reactions with a "granuloma-like" structure engulfing parasitizing copepods, *Pseudomyicola spinosus,* in *M. galloprovincialis* and *M. californianus.*

Rasmussen (1986) found granular leucocytes in *M. edulis* forming clusters (granulocytomas) of different sizes in the hemolymph space and in the vesicular

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connective tissue in the digestive diverticula and in the mantle. A picorna-like virus infection was found associated with the granular leucocytes. Possibly, the virus is first phagocytosed by the granulocytes and then, within them, the virus multiplies and the cells aggregate to form granulocytomas. Because of the multiplication of the virus, the mussel fails to phagocytose it, and the result is, instead, an infection.

In the present study I examined the histopathological conditions of *M. edulis* from three Danish populations. The aim of the study was to test the hypothesis that the prevalence of parasites, bacteria, and granulocytoma differs among blue mussel populations during a year, due to the reproductive cycle and the environment.

MATERIALS AND METHODS

During a year (October 1983 to September 1984) samples of 300 *M. edulis* per sample were collected from each of three populations. The populations were from three localities chosen at random among populations of *M. edulis* in Danish waters: one from Isefjorden in Sjelland and the other two from Hindsgavl and Lyngs odde in Lilla Baelt. The mussels were collected monthly except for February, April, July, and August (eight samples). All 7200 mussels had a shell length of 5–7 cm. At Hindsgavl and Lyngs odde, the mussels were collected at a depth of 1 m, and in Isefjorden (Vellerup vig), the mussels were collected with a trawl at a depth of 4 m. Sampling of the mussels took place during the first week in each month and was done by people from the University of Odense.

The mussels were transported in a cool-bag to the laboratory. In 2–3 days the mussels were cut from the shell and transverse sections containing the stomach and parts of the gill were placed in Baker's fixative. The sections, approximately 5 mm thick, were then rinsed briefly in distilled water, embedded in wax, and dehydrated through an ascending alcohol series. The embedded tissues were cut into 7-µm sections using a rotary microtome and transferred to microscope slides. The tissues were stained with Harris' hematoxylin/eosin. All preparations were done at the laboratory of the University of Odense and I received the mussel slides from Dr. Rasmussen. From each population and each month I then randomly picked slides from 20 mussels, which makes 480 slides, each prepared from 1 mussel.

I examined the slides with a light microscope and histopathological changes of the tissues were assessed. The whole area of each preparation was studied and all bacteria colonies, granulocytomas, and metacercariae were counted. The identification of bacterial infections, granulocytomas, and metacercariae were confirmed to species by Dr. Rasmussen (pers. comm.) and were consistent with the descriptions given in the literature of *Ricketsia* (Lauckner, 1983; Azevedo and Villalba, 1990), *Chlamydia* (Lauckner, 1983), granulocytomas (Lauckner, 1983; Rasmussen, 1985), and metacercariae (Lauckner, 1983). I use the terminology defined by Margolis *et al.* (1982) (in Calvo-Ugarteburu and McQuaid, 1998) in which prevalence refers to the number of mussels containing metacercariae, bacteria, or granulocytomas, divided by the number of mussels examined; and intensity refers to the number of metacercariae, defined bacterial infections, or defined granulocytomas per mussel.

Statistical Analysis

Three orthogonal factors were tested: the random factor "population" with three levels and the fixed factors "month" and "sex" with eight and two levels, respectively. I analyzed the results from the observations using a three-way ANOVA, testing for effects of the individual factors and their interactions. If there were no interactions, I tested the effects of sex, month, and population on prevalence and intensity of infections. Post-hoc (SNK) tests were used to assess differences among levels of a significant factor.

RESULTS

The bacterial infections observed were all inclusions of *Chlamydia* and were found in the tubuli wall of the digestive gland. Only single infections of each tubule were observed. The infection seemed to replace one of the cells in the tubuli wall. The intensity of *Chlamydia* was very low (Fig. 1). In the Isefjord population, only one male mussel from October was infected. In both populations from Lilla Baelt 3% of the mussels were infected. The analysis of variance revealed no significant factor effect or interaction, and thus neither sex, month, nor population were important determinants of the intensity of bacterial infections (Table 1).

The intensity of granulocytomas revealed no significant interactions of month and sex, nor among the

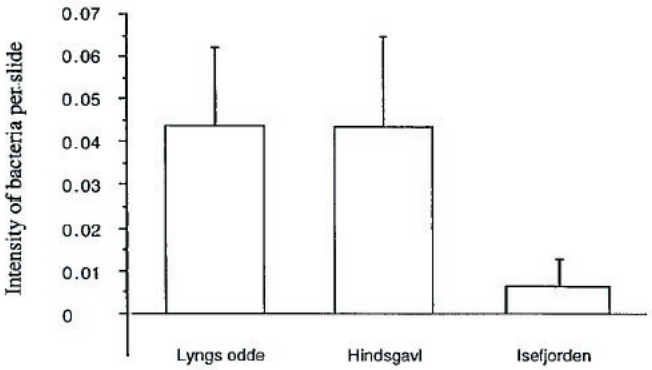

FIG. 1. Intensity of *Chlamydia* infections in *Mytilus edulis* from three populations in Denmark. Mean values and standard error bars. $N = 160$ for each population.

TABLE 1

Effects of Population, Sex, and Month on the Prevalence of *Chlamydia* Infection in 480 Individuals of *Mytilus edulis* from Three Populations in Denmark, Analyzed by a Three-Factor ANOVA

Source	*df*	SS	MS	*F*	*P*	Error term
Population, P	2	0.13	0.07	1.53	0.22	Residual
Sex, S	1	0.09	0.09	6.25	0.13	P × S
Month, M	7	0.24	0.03	0.55	0.78	P × M
P × S	2	0.03	0.01	0.35	0.71	Residual
P × M	14	0.88	0.06	1.47	0.12	Residual
S × M	7	0.15	0.02	0.69	0.68	P × S × M
P × S × M	14	0.43	0.03	0.71	0.76	Residual
Residual	432	18.54	0.04			

factors population, month, and sex, but there was a difference in intensity among the populations (Table 2). The lowest prevalence was found in mussels from Isefjorden (0.6%) and the highest in mussels from Lyngs odde (17.5%) (Fig. 2).

The Lyngs odde population had a peak in the intensity of metacercariae in September (Fig. 3), and in this case the interaction between the factors population and month was significant (Tables 3 and 4). The metacercariae found were larvae of the trematode *R. roscovita.* The prevalence was lowest in the population from Isefjorden (4.4%) and highest in the Lyngs odde population (53.8%). In mussels with metacercariae, the intensity of infection varied from 22 metacercariae per slide (in a female from Lyngs odde in June) to 1 per slide.

In none of the tests did sex have a significant effect on the occurrence of the different tissue changes. There was, furthermore, no significant interaction between sex and any other factor.

DISCUSSION

The granulocytoma condition is believed to result from chronic exposure to domestic and industrial waste products (Bayne *et al.,* 1980) and several studies support this theory. Neff *et al.* (1987) studied histopathological effects on mussels after experimental oil spills and found, among other effects, that 1 year after the spill, a few specimens of *Mya truncata* had granulocytomas throughout the tissues. The authors concluded that acute effects of dispersed oil are greater than those of undispersed oil but that the effects of undispersed oil on the infauna develop more slowly and persist longer than those of dispersed oil. In studies of the acute and toxic effects of *N*-nitroso compounds (which have been found as contaminants in comercially important products) on *M. edulis,* Rasmussen (1983) found granulocytomas in the digestive gland of mussels examined 12 weeks after injections with *N*-nitrosodimethylamine and in mussels 8 and 10 weeks after injections with the vertebrate carcinogen *N*-nitrosodipropylamine. Granulocytomas seems to be a chronic rather than an acute effect of toxins. In Rasmussen's study some acute tissue destruction was seen to lead to the formation of granulocytomas in the long-term experiments (Rasmussen, 1985). Figueras *et al.* (1991) also found granulocytomas

TABLE 2

Effects of Population, Sex, and Month on the Prevalence of Granulocytomas in 480 Individuals of *Mytilus edulis* from Three Populations in Denmark, Analyzed by a Three-Factor ANOVA

Source	*df*	SS	MS	*F*	*P*	Error term
Population, P	2	2.48	1.24	16.91	<0.05	Residual
Sex, S	1	0.07	0.07	4.63	0.16	P × S
Month, M	7	0.66	0.09	0.86	0.56	P × M
P × S	2	0.03	0.01	0.19	0.82	Residual
P × M	14	1.53	0.11	1.49	0.11	Residual
S × M	7	0.36	0.05	0.64	0.71	P × S × M
P × S × M	14	1.12	0.08	1.09	0.36	Residual
Residual	432	31.67	0.07			

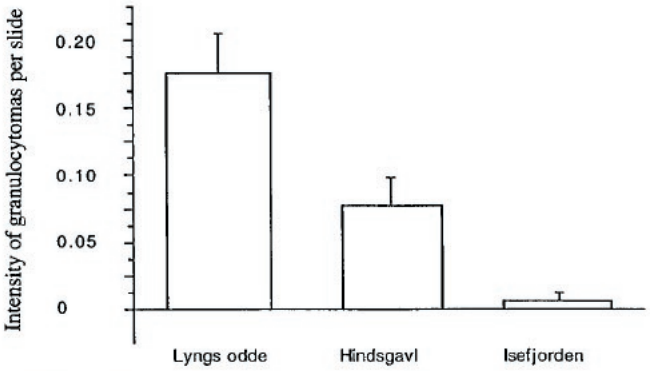

FIG. 2. Intensity of granulocytomas in *Mytilus edulis* from three populations in Denmark. Mean values and standard error bars. $N = 160$ for each population.

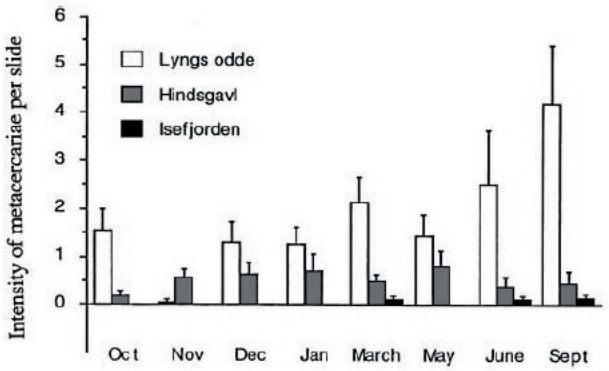

FIG. 3. Intensity of *Renicola roscovita* metacercariae in *Mytilus edulis* from three populations in Denmark. Interactions between month and population. Mean values and standard error bars. $N = 20$ per month.

TABLE 3

Effects of Population, Sex, and Month on the Prevalence of *Renicola roscovita* Metacercaria in 480 Individuals of *Mytilus edulis* from Three Populations in Denmark, Analyzed by a Three-Factor ANOVA

Source	*df*	SS	MS	*F*	*P*	Error term
Population, P	2	264.21	132.11	38.59	0.00	Residual
Sex, S	1	1.24	1.24	0.99	0.42	P × S
Month, M	7	66.07	9.44	1.05	0.44	P × M
P × S	2	2.50	1.25	0.37	0.70	Residual
P × M	14	126.44	9.03	2.64	0.00	Residual
S × M	7	29.79	4.26	0.99	0.47	P × S × M
P × S × M	14	59.95	4.28	1.25	0.24	Residual
Residual	432	1478.83	3.42			

in studies of rafted mussels from the northwest of Spain. Six percent of the mussels had granulocytomas and the condition did not appear to be associated with a detectable pathogenic agent. The samples were taken from commercial mussel rafts in the Ria de Arousa area of Galicia. Furthermore, the mussels were heavily infested by parasites but the authors did not associate this with the granulocytomas. Real *et al.* (1993) found considerable discharges of chrome and copper in sediments in the Ria de Arousa estuary. In the present study, the populations differed significantly in prevalence of granulocytomas. Possibly, the differences between the localities in degree of anthropogenic contamination are important. Although the Lilla Baelt localities were in the same area, there were more industrial plants at Lyngs odde than at Hindsgavl at the time of sampling, e.g., chemical, metal, and cellulose industries and an oil refinery and tanks with ammonia (Rasmussen, pers. comm.). Isefjorden had the clearest water and was used for collecting mussels for consumption and for an oyster hatchery. There might be a correlation between the prevalence of granulocytomas and anthropogenic contaminants. A further study in which these correlations will be tested is in progress.

There was an interaction between the effects of month and population on the intensity of metacercariae of *R. roscovita*. This suggests that there is a variation in the intensity of metacercaria over the year, which probably can be associated with the reproductive cycle of mussels, but the variation is not the same in all populations. Differences among mussel populations include environmental differences in, e.g., water temperature and currents and in the occurence of gulls and snails. In a study from 1975 to 1976 of trematod infection in *L. littorea* from an exposed shore in North Wales, samples were taken monthly. *R. roscovita* was the most frequent parasite with the highest occurence in July–August and the lowest in December–February (Hughes and Answer, 1982). According to Fjälling *et al.* (1980), sporocysts can produce cercariae throughout the year but the cercariae have a lifetime of only a few days. Pekkarinen (1988) studied the development from cercaria to metacercaria of the gymnophallid trematode *Lacunovermis macomae* in *Macoma baltica* and she found that the penetration capacity was good for at least 4–6 days after release from sporocysts. The experiments were done at room temperature. When the temperature was changed to 4–8°C, the invasive behavior persisted for at least 2 weeks. So, mussels can be parasitized throughout the year if the environmental conditions favor host finding. Metacercariae can live for a long time, a year or more, in the second intermediate host (Pekkarinen, 1988) but in the spring one can find dead metacercariae in the labial palps of mussels and the cause of that is probably freezing (Lauckner, 1983). All three localities in my study have low winter temperatures (1–4°C) and high summer temperatures (17–19°C) but the mussels from Isefjorden were taken from a deeper-living population and maybe those mussels are affected by less variation in temperature.

The cercariae use different swimming behaviors and orientation mechanisms to find their hosts, and these host-finding strategies are highly specific and sensitive to various chemical and physical cues of the hosts (Haas *et al.,* 1990). The cercaria of *R. roscovita* is a bad swimmer, according to Werding (1969), and probably relies on water currents to approach mussels. *R. roscovita* cercariae released from snails just circulate, as if

TABLE 4

Renicola roscovita Metacercariae in *Mytilus edulis* from Three Populations in Denmark

Population	Month	Number of ind.	Mean	SE
Lyngs odde	January	20	1.25	0.37
	March	20	2.15	0.50
	May	20	1.45	0.44
	June	20	2.50	1.12
	September	20	4.20	1.20
	October	20	1.55	0.44
	November	20	0.05	0.05
	December	20	1.30	0.43
Hindsgavl	January	20	0.70	0.37
	March	20	0.50	0.14
	May	20	0.80	0.33
	June	20	0.40	0.17
	September	20	0.45	0.26
	October	20	0.20	0.09
	November	20	0.55	0.20
	December	20	0.65	0.22
Isefjorden	January	20	0	0
	March	20	0.10	0.07
	May	20	0	0
	June	20	0.10	0.07
	September	20	0.15	0.08
	October	20	0	0
	November	20	0	0
	December	20	0	0

Note. Mean values and standard error of the means among the effects of population and month.

searching the surrounding water for something to penetrate (pers. obs). According to Lauckner (1983), the location of *R. roscovita* metacercaria in *M. edulis* is determined by the size of the mussel and the space in the organs available for encystment. The space available, in my opinion, is influenced by age, sex, and reproductive phase of the host. Depending on the time of the year and the reproductive phase of the mussel, there is more or less space in the tissues due to the thickness of the mantle. The genital tissue invades the whole body of the mantle and in the fully ripe condition, the eggs and sperm fill up most of the connective tissue.

Metacercariae of the digenetic trematode *Proctoeces* sp. infected significantly more females than males in a study of parasites in the mussel *Perna perna* from the South African coast, and the prevalence was significantly dependent on the size of the females but not on the male size (CalvoUgarteburu and McQuaid, 1998). Possibly, it is advantageous for parasites to target individuals which have sequestered substantial energy stores in a discrete organ (McQuaid pers. comm.). In my study mussel sex had no effect on intensity of *R. roscovita*. But, unlike CalvoUgarteburu and McQuaid, I looked at just the digestive gland, not at the labial palps or the gonads. The mussels were rather large (5–7 cm) and they represented all reproductive stages. Therefore, I cannot say anything about the total metacercarial occurence over a year in this mussel. To do that, it is necessary to know how to separate the newly encysted metacercariae from "old" ones and to study all organs in the mussels.

In *Mercenaria mercenaria* from Chesapeake Bay in the USA, chlamydial infections were found and no defending response of the host was observed. Bacterial inclusions were very rare and at the level of the microscopic section only one inclusion in a single tubule was seen. The bacterial inclusions are described as "up to 100 μm in greatest diameter, which had dilated the host cell to nearly 5 times its normal width and had pressed the nucleus and normal cytoplasmic organelles against the plasma membrane." In another study of clams from Long Island, heavier chlamydial infections were found. In females the average number of chlamydial colonies was 32 per microscopic section and in the males the average number was 23. In the most heavily infected clams, 15 to 20% of the tubuli were damaged. Despite that, no sign of reduced absorptive efficiency of the digestive tubuli was seen (for references see Lauckner, 1983). In my study I saw the bacterial infections replacing one of the tubuli cells but I did not see the nucleus or the organelles. As in the Chesapeake Bay clams, there were few bacterial infections and only one in each tubule and it seemed that no harm was done to the hosts at the tissue level. As with metacercariae, it is probably the intensity of bacterial infections that decide whether the bacteria will damage the tissues or not. It is very important to control the presence of *Chlamydia* in shellfish, because, when adults are infected, there is a great risk that the bacterial agents will spread to the larvae *en masse,* and that can lead to infections of great harm for seafood farming (Leibovitz, 1989). Perhaps, there is also a connection between chlamydial infections in mussels and in shorebirds and a possibility that bivalves are carriers of potential pathogens that can be transferred to birds and mammals. Shorebirds are common in mussel beds and since avian chlamydiosis can be transmitted to humans (Schlossberg *et al.,* 1993) we should, perhaps, be more concerned about the occurence of chlamydial infections in mussels.

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REFERENCES

Azevedo, C., and Villalba, A. 1990. "Fine Structure of *Ricketsiae* with Associated Bacteria in the Oyster Gill Epithelium." 4th Int. Colloq. Pathol. Marine Aquacul., Vigo, Spain.

Bayne, B. L., Brown, D. A., Harrison, F., and Yevich, P. P. 1980. Mussel health. *In* "The International Mussel Watch," pp. 163–235. National Academy of Sciences, Washington DC.

Bell, J. F. 1971. Q (Query) fever. *In* "Infectious and Parasitic Diseases of Wild Birds" (Anderson, Karstad, and Trainer, Eds.), pp. 170–171. The Iowa State Univ. Press, Ames, IO.

Cáceres-Martínez, J., and Vásquez-Yeomans, R. 1997. Presence and histopathological effects of the copepod *Pseudomyicola spinosus* in *Mytilus californianus. J. Invertebr. Pathol.* **70,** 150–155.

Cajaraville, M. P., and Angulo, E. 1991. Chlamydia-like organisms in digestive and duct cells of mussels from the Basque coast. *J. Invertebr. Pathol.* **58,** 381–386.

Calvo-Ugarteburu, G., and McQuaid, C. D. 1998. Parasitism and introduced species: Epidemiology of trematodes in the intertidal mussels *Perna perna* and *Mytilus galloprovincialis. J. Exp. Mar. Biol. Ecol.* **220,** 47–65.

Cheng, T. 1983. Triggering of immunologic defence mechanisms of molluscan shellfish by biotic and abiotic challenge and its applications. *J. Mar. Tech. Soc.* **17,** 18–25.

Comps, M., and Duthoit, J.-L. 1976. Infection virale associée à la "maladie des branchies" de l'huître portugaise *Crassostrea angulata* Lmk. *C. R. hebd. Séanc. Acad. Sci.,* Paris (Ser. D) **283,** 1595–1596.

Figueras, A. J., Jardon, C. F., and Caldas, J. R. 1991. Diseases and parasites of rafted mussels (*Mytilus galloprovincialis* Lmk): Preliminary results. *Aquaculture* **99,** 17–33.

Fjälling, A., Kolsäter, L., and Thulin, J. 1980. A parasitological investigation of wild and cultured mussels, *Mytilus edulis,* in the Tjärnö area, Bohuslän, Sweden. *Medd. Fr. Havsfiskelab. Lysekil* **263,** 1–24.

Gulka, G., and Chang, Pei Wen. 1984. Host response to ricketsial infection in blue mussel, *Mytilus edulis* L. *J. Fish Diseases* **8,** 319–323.

Haas, W., Granzer, M., and Brockelman, C. R. 1990. Finding and

recognition of the bovine host by the cercariae of *Schistosoma spindale*. *Parasitol. Res.* **76,** 343–350.

Hughes, R. N., and Answer, P. 1982. Growth, spawning and trematode infection of *Littorina littorea* (L.) from an exposed shore in North Wales. *J. Moll. Stud.* **48,** 321–330.

Lauckner, G. 1983. *In* "Diseases of Marine Animals" (O. Kinne, Ed.), Vol. I., Chap. 12. Biologische Anstalt Helgoland, Hamburg, Germany.

Lauckner, G. 1983. *In* "Diseases of Marine Animals" (O. Kinne, Ed.), Vol. II, Chap. 13. Biologische Anstalt Helgoland, Hamburg, Germany.

Leibovitz, L. 1989. Chlamydiosis: A newly reported serious disease of larval and postmetamorphic bay scallops, *Argopecten irradians* (Lamarck). *J. Fish Diseases* **12,** 125–136.

Neff, J. M., Hillman, R. E., Carr, R. S., Buhl, R. L., and Lahey, J. I. 1987. Histopathologic and biochemical responses in Arctic marine bivalve molluscs exposed to experimentally spilled oil. *Arctic* **40 (Suppl. 1),** 220–229.

Pekkarinen, M. 1988. "Gymnophallid Trematode Parasites in the Baltic Clam *Macoma balthica* (L.) off the Southwest Coast of Finland." Academic dissertation, Univ. of Helsinki, Finland.

Rasmussen, L. P. D., Hage, E., and Karlog, O. 1983. Light and electron microscopic studies of the acute and chronic toxic effects of *N*-nitroso compounds on the marine mussel, *Mytilus edulis* (L.). I. *N*-nitrosodimethylamine. *Aquat. Toxicol.* **3,** 285–299.

Rasmussen, L. P. D., Hage, E., and Karlog, O. 1985. Light and electronmicroscopic studies of the acute and long-term toxic effects of *N*-nitrosodipropylamine and *N*-methylnitrosurea on the marine mussel *Mytilus edulis*. *Mar. Biol.* **85,** 55–65.

Rasmussen, L. P. D. 1986. Occurence, prevalence and seasonality of neoplasia in the marine mussel *Mytilus edulis* from three sites in Denmark. *Mar. Biol.* **92,** 59–64.

Real, C., Barreiro, R., and Carballeira, A. 1993. Heavy metal mixing behaviour in estaurine sediments in the Ria de Arousa (NW Spain). Differences between metals. *Sci. Total Environ.* **128,** 51–67.

Santarem, M. M., Robledo, J. A. F., and Figueras, A. 1994. Seasonal changes in hemocytes and serum defense factors in the blue mussel *Mytilus galloprovincialis*. *Dis. Aquat. Org.* **18,** 217–222.

Schlossberg, D., Delgado, J., Moore, M. M., Wishner, A., and Mohn, J. 1993. An epidemic of avian and human psittacosis. *Arch. Intern. Med.* **153,** 2594–2596.

Villalba, A., Mourelle, S. G., Carballal, M. J., and Lopez, C. 1997. Symbionts and diseases of farmed mussels *Mytilus galloprovincialis* throughout the culture process in the Rias of Galicia (NW Spain). *Dis. Aquat. Org.* **31,** 127–139.

Werding, B. 1969. Morphologie, entwicklung und ökologie digener trematoden-larven der strandschnecke *Littorina littorea*. *In* "Diseases of Marine Animals," Vol. II, Chap. 13. Biologische Anstalt Helgoland, Hamburg, Germany.

IS THE GLYCOGEN CONTENT OF BLUE MUSSELS (*MYTILUS* SPP.) A GOOD INDICATOR OF MUSSEL REPRODUCTIVE ACTIVITIES?

Abstract

The bivalve Mytilus edulis has specific tissue in the mantle for storage of glycogen. The glycogen is stored during periods of high primary production and is then used for the gametogenesis. In this way the glycogen content is linked to the reproductive cycle. The purpose of the present study was to assess the relationship between glycogen content and reproductive stage of blue mussels over different seasons, in order to be able to predict reproductive stages from glycogen content. Over one year of measurements the mean glycogen content of mussels from two Swedish (Mytilus edulis) and one Spanish (M. galloprovincialis) population correlated well with the stage of the reproductive cycle. The mussels in resting stages had the highest glycogen values while the mussels in the stage just before spawning had the lowest values. It is suggested that glycogen content may be used as an indicator of gonad ripeness, and as such useful in mussel aquaculture. However, further details of the mechanisms relating glycogen synthesis and gonad development must first be disclosed.

Keywords: aquaculture; glycogen; *Mytilus edulis*; *Mytilus galloprovincialis*; reproduction; season

Introduction

Along the Swedish west coast, the blue mussels *(Mytilus edulis)* have been observed to spawn mainly at one occasion of the year (late spring), when the water temperature has reach about 10°C, but additional spawnings might follow during summer. The meat content of the mussels may vary greatly between pre- and post-spawning stage (Loo and Rosenberg 1983), and for mussel farmers it is therefore very important to predict the time of spawning. In theory, the maximum meat content occurs just before spawning and to optimize harvesting the pre-spawning stage must be predicted. Another period of large meat content in blue mussels in western Sweden is during the late autumn, probably as an effect of the autumn phytoplankton blooming. Loo and Rosenberg (1983) recommend harvest during late autumn, but, in recent years this autumn harvest has been cancelled, due to blooms of harmful toxic algae that contaminate the mussels.

Mytilus edulis has specific tissues in the mantle for storage of glycogen and there are two types of cells for this purpose. Adipogranular (ADG) cells are developed from granulocytes and these cells synthesise proteins and glycogen. Vesicular connective tissue cells (VCT) are specialised for glycogen storage, with a specific enzyme probably involved in the storage. During the gametogenesis (development of reproductive tissue including eggs and sperm) the energy is supplied from the stored glycogen and therefore the glycogen content in the tissues decreases during the formation of gametes (Mathieu and Lubet 1993). In blue mussels from the Waddenzee the glycogen content increased during periods of abundant food (summer and early autumn) and the variation in glycogen content among individual mussels was greatest in the mantle (de Zwaan and Zandee 1972). It seems probable that the variation in mantle content of glycogen was due to the mantle being the tissue for gonad development and different individuals will have reached different reproductive stages. A comparison between glycogen content and gonad development suggested that the metabolism of glycogen and the gametogenesis are linked and both controlled by food supply and temperature (Gabbott 1975). Consequently, as an alternative to histological analysis, it would be possible to determine the reproductive stage in mussels by measuring the glycogen content.

The main purpose of the present project was to relate the glycogen content to the reproductive stage of Swedish blue mussels, with the specific aim of gathering information for the construct of a type of standard curve relating mean glycogen content per month to the reproductive stage of Swedish blue

mussels. In this study also a population of Spanish mussels *(M. galloprovincialis)* was included to assess how the relationship between glycogen and reproductive stage is established in populations with repeated spawning periods over the year. The timing of the reproductive cycle in populations of Swedish *Mytilus edulis* has not been described before, and this study is the first to report the pattern of this cycle together with the correlation to the glycogen content.

Material and methods

During ten months (August 2000 – June 2001), blue mussels were collected at two locations in Sweden (*Mytilus edulis*, Fig. 1) and one in Spain (*M. galloprovincialis,* Fig. 1). All mussels were from aquacultures and two years old at the start of the experiment. The mussels were transferred from the farming ropes to cages, and the cages in Sweden were placed in two bays (Tjärnö and Grebbestad, Fig. 1). In Spain the cages were hung from a raft in the Arousa Bay (Fig. 1). Two sets of five cages, each cage containing twenty mussels, were placed at each locality. In each set, the cages were placed on top of each other.

Every two months, mussels were sampled from the sets, starting from the top cages in August 2000, to bottom of the sets in June 2001 (1 m of depth). Samples were transported to the laboratory for measurements and tissue sampling. Pieces of tissue were cut from the digestive gland and the mantle. Part of the tissue was frozen (-70°C) for glycogen analysis and the remaining part fixed in Davidsen´s solution for histological examination of gonad development. The glycogen content was analyzed with a spectrophotometric method (starch kit, cat.no. 207748, Boehringer, Mannheim). The gonad development was determined from histological cuts (according to the methods described in Svärdh and Johannesson 2002) using the gonad index by Seed (1969) indicating four more or less discrete reproductive periods (Table 1).

After data have been balanced by random displacement of observations, differences in effects on the glycogen content between cages and between sets were analysed, using an analysis of variance (one-factor ANOVA). Using a balanced two-factor ANOVA, interaction effects of locality (a random factor with two levels) and reproductive stage (fixed, four levels) (Table 2 a) were analysed regarding the Swedish mussels. Also the interaction effects of locality and sampling month were analysed (Table 2 b). A one-factor ANOVA was used to analyse differences of the factor reproductive stage on glycogen content within months.

Regarding the Spanish mussels, effects of sampling month and reproductive stage were analysed (one-factor ANOVA). (Table 3 a and 3 b).

Results

In both the Swedish and the Spanish populations, there is large variation in reproductive development among individual mussels (Fig. 2 a, 2 b and 2 c). However, the interactions between the effects of locality, reproductive stage and month on the glycogen content of mussels were all non-significant (Tables 2 and 3). On the other hand, there were significant effects of the two single factors, reproductive stage and month, on the glycogen content on the mussels from all three localities. Thus, for example, although most mussels of the two Swedish populations are in post-spawning or resting stage in August and October, a number of mussels are in the pre-spawning stage. This indicated that the mussels were mostly at the end of the gametogenesis, but there were still some individuals that had not yet spawned in October. The developing phase is mainly expressed during winter and spring months (January and April) in the Swedish populations (Fig. 2a and 2 b). On the other hand, the Spanish population had this year the main period of gametogenesis during the autumn (October) (Fig. 2 c). A further difference between

Sweden and Spain is that Spanish mussels seem to have a less established period of histological resting (Fig 2 c), while large proportions of the Swedish mussels rest in August-January. These differences in the timing of gametogenesis are reflected also in differences in glycogen contents of the mussels over the sampling-period. While the Swedish mussels increase in glycogen content during the summer (June-October, Fig. 3), the Spanish mussels have a period of glycogen storage during the winter and early spring (January-April, Fig. 3).

Grouping mussels by reproductive stage, the relationship between glycogen content and reproduction becomes even clearer with very similar curves among populations in both Sweden and Spain (Fig. 4). In all three populations the glycogen content was highest in the mussels during the resting stage (96 – 120 mg glycogen g^{-1} wet weight) and lowest in the pre-spawning stage (13 – 33 mg g^{-1} w.w.). On a small scale, there were differences in glycogen content between mussels of different cages in all localities ($p_{Sweden} < 0.01$, $p_{Spain} < 0.01$) although there was no effect of set of cages.

Thus overall, reproductive stage of individual mussels rather than season explained most of the variation in glycogen content in the populations from both Spain and Sweden, and the seasonal variation in glycogen content is mostly explained by the seasonality (although not absolute) of the reproductive cycle.

Discussion

Adipogranular (ADG) cells store energy reserves (e.g. glycogen) in blue mussels. When Villalba (1995) studied the variation of ADG cell abundance in the mantle of Spanish *M. galloprovincialis* from Arousa Bay, he found glycogen content in mussels from different bays to be strongly correlated to the reproductive stages. In another study of Spanish *M. galloprovincialis*, Cáceres-Martínez and Figueras (1998) compared the stored reserves with the reproductive pattern and they also found these two parameters to have a strong positive correlation in mussels from different localities in one bay. In the present study of Swedish populations of *M. edulis*, the glycogen content correlated well with the different reproductive stages in both localities studied, as well as in the Spanish population of *M. galloprovincialis*. Thus my study supports the hypothesis that the reproductive cycle follows the pattern of storage reserves regardless of species or locality.

There is no description in the literature about the spawning period of Swedish blue mussel populations, but studies of larval settling (Wiigh-Mäsak 1982, Loo and Rosenberg 1983) indicate that spawning occurs during April – July. The same results have been reported from other studies of mussels from high latitudes. Norwegian *M. edulis* have their main spawning period from April to July, but a second spawning may occur (see e.g. Barkati 1989 – 1990). In the present study, large parts of the Swedish individuals were still in the pre-spawning stage in October 2000, and this might indicate that some mussels delay their spawning, or alternatively, that a minor amount of the mussels spawn twice each year. In general, however, the mussels of the present study spawned during the summer and the gonad development and consequently glycogen reduction proceeded over much of the autumn, winter and spring seasons. Cáceres-Martínez and Figueras (1998) found an increase in the volume of storage cells in the mantle of Galician *M. galloprovincialis* during summer and early autumn. Similarly, Villalba (1995) concluded that the gametogenesis of *M. galloprovincialis* from Galician bays is in progress during the late autumn and early winter. The Spanish population I studied showed decreasing glycogen levels and a high proportion of the mussels in the developing stage of reproduction late autumn and winter, that is, the same period of the year as suggested by Villalba (1995). Thus, in summary, Swedish and Spanish mussels all start the gametogenesis in autumn, but the gametogenesis of the Swedish mussels seems to progress more slowly over the whole winter period, while the gametogenesis of the Spanish mussels ended in early winter and then started again after a winter spawning period. This conclusion is also supported by the observation that no developing gonads are visible in the Swedish mussels during the autumn, while the Spanish mussels show developing gonads already in August. A rapid build up of new gametes may occur following the first spawning of Spanish *M. galloprovincialis*,

or if spawning conditions is restored repeatedly, several spawns might follow (Cáceres-Martínez and Figueras 1998).

Overall it seems as if the Spanish*M. galloprovincialis* rely more on a winter to early spring primary production, while the Swedish mussels use the summer peaks of primary production to rebuild glycogen stores. The increase in glycogen content of mussels is obviously dependent on food availability, which in turn is dependent on the availability of nutrients and light. The difference in spawning time of blue mussels between Sweden and Spain is likely a difference between seasonal peaks of primary production and temperature. However, *Mytilus edulis* from Iceland, spawned between mid July and mid August during the years 1986 and 1987, despite low chlorophyll *a* levels (< 1 □g l^{-1}) during the six months preceding the gametogenesis (Thorarinsdóttir 1996). As a comparison, the mean levels of chlorophyll at the Swedish sites, were 4 – 8 □g l^{-1} during August to October 2000 (BVVF 2000), the period before the onset of the gametogenesis. The chlorophyll content in Swedish waters varies over seasons and between years, and thus the length and quality of the period of gametogenesis is likely to be unpredictable. Moreover, the chlorophyll supply probably varies on a spatial scale, resulting in corresponding variation in glycogen content. Rightly, average availability of primary production of a site is already recognized as an important factor when locating mussel farms, and the glycogen content of mussels might be a good indicator of this variation. Thus extended assessments of mussel glycogen content and reproductive development is essential both on spatial and temporal scales, as well as further studies of the mechanisms of the glycogen synthesis and the gonad development.

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References

Barkati S. 1989 – 90. Reproduction of the mussel *Mytilus edulis* L. from Lindaspollene, western Norway. Oebalia XVI: N.S., 1 – 14.

BVVF, 2000. Rapport från Bohuskustens Vattenvårdsförbund, Uddevalla, Sweden.

Cáceres-Martínez J. and **Figueras A.** 1998. A long-term survey on wild and cultured mussels (*Mytilus galloprovincialis* Lmk) reproductive cycles in the Ria de Vigo (NW Spain). Aquaculture 162: 141 – 156.

Gabbott P. A. 1975. Storage cycles in marine bivalve molluscs: A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: H. Barnes (Ed.), Proc. 9th Europ. Mar. Biol. Symp., Aberdeen University Press, pp. 191 – 211.

L.-O. Loo, *Benthic-pelagic coupling in a boreal marine ecosystem.* (Academic dissertation). Göteborg: Göteborg University, 1991.

Loo L.-O. and **Rosenberg R.** 1983. Settling of *Mytilus edulis* at some localities on the Swedish west coast in 1981 and 1982. Medd. fr. Havsfiskelab., Lysekil, Sweden 290: 1 – 11. In Swedish with an abstract in English.

Seed R. 1969. The ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. I. Breeding and settlement. Oecol. 3: 277 – 316.

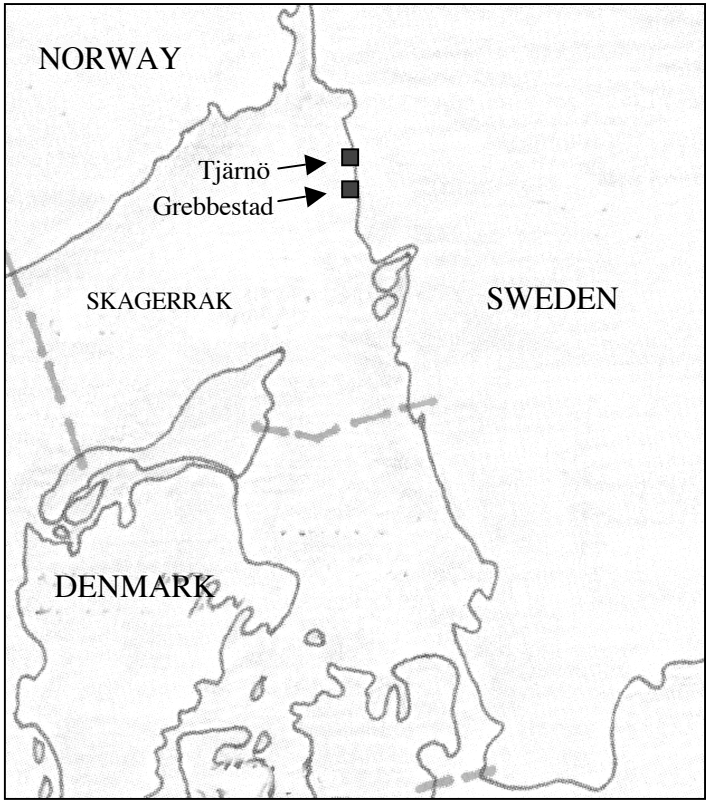
Svärdh L. and **Johannesson K.** 2001. Hemocyte and parasite intensities in coastal populations of blue mussels (*Mytilus edulis*) – testing correlations with area, season and distance to industrial impacts. J. Invertebr. Pathol. 80: 22 – 28.

Thorarinsdottir G. G. 1996. Gonad development, larval settlement and growth of *Mytilus edulis* L. in a suspended population in Hvalfjördur, south-west Iceland. Aquacult. Res. 27: 57 – 65.

Villalba A. 1995. Gametogenic cycle of cultured mussel, *Mytilus galloprovincialis*, in the bays of Galicia (N.W. Spain). Aquaculture 130: 269 – 277.

Wiigh-Mäsak P. 1982. Season-dependent dynamics in mussel cultures in Mollösund and Lyresund, Bohuslän, Sweden. Medd. fr. Havsfiskelab., Lysekil, Sweden 284: 1 – 25. In Swedish with an abstract in English.

de Zwaan A. and **Zandee D. I.** 1972. Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis*. Comp. Biochem. Physiol. 43A: 53 – 58.



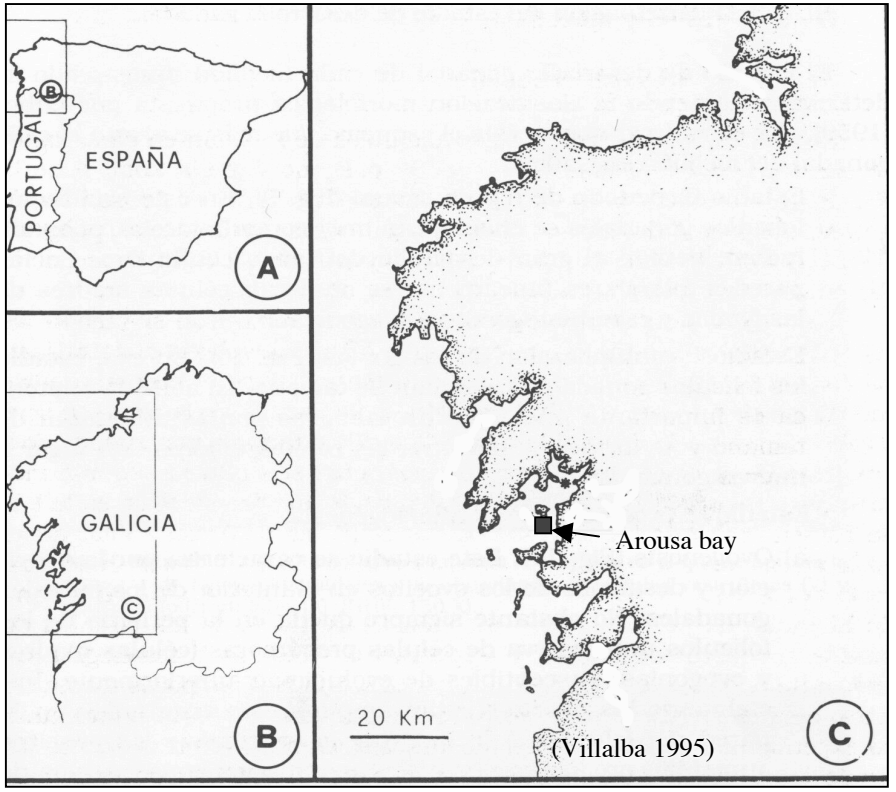


Fig. 1. The locations in Sweden and Spain.

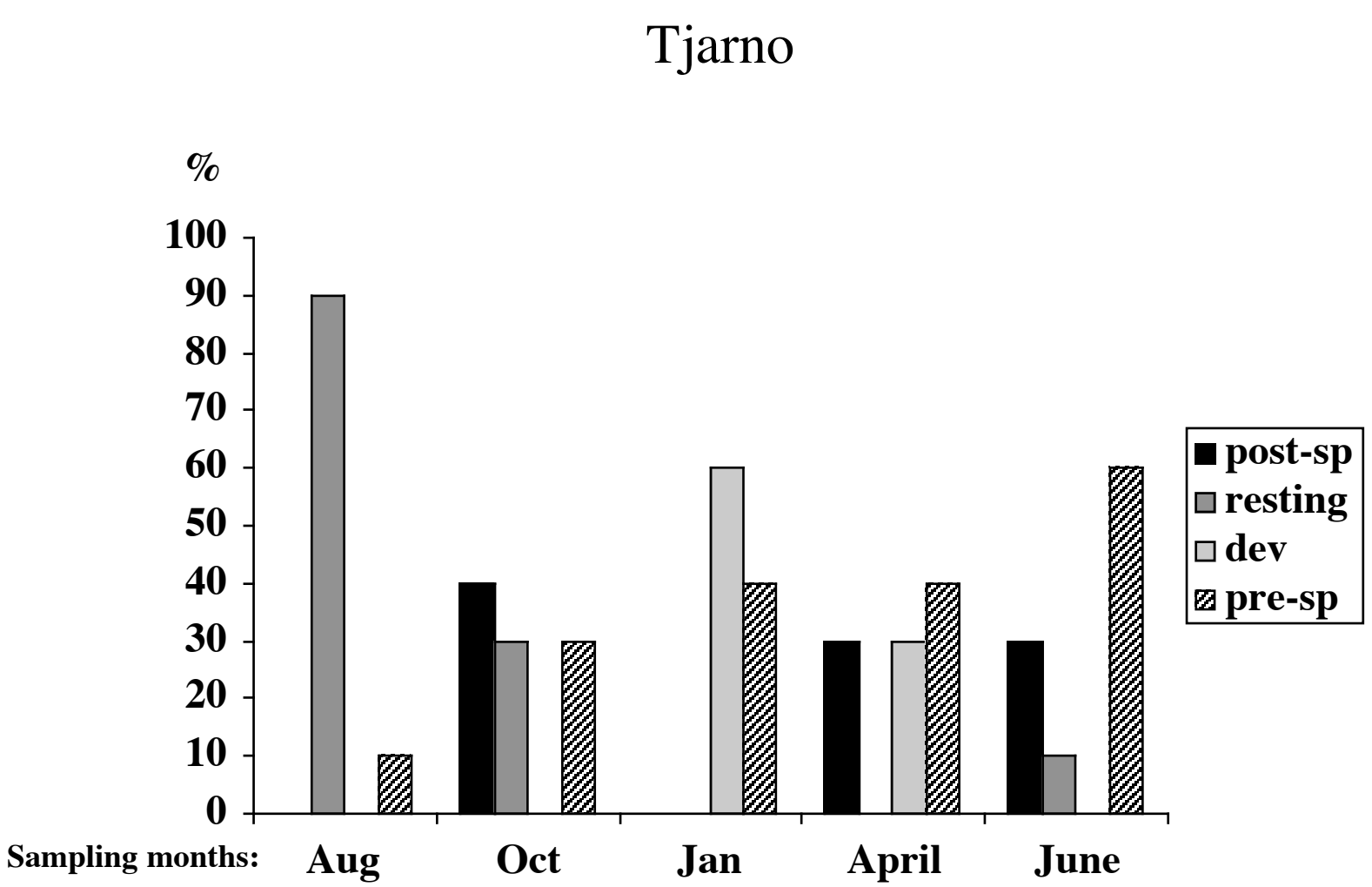


Fig. 2 a. Blue mussel (*Mytilus edulis)* populations from the Tjarno area in Sweden. Percent individuals in different reproductive stages. (n = 10).

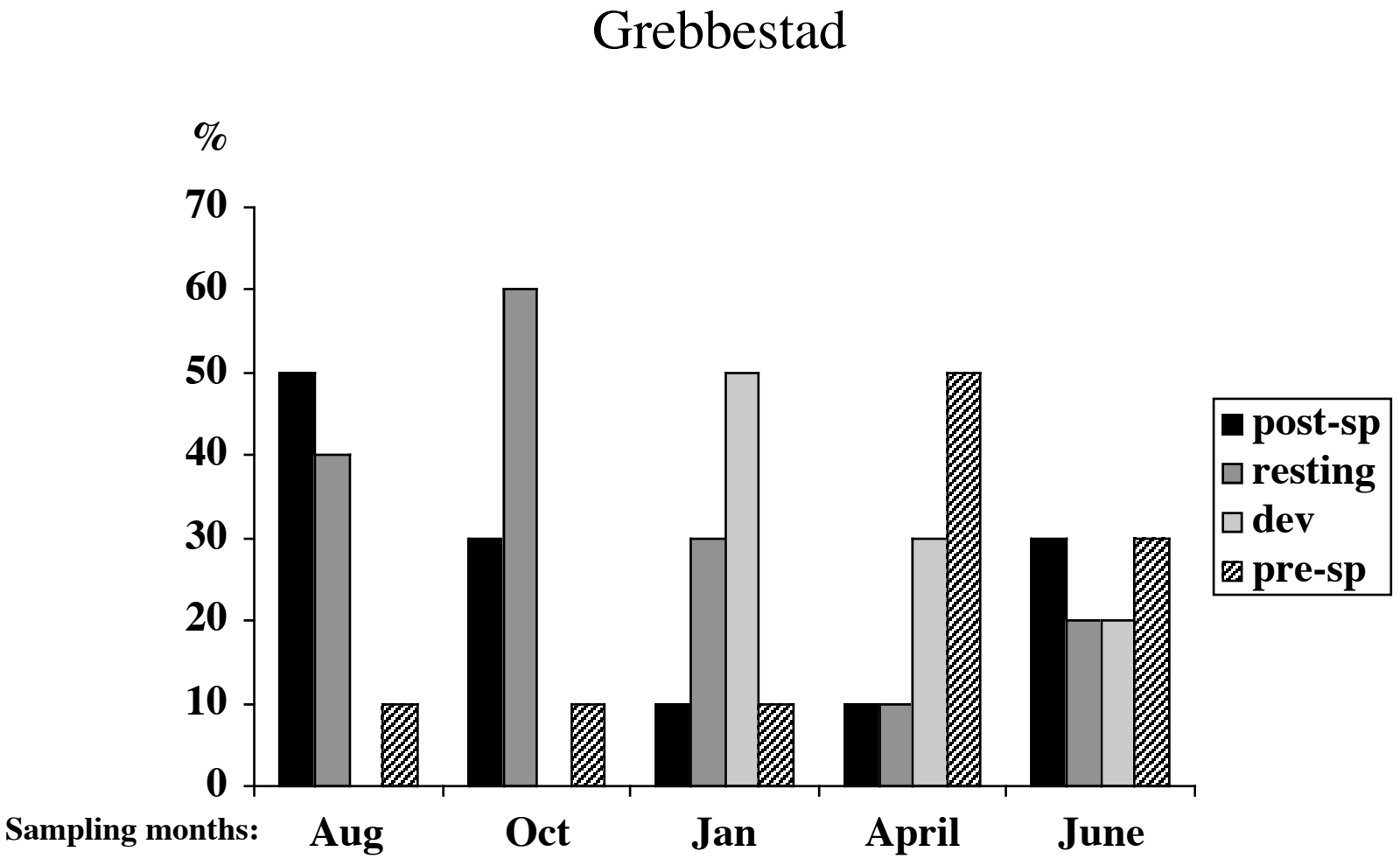


Fig. 2 b. Blue mussel (*Mytilus edulis)* populations from the Grebbestad area in Sweden. Percent individuals in different reproductive stages. (n = 10).

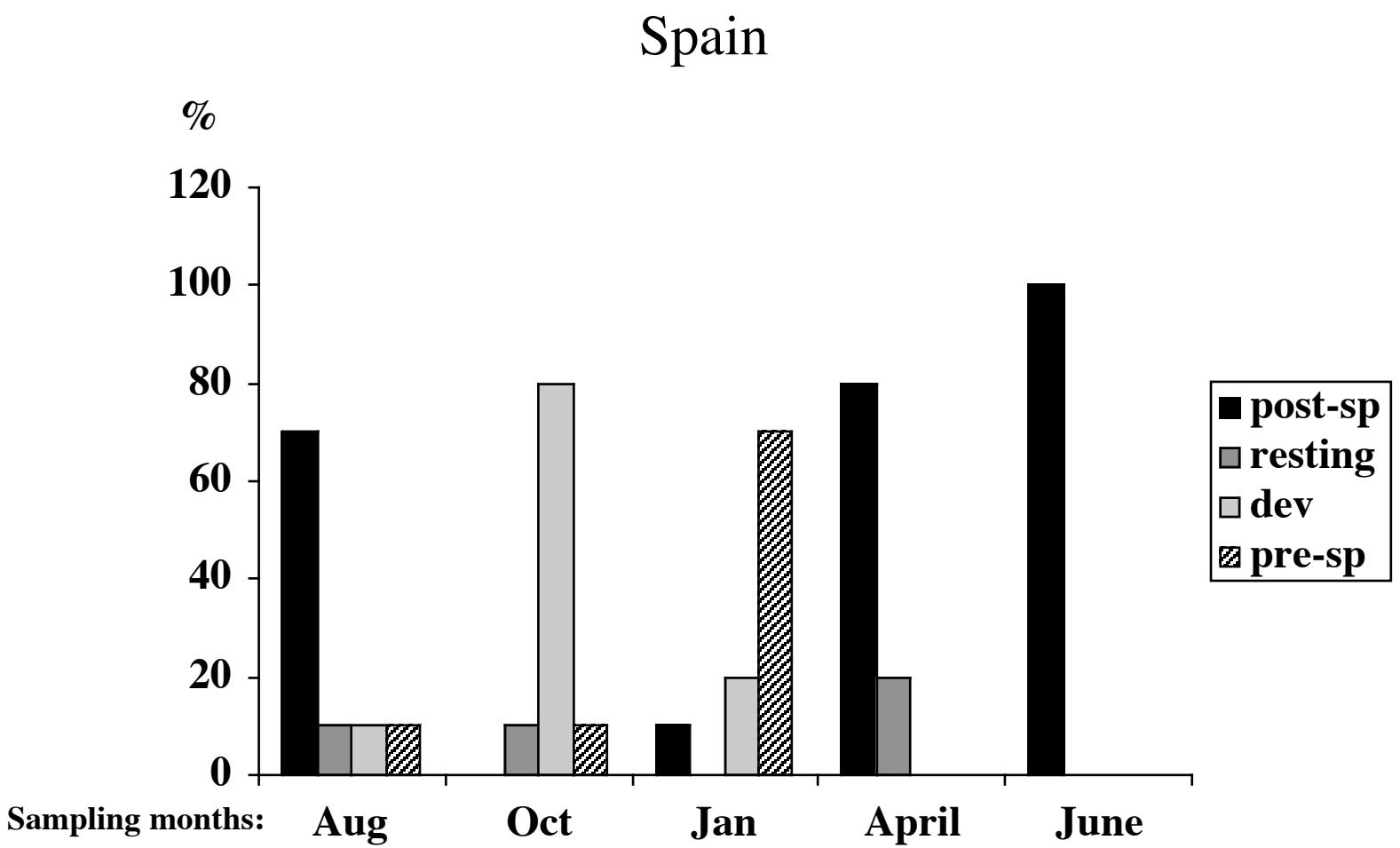


Fig. 2 c. Blue mussel (*Mytilus galloprovincialis)* populations from the Arousa Bay area in Spain. Percent individuals in different reproductive stages. (n = 10).

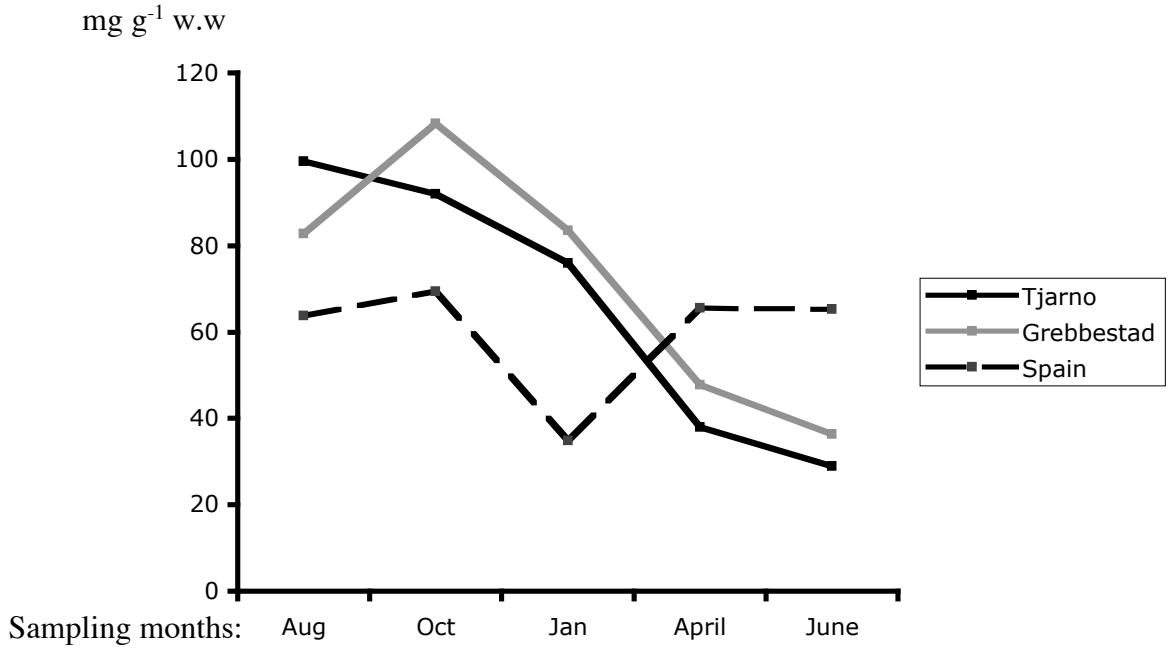


Fig. 3. Mean glycogen content in three populations of *Mytilus* spp. from Sweden and Spain sampled in five different months (n = 10).

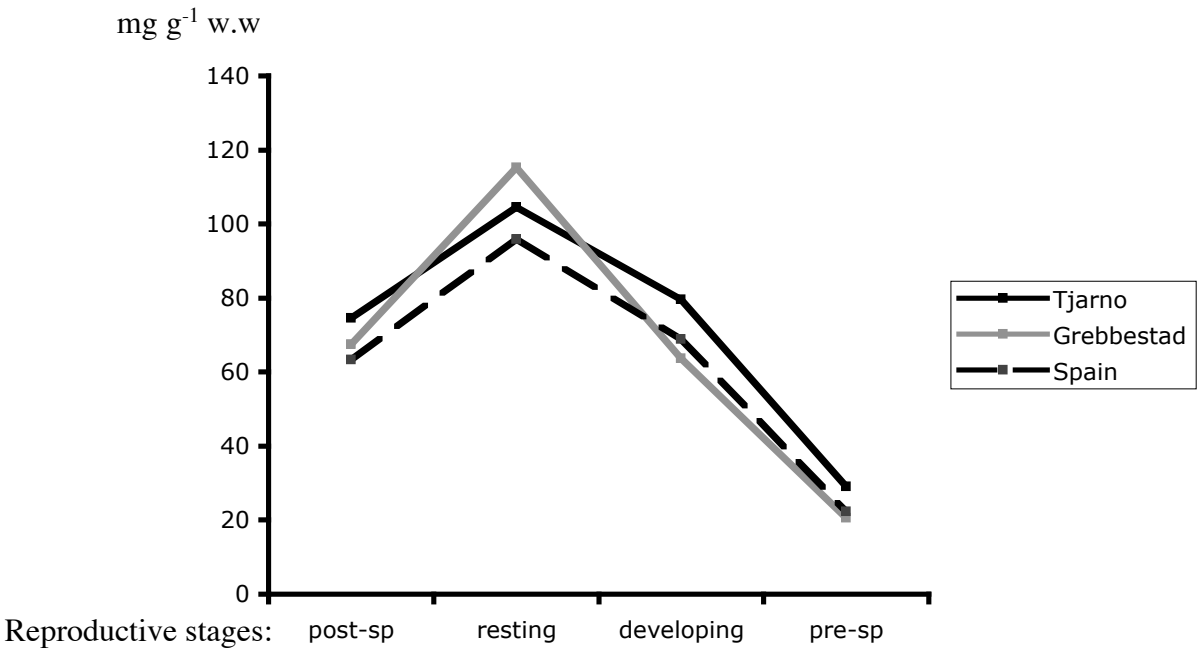


Fig. 4. Mean glycogen content in three populations of *Mytilus* spp. from Sweden and Spain in four different reproductive stages (n = 10).

Table 1. The reproductive stages.

Gonad development	Description
Resting	No trace of sexuality observed, resting gonads
Developing	Ripe gametes appear, but gametogenesis still in progress
Pre-spawning	Fully ripe condition
Post-spawning	Follicles less than half full of mature gametes

Table 2 a. Effects of reproduction stage (R) and locality (L) on the glycogen content in 72 individual of blue mussels (*Mytilus edulis*) from Sweden. Results from a two-factor ANOVA.

Source	df	SS	MS	F	P
Reproduction stage (R)	3	59750	19917	28.00	**0.011**
Locality (L)	1	917	917	0.65	0.42
R x L	3	2134	711	0.50	0.68
Residual	64	90593	1416		

Table 2 b. Effects of sampling month (M) and locality (L) on the glycogen content in 100 individuals blue mussels (*Mytilus edulis*) in Sweden. Results from a two-factor ANOVA.

Source	df	SS	MS	F	P
Month (M)	4	71623	17906	22.36	**< 0.01**
Locality (L)	1	595	595	0.39	0.5348
M x L	4	3203	801	0.52	0.7197
Residual	90	138041			

Table 3 a. Effects of sampling month on the glycogen content in 50 individuals of blue mussels (*Mytilus galloprovincialis*) in Spain. Results from a one-factor ANOVA.

Source	df	SS	MS	F	P
Month	4	7898	1975	7.15	**< 0.01**
Residual	45	12435	276		

Table 3 b. Effects of reproduction stage on the glycogen content in 50 individuals of blue mussels (*Mytilus galloprovincialis*) in Spain. Results from a one-factor ANOVA.

Source	df	SS	MS	F	P
Reproduction stage	3	19036	6345	225	**< 0.01**
Residual	46	1297	28		

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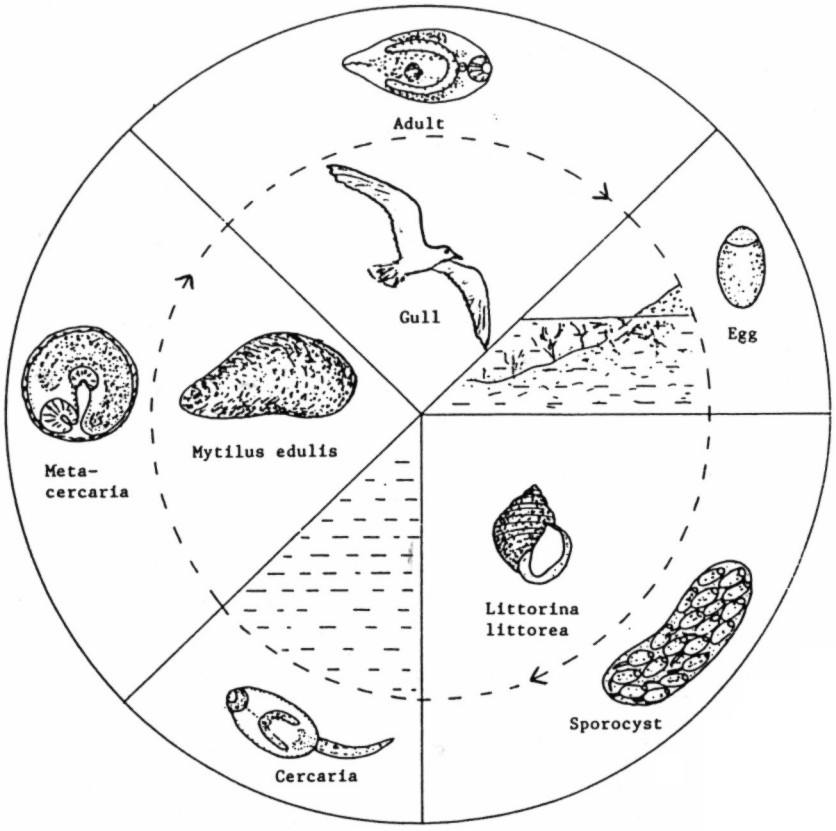

THE PARASITE FAUNA OF NATURAL AND FARMED *MYTILUS EDULIS* FROM THE WEST COAST OF SWEDEN, WITH SPECIAL REFERENCE TO *RENICOLA ROSCOVITA*

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Summary

During the spring 1984 mussels from a natural and a farmed population in an area investigated earlier (1979), Tjärnö, and from a second area, Vrångö, situated about 180 km further south, were investigated. The parasite fauna of the mussel populations in the Tjärnö area was the same in 1984 as that registered in 1979 and was identical to that in the Vrångö area. Mytilicola intestinalis was not found and it seems that the limits of the northeastward distribution of this parasite is reached along the northwest coast of Denmark. Occasional Modiolicola sp and small unidentified nematodes and ciliates were found in mussels from all localities.
Of the 25 mussels from natural populations at Tjärnö and Vrångö investigated with regard to all parasites, 96% and 100% respectively were infested with metacercariae of Renicola roscovita. Corresponding figures for the farmed mussels were 12% and 4% respectively. The distinct difference in the infestation rate is explained by the unfavourable biotope in the farm for the life cycle of the parasite.
The labial palps were found to be the most heavily infested organ. It is obvious that a large number of metacercarial cysts in this organ seriously impairs its function. In a histological examination of the labial palps dead, disintegrating metacercaria were found. Around these a distinct tissue reaction with numerous macrophages could be found. The death of the parasites might have been caused by low winter temperature. The results indicate that the dead parasite and its cyst disintegrate and disappear during spring without leaving any scars or other remains observable in the tissue of the mussel.

Sammanfattning

Under våren 1984 undersöktes musslor från ett odlat och ett naturligt bestånd i ett tidigare (1979) undersökt område, Tjärnö, och ett nytt, 180 km söderut beläget område, Vrångö. Parasitfaunan hos musselbestånden vid Tjärnö var densamma 1984 som den som registrerades 1979 och var även identisk med den som påträffades i Vrångö-området. Mytilicola intestinalis påträffades inte och det tycks som om gränsen för denna parasits nordöstliga utbredning går vid danska nordvästkusten. Enstaka exemplar av Modiolicola sp och små nematoder och ciliater påträffades i musslor från samtliga lokaler.
Av de 25 med avseende på samtliga parasiter undersökta musslorna från naturliga bestånd vid Tjärnö och Vrångö var 96% respektive 100% infesterade med metacerkarier av Renicola roscovita. Motsvarande siffror för de odlade musslorna var 12% respektive 4%. Den markanta skillnaden i infesteringsgrad förklaras av den för parasitcykelns genomförande ogynnsamma odlingsbiotopen. Musslans munflikar var det kraftigast infesterade organet. Det är tydligt att en riklig parasitförekomst i detta organ rent mekaniskt kan nedsätta dess funktion. Vid histologisk undersökning av munflikar påträffades även döda, disintegrerande metacerkarier. Vävnaderna runt dessa döda parasiter och deras cystor var tydligt förändrad och innehöll ett stort antal makrofager. Parasitens död kan ha orsakats av låg vintertemperatur. Resultaten indikerar att den döda parasiten och dess cysta under våren upplöses och försvinner utan att efterlämna ärr eller andra spår i musslans vävnader.

INTRODUCTION

The European mussel, Mytilus edulis L., has long been used as food and the increasing farming of this species during recent years has accentuated the need for more detailed knowledge of its biology. Growth, mortality, population dynamics and general ecology of the mussel have been studied in great detail throughout the years in various countries. However, the different types of diseases occurring in the European mussel are still incompletely known and among these we also find diseases caused by parasites.

The parasite of the mussel which has been studied in the greatest detail is the copepod Mytilicola intestinalis which lives in the intestines. This parasite is capable of a dispersal which may assume epidemic proportions and has been reported to be the cause of severe damage and mortality among mussels. During recent years, however, the opinion of the pathogenic effect of this parasite has been modified and today M. intestinalis alone is not considered to cause more than local modifications to the tissue in the intestine of the mussel (Lauckner, 1983).

There is no doubt that the most important and dominating multi-cellular parasites in mussels are the flukes (Trematoda) which frequently occur as metacercariae, i.e., in their second larval stage. In cases of abundant occurrence of metacercariae they have been found to cause mechanical damage, for instance. Examples of such damage are displacements of organs, disruption of tissues and occlusions of ducts and blood sinuses which can reduce the growth of the mussel and its ability to reproduce and survive. Bearing in mind that these common parasites are capable of causing such extensive damage, it is surprising that mussel ecologists so rarely mention parasites and their possible damaging effect. As proposed by Lauckner (1983), the main cause of the total unawareness of the "trematode problem" in field biology is that these parasites typically cause a slow but certain decrease in the number of adult host animals and thus do not result in a spectacular epizootic mortality.

In Sweden only one parasitological investigation of the mussel has previously been conducted (Fjälling et al., 1980, Billgren & Håkansson, 1980). In this investigation the mussels were investigated from natural and farmed populations in the Tjärnö area on the north-west coast and from two natural populations in the Lysekil area about 80 km further south. The above-mentioned parasite, M. intestinalis, was not found among any of the 400 mussels investigated whereas metacercariae of the trematode Renicola roscovita were observed in large numbers in most of the mussels from the natural populations. On the other hand, only a few individuals of this parasite were found among the farmed mussels. The labial palps of the mussel were, according to Fjälling et al. (1980), the organ which was most severely infested with metacercariae but no histological investigation was conducted.

The present report deals with the results of a parasitological investigation of European mussels conducted in order to examine

1. whether the parasite fauna in the Tjärnö area had changed since the previous investigation,
2. whether this parasite fauna differs from that in a farmed and a natural population in an area about 180 km further south, and
3. whether any reaction in the tissue occurred as a result of parasitic attacks in the most severely infested organ in the mussel.

MATERIAL AND METHODS

The investigation was conducted during March-May, 1984, in two areas, Tjärnö and Vrångö, on the west coast of Sweden (Figs 1-3). From each area 125 mussels were collected from a farmed and from a natural population.

In the Tjärnö area the sampling locality for mussels from the natural population is situated in the sound between Yttre Tenskär and Inre Tenskär (Figs 1 and 2). The depth is 1-2 m and the bottom consists of clay and sand. The shores are rocky and the locality relatively well protected from winds and currents. The locality for the farmed mussels is M. Håkansson´s farm in Nyckelbyviken between S. Öddö and

Tjärnö (Fig. 2). It is located in relatively fast flowing water, the depth varies between 5 and 20 m and the nearby shores are rocky. The mussels are grown on vertically suspended ropes ending a few metres above the bottom. Samples were taken from ropes in the centre of the farm about 300 m from the shore and 3-7 metres from the surface where the depth was 10 m. The water temperature at sampling was 2°C.

In the Vrångö area mussels were taken from a natural population at 0.5 m depth at S. Varskär just to the west of Vrångö (Figs 1 and 3). The locality is exposed to winds and currents, the bottom is stony and the neighbouring shores are rocky. The farmed mussels in this area were taken from O. Johansson´s farm situated to the east of S. Varskär. Here the water is relatively fast-flowing and the depth 4-5 m. Samples were taken from ropes 20 m from the shore and 1 m from the surface. The water temperature at sampling was 10°C.

The mussels were stored in a cold bag during the transports and then in a cold chamber (10°C) for maximally 7 days until they were investigated. All 500 mussels were investigated for M. intestinalis. Several reference specimens of this parasite had kindly been sent to us by Dr. Theisen from Denmark. The mussel was measured for length and opened after the dorsal sphincter had been severed. The digestive gland with the intestine were carefully removed and transferred onto a glass sheet measuring 0.3 x 10.0 x 10.0 cm and covered with a similar sheet. The two sheets of glass were then pressed carefully together and two clamps were applied to keep the samples compressed. The glass sheets were divided into 1 x 1 cm squares and were marked on one end with "A" for anus and on the opposite end with "M" for mouth in order to easily identify the different parts of the intestine once the sample has been compressed. The sample was then studied in a stereo microscope with illumination from below.

In 25 of the 125 mussels taken from each locality the examination also concerned the presence of other parasites, e.g., metacercariae. These mussels were opened in the same way as the others but only the gills and mantle from one side were investigated since random samples had indicated that the number of metacercariae in these organs

were uniformly distributed on both sides. The digestive gland was carefully removed in order to avoid damaging the kidney tissue and the labial palps were cut free. The removed organs were placed one at a time between the glass sheets and any parasites present were counted in each 1 x 1 cm square. The number of parasites from gills and the mantle were doubled when entered in the records. The digestive gland and the labial palps sometimes contained such a large number of uniformly distributed metacercariae that an estimation of the total number was made by counting the metacercariae in a square and then multiplying the number by the number of squares covering the gland. These estimations considered the following aspects: Individual parasites were counted up to 30; in cases of larger numbers (up to 250) the estimation was ± 10 and thereafter the estimation was ± 100.

Labial palps from mussels taken from farmed and natural populations were fixed in Bouin´s fluid and transferred after 24 hours to 70% alcohol. The samples were embedded in paraffin, sectioned (8 µm) and stained in Mallory´s triple stain before being analysed in a light microscope.

RESULTS

Parasites

None of the 500 mussels investigated were infested with the copepod M. intestinalis.

Of the 25 mussels from natural populations at Tjärnö and Vrångö investigated with regard to all parasites, 24 (96%) and 25 (100%) respectively were infested with metacercariae of Renicola roscovita. Corresponding figures for the farmed mussels were 3 (12%) and 1 (4%), respectively. Tables 1 and 2 illustrate the length of the mussels and the number of R. roscovita found in the different organs of mussels from natural populations in the two localities. We can see that mussels from the Tjärnö area had a considerable higher infestation intensity, on average 778 R. roscovita per mussel, than the mussels from Vrångö, on average 98 individuals per mussel. The percentage distribution of R. roscovita in different organs of mussels from the two natural

populations is shown in Figure 4. This shows that labial palps are the most severely infested organ and contain about 70% of the total number of metacercariae in mussels from the two localities. The next most infested organ is the digestive gland with about 20%, and the remaining metacercariae are distributed among the gills, intestine, kidney and mantle.

In the farmed mussels only 1 individual of R. roscovita was found in each of 3 mussels from Tjärnö and 2 individuals in one single mussel from Vrångö. Of these metacercariae, 4 were found in the labial palps and 1 in the digestive gland.

Occasional specimens of Modiolicola sp and small unidentified nematodes and ciliates were found in mussels from both farmed and natural populations in both areas of investigation.

Tissue reaction

The metacercaria of R. roscovita is encapsulated in a cyst with walls which are ca 15 µm thick (Fig. 6). No tissue reaction was found around parasite cysts containing living metacercariae. A number of dead metacercariae were also found in the sectioned material. These were in different stages of disintegration (Figs 7-10) and in the tissue of the host a tissue reaction was found around the cyst, including an abundance of macrophages. At the same time as autolysis of the larva itself occurs, the outer cyst membrane is dissolved and macrophages are found both inside and between the outer and inner cell membrane (Fig. 8). In a later stage of disintegration (Fig. 9) the metacercaria is almost completely dissolved whereas parts of the cyst membrane remain among numerous macrophages and residual products. In a more advanced stage (Fig. 10) only residues of the inner cyst membrane remain and the tissue reaction and concentration of macrophages around the former parasite cyst are now less intensive. Scars or other remaining indications of earlier parasitic infestations were not found.

DISCUSSION

Mytilicola intestinalis has been reported as a common parasite in mussels in the Mediterranean and most North European waters. It has been reported in mussels from western Limfjord, Denmark (Theisen, 1964) but Lauckner (1983) states that it is rarely found north of the Elbe estuary, Germany. Judging from information now available it thus seems that the limits of the northeastward distribution of this parasite is reached along the northwest coast of Denmark.

The parasite fauna of the mussel populations in the Tjärnö area was the same in 1984 as that registered in 1979 (Fjälling et al., 1980). In comparison with the earlier investigation, samples were now taken of farmed mussels from a site in the bay situated further in, whereas samples from natural stands were taken in an archipelago area further out (Fig. 2). Despite the change of localities, the same marked difference was obtained in the infestation of Renicola roscovita metacercariae between the farmed and the natural populations, and a similar difference was also registered in the Vrångö area where the parasite fauna did not differ from that in the Tjärnö area. Biotope differences between the localities for the natural and the farmed populations respectively are, on the other hand, small and the results thus verify the statement that in-shore mussel populations living in shallow water are severely infested by R. roscovita, whereas farmed mussels in deep water have very low degrees of infestation (Fjälling et al., 1980, Thulin, 1983). This relationship is directly related to the occurrence of the parasite´s other hosts, which are the gulls and the snail Littorina littorea (Fig. 11). In the latter, the parasite´s free-swimming larval stage is developed, the cercaria, which infests the mussel during the summer. In the artificial pelagic farming biotope there are few snails and thus also little opportunity for development and infestation of parasitic larvae. The almost 8-fold larger average occurrence of metacercariae of R. roscovita among the natural mussel population in the Tjärnö area in comparison with the corresponding occurrence in the Vrångö area may be explained by a greater abundance of both gulls and L. littorea.

According to Lauckner (1983). the location of R. roscovita metacercariae in M. edulis is largely determined by the size of the host and the space available for encystment. Thus, in younger mussels with small labial palps most metacercariae can be found in the visceral mass. In larger mussels, however, the relative abundance of the parasites was noted to change in favour of the palps. The infestation preference for this organ was confirmed in both the present and earlier investigations along the Swedish coast where all mussels examined were more than 60 mm in length (Fjälling et al., 1980, Billgren & Håkansson, 1980). However, it is most interesting to note that although the abundance of metacercariae in the Tjärnö material from the natural populations was nearly eight times larger than in the Vrångö material, the relative abundance within different organs being similar. This indicates that a mussel in a biotope allowing a high production and infestation efficiency of cercariae may continuously accumulate very high numbers of metacercariae, preferably in the labial palps. It is obvious that a large number of metacercarial cysts in such an organ as the palps, with its proper function as a transport and sorting device for food particles, seriously impairs the function of that organ. High abundances of these trematode larvae may not only be detrimental to single specimens of bivalves but there is in fact evidence that the cockle, Cardium edule, populations on the tidal flats of Sylt, on the German North Sea coast, are largely controlled by the trematodes Himasthla elongata and R. roscovita (Lauckner, 1983).

Even if the effect of the metacercarial infestation of R. roscovita in mussels is mainly mechanical, the present investigation has shown that a host tissue reaction may occur around the cyst of a dead and disintegrating parasite specimen. Although this tissue reaction may be very distinct and affect a considerable region around the cyst the results also indicate that this reaction is one stage in a process of resorption of a dead parasite and its cyst and that no scars or any other signs of earlier tissue damage remain. The reasons for the death of the parasites is unknown. Lauckner (1983), however, refers to preliminary experiments showing that larval trematodes are generally less resistant to freezing than their molluscan hosts. In both the natural mussel population localities studied the water

temperature may decrease to or even below 0°C during the winter months. This low temperature may well cause the death of the parasites. As mentioned earlier, a mussel population living in a shallow and in-shore locality may become heavily infested by cercariae of R. roscovita during the summer. However, the same biotope may thus also offer a regulating factor during the winter months, thereby preventing a high and detrimental parasite burden.

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REFERENCES

Billgren, O. and N. Håkansson. 1980. Pilotundersökning av parasitförekomst hos Mytilus edulis i Lysekilsområdet, 1979. Medd. fr. Havsfiskelaboratoriet, Lysekil, nr 263:25-35.

Fjälling, A., L. Kolsäter, and J. Thulin. 1980. En parasitologisk undersökning av vilda och odlade musslor, Mytilus edulis, i Tjärnöområdet, Norra Bohuslän, 1979. Medd. fr. Havsfiskelaboratoriet, Lysekil, nr 263:1-24.

Lauckner, G. 1983. Diseases of Mollusca: Bivalvia. In O. Kinne (Ed.), Diseases of Marine Animals, Biologische Anstalt Helgoland, Hamburg, FRG. Vol.2:477-961.

Theisen, B. 1964. Mytilicola - En snylter i blåmuslinger. Danmarks Fiskeri- og Havundersögelser 25:21-24.

Thulin, J. 1983. Parasiter i blåmussla. In R. Rosenberg (Ed.), Odling av blåmusslor. Bokförlaget Signum i Lund AB. 84-88.

Table 1. Number of metacercariae of Renicola roscovita in different organs of mussels from a natural population at Tjärnö, Sweden.

Length (mm) of mussel	Number of metacercariae						
	Labial palps	Gills	Digestive gland	Intestine	Kidney	Mantle	Total sum
74	2	0	0	0	0	0	2
75	2	0	0	0	0	0	2
72	180	2	8	16	13	0	219
85	40	0	2	2	2	0	46
82	1300	8	50	70	17	70	1515
73	2300	14	250	170	60	160	2954
81	210	6	7	9	2	0	234
75	950	0	400	140	7	80	1577
75	160	0	6	5	0	6	177
70	1500	0	300	5	5	110	1920
73	340	22	40	0	0	10	412
71	400	24	130	3	3	40	600
70	1000	120	1100	24	0	24	2268
67	480	0	3	0	0	22	505
80	200	6	3	0	0	160	369
74	600	16	0	0	0	0	616
74	920	70	180	50	0	40	1260
80	600	90	40	0	0	6	736
75	520	150	280	60	6	30	1046
79	30	0	0	0	0	0	30
82	220	0	210	5	0	0	435
72	0	0	0	0	0	0	0
72	600	160	90	10	0	100	960
74	390	300	140	20	27	0	877
71	520	50	0	0	0	110	680

Average length of examined mussels: 75 mm
Average number of metacercariae per mussel: 778

Table 2. Number of metacercariae of Renicola roscovita in different organs of mussels from a natural population at Vrångö, Sweden.

Length (mm) of mussel	Number of metacercariae						
	Labial palps	Gills	Digestive gland	Intestine	Kidney	Mantle	Total sum
66	23	14	0	0	0	0	137
65	90	0	21	0	0	8	119
68	27	0	11	0	0	0	38
66	160	10	90	5	0	0	265
67	70	2	0	0	0	0	72
63	17	0	0	0	0	0	17
67	15	0	0	0	0	0	15
64	28	0	7	0	0	0	35
67	13	0	3	0	0	0	16
64	9	0	18	0	3	0	30
68	60	0	0	1	0	0	61
69	320	8	12	0	3	0	343
67	130	0	0	0	0	0	130
65	3	0	0	0	0	0	3
68	100	2	7	0	0	0	109
63	10	0	6	0	0	0	16
66	50	16	100	0	0	0	166
64	220	8	140	0	0	0	368
69	80	0	13	0	0	0	93
63	70	0	0	0	0	0	70
64	100	6	23	0	0	0	129
69	30	0	0	0	0	0	30
67	120	10	70	0	0	0	200
66	13	0	3	0	0	0	16
67	50	8	15	0	0	0	73

Average length of examined mussels: 66 mm
Average number of metacercariae per mussel: 98

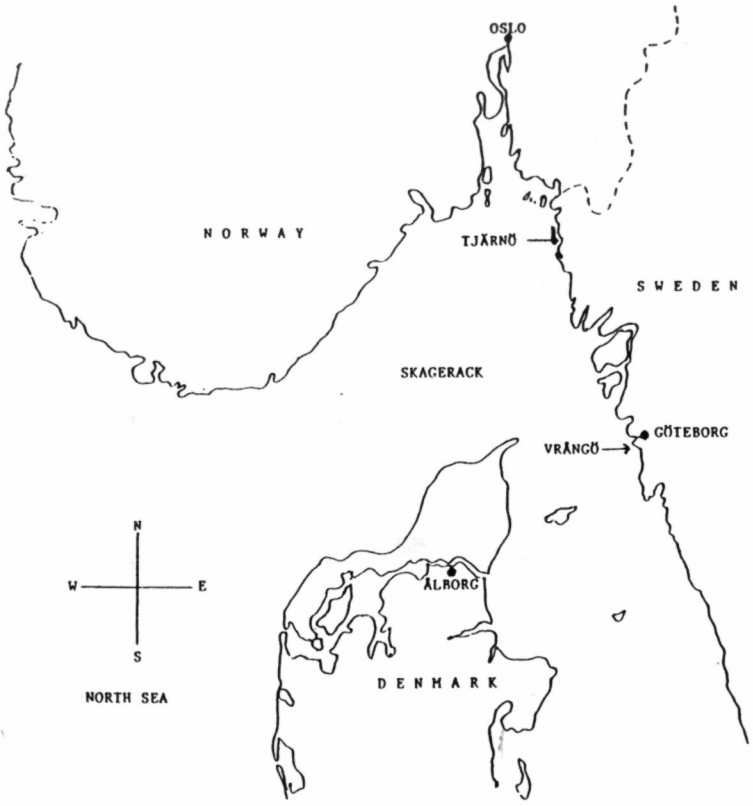


Figure 1. General map to show the positions of the two areas of investigation, Tjärnö and Vrångö, on the west coast of Sweden.

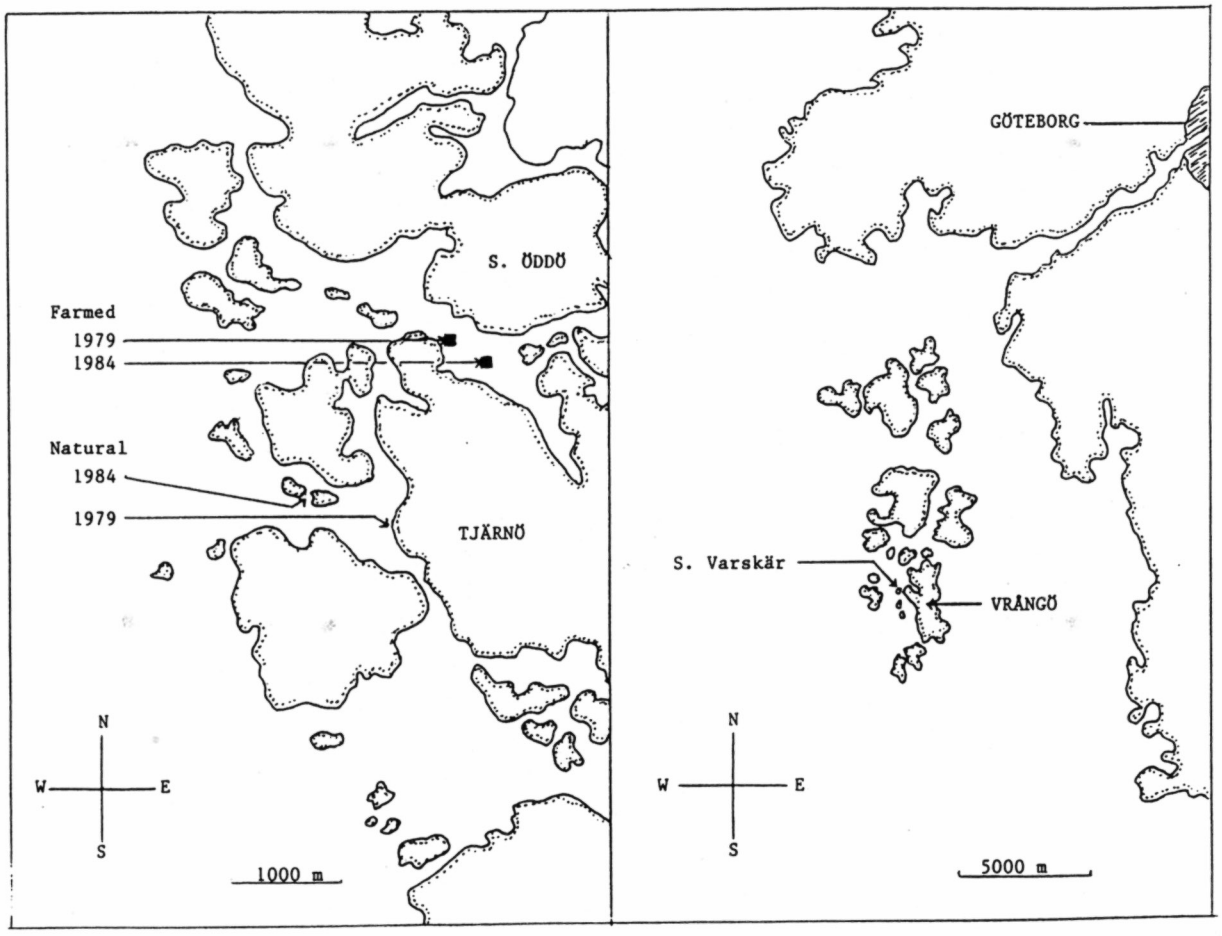


Figure 2. Sampling localities for farmed and natural population of mussels in the Tjärnö area in 1979 an in the present investigation, 1984.

Figure 3. Sampling localities for farmed and natural population of mussels in the Vrångö area situated east and west respectively of S. Varskär.

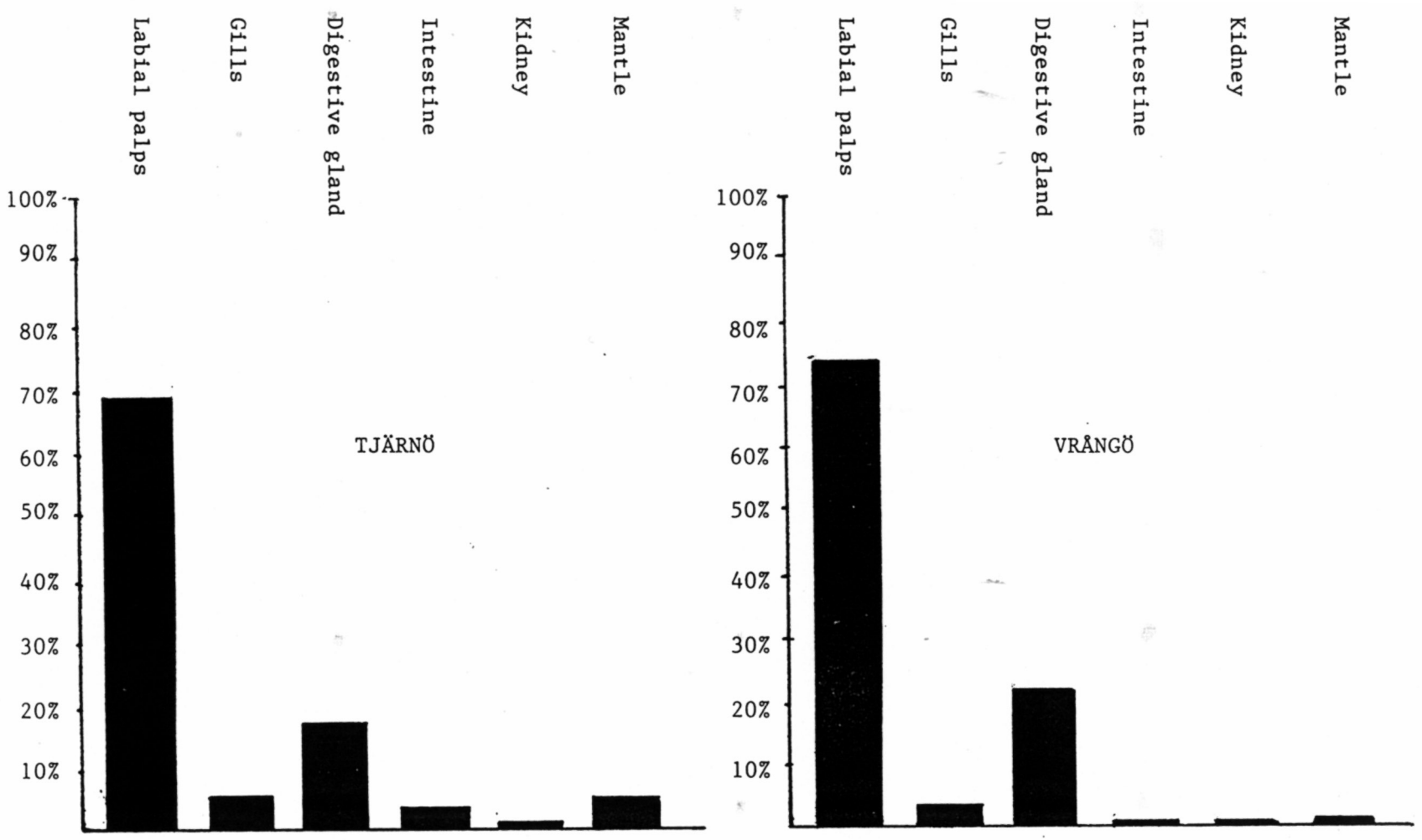
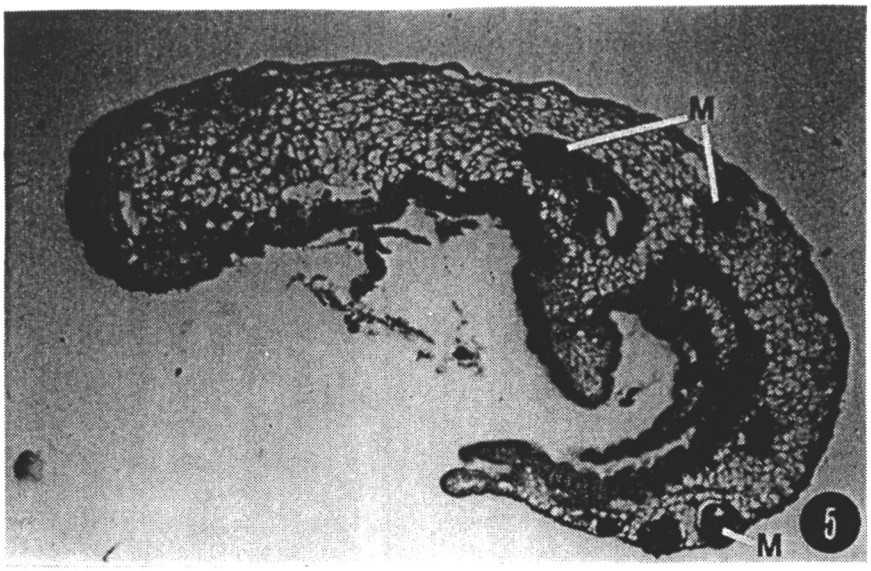
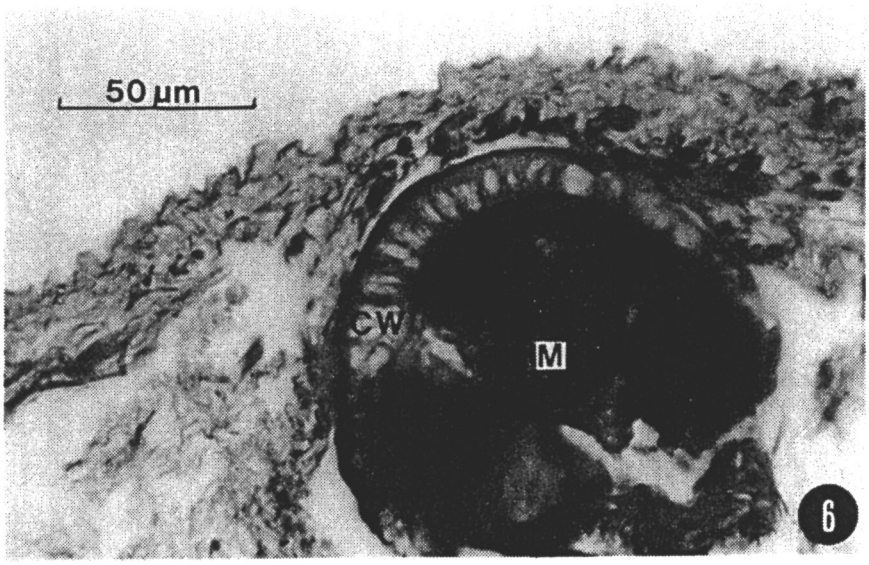


Figure 4. The percentage distribution of metacercariae of Renicola roscovita in different organs of mussels from natural populations at Tjärnö and Vrångö on the west coast of Sweden.





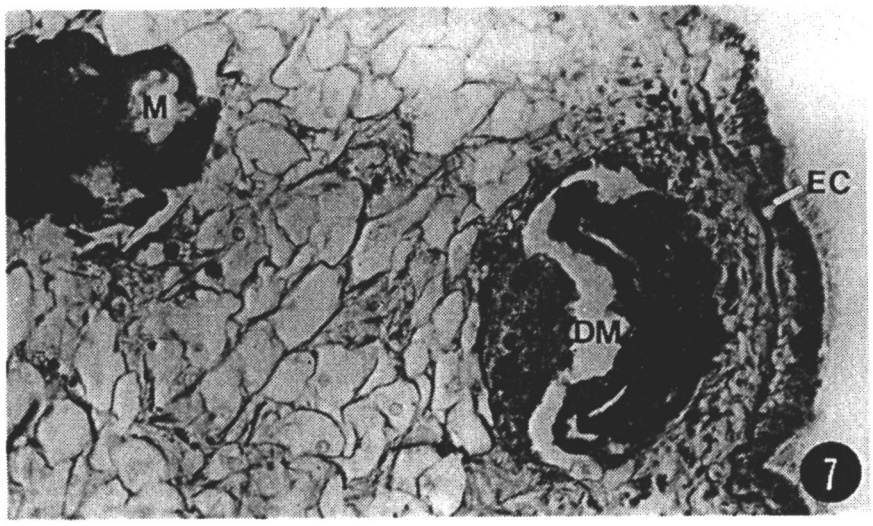


Figure 5. Section of a labial palp of a mussel from a natural population. The palp tissue contains several metacercariae (M) of Renicola roscovita.
Figure 6. The cyst wall (CW) of a living metacercaria of R. roscovita.
Figure 7. A living (M) and a dead, disintegrating (DM) metacercaria of R. roscovita. EC = epithelial cells with ciliae.

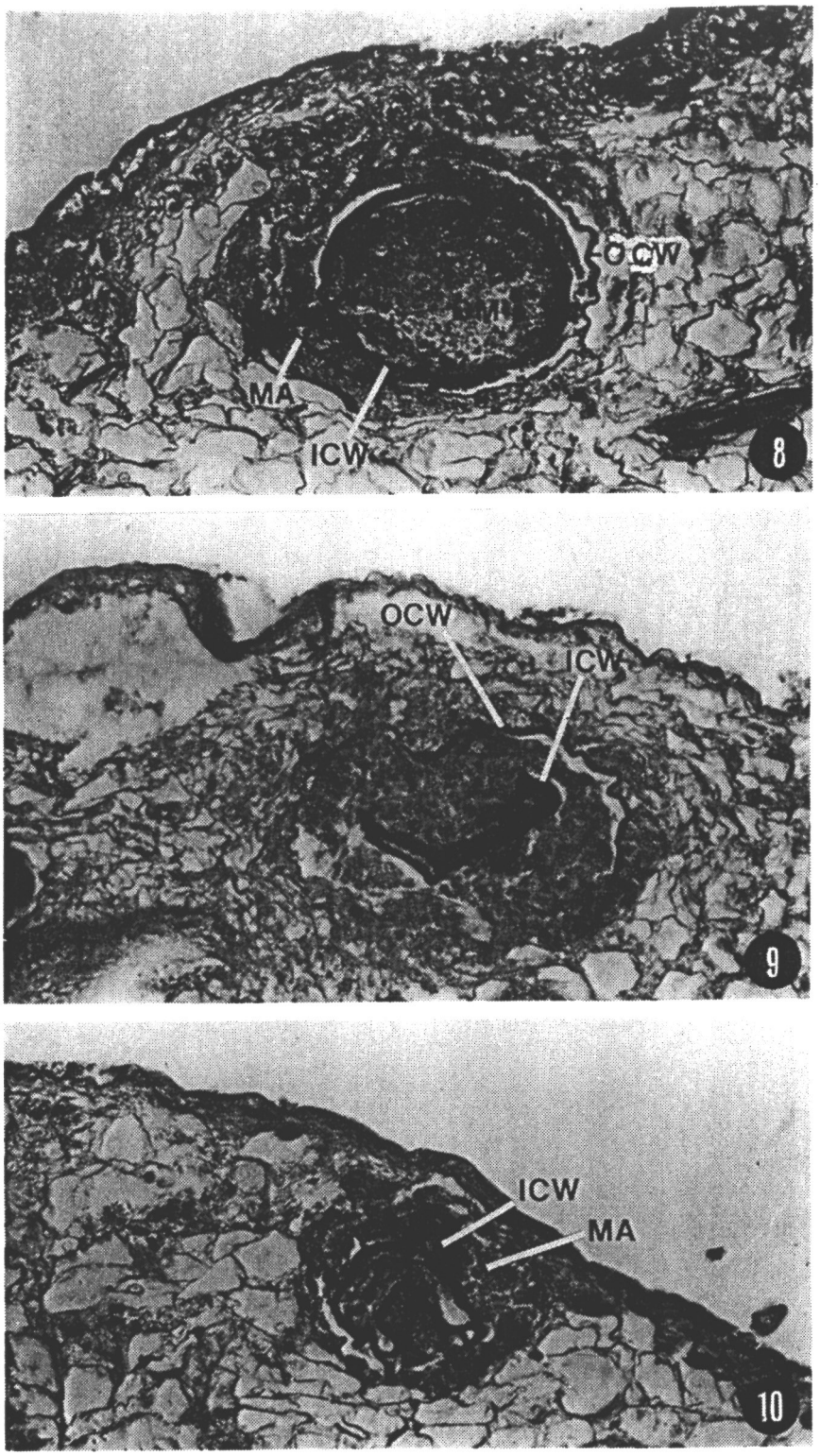


Figure 8. A disintegrating metacercaria. Note the distinct host tissue reaction with its numerous macrophages (MA), and the outer (OCW) and inner (ICW) membrane of the former cyst wall.
Figure 9. The metacercaria is almost entirely disintegrated and the inner and parts of the outer cyst wall membrane are still present.
Figure 10. The inner cyst wall membrane still remains while the outer has disappeared.

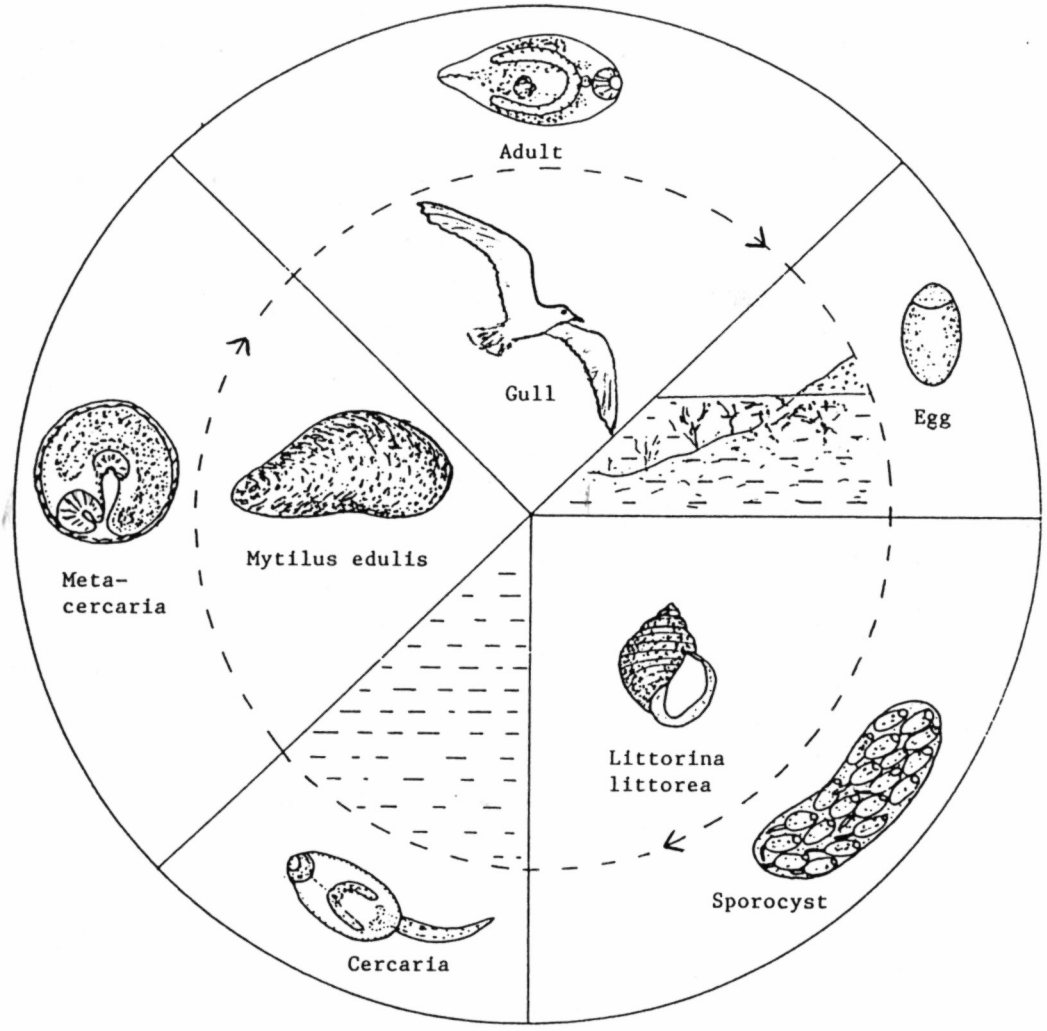


Figure 11. The life cycle of Renicola roscovita. The outer circle corresponds to different developmental stages of the parasite. The inner circle corresponds to host species in which these stages may occur.

PREVALENCE OF TREMATODE LARVAE IN WILD AND CULTURED BLUE MUSSELS, *MYTILUS EDULIS* L..

Abstract. *A pilot study showed differences in the prevalence of trematode metacercariae between a natural and a farmed population of blue mussels. Here I test the hypothesis that these differences are explained by the different microhabitats of the populations. Wild mussels being bound to bottom substrates, usually close to rocky shores, while farmed mussels hang on ropes in the free water column. During their life-cycle, trematodes use rocky shore snails as intermediate hosts prior to the infestation of mussels. If the trematode cercariae that are released from the snail host have a restricted dispersal, bottom-living mussels close to the rocky shore will be more available than farmed mussels as secondary hosts.*

In the present study I compared the prevalence of metacercariae in mussels at different distances from rocky shores and at different depths.

The results indicated that cercariae of Renicola roscovita infected mainly mussles at short distances from rocky shores and this supports a short dispersal of cercariae released from rocky shore snails (e.g. Littorina littorea). This also explains why natural populations of mussels living intertidally and subtidally close to populations of snails are much more infected than mussels farmed on ropes at greater distances from the shore.

Key words: Cercaria, Dispersal, Metacercaria, Mussel culture, *Mytilus edulis*, *Renicola roscovita*, Trematode

Introduction

Metacercariae encysted in marine mussels are in general not lethal to the mussels but they might cause severe mechanical damage of the tissues. Such damage will decrease food uptake and a mass infestation of metacercariae in, for instance, the labial palps (as the one reported by Svärdh and Thulin 1985) probably causes an important reduction in mussel growth. If farmed mussels are infected to a large extent, a loss of mussel production might give economical consequences. In Spain, where farming of mussels constitutes an important industry, a programme was started in 1988 in order to assess the pathological conditions of mussels. A large part of this programme deals with effects of parasites (Figueras et al., 1991; Robledo and Figueras, 1995; Villalba et al., 1993). Although, the effects of metacercariae have not been studied, Villalba et al. (1997) reported host castration and loss of storage tissue in individuals of *Mytilus galloprovincialis* as a consequence of sporocyst infection by the trematode *Proctoeces maculatus.* During the summer 1991 a mass mortality of *Cerastoderma edule* on the Swedish west coast was probably caused by a heavy trematode infection that damaged, for example, the mussel gonads (Jonsson and André, 1992). According to

Lauckner (1983) heavy metacercarial infections impair the byssus-thread production in mussels and affect the burrowing ability of cockles.
Thus, overall, mass infections by trematode larvae might seriously damage mussel populations and is therefore a potential problem for the mussel industry. In light of this, we need knowledge about the parasite ecology and the risks of mass infections of farmed mussels.

The prevalence of trematode parasites of natural and farmed populations of blue mussels on the Swedish west coast differed considerably (Svärdh and Thulin 1985). Mussels of the farmed populations had a much lower prevalence (4% and 12%) of metacercarial larvae from the digenean trematode *Renicola roscovita* than mussels from natural populations (96% and 100%). This might be explained by the different microenvironments of natural and farmed populations. However, as the habitats were not replicated, differences might just be accidental. In the present study I test the hypothesis of metacercarie infections being related to environmental differences with a design replicating both area and habitat.

Thedigenean trematodes *Himasthla elongata* and *R. roscovita*, use species of *Littorina* as their first intermediate host. They are both present in the northern part of the Swedish west coast (Granovitch and Johannesson, 2000). They use mussels as second intermediate host and sea gulls as final host.

In general cercariae have specific host-finding strategies, that are sensitive to various chemical and physical cues of their second intermediate host (Haas et al., 1990; Haas, 1992). However, according to Combes et al. (1994) cercariae seem to have invested relatively little in the localization of individual hosts, but more to ensure that the dispersion takes place under conditions which will increase the probability of an encounter. Bartoli and Combes (1986) in a study of twelve species of trematode cercariae, found the behaviour closely adapted to the ecology of their targets. For instance, the species that develops on fish living in open water spread markedly in the vertical plane and species whose development has to continue on benthic organisms did not spread actively or did so only on the sea bed. Possibly, species like *H. elongata* and *R. roscovita* also have cues to localize their second host, after being released from the first host. However, I also predict that the distances between first and second hosts are important for the trematode survival. To test the importance of mussel environments at different distances from the shore, to the distribution of *R. roscovita* and *H. elongata*, I recorded the presence and densities of metacercariae in natural and farmed blue mussels at various distances and depths from rocky shore habitats inhabited by *Littorina*.

Material and methods

Blue mussels were collected from two areas at the Swedish west coast during April – May 2002. In each area mussels were sampled at four sites: A rocky shore with snails (benthic), a piling without snails (10 m from the shore) (pilings) and two different sites on vertically suspended ropes in a mussel farm (100 m and 120 m from the shore) (inner and outer respectively). At each site mussels were sampled from two depths (Fig. 1). The farmed

mussels were three years old, while the exact age of the natural mussels was unknown, although their sizes suggested they were of similar age as the farmed ones.

The mussels were stored in aquaria for two days and thereafter prepared for histological sectioning. The frontal part of the body, including parts of the gills and the mantle, was placed in Davidsen´s fixative, washed and dehydrated through an ascending series of alcohol and tetrahydrofuran (THF) and finally embedded in solid paraffin. Tissues from 240 individuals were cut into 7 m sections using a rotary microtome and a randomly chosen sequence of 4 – 5 cuts were transferred to microscope slides. The tissues were stained in Ehrlich hematoxylin/eosin. One randomly chosen cut from each slide was examined with a light microscope and all metacercariae appearing were counted. (There was no risk of counting a parasite individual more than once.) Parasite identification was confirmed by Dr. Andrey Granovitch, St. Petersburg.

Statistical analysis

To assess how area, habitat, depth and the interactions between these factors related to the occurrence of metacercariae in the mussels, I used an analysis of variance (ANOVA). All three factors were orthogonal with Area being a random factor with two levels, while both Habitat and Depth were fixed with four and two levels respectively. Post-hoc (Student-Newman-Keul, SNK) tests were used to assess differences among levels of a significant factor.

Results

In the examined cuts a total of 59 metacercariae were found and all were larvae from *R. roscovita*. None of the interactions among the three factors Area, Habitat and Depth was significant. There was, furthermore, no significant differences in parasite prevalence between the different areas and depths (Table 1). Metacercariae numbers were, however, different among the four habitats. According to the SNK-test, the mussels sampled close to rocky shores had significantly higher numbers of metacercariae than the mussels of the other habitats (Fig. 1). Indeed, the mussels sampled in the two farmed sites had no metacercariae at all, except for one individual that was infected by five trematode larvae.

Discussion

The prevalence of metacercariae in *Mytilus edulis* varies substantially at the Swedish west coast, but neither area (100 km apart), nor depth contributed significantly to this variation. It was instead the mussel habitat that explained the variation to a significant degree with mussels of rocky areas being more infected mussels of the other microhabitats.

An earlier study of digenean trematodes in four species of *Littorina* from the west coast of Sweden, showed that *Renicola roscovita* was most frequent in *L. littorea* but was also found at a low prevalence in *L. saxatilis*, *L. fabalis* and *L. obtusata* (Granovitch and Johannesson, 2000). Both *Littorina spp.* (first intermediate host) and *M. edulis* (second intermediate host) and seagulls (final hosts for *R. roscovita*) are frequent in both the areas of the present study year round. It is not known if the emission of *R. roscovita* cercariae from snails occurs continuously or merely during restricted seasons along the Swedish west coast. According to Lauckner (1983) the major attack of cercarial emission (*H. elongata* and *R. roscovita*) from snails at the German North Sea coast, occurs in late May to early June and coincides with the settling of young bivalves. In October the cercarial attack has ceased. Svärdh (1999) found that metacercariae of *R. roscovita* in adult individuals of *M. edulis* appear all year round, but this could be explained by seasonal infection followed by a prolonged encystation of the metacercariae. Indeed, Pekkarinen (1988) found that the development of cercariae of the gymnophallid *Lacunovermis macomae* into metacercariae takes many months and when once encysted in the second intermediate host, they could live for years. In my study the sampled mussels were 2 – 3 years of age and it is possible that the metacercariae found in the mussels infected soon after the mussel settlement. The metacercariae were all dead and in a disintegrating stage, that is, the cyst wall was broken and the cyst filled with dead larva and phagocyting hemocytes. According to Lauckner (1983) dead and disintegrating metacercariae are found in mussels sampled in spring and the author suggest this to be an effect of partly frozen tissues rather than of the host´s internal defence. Freezing temperatures could however not be the explanation in my study as the water temperature during the winter preceding the sampling (2001/2002) did not decrease below 2°C in the sampling areas. Only mussels sampled close to rocky shores could have been exposed to freezing temperatures when emerged, but dead metacercariae were found also in the subtidal mussels. Thus it seems more likely that the dead metacercariae are the result of an active defense from the mussel host. This is also supported by the results from Svärdh and Thulin (1985) who found numerous hemocytes in the cysts of dead trematode metacercariae in *M. edulis.* Likewise from Villalba et al. (1997) who found trematodes in *M. galloprovincialis* to be frequently destroyed by hemocytes.

Metacercariae were found mainly in mussels close to the shores with snails present, which is in accordance to Werding (1969) who found the *R. roscovita* cercaria to be a bad swimmer and to use its tail only for keeping the larvae floating in the water. Cercarial contact with the second intermediate host will probably be faciliated via water propulsion and the filtration of the host. Blue mussels generate currents by sucking in water through the inhalant siphon and then expelling it out through the exhalant siphon. An inactive cercaria could either be lucky to enter the mussel mantle cavity or be carried away by the expelled water current. In a study of unspecified trematode cercariae, Stunkard (1964) describes a laboratory experiment where different bivalves were exposed to thousands of cercariae, and he observed "a stream of cercariae sucked into the incurrent siphon of *M. edulis*". In the field the filtration rate in *M. edulis* various a lot, depending on the size, age and weight of the mussel (see e.g. Thompson et al., 1974), the water temperature (Jorgensen et al. 1990) and the density of

particles in the water (Clausen and Riisgard, 1996). Trematode cercariae behaviour suggests that cercariae in general are more specific to the host´s environment than to the host itself (Combes et al., 1994), but this conflicts with the results from a water flow experiment with *Himasthla elongata* infecting cockles *Cerastoderma edule* (de Montaudouin et al., 1998). The results suggest a passive infection mechanism based on the filtration activity of the hosts.

Conclusion

In spite of the presence of filtering mussels at all sites, the results of my study suggest a restricted dispersal for *Renicola roscovita* cercariae released from snails. The lack of metacercariae in the farmed mussels is probably an effect of the distance from the snail populations. Only occasionally, cercariae are spread to these mussels. Therefore, mussel farms should not be placed close to rocky shores or environments occupied by snails of *Littorina*. Also farming equipment that easily become a suitable substratum for the snails should be avoided.

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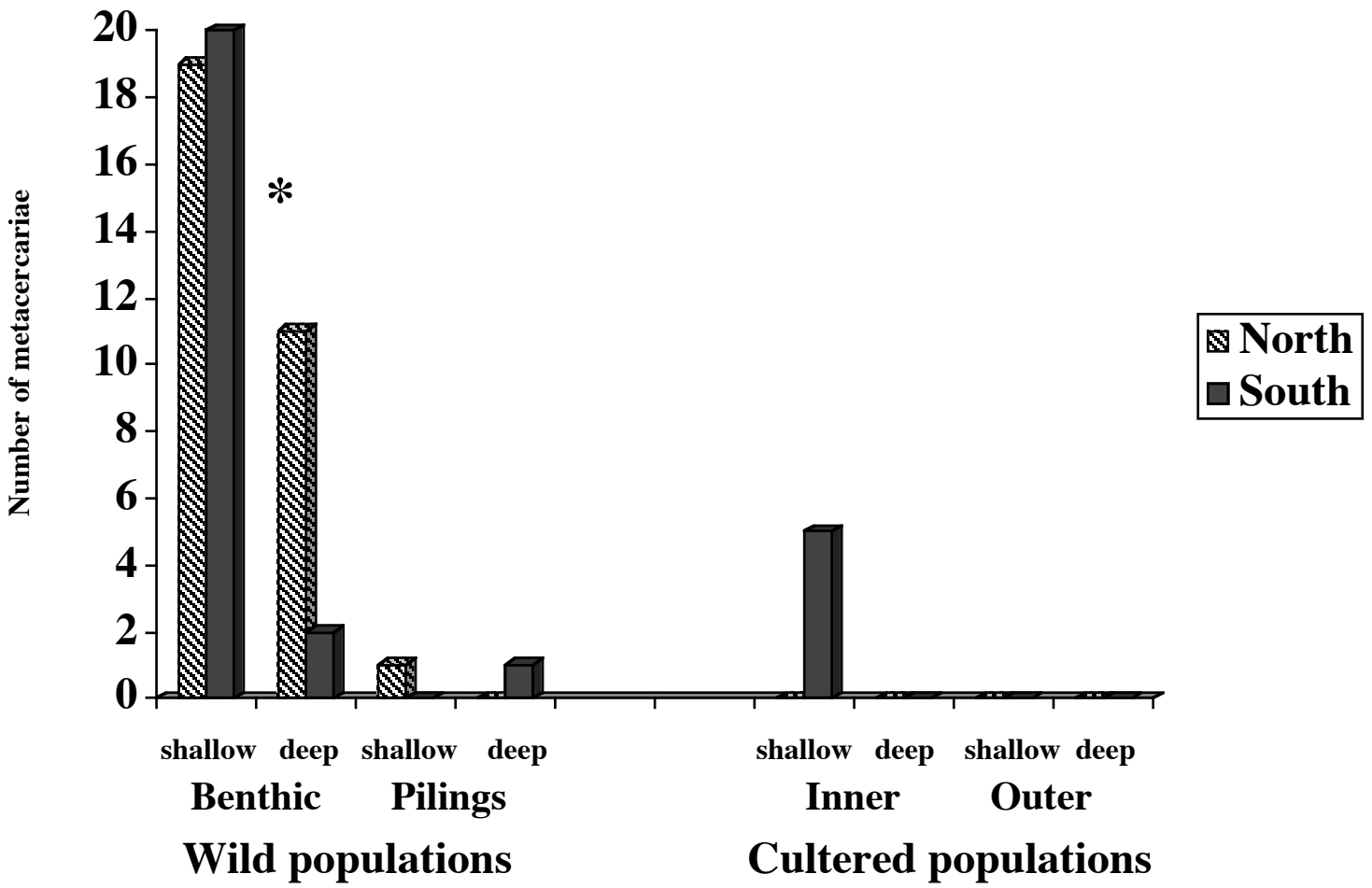
References

Bartoli, P. and **Combes, C**. (1986) Stratégies de dissémination des cercaires de trématodes dans un écosystème marin littoral. Acta AEcologica/AEcologia Generalis **7** (2), 101 – 114.
Clausen, I and **Riisgard, H.U.** (1996) Growth, filtration and respiration in the mussel *Mytilus edulis*: No evidence for physiological regulation of the filter-pump to nutritional needs. Marine Ecology Progress Series **141** (1 – 3), 37 – 45.
Combes, C., Fournier, A., Moné, H. and **Théron, A.** (1994) Behaviours in trematode cercariae that enhance parasite transmission: patterns and processes. Parasitology **109**, 3 – 13.
Figueras, A.J., Jardon, C.F. and **Caldas, J.R.** (1991) Diseases and parasites in rafted mussels (*Mytilus galloprovincialis* Lmk): preliminary results. Aquaculture **99**, 17 – 33.
Granovitch, A. and **Johannesson, K**. (2000) Digenetic trematodes in four species of *Littorina* from the west coast of Sweden. Ophelia **53** (1), 55 – 65.
Haas, W. (1992) Physiological analysis of cercarial behaviour. Journal of Parasitology **78** (2), 243 – 255.
Haas, W., Granzer, M. and **Brockelman, C.R.** (1990) Finding and recognition of the bovine host by the cercariae of *Schistosoma spindale*. Parasitology Research **76**, 343 – 350.

Jonsson, P.R. and **André, C**. (1992) Mass mortlity of the bivalve *Cerastoderma edule* on the Swedish west coast caused by infestation with the digenean trematode *Cercaria Cerastodermae* I. Ophelia **36** (2), 151 – 157.
Jorgensen, C.B., Larsen, P.S. and **Riisgard, H.U.** (1990) Effects of temperature on the mussel pump. Marine Ecology Progress Series **64**, 89 – 97.
Lauckner, G. (1983) Bivalves as second intermediate hosts for Digenea. In: Diseases of Marine Animals, Vol. II (ed. O. Kinne). Biologische Anstalt Helgoland, Hamburg, Germany, pp. 707 – 714.
de Montaudouin, X., Wegeberg, A.M., Jensen, K.T. and **Sauriau, P.G**. (1998) Infection characteristics of *Himasthla elongata* cercariae in cockles as a function of water current. Diseases of Aquatic Organisms **34**, 63 – 70.
M. Pekkarinen. Gymnophallid trematode parasites in the baltic clam *Macoma balthica* (L.) off the southwest coast of Finland (Academic dissertation). Helsinki: University of Helsinki, 1988.
Robledo, J.A.F. and **Figueras, A.** (1995) The effects of culture-site, depth, season, and stock source on the prevalence of *Marteilia refringens* in cultured mussels (*Mytilus galloprovincialis* Lmk) from Galicia, Spain. Journal of Parasitology **81** (3), 354 – 363.
Stunkard, H.W. (1964) Studies on the trematode genus Renicola: Observations on the life-history, specificity, and systematic position. Biological Bulletin, Woods Hole **126**, 467 – 489.
Svärdh, L. (1999) Bacteria, granulocytomas, and trematode metacercariae in the digestive gland of *Mytilus edulis*: Seasonal and interpopulation variation. J. Inv. Pathol. **74**, 275 – 280.
Svärdh, L. and **Thulin, J**. (1985). The parasite fauna of natural and farmed *Mytilus edulis* from the west coast of Sweden, with special reference to *Renicola roscovita*. Medd. fr. Havsfiskelaboratoriet, nr 312, Lysekil.
Thompson, R.J. and **Bayne, B.L**. (1974) Some relationships between growth, metabolism and food in the mussel *Mytilus edulis*. Marine Biology **27**, 317 – 326.**Werding, B**. (1969) Morphologie, Entwicklung und Ökologie digener Trematoden-Larven der Strandschnecke *Littorina littorea*. Marine Biology **3**, 306 – 333.
Villalba, A., **Mourelle, S. G. Carballal, M.J**. and **López, M.C** (1993) Effects of infection by the protistan parasite *Marteilia refringens* on the reproduction of cultured mussels *Mytilus galloprovincialis* in Galicia (NW Spain). Diseases of Aquatic Organisms **17**, 205 – 213.
Villalba, A., Mourelle, S.G., Carballal, M.J. and **López, C** (1997) Symbionts and diseases of farmed mussels *Mytilus galloprovincialis* throughout the culture process in the Rías of Galicia (NW Spain). Diseases of Aquatic Organisms **31**, 127 – 139.

Fig. 1. Number of *Renicola roscovita* metacercariae found in blue mussel populations from two areas at the Swedish west coast.



***Pooled data revealed a significant difference between the level "benthic" and the other levels (SNK-test).**

Table 1. Effects on metacercariae occurence in 240 individuals of *Mytilus edulis*. Results from a three-factor ANOVA.

Source	df	SS	MS	F	p
Area	1	0.04	0.04	0.05	0.08
Depth	1	4.00	4.00	5.69	0.25
Habitat	3	31.05	10.35	21.47	**0.02**
A x D	1	0.70	0.70	0.93	0.34
A x H	3	1.45	0.48	0.64	0.59
D x H	3	7.68	2.56	5.31	0.10
A x D x H	3	1.45	0.48	0.64	0.59
Residual	224	170.13			