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

Lillemor Svärdh

Department of Marine Ecology
Göteborg University
2003

Göteborg University
Faculty of Natural Sciences
2003

Dissertation

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

Lillemor Svärdh

Department of Marine Ecology
Göteborg University
Tjärnö Marine Biological Laboratory
SE – 452 96 Strömstad
Sweden

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.

Svärdh, L. Histological and parasitological studies of the blue mussel *Mytilus edulis* L.. Department of Marine Ecology, Tjärnö Marine Biological Laboratory, SE 452 96 Strömstad.

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.*

Inst. för Marin Ekologi, Göteborgs Universitet
Göteborg 2003
ISBN 91-89677-08-0
Printed in Sweden
Vasastadens Bokbinderi AB, Göteborg

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 μm 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.

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisor **Kerstin Johannesson** and my assistant supervisor **Åke Granmo**, for all theirsupport during the years. I am also grateful to **Jan Thulin** and **Lars Rasmussen** who supervised me in the beginning of my research work. Many thanks to **Gunnar Berg,** who, with all his kindness, gave me such great support during the years at the Zoological Department. I am also grateful to **Zbigniew Uggla** who always has been willing to help me with the sampling of farmed mussels. A special thanks to **Andrey Granovitch** and **Natalia Michailova** in St. Petersburg, who besides "parasite talk" also invited me to an unforgettable performance of the ballet "Swan Lake".

I am very happy that I got the possibility to do my work at the Tjärnö Laboratory. All the kindness and friendship that I have experienced there every day, has been a great privilege. I am also grateful to all my "PhD-student" colleagues at the Department of Marine Ecology for that special feeling of solidarity among you all.

Financial support was provided by the Faculty of Natural Sciences, Göteborg University, the Royal Swedish Academy of Sciences, Swedish Council for Forestry and Agricultural Research, and by the foundations of Adlerbertska, Ax:son-Johnson, Berghaus, Colliander, Kungliga och Hvitfeldtska, Lundgren, Lundström, Nybelin, Wallenberg, and Wåhlström.

Tackalla goa vänner och tillika kollegor på Tjärnö, för att ni fått mig att känna mig som en del i en stor gemenskap! Tack alla ni i forskarkorridoren för att ni alltid har gett mig en del av er tid. Några på labbet måste jag nämna speciellt. **Kerstin**, tack för att du trodde på mig och erbjöd dig att bli min handledare hösten 1997. **Annci, Maria, Gunilla** och **Yvonne**; hur många gånger har jag stått inne hos er och bett om hjälp med praktiska ting? Ni har alltid haft tid och ett vänligt ord, tack för det. **Kalle, Ingemar** och **Anders**, som stått ut med mig alla år, trots att jag ännu inte lärt mig att "knyta knuten på skrapan". Kalle har dessutom bidragit med diverse utstyrsel till den gamla snittmaskinen. **HansG** och **Lars-Ove**, tack för all support! Och **Daniel** och **Mats**, grabbarna i rummet bredvid, som ofta sett mig stå i dörren och säga "har ni tid en minut" och sedan ändå ställt upp och hjälpt mig med statistikproblem, trots att det tagit längre tid.

Gunilla och **Ann**, mina forna rumskompisar! Tack för att ni velat delge mig era tankar i både ljusa och mörka stunder och tack för att ni tagit er tid till att lyssna på mig. **Anette**, som sista året varit mitt stöd då det gällt problem med Power-point och Excel; en jättekram till dig! Tack också till **Lisbeth**, som inte gett upp sina försök att få upp mig på hästryggen igen.

Naturligtvis hade det inte blivit någon avhandling utan mina närmaste, som stöttat mig i alla lägen. **Simmenäsklanen,** min **mor** och mina **systrar, svågrar** och **syskonbarn,** - tack för att ni alltid finns som min trygghet. Tack vare er har jag vågat hoppa!

Kit, tack för alla kramar och kloka ord du gett mig och tack **Tomas**, min allra käraste vän, för ditt stöd och din uppmuntran.

Fig. 1

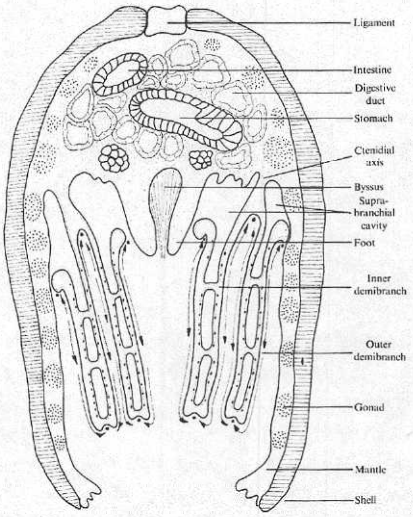


Fig. 2

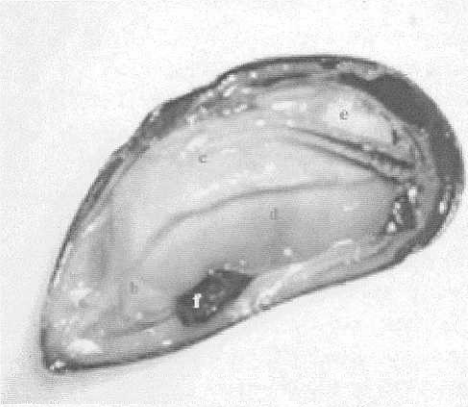


Fig. 1 Cross-section of the mantle, the gills and the digestive gland (Bayne 1976).

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

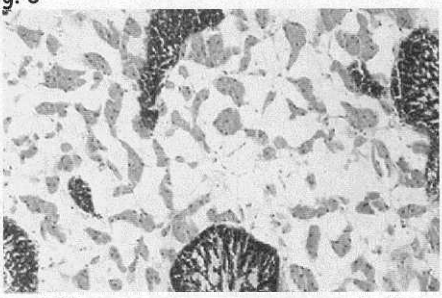
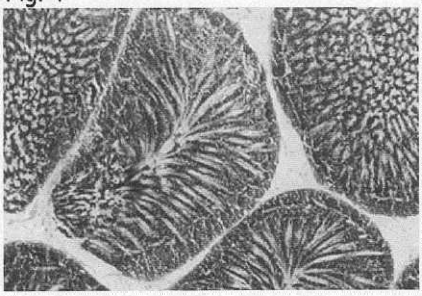


Fig. 4



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 Mediterranean 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.

GÖTEBORGS UNIVERSITET
GÖTEBORGS UNIVERSITETSBIBLIOTEK

Digitaltryckt & Bunden
Vasastadens Bokbinderi AB
Göteborg 2003