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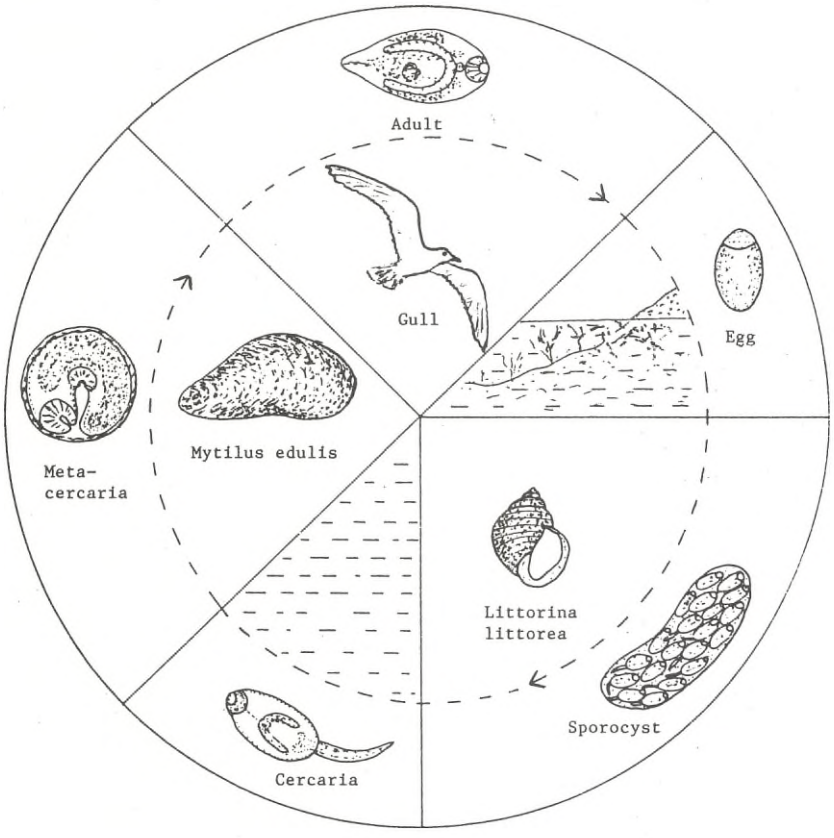

THE PARASITE FAUNA OF NATURAL AND FARMED *MYTILUS EDULIS* FROM THE WEST COAST OF SWEDEN, WITH SPECIAL REFERENCE TO *RENICOLA ROSCOVITA*

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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 $2^{\circ}C$.

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 $10^{\circ}C$.

The mussels were stored in a cold bag during the transports and then in a cold chamber ($10^{\circ}C$) 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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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

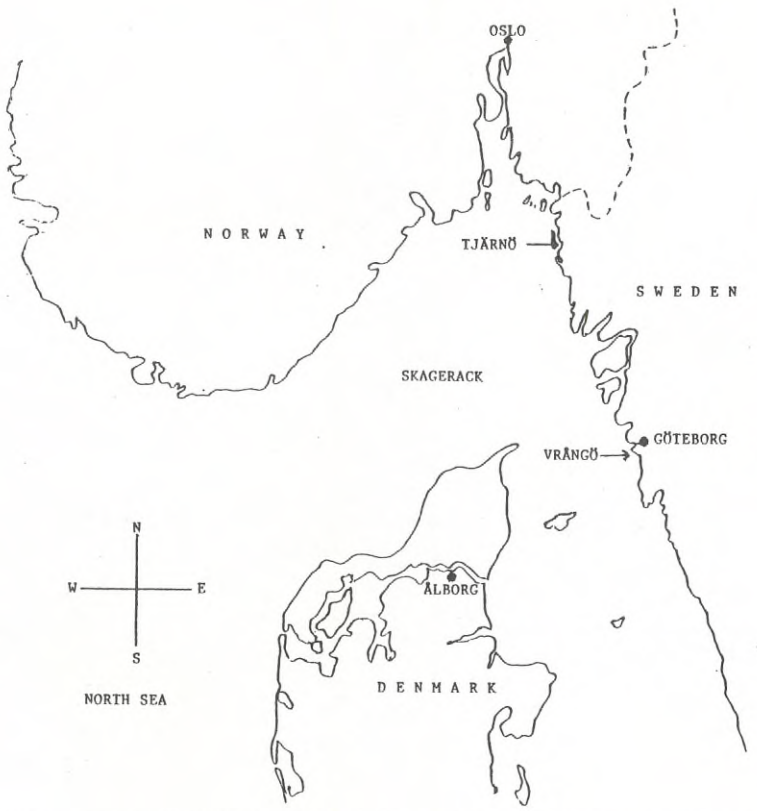


Figure 1. General map to show the positions of the two areas of investigation, Tjärnö and Vrångö, on the west coast of Sweden.

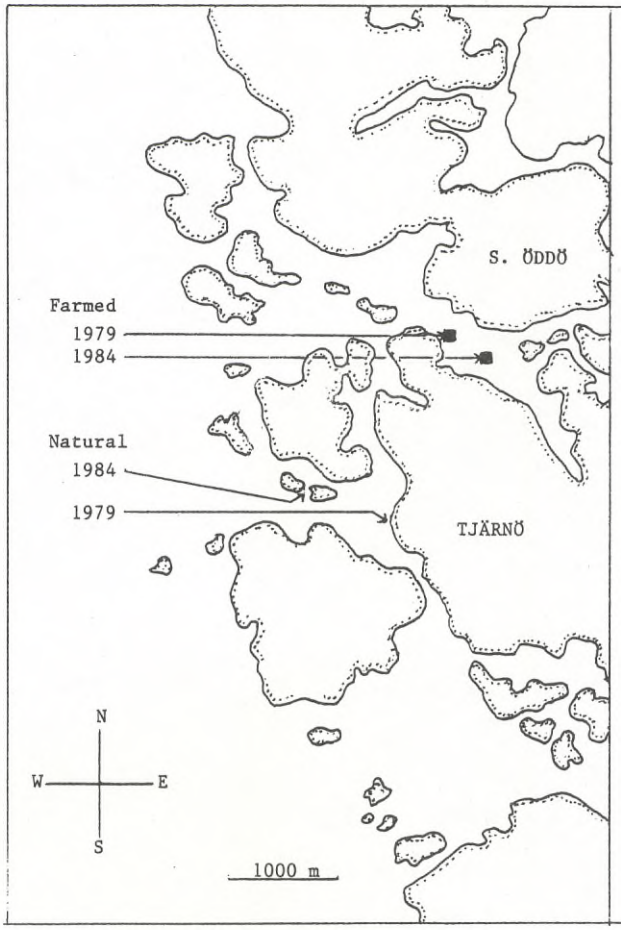


Figure 2. Sampling localities for farmed and natural population of mussels in the Tjärnö area in 1979 an in the present investigation, 1984.

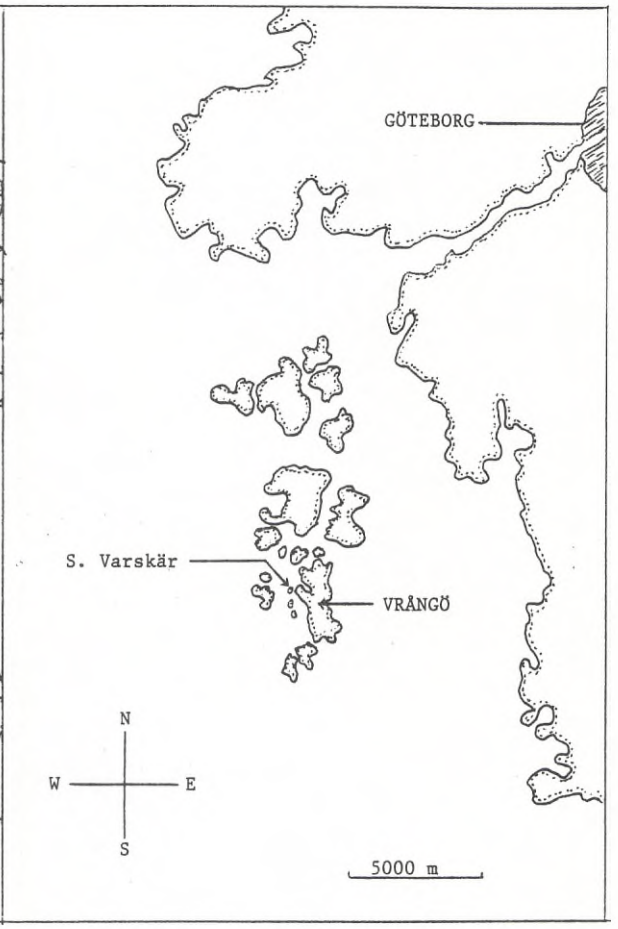


Figure 3. Sampling localities for farmed and natural population of mussels in the Vrångö area situated east and west respectively of S. Varskär.

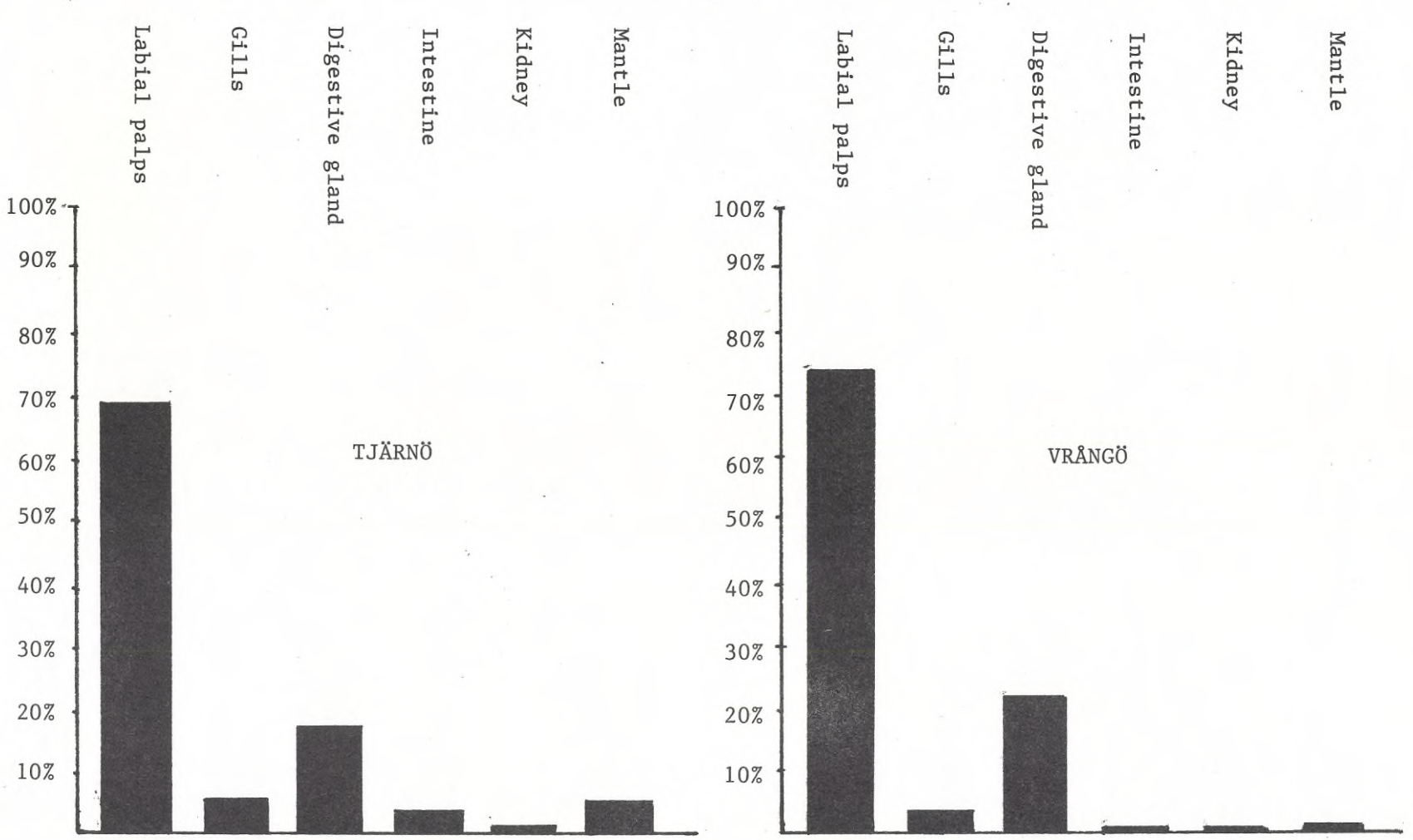
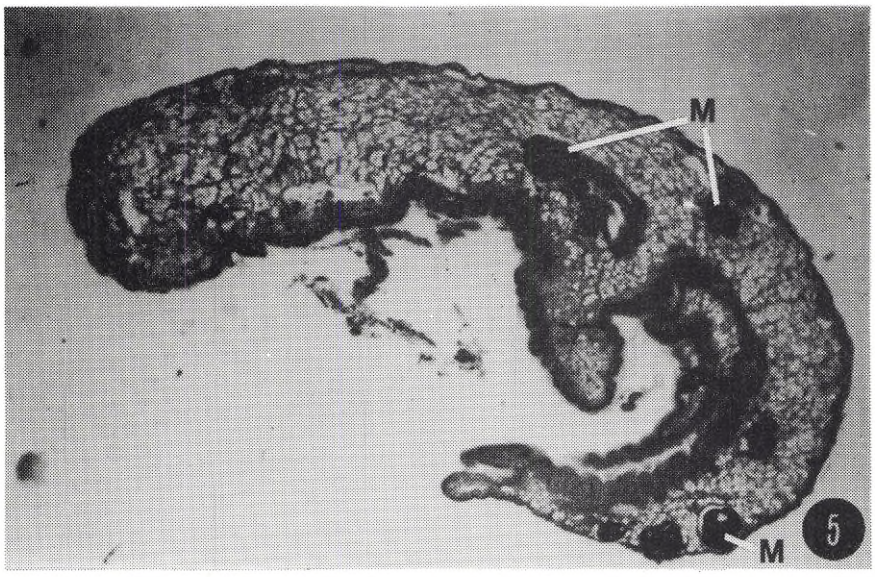
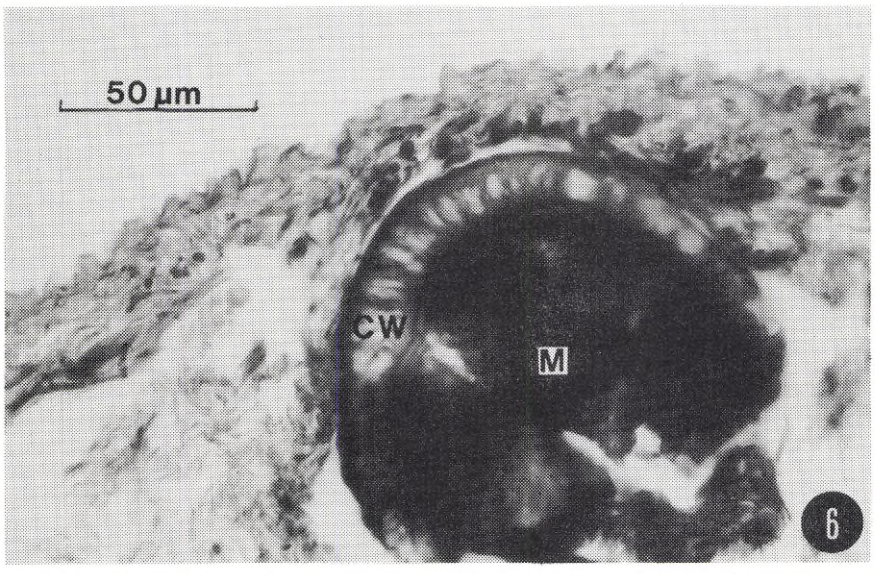


Figure 4. The percentage distribution of metacercariae of Renicola roscovita in different organs of mussels from natural populations at Tjärnö and Vrångö on the west coast of Sweden.





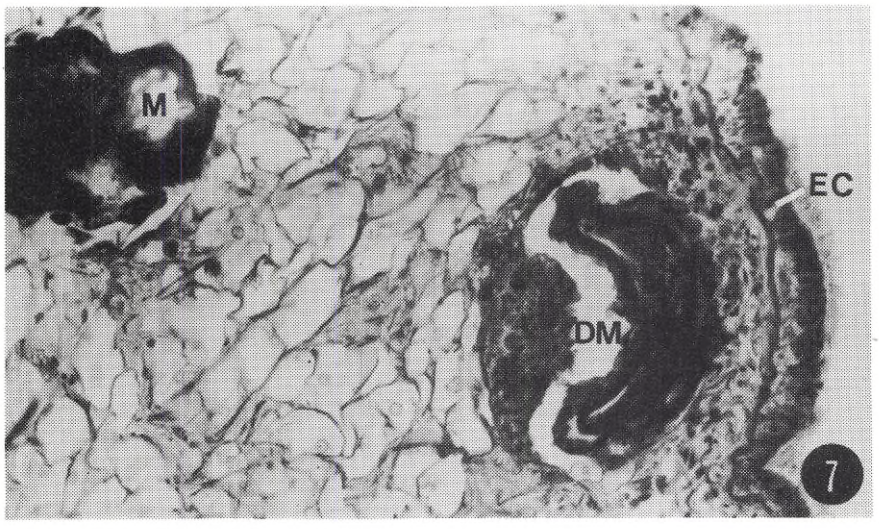
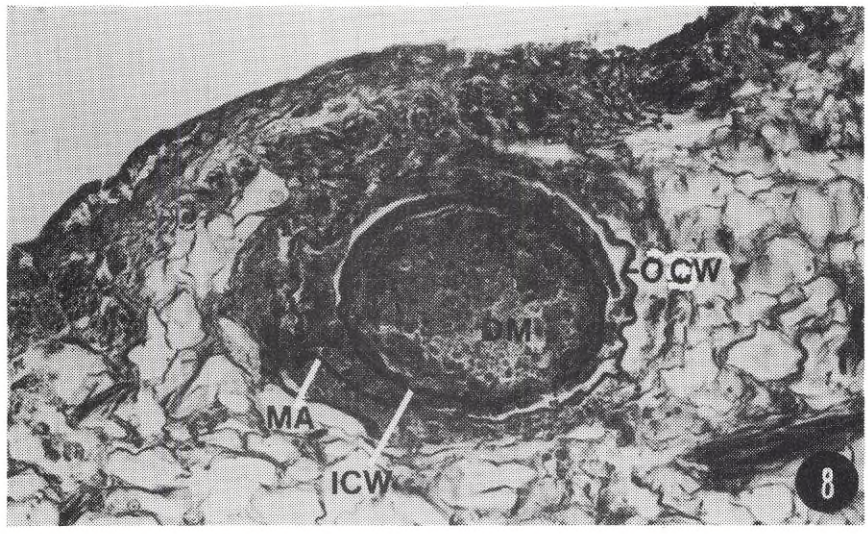
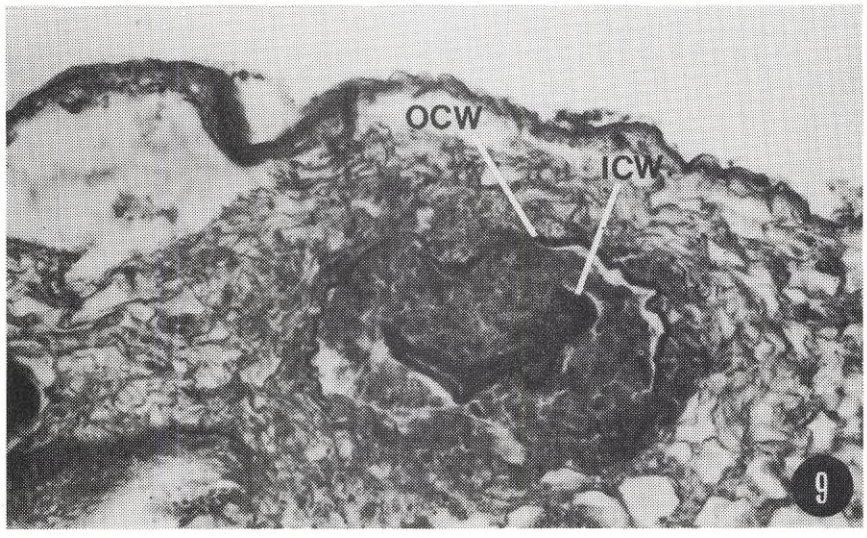


Figure 5. Section of a labial palp of a mussel from a natural population. The palp tissue contains several metacercariae (M) of Renicola roscovita.
Figure 6. The cyst wall (CW) of a living metacercaria of R. roscovita.
Figure 7. A living (M) and a dead, disintegrating (DM) metacercaria of R. roscovita.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





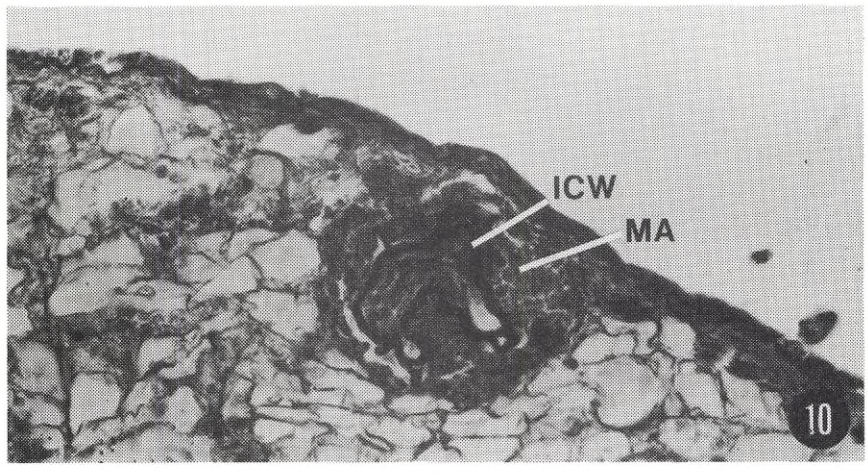


Figure 8. A disintegrating metacercaria. Note the distinct host tissue reaction with its numerous macrophages (MA), and the outer (OCW) and inner (ICW) membrane of the former cyst wall.
Figure 9. The metacercaria is almost entirely disintegrated and the inner and parts of the outer cyst wall membrane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

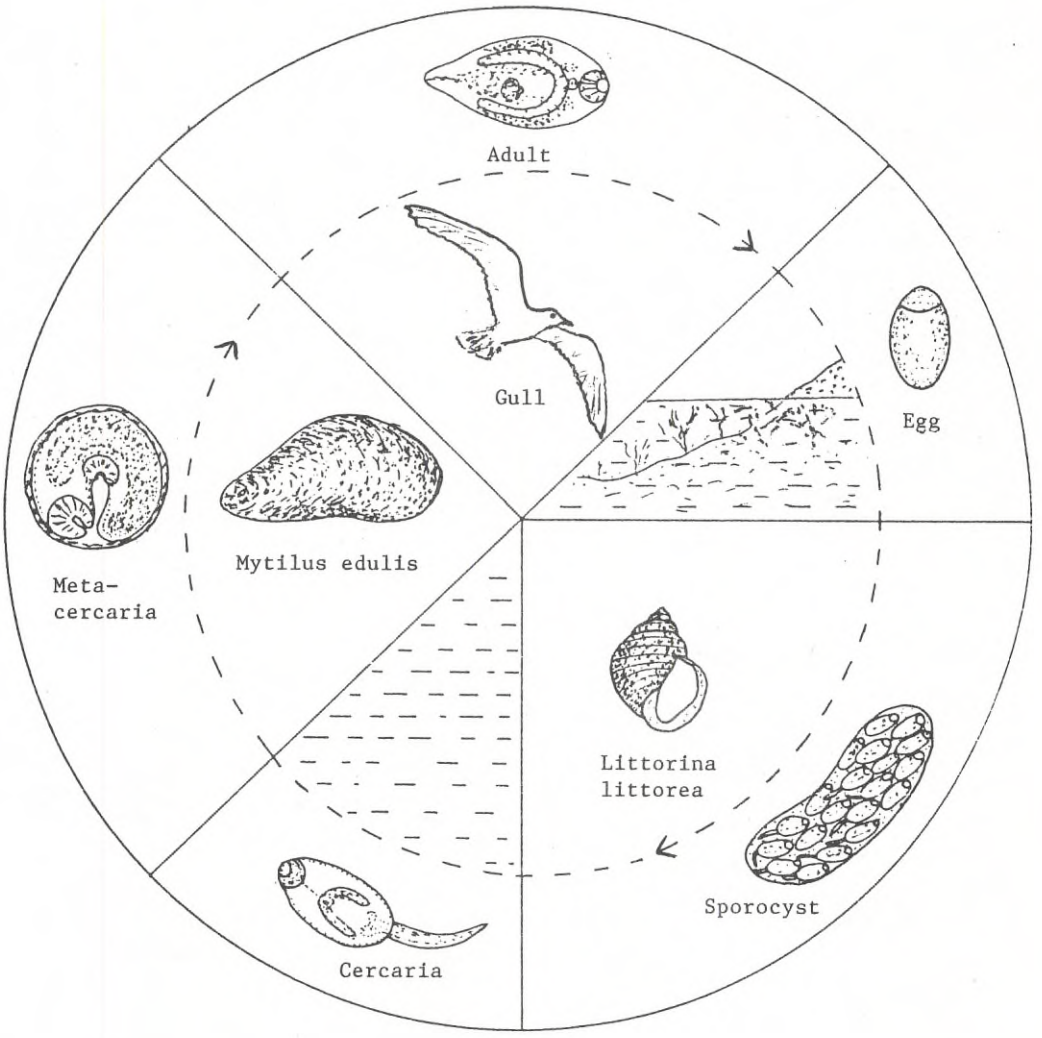


Figure 11. The life cycle of Renicola roscovita. The outer circle corresponds to different developmental stages of the parasite. The inner circle corresponds to host species in which these stages may occur.