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Development of the cercaria of *Lacunovermis macomae* (Trematoda: Gymnophallidae) to the metacercaria in brackish-water *Macoma balthica* (Bivalvia)

Marketta Pekkarinen

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Practically trematode-free *Macoma balthica* (L.) were infected experimentally with cercariae which were released from sporocysts obtained from a few infected individuals of *M. balthica*. The metacercariae which developed from the cercariae in the clams in laboratory conditions, and thus also the cercariae, were shown to be those of *Lacunovermis macomae* (Lebour).

During the first 1–2 months after the penetration of the cercariae some cercarial characteristics, i.e. the penetration glands, microvilli in the suckers and spines in the ventral sucker, disappeared. The lumen of the digestive caeca soon dilated and the caeca grew in size for three months. Fettrot-stainable lipids appeared in the gastrodermal cells, and β-glucuronidase activity appeared in the digestive caeca. The activity of acid phosphatase increased in the distal margins of the gastrodermal cells.

Three to four months after the infection a remarkable development of many organs (e.g. growth of the gonads) of the metacercariae was apparent. Vitelline globules began to appear in the vitelline cells. The ventral pit, genital atrium and papillae of the genital pore were forming. The tegumental spines, which had first divided into doublets, began to broaden. Usually two new individual spines grew under and between the former members of the doublets, and so four-pointed spines were formed. The spine bases were undivided.

After four months, at 7–15°C, the metacercariae were still slightly smaller than aged metacercariae measured earlier, and their genital organs were usually less developed than those in the latter. The sucker ratio (oral sucker length ventral sucker length) of the metacercaria became greater than it was in the cercaria. The oral sucker was proportionally small during the growth of the metacercaria.

Infestation with great numbers of developing metacercariae only slightly depressed the general condition of the host clam in the laboratory during the first three months of a six-month experiment. The mean number of metacercariae per clam decreased during the experiment. In a collecting site where *M. balthica* are generally infected by metacercariae of *L. macomae* an increase of young metacercariae was noticed in the clams in July. This, with the age assessment of the metacercariae, suggests mass emergence of cercariae coincident with the spawning season of the clam.

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1. Introduction

The often complicated life cycles of digenetic trematodes have made it difficult to distinguish different species and to identify different life stages belonging to a single species. *Lacunovermis macomae* (Lebour) is one gymnophallid species for which the whole life cycle has not yet been worked out (Loos-Frank 1971). Its metacercariae have been found several times since Lebour (1908) in the extrapallial space of *Macoma balthica* (L.) (Markowski 1936, Reimer 1962, Loos-Frank 1970, Pekkarinen 1983, 1984a, b). Adults of *L. macomae* have been found from waders and ducks (references in Pekkarinen 1984a). It has been possible to cultivate metacercariae of *L. macomae* to the adults in test animals (birds or

Cunningham's method as modified by Michel & Chretien (1975). Different buffers and pH values were tried. For the detection of calcium salts in the excretory concretions metacercariae were put in a dilute solution of alizarin red S (Chroma) in 0.6% NaCl. Further, aged metacercariae were fixed in glutaraldehyde (see above) and treated with 1.5% OsO_4 in the phosphate buffer and after dehydration embedded in Epon. Sections from them were stained with uranyl acetate and lead citrate for TEM.

2.2. Effect of heavy infestation of developing metacercariae on the host clam

Cercariae obtained from seven clams were allowed to infect as above 38 clams in late September 1984. These infected clams, as 38 control clams, were kept in aquaria in sifted (1 mm mesh) and washed dune sand like those in the experiment above for six months. The water temperature was 10—12°C at the beginning and about 7°C at the end. Condition characteristics of the clams — water content, "fatness" index, condition factor CF_1, according to Pekkarinen (1983); and CF_2, which is calculated as 100× dry weight of the soft part / (total fresh weight — shell weight) — were examined, and trematode counts were made three and six months later and in 15 additional control clams at the beginning.

2.3. "Small metacercariae" in naturally infected clams

The occurrence of metacercariae in the extrapallial space of *M. balthica* at site I was routinely investigated in other experiments during 1984 by staining the shells with Trypan Rot. In July—August a comparison was made between clams from sites I and IV. Metacercariae of about six clams were isolated and counted at one time in a small Petri dish. They were classified as "small metacercariae" (Pekkarinen 1984b), large *L. macomae*, and others and they were added to the numbers obtained from other series of six clams.

3. Results

3.1. Development of metacercariae

Results from the infection experiment are shown in Table 1. Occasional large metacercariae of *L. macomae* and *Gymnophallus gibberosus* (?), which had invaded the clams before the experiment, occurred in the shells in both the infected and control groups. They could, however, be distinguished from the recently developed metacercariae (small metacercariae). Although imprints of small metacercariae could be seen in the infected shells stained with Trypan Rot after one month, the metacercariae did not begin to attach to the shells earlier than the first two months (Fig. 1). Stainable "migration paths" were sometimes visible in the shells during the

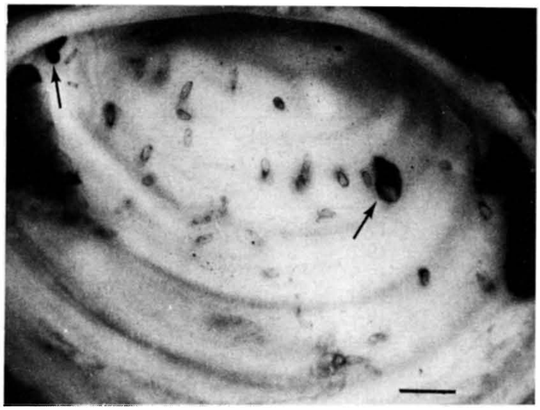

Fig. 1. The shell (stained with Trypan Rot) of a *Macoma* individual (shell length 14.2 mm, see Table 1) two months post infection by cercariae. There are small metacercariae and their imprints and two large imprints (arrowed) without any metacercariae on the inner surface of the shell. Scale bar 1 mm.

first three months after infection. In the most heavily infected clams over 200 metacercariae could be found. Two clams in the three-month sample harboured an oligochaete, over 2 mm in length, in their tissues. Much tissue debris burst out with the oligochaetes. In the four-month sample the interior surface of the shell of one infected individual had an odd appearance and so imprints were not visible, and the shell interior of another individual was covered by mucus and membranes. One control clam was dead.

The penetration glands of the cercariae did not degenerate rapidly after penetration. Nine days after infection the glands had the same appearance as before penetration. Most of the two-week-old metacercariae had their penetration glands unchanged as viewed under the light microscope. In three- to four-week-old metacercariae remnants of the glands were observed (Fig. 2a). The contents of the glands had become reduced, or only 1—6 detached balls were present. The penetration glands were absent in metacercariae two months old. Microvilli on the forebody and around the suckers had disappeared within one month. Spines in the ventral sucker remained visible in some individuals for up to two months (Figs. 3a and 4b), after which they disappeared.

Measurements of the developing metacercariae and their organs are given in Table 2. For comparison the measurements of cercariae (Pekkarinen 1986b), and metacercariae of *L.*

Table 1. Former large (*Lacunovermis macomae* = Lm and *Gymnophallus gibberosus*? = Gg) and recently reared small metacercariae in the extrapallial space and imprints inside the right (R) and left (L) shell valves of *Macoma balthica* infected initially with cercariae in a four-month experiment. In the control clams no small metacercariae were found.

Time (months)	Infected clams						Control clams		
	Shell length (mm)	Large metacercariae Lm	Large metacercariae Gg?	Small imprints R	Small imprints L	Small metacercariae	Shell length (mm)	Large metacercariae Lm	Large metacercariae Gg?
1.5	17.3	2		2	11	>100	17.8		
	19.1			24	14	53	16.6		1
	17.0	4		20	15	>100	15.3		1
	17.5			>30	>30	>100	16.2	1	
	18.1	1		14	18	34	14.9	1	
2	16.5			3	5	>100	16.7		
	16.9	1		13	>30	>100	16.6		
	19.3	3		12	12	42	17.1		
	17.5			0	7	23	17.8	1	
	14.2			>30	>30	>100	17.4	1	
3	18.3	1		19	24	66	17.9	1	1
	15.4			>30	>30	91	20.3	1	
	15.6			>30	>30	43	19.1	1	
	17.2	1	1	>30	>30	38	17.0	4	
	16.2	2		>30	>30	>100	13.3	2	
4	15.8	1		>30	>30	>100	18.3	1	1
	19.0		1	–	–	>100	18.8		
	15.7	1		25		74	17.5	2	
	16.6	1		>30	26	40	16.4	2	
	15.0	1		>30	>30	52	16.4 (dead)		

macomae from natural infections (Pekkarinen 1984b) are also given in the Table. After four months' growth the mean size of the metacecariae was still slightly smaller than that of the metacercariae of *L. macomae* measured earlier. The sucker ratio OSL/VSL was quite small in the cercaria (Table 2). During the first month the oral sucker began to grow, but the ventral sucker remained at its initial size (OSL/VSL increased). During the second and third months the suckers grew in a constant proportion. During the fourth month the sucker ratio slightly grew again, but it still remained smaller than that in aged *L. macomae*. The oral sucker length in relation to body length was smallest (BL/OSL greatest) in metacercariae 1.5–2 months old. Even after four months the length of the oral sucker in relation to body length was smaller than that in the aged metacercariae.

The digestive caeca dilated at, or soon after, penetration (Figs. 2a, b and 5a). They were full of material, and they grew in size until the metacercariae reached the age of three months (Fig. 2c, Table 2). After four months the digestive caeca were still larger than the mean measured earlier for *L. macomae*. There were prominent "salivary glands" around the oesophagus. The gross structure of the gastrodermal cells of the metacercariae is similar to that of the cercariae. The distal parts of the cells are supplied with microvilli or lamellae (Fig. 5f). These processes are visible on the gastrodermal fragments which younger metacercariae often discharge during examination under the light microscope. The proximal margins of the cells have larger processes and small pedicels (Fig. 5e).

Fettrot-stainable lipids, which were not detected in the cercaria, appeared in the gastrodermis of young metacercariae. Most gastrodermal cells of aged metacercariae are full of large lipid globules (Fig. 5e). Acid phosphatase activity increased in the digestive caeca and in the tegument of developing metacercariae as compared to the cercariae (Pekkarinen 1986b). The activity was localized in the distal parts of the gastrodermal cells (Fig. 5a, b), and sometimes also in the lumen (Fig. 5a). Such activity occurred in the pharynx and in some subtegumental cells, as also in the cercariae. The vitellaria stained orange-brown. Gelatinolytic activity was detected in the digestive caecum of one aged

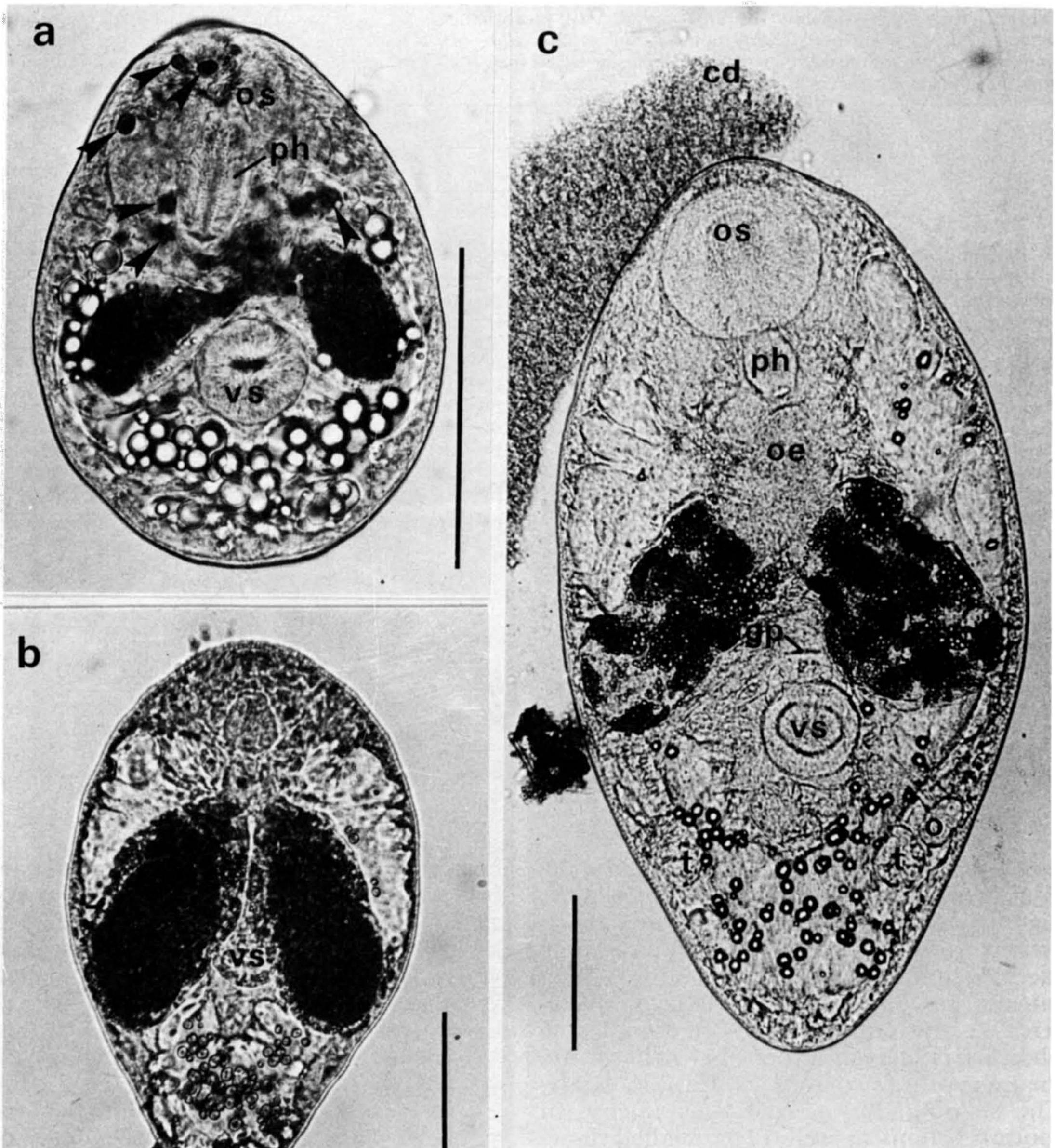

Fig. 2. Metacercariae developed from cercariae after experimental infection in *Macoma balthica*. — a. A metacercaria three weeks post infection. Dilute neutral red solution stained the contents of the digestive caeca and the remnants of the penetration glands (arrowed). The pharynx (ph) is quite large and the excretory concretions are prominent. — b. A two-month-old metacercaria stained with neutral red. Note the proportionally large digestive caeca. — c. An unstained metacercaria three months old (cf. fig. 1a in Pekkarinen 1984b). The bars are 100 μm in all pictures. Note that the metacercariae are more or less pressed under cover glasses. cd = contents of digestive caeca, gp = genital pore, o = ovarium, oe = oesophagus, os = oral sucker, ph = pharynx, t = testis, vs = ventral sucker.

Table 2. Basic measurements (in μm) and derived measurements of cercariae (Pekkarinen 1986b), developing metacercariae (experimental, 1.5–4 months), and metacercariae of *Lacunovermis macomae* (Pekkarinen 1984b). The range of the means of the figures in cercariae/metacercariae from *N* individual clams is given except for the mean figures from the 20 metacercariae of *L. macomae* measured earlier.

	Cercariae	Metacercariae				
		Experimental				*L. macomae*
	(Pekkarinen 1986b)	1.5 months	2 months	3 months	4 months	(Pekkarinen 1984b)
N	5	3	4	5	5	
Individual worms	8+11+10+10+9	7+9+5	10+10+5+10	8+8+10+7+9	10+10+12+10+12	20
Body length (BL)	169–209	239–294	299–371	308–420	387–400	409
Body breadth (BB)	83–96	110–147	141–155	129–195	172–201	238
Oral sucker length (OSL)	41–43	44–58	52–60	52–75	70–76	110
Oral sucker breadth (OSB)	35–38	44–57	49–61	51–75	69–84	115
Pharynx length	26–32	38	37–41	40–45	36–40	43
Pharynx breadth	17–20	27–30	29–32	28–34	27–33	35
Ventral sucker length (VSL)	32–36	32–36	37–39	38–47	43–48	62
Ventral sucker breadth (VSB)	36–40	33–37	39–42	42–52	44–52	71
Ventral sucker distance (VSD)	115–131	155–197	192–238	213–289	234–265	261
Digestive caecum length	35–40	79–97	78–148	112–151	113–151	114
Digestive caecum breadth	25–29	45–50	64–69	66–85	69–89	82
Ovarium length				14–27	18–35	48
Ovarium breadth				14–22	17–23	38
Testis length				16–28	26–36	45
Testis breadth				16–22	20–23	37
Vitellarium length					21–29	40
Vitellarium breadth					21–25	38
$BL \times BB \times 10^{-3}$	15–18	29–43	49–52	48–82	69–82	97
BL/BB	1.78–2.59	1.79–2.59	1.99–2.59	2.00–2.27	1.81–2.38	1.72
BL/OSL	4.10–5.06	5.07–6.82	5.27–7.17	5.35–5.87	4.78–5.75	3.72
OSL/VSL	1.18–1.30	1.36–1.64	1.31–1.62	1.37–1.66	1.54–1.66	1.77
VSD/BL	0.62–0.68	0.65–0.68	0.64–0.69	0.67–0.71	0.64–0.66	0.64

metacercaria (Fig. 5c). In contrast to cercariae, faint or medium β-GU activity occurred in the digestive caeca of young metacercariae. Such activity was often detected in the lumen of the caeca. In some old metacercariae it was quite intense (Fig. 5d). The vitellaria of old metacercariae also stained orange. In the alkaline phosphatase demonstration the vitellaria and the excretory concretions stained very darkly. The staining, however, was as intense in control sections as well. (The method, however, revealed net activity over the control colour, e.g. in the outer margins of the host digestive diverticula and in the free margins of the siphon epithelia.) The excretory concretions of metacercariae, as those of cercariae, stained dull red with alizarin red S.

In three-month-old metacercariae the flame cells were noticed to have increased in number to twelve at each side. In younger metacercariae they were difficult to count. The gonads were difficult to measure in metacercariae up to the age of two months (cf. Fig. 2a, b), while even among older metacercariae they could not be measured in all individuals. The ovarium was kidney-shaped and its size was similar to that of the testis, or smaller (Fig. 2c, Table 2). The gonoducts had become more clearly visible. In three-month-old metacercariae a small ciliated fertilization space (?) was noticed in the oviduct. The Mehlis' gland was visible. The cells of the prostatic gland were still short. None, or a few, of the vitelline cells contained globules. The genital pore was small or large, probably depending on whether the genital atrium had developed and retracted (cf. Figs. 3c and d). The papillae of the genital pore were lacking. The ventral pit was sometimes visible. At the age of four months the papillae of the genital pore in the metacercariae were present in some individuals (Fig. 3d). The number of vitelline globules had increased in the vitelline gland cells. The mean sizes of the gonads and vitellaria were still smaller than those measured earlier in aged metacercariae of *L. macomae* (Table 2).

The lateral papillae of the oral sucker were

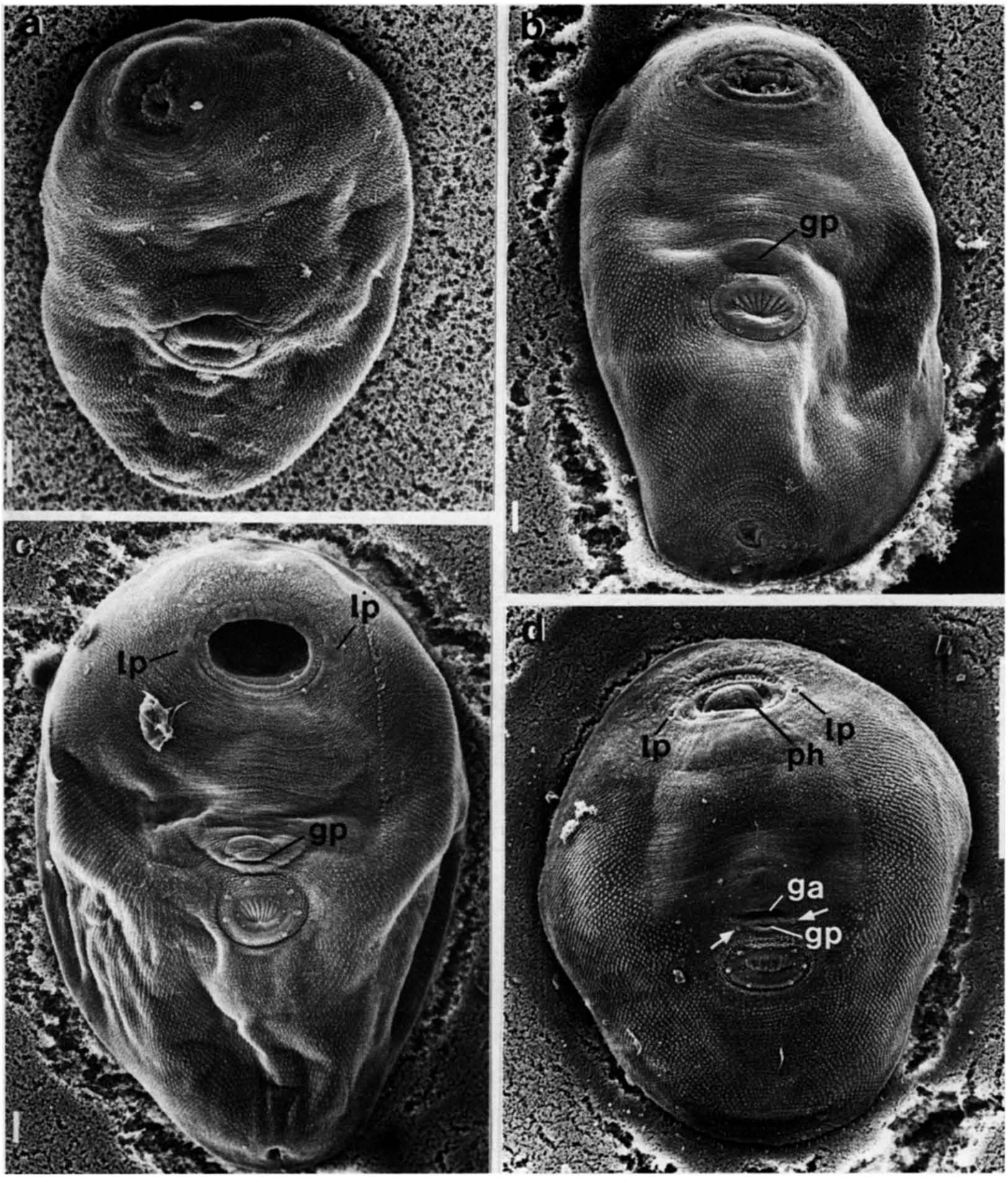

Fig. 3. Metacercariae developed from cercariae after experimental infection in *Macoma balthica*: — a. 1.5 months post infection, — b. two months p.i., — c. three months p.i. and — d. four months p.i. ga = genital atrium, gp = genital pore, lp = sites of the lateral papillae of the oral sucker, ph = pharynx. In the metacercaria of picture d the genital atrium is forming and has not fully retracted (cf. fig. 5 in Pekkarinen 1984b). The arrows indicate the papillae of the genital pore. The scale bars are 10 μm in a—c and 100 μm in d.

visible even in two-month-old metacercariae which had been fixed in formaldehyde, but not in living specimens.

The tegumental spines of 1.5-month-old metacercariae were similar to those of the cercariae, i.e. they were single spines, which sometimes had a shallow longitudinal groove on the face. These young metacercariae had a

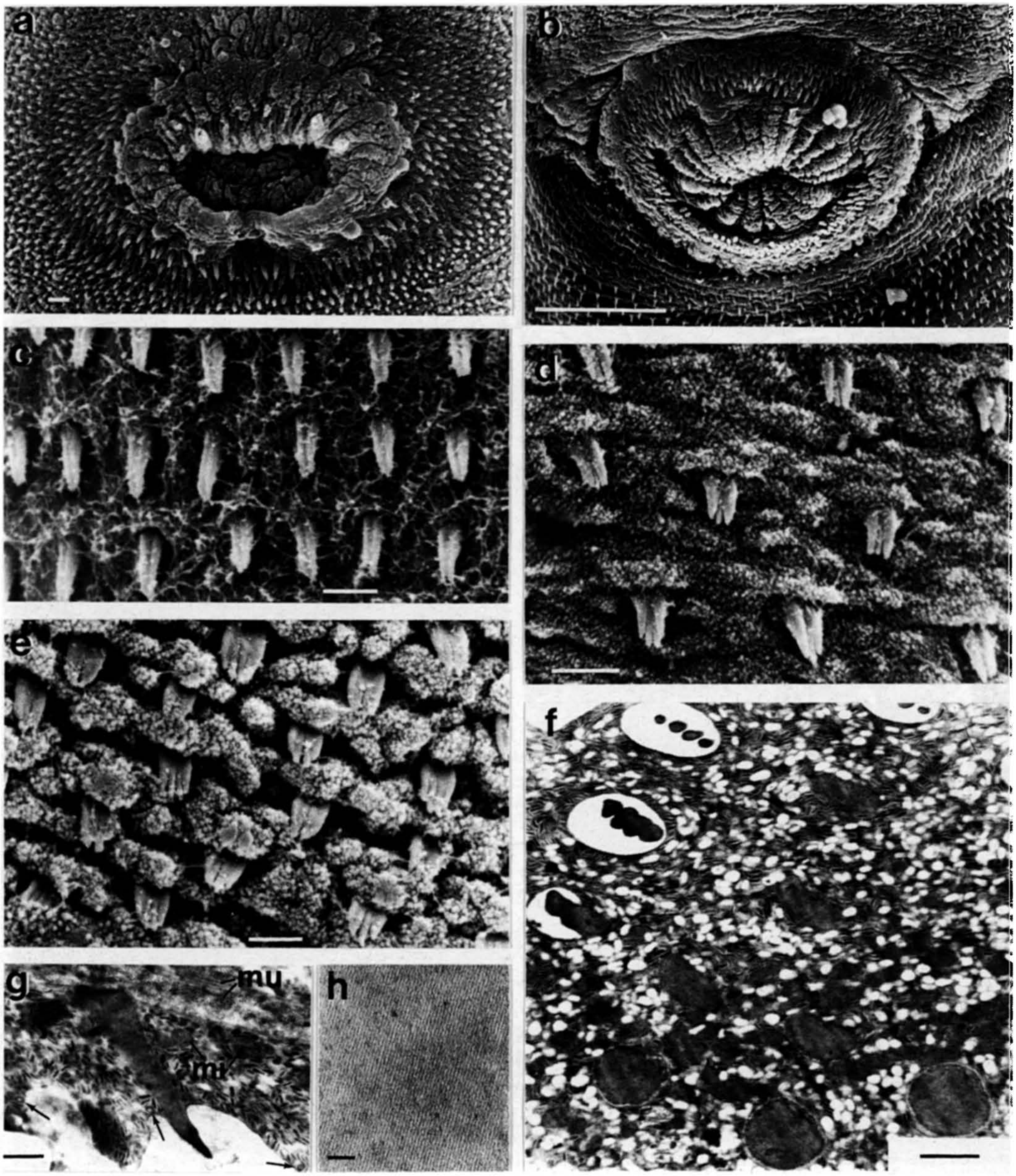

Fig. 4. Tegumental structures of different-aged metacercariae of *Lacunovermis macomae*. — a. The "head" of a metacercaria two months post infection in *M. balthica*. The border of the oral sucker within is indicated by a circular depression around the mouth. — b. A ventral sucker which still has the ring of heavy spines, characteristic of cercariae, of a metacercaria developed for two months. The tegumental spines behind the sucker are only shallowly grooved longitudinally. — c. Deeply grooved tegumental spines of a two-month-old metacercaria. — d. Double spines on the point of broadening of a three-month-old metacercaria. 1—2 new individual spines are growing between and under the former individuals of the doublets. — e. Four-pointed tegumental spines of a metacercaria reared experimentally for four months in *M. balthica*. — f. Cross sections of tegumental spines of an aged metacercaria from naturally infected *M. balthica*. Separated points, undivided shafts and round bases of multi-pointed spines are visible. The tegument proper contains dense rods, light vesicles and mitochondria. — g. Tegument sectioned longitudinally through a spine from another aged metacercaria. Note circles (arrowed) near the outer plasma membrane. mi = mitochondrion, mu = muscle. — h. Longitudinal striation in the spine material under high-power magnification. Bars 1 μm in a and c—g, 10 μm in b and 100 nm in h.

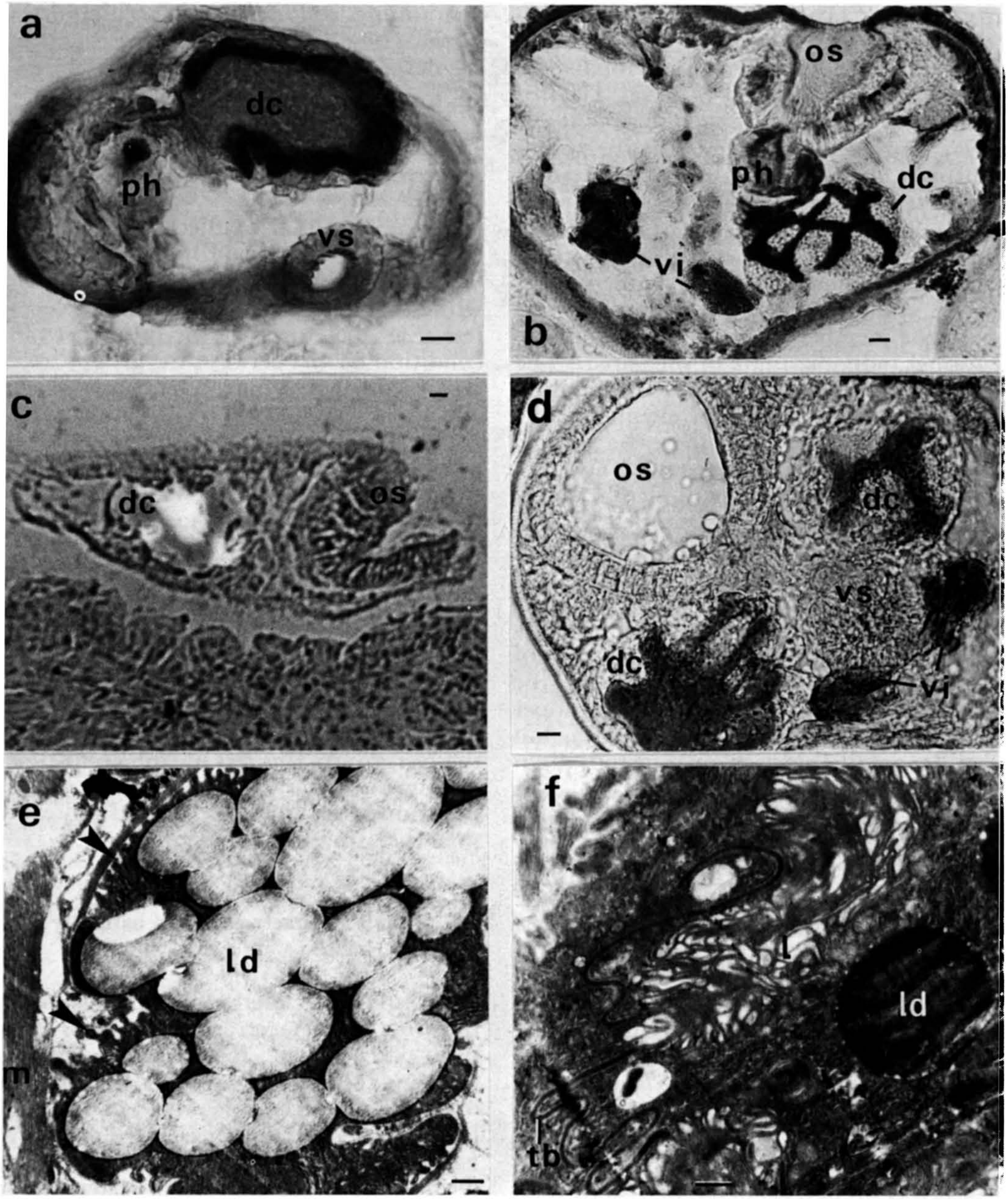

Fig. 5. Histochemistry, and structure of the digestive caeca, of the metacercariae of *Lacunovermis macomae*. — a. In this three-week-old metacercaria acid phosphatase activity can be detected in the pharynx (ph) and in the distal margins of the gastrodermal cells. Some activity is also present in the lumen of the digestive caecum (dc) and in the head region of the tegument. vs = ventral sucker. — b. Acid phosphatase activity in an aged metacercaria. The free margins of the gastrodermal cells stained darkly. The activity was also apparent in the pharynx, tegument and some tegumental cell bodies (also among the oral sucker muscles). The vitellaria (vi) also stained. — c. Lysis of pre-stained gelatine film at the site of the digestive caecum of an aged metacercaria. The pH of the buffer where the film was dipped was 7.0. — d. β-GU activity in the digestive caeca of an aged metacercaria. The vitellaria also stained. — e. Part of gastrodermis showing lipid droplets (ld), and small pedicels (arrowed) and larger projections in the basal region. m = muscle. — f. The free margins of the gastrodermal cells are supplied with irregularly arranged microvilli or lamellae (l). tb = terminal bar. Scale bars 10 μm in a—d and 1 μm in e and f.

"hairy" appearance (Fig. 3a). In two-month-old metacercariae (Fig. 3b) the spine grooves had deepened and in some individuals the spines appeared as "double" (Fig. 4c). The tips of such spines were no longer sharp. At the metacercarial age of three months the spines were usually broad doublets and the formation of the first triplets and quartets had begun (Fig. 4d). The broadening was at first visible on the sides of the metacercariae lateral to the ventral sucker. One to two new members of the multiple spines were growing under and between the members of the former doublets. The former individuals are pushed apart. The new individual spines grow to a slightly longer length than the lateral members; thus the length of the total spine increases and the tip becomes rounded, but serrated (Fig. 4e). Some reformation of the lateral members may also occur. The spine base is single and probably round (Fig. 4f). A high power micrograph shows striation in the spine material (Fig. 4h). An inward process of the basal plasmalemma of the syncytial tegument was observed to accompany the posterior surface of the spine to approximately one-third the height of the syncytium. The tegument proper is quite similar to that of the cercaria, but thicker. It contains electron-opaque rods, light bodies and mitochondria (Figs. 4f, g). The proportion of the rods and light bodies varies between different individuals. Besides the tegumental cell bodies, there are also granular cells containing larger electron-opaque oval or rod-like bodies (0.4—1.0 μm in length and 0.1—0.6 μm in breadth) beneath the tegumental syncytium and muscle layer. The duct of one such cell was seen to open to the exterior. Such cells were present also in the cercariae. The "meshwork figure" of the tegument (Pekkarinen 1984b) begins to form in three- to four-month-old metacercariae. Probably due to the puckering and thickening of the tegument the spines are hardly visible around the oral sucker. There is a spineless area or an area with less developed spines on the ventral surface between the suckers (Fig. 3d). Also the spines of the middorsal surface are less developed.

In addition to individual differences the developing metacercariae exhibited host-dependent differences. Batches of metacercariae that had developed in certain host clams sometimes had some characteristics in common, e.g. the four-month-old metacercariae in one clam had numerous oblique quadrangles or long crystals in their digestive caeca. These metacercariae were smaller and less developed than the others.

3.2. Effect of heavy infestation of developing metacercariae on *Macoma balthica*

Three months after infection the dry matter of the clam body (condition factors CF_1 and CF_2) had decreased significantly ($p<0.05$), while that of the control clams had decreased less (Fig. 6). However, the differences in the condition factors between the groups were not significant at that time. The "fatness" indices and water contents did not differ significantly either. The mean number of developing metacercariae in the infected clams was 114 after three months, and during the subsequent three months it had decreased to about half of this, i.e. 54. At the end of the experiment the dry matter of the body was very similar in the two groups. The only difference lay in the tissue water content, which was smaller ($p<0.05$) in the infected clams than in the control clams. The mean numbers of former, large metacercariae were 0.9—1.2 in the test clams and 0.7—1.7 in the control clams. Six clams died in the test group and five in the control group during the experiment. Metacercariae in this experiment grew and developed more slowly than those in the experiment above.

3.3. "Small" metacercariae in naturally infected *Macoma balthica*

In July 1984 increased numbers of small imprints of metacercariae and their "migration paths" were found inside the shells of clams collected from site I. The proportion of small metacercariae was 44% among the total number of metacercariae isolated from *M. balthica* in July—August (Table 3). The small metacercariae had rudimentary lateral papillae on the oral sucker. The genital atrium was forming, but lacked its papillae. The ventral pit was observed only in contracted individuals. The vitellaria had just a few globules or none at all. The tegumental spines were narrow. At site IV the mean number of metacercariae per clam was greater, but the mean shell length of these clams was larger. The proportion of small metacercariae was, however, much smaller. Dead metacercariae,

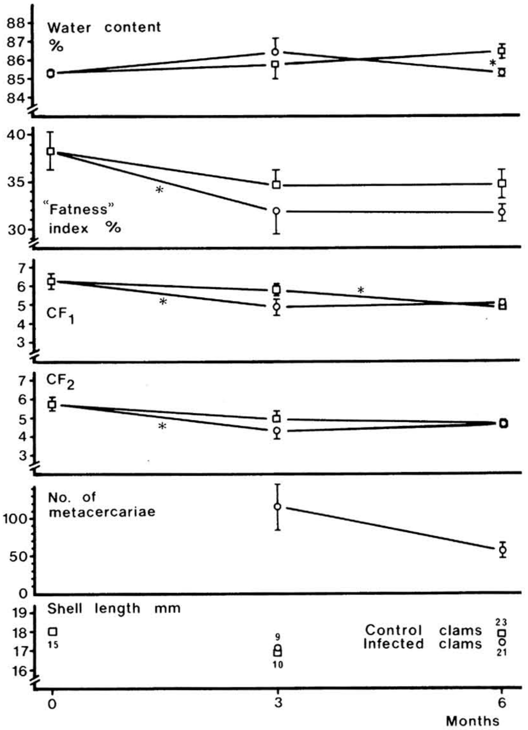

Fig. 6. Condition characteristics (water content, "fatness" index, condition factors CF_1 and CF_2) of control clams and clams infected initially with cercariae in a six-month experiment in the laboratory at 7–12°C. Means (quadrangles = control clams, circles = infected clams) and SEs of the figures are given and they are compared using Student's *t*-test. Mean shell lengths, numbers of the clams and mean numbers of developing metacercariae per clam are also given.

Table 3. Percentages of small, large (= *Lacunovermis macomae*) and other (= *Gymnophallus gibberosus*?) metacercariae among the total number of metacercariae isolated from the extrapallial space of *N* individual *Macoma balthica* at two different collecting sites in July–August 1984.

Collecting site	I	IV
Clams		
N	40	18
Shell length, mm		
mean ± *SD*	14.4 ± 2.6	16.4 ± 1.6
range	7.8 – 19.9	13.1 – 19.7
Metacercariae		
Mean No./clam	34	68
Total No.	1368	1226
% large metacercariae	54.5	91.0
% small metacercariae	44.5	7.3
% other metacercariae	1.0	1.7

with darkened vitellaria, were common inside the shells at this collecting site.

4. Discussion

Although the four-month-old metacercariae experimentally reared from cercariae were genitally less developed and slightly smaller than the metacercariae of *L. macomae* measured in natural infections, they had many of the characteristics of *L. macomae* described by Loos-Frank (1970) and Pekkarinen (1984b). Among these were the number of flame cells, the lateral papillae of the oral sucker and the broad, serrated spines of the tegument. The furthest developed individuals had the ventral pit, large genital pore and the papillae of the genital pore. Thus, the developing metacercariae, and the preceding cercariae, were shown to be those of *Lacunovermis macomae.*

The penetration glands of *L. macomae* do not empty or degenerate at, or soon after, penetration, as they do in *Schistosoma mansoni* (Stirewalt & Kruidenier 1961). In some other metacercariae, too, the glands degenerate only gradually. Six-day-old metacercariae of *Meiogymnophallus strigatus* (Lebour) usually have the voluminous penetration glands left (Bartoli 1983a). The bodies of the gland cells degenerated first. In some of the eighteen-day-old metacercariae of *M. strigatus* the ends of the ducts could still be detected at the oral sucker. In fifteen-day-old metacercariae of *Zoogonoides viviparus* (Olsson) the penetration glands were reduced but still recognizable (Køie 1976). Remnants of the microvilli around the oral sucker of *Z. viviparus* also persisted for at least fifteen days.

During intense growth of the body the oral sucker was proportionally small (BL/OSL was great). Even after four months the oral sucker in relation to body length was smaller than as measured in aged *L. macomae.* The sucker ratio (OSL/VSL) became larger in metacercariae than it had been in the cercariae. The sucker ratio of *Meiogymnophallus strigatus* also grew larger during the development of the metacercaria (Bartoli 1983a).

Proportionally large digestive caeca did coincide with the intense growing phase of *L. macomae,* as was suggested in Pekkarinen (1984b). About three to four months after infection a remarkable development of different organs in the metacercariae was noticed. The broadening of the tegumental spines, growth of the gonads, gonoducts and associated glands, formation of vitelline globules and formation of the ventral pit, genital atrium and genital papillae coincide with this period.

Acid phosphatase activity occurs consistently within the digenean gastrodermis but alkaline phosphatase activity does not occur generally in the gastrodermis (Erasmus 1977). Acid phosphatase has often been reported as being associated with the microvilli or lamellae of the gastrodermis. In the present study the activity was located in the apical parts of the cells, but on the basis of the light microscopical observations it could not be localized in these projections with certainty. The basal "pseudopodia" and pedicels in the gastrodermal cells of *L. macomae* resemble the foldings described by Robinson & Threadgold (1975) in *Fasciola hepatica*.

Alkaline phosphatase activity has been detected in the gonads, vitellaria and excretory ducts of many digenean species (Halton 1967). The vitellaria and excretory concretions of *L. macomae* stained darkly in the present study. A dark control colour, however, hid any activity occurring. The interference of Ca^{2+} in tissues is a disadvantage of this method. Staining with alizarin red S suggests the presence of calcium salts in the concretions of *L. macomae*. The concretions of *Acanthoparyphium spinulosum* Johnston are composed chiefly of calcium carbonate with a trace of phosphate (Martin & Bils 1964). The vitellaria of *L. macomae* darken after death. Such darkening may also be involved in the sections in acid phosphatase and β-GU demonstrations.

Multi-pointed (serrated) tegumental spines are not uncommon within the Digenea. Spines in the anterodorsal part of the oral cone of a strigeoid, *Diplostomum phoxini* Faust, have four to five points (Erasmus 1970). The serrated spines of an acanthocolpid, *Neophasis lageniformis* (Lebour), are most well developed on the anterior end of the body (Køie 1973). On the posterior body of adult *Fasciola hepatica* L. spines can have up to thirty points (Bennett 1975). In older adults of *Leucochloridium* sp. spines could have up to twenty points, which were generally arranged in semi-circles (Bakke 1976). The scale-like spines of a heterophyid, *Cryptocotyle lingua* (Creplin) had up to ten points in the metacercaria and up to twenty in the adult (Køie 1977). The adults of a lepocreadiid, *Opechona bacillaris* (Molin 1859) Looss 1907, have flattened, serrated spines, while those of its cercariae and metacercariae are simple (Køie 1977). Individual spines of *Cephalogonimus retusus* (Dujardin) form crown-like multiple spines or comb-like partially fused spines with four to nine points (Oliver et al. 1984).

Among gymnophallid species, metacercariae of *Gymnophallus rebecqui* Bartoli have crown-like tegumental spines (Bartoli 1983b). According to Pekkarinen (1984b and this study), *Lacunovermis macomae* also possesses multi-pointed spines, the latter usually having four points. The spines of *Parvatrema affinis* (Jameson & Nicoll) are similar (Pekkarinen 1986a). Scanning electron microscopy would reveal whether the spines of *P. borealis* Stunkard & Uzmann, which were reported to be scale-like by Stunkard & Uzmann (1958), are multi-pointed or totally "fused".

In many species the number of points of the spines varies with the site on the body, but also with the age of the individual, increasing with age. The broadening is thought to take place by simple division of the former points (Bakke 1976). Individual spines of some species actually seem to have shallow grooves on their faces (cf. figures in Bakke 1976, Køie 1977, Oliver et al. 1984, Pekkarinen 1986a). The groove formation and subsequent division could be examined in the initially single spines of *L. macomae* in the present study. In addition to this division the broadening also proceeded by the growing of new members between the first ones. The question of how the different ultimate forms of trematode spines are moulded remains to be solved. Bennett (1975) and Bennett & Threadgold (1975) suggested that certain regions of the basal plasma membrane of the syncytium act as organization centres for protein monomers to begin spine formation. The apical plasma membrane may also be involved in the shaping of the spine (Bennett 1975). The striation of the spine material of *L. macomae* was similar to that in the cercaria of *Zoogonoides viviparus* described by Køie (1971).

The tegumental spines of trematodes have been supposed to aid in locomotion and attachment of the fluke and in the abrasion of host tissues in conjunction with the nutrition of the fluke. The change from simple pointed spines in the migratory developmental stages to flattened, multi-pointed spines in the nonmigratory stages is apparently common in trematodes (Køie 1973). The pointed spines of the cercaria of *L. macomae* are of great importance during penetration and migration in the clam tissues (Pekkarinen 1986d). The broadened spines of aged metacercariae may, however, be of little value (cf. Pekkarinen 1986c) as the metacercariae are often enclosed within membraneous cysts.

Digeneans generally have (as in *L. macomae*) some kinds of (e.g. rod-like) dense bodies in their tegument and tegumentary cell bodies. The secretion rods of the granular gland cells of *L. macomae* (cf. Pekkarinen 1984b) resembled in size the spherical or oval secretion droplets in certain gland cells of *Haplometra cylindracea* and *Opisthioglyphe ranae* described by Halton & Dermott (1967).

The measurements and other characteristics of the small metacercariae described in Pekkarinen (1984b) match with those of *L. macomae* 0—2 months in age. Most of the small metacercariae found in the naturally infected *M. balthica* which were collected at site I in July—August in the present study can be categorized as 2—3 months old. Thus, a mass emergence of cercariae may have taken place during the spawning season of the clam in May, although cercariae may also emerge at other seasons. The intense growth period of these metacercariae has coincided with increasing water temperature, and with improving or good general condition of the host clams (Pekkarinen 1983).

Metacercariae in the six-month experiment, which began in September, grew more slowly than those in the foregoing experiment, which began in July. In the later experiment, the temperature of the water and general condition of the clams decreased due to the season earlier than in the foregoing experiment. The heavy infection strained the host's general condition slightly during the first three months, i.e. during the intense growing phase of the metacercariae. Moreover, the number of developing metacercariae may have been quite large at the beginning. Not all metacercariae in the extrapallial space do in fact develop into "full-grown" metaceracariae. Many of them die, as the latter part of the experiment showed.

At site IV the mean number of metacercariae per clam was twice as much as it was at site I. This may partly be due to the larger shell lengths of the clams, because prevalence and intensity of infection increase in conjunction with shell length increase (Pekkarinen 1984a). However, the intensity of infection in these clams may in fact also be greater; a large number of heavy infections at site IV was previously noticed by Pekkarinen (1984a). In the present study young metacercariae were found to be fewer in these clams than in clams at site I. Many aged metacercariae were dead. Metacercariae of *L. macomae* are rarely enclosed by mantle tissue. Although aged metacercariae are usually encysted within a thin membrane on the inner surface of the shell, the extrapallial space may still become overcrowded. The recruitment may thus be limited (cf. Bartoli 1981). There is no knowledge of how long metacercariae of *L. macomae* live. Other reasons for the smaller number of young metacercariae may also be the different time of cercarial emission in that district, unfavourable water currents prevailing during the swimming period of cercariae, and possibly the different general condition of the clams.

The time needed for the development of the "full-grown" metacercaria of *L. macomae* is proportionally long. In the laboratory at 7—15°C it took more than four months. The sudden, simultaneous heavy infection of the parasites in the microhabitat may, however, affect the parasites' size. The twelve-day-old metacercariae reared by Reimer (1962) from *Cercaria duoglandulosa*, although probably having been those of *L. macomae*, were really far too young for identification purposes. The present study has shown that measurements (e.g. sucker ratio) and other characteristics (e.g. tegumental spines) of young metacercariae may be misleading in species determination unless the developmental features are known.

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References

Bakke, T. A. 1976: Functional morphology and surface topography of Leucochloridium sp. (Digenea), revealed by scanning electron microscopy. — Zeitschr. Parasitenk. 51:115—128.

Barka, T. & Anderson, P. J. 1963: Histochemistry. — 660 pp. Harper & Row Publishers, Inc., New York.
Bartoli, P. 1981: Démographie et phénomène de compétition intraspécifique des Gymnophallidae Morozov, 1955 chez les deuxièmes hôtes intermédiaires (Trematoda, Digenea). — Ann. Parasitol. (Paris) 56:33—44.
—"— 1983a: Les stades larvaires de Meiogymnophallus strigatus (M. V. Lebour, 1908) n. comb. (Trematoda: Gymnophallidae). — Ann. Parasitol. Hum. Comp. 58:227—242.
—"— 1983b: Gymnophallus rebecqui n. sp. (syn. Parvatrema sp. 1, J. Rebecq, 1964) (Digenea: Gymnophallidae) parasite intestinal d'Anatidés de Camargue (France). — Ann. Parasitol. Hum. Comp. 58:211—225.
Bennett, C. E. 1975: Scanning electron microscopy of Fasciola hepatica L. during growth and maturation in the mouse. — *J. Parasitol. 61:892—898.*
Bennett, C. E. & Threadgold, L. T. 1975: Fasciola hepatica: Development of tegument during migration in mouse. — Exp. Parasitol. 38:38—55.
Erasmus, D. A. 1970: The host—parasite interface of strigeoid trematodes. IX: A probe and transmission electron microscope study of the tegument of Diplostomum phoxini Faust, 1918. — Parasitol. 61:35—41.
—"— 1977: The host—parasite interface of trematodes. — Adv. Parasitol. 15:201—242.
Halton, D. W. 1967: Studies on phosphatase activity in Trematoda. — J. Parasitol. 53:46—54.
Halton, D. W. & Dermott, E. 1967: Electron microscopy of certain gland cells in two digenetic trematodes. — J. Parasitol. 53:1186—1191.
Køie, M. 1971: On the histochemistry and ultrastructure of the tegument and associated structures of the cercaria of Zoogonoides viviparus in the first intermediate host. — Ophelia 9:165—216.
—"— 1973: The host—parasite interface and associated structures of the cercaria and adult Neophasis lageniformis (Lebour 1910). — Ophelia 12:205—219.
—"— 1976: On the morphology and life-history of Zoogonoides viviparus (Olsson, 1868) Odhner, 1902 (Trematoda, Zoogonidae). — Ophelia 15:1—14.
—"— 1977: Stereoscan studies of cercariae, metacercariae, and adults of Cryptocotyle lingua (Creplin 1825) Fischoeder 1903 (Trematoda: Heterophyidae). — J. Parasitol. 63:835—839.
Lebour, M. V. 1908: Trematodes of Northumberland coast, No. II. — Trans. Nat. Hist. Soc. Northumberland (Durham, Newcastle-upon-Tyne) (N. S.) 3:28—45 + pl. I—III.
Loos-Frank, B. 1970: Zur Kenntnis der gymnophalliden Trematoden des Nordseeraumes. II. Lacunovermis macomae (Lebour, 1908) n. comb. (Syn.: Gymnophallus macroporus Jameson & Nicoll, 1913) und seine Metacercarie. — Zeitschr. Parasitenk. 35:130—139.
—"— 1971: Zur Kenntnis der Gymnophalliden Trematoden des Nordseeraumes. IV. Übersicht über die gymnophalliden Larven aus Mollusken der Gezeitenzone. — Zeitschr. Parasitenk. 36:206—232.
Markowski, St. 1936: Über die Trematodenfauna der baltischen Mollusken aus der Umgebung der Halbinsel Hel. — Bull. Int. Acad. Pol. Sci., Ser. B, Sci. Nat. (II): 285—317 + pl. 13—15.
Martin, W. E. & Bils, R. F. 1964: Trematode excretory concretions: Formation and fine structure. — J. Parasitol. 50:337—344.
Michel, C. & Chretien, M. 1975: Modification of Cunningham's method for the detection of proteases in tissue sections. — Ann. Histochim. 20:39-46.
Oliver, G., Bourgat, R., Mouahid, A. & Touassem, R. 1984: Recherches sur les ultrastructures superficielles de Trématodes parasites d'Amphibiens. — Zeitschr. Parasitenk. 70:499—508.
Pearse, A. G. E. 1968: Histochemistry. Theoretical and applied 1. 3rd ed. — 759 pp. J. & A. Churchill Ltd, London.
—"— 1972: Histochemistry 2, 3rd ed. — 1518 pp. Churchill Livingstone, Edinburgh and London.
Pekkarinen, M. 1983: Seasonal changes in condition and biochemical constituents in the soft part of Macoma balthica (Lamellibranchiata) in the Tvärminne brackish water area (Baltic Sea). — Ann. Zool. Fennici 20:311—322.
—"— 1984a: Trematode metacercariae in the extrapallial space of Macoma balthica (Bivalvia) in brackish water (southwestern Finland, Baltic Sea). — Ann. Zool. Fennici 21:473—480.
—"— 1984b: Anatomy, histology and maturing of the metacercaria of Lacunovermis macomae (Trematoda: Gymnophallidae) from brackish-water Macoma balthica (southwestern Finland, Baltic Sea). — Ann. Zool. Fennici 21:481—498.
—"— 1986a: Notes on Parvatrema affinis (Trematoda: Gymnophallidae) from Macoma balthica (Bivalvia). — In preparation.
—"— 1986b: The cercaria of Lacunovermis macomae (Trematoda: Gymnophallidae) in the bivalve Macoma balthica in the Baltic Sea, southwestern Finland. — In preparation.
—"— 1986c: The cercaria and metacercaria of Gymnophallus gibberosus (Trematoda: Gymnophallidae) in Macoma balthica (Bivalvia) in the Baltic Sea, southwestern Finland. — In preparation.
—"— 1986d: Penetration of the cercaria of Lacunovermis macomae (Trematoda: Gymnophallidae) into Macoma balthica (Bivalvia). — In preparation.
Reimer, L. 1962: Die digenetischen Trematoden der Wasservögel der Insel Hiddensee und ihre Larvalstadien aus den die Insel umgebenden Brackgewässern. — Diss., Univ. Greifswald, 128 pp.
Robinson, G. & Threadgold, L. T. 1975: Electron microscope studies of Fasciola hepatica XII. The fine structure of the gastrodermis. — Exp. Parasitol. 37:20—36.
Stirewalt, M. A. & Kruidenier, F. J. 1961: Activity of the acetabular secretory apparatus of cercariae of Schistosoma mansoni under experimental conditions. — Exp. Parasitol. 11:191—211.
Stunkard, H. W. & Uzmann, J. R. 1958: Studies on digenetic trematodes of the genera Gymnophallus and Parvatrema. — Biol. Bull. 115:276—302.

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