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Physiology and lipid metabolism of *Littorina saxatilis* infected with trematodes

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ABSTRACT: Physiological and biochemical alterations in *Littorina saxatilis* infected with larval trematodes were investigated and compared with the metabolism of non-parasitized snails. Oxygen consumption rates of infected snails differed from those of non-infected controls in medium sized individuals (30 to 130 mg) but not in very large infected individuals (>200 mg). Small snails (0.5 to 8.5 mg) were seldom infected by parasites, and this size-class consisted only of non-infected specimens. The specific oxygen consumption rate of infected snails was not dependent on their mass and remained constant over the size ranges investigated. Alterations in the snail metabolism appeared to be connected to injuries to digestive gland tissues caused by the parasites. The glycogen concentration and fatty acids of neutral lipids and phospholipids in the digestive gland were determined. Infected snails differed from uninfected snails in the complete absence of glycogen in digestive gland and had proportionally higher quantities of eicosenoic (20:1) acid in the total phospholipids. It remains unclear whether infection by trematodes activates enzymes in the snail's digestive gland to synthesize eicosenoic (20:1) acid, or whether the sporocysts themselves possess these enzymes. The role of phospholipid fatty acids in the regulation and maintenance of the parasite's metabolism is briefly considered. Biochemical alterations observed in the fatty acid composition may have an adaptive significance, by helping to stabilize the host-parasite system.

KEY WORDS: *Littorina saxatilis* · Metabolism · Trematodes · Digestive gland · Phospholipids · Fatty acids

INTRODUCTION

At present, the study of adaptive and compensatory mechanisms to determine the physiological capacity of organisms under extreme conditions is one of the main trends in evolutionary physiology. Host-parasite interactions are an example of such mechanisms. Host-parasite interactions are accompanied by species-specific mechanisms that become apparent at infection. In gastropods, pathological changes in digestive gland morphology are reflected in their metabolism. Enzymatic hydrolysis of nutrients occurs as an intracellular process in the digestive gland. Organs affected by parasities, pollution or disease have to compensate to maintain their critical functions, e.g. injured digestive gland cells produce more digestive vacuoles (James 1965).

In *Littorina saxatilis*, heavy infestation by partenites of *Microphallus* spp. (Trematoda: Microphallidae) often leads to complete replacement of the digestive gland by trematode daughter sporocysts. With such extreme pathological changes in structure, the question of digestive gland efficiency can arise. Nevertheless, molluscs and their parasites represent a system where both life cycles of the parasite and the host are usually completed. Despite often heavy infestation by trematodes, gastropods in tidal zone areas are often numerous. In the White Sea, densities of trematode-infected *L. saxatilis* can amount to thousands of individuals m^{-2} (Sokolova 1995).

The effects that parasites can inflict on host physiology remain to some extent ambiguous; it is particularly unclear to what extent parasites stimulate parasitism. Schistosomes interfere with the neuroendocrine sys-

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tems of *Lymnaea stagnalis* and cause castration and gigantism in this species (De Jong-Brink 1995). Trematode parasitism does not have a significant effect on the growth rate of *Littorina saxatilis* but stunts growth in *Littorina obtusata*, and enhances growth rate and size at maturity in *Onoba aculeus* (Gorbushin & Levakin 1999). Infected *Littorina littorea* either show reduced growth, or the parasites do not effect growth rates (Mouritsen et al. 1999). Co-adaptivity within the host-parasite system has been suggested as an explanation for gigantism in parasitized individuals of long-lived snail species (Minchella 1985). According to Curtis et al. (2000), however, the oversized snails in long-lived *Ilyanassa obsoleta* were the result of greater age rather than of faster growth induced by parasites. Thus it is probable that the co-adaptivity theory is not always valid. Most likely, parasites have different effects on growth rate in different species.

With regards to energy metabolism, several studies have shown that alterations in oxygen consumption rates in poikilothermic animals induced by parasitic invasion are inconsistent. For example,the presence of larval trematodes in *Physa occidentalis* induced higher requirements for oxygen in the host (Hurst 1927, Hurst & Walker 1933). A significant decrease in metabolism was noted in (1) infected *Biomphalaria alexandrina* when compared to uninfected individuals (Ishak et al. 1970), (2) *Lithoglyphus naticoides* from the Volga Delta infected by larvae of the trematode *Apophallus muehlingi* (Fam. Heterophyidae) (Arakelova 1999), (3) *Lymnaea stagnalis* infected by redia of Echinostomatidae located in the digestive gland (Stadnichenko et al. 1996), and (4) large parasitized *Pisidium amnicum* (Holopainen & Penttinen 1993). In other studies however, differences between infected and uninfected snails — *Australorbis glabratus* (von Brand & Files 1947), *Littorina obtusata* (Lyzen et al. 1990), *L. littorea* — (Huxham et al. 2001) were not found.

The aim of this work was to measure physiological and biochemical processes in *Littorina saxatilis* Olivi 1792 infected by partenites of *Microphallus piriformis* Galaktionov 1983 (Trematoda, Microphallidae) and to compare results with those obtained for non-parasitized snails.

Table 1. *Littorina saxatilis*. Mean (± SE) shell height (*L*) and wet mass (*W*) used in biochemical analyses

N	Snail group	n	*L* (mm)	*W* (shell and tissue, mg)
1	Non-infected	18	10.5 ± 0.8	266.5 ± 59.5
2	Infected	6	10.4 ± 0.3	253.6 ± 22.0
3	Infected	8	8.8 ± 1.6	160.1 ± 89.0
4	Infected	6	8.1 ± 1.2	131.3 ± 56.6

The oxygen consumption rate during water respiration was used as the main physiological characteristic of energy metabolism. To measure the function of the digestive gland, glycogen and compounds of the total lipid fraction of the digestive gland, especially the fatty acids of neutral lipids and phospholipids of parasitized snails, were compared with those of healthy snails.

MATERIALS AND METHODS

About 150 juvenile and mature *Littorina saxatilis* of both sexes were collected between July and August 2001 from the White Sea littoral zone. Shell height of snails used in respiration experiments varied from 2 to 12 mm. For biochemical analysis, additional samples (about 100 specimens) were taken. Shells were measured, then broken to determine whether the snail was infected by *Microphallus* piriformis Sizes of snails used for lipid analysis are presented in Table 1.

Oxygen consumption rate. Oxygen consumption rate was determined in individuals that were not restricted in movement. Routine metabolism is considered here as similar to standard metabolism according to McMahon (1988), where the aerial:aquatic respiration rates ratio ($V_{O2}a$:$V_{O2}w$) is close to 1 for meso and neogastropod species regardless of zonation. However, for the purpose of this experiment, only the aquatic oxygen consumption rate was determined. Experiments on respiration were carried out during flood tides.

Winkler's method for determining oxygen content in seawater was used (Strickland & Parsons 1972). Periwinkles were placed individually in flasks and exposed *in situ* for 2 to 4 h at seawater temperature 16°C. The volume of the calibrated flask with stopper did not exceed 25 ml. The oxygen content in flasks during the experiments did not sink below 25% of initial value.

Numbers of infected and non-infected snails, as well as brooding females, were determined by dissection after the respiration experiments. Snails were identified as infected when changes in digestive gland caused by daughter sporocysts were recorded. Digestive glands of infected mature individuals (>3.5 mm) were hypertrophied and rose-orange in colour (normal digestive gland cells are yellow-brown) and were almost wholly suppressed by sporocysts capable of developing into metacercaria. Parasites other than *Microphallus piriformis* occurred in the examined snails but their frequencies were very low so 'infected' individuals were defined as those snails with digestive glands either partially or totally altered by *M. piriformis*. In all examined cases, the digestive gland of infected snails looked heavily atrophied.

The ratio between shell height and mass (shell and tissue) of the snails was determined. Wet and dry tissue, shell masses and the mass of the digestive gland, were determined by weighing and drying samples at 60°C.

Lipids. Four groups of rough periwinkles (Table 1) were used for biochemical analysis. Control (non-infected) groups, were similar in size to the large infected group, i.e. 10 to 11 mm. The shells of the snails were broken, digestive glands of both control and infected snails were separated from soft tissue, placed into test tubes containing 10 ml chloroform: methanol (1:1) mixture, covered witha lid and placed in a refrigerator (5°C) until needed.

Lipids were extracted from the digestive gland according to Folch et al. (1957) using a chloroform: methanol (1:1) mixture, then separated into fractions of triglycerids (triacetin) and phospholipids by thin-layer chromatography on silicagel in a mixture of hexane: ether : acetic acid (73:25:2). Chromatograms were developed in iodine vapour. Separated fractions of lipids were exposed to alkaline methanolysis, and fatty acid methyl esters were separated by gas-liquid chromatography using a Pye 104 model 24 gas chromatograph. Identification of individual fatty acids was made by comparing the relative retention time of standard fatty acids and their mass spectra, revealed by gas chromatograph-mass spectrometry. The fatty acid content was estimated as the area under a peak computed by triangulation. The data were expressed as the percentage of the sum of all fatty acids of the given lipid fraction.

RESULTS

Size

Allometric measurements gave the following power equations:

$$W = 0.42L^{2.74} \quad (n = 68;\ R^2 = 0.98) \quad (1)$$

$$DW = 0.506W^{1.04} \quad (n = 17;\ R^2 = 0.99) \quad (2)$$

$$DW_{st} = 0.022L^{2.84} \quad (n = 22;\ R^2 = 0.94) \quad (3)$$

where W is wet weight of snail with shell (mg); L is shell height (mm); DW is dry weight of snail with shell (mg), and DW_{st} is dry mass of soft tissue (mg).

The average wet mass of uninfected digestive gland tissue of *Littorina saxatilis* was 39.15 ± 3.0 mg (n = 5). This value is given for snails of an average shell height of 10.54 ± 0.84 mm and an average weight with shell of 267 ± 59 mg.

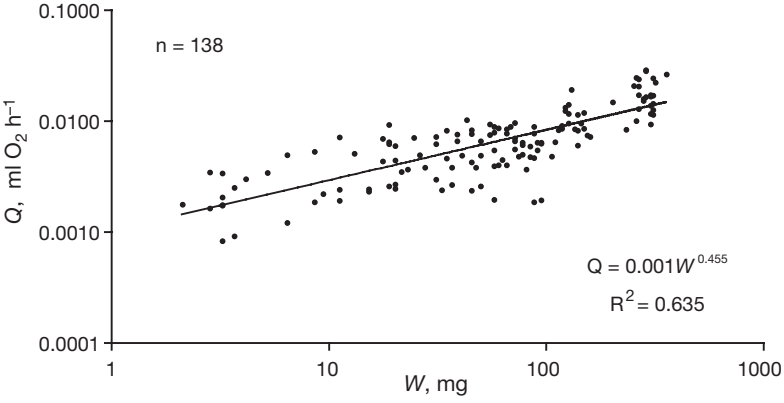

Fig. 1. *Littorina saxatilis*. Relationship between oxygen consumption rate and weight at 16°C

Respiration

The relationship between oxygen consumption rate and mass of both infected and uninfected *Littorina saxatilis* at 16°C (Fig. 1) was approximated by the following equation:

$$Q = 0.001W^{0.455} \quad (n = 138;\ R^2 = 0.635) \quad (4)$$

where Q is the oxygen consumption rate (ml h^{-1}), and W is the wet weight of snail with shell (mg). Q was calculated separately for infected and non-infected snails. Despite the fact that every snail may have been infected with partenites, only individuals with medium and extreme atrophy of the digestive glands were considered parasitized. In this experiment, gravid females (containing up to 100 embryos in different stages of their development (from egg to juveniles)), did not differ in oxygen consumption rate from other non-infected snails, so it was possible to include gravid female data and calculate a common equation. Data analysis showed that the respiration rate of infected specimens differed from non-infected specimens only in the middle size-class. Large infected individuals did not differ in oxygen consumption rates compared with non-infected ones. The relationship between respiration rate (Q_{inf}) and mass of infected snails was close to linear (Fig. 2) and represented by the equation:

$$Q_{inf} = 7 \times 10^{-5}W^{0.986} \quad (n = 32;\ R^2 = 0.788) \quad (5)$$

An analysis of variance was used to test whether there was any difference in the regression coefficients of 2 compared groups of matures (those individuals able to reproduce), infected (b_1) and 'controls' (b_2). The differences in the 2 regression line variances were not significant ($F_{(30, 48)}$ 1.16 < $F_{0.05}$ 1.45); the t-test was then used to compare regression coefficients b_1 and b_2 and test for significance (p 99% 5.11 > t_a 3.42). Student's t-

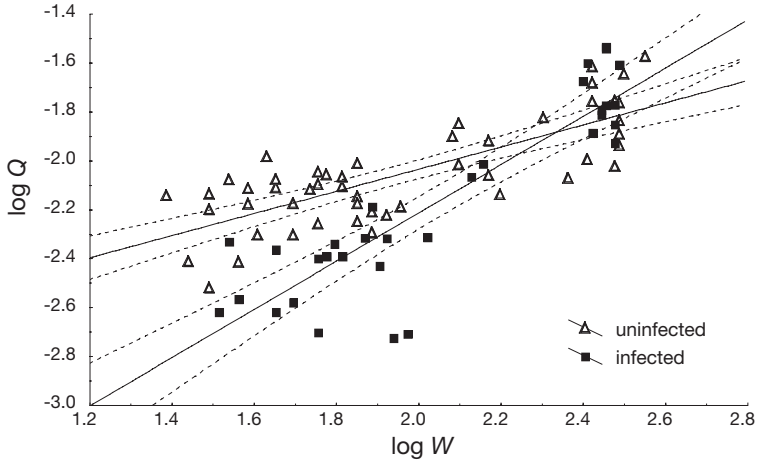

Fig. 2. *Littorina saxatilis*. Relationship between oxygen consumption rate and weight in infected and non-infected periwinkles at 16°C (log-transformed data). Equations for the linear regression lines: log Q_{norm} = –2.94 + 0.45 · log W (n = 50; R^2 = 0.61) and log Q_{inf} = –4.18 + 0.99 · log W (n = 32; R^2 = 0.79). Dotted lines are 95% CI

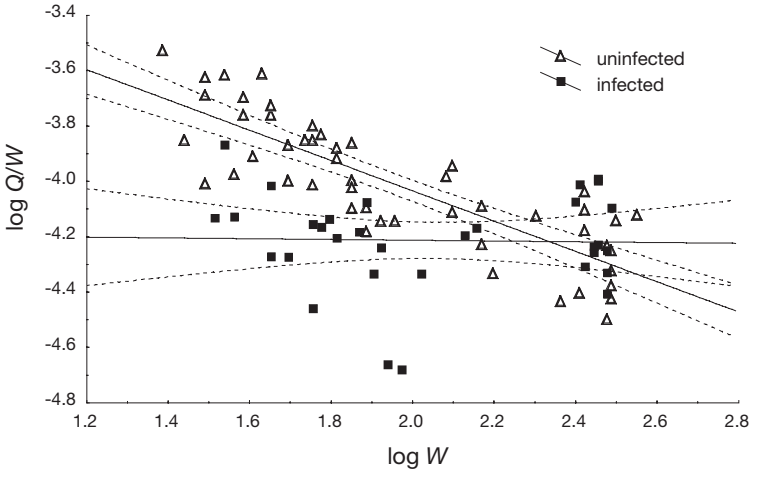

Fig. 3. *Littorina saxatilis*. Relationship between the specific rate of oxygen consumption and weight in periwinkles (log-transformed data). Equations for the linear regression lines: log Q/W_{norm} = –2.94 – 0.55 · log W (n = 50; R^2 =0.69) and log Q/W_{inf} = –4.18 – 0.014 · log W (n = 32). Dotted lines are 95% CI

test showed significant differences in b_1 and b_2. The specific respiration rate of young parasitized snails is much lower than that in young non-infected snails, but such a difference was not evident when large infected and presumed large uninfected individuals were compared (Fig. 3).

Biochemical analysis

The fatty acid composition of common lipids of the digestive gland of non-infected and infected snails is given in Table 2. Because the digestive gland becomes atrophied by the presence of numerous sporocysts, the fatty acid content presented in Table 2 reflects a change in digestive gland mass with increased parasite density. Substantial differences between infected and uninfected snails and also between individuals of various size groups were detected.

All indices obtained for large individuals were closer in value to the control group than to the smaller size-class. Large individuals appeared to have more intact digestive gland tissue than other groups.

Fig. 4 shows some of the fatty acids present (% of sum of total lipids) in examined tissue (i.e. digestive gland in uninfected group, and sporocysts and digestive gland in infected snails). Biochemical analysis revealed a higher saturated stearic acid and lower monounsaturated oleic acid content in small individuals when compared with the control group. The quantity of essential linoleic and linolenic acids, which are not synthesized by the snails but derived from food, was considerably lower in infected small individuals compared with non-infected.

The content of saturated fatty acids in infected individuals was slightly higher in comparison with controls (Table 2). For long-chain unsaturated acids, linolenic type acids did not increase over the linoleic type acids which are considered as unique to marine organisms.

Along with the fatty acid content of total lipids, the fatty acid content of total phospholipids of infected digestive glands was determined (Table 3). In parasitized individuals, the amount of eicosenoic (20:1) acid present increased nearly two-fold, and polyunsaturated arachidonic (20:4ω6) and eicosapentaenoic (20:5ω3) acids were reduced (Fig. 5). On the whole, however, both saturated : unsaturated and ω3 : ω6 acids ratios were similar for all test groups (control and infected) which indicates a normal maintenance of membranes' fluidity.

Digestive gland chromatograms (n = 6) showed that glycogen was absent in the digestive gland of parasitized snails (Fig. 6).

DISCUSSION

As is well known, the slope of the regression line of respiration rate of poikilothermic animals of many taxa is close to 0.75 (Zeuthen 1947, Hemmingsen 1960). However, many factors (including tissue mass

Table 2. *Littorina saxatilis*. Fatty acid content of total lipids (triglycerides and phospholipids) of digestive glands infected with trematodes (% of fatty acid sum). The numeration indicates the size group of snails and corresponds to that in Table 1

Fatty acids	Non-inf. 1	Inf. 2	Inf. 3	Inf. 4
Miristic 14:0	8.0	7.0	7.0	6.0
Myristoleic 14:1	0.5	<0.5	<0.5	<0.5
Palmitic 16:0	11.0	10.0	10.0	10.0
Palmitoleic 16:1	<0.5	<0.5	1.0	3.0
Stearic 18:0	3.0	6.0	9.0	10.0
Oleic 18:1	26.0	19.0	16.0	9.0
Linoleic 18:2ω6	10.0	9.0	7.0	4.0
Linolenic 18:3ω3	4.0	3.0	2.0	2.0
Eicosenoic 20:1	11.0	12.0	13.0	15.0
20:2	2.0	2.0	3.0	2.0
Arachidonic 20:4ω6	13.0	15.0	13.0	16.0
Eicosapentaenoic 20:5ω3	11.0	13.0	15.0	18.0
Docosatetraenoic 22:4ω6	–	1.0	1.0	1.0
Docosapentaenoic 22:5ω3	0.5	3.0	3.0	4.0
Docosahexaenoic 22:6ω3	–	–	–	–
Saturated	22.0	23.0	26.0	26.0
Unsaturated	78.0	77.0	74.0	74.0
Saturated /Unsaturated	0.28	0.30	0.35	0.35
ω3/ω6	0.67	0.76	0.95	1.14
Monoenoic	37.5	31.0	30	27.0
Dienoic	12.0	11.0	10	6.0

Table 3. *Littorina saxatilis*. Fatty acids of total phospholipids of digestive glands infected with trematodes (% of fatty acids sum). The numeration indicates size group of snails and corresponds to that in Table 1

Fatty acids	Non-inf. 1	Inf. 2	Inf. 3	Inf. 4
Miristic 14:0	9.1	6.4	6.4	4.2
Myristoleic 14:1	–	traces	0.3	0.6
Palmitic 16:0	8.8	7	9.5	10.2
Palmitoleic 16:1	–	traces	traces	2
Stearic 18:0	7.5	11.6	12.0	12.5
Oleic 18:1	7.5	10	11.0	8
Linoleic 18:2ω6	5.7	6	5.5	3.5
Linolenic 18:3ω3	2.7	2.0	~1.8	~1
Eicosenoic 20:1	9	15.5	16.0	17
20:2	4.8	4	4.0	3
Arachidonic 20:4ω6	19	15.3	13.6	14.3
Eicosapentaenoic 20:5ω3	22.3	16	16.5	17
Docosatetraenoic 22:4ω6	0.5	1.4	1.0	1.0
Docosapentaenoic 22:5ω3	2.0	4.0	2.7	4.4
Unidentified	~1.1	0.8	traces	1.3
Saturated	25.4	25	27.9	26.9
Unsaturated	73.5	74.2	72.4	71.8
Saturated/unsaturated	0.34	0.34	0.38	0.37
ω3:ω6	1.07	0.97	1.0	1.2
Monoenoic	16.5	25.5	27.3	27.6

and temperature) can cause this coefficient to vary. Ecological characteristics of animals, such as activity and type of feeding, also influence the slope of regression and the rates of oxygen consumption. In *Patella granularis* and *P. oculus* the differences in rates of oxygen consumption span an order of magnitude and may reflect different adaptations to variable habitats in the tidal zone (Branch et al. 1988). In these intertidal snails the rate of metabolism differs by a factor of 2 depending on the availability of food. In *Thais (Nucella) lapillus* the regression slope (b) depends both on season and foraging, and can vary widely (0.34 to 0.96) (Bayne & Scullard 1978). Significant changes in the character of regression of respiration rate were registered in relation to season. In one study, the specific rate of oxygen consumption in *Littorina littorea* was found to be strongly dependent on the size of winkles, showing a wide range of regression coefficients ($b-1$) from −0.755 to −1.033. This link between oxygen consumption rate and winkle size continued until May of the sampling period, but with the onset of warmer conditions, the value decreased to −0.398 (Newell

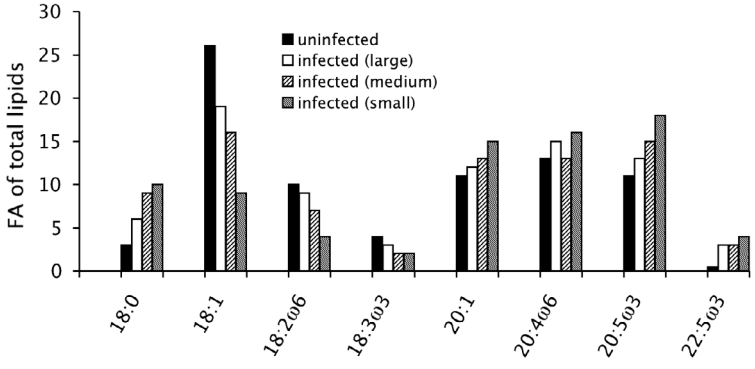

Fig. 4. *Littorina saxatilis*. Influence of infection on the amount of some digestive gland fatty acids (FA) (% of sum of FA in total lipids)

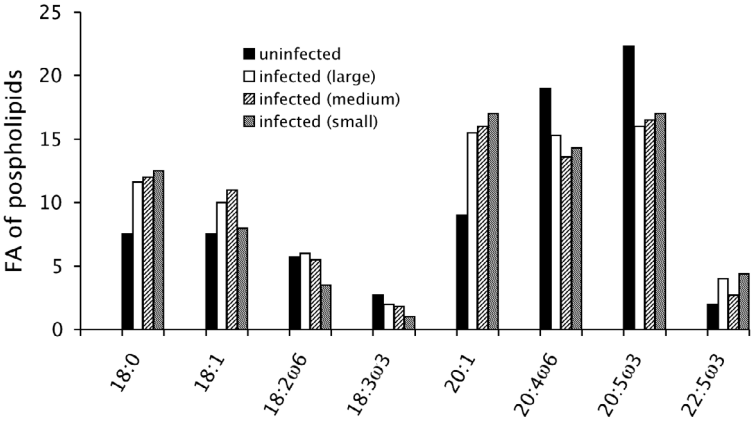

Fig. 5. *Littorina saxatilis*. Influence of infection on the amount of some digestive gland fatty acids (FA) (% of sum of FA of phospholipids)

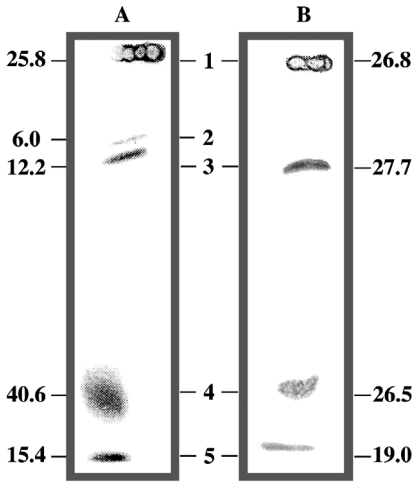

Fig. 6. *Littorina saxatilis*. Components of lipid extract of digestive glands revealed by separation of lipids by thin-layer chromatography on silicagel. (A) uninfected; (B) infected snails. Numbers to the extreme left and right of A and B represent components as percentages of their sum in lipid extract. Numbers between A and B identify components: 1 = phospholipids; 2 = glycogen; 3 = cholesterol; 4 = triglycerides; 5 = cholesterol esters

& Pye 1971). Thus, the slope *b* of the regression line of oxygen consumption rate with increasing mass of *L. littorea* does not exceed 0.6.

For *Littorina saxatilis*, a relatively low value of regression coefficient ($b = 0.45$) was obtained. In brooding females weighing between 15 and 152 mg, the slope was slightly, but not statistically, higher ($b = 0.53$). In a separate equation calculated for infected individuals, the slope of regression was 0.98, which means that the relationship between respiration rate and mass in infected *L. saxatilis* is close to linear. In contrast to what was mentioned above for *L. littorea* (Newell & Pye 1971), the specific oxygen consumption rate did not depend on mass in *L. saxatilis*, because rates remained constant over all infected snail sizes investigated. (Fig.3). This phenomenon may be correlating the degree to which snail growth is stunted by trematode infection with the amount of damage this causes to the snails (Sousa 1983). Individuals parasitized at an early age do not reproduce, show low indices of metabolism and possibly have a shorter life history compared to those infected after they become fully mature. The latter have grown to maximal size, and, as it appears from the results of the respiration experiment for large snails, maintain their energy metabolism at the same level as non-infected animals of the same size (Fig. 2). At the time of spawning, to all appearances, snails appeared not to be intensively infected. After spawning they further lost reproductive function because of the reallocation of energy to growth metabolism and plasticity.

Whether the shortened life cycle in the youngest infected snail group (> 3 mm) is due to trematodes is debatable, but heavy infection of the digestive gland may have interfered with food assimilation and hence the nutrient requirements of this size group may not be entirely satisified.

In non-infected individuals nutrients are absorbed by the digestive gland and released for general metabolism. In infected snails the effect of parasites is severe. Studies of the biochemical and physiological effects of trematodes on their hosts have shown decreased host glycogen and amino acids and an alteration of the fatty acid content of lipids in host tissues (Thompson 1983). Sporocysts reduce the initial digestive function of the digestive gland diverticulum cells and lead to their atrophy. This may be followed by an increase in the digestive and absorptive role of the intestine. Consumed food particles would then be delayed in the mid-oesophagus lateral glandular pouches where they would be exposed to secreted digestive enzymes (Graham 1939). Nutrients would be absorbed across the intestinal wall, which would be important for the metabolism of infected individuals with an injured digestive gland. The entire rectal epithelium of *Littorina saxatilis* is glandular, and possibly produces a number of secretions, which are involved in absorption, removal of nutrients from the blood, and in covering the faecal pellets (Brough & White 1990). Active extracellular amylase produced by both the digestive gland and the crystallin style in sand/mud-flat dwellers, herbivorous snails and bivalves plays an essential role in digestion (Morton 1951, Prosser & Brown 1962).

Although a decrease in oxygen consumption rate may lead to lowered nutrient transportation, the decline in respiration could result in delaying the passage of food to the injured organ (i.e. the digestive gland), enhancing food contact time with the zone to which active digestion has been shifted. Membrane digestion is thought to be possible in the mid-oesophagus region of the digestive system, in the presence of enzymes on the external side of the cell membrane (Ugolev 1963). The increase of enzyme activity in the anterior and posterior parts of the digestive tract should compensate to some degree for the deficit of nutrients caused by the reduced function of digestive gland.

The specific character of enzymes is determined by the substrate, but conformity between enzymes and food quality may be disturbed under the influence of changes induced by parasites in the digestive tract. Because of this, membrane digestion takes on special significance. Described by Ugolev (1972) as the third type of digestion, in addition to intra- and extracellular, membrane digestion occurs when food particles come into contact with enzymes located on the top of mem-

brane surfaces of the intestine. All 3 types of digestion can occur simultaneously and supplement each other. It has been shown that tapeworms, completely deprived of a digestive system, will fix host enzymes which are involved in membrane hydrolysis of carbohydrates and proteins to the upper sides of their integument (Ugolev et al. 1985). In adult worms, membrane digestion occurs in the brush border area, but nothing is known about membrane digestion in sporocysts except the fact that they absorb nutrients osmotically.

It is probable that the cell membranes of sporocysts contain enzymes which split nutrients into monosaccharides and amino acids, which are then partly used by the sporocysts and partly involved in the metabolic process of the host. Higher quantities of eicosenoic (20:1) acid in the total phospholipid content of infected samples,than those present in controls (Fig.5), could mean that synthesis of this fatty acid increases due to trauma suffered by the host. A high content of eicosanoic acid was also found in the digestive gland of the freshwater snail *Biomphalaria glabrata* infected by *Schistosoma mansoni* (Allan et al. 1987). The worm *Fasciola hepatica* cannot desaturate fatty acids obtained from its host but it can elongate chains by using acetate (Oldenborg et al. 1975, 1976). Quantitatively most significant was the elongation of oleinic (18:1) acid to eicosenoic (20:1) acid which is difficult for *F. hepatica* to absorb because of its very low concentration in the host (Tielens 1999).

It remains unclear whether an enzyme in the snail activates the synthesis of eicosenoic (20:1) acid under the influence of parasites, or if sporocysts themselves possess this elongation enzyme, which is necessary for their membranes, but do not have the enzymes for synthesis of 16:0,16:1,18:0 acids. This causes the sporocysts to use host enzymes. Moreover, taking into account that polyenoic fatty acids of membrane phospholipids are involved in maintaining a certain level of cell metabolism (Zabelinskii et al. 1999), the biological meaning of these changes becomes more clear. Unsaturated fatty acids of phospholipids differ by an amount that should reflect condensation in membranes, since monoenoic acids are packed more densely in a membrane, and polyenoic ones on account of being curved chains are packed more loosely. Because the movement of oxygen into a sporocyst is diffusive, the condensation of membranes should limit permeability and oxygen diffusion rate, adjusting the amount of oxygen reaching the tissue of the parasite. The data on metabolism of sporocysts and metacercaria (Pascoe 1972) show that the oxygen consumption rate of the shell of sporocysts is much higher than the metabolic rate of the metacercaria developing in the sporocyst. This was proven not only by direct measurement of consumption of oxygen, but also by detection of an abundance of mitochondria in the integument of the sporocyst. Because of this, it becomes obvious that the role of lipids in the regulation of enzyme activity and permeability of cell membranes is determined by their fluidity and is primarily directed to the maintenance of the parasite metabolism.

Data obtained by thin-layer chromatography showed that infected snails differed from non-infected ones in the complete absence of glycogen in the hepatopancreas. It has been shown that glycogen is decomposed by enzymes in tissues containing parthenites (Cheng & Snyder 1962). When infected by trematodes, *Viviparus viviparus* had low indices of glycogen in all organs and tissues (Stadnichenko 1972). It could be that poor synthesis of glycogen (as a direct result of atropy of the digestive gland) could lead to a less successful over-wintering in a dormant state in a younger group, while the oldest group, having grown to maximum size because of a later infection, exhibits higher resistance to parasites. In *Patella* sp., glycogen is not used to maintain metabolism during the winter (Barry & Munday 1959). In *Biomphalaria glabrata* infected with *Schistosoma mansoni*, the rapidly developing parasites actively utilize the host's glycogen stores and are responsible for carbohydrate depletion in infected snails (Christie et al. 1974). In *Thais lamellosa,* glycogen, of which the peak levels coincide with maximum feeding, is not stored to any great extent, but is either metabolized or converted into lipid or protein (Lambert & Dehnel 1974). Glycogen is used to a much lesser extent than lipids to maintain the metabolism of marine gastropods and is not significant as a reserve (Emerson & Duerr 1967, Blackmore 1969, Webber 1970). In *Littorina saxatilis*, it is possible that stearic acid is deposited by parasites as an energy reserve. Stearic acid originates from palmitic acid and is regulated by mechanisms of biosynthesis of phospholipids of cell membranes (Kreps 1981). These mechanisms are used in adapting to high pressure and low temperatures. Here, possibly, we deal with a biotic factor represented by the parasites.

The effectiveness of *Littorina saxitalis* digestive glands infected by trematodes is debatable. Nevertheless, the host-parasite system described here appears stable, which makes it possible for both the host and parasite to complete their respective life cycles. Observed biochemical alterations in the fatty acid composition of total lipids and phospholipids possibly have an adaptive significance in supporting this stability.

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