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Small-sized euryhaline fish as intermediate hosts of the digenetic trematode *Cryptocotyle concavum*

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ABSTRACT: Cercariae of the trematode *Cryptocotyle concavum*, which encyst in skin and/or kidney of sticklebacks and gobies, were studied in the Schlei Fjord (western Baltic Sea). Mean incidence of dermal cysts was 48 % in *Gasterosteus aculeatus* and 37 % in *Pungitius pungitius*. No cysts were found in the kidneys of sticklebacks. While 97 % of *Pomatoschistus microps* had encysted metacercariae in the kidneys, only 2 % had cysts in the skin. *Pomatoschistus minutus*, however, showed hardly any cyst infestation of either skin or kidney. In *P. microps* the intensity of infestation by metacercariae was frequently more than 50 cysts; in contrast, sticklebacks rarely exhibited more than 5 dermal cysts. Infested fish were larger than 10 mm in total length, the incidence rate increasing with growth. Parasitic infestation depends on ambient salinity: *C. concavum* was not found at salinities below 4 ‰. In contrast to the high incidence in fish, the first hosts – the snails *Hydrobia stagnalis* and *H. neglecta* – showed remarkably low infection rates (3 to 5 %). The findings reported are related to the distribution of *C. concavum*, the mode of life of infested fish, the feeding habits of the final hosts and the infestation of *P. microps* by other parasites. Evidently, *P. microps* represents an optimal second host for *C. concavum*.

INTRODUCTION

In 1975, Möller-Buchner (1981) found among the demersal fish *Pomatoschistus microps* from the River Elbe specimens whose kidneys were interspersed with small cysts which turned out to be metacercariae of the trematode *Cryptocotyle* (Möller-Buchner, pers. comm.). Kollra (1982) identified it as *Cryptocotyle concavum* (Creplin, 1852) which as adults live in the gut of birds such as ducks or gulls.

The life cycle of *Cryptocotyle concavum* was described by Reimer (1970) and Yamaguti (1975) (Fig. 1). Eggs containing developing miracidia larvae are ingested by snails, especially *Hydrobia* species (first host), in which they penetrate the liver and produce large numbers of rediae, each of which then produces large numbers of cercariae; these swim about in the water in search of a fish host. In the second intermediate host, cercariae form cysts, the metacercariae. In fish eaten by a bird the cysts dissolve and the metacercariae develop into ripe flukes which generally live in the intestinal tract of the bird (Fig. 1). Fertilized eggs reach the ambient water together with the bird's faeces.

The related *Cryptocotyle lingua* (Creplin) appears to live preferably in the snail *Littorina littorea* at first and in pelagic fish as second host in which they cause "pigment spots" of the dermis (Sindermann & Farrin, 1962). The final hosts, however, are identical to those of *C. concavum* (Reimer, 1970; Bakke, 1972).

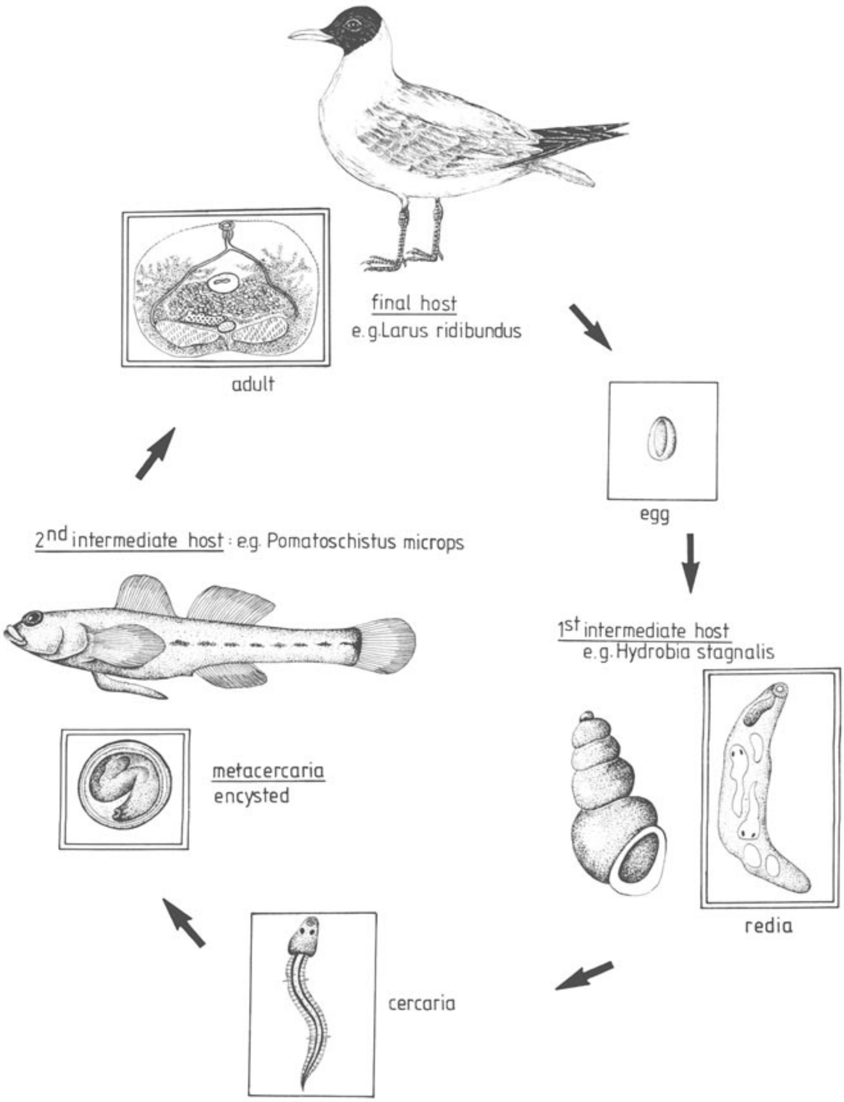

Fig. 1. *Cryptocotyle concavum*. Life cycle in the most frequent hosts. Based on Reimer (1970), Yamaguti (1975) and present study

Until now, only dermal or peritoneal metacercariae cysts of *C. concavum* were known to occur in fish. The results obtained from *P. microps* raise the question whether kidney infestation renders *C. concavum* more successful than dermal infestation.

INVESTIGATION SITE AND METHODS

Field investigations were carried out in the brackish Schlei Fjord which enters the Baltic Sea in Northern Schleswig-Holstein (Fig. 2). Most samples were taken at Olpenitz

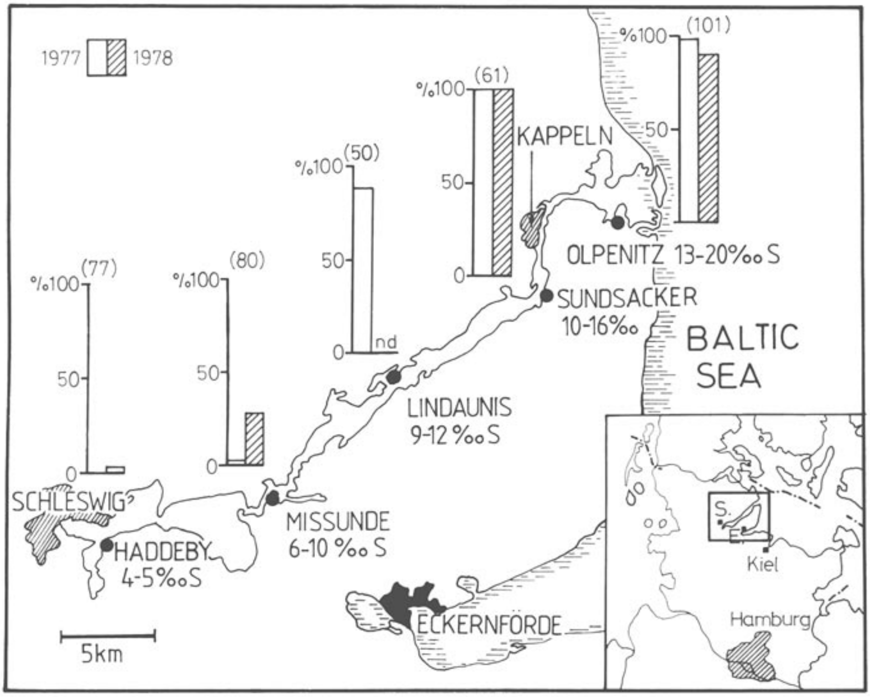

Fig. 2. Map of Schlei Fjord (western Baltic Sea) showing the 5 investigation sites, and their salinity ranges. Columns: incidences of *Pomatoschistus microps* infested by *Cryptocotyle concavum* during 1977 (data from Derksen, 1978) and 1978. Brackets: total number of gobies examined. nd = no data

near the mouth of the Schlei where α-mesohaline conditions prevail (Fig. 2). Less frequently 4 other sites of the Schlei were visited, the innermost of which has α-oligohaline conditions (Fig. 2).

Fish were caught with push-nets of 1 mm mesh size and an opening of 40 × 35 cm (Table 1). Density of gobies was evaluated according to Schmidt-Moser & Westphal (1981), who took into account an efficiency of 20 % with regards to push-nets.

Snails *(Hydrobia)* were sampled from algae and from sediment. In order to obtain quantitative data on species abundance, a meiobenthos core of 20 cm^2 was employed for

Table 1. Infestation by *Cryptocotyle concavum* of small-sized fish species from Olpenitz (Schlei). n = number of fish examined

Species	Observation period	n	% dermal cysts	% kidney cysts
Pomatoschistus microps (Krøyer)	1977–81	1037	2	97
Pomatoschistus minutus (Pallas)	1978–81	112	1	1
Gasterosteus aculeatus L.	1979–81	198	48	0
Pungitius pungitius (L.)	1979–82	646	37	0
Spinachia spinachia (L.)	1979	5	20	0

the sediment. In case of algae, the biomass (DW) of single *Fucus vesiculosus* thalli was measured after *Hydrobia* and other faunal elements had been removed.

Sampled fish and snails were fixed either at the sampling site in 4 % formalin or brought to the laboratory where they were kept in aquaria until examined. Such fish were killed with MS 222 and then examined immediately. Live snails were treated with formalin or ether or vivisected.

The different life stages of *C. concavum* were counted and, for assessment of infestation, grouped according to intensity. Three degrees of metacercariae infestation were identified: (1) = 1 metacercaria, (2) = 2 to 5 metacercariae, (3) = more than 5 metacercariae per fish.

In order to identify the *Cryptocotyle*-species, it was necessary to cause the metacercariae to excyst; this was achieved by keeping the cysts at a constant temperature of 38 °C. The metacercariae then hatched within a few hours.

The kidney region of some infested *Pomatoschistus microps* were cut into 10 µm serial sections and stained with Pasini in order to gain information on the position of cysts within the kidney and to examine the morphology of the cyst covers.

RESULTS

Morphology of metacercariae cysts

Dermal cysts of *Cryptocotyle concavum* are easy to recognize because of their black colour; they are clearly visible on the body surface of the sticklebacks. The black colour is due to a concentration of melanophores, already known from *C. lingua* cysts. The metacercariae were scattered all over the body. More than 5 cysts were rarely found in specimens of *Gasterosteus aculeatus* and *Pungitius pungitius*.

Infested kidneys of common gobies *(Pomatoschistus microps)* are conspicuous due to the small spheres which may be interspersed throughout the whole organ including the foremost part, i.e. the pronephros, which is a lymphatic organ (Derksen, 1978). Cyst diameter was ca 0.2 mm (Fig. 3).

Histological sections reveal that cysts penetrate the whole kidney (Fig. 4) and that the cyst cover consists of a thick secretion of the metacercariae and is surrounded by a layer of connective tissue originating from the host kidney. The parasite lies in these covers in a curved position; at times its oral sucker is clearly visible (Fig. 4). Cysts were seldom found in the bordering muscle tissue. Cercariae settled on the inner side of the peritoneum form dents in the kidney which are covered by an additional, melanophore-bearing layer (Fig. 4).

Infestation of fish by metacercariae of *Cryptocotyle concavum*

Table 1 shows the rates of incidence of metacercariae among all examined fish species from Olpenitz. Only low rates of infestations of both dermis and kidney were found in *Pomatoschistus minutus*. The three stickleback species had dermal cysts with an average incidence of only 48 % *(G. aculeatus)* or 37 % *(P. pungitius)*. In contrast, 97 % of *P. microps* were infested with cysts in their kidney but only 2 % were found with cysts on their skin.

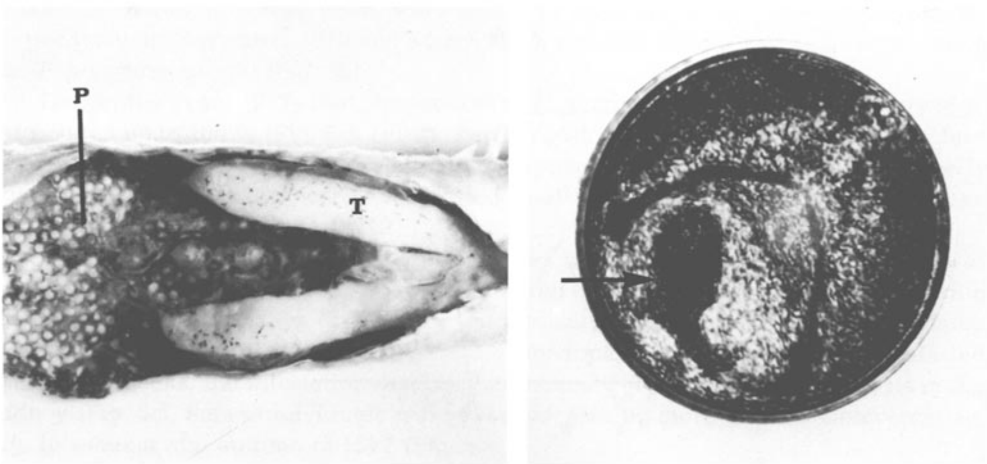

Fig. 3. *Cryptocotyle concavum.* Metacercariae cysts in the kidney of *Pomatoschistus microps.* Left: opened body cavity with the testes (T) and the kidney which is dispersed by sphere-shaped cysts. The anterior lobes are the pronephros (P). Length of kidney: 13 mm (Photo: H.-D. Totzke). Right: single cyst. The two parts of the parasite's cover, the bifid intestine and the bladder (arrow) can be distinguished. Diameter of object ca 0.2 mm

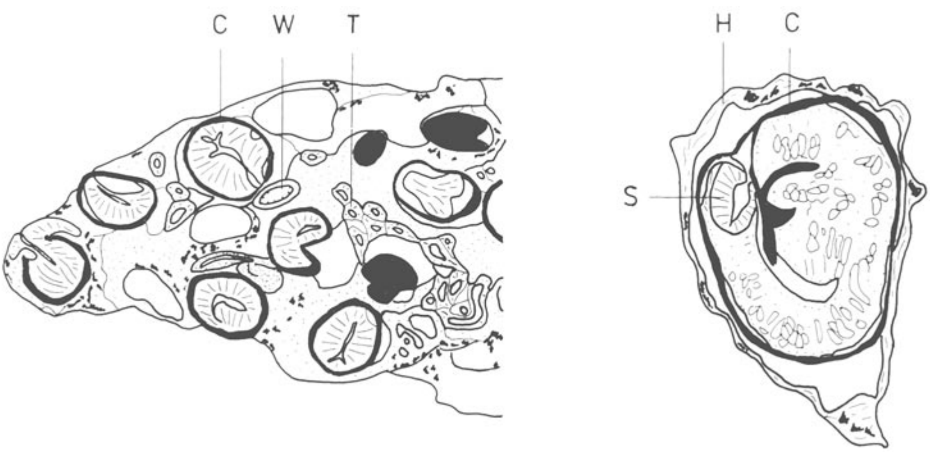

Fig. 4. *Pomatoschistus microps.* Section of the kidney infested by metacercariae cysts of *Cryptocotyle concavum* (diameter 0.2 mm). Left: part of the kidney. Right: excysting metacercaria. C = parasite cover, H = host cover, S = oral sucker, T = tubule, W = urinary duct

Rates of incidences of *G. aculeatus*, sampled monthly during several periods in 1979 to 1981, are presented in Figure 5a. Highest incidence rates were found in October 1981 (87 %), the lowest rates in June 1979 (15 %), December 1979 and August 1981 (0 %). In 1979 and 1981 infestation rates peaked in late summer. Although the time series of samples were incomplete in 1980, the data revealed another peak during spring (Fig. 5a).

Data obtained for *P. pungitius* (Fig. 5b) show two peaks in 1979 (May, 80 %; October, 60 %). Samples from the years 1980 and 1981 may confirm these results:

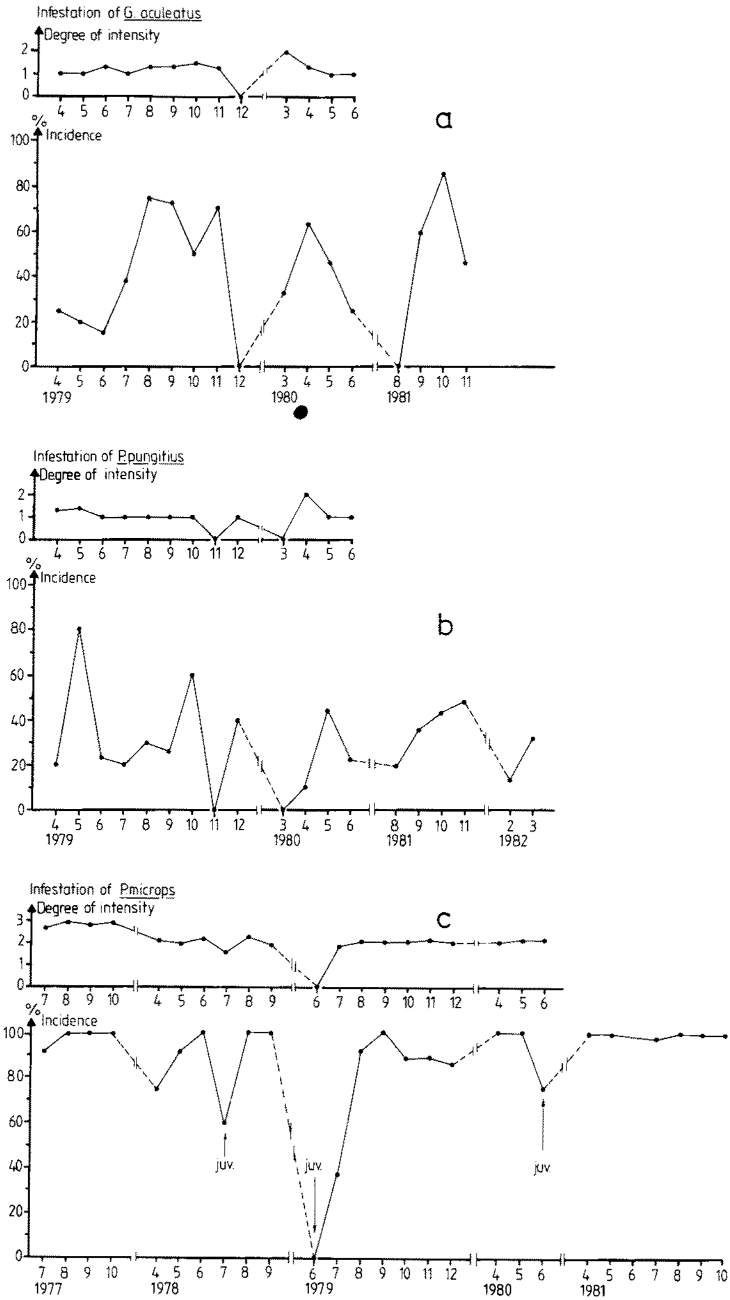

Fig. 5. *Cryptocotyle concavum*. Infestation of 3 fish species (from Olpenitz), recorded monthly over several years (data of 1977 according to Derksen, 1978). (a) *Gasterosteus aculeatus*. Above: degree of intensity, (1) = 1 metacercaria, (2) = 2 to 5 metacercariae, (3) = more than 5 metacercariae per fish; below: infestation rate. (b) *Pungitius pungitius*. (c) *Pomatoschistus microps*. X-axis: months

maximum values for these years were found in May (44 %) and November (48 %), respectively. In November 1979 and March 1980, however, no cysts were present among the *P. pungitius* caught (Fig. 5b).

During the years 1977–1981, incidences of *P. microps* frequently reached very high rates, often up to 100 % (Fig. 5c). During certain periods these rates decreased to values of less than 75 %. This could be related to the appearance of young gobies during early summer; the samples that were not infested at all consisted of young fish of less than 10 mm total length (i.e. June 1979; Fig. 5c).

Average infestation rate in sticklebacks was low. In most cases only one cyst was found on a fish, with rarely more than 5 ever being encountered on an individual during the entire study period (Fig. 5a, b). Peaks in intensity of infestation appeared in March *(G. aculeatus)* and April 1980 *(P. pungitius). P. microps* was usually much more infested than sticklebacks, the infestation reaching an intensity of between 5 and 50 cysts in the kidney (Fig. 5c). Some individuals were even infested by more than 50 metacercariae, e.g. in summer and autumn of 1977 (Fig. 5c).

What is the reason for such changing incidences at different times of the year? As the incidence in *P. microps* decreased when the young gobies appeared, the sampled material was subdivided into size classes each of which comprised 10 mm TL (Fig. 6). No cysts were present in gobies smaller than 10 mm, whereas 35 % of the 10 to 20 mm size group were infested. Almost all specimens of *P. microps* larger than 20 mm total length showed infestation by *C. concavum* (Fig. 6). Similar data were obtained for sticklebacks. The smallest size class of *G. aculeatus* was also not infested (*P. pungitius* less than 10 mm were not taken). The rate of infestation of *P. pungitius* increased from the 10 to 20 mm class in steps to almost 40 % in the 40 to 50 mm class (Fig. 6). In *G. aculeatus*

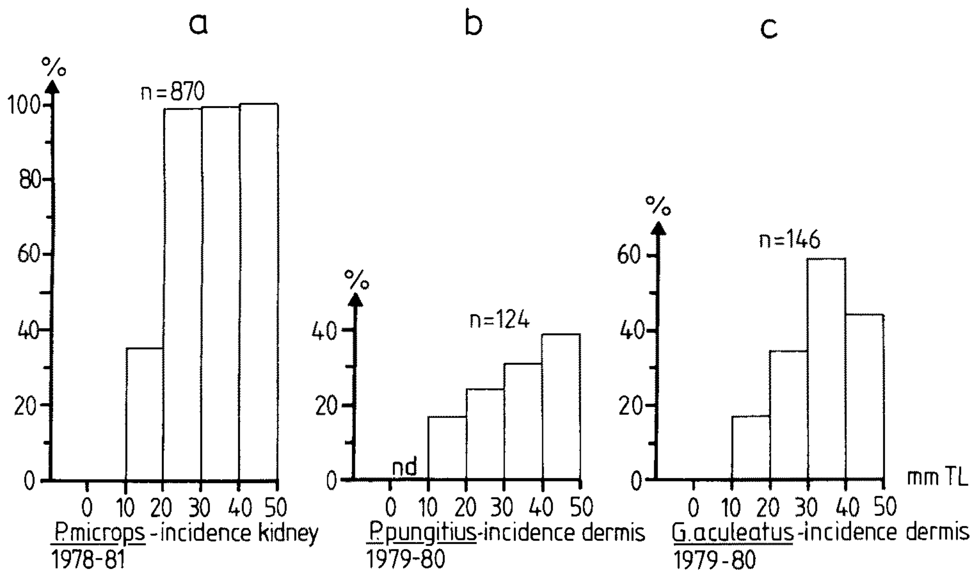

Fig. 6. *Cryptocotyle concavum.* Size dependence of infestation rate in 3 fish species. (a) *Pomatoschistus microps* (combined samples from 1978–81). (b) *Pungitius pungitius* (1979–80). (c) *Gasterosteus aculeatus.* n = total number of fish examined, nd = no data

these steps are steeper, reaching 59 % in the 30 to 40 mm class but decreasing slightly to 44 % in the 40 to 50 mm class (Fig. 6).

When incidences of *P. microps* from the Olpenitz sampling site are compared with those from other sites of the Schlei Fjord, differences of infestation rates apparently depended on salinity. Figure 2 depicts the infestation rates for 5 sites sampled during 1977 (Derksen, 1978) and 1978. At 10 ‰ and higher salinity (Lindaunis, Sundsacker, Olpenitz) there was a very high incidence of 90 to 100 %. In Missunde (8 ‰ S mean, 10 ‰ S maximal) infestation rate decreased to 25 % or even to 5 % as in 1977. At Haddeby (5 ‰ S) no or very low incidences were found (Fig. 2).

Infection of *Hydrobia* by rediae of *Cryptocotyle concavum*

Infection of first intermediate hosts was examined by samples of the snails *Hydrobia stagnalis, H. neglecta* and *H. ulvae* at Olpenitz in 1981 (Fig. 7). The abundance of these

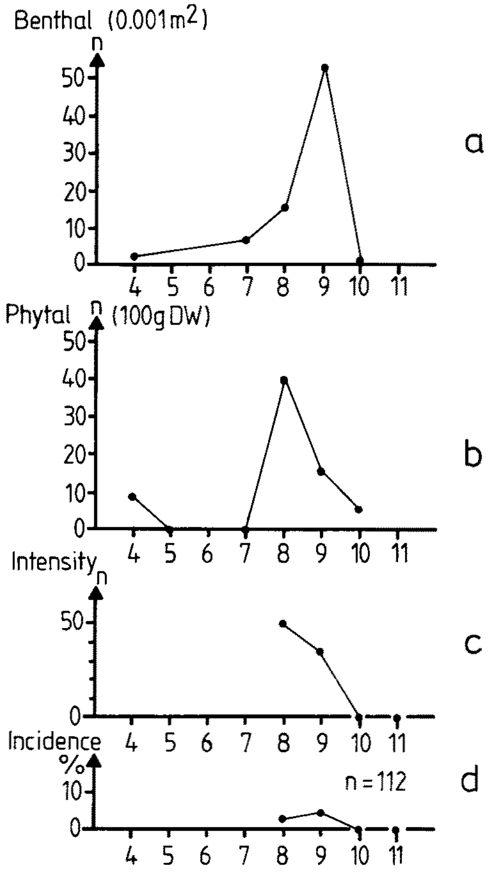

Fig. 7. *Hydrobia* spp. Monthly data of abundance and infection at Olpenitz during 1981. (a) Number of snails per 0.001 m^2 sediment. (b) Number of snails per 100 g (DW) algae. (c) Intensity of *Cryptocotyle concavum* rediae per infected snail. (d) Infestation rate of snails by *Cryptocotyle* rediae. X-axis: months. n = total number of snails examined

species on the sediment was 3000 · m^{-2} in April, increasing slowly to 16 000 in August and rapidly to 53 000 in September. In October, a steep decrease to 2000 · m^{-2} was found. An additional source of *Hydrobia* species were the algae where in August a maximum of snails was present (Fig. 7). Infection rate of snails was very low in August (3 %) and September (5 %). Calculated for 1 m^2, at least 480 (August) or 2650 *Hydrobia* (September) of this area were parasitized. Infected snails revealed from 35 to 50 rediae per host specimen, producing as many as 70 cercariae at a time (Fig. 7).

DISCUSSION

The life cycle of *Cryptocotyle concavum* is complicated; there are three successive hosts – snail, fish, bird – and in addition two free stages – eggs and cercariae (Fig. 1). Hence, distribution and abundance depend on many factors; their interaction in the life cycle of *C. concavum* is summarized in Figure 8. In the following we discuss (1) salinity, (2) relations of first and second hosts, (3) relations of second and final hosts, (4) comparison between *P. microps* and other second hosts.

In German waters, *C. concavum* was described from the Elbe Estuary (Möller-Buchner, 1981) and from the Baltic Sea (Reimer, 1970). In the Elbe, infested *Pomatoschistus microps* were found even in fresh water near Hamburg. Reimer (1970) observed cysts in 5 fish species near Greifswald from the Baltic (6 to 7 ‰ S). However, the distribution of the first host, *Hydrobia* spp., may restrict free cercariae to more than 3 ‰ S as found in the Schlei. This may also be true for the River Elbe. Since young common gobies usually migrate from the sea into the estuaries (Hennig & Zander, 1981), these must be infested at the latest in brackish waters. On the other hand, since juvenile sticklebacks migrate into the sea and return to fresh waters for spawning, infestation of these fish is possible twice during their life span. The upper distribution limit of *C. concavum*, as far as salinity is concerned, is not clear yet. The related *C. lingua* is probably the more successful of the two in sea water (Sindermann & Farrin, 1962) presumably due to its frequent infestation of pelagic fish, while *C. concavum* is mostly found in demersal or

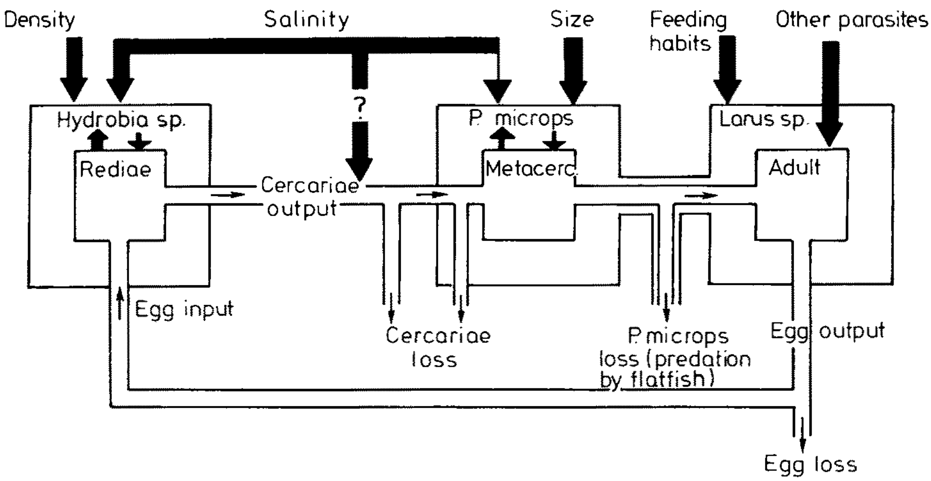

Fig. 8. *Cryptocotyle concavum*. Life cycle in relation to hosts and environmental factors

suprademersal fish. It must be emphasized that both parasites occur together in some brackish waters such as the Baltic Sea or the Wadden Sea and have identical final hosts (Reimer, 1970; Lorch et al., 1982).

C. concavum infection in the first host *(Hydrobia)* can be ascribed to overdispersion (Kennedy, 1975). Generally, such hosts are severely injured and may even be killed (James, 1965; Pennycuick, 1971) so that a mechanism regulating the size of the parasite population is provided. Reimer (1970) found similar incidence and intensity rates in *Hydrobia*. However, the Olpenitz samples revealed that infected snails occur at the rate of 480 (August) or $2650 \cdot m^{-2}$ (September 1981). Such numbers are more than enough to infest almost all common gobies because of the high production rate of cercariae. If the population density of *P. microps* at Olpenitz is $80 \cdot m^{-2}$ in both months, the calculated ratio of first to second intermediate host bearing *C. concavum* is 6 : 1 or 33 : 1, respectively. Late summer seems to be advantageous for infestation of gobies, as the juveniles are then longer than 20 mm. It is not known whether the gobies are damaged by the high intensities of the parasite.

No changes in behaviour of infested *P. microps* are known which would make them more available to potential predators thus ensuring the transmission of *C. concavum*. As the gobies usually live in shallow waters they are easily caught by gulls, terns or ducks. Relations between fish and birds of the Schlei region are unknown. In Norway, 2 % of a population of common gulls *Larus canus* was infested by *C. concavum* with 28 (mean) or 112 (maximally) adult flukes (Bakke, 1972). These rates are low compared to those of 64 % by *C. lingua* in the same population. Blackheaded gulls *Larus ridibundus* from Wangerooge had a higher incidence in that 54 % were infested by both *C. concavum* and *C. lingua* (Lorch et al., 1982). These differences may be due to the specific feeding habits of the two birds: *L. canus* probably feeds more on pelagic fish infested with *C. lingua* than on demersal fish infested with *C. concavum*. These two types of prey might be ingested in similar numbers by *L. ridibundus*.

The significance of sticklebacks for the life cycle of *C. concavum* probably lies in the presence of additional infectional stages for the final hosts although, regarding incidence and intensity, these fish are not as important as *P. microps*. Reimer (1970) found that *Gobius niger*, *Syngnathe typhle* and *Pleuronectes platessa* are also vectors of *C. concavum*. These hosts may ensure infestation of birds where heavy kidney parasitation of *P. microps* causes death of the fish.

P. microps seems to be an ideal second host for *C. concavum* because it (1) is probably infested easily by the cercariae of this fluke (fish investigated by us had mean incidences of only 18 % by *C. lingua* or *Thynnascaris* for which *P. microps* is also an intermediate host); (2) can tolerate high numbers of the parasite by harbouring them in the kidney at least when living in salt or brackish waters; (3) inhabits shallow waters and is small in size, and thus easily available to predating birds.

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