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Studies on bucephalid digeneans parasitising molluscs and fishes in Finland I. Ecological data and experimental studies

Jouni Taskinen[1], E. Tellervo Valtonen[1] and David I. Gibson[2]
[1]Department of Biology, University of Jyväskylä, SF-40100 Jyväskylä, Finland
[2]Department of Zoology, The Natural History Museum, London SW 7 5BD, UK

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Abstract

Two types of bucephalid cercariae are reported from the bivalve *Anodonta anatina* in two Finnish lakes. One, Type A, resembles in gross morphology the cercaria of *Bucephalus polymorphus*, and the other, Type B, resembles the cercaria of *Rhipidocotyle campanula*. Type A daughter-sporocysts develop more slowly, have a greater cercarial productivity and exhibit a differential diurnal rhythm to that of Type B. Cercariae of Type A have a shorter longevity than Type B and tend to encyst in the fins rather than the gill-arches of fish intermediate hosts. The main definitive host of Type A is pike *Esox lucius* and, in the case of Type B, perch *Perca fluviatilis*. Adults of Types A and B are morphologically very similar and both species belong to the genus *Rhipidocotyle*.

Introduction

During the course of a survey of the parasites of the bivalve *Anodonta anatina* L. (=*piscinalis* Nilsson) in Lake Saravesi in central Finland two different bucephalid digenean infections were encountered. One form (herein referred to as Type A), which was rather common (prevalence 33.2%), produced cercariae (Fig. 1) with long, filamentous furcae that resembled those described by Baturo (1977) under the name *Bucephalus polymorphus* von Baer, 1827. The second form (herein referred to as Type B), which was rare (prevalence 1.0%), produced cercariae (Fig. 2) with short furcae which resembled those described by Baturo under the name of *Rhipidocotyle illense* (Ziegler, 1883) [now *R. campanula* (Dujardin, 1845)]. Subsequently, *A. anatina* infected with the latter form only were found in Lake Kuivasjärvi in northern Finland. The only previous records of bucephalids in Finland are those of Järnefelt (1921), who claimed to have found *B. polymorphus* in 1.2% of *Perca fluviatilis* in Tuusulanjärvi in southern Finland, and Hakala (1979), who studied eight *Anodonta* populations in southern Finland and found *Bucephalus* sp. in prevalences varying from 0–50% in different populations. The species of bucephalid actually found by these authors is, however, questionable. It would have not been possible for Järnefelt to distinguish *B. polymorphus* from *R. campanula* in 1921: in the standard identification manual of the time, that of Lühe (1909), the figure said to be *B. polymorphus* clearly resembles *R. campanula*. In view of the present results, Hakala's identification based on larval material must also be doubtful.

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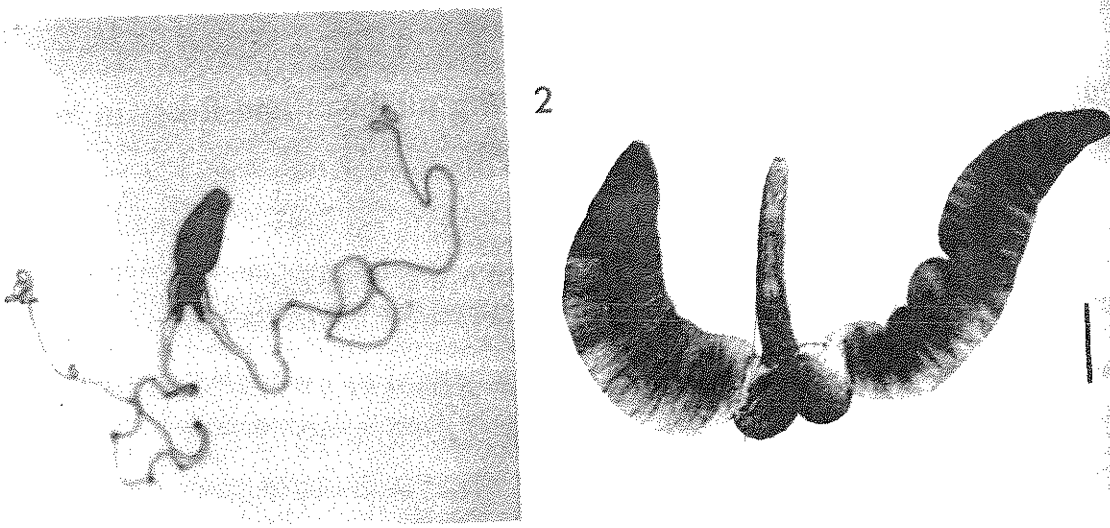

Figs 1,2. 1. Photomicrograph of a Type A cercaria from *Anodonta anatina* from Lake Saravesi. 2. Photomicrograph of a Type B cercaria from *Anodonta anatina* from Lake Kuivasjärvi. *Scale-bar*: 0.1 mm.

The taxonomy and taxonomic history of bucephalids in European freshwater fishes is complex, although most authorities now agree that two species, *B. polymorphus* and *Rhipidocotyle campanula* [=*R. illense*], exist. A major nomenclatorial problem has arisen, however, because Baturo (1977) claimed to have obtained specimens of *R. campanula* (as *R. illense*) from cercariae which resembled the type-material of *B. polymorphus*: the latter species is based upon cercarial material. As a result of her findings, Baturo (1979) applied to the International Commission for Zoological Nomenclature to have *Bucephalus polymorphus* placed on the Official List and based upon an adult neotype: this application is still awaiting a decision from the Commission. Our preliminary experimental studies on the bucephalid cercariae which we found in Finland (Gibson & Valtonen, 1989) appeared to contradict Baturo's results in that cercariae (Type A) resembling those which she considered to be *B. polymorphus* produced an adult form resembling *R. campanula*. In view of these preliminary results more detailed ecological and experimental studies of the bucephalids infecting bivalves in Finnish waters were undertaken.

Materials and methods

Lake Saravesi is situated close to Jyväskylä in Central Finland (Fig. 3) and belongs to the Kymijoki water system which flows into the Gulf of Finland. It is small (7.8 km^2) and shallow (mean depth 5.5 m), and, typically for a river-like lake, its water replacement takes only four days. The water of this lake is eutrophic and contains traces of organic chlorinated compounds from the effluent of paper and pulp mills 25 km upstream. Lake Saravesi is on average ice-covered from 20–25th November to about 10th May and the highest water temperatures are 18–20°C at the end of July or the beginning of August. A dense population of *Anodonta anatina* inhabits the lake and at least 14 species of fish occur in the area. The zander *Stizostedion lucioperca*, originally an inhabitant of the lake, was absent for at least 20 years, but the lake has been successfully restocked in recent years.

Lake Kuivasjärvi is also a small (0.84 km^2), shallow (mean depth 1.9 m), hypereutrophic lake situated close to Oulu in northern Finland (Fig. 3). Its waters flow into the neighbouring Bothnian Bay via a small river. This lake is usually ice-

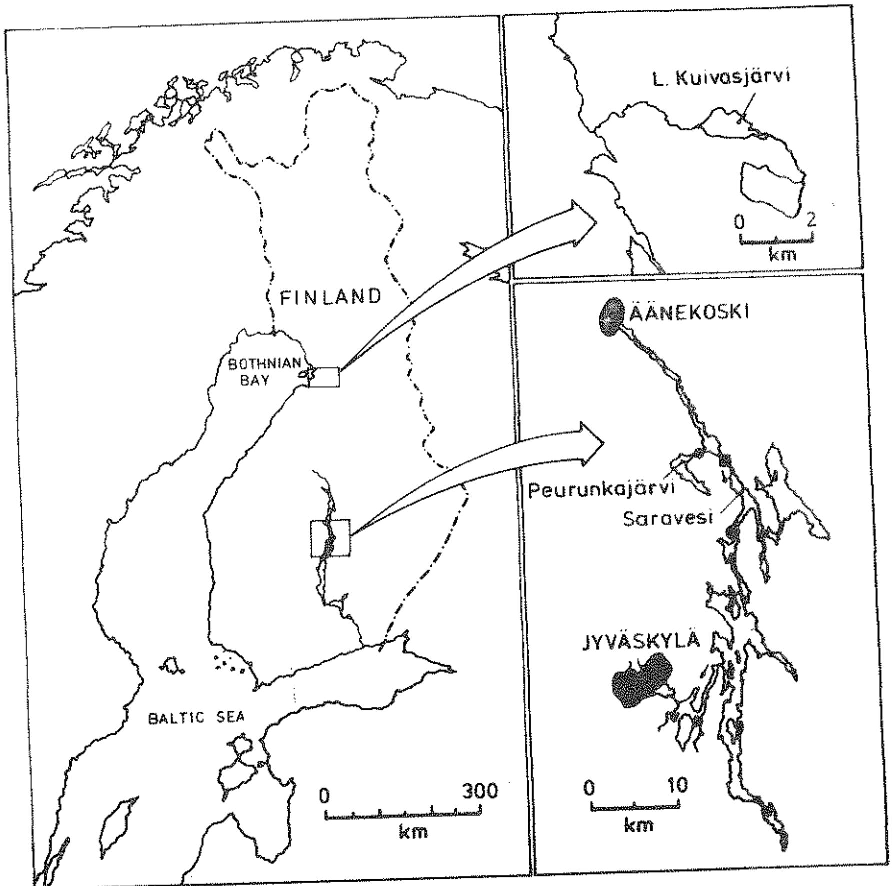

Fig. 3. The location of the study areas in northern and Central Finland. Lake Saravesi is separated from neighbouring lakes by a dam (*), rapids (■) and a hydro-electric power station (●).

covered from the end of October until about the 30th May. A dense *A. piscinalis* population inhabits this lake and at least five species of fishes occur, of which roach *Rutilus rutilus*, perch *Perca fluviatilis* and pike *Esox lucius* are the most common. The zander does not occur in this region.

Uninfected piscine hosts were obtained from Lake Peurunka which is situated close to Lake Saravesi; this is a lake in which *Anodonta* has not been found and no glochidia occur on the fish.

Experimental details are given in the sections dealing with each experiment.

Representative metacercarial and adult bucephalid specimens obtained as a result of surveys and experimental infections were forwarded to the Parasitic Worms Section of the Natural History Museum, London for identification. Worms were fixed in glacial acetic acid, stored in 70–80% alcohol, stained in Mayer's paracarmine and mounted in Canada balsam.

Observations

Studies on the natural occurrence of larval bucephalids in molluscan hosts

Methods

Forty-three samples of about 30 molluscs were collected monthly between May and December in both 1987 and 1988 and between January and November in 1989 from the littoral and sublittoral zones of Lake Saravesi. A total of 1,157 *Anodonta anatina*, 29 *Pseudanodonta complanata* and 65 *Unio* spp. (*U. tumidus* and *U. pictorum*) were dissected and examined for the larval stages of

Table I. The occurrence of Type A and Type B bucephalids in bivalves from Lakes Saravesi and Kuivasjärvi.

Lake	Bivalve	No. examined	Type A No. infected (%)	Type B No. infected (%)
Saravesi	*Anodonta anatina*	1,157	384 (33.2)	12 (1.0)
	Pseudanodonta complanata	29	0 (0)	0 (0)
	Unio spp.	65	0 (0)	0 (0)
Kuivasjärvi	*Anodonta anatina*	168	0 (0)	8 (4.7)

bucephalid digeneans. The gonads of the mollusc were studied by pressing the tissues between two glass plates and examined using transmitted light.

A sample of 168 *Anodonta anatina* was studied from the littoral zone of Lake Kuivasjärvi during June, 1988.

Results

Branching bucephalid sporocysts (Type A) producing cercariae with extremely long, filamentous furcae were found only in *A. anatina* from Lake Saravesi. The specimens of *P. complanata* and *Unio* spp. were not infected. 33.2% of the *A. anatina* examined at Lake Saravesi were infected with this parasite (Table I).

Branching bucephalid sporocysts producing cercariae (Type B) with relatively short, stout furcae were found only rarely (prevalence 1.0%) in *A. anatina* from Lake Saravesi. Of the 384 specimens infected with Type A and 12 infected with Type B only one concurrent infection was found. Parthenitae of *Phyllodistomum* sp. were also found in two specimens of these molluscs.

In the material from Lake Kuivasjärvi no infections of Type A sporocysts were found in *A. anatina*, but about 5% had gonads infected with Type B sporocysts.

Comments

These results indicate that the bivalve *A. anatina* harbours two bucephalid larvae and that the distribution of these infections is variable, with individual species being absent or rare in some lakes. In gross morphology the two types of cercariae resemble those of the two bucephalid species normally occurring in European bivalves (see Baturo, 1977). The results also suggest that these two species are restricted to *A. anatina*, although insufficient numbers of other bivalves have been examined to be certain: this would tend to contradict the impression given in the literature that the two widely accepted species of bucephalid in European freshwaters use a range of bivalves as first intermediate hosts (Table IV).

Observations on the shedding of cercariae

Methods

The numbers of cercariae produced daily by naturally infected molluscs and the frequency and diurnal rhythm of their emergence was studied in the laboratory by monitoring 15 *A. anatina* from Lake Saravesi known to be shedding cercariae of Type A. Each mollusc was kept in its own aquarium in 5 litres of water at room temperature. The number of cercariae emerging from each mollusc was counted visually for 2 minute sessions for an average of 12 times in 24 hours during both August 1st–2nd and August 26th–27th, 1988. The experiment was subjected to natural lighting conditions only, except during the period when cercariae were counted under artificial light. The data collected during the two days involved a total of 312 counts. In order to obtain the average number of cercariae emerging from a mollusc during 2 hour periods of the day, the data from all of the 2 minute sessions on both days were pooled and subdivided into 2 hour periods. The average sum for each mollusc was then calculated.

This experiment was repeated on 13th–14th July, 1988 using 2 specimens of *A. anatina* from Lake Kuivasjärvi known to be releasing Type B cercariae.

Results

During a 24 hour period *A. anatina* from Lake Saravesi shed on average 9,500 Type A cercariae, whereas the average number of Type B cercariae emerging from specimens of *A. anatina* from Lake Kuivasjärvi was only 1,400 (Fig. 4).

There was also a great difference in the diurnal periodicity of emergence between the two species. The most productive time of the day for the release of Type A cercariae was morning between 8 and 10 a.m., when 47% of the total emergence occurred. This was the period of lowest productivity for Type B cercariae (Fig. 4), which increased only between 4 p.m. and 4 a.m.

Comments

These results indicate that Type A sporocysts have a greater cercarial productivity than those of Type B. They also exhibit a quite different diurnal rhythm in relation to the release of cercariae. One possible explanation for these differences may be related to the fact that the two forms may have different preferences regarding the species of fish second intermediate host which may have different diurnal regimes. Since roach are utilised as the second intermediate host for both species in these lakes, this seems unlikely. Alternatively, it may be related to the different mechanisms of transmission to the fish intermediate host (see below) used by the two species and the behaviour of the host at different times of the day. For example, cercariae Type A may stand a better chance of transmission during the day when the fish is more active, whereas Type B may stand more chance during the hours of darkness when the fish is resting. The greater longevity of Type B cercariae (see below) may also be associated with mechanisms and timing of transmission.

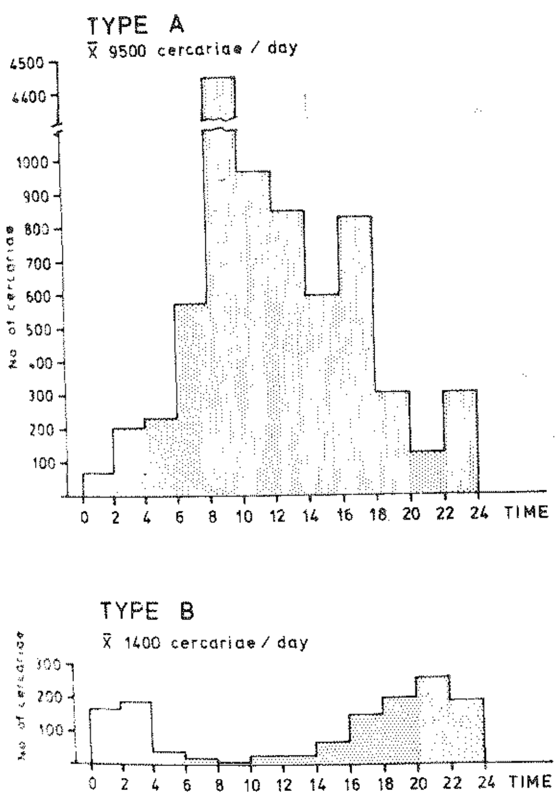

Fig. 4. The emergence of cercariae of Types A and B from naturally infected *Anodonta anatina* in the laboratory in relation to the time of the day (local time).

Baturo (1977) found that her cercariae resembling Type A were produced in numbers of only several hundreds per day and that productivity was greater in the afternoon and evening: this appears to contrast markedly with our results, but the greater day-length in summer in Finland (c. 19–21 vs 16–18 hours) probably means that there is not such a distinct difference when considering the number of hours since dawn. Her cercariae resembling Type B (*R. campanula*) were produced in numbers of 100–300 per day and the main period of emergence was the afternoon and especially the evening: this latter result is similar to ours. The lower daily emergence observed by Baturo for both of her forms of cercariae might be related to the longer period in Poland when the water temperatures are suitable: in Finland this period is restricted to only two months in the year (late July to early September).

Observations of the longevity of bucephalid cercariae

Methods

The longevity of Type A cercariae was studied using 292 newly emerged cercariae from 5 *A. piscinalis* from Lake Saravesi. The cercariae from each mollusc were kept together in separate petridishes at room temperature (c. 19–21°C) and their survival was monitored for up to 29 hours.

The experiment was repeated using 48 Type B cercariae from 3 *A. anatina* from Lake Kuivasjärvi, and they were monitored for 40 hours.

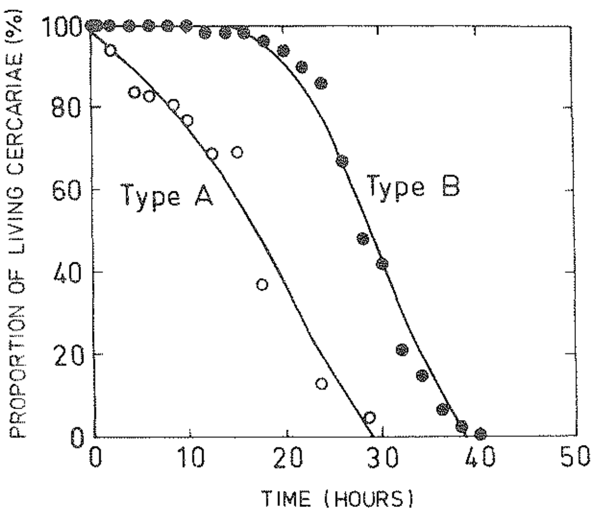

Fig. 5. The longevity of cercariae of Types A and B after emergence from naturally infected *Anodonta anatina* from Lakes Saravesi (Type A) and Kuivasjärvi (Type B) at 19–21°C. The number of cercariae monitored were 292 Type A and 48 Type B.

Results

In the case of Type A cercariae, the first cercariae died during the first two hours, 50% were dead after 17 hours and after 29 hours only 4% of the cercariae were still alive (Fig. 5). In the case of Type B cercariae the first were dead after 12 hours, 50% were dead after 28 hours and the last one died after 40 hours (Fig. 5).

Comments

Baturo (1977) found that her cercariae resembling Type A survived for about 48 hours at 17°C and 28 hours at 22°C. Her cercariae resembling Type B survived for 28 hours at 18°C, 24 hours at 20°C and 18 hours at 25°C. Similarly, Kurandina & Ivantziv (1975) recorded the survival of cercariae said to be *R. illense* as 17–20 hours at 21–23°C. It is not possible to compare our results with those of these workers because it is not clear how they measured longevity (did they record the average longevity or the greatest longevity?) and, as indicated by Baturo's results, longevity is very temperature dependent. Nevertheless, it is clear from our results that cercariae of Type B tend to survive longer than Type A.

During this experiment it was observed that cercariae of both species were active and infective only during the first few hours after emergence. Kurandin & Ivantziv (1975) described the behaviour of cercariae of *R. campanula* (as *R. illense*), and Baturo (1977) has described the behaviour of the cercariae of both this species and *B. polymorphus*: the behaviour of the cercariae of these two species resembles our observations of Type B and Type A cercariae, respectively.

Observations of the response of cercarial emergence to increasing temperature in the laboratory

Methods

The speed of development was assessed in terms of day-degrees: this is a summation of the temperatures on each day of development.

A total of 30 *Anodonta anatina* were collected from Lake Saravesi on 12th May, 15th June and 29th June, 1988, and 168 were collected from Lake Kuivasjärvi on 7th June, 1988. These molluscs were kept at room temperature in the laboratory and cercarial emergence was monitored for four hours daily, between 8 and 10 a.m. for Type A and between 2 and 4 p.m. for type B. The day-degrees were counted from May 1st until the emergence of the first cercaria from each infected mollusc. This date was chosen because of the different collection dates and because the water temperature begins to rise at this time, as the ice-cover is lost, and the rate of development of the parasite may increase. For this purpose the average daily water temperature in the lakes was also monitored.

During the period of ice-cover specimens of *A. anatina* were collected from Lake Saravesi and brought to the laboratory for the same purpose: these were 45 specimens on December 22nd, 1987 and 38 from the same site on 13th January, 1989. In the case of this material, the day-degrees were counted from the date of collection to the first emergence of the cercariae.

Results

Of those collected in the spring 5 molluscs from Lake Saravesi released Type A cercariae and 5 molluscs from Lake Kuivasjärvi released Type B cercariae in the laboratory. Of those collected

Table II. The effects of increased (laboratory) temperatures on the intra-molluscan development of Types A and B bucephalids in terms of degree-days (°C), as indicated by the emergence of the first cercariae.

	Source	Date of collection	No. collected	No. infected (when dissected)	No. producing cercariae	Degree-days (°C) Mean (range)	*t*-test (*p*-value)
Type A	L. Saravesi	12.5–29.6.88	30	8	5	916 (782–1,060)[1]	0.001
Type B	L. Kuivasjärvi	7.6.88	168	8	5	570 (533–675)[1]	
Type A	L. Saravesi	22.12.87 & 13.1.89	45	9	4	615 (321–909)[2]	could not be tested
Type B	L. Saravesi	13.1.89	38	1	1	187[2]	

[1] Day-degrees counted from 1st May in the lake and then in the laboratory until emergence of first cercaria.
[2] Day-degrees counted from date of collection until emergence of first cercaria.

from Lake Saravesi in December, 1987 and January, 1989, 4 of the former released Type A cercariae and one of the latter released Type B cercariae (Table II).

As indicated in Table II, the release of the Type B cercariae occurred much sooner than that of Type A, both in the case of those collected in the spring and those collected in the winter. In the Spring the difference was statistically significant (*t*-test, $t = 5.18$, $df = 8$, $p = 0.001$).

Comments

The actual figures in terms of day-degrees tell us little, as conditions are artificial, but the relative figures indicate that Type B bucephalids develop much more quickly than Type A in the molluscan host. It is not possible to relate this data to the biology of the worms until more is known of the taxonomy and distribution of the two forms; but one might expect that a greater sensitivity in terms of speed of development to increasing temperatures might be an adaptation to a more northern distribution (Gibson & Valtonen, 1984).

The results of those collected in winter and spring cannot be compared directly because of the fact than those collected in the spring may or may not have undergone some development prior to collection. It is not known whether or not there is a proportionate increase in development in relation to temperature or whether temperature thresholds are involved. It is worth noting, however, that some well-formed cercariae of both species can be found in the molluscs throughout the winter which presumably require a temperature stimulus for emergence.

Observations on naturally infected second intermediate hosts

Methods

Ninety roach from Lake Saravesi in the form of 6 bi-monthly samples of 15 fish in 1987, and 12 roach from Lake Kuivasjärvi caught in June, 1988 and April, 1989, were examined for metacercariae using the technique described in the experiment below.

Results

The results of the survey of bucephalid metacercariae occurring in naturally infected roach gave similar results to the experimental study described below (Table III). From the 90 roach from Lake Saravesi 8,703 metacercarie were recovered of which 78% were in the fins and the remainder in the gill-arches. The prevalence of infection in these roach was 95%. The 12 roach from Lake Kuivasjärvi were all infected and revealed 1,104 metacercariae of which 97.5% were located in the region of the gill-arches, with only 28 specimens in the fins.

Comments

These results indicate different penetration site preferences on roach of the bucephalid cercariae from the two lakes, those from Lake Saravesi (mainly Type A) tending to occur in the fins and those from Lake Kuivasjärvi (entirely Type B) in the gill-arches.

Observations on the site of cercarial penetration of the second intermediate host in experimental infections

Methods

Roach were collected from Lake Peurunka, which is located close to Lake Saravesi, but, as far as the fish are concerned, the two lakes are isolated by a dam. Roach from this lake are not infected with bucephalid metacercariae (Valtonen & Koskivaara, 1989). The fish were kept at room temperature and exposed to bucephalid cercariae of both types separately by keeping them in the same aquarium as naturally infected *A. anatina* known to be actively shedding cercariae. In each case the type of cercaria released by the mollusc was known. The number of molluscs per roach averaged 1.1 (range 0.9–3.2) and the period of exposure varied from 6 hours to 4 days but was in most cases 2 days. 148 roach were exposed to molluscs emitting Type A cercariae in 9 separate experiments (20 of these fishes died during the course of the experiment and are omitted from the results) and 11 roach were exposed to molluscs shedding Type B cercariae in 3 separate experiments between July and November in 1987, 1988 and 1989.

The regions of the body of the roach examined for metacercariae after a period of 1–45 days were: all of the fins, the gill-arches on one side of the body only and, on some occasions, the muscles of the body. The fins were studied on glass plates using transmitted light at a magnification of 10–25×. The gill-arches were pressed between 2 glass plates and examined as above.

Results

All 128 surviving roach exposed to Type A cercariae became infected in all 9 experiments (Table III). A total of 15,955 metacercariae were recovered: metacercariae were also present in the body musculature and especially in the caudal peduncle, but these data are not included here as they were not counted in all cases. The vast majority of the recovered metacercariae (99.4%) were located in the fins, especially in the base of the tail fin, and only a minority (103) of specimens in and around the gill-arches (gills on only one side of the body were examined).

All 11 roach exposed to Type B cercariae survived, became infected and 122 metacercariae were recovered. Only 4 metacercariae were found in the fins, the remainder (96.7%) being located in or around the gill-arches (gills on only one side of the body were examined) (Table III).

Comments

These results confirm the different penetration site preferences of the cercariae of the two species. This difference can be explained, as suggested by Baturo (1977) for *B. polymorphus* and *R. campanula*, by the nature of the cercariae. Type A cercariae possess long, filamentous, sticky furcae which easily attach to the moving fins of the roach. Whereas Type B cercariae possess short, stouter and more muscular furcae and tend to attach to the roach by coiling round the gill-rakers, arches, etc, after being swept there in the branchial current.

It is worth noting that one roach infected with Type A metacercariae still carried live worms after one year in the laboratory at room temperature: this compares with Baturo's (1977) finding that metacercariae resembling this type were dead after 5 months.

It is also worth noting that the metacercariae of Type A recovered after 21 days were infective. Chernogorenko & Ivantziv (1980) obtained infective metacercariae from cercariae resembling Type B, but referred to as *Rhipidocotyle illense*, after 21–23 days; but these were not yet infective after 15 days. Baturo (1977) obtained infective metacercariae from cercariae resembling Type A after 15 days.

Observations on naturally occurring definitive hosts

Methods

One hundred and thirty-five perch collected in monthly or bi-monthly samples from Lake Saravesi between November, 1985 and November, 1986 were examined for bucephalid trematodes in the intestine. In addition 11 zanders *Stizostedion*

Table III. Bucephalid metacercariae recovered from the gills and fins of roach *Rutilus rutilus* (1) collected from Lake Saravesi (mainly Type A) and Kuivasjärvi (Type B) and (2) infected experimentally.

No. of fish examined		No. of metacercariae found	Worms from gills (on one side of body only)		Worms from fins	
			No.	Mean intensity (range)	No.	Mean intensity (range)
(1) Natural infestations						
Type A	90	8,703	1,944	21.6 (0–220)	6,759	75.1 (0–700)
Type B	12	1,104	1,076	89.7 (4–216)	28	2.3 (0–12)
(2) Experimental infestations						
Type A	128	15,955	103	0.8 (0–7)	15,852	123.8 (2–913)
Type B	11	122	118	10.7 (0–43)	4	0.4 (0–1)

lucioperca, 7 pike *Esox lucius* and 3 burbot *Lota lota* from this lake were also examined between August and September, 1986, plus a further 4 zanders in September, 1989. Nineteen pike have also been examined from Lake Vatia which is situated upstream from Lake Saravesi and separated from it by rapids.

In the case of Lake Kuivasjärvi, 163 perch and 31 pike were examined by T. Mäkelä in a separate study between April and October, 1989, and the results were supplied by her (pers. comm.).

Results

Of the perch from Lake Saravesi only 17 were infected with 90 bucephalids. All of the worms were found between April and November. Most of these were juvenile, but some (<10) were ovigerous in the July to September period. Initial investigation indicated that these worms were morphologically similar to *Rhipidocotyle* in that they lacked tentacles. One of the zanders collected in 1986 was also infected with a similar specimen in which the testes were developed, and in September, 1989 in 4 zanders one had 33 ovigerous bucephalids, 31 of which were full of eggs. Five of the 7 pike from Lake Saravesi were infected with bucephalids, one of them containing several hundred specimens, while the other infections ranged from 0 to 50 specimens. Of the 19 pike from the neighbouring Lake Vatia 7 were infected with 1–594 worms (mean intensity 98). The worms from pike in both lakes were found between May and September, and during July to September many mature (fully developed but without eggs) and ovigerous worms were found, all those from September being full of eggs.

Of the perch from Lake Kuivasjärvi, Mäkela found that 21% were infected with bucephalids (presumably Type B, the only bucephalid in this lake) which again resembled *R. campanula*. The vast majority of these were found between April and June (with prevalence up to 59%), none were found in August and a low prevalence (10%) occurred in September and October. Worms collected in April were immature, in May and June mature (without eggs) and most in September were ovigerous. Of the pike from this lake only 4 (13%) were infected with bucephalids: one in May had 126 immature worms, but infected fishes later in the year had only 1–2 specimens without eggs.

No bucephalids were recovered from the burbot examined.

Comments

These results suggest that pike is the main natural definitive host of Type A bucephalids in Lake Saravesi, although small numbers of this species are capable of surviving, but not developing, for a limited period in perch. Mature specimens were also recovered from zanders in this lake, but it is not known for certain whether these represent Type A or Type B bucephalids, although preliminary observations indicate some similarities with Type B. It is worth noting that the number of zanders in the lake is small, since the lake has only recently been restocked with this species. On the other hand, perch would appear to be the preferred host of Type B adults in Lake Kuivasjärvi where type A is missing. In order to clarify the apparent host-specificity of the worms with regard to their main hosts, an experimental study was undertaken (see below).

It is clear from the results that both species are immature in their primary definitive host in the spring, when they may be present in very large numbers and after which their numbers fall, they mature during the summer and are gravid in about September, when they tend to be present in relatively small numbers. This rate of development in the definitive hosts compares well with Pojmanska's (1985) results for *Bucephalus polymorphus* in which indicated that they were 'fully mature' (ovigerous) after 4–5 months.

In the literature the commonest definitive host for *R. campanula* (=*R. illense*) would appear to be pike (see Kozicka, 1959, Poland; Rauckis, 1970a, b, Lithuania; Halvorsen, 1972, Norway), whereas the commonest definitive host for *B. polymorphus* would appear to be the zander (see Barysheva & Bauer, 1957, USSR; Kosheva, 1957, USSR; Kogteva, 1957, USSR; Kozicka, 1959, Poland; Komarova, 1964, USSR; Osmanov, 1976, Aral Sea; Terekhov & Safrygina, 1982, Sea of Azov; Pojmanska, 1985, Poland), although, in a recent survey of zanders on the Baltic Sea coast of East Germany, Walter (1988) claimed to have found a heavier level of infection with *R. campanula* than *B. polymorphus*.

The most interesting possibility arising from these results is that two morphologically dissimilar cercariae result in two morphologically similar adults. This is not a unique phenomenon, having been described in other groups, such as the lepocreadiids (e.g. Køie, 1985), and, as discussed by Gibson (1987, p. 431), supports the hypothesis that the shape of the cercarial tail is very dependent upon the nature of the transmission to the next host.

Observations on experimental definitive hosts

Methods

In order to determine the natural definitive hosts of the two bucephalid species, uninfected perch and pike from Lake Peurunka were experimentally infected with Type A metacercariae from the fins of naturally infected roach from Lake Saravesi and experimentally infected roach from Lake Peurunka or Type B metacercariae from the gills of both naturally (from Lake Kuivasjärvi) and experimentally infected roach from Lake Peurunka (the latter infection had been derived 21 days earlier from naturally infected *A. anatina* from Lake Kuivasjärvi).

Fifty-six perch in 8 separate experiments were infected with Type A metacercariae in May, June, August and September, 1988. This was carried out by force-feeding them with pieces of roach caudal fin containing at least 30 metacercariae. Twenty-two perch were infected in 4 experiments during October, 1988 and April, May and November, 1989 with Type B metacercariae by being force-fed with roach gill-arches. In these experiments the roach were from Lake Kuivasjärvi and naturally infected, except for the last experiment in which roach were infected experimentally with Type B cercariae. Subsequent to their infection, perch were force-fed on muscle-tissue of uninfected roach from Lake Peurunka.

Five pike were infected in September and October, 1988 and in November, 1989: 3 pike were permitted to feed on infected live roach containing Type A metacercariae from Lake Saravesi, and two were infected with Type A metacercariae by eating experimentally infected roach which had been exposed to Type A cercariae. Two pike were infected with Type B experimentally by being fed upon roach which had been exposed to Type B cercariae. The pike were subsequently allowed to feed *ad libitum* on uninfected live roach, a new roach being added as one was eaten.

The perch infected with Type A metacercariae were examined between 2 hours and 16 days post-infection (p.i.) and those infected with Type B 4 to 41 days p.i. The pike infected with Type A metacercariae were examined 12, 19, 20, 21 and 22 days p.i. and those with Type B 24 and 32 days p.i.

Results

No adult specimens of the Type A bucephalid were obtained from the perch, but 16 juvenile specimens were recovered from 7 fish dissected between 2 hours and 4 days p.i. Most of the fish dissected within a few days p.i. still contained the residues of digesting roach tail and occasionally dead metacercariae were observed. No worms were found in experimentally infected perch killed after more than 4 days p.i.

Fifteen of the 22 perch infected with Type B metacercariae contained bucephalid worms in the intestine: 291 worms were recovered, the intensity varying from 1–74 (mean 13.2). Gravid worms were found after 15 days at 20°C, but after 27 days at 12°C no mature worms were seen. When the latter temperature was increased to 20°C for one week mature worms were found and after two weeks these were full of eggs.

All 5 experimentally infected pike contained adult and juvenile Type A bucephalids, numbering 29, 71, 900+, 18 and 30 specimens. In one case ovigerous worms were collected 19 days p.i. at 20°C, but in another fish no ovigerous worms were recovered after 21 days at the same temperature. The third fish, examined 12 days p.i., harboured a huge number of live juveniles. In the last two pike mature but not gravid worms were obtained after 20 and 22 days at 17°C.

No bucephalids were obtained from the two pike infected with Type B metacercariae after 24 and 32 days at 17°C.

Comments

From these experiments we can deduce that perch are the main definitive host of the Type B species in the study areas and confirm the field observations that pike are the main definitive host of the Type A species.

Final discussion

Several interesting questions emerge from these results. These questions concern aspects of both the biology and the taxonomy of the worms.

Biology

The dominance of the Type A bucephalid in Lake Saravesi and its absence in Lake Kuivasjärvi along with the presence of the Type B bucephalid is difficult to explain. One possible reason for the preponderance of Type A in the southern lake is its greater cercarial productivity and more efficient transmission mechanism which were commented upon above. Its absence from the northern lake might be because this species has never been introduced. It is clear from our results that Type B sporocysts respond more quickly in terms of cercarial emission and would, therefore, appear to be better adapted to northern conditions, although this does not adequately explain the differences between the results for the two study-areas since in geographical terms they are relatively close (300 km).

The difference between the two forms with regard to prevalence in bivalves in the two lakes is also an interesting problem. Type A in the southern lake (prevalence more than 30%) contrasts with that of Type B in the northern lake (5%). Suitable intermediate and definitive hosts of both species are common in both lakes, although *Anodonta anatina* occurs in greater numbers per square metre in the northern lake. Another explanation for the relatively low prevalence in the northern lake may be the shorter summer; but this argument is not supported by results from more southerly regions of Europe, where Baturo (1977), for example, found only 0.9–3.7% infection in bivalves with cercariae resembling Type A and 2.0–6.5% with cercariae resembling Type B. The low prevalence of Type B (1.0%) in *A. anatina* in the southern lake is difficult to explain. It is possible, however, that the species of bivalve involved might be at least partly responsible for such differences, i.e. *A. anatina* might be less refractive to infections of Type A than Type B. It is worth noting that Kozicka (1959) found *Rhipidocotyle campanula* (as *R. illense*), whose cercaria resembles our Type B, to be considerably more common in Lake Druzno in Poland than *Bucephalus polymorphus*, whose cercaria resembles our Type A.

Table IV. Records of natural infestations of bivalves with bucephalids in European waters where an illustration is included.

Author	Host(s)	Host(s) not infected	Name used
Cercariae resembling Type A in gross morphology			
Kinkelin *et al.* (1968)	*Dreissena polymorpha*		*Bucephalus polymorphus*
Baturo (1977)	*Dreissena polymorpha*	*Unio pictorum* *Anodonta* sp.	*Bucephalus polymorphus*
Wallet & Lambert (1984)	*Dreissena polymorpha*		*Bucephalus polymorphus*
Chernogorenko (1983)	*Anodonta anatina* *Unio pictorum*	*Dreissena polymorpha* *Dreissena bugensis* *Anodonta cygnea* *Unio tumidus*	*Bucephalus polymorphus*
Present study	*Anodonta anatina* (=*piscinalis*)	*Unio tumidus* *Unio pictorum* *Pseudanodonta complanata*	Type A
Cercariae resembling Type B in gross morphology			
von Baer (1827)	*Anodonta mutabilis* *Unio pictorum*		*Bucephalus polymorphus*
Ziegler (1883)	*Anodonta mutabilis*		*Gasterostomum illense*
Koubek (1977)	*Unio* sp.	*Anodonta cygnea* *Anodonta anatina*	*Bucephalus polymorphus*
Baturo (1977)	*Unio pictorum*	*Anodonta* sp. *Dreissena polymorpha*	*Rhipidocotyle illense*
Chernogorenko (1983)	*Anodonta piscinalis*	*Dreissena polymorpha* *Dreissena bugensis* *Anodonta cygnea* *Unio pictorum* *Unio tumidus*	*Rhipidocotyle illense*
Ivantziv & Chernogorenko (1984)	*Anodonta piscinalis* *Unio pictorum*		*Rhipidocotyle illense*
Present study	*Anodonta anatina*	*Unio tumidus* *Unio pictorum* *Pseudanodonta complanata*	Type B

When one considers the intermediate hosts of records where an illustration of the cercaria is included, it is apparent that cercariae resembling Type B in gross morphology have occurred only in unionid bivalves (Table IV), whereas those resembling Type A have occured in both unionids and dreissenids. Furthermore, in cases where the cercariae resembling Type A is known for certain to develop into adults of *Bucephalus polymorphus* (or at least the modern conception of this species – see below), i.e. Kinkelin *et al.* (1968), Baturo (1977) and Wallet & Lambert (1984), the molluscan host is a dreissenid. In relation to this, it is worth noting that, while unionids belong to the subclass Palaeoheterodonta, dreissenids belong to the subclass Heterodonta. In view of the known host-specifcity of digeneans to their molluscan hosts, it would seem unlikely that cercariae resembling Type A from unionids are conspecific with morphologically similar worms from dreissenids. Since dreissenids do not occur in Finland, this supports the results above which indicate that two species of *Rhipidocotyle* occur in Finnish waters.

It is also apparent, as suggested by Kozicka (1959), that there has in the past 100 years been great confusion in the understanding of the adult bucephalids in European waters, although most authors now agree that two species exist, namely *Bucephalus polymorphus* and *Rhipidocotyle campanula*. Kozicka considered it difficult to determine the distribution of these species because of errors of identification in the literature. Nevertheless, she believed *B. polymorphus* to occur in Germany, the Soviet Union, Czechoslovakia and Poland, and to this list can be added France (Kinkelin *et al.*, 1968; Wallet & Lambert, 1984).

R. campanula occurs in Poland, Norway, the UK, the Soviet Union and Finland. As indicated above, the major definitive host listed in the literature for *B. polymorphus* appears to be zander, although there are numerous records from pike, with smaller numbers in perch and burbot. Similarly, the literature indicates that the major definitive host for *R. campanula* is pike (e.g. Kozicka, 1959), with fewer numbers in perch and even smaller numbers of records in burbot, ruff and zander.

Taxonomy

During the early part of this work it was assumed that we were dealing with the two common bucephalids occurring in European freshwater fishes, in spite of the fact that we had not seen adult specimens resembling *B. polymorphus*. This assumption was based upon the fact that we had two different cercariae which resembled those described by Baturo (1977) for *B. polymorphus* and *R. campanula* (as *R. illense*). During the course of the work, however, clear differences emerged between our Type A cercaria and the morphologically similar cercaria, attributed to *B. polymorphus*, described by Baturo (1977). Although these include both biological and minor morphological differences, they pale into insignificance beside the fact that our cercaria developed into a form resembling *R. campanula*. At that time we were concerned that Baturo might have mixed the two cercariae, and that, therefore, her application to the ICZN (Baturo, 1979) to have *B. polymorphus* based upon an adult neotype was invalid. If our hypothesis was correct, then it appeared that the cercaria of *B. polymorphus* did indeed resemble von Baer's original (1827) description. This led us to question Baturo's application (Gibson & Valtonen, 1989) and refer to the presence of *B. polymorphus* in Finland (Taskinen & Valtonen, 1989). More recent experimental work has shown that this is not the case, since, as indicated above, Type B cercariae also produce adults resembling *R. campanula*.

In consequence, it is likely that: (1) Baturo's application to the ICZN is justified: (2) *B. polymorphus* is not present amongst our Finnish material; (3) our Type B cercaria may be that of *R. campanula* and (4) our Type A cercaria represents a second species of *Rhipidocotyle*. A second paper on this subject will include a detailed morphological and taxonomic study of the Finnish forms of this genus.

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