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Site selection and attachment duration of *Anodonta woodiana* (Bivalvia: Unionacea) glochidia on fish hosts

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(With 2 figures in the text)

The relationship between parasitic glochidia of *Anodonta woodiana* (Unionidae: Anodontinae) and potential fish hosts was investigated in the laboratory. Intensity of parasitism was highest on the exotic fish *Gambusia affinis*, lower on the native species *Puntius semifasciolatus* and *Metzia takakii*, and least on *Rhodeus sinensis*. Glochidia generally attached at fin margins, particularly the pectorals and caudal. In *Gambusia affinis*, the incidence of glochidia on the pectorals was higher than would be expected on the basis of ratios between fin margin lengths. Apparently, the rôle of the pectoral fins in locomotion makes them more liable to glochidial contact, thereby increasing their susceptibility to attachment.

The duration of glochidial attachment was shortened as temperatures increased. Mean values ranged from 14.4 days at 15°C to 6 days at 27°C. At 33°C glochidia rapidly detached and metamorphosis was unsuccessful. Significantly, water temperatures in *A. woodiana* habitats in Hong Kong rarely exceed 30°C.

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Introduction

The glochidium of *Anodonta woodiana* (Lea, 1834) (Syn. *Cristaria discoidea* Lea, 1834; Dudgeon & Morton, 1983) has been described by Dudgeon (1980), and is very similar to that of *Anodonta cygnea* L. from Europe (Wood, 1974a; Giusti *et al.*, 1975). It is anatomically specialized for an ectoparasitic existence on fish hosts. The parasitic phase of unionids is generally considered to be obligate (though Isom & Hudson (1982) have obtained glochidial metamorphosis to juveniles in *in vitro* cultures) and of short duration (Dartnall & Walkey, 1979), but detailed studies of glochidial ecology are scarce. Methods of host detection are described by Wood (1974b), but little is known of the duration of attachment time, the range of fish hosts utilized by individual species, or the extent to which glochidia occupy specific sites on the host's body. While some limited information on these topics are available from Europe and North America (Giusti *et al.*, 1975; Dartnall & Walkey, 1979; Zale & Neves, 1982), such data are lacking for Asia.

Anodonta woodiana is widespread in Asia. Its reproductive biology and population dynamics have been investigated by Dudgeon & Morton (1983). In this paper, the settlement of *A. woodiana* on four potential host species, the topographical distribution of glochidia on the host, and the effect of temperature on attachment time are described.

Materials and methods

The 4 fish species investigated, *Gambusia affinis* (Baird & Girard) (Poeciliidae), *Puntius semifasciolatus* (Günther) (Cyprinidae: Barbinae), *Metzia takakii* (Oshima) (Cyprinidae: Abramidinae) and *Rhodeus sinensis* Günther (Cyprinidae: Rhodeinae), are common in the freshwaters of southern China and are possible hosts for *A. woodiana* glochidia. Their potential as glochidial hosts was assessed by placing a large group ($n=340$) of fish of all species into a 50 l tank containing 8 mature *A. woodiana*: the extent and intensity (no. glochidia/infected fish) of parasitism was recorded shortly after the bivalves released glochidia. In a duplicate trial, the fish were anaesthetized in MS-222 and the position of glochidia on the hosts accurately recorded.

Detailed studies of the topographical pattern of glochidial distribution, and the effect of temperature on attachment duration, were undertaken with *G. affinis* hosts. The former was investigated in a population of *G. affinis* maintained in a 500 l outdoor tank, together with a stock of *A. woodiana* which was supplemented monthly by the addition of specimens collected from Plover Cove Reservoir (Dudgeon & Morton, 1983). Fish (5 in number), were removed every 2 days, anaesthetized, and the position and number of glochidia recorded. Fish were regularly replaced to maintain the population size at about 100 individuals. The procedure was continued over a 12-month period (January—December 1980).

In the investigation of the effect of temperature on duration of glochidial attachment, 100 *G. affinis* were infected with glochidia at room temperature (22°C). The fish were separated into groups, each comprising 5 recognizable individuals (on the basis of size differentials), and the number and position of attached glochidia were recorded. Each group of 5 fish was kept in a separate net cage, 5 such cages being placed in each of 4 incubation tanks maintained at 15, 22, 27 or 33°C, respectively. Temperatures higher than ambient were maintained with combined aquarium heater-thermostat units, while the 15°C incubation tank was placed in a refrigerated water bath.

Each group of fish was transferred daily to a beaker of water, at the appropriate temperature, and examined for the presence of glochidia. Records of the fate of individual glochidia were possible as their numbers and position on each host were recorded at the outset, and the fish in each test group were distinguishable on the basis of size. Observations were terminated when all glochidia had detached.

Results

Glochidial attachment was recorded on all test host species. However, the degree of infection varied between species; all *Gambusia affinis* but only 10% of *Rhodeus sinensis* were afflicted (Table I). The intensity of parasitism also varied between hosts and was highest in *G. affinis* and, again, lowest in *R. sinensis*. The latter species is clearly a poor host; there never being more than one glochidium recorded on each fish.

The number of glochidia/fin on suitable hosts varied markedly (Table II), the caudal and pectoral fins being the most common attachment sites. The pelvic fins were least infected. This pattern was similar for *G. affinis*, *Puntius semifasciolatus* and *Metzia takakii*; records of glochidial settlement on *R. sinensis* were too scanty for further analysis.

A detailed investigation was undertaken of topographic patterns of settlement on *G. affinis*, the most suitable test host for glochidia. The degree of infestation varied throughout the year

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TABLE I
Incidence and intensity of glochidial attachment to fish hosts

	Population size	% Parasitized	Mean no. glochidia/ parasitized fish	Maximum intensity
<i>Gambusia affinis</i>	200	100	10.0	39
<i>Puntius semifasciolatus</i>	60	55	5.6	16
<i>Metzia takakii</i>	30	30	4.8	9
<i>Rhodeus sinensis</i>	50	10	1.0	1

TABLE II
Topographical distribution of glochidia on four host species

Location (fins)	<i>Gambusia</i>	<i>Puntius</i>	<i>Metzia</i>	<i>Rhodeus</i>	Total glochidia
Caudal	319 (33.3%)	27 (35.5%)	14 (34.1%)	0	360 (33.5%)
Pectoral (paired)	450 (47.0%)	16 (21.1%)	14 (34.1%)	0	480 (44.6%)
Dorsal	83 (8.7%)	23 (30.3%)	3 (7.3%)	1	110 (10.2%)
Anal	68 (7.1%)	6 (7.9%)	4 (9.8%)	1	79 (7.3%)
Pelvic (paired)	37 (3.9%)	4 (5.3%)	6 (14.6%)	0	47 (4.4%)
Total no. glochidia	957	76	41	2	1076
No. infected fish	73	12	7	2	

and was greatest in April, May and June (Table III) (Dudgeon & Morton, 1983). However, topographical patterns of glochidial settlement were generally consistent with the greatest proportion (37.17%) of glochidia attaching to the pectoral fins, and a small number (<5%) attaching to the body or operculum. Settlement on the fins was not a random process and most glochidia were found at the margins. Significantly, the modified and thickened pelvic fins (gonopodium) of the male were a less favoured site for attachment than those of the female.

Observed topographical patterns of settlement might simply reflect relative fin margin lengths. In order to test this hypothesis, the marginal lengths of the fins of ten fish were measured and expressed as a ratio, setting the margin length of the pelvic fins of the female as equal to one (males were ignored for the purposes of this analysis) (Table IV). The ratios of the total no. glochidia/fin, again setting the ratio for the pelvic fins as equal to one, were then calculated. If margin lengths were the sole factor determining the number of glochidia attaching to fins, then these two sets of ratios should be approximately equal (i.e. all fins should be similarly attractive and the ratios all equal to one when the former value is divided by the latter). Instead, the ratios of total glochidia/fin were always in excess of the ratios of margin lengths. Dividing the former by the latter, an "attractiveness" factor for each fin was calculated, indicating the degree to which the number of glochidia/fin exceeded that which would be expected on the basis of relative margin lengths (Table IV). The pectoral fins were the most frequent attachment site, followed by the caudal fin. The pelvic fins were

TABLE III
Topographical and seasonal patterns of glochidial attachment to Gambusia affinis

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
Body scales	2	9	14	41	39	30	8	1	3	0	0	0	147 (4.38%)
Opercula	0	8	15	37	35	21	2	1	0	1	0	0	120 (3.58%)
Caudal fin	17	81	98	150	230	187	71	25	10	16	9	8	902 (26.89%)
Anal fin	3	36	58	63	82	88	8	7	5	6	1	3	360 (10.73%)
Pelvic fins	9	13	26	40	49	33	4	4	1	0	1	0	180 (5.37%)
Dorsal fin	6	31	68	83	104	63	23	10	1	6	2	2	399 (11.89%)
Pectoral fins	17	101	156	336	304	219	54	18	8	16	7	11	1247 (37.17%)
Total	54	279	435	750	843	641	170	66	28	45	20	24	3355

TABLE IV
Gambusia affinis. Relative "attractiveness" of different fins to glochidial attachment, as indicated by ratios of margin lengths

Fin	Mean margin length (mm)	Ratio of margin lengths	Total no. glochidia/fin	Ratio of glochidia/fin	"Attractiveness" factor
Caudal	15.88	1.56	902	5.64	3.62
Pectoral	20.62	2.03	1247	7.79	3.84
Anal	9.64	0.95	360	2.25	2.37
Dorsal	9.38	0.92	399	2.49	2.71
Pelvic	10.16	1	180	1	1

least favoured. Note that the margin length, and number of attached glochidia on both fins in the pectoral and pelvic pairs, were taken into account in all calculations.

Glochidial attachment time was strongly influenced by temperature (Table V). The mean and maximum durations were longest at 15°C (14.42 and 25 days, respectively) and shortest at 33°C (3.49 and 7 days, respectively). Detachment of glochidia from the host occurred in two main phases (Fig. 1). There was an initial period around day 3 when many glochidia detached regardless of ambient temperature. These represented glochidia which failed to encyst and were sloughed off by the host. A second major period of detachment occurred several days later, usually about two days before the last glochidium fell off. This latter group represents metamorphosed larvae entering the free-living juvenile stage.

A strong negative correlation between mean duration of glochidial attachment and water temperature was noted ($r = -0.97$, significant where $P < 0.05$ with 2 d.f.) (Fig. 2). Significant differences between the mean glochidial attachment time in all between-temperature comparisons were also recorded (Table VI).

Discussion

Anodonta woodiana glochidia show host discrimination and preferentially attach to certain fins. Host preferences are not easily explained in the light of available data on

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TABLE V
Temperature and the duration of glochidial settlement

Temperature (°C)	Mean duration (days) (±s.d.)	Maximum duration (days)
15	14.42 ± 8.52	25
22	7.54 ± 2.41	12
27	5.98 ± 2.52	11
33	3.49 ± 3.49	7

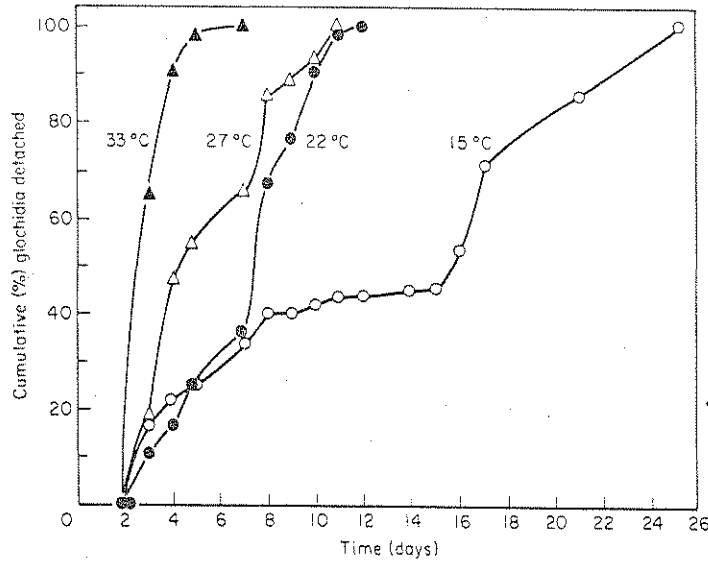


FIG. 1. Increases in the cumulative percentage of detached glochidia over time at different water temperatures.

glochidial ecology. However, it may be presumed that fins which are free of mucus, with a delicate epidermis between the rays, would enable the marginal spines of the glochidium (Wood, 1974a, b; Dudgeon, 1980) to gain a hold. Significantly, the delicate fins of *Gambusia affinis* are more attractive than those of other potential hosts investigated. It should be noted that *G. affinis* is an exotic species in southern China, although this would not prevent it from acting as a host for *A. woodiana* where it and endemic species coexist (cf. Tompa, 1979).

The relative unsuitability of *Rhodeus sinensis* as a glochidial host is worthy of mention. In Europe, *R. sericeus* (the Bitterling) lays its eggs within the mantle cavity of *Anodonta* spp. (Nikolsky, 1963). The cloaca of the female develops into a tubular ovipositor for this purpose, and such a structure is also present in *R. sinensis*, thereby implying a similar breeding habit. The bivalve recipient of *R. sinensis* eggs is unknown, but may be *A. woodiana* in view of that species' wide distribution (Dudgeon & Morton, 1983). Considering the dependence of *Rhodeus* on unionids for successful reproduction, it is surprising that *R. sinensis* does not readily serve as a glochidial host in the laboratory.

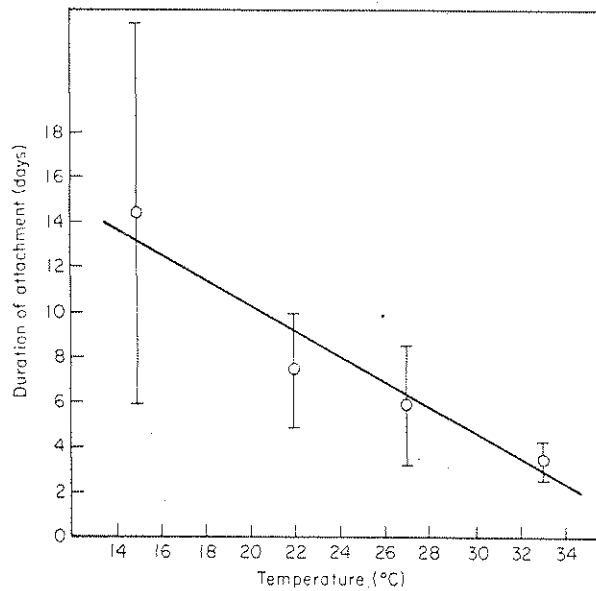


FIG. 2. The relationship between duration of glochidial attachment ($\bar{x} \pm s.d.$) and water temperature, $y = 22.90 - 0.627x$.

TABLE VI
Student's *t* values for comparisons of duration of glochidial attachment to fish hosts maintained at different temperatures

	22°C	27°C	33°C
15°C	8.31**	7.00**	9.22**
22°C		3.10*	11.87**
27°C			6.82**

* $P < 0.01$.

** $P < 0.001$.

The topographical distribution of glochidia on hosts was not random. Most glochidia were found on the fins, the pectorals being the commonest attachment site in *G. affinis*. The caudal fin was more commonly infected in *Puntius semifasciolatus* and *Metzia takakii*, but the pectorals were also heavily parasitized. Dartnall & Walkey (1979) and Tompa (1979) also record extensive glochidial attachment to the fins of fish hosts. The paucity of glochidia on the host's body probably reflects attachment difficulties. Glochidia may also attach to the gill lamellae of certain fishes (Giusti *et al.*, 1975; Dartnall & Walkey, 1979; Zale & Neves, 1982). This phenomenon was not observed in the present study and probably reflects the

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small size of the host fishes (Zale & Neves, 1982), and a lack of host predation on glochidia. Fish that feed upon glochidia are prone to parasitism of the gills and buccal cavity (Dartnall & Walkey, 1979; Zale & Neves, 1982).

Glochidia cannot swim and have no powers of independent movement (Wood, 1974a, b). Consequently, their settlement on a particular part of the host's body is a function of the ease of attachment at that site, and the volume and speed of water flowing over it. As mentioned above, glochidial attachment can be best effected at fin margins and on gill lamellae where the glochidial spines are able to grip the host. Paling (1968) observed that settlement of glochidia on the gills of Brown trout was related to the regions of maximum water flow. Differences in the distribution of glochidia on fins can probably also be attributed to the same factors. *Gambusia affinis* swims using mainly the action of the pectorals, these fins also being employed in braking, steering and possibly maintaining balance. Since they are actively moving, even when the fish is stationary, it is clear that they will be relatively more likely to come into contact with glochidia which are passively transported in water. As a consequence, they are a common site of glochidial attachment. The relative abundance of glochidia on other fins probably reflects differential patterns of water flow over the body.

The negative relationship between duration of glochidial attachment and temperature is similar to that recorded in previous studies (Howard & Anson, 1922; Zale & Neves, 1982). The mean and maximum periods of attachment were both shorter at higher temperatures, and this relationship could account for the relative abundance of glochidia on host fish during late spring and early summer in Hong Kong (Table III and Dudgeon & Morton, 1983). In the latter part of the summer, water temperatures can exceed 30°C and, significantly, glochidial settlement is then reduced (Dudgeon & Morton, 1983). However, such temperatures are not long sustained. In the laboratory, 64% of the glochidia detached after three days at 33°C. This percentage is 2.5 times greater than detachment over the same period at 27°C, and can almost certainly be attributed to glochidia being sloughed off by the host, and can be identified at all temperatures. It is possible that, at these high temperatures, glochidia fail to attach properly, or die, and thus rapidly detach. Breeding of *A. woodiana* in Plover Cove takes place over a single extended phase in early summer, when moderate temperatures between a winter low and a summer high apparently optimize the period of larval attachment.

The duration of *A. woodiana* glochidial attachment is broadly comparable with figures reported in the literature. Tompa (1979) records that *Lasmigona compressa* larvae remained attached to *Poecilia* (= *Lebistes*) *reticulata* hosts for 10–12 days at 20°C. Zale & Neves (1982) investigated glochidial attachment of *Villosa nebulosa* and found that detachment occurred after 10–21 days at 24.7°C; detachment at lower temperatures was delayed. A longer parasitic period of 40–50 days at 15°C is recorded by Telda & Fernando (1969) for *Lampsilis radiata siliquoidea*. Isom & Hudson (1982) induced *in vitro* glochidial metamorphosis of six unionid species, but only record that *Fusconaia ebena* cultures contained transformed juveniles after 18 days. This technique holds promise for meaningful comparisons of unionacean metamorphosis times and the modifying effects of environmental variables.

In conclusion, it is clear that the glochidia of *A. woodiana* exhibit host and site-specific attachment preferences which may be related to ease of attachment and the pattern of water flow over the host's body. Attachment time is negatively related to temperature; glochidial development is, however, impaired at high temperatures. This observation is reflected by the timing of reproduction in a natural population of *A. woodiana*.

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