



Effects of light and presence of fish on lure display and larval release behaviours in two species of freshwater mussels

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We investigated how two sympatric species of freshwater mussels transmit their parasitic larvae to fish hosts. We found that *Villosa nebulosa* and *V. vibex* both display large mantle lures to attract potential host fish, but *V. nebulosa* displayed only at night and *V. vibex* displayed mostly by day. Display periods were similar in the laboratory and in the field. In two laboratory experiments, we found that the frequency of lure display in both mussel species was unrelated to the presence of fish or to the species of fish present. However, both species released more larvae in the presence of a suitable host fish (*Micropterus* spp.) and a nonhost species (*Cyprinella camura*) than in the absence of fish. In all treatments, females released low numbers of larvae on a daily basis throughout the experiment. We also observed several, irregularly occurring major release events in which numbers of larvae released were from one to three orders of magnitude larger than minor, daily releases. In *V. nebulosa*, major releases occurred with suitable and unsuitable host species; in *V. vibex* major releases occurred mostly with suitable host species. In an additional laboratory experiment, we found that *V. vibex* released large numbers of larvae only when the host fish was able to make physical contact with the mussel. Few larvae were released when no fish were present or when host fish were present but physical access to the mussel was restricted. These results show that, in mussel species that display lures, physical interaction with a fish is necessary to stimulate large releases of larvae and suggest that interactions with a suitable host species stimulate larger and more frequent releases than with nonhosts.

Freshwater mussels (family Unionidae) are free-living, filter-feeding animals except for a period of several weeks when mussel larvae (glochidia) are obligate ectoparasites on the gills of fish. Recent work has revealed a wide array of anatomical modifications and behaviours in reproductive females that facilitate transmittal of parasitic larvae to host fish (Kat 1984; Haag et al. 1995; Haag & Warren 1999). There is wide variation in host use and host specificity among mussel species and strategies for glochidial transmittal often appear to target specific host species. Mussel species that are generalists in host use release glochidia in mucous webs that may indiscriminately entangle a wide variety of fish species (Wood 1974; Haag & Warren 1997). In contrast, species that specialize in using only minnows (Cyprinidae) or darters (Percidae) as hosts release glochidia in small packets (conglutinates) that mimic food items of these fish (Bruenderman & Neves 1993; Hove & Neves 1994; Hartfield & Hartfield 1996). In mussel species that use large, predaceous fish such as bass and sunfish (Centrarchidae) as hosts, gravid

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females display modified mantle margins that strongly resemble small fish, caterpillars, or large aquatic insect larvae (Haag et al. 1999), all of which are major food items of bass. These structures act as lures that elicit attacks from potential host fish upon the gravid female, facilitating infection with glochidia (Haag & Warren 1999).

Despite the short duration of the parasitic stage, host relationships and modes of larval transmission may have profound effects on the distribution and abundance of adults (Watters 1992; Vaughn 1997; Haag & Warren 1998) and may have been important in speciation in this diverse group (Graf 1997). It has been suggested that display of a lure and attraction of a host to the gravid mussel is a strategy that allows mussels to infect hosts even when hosts are present at low densities (Haag & Warren 1998). Under this hypothesis we would expect gravid females to display lures regardless of the presence of fish; however, nothing is known about this aspect of display behaviour. Similarly, although these lures do elicit attacks from host fish, the degree to which these strategies reduce the incidence of glochidial transmission to nonhosts is unknown.

We studied the glochidial transmission strategy of two congeneric species, *Villosa nebulosa* and *V. vibex*, that display modified mantle margins which are thought to serve as lures for host fish. In this study, we asked four questions. What are the daily rhythms of lure display and do they differ among closely related, sympatric species? Is lure display influenced by the presence or absence of a fish or by the species of fish present? Is the release of glochidia influenced by the presence or absence of a fish or by the species of fish present? What is the stimulus for glochidial release and transmittance to the host?

STUDY ANIMALS

Villosa nebulosa and *V. vibex* are both locally common, often co-occurring members of clear, upland stream mussel communities in the Mobile Basin of Alabama, Georgia, Mississippi and Tennessee. Females of both species have highly modified mantle lures. The lures consist of approximately 15 pairs of unbranched, tentacle-like papillae about 15 mm in length (Haag et al. 1999). In *V. vibex*, the lure is inky black to rusty orange with numerous fine black spots; in *V. nebulosa*, the lure is pale to pure white. During female displays in both species, papillae are pulsed rapidly in bursts lasting ca. 2–4 s (Haag & Warren 1997; Haag et al. 1999). Host fish use in both *V. nebulosa* and *V. vibex* is restricted to bass and sunfish (family Centrarchidae) (Haag & Warren 1997; Haag et al. 1999).

FIELD OBSERVATIONS OF LURE DISPLAY

We made field observations of day-night frequency of lure displays by gravid female *V. nebulosa* and *V. vibex*. Previous casual laboratory and field observations by us and others (P. Hartfield, U.S. Fish and Wildlife Service, Jackson, Mississippi, personal communication) suggested that peak display times differed between these two species. Based on these observations, we tested the prediction that *V. nebulosa* displays mostly at night while *V. vibex* displays mostly by day.

Methods

Observations of *V. vibex* were made in Shoal Creek (Coosa River system, Mobile Basin, Cleburne County, Alabama, U.S.A., 33°43'N, 85°36'W) on 3 March 1998; observations of *V. nebulosa* were made in Flannagin Creek (Black Warrior River system, Mobile Basin, Lawrence County, Alabama, 34°20'N, 87°23'W) on 25 March 1998. Although these species co-occur in both streams, we were unable to locate sufficient numbers of individuals for comparisons of both species at the same site. Both creeks are clear, third-order, gravel- and sand-bottomed streams. At both sites, we first located as many females as possible in the daytime, made observations on the displays of each female, confirmed gravid status of each individual, then marked the location of each individual with a numbered red flag placed in the substrate

approximately 50 mm away from the mussel. Gravid status was confirmed by gently prying apart the valves and peering inside the shell; in gravid females, the posterior portion of the outer gills is greatly distended by the presence of glochidia, making these individuals easily distinguishable from nongravid females and males. Only gravid females were observed displaying mantle lures. The flags facilitated relocation at night and allowed direct comparisons of daytime versus nighttime behaviours of individual mussels. We made daytime observations in late afternoon (Flannagin Creek, 1500–1700 hours, water temperature 15°C; Shoal Creek, 1400–1700 hours, water temperature 11°C) and nighttime observations near midnight (Flannagin Creek, 1030–1130 hours, water temperature 14°C; Shoal Creek, 2330–0130 hours, water temperature 9°C). We made observations using a glass-bottomed bucket to reduce surface glare; we also used a submersible dive light at night. After switching on the dive light, we noticed no immediate response from the mussels; after about 15–30 s, the animals began to slowly retract their mantles. We saw a similar response to light in animals in the laboratory as well (see laboratory experiments below). Because mussel response to the light at night is slow, and because our observations were made within 15 s of switching on the light, we feel confident that our observations represent display behaviours unaffected by the dive light. We noted the degree of lure display for each individual encountered and scored displays as one of three states: 0=no display; 1=partial display; 2=full display. 'No display' was defined as a mussel that was filtering normally with the shell only slightly agape and the siphons being the only portion of the mantle readily visible. 'Partial display' was defined as a mussel that was siphoning normally with the shell only slightly agape but with semi-extended papillae readily visible beyond the shell margin. 'Full display' was defined as a mussel with the shell widely agape, the modified mantle lure fully extended, and the gravid gills visible through the shell aperture. We tested for the effects of time of day on field display behaviour separately for each species using two-tailed Wilcoxon signed-ranks tests to compare display scores for day versus night.

Results

Display behaviour of *V. nebulosa* and *V. vibex* in the field differed between the two species and differed among observation times for both species. *Villosa nebulosa* displayed only at night; the difference in display scores was marginally significant (daytime: $\bar{X} \pm SE = 0 \pm 0$; nighttime: $\bar{X} \pm SE = 0.85 \pm 0.34$; Wilcoxon signed-ranks test: $T = 0.00$, $N = 7$, $P = 0.06$), and a higher percentage of individuals were in full display at night (night=28%, day=0%). *Villosa vibex* displayed during both day and night, but the display score was significantly higher in the daytime (daytime: $\bar{X} \pm SE = 1.22 \pm 0.28$; nighttime: $\bar{X} \pm SE = 0.33 \pm 0.24$; Wilcoxon signed-ranks test: $T = 21.0$, $N = 9$, $P = 0.03$), and a higher proportion of individuals were in full display during the day (day=44%, night=11%).

EXPERIMENT 1: LURE DISPLAY AND GLOCHIDIAL RELEASE

We conducted two similar experiments, one with *V. nebulosa* and another with *V. vibex*, to determine the effects of light and presence of fish on lure displays and release of glochidia. With these experiments, we tested a series of predictions. First, we predicted that peak display times for these two species would be similar to those observed in the field (i.e. that *V. nebulosa* would display mostly at night and *V. vibex* would display mostly by day). Second, we predicted that, during peak display periods, gravid mussels would display lures regardless of the presence or absence of fish, and regardless of whether fish were suitable or unsuitable host species. Third, we predicted that releases of glochidia would occur most frequently during peak display periods and in the presence of a suitable host species, but rarely in the presence of an unsuitable host species or in the absence of fish.

Methods

After making field observations of lure displays, we brought females of *V. nebulosa* and *V. vibex* to the laboratory. Previously, we had collected additional females of each species from Flannagin Creek on 25 February 1998 (water temperature 13°C). In the field, we wrapped all collected individuals in moist cloth, transported them to the laboratory in an ice chest, and housed them in aerated aquaria at 8°C to prevent release of glochidia before experiments were initiated. Mean length of *V. nebulosa* used in experiments averaged ca. 42 mm; mean length of *V. vibex* averaged ca. 53 mm.

We used two species of bass and one species of minnow in the experiments. Spotted bass, *Micropterus punctulatus*, were collected by electrofishing from Taylor Creek, Tallahatchie River system, Lafayette County, Mississippi (34°15'N, 89°35'W) and Clear Creek, Black Warrior River system, Winston County, Alabama (34°06'N, 87°24'W). Largemouth bass, *Micropterus salmoides*, were obtained from hatchery stock. Bluntnose shiners, *Cyprinella camura*, were collected from Taylor Creek. Before the experiments, we acclimated fish to laboratory conditions until they fed readily on earthworms (bass) or bloodworms (shiners). Bass were held in 15-litre aquaria at a density of approximately 0.03 fish/litre; shiners were held in 76-litre aquaria at a density of approximately 0.25 fish/litre. Mean total length of bass used in experiments averaged ca. 120 mm; mean total length of shiners averaged ca. 80 mm.

For *V. nebulosa*, we randomly assigned 12 fully gravid females to three fish treatments (four females per treatment): (1) no fish present; (2) a suitable host species present (*M. punctulatus*); and (3) an unsuitable host fish species present (*C. camura*). For *V. vibex*, we assigned three females to the unsuitable host treatment and four each to the no-fish and suitable host treatments. All treatment combinations were contained in identical 12.5-litre aerated aquaria. Three sides of each chamber were painted black to eliminate stress to fish resulting from visual interactions between fish in adjacent chambers,

and the chamber floor was covered with fine-mesh, black plastic to reduce fish disorientation caused by reflections in the glass bottom. Both experiments were run for seven 24-h cycles under fluorescent light on an approximate 12:12 h light:dark cycle (lights on: 0600-1800 hours) at room temperature (21–25°C). Before initiating observations, animals were allowed to acclimate to experimental conditions for 24 h. Fish were not fed during the experiment.

We observed each mussel four times in a 24-h cycle, at approximately 1200 hours (light), 1800 hours (light), 2400 hours (dark) and 0600 hours (dark). We made observations at 2400 hours and 0600 hours using a small flashlight; we found that brief, oblique lighting at night did not cause noticeable changes in display behaviour. Displays for each individual were scored as described for the field observations. On each day, after making observations at 0600 hours and 1800 hours, we siphoned the bottom of each chamber to collect glochidia released during the previous two sample periods. Glochidia collected at 0600 hours were released during the preceding 12-h dark period, and glochidia collected at 1800 hours were released during the preceding 12-h light period. Siphonates were washed over a 100- μ m screen and preserved in separate jars in 95% ethanol. Glochidia were later counted under a stereomicroscope.

We tested for the effects of observation time and fish presence on display behaviour for each species separately using two-factor analysis of variance (ANOVA) with orthogonal contrasts of mean display scores. Our response variable, mean individual display score, was calculated as the sum of scores for each individual for each observation time divided by the number of days of the trial. For contrasts of observation times, we hypothesized a priori that display behaviour would be most affected by light (mid-day and late afternoon) versus dark (mid-night and predawn) conditions. For contrasts of fish treatments, we tested the a priori hypothesis that display behaviour would not be affected by fish presence (Table 1). To graphically present these data, we calculated the percentage of full displays for each fish and observation time combination.

We tested for the effects of light conditions and fish presence on glochidial release for each species separately using two-factor analysis of variance with orthogonal contrasts of mean number of glochidia released per 12-h sample period over 7 days. We used a log_e transformation for the response variable, individual mean number of glochidia released, to achieve equality of variances among treatments (F_{\max} test for homogeneity of variances, $P < 0.05$, Sokal & Rohlf 1995). For contrasts of fish treatments, we hypothesized a priori that presence of a suitable host fish (bass) would most affect release of glochidia, and that mussels exposed to either a nonhost species (minnows) or no fish would release few glochidia (Table 2).

We observed a low level of glochidial release in all treatment combinations on a daily basis, but this was punctuated on an irregular basis by major release events that were one to three orders of magnitude greater than minor, daily releases. Because of the apparent bimodality

Table 1. Results of two-factor analysis of variance for effects of presence of fish (host, nonhost and no-fish) and observation time (day: mid-day, afternoon; night: mid-night, predawn) on display behaviour (mean display score) of *Villosa nebulosa* and *V. vibex*

Source of variation	<i>V. nebulosa</i>			<i>V. vibex</i>		
	df	F	P	df	F	P
Fish treatment	2	1.90	0.1649	2	0.50	0.6127
Observation time	3	54.10	0.0001	3	41.35	0.0001
Interaction	6	1.16	0.3490	6	1.32	0.2752
Orthogonal contrasts						
Day versus night	†	157.46	0.0001	†	119.16	0.0001
Predawn versus mid-night	†	4.80	0.0350	†	1.18	0.2858
Mid-day versus afternoon	†	0.05	0.8278	†	3.72	0.0626
Bass/minnow versus no-fish	†	2.03	0.1630	†	0.80	0.3764
Bass versus minnow	†	1.76	0.1925	†	0.13	0.7236
Error	36			32		
Total	47			43		

Table 2. Results of two-factor analysis of variance for effects of presence of fish (host, nonhost and no-fish) and observation time (day: mid-day, afternoon; night: mid-night, predawn) on release of glochidia (mean daily number of glochidia released, log_e, transformed) in *Villosa nebulosa* and *V. vibex*

Source of variation	<i>V. nebulosa</i>			<i>V. vibex</i>		
	df	F	P	df	F	P
Fish treatment	2	10.24	0.0011	2	10.69	0.0011
Observation time	†	7.17	0.0154	†	1.01	0.3295
Interaction	2	0.46	0.6400	2	0.29	0.7514
Orthogonal contrasts						
Bass versus minnow/no-fish	†	6.16	0.0231	†	14.27	0.0016
Minnow versus no-fish	†	14.32	0.0014	†	5.42	0.0334
Error	18			16		
Total	23			21		

of release data, we were interested in identifying and characterizing major and minor release events in order to consider their possible biological significance. We considered releases of 500 or more glochidia to be major releases and those less than 500 to be minor releases. We established this criterion based on two considerations. First, based on our experience in handling and observing gravid mussels, we felt that 500 glochidia represented a conservative, minimum estimate of an unusually large release. Second, we constructed half-normal plots of glochidial counts for each 12-h sample period for each species. Release events of 500 glochidia approximated the magnitude at which outlier points deviated from a straight line expected from a normal distribution (Sokal & Rohlf 1995; Milliken & Johnson 1992). In both experiments, we observed moderate numbers of glochidia (215-859) in sample periods immediately after very large releases (>3000). We felt it was likely that these moderate numbers represented glochidia missed in the previous sample. Two such releases were greater than 500 (551 and 859, both in the *V. vibex*-bass treatment), and these two events were not considered to be major releases. To graphically present these data, we constructed stacked histograms using retransformed 12-h mean release data (Sokal & Rohlf 1995) in which we partitioned total 12-h releases into major and minor release events.

Results

For *V. nebulosa*, display behaviour was affected by time of day but not by fish treatment (Table 1). No differences in mean display score were found among fish treatments, and the interaction was not significant. Contrasts indicated mean display scores were significantly higher during the night (mid-night and predawn) than during the day (mid-day and afternoon), and at night, scores were significantly higher at predawn than mid-night. Display scores between mid-day and late afternoon observation times were not significantly different. Full displays in the laboratory occurred almost exclusively at night with a peak at predawn (Fig. 1).

Similarly, for *V. vibex*, display behaviour in the laboratory was affected by time of day but not by fish treatment (Table 1). No differences in mean display score were found among fish treatments, and the interaction was not significant. Contrasts indicated mean display scores were significantly higher during the day (mid-day and late afternoon) than at night (mid-night and predawn). No differences were detected between mid-night and predawn or mid-day and late afternoon displays. Full displays occurred both during the day and at night, but the highest percentage of individuals displayed during the day (mid-day and late afternoon)

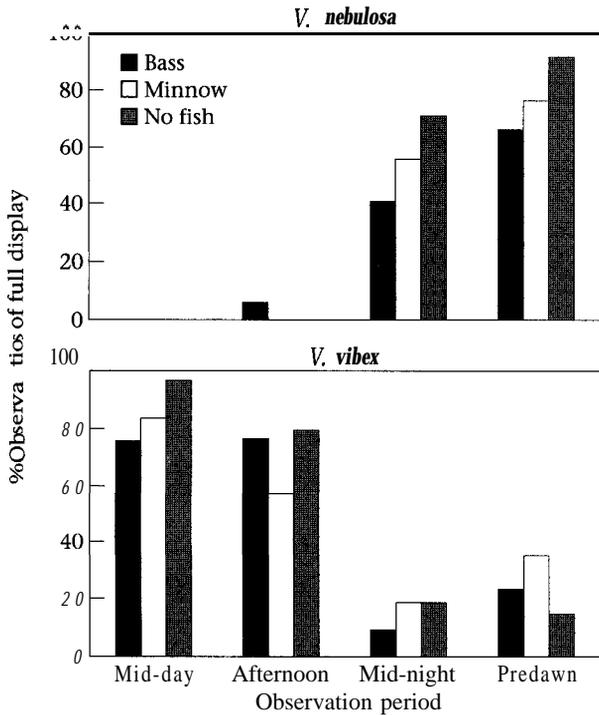


Figure 1. Occurrence of full mantle displays by female *Villosa nebulosa* and *V. vibex* in the laboratory in response to presence of fish and observation time.

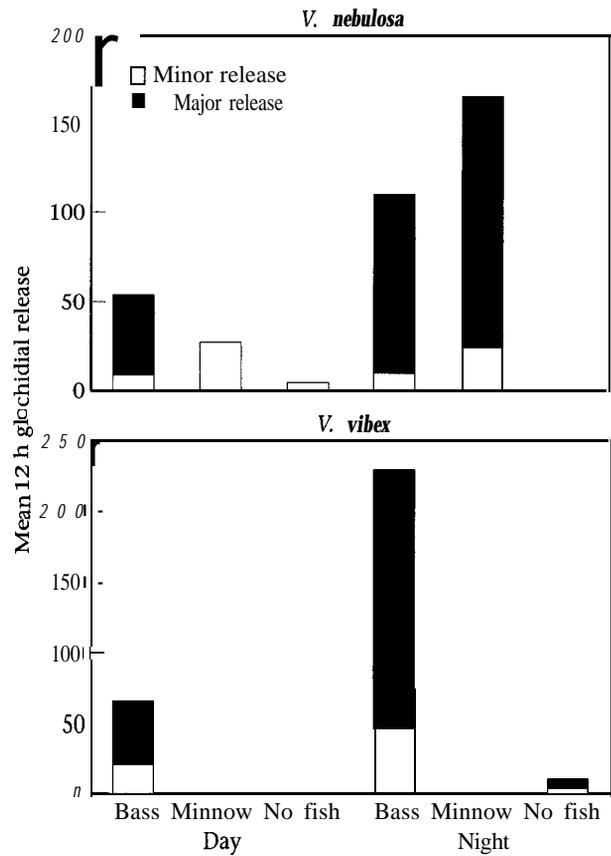


Figure 2. Mean number of glochidia released in major and minor release events per 12-h sample period by female *Villosa nebulosa* and *V. vibex* in the laboratory in response to presence of fish and observation time. Data are retransformed into linear scale from log₁₀ transformed means.

with marked decreases at night (mid-night and predawn; Fig. 1).

Time of day and fish treatment both had a significant effect on release of glochidia for *V. nebulosa* (Table 2, Fig. 2). The interaction was not significant. Releases were significantly higher at night than in the day. Contrasts indicated that the mean 12-h release of glochidia was significantly higher with bass than with minnows and with no fish present, and that release with minnows was significantly higher than when no fish were present.

Fish treatment also had a significant effect on release of glochidia in *V. vibex* (Table 2, Fig. 2). There was no significant effect of time of day on release of glochidia, and the interaction was not significant. Contrasts indicated release of glochidia was significantly higher with bass than with minnows and when no fish were present, and that release with minnows was significantly higher than when no fish were present.

In both species, major releases constituted less than 5% of all 12-h release events, but accounted for more than 75% of total glochidia released during the course of the experiment. Major releases occurred predominantly in the presence of fish in both mussel species (Fig. 2). In *V. nebulosa*, three major releases occurred in the presence of bass (mean release=3391 glochidia, range 1754–5200), four major releases occurred in the presence of minnows (mean release=1370 glochidia, range 749–2484), and no major releases occurred in no-fish treatments. In *V. vibex*, five major releases occurred in the presence of bass (mean release=6262 glochidia, range 1680–19720), no major releases occurred in the presence of minnows, and

one major release occurred in no-fish treatments (529 glochidia). In both species, major releases occurred irregularly throughout the duration of the experiment from day 2 to 7.

EXPERIMENT 2: STIMULUS OF GLOCHIDIAL RELEASE

Although the display of mantle lures may elicit attacks from fish, resulting in release of glochidia (Haag & Warren 1999), the factors which may stimulate release of glochidia are poorly known. It has been suggested that physical contact with a fish is not necessary to stimulate release of glochidia; rather, displaying mussels may release glochidia in response to chemical, hydrological, or light cues that indicate the proximity of a fish (Kraemer 1970; Oesch 1984). We conducted an experiment using *V. vibex* to test the hypothesis that release of glochidia by the female mussel is stimulated by physical contact with a host fish. We predicted that release of glochidia would occur only when fish were able to make physical contact with displaying mussels and not when physical access was restricted or when no fish were present.

Methods

We collected mussels and fish as described in experiment 1. We randomly assigned nine fully gravid *V. vibex* to one of three treatments (three females per treatment): (1) no fish present (no-fish); (2) suitable host fish (*M. salmoides*) present, but physical access to mussel restricted (host-no access), and (3) suitable host fish present, physical access to mussel not restricted (host-access). We restricted access of the potential host fish to the mussel by placing the mussel in a clear Plexiglas box (145 × 85 × 85 × 85 mm), into which we drilled 54 5-mm diameter holes. With this design, the boxes allowed a free exchange of water but prevented the fish from coming into physical contact with the gravid mussel. The experiment was run for seven 24-h cycles under the same laboratory conditions as experiment 1. Before initiating observations, we allowed the animals to acclimate to experimental conditions for 24 h. Fish were not fed during the experiment.

We observed each mussel twice in each 24-h period, at approximately 0600 and 1800 hours. In this experiment, we scored the display of each female as described for experiment 1, to assess whether or not females in all treatments had similar frequencies of lure display. There was no significant difference in the mean display score of females among the three treatments ($X \pm SE = 1.19 \pm 0.28$, 1.14 ± 0.32 , 0.97 ± 0.12 ; ANOVA: $F_{2,6} = 0.20$, $P = 0.82$). On each day, at 1800 hours, we siphoned the bottom of each chamber to collect glochidia released during the previous 24-h period. Siphonates were processed as described in experiment 1.

We tested for the effects of fish presence and physical access to gravid mussels on glochidial release using one-factor analysis of variance with orthogonal contrasts of mean daily number of glochidia released over the 7-day experiment. Because variances of the response variable were equal (F_{\max} test for homogeneity of variances, $P > 0.05$, Sokal & Rohlf 1995), we did not transform these data as in the previous experiment. For contrasts of fish treatments, we hypothesized that physical access of the fish to the mussel would most affect glochidial release and that mussels exposed to fish while in cages or to no fish would release few glochidia. We tested for differences in the frequency of major releases among treatments using an $R \times C$ G test (Sokal & Rohlf 1995).

Results

Physical access of a fish to a gravid mussel had a significant effect on release of glochidia in *V. vibex* (ANOVA: $F_{2,6} = 8.81$, $P < 0.05$; Fig. 3). Contrasts indicated mean daily release of glochidia was significantly higher in the host-access treatment than in host-no access and no-fish treatments (ANOVA: $F_{1,6} = 16.87$, $P < 0.01$). No differences in glochidial release were detected between host-no access and no-fish treatments (ANOVA: $F_{1,6} = 0.76$, $P = 0.42$). Major releases occurred in all treatments but were unequally distributed among treatments (G test: $G_2 = 12.41$, $P < 0.001$; Fig. 3). Ten major releases occurred in the host-access treatment (mean = 4285,

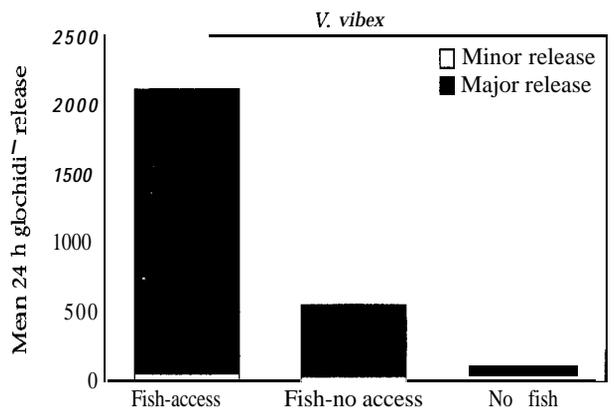


Figure 3. Mean number of glochidia released in major and minor release events per 24-h sample period by female *Villosa vibex* in the laboratory in response to presence of fish and physical access of the fish to the displaying mussel.

range 749–14 800), three major releases occurred in the host-no access treatment (mean = 3320, range 1220–4800), and one major release occurred in the no-fish treatment (1000 glochidia). In the host-access treatment, major releases occurred throughout the duration of the experiment from day 1 to 7. In host-no access and no-fish treatments, major releases occurred only on the last 2 days of the experiment.

GENERAL DISCUSSION

We confirmed the prediction that gravid female mussels display lures regardless of whether or not fish are present. Sustained lure display may increase the likelihood of attracting a host fish and may be particularly important for glochidial transmission at low host-fish densities. Even though mussels in our experiments displayed consistently with or without fish, there are probably effects of fish on display behaviour that we did not evaluate. We often observed increases in pulsation rate of the mantle lure when a fish (bass or minnow) passed near a displaying mussel. Hence, mussels may be able to sense the presence of a fish and accelerate lure pulsations in an attempt to elicit an attack.

We demonstrated consistent differences in rhythms of lure display between *V. nebulosa* and *V. vibex*. Prior to this study, nothing was known about rhythms or interspecific differences of display behaviour in any freshwater mussel species. Our experimental design could not discern whether these patterns reflect endogenous rhythms or responses to light, but diel rhythms of lure display clearly differed between the species. Within a freshwater mussel community, the fish-host resource is often partitioned so that different mussel species use different fish-host species (Haag & Warren 1997). In contrast, *V. nebulosa* and *V. vibex* share a common host resource, both using primarily black bass (*Micropterus* spp.). We suggest that segregation of peak display times and differences in mantle lure coloration may reduce competition between these species for their shared host resource. This assertion is supported by three ecological observations. First,

densities of black bass are often low (Schlosser 1987; Matthews 1998) in headwater streams inhabited by these mussels. Second, fish infected with glochidia from a single mussel acquire at least temporary immunity to parasitization by glochidia from other individuals (O'Connell & Neves 1999). Both low host density and immunity to infection may limit host availability. Finally, species of *Micropterus* show interspecific and ontogenetic differences in diurnal activity and feeding behaviours (Hubbs & Bailey 1938; Becker 1983). The lure of *V. nebulosa* is white, which may render it more visible during nocturnal displays, in contrast to the black lure of *V. vibex*, which displays mostly during the day. Thus, even though these species share a common taxonomic host resource, partitioning may occur through different rhythms of lure display that target distinct ecological subsets of the host resource.

We demonstrated that interaction of gravid female mussels with fish stimulates large releases of glochidia. In other studies, fish attacks on mantle lures and subsequent glochidial transmission were documented (Haag & Warren 1999). Together, this is strong evidence that glochidial transmission is predominantly an active process in lure-displaying mussels. Occurrence of glochidia in stream drift and lake sediments and generally low intensities of infestation on host fish led previous workers to assume transmission primarily occurs passively by haphazard encounters of fish and glochidia (Trdan 1981; Neves & Widlak 1988; Jansen 1991). The importance of fish presence as a stimulus and the magnitude of the effect of fish on glochidial release were previously unknown. We found daily glochidial release rates were 20-80 times higher in the presence of fish. Importantly, female response to fish was not a simple, regular increase in glochidial release over the duration of experiments. Rather, fish presence produced discrete major releases of irregular occurrence. Of 27 major release events observed in our experiments, only two occurred in the absence of fish and both of these releases were among the smallest observed. Our observation of frequent minor glochidial releases regardless of fish presence suggests that host infection may also occur by passive transmission to fish. The continual release of low numbers of glochidia could be a secondary strategy for glochidial transmission or a simple physiological response representing 'leakage' of glochidia during respiration in fully gravid gills. Nevertheless, mantle lures, display behaviours and response to fish attacks on lures all signify a strategy strongly oriented towards active transmission of propagules.

The degree to which this host attraction strategy reduces glochidial transmission to nonsuitable hosts is unclear from our laboratory study. We found that encounters with nonsuitable hosts may stimulate major releases of glochidia. However, our a priori hypotheses were not designed to evaluate differences in releases between suitable and nonsuitable host treatments. Furthermore, small sample sizes prevented us from conducting an a posteriori statistical analysis that could convincingly discount the possibility that release patterns differ with different fish species.

In nature, ecological attributes of host attraction and glochidial release may effectively reduce transmittance of glochidia to nonsuitable host species. We postulate that the physical mechanism of glochidia release is not species specific, and individual mussels are not able to identify an attacking fish. However, fish feeding mechanisms and predator-prey relationships may result in host specificity in two ways. First, the force of the attack may determine the number of glochidia released, therefore, attacks by smaller fish (e.g. minnows) will result in the release of fewer glochidia than attacks by larger fish (e.g. bass). Although we found no significant differences in mean daily rates of glochidial release between bass and minnows, major releases were up to an order of magnitude larger with bass than with minnows. Second, encounters between mussel lures and nonhost species may be rare in natural situations. *Cyprinella camura* and many other minnows feed on small invertebrates in mid-water to surface areas and may not be attracted to benthic mussel lures that mimic large invertebrates or fish. In our experiments, unfed minnows were confined closely and for long periods with displaying mussels, perhaps resulting in unnaturally high rates of encounters and glochidial release. In contrast, these lures are likely to be attractive to top predators such as bass, in the wild as well as in a laboratory situation.

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