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Effects of temperature and photoperiod on lure display and glochidial release in a freshwater mussel

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Abstract. Freshwater mussels use an array of strategies to transfer their parasitic larvae (glochidia) to fish hosts. We examined the effects of temperature, photoperiod, and female gravidity on mantle lure display and conglutinate release by *Ligumia subrostrata* (Say, 1831) in 2 laboratory experiments. In the 1st experiment, we examined the use of these strategies in 4 temperature treatments (5, 15, 25, 35°C) and 3 photoperiods (10:14, 12:12, and 14:10 h light: dark). In the 2nd experiment, we observed infection strategies under ambient conditions with flow-through pond water. Water temperature appeared to be the primary cue governing use of these strategies. Lure display occurred over a protracted period, but the highest display frequency occurred between ~11 and 20°C. Lure display declined rapidly above this range and ceased altogether >28°C. Release of conglutinates increased coincident with the decrease in lure display but, at ambient temperatures, occurred over a protracted period similar in duration to lure display. Females that were not gravid at the beginning of the experiment did not display lures, and gravid females whose gills were flushed of glochidia displayed for only a short period during which frequency of display was much lower than gravid individuals. *Ligumia subrostrata* exhibits a temperature-mediated switch between alternate host strategies. We present evidence that lure display is a primary strategy for host infection and conglutinate release is a secondary, bet-hedging strategy to reduce wastage of glochidia that ultimately must be cleared from the gills at the end of the reproductive season.

Key words: life-history strategies, parasites, conglutinates, Unionidae, *Ligumia subrostrata*, host infection, thermal regime.

Reproductive behavior in exothermic, aquatic organisms often is cued by environmental factors. Temperature and photoperiod initiate gonadogenesis and spawning behavior in many taxa including fish, crustaceans, and mollusks (Aiken and Waddy 1989, Fong et al. 1995, Carscadden et al. 1997, Verween et al. 2009). These seasonal cues are important because the timing of spawning and other reproductive behaviors is strongly related to recruitment success (Kautsky 1982, Wieland et al. 2000). For example, in freshwater mussels, spring recruitment can maximize growth in the 1st y of life allowing greater accumulation of energetic reserves necessary for winter survival and attainment of reproductive maturity (Beaty and Neves 2004). However, if reproduction occurs too early, cold temperatures and low food availability may reduce growth and survival of recruits (Hanlon and Neves 2006). Understanding how abiotic factors control reproductive behaviors can help identify conditions necessary for successful recruitment.

Freshwater mussels have an unusual life history in which larvae (glochidia) are obligate parasites on the gills or fins of fishes. Female mussels brood fertilized eggs in their gills until they develop into mature glochidia. After maturity, glochidia are brooded for an additional period that ranges among species from short-term brooders that retain glochidia for only a few weeks to long-term brooders that retain glochidia for up to 6 to 7 mo, usually over the winter (e.g., Watters and O'Dee 2000). In both brooding strategies, glochidia infect host fishes after they are released

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from the female or extracted from the female gill by fish attacks on mantle lures (see below). The factors that trigger glochidial release are poorly known in most species but are assumed to include a combination of water temperature and photoperiod (Watters and O'Dee 2000, Hastie and Young 2003). The timing of glochidial release probably is crucial for successful recruitment. Temperature and photoperiod may influence the location, abundance, and activity level of potential hosts (Martel and Lauzon-Guay 2005), and the strength of the host immune system, which determines how well glochidia can transform into juvenile mussels (Roberts and Barnhart 1999).

Mussels have a variety of strategies to increase the likelihood that glochidia will encounter suitable host fishes (Barnhart et al. 2008). The best known of these strategies are mantle lures and conglutinates. Mantle lures are modifications of the mantle margins that are variously pigmented and elaborated to resemble small fishes, crayfish, insect larvae, or other prey items of host fishes. These lures elicit attacks from fishes during which fishes become infected with glochidia. Conglutinates are small, discrete packets containing glochidia and usually represent the contents of a single gill water tube. Similar to mantle lures, conglutinates resemble small worms, insect larvae, or other fish prey items, and attacks from host fishes result in infection by glochidia. Both release strategies involve mimicry of host prey items, but they differ in the factors that trigger glochidial release. The factors that trigger conglutinate release have not been studied directly, but release appears to occur in response to water temperature or photoperiod cues, and conglutinates are often released en masse within a few days (Neves and Widlak 1988, Hove and Neves 1994). In contrast, species that display mantle lures release glochidia primarily in response to attacks on the lures by fishes during which fishes rupture the gravid gills (Haag and Warren 2000, Barnhart et al. 2008). These species often display lures for months (Kramer 1970, Haag and Warren 2003), during which time they may infect multiple host individuals. Some species display lures at night and others by day (Haag and Warren 2000), but the factors that influence seasonal periods of lure display have not been studied.

Many mussel species appear to rely on a single host-infection strategy, but others may use both primary and secondary strategies. For example, *Hamiota* infects hosts primarily by releasing large conglutinates (superconglutinates) that resemble small fishes, but conglutinate release is preceded by a short period of display of mantle lures with lures being reduced in most species (Haag et al. 1995, 1999). Many species in the tribe Lampsilini that infect hosts primarily by display of mantle lures also may release conglutinates. These conglutinates contain mature glochidia but are often loose and poorly formed and appear to be released mostly late in the brooding season. Consequently, whether this behavior is necessary simply to make room for the next brood or represents a secondary infection strategy is not known (Corey et al. 2006, Barnhart et al. 2008).

We examined factors that influence mantle lure display and conglutinate release in the freshwater mussel *Ligumia subrostrata* (Say, 1831). First, we ran a laboratory experiment in which we manipulated temperature and photoperiod to examine the influence of these factors on lure display and conglutinate release. Second, we examined temporal patterns of lure display and conglutinate release under ambient conditions reflective of the natural period of host infection for this species (late winter–spring) and how lure display is affected by gravidity. We discuss how these results extend our understanding of the timing of mussel reproduction and the relative benefits of alternate host infection strategies in mussels.

Methods

Study species

Ligumia subrostrata occurs in lentic conditions, including ponds and backwater habitats of streams, throughout central North America (Cummings and Mayer 1992, Williams et al. 2008). Like most species in the tribe Lampsilini, *L. subrostrata* is a long-term brooder that broods glochidia over the winter and releases them in late winter and spring. Known or suggested fish hosts for *L. subrostrata* are sunfishes (*Lepomis* spp.) and black basses (*Micropterus* spp.) (Lefevre and Curtis 1912, Stern and Felder 1978, WRH, unpublished data). Gravid females attract hosts by displaying a mantle lure consisting of small papillae that are fluttered to reveal the gravid gills within. Displays occur primarily during the day (Corey et al. 2006). *Ligumia subrostrata* also releases conglutinates that are proposed to represent an alternate host-infection strategy (Corey et al. 2006).

We obtained gravid female *L. subrostrata* from ponds at South Auburn Fisheries Research Station, Department of Fisheries and Allied Aquaculture, Auburn University. All mussels were young-of-the-year individuals that recruited in late winter or spring 2009 as part of a separate, ongoing experiment (JAS and WRH, unpublished data). Mussels were collected from ponds in November 2009. Despite their young age, 90% of female mussels in the ponds were fully gravid at the time of collection. Length of female

mussels ranged from 43.3 to 53.3 mm (mean = 48.6 mm). After collection, mussels overwintered in the laboratory in flow-through tanks supplied with pond water at ambient outdoor temperature.

Experiment 1: temperature and photoperiod manipulation

To examine the influence of temperature and photoperiod on infection strategies, we held mussels in 4 temperature-controlled water baths (~100 L) and regulated day length with a timer. We tested 4 temperature (5, 15, 25, 35°C), and 3 photoperiod (10:14, 12:12, 14:10 h light:dark [L/D]) treatments. We maintained the 5 and 15°C treatments with chillers and the 25 and 35°C treatments with aquarium heaters. All temperatures remained within ±1°C of the treatment temperature throughout the experiment.

We randomly assigned 10 female mussels to each treatment. However, to minimize stress on the mussels, we did not examine females for gravidity prior to start of the experiment. We examined all mussels for gravidity at the end of the experiment or immediately upon death. Mussels that were not gravid upon examination and had not released any conglutinates during the course of the experiment were considered to have been nongravid at the beginning of the experiment and were not considered in subsequent data analysis. The final number of gravid mussels in each treatment was 9, 8, 7, and 9 in the 5, 15, 25, 35°C temperature treatments, respectively.

Within each temperature treatment, we held each mussel individually in a 1-L cup filled with artificial fresh water (AFW: 50 mg $CaCO_3$, 25 mg CaCl, 50 mg $NaHCO_3$, and 5 mL 30‰ salt water/L deionized water). A preliminary trial showed no difference in display activity in AFW and pond water. We used a foam platform to float cups in the water bath with ~90% of the cup suspended below the waterline. Holding mussels in individual cups isolated individuals from each other in the water bath and allowed us to record glochidia released during the experiment. We fed mussels 0.05 mL of Reed Mariculture shellfish diet (Reed Mariculture, Campbell, California) every other day as AFW was replaced in each cup. We brought replacement water to treatment temperature before adding it.

We initiated the experiment on 27 February 2010 when ambient pond water was at 10 to 12°C. We moved animals from flow-through holding tanks to the experimental containers and acclimated them to experimental temperatures by setting all water baths initially at 12°C and subsequently adjusting by 1°C/d until treatment temperatures were reached. We acclimated mussels at their respective temperature treatments for 3 to 6 d before the start of the experiment and held them at a constant temperature throughout the remainder of the experiment. In each temperature treatment, we manipulated photoperiod to simulate increasing day length progressing from 10:14 to 12:12 to 14:10 h L:D, similar to what mussels would experience in late winter and spring. Unlike gradual changes in day length in nature, our transitions were abrupt and were designed to evaluate obvious behavioral differences among distinctly different light regimes. We observed mussels for 7 d at each photoperiod. At the end of 7 d, we increased day length and allowed mussels to acclimate for 3 d before beginning the next observation period. We made observations of lure display during acclimation periods, but these observations were not used in data analysis (see Fig. 1A–D).

We observed mussels 3 times/d during each temperature/photoperiod combination. We focused on daytime display behavior because Corey et al. (2006) found that *L. subrostrata* did not display at night. We did not make night-time observations, but our results confirmed that this species displays vigorously during the day. We made observations 1 h after lights came on (0700–0900 h, dawn), at midday (1200–1400 h), and 1 h before lights turned off (1900–2100 h, dusk). We scored mussel display activity following methods published by Haag and Warren (2000) where 0 = no display, 1 = partial display, and 2 = full display. A 0 score was given when a mussel was filtering normally with the valves slightly open, but the mantle lure was not extended. A 1 score was given when the valves were slightly more agape and the mantle papillae were extended beyond the shell margin, but papillae were not actively moving. A 2 score was given when the valves gaped widely, the mantle lure was fully extended, and papillae fluttered actively. In full displays, gravid gills often were visible between the shell valves. We used analysis of variance (ANOVA) to examine differences in the number of animals in full display across the 3 daily observations (dawn, midday, and dusk) for each temperature and photoperiod (n = 12 comparisons). We grouped observations for specific time periods (dawn, midday, and dusk) across all days in each temperature treatment.

Full displays are most likely to elicit fish attacks and result in transfer of glochidia (Haag and Warren 2000), so we focused on this display category in our analysis. We calculated the proportion of animals in full display (number in full display/total number of animals per treatment) 3 times/d (morning, noon,

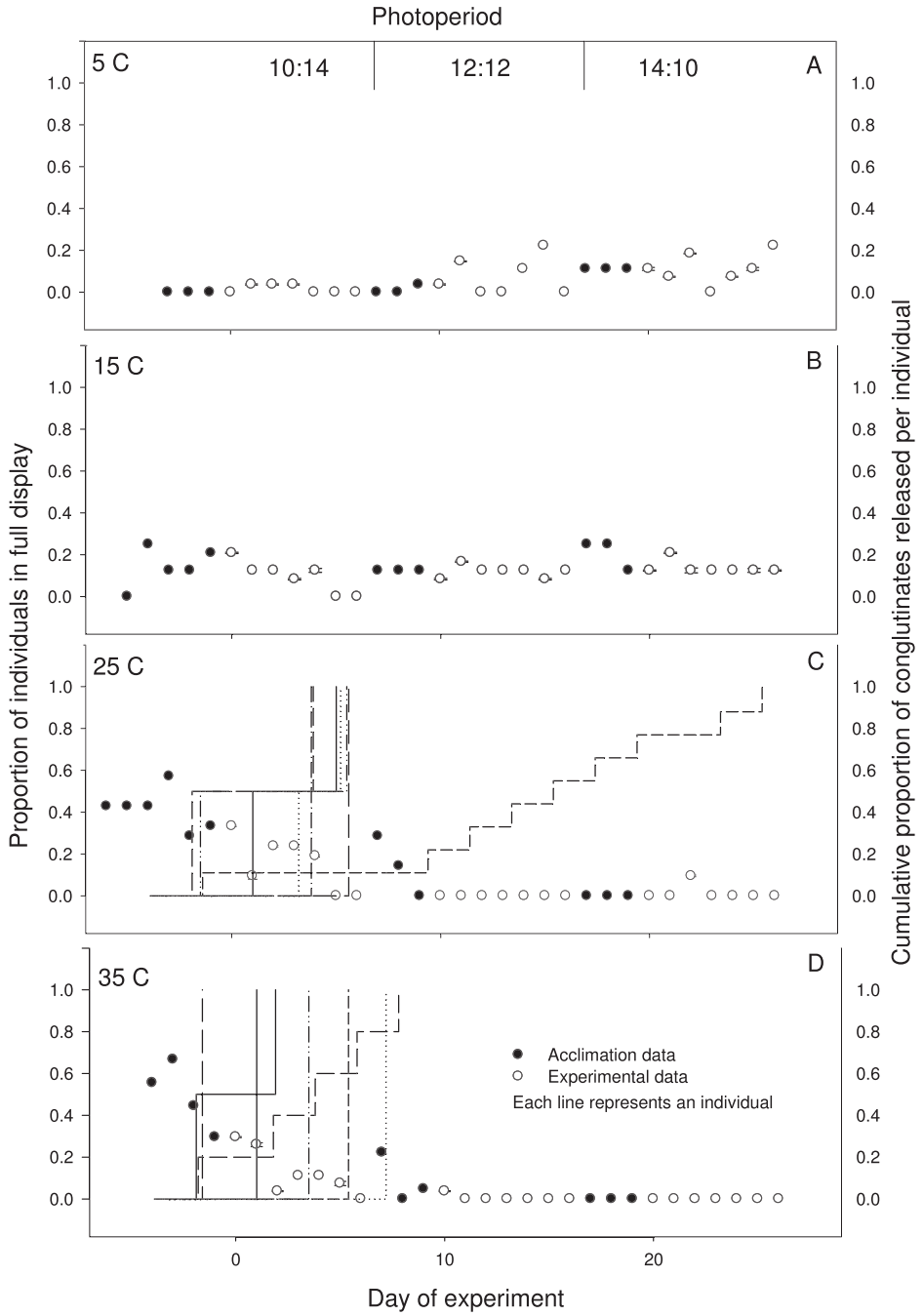

FIG. 1. Experiment 1. Mean (±1 SE) proportion of mussels in full display (circles) and proportion of conglutinates released (lines) with increasing photoperiod and time while maintained at 5 (A), 15 (B), 25 (C), or 35°C (D). Lines represent the cumulative proportion of conglutinates released by individual mussels.

evening) for each of the 4 temperature treatments. We averaged these proportions to obtain the daily mean proportion of mussels in full display for each temperature treatment. If a mussel died during the experiment, we adjusted the total number of animals per treatment accordingly. We used a Kruskal–Wallis test to compare average proportion in full display across temperature treatments for each photoperiod and across photoperiods separately for each temperature treatment (SYSTAT 12; Systat, Chicago, Illinois). We excluded the 35°C treatment from the analysis of the 12:12 and 14:10 photoperiods because of high mortality. We were unable to assign photoperiod randomly to individuals in each temperature treat-

ment because the number of water baths available was limited. Therefore, we were unable to distinguish directly between the effects of time in experimental conditions and photoperiod. In addition to observing display behavior, we collected expelled conglutinates every other day coincident with water changes. From these data, we examined: 1) the number of days at experimental temperatures before each individual first released conglutinates, 2) the occurrence of glochidial dumping by individual mussels (all glochidia released in 2 d), and 3) the number of days until 100% of conglutinates were released.

Display frequency was low and conglutinate release did not occur at 5°C regardless of photoperiod, so we conducted an additional trial with these animals to examine the effect on display behavior of manipulating water temperature while holding photoperiod constant. Upon completion of the longest daylight trial, we held photoperiod at 14:10 h L:D but raised water temperature in the 5°C treatment by 1°C/d up to 12°C, and then held temperature constant for 7 d. We scored display behavior for each mussel once daily throughout the 14-d trial. Successive observations were not independent, so we used a nonparametric runs test to assess if the daily progression of display scores occurred in a random or nonrandom sequence (Zar 1999).

Experiment 2: ambient temperature and photoperiod

We conducted a 2nd experiment to examine: 1) how gravidity status affects lure display behavior, and 2) how the relative use of mantle display and conglutinate release strategies changes over time at ambient conditions during the natural period of host infection for this species (late winter–spring; JAS and WRH, personal observation). We obtained 20 gravid female mussels from our flow-through tanks used to overwinter mussels in the laboratory. Mussels were of similar size and age to those used in Experiment 1, but none were used in previous experiments. We allowed 10 mussels to retain glochidia and emptied 10 of glochidia by using a syringe with a 22-gauge needle to flush water through the gills (Khym and Layzer 2000).

We held all mussels in the laboratory in flow-through tanks from 27 February to 9 July 2010. We randomly assigned 1 gravid and 1 flushed mussel to each of ten 3-L Aquatic Habitat Animal Bank (AHAB) tanks that received unfiltered pond water. In the tanks, we held each mussel in a shallow cup made of polyvinyl chloride pipe (19 mm tall × 25 mm diameter) that was glued to acrylic sheeting. This shallow cup allowed mussels to be positioned upright in a natural filtering position and enabled us to observe the mussels easily without disturbing them. Water temperature closely mimicked that of ambient, outside conditions because tanks received pond water continually. On average, tank temperatures stayed within 0.4 ± 1.2°C (SD) of ambient temperature in an adjacent 0.1-ha pond. We used fluorescent lights and timers to adjust photoperiod continually to match ambient, outside conditions. Photoperiod progressed from 11.25:12.75 to 14.25:9.75 h L:D over the duration of the experiment. We cleaned tanks weekly to remove pseudofeces and other solids that settled from the pond water. We recorded temperature once daily between 1200 and 1500.

We recorded mantle displays once daily (between 1200 and 1500) for the duration of the experiment and used the scoring system described previously. For each day, we determined the proportion of individuals in full display for both gravid and flushed mussels. We used nonlinear regression to fit the following logistic model to the relationship between proportion of individuals displaying (arcsine[x]-transformed) and day of the experiment:

$$y = a/(1 + \exp[-(x - x_0)/b])$$

where y is proportion in full display, a is the y-intercept, x is day of the experiment, x_0 is a threshold value (day of experiment), and b is the slope of the decay of display proportion from the baseline to 0. The y-intercept represents the mean maximum proportion of individuals in full display throughout the experiment, and the threshold value is the day of the experiment at which the proportion of full display decayed to ½ the mean maximum value. We estimated logistic models separately for gravid and flushed females. For both groups, we estimated water temperature on the threshold day with a linear regression describing the relationship between temperature and day of experiment (R^2 = 0.948, temperature = 0.171[day] – 12.6).

We also collected and counted conglutinates from the bottom of each tank every 1 to 2 d. Most glochidia were released in tightly bound conglutinates and not as free glochidia. We assumed that all conglutinates in the tanks were from the gravid individuals. We calculated conglutinate production for a given day as the proportion of the total production over the course of the experiment. Temperatures reported for conglutinate release are those recorded between 1200 and 1500 h. We terminated the experiment when no conglutinates were found in any of the mussel tanks for 7 consecutive days (9 June 2010). We then examined all mussels for glochidia remaining in the gills.

We quantified the time that displaying individuals spent actively moving their lures. Lure movement occurs sporadically in displaying individuals, and this measurement provided an additional and more fine-scaled measure of how much time was devoted to attracting hosts actively. We quantified lure movement by observing each mussel once weekly and recording the proportion of 10 min spent in full display. Observations began 6 d after the start of the experiment and continued weekly for 105 d. We were interested in measuring lure movement only for individuals in full display. We measured lure movement initially on Wednesdays (1200–1400 h). If an individual was not in full display on that day, we attempted observation on the following 2 d (Thursday and Friday). If a mussel did not display on any of the 3 visits, we excluded it from the analysis. We made these measurements only for gravid mussels and used linear regression (arcsine[x]-transformed proportion) to examine the relationship between proportion of time spent in full display and temperature.

Results

Experiment 1: temperature and photoperiod manipulation

Thirty of 33 gravid females were in full display at some point during the experiment. The 3 individuals not in full display were in the coldest (5°C, 1 individual) and hottest (35°C, 2 individuals) treatments. None of the mussels that were considered to have been nongravid at the beginning of the experiment ($n = 7$ across all treatments; see Methods) ever displayed mantle lures (partial or full displays). Mussels held at 35°C had high mortality. Eight of the 10 individuals died between days 19 and 25, and only 2 survived to the end of the experiment. No mussels died in the 5, 15, and 25°C temperature treatments. No significant differences in number of mussels in full display were found among dawn, midday, and dusk within temperature treatments at each photoperiod (ANOVA, all $p > 0.37$).

Mean and maximum observed frequency of full display was low at 5°C but increased at successively higher temperatures (Fig. 1A–D). However, the temporal pattern of display differed widely among temperatures. The proportion of individuals in full display differed significantly among photoperiods in the 5, 25, and 35°C treatments ($p = 0.058$, 0.007, and 0.001, respectively; Fig. 1A, C, D), but not at 15°C ($p = 0.313$; Fig. 1B). In the 5°C treatment, display differed only between the 10:14 and 14:10 h photoperiods (higher at 14:10 h, Kruskal–Wallis post hoc analysis, $p = 0.01$), and showed a gradual but modest increase over the duration of the experiment (Fig. 1A). At both 25 and 35°C, display differed between 10:14 h and both 12:12 and 14:10 h, but not between the latter 2 photoperiods (Fig. 1C, D). In contrast to the 5°C treatment, display frequency at 25 and 35°C was highest at the beginning of the experiment but declined rapidly to low levels by about day 10, before the end of the 10:14 h photoperiod. Display frequency remained essentially at 0 throughout the 12:12 and 14:10 h photoperiods (Fig. 1C, D).

All gravid mussels released conglutinates or free glochidia in the 25 and 35°C treatments, but no individuals released conglutinates at 5 or 15°C (Fig. 1A–D). Conglutinates were white and teardrop shaped, and averaged 6.9 ± 1.2 mm (SD) in length (range: 4.4–9.1 mm, $n = 25$; Fig. 2). Conglutinates were composed primarily of mature glochidia (mean = 99% of total propagules, $n = 5$ conglutinates) and broke apart easily because of the weak cohesion between degenerated egg membranes surrounding the glochidia. At 25°C, individuals initiated conglutinate release 8 to 14 d after the experimental temperature had been reached (Fig. 1C). At 35°C, individuals initiated conglutinate release between days 3 and 13 (Fig. 1D). By the end of the experiment, all surviving individuals in the 25 and 35°C treatments had released all of their glochidia and had empty marsupia. One mussel that died during the experiment (day 21) still retained a portion of its glochidia. The duration of glochidial release differed among temperatures. At 25°C, only 2 of the 7 individuals dumped all conglutinates in a single episode (i.e., within 2 d, corresponding to the interval between conglutinate collection). All but 1 of the other individuals released conglutinates in 2 episodes over 4 to 6 d, with each episode representing ~½ of total conglutinate production. One individual released conglutinates 7 times over 14 d. At 35°C, 75% of individuals dumped all conglutinates in a single episode. For all individuals at both temperatures, conglutinate release began as the proportion of individuals in full display began to decline, and for most individuals, release was complete by the end of the 10:14 h photoperiod (Fig. 1C, D).

Increasing the temperature of the 5°C treatment to 12°C while photoperiod remained constant (14:10) resulted in a marked increase in the proportion of mussels in full display (Fig. 3). The runs test indicated a significant nonrandom pattern in display activity ($p < 0.05$). The proportion of individuals in full display increased rapidly when the temperature reached about 11°C (day 7), and remained high for the remainder of the experiment. No conglutinates were released by any mussel during this component of the experiment.

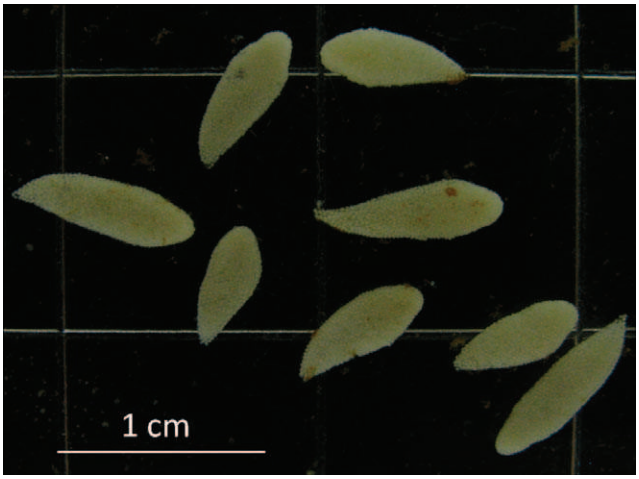

FIG. 2. *Ligumia subrostrata* conglutinates (cohesive conglutinates from Experiment 2).

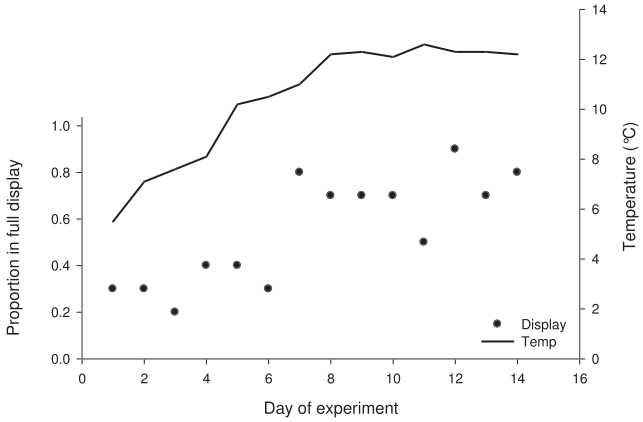

FIG. 3. Experiment 1. Frequency of *Ligumia subrostrata* lure display during an increase in water temperature from 5 to 12°C under a constant photoperiod of 10:14 h light:dark (L:D). Data points represent the proportion of individuals in full display on each day. The solid line is daily water temperature.

Experiment 2: ambient temperature and photoperiod

All mussels survived the 139-d study as ambient temperature increased from 10 to 32°C. Both gravid and flushed (nongravid) mussels displayed mantle lures during the experiment, but the duration and frequency of full display was much lower in nongravid mussels (Fig. 4A). Ninety percent of flushed mussels exhibited full display at some point. From the logistic model (overall model $R^2 = 0.58$), the maximum proportion of flushed individuals in full display was only 0.25, but daily observations ranged as high as 0.60. Maximum proportion of full displays occurred at temperatures <15°C. The threshold value indicated that the proportion declined to 50% of the mean maximum value on day 24 at 16.5°C. Full displays by flushed mussels ceased mostly by day 48 (15 April, 22°C) with the exception of 1 individual that displayed on day 111 (17 June). In contrast, all gravid mussels displayed during the experiment, and the maximum proportion of individuals in full display was 0.86 (overall logistic model $R^2 = 0.86$). The frequency of full display was consistently high throughout the first 46 d of the experiment (27 February–13 April, 11–20°C) and did not decline to 50% of the mean maximum value until day 56 (22.1°C). Display did not cease until day 116 (22 June, 32°C).

Release of conglutinates coincided with a decrease in lure display frequency (Fig. 4B). Conglutinate release began as early as day 25 (23 March, 14.4°C), but some individuals did not release conglutinates until as late as day 68 (5 May, 25.6°C). The mean date of onset of conglutinate release across individuals was day 40 (21.2°C), which corresponded closely to the end of the period of maximum lure display. Within individuals, the rate of conglutinate release over time was roughly linear and occurred incrementally over a protracted period in sharp contrast to the episodic dumping of conglutinates by most individuals at higher temperatures in Experiment 1. Across individuals, only 25% of conglutinates were released by day 65 at water temperatures ~23°C, at which time lure display had declined to <30%. Conglutinate release spanned an average of 88 d across individuals (range 52–99 d) and ceased by day 139 (9 July, 31.8°C). Individual mussels released an average of 140 conglutinates (range = 69–201) during the experiment. Conglutinates were similar in appearance to those released in Experiment 1, but differed in that they did not break apart easily and more readily maintained their shape (Fig. 2). No free glochidia were observed. At the end of the experiment, gills of all individuals were completely empty.

The amount of time that displaying individuals spent actively moving their lures showed a temporal pattern similar to the proportion of individuals in full display. The frequency of lure movement declined exponentially with increasing temperature ($R^2 = 0.59$, $p < 0.001$) (Fig. 5). Frequency declined from 0.33 to 0.13 as temperatures increased to 22.1°C and declined further to 0.05 as temperatures increased to 32°C.

Discussion

Lure display and conglutinate release by *L. subrostrata* are strongly influenced by water temperature. In both the manipulative and ambient experiments, the highest lure display activity occurred within a narrow range of water temperatures from ~11 to

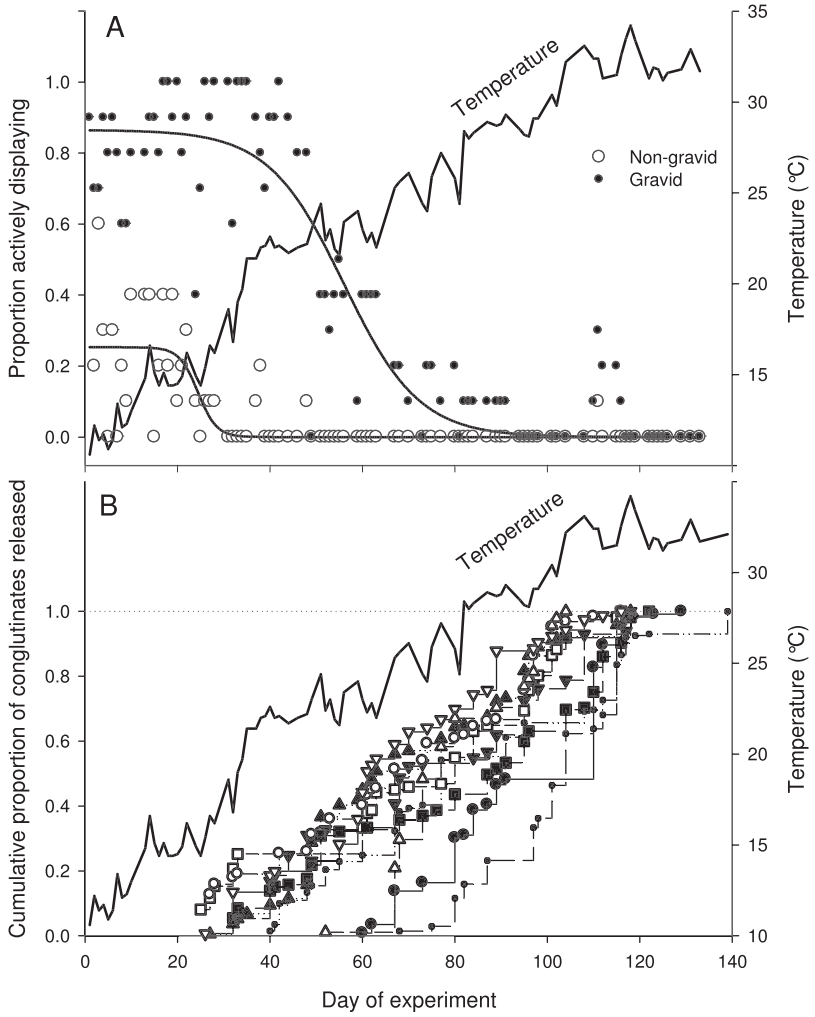

FIG. 4. Experiment 2. A.—Proportion mussels in full display and temperature vs time under ambient increases in photoperiod and temperature. Trend lines for gravid and nongravid mussels were derived from a logistic model (see text). B.—Cumulative proportion of released conglutinates and temperature vs time. Each symbol and line combination represents an individual mussel.

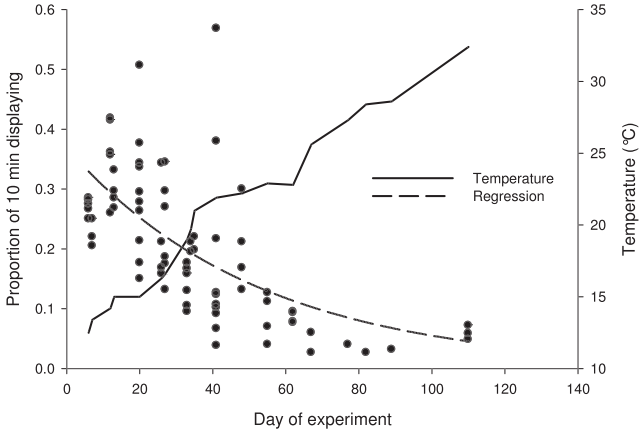

FIG. 5. Experiment 2. Proportion of time spent in full display at ambient increases in temperature and photoperiod from 27 February to 9 July 2010.

20°C. Lure display appeared to increase abruptly when the lower end of this temperature range was reached, but declined quickly above this range. Proportion of time spent in full display also declined steadily as temperatures increased. Similar patterns of lure display were seen for individuals in artificial freshwater and in natural pond water, a result suggesting that display is cued directly by temperature and not by algal abundance or other factors correlated with water temperature. The low display frequency of individuals in the static 15°C treatment was surprising but suggests that periods of changing temperatures are also important cues for lure display, as demonstrated by the temperature increase experiment. In the ambient experiment, conglutinate release occurred over a broad temperature range (15–32°C) but predominantly >20°C, and its duration

was similar to that of lure display. This result suggests that the switch from lure display to conglutinate release and the duration of release are strongly mediated by temperature.

We were unable to evaluate directly the influence of photoperiod on lure display or conglutinate release, but photoperiod appeared to have only a small influence on reproductive behavior. Display increased dramatically in Experiment 1 when temperature was increased from 5 to 12°C even though photoperiod was held constant. In the static 5°C treatment, lure display increased modestly with increasing photoperiod, but no corresponding increase in display occurred in the 15°C treatment. Increasing photoperiod could possibly cause a hormonal or other physiological response in anticipation of increased temperatures normally associated with increasing day length, and this response may stimulate lure display to some extent. However, conglutinates were never released at cooler static temperatures regardless of photoperiod, and at high temperatures, conglutinates were released abruptly before an increase in photoperiod.

Display of mantle lures was strongly associated with gravidity. All gravid females displayed lures at some point in both experiments, but individuals that were not gravid (naturally) at the beginning of the experiment never displayed. Gravid individuals whose gills were flushed artificially at the beginning of the experiment had much lower frequency of display and displayed for only a brief period compared to gravid individuals that were not flushed. This residual display behavior suggests that either some glochidia remained in the gills after flushing, or elevated hormonal levels resulted in a continued display even after gills were emptied. Display behavior can be affected by pharmaceutical hormones in wastewater discharge (Bringolf et al. 2010), but the natural role of hormonal or other physiological mediators of lure display and glochidial release are uninvestigated in freshwater mussels.

The timing of lure display and conglutinate release and its relationship to water temperature probably has evolved to correspond with optimal conditions for glochidia encystment and development. At low temperatures, glochidia can transform successfully on hosts, but glochidial development is slowed or suspended and requires a lengthy period of encystment (Howard and Anson 1922, Watters and O'Dee 1999, Steingraeber et al. 2007) during which chances of host mortality increase. However, glochidial survival decreases rapidly with increasing temperature (Fisher and Dimock 2000, Zimmerman and Neves 2002), and transformation success and the breadth of suitable host species can decrease at higher temperatures (Roberts and Barnhart 1999).

Release of conglutinates by *L. subrostrata* appears to represent an alternate strategy for host infection. Secondary or alternate host-infection strategies of mussels are best known in the genus *Hamiota*, in which females first display mantle lures and then release the entire remaining glochidial contents of the gills in elaborate superconglutinates (Haag et al. 1999). However, unlike *L. subrostrata*, mantle lures of most *Hamiota* are greatly reduced and displayed only briefly, suggesting that superconglutinates represent a primary strategy for host infection (Hartfield and Butler 1997, Roe and Hartfield 2005). Other species that infect fishes primarily with conglutinates produce conglutinates that may represent a considerable cost to the female, either by encasing glochidia in elaborate and highly pigmented membranes (*Ptychobranchus*), or by the presence of nondeveloping structural eggs that decrease fecundity by up to 50% (e.g., *Cyprogenia*, *Dromas*, *Fusconaia*, and *Pleurobema*; Barnhart et al. 2008). In contrast, conglutinates of *L. subrostrata* appear to be formed simply as unelaborated molds of the interlamellar gill spaces in which they are brooded and receive no additional female investment in structure or pigmentation. However, *L. subrostrata* conglutinates are similar in shape and size to those of species that use conglutinates as a primary infection strategy (e.g., *Fusconaia, Pleurobema*; see Haag and Warren 2003), and it is plausible that *L. subrostrata* conglutinates also elicit attacks from fishes. We did not test the viability of glochidia in conglutinates, but glochidia were fully formed and largely free of egg membranes similar to mature glochidia of other lampsilines.

In other lampsiline species that display mantle lures, including *Ligumia*, release of unelaborated conglutinates has been explained primarily as a necessity to clear the gills for deposition of the subsequent brood in late summer or autumn, but also potentially as a secondary infection strategy. Our results are consistent with this idea. At ambient temperatures, mantle lures of *Ligumia* were displayed for an extended period spanning $\geq$2 to 3 mo, showing the importance of this behavior. Conglutinate release occurred primarily during the decline or cessation of lure display. However, individuals released cohesive conglutinates over a protracted period roughly equal to the duration of lure display, rather than dumping all glochidia in a relatively short burst. Many species release puerile conglutinates composed of unfertilized eggs or immature glochidia in response to handling or stress, but these releases occur rapidly, over a few hours or days, and do not appear to be involved in

host infection (Aldridge and McIvor 2003, Haag and Warren 2003, Barnhart et al. 2008). In the static 25 and 35°C treatments, individual *L. subrostrata* released conglutinates rapidly. The release period was shortest at 35°C, and conglutinates disassociated readily, suggesting that this release may have been a stress response to clear the gills quickly and to improve respiratory efficiency at high temperatures (see Aldridge and McIvor 2003). In contrast, at ambient temperatures, conglutinate release was protracted, occurred primarily at temperatures >23°C, and conglutinates were more cohesive, suggesting that this behavior represents a secondary infection strategy and not a stress response.

Ligumia subrostrata exhibited a temperature-mediated switch in infection strategies from display of mantle lures to release of glochidia. The relative importance to population growth of lure display vs conglutinate release is unknown. However, several pieces of evidence suggest that lure display is the primary mode of host infection, and release of conglutinates represents a secondary, bet-hedging strategy. First, lures of *L. subrostrata* are elaborate structures that effectively mimic fish prey items and are displayed for extended periods, but conglutinates are simple, unadorned structures that appear to be mainly artifacts of compaction within the gills and receive no additional female investment. Second, lure display always preceded conglutinate release, even at high temperatures that may be stressful to adult mussels. Infection of hosts via mantle lures and subsequent recruitment of juvenile mussels earlier in the growing season probably increases the fitness of these individuals by maximizing growth and allowing attainment of reproductive maturity in their 1st y (WRH and JAS, unpublished data). Release of glochidia remaining in the gills after lure display must occur eventually to make room for the subsequent brood and perhaps also to minimize O_2 stress associated with brooding or lure display at high summer temperatures. However, an extended period of release of cohesive conglutinates may be a bet-hedging strategy to reduce wastage of residual glochidia.

Other aspects of mussel reproductive biology including gametogenesis, fecundity, and recruitment success are intimately linked to temperature, and human-caused changes in stream temperatures can disrupt these processes (Layzer et al. 1993, Moles and Layzer 2008, Galbraith and Vaughn 2009). Changes in stream temperature may alter dynamics of host infection. In streams with depressed temperatures from hypolimnetic dam release, gravid females may not display or may display later in the season resulting in lower juvenile fitness. Increases in stream temperature associated with global climate change or other factors could shorten the display period or shift the conglutinate release period to earlier in the year. A shorter period of lure display could cause greater reliance on conglutinate release. If conglutinate release is less efficient than mantle lures in transferring glochidia to hosts (e.g., Haag and Warren 1998), such a switch could negatively affect recruitment and population growth.

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