

Thermal Criteria for Early Life Stage Development of the Winged Mapleleaf Mussel (*Quadrula fragosa*)

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ABSTRACT.—The winged mapleleaf mussel [*Quadrula fragosa* (Conrad)] is a Federal endangered species. Controlled propagation to aid in recovering this species has been delayed because host fishes for its parasitic glochidia (larvae) are unknown. This study identified blue catfish [*Ictalurus furcatus* (Lesueur)] and confirmed channel catfish [*Ictalurus punctatus* (Rafinesque)] as suitable hosts. The time required for glochidia to metamorphose and for peak juvenile excystment to begin was water temperature dependent and ranged from 28 to 37 d in a constant thermal regime (19 C); totaled 70 d in a varied thermal regime (12–19 C); and ranged 260 to 262 d in simulated natural thermal regimes (0–21 C). We developed a quantitative model that describes the thermal-temporal relation and used it to empirically estimate the species-specific low-temperature threshold for development of glochidia into juveniles on channel catfish (9.26 C) and the cumulative temperature units of development required to achieve peak excystment of juveniles from blue catfish (383 C·d) and channel catfish (395 C·d). Long-term tests simulated the development of glochidia into juveniles in natural thermal regimes and consistently affirmed the validity of these estimates, as well as provided evidence for a thermal cue (17–20 C) that presumably is needed to trigger peak juvenile excystment. These findings substantiate our model and provide an approach that could be used to determine corresponding thermal criteria for early life development of other mussel species. These data can be used to improve juvenile mussel production in propagation programs designed to help recover imperiled species and may also be useful in detecting temporal climatic changes within a watershed.

INTRODUCTION

The winged mapleleaf mussel [*Quadrula fragosa* (Conrad)], which historically ranged in parts of 11 midwestern states and three major river drainages, was listed as a Federal endangered species in 1991 as a result of range restriction, small population sizes, poor recruitment and habitat alterations caused by changes in watershed land-use practices (U.S. Fish and Wildlife Service, 1991). This mussel currently has only four known populations which are distributed from portions of the St. Croix National Scenic Riverway (Minnesota and Wisconsin) in the north to the Saline River (Arkansas) in the south. Active

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reproduction has been confirmed within only two populations, one each at the northern and southern extremes of the current range. Efforts to recover the species have been focused on the St. Croix River (SCR) population which is jeopardized by several factors including the spread of invasive zebra mussels [*Dreissena polymorpha* (Pallas)] by watercraft, rapid water level changes from dam operations and water quality deterioration as urban development expands in the SCR watershed.

Greater knowledge of fundamental mussel biology and habitat requirements is needed to better manage and conserve members of this faunal group (National Native Mussel Conservation Committee, 1998). A common impediment to the recovery of many imperiled mussels has been a lack of early life-history information, especially the identity of host fish species on which parasitic mussel glochidia (larvae) metamorphose into juveniles. Initial actions to recover the winged mapleleaf began with descriptive observations of reproductive activities. For example, Heath *et al.* (2000) observed winged mapleleaf in the SCR for 3 y before they described it as a late season, short-term (~5 wk) brooder that typically released its glochidia as water temperature decreased to ~15 C in early autumn. Most other *Quadrula* species are reported to brood and release glochidia over longer periods during spring and summer (Oesch, 1995; Heath *et al.*, 2000). Therefore, the winged mapleleaf may be a host-overwintering mussel (Watters and O'Dee, 2000) throughout much of its range.

Discovery of the reproductive timing of winged mapleleaf in the SCR facilitated laboratory trials at the University of Minnesota that screened more than 60 potential host fish species during a 5-y period (M. Hove, pers. comm.). Several channel catfish [*Ictalurus punctatus* (Rafinesque)] trials produced a few juveniles, while others did not. We subsequently collaborated in the host fish identification program to accelerate the screening process and expand biological research opportunities with the winged mapleleaf.

Rates of early life development for poikilotherms such as embryonic fish have been quantified such that dates of peak egg hatching can be accurately predicted if the water temperature remains constant during incubation (Piper *et al.*, 1982). If the water temperature fluctuates, a correction factor can be introduced to account for periods when the temperature drops below the minimum threshold for development. Such a species-specific low-temperature threshold for early life development can be empirically derived from repeated embryological observations made over a range of environmentally relevant temperatures. For example, Kempinger (1988) estimated a low-temperature threshold of 5.8 C for development of embryonic lake sturgeon [*Acipenser fulvescens* (Rafinesque)] based on field observations gathered over several spawning seasons and used this to reliably predict the date of egg hatching despite widely fluctuating river water temperatures.

If thermal relations like those used to predict the development of embryonic fish similarly influence the rate at which encysted glochidia develop into juveniles, then knowledge of the low-temperature threshold for mussel development could be used to predict the time needed for glochidia to complete metamorphosis. We, therefore, evaluated daily water temperature and juvenile excystment data from a series of successful host fish tests to identify thermal criteria for early life stage development of the winged mapleleaf.

MATERIALS AND METHODS

Adult winged mapleleaf mussels were stockpiled in the SCR by divers in July 2003 to increase chances for successful reproduction. Divers returned in September 2003 and collected four gravid females that later released glochidia in the laboratory. The viability of glochidia was confirmed (Zale and Neves, 1982) before they were used (within 24 h of release) to infect the gills of naïve, thermally acclimated test fish during four host fish trials

initiated at the Upper Midwest Environmental Sciences Center (UMESC) on 29 Sep., 1 Oct., 3 Oct. and 6 Oct. 2003. One group of blue catfish [*Ictalurus furcatus* (Lesueur)] and one group of channel catfish (five fish per group) were exposed to glochidia on each date by placement in separate 10-L buckets, each containing 2 L of vigorously aerated well water and equal volumes of the available glochidial slurry. One fish was removed from each bucket at 10-min intervals, anesthetized with tricaine methanesulfonate and briefly examined with a dissection microscope to enumerate gill-encysted glochidia. The exposure was terminated when 25 or more glochidia were found attached to gill lamellae. Two additional groups of channel catfish (six fish per group) were similarly exposed on 3 Oct. because of an abundance of remaining glochidia. Infected fish were subsequently assigned to one of three test temperature regimes (Tests I, II and III; Table 1).

TEST I

Each five-member group of infected fish was randomly assigned to one of eight 38-L glass aquaria. All aquaria received a continuous flow (480–540 mL/min) of aerated well water that was maintained at an unseasonably warm temperature of 19.6 C to promote the rapid metamorphosis of glochidia into juveniles during the four trials. A removable plastic mesh screen (25-mm² openings) was positioned 3.5 cm above the bottom of each aquarium to minimize fish predation on juveniles. Fish were offered food at least once a week. Water temperature and dissolved oxygen were measured (YSI model 58, Yellow Springs, Ohio) daily in each aquarium throughout most of the test. Given the unknown time required for glochidia to develop into juveniles and excyst at this temperature, all fish and the screen were briefly removed from each aquarium once or twice a week while water was siphoned from along the bottom (Waller and Holland-Bartels, 1988) and filtered through a series of sieves (202-, 153- and 53- μ m Nitex[®] mesh) to facilitate the collection of detached mussels. Materials retained on each sieve were rinsed into a partitioned glass Petri dish, illuminated with cross-polarized light (Johnson, 1995) and examined thoroughly using a dissection microscope to enumerate sloughed glochidia and excysted juveniles. Juveniles were considered alive if foot, cilia or valve movement was observed. Once successful metamorphosis was observed, aquaria were siphoned most days throughout the remainder of a trial. The consecutive 5-d period when most live juveniles were recovered from an aquarium was considered the period of peak excystment. Photographic images of representative mussels were collected intermittently and measured for valve dimensions (Image-Pro[®] Express software, Media Cybernetics, Silver Spring, Maryland).

TESTS II AND III

The two remaining groups of channel catfish (12 individuals total) that were exposed to glochidia at the start of the third trial (3 Oct.) were subsequently combined in a 1900-L tank and supplied with a continuous flow of 12.5 C well water. After 45 d, five of these fish were selected for Test II and acclimated to higher temperatures over a 2-d period before being placed into individual 38-L aquaria that received a continuous supply of aerated well water (480–540 mL/min) maintained at a constant temperature of 19.5 C (*i.e.*, nearly identical conditions to Test I). Meanwhile, the seven remaining fish were used for Test III and subsequently maintained from mid-November 2003 through June 2004 in a thermal regime that ranged from 0 to 21 C and followed the daily mean water temperature of the SCR at the U.S. Geological Survey gauging station in St. Croix Falls, Wisconsin (45°24'25"N, 92°38'49"W). These fish were first placed in a cage that was submerged (3–4 m) in an earthen pond at the UMESC for 5 months (*i.e.*, the 0 to 12 C overwinter period) before they were retrieved and returned to the laboratory. Each fish was then placed in a 38-L aquarium

TABLE 1.—Experimental design of tests used to identify and confirm host fish species for winged mapleleaf mussel (*Quadrula fragosa*) glochidia

Test	Fish species	Total length (range; mm)	Wet weight (range; g)	Number of fish per trial	Number of trials	Exposure date(s)	Mean daily water temperature and duration
I	Blue catfish	163–213	30–78	5	4	29 Sep.; 1, 3 and 6 Oct. 2003	19.6 C for 49 d
	Channel catfish	125–161	11–27	5	4	29 Sep.; 1, 3 and 6 Oct. 2003	19.6 C for 49 d
II	Channel catfish	263–300	121–201	6 ^a	1	3 Oct. 2003	12.5 C for 45 d, 16.0 C for 2 d, then 19.5 C for 48 d
III	Channel catfish	237–263	92–114	6 ^b	1	3 Oct. 2003	12.5 C for 45 d, then mimic St. Croix River temperatures for 225 d
IV	Channel catfish	212–245	62–105	6	1	22 Sep. 2004	Mimic St. Croix River temperatures for 270 d

^a Although six fish were originally exposed in Test II, only five fish were maintained in this thermal test regime after 45 d

^b Although six fish were originally exposed in Test III, one additional fish (originally exposed in Test II) was maintained in this thermal test regime after 45 d

and supplied with a continuous flow (480–540 mL/min) of well water (heated as required) for the remaining time. Fish were fed, water temperatures were measured and juveniles were counted in each aquarium during Tests II and III following the same procedures used for Test I.

TEST IV

This test was initiated about 1 y after the start of the preceding series of tests and was performed as an independent repetition of Test III. Six naïve channel catfish were exposed to recently released glochidia on 22 Sep. 2004 and subsequently held in a thermal regime that followed the daily mean water temperature of the SCR through June 2005 by the same means used for Test III (Table 1).

DATA ANALYSIS

Given a nearly complete daily record of the water temperature and the number of live juveniles recovered from each aquarium during all tests, we compared the cumulative daily water temperature units required to initiate peak excystment of juveniles from channel catfish (exposed 3 Oct. 2003) during Tests I and II. This allowed us to empirically estimate the low-temperature threshold (*i.e.*, the minimum mean daily water temperature) required for daily development of encysted glochidia. However, rather than determine this value through an indirect statistical means (Kempinger, 1988), we derived it by solving the following algebraic equation for x

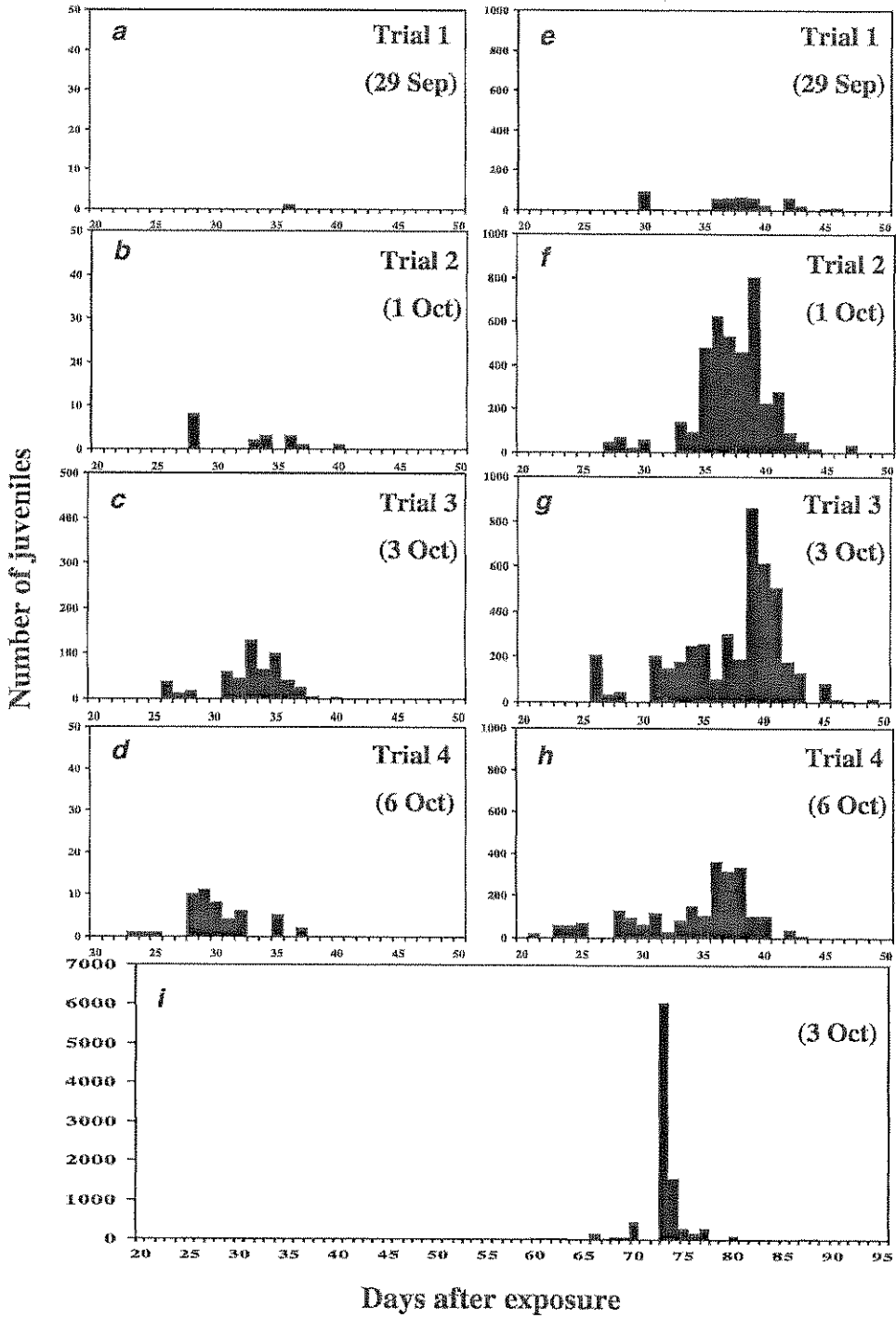
$$(T_1 \cdot d_1) = (T_2 \cdot d_2) + \sum_{i=1}^{n-1} (W_i - x) d_i \quad (1)$$

when $T_1 \approx T_2$ where T_1 is the constant water temperature during Test I (*i.e.*, 19.6 C), T_2 is a constant water temperature during Test II (*i.e.*, 19.5 C), d_1 is the number of days from exposure to the start of peak excystment during Test I, d_2 is the number of days T_2 was maintained until the start of peak excystment during Test II, W_i is a mean daily water temperature $\neq T_2$ during Test II (*i.e.*, 12.5 C and 16.0 C); d_i is the number of days a W_i value occurred during Test II (*i.e.*, 45 and 2); n is the number of different mean daily water temperatures observed during Test II (*i.e.*, 3); and x is the low-temperature threshold (C) for daily development of winged mapleleaf glochidia on channel catfish. The left-side of this equation reflects the amount of time that glochidial development occurred at a constant temperature during Test I; the right-side of this equation reflects the shorter period of time that glochidial development occurred at a similar temperature during Test II plus the cumulative temperature-time interactions when water temperatures were lower (*i.e.*, dissimilar) during Test II.

After the low-temperature threshold (x) was derived, this constant was subtracted from all of the mean daily water temperature values that exceeded it during Tests I and II. The ensuing values were then summed to evaluate the cumulative temperature units of development (CTUD) needed for glochidia to complete metamorphosis on channel catfish (or blue catfish) from the date of exposure until the start of peak excystment, as in the equation

$$\text{CTUD} = \sum_{i=1}^n (T_i - x) d_i \quad (2)$$

where x is the low-temperature threshold (C) for daily development of winged mapleleaf glochidia on channel catfish, T_i is a mean daily water temperature $> x$, n is the number of different mean daily water temperature values $> x$, and d_i is the number of days this T_i value



occurred. Mean daily water temperature data from blue and channel catfish exposures (Tests I and II only) were normalized and summed in this manner to quantitatively estimate the CTUD necessary for peak excystment to begin on each host fish species. Based on these estimates, data from Tests III and IV were used to evaluate the predictive performance of this model under realistic thermal regimes by recording the CTUD daily throughout each long-term test to predict the date when peak juvenile excystment from channel catfish should begin and compare this to the actual start date.

RESULTS

TEST I - CONSTANT THERMAL REGIME

About 11,600 live juveniles were recovered from groups of blue and channel catfish during four trials (Fig. 1a-h). Most of these juveniles (95%) were produced by blue catfish. The period of peak juvenile excystment began 28 to 33 d and 34 to 37 d after channel and blue catfish, respectively, were exposed and maintained at 19.6 C. Peak excystment from groups of fish exposed on 3 Oct. began after 31 d for channel catfish and 37 d for blue catfish. Based on the total number of juveniles and sloughed glochidia recovered during the four trials, the rate of successful metamorphosis was substantially greater for blue catfish (50%) than for channel catfish (7%). Mussels that developed on both of these host fish species exhibited considerable growth during metamorphosis as valve dimensions of developing juveniles were enlarged 2 to 3 times beyond the glochidial valve (Fig. 2a). Juveniles recovered from this test also had valves that typically exhibited nonsymmetrical growth beyond the glochidial valve and a uniform convex surface that displayed little or no topographic variation (Fig. 2b).

TEST II - VARIED THERMAL REGIME

About 9300 live juveniles were recovered from five channel catfish during Test II (Fig. 1i). Juvenile growth and valve appearance during this test resembled those of juveniles recovered during Test I. The period of peak juvenile excystment began 70 d after exposure and 23 d after these infected fish were continuously maintained at 19.5 C. Substitutions of all known values for terms in the first equation are as follows

$$(19.6 \text{ C} \cdot 31 \text{ d}) = (19.5 \text{ C} \cdot 23 \text{ d}) + (12.5 \text{ C} - x) 45 \text{ d} + (16.0 \text{ C} - x) 2 \text{ d} \quad (3)$$

and solving for the unknown variable (x) yielded 9.26 C as the estimated low-temperature threshold for daily development of winged mapleleaf glochidia on channel catfish (*i.e.*, there is no net development for glochidia at or below this temperature). A CTUD-based comparison of the periods of peak excystment for fish that were exposed on 3 Oct. 2003 and used in Tests I and II revealed a near alignment or broad overlap in the recovery of juvenile mussels among these groups of host fish (Fig. 3). Based on the large number of juveniles recovered from blue catfish in Test I and channel catfish in Test II and the similarity of peak excystment for these two groups of fish (*i.e.*, when the data were normalized and expressed as a function of thermal-related development), we concluded that peak excystment of

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FIG. 1.—Daily recovery of live winged mapleleaf juveniles from channel catfish (a-d) and blue catfish (e-h) held at 19.6 C for 49 d during Test I; and from channel catfish (i) held in succession at 12.5 C for 45 d, 16.0 C for 2 d, and 19.5 C for 48 d during Test II. Date of initial exposure (2003) in parentheses

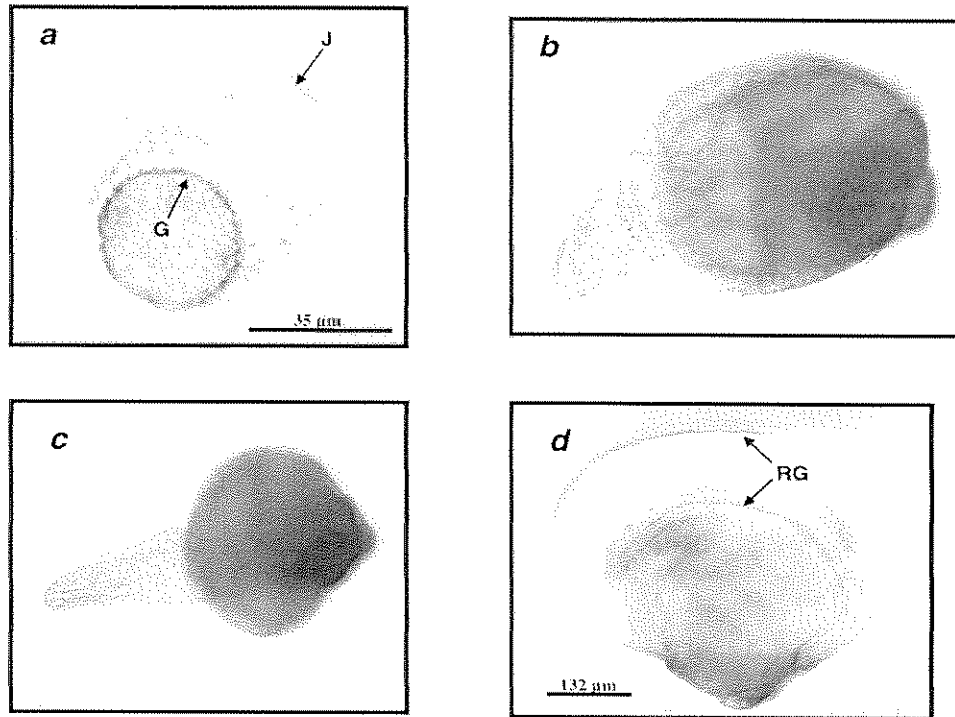


FIG. 2.—Dorsal views of winged mapleleaf valves: (a) outermost margin of the glochidial (G) and juvenile (J) portions of a valve that was sloughed after 21 d of development on a blue catfish held at 19.6 C during Test I; (b) juvenile recovered after 36 d of development on a blue catfish during Test I; (c) juvenile recovered after 266 d of development on a channel catfish held in a thermal regime that simulated the St. Croix River during Test III; and (d) recent growth (RG) near the outer margin of a juvenile 56 d after the start of peak excystment from channel catfish during Test III

juveniles from these host fish should start when cumulative development exceeds 383 C•d and 395 C•d, respectively.

TESTS III AND IV – SIMULATED NATURAL THERMAL REGIMES

A total of 3450 live juveniles were recovered from six channel catfish during Test III (Fig. 4a). These mussels were recovered in June 2004 during the final 27 d of this 270-d test. Peak excystment of juveniles began 262 d after infection (21 Jun. 2004) at 442 C•d of cumulative development; we predicted this excystment would begin 258 d after infection (17 Jun. 2004). The following year, 12,359 live juveniles were recovered from six channel catfish during Test IV (Fig. 4b). These mussels were recovered in June 2005 during the final 14 d of this 270-d test. Peak excystment of juveniles began 260 d after infection (9 Jun. 2005) at 382 C•d of cumulative development; we predicted this excystment would begin 261 d after infection (10 Jun. 2005). Juveniles recovered from both tests had valves with symmetrical growth beyond the glochidial valve and unique, irregular, convex surfaces with a variety of topographic features (*e.g.*, pustules, ridges, sulcus) characteristic of more mature juveniles (Fig. 2c). Some of these mussels were held in the laboratory for several weeks following excystment and notable valve growth was observed (Fig. 2d).

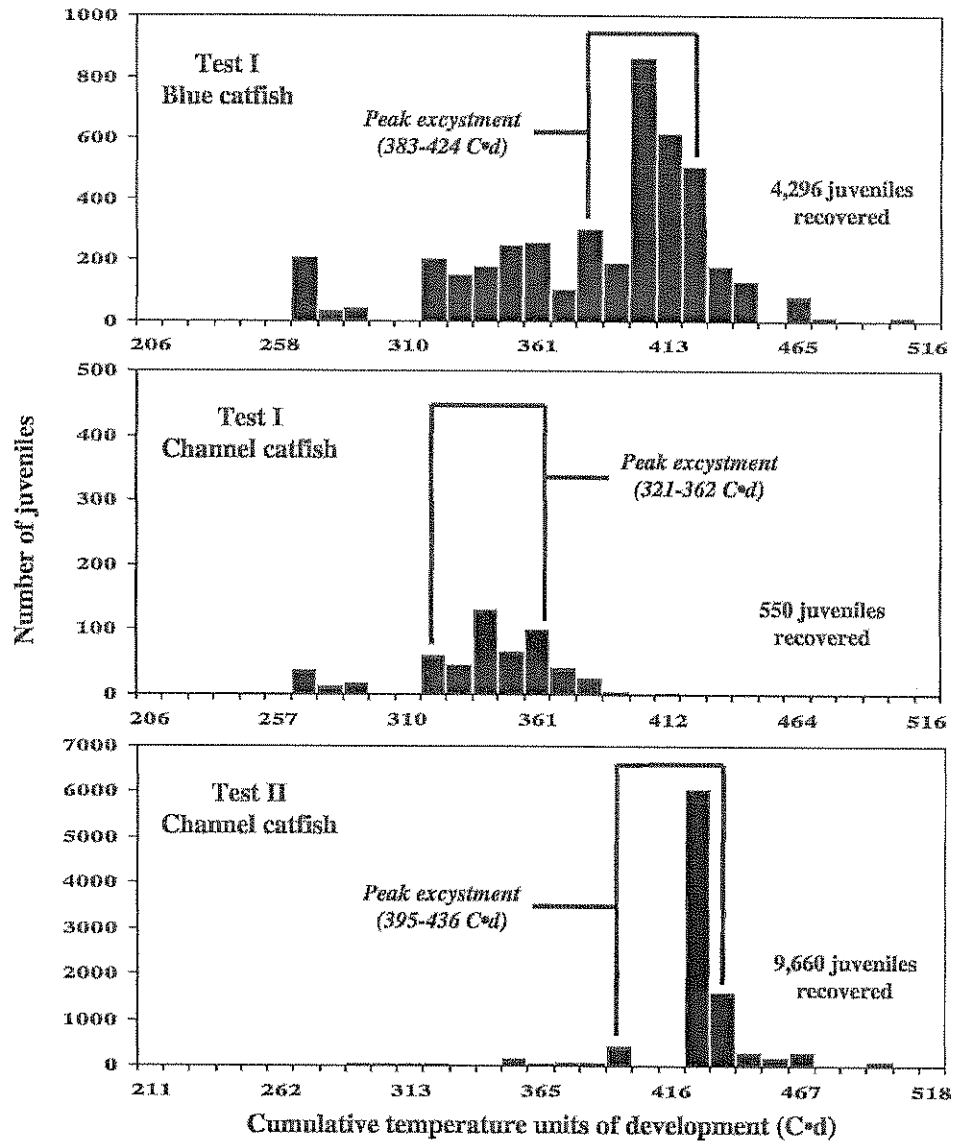


FIG. 3.—Recovery of live winged mapleleaf juveniles from blue catfish and channel catfish exposed to glochidia on 3 Oct. 2003 during Tests I and II as a function of cumulative thermal development at mean water temperatures that exceeded the estimated low-temperature threshold (9.26 C) for daily development

DISCUSSION

TEST I – CONSTANT THERMAL REGIME

Findings from Test I suggest that although both blue and channel catfish are hosts for winged mapleleaf, blue catfish are a more prolific host. However, two inconsistencies may

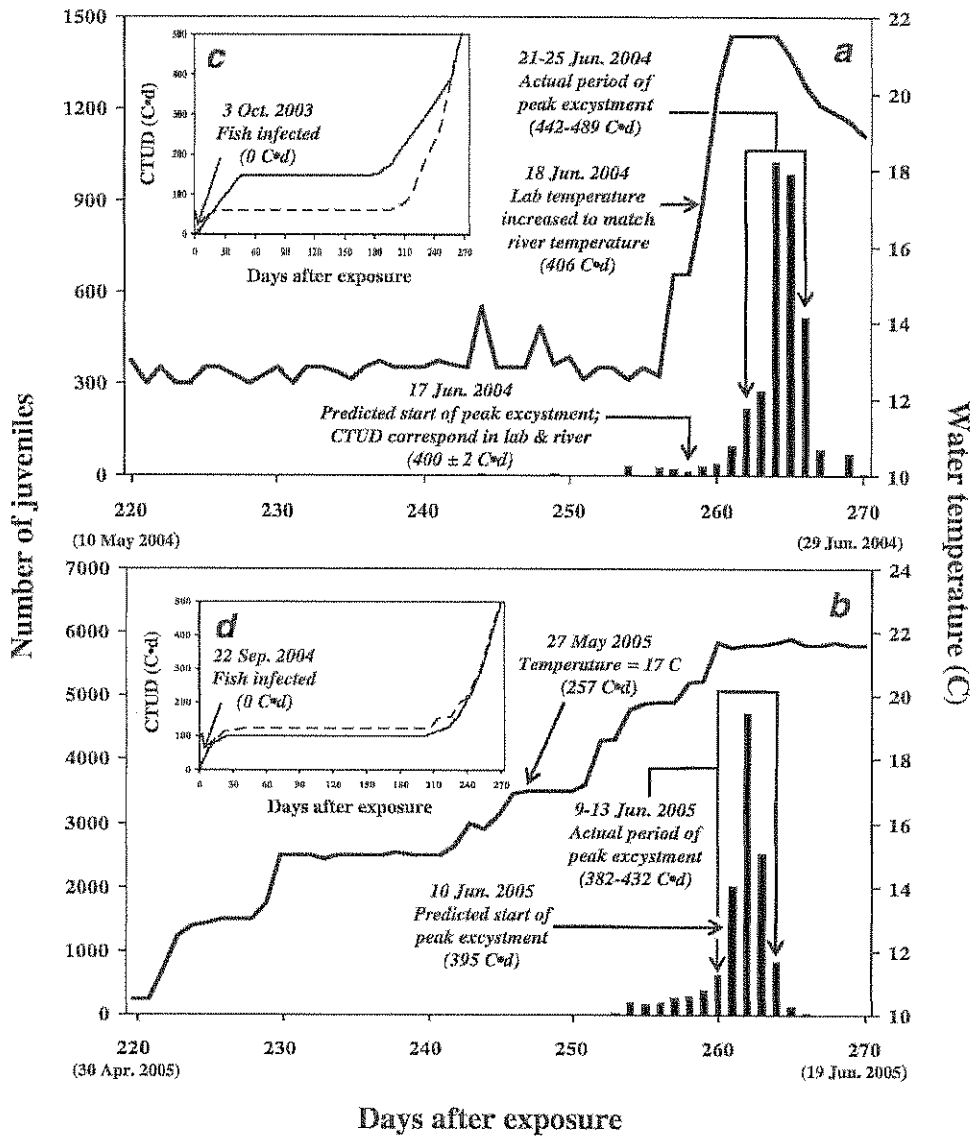


FIG. 4.—Daily recovery of live winged mapleleaf juveniles from: (a) six channel catfish exposed to glochidia on 3 Oct. 2003 (vertical bars) and held for 270 d in a thermal regime (solid line) that simulated the St. Croix River during Test III; and (b) six channel catfish exposed to glochidia on 22 Sep. 2004 (vertical bars) and held for 270 d in a thermal regime (solid line) that simulated the St. Croix River during Test IV; and, predicted cumulative temperature units of development (CTUD) for mussels encysted on channel catfish during: (c) Test III in the laboratory (solid line) and in the St. Croix River (*i.e.*, a concurrent hypothetical exposure; dotted line) based on mean daily water temperature observations from 4 Oct. 2003 to 29 Jun. 2004; and (d) Test IV in the laboratory (solid line) and in the St. Croix River (*i.e.*, a concurrent hypothetical exposure; dotted line) based on mean daily water temperature observations from 23 Sep. 2004 to 19 Jun. 2005

have influenced trial outcomes. First, the channel catfish were considerably smaller than the blue catfish (mean wet weight = 18 and 60 g, respectively). Secondly, the number of juveniles produced varied as much as sevenfold and appeared to reflect large fluctuations in the estimated number of glochidia available to expose groups of fish among trials. Thus, future studies should be conducted with equivalent-sized host fish and numbers of glochidia.

The growth of developing juveniles that we observed during glochidial metamorphosis is rare within the Subfamily Ambleminae (Howard, 1913; Howard and Anson, 1922). In addition, the period of peak juvenile excystment during each of the trials occurred between 4 to 5 wk and 5 to 6 wk after channel and blue catfish, respectively, were exposed and maintained at 19.6 C. These thermal-dependent rates of metamorphosis were reproducible at this constant temperature and provided a reliable basis to estimate several thermal criteria for early life stage development of winged mapleleaf at the conclusion of Test II.

TEST II - VARIED THERMAL REGIME

There was a 62-fold increase in the mean number of juveniles produced per channel catfish from Test I to Test II (*i.e.*, 30 juveniles/fish in Test I trials vs. 1867 juveniles/fish in Test II). This difference may be explained by several factors. First, the mean wet weight of channel catfish in Test II (156 g) was greater than that of the channel (18 g) and blue catfish (60 g) in Test I. Larger fish likely provided greater gill surface area for glochidia to encyst (Rogers and Dimock, 2003). In addition, more glochidia were used to infect fish for Test II (and Test III) than for Test I. Regardless of the reasons, these results are useful to resource managers who want to develop ecologically relevant propagation strategies to recover winged mapleleaf populations throughout its historical range, including the SCR and other northern sites where channel catfish typically occur year-round while blue catfish are normally absent or rare.

A comparison of the time required to initiate peak excystment of juveniles from channel catfish exposed on 3 Oct. during Tests I and II reflects the influence that water temperature had on the rate of metamorphosis. Peak excystment of juveniles during Test II began 39 d later than that observed for channel catfish during Test I and approximated the length of time (45 d) that fish were maintained at 12.5 C immediately after exposure during Test II. Once these fish were subsequently maintained at 19.5 C however, juvenile excystment started to peak 8 d sooner than it had during Test I. This suggests that the physiological mechanisms responsible for the metamorphosis of glochidia into juveniles on channel catfish did not stop but proceeded slowly at this lower temperature. Meanwhile, comparison of the CTUD needed to initiate peak excystment of juveniles from channel catfish (and blue catfish) during Tests I and II demonstrates the potential use of a biological temperature constant to normalize widely varying water temperature data when evaluating early life stage development of an aquatic poikilotherm. These findings should facilitate relatively accurate predictions of the time required for metamorphosis of glochidia into juveniles in either artificial or natural settings and could be used to improve mussel production.

TESTS III AND IV - SIMULATED NATURAL THERMAL REGIMES

The physical appearance of juveniles recovered from tests that followed seasonal temperature variations in SCR was remarkably different from those recovered from unnatural thermal patterns. Regardless of the thermal regime however, few juveniles from any test survived beyond 4 wk. Therefore, it is unknown whether the valves of juveniles that developed rapidly at warm temperatures during Tests I and II would have eventually developed a more characteristic winged mapleleaf appearance, like those that developed at slower rates in seasonally realistic thermal regimes during Tests III and IV.

The final tests provided opportunities to evaluate the predictive capability of the empirically derived low-temperature developmental threshold value in wide ranging thermal regimes that simulated the SCR. Based on findings from Tests I and II, the predicted date for peak excystment during Test III was 4 d premature of the actual date, representing an error of 1.5% over a 262-d period. The following year during Test IV, the predicted date of this event came 1 d after the actual date, representing an error of 0.4% over a 260-d period. These results validate this approach for quantifying the period of thermal development required for the metamorphosis of winged mapleleaf glochidia into juveniles on channel catfish and, perhaps, the metamorphosis of other mussels on their host fish.

Although peak excystment during Test III began 4 d later than predicted and apparently required ~ 47 C·d of additional development, live juveniles were recovered for six consecutive days immediately preceding the peak. This prepeak excystment began 256 d after infection (15 Jun. 2004) at 386 C·d of cumulative development when the water temperature was 12.6 C (Fig. 4a). Exposed fish were purposely maintained at low temperatures (12.4–15.3 C) for 60 d after retrieval from the pond until predicted glochidial development in the laboratory corresponded to that in the SCR (Fig. 4c). This did not occur until 258 d after infection (*i.e.*, 17 Jun. 2004) when the estimated CTUD was 400 ± 2 C·d at both locations. Thereafter, the laboratory water temperature was adjusted to match that of the SCR for the remainder of the test. Thus, although the metamorphosis of most glochidia into juveniles may have been completed by the date when peak excystment was predicted to start (*i.e.*, 17 Jun. 2004 when CTUD exceeded 395 C·d), most juveniles did not excyst from host fish until daily mean water temperatures remained in the 17–20 C range. Repetition of this test in 2004–05 provided corroborating evidence that the 17–20 C cue and ≥ 395 C·d of cumulative development are needed to trigger peak excystment of juveniles from channel catfish (Fig. 4d). Thermal cues and summation effects (analogous to CTUD) can initiate distinct events necessary for successful mussel reproduction (Hastie and Young, 2003). An early life history strategy that requires a combination of thermal criteria to initiate peak excystment (*i.e.*, as we describe for SCR winged mapleleaf) may help increase natural survival by ensuring the availability of an adequate food supply in biologically productive waters.

Our findings also indicate that prevailing water temperatures in the SCR are typically below the empirically derived low-temperature threshold for five to six consecutive months. The continued early life development of winged mapleleaf is thus precluded here during portions of three successive seasons. Therefore, at northern latitudes like this and conceivably throughout much of its historical range, the winged mapleleaf represents one species that is an exception to the claim that many overwintering glochidia complete a portion of their development on the host at winter temperatures (Watters and O'Dee, 1999). Furthermore, 2005 water temperatures in the SCR (U.S. Geological Survey, 2006) suggest that winged mapleleaf may attain up to 46% of the CTUD needed to initiate peak juvenile excystment within 5 wk after host excystment. These encysted glochidia would require less time to complete metamorphosis in the spring and are likely to excyst sooner than other mussel species (*e.g.*, those that infect host fish with glochidia in the spring) which may give this cohort of juvenile winged mapleleaf an advantage in competing for critical resources (*e.g.*, food, habitat) during the initial year of growth.

The period of encystment preceding the peak recovery of juveniles held under a natural thermal regime decreased by 2 d from Test III (261 d in 2003–04) to Test IV (259 d in 2004–05). Likewise, the predicted period of encystment for winged mapleleaf that developed in the SCR decreased by 19 d (from 265 d in 2002 to 246 d in 2006) among a group of recent cohorts when equation 2 was applied to appropriate portions of the

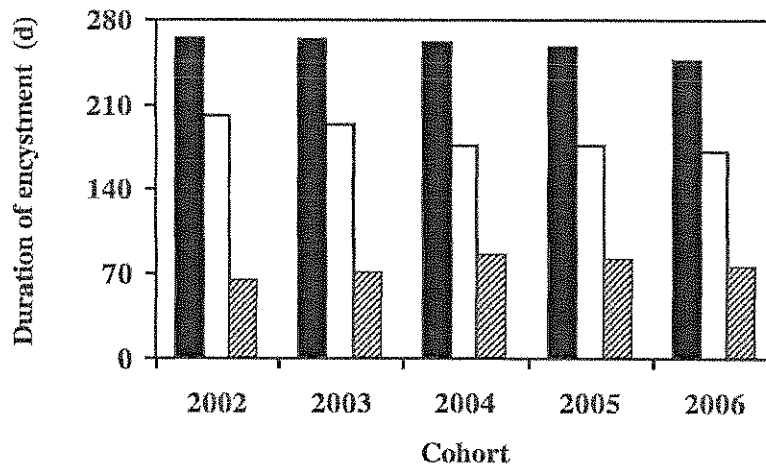


FIG. 5.—Predicted duration of glochidial encystment for recent cohorts of winged mapleleaf mussels that may have developed on channel catfish in the St. Croix River at St. Croix Falls, Wisconsin (black bar – calculated total duration of encystment; white bar – portion of total duration when mean daily water temperature ≤ 9.2 C; hatched bar – portion of total duration when mean daily water temperature ≥ 9.3 C). Calculations are based on a hypothetical annual exposure date of 30 Sep., mean daily water temperatures reported by Waschbusch *et al.* (2003, 2004, 2005, 2006) and the U.S. Geological Survey (2006), and empirically derived thermal criteria for development of encysted glochidia (present study)

historical record of daily mean SCR water temperatures (Fig. 5). Periods that accounted for no glochidial development (*i.e.*, ≤ 9.2 C) decreased by 30 d (from 201 d in 2002 to 171 d in 2006) over the 5-y span of this comparison, meanwhile periods that accounted for glochidial development (*i.e.*, ≥ 9.3 C) increased by as much as 21 d (from 64 d in 2002 to 85 d in 2004) during this time. These trends reflect small increases in the average temperature of the SCR (which accelerated glochidial metamorphosis) during five consecutive autumn through spring periods. Reduced periods of seasonal ice cover have been noted for many rivers and lakes in the Northern Hemisphere since the mid-19th Century and are regarded as evidence that freshwater ecosystems are responding to warming trends that reflect patterns of climate change and global warming (Magnuson *et al.*, 2000; Sagarin and Micheli, 2001). Significant phenological changes have also been noted for a variety of terrestrial species in Wisconsin since the mid-20th Century that indicate an advanced onset of warm weather conditions early in spring throughout much of this state, including the SCR watershed (Bradley *et al.*, 1999; Zhao and Schwartz, 2003). Therefore, an early life-history strategy with indices of thermal precision as great as those which we have described for a sedentary poikilotherm, like the winged mapleleaf, may also represent quantifiable, site-specific standards that could be used as biomonitoring tools to detect phenological changes in the aquatic environment and may reflect seasonal climatic changes within a watershed (Stefan and Sinokrot, 1993; Langan *et al.*, 2001).

IMPLICATIONS FOR MUSSEL RECOVERY

Limited information is available for the early life stages of mussels, particularly species that remain encysted on host fish throughout winter. Watters and O'Dee (1999) infected largemouth bass [*Micropterus salmoides* (Lacepède)] with plain pocketbook [*Lampsilis cardium* (Rafinesque)] glochidia and reared them in a wide range of temperatures (5–21 C)

that were maintained for 66 to 86 d in regimes similar to those which we followed during Tests I and II. Based solely on the recovery of juveniles, they concluded the low-temperature threshold for plain pocketbook metamorphosis on largemouth bass was between 10 and 15 C. A more precise and absolute (*i.e.*, independent of the observational test period) estimate of the low-temperature threshold for continued early life mussel development could be derived by applying equation 1 to data from this past study and other suitable thermal investigations. This may reveal similarities and differences in thermal criteria for metamorphosis of encysted glochidia on a variety of host fish species that could be used to improve mussel propagation efforts.

Our results highlight the importance of learning more about the early life development of mussels to help establish successful propagation programs. Although we determined both blue and channel catfish are hosts for winged mapleleaf, the composition of these species in localized fish communities should be evaluated to select the most appropriate host, or combination of hosts, for each site where juvenile introductions are planned. Likewise, the natural thermal regime should be documented at each introduction site to help select appropriate water temperatures during controlled propagation. Certain procedures used to propagate the winged mapleleaf are similar to those used to propagate the Federal endangered Higgins' eye pearl mussel [*Lampsilis higginsii* (Lea); U.S. Fish and Wildlife Service, 2002], including the movement of exposed host fish from hatchery raceways to cages in rivers before juveniles excyst (R. Gordon, pers. comm.). The thermal development data derived from this study can be used to predict when peak excystment will occur and reduce the length of time that exposed host fish are caged in rivers where they are vulnerable to mortality from stochastic events. These practices should ensure the timely introduction of juveniles to the benthic community and increase chances for their survival because they are synchronized phenologically to their local environment. Determination of thermal criteria for early life stage development of other threatened and endangered mollusks may likewise benefit the recovery of additional species with propagation programs.

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