

## **Winged Mapleleaf Mussel Early Life History Investigations Conducted by the U.S. Department of the Interior in FY 2004**

### ***Executive Summary***

The implementation of plans to restore populations of the winged mapleleaf mussel (*Quadrula fragosa*) has been hindered for years by a lack of adequate early life history information for this federal endangered species, including the identity of its host fish species. Early life history investigations conducted by agencies of the U.S. Department of the Interior in fiscal year 2004 identified two suitable host fish species upon which winged mapleleaf glochidia will transform into juvenile mussels: the blue catfish (*Ictalurus furcatus*) and the channel catfish (*I. punctatus*). Analysis of thermal data collected during these studies also revealed apparent threshold temperatures necessary for transformational development to proceed without interruption (>9.24°C) and for transformed juveniles to release from host fish (17-20°C). These fundamental early life history findings can be applied to develop ecologically relevant, site-specific propagation strategies to help restore winged mapleleaf populations throughout the species historic range and may guide the development of improved propagation strategies to help recover other listed mussel species.

### ***Introduction***

The winged mapleleaf mussel, *Quadrula fragosa* (Conrad 1835), is a federal endangered species that has received considerable management attention in recent years. This species historically inhabited at least 34 river systems in 12 Midwestern states (U.S. Fish and Wildlife Service, 1997). However, just four populations are currently known to exist and the only confirmed reproducing population inhabits a 10-mile stretch of the St. Croix National Scenic Riverway bordering Minnesota and Wisconsin. Efforts to recover the species are presently focused on the St. Croix River population which is at risk due to zebra mussel infestation, the effects of variable water releases at an upstream hydropower dam, and an incomplete knowledge of its life history. Among the recognized factors that are limiting recovery efforts for members of this apparent host overwintering (i.e., fall-released glochidia) mussel population (Watters and O'Dee, 2000; David Heath, Wisconsin Department of Natural Resources, pers. comm.) is a lack of early life history information, particularly regarding which species of fish can serve as a host for its parasitic glochidia (larvae; U.S. Fish and Wildlife Service, 1997).

Since 1997, a team of biologists working at the University of Minnesota has conducted research to identify potential host fish for the winged mapleleaf mussel. Beginning in 2001, Department of the Interior colleagues working in western Wisconsin at the U.S. Fish and Wildlife Service's La Crosse Fishery Resources Office and Genoa National Fish Hatchery, the National Park Service's St. Croix National Scenic Riverway in St. Croix Falls, and the U.S. Geological Survey's Upper Midwest Environmental Sciences Center (UMESC) in La Crosse joined other team members in annual cooperative efforts to expand and accelerate the laboratory host fish identification program by making use of the well-equipped aquatic research facilities at the UMESC. This report summarizes the results of winged mapleleaf mussel early life history studies at the UMESC that were initiated in autumn 2003 by an interagency team of Interior Department employees and which concluded nine months later.

### ***Materials and Methods***

Prior to autumn 2003, more than 60 species of fish in 14 taxonomic families had been investigated at the University of Minnesota as potential hosts for the parasitic glochidia of the winged mapleleaf mussel. These earlier efforts achieved limited success because the long-term tests conducted here were often beset by problems (e.g., fish mortality) that yielded inconclusive results (Mark Hove, University of Minnesota, pers. comm.). This work was also limited by the ability of divers to locate sufficient numbers of female mussels in the fall that were brooding mature glochidia and willing to release them. Despite such difficulties, results from some of these earlier investigations suggested that one or more Ictalurid species might successfully transform large numbers of winged mapleleaf glochidia into juveniles.

In July 2003, an interagency dive team stockpiled adult winged mapleleaf mussels near one another in the St. Croix River to increase chances for successful reproduction. Divers returned in September and collected several gravid females that later released large numbers of glochidia for testing.

These glochidia were subsequently determined to be viable (Zale and Neves, 1982) and used to infest the gills of four Ictalurid species (*Ictalurus furcatus* - blue catfish, *I. punctatus* - channel catfish, *Pylodictus olivarius* - flathead catfish, and *Noturus exilis* - slender madtom) on four trial dates in autumn 2003 (Table 1). One group of blue catfish and one group of channel catfish (5 fish

per group) were infested each day by placement in separate 10-L buckets, each containing 2 L of vigorously aerated well water and an adequate volume (120-175 mL) of a glochidial slurry. One fish was removed from the bucket at 10-min intervals, anesthetized with tricaine methanesulfonate, and briefly examined with a dissection microscope to enumerate gill-encysted glochidia. The infestation was terminated when 25 or more glochidia were found attached to lamellae on either the right or left gill arches. This procedure was typically completed in little more than 10 min for blue catfish and 20 min for channel catfish, however both species were heavily infested (30-50 glochidia or more) after just 10 min on the third date (3 October). Several groups of flathead catfish and slender madtom (2 fish per group) were likewise exposed to glochidia using the bucket method, but none of these fish attained an infestation level comparable to the blue catfish or channel catfish in a similar amount of time. Therefore, remaining groups of flathead catfish and slender madtom were anesthetized and then infested by pipetting a small volume (5-15 mL) of the glochidial slurry directly onto lamellae on both the right and left gill arches. Infested fish were subsequently assigned to one of three water temperature test treatments (Tests I, II, and III; Table 1).

*Test I* – In order to accelerate the glochidial transformation process during this test, each group of infested fish was placed into a 38-L glass aquarium and received a continuous flow (480-540 mL/min) of heated, aerated well water that was thermostatically controlled to maintain an unseasonably warm and constant temperature of 19.6°C. A removable plastic mesh screen (25-mm<sup>2</sup> openings) was positioned 3.5 cm above the bottom of each aquarium as a false-bottom to minimize direct interactions of fish with detached mussels. Fish were offered food at least once per week. The temperature and dissolved oxygen content of the water was measured in each aquarium daily throughout most of the test period using a calibrated dissolved oxygen meter (YSI model 58). Given the uncertain length of time required for winged mapleleaf to transform from the glochidial to the juvenile stage at this test temperature, the screen and all fish were briefly removed from each aquarium once or twice a week to facilitate the collection of detached mussels by filtering water siphoned from along the glass bottom (Waller and Holland-Bartels, 1988) through a series of sieves (202- $\mu$ m, 153- $\mu$ m, and 53- $\mu$ m Nitex® mesh). Materials retained on each sieve were rinsed into a glass Petri dish, illuminated with cross-polarized visible light, and examined using a dissection microscope to enumerate sloughed glochidia and transformed juveniles. Juveniles were considered viable if foot movement, ciliary action, or valve opening

and closing was observed. Once successful transformation was detected for a group of test fish, siphoning to collect juveniles in the aquarium was performed almost daily throughout the remainder of the trial. The consecutive 5-d period during each trial when the maximum number of viable juveniles was recovered from an aquarium was considered the period of peak juvenile excystment. Photographic images of representative mussels collected intermittently during juvenile development were analyzed with Image-Pro Express® software to measure valve dimensions as an index of growth.

*Test II and III* – Only channel catfish were used as host fish in Tests II and III (Table 1). Given an abundant supply of glochidia available for use on the third infestation date (3 Oct 2003), we infested an additional 10 channel catfish that day and subsequently held them in a large (1900-L capacity) tank supplied with a continuous flow of 12.5°C well water for more than 6 weeks. After 45 d, five fish were selected for Test II and acclimated to warmer temperatures over a 2-d period before placement in individual 38-L aquaria continuously supplied with heated, aerated well water (480-540 mL/min) that maintained a constant temperature of 19.5°C (i.e., conditions similar to Test I). Meanwhile, the five remaining fish were used for Test III and subsequently maintained in a thermal regime that closely followed the reported daily mean water temperature of the St. Croix River at the U.S. Geological Survey gauging station in St. Croix Falls, Wisconsin, from mid-November 2003 through June 2004. To achieve this, the fish were first placed in a cage that was submerged (3-4 m) in a constructed earthen pond for five months (overwinter period) before they were retrieved and returned to the laboratory. Each fish was then placed in a 38-L aquarium 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 juvenile mussels were counted in each aquarium during Tests II and III following procedures and schedules similar to those used in Test I.

### ***Results and Discussion***

*Test I* - Approximately 11,600 viable winged mapleleaf juveniles were recovered from groups of blue catfish and channel catfish during Test I. Peak excystment of juveniles from channel catfish and blue catfish occurred 4 to 5 weeks and 5 to 6 weeks after infestation, respectively, at 19.6°C (Figure 1). However, 95% of the juveniles were produced by 20 blue catfish while the 20

channel catfish accounted for the remainder. Based on the total number of juveniles and sloughed glochidia that were recovered during the four trials, the rate of successful glochidial transformation was 50% on blue catfish and only 7% on channel catfish. Mussels that developed on both of these host fish species exhibited considerable growth during the transformational period (a rarely known event for other mussel species) when dimensions of the juvenile valve typically enlarged 2 to 3-times beyond those of the existing glochidial valve (Figure 2). Meanwhile, no juveniles were recovered from either flathead catfish or slender madtom.

These results suggest that although both blue catfish and channel catfish can act as a host fish for winged mapleleaf glochidia, blue catfish appear to be a more suitable host. However, the channel catfish used in this test were considerably smaller in size than the blue catfish (mean wet weight = 18 g and 60 g, respectively). Consequently, it is unknown to what extent differences in initial glochidial attachment and subsequent transformation success may reflect natural differences in the host-parasite relationship among these species, a confounding bias due to the non-uniform size of fish used, or a combination of these factors. Therefore, it is recommended that this test be repeated with equivalent-sized hosts (i.e., only blue catfish and channel catfish) to resolve this uncertainty.

The number of juveniles recovered during Test I trials varied as much as 7-fold with more mussels recovered from fish infested on 3 October (Trial 3) than on any other date (Figure 1). This suggests that glochidia used to infest fish on 3 October were the most physically fit individuals tested and that results achieved with this collection of glochidia were likely more reliable than any other. By chance, these were the same glochidia used to infest fish for Tests II and III.

*Test II* - About 9,300 viable winged mapleleaf juveniles were recovered from five channel catfish during Test II (Figure 3). When compared to Test I results, this finding represented a 62-fold increase in the mean number of juveniles produced per channel catfish (30 juveniles/fish in Test I vs. 1,867 juveniles/fish in Test II). This large difference may be explained by a combination of factors. First, the mean wet weight of channel catfish used in Test II (156 g) was substantially greater than that of the channel catfish (18 g) and blue catfish (60 g) used in Test I.

This size difference likely provided greater gill tissue surface area for more glochidia to successfully encyst on the larger host fish. In addition, we presume that many more glochidia were used to infest fish for Test II than for Test I. Regardless of the reasons, these results are useful to resource managers who want to develop ecologically relevant propagation strategies to help recover winged mapleleaf populations throughout the species historic range, including the St. Croix River and other northerly sites where channel catfish are typically abundant year-round while blue catfish are usually absent or rare.

Peak excystment of juvenile mussels during Test II occurred 10 to 11 weeks after infestation (Figure 3). This was 6 weeks later than that observed for channel catfish during Test I. This additional period corresponded closely to the length of time (45 d) that fish in Test II were maintained at 12.5°C immediately after infestation. Once these fish were subsequently maintained in 19.5°C water however, juvenile excystment peaked about one week sooner than it had during Test I (i.e., within four weeks rather than five weeks). These findings suggest that the physiological mechanisms responsible for the transformation of winged mapleleaf glochidia into juveniles on channel catfish did not stop but proceeded more slowly at colder temperatures.

Rates of early life development for poikilotherms such as embryonic fish have been quantified for many species commonly reared in hatcheries 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 however, a correction factor may need to be introduced to account for periods when the temperature drops below the developmental threshold for a particular species. A species specific threshold temperature 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 developmental threshold temperature of 5.8°C for embryonic lake sturgeon (*Ascipenser fulvescens*) based on field observations gathered over several spawning seasons and applied it to provide a highly reliable means of predicting the date of egg hatching despite widely fluctuating water temperatures.

If thermal relationships like those used to predict the development of embryonic fish similarly influence the rate at which encysted glochidia develop into juvenile mussels, then knowledge of the developmental threshold temperature for a particular mussel species could be used to help predict when its glochidia will complete transformation and detach from host fish as free-living juveniles. Given a nearly complete record of the daily water temperature and the total number of juveniles recovered from each aquarium (i.e., group of fish or individual fish) during Tests I and II, a comparison was made of the cumulative daily water temperature units required to initiate peak excystment of winged mapleleaf juveniles from channel catfish reared in these two thermal regimes. This allowed us to empirically estimate the temperature threshold (i.e., the minimum mean daily water temperature) required for these channel catfish-encysted glochidia to transform into free-living juveniles. However, rather than determine this value through an indirect statistical means as Kempinger (1988) did, we noted that the value could be derived logically by solving this algebraic equation

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

for one unknown variable ( $x$ ) where

$T_1 \approx T_2$ , and

$T_1$  = the constant water temperature in Test I (i.e., 19.6°C);

$T_2$  = a constant water temperature in Test II (i.e., 19.5°C);

$d_1$  = the number of days from infestation to start of peak juvenile excystment in Test I (i.e., 31);

$d_2$  = the number of days that  $T_2$  was maintained in Test II (i.e., 23);

$W_i$  = the mean daily water temperatures  $\neq T_2$  in Test II (i.e., eight values from 12°C to 17°C);

$d_i$  = the number of days a  $W_i$  value occurred in Test II (i.e., two values: 1 and 40);

$n$  = the number of different mean daily water temperatures observed in Test II (i.e., 9); and,

$x$  = the mean daily threshold temperature (°C) for development of winged mapleleaf glochidia on channel catfish.

The left-side of this equation reflects the difference in the length of time that glochidial development occurred at the same (or very similar) constant temperature(s) during Tests I and II; meanwhile, the right-side of this equation reflects the cumulative temperature-time interactions that account for this difference during Test II when water temperatures were cooler. This equation could thus be rewritten as

$$(19.6^{\circ}\text{C} \cdot 31\text{d}) - (19.5^{\circ}\text{C} \cdot 23\text{d}) = \sum (12.5^{\circ}\text{C} - x)40\text{d} + (11.8^{\circ}\text{C} - x)1\text{d} + (12.0^{\circ}\text{C} - x)1\text{d} + (13.7^{\circ}\text{C} - x)1\text{d} \\ + (12.8^{\circ}\text{C} - x)1\text{d} + (14.0^{\circ}\text{C} - x)1\text{d} + (13.0^{\circ}\text{C} - x)1\text{d} + (17.0^{\circ}\text{C} - x)1\text{d}$$

by substituting observed test values for all known terms and solved for the unknown variable ( $x$ ), yielding  $9.24^{\circ}\text{C}$  as the estimated mean daily temperature threshold for development of winged mapleleaf glochidia on channel catfish (i.e., there is no net daily development for glochidia at or below this temperature).

Depictions of the time required to achieve peak excystment of juveniles from blue catfish and channel catfish infested on 3 October during Tests I and II illustrate the substantial influence that water temperature has on the rate of glochidial development (Figure 3). This thermal-based influence is likewise apparent when the peak excystment of juveniles from these fish is compared based on the cumulative temperature units (CTU) of incubation (Figure 4). However, when the threshold temperature constant we derived is applied to (i.e., subtracted from) all of the mean daily water temperature values that exceeded it during each of these tests and the ensuing values are summed to account for the CTU of anticipated glochidial development while encysted on channel catfish (or blue catfish), as in the equation

$$\text{CTU}_{\text{development}} = \sum_{i=1}^n (T_i - 9.24^{\circ}\text{C})d_i$$

where  $T_i$  = a mean daily water temperature  $> 9.24^{\circ}\text{C}$ ,

$n$  = the number of different mean daily water temperature values  $> 9.24^{\circ}\text{C}$ , and

$d_i$  = the number of days this  $T_i$  value occurred,

it results in the near alignment or broad overlap of the peak juvenile excystment periods for these different groups of host fish (Figure 5). This outcome demonstrates the potential utility of a biological temperature constant, like that which we empirically derived, to normalize widely varying water temperature data when evaluating early life development of an aquatic poikilotherm. Based on the large number of juveniles recovered from blue catfish in Test I and channel catfish in Test II and the remarkable similarity in the peak juvenile excystment periods for these groups of fish when the data are normalized and expressed as a function of thermal-related development, we conclude that peak excystment of juvenile winged mapleleaf from blue catfish and channel catfish should begin at about  $382^{\circ}\text{C}\cdot\text{d}$  and  $395^{\circ}\text{C}\cdot\text{d}$  of development,

respectively. These findings should facilitate relatively accurate predictions of the time required for winged mapleleaf glochidia to transform into juveniles while attached to host fish in laboratories, hatcheries, or natural settings and can thus be used as a valuable tool in recovery efforts for this endangered species throughout its historic range.

*Test III* – A total of 3,450 viable winged mapleleaf juveniles were recovered from five channel catfish in during Test III. All of these mussels were recovered in June 2004 during the final 27 d of this 270-d test (Figure 6) when estimated cumulative thermal development ranged in excess of 355°C•d (Figure 7). Peak excystment occurred 262-266 d after infestation (i.e., 21-25 June 2004) when transformed glochidia were estimated to have attained 453-501°C•d of cumulative development. Based on findings from Tests I and II that peak excystment begins at 395°C•d of cumulative development, we predicted that peak juvenile excystment during Test III should begin 255 d after infestation (i.e., 14 June 2004). Thus by applying the threshold temperature constant for development of winged mapleleaf glochidia on channel catfish that we derived from Tests I and II to the daily mean water temperature data collected during Test III, we successfully predicted the dates of peak juvenile excystment to within 1 week during a 37-week developmental period, representing an accuracy error rate of <3%. These results confirm the validity of this approach as a useful tool to quantify the period of thermal development required to account for the transformation of winged mapleleaf glochidia into juveniles on channel catfish and may likewise be useful to quantify such early life changes for other mussel species on their host fish.

Although the peak period of juvenile mussel excystment during Test III began 7 d later than we expected and correspondingly appeared to require an additional 58°C•d of development, viable juveniles were recovered for 6 consecutive days immediately preceding the peak and began to do so at 398°C•d of development when the water temperature was still relatively cold (12.6°C). Fish were purposely maintained at this cold level for 2 months after they were retrieved from the pond to slow the rate of glochidial development in the laboratory until cumulative development here matched the estimated level for the St. Croix River (Figure 7). Due to cold spring weather however, this did not occur until 259 d after infestation (i.e., 18 June 2004) when cumulative development was approximately 429°C•d at both locations and the laboratory water temperature

was finally adjusted to match that of the river for the remaining 11 d of the test (Figures 6, 7). These data thus suggest that although the physiological transformation of most glochidia into juveniles may have been completed by the time we predicted peak juvenile excystment to begin (i.e., on 14 June 2004 at 395°C•d of development), these individuals did not release from their host fish in large numbers until daily mean water temperatures were maintained in the 17-20°C range, which seemed to act as a thermal cue and trigger the start of this long anticipated event. A life-history strategy with such a thermal-linked release mechanism may help to increase early life survival by ensuring that an adequate food supply is available (a result of increased biological productivity with sustained increases in water temperature) when free-living juveniles deplete stored nutritional reserves derived from their host fish and must begin filter feeding to survive.

Finally, the physical appearance of juveniles recovered during Test III was remarkably different from that of juveniles recovered during Tests I and II. Regardless of the host fish species, juveniles recovered during Tests I and II (conducted at unusually warm temperatures that required exceptionally brief developmental periods for a host overwintering mussel species) had valves that typically exhibited non-symmetrical (i.e., lop-sided) growth beyond the original glochidial valve and a uniform convex surface that displayed little or no topographic variation (Figure 8A). Meanwhile, juveniles recovered during Test III (conducted over a span of three seasons in a more natural thermal regime for a host overwintering mussel) had valves that regularly displayed symmetrical growth beyond the original glochidial valve and unique, irregular convex surfaces with a variety of topographic features (e.g., pustules, ridges, sulcus) characteristic of more mature winged mapleleaf (Figure 8B). Juveniles produced from all three tests were held under a variety of experimental conditions (i.e., feeding and temperature regimes) in attempts to maintain them. Notable valve growth was observed for many individuals during these efforts (Figure 9) but most juveniles died within 4-6 weeks after excystment, likely due to nutritional deficiencies. Therefore, it is unknown whether the valves of juveniles produced during Tests I and II would have eventually developed a more characteristic appearance.

### ***Conclusions***

The results of this study highlight the importance of learning more about the early life development of endangered mussels (i.e., besides host fish identity) to aid in establishing

successful propagation programs, especially for species with wide-ranging historic distributions and multiple hosts like the winged mapleleaf. Although we determined that blue catfish and channel catfish can both serve as hosts for winged mapleleaf glochidia to transform into juveniles, the composition of these species in localized fish communities should be carefully considered to select the most appropriate host, or combination of hosts, for each site where juvenile mussel introductions are planned. Likewise, the natural thermal regime should be documented at each introduction site to guide in selecting the duration of seasonally appropriate water temperatures during controlled propagation in hatcheries. Certain procedures used to propagate the winged mapleleaf are likely to be similar to those already used to propagate the Higgins' eye pearl mussel (*Lampsilis higginsii*; U.S. Fish and Wildlife Service, 2002), including the movement of infested host fish from hatchery raceways to cages in rivers prior to juvenile excystment (Roger Gordon, Genoa National Fish Hatchery, pers. comm.) The thermal development information derived from this study can thus be used by hatchery managers to predict when peak excystment will occur and reduce the length of time that infested host fish must be caged in rivers, where they are vulnerable to a variety of potentially catastrophic events (e.g., disease, flooding, siltation). Propagation practices like these should ensure the timely introduction of vigorous juveniles to the benthic community (in as natural a manner as possible) that will have good chances for early life survival because they are genetically well adapted and phenologically synchronized to their surrounding environment. The determination of corresponding early life thermal requisites for other threatened and endangered mollusks could likewise benefit the recovery of additional species with active propagation programs.

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Table 1. Experimental design of tests to identify host fish species for winged mapleleaf mussel glochidia at the UMESC.

| Test | Fish species     | Total length<br>(range; mm) | Number of<br>fish per trial | Number<br>of trials | 2003 Trial<br>start date(s) | Mean daily water<br>temperature and duration                             |
|------|------------------|-----------------------------|-----------------------------|---------------------|-----------------------------|--|
| I    | Blue catfish     | 163 - 213                   | 5                           | 4                   | 29 Sep; 1, 3, 6 Oct         | 19.6°C for 49 d  |
|      | Channel catfish  | 125 - 161                   | 5                           | 4                   | 29 Sep; 1, 3, 6 Oct         | 19.6°C for 49 d  |
|      | Flathead catfish | 238 - 286                   | 2                           | 4                   | 29 Sep; 1, 3, 6 Oct         | 19.6°C for 49 d  |
|      | Slender madtom   | 108 - 122                   | 2                           | 4                   | 1, 3 Oct                    | 19.6°C for 49 d  |
| II   | Channel catfish  | 263 - 300                   | 5                           | 1                   | 3 Oct                       | 12.5°C for 45 d, 16.0°C for<br>2 d, then 19.5°C for 48 d                 |
| III  | Channel catfish  | 237 - 263                   | 5                           | 1                   | 3 Oct                       | 12.5°C for 45 d, then mimic<br>St. Croix River<br>temperatures for 225 d |

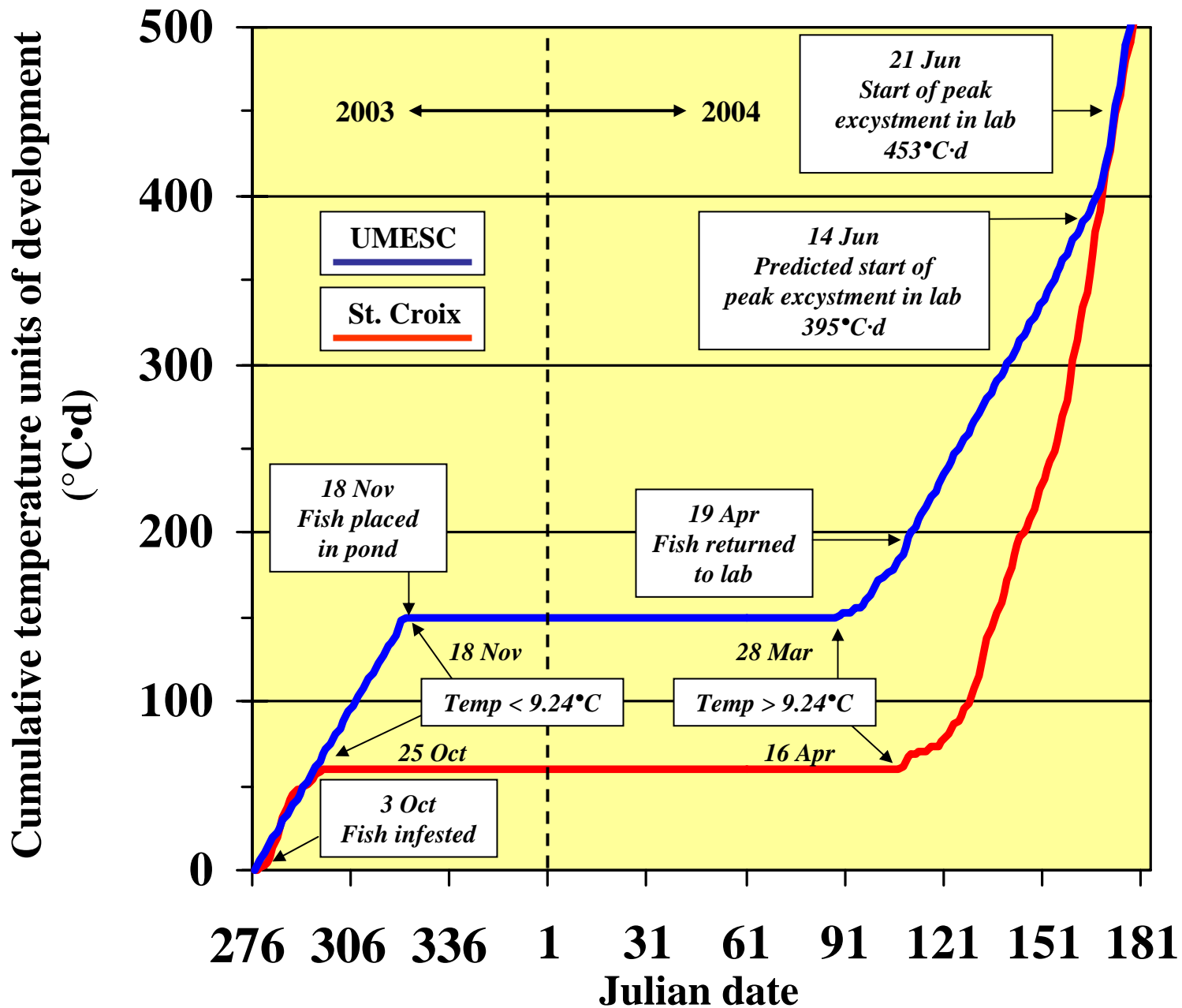


Figure 7. Predicted cumulative thermal development of winged mapleleaf mussels encysted to channel catfish at the UMESC during host fish identification Test III and in the St. Croix River (hypothetical infestation) based on mean daily water temperature observations from 3 Oct 2003 to 26 Jun 2004. Note the chronology of major test events during this period.

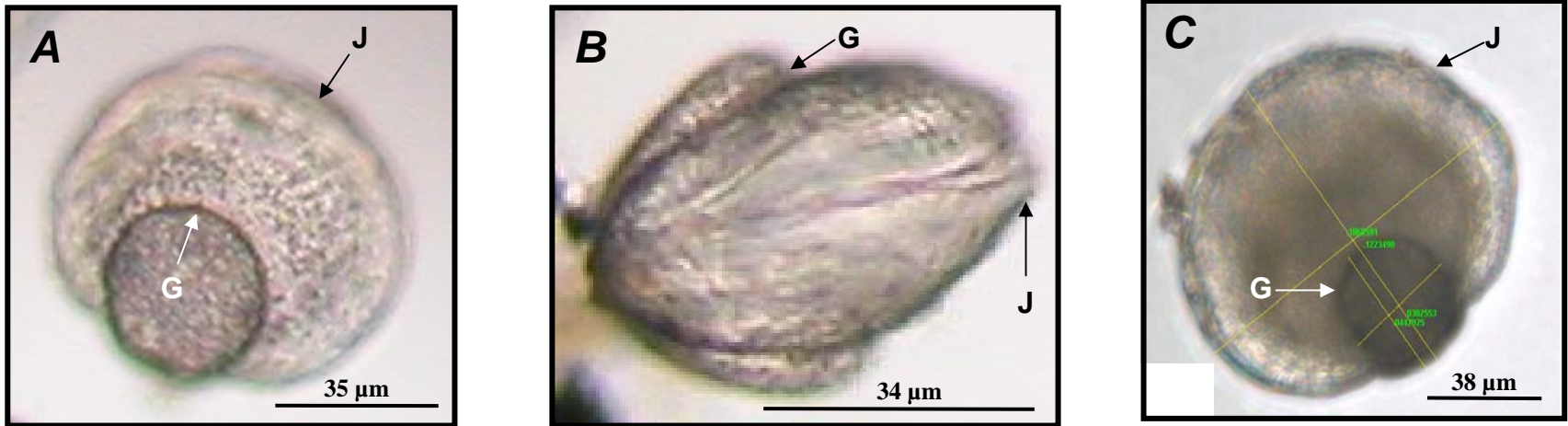


Figure 2. Valve growth exhibited by winged mapleleaf mussels during transformation from glochidia into juveniles after 21 days (**A**-dorsal view and **B**-side view) and 27 days (**C**-dorsal view) of development on blue catfish at 19.6°C. Arrows indicate the outermost margin of glochidial (**G**) and juvenile (**J**) portions of valves on these developing mussels.

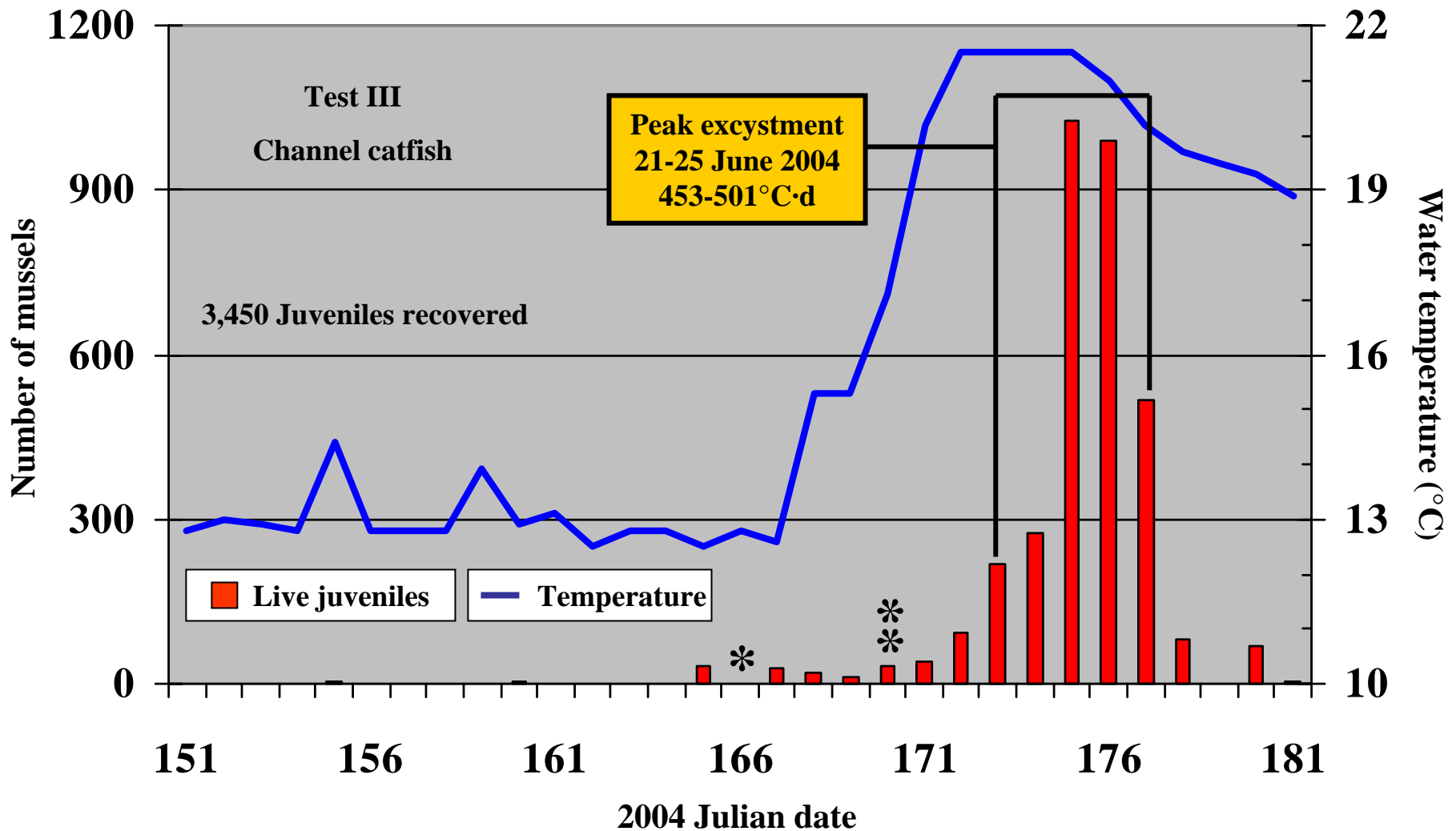


Figure 6. Temporal (30 May – 29 Jun 2004) recovery of juvenile winged mapleleaf mussels from five channel catfish infested with glochidia on 3 Oct 2003 and held for 9 months in a thermal regime that principally simulated the St. Croix River during host fish identification Test III at the UMESC. The single asterisk indicates the date (14 Jun 2004) when peak juvenile excystment from test fish was predicted to start (i.e., at 395°C•d of development); the double asterisk indicates the date (18 Jun 2004) when the predicted development of channel catfish-encysted glochidia at the UMESC and in the St. Croix River were equivalent ( $\pm 3^{\circ}\text{C}\cdot\text{d}$  or less) for the first time in 8 months. Note the total number of juvenile mussels recovered and the cumulative temperature units of development during peak juvenile excystment.



Figure 8. Winged mapleleaf juveniles recovered 36 d after infestation and development on a blue catfish held at 19.6°C during Test I (**A**) and 266 d after infestation and development on a channel catfish held at a thermal regime that simulated St. Croix River water temperatures from November 2003 to June 2004 during Test III (**B**).

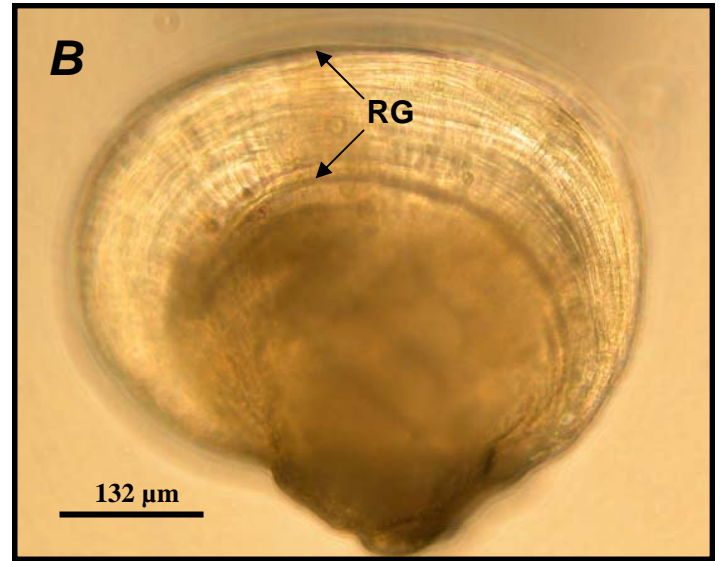
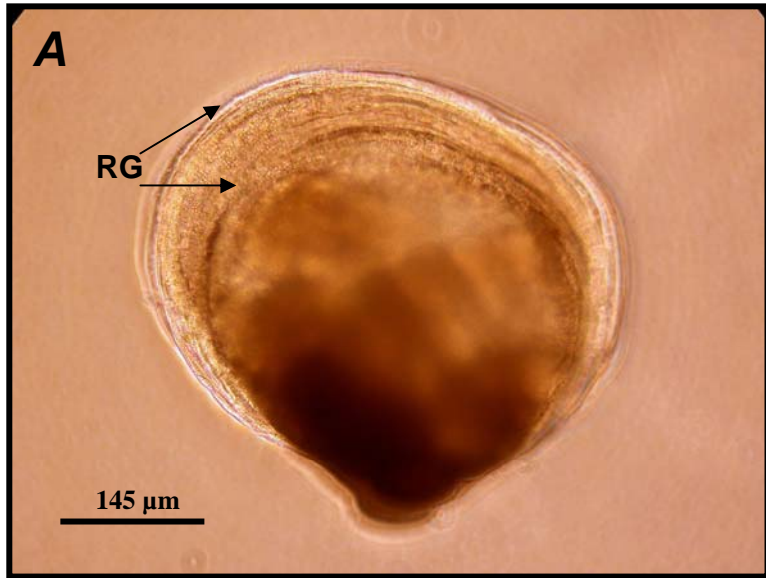


Figure 9. Winged mapleleaf juveniles that developed on channel catfish during Test III exhibit a region of recent growth (**RG**) near the outer margin of the valve 8 d (**A**) and 56 d (**B**) after peak recovery.



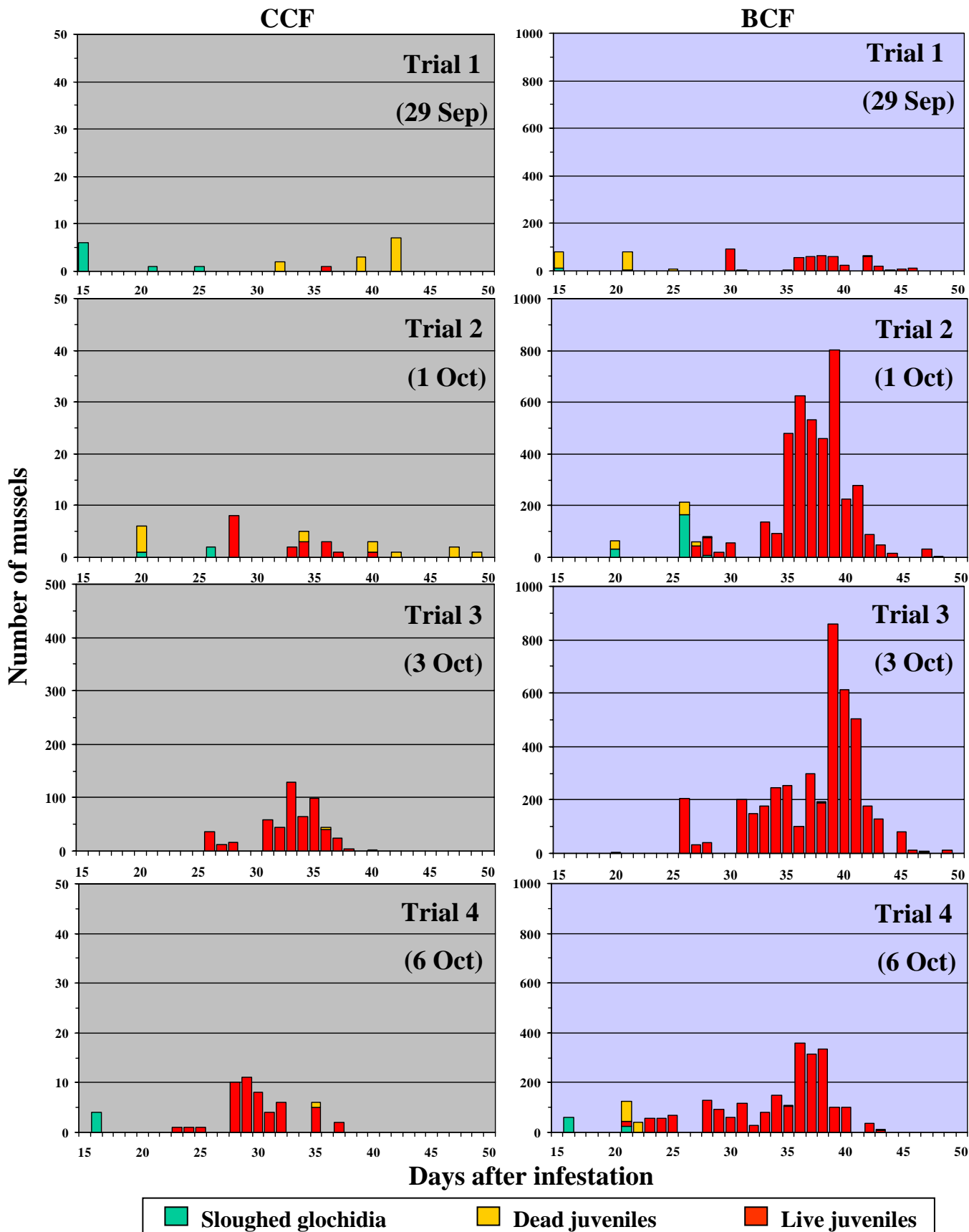


Figure 1. Temporal recovery of early life-stage winged mapleleaf mussels from channel catfish (CCF) and blue catfish (BCF) held at 19.6°C during Test I host fish identification trials at the UMESC. The trial start date (2003) when fish were infested is listed in parentheses. Mussels recovered 1-14 days after infestation are not depicted.

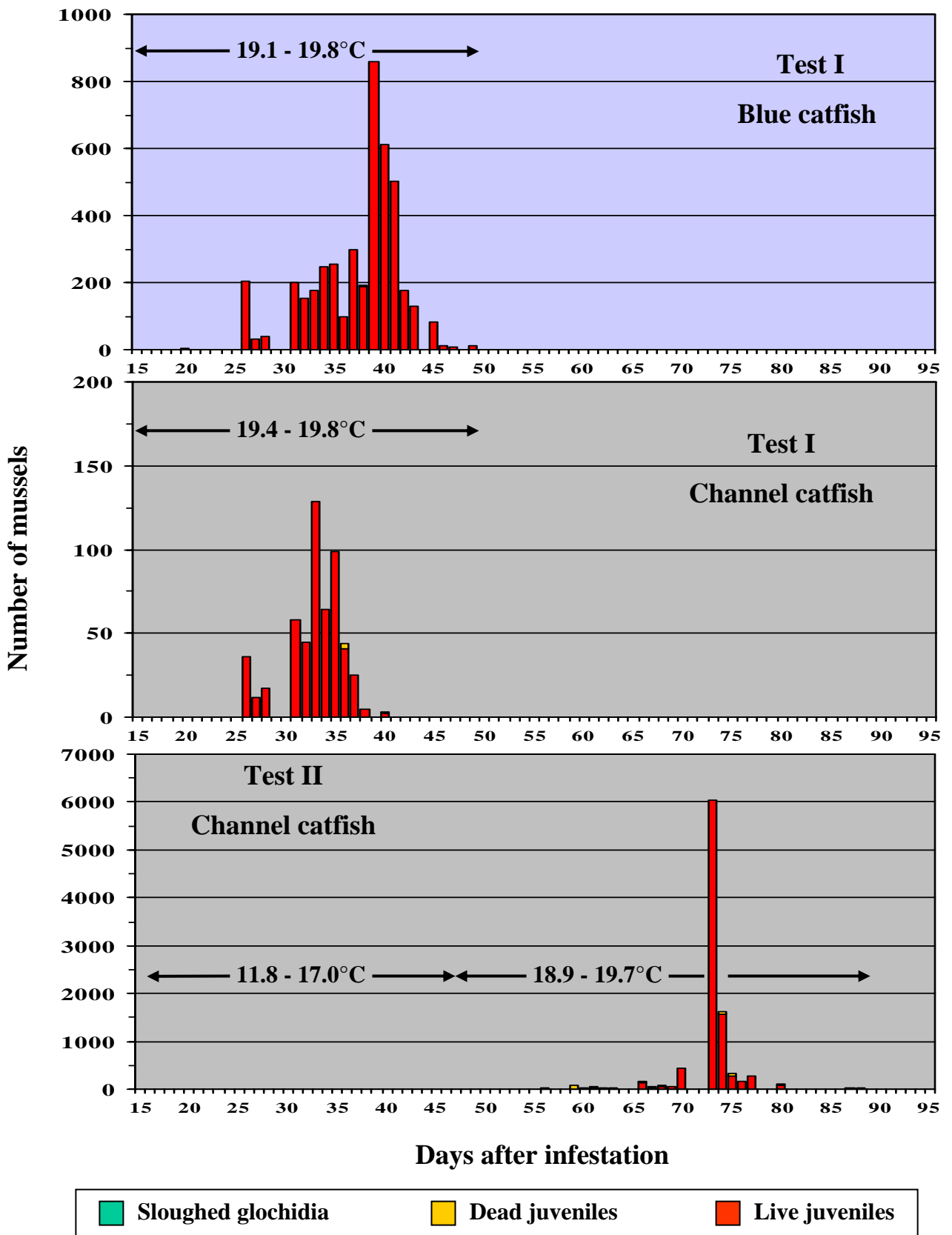


Figure 3. Temporal recovery of early life-stage winged mapleleaf mussels from three groups of test fish (five fish per group) infested with glochidia on 3 Oct 2003 during host fish identification Tests I and II at the UMESC. Note the range(s) of mean daily water temperatures during these tests.

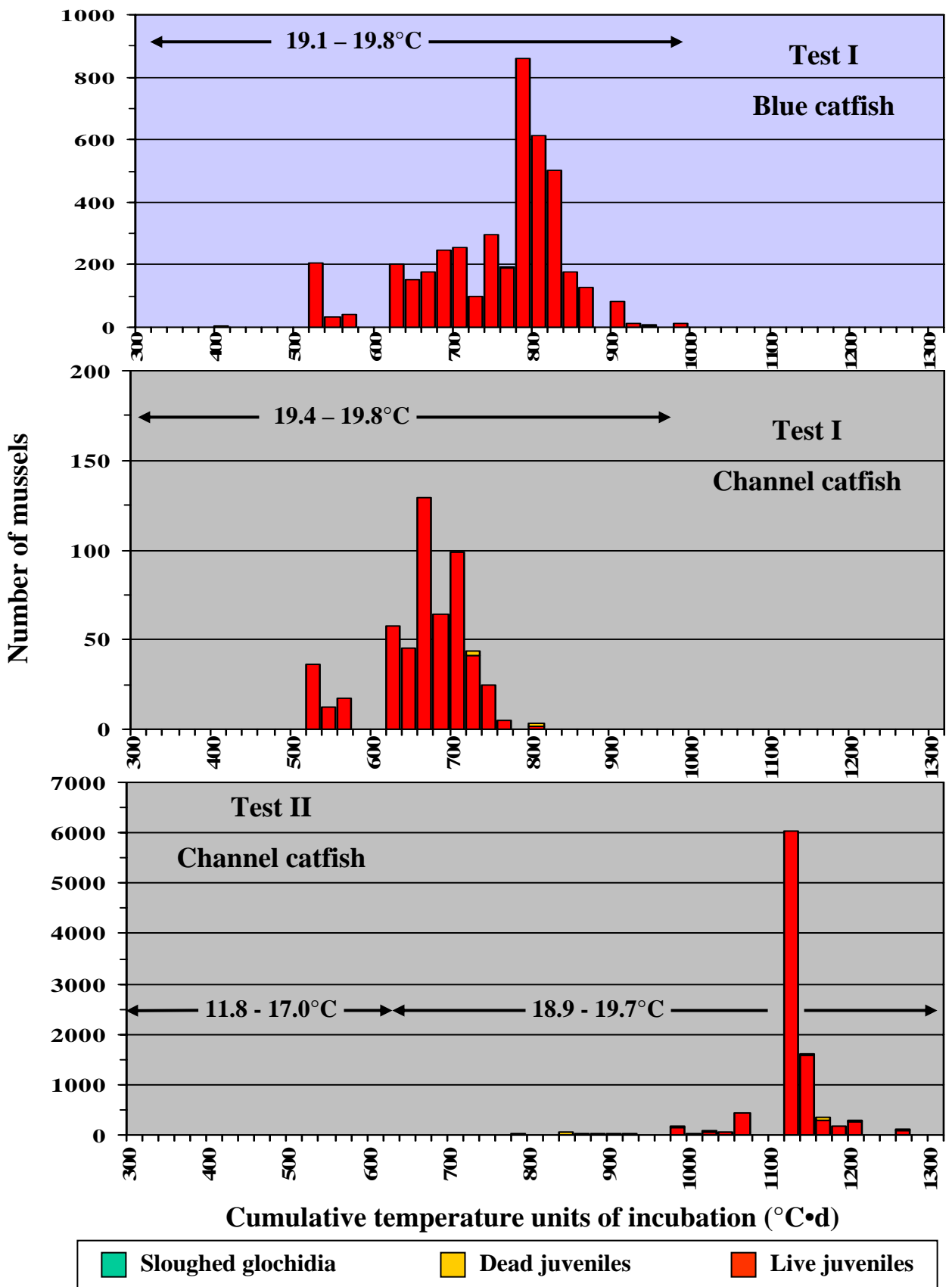


Figure 4. Thermal incubation-based recovery of early life-stage winged mapleleaf mussels from three groups of test fish (five fish per group) infested with glochidia on 3 Oct 2003 during host fish identification Tests I and II at the UMESC. Note the range(s) of mean daily water temperatures during each of these tests.

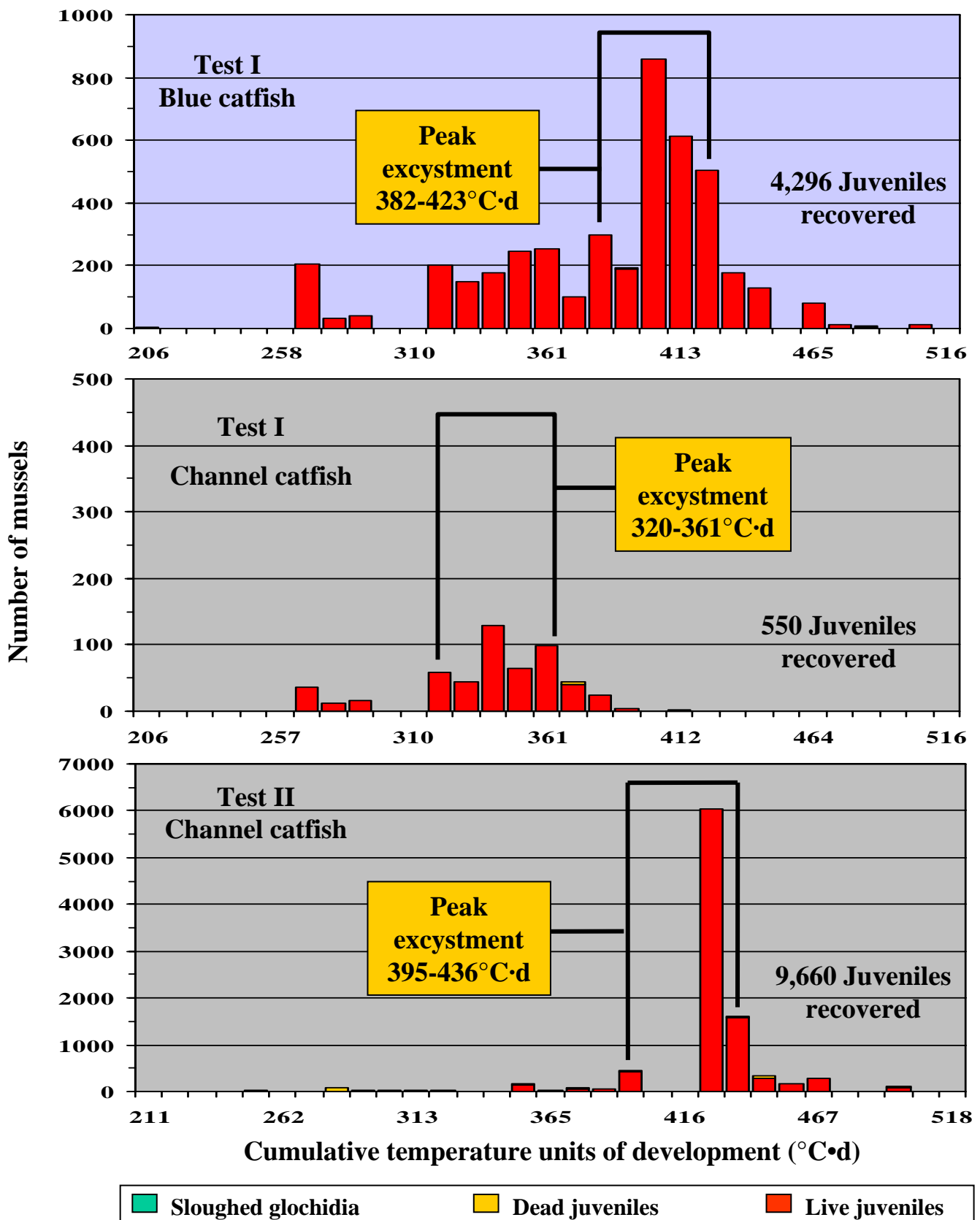


Figure 5. Thermal development-based recovery of early life-stage winged mapleleaf mussels from three groups of test fish (five fish per group) infested with glochidia on 3 Oct 2003 during host fish identification Tests I and II at the UMESC. Note the total number of juvenile mussels recovered during each test and the cumulative temperature units of development during periods of peak excystment.