Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium, 1998, pages 93-102

Differential sensitivity of hooked (*Utterbackia imbecillis*) and hookless (*Megalonaias nervosa*) glochidia to chemical and mechanical stimuli (Bivalvia: Unionidae)

Melanie K. Shadoan and Ronald V. Dimock, Jr.[1]

Department of Biology, Wake Forest University, Winston-Salem, NC 27109

ABSTRACT: The stimuli involved in the attachment of glochidia larvae of freshwater mussels to fish hosts are largely unknown, with a few observations suggesting that fish mucus or body fluids may be important cues. Specificity in the site of attachment, with hookless glochidia generally encysting on gills and hooked forms attaching to fins or opercula, may also involve differential sensitivity to chemical or mechanical stimuli. To test this hypothesis, individual glochidia of *Utterbackia imbecillis* (hooked) and *Megalonaias nervosa* (hookless) were exposed to epithelial mucus of three species of fish, to size-fractionated mucus of bluegill, and to three putative components of mucus. Larvae also were exposed to fish-conditioned water, two fish sex pheromones, and 11 amino acids. Sensory hairs of gaping glochidia were mechanically stimulated, both with and without amino acids in the test medium. Results were assessed from the number of valve adductions min^{-1} and the duration of prolonged (tonic) valve closure. Fish mucus elicited a significant increase in the rate of valve adduction in five of six fish-mussel combinations, and induced tonic closure in all combinations. Larvae of *Megalonaias* responded more actively to the fraction of bluegill mucus of < 3000 Daltons than to intact mucus or two larger molecular weight fractions. *Utterbackia* responded equally to whole mucus and the molecular weight fractions. Of the remaining chemical preparations tested, only sialic acid and the amino acids induced a significant response. *Megalonaias* and *Utterbackia* both were weakly responsive to sialic acid, but only *Megalonaias* was stimulated by the amino acids alone. A synergistic interaction between chemical and mechanical stimuli was evident for both species. In contrast to an overall greater chemical sensitivity of *Megalonaias*, *Utterbackia* was significantly more sensitive to mechanical stimulation.

Keywords: chemoreception, sensory, parasitism, mussel, freshwater, host.

The life cycle of unionid mussels includes the brooding of developing embryos to the shelled glochidium larva within modified water tubes of parental gills. Mature larvae are released and become parasitic on fish, which serve as hosts during subsequent metamorphosis to juvenile mussels. Of the nearly 300 species of unionids in North America, fish hosts are known for only about 25% (Watters 1994). However, it has recently been demonstrated that fish might not be the only suitable vertebrate hosts upon which glochidia effect successful metamorphosis (Watters and O'Dee 1998).

Morphological and behavioral features of some gravid unionids (Kraemer 1970, Hartfield and Butler 1997) probably function to elicit visually mediated responses by potential host fish that may increase the probability of successful parasitism by glochidia. It has also been implied (Davenport and Warmuth 1965), and recently demonstrated experimentally (Jokela and Palokangas 1993), that gravid mussels can detect chemical effluents from fish and respond by releasing glochidia.

The behavior of glochidia is limited to opening and closing the valves (shells). This valve adduction, although occasionally vigorous and repetitive ['clapping' or 'winking' to Lefevre and Curtis (1912)], is insufficient to generate swimming or any other locomotion. Valve adduction typically is phasic (rhythmic) but also may lead to tonic adduction, or prolonged closure of the valves (Labos *et al.* 1964). The abundance of glochidia and ease of quantifying their behavior have led to their use in physiological, pharmacological (Labos and Salanki 1963, Labos *et al.* 1964), and toxicological studies (Varanka 1986, Huebner and Pynnonen 1992, Hansten *et al.* 1996, Keller and Ruessler 1997, Jacobson *et al.* 1997). A novel application of their behavior employed the passive entry of live glochidia into the oral cavity of fish to map the dynamics of flow over the gills by monitoring the pattern of attachment of larvae to gill filaments (Paling 1968).

One feature of mussel-host associations that has not been well studied is the potential for sensory-mediated interaction between glochidia and a host that might facilitate successful attachment of larvae prior to encystment. Lefevre and Curtis (1912) suggested that hookless glochidia that typically attach to gill filaments were less responsive to mechanical stimulation than were hooked species that generally attach to external surfaces such as fins and opercula. Hookless larvae were especially responsive to

[1] For correspondence contact R.V. Dimock (Email: Dimock@wfu.edu)

chemical stimuli, responding with vigorous contractions to the blood of fish and other vertebrates (Lefevre and Curtis 1912). Since that early work, glochidia of several species have been shown to respond to fish mucus and epithelial tissue (Lukasovics and Labos 1965, Wood 1974, Young and Williams 1984) as well as to selected amino acids (Heard and Hendrix 1964). The mechanics of valve closure and attachment by glochidia have been described by Hoggarth and Gaunt (1988).

The present study examines responses of the hooked glochidia of *Utterbackia imbecillis* and the hookless larvae of *Megalonaias nervosa* to chemical and mechanical stimuli. Glochidia were exposed to epithelial mucus of fish, as well as to size-fractionated mucus, putative components of mucus, two fish sex pheromones, fish-conditioned water, and a series of amino acids. They also were subjected to mechanical stimulation of larval sensory hairs. The two species of mussels responded differently, with *M. nervosa* in general being more sensitive to chemical stimuli, while *U. imbecillis* was more responsive to mechanical stimulation. The response of both species to mechanical stimulation was more pronounced in the presence of amino acids.

Methods

Glochidia were obtained from *Utterbackia imbecillis* (Davis Pond, Mecklenburg County, NC, Aug to Oct) and from *Megalonaias nervosa* (Kentucky Lake, Benton County, TN, Nov to Jan). Adult *U. imbecillis* were maintained in the laboratory at 20 °C. Excised brooding demibranchs of *M. nervosa* were shipped chilled overnight from Tennessee, and the glochidia were used within 18-20 h after excision of the gills from live *Megalonaias*. In contrast to the observation of Keller and Ruessler (1997), glochidia of *M. nervosa* remained viable well beyond the maximum time required to complete the relevant experiments with that species.

Approximately 3 ml aliquots of mature glochidia were obtained as needed by inserting a Pasteur pipet into a gravid demibranch. Larvae were gently aerated in 25 ml of artificial pond water (APW, Dietz and Alvarado 1970) at 20 °C to separate glochidia from parental tissue and mucus. Glochidia were washed three times in APW and then were held at 20 °C until use. A subsample of each preparation of glochidia was tested for viability as indicated by rapid valve closure in the presence of 0.4 M KCl. All glochidia were used within 8 h of removal from a marsupial demibranch. Assays were conducted at 20 °C with a minimum of 30 larvae of each species. Individual glochidia were used only once, and all experimental treatments were paired with a corresponding APW control.

For experimental observation, a single larva was transferred by micropipet in 10 µl of APW to a depression slide on a Nikon SMZ-2T dissecting microscope equipped with a video camera. Responses were quantified from recordings made with a JVC Model BR-9000U video recorder with on-screen time display. The parameters evaluated included the number of rhythmic valve adductions min^{-1} and the duration of any resulting tonic closure (> 5 s), within a total observation period of 60 s.

The experimental treatments to which glochidia were subjected are summarized in Table 1. The preparation and presentation of these stimuli were as follows. Mucus was tested from *Lepomis macrochirus* (bluegill) and *Micropterus salmoides* (largemouth bass) from Belews Lake, Rockingham County, NC, and from commercially acquired *Carassius auratus* (goldfish). Epithelial mucus (pH 6.2-6.5) was obtained by gently rubbing the lip of a 50 ml Erlenmeyer flask along the side of a fish. Approximately 5 µl of mucus then was placed within about 100 µm of a glochidium in APW on a depression slide with a sterile 5 µm diameter glass micropipet (pulled with a Narishige PN-3 Electrode Puller). A new micropipet was used with each glochidium. Control larvae were presented with about 5 µl of APW.

Bluegill mucus was fractionated by ultrafiltration using Amicon Centriplus Microconcentrators (Amicon Corp). A 1 ml sample of epithelial mucus was placed in a 10 kD (kilo-Dalton) concentrator (Centricon-10) and overlaid with 9 ml of APW before centrifugation (Sorvall RC2-B) at 3000 x *g* for 2 h at 4 °C. The tube then was inverted and was centrifuged at 2000 x *g* for 4 min to recover the retentate. The filtrate from the 10 kD concentrator was fractionated further with a 3 kD filter (Centricon-3) by centrifuging 0.5 ml of the filtrate with 4.5 ml of APW at 7500 x *g* for 2 h. This procedure yielded three fractions: >10 kD, 3-10 kD, and <3 kD, each of which was tested in the behavioral assay.

Larvae also were exposed to several reported components of mucus, two fish sex pheromones, 11 amino acids and to 'fish-conditioned water' (Table 1). Stock solutions of all chemicals were made up in APW from reagents from Sigma Chemical Company. The pH was adjusted to 6.2 - 6.5, and the pheromones were initially dissolved in 1 ml of methanol. All solutions were presented as 10 µl aliquots to a glochidium that previously had been placed in 10 µl of APW. Controls received 10 µl of APW.

Table 1. Stimuli to which individual glochidia of *U. imbecillis* and *M. nervosa* were exposed. See text for preparation and delivery of experimental treatments.

Chemical stimuli	Mechanical stimuli
Epithelial mucus from:	Stroking sensory hairs:
Largemouth bass	In Artificial Pond Water
Goldfish	In amino acids (10^{-2} M):
Bluegill sunfish	Glutamic acid
Size-fractionated mucus of bluegill (kilo-Daltons)	Histidine
>10 kD	Hydroxyproline
3-10 kD	Threonine
<3 kD	
Components of mucus	
Sialic acid (10^{-3} M)	
Galactose (10^{-2} M)	
Heparin (10 units ml^{-1})	
Fish pheromones	
17α,20β-Dihydroxy-4-pregnen-3-one (10^{-5} M)	
prostaglandin F2α :	
[5Z,9α,11α,13E,15S]-9,11,15-Trihydroxyprosta-5,13-dienoic acid (10^{-5} M)	
Fish-conditioned water	
One 12 cm bluegill in 2 l of APW for 24 h	
Amino acids: (10^{-2} and 10^{-3} M)	
Alanine	
Arginine	
Asparagine	
Aspartic acid	
Glutamic acid	
Glutamine	
Histidine	
Hydroxyproline	
Isoleucine	
Serine	
Threonine	

The response of larvae to mechanical stimulation was examined by gently stroking individual sensory hairs and adjacent mantle tissue of gaping glochidia with a 5 µm micropipet held in a micromanipulator until tonic closure of the shells occurred. Control animals were left undisturbed and observed for 1 min. Possible additive effects of chemical and mechanical stimulation were tested using the micromanipulator-micropipet system with larvae that were in APW and were simultaneously exposed either to an additional 10 µl of APW or to 10 µl of each of four amino acids (Table 1).

The data were analyzed by t-tests and one-way and two-way ANOVA, followed by Tukey's or Dunnett's post hoc comparisons as appropriate. Analyses were performed with BMDP statistical software (Dixon 1990) and Microsoft Excel, following the procedures of Zar (1984).

Results

The rate of valve adduction by glochidia of both species was significantly greater than that of controls in response to epithelial mucus of the three species of fish (two-way ANOVA, $F_{3,232} = 25.7$, $P < 0.001$, Fig. 1A). The larvae of *Utterbackia* responded similarly to the mucus of all three species (Tukey, $P > 0.05$) whereas *Megalonaias* differed significantly from the control only in response to mucus of bluegill and goldfish (Tukey, Fig. 1A). Upon exposure to all three types of mucus, 100% of the glochidia of *Utterbackia*

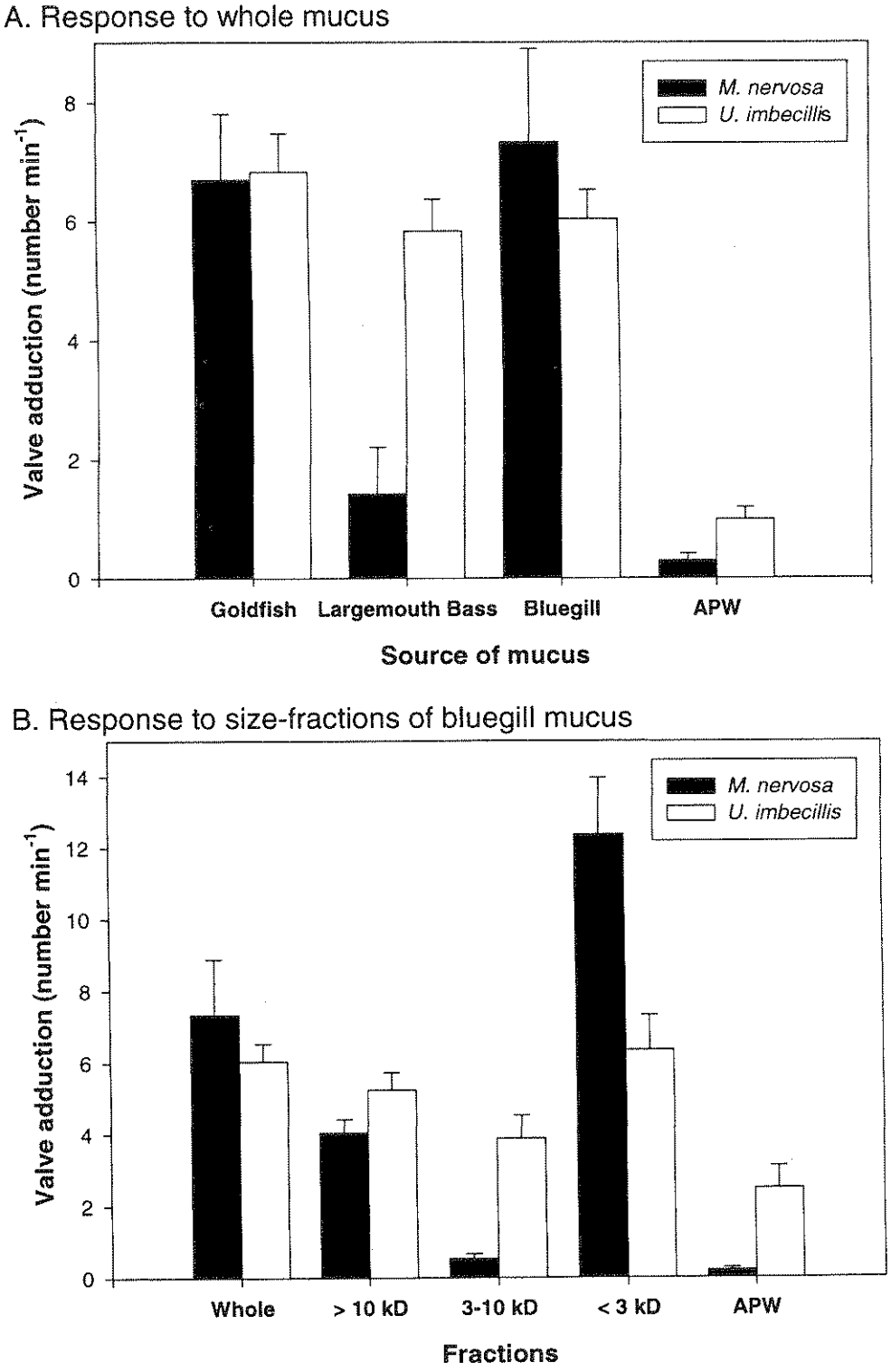

Figure 1. Rate of rhythmic valve adduction of glochidia larvae of *Megalonaias nervosa* and *Utterbackia imbecillis* exposed (A) to epithelial mucus of three species of fish and (B) to whole and size-fractionated epithelial mucus of *Lepomis macrochirus* (bluegill sunfish) (kD = kilo-Dalton; bars depict ± SE, N = 30 for each treatment).

subsequently underwent tonic adduction of their valves and remained closed for the duration of the observation period, while only 30% of *Megalonaias* entered prolonged closure.

Valve adduction by *Utterbackia* was significantly greater than the control in response to whole bluegill mucus and to the < 3 kD and > 10 kD fractions (ANOVA, $F_{4,145} = 5.7$, Tukey, $P<0.05$; Fig. 1B). The response to the 3-10 kD fraction was not significant (Fig. 1B). In contrast *Megalonaias* was highly stimulated by the < 3 kD fraction (Fig. 1B), exhibiting a rate of adduction that significantly exceeded the response to whole bluegill mucus (ANOVA, $F_{4,145} = 25.0$, $P<0.001$, Tukey $P<0.001$). The response by *Megalonaias* to the two larger fractions was not significantly different from the control (Tukey, $P> 0.05$). The differences between the two species were underscored by the significant interaction (species *x* mucus-fraction) in a two-way ANOVA ($F_{4,290} = 9.4$, $P< 0.001$).

Neither the fish pheromones, fish-conditioned water, nor the putative components of mucus, heparin or galactose, elicited a significant response in either species. However, sialic acid significantly increased the rate of valve adduction by *Megalonaias* from a control rate of 1.1 ± 0.1 adductions min^{-1} to 2.5 ± 0.7 ($\bar{x} \pm$ SE, N = 30, $P<0.05$, t-test). The rate also was significantly greater for *Utterbackia* relative to its control (1.8 ± 0.2 vs 1.1 ± 0.1 adductions min^{-1}), but the rates were not different between the two species (two-way ANOVA, $P= 0.18$). None of the amino acids at any concentration stimulated rhythmic adduction by *U. imbecillis* at a rate that was different from the APW control (data not shown). In contrast, 7 of 11 amino acids (Fig. 2) induced significant increases in valve adduction by *Megalonaias* (ANOVA, $F_{11,348} = 15.5$, $P<0.001$). *Megalonaias* was maximally stimulated by histidine, with a few glochidia exceeding 25 adductions min^{-1} (Fig. 2). Results were similar at both 10^{-2} and 10^{-3} M but only data for 10^{-2} M are presented.

While no amino acid induced a significant increase in the rate of rhythmic valve adduction by *U. imbecillis*, exposure of both species of glochidia to isoleucine, arginine, serine, glutamic acid, and histidine resulted in tonic valve closure that was of longer duration than

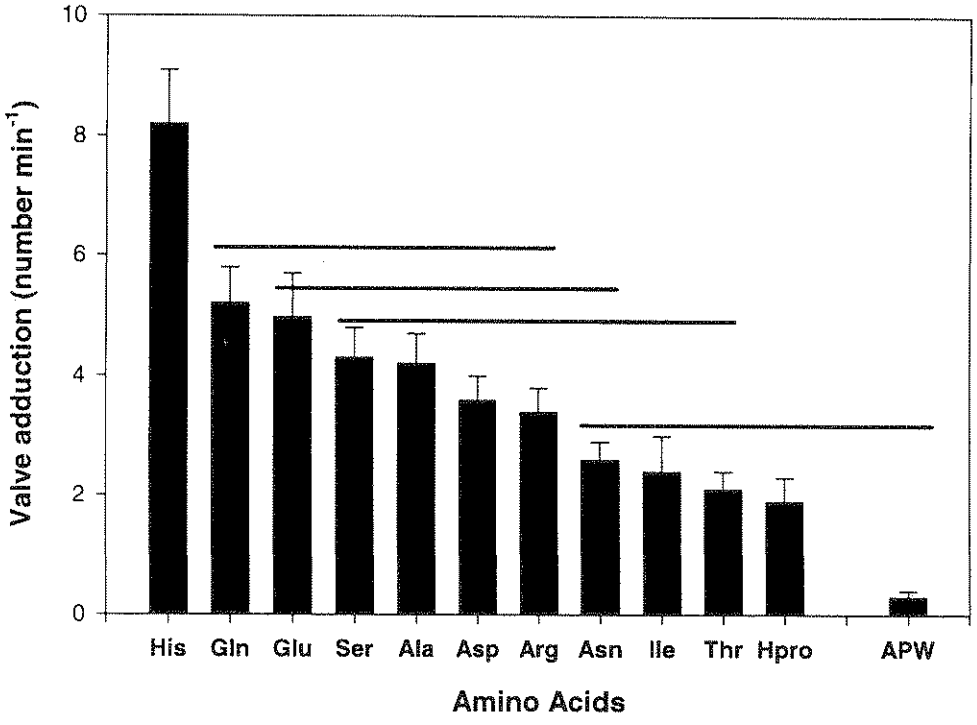

Figure 2. Rate of rhythmic valve adduct ion ($\bar{x} \pm$ SE) of glochidia larvae of *Megalonaias nervosa* exposed to the amino acids histidine, glutamine, glutamic acid, serine, alanine, aspartic acid, arginine, asparagine, isoleucine, threonine and hydroxyproline at 10^{-2} M. Bars under the same line are not significantly different (ANOVA, Tukey, $P< 0.05$, N = 30 for each treatment).

Table 2. Response of glochidia of *M. nervosa* and *U. imbecillis* to mechanical stimulation of larval sensory hairs. [$\bar{x} \pm$ SE, N = 30]

Species	Response	
	Stimuli to Induce Tonic Closure	Duration of Tonic Closure (seconds)
M. nervosa	8.3 ± 1.50	38.5 ± 5.0
U. imbecillis	4.3 ± 0.25	23.0 ± 5.0

in the APW controls (Dunnett's, $P<0.05$). The duration of this prolonged closure was not significantly different between the species (two-way ANOVA, $F_{1,58} = 2.37, P=0.13$).

The responses of glochidia to mechanical stimulation in APW are depicted in Table 2. *Utterbackia* closed in response to significantly fewer mechanical strokes than *Megalonaias* but remained closed for a significantly shorter time (Table 2, *t*-tests, $P<0.05$). However, the effects of mechanically stimulating glochidia in the presence of four amino acids differed in several respects from the results of mechanical stimulation alone, both between the two species and among the controls and specific experimental treatments (Fig. 3A,B). *Megalonaias* closed its valves after significantly fewer mechanical strokes in all four amino acids relative to the APW control (one-way ANOVA, $F_{4,145} = 6.3$, $P<0.001$, Dunnett's, $P<0.05$). Although the difference was not as evident for *Utterbackia* (Fig. 3A), that species was significantly affected by each of the amino acids ($F_{4,145} = 2.9$, $P< 0.002$, Dunnett's, $P<0.05$). The greater sensitivity of *Megalonaias* was underscored by a significant species-effect in a two-way ANOVA ($F_{1,290} = 15.7$, $P< 0.001$).

The duration of the resulting tonic closure that occurred in all four amino acids (Fig. 3B) was significantly increased for both species (ANOVA and Dunnett's, $P< 0.05$). However, *Megalonaias* was more markedly affected than *Utterbackia*, with, for example, histidine causing 100% of the glochidia of *Megalonaias* to remain closed for the full 60 s observation period (Fig. 3B). This difference between the species also was evident from the significant species-effect in a two-way ANOVA ($F_{1,290} = 69.0, P<0.001$).

Glochidia in all the APW controls employed in this study exhibited an overall mean rate of rhythmic valve closure of 1.23 ± 0.4 ($\bar{x}$± SE) adductions min^{-1} for *U. imbecillis* and 0.28 ± 0.07 for *M. nervosa*. These differences were significant (*t*-test, $P< 0.05$, $N = 120$ for each species), and indicate that larvae of *Megalonaias* are more quiescent following their removal from the marsupial chambers of parental gills than are glochidia of *Utterbackia*.

Discussion

The rate of valve adduction for undisturbed glochidia has been reported as about 0.1 adductions min^{-1} for *Anodonta cygnea* (temperature unknown) (Labos 1966), 0.18 min^{-1} for *Margaritifera margaritifera* (20 °C) (Young and Williams 1984) and 0.36 min^{-1} for *Pyganodon grandis* (20 °C) (Jacobson *et al.* 1997). In the present study spontaneous rhythmic valve closure occurred at 0.3 adductions min^{-1} for *Megalonaias* and 1.2 min^{-1} for *Utterbackia* (20 °C). These very low frequencies contrast with the potential rate of shell closure. For example, exposure to exogenous inorganic and organic substances including the neurotransmitters serotonin and tryptamine elicits on the order of 35 to 40 adductions min^{-1} for glochidia of *Anodonta cygnea* (Labos and Salanki 1963, Labos *et al.* 1964). The rate of adduction of a few *U. imbecillis* exceeded 15 min^{-1} in response to the <3 kD fraction of bluegill mucus, and some *Megalonaias* approached 40 adductions min^{-1} in both whole bluegill mucus and the <3 kD fraction. Since glochidia are limited to the opening and closing of their valves by contraction and relaxation of a single adductor muscle, the pattern and rate of such shell movements are likely to be critical to their successful attachment to a vertebrate host (Arey 1921, Wood 1974, Hoggarth and Gaunt 1988).

Glochidia of *U. imbecillis* and *M. nervosa* exhibited an increase in rhythmic valve adduction and the duration of tonic shell closure in response to intact fish mucus. Larvae of *U. imbecillis* responded equally to mucus from all three species of fish, and 100% subsequently underwent tonic closure. This observation is consistent with the attachment of hooked glochidia to external surfaces of a fish where contact

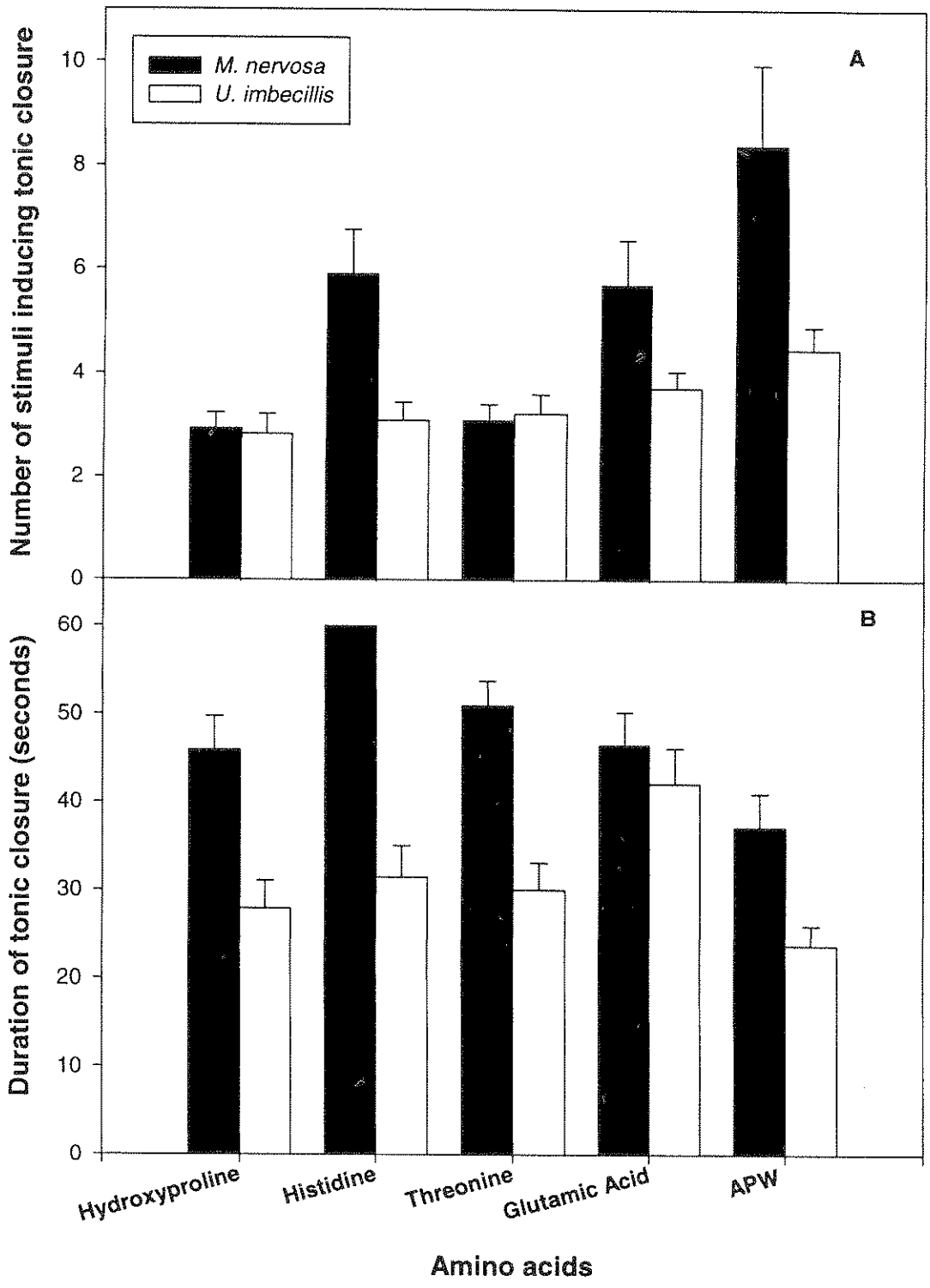

Figure 3. (A) The number of mechanical contacts of larval sensory hairs required to induce tonic valve closure by glochidia larvae of *Megalonaias nervosa* and *Utterbackia imbecillis* in the presence of selected amino acids (10^{-2} M). (B) The duration of tonic closure resulting from that stimulation (bars depict $\bar{x} \pm$ SE, N = 30 for each treatment).

with epithelial mucus would occur, and the relative lack of host specificity exhibited by this species (Watters and O'Dee 1998).

Lukasovics and Labos (1965) reported tonic closure by *A. cygnea* larvae in response to mucus from a variety of fish, while Wood (1974) observed significant closure by the same species in goldfish mucus in just one of two experiments and only after 30 min exposure. Data from these studies perhaps are confounded by the trauma to epithelial tissue by the maceration techniques the authors used to prepare the mucous solutions. While Lefevre and Curtis (1912) demonstrated that other species of hooked glochidia respond to epithelial mucus with increased rhythmic and tonic adduction, this is not exclusively a response of hooked glochidia since hookless *M. margaritifera* shows similar behavior (Young and Williams 1984). All previous studies of glochidial behavior have employed groups of larvae (often >25 simultaneously), adding the complication of potential interaction among glochidia to an interpretation of the results.

Megalonaias responded maximally to the <3 kD fraction of bluegill mucus, implying that the principal stimulatory component(s) in the mucus is of relatively small molecular weight. Wood (1974) found that glochidia of *A. cygnea* responded to epithelial mucus of *C. auratus* that had been subjected to filtration, variation of pH, boiling and dialyzation, and that the active component is thermostable, uncharged and of small molecular weight. Her observation of the effectiveness of small components of mucus in eliciting rhythmic adduction is consistent with the results for *Megalonaias*.

The individual components of fish epithelial mucus have not been extensively characterized. Wessler and Werner (1957) determined that the major constituent of external mucus of several species of freshwater fish was protein that yielded most of the normal assortment of amino acids upon hydrolysis. They also found lesser quantities of simple sugars, nucleic acids and glycoproteins. Sialic acid has been reported in glycoproteins from mucus of *Anguilla vulgaris* (Faillard and Schauer 1972) and *Allolepsis hollandi* (Hashimoto and Yoshimura 1977). Other prevalent components of *A. hollandi* mucus were galactose, mannose, glutamic acid, aspartic acid, threonine and serine (Hashimoto and Yoshimura 1977). The significant but relatively moderate response of both *Utterbackia* and *Megalonaias* to sialic acid is consistent with the occurrence of this substance in vertebrate mucus.

Although there are few reports of the amino acid concentration of fish fluids, McDonald and Milligan (1992) determined that the blood of *Cyprinus carpio* (common carp) included less than 10^{-6} M total amino acids. *Salmo gairdnerii* (rainbow trout) and *C. auratus* mucus also contains less than 10^{-6} M amino acids (Gras *et al.* 1978, Saglio and Fauconneau 1985), with slight fluctuations in concentrations prior to spawning (Fontaine *et al.* 1978) and following feeding (Saglio and Blanc 1989). In the present study amino acids at both 10^{-2} and 10^{-3} M were highly excitatory to *M. nervosa* glochidia, but failed to elicit any rhythmic response in *U. imbecillis*. Wood (1974) noted that *A. cygnea* responded to amino acids at 10^{-3} M but not 10^{-4} M. Four of the seven amino acids that were stimulatory for *M. nervosa* have been reported from fish mucus (Wood 1974) but at less than 10^{-6} M. Heard and Hendrix (1965) subjected several species of lampsiline glochidia to individual amino acids at 10^{-1} M, and reported that tonic contraction occurred among all the species in response to all amino acids except arginine, histidine and lysine. However, their study is difficult to interpret since it is a brief report without data.

Larvae of *U. imbecillis* were more sensitive than *M. nervosa* to mechanical stimulation as indicated by fewer stimuli being required to initiate valve adduction. Although 100% of the glochidia of both species ultimately underwent tonic closure in response to mechanical stimulation of the larval sensory hairs, *Megalonaias* remained closed significantly longer than *Utterbackia*. These data perhaps indicate that hooked larvae quickly re-open the valves if mechanical stimulation is not accompanied by chemical stimuli. The observation by Wood (1974) that fewer than 2% of the glochidia of *A. cygnea* (a hooked form) underwent tonic closure when mechanically stimulated is in contrast to the behavior of *U. imbecillis* and *M. nervosa*. Furthermore, glochidia of *M. margaritifera*, which are hookless and typically attach to gill filaments, respond to mechanical stimulation with immediate closure (Young and Williams 1984).

The larvae of both *Utterbackia* and *Megalonaias* required significantly fewer mechanical contacts to induce shell closure in the presence of amino acids than in APW. Once closed, however, glochidia of *Megalonaias* stayed closed significantly longer than *Utterbackia*. This heightened sensitivity to mechanical stimulation in the presence of amino acids is consistent with the greater sensitivity of *Megalonaias* to the small molecular weight fraction of bluegill mucus and to the series of amino acids. Perhaps hooked glochidia, such as those of *Utterbackia*,

require continuous exposure to both chemical and mechanical stimulation to remain closed. Hoggarth and Gaunt (1988) suggested that mechanical stimulation that causes valve adduction would increase tactile stimulation of the sensory hairs on the opposing larval mantle surfaces and would produce continuous contraction of the adductor muscle. However, the induction of prolonged shell closure seems to be more complex than a response to either mechanical or chemical signals operating alone.

The failure of either species to respond to fish pheromones or to fish-conditioned water may have been a concentration-dependent result, or may simply indicate that glochidia are unresponsive to these substances. Perhaps brooding adult mussels are affected by these agents during release of mature glochidia in the presence of host fish (Davenport and Warmuth 1965, Jokela and Palokangas 1993).

This study is the first systematic analysis of the behavior of individual glochidia in response to defined stimuli. The results reveal significant behavioral and physiological differences between the two species. The relatively greater chemical than mechanical sensitivity of *Megalonaias* may be related to these hookless larvae attaching to highly vascularized gills and being in intimate contact with substances in fish blood. In contrast the hooked glochidia of *U. imbecillis* may be adapted to rapid closure on a chemo- and mechano-stimulatory surface such as a moving fin or operculum. However, the kinetics of the interaction between hookless larvae and the gill filaments of a fish as the glochidia are drawn through the buccal cavity clearly are unknown. It may be instructive to examine the behavior of larvae of other mussel species that exhibit different combinations of host specificity and site of attachment. Until a more direct measure of sensory/ neuronal involvement in the control of valve adduction is available, the role of excitatory (or inhibitory) agents in the interactions between glochidia and their fish hosts will remain to be more fully elucidated.

Acknowledgements

We would like to thank Mr. Don Hubbs, Tennessee Fisheries and Wildlife Resource Agency, for providing glochidia of *Megalonaias nervosa*. Constructive editorial comments from anonymous reviewers also are appreciated.

Literature Cited

Arey, L.B. 1921. An experimental study on glochidia and the factors underlying encystment. Journal of Experimental Zoology 30: 463-492.

Davenport, D. and M. Warmuth. 1965. Notes on the relationship between the freshwater mussel *Anodonta implicata* Say and the alewife *Pomolobus pseudoharengus* (Wilson). Limnology and Oceanography 10: R74-R78.

Dietz, T.H. and R.H. Alvarado. 1970. Ionic regulation in *Lumbricus*. Biological Bulletin 138: 247-261.

Dixon, W.J. 1990. BMDP statistical software manual. Vol.1. University of California Press, Berkeley, CA. 629 pp.

Faillard, H. and R. Schauer. 1972. Glycoproteins as lubricants, protective agents, carriers, structural proteins, and as participants in other functions. Pages 1246-1267. *In* A. Gottschalk, (editor) Glycoproteins. Their composition, structure, and function. Elsevier, New York, NY.

Fontaine, M., J. Marchelidon and A. Momzikoff. 1978. Study of riboflavin, pterins, and free amino acids dissolved in the seawater of a herring spawning ground. Oceanology Acta 1: 415-416.

Gras, J., Y. Gudefin and F. Chagny. 1978. Free amino-acids and ninhydrin-positive substances in fish. I. Muscle and skin of the rainbow trout (*Salmo gairdnerii* Richardson). Comparative Biochemistry and Physiology 60B: 369-372.

Hansten, C., M. Heino and K. Pynnonen. 1996. Viability of glochidia of *Anodonta anatina* (Unionidae) exposed to selected metals and chelating agents. Aquatic Toxicology 34: 1-12.

Hartfield, P. and R. Butler. 1997. Observations on the release of superconglutinates by *Lampsilis perovalis* (Conrad 1834). Pages 11-15 *In* K.S. Cummings, A.C. Buchanan, C.A. Mayer and T.J. Naimo, eds. Conservation and management of freshwater mussels II: Initiatives for the future. Proceedings of a UMRCC symposium, 16-18 October 1995, St. Louis, MO. Upper Mississippi River Conservation Committee, Rock Island, IL.

Hashimoto, H. and J. Yoshimura. 1977. Studies on body-surface mucin of a fish, "Norenge" *Allolepis hollandi* I. Chemical compositions of glycoproteins and glycopeptide. Bulletin of the Japanese Society of Scientific Fisheries 43: 1319-1325.

Heard, W.H. and S.S. Hendrix. 1964. Behavior of unionid glochidia. American Malacological Union Annual Report 31: 2-3.

Hoggarth, M.A. and A.S. Gaunt. 1988. Mechanics of glochidial attachment (Mollusca: Bivalvia: Unionidae). Journal of Morphology 198: 71-81.

Huebner, J.D. and K.S. Pynnonen. 1992. Viability of glochidia of two species of *Anodonta* exposed to low pH and selected metals. Canadian Journal of Zoology 70: 2348-2355.

Jacobson, P.J., R.J. Neves, D.S. Cherry and J.L. Farris. 1997. Sensitivity of glochidial stages of freshwater mussels (Bivalvia: Unionidae) to copper. Environmental Toxicology and Chemistry 16: 2384-2392.

Jokela, J. and P. Palokangas. 1993. Reproductive tactics in *Anodonta* clams: Parental host recognition. Animal Behaviour 46: 618-620.

Keller, A.E. and D.S. Ruessler. 1997. The toxicity of malathion to unionid mussels: relationship to expected environmental concentrations. Environmental Toxicology and Chemistry 16: 1028-1033.

Kraemer, L.R. 1970. The mantle flap in three species of *Lampsilis* (Pelecypoda: Unionidae). Malacologia 10: 225-282.

Labos, E. 1966. Contributions to the mechanism of tryptamine effect on the adductor activity of fresh-water mussel larvae. Annales Biologie Tihany 33: 13-35.

Labos, E. and J. Salanki. 1963. The effect of alkali metal ions and alkaline earth metal ions on the rhythmic activity of glochidia of the freshwater mussel *Anodonta cygnea* L. Annales Biologie Tihany 30: 45-57.

Labos, E., J. Salanki and K.S. Rozsa. 1964. Effect of serotonin and other bioactive agents on the rhythmic activity in the glochidia of fresh-water mussel (*Anodonta cygnea* L.). Comparative Biochemistry and Physiology 11A: 161-172.

Lefevre, G. and W.C. Curtis. 1912. Studies on the reproduction and artificial propagation of fresh-water mussels. Bulletin of the Bureau of Fisheries 30: 109-201.

Lukacsovics, F. and E. Labos. 1965. Chemo-ecological relationship between some fish species in Lake Balaton and the glochidia of *Anodonta cygnea* L. Annales Biologie Tihany 32: 37-54.

McDonald, D.G. and C.L. Milligan. 1992. Chemical properties of the blood. Pages 80-113. *In* W.S. Hoar, D.J. Randall, A.P. Farrell (editors). The cardiovascular system. Fish Physiology, Vol. 12B. Academic Press, New York, NY.

Paling, J.E. 1968. A method for estimating the relative volumes of water flowing over the different gills of a freshwater fish. Journal of Experimental Biology 48: 533-544.

Saglio, P. and J.M. Blanc. 1989. Intraspecific chemocommunication in immature goldfish, *Carassius auratus* L.: Attraction in olfactometer to free amino acid fractions from skin extracts. Biological Behaviour 14: 132-147.

Saglio, P. and B. Fauconneau. 1985. Free amino acid content in the skin mucus of goldfish, *Carassius auratus* L.: Influence of feeding. Comparative Biochemistry and Physiology 82A: 67-70.

Varanka, I. 1986. Toxicity of mosquitocides on freshwater mussel larvae. Acta Biologica Hungarica 37: 143-158.

Watters, G.T. 1994. An annotated bibliography of the reproduction and propagation of the Unionoidea (Primarily North America). Ohio Biological Survey Miscellaneous Contributions 1: 1-158.

Watters, G.T. and S.H. O'Dee. 1998. Metamorphosis of freshwater mussel glochidia (Bivalvia: Unionidae) on amphibians and exotic fishes. American Midland Naturalist 139: 49-57.

Wessler, E. and I. Werner. 1957. On the chemical composition of some mucous substances of fish. Acta Chemica Scandinavica 11: 1240-1247.

Wood, E.M. 1974. Some mechanisms involved in host recognition and attachment of the glochidium larvae of *Anodonta cygnea* (Mollusca: Bivalvia). Journal of Zoology, London 173: 15-30.

Young, M. and J. Williams. 1984. The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn.) in Scotland. II. Laboratory studies. Archiv fur Hydrobiologie 100: 29-43.

Zar, J.H. 1984. Biostatistical analysis. 2nd ed. Prentice Hall, Englewood Cliffs, NJ, 718 pp.