

BIOCHEMICAL COMPOSITION OF THREE SPECIES OF UNIONID MUSSELS AFTER EMERSION

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ABSTRACT

Freshwater mussels are emersed (exposed to air) during conservation activities such as surveys and relocations. Success of these activities depends upon the ability of mussels to survive emersion and to re-burrow in the substratum. We evaluated the acute sublethal effects of emersion on three species of unionid mussels [pocketbook, *Lampsilis cardium* (Rafinesque, 1820); pimpleback, *Quadrula pustulosa pustulosa* (I. Lea, 1831); spike, *Elliptio dilatata* (Rafinesque, 1820)] by measuring three biochemicals (carbohydrate, lipid, protein) indicative of biochemical function and energy storage. Mussels were acclimated in water at 25°C and exposed to five air temperatures (15, 20, 25, 35 and 45°C) for 15, 30 and 60 min. After emersion, mussels were returned to water at 25°C and observed for 14 days. Samples of mantle tissue were taken after the 14-day postexposure period and analysed for carbohydrate, lipid and protein. Three-way analysis of variance (ANOVA) did not reveal consistent trends in carbohydrate, lipid or protein concentrations due to sex of mussels, duration of emersion, air temperature or their interaction terms that indicated biological compensation to stress. Overall mean carbohydrate concentrations were greatest (range 447–615 mg/g dry wt) among the species, followed by protein (179–289 mg/g dry wt) and lipids (26.7–38.1 mg/g dry wt). These results have positive implications for conducting conservation activities, because emersion over the range of temperatures (15–35°C) and durations (15–60 min) examined did not appear acutely harmful to mussels.

INTRODUCTION

There is growing concern worldwide about the conservation of unionid mussels, because of their recent widespread declines and their importance as indicators of healthy aquatic ecosystems. For example, unionid populations in North America, which are among the most diverse in the world, have declined over the past 50 years such that about 70% of its nearly 300 species are listed as extinct, endangered, threatened or of special concern (Williams, Warren, Cummings, Harris & Neves, 1993). Consequently, a Strategy for the Conservation of Native Freshwater Mussels was developed in the United States to provide a framework for preventing further mussel extinctions and population declines (National Native Mussel Conservation Committee, 1998). Many federal, state and private natural resource agencies and institutions have begun investigating approaches outlined in the Strategy to conserve this declining fauna. Among the approaches listed were the recommendations to initiate comprehensive status surveys and to investigate relocation of native mussels to various types of refugia. In conducting these types of activities, it is often necessary to emerse mussels (i.e. expose them to air) for some period of time during collection and processing. The subsequent replacement and survival of the mussels in the system depends on their ability to survive emersion at various water and air temperatures and to re-burrow in the substratum.

While the effects of collection and handling on mussels in the field are generally considered benign and inconsequential relative to most threats (e.g. construction projects, zebra mussel *Dreissena polymorpha* infestation, habitat loss), Cope & Waller (1995) found that mortality of mussels after relocation can be substantial (average mortality of 49% in 37 mussel relocations

conducted between 1967 and 1994). In their review, mortality was often highest within 1 year of the event, suggesting that the effects of collection, handling, and displacement of mussels may be greater than previously considered. For example, Waller, Gutreuter & Rach (1999), who evaluated the ability of four unionid species to orientate themselves upright, move, and burrow following displacement at three water temperatures (7, 14 and 21°C), found that water temperature had a significant effect on these three behaviours, with the greatest probability of re-establishment in the substratum occurring at the highest temperature tested (21°C). The environmental conditions that mussels experience during surveys and relocations may contribute to low survival, but can also be controlled to some extent. Determining the effects of temperature and emersion on unionid mussels would provide guidance on the conditions in which surveys and relocations should occur to enhance mussel survival and overall success.

In a companion study to this one, Bartsch, Waller, Cope & Gutreuter (2000) examined the short-term survival and behavioural effects of extreme temperatures and moderate emersion duration on the same three species of mussels studied here [pocketbook, *Lampsilis cardium* (Rafinesque, 1820); pimpleback, *Quadrula pustulosa pustulosa* (I. Lea, 1831); spike, *Elliptio dilatata* (Rafinesque, 1820)] and found that emersion at moderate temperatures (15–35°C) and durations (15–60 min) was not acutely detrimental to mussels. However, recent evidence suggests that certain physiological and biochemical endpoints may be adversely affected in unionids long before changes in survival are observed (Haag, Berg & Garton, 1993; Patterson, Parker & Neves, 1997; Naimo & Monroe 1999; Baker & Hornbach, 2000, 2001; Chen, Heath & Neves, 2001). Therefore, we evaluated changes in total carbohydrate, lipid and protein, all indicators of biochemical condition and energy status in mussels, as related to differences in air temperature, duration of emersion and sex.

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MATERIAL AND METHODS

Test organisms

Mussels were collected from the Wolf River at Shawano, Wisconsin, USA in July 1995 and were transported in holding tanks containing Wolf River water (25°C) to the Upper Midwest Environmental Sciences Center, in La Crosse, Wisconsin, USA. Holding tank water temperatures were maintained at $25 \pm 3^\circ\text{C}$ (with addition of non-chlorinated ice as needed), and dissolved oxygen concentration was maintained by aeration at $>60\%$ saturation. Water temperature and dissolved oxygen (Yellow Springs Instrument Model 58 oxygen meter) were measured at 1-h intervals. At the laboratory, mussels were placed into submerged cages held on the bottom of the Black River (water temperature, 27°C), near La Crosse, Wisconsin, for 6–47 days until each test was initiated (July–September). Mussels were continuously immersed in river water during collection, transport and allocation to cages.

Experimental design and exposure system

Three sequential laboratory tests were performed, one each with *L. cardium*, *Q. p. pustulosa* and *E. dilatata*, in a completely randomized design. For each mussel species tested, there was one acclimation water temperature treatment (25°C), five air temperatures (ranging within $\pm 20^\circ\text{C}$ of the water temperature), three durations of aerial exposure (15, 30 and 60 min), and a no-emersion control (Fig. 1). The range of emersion temperatures and durations tested encompass the extreme air temperature and water-air thermal differentials commonly encountered during mussel surveys and relocations throughout summer in the upper Midwestern USA. All treatments, including controls, were duplicated, with 10 organisms per emersion time and temperature ($n = 320$ per test), for a total of 32 experimental units.

An experimental unit consisted of a flow-through, stainless steel tank containing sand (13 ± 0.5 cm in depth) and 42 ± 0.5 l of well water. Each tank was placed into one of six water baths maintained at the test temperature ($25 \pm 1^\circ\text{C}$) with a Remcor Model CFF-501 thermostatically-controlled, liquid circulation pump (Remcor Products Co.) connected to a water bath. Tanks were aerated to maintain dissolved oxygen concentrations at $>60\%$ saturation. The flow rate of well water to each tank was 200 ml/min, yielding a renewal rate of seven times per day. The photo period was 16-h light and 8-h dark.

Tests were initiated when the water temperature of the Black River, where the test animals were being held, reached the

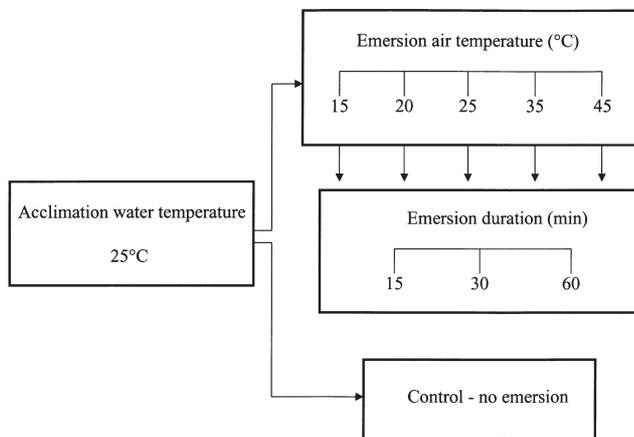


Figure 1. Experimental design for the thermal and emersion exposure tests with each of the three species of unionids.

target test water temperature of 25°C (July–September). Each mussel species was tested individually and a given species was transported in coolers containing Black River water to the laboratory. A total of 10 mussels was randomly allocated to each experimental unit (tank). Mussels were acclimated in their respective tanks at a water temperature of $25 \pm 1^\circ\text{C}$ for 2 days and were not fed during the acclimation period.

For each treatment, 20 mussels (10 from each duplicate) were removed from the test water (25°C), transported in water (held at test temperature), and placed into an environmental chamber (Hotpack® Biological Chamber, Hotpack Corp.) at a given air temperature (e.g. 15, 20, 25, 35, 45°C) for a duration of 15, 30 or 60 min. Treatments were sequentially conducted in order of increasing air temperature and duration of emersion. The target relative humidity in the environmental chamber was $60 \pm 5\%$. This relative humidity was similar to average summer ambient air conditions in the upper Midwestern USA (S. Thompson, 1995, personal communication). Following emersion, mussels were removed from the environmental chamber, transported in water (at test temperature of 25°C), and returned to their respective tanks. Test organisms were fed a mixture of C4 algae diet (Coast Seafoods Co., Bellevue, WA; 0.2 ml per mussel per day) and dry *Chorella* (0.013 g dry weight per mussel) daily. Mussels were monitored for mortality and behavioural responses (uprighting and burrowing) for 14 days postemersion (Bartsch *et al.*, 2000). After 14 days, mussels were recovered from each tank and measured for total length and whole animal wet weight. Sex of *L. cardium* was determined by shell dimorphism. *Elliptio dilatata* and *Q. p. pustulosa* are not sexually dimorphic, and for these species we examined histological sections of five mussels from each duplicate treatment to determine sex.

Mantle tissue from the same five mussels used to determine sex was dissected, weighed, placed into 2-ml cryovials, and stored at -84°C until analysis. The dissected mantle tissue consisted of a 5–10 mm cross-sectional, rectangular piece that corresponded to section locations shown in Figure 2. We used only mantle tissue in this study, because (1) concentrations of biochemical indices vary among tissues (Giesy & Graney, 1989; Naimo & Monroe, 1999), (2) mantle is important in physiological function and energy storage (de Zwaan & Zandee, 1972; Gabbott & Bayne, 1973; Fraser, 1989), and (3) mantle is responsible for shell growth (McMahon, 1991). Unionid mussels store carbohydrate, primarily in the form of glycogen, for their primary energy reserve (Haag *et al.*, 1993). Lipids are also generally considered to be used for energy storage (Fraser, 1989); therefore, changes in lipid content may indicate altered use of energy reserves. Lipid type and concentration in tissues are heterogeneous; however, by examining only mantle tissue, the types of lipids examined should be similar between mussels (Giesy &

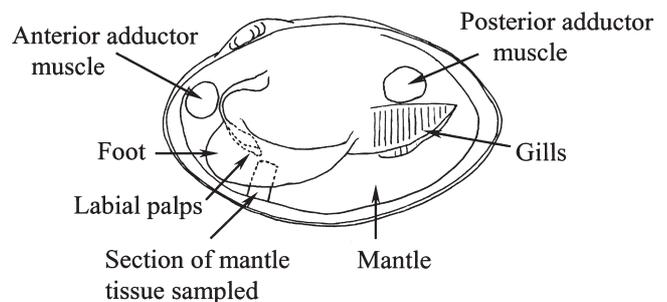


Figure 2. Cross-section of unionid mussel showing the section (10 × 20 mm) of mantle tissue sampled (posterior to labial palps) for biochemical analysis in this study.

Graney, 1989). Proteins are involved in all biochemical processes and may also be used as an energy source (Bayne & Thompson, 1970).

Sample processing and analysis

Each cryovial of mantle tissue was lyophilized at -50°C and 150 mTorr for 24 h, then ground to a fine powder with a ceramic mortar and pestle, returned to the cryovial, and stored at -40°C until analysed for carbohydrate, lipid and protein. Before analyses, composite samples were made by combining equal amounts (10 ± 1 mg) of mantle tissue from mussels grouped by sex from each experimental unit. All composite samples were divided into two 10-mg subsamples; one subsample was used for protein determination, and the other was used for determination of lipid and carbohydrate.

Protein was determined by the bicinchoninic acid method (Sigma, 1997). The vanillin-phosphoric acid method (van Handel, 1985) was modified as follows for lipid determinations. To extract lipids, 500 μl of the 1:1 chloroform-methanol solvent was added to the mantle tissue and vortexed for 20 sec. After 30 min, samples were centrifuged for 7 min at full speed in a clinical benchtop centrifuge. The supernatant, which was used for lipid analysis, was poured into a 16×150 -mm test tube and vortexed for 5 sec; the pellet in the cryovial was used for carbohydrate determination with the phenol-sulphuric acid method (Naimo, Damshen, Rada & Monroe, 1998). Analysis of mantle tissue for water content was conducted according to standard methods (APHA, AWWA & WPCF, 1995).

Precision and bias (i.e. accuracy) of each method was determined by triplicate analysis (analytical triplicates) of all samples, spikes, procedural blanks and procedural calibration standards. Approximately 5% of all samples were spiked with known amounts of carbohydrate, lipid and protein to evaluate the precision and bias (accuracy) of each method. Percentage relative standard deviations (RSD) for samples averaged 5.3% (range 0.09–24%) for carbohydrate; 9.6% (range 0.23–55%) for lipid; and 11% (range 0.60–42%) for protein. Percent RSD for procedural blanks and standards averaged 11% (range 0.00–88%) for carbohydrate; 7.3% (range 1.2–13%) for lipid; and 5.1% (range 0.50–18%) for protein. Recovery of spikes averaged 96% (range 91–103%) for carbohydrate; 112% (range 101–127%) for lipid; and 101% (range 95–107%) for protein. Method detection limits (MDL) and limits of quantification (LOQ) were determined for each daily batch of samples from seven replicate analyses of a sample with low-analyte concentration (APHA *et al.*, 1995). The mean MDLs and LOQs, respectively, for each method were as follows: carbohydrate, 61.7 and 154 $\mu\text{g}/\text{ml}$; lipid, 190 and 475 $\mu\text{g}/\text{ml}$; and protein, 132 and 332 $\mu\text{g}/\text{ml}$.

Statistical analyses

Statistical analysis was performed with Statistical Product and Service Solutions software (SPSS, 1998). Data were tested to determine if distributions were normal and variances were homogeneous; no transformations were required to meet assumptions of statistical tests. Three-way analysis of variance (ANOVA) was used to determine whether carbohydrate, lipid and protein concentrations differed due to sex, duration of emersion, air temperature or their interaction terms. One-way ANOVA was used to determine whether carbohydrate, lipid and protein concentrations differed among species. Differences between treatment means were examined with Tukey's honestly significant difference (*hsd*) test. An experiment-wise Type-I error (α) of 0.05 was used to judge significance of all statistical tests.

RESULTS

Physiochemical characteristics of water measured daily in each experimental unit during the three tests averaged 24.5°C (standard deviation, SD = 0.8) for temperature, 8.2 mg/l (0.4) for dissolved oxygen, and 8.10 standard units (0.06) for pH. Mean relative humidity in the environmental chamber during emersion was 64% (2.2).

Mean length of the three mussel species tested was 80.6 mm (SD 9.7) for *E. dilatata*, 61.8 mm (13.2) for *Q. p. pustulosa* and 100.3 mm (10.7) for *L. cardium*. Wet weight of the three mussel species averaged 54 g (SD 18) for *E. dilatata*, 78 g (42) for *Q. p. pustulosa* and 186 g (54) for *L. cardium*. The sex ratios (male:female) of *E. dilatata* and *Q. p. pustulosa* were similar (66:67 and 74:65, respectively), whereas for *L. cardium* there were almost twice as many males (209) as females (111). Mortality and behavioural responses (uprighting and burrowing) of mussels after the 14-day post-emersion period were presented by Bartsch *et al.* (2000). Briefly, mussel survival averaged 93% across all air temperatures and durations of emersion. Survival did not differ among treatments for *L. cardium* and *Q. p. pustulosa*; however, *E. dilatata* experienced significant mortality after emersion at 45°C (50% mortality at 30 min and 100% mortality at 60 min of emersion). Therefore, no biochemical determinations were made for *E. dilatata* at the 45°C , 60-min emersion treatment. The percentage of mussels that burrowed during the 14-day post-emersion period was 80% for *E. dilatata*, 83% for *Q. p. pustulosa* and 96% for *L. cardium*.

Concentrations of carbohydrate, lipid and protein did not differ between sex of any of the three species tested or among durations of emersion within an air temperature emersion treatment. Therefore, each of the biochemical response variables was averaged over sex for a given species and over all durations of emersion within an air temperature (Table 1). Carbohydrate concentrations varied significantly among air temperature treatments for *Q. p. pustulosa*, but not among treatments for *L. cardium* and *E. dilatata* (Table 1). Emersion temperature significantly affected lipid concentrations in the mantle tissue of *E. dilatata*, but not of *Q. p. pustulosa* and *L. cardium*. Protein content of mantle tissue did not vary significantly among emersion temperatures for any of the three species tested (Table 1).

Although three-way ANOVA showed statistical significance for two of the main effects variables within a species over the various emersion air temperatures (e.g. carbohydrate in *Q. p. pustulosa* and lipid in *E. dilatata*, Table 1), there were no consistent trends in mussel response that would indicate a biologically significant compensation to emersion and temperature stress. Therefore, inferences about the biological relevance of the two statistically significant variables were not made.

Among the three species, carbohydrate concentrations averaged over all treatments ranged from 447 mg/g dry wt in *E. dilatata* to 615 mg/g dry wt in *Q. p. pustulosa* (Fig. 3). Average lipid concentrations ranged from 26.7 mg/g dry wt in *Q. p. pustulosa* to 38.1 mg/g dry wt in *L. cardium* and mean protein concentrations ranged from 179 mg/g dry wt in *Q. p. pustulosa* to 289 mg/g dry wt in *E. dilatata* (Fig. 3). The total residual components (expressed as ash-free dry weight, AFDW), not accounted for by the three main biochemicals of energy storage, ranged from 179 mg/g dry wt in *Q. p. pustulosa* to 268 mg/g dry wt in *L. cardium* (Fig. 3). Concentrations of carbohydrate in mantle tissues of *E. dilatata* and *L. cardium* were similar, but both species had significantly lower concentrations than *Q. p. pustulosa* ($P < 0.01$). Lipid, protein, and AFDW concentrations in mantle tissue were significantly different ($P < 0.01$) among all three species. In relative abundance, carbohydrate concentrations were greatest for all species, followed by protein, and lipid. The mean water content of mantle tissue from the three species of mussels in our test was 86% (range 83–89%) and may be used to

Table 1. Mean concentrations (standard error in parentheses) of carbohydrate, lipid and protein in mantle tissue from three species of unionid mussels emersed at five air temperatures (no-emersion control, 15, 20, 25, 35 and 45°C). Means were averaged over sex and three emersion durations (15, 30 and 60 min) per air temperature ($n = 30$ mussels for each constituent and emersion air temperature for a given species, except at the 45°C emersion air temperature in the test with *Elliptio dilatata*, in which $n = 20$ mussels due to 100% mortality in the 60-min emersion duration treatment; $n = 10$ mussels for each constituent and the no-emersion control for a species).

Species	Constituent	ANOVA <i>P</i> -value	Mean concentration (mg/g dry weight)*					
			Control	Emersion air temperature (°C)				
				15	20	25	35	45
<i>Lampsilis cardium</i>	Carbohydrate	0.08	388 (12)	411 (18)	425 (38)	486 (38)	473 (32)	530 (31)
	Lipid	0.29	39.3 (1.9)	37.8 (1.1)	40.3 (2.2)	36.5 (1.1)	40.8 (2.2)	34.4 (1.7)
	Protein	0.15	224 (8)	216 (11)	260 (13)	240 (14)	260 (15)	254 (18)
<i>Quadrula p. pustulosa</i>	Carbohydrate	0.02	640 ^a (31)	564 ^{a,b} (24)	603 ^{a,b} (17)	670 ^{a,b} (22)	616 ^{a,b} (22)	598 ^b (11)
	Lipid	0.06	22.9 (0.7)	27.9 (0.9)	25.0 (0.8)	26.6 (1.2)	28.5 (1.2)	29.7 (1.3)
	Protein	0.65	175 (13)	187 (14)	184 (8)	186 (7)	169 (9)	175 (11)
<i>Elliptio dilatata</i>	Carbohydrate	0.32	473 (31)	452 (41)	423 (17)	493 (23)	435 (26)	403 (25)
	Lipid	0.04	26.3 ^a (2.3)	28.0 ^{a,b} (1.2)	30.6 ^{a,b,c} (1.3)	34.4 ^{b,c} (1.6)	31.8 ^{a,b,c} (0.8)	36.4 ^c (1.7)
	Protein	0.15	291 (20)	285 (16)	277 (23)	258 (32)	281 (15)	399 (18)

*Means accompanied by a different letter within a row for a species and constituent with significant ANOVA were judged to be significantly different with Tukey's *h*_{sd} test at $\alpha = 0.05$.

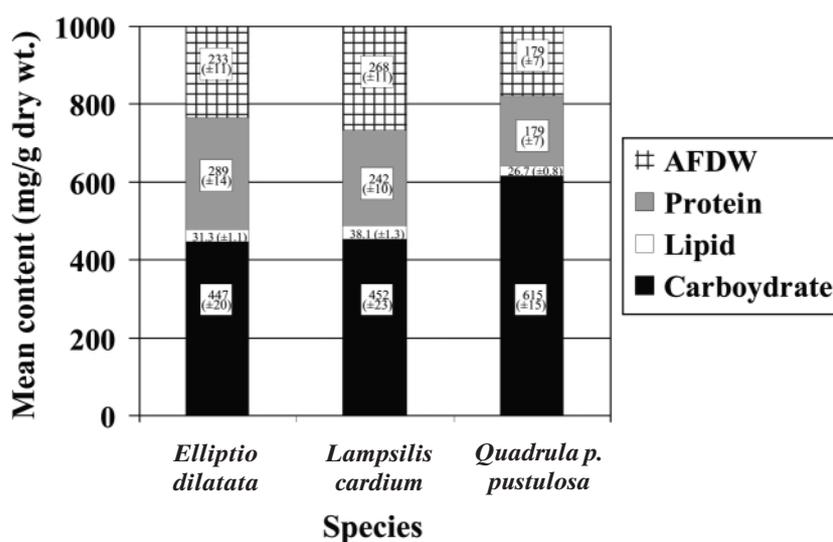


Figure 3. Mean content of carbohydrate, lipid, protein and ash-free dry weight (AFDW) in mantle tissue from three species of unionid mussels, averaged over all five air temperature (no-emersion control, 15, 20, 25, 35 and 45°C) and three emersion duration (15, 30 and 60 min) treatments. Mean and 95% confidence interval (in parentheses) are presented inside each constituent bar; $n = 32$ experimental units and 160 mussels analysed for each constituent and species, except for the test with *Elliptio dilatata*, in which $n = 30$ experimental units and 150 mussels.

convert the dry weight units of expression to a wet weight basis (Naimo & Monroe, 1999).

DISCUSSION

The results of this study have positive implications for conducting mussel conservation activities such as status surveys and relocations because emersion over the range of temperatures (15–35°C) and durations (15–60 min) examined did not cause acute sublethal effects in mussels. We found that there were no consistent trends (results of three-way ANOVA) among the three (carbohydrate, lipid or protein) biochemical indices measured in *E. dilatata*, *L. cardium* or *Q. p. pustulosa* that would indicate a biologically relevant compensation to emersion and thermal stress. For example, mean carbohydrate concentrations (averaged over all treatments) in mantle tissue from the

three mussel species studied ranged from 447 to 615 mg/g dry wt. These concentrations were not different from those of mussels in the no-emersion controls from each test. Moreover, the observed carbohydrate concentrations were slightly greater, but similar to mean glycogen concentrations in mantle tissue of undisturbed or 'native' *Amblema plicata* (Say, 1817) (range 243–434 mg/g dry wt) sampled from the upper Mississippi River (Naimo & Monroe, 1999; Newton, Monroe, Kenyon, Gutreuter, Welke & Thiel, 2001). Likewise, the mean lipid concentrations from the mussels studied ranged from 26.7 to 38.1 mg/g dry wt and were similar to those of a natural population of *A. plicata* (30 mg/g dry wt) taken from the upper Mississippi River (Baker & Hornbach, 2000). The similarity in range of these biochemical indices between studies suggests that the exposed mussels from our study were not biochemically compromised and did not differ from presumably unstressed natural populations.

Although the specific time course for alteration of biochemical indices in unionids due to relocation and associated stresses (like those studied here) is unknown, several recent studies have shown that biochemical responses may be detectable within 7–35 days of the stress event. For example, Patterson *et al.* (1997), who studied *Q. p. pustulosa* held in quarantine (done to prevent the accidental introduction of zebra mussels during unionid relocations), found that glycogen concentrations in mantle tissue had decreased about 56% from initial concentrations in as few as 7 days. Likewise, glycogen concentrations in both foot and mantle tissue of *A. plicata* decreased 15–44% from initial concentrations during a 30–35 days quarantine time (Patterson *et al.*, 1997; Newton *et al.*, 2001). Based on the results of these studies, it therefore, seems probable that emersion and thermal stress on mussels from our study, had it occurred, should have been detectable within the 14-day post-exposure period.

Alternatively, the lack of difference in biochemical response to emersion and thermal stress in our study may have been due to the relatively brief exposure duration (maximum of 60 min) and relatively long post-exposure period (14 days) before tissue samples were taken. However, Chen *et al.* (2001), who evaluated exposure durations up to 24 h in both air and water for five species of unionids, found that glycogen concentrations did not change significantly in any of the species over the tested time period in either exposure medium. Their study, however, was only conducted at one unspecified aerial and water temperature differential. Therefore, the possibility remains that mussels from our study that may have been stressed and had altered biochemical indices were able to recover to pre-treatment levels during the 14-day post-exposure period.

The peer-reviewed literature on unionid physiology and biochemistry has expanded greatly in recent years, facilitating comparisons that were previously impossible. Most of these contributions have focused on glycogen, lipid or cellulase activity as indicators of baseline or stressed condition (Haag *et al.*, 1993; Farris, Grudzien, Belanger, Cherry & Cairns, 1994; Naimo *et al.*, 1998; Patterson, Parker & Neves, 1999; Hallac & Marsden, 2000). However, only our study and those of Baker & Hornbach (2000, 2001), have simultaneously measured the holistic biochemical contributors (carbohydrate, lipid and protein) to overall energy status in unionids. In their study of the biochemical composition of a natural population of *A. plicata* infested by zebra mussels, Baker & Hornbach (2000) found that two of the three indices (carbohydrate and protein) were sensitive indicators of stress caused by zebra mussel infestation. They found that infested unionids had first catabolized carbohydrate and then protein from their energy stores as compensation to zebra mussel attachment, and were indicative of concentrations in mussels experiencing starvation. In contrast, our study of relatively short-term aerial and thermal exposures that are indicative of the situations commonly encountered in field situations, did not cause such compensatory changes in biochemical composition of the three tested species.

Comparison of biochemical endpoints among studies is useful, but tenuous and complicated by differences in mussel species, seasonal cycles in life history status, tissues analysed, and analytical methods. For example, Baker & Hornbach (2000) found that non-zebra mussel infested *A. plicata* had an average protein concentration of 450 mg/g dry wt and an average carbohydrate concentration of 250 mg/g dry wt. The protein concentration given by Baker & Hornbach (2000) for *A. plicata* was greater than those for *E. dilatata*, *L. cardium* or *Q. p. pustulosa* in our study (179–289 mg/g dry wt) and their carbohydrate concentration was less than we observed (447–615 mg/g dry wt). The differences between the concentration range for protein and carbohydrate in our study and theirs may be partially attributed to differences among mussel species. Likewise, Chen

et al. (2001) found that baseline glycogen concentrations in mantle tissue varied over 24-fold among five unionid species. Considerable seasonal variation in biochemical status within a single species (Baker & Hornbach, 2001; Newton *et al.*, 2001), presumably due to changes in life history, nutrition or reproductive status, also further complicates point-in-time comparisons of endpoints among studies.

The type of tissue analysed greatly influences the range of biochemical concentrations obtained. For example, Naimo & Monroe (1999), who assessed variation in glycogen concentrations between mantle and foot tissue in *A. plicata*, found that mantle yielded greater concentrations (243–434 mg/g dry wt) than did foot (47–101 mg/g dry wt), and hypothesized that the greater concentrations measured in mantle may be a better indicator of stress due to its high metabolic activity associated with shell formation. In contrast, other studies that have also analysed mantle tissue for glycogen have reported relatively low concentrations compared to Naimo & Monroe (1999), primarily because of differences in methodology (Patterson *et al.*, 1997, 1999; Hallac & Marsden, 2000). Yet other studies have analysed whole-body homogenates for carbohydrate and obtained a wide range of concentrations, even when using similar analytical methods (Haag *et al.*, 1993; Baker & Hornbach, 2000, 2001). In our study, we analysed 5–10 mm cross-sectional, rectangular pieces of mantle tissue (Fig. 1) similar to mantle locations analysed by Naimo & Monroe (1999), thus our carbohydrate concentrations are most comparable to theirs.

Analytical methods for biochemical endpoints, especially carbohydrate (glycogen), have differed greatly among recent studies and have resulted in reported concentrations varying almost 40-fold, even among those evaluating the same species (Haag *et al.*, 1993; Naimo & Monroe, 1999). Methods for glycogen analysis used in some studies (e.g. Patterson *et al.*, 1997, 1999; Hallac & Marsden, 2000) preserve the tissue in 95% ethanol before analysis, whereas others (Naimo *et al.*, 1998; Naimo & Monroe, 1999; Baker & Hornbach, 2000; Newton *et al.*, 2001) use fresh, or fresh frozen and freeze-dried samples for analysis. Glycogen concentrations in 'untreated' mussels measured from preserved tissue are comparable among studies (range 2.52–9.40 mg/g preserved tissue; Patterson *et al.*, 1997, 1999; Hallac & Marsden, 2000), but are markedly less than those measured from wet or fresh frozen tissue (range 131–434 mg/g dry wt; Naimo *et al.*, 1998; Naimo & Monroe, 1999; Baker & Hornbach, 2000; Newton *et al.*, 2001). A side-by-side comparison of ethanol-preserved tissue and fresh wet tissue, along with providing conversion factors for tissue water content and dry weight for common units of expression, would be useful in assessing the potential effects of preservation and water content on glycogen concentrations. We used fresh frozen, freeze-dried samples for carbohydrate analysis in this study with methods similar to those of Naimo *et al.* (1998) and Baker & Hornbach (2000); therefore, our concentrations are most comparable to theirs.

In conclusion, this study provides new and useful baseline information on three biochemical indices that describe the relative health and condition of three previously unstudied unionid species. Moreover, our findings coupled with those of Bartsch *et al.* (2000), who performed a companion study of the effects of temperature and emersion on survival and behaviour with the same three species, suggests that handling these mussel species over the range of environmental conditions examined (air temperatures of 15–35°C and emersion durations of 15–60 min) should not impair biochemical condition, survival or behaviour (burrowing or uprighting). Therefore, conservation activities aimed at protecting the remaining mussel fauna that are conducted at or near these conditions should improve the survival and re-establishment of mussels in the substratum in good biochemical condition.

Because total carbohydrate, lipid and protein concentrations in mantle tissue did not vary consistently in response to the range of emersion temperatures and relatively short exposure durations in this study, we believe that future studies should examine specific enzymes, proteins, or lipids that may be more responsive to short-term stresses that are associated with relocation or other activities, evaluate analytical methods in side-by-side comparisons with rigorous quality assurance protocols, provide conversion factors for expression of data in multiple formats (e.g. wet, dry or preserved weight), provide greater description of the tissue type and its exact sampling location within the organism (Naimo & Monroe, 1999), and assess the effects of sex, reproductive status and seasonality (Baker & Hornbach, 2001) on biochemical response. In addition, an examination of other organismal and environmental variables that may have influenced the relatively poor success rate of past unionid relocations (Cope & Waller, 1995) is also warranted.

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