Chapter 4: Changes in glycogen stores of freshwater mussels (Bivalvia:Unionidae) during starvation and controlled feeding in captivity: an assessment of protocols for quarantine.

Abstract: The effects of controlled feeding and starvation in captivity on the glycogen stores of two mussel species were compared. Starved individuals were not provided with supplemental food in captivity, while specimens under controlled feeding were provided with 1 x 10^5 algal cells·ml^{-1}, twice per day. Initial mean glycogen levels for *Amblema p. plicata* (9.4 ± 2.4 mg/g) and *Quadrula p. pustulosa* (7.9 ± 1.8 mg/g) collected from Ohio River Mile (ORM) 175.5 in July 1997 were not significantly different ($p>0.3$) than the mean glycogen levels of *A. plicata* (8.1 ± 4.2) and *Q. pustulosa* (6.2 ± 2.9) collected from the same site in July 1996. Glycogen stores of unionids entering quarantine, therefore, were similar in both the starvation and controlled feeding experiments. After 7 days of controlled feeding in quarantine, mean glycogen levels of *A. plicata* (12.3 ± 2.3 mg/g) and *Q. pustulosa* (7.1 ± 3.7 mg/g) did not change significantly ($p>0.1$), while the mean glycogen levels of starved individuals (3.6 ± 1.8 mg/g and 3.5 ± 2.3 mg/g, respectively) declined nearly 50%. Similarly, the mean glycogen levels of *A. plicata* and *Q. pustulosa* after 14 days (8.1 ± 3.3 mg/g and 7.7 ± 3.3 mg/g, respectively) and 30 days (9.9 ± 4.8 mg/g and 8.4 ± 2.7 mg/g, respectively) of controlled feeding were not significantly different ($p>0.1$) than the mean glycogen level of wild caught specimens. After 30 days of starvation in 1996, however, the mean glycogen levels of *A. plicata* (1.2 ± 0.5 mg/g) and *Q. pustulosa* (1.9 ± 1.4 mg/g) were reduced by nearly 80% relative to wild caught specimens. Thus, the provision of adequate food resources is critical to maintaining unionid glycogen levels and body condition in quarantine.

INTRODUCTION

Relocation has been widely used as a management tool to re-establish or assist in the recovery of declining populations of terrestrial and aquatic organisms (Griffith et al. 1989; Cope and Waller 1995). Due to recent declines in the unionid fauna of North America (Williams et al. 1993), relocation is being tested as a management technique to 1) re-introduce extirpated populations, 2) remove unionids from project construction zones, 3) augment populations to increase genetic diversity, and 4) salvage unionids from zebra mussel-infested areas (Cope and Waller 1995). The ultimate goal of any relocation project is to establish a self-sustaining population of the target organism, and several authors have developed a list of factors that should extend population persistence after relocation: 1) the presence of suitable habitat and refugia, 2) the release of large founder groups with high genetic diversity, 3) low levels of competition, and 4) high rates of population growth (Griffith et al. 1989, Cope and Waller 1995). Little attention, however, has been given to the biochemical and physiological condition of an organism prior to relocation and its influence on survival and the ability to develop reproductively viable populations after relocation.

Condition, as defined by Mann (1978), is the "ability of an animal to withstand an adverse environmental stress, be this physical, chemical or biological". Glycogen is the primary energy store in bivalves (Bayne 1976, Gabbott 1983), and the relative amount of glycogen stored in bivalve tissues is considered a good indicator of body condition (Galtsoff 1964, Walne 1970). Significant reductions in unionid glycogen stores prior to and during relocation, therefore, may greatly reduce their ability to cope with natural stressors present in the new environment.

Collection, handling, aerial exposure, abrupt temperature changes, holding, and transport of unionids during relocation can result in varying degrees of stress, depending on the length of exposure, that may adversely affect body condition (Cope and Waller 1995). Relocation of unionids to avoid the adverse effects of bridge construction, for example, may have little or no effect on condition if individual specimens are removed from their natural habitat for short time periods (several hours or days). Zebra mussel-infested unionids, however, presently require a full 30 days of quarantine to ensure that zebra mussel adults and juveniles are removed prior to relocation (J. Clayton, pers. comm.). In addition to the stress of relocation, unionids may experience nutritive stress in quarantine because little or no information exists on their nutritional requirements (Gatenby et al. 1997). Under laboratory or hatchery conditions, bivalve energy stores have been shown to decline without proper feeding (Calvin 1931, Pora et al. 1969, Bayne and Thompson 1970, Gabbott and Walker 1971), and recent studies reveal that unionid glycogen stores may decline as much as 80% after only 30 days of starvation in quarantine (Patterson et al.

1997). Consequently, the development of a feeding regime for mussels held in captivity may be a critical link in the success of future relocation projects. The objective of this experiment was to 1) monitor the glycogen levels of unionids during controlled feeding in captivity and 2) compare these results to changes in unionid glycogen levels during starvation in captivity, as reported by Patterson et al. (1997).

METHODS

On July 20, 1997, ten specimens each of *Amblema p. plicata* (Say, 1817) and *Quadrula p. pustulosa* (I. Lea, 1831) were collected from Ohio River Mile (ORM) 175.5 near Parkersburg, WV. Mussels were removed from the shell and placed in 95% ethanol for the determination of initial glycogen levels. Additional specimens of *A. plicata* and *Q. pustulosa* (200 and 80, respectively) were collected from ORM 175.5, scrubbed free of zebra mussels, and placed in individual quarantine tanks, as described by Patterson et al. (1997), on Middle Island National Wildlife Refuge near St. Mary's, WV. Mussels were quarantined for 30 days and hand-inspected according to quarantine protocol to ensure that all zebra mussels were removed. Ten specimens of *A. plicata* and *Q. pustulosa* were sacrificed after 7, 14, and 30 days of quarantine, and preserved in 95 % ethanol for subsequent glycogen analysis. After the experiment, all remaining specimens were returned to the Ohio River.

In 1997, unionids were fed cultures of the chlorophyte, *Neochloris oleoabundans* (Chantanachat and Bold 1962). This species was chosen because previous experiments indicated that adult and juvenile unionids readily ingest and assimilate this alga (Gatenby et al. 1997, Patterson et al. in review). Initial stock cultures of *N. oleoabundans* were grown in Bold's Basal Medium (Nichols 1973) under continuous cool white fluorescent light (photon flux: 60-100 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 20^0C. Stock cultures were then transferred to the quarantine facility and used to inoculate a 20 L carboy containing Fritz F2 algae media (Fritz Aquaculture, Mesquite, TX). When cell densities in the carboy reached 1 x 10^6 cells $\cdot mL^{-1}$, 7L aliquots were used to inoculate three-250L algae culture tanks (Aquatic Ecosystems, Inc., Apopka, FL), that also were fertilized with Fritz F2 algae media, aerated, and placed outside the quarantine facility in direct sunlight. Culture tanks were placed outside because algae cultures continually crashed inside the quarantine facility, possibly due to insufficient light or high water temperatures. Algal cell densities in the 250L culture tanks reached 1 x 10^6 cells $\cdot mL^{-1}$ in ca. 4 days. Water samples from the culture tanks were collected daily and fixed with acid Lugol's for enumeration and identification of the algae. Water samples from the Ohio River in 1996 and 1997 showed that algal cell densities ranged from 1 x 10^4

- 1 x 10^5 cells · mL^{-1} during the summer (Parker et al. in press); thus, unionids in quarantine were fed ca. 1 x 10^5 cells · mL^{-1} twice per day, at 8am and 5pm. Each day, 75% of the water in the quarantine tanks was drained; fresh water and food were added, and vigorous aeration was applied to maintain algal cells in suspension.

The glycogen content of all preserved specimens was determined from mantle tissue using the technique of Keppler and Decker (1974) as described in Patterson et al. (1997). Mean glycogen levels were expressed in milligrams glycogen per gram preserved mantle tissue. It should be noted that preserved tissue weights overestimate dry weights and underestimate wet tissue weights because 95% ethanol dehydrates tissue. Dehydration by 95% ethanol also reduces error that may result from changes in tissue water levels during stress. Mean glycogen levels were then compared using ANOVA and, if significant differences were detected, the Scheffe F-test was used to determine the statistical significance of individual treatments.

RESULTS AND DISCUSSION

During the first 14 d of quarantine, *N. oleoabundans* comprised > 95% of the algae in the 250 L culture containers. Since culture tanks were maintained outside the quarantine facility, cultures were contaminated with low densities of two green algal genera, *Scenedesmus* and *Ankistrodesmus*. Once cultures were contaminated, densities of these genera continued to increase. After 30 days, the algal community in the culture containers had changed significantly with *Scenedesmus* and *Ankistrodesmus* comprising ca. 40% of the available algae, and *N. oleoabundans* comprising the remaining 60%. Despite changes in the algal community, cell densities remained at 1 x 10^6 cells · mL^{-1} throughout the experiment.

Mean glycogen levels of *A. plicata* and *Q. pustulosa* during controlled feeding in quarantine remained the same (Figure 1), whereas mean glycogen levels declined in a previous starvation experiment by Patterson et al. (1997) (see Figure 2 for comparison). Initial mean glycogen levels for *Amblema plicata* (9.4 $\pm$ 2.4 mg · g^{-1}) and *Quadrula pustulosa* (7.9 $\pm$ 1.8 mg · g^{-1}) collected from ORM 175.5 in July 1997 were not significantly different ($p>0.3$) from the mean glycogen levels of *A. plicata* (8.1 $\pm$ 4.2 mg · g^{-1}) and *Q. pustulosa* (6.2 $\pm$ 2.9 mg · g^{-1}) collected from the same site in July 1996 (Patterson et al. 1997). Glycogen stores of unionids entering quarantine, therefore, were similar in both the starvation and controlled feeding experiments. After 7 d of feeding in quarantine, mean glycogen levels of *A. plicata* (12.3 $\pm$ 2.3 mg · g^{-1}) and *Q. pustulosa* (7.1 $\pm$ 3.7 mg · g^{-1}) did not change significantly ($p>0.1$), while the mean glycogen levels of starved individuals (3.6 $\pm$ 1.8 and 3.5 $\pm$ 2.3 mg · g^{-1}, respectively) declined nearly 50% in 1996. Similarly, the mean glycogen levels of *A. plicata* and *Q. pustulosa* after 14 d (8.1 $\pm$ 3.3 and 7.7 $\pm$ 3.3 mg · g^{-1}, respectively) and 30 d (9.9 $\pm$ 4.8 and 8.4 $\pm$ 2.7 mg · g^{-1}, respectively) of

feeding were not significantly different ($p>0.1$) from the mean glycogen level of wild-caught specimens. After 30 d of starvation in 1996, however, the mean glycogen levels of *A. plicata* (1.2 ± 0.5 mg $\cdot$ g^{-1}) and *Q. pustulosa* (1.9 ± 1.4 mg $\cdot$ g^{-1}) were reduced by nearly 80% relative to wild-caught specimens.

Glycogen, the primary energy reserve in bivalves, drives many important physiological processes and may be used to endure short-term exposure to anoxia, emersion, and reduced food supplies (Bayne 1976, Gabbott 1983, Bayne et al. 1985, Hummel et al. 1988). Although exposure to anoxia and emersion may be limited if unionids are relocated to suitable habitat, unionids will likely experience short-term, localized shifts in food abundance and long-term food shortages during the winter months. Normally, by accumulating glycogen when food is abundant, bivalves are able to withstand these food shortages (Gabbott 1983, Hummel et al. 1988). However, unionid survival after relocation may be greatly reduced if glycogen stores are depleted in quarantine and do not recover prior to the onset of winter. Regardless of effects on survival, decreased glycogen levels in adult bivalves also may have sublethal effects including reduced fecundity and reduced growth rates of developing offspring (Bayne 1972, Helm et al. 1973, Bayne et al. 1975). Thus, the provision of adequate food resources for unionids in quarantine is pertinent to maintaining glycogen levels and enabling mussels to develop reproductively viable populations after relocation.

Results from this study indicate that relatively high cell densities of the green alga, *Neochloris oleoabundans* , in combination with smaller amounts of *Scenedesmus* and *Ankistrodesmus* , is an adequate food resource for the maintenance of unionid glycogen stores in the short term (30 days). Currently, no information exists on the ability of adult unionids to digest and assimilate *Scenedesmus* and *Ankistrodesmus* , but recent studies show that unionids assimilate *Neochloris oleoabundans* with relatively high efficiency (>50%, Patterson et al. in review). Additional studies to determine which algal species are most suitable as food for unionids are critical, because some algal species may not be readily digested and assimilated in the bivalve gut (Peirson 1983). Regardless of the digestibility of a particular algal species, large amounts of algae will be required if management agencies hope to relocate large numbers of native mussels away from zebra mussel-infested areas. In this study, the quarantine of 300 mussels required constant culture of 750 L of live algae. Although it may not reduce the volume of food required, discovering ways to shorten the quarantine period will decrease the time unionids must be maintained in captivity, outside their natural habitat. Studies dealing with more efficient methods of removing zebra mussels from the shells of unionids may prove to be the best avenue for shortening the quarantine period. Ultimately, a short quarantine period along with the provision of food will maintain the body condition of unionids and improve the success of future relocations.

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Figure 1. Glycogen levels (mg . g $^{-1}$) of *A. plicata* and *Q. pustulosa* at 1, 7, 14, and 30 days of controlled feeding in quarantine (n=10/sampling period).

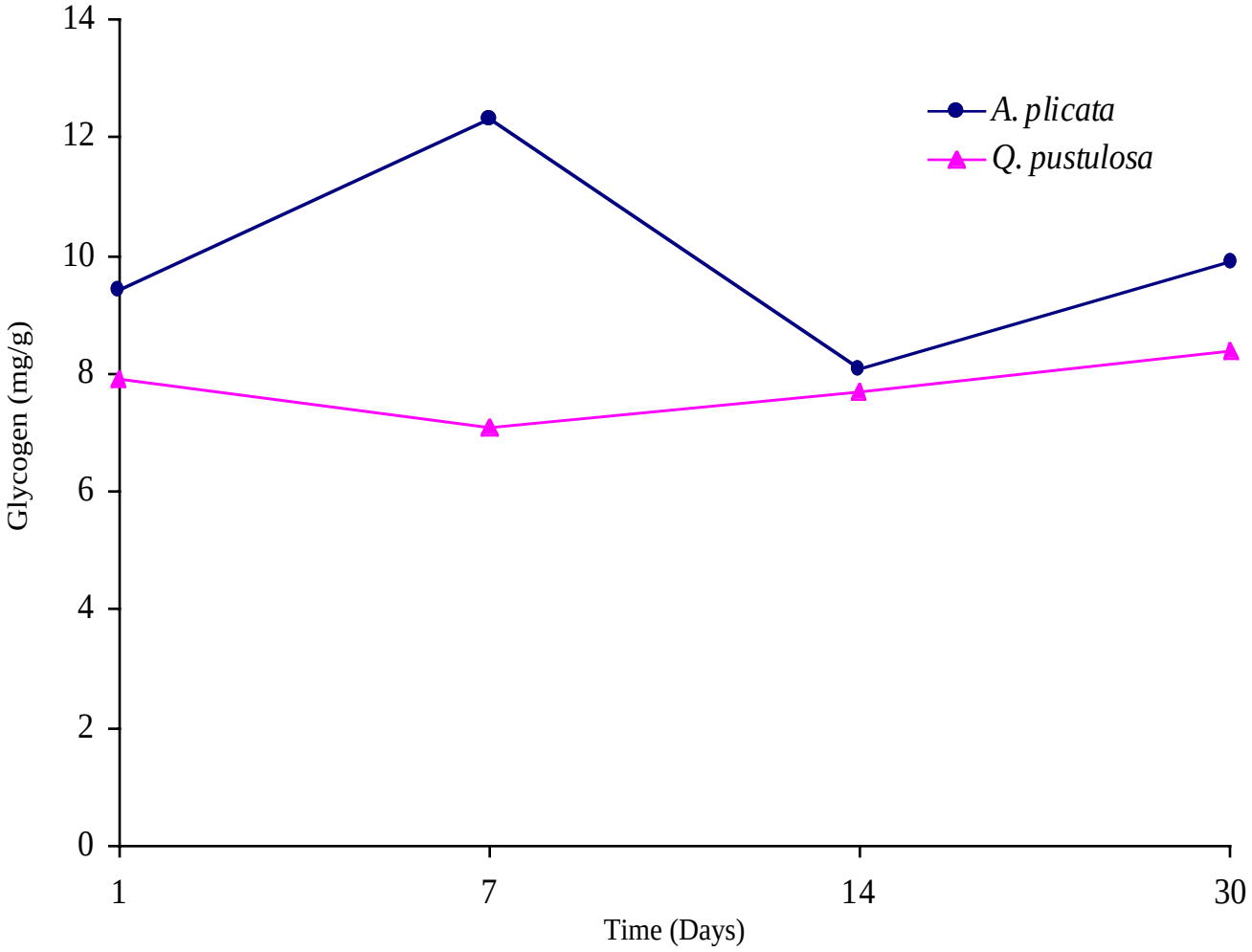
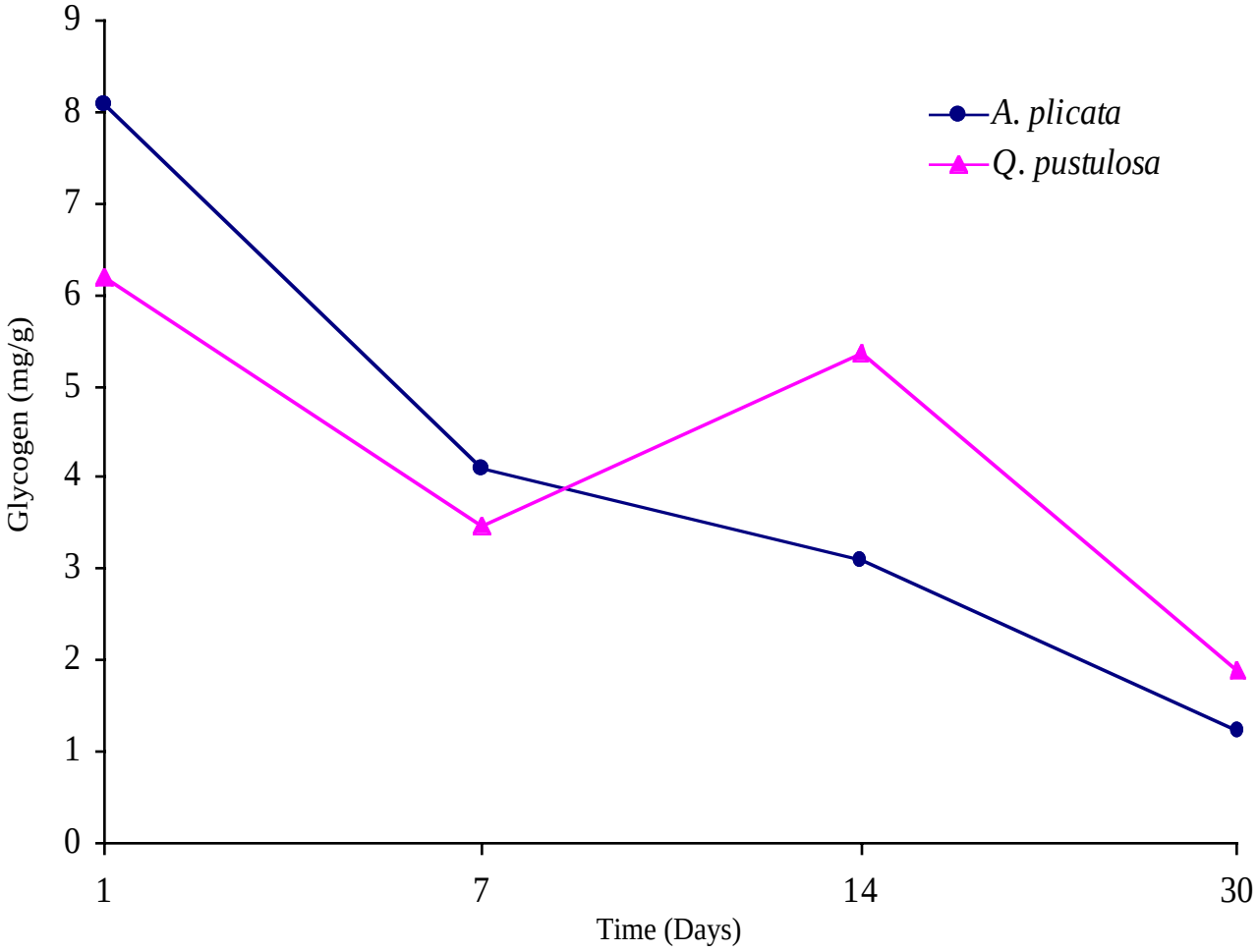


Figure 2. Glycogen levels (mg · g $^{-1}$) of *A. plicata* and *Q. pustulosa* at 1, 7, 14, and 30 days of starvation in quarantine (n=10/sampling period).



CONCLUSIONS

1) Specimens of *Amblema p. plicata* and *Quadrula p. pustulosa* , heavily infested with zebra mussels, experienced greater than 60% reductions in the storage of glycogen realtive to lightly infested specimens.

2) Significant reductions in glycogen storage seemingly result from significant reductions in unionid ingestion caused by reduced food resources and fouling by zebra mussels.

3) The provision of adequate food resources during the mandatory quarantine period is critical to the maintenance of unionid condition and should increase the chances of successful relocation.

4) The green alga, *Neochloris oleoabundans* , is assimilated by the freshwater mussel, *Villosa iris*, with relatively high efficiency (~ 50%) and therefore may serve as a good food resource for freshwater mussels held in captivity.

5) The recommended concentration to feed in captivity should be no less than 1×10^5 cells $\cdot$ mL^{-1} (3 mg dry weight $\cdot$ L^{-1}), because this amount lead to the highest total assimilation of carbon.

6) By maximizing total carbon assimilation, *V. iris* will have the greatest amount of energy available for growth, reproduction, and maintenance of condition in captivity.

7) The provision of 1×10^5 cells $\cdot$ mL^{-1} of *N. oleoabundans* twice per day in quarantine allowed for the maintenance of unionid glycogen stores.

8) Because glycogen levels in the natural environment are typically lowest in the summer during the reproductive season, relocation efforts should avoid removing and quarantining freshwater mussels during the summer months.

VITA

Matthew Alan Patterson was born in Flint, Michigan on June 6, 1973. In 1975, his family moved to Flatwoods, Kentucky where he attended and graduated from Russell High School in 1991. After spending two years at Georgetown College in Georgetown, Kentucky, he tranfered to Eastern Kentucky University where he received a Bachelors degree in Biology in December 1995. After graduation he was accepted in May 1996 as a graduate student at Virginia Polytechnic Institute and State University in Blacksburg, Virignia. In June 1998, he passed his thesis defense and completed the requirements for a Masters Degree in Biology. Below are a list of manuscripts either published, in press, or in review as a result of his masters research.

Patterson, M. A., B. C. Parker, and R. J. Neves. 1997. Effects of quarantine times on glycogen levels of native freshwater mussels (Bivalvia:Unionidae) previously infested with zebra mussels. American Malacological Bulletin 14(1):75-79.

Parker, B. C., M. A. Patterson, and R. J. Neves. 1998. Feeding interactions between native freshwater mussels (Bivalvia: Unionidae) and zebra mussels (*Dreissena polymorpha*) in the Ohio River. American Malacological Bulletin.

Patterson, M. A., C. M. Gatenby, B. C. Parker, R. J. Neves, and D. A. Kreeger. 1998. Ingestion and assimilation of ^{14}C labeled algae by the freshwater mussel, *Villosa iris* (Lea, 1829) at three cell concentrations. Journal of Shellfish Research

Patterson, M. A., B. C. Parker and R. J. Neves. 1998. Changes in glycogen stores of freshwater mussels (Bivalvia:Unionidae) during starvation and controlled feeding: An assessment of protocols for quarantine. American Malacological Bulletin

Following graduation Matthew plans to continue research on freshwater mussels under Dr. Richard Neves of the Fisheries and Wildlife Deaprtment of Virginia Polytechnic Institute and State University.