Effects of quarantine times on glycogen levels of native freshwater mussels (Bivalvia: Unionidae) previously infested with zebra mussels

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Abstract: The effects of zebra mussel infestation and subsequent quarantine on three mussel species were evaluated through glycogen analyses of mantle tissue. Specimens of *Amblema plicata* (Say, 1817) and *Quadrula pustulosa* (I. Lea, 1831), collected from a heavily infested (> 350 zebra mussels/m^2) reach of the Ohio River in 1996, had significantly lower glycogen levels (2.73 and 1.84 mg/g, respectively) than specimens collected from a lightly infested (< 5 zebra mussels/m^2) reach upstream (8.08 and 6.20 mg/g, respectively). Levels of glycogen after 7, 14, and 30 d of quarantine in tanks declined dramatically with length of quarantine. After 30 d without supplemental feeding, mean glycogen levels of *A. plicata* collected from the low density reach had dropped to 15% of that of wild caught specimens (1.22 versus 8.08 mg/g, respectively). After 30 d, mean glycogen levels of *Q. pustulosa* also dropped significantly to 30% of that of wild caught specimens (1.90 versus 6.20 mg/g, respectively). Mean glycogen levels of *Fusconaia ebena* (I. Lea, 1831), collected from the heavily infested reach of the Ohio River dropped to extremely low levels (from 2.75 to 0.53 mg/g) after 30 d of quarantine. Specimens of *F. ebena* were quarantined for an additional 100 d because zebra mussels were found on unionids after 30 and 60 d of quarantine. Feeding every three days between 30-130 d of quarantine was insufficient to allow for recovery after 100 d (0.30 mg/g) or 130 d (0.34 mg/g). A 30 d quarantine of unionids removed from zebra mussel-infested waters causes a significant reduction in glycogen levels which are further reduced if additional quarantine time is required. Feeding of unionids is necessary to maintain their condition during lengthy quarantine, or more effective methods are needed to remove zebra mussels and thus shorten the required quarantine period.

Key words: glycogen, zebra mussels, Unionidae, Ohio River, quarantine

Freshwater mussels of the family Unionidae reach their greatest diversity in North America with nearly 300 species (Williams *et al.*, 1993). However, increased habitat alteration, siltation, and pollution have caused dramatic declines in both species diversity and richness (Bogan, 1993). Populations of freshwater mussels are now at further risk of extirpation or extinction from the exotic zebra mussel, *Dreissena polymorpha* (Pallas, 1771). Since the zebra mussel's introduction into Lake St. Clair around 1985, this exotic mollusk has decimated local populations of freshwater mussels throughout the Great Lakes (Hunter and Bailey, 1992; Gillis and Mackie, 1994; Schloesser and Nalepa, 1994). The pelagic veliger stage has enabled zebra mussels to colonize many of the large river systems of the southeastern United States, and extremely high fecundities have allowed populations to increase exponentially after settlement of veligers (Sprung, 1991). Adult zebra mussels were first collected from the lower Ohio River in 1991 (USACOE, 1993), and densities reached nearly 100,000/m^2 in the lower river by July 1994 (A. Miller, pers. comm.). In 1995, the Ohio River Valley Ecosystem Team identified the potential decline of native aquatic mollusks as its top management priority (USFWS, 1995).

Resource agencies and universities are currently testing removal and translocation as a management tool to conserve declining numbers of unionids from zebra mussel-infested waters. The current protocol in West Virginia for unionid salvage from zebra mussel infested waters requires that all unionids be thoroughly scrubbed to remove zebra mussels (J. Clayton, pers. comm.). Cleaned unionids are then hand-inspected before being placed in aerated quarantine tanks for a minimum of 30 d to allow juvenile zebra mussels missed during the scrubbing procedure to become visible. During quarantine, water quality parameters (*i. e.* temperature, dissolved oxygen, and pH) are monitored to provide suitable conditions for unionid survival. At the end of 30 d, individual unionids are inspected under 10X magnification, and if zebra mussels are found, all specimens must be rescrubbed, placed in clean tanks, and quarantined for an additional 30 d. Finally, translocation can occur only

when the native mussels are certified free of zebra mussels.

Zebra mussel infestations in combination with collection, transport, and handling during quarantine, can lead to increased stress in freshwater mussels. Glycogen, an important energy reserve for animals, especially bivalves (de Zwann and Zandee, 1972; Barber and Blake, 1981; Bayne and Newell, 1983; Haag *et al.*, 1993), has been shown to change in response to environmental perturbations such as temperature extremes, anaerobiosis, pollutants, or starvation (de Zwann and Wijsmann, 1976; Hummel *et al.*, 1989). In marine bivalves, glycogen levels also have been shown to change seasonally (Hummel *et al.*, 1988) in response to such factors as gametogenesis (Gabbott, 1983) and winter food shortages (Gade, 1983). Haag *et al.* (1993) showed that the mean glycogen content of *Amblema plicata* (Say, 1817) and *Lampsilis radiata* (Gmelin, 1791) from Lake Erie was significantly lower in zebra mussel-encrusted versus unencrusted control specimens. Thus, a glycogen assay was used in this experiment to assess the impact of zebra mussel infestation, removal, and 30 d quarantine on the physiological condition of freshwater mussels collected from the Ohio River. Specific research objectives were to (1) quantify the glycogen levels of freshwater mussels infested with zebra mussels in high versus low density areas, and (2) assess the change in glycogen levels of unionids during quarantine periods ranging from 30 to 130 d.

METHODOLOGY

The effect of zebra mussel infestation on unionid glycogen levels was compared between specimens collected from high versus low zebra mussel-infested sites on the Ohio River. To minimize the natural, seasonal fluctuation in glycogen levels, specimens were collected from the study sites between 23 July and 21 August 1996. Ten specimens each of *Amblema plicata* and *Quadrula pustulosa* (I. Lea, 1831) were collected from Ohio River Mile (ORM) 175.5 on 23 July 1996. This low infestation site near Parkersburg, West Virginia, had a mean density of 0.3 zebra mussels/m^2, and a maximum of one zebra mussel/unionid (P. Morrison, pers. comm.). On 16 August 1996, ten specimens of *A. plicata* were collected from ORM 967. This heavily infested site near Paducah, Kentucky, had a mean density of 3,600 zebra mussels/m^2 (A. Miller, pers. comm.). Because *Q. pustulosa* was uncommon at ORM 967, ten specimens were collected from ORM 397 on 21 August 1996. Zebra mussel densities at this site near Maysville, Kentucky, increased 30-fold between 1995 and 1996. With a mean density of 360 zebra mussels/m^2 and a maximum of 92 zebra mussels/unionid (P. Morrison, pers. comm.), ORM 397 also was considered to be a heavily infested site. All specimens collected in the field were sacrificed on the day of collection, shucked, weighed, preserved in 95% ethanol, and transported to the laboratory for analysis.

To assess the effect of quarantine on unionid condition, additional specimens of *Amblema plicata* and *Quadrula pustulosa* (250 and 80, respectively) were collected from ORM 175.5. All specimens were aged, measured, tagged, and transported in well water to 300 l, aerated quarantine tanks on Middle Island, Ohio River Islands National Wildlife Refuge, in St. Mary's, West Virginia. Because the quarantine tanks did not provide flow-through conditions, tank water was drained and filled with well water every 2 d. Specimens of *A. plicata* were placed in individual quarantine tanks at densities of 250/m^2 and 65/m^2, respectively, to determine possible density effects on glycogen stores. The 80 specimens of *Q. pustulosa* were placed in a third tank. During the 30 d quarantine, unionids were not fed, simulating likely conditions during recovery, quarantine, and relocation of threatened unionids. Ten specimens of each species were sacrificed from each tank at 7, 14, and 30 d of quarantine, and preserved in 95% ethanol for subsequent glycogen analysis.

Heavily infested individuals of *Amblema plicata* and *Quadrula pustulosa* could not be used to monitor glycogen levels during quarantine because specimens could not be collected in sufficient numbers from the lower Ohio River. Instead, 250 specimens of *Fusconaia ebena* (I. Lea, 1831) were collected from ORM 967 on 16 August 1996, to determine the effect of quarantine on heavily infested unionids. Ten specimens were sacrificed in the field, and the remainder transported to the quarantine site. Again, ten specimens were sacrificed after 7, 14, and 30 d of quarantine, and preserved in 95% ethanol for subsequent glycogen analysis. At the end of 30 d, zebra mussels (3 mm in length) were discovered attached to the umbonal region of five *F. ebena* in quarantine. All specimens were removed, rescrubbed, hand-inspected, and placed in clean quarantine tanks for an additional 30 d. After the initial 30 d, mussels were fed from a fertilized algae tank every 3 d. After 60 d, zebra mussels again were found attached to the umbonal region of five specimens of *F. ebena*, and all specimens were rescrubbed, inspected, and placed in clean quarantine tanks. At the end of 100 d, no zebra mussels were found during inspection but an additional 30 d period was required to assure that no zebra mussels would be transported out of quarantine. After 130 d, unionids were certified free of zebra mussels and removed from quarantine. To assess the effect of this long-term quarantine period, ten specimens were sacrificed after 100 d and 130 d and preserved in 95% ethanol for subsequent glycogen analysis.

The glycogen content of all preserved specimens was determined using the technique described by Keppler

and Decker (1974). A 50-100 mg sample of preserved mantle tissue was dissected, blotted dry to remove the ethanol and weighed. Tissue samples were homogenized for 2 hr in 3M perchloric acid and neutralized with 2M $KHCO_3$. Glycogen was converted to glucose with amyloglucosidase (Sigma Chemical Co., St. Louis, Missouri), combined with a dye solution containing o-dianisidine dihydrochloride, and absorbance measured in a spectrophotometer at 450 nm. Total glycogen was determined from a standard curve of glycogen extracted from the blue mussel, *Mytilus edulis* (Linné, 1758), and expressed in milligrams glycogen/gram preserved mantle tissue. It should be noted that 95% ethanol dehydrates tissue and preserved tissue weights likely underestimate wet tissue weights. However, dehydration also reduces error that can result from any change in tissue water levels during stress. Mean glycogen levels were not standardized by total body weight because simple regression revealed no correlation between wet weight and glycogen content ($r^2 < 0.10$). The mean glycogen levels of all treatments (high versus low zebra mussel density and 7-30 d of quarantine) were normally distributed according to the Kolmogorov-Smirnov goodness of fit test ($\alpha = 0.05$). However, zebra mussel infestation and starvation during quarantine uniformly decreased the glycogen levels of all specimens and consequently decreased overall variance. Following Lentner (1993), the sample variances were equalized using the square root of each individual glycogen value. Converted mean glycogen levels were then compared using ANOVA. If significant differences were detected, Scheffe F-test was used to determine the statistical significance of individual treatments.

RESULTS

Initial mean glycogen levels of *Amblema plicata* collected from the heavily infested site (ORM 967) were significantly lower ($p < 0.05$) than those collected from the upper river at ORM 175.5 (2.73 ± 2.81 mg/g versus 8.08 ± 4.26 mg/g, respectively). The initial mean glycogen level of *Quadrula pustulosa* collected from ORM 397 also was significantly lower ($p < 0.05$) than that collected from ORM 175.5 (1.84 ± 1.23 mg/g versus 6.20 ± 2.89 mg/g, respectively). During quarantine, the mean glycogen level of *A. plicata* collected from ORM 175.5 dropped significantly ($p < 0.05$) after 7 d (Fig. 1). While significant differences were not observed between 7 and 14 d ($p > 0.3$), the mean glycogen level continued to drop significantly ($p < 0.05$) between 14 and 30 d until reaching 15% of that measured in wild-caught specimens (Fig. 1). The mean glycogen level of *Q. pustulosa* collected from ORM 175.5 also dropped significantly ($p < 0.05$) after 7 d of quarantine (Fig. 1). Between 7 and 14 d, the mean glycogen level increased; however, the increase was not statistically significant ($p > 0.1$). At 30 d, the mean glycogen level dropped significantly ($p < 0.05$) to only 31% of that measured in wild-caught specimens (Fig. 1). There was no significant difference ($p > 0.3$) in mean glycogen levels between mussels held at 250/m² and 65/m² after 7 d (3.56 ± 1.78 mg/g and 4.09 ± 2.18 mg/g, respectively) or 14 d (3.27 ± 1.74 mg/g and 3.10 ± 1.57 mg/g, respectively).

Specimens of *Fusconaia ebena* collected from ORM 967 also showed a significant decline ($p < 0.05$) in the mean glycogen level after 7 d of quarantine (Fig. 2). However, significant changes were not detected for the remainder of the quarantine period. After 30 d, the mean glycogen level was only 20% of that measured in wild-caught specimens (Fig. 2). Feeding of unionids every 3 d between 30 and 130 d was not sufficient to allow unionid glycogen levels to recover. After 130 d, the mean glycogen level was only 12% of that measured in wild-caught specimens (Fig. 2).

DISCUSSION

While different densities (up to 250 unionids/m²) in quarantine had no significant effect on the glycogen stores of *Amblema plicata*, it is clear that previous levels of zebra mussel infestation and starvation during quarantine significantly reduce unionid energy stores. By attaching in great densities to the outer shell of living unionids, zebra mussels reduce glycogen stores, presumably by reducing vital food resources, disrupting proper feeding and respiration, and preventing valve opening and closing (Mackie, 1991).

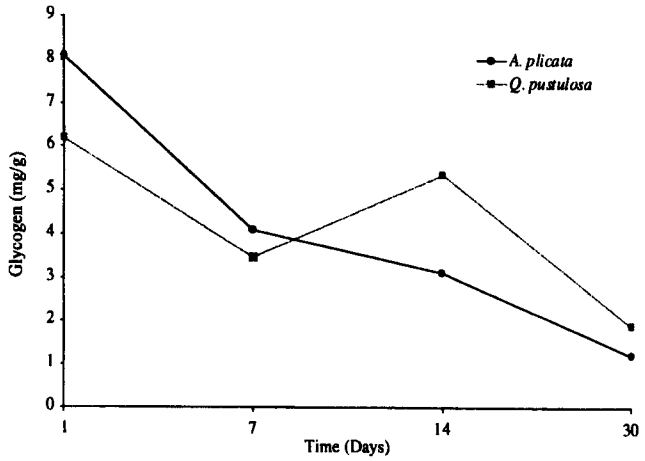

Fig. 1. Glycogen levels (mg/g) of *Amblema plicata* and *Quadrula pustulosa* at 1, 7, 14, and 30 d of quarantine (n = 10/sampling period).

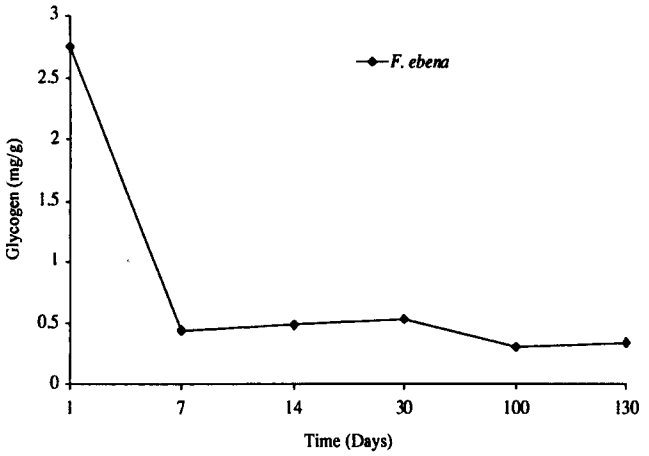

Fig. 2. Glycogen levels (mg/g) of *Fusconaia ebena* at 1, 7, 14, 30, 100, and 130 d of quarantine (n = 10/sampling period).

Thus, energy stores were already at low levels when unionids entered the 30 d quarantine period. Assay results from *Fusconaia ebena* revealed that low glycogen levels of unionids removed from areas with high densities of zebra mussels reach dangerously low levels during a 30 d quarantine period. It is unclear whether a threshold level of glycogen is required to cause mortality, but low energy stores after quarantine can decrease the likelihood that unionids will survive the relocation process. Unionids collected from high quality habitat with low zebra mussel densities can have sufficient energy stores to survive a quarantine period and subsequent translocation, however, unionids in areas with high densities of zebra mussels are the primary candidates for relocation. Thus, when unionids from zebra mussel-infested waters are translocated, a major limiting factor could be the physiological condition and energy reserves of unionids at the time of relocation.

In a review of the literature, Cope and Waller (1995) reported that survival of translocated unionids is typically low (< 50%) and is influenced by many factors. Factors affecting translocation success such as habitat suitability, numbers of individuals released, and the frequency of release have been given significant attention in recent years for both terrestrial and aquatic organisms (Griffith *et al.*, 1989; Cope and Waller, 1995). However, no attention has been given to the physiological condition or energy reserves of relocated organisms. In order to reduce the likelihood of latent mortality of mussels salvaged from zebra mussel-infested waters, it is necessary to either provide sufficient food and favorable water quality conditions during quarantine or to have a brief quarantine period to ensure that unionids have sufficient energy stores to recover from the stressful relocation to new environments.

As judged by the energy reserves in specimens of *Fusconaia ebena* from 30-130 d of quarantine, starved unionids can reach a point where supplemental feeding contributes little to the recovery of energy reserves. Thus, a detailed study to determine the amount of food required to maintain unionid condition during quarantine is needed. In addition to maintaining unionid condition, food supplements also will increase the growth rate of juvenile zebra mussels that are missed during the scrubbing procedure. It is evident that small zebra mussels can avoid extensive scrubbing and inspection, possibly by residing in the crevices of damaged shells. Because the purpose of quarantine is to guarantee the absence of zebra mussels, increased growth rates would enhance detection and justify a reduction in the quarantine period. More effective techniques of zebra mussel removal also should be developed to reduce or perhaps eliminate the need for a lengthy quarantine period.

Assay results from this study reveal that the glycogen levels of all three species decreased significantly after 7 d, and then stabilized between 7 and 14 d. Thus, a reduction in the quarantine period from 30 to 15 d would greatly improve the overall condition of unionids prior to translocation. However, under current protocol standards, unionids must endure a minimum of 30 d of quarantine and a total of 60 d if zebra mussels are detected, which can cause glycogen levels of unionids to decline to life-threatening levels. Thus, one of the greatest concerns during the salvage of zebra mussel-infested unionids should be the physiological condition of unionids at the time of their final relocation.

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