An Evaluation of Air and Water Transport of Freshwater Mussels (Bivalvia: Unionidae)

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Abstract: To compare the stress of transportation of mussels in water versus in air, we collected in the field fifty specimens of each of five species: *Elliptio complanata, Villosa iris, Fusconaia ebena, Quadrula quadrula,* and *Amblema plicata*. We froze subsamples of each species in liquid nitrogen and the rest of the animals were divided into two groups. One group was placed in a water-filled, aerated tank and the other group was placed in coolers with wet burlap before all were transported to the laboratory. We removed a subsample of each group at 6 hr intervals up to 24 hr, and froze it in liquid nitrogen. We used changes in the concentration of glucose and glycogen in the posterior adductor muscle, gill, and mantle to estimate the stress caused by the transport and holding methods.

Villosa iris was the most sensitive to the effects of transportation, particularly as reflected by glucose concentrations in the mantle and posterior adductor muscle. Glucose became elevated in both air-transported specimens of this species, but the change was significantly greater in those transported in air. *V. iris* is found in well aerated habitats, and previous studies have documented its greater sensitivity to reduced oxygen than most other species. The other unionids we tested tended to show significant elevations in glucose only in air-transported specimens, with the extent of increase varying with the species. Glycogen concentrations did not change significantly in any of the species during transportation in either mode. Overall, transportation in water appeared to be less stressful than in air, but for short intervals of time, it may not matter for species such as *Fusconia ebena*, which are relatively tolerant of oxygen lack.

Key Words: Unionids, transportation, stress, glycogen, glucose, mollusks, bivalves

The freshwater mussel fauna (Superfamily Unionacea) in the United States has experienced a precipitous decline in abundance during this century, the decline stemming principally from anthropogenic alterations of physical habitat and water quality. Because of continuing declines in mussel populations and the recognition of 69 federally endangered and threatened mussel species, protected under the Endangered Species Act of 1973, state and federal resource agencies have become actively involved in conservation efforts. Relocation of mussels has become a widely accepted technique to recolonize stream reaches decimated by previous pollution events (Ahlstedt, 1979; Sheehan *et al.,* 1989), to salvage specimens from construction projects (Oblad, 1980; Dunn, 1993), to restore populations of endangered species (O'Beirn *et al.*, 1998), and to prevent loss of populations through colonization by zebra mussels [*Dreissena polymorpha* (Pallas, 1771)]. In a recent review of relocation projects, Cope and Waller (1995) noted high mortality in most relocation efforts and little guidance on standard methods for relocation or for monitoring relocation success. While high mortality following transportation could be due to poor conditions in the receiving waters (*e. g.*, low dissolved oxygen), the physiological impact of the transportation process itself has seemingly not been investigated.

Although mussel relocations have been conducted routinely for nearly 30 years, there has been no adequate assessment of the modes of transport from one location to another. Waller *et al.* (1995) conducted a relocation of mussels in the upper Mississippi River and noted a decreasing trend in survival with duration of aerial exposure in June compared with September. These authors concluded that handling and aerial exposure should have no major effect on survival of mussels in moderate air temperatures, if mussels are collected and processed within several hours. However, no physiological indicators of condition or stress in their mussels were monitored, only survival. The subsequent survival of mussels following translocation is affected by the stress of the transport itself as well as the charac-

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teristics, such as dissolved oxygen, temperature, pollution, etc. of the habitat to which they were moved.

Our study was conducted to compare the degrees of stress caused by transporting mussels in air versus in water, and to determine to what extent different species vary in their responses. Biochemical analyses of free glucose and glycogen in body tissues were used to assess the level of stress in five species from diverse habitats by the two transport methods.

METHODOLOGY

We collected unionids either by SCUBA diving in lakes, or by snorkeling in streams. Three freshwater mussel species, ebonyshell (*Fusconaia ebena* I. Lea, 1831), mapleleaf (*Quadrula quadrula* Rafinesque, 1820), and threeridge (*Amblema plicata* Say, 1817) were obtained from Kentucky Lake, Tennessee in cooperation with the Tennessee Shell Company and the Tennessee Wildlife Resources Agency in July 1996. We collected specimens of the rainbow mussel (*Villosa iris* I. Lea, 1829) from Copper Creek, Virginia and the eastern elliptio (*Elliptio complanata* Lightfoot, 1786) from the Nottoway River, Virginia in September 1996.

Fifty specimens of each species were collected, and a subsample (5-6) of these was frozen in the field by opening the shell and immersing specimens in liquid nitrogen (-195°C) for a few seconds until thoroughly frozen. For technical reasons, it was not practical to freeze the mussels immediately after bringing them up from the lake or river bottom. The subsequent delay of 15 to 60 minutes (depending on species) means that the zero-time glucose concentrations may be higher than in situ, although the animals were kept in buckets of water during this delay. All frozen mussels were then transferred to coolers with dry ice (-56°C) and subsequently placed in a freezer (-80°C) upon arrival at the laboratory. Half of the remaining animals were placed in a water-filled, aerated transport tank (transportation in water), and the other half were placed in coolers with wet burlap (transportation in air) and transported to the laboratory.

For the unionids transported in water and air, at intervals of 6, 12, 18, and 24 hr, a subsample of each species was removed and quickly frozen in liquid nitrogen, then stored at -80°C until analyzed. On the basis of pilot studies (Chen *et al.*, 1996), we chose three tissues (posterior adductor muscle, gill and mantle) and two metabolites (glucose and glycogen) for estimating the stress response of the mussels. Increases in glucose probably reflect short-term stress (Pekkarinen and Suoranta, 1995), and glycogen represents the primary energy store and is one measure of the relative health of mussels (deZwaan, 1983).

In preparation for biochemical analysis, the three tissues were dissected out, weighed, homogenized in 6% perchloric acid, neutralized with potassium bicarbonate, and the extract was stored at -80°C. Free glucose was measured first on the extract, and then glycogen was determined by the enzymatic method of Keppler and Decker (1984). Sigma kits (Catalog No. 510-A, Sigma Chemical Co., St. Louis, Missouri) were used for measuring the free glucose and that released after enzymatic breakdown of the glycogen.

Statistical analyses were conducted by unpaired Student's t-test, comparing pre-transport and post-transport results, and the results of transportation in water versus transportation in air. The criterion for statistical significance was $p < 0.05$.

RESULTS

Glucose

Villosa iris. Both transportation in water and in air caused an initial elevation in glucose concentrations in the mantle tissue (Fig. 1). However, for those mussels transported and held in water, the mantle glucose concentrations recovered after they reached a peak at 12 hr, whereas those transported in air did not recover. Similarly, mussels transported in air had glucose concentrations in the posterior adductor muscle that were significantly higher than initial levels. Glucose concentrations were significantly higher in air-held versus water-held mussels at 18 hr and 24 hr.

Elliptio complanata. Transportation in water caused no significant elevation in glucose concentrations (Fig. 2), but there were obvious increases in glucose in mussels transported in air. The glucose concentrations of animals transported in air were significantly higher than those transported in water for the mantle tissue at all sample periods, and for the posterior adductor muscle at 18 hr.

Fusconaia ebena. Glucose concentrations of the three different tissues in this species did not change during the transportation in water, but increased significantly in specimens transported in air (Fig. 3). The changes in the posterior adductor muscle were the most noteworthy at 12 hr and 18 hr.

Quadrula quadrula. The glucose concentrations of specimens transported in air were significantly higher than those transported in water for some of the tissues at particular time periods; however, results were too inconsistent to provide an obvious trend (Fig. 4). Glucose levels in mantle tissue actually decreased during the 24 hr holding period.

Amblema plicata. The glucose concentrations of those transported in water increased significantly in the gill tissue after 18 hr, but there was a trend of decreasing glucose in the mantle and posterior adductor muscle (Fig. 5). In those specimens transported in air, glucose increased and then recovered to initial levels in gill and mantle tissue.

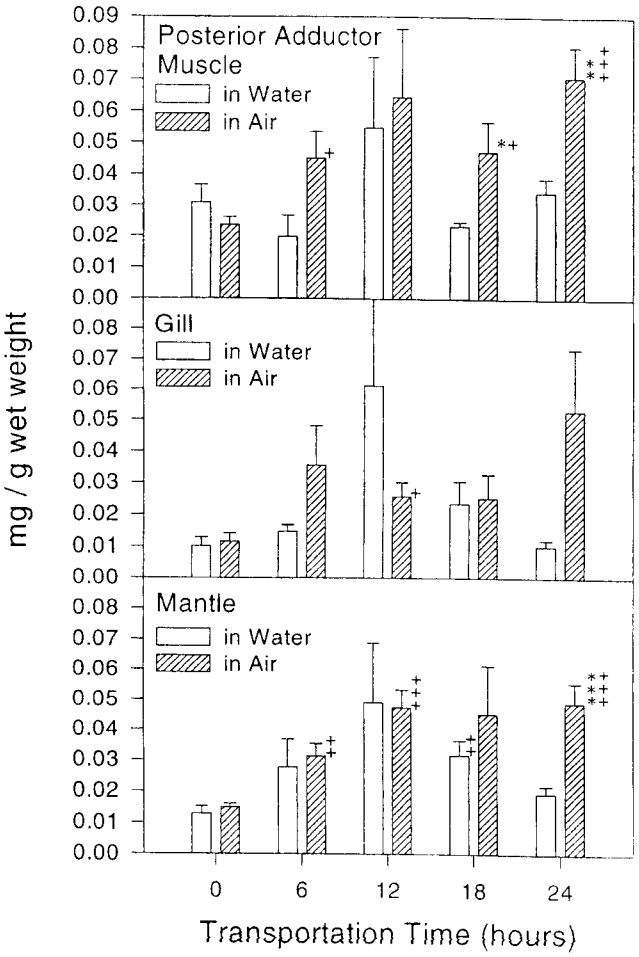

Fig. 1. Changes in glucose concentration in *Villosa iris* for three tissues under two different modes of transport. Error bars = ± SEM. N= 5. For the unpaired t-test, * $P < 0.05$, **$P < 0.01$, and ***$P < 0.005$ were used for the comparison between the glucose levels of mussels transported in air and water; + $P < 0.05$, ++ $P < 0.01$, and +++ $P < 0.005$ were used for the comparison between time 0 and the other transportation times.

Glycogen

Glycogen concentrations were variable, with no significantly consistent trends evident during the 24 hr of either transportation mode (Table 1). It is noteworthy that the mantle tissues have considerably higher concentrations of glycogen than the other tissues in all of the species tested.

DISCUSSION

Our results indicate that transportation in air generally was more stressful than transportation in water for all of the five species tested. The severity of the stress response by the animals, as reflected in the extent of glucose elevation, was seemingly related to the availability, or lack thereof, of dissolved oxygen in the typical habitat of a given species. *Villosa iris*, which is usually found in riffle areas of rivers having high dissolved oxygen concentrations, was the most sensitive to the transportation stress. Even transportation in water resulted in elevated glucose levels in mantle tissue of this species for the first 12 hr. On the other hand, species that live in areas experiencing relatively low dissolved oxygen conditions seemed to be more tolerant of transportation. Transportation in water did not result in obvious changes in glucose for *Elliptio complanata* (found typically in slowly flowing rivers) and the other three species sampled from Kentucky Lake, Tennessee. The mapleleaf (*Quadrula quadrula*) is found in medium to large rivers and reservoirs with a mud, sand, or gravel bottom; the ebonyshell (*Fusconaia ebena*) is found in sand and gravel of large rivers and reservoirs; and the threeridge (*Amblema plicata*) is found in small to large rivers and impoundments in mud, sand, or gravel.

Elevated glucose concentrations in hemolymph or tissues during environmental hypoxia or handling stress (as

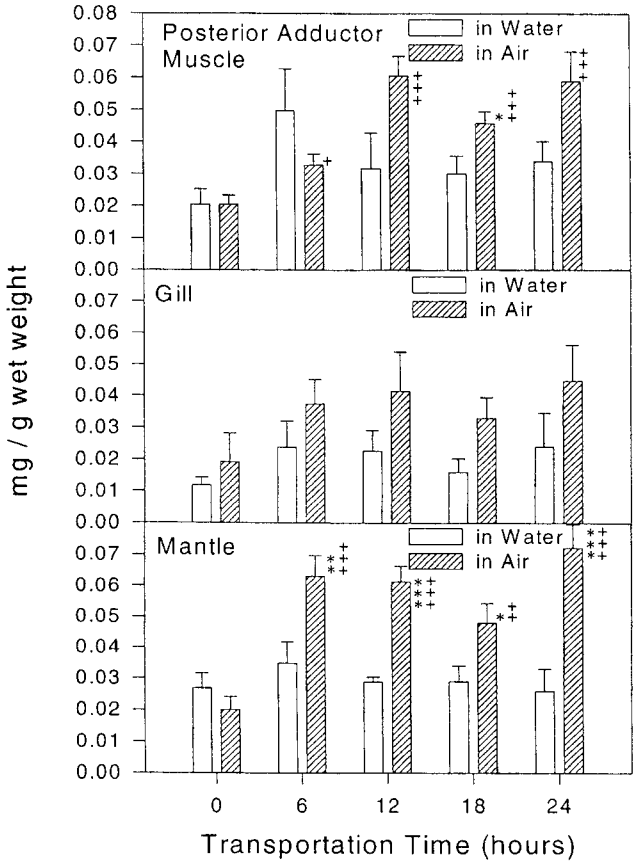

Fig. 2. Changes in glucose concentration in *Elliptio complanata* for three tissues under two different modes of transport. Error bars = ± SEM. N = 5. Statistical comparisons as in figure 1.

Table 1. Mean glycogen concentrations in unionids before and during transportation in water and in air. Values are in mg/g wet weight with SEM in parentheses. N = 5; PAM = posterior adductor muscle.

Method	Tissue	Sample Period				
		0 hr	6 hr	12 hr	18 hr	24 hr
			Villosa iris			
Water	Gill	1.73(0.35)	1.20(0.28)	0.98(0.26)	1.11(0.23)	1.14(0.21)
Air	Gill	1.18(0.29)	1.12(0.29)	0.95(0.17)	1.30(0.39)	2.40(0.67)
Water	Mantle	5.65(2.34)	11.08(2.69)	5.23(1.61)	10.17(2.15)	5.44(2.22)
Air	Mantle	5.84(1.52)	8.48(3.22)	5.59(1.14)	10.66(3.00)	3.37(0.89)
Water	PAM	3.45(0.70)	3.59(0.83)	3.46(0.73)	4.86(0.31)	2.08(0.52)
Air	PAM	2.28(0.51)	3.55(0.45)	3.00(0.68)	4.92(1.46)	3.33(0.32)
			Elliptio complanata			
Water	Gill	1.52(0.17)	1.24(0.22)	2.21(0.29)	2.30(0.28)	2.13(0.28)
Air	Gill	2.07(0.45)	1.08(0.10)	2.03(0.46)	2.66(0.10)	2.78(0.17)
Water	Mantle	11.77(1.38)	27.80(7.26)	19.78(2.80)	25.74(0.77)	20.03(2.75)
Air	Mantle	18.66(5.06)	23.76(4.78)	20.81(2.90)	24.06(6.28)	39.557(10.37)
Water	PAM	5.97(0.83)	7.51(0.54)	6.41(0.50)	7.36(0.59)	6.80(0.60)
Air	PAM	5.88(0.37)	6.84(0.91)	7.59(0.88)	9.18(1.13)	7.64(0.67)
			Fusconaia ebena			
Water	Gill	4.97(1.25)	4.16(0.91)	3.03(0.25)	4.99(1.86)	8.42(2.53)
Air	Gill	4.34(0.79)	3.53(0.71)	4.36(1.00)	2.55(0.43)	5.35(2.75)
Water	Mantle	137.88(12.62)	120.68(7.75)	113.35(7.32)	122.86(14.94)	125.02(8.31)
Air	Mantle	127.16(4.09)	119.19(7.66)	131.27(11.1)	134.22(14.04)	116.48(7.02)
Water	PAM	13.13(0.92)	11.93(1.87)	10.9(1.53)	12.42(0.68)	13.15(2.26)
Air	PAM	9.93(0.41)	14.79(1.61)	13.93(2.91)	15.78(2.89)	16.84(1.41)
			Quadrula quadrula			
Water	Gill	3.53(0.26)	4.03(1.23)	6.72(2.98)	4.23(0.85)	4.87(1.59)
Air	Gill	3.51(0.29)	6.11(2.44)	5.30(0.43)	2.48(0.55)	2.80(0.52)
Water	Mantle	75.71(3.48)	64.85(17.8)	61.20(12.95)	66.27(17.06)	49.38(12.58)
Air	Mantle	68.61(5.39)	65.44(8.11)	76.46(14.13)	63.66(16.31)	14.50(11.91)
Water	PAM	12.62(2.59)	9.17(1.95)	14.23(2.82)	7.09(1.67)	12.14(2.76)
Air	PAM	11.80(1.73)	15.09(3.29)	14.12(3.03)	13.24(2.49)	11.56(3.54)
			Amblema plicata			
Water	Gill	22.8(0.34)	3.26(0.81)	4.36(1.50	3.86(0.37)	4.28(1.33)
Air	Gill	2.12(0.68)	2.42(0.19)	2.84(0.58)	2.95(0.31)	3.52(0.57)
Water	Mantle	38.68(7.33)	44.03(7.86)	34.49(7.48)	36.17(6.45)	38.50(5.80)
Air	Mantle	26.22(8.03)	35.85(5.35)	43.99(9.40)	31.61(5.50)	52.33(12.82)
Water	PAM	7.52(0.53)	9.28(1.52)	8.54(1.36)	11.85(1.09)	7.28(1.24)
Air	PAM	7.54(2.12)	10.61(1.96)	11.39(2.34)	9.39(1.27)	13.64(3.44)

was seen in this study) have been observed in various invertebrates such as arcid clams (de Vooys *et al.*, 1991; de Zwaan *et al.*, 1995), terrestrial snails (Marques and Falkmer, 1976), freshwater snails (Wijsman *et al.*, 1988) and arthropods (*e. g.*, Kleinholtz and Keller, 1979; Mordue and Stone, 1979). The effects of transportation stress in water (duration not specified) were studied in *Anodonta anatina* by Pekkarinen and Suoranta (1995), who found that the glucose concentrations in the hemolymph and extrapallial fluids rose about 5-fold when mussels were transferred to the laboratory from the collecting site. The elevations of glucose in hemolymph and tissues are probably induced by hyperglycemic neurohormones that have been found in molluscs such as the marine bivalve *Mytilus edulis* (Robbins *et al.*, 1990) and freshwater snail *Lymnaea stagnalis* (Wijsman *et al.*, 1988; Hemminga *et al.*, 1985). These hormones act in a manner similar to the stress hormones (epinephrine and cortisol) of vertebrates, causing a mobilization of glycogen into glucose (Sumpter, 1997).

Changes in the concentrations of glycogen in the tissues of molluscs have been widely used as an assessment of their energy status and general physiological condition (Holopainen, 1987; Hemelraad *et al.*, 1990; Patterson *et al.*, 1997; Naimo *et al.*, 1998). Furthermore, the ability to tolerate acute hypoxia, and the anaerobic metabolic capacity of an animal, are seemingly related at least in part to the

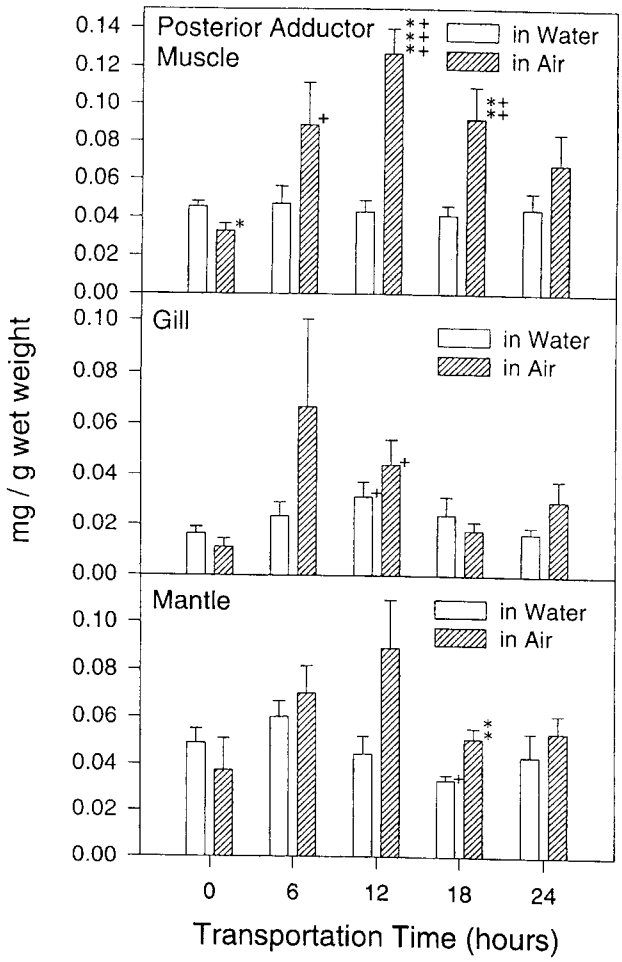

Fig. 3. Changes in glucose concentration in *Fusconaia ebena* for three tissues under two different modes of transport. Error bars = ± SEM. N = 5. Statistical comparisons as in figure 1.

glycogen concentrations in tissues. Hochachka (1982) compared the glycogen content of a variety of animals including *Mytilus edulis*, turtles, and some fish species and concluded that animals with high anaerobic capacity normally have high glycogen levels in different tissues. In other studies done in our laboratory, we also found that species [such as *Elliptio complanata* and *Pyganodon grandis* (Say, 1829)] which are more tolerant of low dissolved oxygen, usually have higher concentrations of glycogen in the tissue. The glycogen content of the mussels in our study did not decline during the 24 hr in either transportation mode. This result is likely because the glycogen consumed through anaerobic metabolism was small in comparison to the high storage concentrations of glycogen. It is also reassuring that glycogen did not decline during transport of the duration we tested.

The ebonyshell specimens exhibited increasing glucose levels in the posterior adductor muscle, which reached a maxium at 12 hr but declined thereafter. The results imply that *Fusconaia ebena* uses considerable energy in closing the valves during the first few hours of transport. The other two species collected from Kentucky Lake also seemed to tolerate transportation in air and exhibited a rise and subsequent decrease in glucose, somewhat similar to that of *F. ebena*. There are two possible explanations for the decline in glucose in the later period; one is the shift to alternative anaerobic metabolic pathways that use amino acids. For example, aspartate is used in the anaerobic metabolism of many marine bivalves (Kreutzer *et al.*, 1985; Demers and Guderley, 1994). However, it was found that the Asian clam *Corbicula fluminea* (Müller, 1774) does not catabolize amino acids during emersion (Byrne, 1988); hence, freshwater mussels might not use aspartate for anaerobiosis. The other possible explanation for the decline of glucose over time is the reduction of overall metabolism. We determined previously that the giant floater (*Pyganodon*

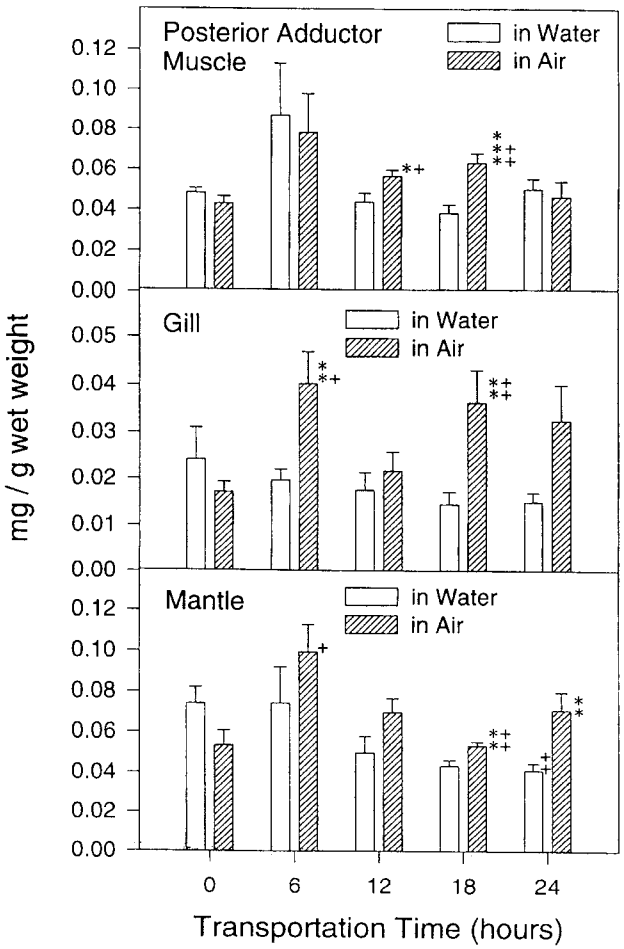

Fig. 4. Changes in glucose concentration in *Quadrula quadrula* for three tissues under two different modes of transport. Error bars = ± SEM. N = 5. Statistical comparisons as in figure 1.

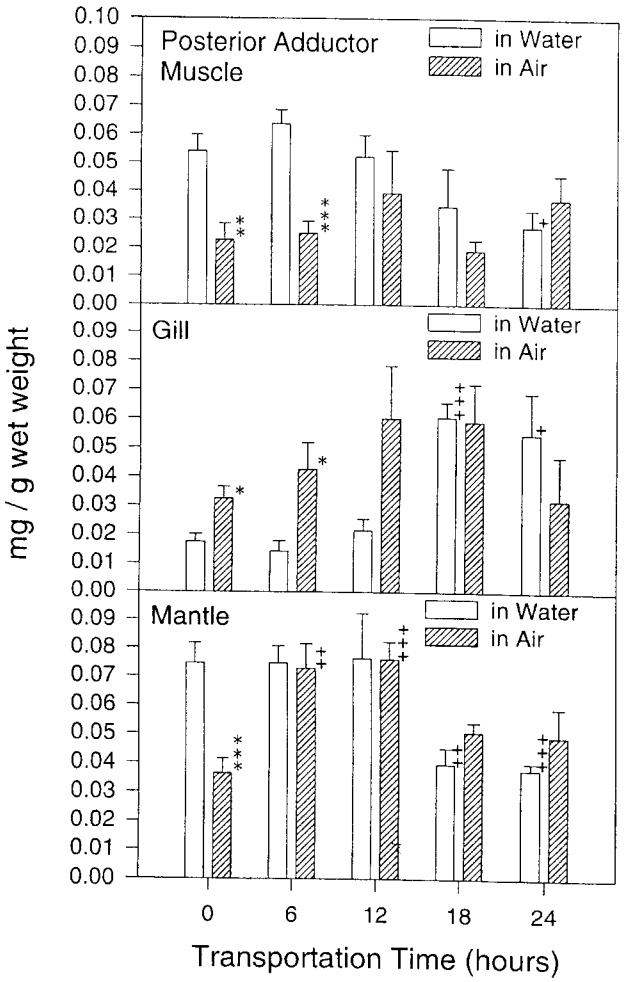

Fig. 5. Changes in glucose concentration in *Amblema plicata* for three tissues under two different modes of transport. Error bars = ± SEM. N = 5. Statistical comparisons as in figure 1.

grandis), an hypoxia-tolerant mussel found in the benthic area of lakes, can drastically reduce its heart rate when dissolved oxygen becomes very low (Heath and Chen, 1996). Some marine bivalves can reduce energy metabolism to increase resistance to emersion in air (Storey, 1988; Wang and Widdows, 1993).

As mussels are strictly aquatic, it can be assumed that transportation in water would be less stressful, especially since the water for transport was aerated. However, transportation in water requires more space, and aeration facilities may be required. In contrast, transportation in air with wet burlap does not require as much equipment and is not especially stressful for the oxygen-tolerant species for up to at least 24 hr. It has been reported that *Fusconaia ebena* has > 94% survival after four months following 24 hr exposure to air in wet burlap and > 90% survival when in air for 48 hr (Dunn and Layzer, 1996). Incidentally, that study also showed that mussel transport on ice in wet burlap was more harmful than just transport in air with wet burlap. Therefore, transport of specimens in air solely with cool, wet burlap seems to be a feasible method for oxygen-tolerant species.

Some species of mussels can exist in air for days suggesting that they can obtain some oxygen from the air, although at a greatly reduced rate (Hochachka and Guppy, 1987). However, there is a likelihood that ammonia would accumulate in the tissues more during such air exposure because mussels normally excrete this toxic substance through the gills into the water (Withers, 1992). Indeed, this ammonia could contribute to the stress of emersion although there seemingly are no data available on this possibility.

There are several additional factors that might be considered for optimizing the transportation of freshwater mussels. For example, Waller *et al.* (1995) found that *Amblema plicata* (Say, 1817) and *Obliquaria reflexa* (Rafinesque, 1820) had high survival rates after several hours of aerial exposure, when the exposure was conducted in October. However, a trend of decreased survival rate was found when aerial exposure occurred in June. They suggested that these results might be related to the wetness and temperature of the season, thickness of the shell, reproductive status, and level of metabolic activity. *Villosa iris*, a thin-shelled species, was the most sensitive to handling stress in our study, which would make it less able to withstand desiccation than thicker-shelled species (Matteson, 1955; Heming *et al.*, 1988). The effects of ambient temperature are also important, as it was found in pilot studies in our laboratory that *V. iris* has a better ability to regulate its oxygen consumption under hypoxia and is more tolerant of low dissolved oxygen when the temperature was lower (Heath and Chen, 1996). It has also been reported that the survival of mussels after emersion decreases as the relative humidity decreases (Byrne and McMahon, 1991). Finally, since air exposure or hypoxia may cause the release of sperm from males and premature release of embryos or glochidia by females (Waller et al., 1995), the reproductive season may be an inopportune time to transport freshwater mussels, even though glycogen reserves are usually high during that period (Jadhav and Lomte, 1982).

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