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Effects of Handling and Aerial Exposure on the Survival of Unionid Mussels

Diane L. Waller[a], Jeffrey J. Rach, and W. Gregory Cope
National Biological Service
Upper Mississippi Science Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54602-0818
and
Glenn A. Miller
Great Lakes Indian Fish and Wildlife Commission
P.O. Box 9, Odanah, Wisconsin 54681

Abstract

We conducted a relocation study of unionid mussels in Navigation Pool 7 of the upper Mississippi River (river mile 713.2) to evaluate survival after handling and aerial exposure. Two separate studies were conducted to compare seasonal differences in mussel survival; the first was initiated in June and the second in October. Amblema plicata plicata (subfamily Ambleminae) and Obliquaria reflexa (subfamily Lampsilinae) were studied. Mussels were marked, held out of water for either 0, 1, 4, or 8 h, and then placed into a 3 x 3 m grid (divided into nine 1-m^2 units). The mussels were re-examined after four-five months to measure mortality in the control and treatment groups. Mussels of both species had >90% survival after aerial exposure up to 4 h in both studies. However, survival (number recaptured live / number recaptured live and dead) of mussels showed a decreasing trend with duration of exposure in the first study, but not in the second study. The overall recovery of marked mussels (number recaptured/number marked) was 91% in the first study and 87% in the second study. However, only 37% of O. reflexa mussels in the 8-h treatment were recovered in the first study; the adjusted survival (number live recaptured/number marked) of this treatment group was significantly ($p < 0.05$) lower (35%) than all other treatments.

Introduction

Presently, the North American unionid mussel (Superfamily Unionacea) fauna is rapidly declining as a result of anthropogenic activity and is threatened with widespread extirpation by the exotic zebra mussel, Dreissena polymorpha (Williams et al. 1993). State and federal agencies are actively conducting status surveys and relocation operations in an effort to preserve the remaining unionid fauna. Information on threshold and tolerance limits of different mussel species to collection and handling conditions is especially critical at this time for planning management and conservation activities for unionid mussels.

The effects of handling and aerial exposure on mussels are often considered minimal, but the condition and survival of mussels after disturbance are seldom assessed. Handling methods for unionid mussels have not been systematically evaluated in controlled studies to isolate individual variables that affect mussel survival. Variables that may potentially influence mussel survival during collection include duration of aerial exposure, water-air temperature differential, relative humidity, and collection and marking methods. Additionally, tolerance to handling may vary among mussel species, size, and with the metabolic and reproductive condition of the mussel.

[a]To whom correspondence should be addressed.

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Our objective was to evaluate the survival of two unionid mussel species after handling and aerial exposure at two different times of the year. We selected treatments of 0-, 1-, 4-, and 8-h to encompass probable minimum and maximum periods of aerial exposure. During status surveys, mussels are usually held out of water for less than 1 h, the time needed to identify, mark, and measure a group of 50-75 mussels (Robert Whiting, U.S. Army Corps of Engineer, St. Paul, MN; David Heath, Wisconsin Department of Natural Resources, Rhinelander, WI, personal communication). However, during commercial clamming operations and relocation projects that involve hundreds of organisms, mussels may be left out of water for several hours. We chose June and October to conduct our studies because both are months in which mussel survey and collection work is common (Cope and Waller 1995), but environmental influences on the physiological condition of mussels differs. Early summer is a period of increasing metabolic activity, coinciding with increasing water temperatures and food availability. In contrast, fall is a period of decreasing metabolic activity, coinciding with decreasing water temperatures and decreasing food availability.

Amblema plicata plicata from the subfamily Ambleminae and Obliquaria reflexa from the subfamily Lampsilinae were used in the study. Both species are common in the upper Mississippi River basin, but A. plicata is a thick-shelled mussel relative to the small, thin-shelled O. reflexa mussel. The reproductive conditions of A. plicata and O. reflexa are distinctly different during June and October. A. plicata, a tachytictic breeder, undergoes gametogenesis in June and completes glochidial development and release by late summer (Holland-Bartels and Kammer 1989); Obliquaria reflexa is a bradytictic breeder, (Utterback 1915) and overwinters glochidia in the marsupia for release in the spring.

Methods

Study Area

Studies were conducted at an existing mussel bed in Navigation Pool 7 (river mile 713.2) of the upper Mississippi River. The first study was conducted on June 12, 1992 with follow-up on October 5, 1992. The second study was conducted on October 6, 1992 with follow-up on March 29, 1993. Study sites were in a main channel border habitat at a depth of about 2 m during normal flow conditions. The substrate was mixed sand, silt, and clay. The site of the second study was 100 m directly downstream of the first study site and thus had similar flow, depth, and water chemistry. Study sites were about 50 m offshore from an island. Human activity in the immediate area was minimal with the exception of commercial fishing near the study sites. We also saw evidence of muskrat predation on mussels, as evidenced by scarred empty shells around the island.

Mussels were collected on the west side of the main navigation channel by a SCUBA diver, placed in mesh bags, and immediately transferred into coolers of river water. We transported mussels to the study site at the east side of the navigation channel and placed the animals in wire cloth cages that were sumberged in the river.

Size of mussels collected for the studies ranged from 41 to 119 mm in shell length for A.plicata and from 23 to 35 mm in shell length for O. reflexa. Shell length was measured during the resurvey period to minimize handling of mussels during aerial exposure. Mean shell length of A. plicata in both studies was significantly greater than that of O. reflexa (Table 1). Mean shell length of mussels within a species was not significantly different between studies (Table 1).

The experimental design was a randomized block that included four treatments and three replicates per treatment each consisting of 25 mussels (Waller et al. 1993). A 3-x-3-m PVC pipe grid was used to separate nine 1-m^2 units that delineated treatment and control squares (Figure 1). Upon placement of the grid, three squares served as controls and resident mussels within these squares were left undisturbed. All resident mussels in the remaining six squares were removed. These mussels were counted and identified for a baseline measure of natural density and mortality.

Three of the six empty squares were randomly assigned to each of the two mussel species for placement of the treatment groups. Each treatment square contained 25 mussels from each of the four exposure treatments; total density in the treatment squares of the grid was 100 mussels/m^2.

Table 1. Mean shell length and range of A. plicata and O. reflexa mussels recovered following handling and aerial exposure studies in June and October.

	A. plicata			O. reflexa		
Study Period	N	Mean shell length (mm)	Range (mm)	N	Mean shell length (mm)	Range (mm)
June	290	72	42-110	243	35	23-55
October	282	62	41-119	269	34	23-41

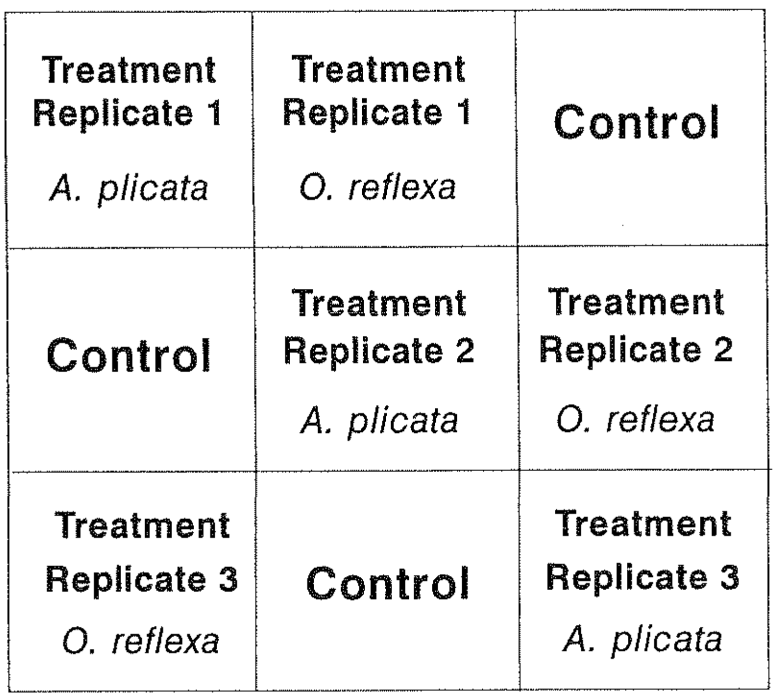

Figure 1. Sampling grid and assignment of experimental treatments. A treatment square contained 100 mussels which consisted of 25 mussels from each of four treatments (0-, 1-, 4-, and 8-h aerial exposure). Control squares remained undisturbed until the resurvey period.

Handling Treatments

The treatments for aerial exposure of mussels in our study were 0, 1, 4 or 8 h. Mussels in the 0-h treatment (control) were brought to the surface in the cage, briefly emersed in the atmosphere(< 5 sec), marked, and immediately returned to the water. We initiated the exposure treatments in descending order (e.g., 8-h, 4-h and 1-h) so that shorter exposure treatments occurred during the longer exposure treatments. Groups of 75 mussels were removed from the submerged cages for each exposure treatment. Mussels were marked by etching the periostracum with a motorized grinding wheel; a unique mark was assigned to each replicate and treatment. Mussels were then placed in the shade on the riverbank for exposure to the atmosphere. Air temperature directly over the mussels was measured hourly during the aerial exposure period. Water temperature at the substrate-water interface was measured hourly throughout the day.

Following the exposure period, mussels were placed in the appropriate treatment square by a diver. The diver first loosened the substrate and then positioned the mussels into the stubstrate with the anterior one-fourth of the animal buried.

Assessment of Mortality

Mussels were re-surveyed in October following the first study and in March following the second study. All mussels in each of the nine squares were collected by a diver, placed in separate mesh bags, and taken to the surface to record mussel mortality. The diver also searched for migrant mussels in a zone 5 m wide surrounding the grid.

Mortality was defined as an empty shell or gaping valves that did not respond to stimulation with a blunt probe. Our assessment of recovery and survival was based on the following definitions:

Recovery = the number of live and dead marked mussels that were recaptured during the re-survey divided by the number originally marked.

Survival = the number of live recaptured mussels divided by the total number of live and dead recaptured mussels.

Adjusted survival = the number of live recaptured mussels divided by total number of mussels. This definition of survival attributes loss of marked mussels to mortality.

Natural mortality = the fraction of empty shells/live mussels taken from the control squares during re-survey minus the fraction of empty shells/live mussels collected from the placement squares at the beginning of the study.

Emigration = the number of recaptured mussels found outside their original placement square. Cumulative migration, such as movement out of and back to the original square, could not be measured.

Data could not be transformed to fit a normal distribution (Zar 1984). Therefore, we tested for statistical significance among treatments with the NPAR1WAY procedure(GLM) with PC-SAS (SAS Institute 1987). Differences among treatments were judged significantly different at the $P \leq 0.05$ level.

Results

Treatment Period

Mortality of mussels did not occur during the aerial exposure period in either of our studies. During the 4- and 8-h exposures, mussels showed signs of stress including gaped valves, protruded foot, and copious mucus production. After replacement of mussels to the river bottom, the diver conducted a final qualitative examination of the study grid and reported that about 90% of the mussels had repositioned in the substrate and were actively siphoning within several hours.

Water temperature did not vary appreciably ($\pm$ 1°C) during the aerial exposure periods for either study. However, air temperature during the aerial exposure period increased as much as 10°C in June and 13°C in October (Table 2). Water and air temperatures also differed between studies. Water temperature was 7.5°C higher in June than in October, and air temperature was 3.5 to 6.5°C higher in June relative to October (Table 2).

Mean density of resident mussels at the first study site was 8/m^2 and at the second study site was 12/m^2. Mean percentage of empty shell/live mussels of resident mussels of all species at the first study site was 42% and at the second study site was 17%. Although mussel density in the study grids was much higher (100/m^2) than that of the resident population, it was similar to the reported density of mussels in other areas of the upper Mississippi River (Holland-Bartels 1990).

Table 2. Mean water temperature at substrate-water interface, air temperature, and air temperature change during aerial exposure treatments with unionid mussels in June and October.

Study Period	Water temperature (°C)	Exposure duration (h)	Air Temperature (°C)		
			Begin	End	ΔT
June	23.0	1	19.3	20.6	1.3
		4	18.5	23.4	4.9
		8	18.5	28.9	10.4
October	15.5	1	13.0	15.0	2.0
		4	12.0	15.8	2.8
		8	12.0	25.1	13.1

Resurvey Period

The overall recovery of marked mussels was 91% following the first study and 87% following the second study. The recovery rates were not significantly different among treatments or studies, with one exception; in the first study, the recovery of *O. reflexa* in the 8-h treatment was 37%.

Overall, emigration was lower in the second study than in the first study. Following the October study, eight mussels (1%) were found outside their placement squares, whereas, 9% (n=48) of the mussels collected had emigrated during the first study. All of the migrant mussels that we recovered were in an adjacent grid square or within 1 to 2 m of the grid.

Natural mortality of resident mussels was low in both studies, as inferred from the difference between the percent empty shells/live mussels at study initiation and at the re-survey period, and likely did not contribute to mortality of mussels in experimental treatments. At the start of the first study, empty shells comprised 25% of the 40 resident *A. plicata* specimens collected from the six treatment squares. At the end of the first study, empty shells comprised only 11% of the 19 resident specimens collected from the control squares. Natural mortality of resident *O. reflexa* mussels could not be estimated in the first study because none were found in the control squares. In the second study, the percent empty shell of *O. reflexa* decreased from 50% (n=10) to 0% (n=2) and of *A. plicata* from 9% (n=54) to 6% (n=16).

Mean survival of A. plicata and O. reflexa mussels did not vary significantly among treatments or between studies. However, there was an decreasing trend in survival for both species with the duration of aerial exposure during the first study (Figure 2). Survival of O. reflexa in the 8-h treatment was relatively low in two of the three replicates (82% and 86%). We also observed large variation in survival among the three replicates of A. plicata in the 8-h treatment (i.e., 44%, 92%, and 100%); Adjusted percent survival was significantly lower only for O. reflexa mussels in the 8-h treatment in the first trial (Figure 2). The large decrease in the adjusted survival estimate was a direct result of low recovery of mussels in this treatment.

There was no significant difference in mean shell length among treatments of mussels of the same species. Additionally, there was no significant difference in the the mean shell length between dead and live mussels in the treatment groups.

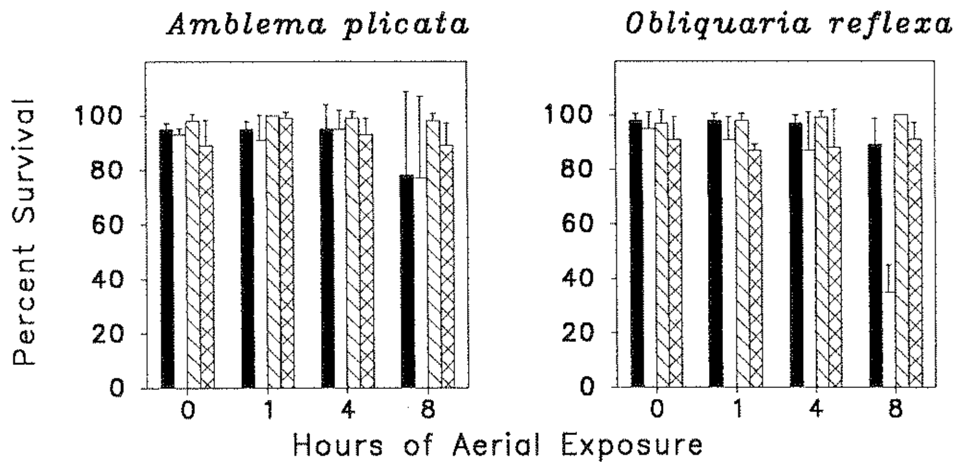

Figure 2. Mean and standard deviation of percent survival of 0-, 1-, 4-, and 8-h treatment groups of A. plicata and O. reflexa mussels from June to November (study 1) and October to March (study 2). Study 1 survival (black bar), study 1 adjusted survival (white bar), study 2 survival (single hatch line) and adjusted survival (cross-hatch line), n = 75 mussels/treatment.

Discussion

Based on the results of our studies, handling and aerial exposure should not be a major cause of mortality of mussels in surveys and relocations during moderate air temperatures if mussels are collected and processed within several hours. However, our data represent a very limited range of environmental conditions to which mussels may be exposed. Survey and collection work is also common during periods of extreme environmental conditions (Cope and Waller 1995). More importantly, the microenvironment of a mussel during aerial exposure can vary greatly, even among mussels collected in the same sample. For example, conditions on the bottom of an aluminum boat differ significantly from those on a shaded riverbank. Survival of mussels after emersion also decreases as the relative humidity decreases (Byrne and McMahon 1991). Accordingly, a mussel's chances of surviving exposure in warm air are greater on humid, rainy days or when the mussel is covered with a wet towel during exposure.

Given the wide fluctuations in environmental conditions that can occur during a field survey, it appears impractical to establish an absolute limit on the duration of aerial exposure for mussels. However, we may establish guidelines using behavioral cues as indicators of stress in mussels during exposure. Many behavioral adaptations are used by mussels during emersion to compensate for physiological changes that result from the need to conserve water by valve closure versus respire by valve opening (Byrne et al. 1991, Heming et al. 1988). Mussels in the present study demonstrated several of these behaviors after 1-2 h of emersion, including periodic valve gaping and exposing mucus-sealed mantle edges between long periods of valve closure.

Generally, thin-shelled, toothless mussels do not withstand desiccation as well as thicker shelled species (Matteson 1955, Miller and Nelson 1983). We expected A. plicata, a thick-shelled mussel with a tight valve closure, to withstand emersion longer than O. reflexa, a moderately thin-shelled mussel that maintains a slight valve gape. Although, there was no significant difference in survival between the two species after 4 h emersion, O. reflexa mussels gaped about 3 h before A. plicata mussels during aerial exposure. These behavioral differences suggest that differential mortality among species would be seen as exposure periods increased and relative humidity decreased.

Because mussels are poikilotherms, their activity level is dependent on water temperature. Handling mussels when water temperatures are relatively warm is considered advantageous because mussels are most active and will reposition and reburrow more quickly. In one of the few papers to address seasonal and temperature related effects on mussels, Imlay (1972) reported that mussels displaced in fall were slower to reposition and reburrow than those displaced in summer. Conversely, mussels may experience less metabolic and reproductive stress if handled when water temperatures are relatively low and mussels are quiescent. Overall, we saw greater survival of mussels that were handled in October compared to those handled in June. This slight difference may be due to the higher air temperature in June or to differences in physiological and reproductive condition of the mussels.

The condition of the mussel (e.g., reproductive status and level of metabolic activity) is influenced by the time of year and water temperature (McMahon 1991). Therefore, the optimal season for handling a mussel probably varies among species. For example, we observed lampsiline females aborting glochidia during aerial exposure in October, whereas, A. plicata males responded to exposure by releasing sperm. Thus, handling different species at the same time of year elicited different sublethal responses.

One of the primary problems with evaluating mussel survival in field studies has been the lack of reliable survival estimates (Cope and Waller, 1995). The accuracy of the mortality estimate is dependent on the percent recovery of marked mussels; if recovery rates are low, the estimate of mortality will be unreliable. For example, we found that low (37%) recovery of O. reflexa mussels in the 8-h treatment in the first study greatly skewed the adjusted survival estimate for that treatment from 89% to 35%. One possible explanation for the low recovery of O. reflexa mussels in this treatment may be that the mussels emigrated from the grid and immediate area. This species had a high rate of emigration relative to A. plicata. However, the greater rate of emigration should have produced a lower percent recovery in all treatment groups, not just the 8-h treatment.

Alternatively, low recovery was probably due to mortality related to the aerial exposure treatment. Obliquaria reflexa is a small species that does not burrow deeply into the substrate (personal observation). Live relocated mussels are commonly found within several meters of their placement site (Sheehan et al. 1989, Imlay 1972), whereas, dead mussels would be easily transported downstream with water currents. Unfortunately, we were not able to recover any of these animals even after a later search further downstream.

A second indication of a treatment effect due to 8-h exposure was the low survival (44%) and high recovery (92%) of Amblema plicata in grid square one. The large variation among 8-h treatment replicates of A. plicata may indicate differences in the microhabitat among grid squares. For example, in the first study we noted that a log had lodged in the upper left corner of the grid (square one) and created turbulence, scouring and depositional activity in this area of the grid. Thus, secondary stressors in some squares of the grid, such as sediment deposition, may have compounded effects of the 8-h treatments resulting in higher mortality in those squares. Nevertheless, minimal handling and brief exposure of mussels to the atmosphere in moderate air temperatures and should not cause significant levels of mortality in unionid mussels.

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