GROWTH AND SURVIVAL OF THE FRESHWATER MUSSEL, *ANODONTITES TRAPESIALIS* (LAMARCK 1819), IN A FLOW-THROUGH SYSTEM FOR LONG-TERM HOLDING

EL CRECIMIENTO Y LA SUPERVIVENCIA DEL MEJILLÓN DE AGUA DULCE, *ANODONTITES TRAPESIALIS* (LAMARCK 1819), EN UN SISTEMA DE FLUJO CONTINUO A LARGO PLAZO

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ABSTRACT

As with other freshwater mussels, *Anodontites trapesialis* is an endangered and threatened species. Artificial culture has been strongly recommended in recovery plans as a strategy to bolster declining populations, as well as the reintroduction of species to sites within their historic ranges. Our project compares two methods of adult animal management: buried and suspended, focusing on growth and survival of *A. trapesialis* in a captive environment. Animals were fed with *Chlamydomonas spp.* After 120 days, weight (soft and hard body) increased by 2.1% in the suspended group and decreased by 1.4% in the buried group. Suspended animals showed higher survival rates than those that were buried. The information provided may be of particular interest to develop future conservation measures for this and other similar endangered species.

Keywords: Freshwater mussel, Mycetopodidae, aquaculture system, adult management, biodiversity.

RESUMEN

Así como otras especies de almejas de agua dulce, *Anodontites trapesialis* se encuentra en peligro y amenazada de extinción. El cultivo artificial ha sido muy recomendado en los planes de recuperación como una estrategia para mejorar las cifras de población en declive, así como la reintroducción de especies a sitios dentro de sus rangos históricos. Nuestro estudio compara dos métodos de manejo de animales adultos, enterrados y suspendidos, con un enfoque en el crecimiento y la supervivencia de *A. trapesialis* en un ambiente de cautiverio. Los animales fueron alimentados con *Chlamydomonas spp*. Al comparar los porcentajes de peso corporal (concha y tejido blando) después de 120 días, un aumento medio del 2.1% se observó en el grupo suspendido y una pérdida media del 1.4% en el grupo enterrado. Los animales suspendidos tuvieron mejores tasas de supervivencia que aquellos que fueron enterrados. La información proporcionada puede ser de especial interés para el desarrollo de las futuras medidas de conservación para esta y otras especies similares en peligro de extinción.

Palabras claves: Mejillón de agua dulce, Mycetopodidae, sistema de acuicultura, manejo de reproductores, biodiversidad.

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INTRODUCTION

The decline of many mussel populations has led to several conservation and management programs aimed at preventing mussel extirpation and extinction. Studies have been conducted to determine the feasibility of relocating risk mussel species into aquaculture and hatchery facilities (Ramírez, 2005), and translocating species to areas within their natural range (Martel *et al.* 2003). Artificial culture of endangered and threatened mussel species has been strongly recommended in recovery plans as a strategy to enhance declining populations, as well as the reintroduction of species to sites within their historic ranges (O'Beirn *et al.* 1998).

However, standard protocols for cultivating unionids have not been completely developed, even though many populations of threatened and endangered mussel species would benefit from the release of cultured juveniles. The speed with which culture protocols are developed and employed is crucial for preventing the extinction of rare species (Beck, 2001).

Historically, reports on the aquaculture of freshwater mussels have conveyed little detailed empirical information. The studies done thus far have been focused mainly on larvae and juvenile stage (Buddensiek, 1995; O'beirn *et al.* 1998; Beck, 2001; Henley, 2002; Araujo *et al.* 2003; McIvor, 2004), and no study has included broodstock conditioning in captive environment.

Anodontites trapesialis (Lamarck 1819), freshwater mussels belonging to the Mycetopodidae family, are found in all main hydrographical basins of South America, east of the Andes, with the exception of the lower São Francisco River and basins in the extreme south. Typical habitat consists of substrates of varying size from sand or muddy terrain to compact clay. *A. trapesialis* are found in depths less than 20 m, but are generally collected from depths of 1 to 2 m. Unlike other members of Unionoida, mycetopodidae mussels produce a lasidium rather than a glochidium larvae (Simone, 1994; Callil & Mansur, 2007).

Our project focused on factors influencing the growth and survival of *A. trapesialis* in a captive environment. To this end, we compared two treatments of broodstock management: buried and suspended.

MATERIALS AND METHODS

Adults of *A. trapesialis* were collected from the marginal region of the Galo Bravo Reservoir, at Ribeirão Preto, SP, Brazil (21º 07' 06.9" S, 47º 49' 32.1" W). Animals were located by probing the bottom of the reservoir with feet and hands. Bivalves were transported alive to the laboratory in insulated boxes with enough local water to cover them.

Two hundred mussels were randomly divided into two treatments (two replicates). A control treatment (N = 50) was kept in tanks (40x60x50 cm), buried by a 15 cm layer of sediment (particle size < 600 μm). The other treatment (N = 50) was suspended in 32x28 cm nylon bags (mesh size of 10x3 mm), with three animals each, at intervals of 15 cm between bags, at 50 cm off the bottom, in the same tanks as buried mussels. All treatment units, consisting of flow-through water systems (mean flow was 10.0 L/min), were located in a room with constant temperature and a 12 hour light-12 hour dark cycle. To promote the suspension of algae cells in the water column, two 5.0 cm air diffusers were used in each treatment during 120 days of controlled culture.

Mussels were fed the unicellular green alga *Chlamydomonas spp*., at a density of 200 000 cells/ml/day once daily, and water was changed in all treatments on a weekly basis. This microalgae species was chosen due to its availability, easy culture, and fast growth in the laboratory.

Dechlorinated deep-well system water from the Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, São Paulo, Brazil (Table 1) was used for algal cultures and flow-through system. Living algal cultures were maintained in bath cultures in 20 l plastic bottles. Unialgal cultures were not axenic. Enrichment of the algal culture was achieved by the addition of fertilizer (NPK 12:6:6) at a ratio of 1/200.

On days 0, 30, 60, 90 and 120, the wet weight (fresh weight) of each mussel was measured to 0.01 g using a Gehaka BG 400 electronic balance.

Mortality rates of both groups were compared using Pearson´s chi-square test. ANOVA was used with repeated measures considering two factors: treatments (buried and suspended) and time (days 0, 30, 60, 90 and 120). Comparisons between and inter-groups were evaluated by orthogonal contrasts. All tests for significance were done at the 95% confidence level and calculations were performed using SAS software (9.0).

RESULTS

Survival was higher for suspended mussels (96%) than for buried mussels (78%; $P = 0.007$).

Despite the random assignment of mussels to treatments, initial mean weight of buried mussels was heavier than the initial mean weight of suspended mussels ($P = 0.0005$).

The ANOVA model demonstrated that the interaction effect between treatment and time was significant ($P < 0.001$), indicating that the growth patterns of mussels were different between the treatments (Table 2). The suspended group lost weight during the first 30 days ($P < 0.001$) but later gained it with all differences being statistically significant ($P < 0.001$). In the buried treatment, there was a significant loss of weight at days 30 and 60 ($P < 0.001$); however, no differences were observed between days 60 and 90 ($P = 0.1226$) and between days 90 and 120 ($P = 0.6963$). Over the 120 day experiment, the mean weight of the buried treatment was higher than the suspended treatment ($P < 0.01$).

Both treatments lost weight during the first month. However, the suspended mussels gradually regained weight, while the control group initially lost more weight than the suspended mussels and did not regain the initial loss (Table 2). Comparing the experiment over the 120 day, weight of the suspended treatment increased by 2.1% and the buried treatment lost 1.4% of initial mean weight.

DISCUSSION

Most attempts to rear freshwater mussels have focused on North American species, with a few studies on European species (Buddensiek, 1995; Henley, 2002; Araujo *et al.* 2003; McIvor, 2004). This study represents the first attempt to rear a Brazilian species of freshwater mussel: *A. trapesialis*, which is of particular importance because this species is currently listed as threatened (Amaral *et al*. 2008). Its culture may offer both the possibility of reintroducing this species to sites where it has been lost and the opportunity to study its requirements for future laboratory research (McIvor, 2004).

Table 1. Chemical composition of deep-well system water at the Faculdad de Filosofia, Ciencias e Letras de Ribeirão Preto
Cuadro 1. Composición química del agua de los sistemas de pozo profundo de la Facultad de Filosofía, Ciencias y Letras de Ribeirão Preto

PARAMETER	UNIT	RESULT
pH		6.11
Turbidity	uT (NTU)	0.98
Conductivity	µS/cm	113.6
Residual chlorine	mg/L Cl	<0.1
Chloride	mg/L Cl^-	1.8
Total dissolved solids	mg/L	91
Total hardness	mg/L $CaCO_3$	25
Ammonium	mg/L N-NH_3	<0.001
Nitrite	mg/L N-NO_2	<0.001
Nitrate	mg/L N-NO_3	2.039
Carbonate Alkalinity	mg/L $CaCO_3$	<0.1
Bicarbonate Alkalinity	mg/L $CaCO_3$	20
Sulphate	mg/L SO_4	0.5
Alum	mg/L Al	0.002
Cadmium	mg/L Cd	<0.001
Copper	mg/L Cu	0.035
Total Chromium	mg/L Cr	<0.001
Total Iron	mg/ L Fe	0.020
Manganese	mg/L Mn	0.004
Silver	mg/L Ag	0.001
Zinc	mg/L Zn	<0.001

The analytical methods are in agreement with APHA-AWWA-WPCF (1998). "Standards Methods for the Examination of Water and Wastewater", 20th edition. Washington, USA.

Table 2. Mean wet weight (g; standard deviation) of freshwater mussels, over a 120 day period, buried and suspended
Cuadro 2. La media de peso húmedo (g; desviación estándar) de mejillones de agua dulce, en el plazo de 120 días, mantenidos enterrados y suspendidos

	Mean Wet Weight (g)				
Treatment	Day 0	Day 30	Day 60	Day 90	Day 120
Buried	140.1 (44.3)	138.5 (44.4)	133.4 (41.0)	136.2 (41.2)	134.2 (40.5)
Suspended	113.3 (31.8)	111.6 (31.9)	113.5 (32.2)	114.4 (32.1)	115.7 (31.8)

The quality and quantity of suspended food are important to the physiological condition of marine and freshwater bivalves (Henley, 2002), although the diet of adult freshwater mussels is poorly known. It has generally been assumed that adult mussels primarily feed on phytoplankton, the same way as marine bivalves (Gosling, 2003), and most culture studies to date have fed juvenile mussels with a suspension of algae (Hudson & Isom, 1984; Yeager *et al*. 1994; Gatenby *et al.* 1997; O'Beirn *et a*l. 1998; Dimock, 2000; Henley *et al*. 2001; Jones *et al*. 2005). However, Nichols & Garling (2000) showed that freshwater mussels gained most of their carbon from bacterial sources.

Gatenby *et al.* (1997) looked at a variety of foods for juveniles, including various algal combinations, bacteria, and sediment. They found that sediment alone supported *Villosa iris* juveniles, but the addition of algae increased survival and growth. The addition of bacteria to their diet did not increase survival and growth. However, a different study by Yeager *et al.* (1994) found that three to five-day-old *V. iris* juveniles contained flagellated bacteria in their guts, with smaller quantities of algal cells. Therefore, it is likely that juveniles gain their nutrition from both small algal cells and bacteria.

The unialgal diet offered, *Chlamydomonas spp*., supported growth of *A. trapesialis*, at least for the suspended treatment. Based on the results of this study, the main problem for buried mussels was the high mortality level, which could be due to an anoxic environment within the substrate. It was observed that when a mussel died the substrate close to it turned gray and caused the death of the other neighboring mussels.

The feeding rate for juvenile mussels varies among authors, from 10,000 cells/ml/day (O'Beirn *et al.* 1998) to 30 000 cells/ml/day (Jones *et al*. 2005; Henley, 2002). Beck (2001) examined the particle selection by *Villosa iris* at three different ages and proposed that feeding rations should be increased every 10 days, starting with 30 000 cells/ml/day until reaching the rate of 90 000 cells/ml/day. There is little data on the feeding rate for maintaining captive broodstock freshwater mussels; however, the rate used in this study, 200 000 cells/ml of *Chlamydomonas* spp., sustained the adults of *A. trapesialis*, as the animals gained weight.

Martel *et al.* (2003) working with *Elliptio complanata* showed that mussels grow better when kept in steel cages rather than individual chambers tied off with a mesh bag, possibly due to their biology. In their natural environment, these mussels are partly imbedded in soft substrate and are mobile. In a steel cage, mussels left unrestricted can orient their position thereby maximizing their ability to obtain nutrients. In contrast, in the study by Martel *et al.* (2003), the mesh bag supported by the PVC frame may have restricted the ability of *E. complanata* to orient themselves or open adequately and therefore reduced their ability to feed properly. In the present experiment a nylon mesh bag was used allowing mussels to orient themselves and not disturbing their growth.

Management of suspended mussels is much easier and provides better water conditions based on survival rate. Additionally, microalgae were more available to them due to the use of aeration and because bags were hanging up within the water column. This study demonstrates that growth and survival of *A. trapesialis* in a captive environment for long-term holding is possible when they are kept suspended, which seems to promote healthier animals for propagation programs. The information provided may be of particular interest for developing future conservation measures for this and other similar endangered species.

It should be noted that there are many possible levels of replication in these experiments, including the different number of juveniles held in each mesh bag or tray, the different number of bags and trays within each tank, and the different sizes of tanks. Where possible in these experiments, replication was at the level of different containers (trays/mesh bags). However, there has been little replication at the level of tanks because it was not known which tanks would support

juvenile survival and growth; therefore, it was considered more important to place juveniles in many different tanks, rather than in many tank replicates. This has resulted in some pseudoreplication (as described by Hurlbert, 1984), which is a common problem for aquaculture experiments (Smart *et al.* 1997, 1998; Riley & Edwards, 1998), and many previous freshwater mussel culture experiments have suffered from the same low levels of replication (e.g. O'Beirn *et al.* 1998; Jones & Neves, 2002). In future studies, replication at the level of tanks or recirculating systems should be attempted where possible.

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