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A floating cage system for rearing freshwater mussels *Anodonta anatina* and *Unio mancus* in Piedmont region (NW Italy)

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Abstract

This research represents the first attempt in Italy to develop a cage system for adult freshwater mussel stocking, for extensive farming, that was realized from 2003 to 2005 in NW Italy. Two autochthonous species of Italian freshwater mussel were used: *Anodonta anatina* and *Unio mancus*. 5133 mussels were collected and successively stocked in floating cages in 5 different sites in a lake. This study was planned in order to determine the effect of main rearing factors for potential farming: stocking density and cage position in the water column (depth). For each species, the effects of three rearing densities and two water depths were tested. Mussel growth was measured on a monthly basis in each experimental unit throughout the study. The survival rate at the end of the experiment for both species considered was 98.4%. *U. mancus* resulted to be more resistant to rearing conditions and during the summer the highest mortality was registered for *A. anatina*. Summer mortality was between 4.8% and 20.5%. Highest mortality in *U. mancus* was recorded in September 2004 at 1.5 m (3.6±2.4%), while the highest mortality for *A. anatina* was recorded in September 2003 as 13.3%±3.8% at a depth of 1.5 m. This research showed that cage stocking is a suitable method and the optimal position of cages is at the lake thermocline. The proposed species proved to be easily utilizable for future farming or bioremediation projects, and can readily be reared up to 135 kg m^{-3}.

Keywords: Freshwater mussels, Mussel farming, *Anodonta anatine*, *Unio mancus*, Conservation aquaculture

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Introduction

The study of freshwater mussels is traditionally carried out for conservation of endangered species and for restocking natural population (Neves, 1999; O'Connor and Wang, 2001; Dan and Ruobo, 2002; Araujo *et al*., 2003; Hastie and Young, 2003; Neves, 2004; Bogan, 2008). The presence of exotic species as *Corbicula fluminea* and *Dreissena polymorpha* in North America and in Europe represents a serious threat for native species and several researches are currently being carried out for the protection of autochthonous species (Williams *et al*., 1993; Neves, 2004; Lima *et al*., 2012), as already stated by a letter to Nature in 1997, "Freshwater unionid clams in North America have been virtually eliminated from waters that are colonized by zebra mussels" (Nichols and Wilcox, 1997). Unionids are also farmed for freshwater pearl production in China (Young and Williams, 1983; Buddensienk, 1995; Monteforte and Carino 2013), European freshwater pearl mussel (*Margaritifera margaritifera*) has been recently studied for conservation in Europe (Gatenby *et al*., 1994; Hastie, 2006; Englund *et al*., 2008), in Turkey (Başçinar *et al*., 2009a; Başçinar *et al*., 2009b; Ersoy and Şereflişan, 2010) and other pearl mussel species in North America (Hua *et al*., 2007; Xu *et al*., 2011). Freshwater mussels are locally farmed for human consumption in some areas of the Far East (FAO, 1986; Wagner and Boman, 2004; Chakraborty *et al*., 2008). Triangle shell mussel (*Hyriopsis cumingii*) is the most reared mussel species in China (Xu *et al*., 2011), while to a much less extent crown mussel (*Cristaria plicata*) and the swan mussels (*Anodonta sp*.) are reared for pearl culture. Bioremediation is another application of bivalves farming (Ward *et al*., 1998; Soto and Mena, 1999; Vlamis *et al*., 2010; MacDonlad *et al*., 2011) and they are successfully utilized in integrated production with commercial aquaculture shrimp farms (Jones and Preston, 1999; Jones *et al*., 2002), in polyculture (Hopkins *et al*., 1993; MacDonald *et a*l., 2011) and in recirculation systems (Huang *et al*., 2013). Considering the application of the freshwater mussel farming, several uses have been suggested: in human diet for their high content in polyunsatured fatty acids (Bascinar *et al*., 2009a; Ekin and Başhan 2010; Ersoy and Şereflişan 2010), and as a mussel meal in fish feeds or as a source of additives for animal feeds (Sicuro *et al*., 2010). Moreover, Indian freshwater mussel is a traditional food for the rural human population in the Indian subcontinent and it is an ingredient of artificial feed for fish and poultry (Chakraborty *et al*., 2008). Crushed blue mussels are usually used in lobster nutrition as feed attractants and in the fish broodstock nutrition (Myers and Tlustly 2009). Following this application and considering their content of polyunsatured fatty acids, freshwater bivalves could be readily used in broodstock nutrition for freshwater aquaculture ofboth fish and crayfish. With regard to the very well known filtration ability of bivalves to filter 30–50 times their volume of water per hour, this research was a part of a bioremediation project where the use of freshwater bivalves was conceived as a possible system utilizable for bioremediation in polluted freshwater ecosystems or in integrated freshwater aquaculture. In order to investigate the potential application of integrated aquaculture with freshwater mussels, it was necessary to preliminarily verify the feasibility of freshwater mussel stocking in floating cages. Moreover the most important rearing factors affecting freshwater mussels were investigated: possible differences between main autochthonous freshwater mussel Italian species, stocking density and position of stocking cages in the water column (depth).

The present study was primarily aimed to evaluate the suitability of a cage culture method for adult freshwater bivalves rearing.

Materials and methods

The research was realized utilizing two autochthonous species of freshwater mussels: *A. anatina* and *U. mancus* (Unionidae: Bivalvia). This study was composed of two parts: a mussel stocking trial and a second part focused on investigation of juvenile mussel growth. The second part was carried out only with *U. mancus*. The present study started in 2003 and finished in 2005. During the research, water chemical parameters were measured: chemical (total phosphorus, reactive phosphate, total nitrogen and ammonium) and physical parameters (dissolved oxygen % and water temperature) at site 5 (1.5 m and 5 m of depth) were recorded on a monthly basis. Water transparency was also monitored with Secchi disk every month.

Site description

This research was carried out in Avigliana lake that is located in Piedmont region, Torino Province, NW Italy (0.83 km^2). 5133 *A. anatina* and *U. mancus* (Unionidae: Bivalvia) adult mussels were collected from April to November 2003: 294 kg of *A. anatina* (12.38 ± 1.36 cm in length) and 129 kg of *U. mancus* (7.37 ± 0.96 cm in length) (Tables 1, 2).

Table 1: Culture conditions of *Anodonta anatina* (n=5).

Rearing density	**LD**			**HD**		
Site	**3**		**1**		**5**	
Depth (m)	1.5	5	1.5	5	1.5	5
Density (kg/m^3; mean ±SD)	25.9 ± 0.7	29.6 ±0.4	98.2 ± 14.5	83.1 ± 7.6	89.6 ± 0.4	89.3 ± 0.9
Specimens (n)	191	154	450	375	553	564
Biomass (kg)	20.7	20.7	68.7	58.2	62.8	62.5
Individual weight (g; mean±SD)	108.5 ± 5.2	134.8 ± 8.4	153 ± 7.9	155.2 ± 14.2	114.4 ± 11.0	110.9 ± 4.4

Table 2: Culture conditions of *Unio mancus* (n=5; except in site 4, HD: n = 2)

Rearing density	**LD**		**HD**	
Site	**2**		**4**	
Depth (m)	1.5	5	1.5	5
Density (kg/m^3; mean ± SD)	29.9 ± 2.7	28.8 ±4.8	89.1 ± 0.3	89.4 ± 0.8
Specimens (n)	497	501	499	1349
Biomass (kg)	20.9	20.1	24.9	62.5
Individual weight (g; mean ± SD)	42.2 ± 3.6	40.2 ± 6.7	50.1 ± 2.8	47.3 ± 7.7

First stocking trial

Mussels were collected utilizing nets and by scuba diving. Seven floating cages (0.6 m x 0.3 m x 0.7 m) were set up holding together the plastic trays (Fig. 1) and successively placed in 5 sites of the lake. Ten cages were placed in each site, (except site 4, having only 7 cages). Stoking density and cage position in the water column (depth) were the experimental factors investigated in each species. Two different rearing densities (low density: LD; high density: HD) were initially tested: LD (26- 30 kg m^{-3}), HD (83 - 98 kg m^{-3}) (Tables 1, 2). In each site, 5 cages were placed at depths of 1.5 and 5 m depth (Fig. 1); except at site 4, where only 2 cages were placed at 5 m. This part of experimentation started in June 2003 and finished in September 2004. Mussel mortality rate was measured on a weekly basis from May to August 2003 in each trestle, every two weeks from September to November 2003 and monthly from December 2003 to September 2004. During spring and summer the cages were cleaned every month from fouling and dead mussels were eventually removed.

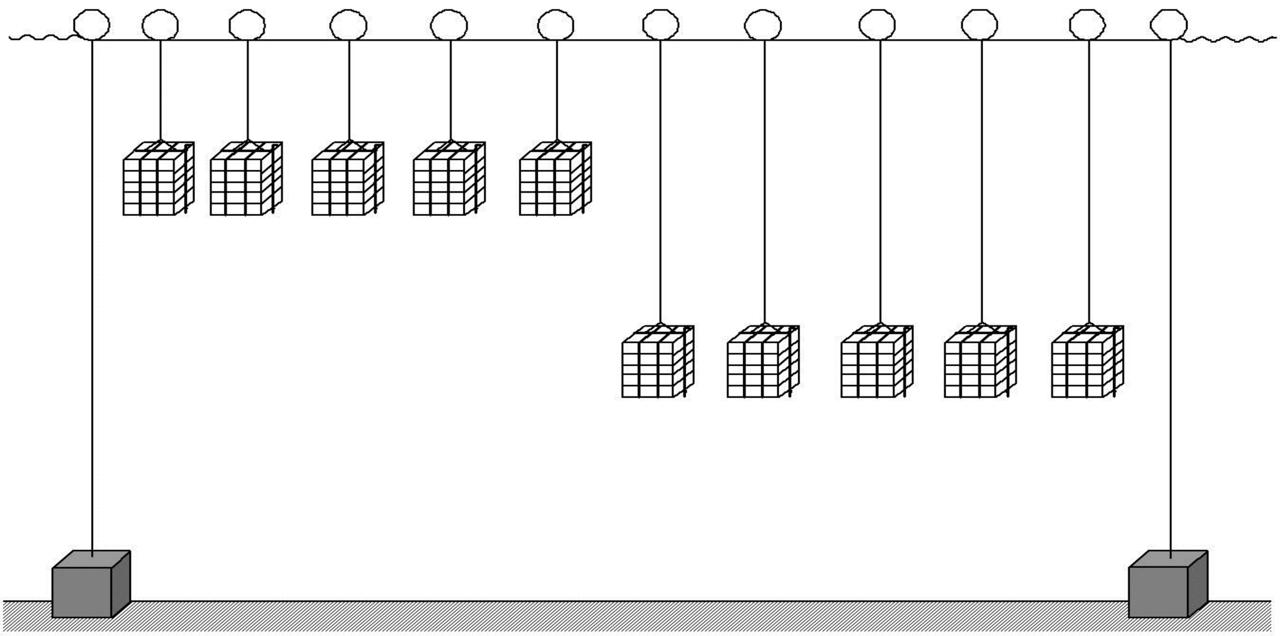


Figure 1: Floating cage structure used for mussel farming.

High density stocking trial

In order to increase the filtration efficiency, from November 2004 to May 2005, the study was conducted increasing rearing density up to 135 kg m^{-3}. In light of the results previously obtained, only two stations with 4 and 5 cages were utilized at 5 m depth. Survival rate was measured once every month.

Growth trial

From July 2004 to June 2005 a study on juvenile mussel growth was conducted. This trial on mussel growth was conducted utilizing 45 bivalves (*U. mancus*) divided into 4 age classes (2 years old, n=10; 3 years, n=15; 4 years n= 15; 5 years n=15), individually marked and monthly weighted.

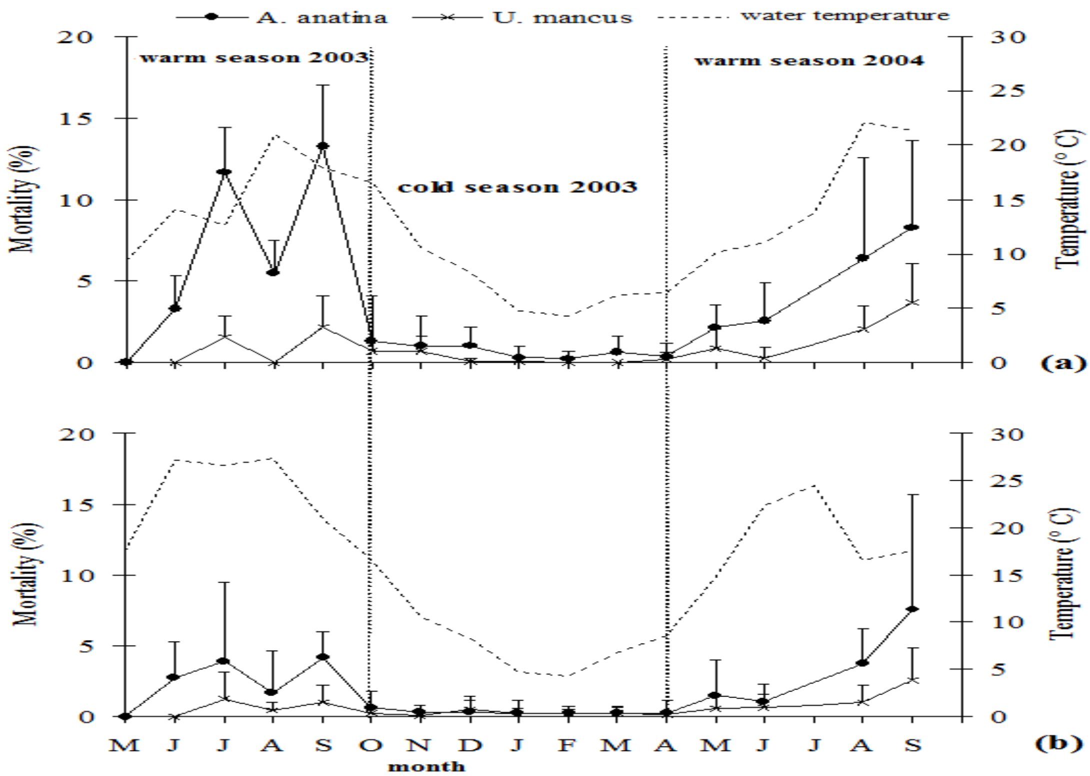
Statistical analyses

Mussel mortality rate was used for statistical data elaboration, ANOVA was utilized and the data were previously elaborated with quality analysis in order to verify the correct utilization of parametric statistics. Tests for normal distribution of data (Shapiro -Wilks test) and for homogeneity of variances (Bartlett test) were preliminarily performed. The relation between mussel mortality and water temperature, dissolved oxygen and water transparency was investigated for each species in all the experimental sites, with Pearson correlation coefficient. Statistical analyses were conducted using R version 1.6.2 software.

Results

The mussel rearing period lasted 499 days for *A. anatina* and 446 days for *U. mancus*. Data quality analysis confirmed that ANOVA test was correctly applied. Dissolved oxygen was higher than 100% of saturation level during the experimental period, except from October 2003 to February 2004, when the oxygen level ranged between 67% and 90% of saturation. At the end of the research period the average monthly mortality rate was 1.6% and was lower for *U. mancus* than for *A. anatina* (Fig. 2). Highest mortality of *U. mancus* was recorded in September 2004 both at 1.5 m (3.6±2.4%) and 5 m depths (2.5± 2.3%), while the highest mortality for *A. anatina* was in September 2003, both at 1.5 m and 5 m depths (13.3%±3.8% and 4.2%±1.9%, respectively), a successive peak of mortality for *A. anatina* was registered in September 2004, at 1.5 m and 5 m of depth. In order to investigate in deep the effect of water temperature, the period of study was separately analyzed, dividing into three different phases: warm season of 2003 (from May 2003 to October 2003), cold season of 2003-2004 (from October 2003 to April 2004) and warm season of 2004 (from April 2004 to September 2004) (Fig. 2). During the cold season no differences in mortality were found considering species, rearing density and cage position in the water column and mortality rate ranged between 1.2% and 4.6%. During the warm season, mortality ranged between 4.8% and 20.5%. Survival rate during the cold season of 2003-2004 was significantly higher ($p<0.01$) than in the warm season of 2004 (Fig. 3a). Considering that during the winter mussel survival was very high and comparable between the two species studied, successive data elaboration was only focused on warm season of 2004. In particular the differences between mussel species were investigated. Survival rate was higher for *U. mancus* than for *A. anatina* ($p<0.01$) (Fig. 3b). No differences between stocking density were found for *U. mancus*. Survival rate was lower ($p<0.05$) in site 1 for *A. anatina* at high density (Fig. 3c). Considering the position of floating cages in the water column (depth), there was no significant difference both for *A. anatina* and for *U. mancus*; however survival rate at 5 m depth was fairly higher, especially for *A. anatina* (Fig. 3d). Algal blooms, measured by means of water transparency, occurred from May to June of 2003 and from July to September 2004. The correlation between mussel mortality rate and dissolved oxygen, temperature and water transparency was calculated for each species in all the experimental sites. Even if observing the time trend of mussel survival during the experimentation (Fig. 2) a graphical relation between water temperature and mussel survival is visible, the statistical results showed that correlation between mussel survival and environmental conditions was low, always ranging between -0.3 and +0.5. In the high density (135 kg m^{-3}) stocking trial, the survival rate was still high ranging from 95.8±2.5 (%) and 92.7±2.3 (%) respectively in *Anodonta* and *Unio*, similar to the previous phase.

Juvenile mussels are rare and difficult to find, and throughout the period of research less than 0.01 % of juvenile specimens were found and collected for the final experiment that indicated that their growth in experimental cages was comparable with that in natural conditions. Similar to natural conditions, the biomass increase occurred only during the summer (Fig. 4), from July to September and the growth was particularly evident in the group of mussels with less than two years of age.



Fiure 2: Mortality trend of *Anodonta anatina* and *Unio mancus* (average + S.D.) during the experimental period. Warm season 2003 (from May to October 2003), cold season 2003-2004 (from October 2003 to April 2004) and warm season 2004 (from April to September 2004). Mortality rates at 1.5 m (a) and 5 m (b).

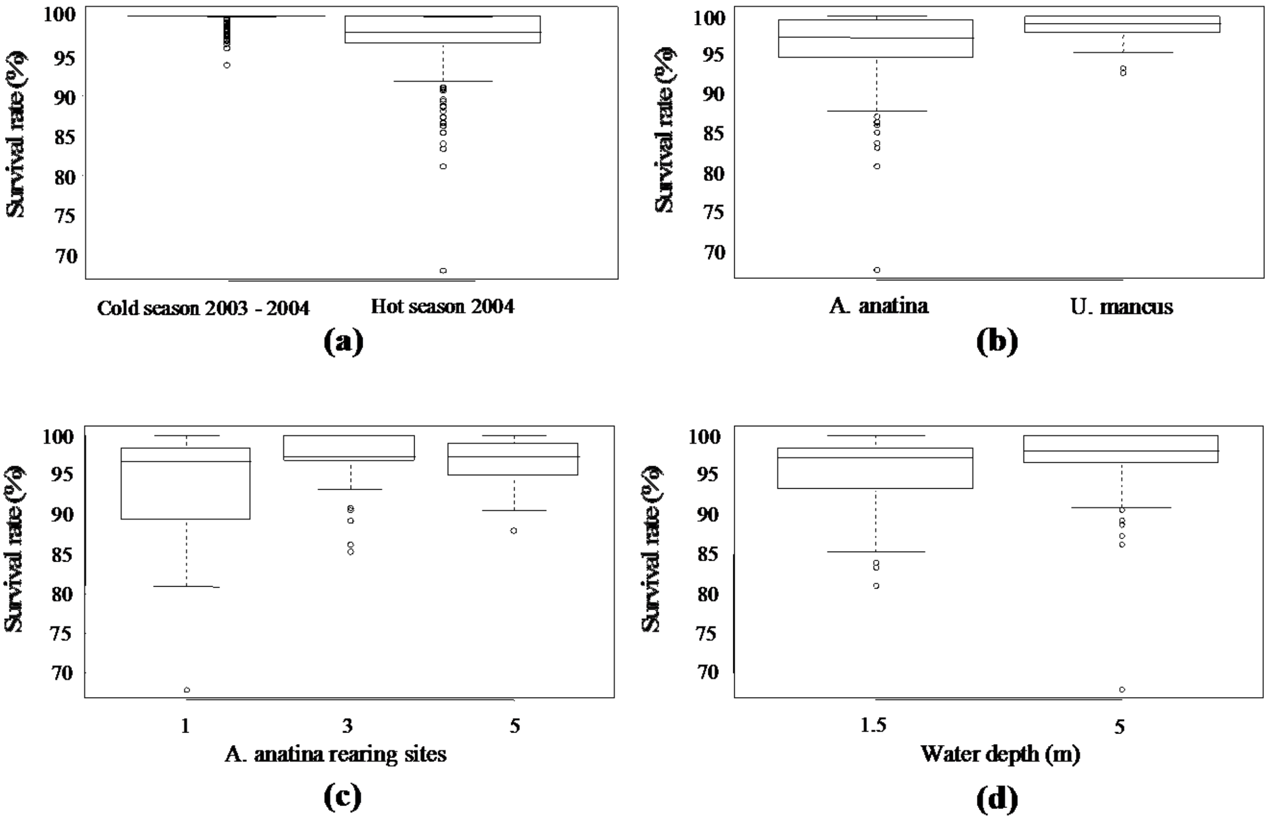


Figure 3: Mussel survival rates (%). (a) Seasonal comparison; (b) Species comparison during warm season 2004; (c) *Anodonta anatina* in different density conditions (HD: site 1, 5; LD: site 3) during warm season 2004; (d) *Anodonta anatina* in different water depth during warm season 2004.

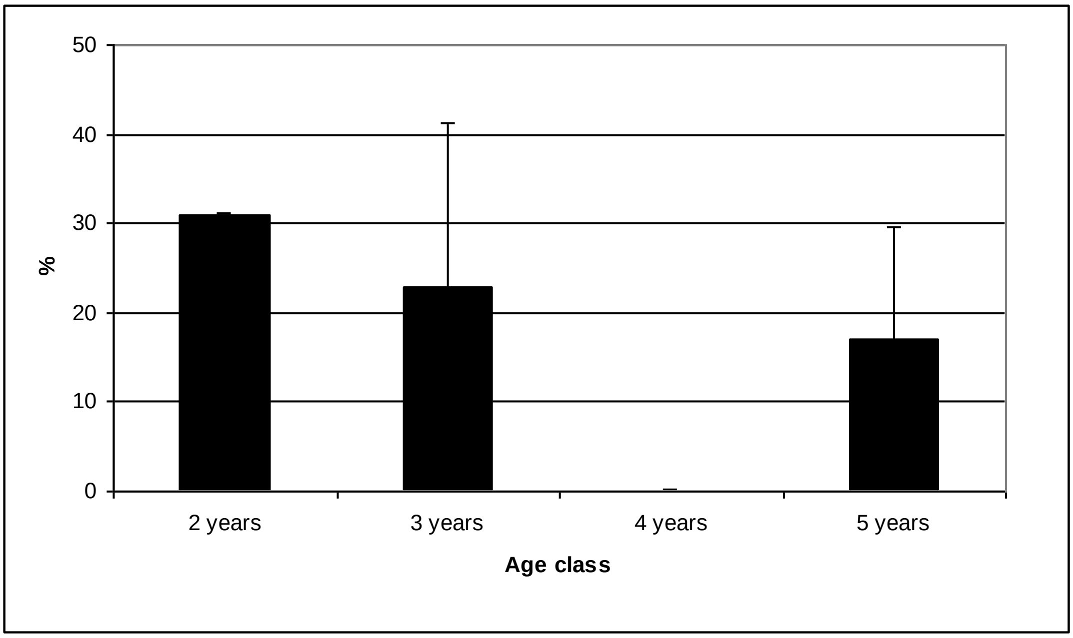


Figure 4: Weight gain in *Unio mancus* during summer period.

Discussion

This research represented, up to our knowledge, the first attempt to set up a freshwater mussel farming system in Europe and even if Unionids are organisms tolerant to a wide range of environmental conditions, in the wild they live partially burrowed into the sediment in the shallow waters (Pusch *et al*., 2001; Zotin and Vladimirova, 2001) and the stoking in the floating cages was the preliminary condition to be verified for possible rearing. Even if the proposed systems of floating cages has never been proposed, comparable rearing systems, set up with suspended nets, have been proposed for freshwater pearl mussel rearing (Hua *et al*., 2007; Yan *et al*., 2009) or in blue mussels (Karayucel and Karayucel, 2000) and other researches on freshwater mussel farming have been essentially focused on larval rearing that is the critical phase (Buddensienk, 1995; Jones *et al*., 2002; Hastie *et al*., 2003;). Moreover few studies have been carried out on juvenile rearing, focusing on toxicity tests or freshwater pearl propagation (Barnhart, 2006; Kovitvadhi *et al*., 2008). The main finding obtained from this research is that the mortality observed in the floating cages adopted in this experiment was extremely low thus confirming their suitability to future rearing of *A. anatina* and *U. mancus*. The experimental factors tested: mussel species, stocking density and position of cage (depth) for a long period of time that included seasonal variability, confirmed that the adopted techniques are utilizable for these species, at least for adult mussels. The low mortality measured in this research is in agreement with previous studies on *M. margaritifera* in North Germany (Buddensienk, 1995), on other freshwater mussels held suspended in pocket nets and net bags in China (Hua and Neves, 2007; Yan *et al*., 2009) and in blue mussels in Scotland (Karayucel and Karayucel, 2000). Besides, *U. mancus* proved to be more resistant to the rearing conditions and the summer appeared the most critical season for intensive rearing. *U. mancus*, showed good adaptation to rearing conditions also at the highest density tested and mortality was not affected by manipulation as registered in blue mussel (Karayucel and Karayucel, 2000). As regard to stocking density, it is clear that the maximum level of density was not reached and these mussels can be presumably reared in densities higher than 135 kg m^{-3}. This finding strongly differs from a previous study on density rearing of *Hyriopsis cumingii* in China where much lower stoking density (1.5 individuals m^{-3}) was proposed in experimental ponds (Yan *et al*, 2009; Jing *et al*., 2011). This fact is particularly interesting for future applications for integrated aquaculture or bioremediation projects. Between the two tested water depths there were no significant differences in mortality, even though the survival rate was higher in the floating cages at 5 m of depth (Fig 3d). In the wild, summer is the better season for mussel growth, while under rearing conditions it appeared to be the critical season and the slight mortality registered during warm seasons can be explained by fouling and organic material deposited on rearing cages. During summer, freshwater sponges (*Spongilla lacustris*) were found on the bottom of the cages (Fig. 5). These organisms are uncommon in the considered area and this observation could lead to future applied researches for integrated systems as fish-bivalves-sponges in freshwater systems. After this research, the bathing activity was restored after 15 years of prohibition in the Avigliana Lake because of the significant decrease in coliform bacteria in the water. Even if this was unexpected, it was one of the most important beneficial effects observed in the lake after the completion of the project. Finally, this research serves as the basis for the possibility of future use of freshwater mussels with particular emphasis on possible integrated farming and bioremediation projects.

Figure 5: Freshwater sponge (*Spongilla lacustris*) found on the bottom of the cages.

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