

Experimental demonstration of viability for the endangered giant pearl mussel *Margaritifera auricularia* (*Bivalvia: Unionoida*) in its natural habitat

Cristian R. ALTABA and Miguel A. LÓPEZ

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The giant pearl mussel *Margaritifera auricularia* is a critically endangered species, surviving in natural habitats only in the lower Ebro (Catalonia, Spain). The viability of this population was assessed by addressing whether the adults are producing larvae, if any host fish exists in the area, and what recruitment is actually taking place. All adults monitored released larvae during Spring, showing that they are fertile hermaphrodites. Artificial infections of a wide array of fishes were performed under controlled conditions, and it was found that the only host is the freshwater blenny *Salaria fluviatilis*, which lives in the same habitat and is also endangered. Young individuals were located, the smallest one with an age just above 2 years. It appears that this population is indeed viable, although it needs urgent and effective conservation action.

Keywords: *species interactions, limnology, conservation, Ebro, Margaritifera auricularia, Salaria fluviatilis.*

VIABILITAT DE LA NÀIADE AMENÇADA *Margaritifera auricularia* (BIVALVIA: UNIONOIDA) AL SEU HÀBITAT NATURAL. La nàiade *Margaritifera auricularia* és una espècie que es troba en perill crític, i que sobreviu en hàbitats naturals únicament al curs inferior de l'Ebre (Catalunya, Espanya). La viabilitat d'aquesta població ha estat avaluada investigant si els adults estan produint larves, si hi ha cap peix hoste a l'àrea, i si té lloc un reclutament efectiu. Tots els adults que foren objecte de seguiment alliberaren larves a la primavera, demostrant així que són hermafrodites fèrtils. Es realitzaren infeccions artificials d'un ampli ventall de peixos sota condicions controlades, i es determinà que l'únic hoste és la bavosa de riu (*Salaria fluviatilis*), que viu al mateix hàbitat i es troba igualment amenaçada. Es localitzaren individus joves, el més petit dels quals tenia una edat just per damunt dels dos anys. Aquesta població, doncs, sembla del tot viable, encara que necessita mesures de conservació urgents i efectives.

Paraules clau: *interaccions entre espècies, limnologia, conservació, Ebre, Margaritifera auricularia, Salaria fluviatilis.*

Cristian R. ALTABA and Miguel A. LÓPEZ, Institut Mediterrani d'Estudis Avançats (CSIC-UIB), 07071 Palma de Mallorca. E-mail: iecra4@clust.uib.es. Actual adreça: CRA Conselleria de Medi Ambient, Govern de les Illes Balears, carrer Reina Constança s/n, 07006 Palma de Mallorca and MAL Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 242, 08017 Barcelona.

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Introduction

One of the most endangered species in Europe is the giant pearl mussel *Margaritifera auricularia* (Spengler, 1793), a very large freshwater mussel that lives only in big rivers. Its original range extended throughout Western Europe and North Africa, but this species now survives only in the Ebro river and adjacent channels in Spain (Altaba, 1990; 1992; 1997; 2000; Primack, 1998). Initially, only recently dead specimens were located (Altaba, 1990; 1993). Later on, during public works in the lower Ebro in Catalonia (Lorán, 1993), a sampling project of freshwater bivalves allowed the finding of live individuals and the assessment of their distribution (Altaba, 1996a).

The lower Ebro has experienced important changes in hydrology as a consequence of dam construction, water diversion, and pollution (Ibáñez *et al.*, 1996). However, it still keeps many of the features of a wild big river, such as gravel shallows, and it harbors a thriving native fauna. This is clearly shown by the abundance of freshwater mussels belonging to several species. As a result of a large-scale intensive sampling, it was possible to evaluate the size and distribution of their populations (Altaba, 1997; 2000). In contrast, the fish fauna of the lower Ebro, which originally was composed of only 14 species, has been enriched in recent years through the introduction of at least 13 exotics, several of which have become exceedingly successful, displacing the native ones (Sostoa & Lobón-Cervià, 1989; Elvira, 1995; 1996; 1997).

However, the question remained of whether the population of *Margaritifera auricularia* in the lower Ebro is indeed viable. This is a highly relevant issue, because outside of this natural habitat there has been no recruitment for decades. The relic demes living further upstream and in adjacent channels in Aragon have suffered from rampant pollution and massive siltation. These factors may be responsible for those demes being composed of only very old specimens (Altaba, 1997; 2000; Álvarez Halcón *et al.*, 2000).

In the lower Ebro there are sites where the local density of *M. auricularia* is fairly high (Altaba, 1997). This suggests that the limiting factor must be in the larval stages of its complex life history, which involves a parasitic stage on the gills of certain fishes. Indeed, it has been shown in other species of *Margaritifera* that the local density of young and adult pearl mussels depends mainly on the density of available host fishes (Johnson & Brown, 1998; Cunjak & McGladdery, 1991). Thus, identifying the host fishes for such a species has important consequences for its conservation (Neves *et al.*, 1985; Neves & Widlak, 1987). Based on indirect evidence, it was hypothesized that *Acipenser sturio* (Common Sturgeon) could be the host of *M. auricularia* (Altaba, 1990). However, most of the living specimens in the Ebro were certainly not carried by this fish, because its upstream migration was halted by the Xerta weir centuries ago. Furthermore, overfishing caused its disappearance 30 years ago (Fernández & Farnós, 1999).

In order to test the viability of *M. auricularia* in the lower Ebro it was necessary to assess whether the adults are producing larvae, if any host fish exists in the area, and what recruitment is actually taking place. The first question was addressed by monitoring adults in the wild, the second was tested through experimental infestations on a wide array of fishes, and the third involved exhaustive searches in the field. A detailed account of the laboratory work will be published elsewhere (López & Altaba, 2000, in press), and only the relevant findings are reported here together with the results of field work.

Material and methods

The live specimens that were found during public works in the river were translocated into trays filled with cobbles and suspended in the flowing water from a stable surface. In this manner, they could be monitored daily, independently of water level. No further manipulation of the animals took place, and the release of glochidia larvae was followed by visual inspection. The larvae used in the following experiments were collected with a

syringe from the shell's edge, placed in a larger container, and rapidly carried to the laboratory.

From March to June 2000, a total of 176 fishes belonging to 16 species were infected. This included 8 which are native to the lower Ebro (*Anguilla anguilla*, *Barbus graellsii*, *Barbus haasi*, *Chondrostoma toxostoma*, *Cobitis paludicola*, *Salaria fluviatilis*, *Liza aurata* and *Mugil cephalus*), and 8 which are introduced exotics (*Acipenser baeri*, *Alburnus alburnus*, *Carassius auratus*, *Cyprinus carpio*, *Gobio gobio*, *Scardinius erythrophthalmus*, *Tinca tinca* and *Gambusia holbrooki*). Infections were produced by placing the fish in a container with a high concentration of larvae, or by pipetting larvae directly

into the gills. Infected fish were kept in closed-circuit tanks at the *Centre d'Aqüicultura* (IRTA) facilities in the Ebro Delta. The bottom of these tanks was separated by a mesh, and all particles on it were siphoned, sieved and inspected every day.

The search for young age classes in the field was carried out by careful manual inspection of selected areas. There is a limited number of sites inhabited by *M. auricularia*, so it was considered inappropriate to proceed with any sampling that could be destructive of the natural bottom. The searching process was very slow and tedious. However, it benefited from a catastrophic event: in early summer a portion of one of the areas occupied by the species was left above the water level,

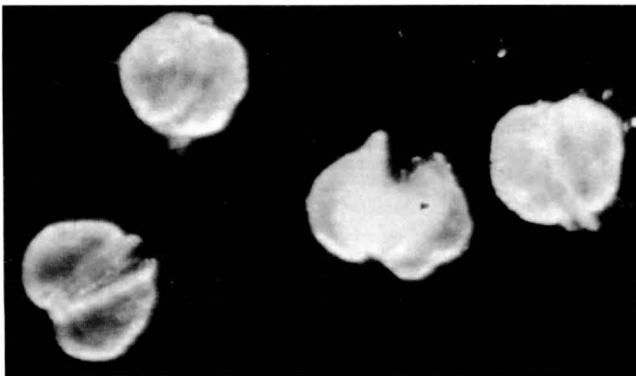


Fig. 1. a) Encysted glochidia of *Margaritifera auricularia* in the filaments of a *Salaria fluviatilis* gill. b) Juveniles released from their host, after less than one day of free life.

Fig. 1. a) Gloquidis de *Margaritifera auricularia* encistats als filaments d'una brànquia de *Salaria fluviatilis*. b) Juvenils alliberats del seu hoste, després de menys d'un dia de vida lliure.

due to the construction of a power station at the Xerta weir. Initially, all adult bivalves in this area were recovered and translocated to safe sites, but no young specimens were found. When this site was dry a second, more thorough search was performed.

Results

The production of glochidia larvae was observed in all the adult individuals monitored. They were released, gradually but almost continuously, in dense masses, which became slowly washed away through the dilution of the hyaline mucus containing the larvae. The time of release extended from March to June, and every individual was productive for at least two months.

The glochidia adhered readily to the gills of most fishes. However, after a few days they were rejected by the vast majority of individual fishes. Juveniles were recovered from only one potential host species, the Freshwater Blenny, *Salaria fluviatilis*. The infection of this species was readily effected, although not all attached glochidia became encysted by the host's epithelium. Later on, numerous encysted larvae were visible on the gill filaments. The cysts appear as pustules with a diameter of 0.2 mm (Fig. 1a). Finally, juveniles were recovered (Fig. 1b). The parasitic stage lasted from over 40 to just 26 days

The juveniles of *Margaritifera auricularia* are very small (175 µm in diameter). Their shape is almost spherical, with a long and almost straight hinge. Their color is whitish, with a faint tinge of golden in the hyaline shell. The shell surface is rough, and the glochidial shell is clearly visible as a hump at the apical part. The edge starts a fast growth just after release, clearly visible as a sharp protruding margin after a few hours. The foot is cylindrical club-shaped, clearly enlarged near the tip. No cilia were visible, and there were no signs of any ability to move reptating. However, the movements of the foot were fast, suggesting that these juveniles are able to burrow readily.

Young specimens were found in the field only in the exposed area. They were already dead, and the soft parts were rotten inside. The smallest

individual found measures only 5.74 mm in length (Fig. 2). According to the annual rings present (and the application of an unpublished growth equation for the population), its age is just above 2 years. The shape of this specimen is reminiscent of the adult's, although the umbos are considerably more central, and the valves are quite flat.

Discussion

The results obtained point all in one direction: the studied population is a viable one. The finding that all adults produce larvae strongly suggests that they are hermaphroditic, a condition that has occasionally been reported in other margaritiferids (Smith, 1976; Ziuganov *et al.*, 1994). It also shows that the precautions taken during the handling of the pearl-mussels were adequate to mitigate the possible stress response (Waller *et al.*, 1995). Furthermore, together with the activity demonstrated by the glochidia, it indicates that these specimens are in good reproductive condition.

The finding of a natural host is crucial. Remarkably, *Salaria fluviatilis* is found in the same microhabitat as *Margaritifera auricularia*: sites with swift current passing over a bottom of coarse (decimetric) consolidated gravels (Altaba, 1997). Other species of *Margaritifera* live in similar habitats (Stober, 1972; Vanotte & Minshall, 1982). Moreover, only from this fish could juveniles reach such a microhabitat in a big river before being carried away by the current. Indeed, *S. fluviatilis* is an ambush predator living in burrows, and lays and takes care of the eggs on the underside of boulders (Côté *et al.*, 1999; Perdices *et al.*, 2000).

The attached glochidia were often shed quite fast by non-hosts, suggesting that a strong immune response is involved (Fustish & Millemann, 1978; Meyers *et al.*, 1980; Bauer, 1987). No sign of such a response was observed in the host species, even if the number of larvae on the gills decreased with time, as happens in related bivalve species (Young & Williams, 1984). Several workers have reported a narrow host specificity for margaritiferids

(Bauer, 1987; Zjuganov *et al.*, 1990; Nezlin *et al.*, 1994; Johnson & Brown, 1998), although in rivers harboring a rich fish fauna there seems to be a wider variation in resistance across fish species (Murphy, 1942; Awakura, 1968; Meyers & Millemann, 1977). The fairly large number of tested fish species supports the hypothesis that *M. auricularia* is host-specific on *S. fluviatilis*.

Live young individuals of *Margaritifera auricularia* had not been reported anywhere for decades. The early and only records were from channels adjacent to the Ebro in Aragon, where they have not been found again (Haas, 1917; Ciria, 1920; Alvarez Halcón *et al.*, 2000). This new finding shows that recruitment is not only possible, but that it is actually taking place.

As in the case of many other freshwater bivalves (Bogan, 1993), the decline and near extinction of *Margaritifera auricularia* was caused by a combination of factors (Altaba, 1990; 2000; Primack, 1998). Its host fish is an endangered species (Elvira, 1996; Perdices *et al.*, 2000). Considering the host-specific relationship reported here, the original distribution of *Salarias fluviatilis* may have been considerably larger than at present. Thus, the disappearance of both interacting species would have occurred through the building of weirs and dams, by the destruction of

shoals by agriculture and settlements along river banks, and finally by pollution. In addition, the freshwater molluscan faunas of the Mediterranean region as a whole are endangered due to widespread habitat destruction (Altaba, 1996b).

The knowledge obtained on the viability of the *Margaritifera auricularia* population in the lower Ebro should be used to promote its conservation urgently and efficiently. It should be incorporated into a sound recovery program (e.g., Beasley & Roberts, 1999), which should be focused both on the strict protection of its habitats, and on rearing juvenile pearl mussels in captivity for reintroduction to the wild. Clearly, the survival of this species is now intimately linked with preservation of the lower Ebro.

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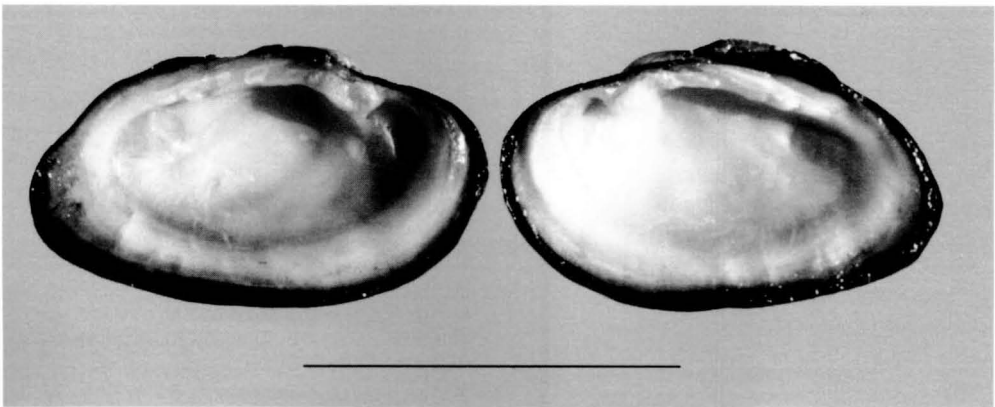


Fig. 2. Young specimen of *Margaritifera auricularia* found recently dead in an exposed gravel bar. The scale bar equals 50 mm.

Fig. 2. Espècimen jove de Margaritifera auricularia trobat mort de poc en una barra de grava emergida. L'escala equival a 50 mm.

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