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Fish host determination for *Margaritifera auricularia* (Bivalvia: Unionoida): results and implications

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Abstract

The fish hosts of *Margaritifera auricularia*, the critically endangered Giant Pearlmussel, have been identified. Natural infestations were searched for in the lower Ebro river (Catalonia, Spain), where the last viable population of *M. auricularia* survives. No encysted glochidia were found on wild fish. In addition, artificial infections were performed on 8 native and 8 exotic fish species. Encystment and growth was observed on the gill filaments of three species: *Acipenser baeri*, an introduced fish (showing that our early hypothesis involving sturgeons cannot be rejected, although it is not sufficient nor necessary); *Gambusia holbrooki*, a widespread exotic that may be useful in captive breeding; and *Salaria fluviatilis*, which is highly susceptible to infection, was the only species where full development was observed, shares the bivalve's habitat, and is also endangered. The dramatic reduction of the Giant Pearlmussel's range can now be explained as due to a combination of habitat destruction and disruption of its life cycle. This knowledge can now be used in a recovery program centered on the rearing of juvenile pearlmussels in captivity for reintroduction to the wild.

Riassunto

Nel corso del presente lavoro sono stati identificati i possibili pesci ospiti per i *glochidia* del bivalve *Margaritifera auricularia* (Fam. Unionidae), specie criticamente minacciata. Al fine di individuare gli ospiti naturali, è stato effettuato uno screening dei pesci presenti nel corso basso del fiume Ebro (Catalogna, Spagna), dove sopravive l'ultima popolazione di *M. auricularia*. In nessun caso è stato possibile rinvenire *glochidia* incistati nelle branchie dei pesci ivi rinvenuti. Parallelamente a questa indagine, sono state effettuate infestazioni artificiali su otto specie di pesci nativi, ed otto specie di pesci esotici. L'incistamento e l'accrescimento dei *glochidia* è stato osservato unicamente in tre specie: *Acipenser baeri*, un pesce introdotto; *Gambusia holbrooki*, specie esotica ubiquitaria (che può risultare pertanto utile per la riproduzione in cattività di *M. auricularia*); e *Salaria fluviatilis*, specie risultata altamente suscettibile all'infestazione e l'unica specie nella quale è stato possibile osservare uno sviluppo completo delle larve di *M. auricularia*. *Salaria fluviatilis* è una specie egualmente minacciata, che condivide l'habitat dell'unionide. La drammatica riduzione nella distribuzione di *M. auricularia* potrebbe pertanto essere spiegata con l'effetto sinergico della distruzione dell'habitat e dell'assenza dell'ospite adatto. I nuovi dati relativi a specie native ed esotiche di pesci, potenzialmente utilizzabili per far sì che *M. auricularia* possa completare il ciclo vitale in cattività, acquisiscono pertanto grande importanza nell'ottica di reintroduzione dell'unionide nel suo ambiente naturale.

Key words

Life history, river ecology, species interactions, parasitism, host specificity, Ebro, Margaritiferidae, *Margaritifera auricularia*, Blenniidae, *Salaria fluviatilis*.

Introduction

The Giant Pearlmussel, *Margaritifera auricularia* (Spengler, 1793) is a critically endangered species. Its original range extended throughout most of Western Europe, where it lived in virtually all big rivers draining into the North Sea, the Atlantic Ocean, the Western Mediterranean and the Adriatic Sea. However, this species experienced a dramatic, steady decline since the late XVth century, and it was thought to have become extinct around 1930 (Preece *et al.*, 1983; Preece, 1988; Altaba, 1990). After almost 70 years without any sightings, *M. auricularia* was rediscovered in the lower Ebro, in Catalonia (Altaba, 1990, 1991, 1992, 1996a, 2000a; Primack, 1998).

Initially, only recently dead specimens were found (Altaba, 1990, 1993), but living individuals were later located during the sampling of freshwater bivalves required for public works in the Ebro (Altaba, 1996a, 1996c). As a result of this large-scale sampling, it was possible to evaluate the size and distribution of the population (Altaba, 1997a, 2000a). It has also been found that this is the last chance for the species, because the lower Ebro harbors a viable population, with active recruitment and an age structure considered near equilibrium (Altaba *et al.*, 2001; Altaba & López, 2001a). This is in stark contrast with the status of relict demes living further upstream and in adjacent canals, where pollution and siltation may be responsible for those being only composed of very old specimens (Altaba, 1997a, b, 1999b, 2000; Álvarez Halcón *et al.*, 2000). Some reports of the species' rediscovery in the Ebro (Araujo & Ramos, 1998b, 2000, 2001) misrepresent the events and give an arguable importance to the assemblages of old, non-recruiting specimens in those artificial canals. Surely such specimens deserve conservation attention, but under the light of findings about the population living in natural habitats.

Live specimens of *Margaritifera auricularia* have also been recently found in some Atlantic drainages in France (Cochet, 2001; Nienhuis, 2003; Bichain, 2005). The demographic status of these surviving populations is now at a critical point, because there seems to have been no recruitment in recent decades. Indeed, major alterations of the natural riverbeds appear to be the cause (Nienhuis, 2003). It is thus of paramount importance for the conservation of freshwater mussels to determine which are its host fishes, and what other fishes can be used in laboratory propagation work (Neves, 2004).

The taxonomy of margaritiferids has been the subject of proposals to split the genus *Margaritifera* Schumacher, 1916 on the basis of few shell features alone into several genus-level taxa, including *Pseudunio* Haas, 1910 for *M. auricularia* (Falkner, 1994; Nagel, 1999; Smith, 2001; Falkner *et al.*, 2002; Nienhuis, 2003). A recent phylogenetic analysis based on molecular data shows that all extant margaritiferids are indeed closely related, and does not support any such scheme (Huff *et al.*, 2004). Furthermore, the conchological features reported as diagnostic of *Pseudunio* and other nominal genera are quite variable and unreliable (pers. obs.). In the absence of any diagnostic traits, and without any evidence of adaptive gaps in support of genus-level distinction (Cela-Conde & Altaba, 2002), we advocate to maintain the name *Margaritifera auricularia* as the most accurate for this species.

The life history of margaritiferids (**Fig. 1**) is a complex adaptation to life in rivers, to which these molluscs are intimately dependent (Ziuganov *et al.*, 1994). The adults are often hermaphroditic (as is the case in *Margaritifera auricularia*; Altaba & López, 2001a; Araujo *et al.*, 2002). Spermatozoa are liberated in spheroidal aggregations that are inhaled by other individuals. Fertilised eggs are incubated within all four gills, where they develop into

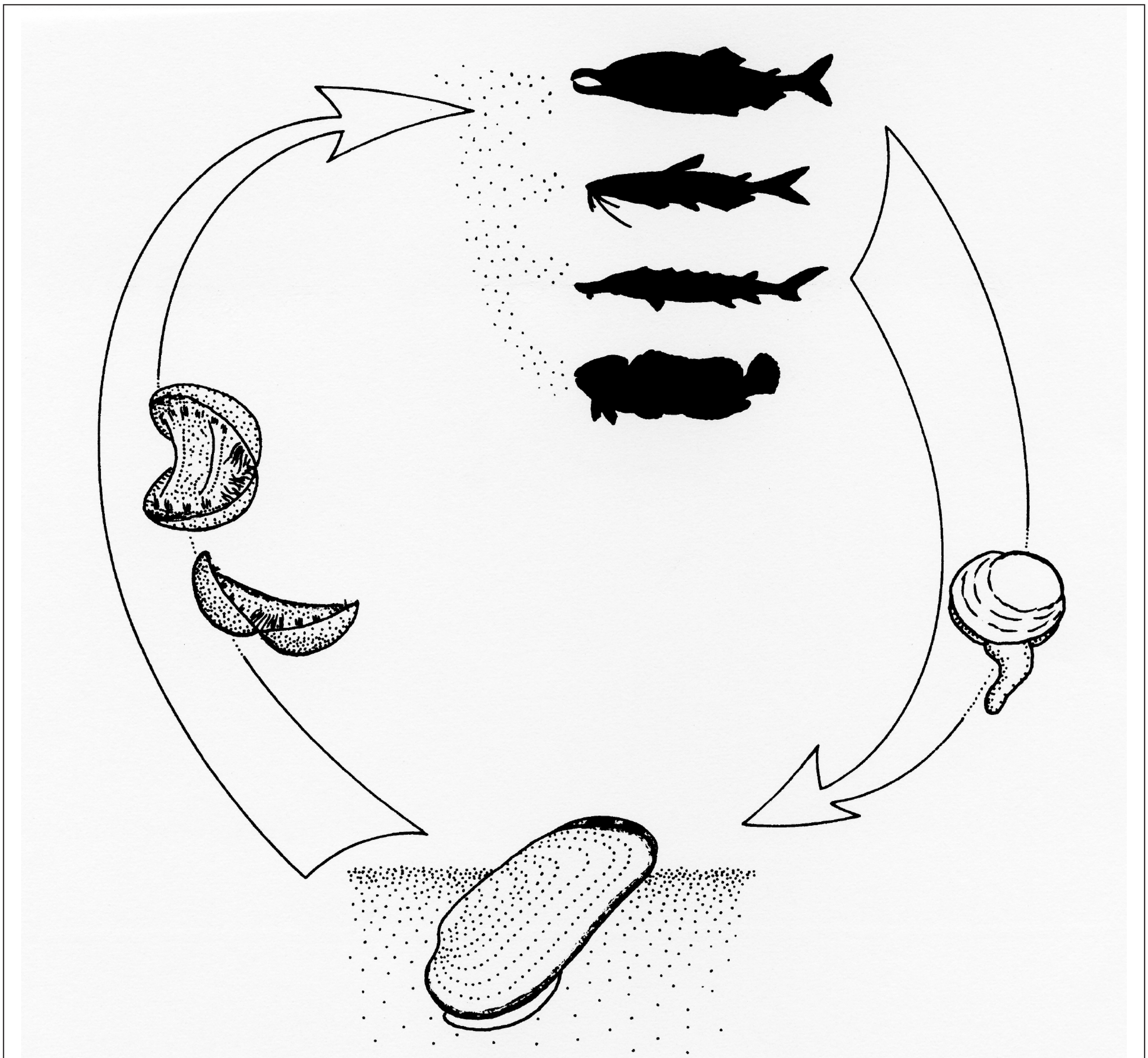

Fig. 1. Life history of margaritiferids. The fish host for members of this family may be (from top to bottom) a salmonid, an ictalurid, perhaps an acipenserid, or – as shown in this paper – a blenniid. See text for further explanation.

Fig. 1. Ciclo biologico dei margaritiferidi. Il pesce ospite per le specie di questa famiglia può essere (dall'alto in basso) un salmonide, un ictaluride, forse un acipenseride, o, come dimostrato in questo lavoro, un blenniide. Si veda il testo per ulteriori spiegazioni.

tiny larvae called glochidia (Nezlin *et al.*, 1994; Araujo & Ramos, 1998a). These are released so that the river flow (and chance) put them in contact with the gill epithelium of a susceptible fish. The parasite develops growing inside a cyst (Karna & Millemann, 1978; Nezlin *et al.*, 1994; Ziuganov *et al.*, 1994), and when metamorphosis into a tiny bivalve is completed it breaks the host's skin and falls to the bottom. There it must encounter a favorable microhabitat, while avoiding the numerous predators. The probability of overcoming all these trials is quite low (Young & Williams, 1984), and thus every single adult produces millions of offspring.

The fact that the Giant Pearlmussel's population in the lower Ebro appears to be viable (i.e., has an age distribution at equilibrium including young specimens), together with its very patchy distribution, shows that the limiting factor must be in the larval stages of its life history. Indeed, it has been shown in related species that the local density of adult pearlmussels 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).

The fish hosts of *Margaritifera auricularia* remained unknown until the work reported here was performed (López & Altaba, 2001b). Based on indirect evidence (coinciding historical range and decline), we had postulated that *Acipenser sturio* (Common Sturgeon) could be a host (Altaba, 1990). However, most of the living specimens were certainly not carried by this fish species, because its upstream movements have been prevented by the Xerta weir at least since the XIIth century. Furthermore, overfishing caused its complete extinction in the Ebro 30 years ago (Fernández & Farnós, 1999), and it is now critically endangered (Gessner, 2000; Litvak, 2003). Clearly, other fish species must be involved.

The fish fauna of the lower Ebro is composed of only 14 native species. However, in recent years it has been enriched 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). The molluscan fauna is also changing (López & Altaba, 1998) Moreover, the lower Ebro has experienced conspicuous hydrological changes, due to the construction of large dams upstream and the alteration of its shores and shoals (Ibáñez *et al.*, 1996; Mejuto & Llamas Madurga, 1996). It was considered urgent to identify the natural host(s) of *M. auricularia*, because the local extirpation of such species would lead to disruption of its life history, and thus would produce the species' complete extinction. The suitability of exotics had to be tested as well, in order to assess whether any of these newcomers could become a host in natural or artificial conditions.

Material and methods

In order to test the suitability of different fish species to serve as hosts for *Margaritifera auricularia*, we performed artificial infections on a wide array of native and exotic fishes. From March to June 2000 we tested under controlled conditions the suitability of fish to host glochidial development in their branchial structures. We infected 176 fishes belonging to 16 species. Eight are native to the lower Ebro: *Anguilla anguilla*, *Barbus graellsii*, *Barbus haasi*, *Chondrostoma toxostoma*, *Cobitis paludicola*, *Salaria fluviatilis*, *Liza aurata* and *Mugil cephalus*. The remaining eight are introduced exotics: *Acipenser baeri*, *Alburnus alburnus*, *Carassius auratus*, *Cyprinus carpio*, *Gobio gobio*, *Scardinius erythrophthalmus*, *Tinca tinca* and *Gambusia holbrooki*. Four other species were not available, either because they are very rare or locally extinct anadromous fishes (*Alosa alosa, Alosa fallax* and *Petromyzon marinus*), or because their presence in the river itself is questionable (*Barbatula barbatulus*).

Infections were produced in three different ways. The simplest procedure was placing the fish in an aquarium where four adult *Margaritifera auricularia* were releasing glochidia. In other cases infestations were enhanced by pipetting glochidia directly on the gills of immobilized fish. Finally, contact of glochidia and potential hosts was ensured by placing the fishes in a beaker containing a concentration of 300 glochidia per mL, for one hour. In order to test for any age-dependent development of resistance in host fishes (Awakura, 1968), individuals of various age classes were used, although most fishes were very young.

Fishes used in artificial infestations came from several sources (**Tab. 1**). Those obtained from a fish hatchery, river Canaleta (a tributary with a Mediterranean regime) or la Carrova (an adjacent, large spring) had not experienced previous contact with glochidia of any species. The fishes obtained from the Ecomuseu at the Ebro Delta Natural Park were fingerlings also naïve to glochidia. Those obtained from aquaria had been captured as young in areas now virtually deprived of unionids, so it is most unlikely that any of them had experienced previous contact with glochidia. The few fishes collected in the river Ebro itself were taken far from where *M. auricularia* now survives, yet if they had developed any immunity to water-borne glochidia this remained undetected because those were the only fishes on which metamorphosis was accomplished.

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 out of the reach of any fish, separated by a mesh that would allow released juveniles to pass by. Periodically, samples of these fish stocks were killed in order to determine the presence of glochidia or cysts on their gills. Every day, the bottom of the tanks was siphoned and the sieved materials inspected under the stereoscope.

In addition, we investigated natural infestations in the lower Ebro river. Wild fishes were captured periodically with lines and nets between March and June 2000. Almost all species present in the Ebro were represented in these catches. Only five species were absent from these samples, because they could not be captured at this time of the year: four anadromous species (inclu-

start	method[1]	species[2]	n[3]	origin[4]	end	duration[5]	yield[6]		
							glochidia	cysts	juveniles
19/03/00	P	*Barbus graellsii*	1	p	20/03/00	22 h	+	0	0
19/03/00	A	*Barbus graellsii*	1	p	21/03/00	2 d	+	0	0
20/03/00	P	*Barbus graellsii*	1	p	21/03/00	24 h	+++	0	0
23/03/00	P+S+A	*Carassius auratus*	1	a	25/03/00	2 d	0	0	0
25/03/00	S	*Chondrostoma toxostoma*	1	c	26/03/00	1 d	0	0	0
23/03/00	P	*Barbus graellsii*	1	p	27/03/00	4 d	0	0	0
25/03/00	A	*Barbus graellsii*	1	p	27/03/00	2 d	++	0	0
23/03/00	S+A	*Carassius auratus*	1	a	27/03/00	4 d	0	0	0
26/03/00	S	*Chondrostoma toxostoma*	2	c	27/03/00	1 d	0	0	0
26/03/00	P	*Barbus graellsii*	1	e	28/03/00	2 d	0	0	0
27/03/00	S	*Anguilla anguilla*	1	p	29/03/00	2 d	++	0	0
27/03/00	S	*Anguilla anguilla*	1	p	29/03/00	2 d	++	0	0
26/03/00	P	*Barbus graellsii*	1	e	29/03/00	3 d	0	0	0
23/03/00	S+A	*Carassius auratus*	1	a	29/03/00	6 d	+	0	0
23/03/00	S+A	*Carassius auratus*	1	a	29/03/00	6 d	0	0	0
26/03/00	P	*Carassius auratus*	1	p	29/03/00	3 d	0	0	0
25/03/00	P+S	*Chondrostoma toxostoma*	1	c	29/03/00	4 d	+++	0	0
25/03/00	P	*Chondrostoma toxostoma*	1	c	29/03/00	4 d	0	0	0
26/03/00	P	*Chondrostoma toxostoma*	1	c	29/03/00	3 d	0	0	0
26/03/00	P	*Chondrostoma toxostoma*	1	c	29/03/00	3 d	0	0	0
26/03/00	P	*Cyprinus carpio*	1	p	29/03/00	3 d	0	0	0
25/03/00	S	*Gobio gobio*	1	a	29/03/00	4 d	0	0	0
26/03/00	P	*Chondrostoma toxostoma*	1	c	30/03/00	4 d	0	0	0
23/03/00	P	*Acipenser baeri*	1	p	31/03/00	8 d	–	+	0
31/03/00	P	*Barbus graellsii*	1	p	31/03/00	2.5 h	++	0	0
26/03/00	P	*Carassius auratus*	1	p	31/03/00	5 d	0	0	0
31/03/00	P	*Barbus graellsii*	1	p	01/04/00	23 h	+	0	0
21/03/00	A	*Barbus graellsii*	1	p	03/04/00	13 d	0	0	0
31/03/00	P	*Barbus graellsii*	1	p	03/04/00	3 h	0	0	0
23/03/00	S+A	*Carassius auratus*	1	a	03/04/00	11 d	0	0	0
31/03/00	P	*Barbus graellsii*	1	p	04/04/00	60 h	0	0	0
27/03/00	S	*Anguilla anguilla*	4	p	05/04/00	9 d	+	0	0
31/03/00	P	*Barbus graellsii*	1	p	05/04/00	4 d	0	0	0
31/03/00	P	*Barbus graellsii*	1	p	05/04/00	6 d	0	0	0
26/03/00	P	*Barbus haasi*	1	c	05/04/00	10 d	0	0	0
26/03/00	P	*Chondrostoma toxostoma*	1	c	05/04/00	10 d	0	0	0
25/03/00	S	*Gobio gobio*	1	a	05/04/00	11 d	0	0	0
12/04/00	P+S	*Barbus graellsii*	1	p	13/04/00	1 d	0	0	0
20/03/00	P	*Barbus graellsii*	1	p	19/04/00	30 d	0	0	0
26/03/00	P	*Chondrostoma toxostoma*	1	c	19/04/00	24 d	0	0	0
26/03/00	P	*Cyprinus carpio*	2	p	19/04/00	24 d	0	0	0
20/04/00	P	*Barbus graellsii*	1	p	20/04/00	1 h	+	0	0
20/04/00	P	*Barbus graellsii*	1	p	20/04/00	1 h	0	0	0
26/03/00	Pt	*Carassius auratus*	1	p	20/04/00	25 d	0	0	0
26/03/00	P	*Chondrostoma toxostoma*	1	c	21/04/00	26 d	0	0	0
26/03/00	P	*Carassius auratus*	1	p	22/04/00	27 d	0	0	0
27/03/00	S	*Anguilla anguilla*	2	p	23/04/00	27 d	0	0	0
26/03/00	P	*Barbus graellsii*	2	e	26/04/00	31 d	0	0	0
26/04/00	A	*Gambusia holbrooki*	4	l	26/04/00	4 h	+	0	0
17/03/00	A	*Salaria fluviatilis*	1	r	26/04/00	40 d	–	++	0
26/03/00	P	*Tinca tinca*	1	e	26/04/00	31 d	0	0	0

continua ☛

start	method[1]	species[2]	n[3]	origin[4]	end	duration[5]	yield[6]		
							glochidia	cysts	juveniles
23/03/00	P	*Barbus graellsii*	1	p	27/04/00	35 d	0	0	0
26/04/00	A	*Gambusia holbrooki*	3	l	27/04/00	1 d	+	0	0
25/03/00	P+A	*Gobio gobio*	1	a	27/04/00	33 d	0	0	0
20/04/00	P	*Liza aurata*	2	p	27/04/00	7 d	0	0	0
20/04/00	P	*Mugil cephalus*	2	p	27/04/00	7 d	0	0	0
26/04/00	A	*Scardinius erythrophthalmus*	1	l	27/04/00	1 d	0	0	0
26/04/00	A	*Scardinius erythrophthalmus*	1	l	27/04/00	1 d	0	0	0
26/03/00	P	*Barbus graellsii*	1	p	05/05/00	40 d	0	0	0
26/03/00	P	*Chondrostoma toxostoma*	3	c	5/05/00	40 d	0	0	0
26/03/00	S	*Cyprinus carpio*	1	p	05/05/00	40 d	0	0	0
26/03/00	P	*Cyprinus carpio*	1	p	05/05/00	40 d	0	0	0
29/04/00	A	*Alburnus alburnus*	10	a	06/05/00	7 d	0	0	0
5/05/00	A	*Barbus graellsii*	2	p	06/05/00	1 d	0	0	0
20/04/00	S	*Gambusia holbrooki*	18	p	08/05/00	18 d	0	0	0
20/04/00	P	*Liza aurata*	7	p	08/05/00	18 d	0	0	0
20/04/00	P	*Mugil cephalus*	2	p	08/05/00	18 d	0	0	0
26/03/00	P	*Chondrostoma toxostoma*	1	p	09/05/00	44 d	0	0	0
23/03/00	P	*Acipenser baeri*	1	p	11/05/00	49 d	0	0	0
23/03/00	P	*Acipenser baeri*	2	p	11/05/00	49 d	–	+	0
26/04/00	A	*Gambusia holbrooki*	4	l	11/05/00	15 d	–	+	0
26/04/00	A	*Gambusia holbrooki*	16	l	11/05/00	15 d	0	0	0
26/04/00	A	*Scardinius erythrophthalmus*	3	l	11/05/00	15 d	0	0	0
4/05/00	S	*Barbus graellsii*	1	e	12/05/00	8 d	0	0	0
12/05/00	S	*Barbus graellsii*	1	e	12/05/00	1 h	+++	0	0
12/05/00	S	*Barbus graellsii*	2	e	12/05/00	1 h	+++	0	0
12/05/00	S	*Barbus graellsii*	2	e	12/05/00	2 h	+++	0	0
12/05/00	S	*Barbus graellsii*	1	e	12/05/00	0.5 h	+++	0	0
12/05/00	S	*Barbus graellsii*	2	e	12/05/00	4 h	++	0	0
12/05/00	S	*Barbus graellsii*	2	e	12/05/00	5 h	++	0	0
26/03/00	P	*Carassius auratus*	1	p	12/05/00	47 d	0	0	0
12/05/00	S	*Chondrostoma toxostoma*	1	c	12/05/00	1 h	+++	0	0
12/05/00	S	*Chondrostoma toxostoma*	1	c	12/05/00	1 h	0	0	0
12/05/00	S	*Chondrostoma toxostoma*	1	c	12/05/00	2 h	++	0	0
12/05/00	S	*Chondrostoma toxostoma*	2	c	12/05/00	1 h	0	0	0
26/03/00	P	*Cyprinus carpio*	2	p	12/05/00	47 d	0	0	0
26/04/00	A	*Cobitis paludicola*	1	l	16/05/00	20 d	+	0	0
21/03/00	P	*Barbus haasi*	1	c	19/05/00	59 d	0	0	0
21/03/00	P	*Gobio gobio*	1	a	19/05/00	59 d	0	0	0
04/05/00	A	*Salaria fluviatilis*	10	r	30/05/00	26 d	–	–	+

Tab. 1. Experimental infestations of individual fishes with glochidia of *Margaritifera auricularia*.

1 P = infestation enhanced by pipetting glochidia over the gills; S = infestation assisted by placing the fish in a solution of glochidia; A = free infestation in aquaria with adult bivalves releasing glochidia.
2 Individuals of *Barbus graellsii* coming from the Ecomuseu were all fingerlings. Most other fishes were young.
3 Number of individuals tested.
4 p = fish hatchery; a = aquarium; c = river Canaleta (a tributary with a Mediterranean regime); e = Ecomuseu at the Ebro Delta Natural Park; l = la Carrova (an adjacent, large spring); r = river Ebro.
5 Time lapse between infestation and extraction, in hours (h) or days (d).
6 Numbers of larvae attached, or juveniles released per individual fish: none (0), < 10 (+), 10-100 (++), > 100 (+++), host not examined at this stage (–).

Tab. 1. Infestazioni indotte sperimentalmente con glochidi di *Margaritifera auricularia* su singoli individui di pesci.

1 P = infestazioni favorita inserendo i glochidi sulle branchie tramite la pipetta; S = infestazione favorita collocando il pesce in una soluzione di glochidi; A = infestazione libera in acquario contenente bivalvi adulti che rilasciavano glochidi.
2 Gli individui di *Barbus graellsii* provenienti dall'Ecomuseu erano tutti molti piccoli. Molti altri pesci erano allo stadio giovanile.
3 Numero degli individui esaminati.
4 p = vasca di incubazione; a = aquario; c = fiume Canaleta (affluente con regime Mediterraneo); e = Ecomuseu nel Parco Naturale del Delta dell'Ebro; l = la Carrova (una grossa sorgente vicina); r = fiume Ebro.
5 Tempo intercorso tra l'infestazione e l'estrazione, in ore (h) o giorni (d).
6 Numero delle larve che si sono insediate, o juvenili rilasciati da un singolo pesce: nessuno (0), < 10 (+), 10-100 (++), > 100 (+++), ospite non esaminato a questo stadio (–).

ding the Common Sturgeon) and one rheophilous species (the Freshwater Blenny, *Salaria fluvuatilis*). In total, over 200 individual fishes have been examined in this way.

Results

The inspection of the captured wild fishes proved unhelpful. Indeed, no larvae of *Margaritifera auricularia* were recorded on any of them. The common presence of attached or encysted glochidia of other unionoids present in the Ebro (Altaba, 1992, 1996c) showed that these fishes were in sufficiently good condition to host young mussels.
During the artificial infections, glochidia adhered readily to gills (and occassionally to gill arches) of several species (**Tab. 1**). However, the ability to retain the parasites varied greatly among species (**Tab. 2**). After a few days they were rejected by the vast majority of individual fishes. The shedding of attached glochidia was quite fast in some cases, probably involving a strong immune response (Bauer, 1987; Fustish & Millemann, 1978; Meyers *et al*., 1980). The strength of the observed immunity could be age-dependent, but this could not be evaluated given the low number of old fish and the data collected. Even on suitable hosts the number of larvae on the gills decreased with time, as happens in related bivalve species (Young & Williams, 1984b). In contrast to other reports (Murphy, 1942; Awakura, 1968; Cunjak & McGladdery, 1991; Karna & Millemann, 1978), no fish deaths were attributable to glochidia, even in heavy infestations.
Juvenile mussels were recovered from only one potential host species, although encystment and growth was observed on the gill filaments of two other species. The Freshwater Blenny, *Salaria fluviatilis*, proved to be an excellent host (López & Altaba, 2000; Altaba & López, 2001a, 2001b; Altaba *et al.*, 2001; also Araujo *et al.* 2001). The infection of this species was readily effected. Later on, numerous encysted larvae were visible on the gill filaments (**Fig. 2 a**). Finally, juveniles were recovered (**Fig. 2 b**). Depending on the time of the year, the parasitic stage lasted from 26 days to over 40 (glochidia still encysted when the experiments were terminated).
The juveniles of *Margaritifera auricularia* are very small, measuring nearly 175 µm. 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.

Fish species[a]	N[b]	Prevalence[c]			Suitability[d]
		attachment	encystment	metamorphosis	
Acipenser baeri	4	4/4	2/3	?	2
Anguilla anguilla	8	6/6	0/2	0	0
Alburnus alburnus	10	?	0/10	0	0
Barbus graellsi	35	13/19	0/16	0	0
Barbus haasi	2	?	0/2	0	0
Carassius auratus	10	1/6	0/4	0	0
Chondrostoma toxostoma	20	3/13	0/7	0	0
Cyprinus carpio	7	0/1	0/6	0	0
Gobio gobio	4	0/2	0/2	0	0
Scardinius erythrophthalmus	5	0/2	0/3	0	0
Tinca tinca	1	?	0/1	0	0
Cobitis paludicola	1	0/1	0/1	0	0
Gambusia holbrooki	45	7/7	4/38	?	1
Salaria fluviatilis	11	11/11	1/1	10/10	3
Liza aurata	9	?	0/9	0	0
Mugil cephalus	4	?	0/4	0	0

Tab. 2. Suitability of different fish species as hosts for larval *Margaritifera auricularia*.

a. Native species shown in boldface.
b. Total number of individuals tested.
c. Proportion of examined fishes carrying or releasing young mussels at each stage. Question marks indicate that attachment may be possible but was not obsereved in the time available, or that metamorphosis may be possible but was not accomplished. The single *Cobitis paludicola* was found still carrying two dead glochidia which had not succeeded in forming cysts. No *Salaria fluviatilis* were killed at attachment stage, but all carried larvae to later stages.
d. Suggested ability of each species to serve as host, from nill (0) to excellent (3).

Tab. 2. Idoneità di differenti specie di pesci ad ospitare larve di *Margaritifera auricularia*.

a. Specie indigene indicate in neretto.
b. Totale degli individui esaminati.
c. Proporzione di pesci esaminati contenenti, o che rilasciano, bivalvi juvenili nei vari stadi. Il punto interrogativo indica che l'attacco può essere possibile, ma non è stato osservato nel periodo disponibile, o che la metamorfosi può essere possibile ma non è stata raggiunta. Solamente *Cobitis paludicola* è stato trovato che conteneva due glochidi morti che non hanno formato cisti. Nessun esemplare di *Salaria fluviatilis* è morto durante lo stadio di attacco, ma tutte le larve hanno raggiunto gli stadi successivi.
d. Valutazione dell'idoneità per ogni specie ad essere utilizzata come ospite, da nulla (0) ad eccellente (3).

The shell surface is rough, and the glochidial shell is clearly visible as a hump at the apical part. It is thus clear that in this species metamorphosis involves also growth. The edge of the valves starts a new stage of fast growth just after release, clearly visible as a sharp protruding margin after a few hours. The foot is cylindroid club-shaped, clearly enlarged near the tip. No cilia were visible, and there were no signs of any ability to move crawling (as sphaeriids or other juvenile unionoids do). However, the movements of the foot were fast, suggesting that these juveniles are able to burrow readily (apparently in a manner similar to solenid razor-clams).
Two other fish species carried glochidia to an encystment stage, but their ability to harbor complete metamorphosis was not confirmed. Consequently it is possible that the Adriatic Sturgeon *(Acipenser baeri)* might make suitable host in aquarium conditions (as reported by Araujo *et al.*, 2002, 2003), as well as the Mosquitofish (*Gambusia affinis*) and this needs further testing.

Discussion

The results obtained, although not as complete as ideally desired, are sufficient to draw relevant conclusions from them. In the first place, it is clear that the early hypothesis involving sturgeons cannot be rejected. However, since most of the extant pearlmussels are younger than the sequential extinction of *Acipenser sturio* in the Ebro, this is no longer a necessary nor sufficient explanation.
Several workers have reported a narrow host specificity for margaritiferids (e.g., Bauer, 1987; Zjuganov *et al.*, 1990; Nezlin *et al.*, 1994; Johnson & Brown, 1998). However, this occurred in rivers with very low local fish diversity. In exchange, research in drainages where a richer fish fauna exists reported a wide variation in the degree of resistance to glochidia across coexisting fish species (Murphy, 1942; Awakura, 1968; Meyers & Millemann, 1977). This appears to be also the case in the Ebro, at least with its current complement of exotic fishes.
The finding of a natural host (*Salaria fluviatilis*) is therefore crucial, especially because it is found also in the same microhabitat as *Margaritifera auricularia* – sites with swift current passing over a bottom of coarse (decimetric) consolidated gravels (Altaba, 1997a; Altaba & López, 2001a). This fish lives in burrows, where it waits for food as an ambush predator, and lays the eggs on the underside of boulders. It is easy to imagine that juvenile mussels could fall from the fish to suitable habitat. Remarkably, this is a habitat quite similar to those where other species of *Margaritifera* live (Stober, 1972; Vanotte & Minshall, 1982). Unfortunately, catching freshwater blennies in the fast-flowing habitat of *M. auricularia* has proven impossible during the period of glochidial release.
The length of the parasitic stage is probably dependent on water temperature This is in accordance with reports for other margaritiferids: overwintering of encysted larvae is usual in Nova Scotia (Cunjak & McGladdery, 1991), Kola (Ziuganov *et al.*, 1994) and Central Europe (Hruska, 1995, 1996), but rare elsewhere in Germany

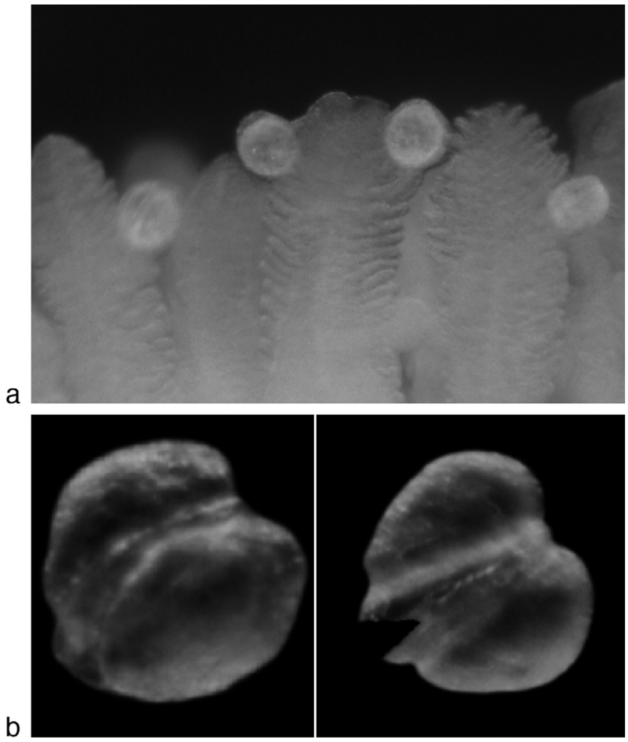

Fig. 2. a. Glochidia encysted in the filaments of a *Salaria fluviatilis* gill. The diameter of the cysts is 0.2 mm; **b.** Juvenile less than 24 h after release from their host (apical and lateral views). Notice the glochidial shell at the apex, and the fast growing shell edge. The diameter of is 175 µm.

Fig. 2. a. Glochidi incistidati nei filamenti branchiali di *Salaria fluviatilis*. Il diametro delle cisti è 0.2 mm; **b.** Esemplari juvenili rilasciati da poco meno di 24 h dal loro ospite (vista apicale e laterale). Si osservi l'apice della conchiglia del glochidio ed il margine che si accresce velocemente. Il diametero è di 175 µm.

(Bauer, 1979) and Scotland (Young & Williams, 1984a). It is unknown in the Western (Murphy, 1942; Karna & Milleman, 1978) and Eastern USA (Smith, 1976), as well as in Japan (Awakura, 1968). The Ebro is a much warmer river than any in these areas, so the short parasitic period should come as no surprise.
It is also noteworthy that the Freshwater Blenny (*Salaria fluviatilis*) is an endangered species (Lelek, 1986; Elvira, 1996; Perdices *et al.*, 2000). Although often considered a circum-Mediterranean species, there are reliable literature and museum records in Atlantic watersheds; it thus seems that its original range is unknown, rendering explanations of its currently fragmented distribution highly speculative (Perdices *et al.*, 2000). Taking into account the tight relationship reported here with *Margaritifera auricularia*, it is possible that its original distribution embraced most of Western Europe. The disappearance of both species would have occurred in parallel, caused mainly by habitat destruction. This was widely done through the building of weirs and dams, and by gaining land for agriculture and settlements along river banks.
It appears that complex interactions with biotic and abiotic factors at various spatial scales affect the ecology of the juvenile *M. auricularia*. This has been shown for other riverine meiofauna (Hakenkamp & Morin, 2000; Robertson *et al.*, 2000; Swan & Palmer, 2000). However, in contrast with other meiofauna (Rundle *et al.*, 2000), freshwater mussels add a level of complexity because

they benefit from dispersal by fishes. The existence of such interacting ecological processes has important consequences for the design and implementation of sound conservation strategies for this species and its riverine ecosystem.

The possibility that *Gambusia holbrooki* might be an adequate host under certain conditions deserves careful attention and further work. In case this could be achieved, then a favorable, cost-effective and large-scale production of juveniles could be feasible.

Conclusions

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 (Preece *et al.*, 1983; Altaba, 1990, 2000a; Primack, 1998). Overfishing and climate change have been hypothesized to be causal for this decline (Fechter & Falkner, 1993). However, the dramatic reduction of the Giant Pearlmussel's range may now be explained as due to a combination of habitat destruction and disruption of its life cycle (Altaba *et al.*, 2001). The original distribution of both *Margaritifera auricularia* and *Salaria fluviatilis* probably were concordant, but this is uncertain. Indeed, as most animals living now in central and north-western Europe did (Hewitt, 1996), both probably spread northwards together during the postglacial.

This knowledge can now be used in a recovery program centered on the rearing of juvenile pearlmussels in captivity for reintroduction to the wild (Altaba, 1996d). The protection of the fluvial ecosystem as a whole is needed, especially given that *Salaria fluviatilis* is very sensitive to alteration of the natural riverbed (Côté *et al.*, 1999). Although conservation has come a long way in recent years in Spain (Morillo & Gómez-Campo, 2000), efforts are often unsuccessful (Altaba, 1999a), with the result that many freshwater molluscs are endangered or already extinct (Altaba, 1996b; 1998; 2000b, 2001, 2003). A sound recovery plan for *Margaritifera auricularia* (as that for *M. margaritifera* by Beasley & Roberts, 1999) should be made and put into practice urgently.

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