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Status and conservation of the giant European freshwater pearl mussel (*Margaritifera auricularia*) (Spengler, 1793) (Bivalvia: Unionoidea)

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Abstract

The geographic range of the freshwater naiad *Margaritifera auricularia* has declined alarmingly since the beginning of the 20th century. The last records of live specimens date from 1933, and this species was thought to be extinct until 1996 when the authors found a population of about 2000 mussels in a channel of the Ebro River basin (Spain). The habitat, biology and host fish of the species are here described for the first time. The habitat is a well oxygenated, subsaline and oligo-mesotrophic water with high values of calcium (150 mg/l) where *M. auricularia* shows a patchy distribution in a substrate of pebbles, gravels and sand. In aquarium experiments all individuals released glochidia and developing embryos during 35 days (February–March), the former becoming attached to the gill filaments of the sturgeon (*Accipenser baeri*) 1 day after emission. One month later, at a temperature of 16–20°C, metamorphosed juveniles were obtained from induced infestations on the sturgeons. We review the current situation of this population and measures that should be urgently implemented to conserve this species through conservation of the habitat (Canal Imperial de Aragón) and long term programmes of artificial propagation.

Keywords: *Margaritifera auricularia*; Conservation; Freshwater pearl mussels; Ebro basin; Spain

1. Introduction

The global decline of freshwater mussel populations in the last 50 years has attracted much concern from national and international conservation organizations. Several causes are suggested. Their complex life cycle requires a fish host during their parasitic larval stage. The microscopic thin-shelled larvae (glochidia) that are brooded and released by the millions usually have hooks to attach themselves to the fins or gills, where they become encapsulated for several weeks before transforming to a free-living juvenile. Therefore, mussel survival depends not only on habitat conservation but also on the availability of the fish host.

Species of the genus *Margaritifera* have been historically the object of intensive exploitation for their nacre and pearls. Three taxa are known to occur in Europe: *M. auricularia*, *M. margaritifera* (L. 1758) and *M. durrovensis* (Phillips, 1928). Validity of the specific or subspecific status of *M. durrovensis* is discussed in relation to *M. margaritifera* by Phillips (1928), Chesney et al. (1993) and Moorkens and Costello (1994).

During this century, *M. margaritifera* has been extirpated from many regions of Central Europe (Buddensiek, 1995), and is now a priority subject for study in many countries. Numerous studies of this species document the precipitous decline and status of remnant populations [Bauer, 1986 (and references therein), 1987a,b; Bauer and Eicke, 1986; Bauer and Vogel, 1987; Hruska, 1992; Chesney et al., 1993; Ziuganov et al., 1994; Valovirta, 1995, 1998; Chesney and Oliver, 1998; Gibson, 1998; Killeen et al., 1998].

The only descriptive report on *M. auricularia* deals with a Spanish population (Haas, 1916a,b, 1917) living in a 70–120-m wide section of the river Ebro between stones and boulders with a predilection for deep areas (5–7 m), as well as in the Canal Imperial de Aragón, an ancient channel of the Ebro. A brief description of the anatomy of the species is also available from Haas (1924), but no information on its reproductive biology and larval morphology was reported. No other living

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specimens of this species were reported in the scientific literature until 1996 (Araujo and Ramos, 1996, 1998; Ramos, 1998). However Azpeitia (1933) documented specimens that were probably collected after 1917 from the Ebro basin, and one specimen from Toledo (Tajo River), and Altaba (1990) reported dead specimens of *M. auricularia* from an irrigation channel of the Ebro River near the estuary (Tarragona, Spain).

The species' former range included the Iberian Peninsula, France, Italy, England, Germany and Morocco, where a local race probably occurred (Haas, 1969). In Spain, from 5000 to 400 BC, it had a much wider distribution (Araujo and Moreno, 1999) as in other European countries (Preece et al., 1983), suggesting a continuing decline during the post-glacial period.

The species has been listed in Appendix IV of the EC Habitats Directive and in Appendix II of the Bern Convention. In the IUCN is listed as Critically Endangered (IUCN, 1996). After the rediscovery of living specimens of *M. auricularia* (Araujo and Ramos, 1996) and the corresponding report to the Dirección General de Conservación de la Naturaleza of Spain, the Comisión Nacional de Protección de la Naturaleza has included this as the first invertebrate species on the National Endangered Species List (Royal Decree 439/90) in the category of "threatened with extinction".

More recently, there have been reports of other *M. auricularia* populations in the main stem Ebro River, in one nearby irrigation channel (Altaba, 1997), in one channel of the Loire River (France) (Bacchi and Gilbert, pers. comm.; Nienhuis, pers. comm.) and in rivers of Morocco (Araujo and Ramos, 2000a).

In this paper, we describe the habitat of *M. auricularia* in the Canal Imperial and the results of several experiments designed on the artificial reproduction of the species. We summarize the legal conservation status of the species, the national and international efforts to conserve and restore the species, and those actions that are urgently needed for species conservation.

2. Materials and methods

The Canal Imperial de Aragón was built in the 18th century and runs parallel to the Ebro River through the Spanish provinces of Navarra and Zaragoza. It belongs to the Ebro river basin and flows across quaternary sediments for 115 km, the last 25 km being a narrow concrete ditch 1–2 m wide. The main part of the channel is 10 m wide and about 3.5 m deep when it is full, with a water velocity of 0.6 m/s. When "empty", the water depth is c. 40–100 cm. There is no bankside vegetation, except some trees and scattered patches of *Typha* sp. No macrophytes occur in the channel.

The Canal Imperial (Fig. 1) was surveyed completely at low flow with special emphasis on sites where the bed has not been artificially covered with concrete. Mussel sampling was conducted by looking for traces in the sediment and shells, using a "viewing glass" in deeper areas. In order to estimate the size structure and population size, specimens of *M. auricularia* were collected in February 1997, measured (length, width and height), marked on the left valve with numbered glue-on shellfish yellow tags (Hallprint tags, Holden Hill, Australia) and released. A recapture survey was conducted 1 year later.

The simple Lincoln (1930) index (= Petersen's method) (Le Cren, 1965) was used to estimate population size, assuming a stable population. According to this index, $Nn/Nc = Nm/Np$, where Nm= number of specimens marked, Nc= number of specimens collected, Nn= number of specimens recaptured, and Np= total population.

For granulometric (particle size) analyses of the substrate, samples were taken from three sites where *M. auricularia* occurs at different densities and at one site where it was absent. Samples were obtained at each site by digging in the substrate (to c. 10 cm depth) to obtain 1 kg of wet sediment.

Repeated physico-chemical measurements of the water column pH, temperature (°C), conductivity (μS/cm), suspended material (mg/l), organic material (mg/l O_2), dissolved oxygen (mg/l), BOD_5 (mg/l O_2), total ammonium (mg/l NH_4), Ca (mg/l), Mg (mg/l), Na (mg/l), K (mg/l), Cl (mg/l), SO_4 (mg/l), NO_3 (mg/l), NO_2 (mg/l), Alkalinity (mg/l CO_3), PO_4 (mg/l), Zn (mg/l), Cu (mg/l), Cd (mg/l) and Hg (mg/l) were taken in the area with the densest population at low- and full-water levels. These analyses were kindly conducted by the Confederación Hidrográfica del Ebro.

Since February 1996, eight specimens of *M. auricularia* were maintained in an aquarium with artificial airation containing 120 liters of water and 10 cm of substrate. Several days later, 11 Siberian sturgeon (*Acipenser baeri* Brandt, 1869), 10–20 cm in length, were placed in the aquarium. Water temperature ranged between 16 and 20°C and pH from 7.5 to 7.7. The mussels were fed with dissolved egg yolk and a nutritional diet for fine-filter feeders (Advanced Invertebrate 1 Formula, Marine Enterprises, Inc., USA) and the fishes were fed with red mosquito larvae. After infestation with glochidia, one or two sturgeon were regularly removed from the aquarium and anaesthetised with 3-aminobenzoic acid ethyl ester (MS222) and their gills examined.

For induced infestation, glochidia were obtained with a pipette from the exhalant aperture of the gravid mussels and placed in aerated water in a glass jar containing isolated fish. To confirm the presence of glochidia gill filaments were excised under a SZH10 Olympus stereomicroscope, and fixed for scanning electron microscopy for 2 h in glutaraldehyde, then dehydrated in a graded ethanol series and transferred to acetone. They were critical-point-dried with liquid CO_2 in a Polaron E-3000

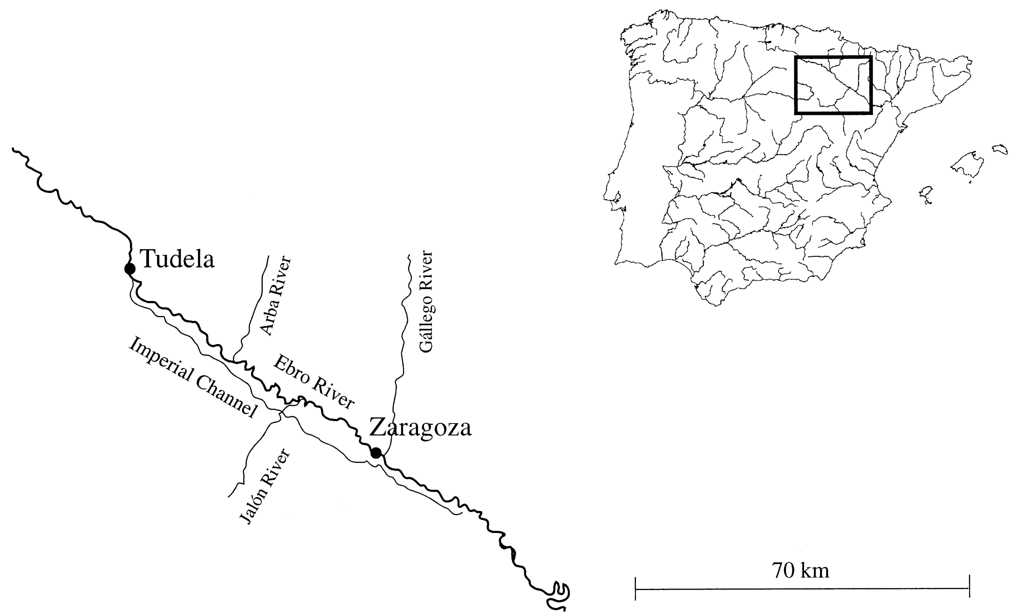

Fig. 1. Map of the Canal Imperial, in north east Spain.

unit and then coated with gold in a BioRad SC515 sputter coating unit, 20 nm thick. Observations were made in a Philips XL20 SEM at accelerating voltages of 20–30 kV.

In order to recover metamorphosed juveniles, a second experiment was conducted. Five sturgeon infested with *M. auricularia* glochidia were isolated in an aquarium (water temperature between 23 and 24°C) without substratum and with a 5 mm mesh plastic net on the bottom. One month after infection, the bottom water layer was pumped through a 60 µm mesh every 2 days.

3. Results

3.1. Natural habitat

Specimens of *M. auricularia* exhibited a patchy distribution along 30 km of the channel, being absent from all areas with concrete covered beds but also many areas with a natural bed. The species coexists with a rich unionid community of *Potomida littoralis* (Lamarck), *Unio elongatulus* C. Pfeiffer and *Anodonta cygnea* (L.), which inhabit most of the channel at various densities. All naiads live throughout the channel width.

M. auricularia lives partially buried in clay-sand and gravel beds, sometimes (especially in the breeding season) nearly vertical with the posterior end exposed and sometimes horizontal, showing only the dorsal margin. The main fraction of the substrate (Table 1) is of pebbles followed by gravel and sand. The finest fraction is always very small. Burrowing activity of the specimens leaves very visible wide ($\bar{X}=7.97$ cm; S.D. $=1.12$; $n=123$) circular traces in the substrate.

Table 1
Particle size analysis (%) of substrate at the Canal Imperial where *Margaritifera auricularia* is very abundant (A and B), rare (C) and lacking (D)

	A	B	C	D
Pebbles (10–100 mm)	64.69	70.54	42.66	37.50
Gravel (2–10 mm)	23.24	15.75	30.43	34.13
Sand (0.063–2 mm)	10.36	10.66	24.83	27.99
Mud (<0.063 mm)	1.69	3.02	2.06	0.36

A total of 565 live specimens were caught, marked and released on February 1997 and 731 were captured 1 year later of which 211 were marked, indicating an estimated number of 1956 specimens in the Canal Imperial. These nearly 2000 specimens are not the total population size because the species probably exists in other unexplored areas in the canal. No dead marked specimens were found.

As regards mobility, 14 of 16 marked specimens released in two parallel rows in the channel bottom were found in the same position 1 year later. In another site with 20 marked specimens, five were in the same position after 1 year, and the rest were no more than 4 m upstream.

All the live specimens were >13 cm in length, except two measuring 10 cm. The population structure, based on length measurements of 438 specimens is highly skewed to large individuals (Fig. 2).

Data on water quality in the channel are presented in Table 2. *M. auricularia* is a hardwater species; calcium levels in the Ebro River were c. 150 mg/l. The temperature in the Canal Imperial ranged from 7.4 (March) to 22.9°C (August).

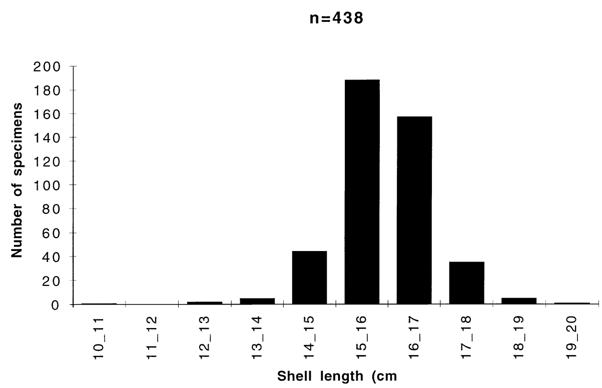

Fig. 2. Size frequencies structure of the *Margaritifera auricularia* population.

Table 2
Physico-chemical analyses of the water at the site of the richest population of *Margaritifera auricularia* at low water level (26 February 1997) and high water level (7 March 1997)

	Low water	High water
pH	8.3	8.1
Temperature (°C)	12.2	12.8
Conductivity at 20°C (μS/cm)	1547	1077
Suspended material (mg/l)	45	51
Organic material (mg/l O_2)	12.7	12.1
Dissolved oxygen (mg/l)	10.6	10.3
DBO5 (mg/l O_2)	2.2	6.2
Total ammonium (mg/l NH_4)	0.13	0.10
Ca (mg/l)	163	114
Mg (mg/l)	49.9	23.8
Na (mg/l)	64.5	98.5
K (mg/l)	4.4	3.3
Cl (mg/l)	103	135
SO_4 (mg/l)	373	196
NO_3 (mg/l)	15.1	25.2
NO_2 (mg/l)	–	0.057
Alkalinity (mg/l CO_3)	253	187
PO_4 (mg/l)	0.28	0.11
Zn (mg/l)	0.01	0.02
Cu (mg/l)	0.00	0.00
Cd (mg/l)	0.00	0.00
Hg (mg/l)	0.00	0.00

3.2. Aquarium results

As soon as the *M. auricularia* specimens were placed into the aquarium, all of them began to release white masses of eggs and developing embryos, with mature glochidia released after 7 days, that is 4 days after the fish were introduced. The masses of glochidia were strongly expelled through the exhalant aperture, and subsequently remained either on the aquarium bottom or lying over the mussel until they were inhaled by a sturgeon. One day after emission of the glochidia, they were observed attached to the gill filaments of fishes. Expulsion of glochidia and immature embryonic stages lasted c. 35 days, with a lapse of 5 days without emission in the middle of the spawning period and with a marked peak of glochidial release on the 28th day.

Four infested fishes were removed from the aquarium, anaesthetized, sacrificed and examined 5, 13, 34 and 60 days after infestation, respectively. On the first three occasions they had gill filaments packed with glochidia, but glochidia were absent at 60 days.

The glochidia became encapsulated on all gill filaments (Fig. 3A). Thirty-four days after infestation, encapsulated glochidia became spherical in shape (Fig. 3B) when compared to those from two fishes sacrificed earlier (i.e. after 5 and 13 days, respectively).

Ten days after the fourth sturgeon was sacrificed, the remaining fishes were removed from the aquarium. Subsequently, several bottom samples were examined and some empty glochidial shells were found, but no juvenile mussels were detected even after all the aquarium substrate was examined.

Exactly 1 month after the five sturgeon were infested in the second experiment, we found 15 live juveniles and many empty juvenile valves in the aquarium without substrate. Only a few empty juvenile shells were found in the following 2–4 days, and no encysted glochidia were found on the surviving sturgeon. Shell measurements of the juveniles were: length 190 μm, height, 187 μm, and width 225 μm.

4. Discussion

4.1. Natural history

Specimens of *M. auricularia* in the Canal Imperial seem to be very sedentary, as suggested by the results of our capture–recapture experiment in the different areas where the species lives. We assume that the whole area for these mussels is very small because adults have very poor locomotion or migration capacity, as reported by Coker et al. (1921) for heavy-shelled mussels and, more recently, by Amyot and Downing (1997) for *Elliptio complanata* (Lightfoot).

Although more thorough studies are needed on habitat, the granulometric data suggest that *M. auricularia* numbers fall when substrates with a predominance of finer materials (gravel, sand, mud) increase and there is a relative decrease in pebbles.

Water conditions vary throughout the year as may be expected in a regulated irrigation channel. However, the range of values in Table 2 indicate that the water is generally subsaline (having moderate conductivity), basic, well oxygenated and mesotrophic with moderate levels of phosphorus and organic matter (BOD_5). This contrasts clearly with the conditions favoured by *M. margaritifera*, which are typically fast flowing, clean and oligotrophic rivers with low calcium levels.

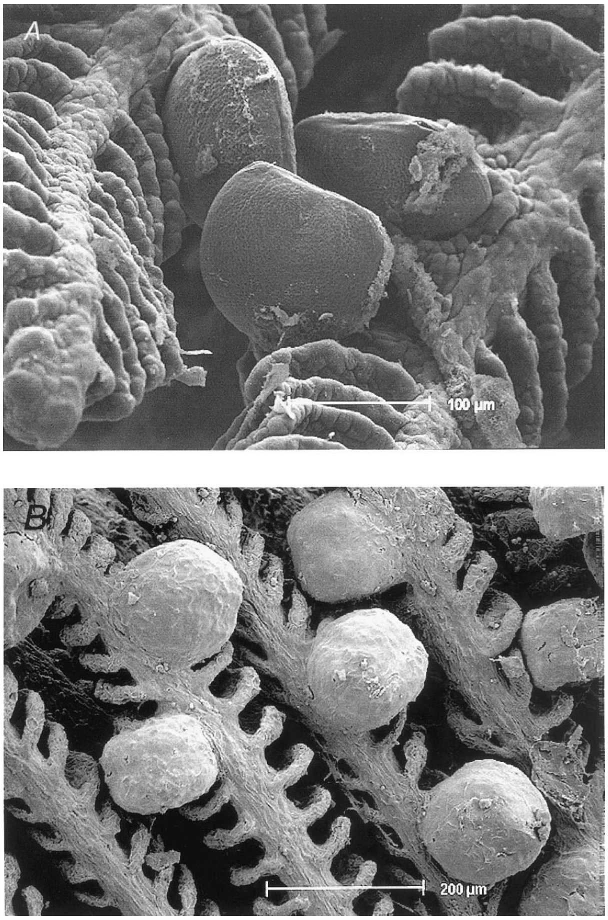

Fig. 3. Sturgeon (*Acipenser baeri*) gill filaments showing encysted larvae (glochidia): (A) 1 h after infestation; (B) 1 month after infestation.

M. auricularia appears to be a short-term brooder (tachytictic) (Araujo et al., 2000), all known species of the genus *Margaritifera* are short-term brooders (Heard, 1970; Smith, 1979; Bauer, 1994). We do not know when fertilization of the ova occurs, but specimens collected in the wild in mid-February had their gills full of embryos, which developed into glochidia which were released from late February until March 22 (peak on March 14).

Although there is a well known affinity between *M. margaritifera* glochidia and salmonid fishes, species of the latter were never common in the middle and lower course of the Ebro Basin. Natural and induced infestations showed that the sturgeon is a suitable host for the glochidia of *M. auricularia*, as previously suggested by Altaba (1990). In our experiments, glochidia metamorphosed on the gills of sturgeon in aquaria, and juveniles were released after 30 days at 23–24°C (690 degree-days). Live juveniles move rapidly when observed under the stereomicroscope, with retraction and protraction of the finely ciliated foot. As regards shell changes during metamorphosis, the juveniles exhibit a nearly spherical shape produced by the addition of a very thin edge of shell material around the glochidial valve. *Acipenser sturio*, probably the only species of sturgeon occurring naturally in Spain, was exploited until the mid-1960s in the Ebro River, but catches declined following the construction of reservoirs (Sostoa and Lobón-Cerviá, 1989). The absence of *A. sturio* specimens in Spain, and especially in the Ebro basin over many years, is a likely reason for the decline of *M. auricularia*.

Our results indicate that *A. baeri* is a good species to be used as a surrogate fish host if a recovery plan for the species is proposed.

4.2. Conservation measures

The unexpected discovery of this *M. auricularia* population in Spain and results reported here revive interest in the species and pose new and puzzling questions. Unionoid juveniles are rarely collected because of their small size and the unknown and different habitats they occupy (Isely, 1911; Hudson and Isom, 1984; Neves and Widlak, 1987; Miller and Payne, 1988; Buddensiek, 1995; Richardson and Yokley, 1996). Only Buddensiek et al. (1990) and Buddensiek and Ratzbor (1995) have studied the chemistry of the interstitial water of bivalve habitats, with emphasis on the juvenile stages of *M. margaritifera*. Data on feeding and burrowing behaviour of juvenile unionids, reported by Yeager et al. (1994) suggest that this stage is the most susceptible to environmental alterations. The fact that neither juveniles nor middle-aged specimens were found in the study channel suggests either that (1) the specific host fish has not lived in the channel or in the river for many years (Araujo et al., 2000) and/or (2) features such as substrate or water quality do not allow juveniles to settle and survive. Nevertheless, the great abundance of the other three species of mussels in the channel, all showing recruitment, mainly in its upper half, clearly suggests that the conditions in this old channel (mainly water quality and bottom composition) are reasonably suitable for a healthy naiad community.

Evaluation of other fish hosts [e.g. eel *Anguilla anguilla* (L.), river blenny (*Salaria fluviatilis* Asso)], through recruitment observations in the wild and experiments with local fishes in aquaria, and electric-fishing in the Canal Imperial, are underway. These studies are essential for subsequent application of species reintroduction policies.

After dead specimens of *M. auricularia* were found near the Ebro estuary in 1990, a set of recommendations for the effective protection of *M. auricularia* and other unionoids in the Ebro Delta Natural Park were proposed (Altaba, 1990): namely (1) restrictions on collecting; (2) appropriate research; (3) protection of fish hosts; (4) protection of key habitats; (5) education; (6) establishment of captive breeding colonies; (7) re-introduction and (8) water quality control.

Points 2, 4, 6 and 8 are of primary importance, but greater knowledge of *M. auricularia* ecology is needed prior to implementing other actions. With our new knowledge, it is time to implement a recovery program.

First, the newly discovered breeding population, threatened by "modernization plans" to cover the channel bed where it lives with concrete slabs, needs to be protected. Its habitat is threatened because the channel, like many others in Spain, is included in government restoration plans to avoid water wastage. The massive dredging of the Ebro River bottom near the estuary to make the river navigable, and the construction of several dams are impending threats to the Catalonian population (Altaba, 1997).

In the case of the Canal Imperial, one option is to stop the proposed work and protect the channel under a comprehensive project of rational water use and sustainable development of the area (Araujo and Ramos, 2000b).

It is essential to increase survey efforts in the main course of the river to locate additional breeding aggregations or cohorts of juveniles. This is not an easy task due to the river's characteristics; it would be necessary for scuba divers to survey different transects and to employ special controlled dredging methods.

Simultaneously, and with the available information on the breeding season and reproductive strategies under study, an experiment close to the natural habitat of the species should be designed to investigate the suitability of different kinds of substrates for adults and juveniles. Alternative fish species, that are likely to host the parasitic larval stage, should be tested. This information is a preliminary step to any plans for species recovery or restocking of former habitats. Similar experiments on artificial propagation of other freshwater mussels were carried out successfully many years ago in the Mississippi River (Lefevre and Curtis, 1921) and currently in several North American rivers (Neves, pers. comun.).

Old reports like that of Coker et al. (1921) and more recent research on endangered unionids (Bruenderman and Neves, 1993; Hove and Neves, 1994; Vaughn and Pyron, 1995) are essential to an understanding of the natural history of freshwater mussels, but essential information about feeding habits, bottom, depth and current of the water bodies suitable for these molluscs, especially species of the genus *Margaritifera,* are still unknown.

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References

Araujo, R., Moreno, R., 1999. Former Iberian distribution of *Margaritifera auricularia* (Spengler) (Bivalvia: Margaritiferidae). Iberus 17 (1), 127–136.

Araujo, R., Ramos, M. A., 1996. *Margaritifera auricularia* re-discovered in Spain: observations on a relict population. In: Convention on the Conservation of European Wildlife and Natural Habitats. Conservation, Management and Restoration of Habitats for Invertebrates: Enhancing Biological Diversity, Killarney, Ireland, 27–28 May.

Araujo, R., Ramos, M.A., 1998. *Margaritifera auricularia* (Unionoidea, Margaritiferidae), the giant freshwater pearl mussel rediscovered in Spain. Graellsia 54, 129–130.

Araujo, R., Ramos, M.A., 2000a. A critic revision of the historical distribution of *Margaritifera auricularia* (Spengler, 1793) (Mollusca: Margaritiferidae) based on museum specimens. Journal of Conchology 37 (1), 49–59.

Araujo, R., Ramos, M.A., 2000b. Action Plan for *Margaritifera auricularia*. Council of Europe, T-PVS (2000) 9.

Araujo, R., Bragado, D., Ramos, M.A., 2000. Occurrence of glochidia of the endangered *Margaritifera auricularia* (Spengler, 1793) and other mussel species (Bivalvia: Unionoida) in drift and on fishes in an ancient channel of the Ebro River, Spain. Archiv für Hydrobiologie 148 (1), 147–160.

Altaba, C.R., 1990. The last known population of the freshwater mussel *Margaritifera auricularia* (Bivalvia, Unionoida): A conservation priority. Biological Conservation 52, 271–286.

Altaba, C.R., 1997. Al límit de l'extinció: *Margaritifera auricularia* (Bivalvia: Unionoida). Butlletí de la Institució Catalana d'Historia Natural 65, 137–148.

Amyot, J.-P., Downing, J.A., 1997. Seasonal variation in vertical and horizontal movement of the freshwater bivalve *Elliptio complanata* (Mollusca: Unionidae). Freshwater Biology 37, 345–354.

Azpeitia, F., 1933. Conchas bivalvas de agua dulce de España y Portugal. Memorias del Instituto Geológico y Minero de España, Vol. 1, Madrid.

Bauer, G., 1986. The status of the freshwater pearl mussel *Margaritifera margaritifera* L. in the south of its European range. Biological Conservation 38, 1–9.

Bauer, G., 1987. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L). II. Susceptibility of brown trout. Archiv für Hydrobiologie, 76 (4) (Suppl.), 403–412.

Bauer, G., 1987. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). III. Host relationships. Archiv für Hydrobiologie, 76 (4) (Suppl.), 413–423.

Bauer, G., 1994. The adaptive value of offspring size among freshwater mussels (Bivalvia: Unionoidea). Journal of Animal Ecology 63, 933–944.

Bauer, G., Eicke, L., 1986. Pilotproject zur rettung der flussperlmuschel. Natur und Landschaft 61 (4), 140–143.

Bauer, G., Vogel, C., 1987. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). I. Host response to glochidiosis. Archiv für Hydrobiologie, 76 (4) (Suppl.), 393–402.

Bruenderman, S.A., Neves, R.J., 1993. Life history of the endangered fine-rayed pigtoe *Fusconaia cuneolus* (Bivalvia: Unionidae) in the Clinch River, Virginia. American Malacological Bulletin 10 (1), 83–91.

Buddensiek, V., 1995. The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* L. in cages: a contribution to conservation programmes and knowledge of habitat requirements. Biological Conservation 74, 33–40.

Buddensiek, V., Ratzbor, G., 1995. Restoration of sedimental quality in a small brook of the Lüneburger heide, northern Germany. Folia Facultatis Scientiarum Naturalium Universitatis Masarykianae Brunensis, Biologia 91, 19–24.

Buddensiek, V., Engel, H., Fleischauer-Rössing, S., Olbrich, S., Wächtler, K., 1990. Studies on the chemistry of interstitial water taken from defined horizons in the fine sediments of bivalve habitats in several northern German lowland waters. Archiv für Hydrobiologie 119 (1), 55–64.

Chesney, H.C.G., Oliver, P.G., Davis, G.M., 1993. *Margaritifera durrovensis* Phillips, 1928: taxonomic status, ecology and conservation. Journal of Conchology 34 (5), 267–299.

Chesney, H.C.G., Oliver, P.G., 1998. Conservation issues for Margaritiferidae in the British Isles and Western Europe. In: Killeen, I., Seddon, M.B., Holmes, A.M. (Eds.), Journal of Conchology Special Publication No. 2, June 1998, pp. 231–242.

Coker, R.E., Shira, A.F., Clark, H.W., Howard, A.D., 1921. Natural history and propagation of fresh-water mussels. Bulletin of the United States Bureau of Fishes 37, 77–181.

Gibson, M., 1998. Issues involved in protecting important populations of *Margaritifera margaritifera*. In: Killeen, I., Seddon, M.B., Holmes, A.A. (Eds.), Journal of Conchology Special Publication No. 2, June 1998, pp. 243–244.

Haas, F., 1916. Sobre una concha fluvial interesante (*Margaritana auricularia*, Spglr.) y su existencia en España. Boletín de la Sociedad Aragonesa de Ciencias Naturales XV(2), 33–45.

Haas, F., 1916. Spanischer Brief III. Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft 48, 32–44.

Haas, F., 1917. Estudios sobre las Náyades del Ebro. Boletín de la Sociedad Aragonesa de Ciencias Naturales XVI, 71–82.

Haas, F., 1924. Anatomische Untersuchungen an europäischen Najaden. I. Archiv für Molluskenkunde 56, 66–82.

Haas, F., 1969. Superfamilia Unionacea. Das Tierreich 88, 1–663.

Heard, W.H., 1970. Hermaphroditism in *Margaritifera falcata* (Gould) (Pelecypoda: Margaritiferidae). Nautilus 83 (3), 113–114.

Hove, M.C., Neves, R.J., 1994. Life history of the endangered James spiny mussel *Pleurobema collina* (Conrad, 1837) (Mollusca: Unionidae). American Malacological Bulletin 11 (1), 29–40.

Hruska, J., 1992. The freshwater pearl mussel in South Bohemia: evaluation of the effect of temperature on reproduction, growth and age structure of the population. Archiv für Hydrobiologie 126 (2), 181–191.

Hudson, R.G., Isom, B.G., 1984. Rearing juveniles of the freshwater mussels (Unionidae) in a laboratory setting. The Nautilus 98 (4), 129–135.

Isely, F.B., 1911. Preliminary note on the ecology of the early juvenile life of the Unionidae. Biological Bulletin 20, 77–80.

IUCN, 1996. IUCN Red List of Threatened Animals. IUCN, Gland, Switzerland/Cambridge, UK.

Killeen, I. J., Oliver, P. G., Fowles, A. P., 1998. The loss of a freshwater pearl mussel (*Margaritifera margaritifera*) population in NW. Wales. In: Killeen, I., Seddon, M.B., Holmes, A.M. (Eds.), Journal of Conchology Special Publication No. 2, June 1998, pp. 245–250.

Le Cren, E.D., 1965. A note on the history of mark–recapture population estimates. Journal of Animal Ecology 34, 453–454.

Lefevre, G., Curtis, W.C., 1912. Studies on the reproduction and artificial propagation of freshwater mussels. Bulletin of the Bureau of Fisheries 30, 105–201.

Lincoln, F.C., 1930. Calculating waterfold abundance on the basis of banding returns. USDA Circular 118, 1–4.

Miller, A.C., Payne, B.S., 1988. The need for quantitative sampling to characterise size demography and density of freshwater mussel communities. American Malacological Bulletin 6, 49–54.

Moorkens, E.A., Costello, M.J., 1994. Imminent extenuation of the Nore freshwater pearl mussel *Margaritifera durrovensis* Phillips: a species unique to Ireland. Aquatic Conservation: Marine and Freshwater Ecosystems 4, 363–365.

Neves, R.J., Widlak, J.C., 1987. Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. American Malacological Bulletin 5 (1), 1–7.

Phillips, R.A., 1928. On *Margaritifera durrovensis*, a new species of pearl mussel from Ireland. Proceedings of the Malacological Society of London 18, 69–73.

Preece, R.C., Burleigh, R., Kerney, M.P., Jarzembowski, E.A., 1983. Radiocarbon age determination of fossil *Margaritifera auricularia* (Spengler) from the River Thames in West London. Journal of Archaeological Science 10, 249–257.

Ramos, M.A., 1998. Implementing the Habitats Directive for mollusc species in Spain. In: Killeen, I., Seddon, M.B., Holmes, A.N. (Eds.), Journal of Conchology Special Publication No. 2, June 1998, pp. 125–132.

Richardson, T.D., Yokley Jr, P., 1996. A note on sampling technique and evidence of recruitment in freshwater mussels (Unionidae). Archiv für Hydrobiologie 137, 135–140.

Smith, D.G., 1979. Marsupial anatomy of the demibranch of *Margaritifera margaritifera* (Lin.) in Northeastern North America (Pelecypoda: Unionacea). Journal of Molluscan Studies 45, 39–44.

Sostoa, A., Lobón-Cerviá, J., 1989. Fish and fisheries of the River Ebro: actual state and recent history. In: Petts, G.E. (Ed.), Historical Change of Large Alluvial Rivers: Western Europe. John Wiley and Sons Ltd., pp. 233–247.

Valovirta, I., 1995. Modelling the occurrence of the freshwater pearl mussel *Margaritifera margaritifera* (L.) by environmental data. 12th Intern. Malcological Congress, Vigo, 1995, pp. 535–537.

Valovirta, I., 1998. Conservation methods for populations of *Margaritifera margaritifera* (L.) in Finland. In: Killeen, I., Seddon, M.B., Holmes, A.M. (Eds.), Journal of Conchology Special Publication No. 2, June 1998, pp.251–256.

Vaughn, C.C., Pyron, M., 1995. Population ecology of the endangered Ouachita rock-pocketbook mussel, *Arkansia wheeleri* (Bivalvia: Unionidae), in the Kiamichi River, Oklahoma. American Malacological Bulletin 11 (2), 145–151.

Yeager, M.M., Cherry, D.S., Neves, R.J., 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). Journal of the North American Benthological Society 13 (2), 217–222.

Ziuganov, V., Zotin, A., Nezlin, L., Tretiakov, V., 1994. The Freshwater Pearl Mussels and their Relationships with Salmonid Fish. VNIRO Publishing House, Moscow.