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Araujo
1998

Madrid, 2, Oct., 1998

Dear Dick,
here I send you our manuscript for revision. Thank you very much for your time. The photographs are paper copies of the slides (good slides) that I want to send to the journal in case you think that the manuscript is worth publishing in *Conservation Biology*..

Other things: the trip to Kola Peninsula was fantastic. I really enjoyed the taiga, the river, the canoes and catamarans and the people. Fifteen nice days in the wild. The populations of salmons and mussels are really big in these rivers. Easy to collect juveniles of *Margaritifera margaritifera*. During the trip Valerij told me about your stay there. In several campaments I think I put my tent in the same place than you did.

Best regards to your wife. And to Braven and Bill, what about their progress?
So, thank you very much again. I hope to see you soon.
Yours,

RAFAEL ARAUJO

Rafael Araujo

CONSERVATION OF THE RELICT GIANT EUROPEAN FRESHWATER PEARL MUSSEL *MARGARITIFERA AURICULARIA*

Short title: Conservation of *Margaritifera auricularia*

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Abstract: The ~~distribution area~~ of the freshwater naiad *Margaritifera auricularia* has declined alarmingly since the beginning of the century. The last records of live ~~mussels~~ date from 1917, and the species is considered to be one of the most threatened in the world. ~~Thanks to the~~ recent finding of a live population in the basin of the Ebro River (Spain) ~~we can~~ describe, for the first time, ~~some~~ characteristics of its habitat, biology, breeding cycle and host fish, ~~for its larval stage (glochidia), basic data for applying conservation measures for this relict species. For the first time~~ metamorphosed juveniles ~~recently released~~ from ~~the branchia of an exotic species of~~ sturgeon ~~are reported~~, ~~which indicates that culture and artificial propagation of this species is now a reality~~. In this paper we examine the history of the species and discuss national and international efforts to conserve it and boost its numbers and those that need to be urgently implemented now that this population, one of the last in the world, has been discovered.

Resumen: El área de distribución de la náyade perlífera gigante de agua dulce *Margaritifera auricularia* ha disminuido de forma alarmante desde el principio de nuestro siglo, de forma que los últimos registros de animales vivos datan de 1917. La especie está considerada como uno de los animales más amenazados del mundo. Gracias al reciente descubrimiento de una población viva en la cuenca del Río Ebro (España) podemos describir, por primera vez, las características de su hábitat, biología, ciclo reproductivo y pez hospedador de su fase larvaria (gloquidio), conocimientos fundamentales a la hora de aplicar medidas de conservación para esta especie relicta. Se han obtenido por primera vez ejemplares juveniles recién liberados de las branquias de una especie exótica de esturión, lo que indica que el cultivo y propagación

artificial de esta especie es ya una realidad. En este trabajo se examina la historia de la especie y se discute sobre los esfuerzos nacionales e internacionales para conservarla y restaurar sus efectivos, así como sobre los que se deben llevar a cabo con urgencia una vez que esta población, una de las últimas del mundo, ha sido descubierta.

Introduction

Margaritifera auricularia (Figure 1) belongs to one of the oldest genera among the naiads, superfamily Unionoidea, the freshwater mussels. Historically, species of the genus *Margaritifera*, known as pearl mussels, have been the object of rather intensive exploitation for their nacre and pearls. Three species are known to occur in Europe: *M. margaritifera* (Linnaeus 1758), *M. auricularia* (Spengler 1793) and *M. durrovensis* Phillips 1928. The specific or subspecific status of the last taxon is discussed in relation to *M. margaritifera* (Phillips 1928; Chesney, Oliver & Davis 1993; Moorkens & Costello 1994). Therefore, only the first two names appear on the list of wildlife species under the Council of Europe's Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention 1979), and the European Union Directive on the Conservation of Natural and Semi-Natural Habitats and of Wild Fauna and Flora (Directive 92/43/EEC, Habitats Directive), as well as on the International Union for the Conservation of Nature and Natural Resources Red Data List (IUCN 1990). *M. auricularia*, the giant European freshwater pearl mussel, is considered to be one of the most threatened animal species in the world.

The extraordinary decline experienced by ~~naiad~~ populations ~~over~~ the last fifty years, ~~as well as their interesting ecology and life cycles,~~ have attracted much attention and concern in recent years ~~in the most diverse~~ conservation ~~forums~~. During this century, *M. margaritifera* has ~~become extinct over~~ extensive ~~areas~~ of Central Europe (Buddensiek 1995) and is now a priority subject for study in many countries. ~~A long list of references~~ dealing with this species ~~is~~ now available (Bauer & Eicke 1986; Bauer 1986 and references therein; Bauer & Vogel 1987; Bauer 1987a,b; Hruska 1992; Chesney et al. 1993; Ziuganov et al. 1994; Valovirta 1995, among others) ~~and active research is still in progress.~~ ~~On the contrary,~~ the only published ~~succinct~~ data on *M. auricularia* ~~biology came from~~ a Spanish population (Haas 1916a, b; 1917a). Since then, no living specimens of this species have been reported, either from Western Europe, the species' former range (Iberian Peninsula, France, Italy, England, Germany) or from rivers in Morocco, where a local race probably occurs (Haas 1940). Only Azpeitia (1933) cited some specimens that were probably collected after 1917, ~~also in~~ the Ebro basin. He also cited one specimen from Toledo (Tajo River) that is ~~stored~~ in the collection of the Museo Nacional de Ciencias Naturales (Madrid). ~~Data~~ on the past distribution of the species in Europe (Czechoslovakia, Germany, ~~and~~ England) from the fossil record ~~are~~ recorded in Preece et al. (1983).

Thanks to Haas ~~above mentioned papers,~~ we know that the species was very abundant in the Ebro River, where the nacre of the empty valves was collected to manufacture knife hilts. At the beginning of this century, there was a small factory in Sástago (Zaragoza, Spain), where the shells were carefully cleaned and buried for months in a hole ~~in the~~

~~ground~~ covered by wet sand. This was the usual way to keep them useable for up to 40 years. The best nacre was obtained from the thick anterior part and middle part of the shell (Haas 1917a).

~~Haas's research provided~~ A brief description of the anatomy of *M. auricularia* (Haas 1924), but no ~~data~~ information on reproductive strategy, breeding season and larval morphology has been reported. ~~He was only able to~~ Haas (1924) noted that specimens were not gravid between mid-July and the beginning of September, and that they lived in the Ebro on a 70-120 ~~m~~ meter-wide section of the river between stones and boulders with a predilection for deep bottoms (5-7 m), as well as in the Imperial Channel ("Canal Imperial"), an ancient channel of the Ebro (Haas 1916a, b; 1917a). ~~He also mentioned the rarification of the species in recent years~~.

In 1990, Altaba reported the presence of a few empty valves of *M. auricularia* in an irrigation channel of the Ebro River near the estuary in Tarragona (Spain), proposing several conservation measures ~~(see below)~~ ~~should~~ live specimens were ever found. ~~Hopefully~~, during a study carried out in 1996 to inventory some Habitats Directive invertebrates living in Spain (Ramos 1998), a population of *M. auricularia* was "re-discovered" in the Imperial Channel of the Ebro River (Araujo & Ramos 1996a, b, 1998). Since then studies have been conducted on the ecology, population size and age, and reproductive strategies of the species. More recently, there has been news of other *M. auricularia* populations living in the main course of the Ebro River and in one of its irrigation channels (Altaba 1997), both in Catalonia. Other news concerning *M. auricularia* populations in the Guadiana River are unconfirmed. ~~plausible~~. As regards an old record for *Unio sinuatus* (= *M. auricularia*) in the Guadalquivir River, no endemic ~~real data~~ exist beyond the "unknown specimen" (Haas 1917b) cited by Bourguignat (1866). In fact, the

sampling we carried out in both rivers detected some naiad populations other than *Margaritifera* spp.

The dramatic decline of *M. auricularia* in Europe has run parallel to that of some North American mussels. Bogan (1993) described the reasons for its extinction as habitat alteration or destruction, decline or extinction of host fishes, commercial exploitation, and introduced species.

This paper deals with both a description of the habitat of the recently discovered population of *M. auricularia* in the Imperial Channel and with the results obtained in an indoor experiment on its biology and reproductive cycle, which are essential for the application of conservation measures. It also summarizes the legal conservation status of the species, and Spain's efforts to implement international recommendations. Finally, we discuss the national and international efforts to conserve and restore the species, and those that must be taken urgently now following the discovery of this population, which is probably one of the last of the species in the world.

International efforts to protect *M. auricularia*

In the background information on invertebrates of the Habitats Directive and the Bern Convention, the status of *M. auricularia* is reported as: "at least vulnerable, probably extinct throughout most of its range" (Woodward 1996, Council of Europe). The species has been listed on Appendix IV of the EEC Habitats Directive, which includes animal and plant species of European interest requiring strict protection.

In the item on conservation, the same document (Woodward 1996) says that "the biology of the species remains unknown until such time as any surviving populations can be located. In the interim, every effort should

be made to ensure that any historical sites, such as the Ebro basin, should undergo the least development or modification as possible until a full survey of the area has been undertaken". This document also recommends: 1) Urgent distribution surveys to determine the location and status of any surviving populations, throughout its former range, but particularly in France, Italy and Spain; 2) Measures to ensure that all sites found to contain surviving populations must receive immediate and adequate protection; 3) A study of the lifecycle of *M. auricularia*; and 4) To determine habitat requirements and to formulate a captive breeding programme.

Following these recommendations endorsed by the Bern Convention Standing Committee (Council of Europe 1996), Spain undertook a research project to investigate the presence of live populations, in order to contribute to knowledge of the species distribution and biology as a preliminary step towards designing a complete plan for its conservation and restoration. In this regard, we had the opportunity to find the species in a well-preserved channel of the Ebro River. In the near future, efforts should be directed at surveying the main course of the river. Studies currently in progress are concentrating on some biological aspects.

Peculiar reproductive behaviour

All freshwater mussels share a complex way of life because they require a vertebrate host, usually a fish, during their larval stage. This microscopic thin-shelled larva (glochidium) that the mussels brood and release by the millions was interpreted in the nineteenth century as a parasite species of fishes (*Glochidium parasiticum*), different from any

~~other mussel species.~~ This ~~larva~~ ~~normally~~ has hooks ~~and/or teeth~~ to attach itself to the fish's body (fins or gills), where it encapsulates ~~and~~ ~~spends~~ several weeks as a temporary ectoparasite, ~~completing its development~~ until its recruitment ~~as a metamorphosed benthic~~ juvenile. ~~This is the mussel's dispersal method.~~

Knowledge of the relationship between mussel and host fish is essential ~~to~~ any attempt to preserve endangered freshwater ~~bivalve species.~~

Altaba (1990) hypothesized about the possible specificity between the glochidium of *M. auricularia* and the ~~Western~~ European sturgeon *Acipenser sturio* (Linnaeus 1758), a relict fish in European rivers and practically extinct in Spain (Elvira et al. 1991; Blanco & González 1992). Both species occur together in Pleistocene deposits (Preece 1988), and both have been declining since the first half of this century.

The glochidium of *M. auricularia* was recently described by Araujo & Ramos (1998).

Material and Methods

The study area

The Imperial Channel of Aragón was built in the eighteenth century and runs parallel to the Ebro river through the Spanish provinces of Navarra and Zaragoza (Fig. 2). It belongs to the Ebro river basin and flows across a quaternary bed over 115 km, the last 25 km being a narrow concrete ditch 1-2 m wide. This area is regularly dredged and cleaned. The main part of the channel is 10 m width and about 3.5 m depth when it is full. When empty, the water layer is around 40-100 cm depth. ~~The stream speed is~~ 0.6 m/sec. ~~It has~~ no shore vegetation except some trees and

scattered patches of *Typha* sp. No aquatic vegetation ~~is found along~~ occurs in the channel.

Methods

The Imperial Channel (Fig. 2) was surveyed ~~from start to end when it was nearly empty~~ completely at low flow, with special emphasis ~~on the areas~~ in reaches whose bed has not been artificially covered. Mussel sampling was ~~made~~ conducted by looking for tracks and shells, using a bathiscope watiscope in deeper areas. Samples taken with a dredge what kind? when the channel was full of water did not collect specimens in areas ~~where they do actually live~~ of known occurrence. In order to estimate the age and density of the *M. auricularia* population, collected specimens were measured (length, width and height), marked, released at the site and recaptured ~~(the exact sites where the species currently lives cannot be published for obvious reasons)~~.

Six areas in the channel were successfully sampled:

- Area 1: a 100 m transect was surveyed on 28 Feb.1997 and on 24 Nov. 1997.
- Area 2: a 2 Km transect was surveyed on 20 Feb. 98.
- Area 3: 12 transects of known length were made along 150 m on 4. Dec. 1996. These transects were sampled again on 26 Feb. 1997.
- Area 4: a 200 m transect was sampled on 14 Feb.1996 and on 4 Dec.1996.
- Area 5: a 200 m transect was surveyed on 3 Dec. 1996.
- Area 6: a 1 ~~Kilometre~~ Km transect was sampled on 28 Nov. 1997.

The simple Lincoln index (Lincoln 1930) was used to estimate population densities, ~~as the population size is expected to be fixed without gains and losses during the experiment~~. According to this index, Nn/Nc=Nm/Np, where Nm= number of specimens marked, Nc= number of specimens collected, Nn= number of specimens marked at Nc, and Np= total population

Specimen track width of the four Unionoid species living in the channel was also measured between the peaks of the track, the section of which is more or less triangular.

In order to ~~discover~~ specimen age, we used the technique of thin-sectioning of valves, which is ~~highly~~ recommended by Neves & Moyer (1988) ~~for~~ Unionoids. Three old empty valves representing three specimen sizes (~~the biggest~~, ~~the medium~~ and ~~the~~ smallest found) and one recently dead specimen were sectioned and the annuli counted. In order to validate age estimates, complementary valves of those ~~that had been~~ thin-sectioned were submerged in aqueous KOH solution and the external shell rings were compared ~~with~~ those observed in thin-sections. With these data, we ~~tried to~~ extrapolate the age of living specimens according to their lengths.

The algae species in the channel water column and in sediment samples taken when the water level was low in Area 3 (the richest in *M. auricularia*) were studied. Samples were studied both *in vivo* and fixed in lugol and formol.

Repeated physico-chemical measurements of the water were ~~monitored~~ in Area 3 ~~with~~ low and full water levels. Other water quality analyses were made at ~~the zero~~ ~~kilometre~~ and 30 km downstream of Area 3. All

You talk about algae and water samples taken but don't say what you did with them. What analyses?

these analyses were kindly provided by the Confederación Hidrográfica del Ebro. What was done with them?

use abbreviations for all units of measure

Eight specimens of *M. auricularia* were maintained in an aquarium containing 120 ~~litres~~ of water and 10 cm of sediment, both from Area 4. Several days later, 11 specimens of the sturgeon species *Acipenser* cf. *baeri* Brandt 1869, ~~(one 20 cm and the rest 10 cm long)~~ 10-20 cm in length were ~~added to~~ 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 nutritional diet for fine-filter feeders (Advanced Invertebrate 1 Formula. Marine Enterprises, Inc.). The fishes were fed with Tetra Diskus Futter (Tetra) and red mosquito larvae. After infection with glochidia, the sturgeons were regularly removed from the aquarium and anaesthetised with MS222. Filament gills were excised and observed under a SZH10 Olympus stereomicroscope.

In a second experiment, five sturgeons infected with *M. auricularia* glochidia were isolated in an aquarium (water temperature between 23-24 °C) without sediment 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 two days in order to recover ~~any released~~ metamorphosed juveniles.

Observations on the Spanish population of *M. auricularia*

Natural habitat

Specimens of *M. auricularia* were only detected in six transects of the channel, being absent from all the areas with artificially covered beds and many of the areas with natural beds. The species coexists with a very rich community of *P. littoralis* (Lamarck), *U. elongatulus* C. Pfeiffer and *A. cygnea* (Linnaeus), which inhabit most of the channel at different densities.

M. auricularia lives partially buried in clay-sand and gravel beds, sometimes (especially in the breeding season) nearly vertical with the hind portion exposed and sometimes horizontal, showing only the umbonal and ligament area. The tracks these bivalves leave (Figure 3) in the sediment are very wide (X=7.97 cm; sd=1.12; n=123) and easily distinguishable from those of other mussels: *U. elongatulus* (X=3,61; sd=1.34; n=53), *P. littoralis* (X=4.5; sd=0; n=2), *A. cygnea* (X=4,8; sd=1.24; n=17), although the largest specimens of *A. cygnea* can leave similar tracks to those of *M. auricularia.*

The number of live *M. auricularia* specimens estimated for the study transects is shown in Table 4. It is not the total number of live specimens because the species probably still exists in other unexplored areas. Indeed, the selected transects do not represent the whole area occupied by the mussels.

In Area 3, we found one specimen which, without any doubt, came from Area 4, two kilometres downstream. All the collected specimens were above 13 cm except one measuring 10 cm. Thin sectioning and reading the rings in valves did not yield accurate results about age, as it was impossible to correlate a specific length with age. The population structure, based on length measurements, is represented in Figure 4.

You cite Table 4 before Table 1-3

The algae species identified in the Imperial Channel are shown in Table 1. In both ~~free~~ water and sediment samples, the main species ~~are~~ of diatoms, which were much more abundant in the sediment. All ~~of them~~ are benthic algae ~~belonging to~~ occurring in alkaline and mineralized waters.

Data on water quality in the channel at zero km and 30 km downstream of Area 3 are given in Table 2. Physico-chemical values of the water in Area 3 are in Table 3.

You give this kind of what value is it? No description of the results!

Aquarium results

As soon as the *M. auricularia* specimens were ~~put~~ placed into the aquarium, they began to release white masses of eggs and developing embryos, starting the true emission of mature glochidia after 7 days ~~after the mussels had been placed~~ and 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 ~~hanging~~ laying over the female mussel ~~shell~~ (Figure 5) until they were ingested ~~and inhaled~~ by the ~~fishes~~ Hungerford. One day after emission of the glochidia, they were observed attached to ~~the fishes'~~ gill filaments. Glochidia Expulsion and ~~other~~ immature ~~developmental~~ embryonic stages lasted approximately 35 days, with a lapse of ~~five~~ 5 days without emission in the middle of the spawning period and with a marked peak of glochidia release on the 28th day. The spawning of the eight specimens was synchronous.

Four infected fishes were removed from the aquarium, anaesthetized, ~~killed~~ sacrificed and examined 5, 13, 34 and 60 days after infection, respectively. The first three had gill filaments packed with glochidia , but glochidia were absent ~~from the latter~~ at 60 days.

Two other parasitized fishes were removed 8 days after infection and transferred to a small 3 litre aquarium without sediment, in order to monitor glochidia development and recover the possible juvenile mussels released. After six days in these conditions (temperature 18-19°C), no glochidia were observed in the fishes gills. A regular survey of the bottom of this aquarium with a suction pump revealed several empty glochidial shells. These two fishes were reintroduced into the aquarium with the mussels being reinfected over a period of seven days. The glochidia encapsulated on all the gill filaments and over their entire length (Fig. 6A). Thirty-four days after infection, encapsulated glochidia became spherical in shape (Fig. 6B) when compared with those from the two former fishes (5 and 13 days, respectively), but they did not increase in length.

Ten days after the last sturgeon was killed, the remaining fishes were removed from the aquarium. Since then, several bottom samples have been examined and some empty glochidial shells were found, but no juvenile mussels were detected even after all the aquarium sediment was examined.

Exactly one month after the five sturgeons were infested in the second experiment, we found 15 live juveniles (Fig. 7A) and many empty juvenile shells (Fig. 7B) in aquarium bottom without substrate. Only a few empty juvenile shells were found in the following 2 and 4 days, and no encysted glochidia were found on the surviving sturgeons. Shell measurements of the newborn were: length 190µm; height 187 µm; and width 225 µm.

We are currently studying samples fixed either in bouin's fluid (for histology) or in glutaraldehidum (for scanning electron microscopy) of

all the fish gill filaments with encysted glochidia and of the juveniles in order to monitor the metamorphosis of the mussel larva.

Discussion

Natural History

The relict population described above is a particularly healthy and fertile one, with mussels that are probably survivors of a very big community thriving in the main river bed of the Ebro. That explains why the channel probably does not represent the natural (ancient) habitat of the species in all its distribution range.

M. auricularia in the Imperial Channel seems to be a very sedentary species, as suggested by a comparison of the results of our capture-recapture experiment in the different areas where the species lives. We assume that the panmictic area for these mussels is very reduced 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 & Downing (1997) for *Elliptio complanata* (Lightfoot). The case of the only specimen recaptured two kilometres upstream of the site where it was released might be explained by an artificial translocation.

M. auricularia can be defined as a short-term brooder (tachytictic) (Araujo, Bragado & Ramos submitted). Our results are supported by those of Haas (1917a), who demonstrated that the species does not incubate between mid-July and the beginning of September. Although in the Lydeard, Mulvey & Davis (1996) paper the *Margaritiferidae*

species are reported as bradytictic, 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 1996 had their four gills full of embryos which developed in the glochidia released one week later and until March 22, with a peak on March 14. As regards the sex ratio of this population, our results suggest that there is a high proportion of hermaphrodites. This idea derives from the fact that all aquarium specimens released glochidia. Histological studies of the gonad of *M. auricularia* specimens will answer this question more accurately.

As regards larval transformation, our results show that the sturgeon is a good candidate as specific host of the glochidium of *M. auricularia*, as was previously suggested (Altaba 1990), without experimental data. In our experiments, glochidia underwent metamorphosis on the gills of the sturgeon fishes in the aquarium, and juveniles were released after 30 days at 23-24 °C (690 days/degrees). 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 present a near spherical shape produced by the addition of a very thin edge of shell material around the old glochidial shell. *Acipenser sturio*, probably the only species of this group occurring naturally in Spain, was exploited until the mid-1960s in the Ebro River, but catches declined following the construction of reservoirs (Sostoa & Lobón-Cerviá 1989). The absence of *A. sturio* specimens in Spain, and especially in the Ebro basin for many years may be one of the reasons for the decline of *M. auricularia*.

Our results indicate that *A.* cf. *baeri* is a good species to be used as ~~an ex situ~~ fish host if a recovery plan for the species ~~were needed~~. Moreover, it seems that reinfected fish specimens do not develop acquired immunity against glochidial infection.

Conservation Measures

The unexpected discovery of this *M. auricularia* population in Spain and ~~the first~~ results ~~of this paper~~ revive interest in the species and pose new and puzzling questions ~~about it.~~ On the one hand, ~~it is true that, compared to adults,~~ unionoid juveniles are ~~hardly~~ collected because of ~~the hidden~~ and different habitat ~~they inhabit~~ (Isely 1911; Hudson & Isom 1984; Neves & Widlak 1987; Miller & Payne 1988; Buddensiek 1995; Richardson & Yokley 1996), ~~which is still unknown for most unionoid species and particularly the margaritiferids~~. Only Buddensiek et al. (1990) and Buddensiek & Ratzbor (1995) studied the chemistry of interstitial water of bivalve habitats, with emphasis on the juvenile stages of *M. margaritifera*. Data ~~about~~ feeding and burrowing behaviour of juvenile unionids reported by Yeager, Cherry & Neves (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 ~~both~~ that 1) the specific host fish has not lived in the channel or in the river for many years and/or 2) features like ~~bottom~~ or water quality do not allow juveniles to settle and survive. Nevertheless, the ~~luxuriant~~ abundance of the other three species of mussels in the channel, mainly in its first half, clearly suggests that the special conditions of this old channel (mainly water quality and bottom composition) are among the best for a healthy naiad community. *In situ*

observations suggest that the species should be more common, as are the other related ~~genera~~ species in the study area.

Evaluation of the possibility of other fish species ~~other than those analysed here~~ being parasitized by its glochidia, and of ~~the success of this unusual cycle~~ recruitment in the wild is pending, although experiments with local fishes in ~~the~~ a aquarium and electric-fishing in the Imperial Channel are ~~currently in process~~ underway. These ~~results~~ studies are essential for subsequent application of species reintroduction policies.

After the dead specimens of *M. auricularia* were found near the Ebro estuary in 1990, a ~~series~~ set of recommendations for the effective protection of *M. auricularia* and other unionoids in the Ebro Delta Natural Park were proposed (Altaba 1990) ~~as follows:~~ 1) Restricted collecting, 2) Research, 3) Protection of fish hosts. 4) Protection of key habitats, 5) Education, 6) Establishment of captive breeding colonies, 7) Repopulation, and 8) Water quality control.

Points 6 and 7 are of primary importance, but as the ~~same~~ author realised, we need to increase our knowledge of *M. auricularia* ecology prior to carrying out these actions. ~~Now that we have managed to acquire this~~ Without this knowledge, it is time to ~~work on these ideas~~. Implement a recovery program.

First ~~of all~~, the newly discovered breeding population, threatened by plans to cover the channel bed where it lives, 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 dredging of the Ebro river bottom near the estuary to make the river navigable and the construction of several dams are ~~also~~ pending threats to the Catalonian population (Altaba 1997).

Is the species successfully reproducing and recruiting juveniles to the population?

In the case of the channel ~~in question~~, the best ~~solution from recreational and cultural points of view~~ is to stop the proposed work and maintain the channel in an "organic" state.

~~In any case,~~ It is ~~necessary~~ to increase efforts ~~to~~ survey the main course of the river ~~with the aim of~~ discovering new breeding colonies or ~~stocks~~ of juveniles ~~in order to confirm whether the species conserves the reproductive potential to complete larval maturation in natural conditions~~. This is not an easy task due to the river's characteristics, ~~and~~ it would ~~probably~~ be necessary for scuba divers to survey different transects and to employ special dredging methods.

Simultaneously, and with the available information on the breeding season and reproductive strategies under study, a ~~macro~~ experiment close to the natural habitat of the species ~~needs to~~ be designed to investigate different kinds of substrates suitable for adults and juveniles, ~~as well as~~ fish species that are likely to host the parasitic larval stage in order to carry out "in situ" and "ex situ" breeding experiments, including artificial infestation of host fishes. This information is preliminary step to any plans for species recovery or restocking of former habitats. Similar experiments on artificial propagation of freshwater mussels were carried out successfully many years ago in the Mississippi River (Lefevre & Curtis 1921) and currently in several North American rivers (Neves pers. com.).

After the rediscovery of *M. auricularia* 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 the ~~species~~ on the National Endangered Species List (Royal Decree 439/90) in the category of "threatened with extinction", ~~being the first invertebrate species on this list~~. Furthermore, the presentation of this

discovery at the "Colloquium on Conservation, Management and Restoration of Habitats for Invertebrates" and at the "Meeting of the Group of Experts on Invertebrate Conservation" of the Bern Convention (Killarney, Ireland, 26-29 May, 1996) (Araujo & Ramos 1996a) led to new recommendations approved by the Standing Committee of the Convention, including most of the measures listed above and also the suggestion to include the species on Appendix II of the Habitats Directive among the species group for which the designation of special conservation areas in the European Community is needed (Council of Europe 1996).

Old reports like that of Coker et al. (1921) and more recent research on endangered unionids (Bruenderman & Neves 1993; Hove & Neves 1994; Vaughn & Pyron 1995) are essential to an understanding of the natural history of freshwater mussels, but essential information about feeding habits, bottom, depth, ~~light and~~ current of the water bodies suitable for these molluscs, especially species of the genus *Margaritifera,* are still unknown. This is further proof of the contingencies prevailing through out the life of naiads, particularly due to their parasitic stage on the body of an extremely active host like fishes.

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Literature Cited

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

Araujo, R., and M. A. Ramos. 1996a. *Margaritifera auricularia* re-discovered in Spain: observations on a relict population. Convention on the Conservation of European Wildlife and Natural Habitats. Conservation, Management & Restoration of habitats for invertebrates: enhancing biological diversity. Killarney (Ireland) 27-28 May. pp ?

Araujo, R., and M. A. Ramos. 1996b. The last living population of *Margaritifera auricularia* (Spengler, 1783). Molluscan Conservation. A strategy for the 21st Century. National Museum & Gallery, Cardiff (Wales, Great Britain) 20-22 November. pp ?

Araujo, R., and M. A. Ramos. 1998. Description of the glochidium of *Margaritifera auricularia* (Spengler, 1793) (Bivalvia, Unionoidea). Philosophical Transactions of The Royal Society of London B. **353**:1-7.

Araujo, R., D. Bragado., and M. A. Ramos. Occurrence of glochidia of *Margaritifera auricularia* (Spengler, 1793) and other mussel species (Bivalvia: Unionoida) in drift and on fishes in an ancient channel of the Ebro River, Spain. Freshwater Biology. Submitted.

Azpeitia, F. 1933. Conchas bivalvas de agua dulce de España y Portugal. Memorias del Instituto Geológico y Minero de España. Madrid. Vol. 1, 458 pp.

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. 1987a. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) II. Susceptibility of brown trout. Archiv für Hydrobiologie, Supplement 76 **4**:403-412.

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

Bauer, G. 1992. Variation in the life span and size of the freshwater pearl mussel. Journal of Animal Ecology **61**:425-436.

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

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

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

Blanco, J. C., and J. L. González. (eds.). 1992. Libro Rojo de los Vertebrados de España. ICONA Colección Técnica, Madrid:113-114.

Bogan, A. E. 1993. Freshwater Bivalve Extinctions (Mollusca: Unionoida): A Search for Causes. American Zoologist **33**:599-609.

Bourguignat, M. J. R. 1866. Mollusques noveaux, litigieux ou peu connus. Revue de Zoologie 2nd. Ser. **18**:6-23.

Bruenderman, S. A., and R. J. Neves. 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., H. Engel., S. Fleischauer-Rössing., S. Olbrich., and K. Wächtler. 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.

Buddensiek, V. and G. Ratzbor. 1995. Restoration of sedimental quality in a small brook of the Lüneburger heide, Northern Germany. Folia Fac. Sci. Nat. Univ. Masarykianae Brunensis, Biologia **91**:19-24.

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

Council of Europe. 1996. Draft Recommendations on the Conservation of *Margaritifera auricularia*. In: Group of Experts on Conservation of Invertebrates. Secretariat Memorandum Appendix 4. T-PVS (96) 33: 66-67.

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

Elvira, B., A. Almodóvar., and J. Lobón-Cerviá. 1991. Recorded distribution of sturgeon (*Acipenser sturio* L., 1758) on the Iberian Peninsula and actual status in Spanish waters. Archiv für Hydrobiologie **121**(2):253-258.

Haas, F. 1916a. 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. 1916b. Spanischer Brief III. Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft **48**(32-44).

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

Haas, F. 1917b. Estudio para una monografía de las náyades de la Península Ibérica. Anuari de la Junta de Ciencias Naturales **2**:131-190.

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

Haas, F. 1940. A tentative classification of the Palearctic Unionids. Field Museum of Natural History, Zoological Series **24**:115-141.

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

Hove, M. C., and R. J. Neves. 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., and B. G. Isom. 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. 1990. 1990 IUCN Red List of Threatened Animals. IUCN, Gland, Switzerland and Cambridge, UK, 228 pp.

Lefevre, G., and W. C. Curtis. 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.

Lydeard, Ch., M. Mulvey., and G. M. Davis. 1996. Molecular systematics and evolution of reproductive traits of North American freshwater unionacean mussels (Mollusca: Bivalvia) as inferred from 16S rRNA gene sequences. Philosophical Transactions of the Royal Society of London Series B **351**:1593-1603.

Miller, A. C., and B. S. Payne. 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., and M. J. Costello. 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., and J. C. Widlak. 1987. Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. American Malacological Bulletin **5**(1):1-7.

Neves, R. J., and S. N. Moyer. 1988. Evaluation of techniques for age determination of freshwater mussels (Unionidae). American Malacological Bulletin **6**:179-188.

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. 1988. A second British interglaciar record of *Margaritifera auricularia*. Journal of Conchology **33**(1):50-51.

Preece, R. C., R. Burleigh., M. P. Kerney., and E. A. Jarzembowski. 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. Journal of Conchology. Special Publication No 2: 125-132.

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

Sostoa, A., and J. Lobón-Cerviá. 1989. Fish and fisheries of the River Ebro: actual state and recent history. Pages 233-247 in G. E. Petts, editor. Historical Change of Large Alluvial Rivers: Western Europe. John Wiley & Sons Ltd.

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.

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

Vaughn, C. C., and M. Pyron. 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.

Woodward, F. R. 1996. *Margaritifera auricularia* (Spengler, 1793). In: Background Information on Invertebrates of the Habitats Directive and the Bern Convention, III. van Helsdingen, P. J.; Willemse, L. & Speight, M. C. D. eds. Nature and Environment (Council of Europe), **81**:495-500.

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

Ziuganov, V., A. Zotin., L. Nezlin., and V. Tretiakov. 1994. The freshwater pearl mussels and their relationships with salmonid fish. VNIRO Publishing House, Moscow, 104 pp.