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A Simple and InexpensiveTechnique for the *Ex Situ* Reproduction of Critically Endangered Cyprinids – *Achondrostoma occidentale* as a Case Study

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Ex situ reproduction of species in risk of extinction has been recognized by several authors (e.g., Gipps 1991; Maitland and Lyle 1992; Xia et al. 2005; Gayton 2008) as a solution for populations whose survival is threatened by an extremely low number of individuals and severe habitat degradation. Under such scenarios, although it may be difficult to preserve the original genetic diversity of the species, *ex situ* reproduction seems to be the only solution to avoid the complete extirpation of a species or population, by preserving their last representatives until their original habitats are restored (Maitland and Morgan 2002).

When establishing an *ex situ* reproduction program, it is necessary to strike a balance between several conflicting demands. On one hand, it is necessary to retain maximum genetic diversity within the population whereas, on the other hand, space, human resources, and funding often are limited. Finally, culturing techniques employed must not negatively affect the behavior of the species which could lead to unintended artificial selection caused by hatchery conditions. Thus, planning aquaculture schemes to breed endangered species imposes a serious challenge for fish conservation.

In this note, we describe a very simple, low cost protocol to breed the critically endangered cyprinid *Achondrostoma occidentale* (Robalo, Almada, Sousa-Santos, Moreira, and Doadrio). With minimal adjustments, this protocol may be applicable to other small freshwater fish.

The Portuguese freshwater fish fauna comprises at least 23 cyprinid species, seven of which are endemic to Portugal and are critically endangered, and the remainder endemic to the Iberian Peninsula (Cabral et al. 2005). The persistence of these species is highly unstable because they inhabit small Mediterranean-type streams that are subjected to high levels of pollution and habitat degradation (Collares-Pereira and Cowx 2004). Moreover, in the last decade, as a result of global warming, these rivers are experiencing summer droughts that clearly are more extended in time and space (Lehner et al. 2006).

The recently described small cyprinid *A. occidentale* (Robalo et al. 2005) occurs in only three small, degraded coastal drainages located north of Lisbon (Sizandro, Alcabrichel and Safarujo Rivers), and exemplifies a case of eminent extinction.

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Materials and Methods

In this pilot study, the population of the critically endangered species *A. occidentale* from the Alcabrichel River was selected for the *ex situ* reproduction as it was clear from the field work that, since the first collection of individuals in 2001, the habitat conditions were more degraded. Indeed, levels of pollution (mainly from industry and sewage discharges) and water scarcity (because of intense summer droughts and illegal water abstraction for agriculture) have increased.

A group of 16 adult *A. occidentale* (unknown sex-ratio) was electrofished and transported to a 1250-L outdoor concrete tank located at the Vasco da Gama Aquarium's facilities in April 2006. One year later, fish were moved to the breeding tank: a larger outdoor 3000 L, epoxy resin coated, concrete tank (500 × 195 × 32 cm). The system was open and sourced by the Lisbon city water supply system. The tank had constant aeration and constant flow – see scheme in Figure 1 – and 25% of the tank was covered by a net to provide shade (Fig. 1). Water temperature was not regulated. As *Iberochondrostoma lusitanicum* (Collares-Pereira 1980), a close relative of *A. occidentale,* spawns adhesive eggs (Carvalho et al. 2003), wool spawning mops similar to the ones used to collect the adhesive killifish eggs were placed against one of the tank walls. A cage (100 × 50 × 50 cm) made with a plastic net (5 mm mesh diameter) was placed in the center of the tank so that the juveniles had a sheltered area to avoid competition for food and predation by the adults. Fish were fed daily with a mixture prepared by blending equal portions of finely chopped mussels, peas and shrimp. This mixture was stored at −18 C. Occasionally, live artemia and red mosquito larvae were also included in the diet.

Results

From March to June, nitrites and ammonia remained below 0.01 and 0.1 mg/L, respectively; pH values varied between 8.2 and 8.5; dissolved oxygen was between 8.7 and 11.00 mg/L; total water hardness was of 106 ppm; and maximum water temperature varied between 16 and 22 C.

The setup for the captive breeding program was designed to minimize human intervention, not only to minimize the stress of the individuals but also to allow them to react to natural stimuli (water temperature and number of daylight hours). Thus, spawning was achieved naturally, without hormone induction or artificial fertilization. In addition, there was no human intervention in the incubation of the fertilized eggs or rearing of the young.

One month after transferring the adults to the breeding tank, several 15 mm (TL) juveniles were seen swimming in the water column, indicating that egg laying had occurred shortly after the adults were moved to the breeding tank, with a water temperature reaching

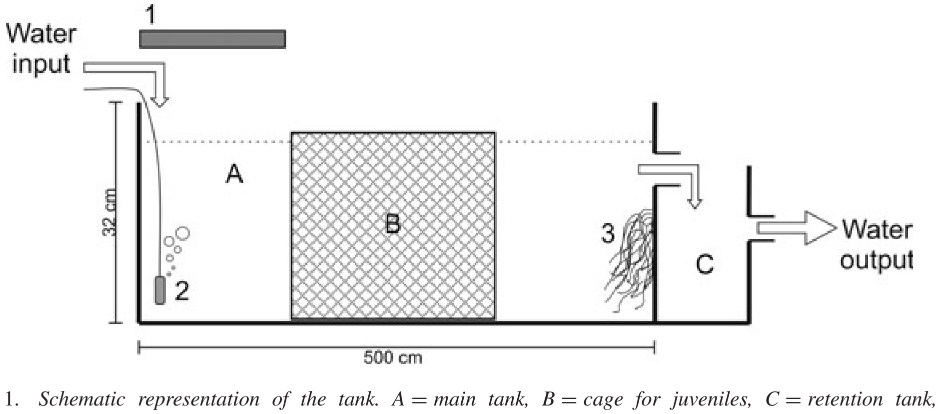

FIGURE 1. *Schematic representation of the tank. A = main tank, B = cage for juveniles, C = retention tank, 1 = suspended net for shadow, 2 = aeration, 3 = spawning mop.*

TABLE 1. *Standard length, weight and condition factor of adult* A. occidentale *and F1 individuals bred in captivity, measured in their first 2 yr of life.*

	N	Standard length (mm)		Weight (g)		Condition factor (g/mm^2)	
		$x \pm$ SD	Min–max	$x \pm$ SD	Min–max	$x \pm$ SD	Min–max
Adult *A. occidentale* (4+ age class)	24	102.79 ± 6.78	91.00–119.00	17.05 ± 4.08	10.50–26.50	1.59 ± 0.18	1.27–1.97
F1 age 1	32	70.33 ± 5.27	58.00–80.50	5.27 ± 1.23	3.30–8.10	1.05 ± 0.13	0.84–1.35
F1 age 2	20	88.71 ± 5.35	81.00–98.00	11.25 ± 2.58	7.90–18.00	1.41 ± 0.18	1.20–1.87

a maximum of 22–23 C during this period. Five months later, the number of juveniles (F1) was estimated to be >200. From these, approximately 100 juveniles were transferred to another tank with the same volume and conditions. At the end of May 2008, approximately 60 individuals (F2) measuring an average of 20 mm total length were detected in the new tank, indicating that the captive born 1-yr-old fish had spawned.

Subsets of sexually mature F1 individuals were analyzed in two consecutive years for average standard length, average weight and average condition factor, expressed as (weight/length^2) × 1000 – Table 1. To characterize the adult stage of this species, a sample of 24 adult *A. occidentale* from Sizandro River (age 4+) was analyzed in October during the non-breeding season (Table 1). Although growth curves are not available for this recently described species, based on other small cyprinids of the same geographic region (Collares-Pereira 1980), it is unlikely that *A. occidentale* grow much after age 4.

In its first year, *A. occidentale* attained 68.42% of the average adult standard length and 17.89% in its second year (Table 1). In contrast, the average adult weight increment was higher during the second year than during the first year: 30.92% and 35.05%, respectively. Thus, age 2 *A. occidentale* attained 86.30% and 65.97% of the average adult standard length and weight, respectively.

Discussion

This first essay of *ex situ* reproduction of *A. occidentale* was extremely encouraging. The maintenance and breeding of this species in captivity were inexpensive and involved only minor requirements concerning habitat, water quality and diet, whereas the breeding cycle was achieved without environmental or hormonal manipulations. In addition, this study showed that *A. occidentale* attains sexual maturity at age 1, a feature that will maximize the number of juveniles produced each year and, ultimately, the number of fish that will be returned to the natural habitat as soon as it is properly restored.

Based on the available information on the behavior of other Iberian cyprinids (Almada et al. 2003; Carvalho et al. 2003; Sousa-Santos et al. 2006; Pereira 2007), this breeding protocol can be scaled up easily and applied to other species with minimal adjustments. As a result, preliminary breeding essays with other Portuguese endangered species (*Squalius pyrenaicus* and *I. lusitanicum*) are already being conducted by our team.

As many critically endangered cyprinids attain small sizes, *ex situ* reproduction and maintenance are easier than with larger sized species. With small fish, a considerable number of individuals can be maintained and produced in a relatively small tank volume, allowing for larger captive breeding populations which is critical for retaining genetic diversity.

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