Embryonic development and larval stages of *Achondrostoma occidentale*

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1. INTRODUCTION

Achondrostoma occidentale (Western Ruivaco) is a strictly freshwater fish endemic to Portugal, listed as **'Endangered'** by IUCN (Freyhof & Kottelat, 2008). This species **has a reduced distribution range**, being present in only three independent rivers basins located at the western coast of Portugal: Alcabrichel, Sarafujo and Sizandro.

A. occidentale populations face a **considerable risk of extinction** mainly due to **increasingly severe summer droughts and habitat degradation (pollution, water abstraction for agriculture, destruction of riparian vegetation and proliferation of exotic species)**. As an extreme protection measure, *A.occidentale* has been **one of the target species of the *ex-situ* conservation program** held at Aquário Vasco da Gama (Sousa-Santos et al. 2014; Gil et al. 2010).

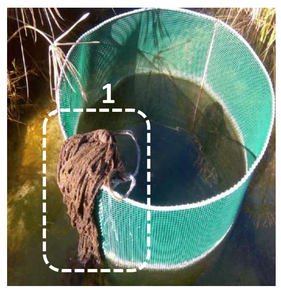
Besides providing fish to restock threatened populations, this breeding program is also a valuable tool to obtain multidisciplinary data on endangered species, such as those related to the first life stages, which remain lacking for most freshwater fish species (Aral et al. 2011). Studies on the **early development of fish are important** as they yield information **on the development mechanisms, nutritional needs and influence of environmental variables** (i.e **temperature ranges, photoperiod and oxygen concentration**) (Korwin-Kossakowski et al. 2008). **Disruptions and/or abnormalities in development stages of embryos and larvae are frequently considered as indicators of alterations in the environment** (Aral et al. 2011). Early life history data are also useful to identify eventual development differences between wild and captive broodstocks (Park et al. 2017).

2. RESEARCH GOAL

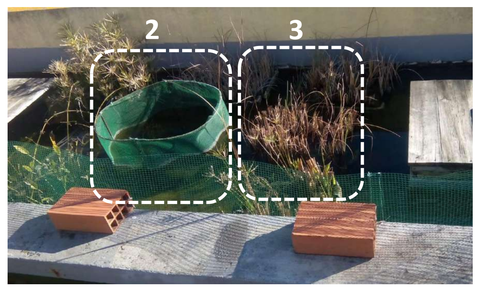
The present study aims to **provide the first description of the early life stages of *A. occidentale*** (Sizandro population) raised in captivity, **using a semi-naturalistic approach** (*i.e.* natural conditions of light and temperature; and absence of hormonal induction, artificial fertilization and artificial selection). This description **will enable future identifications of larvae from this endangered species**.

3. METHODOLOGY

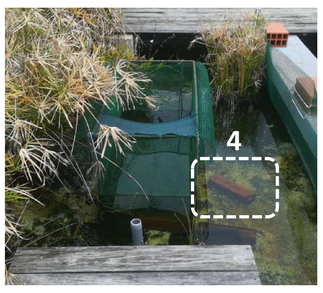
ENVIRONMENTAL ENRICHMENT



(1) Spawning mops



(2) Cages with small mesh size (shelter for larvae)
(3) Abundant aquatic plants (shelter for juveniles)



(4) Bricks (shelter for adults)

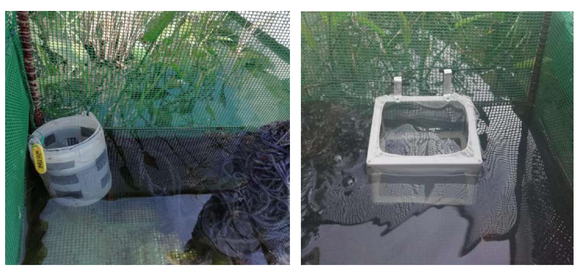
SPAWNING

Spawning	Number of *A.occidentale* adults	Mean fork-length (min – max, mm)
1° - March 26th	27	123 (88 – 150)

1250 L tank | *Natural Photoperiod*

EGG AND LARVAE COLLECTION

Eggs detected in the synthetic wool mops



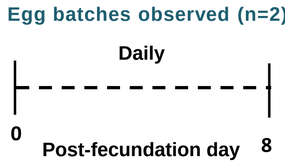
A subset of the laid eggs/larvae born was kept in containers inside the breeding tank to facilitate periodical collection for microscopic analyses

OBSERVATIONS

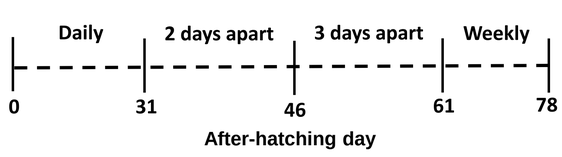
Microscopic Analysis: equipped with a digital camera and software Motic Cam 5+

Water Temperature recorded (Galvanic sensor)

Egg batches observed (n=2)



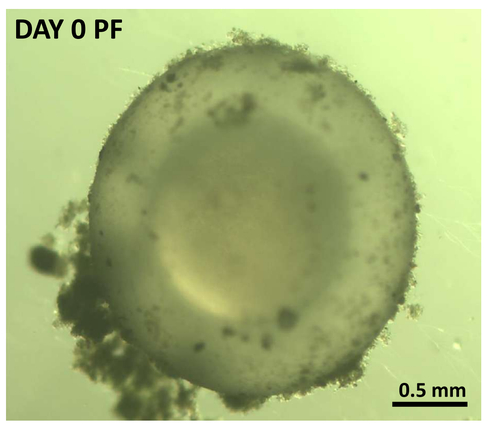
Larvae batches observed (n=2)



4. RESULTS

PRE – HATCHING DEVELOPMENT

ZYGOTE AND CLEAVAGE STAGE

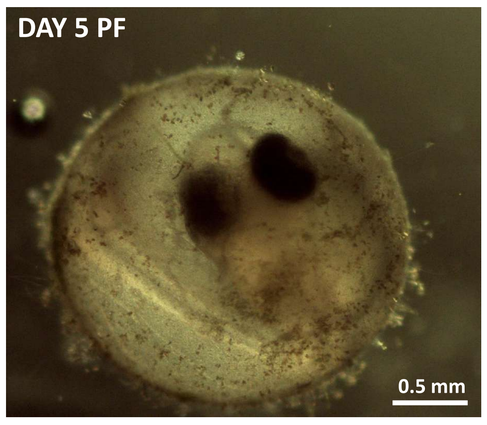


Diameter: **1.8 mm**

Perivitelline space

Yellow and spherical

ORGAN DIFFERENTIATION

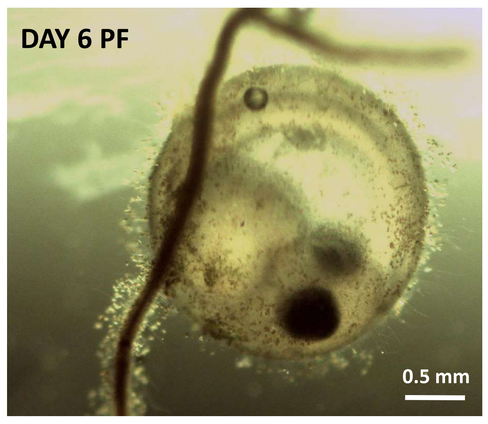


Cephalic differentiation

Eyes lens and nostrils

Red cells blood fluid

ORGAN DIFFERENTIATION

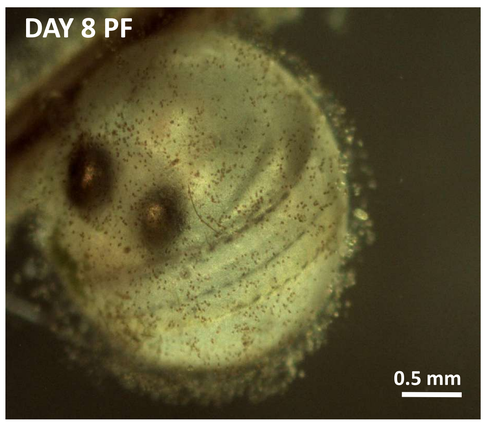


Embryo tail passed over the brain

Slower movements

Smaller yolk mass

PRE-HATCHING



Rows of melanophores over the dorsal, midline and ventral line

Increased heartbeat

Reduced yolk mass

Embryonic Development time: 8 days | Mean Temperature: 16.7 ± 1.9 ºC

LARVAL DEVELOPMENT

Total Length: **7 mm**

Larvae with pyriform yolk sac

Developed mouth

Melanophores over the head and dorsal area

Total Length**: 7.9 mm**

Transitional yolk-sac larval stage

Increased number of melanophores

Total Length: **14 mm**

Swim bladder division

Increased iridophores

Total Length: **21.5 mm**

Fins segmented

Completed fins rays

Total Length: **29.5 mm**

Completed scales

Similar to a juvenile

Larval Development time (until juvenile) : 78 days | Mean Temperature: 16.7°C ± 1.8 ºC

5. CONCLUSIONS

- **Environmental enrichment structures are important to promote the reproduction and to maximize recruitment of *A. occidentale* in captivity.**
- ***A. occidentale* eggs are spherical, yellowish and sticky to each other and to surfaces, especially vegetation and spawning mops.**
- **Hatching occurred after 8 days and the newly hatched larvae had about 7 mm total length and a pyriform yolk sac. Complete larval stage lasted about 80 days.**
- **Larvae morphology was identical to that of captive bred *Iberochondrostoma lusitanicum* and *Iberochondrostoma almacai* (Calado, 2018) but more detailed comparisons are planned.**
- **The 'semi-naturalistic approach' adopted for the *ex-situ* conservation program likely prevents bias in the description of the first stages of the life cycle of *A. occidentale* due to rearing conditions.**
- **These observations are baseline data for establishing comparisons with embryonic development of wild specimens and for future larvae identification guides.**

REFERENCES:

Aral, F., Şahinöz, E., & Doğu, Z. (2011). Embryonic and Larval Development of Freshwater Fish, Recent Advances in Fish Farms, Dr. Faruk Aral (Ed.), ISBN: 978-953-307-759-8, InTech, Available from: http://www.intechopen.com/books/recent-advances-in-fish-farms/embryonic-and-larval-development-offreshwater-fish; **Calado, R. (2018)**. Relatório de Estágio no Aquário Vasco da Gama (Master's thesis, Escola Superior de Turismo e Tecnologia do Mar – Peniche Instituto Politécnico de Leiria, Portugal; **Freyhof, J., & Kottelat, M. (2008)**. *Achondrostoma occidentale*. The IUCN Red List of Threatened Species 2008; **Gil, F., Sousa Santos, C., & Almada, V. (2010)**. A simple and inexpensive technique for the *ex-situ* reproduction of critically endangered cyprinids - *Achondrostoma occidentale* as a case study, Journal of the World Aquaculture Society, 41:661-664; **Korwin-Kossakowski, M. (2008)**. The influence of temperature during the embryonic period on larval growth and development in carp, *Cyprinus carpio*, Archives of Polish Fisheries, 16(3):231-314; **Park, J. M., Mun, S. J., Yim, H. S., & Han, K. H. (2017)**. Egg Development and Larvae and Juveniles Morphology of Carp, *Cyprinus carpio* in Korean, Development & Reproduction, 21(3):287-295; **Sousa-Santos, C., Gil, F., & Almada, V.C. (2014)**. *Ex-situ* reproduction of Portuguese endangered cyprinids in the context of their conservation. Ichthyological Research, 61:193–198.