

Abstract

Sperm Characterization of Endangered Leuciscids Endemic from the Iberian Peninsula: Gamete Storage as a Tool for Helping Ex-Situ Breeding Programs [†]

Ana Hernández ¹, Carla Sousa-Santos ², Fátima Gil ³, Pedro M. Guerreiro ¹, Elsa Cabrita ¹
and Victor Gallego ^{1,*}

¹ Center of Marine Sciences (CCMAR), University of Algarve, 8005-139 Faro, Portugal; ana.hernandez149@alu.ulpgc.es (A.H.); pmgg@ualg.pt (P.M.G.); ecabrita@ualg.pt (E.C.)

² MARE—Marine and Environmental Sciences Centre, ISPA, 3004-517 Coimbra, Portugal; carla_santos@ispa.pt

³ Aquário Vasco de Gama, 1495-718 Cruz Quebrada, Portugal; avg.aqua@marinha.pt

* Correspondence: vgalbiach@ualg.pt

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[‡] Presenting author (Oral communication).

Abstract: Populations of freshwater fish species endemic to the Iberian Peninsula have been declining since the mid-20th century, and several types of actions (from in situ to ex situ measurements) have been applied over the past decades for preserving these species. However, limited knowledge about their reproductive biology makes it necessary to investigate different aspects of the reproductive cycle for improving breeding programs. The main objectives of this work were to advance knowledge concerning sperm kinetics and spermatozoa morphology and to develop protocols for the short- and long- term storage of gametes. Populations of different endangered leuciscid species (*Anaecypris hispanica*, *Iberochondrostoma lusitanicum*, *Achondrostoma occidentale*, and *Squalius aradensis*) were sampled during the spring of 2022 both in captive populations kept at Aquário Vasco da Gama (AVG) and in wild populations from different Portuguese rivers. Sperm samples were collected and sperm motion parameters were assessed, for the first time, for these four species. Sperm kinetics differed between species in motility and velocity traits, also showing a different number of sperm subpopulations. The longevity of sperm (swimming period) was also different among species: the shortest period was obtained for the wild population of *S. aradensis* (values close to zero at 40 s), and the longest swimming period for the captive population of *I. lusitanicum* (values close to zero at 120 s). Furthermore, different storage trials were carried out diluting the sperm in a extender solution (75 mM NaCl, 70 mM KC1, 2 mM CaCl₂, 1 mM, MgSO₄, 10 mM Hepes, pH 8) at a ratio 1:20 (sperm:extender). Sperm quality (>40% of motile cells) was kept for a maximum of four days of storage, depending on the species. In addition, new cryopreservation protocols (using DMSO, Methanol and/or egg yolk) were tested for cryobanking the sperm of these threatened species. Cryopreserved samples showed significantly lower motility when compared with fresh samples, and the best results were obtained for *I. lusitanicum*, reaching 20% of motile cells after thawing using 10% of DMSO supplemented with 10% of egg yolk. This study is the first of its kind to successfully achieve gamete cryopreservation of Iberian endemic and endangered freshwater fish species, developing new and useful tools to complement the management and conservation programs.

Keywords: gamete quality; cryopreservation; sperm subpopulations



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