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Abstract

Assessment of the Reproductive Status of Captive Populations of Endangered Leuciscid Species from the Iberian Peninsula: *A. hispanica, I. lusitanicum* and *A. occidentale* †

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Abstract: Populations of freshwater fish species endemic to the Iberian Peninsula have been declining since the mid-20th century, and the captive breeding of highly endangered species is considered to be a useful tool to restock declining populations. A pioneer project of supportive breeding of critically endangered fish started in 2007 at the Aquário Vasco da Gama (AVG), and this work aims to show the reproductive status of the breeders which make up the current captive broodstoks. Populations of different endangered leuciscid species (*Anaecypris hispanica, Iberochondrostoma lusitanicum and Achondrostoma occidentale*) were sampled at AVG during the spring of 2022. Breeders were counted and sexed, and males were stripped to check for the presence of spermatozoa. The sperm volume was assessed visually, and spermatozoa motility was assessed by a CASA system. Sperm samples were classified into four classes based on the percentage of motile cells: C-I $\leq$ 25%, C-II = 25–50%, C-III = 50–75%; and C-IV > 75%. The captive population of *A. hispanica* consisted of 63 individuals and showed a 40% of spermiating males, with an average volume of 5–10 µL. The histogram of sperm quality reported that 15% males had sperm motility of C-II, 50% of males had sperm motility of C-III and, finally, 35% of males had sperm with the high-quality class (C-IV). The population of *I. lusitanicum* consisted of 599 individuals and showed 93% of spermiating males, with an average volume of 15–20 µL. The histogram of sperm quality reported that most part of the males had good sperm quality belonging to C-III and C-IV class (26% and 71%, respectively), while just 1 male showed bad quality sperm (C-II). The captive population of *A. occidentale* consisted of 193 individuals, showing a 62% of spermiating males with an average volume of 20–25 µL. The histogram showed that 6% males had sperm motility of C-I, 26% of males had sperm motility of C-II, the most part of the males (45%) showed a sperm quality of C-III and, finally, 23% of males had sperm with the high-quality class (C-IV). Since the project began in 2007, more than 12,000 fish of these three critically endangered species have been released to restock the populations from which the respective wild breeders were caught. All captive fish were released after a maximum of three consecutive generations in captivity, and new stocks were established with wild adults from the target populations, to avoid the negative effects of inbreeding and genetic drift on the original genetic pool.

Keywords: gamete quality; ex-situ conservation; sperm kinetics

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