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# DESCRIPTION OF THE REPRODUCTIVE STRUCTURE IN EUROPEAN HAKE (Merluccius merluccius) BROODSTOCK USING MICROSATELLITE MARKERS

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## Introduction

European hake (*Merluccius merluccius*) is one of the most important European fisheries species, with a great potential as aquaculture species, which is still under development (Pérez, 2015). The use of genetic markers in the management of breeding stocks, can truthfully establish kinship relations between the parents and the different spawns obtained, allowing know which individuals are the main contributors to produce the next generations. This study analyzes, using microsatellites, the relationships between breeders and different spawn batches, within and between years, in the only stock of hake stabled in the world (Iglesias et al. 2010), in order to identify the number of breeders involved in each spawn.

# Material and methods

The stock consists of 22 breeders (14 females, 8 males), is located on one tank of 32,000 liters. Tissue samples (from tail fin) of breeding stock, and larvae obtained in 10 different set-ups, for the years 2011-2014 (50 larvae per set) were preserved in absolute alcohol. The extraction and purification of DNA from these samples was performed following the FENOSALT method (Perez and Presa, 2011). 5 microsatellite markers were amplified, 3 neutral described by Moran et al. 1999 (HK-3b, HK and HK-9-20) and 2 expression markers (EST 6.3 and EST 13.3) described by Santafé et al. (submitted) following a pentaplex amplification protocol (Santafé et al., submitted). The PCR products were genotyped in an ABI PRISM 3130 sequencer at the CACTI (Servicio de Genómica, Universidad de Vigo). The allele size was calculated using ROX 500 GeneScan as a reference standard. Genotypic data were analyzed with software GeneMarker v2.4.2 (SoftGenetics) and ML-RELATE (Kalinowsky et al. 2006). VITASSIGN program (Vandeputte et al. 2006) was used for the parent assignment.

# Results

Parental assignment analysis shows that a single couple participates in each spawn in the most cases (Table I), although the pairings vary in different spawns and sometimes simultaneous spawning of more than one pair occurs. The participation of 7 males and 4 females within the stock was detected for the period of time analyzed. Regarding the quality of the spawns, there is a notable decrease in the percentage of fertilized eggs in those produced during the summer months (Figure 1)

# Discussion

Obtaining spontaneous spawns in captivity without hormonal induction processes, is one of the best indicators of the potential of a species for its culture. In our study only 10 hake spawns of the total 167 which were obtained spontaneously between 2011 and 2014 were analyzed, showing that breeding in culture conditions does not represent one of the main obstacles for this species. Most of the spawns are produced by a single couple, although there are exceptions (spawns 0212 and 3912). This fact, together with the participation of only part of all breeders, especially in the case of males, might seem an indication of the existence of dominance relations within the stock. However, different permutations observed suggest that the intervention of each individual would primarily respond to their physiological and sexual maturity during the breeding season. The analysis of a larger number spawns in the future, will allow confirming these initial assumptions, and detecting possible pairing patterns which could remain underlying.

Spawn	2211	0212	0512	0612	0712	3912	4112	4612	0313	0314
Parent Pairs	M11xF10	M1xF6 M16xF6	M17xF6	M1xF7	M11xF5	M19xF7 M1xF12	M11xF5	M1x F14	M19xF22	M11xF7

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