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First-generation genetic drift and inbreeding risk in hatchery stocks of the wreckfish *Polyprion americanus*

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ABSTRACT

The wreckfish *Polyprion americanus* is a component of directed and admixed species fisheries throughout its world range and has recently been identified for its aquaculture potential. Several wreckfish broodstocks were founded in Galician hatcheries in the last decade and are now approaching maturity. The combination of genetic markers with classic domestication strategies is a pertinent approach to preserve genetic variation and to slow down consanguineous-derived adverse effects. Genetic data from microsatellites used to characterize four Galician wreckfish stocks provide evidence of both, a significant interstock differentiation (7.60%) and a pronounced loss of allele diversity (26%) in 80% of the stocks. Such artificial sub-structuring linked to the erosion of gene diversity is most likely caused by sampling drift that stepped up from the North Atlantic population. Computer simulation of relatedness helps to appraise alternate breeding protocols to avoid uncontrolled inbreeding within stocks. We show that under a fixed number of breeders and a sex balanced contribution, two protocols consisting on random mating among broodstocks ($\Delta Rxy = 2.21\%$) or enhanced with a wild sample ($\Delta Rxy = 0.97\%$), are far more advantageous strategies to slow inbreeding in F1 and F2 than breeding protocols within stock ($\Delta Rxy = 6.6\%$).

Statement of relevance: In silico scenarios upon real data help slowing inbreeding

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1. Introduction

The industrial profit from feeding a growing human population has made marine fisheries expand beyond natural fish resilience to predation (e.g. Swartz et al., 2010). Consequently, fisheries catches are now declining (Fisheries FAO, 2012) and their sustainability is seriously questioned in many fish stocks from European waters (Baudron and Fernandes, 2014). Aquaculture has been politically focused, as a worthy alternate to fisheries for it would come to palliate wild fish scarcity and reallocating fishing jobs from the fishing industry. Nonetheless, a massive implementation of aquaculture is not warranted since many concerns on ecosystem and health-related issues are in play, e.g. coastal industrialization, genetic escapes, increasing demand for fishmeal, animal health in intensive culture and its suitability for human consumption, etc. Therefore, global and wise management plans seem to be essential for aquaculture developments expected this century (Asche and Tveterås, 2004).

A currently neglected genetic issue in the management of aquaculture stocks is the prior foundation criteria used upon the goal of the hatchery (Allendorf, 1986). The amount and distribution of genetic diversity determines the minimum number of founders and their origin in order to capture the maximum genetic background to maintain a wealthy hatchery stock. While high levels of variation imply higher additive variance in productive traits (e.g. Reed and Frankham, 2001; Overturf et al., 2003), breeding a small broodstock drawn from a large wild population followed by inbreeding leads to a rapid increase in the expression of deleterious alleles. That inbreeding depression (Falconer, 1989; Hahn, 1989) is characterized by depressed fitness-related traits such as growth rate, fry survival, e.g. salmonids (Kincaid, 1976; Pante et al., 2001), survival and reproductive performance, e.g. shrimps (Sbordoni et al., 1986; De Donato et al., 2005; Moss et al., 2007) and fish fitness in general (Danzmann et al., 1989).

The effective size (*Ne*) of founder stocks is constrained by farming facilities, which results in the use of few breeders. The usual crossbreeding systems also erode the genetic diversity, either deliberately in selection programs or inadvertently through reproductive bottlenecks and inbreeding among relatives, even in first generation hatchery stocks (e.g. Taniguchi et al., 1983). If *Ne* is too low, the genetic drift will dramatically change the genic composition of the stock regarding the source population. Such genetic diversity will be further reduced by

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consanguinity as soon as in the F1 generation (remnant as little diversity as $1 - 1/2\ N$ when the size of the founder stock is low). Therefore, minimization of inbreeding and control of the genetic structure are key issues to sustain a healthy hatchery stock (e.g. clams, Benzie and Williams, 1996).

From an aquaculture perspective, the implementation of genotyping in early stages of domestication not only has a significant impact to prevent or slow down inbreeding but also to improve broodstock management and larval culture (Liu and Cordes, 2004; Le François et al., 2010). Diversifying efforts in aquaculture include the domestication of new species, a foundation time where future genetically related problems can be prevented. One of such species is the wreckfish *Polyprion americanus* (Bloch and Schneider, 1801), a long-lived pelagic fish (Roberts, 1977) that can be found in the Mediterranean Sea and the Atlantic Ocean (from Norway to South Africa and from Canada to Argentina), the Indian Ocean, the South Pacific, New Zealand, and Western Australia (Ball et al., 2000). This species reaches sexual maturity between 60 and 90 cm at the age of 10 (Papandroulakis et al., 2004), shows a demersal adult phase in the Atlantic (from depths between 42 m and 1,000 m) and reaches up to 100 kg weight and 2 m length (Peres and Haimovici, 2004; Jolivet et al., 2012) at 32 years old (Sedberry et al., 2006). Spawning seems to take place preferentially in the Blake Plateau, from February to March and has occasionally been reported in the Mediterranean Sea from January to April (Hardy, 1978). In the eastern Atlantic, 1–7 yr old juvenile wreckfish 45–55 cm in length (Goujon, 2004) are found off the Norwegian coast along shallow portions of the Mid-Atlantic ridge and associated islands (Macaronesia Islands) and along northern and southern Africa, including the Mediterranean. Namely, in Macaronesia this species is usually found at depths 50–1000 m (Menezes et al., 2013) and is often associated with floating objects which are used as bait to capture them (e.g. Machias et al., 2003). Those demographic and fishery data have led to hypothesize that Atlantic fish grounds are systematically connected through juvenile wreckfish migrating from Blake Plateau to southwestern Euro-African waters, a key phenomenon to understand the dynamics of the single North Atlantic wreckfish population and to design appropriate multinational management plans (Sedberry et al., 1999).

While large-scale biological data on *P. americanus* are needed to properly deal with the complexity of its domestication process, e.g. population dynamics, behavior, spawning regimes, nutrition, etc. (Goldman and Sedberry, 2011), its aquaculture properties are acknowledgedly remarkable, e.g. taming, low mortality and high growth rate during the pelagic life ($k = 0.03$–0.08 yr-1, combined sexes, Sedberry et al., 1999; Vaughan et al., 2001) as well as in culture ($k = 0.03$ yr-1; combined sexes, Peleteiro and Bruzón, 2013). Such biological properties and its market price have prompted an increasing interest for initiating an aquaculture industry of wreckfish in Europe (Papandroulakis et al., 2004). The genetic consequences of inbreeding, domestication, genotype-environment interactions and selection are well known in many species, so preliminary studies are needed for setting up suitable guidelines for founding and maintaining cultured wreckfish stocks. Genetic erosion can be detected at early stages of domestication by the loss of rare alleles and by departures from Hardy-Weinberg equilibrium (HWE). Those and other phenomena can be tracked with microsatellites that show high sensitivity to measuring changes in the genetic load both, between hatchery stocks and wild sources (e.g. Tessier et al., 1995) and vice versa, between transferred stocks used to the enhance natural populations (Presa et al., 1994).

This study aims to measure the amount of genetic change enforced on first generation Atlantic wreckfish stocks in Europe since 2007 (Galicia, NW Spain), previous to its reproduction and transfer among farms. Since a small number of fish has been collected as candidate breeders for aquaculture, we pursued the determination of relatedness within each stock as a decisive step to avoid inbreeding when these fish were ultimately bred. We also aimed to explore the likely evolving scenario of the relatedness coefficient following simulation of random mating under three breeding protocols. We hypothesize that if the foundation of stocks was correctly made, then a) a minimal genetic divergence should exist among captive wreckfish stocks as well as between them and the wild source, and b) no better minimization of inbreeding is expected in optimized breeding protocols within stock as compared to those among stocks and external enhancement.

2. Materials and methods

2.1. Sample collection and DNA extraction

The Aquarium Finisterrae hatchery (D_AQUA, n = 12) and the Luso-Hispana de Acuicultura hatchery (D_LHA, n = 39) founded their stocks in June 2007 from specimens caught in Northwest Cantabrian Sea grounds, between Cape Finisterrae and Cedeira. The 51 specimens from D_AQUA and D_LHA were captured at the age of *ca.* 3–4 and sampled in 2012 when they were *ca*. 8–9 yr old and averaged 92.00 ± 15.56 cm in length and 23.45 ± 14.35 kg in weight.

The IGAFA hatchery (D_IGA, n = 12) and the IEO hatchery (D_IEO, n = 10) founded their stocks in 2007 from specimens caught in Ría de Arousa fishery grounds (Southern Galician estuaries, NW Spain). The 22 specimens from D_IGA and D_IEO were captured at the age of *ca.* 3–4 yr old and sampled in 2012 when they were *ca.* 8–9 yr old and averaged 78.50 ± 13.44 cm in length and 11.72 ± 6.86 kg in weight.

The wild Atlantic population (W_AZO) was sampled from a fishing vessel working off the Azorean Archipelago and landing at Vigo Port on 26 April 2012. Its 40 specimens had about 4–5 yr old and averaged 67.40 ± 9.25 cm in length and 7.01 ± 4.73 kg in weight.

All 113 wreckfish specimens from the four stocks and the wild sample were sampled in spring 2012 (Fig. 1) by collecting a piece of the dorsal fin and preserving it in pure ethanol until genomic DNA extraction, isolation and purification following the method FENOSALT (Pérez and Presa, 2011). Clean DNA was resuspended in 50 μL 0.5× TE buffer and kept at −20 °C until its direct use as a PCR amplification template.

2.2. PCR amplification of microsatellite markers and genotyping

DNA from 113 specimens of wreckfish was amplified using five microsatellites, two of them (Pam006-$(GT)_{17}$ and Pam021-$(AC)_{15}$) showed a relative high divergence among North Atlantic wild samples (Ball et al., 2000) and three ones reported herein were isolated from this species following the enrichment technique FIASCO (Zane et al., 2002) (PamD1-$(GACA)_9$, PamA5-$(GTCT)_{13}$ and PamD2a-$((GA)_5CA(GA)_7)$. Markers Pam006 and Pam021 were co-amplified in a single reaction using 0.3 μM of each primer, 55 °C annealing temperature and 3.0 mM $MgCl_2$ (Ball et al., 2000). Amplification of locus PamD1 was performed in a single reaction using 0.27 μM of each primer (Forward 5′-CCCTGTTATCATCGCTTCCT-3′; Reverse 5′-GCCATCACACCC TTCTGTTT-3′), 1.7 mM $MgCl_2$ and 58 °C annealing temperature. Markers PamA5 (Forward 5′-TGGCCTTTGACTTTGAGGAC-3′; Reverse 5′-TCATAT GACCCCTCCTGCTC-3′) and PamD2a (Forward 5′-ACAGACAGCCAGGG AGAGAG-3′; Reverse 5′-GACAGTCCGTGTGACAGTCG-3′) were co-amplified using 0.33 μM and 0.27 μM of each primer pair, respectively, 1.4 mM $MgCl_2$ and 58 °C annealing temperature. Forward primers of each locus were labeled with fluorophores as FAM (locus-modal allele size: PamD1-171 bp and Pam021-112 bp) and HEX (Pam006-112 bp, PamA5-157 bp and PamD2a-198 bp). PCR amplifications were performed in a Mastercycler Gradient Thermocycler (Eppendorf) using the following routine: one step at 95 °C for 10 minutes followed by 35 cycles at 95 °C for 80 s, locus-specific annealing temperature for 50 s and extension at 72 °C for 70 s; a final extension step was enforced at 72 °C for 15 minutes. One microliter of each locus-specific PCR amplification were pre-mixed and one microliter of such admixture was added to 0.25 μL ROX fluorophore as an internal marker (GeneScan-500, Applied Biosystems) and 10 μL of deionized formamide. That multiplexed PCR mix was used to genotype the 113 wreckfish

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