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Trends of the genetic effective population size in the Southern stock of the European hake

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ABSTRACT

Scarce evidence exists on the amount of genetic diversity remaining after fishing overharvest, on how the genetic effective population size (N_e) behaves under counteracting forces such as drift and migration, and on N_e predictability of population status and trends. This study aims testing both, the temporal stability of the genetic diversity among samples from the Southern stock of European hake and the influence of a high fishing intensity on its N_e. Such goals were addressed with variation of microsatellites and Cytochrome *b* haplotypes on an 82 sample collection taken in years of deep population depletion (2000–2007) plus a historical anchoring sample from 1976 which predates the industrial exploitation of this fishery. Despite the Southern stock exhibits similar levels of variation at microsatellites and Cytochrome *b* across the historical window addressed, N_e experienced a 43-fold reduction in parallel to an 80% biomass (N_{ssb}) loss between the historical estimates of 1976 ($N_{ssb} = 20.55{*}10^6$ Mi, $N_e = 12{,}480$) and their minimums of 2004 ($N_{ssb} = 4.68{*}10^6$ Mi, $N_e = 291$). Neither N_e nor N_e/N_{ssb} matched SSB trends at overharvest in 2000–2007, when a faster N_e reduction was patent. Nevertheless, N_e correlated well with SSB and the N_e/N_{ssb} ratio was maximal at population equilibrium and minimal at population depletion. Current results suggest that the short-term unpredictability of N_e on population trends following overexploitation can be circumvented by increasing the number of point estimates from pre-harvest and postharvest times. We show that the Southern stock has passed through a genetic throat of $N_e \approx 300$ between 2002 and 2005 upon which biomass rebounded to 1/3 in 2007 and to ½ in 2014 of its N_e size from equilibrium in 1976. Such recovery was likely helped by EU policies enforced to make this fishery sustainable, but collateral enhancers such as maintenance of a minimum N_e threshold and the enriching input of occasional gene flow from the Northern stock of hake, have presumably played a synergic role at triggering overfishing resilience. Incorporating both, structural genetics and genetic metrics such as N_e into assessment can provide more precision on the rate of genetic erosion and improving assessing risks of extinction by genetic factors.

1. Introduction

The European hake (*Merluccius merluccius*, Linnaeus, 1758) occupies the widest longitudinal and latitudinal ranges among hakes, i.e. from Mauritanian coasts to Irish and Norwegian ones, including the Mediterranean Sea and the southern coast of the Black Sea (Lloris et al., 2003). Such ample distribution and high biomass make hake play a key trophic role in NW Atlantic and Mediterranean marine ecosystems (e.g., Casey and Pereiro, 1995). ICES recognizes a Northern stock distributed from the western coast of Norway (62°N) to Capbreton Canyon (43°38′N) (ICES Division IIIa, Sub-areas IV, VI, and VII, and Division VIIIa, b, and d) and a Southern stock distributed from southern Bay of Biscay to the Gulf of Cadiz (ICES Divisions VIIIc and IXa) (e.g., ICES, 2009a,b). While occasional spatial genetic divergence has been reported between the Cantabrian Sea and both, other Southern stock grounds such as northern Portugal (e.g., Milano et al., 2014) or Northern stock grounds such as Irish waters (Roldán et al., 1998), a non-structuralistic Atlantic scenario showing a limited connectivity only with the North Sea grounds has been described so far with

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microsatellites and mtDNA markers (e.g. Lundy et al., 2000), otolith core geochemistry (e.g., Tanner et al., 2014), classic allozymes (Mangaly and Jamieson, 1979) and morphometric traits (Jones and Mackie, 1970). Such a pan-Atlantic scenario in the European hake is congruent with the absence of population substructures within management Division VIIIc comprising the Cantabrian Sea (Southern stock) as inferred from several marker types (e.g., Pita et al., 2011), except one study reporting a stable fine spatial and temporal differentiation within the Cantabrian hake population (Castillo et al., 2004).

The European hake is highly appreciated for human consumption, has been harvested since the 18th century (e.g. see C. F. Hickling studies cited in Casey and Pereiro, 1995) and the two Atlantic stocks have been heavily exploited over the last three decades (ICES, 2009a,b). The depleted Southern stock (EC, 2005) exhibited a biomass minimum of 9000 t in 2004 and a recovery plan was implemented to regain its historical SSB biomass up to 35,000 t by 2016, i.e. a 400% SSB increase. Pursuing such a goal, the implementation of regulatory measures on this fishery since 2005 has led to good recruitments and to a SSB increase close to that objective. However, high uncertainty exists on the causation of such recovery, i.e. the influence of palliating fishery policies *versus* the contribution of environmental facilitation on population growth (Díez et al., 2012) and/or demographic enrichment lent by migration (Pita et al., 2011). Investigation on causes of biomass recovery requires an ecosystem approach where environmental variables, assessment parameters and the genetic background of the species were combined under the widest temporal framework affordable. A key issue barely shuffled in fisheries management is the genetic diversity remnant after overharvesting which is a necessary condition for population viability, bottleneck recovery and adaptation. Soundly unknown is how the genetic background of the European hake has responded to decades of exploitation, the amount of erosion exerted on its genome or the minimum genetic threshold required to regain equilibrium. In this regard, genetics provides biologically-based candidate-parameters to help assessing the goodness of population estimates from fishery data. Incorporation of genetics into fishery science has gone from the basic description of intrapopulation genetic variation of neutral markers (Utter, 1991) to the molecular traceability of species for ecumenical purposes (e.g., Pérez et al., 2005). A general concern exists on the need for a deeper analytical horizon in fisheries comprising long term data sets of genetic metrics (e.g., Cuéllar-Pinzón et al., 2016) allowing to distinguish meaningful genetic changes from sampling errors and environmental variability (e.g., Shapiro and Hofreiter, 2012).

Maintaining the maximum amount of genetic variation needs to become a central goal of long term management plans for fisheries, irrespective what the population census size might be (e.g., McNeely et al., 1990). Theoretically, a low census size can bear the same amount of genetic variation than a large census size, thus allowing population rebound from a reduced population size and *vice versa*, a large census size can bear an extremely low genetic variation after overharvest or natural bottlenecks (e.g., Pinsky and Palumbi, 2014). The erosion of genetic diversity of exploited fisheries can be addressed with population metrics drawn from large-scale genotyping approaches, such as the genetic effective population size (N_e). This parameter was defined by Wright (1931) as the size of an ideal population which had the same rate of change in allele frequencies or heterozygosity as the observed population, and introduced this concept to calibrate the amount of genetic drift in populations with a binomial distribution of progeny numbers, segregated sexes or fluctuations in population size. The N_e concept was later extended to populations with overlapping generations. A low N_e is related to a low-fitness risk, so it becomes a critical parameter in both evolutionary biology and fishery management. Estimates of N_e can be theoretically useful to predict the impact of management practices on the loss of genetic variability due to the fishery-induced effects of random drift over many generations. While predictions based on N_e equations may prove inaccurate for a number of reasons (e.g., estimates with a large variance and error or approximate formulation for neutral loci; Lande and Barrowclough, 1987) a trend of loss in temporal estimates of N_e can serve as a warning of fishing-induced drift (e.g., Waples et al., 2008).

In this study we have addressed the spatial scale of the biomass core of the Southern stock of hake aiming to assess the fingerprint of the historical overexploitation on its genetic variation. We also aimed to assess the putative role that the post-overharvest genetic background could have played on the fishery recovery after 2005. Genetic inference was performed after analyses of the inter-annual genetic variation of microsatellites and Cytochrome *b* sequences, as well as on trends of the genetic effective population size and its correlation with demographic metrics. In this scenario, we hypothesize that if the Southern stock were self-contained a patent irreversible erosion of its N_e should be expected despite the success of fishing policies based on leveling fishing pressure. If opposite, the Southern stock is interdependent of other Atlantic grounds, the observed SSB rebound would have been enhanced not only by alleviation of fishing intensity but also by gene flow-dependent genetic enrichment from the Northern stock, i.e. a N_e rebound would be expected after a SSB rebound.

2. Material and methods

2.1. Sampling and molecular analyses

Eighty-two samples comprising 1833 mature hakes (fork-length ≥ 35 cm) were taken from commercial vessels as well as from annual surveys conducted by the Spanish Institute of Oceanography (IEO) during autumn campaigns on ICES Areas VIIIc and IXa. Those samples were taken in seven years (1976, 2000–2005 and 2007) from two main areas of the Southern hake stock, *i.e.* the Cantabrian Sea and North Atlantic Iberia (Appendix A in Supplementary material). Individual gill and muscle tissue were collected on board and preserved in absolute ethanol while otoliths that were stored dried in envelops from 1976 were individualized in test tubes and moisturized by immersion in pure water. Few picograms of tissue were recovered after dipping otoliths at 40°C for 24 h in a lysis solution (100 mM Tris pH = 8.0, 100 mM NaCl, 10 mM EDTA, 2% SDS and 200 μg/ml of proteinase-K). DNA was subsequently extracted as described for Atlantic cod otoliths (Hutchinson et al., 1999). DNA from 20 mg of gill or muscle tissue was extracted and purified using the method FENOSALT (Pérez and Presa, 2011). A DNA aliquot was diluted 1:5 before PCR amplification of five polymorphic microsatellites (Morán et al., 1999) used to genotype all individuals following conditions described in Pita et al. (2011). The forward primer of each marker was labeled with *Cy5* fluorophore (5-*N*-*N*-diethyl-tetramethylindodicarbocyanine) for laser detection and amplified fragments were electrophoresed in an ALF-expressII fragment analyzer (GE Healthcare). Molecular ladders (80 bp, 180 bp, 230 bp, and 402 bp) were co-migrated in parallel to samples for allele sizing. Genotypic data was checked twice by two researchers and individuals showing dubious gel bands were re-amplified and re-genotyped. The consistency of the scored allelic series and the genotypic adjustment to Hardy-Weinberg Equilibrium (HWE, the mathematical principle by which allele frequencies of a population will remain constant from generation to generation in the absence of destabilizing, evolutionary or exploitation forces) were tested with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). The PCR amplification of a 465 bp fragment spanning 37 bp from the 3′-end of the tRNA-*Glu* gene and 428 bp from the