Evidence of stock connectivity, hybridization and misidentification in white anglerfish support the need for adopting a genetics-informed fisheries management approach

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Abstract

Genetics analyses can reveal mismatches between biological and management units and thus prevent derived ineffective management actions that could lead to serious consequences for fisheries management. Additionally, genetics could reveal other relevant but hidden information such as species misidentification or hybridization. Here, we have assessed the power of genetics to better understand the population connectivity of white anglerfish (*Lophius piscatorius*) and its interaction with its sister species, the black anglerfish (*L. budegassa*). Our analyses, based on thousands of genome-wide single nucleotide polymorphisms, show three thus far unknown findings that are crucial for white anglerfish management. We found i) that white anglerfish is composed by a single panmictic population throughout the Northeast Atlantic, challenging the three-stock based management, ii) that a fraction of specimens classified as white anglerfish using morphological characteristics are genetically identified as black anglerfish (*L. budegassa*) and iii) that the two *Lophius* species naturally hybridize leading to a population of hybrids of up to 20% in certain areas. Our results set the basics for a genetics-informed white anglerfish assessment framework that accounts for stock connectivity, revises and establishes new diagnostic characters for *Lophius* species identification and evaluates the effect of hybrids in the current and future assessments of the white anglerfish.

Key-words: *Lophius piscatorius*, *Lophius budegassa*, genetic connectivity, hybridization, misidentification, SNPs, stock delimitation

Introduction

Sustainable exploitation of fisheries resources relies on effective fisheries management actions, which in turn rely on accurate fisheries assessment, that is, the process of synthesizing information on life history, fishery monitoring, and resource surveys for estimating stock size and harvest rate relative to sustainable reference points (Methot and Wetzel, 2013). The process of fisheries assessment is usually applied independently to pre-established management units (so-called stocks), of which parameters such as growth, recruitment, and natural and fishing mortality are assumed to be intrinsic and not dependent on emigration or immigration rates (Cadrin, 2020). Genetic data has demonstrated ability to delineate populations within a species, that is, to identify groups of sexually interbreeding individuals which possess a common gene pool, but has also revealed hidden phenomena such as species misidentification (Garcia-Vazquez et al., 2012) or hybridization (Pampoulie et al., 2020).

Fisheries stocks are often defined upon political and administrative considerations (Stephenson, 2002); yet, failure to align stocks with natural populations results in unfeasibility to establish an accurate relationship between productivity and harvest rates and can result in local reduction of populations and, in extreme cases, to local population collapse (Bonfil, 2005). Mislabeling can be a common phenomenon when morphologically similar species are caught simultaneously, and results in misled exploitation rate estimations when those are based on reported catches (Marko et al., 2004). Hybridization, the successful reproduction between different species (Arnold, 1997; Stronen and Paquet, 2013), has been reported in teleost fishes (Yaakub et al., 2006; Schwenke, 2013), but incidence and associated consequences in commercial fisheries has been scarcely explored (Epifanio and Nielsen, 2000); yet, hybridization could play a key role in diversity loss and even parental species extinction, which has important consequences for management and conservation (Allendorf et al., 2001). An example of species whose management could largely benefit from genetic-derived information is the white anglerfish (*Lophius piscatorius*, Linnaeus, 1758), a bottom-living fish that moved from being a bycatch species in the last century (Hislop et al., 2001) to become one of the most valuable demersal species in southern and western Europe (ICES, 2010).

Within the Northeast Atlantic, the white anglerfish is managed as three stocks: the Northern Shelf stock (Skagerrak, Kattegat, North Sea, West of Scotland and Rockall), the Northern stock (Celtic Seas and Northern Bay of Biscay) and the Southern Stock (Atlantic and Iberian waters) (ICES, 2019a; ICES, 2019b); yet, the few studies assessing the population structure, based on otolith shape analysis (Cañas et al., 2012), tagging surveys (Laurenson et al., 2005; Landa et al., 2008) and molecular markers, including allozymes (Crozier, 1987), mitochondrial DNA (Charrier et al., 2006) and microsatellites

(Blanco et al., 2008), did not find differences between stocks. However, this needs to be confirmed with the analysis of a large number of genomic markers, which has been effective in resolving the population structure of marine fish when other markers failed (Rodríguez-Ezpeleta et al., 2016; Leone et al., 2019; Rodríguez-Ezpeleta et al., 2019). The white anglerfish coexists with its sympatric sister species, the black bellied anglerfish (*Lophius budegassa*), which has a more southern distribution (Relini, 1999; Ungaro et al., 2002). The outer morphology of both species is similar which is why they are often confused, although the color of the epithelium that covers the abdominal cavity, called peritoneum (white in *L. piscatorius* and black in *L. budegassa*) is considered a unequivocal diagnostic character for individuals larger than 15 cm (Caruso, 1986). Yet, genetic analyses based on polymerase chain reaction amplification of restriction fragment length polymorphisms (PCR-RFLP) used for species identification have revealed mislabeling among the two species (Armani et al., 2012).

There is thus a need for resolving the population structure of white anglerfish within the Northeast Atlantic and for understanding its interaction with its sister species, the black anglerfish. For that aim, we have used thousands of genome-wide distributed SNP markers discovered and genotyped through restriction site associated DNA sequencing (RAD-seq) and found i) that white anglerfish from the three stocks within the northeast Atlantic are genetically connected, ii) first evidence of hybridization between white and black anglerfish and iii) records of black anglerfish misidentified as white anglerfish due to a lack of black peritoneum. These findings have important implications for white anglerfish management and conservation, while revealing the enormous advantage of including genomics in fisheries assessment in general.

Material and methods

Tissue sampling, DNA extraction, and species identification

Lophius piscatorius (n=693) and *Lophius budegassa* (n=31) samples were collected from Northeast Atlantic Ocean and Mediterranean Sea locations using scientific surveys and commercial fisheries (Table S1; Figure 1). Sampling of *L. piscatorius* was carried out so as to cover a large part of the geographic range of this species within the Atlantic, whereas samples of *L. budegassa* were collected opportunistically as they were only used for comparison purposes. Each individual was assigned to either species by the color of the peritoneum, white for *L. piscatorius* and black for *L. budegassa* (Caruso, 1986). Maturity was assigned following the 5 stages key (ICES, 2007). From each individual, a ~1 cm³ muscle tissue sample was excised and immediately stored in 96% molecular grade ethanol at -20 °C until DNA extraction. Genomic DNA was extracted from about 20 mg of muscle tissue using the Wizard® Genomic DNA Purification kit (Promega, WI, USA) following the manufacturer's instructions. Extracted DNA was suspended in Milli-Q water and concentration was determined with the Quant-iT dsDNA

HS assay kit using a Qubit® 2.0 Fluorometer (Life Technologies). DNA integrity was assessed by electrophoresis, migrating about 100 ng of Sybr™ Safe-stained DNA on an agarose 1.0% gel. A polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method (Armani et al., 2012) was used for authentication of all specimens collected. In order to further validate the PCR-RFLP results, for 122 of the samples, a fragment of the cytochrome b (cytb) gene was amplified with the primers GluFish-F and THR-Fish-R (Sevilla et al., 2007) using the following amplification conditions: denaturation at 95 °C for 3 min; 35 cycles at 95 °C for 30 s, 57 °C for 30 s, 72 °C for 60 s; and final extension at 72 °C for 10 min. The PCR products were purified and sequenced using Sanger.

RAD-seq library preparation and sequencing

Restriction-site-associated DNA libraries were prepared for 306 *L. piscatorius* and 27 *L. budegassa* individuals (Table S1) following the methods of Etter et al. (2012). 5 individuals were run by duplicate starting from the tissue sample to check for replicability. Between 300 and 600 ng of starting DNA (depending on integrity) was digested with the *SbfI* restriction enzyme and ligated to modified Illumina P1 adapters containing 5bp sample-specific barcodes. Pools of 32 individuals were sheared using the Covaris® M220 Focused-ultrasonicator™ Instrument (Life Technologies) and size

selected to 300-500 bp with magnetic beads. Following the Illumina P2 adaptor ligation, each library was amplified using 12 PCR cycles and batches of three pools were paired-end sequenced (100 bp) on an Illumina HiSeq4000.

SNP genotype table generation

Generated RAD-tags were analyzed using *Stacks* version 2.4 (Catchen et al., 2013). Quality filtering and demultiplexing were performed using the *Stacks* module *process_rad-tags* removing reads with ambiguous bases and with an average quality score lower than 20 in at least one stretch of 15 nucleotides and using a maximum of 1 mismatch when rescuing single-end barcodes. Only reads whose forward and reverse pair passed quality filtering were kept and the module *clone_filter* was applied to remove PCR duplicates. *De novo* and reference-based assemblies were performed for consistency. For the *de novo* assembly, the module *ustacks* was used to assemble filtered reads into putative orthologous loci per individual, with a minimum coverage depth required to create a stack (parameter -m) of 3, and a maximum nucleotide mismatches allowed between stacks (parameter -M) of 4. RAD-loci were then assembled using the module *cstacks* with a maximum of 6 allowed mismatches between sample loci when generating the catalogue (parameter -n). Matches to the catalogue for each sample were searched using *sstacks* and transposed using *tsv2bam*. For the reference mapped assembly, the filtered reads were mapped against the available draft white anglerfish genome (Dubin et al., 2019) using the BWA-MEM algorithm (Li, 2013), and the resulting SAM files converted to BAM format, sorted and indexed using SAMTOOLS (Li et al., 2009). Mapped reads were filtered to include only primary alignments and correctly mate mapped reads. The following steps were applied to both the *de novo* and reference mapped catalogs including only samples with a minimum of 30,000 RAD-loci. Paired-end reads were assembled into contigs and SNPs derived were identified and genotyped using the module *gstacks*. For each subset of individuals to be analyzed (Table S2), the module *populations* was used to select the tags present in RAD-loci found in at least 90% of the individuals, and *PLINK* version 1.07 (Purcell et al., 2007) was used to select samples with a minimum of 0.85 genotyping rate, and SNPs with a minimum of 0.95 genotyping rate and a minimum allele frequency (MAF) bigger than 0.05. Tags on which the selected SNPs were located were mapped against the complete mitochondrial genome of *Lophius piscatorius* (NC_036988.1) with the BWA-MEM algorithm (Li, 2013) to exclude the possibility that the final genotype dataset contained mitochondrial SNPs.

Genetic diversity, population structure and hybridization analyses

The following analyses were performed using only the first SNP per tag. Expected (H_e) and observed (H_o) heterozygosity and average pairwise F_{ST} values were computed with *PLINK* (Purcell, 2009) and *GENEPOP* (Rousset, 2014) respectively. Principal component analysis (PCA) was performed without any *a priori* assignment of individuals to populations using the package *adegenet* (Jombart and Ahmed, 2011) in R version 3.5.0

(Team, 2018). The genetic ancestry of each individual was estimated using the model-based clustering method implemented in ADMIXTURE (Alexander et al., 2015) assuming from 2 to 5 ancestral populations (K) and setting 1000 bootstrap runs. The value of K with lowest associated error value was identified using the ADMIXTURE's cross-validation procedure assuming from 1 to 5 ancestral populations. Using *NewHybrids* (Anderson and Thompson, 2002), posterior probabilities of each individual's membership as a pure parent, first (F1) or second (F2) generation hybrids or backcrosses were calculated applying default parameters to the 200 SNPs with less than 1% of missing data that presented the highest F_{st} between genetically assigned *L. piscatorius* and *L. budegassa* samples.

Abundance distribution maps

White and black anglerfish catch data from trawl surveys targeting anglerfish (BITS, NS-IBTS, SWC-IBTS, EVHOE, SP-NORTH, SP-ARSA, ROCKALL, PT-IBTS, IE-IGFS, NIGFS, DWS, SCOROC, SCOWCGFS, IE-IAMS, FR-CGFS) conducted between 1995 and 2019 was retrieved from the ICES Database of Trawl Surveys using the icesDatras R library (<https://github.com/ices-tools-prod/icesDatras>). Using information for each haul (latitude, longitude, haul duration and catch weight per species), data was aggregated by five consecutive years and ICES statistical rectangles and converted to g/min per species. For those hauls with catch data as number of individuals instead of weight, weight was estimated using weight-length relationships. For each five-year period and rectangle, g/min per species was plotted in a gradient from white (minimum abundance) to red (maximum abundance) using grey to represent areas where sampling was performed but no catch was reported.

Results

Genetically distinct groups that are not related to sampling location

The genotype table including individuals from both species resulted in 323 and 327 individuals (of which 27 were *L. budegassa*) and 16712 or 23126 SNPs after filtering when using a de novo or reference mapped assembly respectively (Table S2). Replicates resulted in 0.9947% identical genotypes. The PCA and ADMIXTURE analyses based on these datasets grouped most samples in three clearly distinguishable groups and results were virtually identical among the de novo and reference assembly datasets (Figure 2; Figure S1). In the PCA (Figure 2A), all samples except five of them that could not be clearly assigned to any of the three groups, are disposed along the first principal component (accounting for 80.82% of the total variance): the first group includes *L. piscatorius* individuals from all locations, the second group includes *L. piscatorius* individuals from all locations except the Southern Stock, and the third group includes *L. piscatorius* individuals from all locations except the Northern Shelf Stock and *L. budegassa* individuals. These groups are coherent with the different types of genetic

admixture patterns observed, which supports two ancestral populations (Figure 2B; Figure S2): samples classified in Group 1 and Group 3 in the PCA are not admixed and belong to different ancestral populations and samples classified in Group 2 in the PCA are admixed with equal contribution from both ancestral populations. Samples located between Groups 1 and 2 and between Groups 2 and 3 show consistent patterns in admixture analyses with about 75% or 25% of contribution from Groups 1 and 3, respectively.

Misidentification and hybridization

Together, these results indicate that some of the samples that were morphologically identified as *L. piscatorius* (Group 3) are actually *L. budegassa*, which is confirmed by mitochondrial DNA analyses that identify all samples in Group 1 as *L. piscatorius* and all samples in Group 3 as *L. budegassa*. Samples in Group 2 and between this group and the other two have a significantly higher observed average heterozygosity (0.77) than Groups 1 and Groups 3 (0.05 and 0.03, respectively), which could indicate that these individuals are hybrids. This was confirmed by NewHybrids, which assigned all individuals in Group 2 as new generation hybrids between the two species (F1), and the five remaining individuals as backcrosses between F1 hybrids and white or black anglerfish. Mitochondrial DNA from all hybrids except for 3 F1 was of *L. budegassa*. Overall, we found about 4 and 10% misidentified and hybrid individuals, respectively. However,

these were not distributed homogeneously across the study area (Figure 3). Misidentified individuals were more present in the most southern region of the Southern stock (Portuguese coast) and Mediterranean Sea, with 1/3 of the individuals identified as *L. piscatorius* being *L. budegassa*, less frequent in the Northern stock (2%), and absent in Northern Shelf stock. Hybrid individuals were absent in the Southern stock and North Sea (belonging to the Northern Shelf stock) and were most frequent in the northern Bay of Biscay and Celtic seas (13%).

Connectivity of white anglerfish within the Atlantic

The genotype table including only those individuals genetically identified as *L. piscatorius* include 238 or 232 individuals and 6233 or 6246 SNPs when including or not Mediterranean samples, respectively. PCA and ADMIXTURE analyses based on those datasets (Figure 4) reveal strong differentiation between Mediterranean and Atlantic samples ($F_{ST} = 0.057$) and no genetic differentiation within the Atlantic ($F_{ST} = 0$).

Discussion

What's in a white anglerfish sample?

We found that several of the samples provided as white anglerfish (*Lophius piscatorius*) by expert scientists involved in surveys targeting anglerfish were indeed black anglerfish or hybrids. This implies that the commonly used diagnostic character for species identification, the color of the peritoneum, is not discriminative as all the black anglerfish and hybrid individuals provided as white anglerfish had white peritoneum. Indeed, it has already been suggested that some young individuals cannot be distinguished by the color of the peritoneum (e.g. some that show a white peritoneum with small black dots can be assigned to either black or white anglerfish when using another diagnostic character such as the number of dorsal fins rays (J. Landa and A. Antolínez, unpublished data). Here, we found misidentified individuals that are large (up to 75cm) (Figure 5), suggesting that the reason for misidentification is not related to the size of the specimens. In light of these findings, alternative characteristics for species identification are needed (e.g. dorsal and anal fin ray counts or length of the cephalic dorsal fin spines (Caruso, 1986)), so that misidentification does not affect data

collection. Additionally, a way of identifying hybrids would be needed. Notably, in our dataset, the hybrid size distributions was significantly smaller than that of the *L. piscatorius* individuals ($p < 0.05$ in the Mann-Whitney test), although the small sample size of hybrids and confounding factors (e.g. different proportion of hybrids in the different stocks, age classes) prevent drawing conclusions from this fact at this stage.

Causes and consequences the interspecific hybridization

We provide, to the best of our knowledge, the first evidence of natural hybridization between *Lophius piscatorius* and *L. budegassa*, a phenomenon that can occur between closely related species sharing morphological, ecological and reproductive compatibilities (Montanari et al., 2014). *Lophius spp.* hybrids were predominant in Northern Bay of Biscay and Celtic seas, were both species' distribution range overlap and have been historically abundant (Figure 6). There is also overlap in both species' bathymetric distribution (20-1000m and 100-500m depth respectively for white and black anglerfish)(Caruso, 1986; Azevedo, 1995; Quincoces et al., 1998; García-Rodríguez et al., 2005), and in spawning periods, both spawning in winter and spring (Quincoces, 2002.; Ofstad et al., 2013). This overlap in space, depth and time could have even been increased by recent changes of geographical and bathymetric distributions of both species, potentially as consequence of climate change (Maltby et al., 2020). According to mitochondrial DNA, which is maternally inherited in most animals (Moritz et al.,

1987), we found that most hybrids (27 out of 30) resulted from a black anglerfish mother and a white anglerfish father. We found a few backcrosses (hybrids crossed with parental species) and no crosses involving two hybrids. This could be due the presence of a stable hybrid zone with hybrids being less able to produce offspring (Hayden et al., 2010; Mirimin et al., 2014) or to recent changes that have induced hybridization between both species so newly that they did not have time to produce a hybrid-derived population. The presence of a stable hybrid zone between black and white anglerfish does not forcedly imply changes in the evolution of the parental species but could imply management uncertainties. However, if hybridization is recent, we cannot discard a process of evolutionary novelty (Budd and Pandolfi, 2010), through parental species acquiring new functions (Anderson et al., 2009) and even producing new species (Verheyen et al., 2003), or of biodiversity loss, through extinction of parental species (Seehausen, 2006). Thus, monitoring the hybrid zone and adjacent areas is crucial to understand the role of hybridization in management and conservation of white and black anglerfish.

Management implications of stock connectivity, hybridization and misidentification

Our results suggest that the white anglerfish within the Northeast Atlantic Ocean constitutes a genetically homogeneous population, which confirms previous findings based on genetic and non-genetic data (Crozier, 1987; Laurenson et al., 2005; Charrier

et al., 2006; Blanco et al., 2008; Landa et al., 2008; Cañas et al., 2012) and point towards the necessity to harmonize current stock definitions. Morphometric characteristics such as length of maturity (Duarte et al., 2001) or weight-length relationships (Landa and Antolínez, 2018) differ between stocks. These differences are potentially due to sampling biases, differences in scales and/or measurement methods or interannual differences among studies, as well as the possible influence of different fishing pressure or environmental conditions between areas. However, they could also be due to the inclusion of different proportions of misidentified and hybrid individuals in each stock. Although in the Northern and Southern stocks the assessment is done separately for white and black anglerfish, they are combined for management, being Total Allowable Catches (TAC) for *Lophius spp.* In the Northern Shelf stock, the two species are assessed and managed as one assuming that black anglerfish in this area are rare. However, the obvious increase of black anglerfish in this stock (Figure 6) has created concern and suggestions to separate species have been made (ICES, 2018). The deliberate (in the North Shelf stock) or non-deliberate (in the Northern and Southern stocks) inclusion of an unknown (and perhaps variable) proportion of black anglerfish in the white anglerfish assessment will likely lead to some bias. Therefore, some corrections could be applied to the total catches and the length composition data. However, the proportions of black anglerfish included in these assessments seem relatively small (<10%) and would likely not have a major impact on the assessments. The implications of including hybrids in the stock assessment are more difficult to anticipate as biological characteristics of hybrids are unknown. The main concern is that inclusion of hybrids would lead to erroneous inferences of reproductive potential because this feature is inferred from the biomass of mature fish and the reproductive output of anglerfish hybrids is unknown. If, as in many other species (Mallet, 2007), they have no or neglectable offspring, they should not be included in the spawning biomass as otherwise they could lead to an overestimation of productivity, even to a level below of required to support a sustainable fishery (Morgan et al., 2012). On the other hand, if, as the presence of backcrossed individuals suggest, they are reproductively viable, further studies are required to assess hybrid fitness compared to pure individuals.

Despite supporting stock merging, the analyses presented here suggest a series of concerns that should be considered for white anglerfish management and which affect differently each of the stocks. The southernmost locations of the Southern Stock and the Mediterranean Sea are more affected by misidentification, the northernmost locations of the Northern stock are more affected by hybridization, and the North Shelf stock does not seem to be affected by any of them. The over and under representation of misidentified individuals (black anglerfish with white peritoneum) in the south and north respectively could be simply explained by differences in black anglerfish abundance. However, both species are moving northwards, and so the proportion of misidentified individuals could increase in northern areas as black anglerfish becomes more abundant. This northward movement of both species could also contribute to

enlarge the hybrid zone towards northern areas. If the proportion of hybridization and mislabeling varies significantly over time, then this will have implications for the robustness of the assessment for these stocks. Thus, genetic studies monitoring white and black anglerfish populations across the Northeast Atlantic and Mediterranean Sea through time are needed.

Outlook

Our study shows that genetic analyses can be used to confirm or reject existing hypothesis about, for example, stock connectivity but, most importantly, that they can reveal hidden phenomena that were not foreseen, such as the thus far unknown hybridization between white and black anglerfish. Yet, despite the power of genetics to provide fisheries assessment relevant information, there are still barriers for the uptake of genetic data by fisheries management, which can be due to a variety of factors (Ovenden Jennifer et al., 2013; Bernatchez et al., 2017), such as lack of clear communication of genetic concepts by geneticists to end-users and reluctance to change or adapt established assessment and management procedures. Being the white anglerfish a high valuable species (around 30K tons with a corresponding value of around 142 million euro; data extracted from <https://stecf.jrc.ec.europa.eu/dd/fdi>) with a well established data collection, assessment and management frameworks, we anticipate that these results will set the basics for an imminent genetics informed fisheries management for this species, without which the foundations of fisheries science, whereby maximum sustainable yield can only be reached by efficient management of distinct populations, is threatened.

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Supplementary Tables

Table S1. Samples used for this study with collection data (survey, responsible institute, date, location), biological variables measured (morphological identification, depth in meters, length in cm, weight in g, sex and maturity) and molecular analysis information (used for RAD-seq or not, replicate or not, genetic identification and hybrid class).

[excel file available upon request]

Table S2. Number of individuals and SNPs including in the catalog and remaining after each filtering steps for each dataset (All: all individuals included; L. pis: only those individuals genetically identified as *L. piscatorius*; L. pis without MED: L. pis dataset without Mediterranean Sea individuals).

	All				L. pis		L. pis without MED	
	de novo		Ref. mapped		de novo		de novo	
	Inds	Tags/SNPs	Inds	Tags/SNPs	Inds	Tags/SNPs	Inds	Tags/SNPs
Catalog	327	25892	327	35561	241	23301	235	23095
Genotyping rate	323	24346	327	31545	238	21705	232	21579
MAF	323	16712	327	23126	238	6233	232	6246

Supplementary Figures

Figure S1. A. Principal Component Analysis (PCA) where main differentiated groups are circled and number of individuals per group for each stock (*L. piscatorius*) and for *L. budegassa* are indicated. B. Individual ancestry proportions estimated by ADMIXTURE when assuming two ancestral populations. These results are derived from the reference mapped catalog.

Figure S2. Associated error (y axis) for each assumed value of K (x axis) following the cross-validation method described in the ADMIXTURE manual for the de novo mapped dataset. Cross-validation method described in the ADMIXTURE manual suggests that the most likely number of ancestral populations (K) associated with the lowest error rate is 2.