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Mislabelling in megrims: implications for conservation

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Abstract — Mislabelling of fish catch and commercial seafood products is relatively frequent worldwide and can affect fisheries management exploitation when stock estimates are based on landings. In this study we have analyzed genetically 239 commercial lots of two morphologically similar species of megrims (genus *Lepidorhombus*) that are caught together in mixed fisheries. A high proportion of mislabelling was detected, suggesting enormous underreported exploitation of one of the species, which can be endangered if the problem persists. These results highlight the urgency of applying currently available species-specific molecular tools in fisheries sciences for preventing biodiversity losses in exploited species.

Index Terms — exploitation, genetic identification, megrims, mixed fisheries, species-specific markers.

1 Introduction

Industrialized fisheries typically reduce community biomass by 80% within 15 years of exploitation, and, as a consequence, large predatory fish biomass today is only about 10% of pre-industrial levels [1]. Depletion of fish stocks is due to many different factors, some of them anthropogenic. For example, due to factors ranging from climate change [2] to pollution or overfishing, exploited natural populations are in decline in many marine areas. Possible solutions for environmental challenges fall out of the scope of this study. Solutions for overfishing, however, exist and are relatively simple, although they may have a short-term socioeconomic cost for the fishery sector. Some solutions really work, as demonstrated by the recovery of Atlantic herring after its depletion in the late 70s and further implementation of protective measures [3]. Restricted fisheries effort and protection of spawning areas and juveniles are some of the

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possible approaches for allowing natural stocks to recover. However, population estimation techniques are not exact, leading to inaccurate estimates of stock size. Eggs and larvae of different species with overlapping spawning areas are often morphologically similar, and methods of species identification in addition to visual identification are needed for accurate stock assessment [4], [5], [6].

The same problem exists when estimates of fishery effort are based on reported catch data. In some cases, such as for sharks in Hong Kong markets, high concordance between trade and specific names may allow the use of market records for monitoring species-specific trends in trade and exploitation rates [7], [8]. This method, however, cannot be generalized. Sometimes the adults of two species caught simultaneously, for example in trawl fisheries, are so similar that it is difficult to identify them so that mislabelling may occur, as shown for example in hakes [9]. Once mislabelled at landing, the error persists along the entire seafood chain to the consumer, who buys a marketed product which does not correspond to the species marked on the label.

DNA variants can be revealed employing many different techniques. Here it is almost impossible to describe all of them in detail, but some specific examples of molecular techniques used for revealing species-specific variations in fish are listed in Tab 1. They could be useful to fisheries if assessment of trade fish products is used to estimate stock exploitation.

The aim of this study was to analyze in detail a case where application of species-specific markers to fisheries science seems necessary and likely urgent. Species misidentification was detected from landings to commercial products, suggesting underreported exploitation of megrim species (genus *Lepidorhombus*), whose exploitation rates are largely based on catch reports. We also assessed the possible consequences of these likely inadvertent errors for long-term sustainability of fish stocks.

2 Material and methods

2.1 Mixed-fisheries case studies

We have focused our study on two morphologically similar fish species that are caught in different areas of the Atlantic Ocean. In Europe, the two species of megrim, *Lepidorhombus whiffiagonis* (megrim) and *L. boscii* (four-spotted megrim), are flatfishes of the Scophthalmidae family (Pleuronectiformes) having overlapping distributions (Fig. 1).

As with other species, they are caught together in trawl fisheries. Most landings correspond to Spain, followed by the UK, the two countries having together approximately 70% of European catches. In the period 2000-2004, catches of 58,180 tons of *L. whiffiagonis* were reported (FAO catch statistics, available at http://www.fao.org/fishery/statistics/global-capture-production) compared to only 40,187 tons of *L. boscii* (40.85 % of megrim catches). Little is known about population structure and/or population size of megrims, although the existence of a separate stock of *L. whiffiagonis* in the Mediterranean sea has been demonstrated employing genetic markers [18] and differences in growth

among distributional areas have been described [19], [20].

2.2 Samples analyzed

Reference samples for each species (Tab. 2) were obtained in the context of research cruises for the European Project MARINEGGS. The specimens were obtained from at least four different locations covering roughly the Atlantic distribution range of each species and identified by local experts in fish taxonomy. A piece of gill or muscle tissue (about 3 g) was taken from each specimen and stored in absolute ethanol. The reference samples are deposited in the laboratory of the research team at the University of Oviedo (Spain).

Spain was chosen for the survey because it is the top country in megrim fisheries (43% of total landings, FAO catch statistics 2008). Marketed products of both megrim species, labelled with species names, were directly purchased from landings in Asturias (North Spain) in 2004. A total of 239 landings were analyzed. A piece of tissue (approx. 3 g) was taken from each sample and stored in absolute ethanol until analysis.

2.3 DNA analyses

DNA extraction was carried out employing the resin Chelex [21]. For identification of megrims we used differences in sequence length of the PCR-amplified fragment of a conserved locus, the 5S rDNA coding for small ribosomal RNA (5S rRNA), as the species-specific marker. The locus is composed of the coding sequence, typically 120 base pairs (bp) long and highly conserved among species, and the non transcribed spacer (NTS) which can differ in sequence and length among closely related species. PCR amplification of the 5SrDNA locus was carried out in a GeneAmp PCR system 9700 (Applied Biosystems), employing the primers designed by Pendas et al. [22], in a total volume of 20 µl containing 0.5 µl GoTaq Polymerase at 5U/ml (Promega), 2µl of 10x Buffer, 2µl of 25 mM $MgCl_2$, 2µl of dNTPs, 100 pmol of each primer and approximately 5 ng of genomic DNA. PCR amplification conditions were: initial denaturation at 95ºC for 5 min, then 35 cycles of denaturation at 95ºC for 20 s, annealing at 65º for 20 s and extension at 72ºC for 30 sec, and a final extension at 72ºC for 20 min. When agarose methodology was employed, products were run in 2.5% agarose gels at 100 V and visualized by staining with 2 µl ethidium bromide (10 mg/ml). The size of the amplified fragments was estimated by comparison with a standard 100 bp DNA marker (Promega). In the Genetic Analyzer (Sequencing Unit, University of Oviedo), fragment sizes were also directly visualized in a chromatogram employing the GeneScan 3.7 Analysis Software (Applied Biosystems).

3 Results

3.1 Species-specificity of the markers assayed

Species-specificity of the marker was confirmed for the two species studied. All the individuals morphologically identified as belonging to a given species yielded the same genetic pattern. The 5S rRNA locus amplification yielded two DNA fragments of 435 and 217 base pairs (bp) for *Lepidorhombus whiffiagonis* and two main fragments 331 and 233 bp long (plus some secondary heavier shorter fragments) for *L. boscii* (Fig. 2, amplification fragments visualized in an agarose gel).

3.2 *Mislabelling in landings*

A high level of mislabelling was found in the 239 commercial landings analyzed. Although declared landings were 60% *L. whiffiagonis* and 40% *L. boscii*, the actual proportion of each species was 49 and 51% for *L. whiffiagonis* and *L. boscii,* respectively (Tab. 3).

4 Discussion

The 5S rDNA can be considered a good species-specific marker. It has already been used for example in fishes like the Genus *Leporinus* [23], the Sciaenidae family [24] or in shark species [25], and also in many other taxa. The patterns obtained here for both megrims are in concordance with patterns described for this species by Garcia-Vazquez et al. [26]. Mislabelling in these megrim species is likely accidental, as they are morphologically similar and often difficult to separate by visual inspection. The trade price is the same for the two species, therefore intentional mislabelling for purposes of commercial fraud cannot explain the detected differences between declared and real commercialized species. Although inadvertent, this type of mislabelling could however produce serious errors in fisheries assessments. If we assume that the individuals analyzed are representative of landings, the divergence between declared and actual catches would be thousands of tons of megrims (Fig. 3). Figures corresponding to estimated “actual” catch can be obtained based on species content in the commercial landings analysed (in percent), multiplied by the total catch (in tons) of each species.

Another point to consider is the direction of mislabelling, which was deviated incrementing the catch data of *L. whiffiagonis* in a high percentage, and so decreasing the catch data corresponding to *L. boscii*. Underreported exploitation of a species leads to overexploitation and, in the long term, to exhaustion of stocks, fisheries decline and eventual extinction of the overexploited species [27]. For purposes of fisheries management, these data should clearly be taken into account.

Stock sizes are not estimated separately for this two species in annual surveys in their respective area of occurrence. The two *Lepidorhombus* are not

genetically distinguished in routine plankton surveys, although there are recent studies describing species-specific markers for these species [5] that clearly demonstrate that visual identification is not accurate. *L. whiffiagonis* was the only megrim species identified in Bay of Biscay plankton samples [28], and was also the megrim species confounded with hake eggs in other plankton surveys [5]. Absence to date of genetically analyzed *L. boscii* in plankton samples could be interpreted as a signal of its scarcity, but those studies were based on a limited number of samples and cannot be taken as an indicator of real abundance of that species.

Genetic identification of specimens in landings is even more important for species like those studied in this work, whose production in aquaculture is not forecasted at short-term. As demersal species, their cultivation is not easy. For megrims, cultivation assays have not been carried out as far as we know. Thus, although aquaculture seems to be a solution for obtaining seafood protein at a global scale, as for other marine species [29], production of megrim at commercial scale will likely rely on extractive fisheries in the forthcoming years. Application of species-specific markers to fisheries science seems necessary and likely urgent, and stock evaluation based on catch records will require application of genetic markers for improving its utility for sustainable exploitation of these valuable marine species.

5 Conclusion

DNA analysis revealed high percentage of mislabelling in megrim landings. These results suggest underreported exploitation of four-spotted megrim *L. boscii*, a species whose exploitation rates are largely based on catch reports and which could become endangered if the problem persists. We highlight the urgency of applying currently available species-specific molecular tools in fisheries sciences.

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