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Species misidentification in mixed hake fisheries may lead to overexploitation and population bottlenecks

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ABSTRACT

Accurate species identification is required for stock assessment to monitor exploited species. Genetic tools are useful for species identification; employing SNP (single nucleotide polymorphisms) as markers, here we describe two cases of species misidentification which may lead to inaccurate estimates of stock size and adversely affect sustainable hake fisheries management in the Atlantic Ocean. North American hakes and African hakes show evidence of erroneous labelling that may obscure exploitation estimates of the species caught together in mixed fisheries (*Merluccius albidus* and *Merluccius bilinearis*, and *Merluccius capensis* and *Merluccius paradoxus* in North America and Africa respectively). Use of genetic methodology for species identification is recommended for improving accuracy of stock estimates in these two pairs of hakes.

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 (Myers and Worm, 2003). Depletion of marine stocks is due to many different factors, some of them anthropogenic. For example, exploited natural populations are in decline in many marine areas due to factors which include climate change, pollution and overfishing (e.g. O'Brien et al., 2000), as well as illegal fisheries (e.g. Baker and Palumbi, 1994). Inaccurate stock size estimates may also lead to incorrect management decisions and endanger exploited populations in the long term. When eggs and larvae are correctly identified, methods based on egg production are useful for estimating stock sizes in marine species and can provide accurate estimates of sustainable catches, that is, a level of catch that does not endanger the self-sustaining capacity of a population. The problem is that eggs and larvae are not always easy to identify. 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 (Fox et al., 2005; Perez et al., 2005; Von der Heyden and Lipinsky, 2007).

Mislabelling can also mislead estimates of exploitation rates when they are based on reported catch data. In some cases, such as in some sharks, mislabelling is not a problem and high concordance between trade and specific names allows the use of market records for monitoring exploitation rates (Abercrombie et al., 2005). But this method 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 and mislabelling may occur. 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. Mislabelling not only defrauds consumers but could also adversely affect estimates of stock size if it influences the reporting of catch data that are used in fisheries management (Marko et al., 2004).

Genetic methods for species identification can help from the beginning (egg production) to the end (commercial label in stores) of the fisheries lifespan. DNA offers many possibilities from the technical point of view (e.g. Rasmussen and Morrissey, 2008; Teletchea, 2009). The potential utility of species-specific markers in assessment of real fisheries has been explored in some cases. For example, illegal whale hunting was detected employing mitochondrial DNA markers (Baker and Palumbi, 1994). Egg identification in plankton surveys is carried out by means of molecular markers for stock assessment of cod, whiting and haddock in the southern North Sea (Taylor et al., 2002). Another example is sharks, for which trade data in combination with species-specific genetic identification are a good fishery-independent estimate of worldwide catches (Clarke et al., 2006).

The aim of this study was to analyze in detail two cases of mixed hake fisheries where application of species-specific markers to fisheries science seems necessary because the species are mor-

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Table 1
Reference samples employed for testing species-specificity of the markers assayed. Year, sampling year; NL, number of sampling locations; N, total sample size.

Species	Year	NL	*N*
Merluccius albidus	2004	10	30
M. bilinearis	2004	10	30
M. capensis	2001	6	96
M. paradoxus	2001	6	65

phologically similar. Species misidentification has been reported for the North American *M. albidus* and *M. bilinearis* (Garcia-Vazquez et al., 2009), and also for juveniles of the South African *M. capensis* and *M. paradoxus* (Von der Heyden and Lipinsky, 2007). In this study we have identified genetically products of these two mixed hake fisheries, and assessed the possible consequences of inadvertent errors for long-term sustainability of hake stocks.

Materials and methods

Samples analyzed

Reference samples for each species (Table 1) were obtained from research cruises for the European Project MARINEGGS (*M. capensis*, *M. paradoxus*), and from the NMFS NEFSC bottom trawl survey (Azarovitz, 1981) (*M. bilinearis* and *M. albidus*; Garcia-Vazquez et al., 2009). The specimens were obtained from at least four different locations covering roughly the Atlantic distributional range of each species and identified by local experts in fish taxonomy: scientists from the Northeast Fisheries Science Center (NMFS, USA), in charge of North American hake samples; and Dr. Robin Tilney in the Department of Environmental Affairs (Cape Town, South Africa) for South African samples. The species was determined based on morphological characteristics, mainly by the number of gill rakers for North American species (Chang et al., 1999), and by differences in pigmentation of the gill rakers as well as pectoral fin shape (Van Eck, 1969) for South African species. Inada, 1981) for Cape hakesA 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 was chosen for the survey of commercialized hake because it is the largest hake market in the world and imports all hake species (Alheit and Pitcher, 1995). Marketed products of all these species, labelled with species names, were directly purchased from markets from five different Spanish regions (Catalonia, Galicia, Asturias, Valencia and Madrid) between 2005 and 2010. A total of 89 commercial sample lots were analyzed for North American silver hakes (imported from USA providers), and 88 for South African deep-water and shallow-water Cape hakes (imported from Namibian providers). Three specimens (or fillets) were analyzed per lot. North American hakes were whole fish and Cape hakes were frozen fillets. A piece of tissue (approx. 3 g) was taken from each sample and stored in absolute ethanol until analysis.

DNA analyses

DNA extraction was carried out employing the resin Chelex (Estoup et al., 1996). For species identification, we employed seven mitochondrial SNPs (single nucleotide polymorphisms) (Machado-Schiaffino et al., 2008). The SNPs are in the mitochondrial control region and the method is a two-step (nested) amplification approach, in which the control region is amplified first, followed by the SNP assay.

The control region for each sample was PCR amplified using the primers Mmer Hk01 and MmerHk02 (Lundy et al., 2000) on reaction mixtures containing approximately 50 ng of extracted hake DNA template, 10 mM Tris–HCl pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton x-100, 40 pmols of each primer, 1 Unit of Taq DNA Polymerase (Promega, Madison, WI) and 250 μM of each dNTP in a final volume of 40 μL. PCR was performed using the GeneAmp PCR system 9700 (Applied Biosystems) with the following conditions: an initial denaturing step at 95 °C for 5 min, followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 53 °C for 30 s and an extension at 72 °C for 30 s, ending with a final extension at 72 °C for 10 min. Products were quantified in 2% agarose gels using a Low DNA Mass Ladder (Invitrogen). SNaPshot multiplex system (ABI PRISM) was employed to amplify seven SNPs, based on determination of fragment sizes and fluorescent label identity by capillary electrophoresis at multiple SNP sites. PCR SnaPshot assays were carried out on reaction mixtures containing approximately 3 μl of control region *amplicons* (0.2 pmol), 5 μl of SnaPshot reaction mix (Taq Polymerase, Buffer and fluorescently labelled ddNTPs), 1 μl of primers mix (final concentration 0.5 μM) in a final volume of 10 μl. PCRs were performed using the GeneAmp PCR system 9700 (Applied Biosystems) with the following conditions: an initial denaturing step at 96 °C for 3 min, followed by 25 cycles of denaturing at 96 °C for 10 s, annealing at 50 °C for 5 s and an extension at 60 °C for 30 s, ending with a final extension at 60 °C for 1 min. After SNP amplification, a treatment with Alkaline Phosphatase (Calf intestinal, CIP) was applied in order to remove phosphoryl groups from non incorporated labelled ddNTPs. PCR products were incubated with 1 unit of CIP (New England BioLabs) at 37 °C for 1 h followed by 15 min of inactivation at 75 °C. The amplicon size and its fluorescent label were determined with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). SNPs were scored employing the GeneScan software (Applied Biosystems).

Table 2
Nucleotides found for each analyzed species at the surveyed SNPs. Sites are nucleotide positions within the mitochondrial control region. Positions yielding differences useful for differentiating the two species within a pair are marked in bold.

Sites	105	145	157	269	280	370	411
Merluccius albidus	A	**T**	A	C	T	**G**	A
M. bilinearis	A	**G**	A	C	T	**C**	A
M. capensis	T	**G**	A	C	**T**	**G**	A
M. paradoxus	T	**T**	A	C	**C**	**C**	A

Results

Species-specificity of the markers assayed

Species-specificity of the markers, already validated by Machado-Schiaffino et al. (2008), was confirmed here with more individuals of the two species pairs. The seven SNPs analyzed in the control region exhibited species-specific nucleotide differences (Table 2). Three sites were informative for differentiating the species within a pair. The sites 145 and 370 allowed us to distinguish *M. capensis* and *M. paradoxus,* and also *M. bilinearis* and *M. albidus*. The site 280 served to differentiate *M. capensis* and *M. paradoxus* (see Fig. 1).

Mislabelling in Atlantic North American hakes

The level of mislabelling for silver and offshore hakes (Fig. 2) was 20.2% in total, with 12.2% of lots erroneously labelled and 8% incomplete. All of these analyzed North American hakes sold in Spain had been named *M. bilinearis* (or just "North American hakes"), although 12.2% were really *M. albidus* (Table 3).

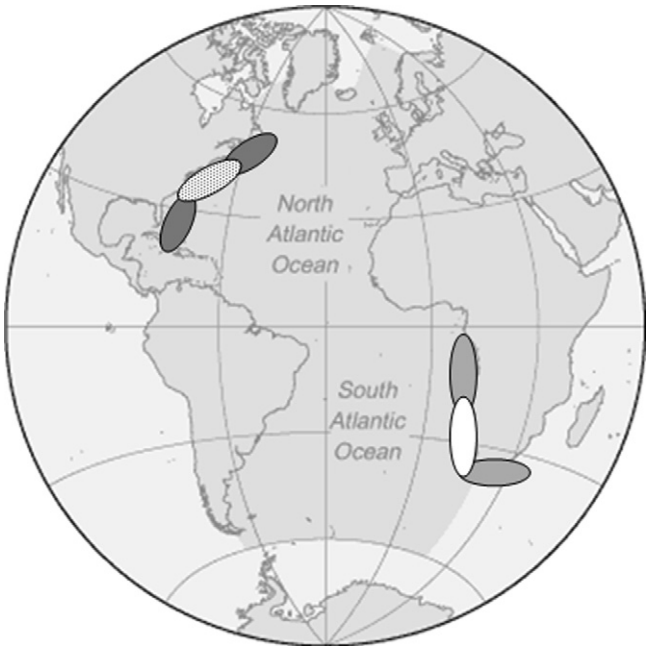

Fig. 1. Map showing fishing areas of the market samples of the two pairs of species analyzed in this study. Dotted – overlapping area and dark grey, North American hakes *Merluccius albidus* (south) and *M. bilinearis* (north); white – overlapping area and light grey, South African hakes *Merluccius capensis* (north) and *M. paradoxus* (south). The species in each pair overlap in these areas.

Mislabelling in African Cape hakes

The proportion of commercial lots of Cape hakes correctly labelled (as *M. capensis* or *M. paradoxus*) was 39.4%. Almost 31% were incompletely (*Merluccius* spp.) or ambiguously (*M. capensis*/*M. paradoxus*, "African hake") labelled, and 29.4% were commercialized under incorrect species names (Fig. 2). From labels (Table 3), *M. capensis* represented 54% of the African hakes commercialized in Spain while *M. paradoxus* comprised the rest (46%). However, the great majority (85%) of marketed Cape hake was actually *M. paradoxus*.

Discussion

The level of mislabelling was different in the two groups of species considered in this study, higher in African and lower in North American hakes. However the potential implications for fisheries science are similar, because in the two cases there was a noticeable difference between estimated catches and actual marketed products.

Mislabelling in these hakes is likely accidental (e.g. Garcia-Vazquez et al., 2009), as the species in each pair are morphologically

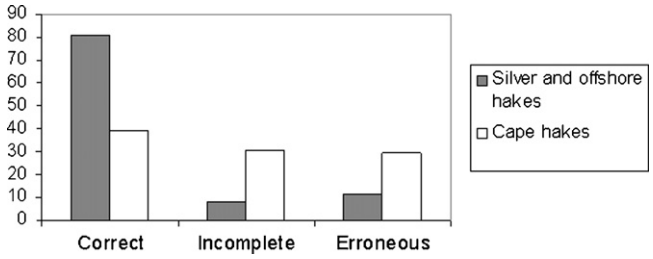

Fig. 2. Mislabelling in marketed seafood of the two groups of species considered (Spanish markets). Percent of lots with correct, ambiguous or incomplete, and erroneous labels for species name/s, for each group of species. North American and Cape hake pairs are represented in grey and white, respectively.

Table 3
Percent of commercial lots of each species within a group (Atlantic North American hakes *Merluccius bilinearis* and *M. albidus*, 89 lots; South African hakes *M. capensis* and *M. paradoxus*, 88 lots), identified from market labels and from genetic markers.

Species	Identification based on labels	Identification based on genetics
Merluccius bilinearis	100	87.8
M. albidus	0	12.2
M. capensis	53.9	14.6
M. paradoxus	46.1	85.4

similar and often difficult to separate by visual inspection. Although probably inadvertent, this type of mislabelling could produce serious errors in stock estimates and should be considered for purposes of fisheries management. Stock size estimates for Atlantic North American hakes are based on landings and on annual NMFS NEFSC bottom trawl surveys (Azarovitz, 1981) where specimens are taxonomically identified based on morphological traits and some misidentification has been recently found (Garcia-Vazquez et al., 2009). On the other hand, Cape hake stock sizes have been estimated by diverse methods that include catch rates (e.g. Punt, 1994). It is evident that errors will be introduced in such estimators if catches are not well identified. If a similar level and direction of misidentification found in our modest sampling (Table 3) occurred in the total catch of each pair of species, the divergence between declared and actual catches would be thousands of tons. Application of species-specific molecular markers in catch estimates and population surveys should be therefore considered for more accurately estimating stock sizes of the two pairs of species studied here. This re-estimation is even more important when signals of population depletion have already been detected, as in the case of *M. paradoxus* (Von der Heyden et al., 2007). Those signals had been detected based on genetic markers, which imply that a population bottleneck occurred in the past and cannot be directly attributed to current overfishing; in any case, warning signals reinforce the need for accurate population estimates for improved management.

The implications of much larger landings for *M. albidus* and *M. paradoxus* than expected are negative for these two species, and may be positive for the other species caught with them in mixed fisheries. For the most exploited species within a pair, if such trend is sustained over time, reduced population size would encompass losses of genetic variability, likely inadvertent selection for size and other adverse effects of overfishing (e.g. Myers and Worm, 2003). In contrast, the other species in the pairs that, in response, experience a lower fishing mortality (*M. bilinearis* and *M. capensis*) would exhibit population recovery. In a hypothetical long-term situation of mixed fisheries, increasing the catch of such recovered (and more abundant) species would in turn imply diminished catch of the formerly more fished species, and so on. Theoretically, such mixed fisheries would reach a cycle of alternate higher exploitation of one or the other species. However, if catches are inadvertently too unbalanced, one species may decline drastically and lose variation, and may even collapse. Lost diversity cannot be recovered unless new variants come from neighboring populations or arise by mutations—an expectedly much longer time frame for fisheries management.

Genetic identification of specimens in landings is even more important for species like the ones studied here, where aquaculture production is not forecast in the short-term. As demersal species, their cultivation is difficult. The main challenges for hake production in captivity are rearing conditions and establishment of feeding (Bjelland and Skiftesvik, 2006). Thus, although aquaculture seems to be a solution for obtaining seafood protein at a global scale, as in other marine species production of hake at a commercial scale will likely rely on extractive fisheries in the forthcoming years

(Goldburg and Naylor, 2005). However, stock evaluation based on catch records will require application of genetic markers for improving its utility for sustainable exploitation of these valuable marine species.

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References

Abercrombie, D.L., Clarke, S.C., Shivji, M.S., 2005. Global-scale genetic identification of hammerhead sharks: application to assessment of the international fin trade and law enforcement. Conserv. Gen. 6, 775–788.

Alheit, J., Pitcher, T.J., 1995. Hakes: Biology, Fisheries and Markets. Chapman and Hall, London.

Azarovitz, T.R., 1981. A brief historical review of the Woods Hole laboratory trawl survey time series. Can. Spec. Publ. Fisheries Aquat. Sci. 58, 62–67.

Baker, C.S., Palumbi, S.R., 1994. Which whales are hunted? A molecular genetic approach to monitoring whaling. Science 265, 1538–1539.

Bjelland, R.M., Skiftesvik, A.B., 2006. Larval development in European hake (*Merluccius merluccius* L.) reared in a semi-intensive culture system. Aquat. Res. 37, 1117–1129.

Chang, S., Berrien, P.L., Johnson, D.L., et al., 1999. Offshore Hake *Merluccius albidus*, Life History and Habitat Characteristics. NOAA Technical Memorandum NMFS-NE-130, pp. 1–24.

Clarke, S.C., McAllister, M.K., Milner-Gulland, E.J., et al., 2006. Global estimates of shark catches using trade records from commercial markets. Ecol. Lett. 9, 1115–1126.

Estoup, A., Largiader, C.R., Perrot, E., et al., 1996. Rapid one-tube DNA extraction protocol for reliable PCR detection of fish polymorphic markers and transgenes. Mol. Mar. Biol. Biotech. 5, 295–298.

Fox, C.J., Taylor, M.I., Pereyra, R., et al., 2005. TaqMan DNA technology confirms likely overestimation of cod (*Gadus morhua* L.) egg abundance in the Irish Sea: implications for the assessment of the cod stock and mapping of spawning areas using egg-based methods. Mol. Ecol. 14, 879–884.

Garcia-Vazquez, E., Horreo, J.L., Campo, D., Machado-Schiaffino, G., Bista, I., Triantafyllidis, A., Juanes, F., 2009. Mislabelling of commercial North American hakes suggests underreported exploitation of *Merluccius albidus*. Trans. Am. Fish. Soc. 138, 790–796.

Goldburg, R., Naylor, R., 2005. Future seascapes, fishing, and fish farming. Front. Ecol. Environ. 3, 21–29.

Inada, T., 1981. Studies on the Merluciid fishes. Bull. Far Seas Fish. Res. Lab. 18, 1–172.

Lundy, C.J., Rico, C., Hewitt, G., 2000. Temporal and spatial genetic variation in spawning grounds of European hake (*Merluccius merluccius*) in the Bay of Biscay. Mol. Ecol. 9, 2067–2079.

Machado-Schiaffino, G., Martinez, J.L., Garcia-Vazquez, E., 2008. Detection of mislabelling in hake seafood employing mtSNPs-based methodology with identification of eleven hake species of the genus *Merluccius*. J. Agric. Food Chem. 56, 5091–5095.

Marko, P.B., Lee, S.C., Rice, A.M., et al., 2004. Fisheries: mislabelling of a depleted reef fish. Nature 430, 309–310.

Myers, R.A., Worm, B., 2003. Rapid worldwide depletion of predatory fish communities. Nature 423, 280–283.

O'Brien, C.M., Fox, C.J., Planque, B., et al., 2000. Fisheries: climate variability and North Sea cod. Nature 404, 142.

Perez, J., Alvarez, P., Martinez, J.L., et al., 2005. Genetic identification of hake and megrim eggs in formaldehyde-fixed plankton samples. ICES J. Mar. Sci. 62, 908–914.

Punt, A.E., 1994. Assessments of the stocks of Cape hakes *Merluccius* spp. off South Africa. S. Afr. J. Mar. Sci. 14, 159–186.

Rasmussen, R.S., Morrissey, M.T., 2008. DNA-based methods for the identification of commercial fish and seafood species. Compr. Rev. Food Sci. Food Saf. 7, 280–295.

Taylor, M.I., Fox, C., Rico, I., Rico, C., 2002. Species-specific TaqMan probes for simultaneous identification of (*Gadus morhua* L.), haddock (*Melanogrammus aeglefinus* L.) and whiting (*Merlangius merlangus* L.). Mol. Ecol. Notes 2, 599–601.

Teletchea, F., 2009. Molecular identification of fish species; reassessment and possible applications. Rev. Fish Biol. Fish. 19, 265–293.

Van Eck, T.H., 1969. The South African hake: '*Merluccius capensis*' or '*Merluccius paradoxus*'? S. Afr. Shipp News Fish Ind. Rev. 24, 95–97.

Von der Heyden, S., Lipinsky, M.R., 2007. Species-specific genetic markers for identification of early life-history stages of Cape hakes *Merluccius capensis* and *Merluccius paradoxus* in the southern Benguela Current. J. Fish Biol. 70 (Suppl. sb), 262–268.

Von der Heyden, S., Lipinski, M.R., Matthee, C.A., 2007. Mitochondrial DNA analyses of the Cape hakes reveal an expanding, panmictic population for *Merluccius capensis* and population structuring for mature fish in *Merluccius paradoxus*. Mol. Phylogen. Evol. 42, 517–527.