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Genetic markers in marine fisheries: Types, tasks and trends

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ABSTRACT

Thirty years of industrial fishing have led to overexploitation of many species, triggering the urgent need to better assess and manage marine stocks for sustainability. From the very simple definition of stock as "an exploited fishery unit" to its complex delimitation using ecosystem approaches, genetic approaches provide a large number of potential markers to unveil the underlying molecular background of complex biological phenomena in marine species.

The present study is a diagnostic assessment of the number of scientific data published in marine fisheries using genetic technologies since the onset of NGS up to 2014. This state of the art approach would allow to derive causalities of real figures and to hypothesize trends in the use and applications of genetic markers. We first briefly overview pros and cons of current technologies used to delineate fish stocks and address intrapopulation knowledge deficits identified under an open population paradigm. Then, in order to assess the advancement of the application of molecular genetic techniques in "fish stock delineation" we review the work published on spatio-temporal structures of fish stocks. Finally, the datamining effort is enlarged across marine fisheries during the period 2004–20014 to decipher the intensity of use of genetic tools per taxa, the main application of each marker type and the trend of use of classic, modern and new generation techniques in fishery research tasks. Although microsatellites and mitochondrial DNA-based markers have being used at a growing pace in fishery research tasks until 2011, a time when application of gene sequencing and NGS-derived markers to fishery research has significantly grown. NGS technologies are underused in fishery science regarding their wide application in other research fields such as agriculture and aquaculture. So a significant increment of new genetic marker types is expected to be implemented in the near future. Conversely, application of classic marker types such as allozymes, AFLPs, whole mitochondrial genomes, RAPDs and RFLPs on mtDNA has been much less used in the period 2004–2014 following the advent of fast and cheap genotypic screening and NGS-derived markers. Power gain afforded from the combination of different genetic markers to address complex processes is lacking in current fishery research since only 21% of the studies over that period employed two genetic marker types and only 3% employed more than two.

Combinations of methods would allow addressing management issues such as distribution shifts and population expansion in response to climatic fluctuations and global change, as well as overfishing and fishery collapses.

1. Introduction

In this paper we briefly review the stock concept, recap on physical and phenotypic delineation tools before briefly outlining classic genetic approaches including next generation approaches. We later undertake a data-mining project to chart progress in the period 2004–2014 regarding the number of studies and markers and the intensity of use of each marker upon applications and marine taxa. We conclude by discussing trends and challenges in the application of genetic methods in fisheries research.

1.1. *The stock concept*

Delineation of management biological units is essential for the optimal exploitation and sustainability of fish stocks (Cadrin et al., 2014a). Accordingly, delimitation of fish stocks has been much discussed but less efficiently addressed over the last decades. With

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some exceptions, fish management units continue being based on territorial jurisdictions better than on biological evidence (e.g. Maguire et al., 2006; Reiss et al., 2009). The prevailing widely used definition of a fish stock in fisheries management as a "subset of one species having the same growth and mortality parameters, and inhabiting a particular geographic area" (Sparre and Venema, 1998) reflects practical administrative necessities rather than biological reality. This definition oversimplifies the many complex processes involved in shaping the ecology and genetics of populations such as exchange between sub-population units and biological interactions.

The concerns have prompted the need for multidisciplinary approaches to properly unveil the nature of an exploited population, particularly whether is a closed single reiterative selfish spawning aggregation with little or no external connection or part of an open metapopulation (Booke, 1999). The metapopulation as a population of populations (Levins, 1969) advanced classic models of population dynamics, including better understanding of gene flow between adjacent populations. Present day perceptions of fish metapopulations as a swarm of open subpopulation units which genetic background and structuring is temporal unstable within fish grounds (Pita et al., 2011) is gaining traction, not only from a fishery management perspective but also from the wider multidisciplinary comprehension of how connectivity operates in the marine realm. Connectivity is "the demographic linking of local populations through the dispersal among them of individuals as larvae, juveniles, or adults. Successful dispersal requires that individuals move between populations and become successfully incorporated into the recipient population" (Sale et al., 2005). While connectivity is conceptually assessable, knowledge of its spatio-temporal pattern within metapopulations as well as on its causative forces are far from being concretized for marine species (Cowen et al., 2006). In order to prevent mismanagement of different fish stocks as a single management unit (Waldman, 1999) or vice versa (Ying et al., 2011) it is advisable to properly assess them with all the tools so far available (Antoniou and Magoulas, 2014), a task that the continuous scientific and technological advancement is beginning to permit nowadays.

1.2. Physical and phenotypic delineation tools

Mark and recapture method were one of the first methods used to track fish movements and to approach the dynamics of fish populations (Hall, 2014). Those methods rely on external or internal, physical or electronic, tags useful to assess fish movements after tagged individuals are released and recaptured. Migration information from physical tags is limited to end points of the process, i.e. release location and recapture location, so little can be usually inferred on the unrecorded pathway followed by fishes during their free tagged-life span. For instance, migration in the Atlantic cod (*Gadus morhua*) was inferred in Newfoundland after observing the displacement pattern of those individuals that were fished and tagged (external cellulose nitrate and aluminum disc bachelor-button tag, and internal cellulose nitrate tag combined with a slit in the body wall) in the north of the Strait of Belle Isle through the Gulf of St. Lawrence to Newfoundland and near Cabot Strait once they were released (Templeman, 1974, 1982).

This method can also be used to estimate growth rates in different species. For instance, physical tagging (FF-94 Floy® anchor tags) of the European hake (*Merluccius merluccius*) prompted the fast-growth hypothesis for this species (Piñeiro et al., 2007) leading to revision of previous demographic properties of this fishery in the NE Atlantic. Since this method can affect the species growth and increase its mortality rate, its success depends on an extensive tagging effort to ensure good records of recaptured individuals (Hall, 2014), on advertising campaigns to raise awareness among fishermen as to maximize return, and advertise a monetary reward in exchange for returned tagged individuals (Piñeiro et al., 2007).

There are, however, still issues that cannot be resolved by classical mark and recapture methods in open populations, such as intermediate steps of stocks movement from one area to another, including the traceability of progenies from spawning grounds to nursery areas and therefore complementary satellite tracking is badly required to trace the displacement of stocks and to confirm their inner dynamics. Conversely, electronic tags allow tracking of fish movements during the free tagged life span, irrespective of the feasibility of their recapture. Such information on fish movements is, however, restricted to species dwelling or visiting the sea surface to emit reliable satellite signals. For instance, the attachment of electronic tags to adults and sub-adults of cuttlefish (*Sepia officinalis*) has allowed the identification of migratory patterns in wild populations of the English Channel (Bloor et al., 2013). Also, compilation of available data from the use of satellite-tags in whale sharks (*Rhincodon typus*) revealed large migrations across the three main oceanic basins (Sequeira et al., 2013). Although regional mark-recapture studies and electronic chips are useful to elucidate fish movements and precise homing or schooling mechanisms (i.e. the internal dynamics of a geographic subpopulation) the integration of data from all the fishing grounds inhabited by the species is necessary for understanding the structure of each stock and the whole metapopulation its belongs to (e.g. dolphinfish, *Coryphaena hippurus*, Merten et al., 2014). Thus extensive collaboration and spatial coverage is essential for implementation of such approaches.

Parasites have been proven to be applicable as biological markers in fish stock delineation since middle of the last century (e.g. Margolis, 1963). Fishes are infected by specific parasites of an endemic area so it is potentially feasible to identify stocks that live or have lived in that area (MacKenzie and Abaunza, 2014). For instance, significant differences in prevalence, infection intensity and abundance of a metacercarial parasite found between South African sardine populations (*Sardinops sagax*) separated by Cape Agulhas suggested the existence of two putative stocks in that area (Weston et al., 2015). Strengths and weaknesses of this technique are widely discussed in previous reviews (Catalano et al., 2014; MacKenzie and Abaunza, 2014) and a general concern exists about the importance of combining parasites with molecular techniques. Combined multidisciplinary information can allow to identify cryptic parasite species and to detect how recent demographic changes of stocks can modify the delineation signal afforded from parasites.

Body size and shape and calcified structures have also been used to infer population structure in marine fishes using meristic characters (i.e. standard length, total length, head length, etc.) or morphological landmarks (i.e. center of eyes, fin insertion points, otolith outline, etc.) (see Cadrin et al., 2014b). Those phenotypic techniques have been successfully applied to check stock structuring within species (e.g. stocks of the redfish *S. mentella* in the Irminger Sea Trella et al., 2013) or to discriminate between sister species e.g. three sympatric hake species *Merluccius australis*, *M. hubbsi* and *M. patagonicus* Lloris and Matallanas, 2003) in Argentine waters (Díaz de Astarloa et al., 2011). Also, ultra-morphologic studies have been applied to characterize stock-specific gametes such as in male gametes of *Perumytilus purpuratus* where significant differences of location and length of acrosomes as well as the acrosome/head ratio served at separating northern and southern South Pacific stocks of this species (Briones et al., 2012).

The longstanding estimation of age and growth of fish species from calcified structures (i.e. scales, otoliths, etc.) for assessment purposes goes back over 100 years and has also proved to be useful in stock delineation tasks (e.g., Brophy, 2014). The aggregation pattern of otolith rings from many commercial fishes has been used to estimate growth rates (e.g. the European hake, *M. merluccius* Otxotorena et al., 2010; Pattoura et al., 2015; the Atlantic

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