Stock discrimination and connectivity assessment of yellowfin seabream (Acanthopagrus latus) in northern South China Sea using otolith elemental fingerprints

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Abstract

Connectivity between fish stocks is fundamental to the understanding of population dynamics and the implementation of sustainable fisheries management. Otolith microchemistry is a promising tool as it can provide information on the continuous growth of otoliths and the environmental effects on otolith composition. Such elemental fingerprints can help distinguish different stocks or life history stages, identify the origins or nursery areas of fish, and assess population structure. In this study, we examined the stock discrimination and spatial connectivity of cage-cultured and wild stocks of yellowfin seabream (Acanthopagrus latus) from the coastal waters of Shantou, Yangjiang, and Zhanjiang in China southern province Guangdong during 2012–2014, based on otolith trace-elemental signatures using multivariate statistical analysis and machine learning approaches. The concentrations of 13 elements (7Li, 23Na, 24Mg, 40Ca, 55Mn, 56Fe, 59Co, 59Ni, 64Cu, 65Zn, 88Sr, 122Sb, and 137Ba) in the natal spot of fish otoliths, representing the embryonic and paralarval stages of fish, were analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Stepwise discriminant analysis and random forests were used to distinguish the cultured and wild stocks of yellowfin seabream, and non-metric multidimensional scaling (NMDS) and cluster analysis were used to determine the spatial variation and connectivity of yellowfin seabream stocks. Overall, the cultured and wild stocks of yellowfin seabream could be identified with classification accuracy of 80.7% and 99.2% by using stepwise discriminant analysis and random forests respectively. When we compared site difference between cultured and wild stocks (site × stock interactions), the classification success was 60.4% for stepwise discriminant analysis and 85.7% for random forests. The misclassification of cultured and wild stocks within the three sites suggested the spatial connectivity between stocks and among sampling locations. Our findings suggested that the three wild stocks of yellowfin seabream from Guangdong coastal waters could be considered as one unit for management, and the difference between cultured and wild stocks was significant for yellowfin seabream from Shantou and Yangjiang, but less significant for yellowfin seabream from Zhanjiang. This study demonstrated that otolith elemental fingerprints can help improve our knowledge on the spatial connectivity, population structure, and life history of fish stocks, and random forests can be a useful tool for identifying cultured and wild stocks compared to the traditional stepwise discriminant analysis.

1. Introduction

Fish stocks are generally considered as the fundamental units in fisheries management. Marine fish species often exist as mixed stocks because of the complexity of ocean conditions and fish life history (Bacha et al., 2014). Stock identification has been used in fisheries for classifying mixed stocks and tracking species movement or migration. Successful discrimination of stocks is critical for investigating population dynamics and trophic interactions with other marine resources (Pita et al., 2016), and defining stock
boundaries (Cadrin, 2000; Ataa et al., 2017). Stock management based on stock discrimination is increasingly used for providing guidance on fishery resources assessment, optimization of fishing efforts on stocks at spatial and temporal scales, and evaluation of management strategies (Pita et al., 2016). For example, being able to classify mixed spawning stocks can help identify the origins of fish caught, quantify the proportions of different stocks, and improve cross-area fisheries management. Therefore, fish stock discrimination is of long-term importance in understanding fish population structure and dynamics, and developing sustainable fisheries management (Thresher, 1999).

There have been phenotypic and genetic techniques used for fish stock identification to track individual movements and discriminate mixed stocks. Fish otolith is often considered as a good proxy for stock identification or the ontogenetic life history, because it is metabolically inert and the trace elements accumulated to the growing otoliths can reflect species' ambient aquatic environment, especially when populations are just separated by geographic factors or life stages rather than being genetically different (Campana, 1999; Thresher, 1999; Campana et al., 2000). The morphology and microchemistry of fish otoliths have been useful tools for fish stock discrimination, as these otolith characteristics can show intra-specific difference between stocks, which is normally caused by the variation of environmental factors, such as water temperature, trace elements, salinity, dissolved oxygen, and inhibiting water depth (Cardinale et al., 2004). Otolith microchemistry, that is, the elemental composition of otoliths based on the continuous growth and elemental intake of otoliths during the lifetime of fish, provides valuable information on fish's inhabiting environment, therefore can serve as an efficient surrogate of fish life history spent in different aquatic environments, and a tag of stocks (Campana et al., 2000; Halim et al., 2017).

Overexploitation on marine resources and anthropogenic influences on the key habitats of ecologically or economically important species have led to the decline of fisheries resources in China coastal regions, and the declined fisheries resources have negative impact on the recruitment of fish species important to maintaining the resilience of coastal ecosystems. China has put increasing efforts on the restoration of marine resources in coastal regions, mainly through fish stocking and enhancement for maintaining resources and marine protected areas for habitat restoration. The effectiveness of fisheries enhancement is what fishermen and stakeholders are concerned about when stocking and enhancement are carried out for economically important species, such as salmon, flounder, lobster, shrimp, and urchin. Identification of released cultured fish from the wild or mixed stocks is crucial for assessing the effectiveness of stock enhancement and evaluating the biological interactions among released fish, wild fish, and their predators or competitors. Fish marking techniques based on otolith fingerprints using tetracycline (TC), alizarin complexone (ALC), or thermal marking have been utilized for distinguishing released and wild stocks (Wright et al., 2002; Katayama and Ishikii, 2007). Successful discrimination between released fish and wild fish can help improve the efficiency of stock enhancement and fisheries restoration, and provide guidance to fisheries stakeholders to support decision-making (Taylor et al., 2005).

As one of the most important fisheries species for both cage-culturing and marine fishing in coastal waters along the northern South China Sea, wild populations of yellowfin seabream (Acanthopagrus latus) have severely declined because of overfishing and habitat loss (Xia et al., 2008; Ismail and Haron, 2017). Stock-enhancement programs have been conducted for yellowfin seabream in coastal waters of the northern South China Sea since 2005, and there is a need for assessing the effectiveness of stocking and enhancement, as the cultured stocks often mix with wild stocks during the fish landing. In this study, we applied multiple modeling approaches to investigate if the natal spot of fish otoliths can be used as a reliable tool for discriminating the cultured and wild stocks of yellowfin seabream and understanding the spatial connectivity of different stocks. By evaluating the applicability and sensitivity of different approaches, we hope to provide more effective and practical techniques to discriminate fish stocks, and to quantify the contribution of stock-enhancement programs to the rebuilding of coastal fishery. Such techniques could serve as bases for the development of a more efficient batch marking method for stock discrimination and for the tactical management of coastal ecosystems.

2. Material and methods

2.1. Sample collection

Three coastal areas (Shantou, Yangjiang, and Zhanjiang) along the northern South China Sea were selected for this study based on the availability of both cultured and wild stocks of yellowfin seabream (Fig. 1). A total of 119 fish samples were collected from the study areas during 2012–2014, for which the cultured stocks were from the cages in coastal waters and the wild stocks were from the catches using bottom trawls or gillnets. All the sampled fish were measured in situ for body length (BL) and body weight (BW), with a size range of 68.3–239.6 cm and a weight range of 13.0–433.4 g (Table 1). The sagittal otoliths were removed, washed...
using ultra-pure water, and then stored in microcentrifuge tubes with 90% alcohol. The sex of fish was not considered in this study, because yellowfin seabream is a protandrous hermaphrodite (Li and Ou, 1999), and previous studies suggested that sex did not have significant influence on otolith elemental signatures for fish such as roundnose grenadier, Coryphaenoides rupestris (Longmore et al., 2010).

2.2. Otolith preparation and trace elements analysis

As there is typically no significant difference in elemental signatures between the right and left otoliths from the same fish (Rooker et al., 2001; Longmore et al., 2010), the right otolith of each sampled fish was taken, washed using ultra-pure water, and dried overnight in a drying oven at 40 °C. Each otolith was firstly put on a resin bed, and then poured with araldite epoxy resin to generate a solid resin block that enclosed the otolith. Each resin block was encoded and labelled corresponding to the fish sample, and cured for the block was solid for further analyses (Longmore et al., 2010). Each of the solid blocks encasing the otoliths was grounded on a plane using waterproof sandpaper (600, 800, 1200, and 2000 grit) to the nucleus area, and then the block was turned over and grounded to the nucleus area from the other side. Finally, the otolith sample was polished using a diamond lapping board with alumina powder for the following elemental analysis. To minimize the confounding effects of elemental signatures from different testing spots of otoliths due to ontogeny, each otolith sample was measured at the natal centre, representing the original information on the early development of individual fish.

The concentrations of 13 trace elements (7Li, 23Na, 24Mg, 40Ca, 54Mn, 55Fe, 56Co, 56Ni, 64Cu, 65Zn, 88Sr, 122Sb, and 137Ba) in the natal spot of each otolith sample were measured using New wave UP-213 laser ablation system coupled to Agilent 7700a ICP-MS (LA-ICP-MS). The 13 elements were chosen for analysis in this study because this suite of trace elements best represents the microchemical characteristics of yellowfin seabream otoliths and typically exhibits relatively stable concentrations within detection limit of the measuring equipment. The laser ablation procedure for the natal spot of each otolith includes 20-s sample signal and 20–30-s control signal, and the ablation parameters were 30 μm for the spot size and 5 Hz for the laser pulse. Helium-argon mixtures (0.7 L/min helium and 0.8 L/min argon) were used as aerosol carrier to enhance the sensitivity of the ablation system. Data were processed and the concentrations of 13 elements were calculated for each otolith sample using ICPS DataCal software.

2.3. Statistical analysis

Both traditional multivariate statistical analysis and machine learning approaches were used to distinguish the cultured and wild stocks of yellowfin seabream collected from coastal waters of the northern South China Sea. Stepwise discriminant analysis and random forests were compared for their abilities in discriminating the cultured and wild stocks of yellowfin seabream. Stepwise discriminant analysis is a traditional multivariate statistical approach with the assumptions of multivariate normality and linearity and works well when data have covariation among predictors (McCune and Grace, 2002), whereas random forests is a machine learning approach with no assumptions on the relationships between predictors and can give information about the relation between variables and classification (De’ath, 2007). Non-metric multidimensional scaling (NMDS) and cluster analysis were used to examine the spatial variation and connectivity of different yellowfin seabream stocks. To provide equitable comparisons, all the variables were standardized prior to conducting statistical analyses. All analyses were conducted in the R statistical language (R Development Core Team, 2017), except that stepwise discriminant analysis was performed using SPSS 22.

3. Results

3.1. Elemental signatures in otolith natal spot of yellowfin seabream

As 40Ca is the most abundant element in the natal spot of fish otoliths with a near-constant value, the concentrations of other elements are usually expressed as their ratios to 40Ca. Overall, the ratios of trace elements to 40Ca in the otolith natal spot of yellowfin seabream ranged 9.28 × 10⁻³–4.62 × 10² μmol/mol. 24Mg, 23Na, 88Sr, and 56Fe were the four most abundant elements besides 40Ca, with concentrations two or three orders of magnitude higher than 137Ba, 65Zn, 55Mn, and 7Li (Table 2). The ratio of 24Mg to 40Ca was the highest among the elements measured in this study, with a value of 1.56 × 10⁶ μmol/mol, followed by 23Na (8.09 × 10⁵ μmol/mol), 88Sr (3.39 × 10² μmol/mol), and 56Fe (1.32 × 10² μmol/mol).

3.2. Relative importance of elements in stock discrimination of yellowfin seabream

Stepwise discriminant analysis distinguished the cultured and wild stocks of yellowfin seabream with the classification accuracy of 80.7% (Table 3 and Fig. 2). The absolute values of standardized canonical coefficients in the final discriminant function indicated the relative importance of elements in distinguishing different stocks. 137Ba, 24Mg, 7Li, and 56Co were the four elements suggested by stepwise discriminant analysis that showed the greatest contribution towards classifying the cultured and wild stocks (explained 67.5% of the variation, p < 0.01), with standardized canonical coefficients of −0.82, 0.47, 0.40, and 0.28.

Random forests distinguished the cultured and wild stocks of yellowfin seabream with the classification accuracy of 99.2% (Table 3). Random forests performed better than stepwise discriminant analysis for the stock discrimination of yellowfin seabream, and indicated that 137Ba, 24Mg, and 23Na contributed most to distinguishing the cultured and wild stocks, although their relative importance varied with the spatial scales of the analyses (Table 4). Random forests suggested that 137Ba, 59Co, 23Na, and 24Mg ranked as the most important elements for classifying different stocks when the three study areas were considered together, whereas...
137Ba, 55Mn, 24Mg, and 23Na showed the greatest contribution to stock discrimination for pairwise comparisons between areas.

3.3. Stock discrimination and spatial connectivity of yellowfin seabream

When we compared site difference (coastal waters of Shantou, Yangjiang, and Zhanjiang) between wild and cultured stocks (site/C2 stock interactions), the overall classification accuracy was 60.4% for stepwise discriminant analysis and 85.7% for random forests (Table 5). Among all the stocks from the three study areas, the classification accuracy was highest for identifying the cultured stock from Shantou coastal waters (90% by stepwise discriminant analysis and 100% by random forests), but lowest for identifying the wild stock from Shantou coastal waters (45% by stepwise discriminant analysis and 80% by random forests) and the cultured stock from Zhanjiang coastal waters (47.4% by stepwise discriminant analysis and 78.9% by random forests). The misclassification of cultured and wild stocks within the three sites suggested the spatial connectivity between stocks and among study areas (Fig. 3).

4. Discussion

4.1. Otolith elemental fingerprints for stock discrimination of yellowfin seabream

The concentrations of trace elements in fish otoliths are critical fingerprints for stock discrimination, and their ratio to 40Ca and relative importance in classifying stocks varied with fish species and the inhabiting environment. Our findings suggested that 137Ba, 24Mg, and 23Na contributed most to distinguishing the cultured and wild stocks of yellowfin seabream (Table 4). 137Ba, 55Co, 59Ni, and 65Zn ranked as the most important elements for classifying different stocks when the three study areas (coastal waters of Shantou, Yangjiang, and Zhanjiang) were considered together, whereas 137Ba, 55Mn, 24Mg, and 23Na showed the greatest contribution to stock discrimination for pairwise comparisons between areas.
areas. Elemental fingerprints in the natal spot of otoliths can serve as useful tools for distinguishing the cultured and wild stocks of yellowfin seabream, with overall classification accuracy of 80.7% by stepwise discriminant analysis and 99.2% by random forests (Table 3). Random forests performed better than stepwise discriminant analysis in the stock discrimination of yellowfin seabream both for all three study areas (Table 3) and for pairwise comparisons between areas (Table 5).

4.2. Genetic fingerprints versus otolith elemental fingerprints for stock discrimination

Traditional genetic fingerprints based on molecular biology are generally considered as powerful tools for fish population classification at broad spatial and temporal scales, whereas the elemental signatures of fish otoliths provide microchemistry support for stock discrimination at finer scales, especially when fish stocks have different inhabiting environment but no significant genetic difference (Begg and Waldman, 1999; Noor and Ashraf, 2017). The elemental signatures of otoliths often classify fish populations into more and smaller units than genetic fingerprints, and such units (i.e., stocks) are typically not genetically different but exhibiting significant difference in life history characteristics or distributions, which may have great impact on fish recruitment and fishing efforts. Therefore, fish stocks are considered as fundamental units in the exploitation and management of fisheries resources. Genetic and phylogeographical analysis suggested that yellowfin seabream populations in the northern South China Sea could be divided into two major management units, as gene flow was limited by the Qiongzhou Strait and the terrestrial barriers along the Beibu Gulf (Xia et al., 2008). Among the three wild stocks of yellowfin seabream in this study, the classification accuracy was highest for identifying the wild stock from Yangjiang coastal waters, followed by Zhanjiang and Shantou coastal waters (Table 5). The multiple modeling approaches applied for stock discrimination could serve as bases for further investigations on the relative contribution of yellowfin seabream stocks from the three areas to the commercial fishery in the northern South China Sea.

4.3. Spatial connectivity and migration of cultured and wild yellowfin seabream stocks

Connectivity between fish stocks is fundamental to the understanding of population dynamics and recruitment mechanisms, and the implementation of sustainable fisheries management (Gillanders, 2005; Wong, 2017). The elemental fingerprints of otoliths can be used to better understand the spatial connectivity and migration of fish stocks, because otolith microchemistry often exhibit significant difference for life stages spent in different habitats (Campana, 1999; Thresher, 1999). To minimize the confounding effects on otolith fingerprints caused by fish size or age, only the trace elements in the natal spot of otoliths were analysed and used for stock discrimination in this study. The natal spot of fish otoliths represents the embryonic and paralarva stages of fish, and the elemental concentrations in the natal spot can indicate the
inhabiting environment of spawning, therefore provide valuable information on the spawning and migration of fish stocks (Starrs et al., 2016). Yellowfin seabream is a coastal species, which spends the adult stage in coastal waters and estuaries, and makes short-distance spawning migration along coastal waters (Xia et al., 2008; Gao et al., 2017). Although yellowfin seabream is often caught during trawling, gillnetting, or angling in coastal waters of the northern South China Sea, knowledge on the spatial connectivity and spawning grounds of different stocks is still limited.

This study demonstrated that random forests and stepwise discriminant analysis, especially random forests, can effectively distinguish cultured and wild stocks of yellowfin seabream from Shantou, Yangjiang, and Zhanjiang based on the elemental fingerprints of otolith natal spot (Table 5). The multiple modeling approaches provided a reasonable classification for the spatial distributions of yellowfin seabream stocks from the three study areas (Fig. 3). The misclassification of cultured and wild stocks within the three sites suggested spatial connectivity between stocks and among sampling locations (Begg and Waldman, 1999). For cultured stocks of yellowfin seabream from the three study areas, Shantou stock tended to be a more independent stock from Yangjiang and Zhanjiang stocks, as the elemental signatures of otolith natal spot from Shantou cultured stock showed little overlap with Yangjiang or Zhanjiang cultured stock (only two samples from Shantou were misidentified as from Yangjiang). Shantou cultured stock may originate from the coastal waters of Shantou and Fujian Province, which are quite different from the hatcheries of Yangjiang and Zhanjiang cultured stocks; therefore, the trace elements accumulated in the natal spot of otoliths exhibited significant difference. Misclassification occurred more frequently between Yangjiang and Zhanjiang cultured stocks, probably because the two study areas are both within west Guangdong coastal waters, and the larvae of the two stocks are more likely to be from adjacent hatcheries in Shapa Town in Yangjiang with similar trace-elemental levels. For wild stocks of yellowfin seabream from the three study areas, Zhanjiang stock tended to be significantly different from Shantou and Yangjiang stocks, probably because Zhanjiang wild stock has quite different origins from Shantou and Yangjiang wild stocks. The spawning grounds of Shantou and Yangjiang wild stocks may be spatially connected or have similar environmental characteristics, whereas Zhanjiang wild stock may partially originate from the Beibu Gulf.

Non-metric multidimensional scaling (NMDS) and cluster analysis suggested that the three wild stocks of yellowfin seabream from Guangdong coastal waters could be considered as one unit for management, and the difference between cultured and wild stocks were significant for yellowfin seabream from Shantou and Yangjiang, but less significant for yellowfin seabream from Zhanjiang (Fig. 3). Future studies on the stock discrimination of yellowfin seabream or similar fish species utilizing otolith elemental fingerprints could take account of the spatial or temporal scales of the analyses when data are available, as the applicability and sensitivity of otolith elemental fingerprints in stock discrimination can be influenced by the spatial or temporal scales of studies. The elemental levels in waterbodies may differ at local scale but exhibit no significant difference at regional scale (Gillanders, 2002; Eldson and Gillanders, 2004). Seasonal and interannual variability of the elemental concentrations within one waterbody may affect particular fish species as well. Furthermore, the misclassification of cultured and wild stocks from different sampling areas could also be caused by the potential location difference between cage sampling and trawl surveys. Fish samples of cultured stocks are often collected from the cages in coastal waters whereas samples of wild stocks are generally from the catch using bottom trawls or gillnets, and this may lead to the samples of cultured and wild stocks coming from different locations even within the same sampling area.

5. Conclusions

Yellowfin seabream is an important species for the commercial fishery industry and aquaculture in the northern South China Sea. However, wild stocks of yellowfin seabream are undergoing silent and invisible declines, because the species is widely cultured and heavily demanded in the fish markets in southern China provinces. More attentions should be paid on precautionary approaches to the management of species experiencing silent and invisible declines, and special efforts should be made to achieve an improved understanding of such species’ ecological characteristics, inhabiting environment, population connectivity, and recruitment mechanisms.

This study demonstrated that the elemental fingerprints of otolith natal spot can be an effective tool for discriminating cultured and wild stocks of yellowfin seabream. The findings could serve as bases for further investigating the population structure and year-class recruitment of yellowfin seabream, identifying and protecting the spawning grounds of stocks, and quantifying the relative contribution of different spawning grounds to adult fish stocks. Improved knowledge on the spatial connectivity of yellowfin seabream stocks can help identify the ecological boundaries of different stocks, and their migration routes from nursery grounds to fishing grounds and spawning grounds at different life stages. As fish stocking and enhancement are increasingly carried out along China coastal waters since 2000, otolith microchemistry could provide insightful guidance on assessing the effectiveness of stocking and enhancement for other ecologically or economically important fish species or geographical areas.

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