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Unraveling habitat use of *Coilia nasus* from Qiantang River of China by otolith microchemistry

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ABSTRACT

The habitat use and life history characteristics of the estuarine tapertail anchovy, *Coilia nasus* from Qiantang River were studied by examining the environmental signatures of Sr and Ca in otoliths using otolith microchemical analysis. The results indicate the *C. nasus* in the present study could be briefly divided into three types: Types 1/Type 2 of riverine origin and initial relative long-term/short-term freshwater residence, and Types 3 of estuarine origin and initial brackish early life history. The availability and fluctuation of certain levels of Sr:Ca ratios (i.e., Sr:Ca$\times$1000) between salinity scale of 3.00 and 7.00 reveal that the *C. nasus* analyzed in this study spent much more time in brackish habitat of the river's estuary. This study has established that the *C. nasus* of Qiantang River Estuary has a diverse and complex pattern of life history and habitat use which makes their life history and migration pattern vary from those of the Yangtze River.

1. Introduction

The estuarine tapertail anchovy *Coilia nasus* in China is known to be widely distributed in coastal waters, estuaries, and rivers, e.g., East China Sea, Yellow Sea, Yangtze River (Zhong et al., 2007; Jiang et al., 2012; Dou et al., 2012; Jiang et al., 2014), Yellow River (Jiang et al., 2012) and Qiantang River. This fish is a highly valuable commercial species due to its nutritional value and delicacy (Zhong et al., 2007; Jiang et al., 2012). Stocks of the anchovy in Chinese water bodies have been in a steep decline since the 2000s by anthropogenic influences, e.g., water pollution, hydraulic construction and overfishing. Consequently, the price in 2012 jumped up to a maximum of US$1000/kg for common size fish and even $9600 for a single particularly large fish of 45.3 cm total body length weighing 0.325 kg (Jiang et al., 2017). As an anadromous species, *C. nasus* migrates from near-ocean waters to fresh water areas every year during the spawning season. *C. nasus* reaches sexual maturity at 1–2 years of age, breeding once every year (Ma et al., 2004). From early February to the end of April, adult mature *C. nasus* (mostly 1–2 years old) move upstream and spawn in affiliated lakes in the Yangtze's middle and lower reaches (Ma et al., 2004; Li et al., 2007, 2011; Liu et al., 2014), and other rivers, such as the Yalujiang River, that drain into the ocean (Li et al., 2007; Ma et al., 2010). *C. nasus* may also spawn in lakes adjacent to the rivers, including Dongting, Poyang and Taihu Lakes (Cheng et al., 2007; Wang et al., 2015). Qiantang River, just like the Yangtze, Minjiang, and Yalujiang Rivers, are geographically isolated from each other by long distances, such that adaptation to the local environment, overfishing, and environmental pollution may be contributing to genetic divergence of the *C. nasus* populations (Ma et al., 2010) in the individual rivers.

Knowledge of life history and connectivity between essential ecological habitats are relevant for conservation and management of species and some natural geo-elements could be used to study the lifecycles of small or short-lived marine fishes (Zhong et al., 2007; Mateo et al., 2012; Laugier et al., 2015; Uehara et al., 2017). Otolith (ear stones) microchemistry of geo-elements (e.g., Sr, Ca, Mg, and Ba) is used to determine the movement and past life history habitats of fish (Carlson et al., 2016). It is a powerful and useful tool (Campana and Gagné, 1995; Campana et al., 1997; Secor and Rooker, 2000; Elsdon and Gillanders, 2003; DiMaria et al., 2010; Curtis et al., 2014; Jiang et al., 2014; Carlson et al., 2016) for tracking the migratory paths (Yang et al., 2006; Liu et al., 2012; Jiang et al., 2014), assess habitat shifts, population structure/dynamics, and location of origin (Clarke et al., 2015), but can also be used to trace the extent and pattern of movement of individual fish based

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on the reconstruction of environmental histories as recorded in the layering of the otolith (Secor, 1992; Campana, 1999; Elsdon and Gillanders, 2003; Lee et al., 2007; Jaecks et al., 2016). The tool has been proven useful in marine, diadromous, and freshwater fishes (Secor et al., 1995; Pracheil et al., 2014). Otoliths are calcium carbonate concretions with a small amount of organic matter located in the inner ears of teleost fishes and are used for hearing and balance (Campana, 1999; Benson and Fitzsimons, 2002; Arai, 2010; Peacock et al., 2016). Otolith increment analysis is also the most accurate technique for estimating fish age (Steward et al., 2009) which results in better understanding of fish recruitment success (Jones, 1992).

Yang et al. (2006) firstly studied the environmental signatures of Sr and Ca in otoliths of *C. nasus* in the Yangtze Estuary using electron probe microanalyzer (EPMA). The results of their study indicated that the habitat use patterns of the Yangtze estuary *C. nasus* were much more flexible; varying among freshwater, brackish water and marine habitats (Yang et al., 2006; Jiang et al., 2012). Zhong et al. (2007) found a similar tendency in particle-induced X-ray emission (PIXE) analyses on *C. nasus* otoliths collected from the Yangtze River. It is noteworthy that *C. nasus* in Qiantang River might move into the East China Sea where Qiantang Estuary connects with the sea, for feeding.

This study was aimed at determining the life history characteristics of *C. nasus* in Qiantang River Estuary. Otolith microchemistry was chosen in this study because the application of otolith chemical composition satisfyingly ranges from studies for identifying natal origin or nursery areas (Vasconcelos et al., 2007; Clarke et al., 2009; Pangle et al., 2010; Reis-Santos et al., 2012), classifying adults to their areas of origin (Thorrold et al., 2001; Tanner et al., 2013), reconstructing migration patterns (Fairclough et al., 2011; Mercier et al., 2012), to studies estimating population structure and discriminating stocks (Thresher and Procter, 2007; Tanner et al., 2012). It is very important to understand the habitat use strategies and ecology of species and their stocks throughout their life history (Yang et al., 2006). Migration pattern and habitat use studies have been conducted and are known for the *C. nasus* from Yangtze River stock (e.g., Yang et al., 2006; Cheng et al., 2007; Jiang et al., 2012; Liu et al., 2014; Wang et al., 2015) while less is known about habitat use strategies of the fish stocks from other regions, like Oujiang, Qiantang River, and Huanghe River (Jiang et al., 2014). This river is separated from other rivers with long distances and no research has been done to establish the life history and habitat use pattern of its *C. nasus* stock. It was hypothesized in this study that Qiantang *C. nasus* is a separate and different population from that of Yangtze *C. nasus* although the estuaries of these two rivers are near. The evidence on this was revealed by analyzing otoliths of *C. nasus* individuals from Qiantang River using otolith Sr levels with EPMA probe.

2. Materials and methods

A total of 23 *C. nasus* fish samples (total length 17.47 ± 5.05 cm, L_T, mean ± S.D.) were collected at same place at Jiubao Bridge, in the upper part of Qiantang River Estuary, at a location 120°17′28.76″E, 30°17′11.51″N (Fig. 1), on 10 May, 2016.

Measurement of total body length, upper jaw length, head length and body weight was performed on fish samples and the data recorded accordingly for analysis, and then left and right sagittae otoliths of each anchovy sample were extracted and cleaned. Photographs of the otoliths were taken using a digital microscope camera (QX800HD720P3D, China) of resolution 1280 × 720 mounted onto a computer. This study used left sagittae otoliths only where possible. The left sagitta otolith of each anchovy was weighed and then embedded in epoxy resin (Epofix; Struers, Copenhagen, Denmark) in the frontal plane (Yang et al., 2006;

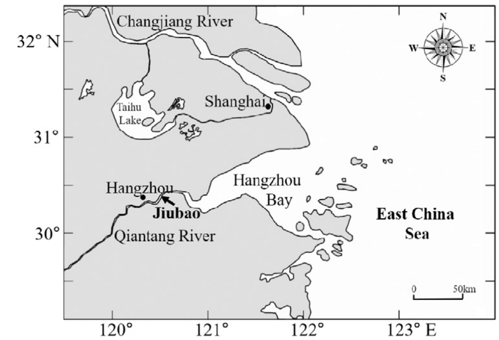

Fig. 1. Map showing the *Coilia nasus* sampling site in upper Qiantang River Estuary.

Jiang et al., 2012, 2014). This process was followed by grinding the otoliths to expose the core by using an automated polishing wheel (LaboPol-35; Struers, Copenhagen, Denmark). All the otolith samples were thereafter cleaned in an ultrasonic bath and rinsed with Milli-Q water to remove any unwanted materials on the samples. The otoliths were dried and then carbon coated using a high vacuum evaporator (JEE-420, JEOL Ltd Tokyo, Japan) (Jiang et al., 2012) before examining them in an EPMA. After EPMA analysis, all the otoliths were re-polished to remove the carbon coating. Thereafter, etching with 5% ethylenediaminetetraacetate (EDTA) was done to reveal the annulus marks on the otoliths to determine age of the individual samples.

All the otoliths were used for life history transect analysis on Sr and Ca concentrations (Jiang et al., 2012). Measurement was along a line down the longest axis from the core to the edge of each otolith using a wavelength dispersive X-ray electron probe microanalyzer (JXA-8100 JEOL Ltd, Tokyo, Japan). Standards used were Calcite ($CaCO_3$) and Tausonite ($SrTiO_3$) (Jiang et al., 2012), with the accelerating voltage and beam current of 15 kV and 2×10^{-8} A, respectively (Yang et al., 2006; Jiang et al., 2014). The electron beam was focused on a point 5 μm in diameter, and measurements were spaced at 10 μm intervals. The X-ray intensity maps of both of these elements were made of the representative otoliths using the same microprobe in accordance with the above mentioned life history transect (Jiang et al., 2012). The beam current was 5×10^{-7} A (Jiang et al., 2017), the counting time was 30 ms, pixel size used was 6 × 6 μm, and the electron beam diameter was focused on a point of 5 μm.

By conventional otolith research, Sr:Ca ratios of concentrations were expressed as Sr:Ca×1000 by a simple conversion based on the molecular weights of SrO and CaO in this study. Based on our previous studies (Yang et al., 2006; Jiang et al., 2014; Chen et al., 2017) on otoliths of *C. nasus* which indicated freshwater (low salinity, Sr:Ca ratio: ⩽3, X-ray intensity mapping color: blue, similarly hereinafter), brackish (medium salinity, 3–7, green or yellow), and seawater habitats (high salinity, >7, red) respectively, by corresponding 16 color map patterns of Sr concentration from blue (lowest) through green and yellow, to red (highest).

To standardize the freshwater dependency, Freshwater Coefficient (F_C) was calculated as Jiang et al. (2014):

$$F_c = \frac{L_f}{L_T}$$

where L_f was the length of first freshwater phase along the line down the longest axis of each otolith from the core, in other words the length of bluish central regions, and L_T was the total length of the otolith, both of which were based on the fluctuation of otolith Sr:Ca ratios.

Statistical analysis was performed using Excel 2013 (Microsoft, Seattle, WA, USA) and IBM SPSS Statistics v.19.0 (IBM Corp., Armonk, NY, USA). A sequential regime shift algorithm (Rodionov,