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Short Communication

Natal origin identification of Pacific bluefin tuna (*Thunnus orientalis*) by vertebral first annulus

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ABSTRACT

Pacific bluefin tuna (*Thunnus orientalis*) has two spawning grounds, and each is used at different times: May–June in the Nansei Islands of western North Pacific Ocean and July–August in the Sea of Japan. Although previous studies developed body-length based methods to differentiate between individuals originating in each spawning ground, these methods are only applicable to the young-of-the-year (YOY). In this study, we evaluate a simple technique for discerning the natal grounds of Pacific bluefin tuna greater than one year in age by observing the first annulus on the vertebrae of fish caught around Japan. We found a significant linear relationship between fork length and vertebral radius, and determined that the first annulus forms in boreal winter. Both the back-calculated fork length at the time of first annulus formation and the measured fork length of YOY in February and March had a bimodal frequency distribution, and the peaks of the two frequency distributions had similar means. The larger fork length individuals appear to have originated near the Nansei Islands, whereas the smaller fork length individuals appear to have originated in the Sea of Japan. This is because PBF hatched around the Nansei Islands will have longer growth time for the YOY to develop the first annulus at longer distance from the vertebrae center. Similarly, we observed bimodal distributions for the vertebral first annulus radius of mature Pacific bluefin tuna (> 120 cm) in both spawning grounds. Our technique offers improved sample treatment efficiency and lower costs. In addition, this method may help reveal the contribution rate of each spawning ground.

1. Introduction

Pacific bluefin tuna (PBF; *Thunnus orientalis*) is a highly migratory species with a complex migratory life history in the Pacific Ocean (Bayliff 1994; Fujioka et al., 2015). PBF is one of the most important fishery resources for several countries managed by the Western and Central Pacific Fisheries Commission based on stock assessments by the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean (ISC). In order to manage the stock sustainably, these fishing entities must understand the population dynamics of PBF and the contribution rate of the different spawning grounds.

In spite of its broad distribution, its spawning grounds are limited to two areas in the western North Pacific, specifically the Nansei Islands (extending from the waters offshore of the Philippine to the Ryukyu Archipelago in southern Japan; Chen et al., 2006; Ashida et al., 2015) and the southern Sea of Japan (Okochi et al., 2016). After PBF larvae grow to young-of-the-year (YOY), their distribution becomes heterogeneous due to migration and life history differences (Ichinokawa et al., 2014). YOY originating from the two spawning grounds mix and stay in the waters around Japan (Fig. 1) until the following spring (Fujioka et al., 2015).

Previous studies have inferred the natal origin of PBF using the difference in the spawning period at each spawning ground: May–June in the Nansei Islands and July–August in the Sea of Japan (Itoh 2009; Ashida et al., 2015; Okochi et al., 2016; Ohshimo et al., 2017). Itoh (2009) used growth curves derived from otolith daily increments until 400 days of age to determine that differences in spawning periods at the two spawning grounds drive corresponding differences in body length. He then divided monthly length data on YOY from July–May into subcohorts originating in each spawning ground, and estimated the contribution rate of each subcohort to the YOY. Although this technique can infer the natal grounds of YOY fish with a high degree of confidence by comparing monthly length data from each subcohort starting at the time of recruitment, it cannot be applied to older PBF (age ≥ 1) because

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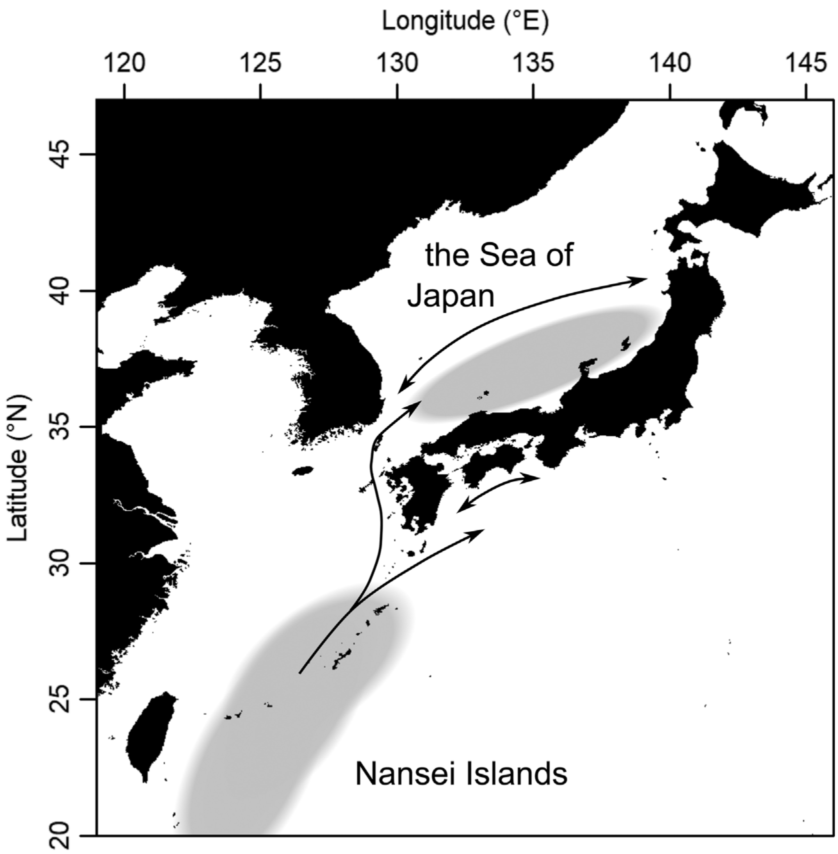

Fig. 1. Spawning grounds and sampling locations (grey shaded regions) of Pacific bluefin tuna around Japan from 1998 to 2015. Arrows indicate migration routes taken by the young-of-the-year.

the difference in body length between subcohorts decreases with growth (Itoh 2009). Shiao et al. (2010) also used the difference in spawning periods to infer the natal origin of YOY, and suggested that the natal origin of older PBF could be inferred by estimating the position of the first winter signal in the otolith using an oxygen stable isotope analysis. Rooker et al. (2001) showed that element concentrations in YOY otoliths differed between nurseries. However, large sample sizes are necessary to estimate the contribution rate of the different spawning grounds, and the high cost and slow speed of these microchemical analyses are not suitable for analyzing numerous samples. To overcome this problem, we aimed to establish a simpler technique for estimating the natal ground of older (age ≥ 1) PBF.

The body length of YOY is markedly different between the natal grounds (Itoh 2009). Therefore, if the body length of older individuals could be back-calculated to a particular time period during the first year of life, this would allow us to predict an individual's natal ground. In general, the hard parts of fish are used as a source of age and growth information (Prince et al., 1985). Vertebrae are especially useful for back-calculating fork length (Lee et al., 1983) because body length is more tightly correlated with vertebral radius (Foreman 1996) than otolith radius (Lee et al., 1983). In addition, the first annulus on bluefin tuna vertebrae is distinct (Prince et al., 1985), allowing us to estimate the body length when the first annulus was formed.

Thus, to establish the simple technique we evaluated two assumptions: (1) the first annuli on the vertebrae of PBF from both spawning grounds form during the same period of the year, and (2) the body lengths of individuals hatched at the two spawning grounds differ at the time of first annulus formation. Finally, we applied this new technique to identify natal origin of older PBF.

2. Materials and methods

2.1. Sample collection

The PBF specimens were caught by commercial fisheries around Japan from 1998 to 2015 (Fig. 1). In the Sea of Japan (January–December 1998–2015), 752 PBF collected by the purse seine fishery were used for vertebral analysis (30–223 cm straight fork length [FL], measured from the tip of the snout to the end of the middle caudal fin rays). Around the Nansei Islands (April–July 1998–2015), 122 long-line caught PBF (151–262 cm FL) were used for the analysis.

All specimens were measured for FL (cm), and the 34th caudal vertebra and sagittal otoliths were removed from each specimen. The caudal vertebrae are easy to collect because the caudal fin of PBF is cut off at the market. On the 32nd to 36th vertebrae, lateral processes form a horizontal bony keel (Gibbs and Collette 1967), and the 34th vertebra can be distinguished by having the largest lateral process (Nakamura, 1994). Age of PBF was determined based on otoliths annuli (Shimose and Ishihara, 2015).

To compare against back-calculated FL, we obtained measured FL from PBF YOY collected in the Sea of Japan in February–March 1998–2015 by the Research Project on Japanese Bluefin Tuna. We chose to sample YOY from the Sea of Japan because this matches the location from which we obtained vertebrae of young PBF, and because YOY originating from both spawning grounds inhabit the Sea of Japan (Itoh 2009).

2.2. Sample treatment

To determine the age of sampled fish, the otoliths were embedded in resin, sectioned, and examined under a light microscope to count the number of annual opaque zones (Shimose and Ishihara, 2015). The vertebrae were simmered in water to remove remnant tissue and soaked in 1.65% (w/v) alizarin red S stain solution for 2–5 h, as described by