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Genetic structure and demographic connectivity of marbled flounder (*Pseudopleuronectes yokohamae*) populations of Tokyo Bay

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ABSTRACT

Marine populations are generally well connected through larval exchange, and juvenile and adult migration. Genetic differentiation and/or local adaptation among populations are sometimes observed at small spatial scales. The marbled flounder (*Pseudopleuronectes yokohamae*) is a commercially important coastal species in Tokyo Bay, Central Japan, exhibiting different spawning phenology at a small scale. Using 30 highly polymorphic microsatellite loci, including the 16 new microsatellite loci developed in the present study, we estimated the genetic structure and demographic connectivity of specimens collected along Tokyo Bay in 2012–2013 and 2015–2016. *F*-statistics indicated a genetic differentiation between adult populations collected within and outside Tokyo Bay during both collection periods, but almost no difference was detected between the populations from the inner and outer parts of Tokyo Bay. Assignment tests directly estimated demographic connectivity between the inner and outer bays via larval dispersal and juvenile and adult migration. The average movement distance of marbled flounder was 13.1–29.6 km, with a maximum distance of 42.8–54.0 km. This movement distance contributed to the strong demographic connectivity between the inner and outer bays. Meanwhile, an indirect method using the isolation by distance (IBD) theory underestimated the average movement distance (5.0–7.8 km) because the isolated setting of Tokyo Bay resulted in a higher IBD slope. Based on the results of *F*-statistics and assignment tests, we suggest that a multilayered management by local and regional organizations may benefit the fishery in Tokyo Bay.

1. Introduction

Most marine populations are well connected through larval exchange (Jones et al., 2005) and ontogenetic, seasonal, and spawning migration of juveniles and adults (Frisk et al., 2014; Gillanders et al., 2003; Thorrold et al., 2001). This has led to a view that marine populations are demographically open (Mora and Sale, 2002). However, recent studies have revealed that the dispersal of larvae of marine fish can be restricted to a few kilometers, and that genetic structure can be significantly heterogeneous at small scales in demersal fishes (Almany et al., 2007; Jones et al., 2005; Taylor and Hellberg, 2003). A comparison of dispersal estimates revealed a large variation in the larval dispersal distance, i.e., the dispersal distance ranged from tens of meters to 35 km for *Amphiprion* and from 1 to 33 km for *Chaetodon vagabundus* (Berumen et al., 2012; Jones et al., 2005; Saenz-Agudelo et al., 2011). Studies on the dispersal of marine populations showed a rapid decline in the dispersal probability within the first few kilometers (Buston et al., 2012; D'Aloia et al., 2013; Saenz-Agudelo et al., 2012). Therefore, the spatial scale of larval dispersal is largely restricted within a certain radius, although some individuals disperse long distances (i.e., > 30 km). Besides, the empirical evidence of adaptive variation among marine populations has grown in recent years (Marshall et al., 2010). For example, differences in survival, growth, and reproduction among local populations have been observed (Barth et al., 2017; Blanchette et al., 2002; Fincham et al., 2013; Yamahira and Conover, 2002). Therefore, the variability in environmental conditions on a small

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scale may lead to local adaptation, thereby limiting demographic connectivity. On the contrary, some demersal fishes show long-distance migration (10s–1000s of km) during juvenile and adult stages (Frisk et al., 2014; Loher and Seitz, 2006; Robichaud and Rose, 2004), which can lead to open and panmictic populations over large spatial scales. Information on such local population characteristics and connectivity is important for the prediction of population dynamics and the management of marine species.

The population genetic approach, which utilizes indices of population differentiation and models of isolation by geographical and environmental distances, is widely used to make indirect inferences on migration and selection in aquatic species (Kurokochi et al., 2015; Nakajima et al., 2014; Nanninga et al., 2014; Orsini et al., 2013; Schunter et al., 2011). Although this approach can estimate connectivity over multiple generations (Waples and Gaggiotti, 2006), it cannot distinguish between contemporary and historical gene flows. Recent advances in molecular and statistical tools enable us to directly estimate the fine-scale contemporary connectivity of marine species using highly polymorphic markers (e.g., microsatellite markers) in conjunction with the assignment tests and/or parentage analysis (Hogan et al., 2012; Saenz-Agudelo et al., 2011; Sato et al., 2017). Although such assignment methods have been applied to several coral reef fishes (Almany et al., 2013; D'Aloia et al., 2013; Jones et al., 2005; Saenz-Agudelo et al., 2009; Sato et al., 2017), only a few studies have estimated the dispersal pathways or demographic connectivity of temperate marine fishes using these methods (but also see, Schunter et al., 2014). The combination of direct and indirect inference methods to determine connectivity can provide more valuable and detailed inferences regarding connectivity in marine species.

Marbled flounder, *Pseudopleuronectes yokohamae* (Günther, 1877) (Pleuronectidae; Actinopterygii), is a commercially important coastal species in Japan (Kume et al., 2006). Unlike other temperate flatfishes, marbled flounder has sticky demersal eggs, which prevent mixing and transport during the embryonic stage. The pelagic larvae disperse during a relatively short period (i.e., three weeks) (Joh et al., 2013), which makes that they are locally retained especially within a peculiar setting such as a semi-enclosed bay (Nakagami et al., 2001). Tokyo Bay is one of the most industrialized coastal areas in Japan and is heavily eutrophied. Nevertheless, coastal fisheries are still active. The annual catch of marbled flounder decreased significantly from > 500 tons in the late 1980s to around 50 tons in the 2000s according to the statistics of the local fisheries cooperation association in Tokyo Bay (Kume et al., 2006; Lee et al., 2009). Tokyo Bay consists of an inner and outer bay; the inner bay is subjected to extreme environmental conditions, including frequent events of hypoxia and red tides in summer and the outer bay is characterized by relatively stable open ocean conditions (Akaba, 2010; Kodama and Horiguchi, 2011). Such extreme conditions can cause habitat shift and escape of juvenile flounder from the most inner parts of Tokyo Bay in summer (Ishii, 1992; Nagayama, 2005). Several marbled flounder spawning grounds are located in the inner and outer bays, and a time gap in spawning peaks has been observed between the two bays (Akaba, 2010; Ishii, 1992). There could be limited gene flow and reproductive isolation between the two bays because of their geographical setting and different environmental conditions. However, adult marbled flounders may travel up to 70 km (Jusan, 1988; Kurono, 2015), such movements can connect local populations around Tokyo Bay. Elucidating the spatial scale of demographic connectivity via larval dispersal as well as juvenile and adult migration is essential to establish effective management strategies for marbled flounders in Tokyo Bay.

Given the difficulty in elucidating the genetic structure of marine fish populations at a small spatial scale, we developed additional microsatellites for marbled flounder and estimated their genetic population structure around Tokyo Bay using datasets obtained from populations sampled during two winter periods. We also estimated the natal (parent) population of juveniles, sub-adults, and two-year-old adults from Tokyo Bay using the assignment tests, which enabled us to track the pathways of larval dispersal as well as juvenile and adult migration. This study was designed to address the following questions: (1) Are the populations in Tokyo Bay, especially between the inner and outer parts connected? (2) Which adult populations around Tokyo Bay contributed to the production of juveniles, sub-adults, and two-year-old adults? and (3) How large are the spatial scales associated with the movement of juveniles, sub-adults, and two-year-old adults?

2. Materials and methods

2.1. Study species

Marbled flounders, (*Pseudopleuronectes yokohamae*) are widely distributed in coastal waters extending from Japan to the Yellow and Bohai Seas. The spawning season of marbled flounders extends from November to March, with December and January being the active spawning period in Tokyo Bay (Kume et al., 2006). The demersal eggs are laid on the bottom of the sea and embryos develop over a period of 10 d until hatching (Ishii, 1992; Kume et al., 2006; Minami, 1981; Tanda and Nagai, 2007). The hatched larvae then disperse in the waters around Tokyo Bay during the pelagic larval phase that lasts 20–23 d (Joh et al., 2013; Lee et al., 2017). The large number of larvae in the inner bay settle on shallow sandy habitats during late winter, and they grow there as juveniles until late spring or early summer (Ishii, 1992; Nagayama, 2005). However, they disappear in the northern part and appear only in the southern part (at > 20 m water depth) during periods of hypoxia in summer (Ishii, 1992; Nagayama, 2005). Therefore, the juvenile marbled flounders are expected to move from northern to southern parts in order to avoid hypoxic condition and grow into sub-adults (Ishii, 1992; Nagayama, 2005), but direct evidence of this movement is limited. After the extreme conditions from June to September, mature flounders (generally > two years old) gather in shallow waters to spawn.

2.2. Microsatellite development

Genomic DNA was extracted from the muscle of marbled flounders collected in Tokyo Bay using a DNeasy Blood & Tissue Kit (QIAGEN). We developed 16 novel microsatellite markers with next-generation sequencing following the protocol described in Kitanishi et al. (2014). Totally, 22,229 sequences containing 2–5 bp repeats were obtained, and 686 microsatellite loci were designed using AutoPrimer (Nakamura et al., 2013). Of these microsatellite loci, we selected 60 candidate microsatellites, containing dinucleotide repeats with perfect motifs and > 10 repeats.

To determine the effectiveness of these 60 candidate microsatellites, eight samples were amplified with PCR. The PCR products were electrophoresed on an ABI PRISM 3730 Genetic Analyzer with GeneScan 600 LIZ (Applied Biosystems), and the allele sizes were determined using GeneMapper ver.4.0 (Applied Biosystems). Of the 60 microsatellite loci, 16 microsatellites that were well amplified and were of appropriate allele size (< 450 bp) were selected for subsequent PCR amplification. Polymorphisms in these 16 microsatellite loci were tested using 88 samples collected from three different regions in Japan. The observed (H_O) and expected heterozygosity (H_E) were calculated using Arlequin 3.5.1.2 (Excoffier et al., 2005). The presence of null alleles, linkage disequilibrium, and deviations from Hardy-Weinberg equilibrium (HWE) were examined using GENEPOP (Rousset, 2008), MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004), and Arlequin, respectively.

2.3. Study sites and sampling procedure

Tokyo Bay is located in Central Japan, and consists of inner (inner bay) and outer areas (outer bay) that are separated by a narrow strait