Evaluation of the Tsunami Impact on the Genetic Diversity of the Marbled Flounder Pseudopleuronectes yokohamae in Sendai Bay, Miyagi, Japan

メタデータ	言語: English 出版者: 水産研究・教育機構 公開日: 2024-10-02 キーワード (Ja): キーワード (En): Marbled flounder; Pseudopleuronectes yokohamae; genetic diversity; management units; Sendai Bay 作成者: Minegishi, Yuki, kedal, Minoru, 栗田, 豊, 冨樫, 博幸, Nakane, Yukinori, Kijima, Akihiro メールアドレス: 所属: 水産研究・教育機構, 水産研究・教育機構
URL	https://fra.repo.nii.ac.jp/records/2010944

水研機構研報，第45号，69－73，平成29年
Bull. Jap. Fish. Res. Edu. Agen. No. 45，69－73，2017

Evaluation of the Tsunami Impact on the Genetic Diversity of the Marbled Flounder *Pseudopleuronectes yokohamae* in Sendai Bay, Miyagi, Japan

Yuki MINEGISHI*1, Minoru IKEDA*1, Yutaka KURITA*2, Hiroyuki TOGASHI*2,
Yukinori NAKANE*2, and Akihiro KIJIMA*1

Abstract: Evaluating genetic diversity is a fundamental step for stock management. However, it can be influenced by environmental and demographic changes, and thus it should be carefully investigated, especially when drastic disturbances have occurred. Marbled flounder *Pseudopleuronectes yokohamae* is a commercially valued fish in Japan, and possibly consists of multiple management units due to its benthic lifestyle including low dispersal ability. In Sendai Bay, the population is thought to form a single management unit, and its spawning ground has been protected for stock conservation. Since this population could have been strongly affected by the huge tsunami associated with the 2011 off the Pacific coast of Tohoku Earthquake, the tsunami impact on the genetic diversity of the population of the marbled flounder in Sendai Bay needs to be evaluated.

We firstly developed genomic resources for this species using next generation sequencing technology and *de novo* assembly, which generated a total of 525,502 contigs and 10,732,070 unassembled reads. We then designed a total of 331,368 microsatellite primer pairs from the genomic data, and characterized sixteen primer pairs as novel microsatellite loci. Using these microsatellite markers, significant genetic divergence was found between the east and west sides of Boso Peninsula (pairwise F_{ST} = 0.02305 – 0.19784). At the east side, genetic homogeneity was observed over the wide area from Onagawa Bay to Choshi including Sendai Bay, except for Mutsu Bay. The genetic variability of the Sendai Bay population did not show any considerable changes after the tsunami from April 2012 to February 2014 (H_E = 0.5061 – 0.5463) and was relatively higher compared to those of the western populations of Boso Peninsula (H_E = 0.3817 – 0.4557). These results suggest that the tsunami impact on the Sendai Bay population of the marbled flounder is considered to be minor but long-term monitoring may be needed.

Key words: Marbled flounder, *Pseudopleuronectes yokohamae*, genetic diversity, management units, Sendai Bay

Introduction

Evaluating genetic diversity of species and / or populations of interest is a fundamental step for appropriate stock management. The information on genetic diversity such as population structure, migration rate between populations and population size helps identify management units and translocation ranges, and monitoring those alterations provides the insights into the population vulnerability

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2017年２月28日受理（Received on February 28, 2017）

[*1] Tohoku Ecosystem-Associated Marine Sciences, Graduate School of Agricultural Science, Tohoku University, Mukai 15, Konorihama, Onagawa, Oshika, Miyagi 986-2242, Japan

[*2] Tohoku National Fisheries Research Institute, Fisheries Research Agency, Shinhama-cho 3-27-5, Shiogama, Miyagi 985-0001, Japan
E-mail: y.minegishi@m.tohoku.ac.jp

and viability (Waples *et al*., 2008). On the other hand, genetic diversity is influenced by environmental and demographic changes like climate change, natural disaster and human activities. Direct effects involve population bottleneck and varied migration between populations, whereas indirect ones cause changes in habitat structure and community composition (Banks *et al*., 2013). Genetic diversity should be, therefore, carefully investigated, especially in the situation where drastic disturbances have occurred.

The 2011 off the Pacific coast of Tohoku Earthquake occurred on March 11, 2011, with a magnitude 9.0 generated a large tsunami that struck the Pacific coastline of eastern Japan (Mori *et al*., 2012). In addition to social, economic and physical impacts, ecological ones have been recently studied and reported in this area. Urabe *et al*. (2015) examined the macrobenthic animal communities at nine intertidal flat in Sendai Bay and the Sanriku Coast and found that 30 – 80% of taxa disappeared after the tsunami. Hata *et al*. (2016) investigated the ecological traits of ayu *Plecoglossus altivelis* with a one-year amphidromous life history and concluded that the tsunami drastically changed the ecological traits of this fish on the Sanriku Coast, such as hatching date and oceanic growth period. It can be, therefore, expected that this environmental event also affects genetic diversity of aquatic organisms in this area.

Marbled flounder *Pseudopleuronectes yokohamae* is a commercially valued fish across Japan. Due to the benthic lifestyle including low dispersal ability of the marbled flounder, this species possibly consists of multiple management units over its geographic distribution. In Sendai Bay, the population is considered to form a single management unit, and its spawning ground has been protected for stock conservation. This population, however, could have been strongly influenced by the great tsunami that occurred along the Pacific coast in Japan in 2011, and consequently the genetic diversity could be changed. Accordingly, the temporal genetic variation of the Sendai Bay population needs to be investigated and the geographic range of the management unit should be determined based on the genetic population structure of this species inferred together with other Pacific Ocean populations.

In this study, we firstly introduce our previous studies to develop the genomic resources and novel microsatellite markers for the marbled flounder, then demonstrate the results of population genetic analyses of this species using newly developed markers, and finally discussed the impact of that tsunami on the genetic diversity of this fish.

Materials and Methods

Development of the genomic resource and novel microsatellite markers

A 200-bp library was prepared using the genomic DNA extracted from a single adult specimen of the marbled flounder collected in Sendai Bay in 2012. IonTorrent PGM sequencing was performed and a single round of *de novo* assembly was conducted. To test if the assembled contigs can function as reference in genomic analyses, IonTorrent RAD-seq was performed using six individuals of this fish and SNPs were discovered by mapping RAD-tag reads to those contigs. The detailed procedure was provided in Genomic Resources Development Consortium *et al*. (2015). Using the software QDD version 3.0 (Meglécz *et al*., 2010), microsatellite sequences were searched for in the obtained genome sequence data of *P. yokohamae* and primers were designed for those microsatellites with a default configuration. The assembled contig sequences and unassembled reads from *de novo* assembly were separately used as input data in the software. Based on PCR amplification, visual confirmation of polymorphisms on an agarose gel and accurate genotyping, the primer pairs were chosen for subsequent population genetic analyses (Minegishi *et al*., 2015).

Population genetic analyses

Temporal change in genetic diversity of the marbled flounder after the tsunami impact was investigated using thirteen populations collected in Sendai Bay from April 2012 to February 2014 (N = 807). Nine populations from the Pacific Ocean coasts (Mutsu Bay, Onagawa Bay, Sendai Bay, Ibaraki, Choshi, Tokyo Bay [Funabashi, Hagiu, Takeoka, and Uraga], Sagami Bay, Hamana Lake, and Mikawa Bay; N = 596) were also analyzed for comparisons (Fig. 1). Genomic DNA was extracted from fin clips and genotyping was performed at the newly developed sixteen microsatellite loci. As the presence of null alleles was suggested at locus Psyo075, this locus was excluded from further analyses. Population genetic analyses were conducted using Arlequin ver. 3.5 (Excoffier *et al*., 2005). The detailed experimental conditions for genotyping were described in Minegishi *et al*. (2015).

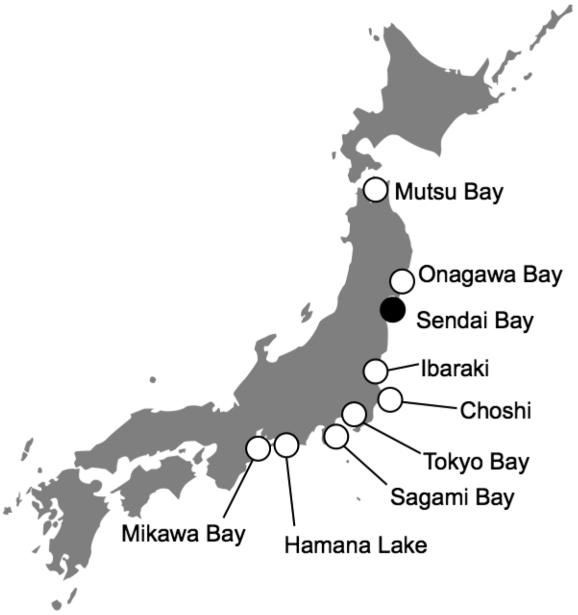

Fig. 1. Distribution of the marbled flounder (bold lines) and sampling sites in the present study. The black circle shows Sendai Bay. Boso Peninsula locates between Choshi and Tokyo Bay.

Results and Discussion

Development of the genomic resource and novel microsatellite markers

IonTorrent PGM sequencing and *de novo* assembly generated a total of 525,502 contigs (N50 = 1994) and 10,732,070 unassembled reads. The summed length of the contigs was 547.8 megabases, corresponding to 81.8% of the genome of this species (670 megabases; Ojima and Yamamoto, 1990). This suggests that this genome sequences data contains most information of the genome of this species. RAD-seq reads (91.92 – 95.34% of the reads) were successfully mapped to these contig sequences, which subsequently yielded 75,472 SNPs at maximum, indicating the utility of this genome data as a reference sequence in the genome-scale analyses (Genomic Resources Development Consortium *et al*., 2015). Microsatellites were found in 781,773 sequences in the unassembled reads, and a total of 331,368 primer sets were successfully designed. In contrast, no microsatellites were detected in the contigs. Screening based on PCR amplification, visual confirmation of polymorphisms on an agarose gel and accurate genotyping resulted in sixteen primer pairs that are polymorphic and applicable for population genetic analyses of this species (Minegishi *et al*., 2015). These results indicate that the obtained genome sequence data can be used as a genomic resource of this species and contributes to finding novel DNA markers such as SNPs and other microsatellites and the genes associated with various useful phenotypes including faster growth and high resistance to virus infection.

Evaluation of the genetic diversity of the Sendai Bay population of the marbled flounder

Population genetic analyses using genotyping data from the microsatellites developed above found significant genetic divergence between the east and west sides of Boso Peninsula (pairwise F_{ST} = 0.02305 – 0.19784). On the east side of Boso Peninsula, the Mutsu Bay population was differentiated (F_{ST} = 0.03829, P < 0.001) from other populations and gene flow was observed over the wide area from Onagawa Bay to Choshi including Sendai Bay. The genetic

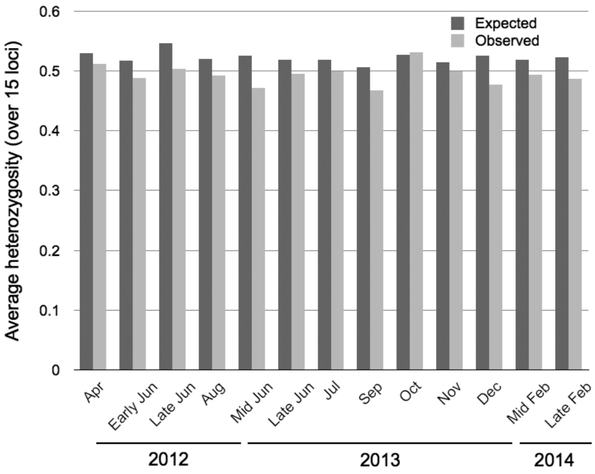

Fig. 2. Temporal change of the average heterozygosity over 15 microsatellite loci of the Sendai Bay population sampled from April 2012 to late February 2014. Dark and light grey indicates expected and observed values, respectively.

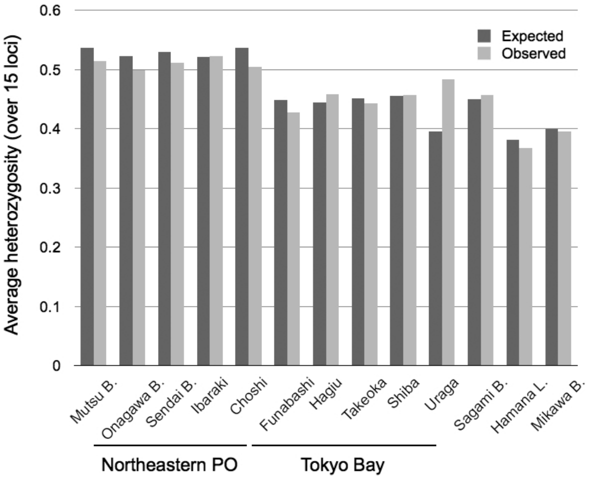

Fig. 3. Comparison of the average heterozygosity over 15 microsatellite loci of nine populations from Mutsu Bay to Mikawa Bay. Dark and light grey indicates expected and observed values, respectively.

variability of the Sendai Bay population did not show any drastic changes during the sampling period from April 2012 to February 2014 (H_E = 0.5061 – 0.5463) (Fig. 2) and no signal of a genetic bottleneck was observed. Moreover, the genetic diversity of the Sendai Bay population was relatively higher compared to those of the western populations of Boso Peninsula such as Tokyo Bay and Mikawa Bay (H_E = 0.3817 – 0.4557) (Fig. 3) . These results indicate that the Sendai Bay population should be treated as a single management unit together with other populations in the area ranging from Onagawa Bay to Choshi for stock management. In addition, unlike macrobenthic animals and ayu mentioned earlier, the tsunami impact on the Sendai Bay population of the marbled flounder is considered to be minor, but further monitoring may be needed since a long-term effect of the tsunami impact on marine ecosystems could emerge in the future.

Acknowledgments

This study was financially supported by Tohoku Ecosystem-Associated Marine Sciences from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Environmental disturbance underpins the dynamics and diversity of many of the ecosystems of the world, yet its influence on the patterns and distribution of genetic diversity is poorly appreciated. We argue here that disturbance history may be the major driver that shapes patterns of genetic diversity in many natural populations. We outline how disturbance influences genetic diversity through changes in both selective processes and demographically driven, selectively neutral processes. Our review highlights the opportunities and challenges presented by genetic approaches, such as landscape genomics, for better understanding and predicting the demographic and evolutionary responses of natural populations to disturbance. Developing this understanding is now critical because disturbance regimes are changing rapidly in a human-modified world.

(2) Waples R. S., Punt A. E., and Cope J. M., 2008: Integrating genetic data into management of marine resources: how can we do it better? *Fish Fish.* **9**, 423–449.

Molecular genetic data have found widespread application in the identification of population and conservation units for aquatic species. However, integration of genetic information into actual management has been slow, and explicit and quantitative inclusion of genetic data into fisheries models is rare. In part, this reflects the inherent difficulty in using genetic markers to draw inferences about demographic independence, which is generally the information of the greatest short-term interest to fishery managers. However, practical management constraints, institutional structures and communication issues have also contributed to the lack of integration. This paper identifies some of the organizational, conceptual and technical barriers that have hampered full use of genetics data in stock assessment and hence fishery management and outlines how such use could be enhanced.