View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by International Journal of Aquatic Biology

International Journal of Aquatic Biology (2015) 3(3): 183-190
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.NPAJournals.com
© 2015 NPAJournals. All rights reserved

Original Article

Genetic variation and population structure of spottybelly greenling (*Hexagrammos agrammus*) in Korean coasts analyzed by DNA markers emphasizing on microsatellites

Kazi Ahsan Habib*[1], Mohammad Nazrul Islam[2], Shirin Sultana[3], Youn-Ho Lee[4]

[1]Department of Fisheries, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.
[2]Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.
[3]Fisheries Biotechnology Division, National Institute of Biotechnology, Savar, Dhaka-1349, Bangladesh.
[4]Korea Institute of Ocean Science and Technology (KIOST), Sadong, Ansan, 426-744, South Korea.

Abstract: Two nuclear microsatellite DNA loci along with partial sequences of mitochondrial DNA $tRNA^{PRO}$-D-loop region were analyzed to assess the genetic diversity and population genetic structure of spottybelly greenling (*Hexagrammos agrammus*). A total of 85 individuals were investigated from two different locations at the East and the South coasts of Korea which are Imwonjin (IM) and Tongyeong (TN), respectively. In the analysis of genetic diversity, nucleotide diversities were low showing 0.01 whereas haplotype diversities were as high as 0.92 and 0.97 in IM and TN populations, respectively indicating population bottleneck after rapid growth of these populations. No significant genealogical branches or clusters were recognized on the neighbor-joining phylogenetic tree. Pairwise population statistics F_{ST} and the exact test of population differentiation from the analysis of microsatellite DNA loci demonstrated no significant genetic difference between two populations investigated in the Korean coasts. In addition, significant deviations from Hardy-Weinberg equilibrium and heterozygote deficiencies were found from the microsatellite DNA loci. The results of the study will help to make a plan for fisheries management of the species.

Article history:
Received 16 January 2015
Accepted 27 February 2015
Available online 25 June 2015

Keywords:
Genetic diversity
Population structure
Mitochondrial DNA
Microsatellite DNA
Spottybelly greenling

Introduction

Spottybelly greenling, *Hexagrammos agrammus* (Temminck and Schlegel, 1843), a member of the family Hexagrammidae, is a commercially important fish species in Korea. This fish is distributed along coastal areas of Japan and Korean Peninsula, and somewhat found in the Yellow Sea. The species lives in shallow seaweed beds and reproduce from September to December on seaweeds as spawning substrates (Chung and Kim, 1994). During the breeding season, multiple females visit in the breeding territory of a male and release eggs. Afterward, male fertilizes the eggs and look after these until hatching (Munehara et al., 2000).

Marine organisms generally show low levels of genetic differentiation over large geographic distances. Lack of physical barriers, high fecundity, dispersal of eggs and larvae by ocean current, and migration of adults in the open ocean contribute to the high gene flow, eventually lead to panmictic populations in many marine fishes (Palumbi, 1994; Walpes, 1998; Grant and Bowen, 1998). On the other hand, physical barriers to dispersal, such as depth, temperature, or salinity boundaries; or hydrodynamic eddies favoring larval retention; or strong philopatry are the factors that can promote population differentiation and genetic structure (Grant and Bowen, 1998). Thus, the reason of forming population genetic structure of many marine organisms is sometimes difficult to understand (Machado-Schiaffino et al., 2009). Assessment of population genetic structure is essential to make strategies for conservation and management of the fisheries' resources. Failure to detect population

* Corresponding author: Kazi Ahsan Habib
E-mail address: ahsan_sau@yahoo.com

units can lead to local overfishing and ultimately to severe declines (Hutchings, 2000; Knutsen et al., 2003). Analysis of genetic diversity and population genetic structure also provide insights into historical demography and evolutionary process of the populations in the species.

In this study, we investigated the genetic diversity and population genetic structure of spottybelly greenling in the East and the South coast of Korea by analyzing microsatellite DNA (msDNA) and partial concatenated tRNA^{PRO}-D-loop region of mitochondrial DNA (mtDNA) markers. Microsatellite markers are very useful for assessing genetic structure of fish populations due to their selective neutrality and high mutation rates providing greater allelic diversity and heterozygosity (O'Connell and Wright, 1997). Mitochondrial DNA D-loop region is particularly sensitive to assess genetic diversity and demographic history of the population in marine fish (Buonnacorsi et al., 2001).

Materials and Methods

Samples collection and DNA extraction: A total of 85 spottybelly greenling individuals were collected from two locations of which 35 fish from Imwonjin (IM) and 50 fish from Tongyeong (TN) of the south and the east coasts of Korea, respectively (Fig. 1) in 2007 and 2008. Fishes were collected with the help of local fishermen and preserved in 95% ethanol until DNA extraction. Genomic DNA was extracted from the tissue of preserved samples using a DNeasy blood and tissue kit (Qiagen, Germany) according to the manufacturer's protocol provided in the kit box.

PCR amplification and sequencing: From the genomic DNA, concatenated 3' region of tRNA^{PRO} gene and 5' region D-loop region (hyper-variable portion) was amplified using the primers HagF2: 5′–CCCTAACTCCCAAAGCTAGGATTC–3′ and HagR2: 5′–ATGCTGGGTCTCTCGGAGATGTA–3′, respectively through polymerase chain reaction (PCR) (Habib et al., 2014). All the PCR reactions contained 50 μℓ reaction mixture having 2.5 unit of Taq DNA polymerase, 5 μℓ of 10× PCR buffer, 10 mM each of the dNTPs (2.5 μℓ each), 25 pmoles of

Figure 1. Map showing sampling locations of *H. agrammus* samples and the major warm currents around the sampling area. Abbreviations as follows: IM– Imwonjin (Sea of Japan) and TN– Tongyeong (South Sea of Korea).

each primer, and 0.5–1.0 μg template DNA. The temperature profile of PCR was set at 95°C for 5 min as preheating, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C, extension at 72°C for 1 min, with a terminal elongation at 72°C for 7 min. PCR products were examined by 1% agarose-gel electrophoresis with a standard-size marker and purified by a PCR purification kit (Takara, Japan). The purified DNA was sequenced bidirectionally using automated DNA sequencer 377 or 3100 (Applied Biosystems, USA).

Microsatellite loci amplification and scoring: Two microsatellite loci (Heot06 and Heot17) developed for the most closely related species *H. otakii* (Chen et. al., 2009) were amplified from the 85 samples of the Korean coasts. PCR amplifications consisted of 3 min pre-heating at 94°C followed by 35 cycles of denaturation at 95°C for 45 sec, annealing at 55°C for 45 sec, and extension at 72°C for 1 min. PCR ended with a final extension at 72°C for 7 min. The PCR reaction mixture contained 1.5 μℓ of 10× PCR reaction buffer, 1.5 μℓ of 10 mM dNTP, 1 μℓ of 0.2 mM of each primer, 3 units of Super Taq polymerase (SUPER BIO, Osong, Korea), and 0.5 μg of template DNA with the total volume of 15 μl brought up with sterilized water. Forward primers were labeled with

Table 1. Genetic diversities of IM and TN populations.

Population	No. of haplotypes	Substitutions		h	π
		ti	tv		
IM	18	15	5	0.92	0.01
TN	35	28	11	0.97	0.01
Pooled	43	30	12	0.95	0.01

* h: Haplotype diversity, ti: transitions, tv: transversions, π: nucleotide diversity

a fluorescent dye (Genotech, Korea) and the amplified products were run for size detection on the ABI PRISM_310 automated sequencer. Data collection and sizing of alleles were carried out using Peak Scanner v31.0 software (Applied Biosystem).

Data analysis: The tRNA^{PRO}-D-loop sequence data were edited and aligned with Clustal W (Thompson et al., 1994) and DNAssist 2.5 (Patterton and Graves, 2000). The level of polymorphism was estimated as the haplotype diversity (h), nucleotide diversity (π), number of haplotypes, transitions (ti) and transversions (tv) for both populations separately and also for pooled samples using the program Arlequin (version 3.5, Schneider et al., 2000). In the microsatellite analysis, microsatellite polymorphism was evaluated by the number of alleles (N), allelic richness (A_R), observed heterozygosity (H_O), and expected heterozygosity (H_E). Population genetic structure was evaluated by pairwise estimates of the F_{ST} values and by the exact test of population differentiation. Exact test gives more weight to rare alleles and can therefore be more sensitive to the detection of weak population differentiation. All of these parameters were computed using the Arlequin software except A_R which was calculated using the FSTAT software (ver. 2.9.3.2, Goudet, 2001). Deviation from the Hardy–Weinberg equilibrium (HWE) was tested using POPGENE (ver. 1.31; Yeh et al., 1999).

Phylogenetic relationship among haplotypes of tRNA^{PRO}-D-loop region was reconstructed with MEGA 3.1 (Kumar et al., 2004) using the neighbor joining (NJ) method (Saitou and Nei, 1987) of phylogenetic inference with 1000 replications. Genetic distances were corrected following the model of Tamura and Nei, which was specifically conceived to reproduce the evolution of the mitochondrial D-loop region (Zardoya et al., 2008).

Results

Genetic diversity: Sequences of all of the 85 individuals of IJ and TN populations were obtained after sequencing. A total of 500 nucleotide long sequences were acquired after removing the confusing sequences near the primer ends. These 500 bp long sequences included a portion of the tRNA^{pro} (31 bp) sequence in the 3′ end and the rest 469 bp of the partial d-loop region at the 5′ end. Most of the nucleotide substitutions were transitional in the studied sequences (Table 1). About 30% of the nucleotide substitutions were transversion. No indels were detected in all the sequences analyzed. Variations in the D-loop region defined 43 haplotypes with 39 polymorphic sites of which ten haplotypes were shared by two populations. The nucleotide diversities (π) were as low as 0.01 nucleotide differences per site. On the other hand, the haplotype diversities (h) were relatively high providing the values of 0.92-0.97 (Table 1).

In the msDNA analysis, two microsatellite loci revealed a moderate level of polymorphism in the Korean populations. In the locus Heot06, 10 and 12 alleles were found from the IM and TN populations, respectively, and 17 and 22 alleles in the locus Heot17. The observed heterozygosities (H_O) were found significantly lower than expected heterozygosities (H_E) for all loci in both populations. The average H_O was around 0.5 and 0.6 for IM and TN populations, respectively whereas H_E was estimated over 0.85 for both populations (Table 2). In all cases, the 1− H_O/H_E values were positive, meaning that IM and TN populations were deficient

Table 2. Summary statistics for two microsatellite loci in the IM and TN populations of *H. agrammus*. (N = No. of alleles, A_R = Allelic richness, H_O = observed heterozygosity and H_E = expected heterozygosity)

Microsatellite locus	Parameters	Population	
		IM	TN
Heot06	N	10	12
	A_R	10.95	11.031
	H_O	0.6	0.764
	H_E	0.804	0.837
	Deviation from HWE	NS	*
Heot17	N	17	22
	A_R	18.959	19.492
	H_O	0.4	0.418
	H_E	0.911	0.915
	Deviation from HWE	*	*
Average value over two loci	H_O	0.5	0.59
	H_E	0.858	0.876
	N	13.5	17

*Significant; NS = not significant

in heterozygosities at both loci. The locus Heot17 in both populations and Heot06 in the TN population showed significant deviations from HWE. Only non-significant deviation (P>0.05) from HWE was detected for the locus Heot06 in IM population. A summary of the statistical data, including N, A_R, H_O, H_E, and agreement to the HWE, for IM and TN populations are presented in Table 2.

Population differentiation: Estimates of F_{ST} between IM and TN populations for msDNA was nearly zero (0.013) with no significant difference (P= 0.07) from random mixing, indicating that no population genetic structure (i.e. a single genetic stock) was established over the range of investigation. Exact test of population differentiation also did not give any significant differentiation between two populations with the P-values for microsatellite loci 0.932 between IM and TN.

Phylogenetic relationships of haplotypes: The topology of the neighbor joining tree of 43 haplotyp sequences of *H. agrammus* was shallow with short branches (sum of branch length, SBL = 0.121), and there were no genealogical clades or internal branches corresponding to any particular sampling locality (Fig. 2). Haplotypes from individuals of a population were scattered throughout the tree being well mixed with those from another population.

Discussion

Spottybelly greenling is a demersal fish which inhabits in coastal reef area. The fish shows restricted movement and releases sticky eggs on the seaweed beds. Such limited dispersal characteristic is responsible for genetic differentiation among populations in several marine fish species (Han et al., 2008a; Mukai et al., 2009). However, the present study clearly indicates genetic homogeneity among two regional population samples, i.e. IM and TN population of spottybelly greenling. The F_{ST} statistics for msDNA markers revealed no significant genetic structure between two sampling sites suggesting substantial genetic exchange along the coast of Korea. Moreover, non-significant exact P-value in the exact test of population differentiation reveals population panmixia for the fish in the Korean coastal water. Lack of clade with a specific geographic location (East coast or South coast) in the phylogenetic tree further supports high rate of gene flow between two populations (Han et al., 2008a). Many marine fishes show lack of genetic differentiation among the geographic regions due to higher dispersal potential during planktonic egg and larvae (Palumbi, 1994). The adult spottybelly greenling lives in the bottom however, the larvae and fry spend planktonic stage for around two months in the shallow or subsurface waters (Cho et al., 2001). Therefore, transportation of planktonic larvae and fry by the ocean currents possibly caused this genetic homogeneity of spottybelly greenling along the Korea coasts. The Tsushima current would carry the

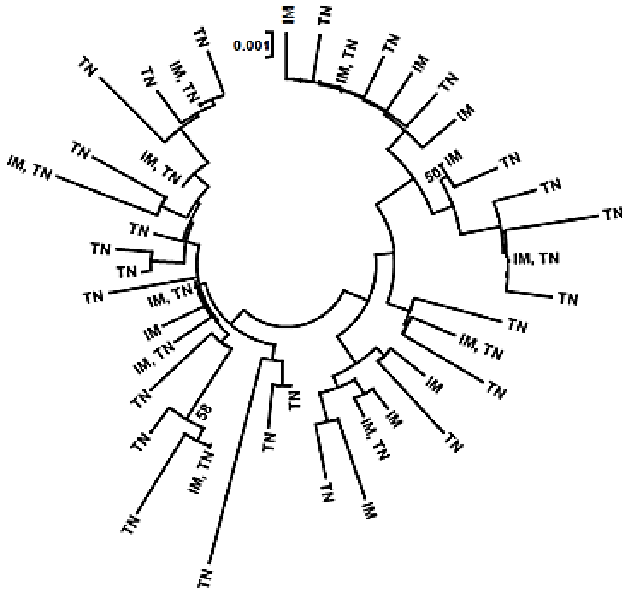

Figure 2. A Neighbor-Joining tree of $tRNA^{PRO}$-D-loop region haplotypes of *H. agrammus*. Bootstrap supports of less than 50% in 1000 replicates are not shown. IM and TN represent geographical distribution of each haplotype.

fries and larvae from the south to the northern parts. Conversely, the coastal and the subsurface counter currents can transport these young fish from the northern to the southern areas and cause extensive genetic exchange between the regions. Several marine fishes around Korea as well as in northwest Pacific exhibited similar pattern of genetic characteristics. As for example, fat greenling (*H. otakii*), a sister species of the spottybelly greenling did not show any genetic separation among the populations of the west coast (Yellow Sea), the south coast and the east coast (East Sea) (Habib et al., 2011). Japanese sea bass *Lateolabrax japonicus* and blackfin flounder *Glyptocephalus stelleri* formed a single genetic population around the Korea and the Japan coastal waters (Liu et al., 2006; Xiao et al., 2010). In an another study, red tilefish (*Branchiostegus uitions*), a subtropical demersal fish showed no population genetic structure among the samples collected from the East China Sea and the Japan coast (Nohara et al., 2010). In all of these fishes, dispersal of pelagic eggs and larvae by the currents were considered as a probable cause for such genetic homogeneity.

In the microsatellite study, no significant genetic structure was found between any of the populations but expected heterozygosity was significantly high across both locations. Strong heterozygote deficiencies also have been reported for a number of fish species (Waldman and McKinnon, 1993) such as common sole *Solea vulgaris* (Kotoulas et al., 1995) and plaice *Pleuronectes platessa* (Hoarau et. al., 2002), and the reasons were mentioned as the result of null alleles, Wahlund effect or biological processes such as inbreeding and selection. Inbreeding would be unlikely for the observed heterozygote deficiency in spottybelly greenling fish with large populations since the fish is widely distributed in the coasts of Korea as well as in Northwestern Pacific Ocean. Wahlund effects or selection might be the reason for observed heterozygote deficiencies for this fish. Wahlund effects occur when two or more subpopulations are unintentionally sampled as a single population. In case of selection, it might have occurred as a very recent event since microsatellites reflect very recent demographic history below last ten thousand years. Null alleles are a common problem with microsatellite loci that can lead to high heterozygote deficiencies (Hare et al., 1996; O'Connell and Wright, 1997). They occur when one allele is not amplified due to mutations in one of the primers, and/or when technical problems associated with amplification and scoring arise. The primers that we used in the present study were originally developed for the sister species, fat greenling (*H. otakii*) which might be one of the causes for heterozygote deficiency. Significant deviations from Hardy-Weinberg equilibrium in the present study were possibly caused due to the loss of heterozygosity in the population.

Very low nucleotide diversity with high haplotype diversity in both IM and TN population of spottytbelly greenling (π = 0.01; h>0.9) indicates that the fish has experienced rapid population growth after bottleneck and accumulation of mutation (Grant and Bowen, 1998). Habib et al. (2014) showed that this population expansion of spottybelly greenling has started during the Pleistocene climatic

changes. In case of sudden population growth, the rate of stochastic loss of haplotypes decelerates and maintains more haplotypes than the loss by genetic drift (Avise et al., 1984). High haplotypic diversity further suggests large, stable and effective population size over time in the spottybelly greenling since it is widely distributed in coastal waters of Korea and the Japanese Archipelago (Stepien, 1999). This kind of genetic make-up has been detected in a number of fish species, including fat greenling (h = 0.69 – 96, π = 0.002 – 0.004) (Habib et al., 2011), redtile fish (h = 0.93, π = 0.008) (Nohara et al., 2010), blackfin flounder (h = 0.99, π = 0.014) (Xiao et al., 2010) and North Pacific light fish, *Maurolicus japonicas* (h = 0.58, π = 0.003) (Habib et al., 2012). On the other hand, the shallow phylogenetic tree reveals very recent origin of the haplotypes and rapid population growth. Moreover, the short branch length in the NJ tree suggests a recent (young) population genetic divergence for the populations of *H. agrammus* (Zhang et al., 2006). Another greenling species, *H. otakii*, a shallow coastal water demersal fish distributed along the coast of Korea, Japan, and China in the northwestern Pacific went through recent expansion (Habib et al., 2011). Since these two sister species, i.e. *H. agrammus* and *H. otakii* are distributed almost in the same geographic region and their habitats are also same, similar demographic pattern i.e. rapid population growth after a bottleneck was found. Population expansion has been also reported for a number of other benthic fishes such as the yellow drum (Han et al., 2008b), willowy flounder (Xiao et al., 2008), blackfin flounder (Xiao et al., 2010).

Assessment of genetic diversity and population genetic structure are essential for maintaining a productive fishery through effective management (Seeb et al., 1990). Different populations with unique genetic structure and/or geographically isolated stock should be managed as distinct units, and such units require separate monitoring and management (Salgueiro et al., 2003). Our study of msDNA variations in spottybelly greenling did not show any evidence for genetic subdivision and revealed a single population between the East and South coast of Korea. Therefore, the study suggests rather simple management strategy for this fish, i.e. 'one stock' management policy in the study areas. However, caution must be taken for one stock management policy until the population genetic structure and the current migration pattern between the populations were thoroughly understood. For this, more microsatellite loci can be developed and analyzed for the samples incorporating more sampling sites.

References

Avise J.C., Neigel J.E., Arnold J. (1984). Demographic influences on mitochondrial DNA lineage survivorship in animal populations. Journal of Molecular Evolution, 20: 99-105.

Buonnacorsi V.P., McDowell J.R., Graves J.E. (2001). Reconciling pattern of interocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Molecular Ecology, 10: 1179-1196.

Chen S.-L., Miao G.-D., Shao C.W., Tian Y.-S., Liao X. (2009). Isolation and characterization of polymorphic microsatellite loci from fat greenling (*Hexagrammos otakii*). Conservation Genetics, 10: 1429-1431.

Cho S.-H., Myoung J.-G., Kim J.-M., Lee J.-H. (2001). Fish fauna associated with drifting seaweed in the coastal area of Tongyeong, Korea. Transactions of the American Fisheries Society, 130: 1190-1202.

Chung E.Y., Kim S.Y. (1994). On the maturity and spawning of the greenling, *Hexagrammos agrammus* (Temminck et Schlegel). Korean Journal of Ichthyology, 6(2): 222-236 (in Korean).

Goudet J. (2001). FSTAT: a computer program to calculate F-statistics. Journal of Heredity, 86: 485-486.

Grant W.S., Bowen B.W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. Journal of Heredity, 89: 415-426.

Habib K.A., Jeong D., Myoung J.-G., Kim M.S., Jang Y.S., Shim J.S., Lee Y.-H. (2011). Population genetic structure and demographic history of the fat greenling *Hexagrammos otakii*. Genes and Genomics, 33: 413-423.

Habib K.A., Jeong D., Myoung J.-G., Lee Y.-H. (2014). Population panmixia and the Pleistocene demographic expansion of spotty belly greenling *Hexagrammos agrammus* in the East Sea and Northwest Pacific. Ocean Science Journal (In press).

Habib K.A., Oh J.D., Kim S., Lee Y.-H. (2012). Divergence and gene flow between the East Sea and the Southeast Atlantic populations of North Pacific light fish *Maurolicus japonicas* Ishikawa. Genes and Genomics, 34: 609-618

Han Z.Q., Gao T.X., Yanagimoto T., Sakurai Y. (2008a). Genetic population structure of *Nibea albiflora* in Yellow Sea and East China Sea. Fisheries Science, 74: 544-552

Han Z.-Q., Gao T.-X., Yanagimoto T., Sakurai Y. (2008b). Deep phylogeographic break among white croaker *Pennahia argentata* (Sciaenidae, Perciformes) populations in North-western Pacific. Fisheries Science, 74: 770-780.

Hare M.P., Karl S.A., Avise J.C. (1996). Anonymous nuclear DNA markers in the American oyster and their implications for the heterozygote deficiency phenomenon in marine bivalves. Molecular Biology and Evolution, 13: 334-345.

Hoarau G., Rimnsdorp A.D., Van der veer H.W., Stam W.T., Olsen J.L. (2002). Population structure of plaice (*Pleuronectes platessa* L.) in northern Europe: microsatellites revealed large-scale spatial and temporal homogeneity. Molecular Ecology, 11: 1165-1176.

Hutchings J.A. (2000). Collapse and recovery of marine fishes. Nature, 406: 882-885.

Knutsen H., Jorde P.E., Andre C., Stenseth N.C. (2003). Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Molecular Ecology, 12: 385-394.

Kotoulas G., Bonhomme F., Borsa P. (1995). Genetic structure of the common sole *Solea vulgaris* at different geographic scales. Marine Biology, 122: 361-375.

Kumar S., Tamura K., Nei M. (2004). Mega 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics, 5: 150-163.

Liu J.-X., Gao T.-X., Yokogawa K., Zhang Y.-P. (2006). Differential population structuring and demographic history of two closely related fish species, Japanese sea bass (*Lateolabrax japonicus*) and spotted sea bass (*Lateolabrax maculatus*) in Northwesternern Pacific. Molecular Phylogenetics and Evolution, 39: 799-811.

Machado-Schiaffino G., Campo D., Garcia-Vazquez E. (2009). Strong genetic differentiation of the Australian hake (*Merluccius australis*) across the species range. Molecular Phylogenetics and Evolution, 53: 351-356.

Mukai T., Nakamura S., and Nishida M. (2009). Genetic population structure of a reef goby, *Bathygobius cocosensis*, in the northwestern Pacific. Ichthyological Research, 56: 380-387

Munehara H., Kanamoto Z., Miura T. (2000). Spawning behavior and interspecific breeding in three Japanese greenlings (Hexagrammidae). Ichthyological Research, 47(3): 287-292.

Nohara K., Takeuchi H., Tsuzaki T., Suzuki N., Tominaga O., Seikai T. (2010). Genetic variability and stock structure of red tilefish *Branchiostegus japonicus* inferred from mitochondrial DNA sequence analysis. Fisheries Science, 76: 75-81.

O'Connell M., Wright J.M. (1997). Microsatellite DNA in fishes. Reviews in Fish Biology and Fisheries, 7: 331-363.

Palumbi S.R. (1994). Genetic divergence, reproductive isolation, and marine speciation. The Annual Review of Ecology, Evolution, and Systematics, 25: 547-572.

Patterton H.G., Graves S. (2000). DNAssist: the integrated editing and analysis of molecular biology sequences in windows. Bioinformatics, 16: 652-653.

Rogers A.R., Harpending H. (1992). Population growth makes waves in the distribution of pairwise genetic difference. Molecular Biology and Evolution, 9: 552-569.

Saitou N., Nei M. (1987). The neighbour-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4: 406-425.

Salgueiro P., Carvalho G., Collares-Pereira M.J., Coelho M.M. (2003). Microsatellite analysis of genetic population structure of the endangered cyprinid *Anaecypris hispanica* in Portugal: implications for conservation. Biological Conservation, 109: 47-56.

Schneider S., Roessli D., Excoffier L. (2000). Arlequin: a software for population genetics data analysis. Ver 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva.

Seeb L.W., Seeb J.E., Polovina J.J. (1990). Genetic variation in highly exploited spiny lobster *Panulirus marginatus* populations from the Hawaiian

Archipelago. Fishery Bulletin, 88: 713-718

Stepien C.A. (1999). Phylogeographical structure of the Dover sole *Microstomus pacificus*: the larval retention hypothesis and genetic divergence along the deep continental slope of the northeastern Pacific Ocean. Molecular Ecology, 8: 923-939.

Thompson J.D., Higgins D.G., Gibson T.J. (1994). Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position specific gap penalties and weight matrix choice. Nucleic Acid Research, 22: 4673-4680.

Waldman B., McKinnon J.S. (1993). Inbreeding and outbreeding in fishes, amphibians and reptiles In: N.W. Thornbel (Ed.). The Natural History of Inbreeding and Outbreeding. University of Chicago Press, Chicago. pp: 250-282.

Walpes R.S. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity, 89: 438-450.

Xiao Y., Gao T., Zhang Y., Yanagimoto T. (2010). Demographic History and Population Structure of Blackfin Flounder (*Glyptocephalus stelleri*) in Japan Revealed by Mitochondrial Control Region Sequences. Biochemical Genetics, 48(5-6): 402-417.

Xiao Y., Takahashi M., Yanagimoto T., Zhang Y., Gao T., Yabe M., Sakurai Y. (2008). Genetic variation and population structure of willowy flounder *Tanakius kitaharai* collected from Aomori, Ibaraki and Niigata in Northern Japan. African Journal of Biotechnology, 7(21): 3836-3844.

Yeh F.C., Yang R.C., Boyle T. (1999). POPGENE, Version 1.31: Microsoft Window-Based Free Ware for Population Genetic. Analysis. http://www.ualberta.ca/~fyeh

Zardoya R., Castilho R., Grande C., Favre-Krey L., Caetano S., Marcato S., Krey G., Patarnello T. (2004). Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*) in the Mediterranean Sea. Molecular Ecology, 13: 1785-1798.

Zhang J., Cai Z., Huang L. (2006). Population genetic structure of crimson snapper *Lutjanus erythropterus* in East Asia, revealed by analysis of the mitochondrial control region. ICES Journal of Marine Science, 63: 693-704.