Mediterranean Marine Science

Vol 16, No 1 (2015)

Vol 16, No 1 (2015)

Loss of genetic variation in Greek hatchery populations of the European sea bass (Dicentrarchus labrax L.) as revealed by microsatellite DNA analysis

D. LOUKOVITIS, B. IOANNIDI, D. CHATZIPLIS, G. KOTOULAS, A. MAGOULAS, C. S. TSIGENOPOULOS

doi: 10.12681/mms.1033

Short Communication

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.1033

Loss of genetic variation in Greek hatchery populations of the European sea bass (*Dicentrarchus labrax* L.) as revealed by microsatellite DNA analysis

D. LOUKOVITIS[1,2], B. IOANNIDI[3], D. CHATZIPLIS[1], G. KOTOULAS[2], A. MAGOULAS[2] and C.S. TSIGENOPOULOS[2]

[1] Department of Agricultural Technology, School of Agricultural Technology, Food Technology and Nutrition, Technological Educational Institute of Thessaloniki, P.O. Box 141, 57400 Sindos, Thessaloniki, Greece
[2] Department of Genetics and Molecular Biotechnology, Institute of Marine Biology and Genetics (IMBG), Hellenic Centre for Marine Research (HCMR), P.O. Box 2214, 71500 Heraklion, Crete, Greece
[3] Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, 38446 Volos, Greece

Corresponding author: dloukovi@hotmail.com

Handling Editor: Marco Oliverio

Received: 14 August 2014; Accepted: 20 October 2014; Published on line: 27 February 2015

Abstract

Genetic variation in four reared stocks of European sea bass *Dicentrarchus labrax* L., originating from Greek commercial farms, was assessed using five polymorphic microsatellite markers and was compared with that of three natural populations from Greece and France. The total number of alleles per marker ranged from 8 to 22 alleles, and hatchery samples showed the same levels of observed heterozygosity with samples from the wild but substantially smaller allelic richness and expected heterozygosity. The genetic differentiation of cultivated samples between them as well as from the wild origin fish was significant, as indicated by F_{st} analysis. All population pairwise comparisons were statistically significant, except for the pair of the two natural Greek populations. Results of microsatellite DNA analysis herein showed a 37 % reduction of the mean allele number in the hatchery samples compared to the wild ones, suggesting random genetic drift and inbreeding events operating in the hatcheries. Knowledge of the genetic variation in *D. labrax* cultured populations compared with that in the wild ones is essential for setting up appropriate guidelines for proper monitoring and management of the stocks either under traditional practices or for the implementation of selective breeding programmes.

Keywords: Genetic variation, hatchery stocks, microsatellites, *Dicentrarchus labrax*.

Introduction

The European sea bass *Dicentrarchus labrax* L. is an economically important marine food fish and along with sea bream (*Sparus aurata*) they are the two leading species of Mediterranean aquaculture in the last three decades; in 2012, almost 155,000 metric tons (mtn) of sea bass were produced with a commercial value of around one billion USD (FAO, http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en). With the increase in hatchery production, selective breeding of sea bass received a growing interest, while several studies reported a medium to high estimated heritability of growth (Chatziplis *et al*., 2007; Dupont-Nivet *et al*., 2008; Vandeputte *et al*., 2009), revealing a great potential of the species for genetic improvement of commercially important traits. One of the requirements for a successful breeding program, however, is the presence of sufficient genetic variation in the base population, which contradicts already existing results from various research surveys that have shown a lower genetic diversity of cultured fish stocks compared to that of wild populations (Norris *et al*., 1999; Kang *et al*., 2006; Loukovitis *et al*., 2012). Small effective population sizes and founder effects, such as the use of only limited individuals for the establishment of broodstocks, are thought to be the major reasons for the substantial loss of genetic variation in hatchery strains.

Microsatellite markers are characterized as codominant and highly polymorphic systems and have quickly become the molecular marker of choice for studies concerning parentage, monitoring changes in genetic variation of hatchery stocks, estimating effective population size in hatcheries (Brown *et al*., 2005) and examining the population structure in marine species (De Innocentiis *et al*., 2004; Guinand *et al*., 2008). They have also been used to produce genetic linkage maps (Chistiakov *et al*., 2005; Franch *et al*., 2006) as well as to unravel links between genetic and phenotypic variation through QTL scans (Chatziplis *et al*., 2007; Massault *et al*., 2010; Loukovitis *et al*., 2011).

In this study, the amount of genetic variation in four Greek reared stocks of sea bass was assessed using five microsatellite markers and compared to that of three natural populations from the Mediterranean Sea. Our aim

was to obtain information about the levels and patterns of genetic diversity for *D. labrax* hatchery stocks in Greece in order to set up the appropriate guidelines for proper management of the stocks, as a precursor towards the implementation of future selection programs.

Materials and Methods

A total of 100 wild *D. labrax* individuals were obtained in 2000 from two different locations in Greece (Thermaikos and Korinthiakos Gulf) and one site in France (Gulf of Lion). Random samples (n = 243) of cultured sea bass were collected in 2006 from different, in all cases, Greek commercial farms located in the north-east part of the Peloponnese. Genomic DNA was isolated from abdominal fin, stored in 100% ethanol, by standard *proteinase K* digestion, following the salting out procedure as described in Miller *et al*. (1988). Geographic locations, sample sizes, numbering and nomenclature of samples are given in Fig. 1.

Multiplex PCR reaction method amplifying simultaneously five microsatellite loci: *DLA0061*, *DLA0075*, *DLA0078*, *DLA0081* and *DLA0086* (Tsigenopoulos *et al*., 2003) was used. All multiplex PCRs were performed in 10 µl volumes containing 1 U of *Taq polymerase* (Genaxxon, Biberach, Germany), 1 × *Taq* buffer, 0.25 mM dNTP mix, 1.5 mM $MgCl_2$, 0.3 pM for each primer and approximately 20 ng of template DNA. Cycling conditions for the multiplex amplification consisted of an initial 95 °C denaturation step for 3 min followed by 35 cycles of 30 s at 94° C, 90 s at 57° C, and 60 s at 72° C, with a final extension at 72° C for 10 min. Fluorescently labelled PCR products were separated on an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems). Alleles were sized and individuals genotyped using the STR and 2.3.79 software (http://www.vgl.ucdavis.edu/informatics/STRand/).

Number of alleles (A), observed and expected heterozygosity (H_o, H_E), allelic richness (R_s) and departure from Hardy-Weinberg equilibrium at each locus and sample were calculated using the Genetix 4.02 (http://www.univ-montp2.fr/~genetix/) and FSTAT 2.9.3.2 (http://www.unil.ch/izea/softwares/fstat.html) statistical packages. FSTAT was also used to calculate population pairwise F_{st} (significance of the pairwise values was computed by 1000 permutations of the data sets) and conventional F-statistics (Weir & Cockerham, 1984). Corrections of the significance level for multiple tests were performed following the strict Bonferroni procedure (Rice, 1989). Furthermore, for each population, the effective population size (N_e) was estimated using the gametic disequilibrium method implemented in LDNe 1.31 (Waples & Do, 2008), while BOTTLENECK 1.2.02 (Piry *et al*., 1999) software was used to test whether the genetic diversity of the aquaculture stocks had the signature of a recent population expansion after a founder effect. The program was run with 1000 iterations assuming a two-phased model of mutation (TPM).

Results and Discussion

Polymorphism of the microsatellite markers was medium to high, ranging from 8 to 22 alleles. Summary statistics for genetic diversity are presented in Table 1. The mean number of alleles over all loci in the wild and reared stocks was 9.7 and 6.1, respectively, and significantly different (Mann-Whitney test, $P < 0.05$), showing that approximately 63 % of the alleles in the wild populations was present in the farmed ones. Average allelic richness (R_s) over all loci ranged from 3.769 to 6.171 in the cultured samples, whereas the respective value for the wild ones was significantly higher (7.419-10.934, Mann-Whitney test $P < 0.05$). Additionally, mean expected het-

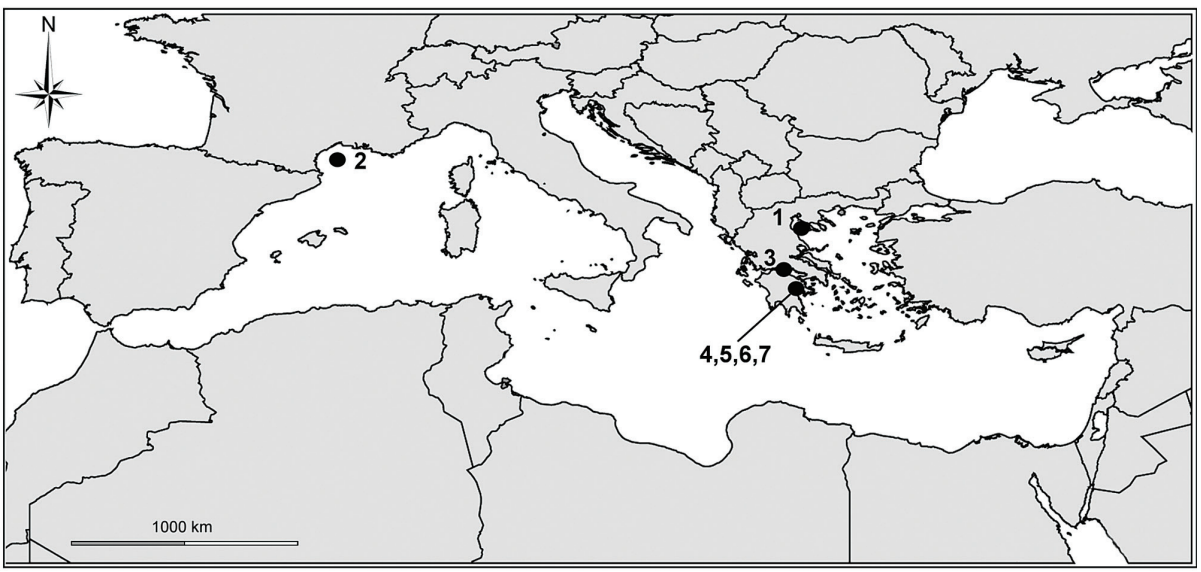

Fig. 1: Numbering, geographic origin and size of *D. labrax* samples. Wild: 1. GR1 Thermaikos Gulf (n = 30); 2. FR Gulf of Lion (n = 30); 3. GR2 Korinthiakos Gulf (n = 40). Cultivated: 4. PEL1 (n = 110); 5. PEL2 (n = 28); 6. PEL3 (n = 60); 7. PEL4 (n = 45). Abbreviation is as follows: GR = Greece; FR = France; PEL = Peloponnese.

Table 1. Number of alleles (A), allelic richness (R_s), observed/expected heterozygosity (H_o, H_E) and number of surveyed specimens (n) at five microsatellite loci genotyped in 7 samples of the European sea bass *Dicentrarchus labrax*.

	1. GR1	**2. FR**	**3. GR2**	**4. PEL1**	**5. PEL2**	**6. PEL3**	**7. PEL4**
Locus	$n = 30$	$n = 30$	$n = 40$	$n = 110$	$n = 28$	$n = 60$	$n = 45$
DLA0081							
A	9	10	7	7	5	4	6
R_s	8.713	9.819	6.556	5.906	4.929	4.000	5.999
H_o	0.633	0.700	0.550	0.782	0.357	0.732	0.886
H_E	0.641	0.825	0.631	0.693	0.474	0.738	0.775
DLA0061							
A	6	6	6	4	4	3	5
R_s	5.718	5.835	5.516	3.847	4.000	3.000	4.998
H_o	0.633	0.400	0.875	0.518	0.571	0.800	0.711
H_E	0.606	0.494	0.645	0.597	0.620	0.610	0.693
DLA0075							
A	5	8	5	5	4	3	4
R_s	4.984	7.467	4.650	4.230	4.000	2.433	3.990
H_o	0.667	0.833	0.675	0.845	0.846	0.483	0.614
H_E	0.742	0.745	0.740	0.694	0.755	0.513	0.573
DLA0078							
A	16	17	13	11	8	7	8
R_s	15.151	16.400	11.427	7.598	7.926	5.300	7.402
H_o	0.967	0.967	0.900	0.752	0.926	0.800	0.911
H_E	0.904	0.918	0.872	0.685	0.798	0.732	0.823
DLA0086							
A	11	16	10	11	10	5	8
R_s	10.551	15.149	8.946	9.127	10.000	4.114	7.800
H_o	0.767	0.800	0.850	0.991	0.961	0.717	0.909
H_E	0.820	0.870	0.857	0.834	0.888	0.606	0.827
Average							
A	9.4	11.4	8.2	7.6	6.2	4.4	6.2
R_s	9.023	10.934	7.419	6.142	6.171	3.769	6.038
H_o	0.733	0.740	0.770	0.778	0.732	0.706	0.806
H_E	0.743	0.770	0.749	0.701	0.707	0.640	0.738

erozygosity (H_E) ranged from 0.640 to 0.738 in the hatchery stocks, and it was significantly (Mann-Whitney test, $P < 0.05$) lower than that in the three wild populations (0.743-0.770). On the contrary, no significant difference was found in observed heterozygosity (H_o) (Mann-Whitney test, $P < 0.05$). However, allelic diversity is a more sensitive measure of genetic variation than overall heterozygosity for short bottlenecks such as a founder event (Norris *et al*., 1999), and allelic loss may occur faster than loss of heterozygosity (Maruyama & Fuerst, 1985).

Average F_{is} values for all loci were negative in five out of seven samples, although none of them significantly different from zero ($P < 0.05$), suggesting that all sample sets are in Hardy-Weinberg equilibrium. Analysis of potential population bottleneck under the TPM revealed that all cultivated stocks might have experienced a recent bottleneck ($P < 0.05$), while estimates of N_e for the specific sets were quite low, ranging from 12.3 to 48.5 individuals. Furthermore, according to pairwise multilocus F_{st} estimates, most genetic differentiation was distributed among hatchery stocks (F_{st} range 0.060-0.203). Differentiation between farmed and wild samples was lower (F_{st} range 0.028-0.154), whereas differentiation between the three natural populations was the lowest (F_{st} range 0.011-0.035). All population pairwise comparisons were statistically significant ($P < 0.05$ after Bonferroni correction), except for the GR1, GR2 pair ($F_{st} = 0.011$).

Understanding the genetic diversity of natural populations is needed for setting up a founder population with high allelic and gene diversity, and in turn that is the first step towards successful selection projects. Results of microsatellite DNA analysis herein showed a 37 % reduction of the mean allele number in the hatchery samples compared to the wild ones, despite the fact that three out of four strains had greater sample size than the wild collections. Significant differences between wild and farmed sea bass were also observed for expected heterozygosity (H_E) and allelic richness (R_s), which constitutes a measure of the number of alleles independent of sample size, suggesting that there is a severe reduction of genetic diversity in *D. labrax* cultivated stocks. Moreover, the genetic differentiation between hatchery samples as well as from the wild origin fish is significant, as indicated by F_{st} analysis. All these changes in the genetic composition of the reared stocks are mainly due to random genetic drift and inbreeding events that might have occurred in

the hatcheries, resulting, e.g. from the use of small sets of breeders for the establishment of founder broodstocks or effective population sizes much lower than the real ones (estimated mean N_e = 23.4). Fish are highly fecund and there is a great temptation for farmers to spawn as few fish as possible in order to reduce costs, something that could lead to genetic drift and inbreeding. Consequently, based on the results of this study about the present genetic status of the examined strains, care should be taken in the design of a selective breeding program. Such a program should take into account any possible loss of functional genetic variation due to genetic drift and/or inbreeding.

This work, based on a relatively small number of markers, offers important insights into the effects of current husbandry practices on sea bass genetic structure and can be easily implemented in any fish farm and offer fast monitoring of genetic diversity. We suggest that genetic screening of potential breeders/stocks that could be used as future founders for a hatchery is the best starting point for a selection project. The basic rule that all hatcheries should follow is that the base population must capture as much genetic variation as possible. This in turn will ensure maximum long term genetic response to the breeding program, aiming at high genetic gain for commercially important traits. However, the results herein are based on neutral genetic variation derived from molecular markers such as microsatellites. The impact of these results (reduction of genetic diversity and/or inbreeding) on the genetic variation of traits that are economically important for the aquaculture industry remains to be investigated.

Acknowledgements

We are grateful to the Research Committee of the Alexander Technological Educational Institute of Thessaloniki (A.T.E.I.Th.) and the Hellenic Centre of Marine Research (H.C.M.R.) for funding this project. The authors would also like to thank all the Greek aquaculture farms and their personnel for providing the cultivated samples.

References

Brown, R.C., Woolliams, J.A., McAndrew, B.J., 2005. Factors influencing effective population size in commercial populations of gilthead seabream, *Sparus aurata*. *Aquaculture*, 247, 219-225.

Chatziplis, D., Batargias, C., Tsigenopoulos, C.S., Magoulas, A., Kollias, S. *et al*. 2007. Mapping quantitative trait loci in European sea bass (*Dicentrarchus labrax*): The BASSMAP pilot study. *Aquaculture*, 272S1, S172-S182.

Chistiakov, D.A., Hellemans, B., Haley, C.S., Law, A.S., Tsigenopoulos, C.S. *et al*. 2005. A microsatellite linkage map of the European sea bass *Dicentrarchus labrax* L. *Genetics*, 170, 1821–1826.

De Innocentiis, S., Lesti, A., Livi, S., Rossi, A.R., Crosetti, D. *et al*. 2004. Microsatellite markers reveal population structure in gilthead sea bream *Sparus auratus* from the Atlantic Ocean and Mediterranean Sea. *Fisheries Science*, 70, 852-859.

Dupont-Nivet, M., Vandeputte, M., Vergnet, A., Merdy, O., Haffray, P. *et al*. 2008. Heritabilities and GxE interactions for growth in the European sea bass (*Dicentrarchus labrax* L.) using a marker-based pedigree. *Aquaculture*, 275, 81-87.

Franch, R., Louro, B., Tsalavouta, M., Chatziplis, D., Tsigenopoulos, C.S. *et al*. 2006. A genetic linkage map of the hermaphrodite teleost fish *Sparus aurata* L. *Genetics*, 174, 851-861.

Guinand, B., Dujardin, E., Dufour, V., Tsigenopoulos, C.S., 2008. Characterization of genetic structure of *Dicentrarchus labrax* larvae in two nurseries of the Gulf of Lions (NW Mediterranean). *Aquatic Living Resources*, 21, 81–87.

Kang, J.H., Noh, J.K., Kim, J.H., Lee, J.H., Kim, H.C. *et al*. 2006. Genetic relationship between broodstocks of olive flounder, *Paralichthys olivaceus* (Temminck and Schlegel) using microsatellite markers. *Aquaculture Research*, 37, 701-707.

Loukovitis, D., Sarropoulou, E., Tsigenopoulos, C. S., Batargias, C., Magoulas, A. *et al*. 2011. Quantitative trait loci involved in sex determination and body growth in the gilthead sea bream (*Sparus aurata* L.) through targeted genome scan. *PLoS ONE*, 6 (1), e16599.

Loukovitis, D., Sarropoulou, E., Vogiatzi, E., Tsigenopoulos, C.S., Kotoulas, G. *et al*. 2012. Genetic variation in farmed populations of the gilthead seabream *Sparus aurata* in Greece using microsatellite DNA markers. *Aquaculture Research*, 43, 239-246.

Maruyama, T., Fuerst, P.A., 1985. Population bottlenecks and nonequilibrium models in population genetics.*Genetics*, 111, 675-689.

Massault, C., Hellemans, B., Louro, B., Batargias, C., Van Houdt, J.K.J. *et al*. 2010. QTL for body weight, morphometric traits and stress response in European seabass *Dicentrarchus labrax*. *Animal Genetics*, 41, 337-345.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, 16, 1215.

Norris, A.T., Bradley, D.G., Cunningham, E.P., 1999. Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture*, 180, 247-264.

Piry, S., Luikart, G., Cornuet, J.M., 1999. Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. *Journal of Heredity*, 90, 502-503.

Rice, R.W., 1989. Analyzing tables of statistical tests. *Evolution*, 43, 223-225.

Tsigenopoulos, C.S., Hellemans, B., Chistiakov, D.A., Libertini, A., Kotoulas, G. *et al*. 2003. Eleven new microsatellites of the sea bass (*Dicentrarchus labrax* L.). *Molecular Ecology Notes*, 3, 352-354.

Vandeputte, M., Dupont-Nivet, M., Haffray, P., Chavanne, H., Cenadelli, S. *et al*. 2009. Response to domestication and selection for growth in the European sea bass (*Dicentrarchus labrax*) in separate and mixed tanks. *Aquaculture*, 286, 20-27.

Waples, R.S., Do, C., 2008. LDNe: A program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*, 8, 753-756.

Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, 38, 135-1370.