

POPSIZE - Estimating effective population size in populations of marine fish: an approach using interannual fluctuations of the genetic composition

Final report ICES Science Fund

Project participants:

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Project Objectives:

This project focused on the comparison of the genetically effective sizes of the populations of four species of coastal fish along the European coast (two of them being intensively exploited). The main objectives aimed at: 1) comparing the population structure obtained by two sets of temporally independent samples; 2) contrasting the current state with the one of at least one generation earlier, under the hypothesis that the commercially exploited species ought to show a decrease in their effective size in the case of fishing; and 3) developing an innovative approach that combines historical and contemporary data on the genetically effective sizes of the study populations.

Updated Status of the Project:

In general, the work took place according to the plan, as the various tasks have been carried out successfully. More details are presented below.

- **Lipophrys pholis (shanny):**

Juveniles of the year of *L. pholis* were collected near Lisbon (S. Pedro do Estoril 38° 42' N, 9° 22' W), in rocky tide-pools. Fin samples were clipped and preserved in 2003 (N = 30), 2013 (N = 97) and 2014 (N = 99) – work done before the beginning of this project. All corresponding laboratory work was performed, and a fragment of the mitochondrial control region was amplified and sequenced. The resulting sequences were deposited in GenBank. Genealogies, genetic diversities, temporal structures and contemporary effective population sizes were assessed.

For the first intron of the nuclear S7 ribosomal protein gene, 20 (2003), 76 (2013) and 84 (2014) shanny individuals were sequenced. Genealogies, genetic diversities, temporal structures and contemporary effective population sizes were also assessed for this marker.

- **Atherina presbyter (sand-smelt):**

Also before the beginning of this project, juveniles of the year of *A. presbyter* were collected near Lisbon (Fonte-da-Telha 38° 34' N, 9° 11' W, and S. Pedro do Estoril 38° 42' N, 9° 22' W), in intertidal channels in rocky shores. Fin samples were clipped and preserved in 2005 (N = 34), 2012 (N = 91), 2013 (N = 61) and 2014 (N = 95). All corresponding laboratory work was performed, and a fragment of the mitochondrial control region was amplified and sequenced. The resulting sequences were deposited in GenBank. Genealogies, genetic diversities, temporal structures and contemporary effective population sizes were assessed.

Sand-smelts were also sequenced for a nuclear fragment, the second intron of the nuclear S7 ribosomal protein gene. Sequences are now ready and editing, alignment and data analyses are being performed. Similarly to what was done for the mitochondrial marker, genealogies, genetic diversities, temporal structures and contemporary effective population sizes will be assessed (work scheduled for the next few months).

- **Comparative analysis of the mitochondrial control region of *L. pholis* and *A. presbyter*:**

Haplotype networks showed deep genealogies with multiple levels of diversification and no temporal structure. Genetic diversity indices showed little variation among sampling periods and were generally high. For *L. pholis* significant genetic differentiation was detected between 2013 and 2014, while no significant differences were detected between sampling periods in *A. presbyter*. The shanny showed lower effective population size per generation (N_e) when compared to the sand smelt (which yielded lack of evidence for genetic drift for the first two periods of the study). In fact, genetic stochasticity may play a smaller role in populations with a N_e as large as *A. presbyter*. On the other hand, the shanny presented lower estimates of N_e per generation, which is in accordance with the temporal structure found for some of the periods and its higher vulnerability to stochastic events. These results highlight the fact that temporal changes in the gene pool composition need to be considered when evaluating population structure, especially for species with long pelagic larval dispersion and, therefore, more vulnerable to fluctuations in the recruitment.

These first results (representing the mtDNA control region only) were published in the Journal of Phylogenetics and Evolutionary Biology, presented at the European Congress of Ichthyology (Porto, Portugal) –Conference Abstract published in Frontiers in Marine Science Journal, and at the ICES Annual Science Conference (Copenhagen, Denmark) (see output list).

- **Diplodus sargus (white-seabream):**

Before the beginning of this project, juveniles of the year of *D. sargus* were collected near Lisbon (S. Pedro do Estoril 38° 42' N, 9° 22' W) in intertidal channels in the rocky shore. Fin samples were clipped and preserved in 2006 (N=30), 2009 (N = 40) and 2011 (N = 33) and 2014 (N = 95). Similarly to the procedure carried out for the non-commercial species, the

corresponding laboratory work was performed and fragments of the mitochondrial control region the first intron of the nuclear *S7* ribosomal protein gene were amplified and sequenced. Genealogies, genetic diversities, temporal structures and contemporary effective population sizes were assessed for both markers.

- **Comparative analysis of *L. pholis* and *D. sargus*:**

A total of 226 shanny specimens (collected in 2003, 2013 and 2014) and 181 white seabreams (collected in 2006, 2009, 2011 and 2014) were screened for genetic variation using the mitochondrial control region and the first intron of the *S7* ribosomal protein gene. For *D. sargus* and *L. pholis* results for the two markers were congruent and highly divergent, respectively. Genetic diversity indices showed little variation among sampling periods and were generally high, with the exception of the shanny's *S7*. No temporal structure was found for either species. However, for *L. pholis* significant genetic differentiation was detected between 2013 and 2014 (CR), and between 2003–2013 and 2003-2014 (*S7*), while no significant differences were detected between sampling periods in *D. sargus*. The shanny showed lower effective population size per generation when compared to the white seabream, which yielded lack of evidence for genetic drift for the 2nd (CR and *S7*) and 3rd (*S7*) periods of the study. This may mean that genetic stochasticity may play a minor role in populations with a large N_e . The fishing pressure may have a strong influence on the N_e , inducing temporal variations in the composition of the genetic pool, although this does not seem to be the case for the white seabream. The lower N_e found for the shanny is also in agreement with the temporal structure found between some periods and their greater vulnerability to recruitment fluctuations due to long PLD.

- ***Diplodus vulgaris* (common two-banded seabream):**

After the beginning of this project, in 2015, samples of *D. vulgaris* were collected near Lisbon (S. Pedro do Estoril 38° 42' N, 9° 22' W), in intertidal channels of the rocky area (N=50). However, doubts on the taxonomic identification of these small juveniles were raised. Fin samples were clipped, preserved and added to our collection - 2007 (N=30) and 2009 (N = 33). Amplification and sequencing of the fragment of the mitochondrial control region (for the 2009 and 2015 periods) were performed. As suspected, only a small fraction of the seabreams sampled in 2015 were correctly identified in the field (N=7). In our opinion the remaining dataset is much reduced (only two sampling periods, with N<50) and, thus, we decided to quit the temporal analysis for *D. vulgaris*.

Conclusions:

A great deal of work was accomplished during this year toward completing the proposed research goals. The results from this project highlight the fact that temporal changes in the gene pool composition need to be considered when evaluating population structure, especially

for species with long pelagic larval dispersion, more vulnerable to recruitment fluctuations, and species subjected to commercial pressure. Extending this approach to species with different ecologic characteristics and distinct fishing pressure can contribute to produce an alternative way of monitoring the status of fish populations. Thus, in the future, this approach may be applied to both the management of stocks of commercial interest, and the genetic monitoring of species representative of a healthy environment. This would be important to achieve goal 3 of ICES Strategic Plan (evaluate and advise on options for the sustainable use and protection of marine ecosystems).

Next steps:

As stated above, next steps include the analyses for the *A. presbyter* S7 dataset. In the next months we will perform comparative analyses among commercial and non-commercial species. For this final stage, the project will greatly benefit from the critical expertise of the Norwegian partners in temporal genetic change and N_e estimating methodologies.

In the future, we are planning the extension of this approach to an estuarine and a freshwater species, as well as adding another marker to enhance the robustness of the applicability of the temporal method.

Research Outputs:

- Francisco, SM; Robalo, JI (2015) Genetic structure and effective population size through time: a tale on two coastal marine species with contrasting life-history patterns. *Journal of Phylogenetics and Evolutionary Biology*. 3: 155 doi:10.4172/2329-9002.1000155 – Appendix 1
- Francisco, SM; Robalo, JI (poster, September 2015) Genetic structure and effective population size through time: a tale on two coastal marine species with contrasting life-history patterns. XV European Congress of Ichthyology, Porto, Portugal – Appendix 2
- Francisco, SM; Robalo, JI (2015) Genetic structure and effective population size through time: a tale on two coastal marine species with contrasting life-history patterns. *Front. Mar. Sci. Conference Abstract: XV European Congress of Ichthyology*. doi: 10.3389/conf.FMARS.2015.03.00248 – Appendix 3
- Francisco, SM; Robalo, JI (Oral communication, September 2015) Genetic structure and effective population size through time: a tale on two coastal marine species with contrasting life-history patterns. Session E: Beyond ocean connectivity: embracing advances on early life stages and adult connectivity to assessment and management challenges. ICES Annual Science Conference, Copenhagen, Denmark

- Obtained sequences were deposited in GenBank (Accession numbers KR028543-KR028984)
- Francisco, SM (June 2016) Population Genetics of coast fish: a temporal approach. Internal Conference Cycle at ISPA University Institute (talk), Portugal – Appendix 4
- Francisco, SM; Robalo, JI (July 2016) Effects of fishing pressure on the genetic structure and effective population size through time: a tale on two coastal marine species. International Meeting on Marine Research, Peniche, Portugal (poster - abstract submitted). If accepted, will be given a DOI and will be published in the Frontiers in Marine Science Journal. – Appendix 5
- Francisco, SM; Robalo, JI (2016) Effects of fishing pressure on the genetic structure and effective population size through time: a tale on two coastal marine species. Front. Mar. Sci. Conference Abstract: IMMR | International Meeting on Marine Research 2016. doi: 10.3389/conf.FMARS.2016.04.00083
- Francisco, SM; Robalo, JI (September 2016) Effects of life-history and fishing pressure on the genetic structure and effective population size through time: a tale on two coastal marine species. Session C: From individuals to ecosystems: their ecology and evolution. ICES Annual Science Conference, Riga, Latvia. (poster, speed-talk) – Appendix 6

Scheduled Research Outputs:

- The remaining sequences obtained for this project will be deposited in GenBank: mitochondrial control region (*D. sargus*) and S7 (*L. pholis*, *D. sargus* and *A. presbyter*)
- Final manuscript with the compared analysis for the target species of this project (2 molecular markers)
- Final results will be shared in a Facebook page in order to widespread the importance of this topic, also allowing a rapid and cost free communication channel with the general public.

Financial report:

The funding from ICES was essential for the development of this project, as the only additional funding available was a post-doctoral scholarship awarded to the PI (Sara Francisco). The funds provided by ICES were used according to the budget stated in the application: sampling campaigns, lab consumables, sequencing service, participation in scientific meetings (XV

European Congress of Ichthyology, Porto, Portugal; ICES ASC 2015 in Copenhagen, Denmark; International Meeting on Marine Research, Peniche, Portugal; ICES ASC 2016 in Riga, Latvia), and publication of first results in Journal of Phylogenetics and Evolutionary Biology.

Publication of final results in an open access scientific journal is also planned for the beginning of 2017.