International Consultations on the Establishment of the
South Pacific Regional Fisheries Management Organisation

Recent Genetic studies of Pacific jack mackerel *Trachurus murphyi* in Russia

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In 2007 in Russia in the Belozersky Sceintific-Research Institute of physico-chemical biology the Moscow State University initiated preliminary genetic research of Pacific jack mackerel. Samples were collected onboard the Dutch fishing vessel "Jan Maria". I'd like to take this opportunity to express my thanks to Dr.Corten and the shipowner for the rendered opportunity for taking of these genetic samples. Since all the samples were taken at the comparatively small area not far from the EEZ of Chile between 37º48 and 38º28 S and 80º49 and 84º28 W, the undertaken activities were of preliminary nature and their objective was to elaborate methodical aspects for search of genetic polymorphous markers of population level. The method of restricted fragments length polymorphism (RFLP) mtDNA was applied in the research. The branded NucleoSpin®Tissue (MACHEREY-NAGEL) set was used for extraction of Pacific jack mackerel's DNA. The DNA was extracted out of 50 samples of liver (Fig. 1). For RFLP analysis variable sectors of mtDNA-cytochromes b (1140 n.p.) and D-loop (800 n.p.) were chosen.

The following primers' sequences were used for amplification of these fragments:

Cytochrome b (Cardenas et al., 2005)

tRNA-Glu-CTB-F: 5`-ATG GCA AAT CTC CGT AAA ACC C-3`

t-RNA-Thr- CTB-R: 5`-AGG CTC ATC CGA GCA TTT TA-3`

D-loop (Poulin et al., 2004)

(tRNA-Pro)-tRNA T1-Dl-F: 5`-CAG AAA AAG GAG ACT CTA ACT CCT **AAA**-3`

(tRNA-Phe)-tRNA T2-Dl-R: 5`-TGC TTG CGG GGC TTT CTA-3`

Primers' sequence (tRNA-Pro)-tRNA T1-Dl-F was changed as per GenBank:

5`-CAG AAA AAG GAG ACT CTA ACT CCT-3`

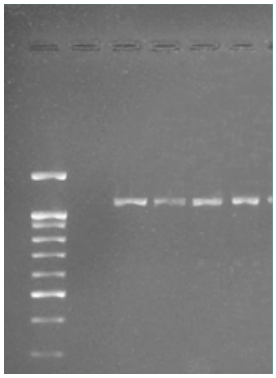
Cytochrome b and D-loop were amplified in the following conditions: initial denaturation at 95°C - 5 min., then 30 cycles consisting of denaturation at 94°C 30 sec, primers' annealing (e'niling) at 56°C - 45 sec, elongations at 72°C - 45 sec and the final elongation at 72°C - 10 min. Amplification of D-loop was carried out in the same conditions except for the elongation stage of 30 cycles: at 72°C - 90 sec. Amplification of mtDNA fragments was conducted in 25 µl of reaction mixture consisting of 2.5 µl of 10X buffer (Dialat Ltd.), 2 units of Taq-polymerase (Dialat Ltd.), 2.5 mM $MgCl_2$ (Dialat Ltd), 0.2 mM dNTPs (Dialat Ltd), 10 pM of each primer and 5-10 ng of DNA. Amplification of D-loop was carried out with the same volume of reaction mixture with increase of $MgCl_2$ concentration to 3.2 mM. (Fig. 2) The amplificated fragments of mtDNA were studied for polymorphism of restricted fragments. Restricted endonucleases (restrictases) were used in this work.

Characteristics of the used restrictases.

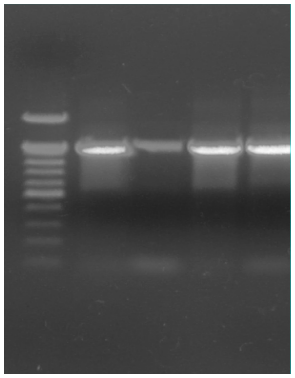
Name of restrictase	T of restriction, °C	Recognition site	Restricted fragments, n.p.	Polymorphism of fragments' lengths
HaeIII (BsuRI)	37	GG^CC	320+300+220+200+100	No
HpaII (MspI)	37	C^CGG	820+210+110	No
NlaIV (PspN41)	37	GGN^NCC	540+350+150+100	No
RsaI	37	GT^AC	540+310+200+90	No
TaqI	60	T^CGA	370+240+220+220+90 610+220+220+90	Yes

Restriction of amplificated fragments of mtDNA took place in 20 µl of reaction mixture, consisting of 2 µl of 10X buffer, 5 µl of the obtained amplificate and 1.5 µl of the appropriate restrictases. The restriction was carried out at optimal temperatures within 2 hours. Determining of lengths of amplificated and restricted fragments was conducted in 2% agarous gel with 1XTAE buffer using 100 bp+1.5 Kb DNA ladder (NPO SibEnzim) marker with further colouring by ethidium bromide (0.5 mg/ml).

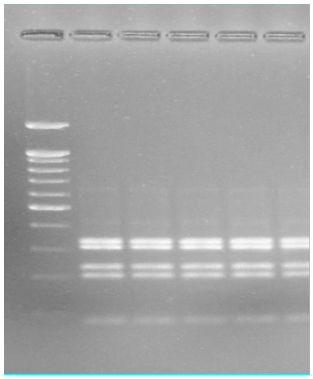
(Fig. 3) As a result of the performed research: DNA was extracted out of 50 samples of Pacific jack mackerel tissue. Variable fragments of mtDNA cytochrome b and D-loop have been amplificated. RFLP analysis of cytochrome b was carried out with use of HaeIII, HpaII, NlaIV, RsaI, TaqI restrictases. Polymorphism of restricted fragments was determined with use of TaqI.



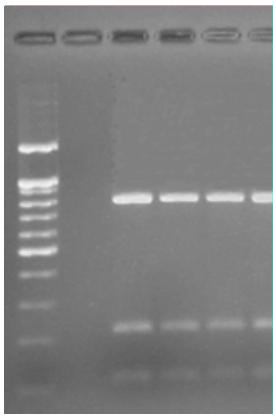
Cytochrome B



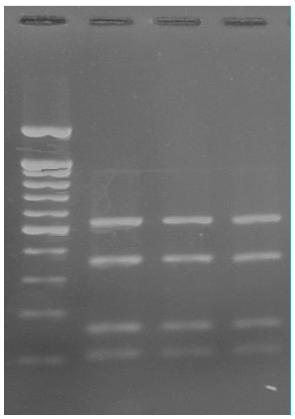
D-loop



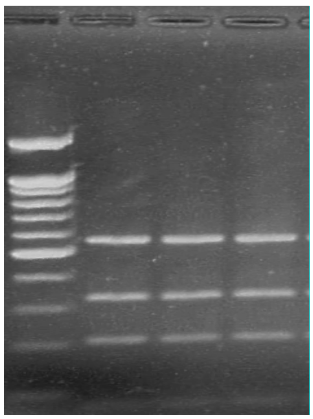
HaeIII



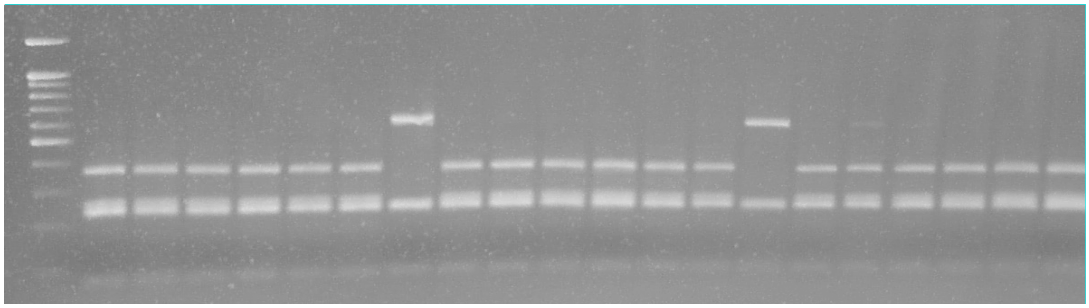
HpaII



NlaIV



RsaI



TaqI

Fragments of Cytochrome b obtained as a result of restriction by the restrictases signed under the figures

In conclusion I'd like to discuss some methodological aspects of future research activities. In the report prepared by Chile reference is made to the work of Ojeda and Poulin, 2002 regarding testing of 4 microsatellite markers for *T. murphyi*. Since until now there has been no information concerning availability of sequences of *T.murphyi* or *T.symmetricus* microsatellite fragments in the Genetics

Bank, it seems that the heterologous primers of related species, i.e. *Gnathonodon speciosus* or *Seriola dumerili* (Feng et al, 2005, Ohara et al, 2003, Babbucci et al, 2006), sequences of microsatellite loci of which are available in the Genetics Bank or published., were used while performing of this work.

At the end of 2007 the nucleotide sequences of some microsatellite loci of *Trachurus trachurus* and *Trachurus japonicus* apeeared in the Genetics Bank, however all of them are dinucleotide (registration numbers are EF 109781-EF 109801 for *T.japonicus* and EU 748-EU 751 – for *T.trachurus*).

For Mediterranean scad and Atlantic jack (blue scad) differentiation there were used four specially developed dinucleotide microsatellite markers (the same as the above mentioned of the Genetics Bank), which proved to be low effective. In the *Fisheries Research* publication (Kasapidis & Magoulas, 2008) there were given characteristics of these loci, including polymorphism – 40-42 and even 65 alleles. Samplings of up to 1000 specimens are necessary for proper use of such markers. There is a probability that inefficiency of microsatellite analysis in this case was caused by non-optimal and not quite sufficient set of microsatellites. This is also pointed out by the authors of that paper, proposing to consider the results of the work to be preliminary.

Tetranucleotide microsatellite sequences are more effective for the purposes of population analysis. However, development of such primers is considerably more expensive compared with the same for dinucleotides.

(Fig. 4) The main conclusions which can be made as a result of studying the existing literature on identification of Horse mackerel stocks are as follows:

The main literature conclusions

1. Mitochondrial DNA is not characterized by sufficient polymorphism in respect of intraspecific jack mackerel's groups.

2. Allozyme analysis is quite perspective, however requires selection of enzymatic systems with more moderate polymorphism in respect of Jack mackerel's populations.

3. At present developing of specific microsatellite markers, the number of nucleotides of which equals to 4 and over, is the most perspective tendency of jack mackerel's population genetic research. However, such research activities require significant temporal and financial inputs.

4. The Single Strend Conformational Polymorphism - analysis is perspective for separation of jack mackerel's populations

(Fig. 5)

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(Fig. 6)

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