



*SCIENTIFIC COOPERATION TO SUPPORT
RESPONSIBLE FISHERIES IN THE ADRIATIC SEA*

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Genetic stock structure analysis revealed single population units in the
shared stocks of Adriatic demersal species

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Genetic stock structure analysis revealed single population units in the shared stocks of Adriatic demersal species*

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Abstract

Two genetic research projects (financed in parallel by FAO-AdriaMed and by the Italian Ministry of Agriculture and Forestry Policies) have been carried out to identify population status and structure within the Adriatic shared demersal fish stocks, which are bio-economically relevant for SCSA in the Mediterranean (see Annexes 1 and 2).

The sampling and genetic methodologies adopted by both projects were standardised as much as possible for all species by the use of MEDITS samplings and microsatellite marker analyses. Genetic estimators for population status (i.e. genetic polymorphism in terms of allelic diversity, heterozygosity, and fitting of Hardy-Weinberg equilibrium) and structure (i.e. genetic differentiation in terms of fixation indexes and gene flow rates) were applied using specific statistical software.

High levels of genetic polymorphism and correlation with Hardy-Weinberg equilibrium were scored in most species, implying good status of stocks. Similarly, high levels of genetic homogeneity were observed among geographical samples in most species, clearly suggesting the occurrence of single population units within the Adriatic shared stocks. Low genetic polymorphism and weak genetic divergences were occasionally found, probably related to specific bio-ecological and behavioural features, and/or to methodological artefacts.

In populations of marine species, such a pattern of genetic features can be related to large-size and unfragmented populations (a fishery question: Can it also be associated with undepleted stocks?). This initial picture of the population structure of several Adriatic marine fishery resources needs to be integrated by further genetic analyses carried out at a finer scale as well as by data obtained from other methodological approaches.

*The opinions, interpretations, conclusions, or recommendations expressed in this document are entirely those of the authors and do not necessarily reflect the view or position of FAO or of the Countries and Institutions participating in the AdriaMed Project.

1. Introduction

An understanding of the number and boundaries of population units within a given fishery stock is relevant to address fruitful stock assessment activities. In addition, where fishery stocks are transboundary and exploited by more than one country, the knowledge of population biology factors assumes international relevancy (Vrgoc *et al.* 2004).

In the Adriatic Sea, several demersal and pelagic fishery resources are co-exploited by fleets of five countries: Albania, Croatia, Italy, Montenegro and Slovenia. Within the international AdriaMed Project and the 6th Three-year Plan for Fishery and Aquaculture (VI Piano Triennale della Pesca e Acquacoltura) of the Italian Ministry of Agriculture and Forestry Policies, two research projects (IPUAS by FAO-AdriaMed; GENPOPADR by MIPAF) were financed to address the genetic characterisation and the identification of population units of the Adriatic shared stocks of several demersal and pelagic fishery resources. Both projects were based on genetic methods that use indirect analytical tools to assess population status and structure of the fishery resources in the Adriatic area. The specific goals of these projects were *i*) to develop species-specific genetic markers useful for the stock structure analysis (i.e. microsatellite and mitochondrial loci), *ii*) to define the genetic polymorphism of Adriatic stocks, *iii*) to identify the number and boundaries of population units in the area investigated. The definition of a multi-species genetic pattern for the Adriatic Sea represented the general focus of this work, a pattern that could contribute to the improvement of responsible fishery and sustainable management of stocks in the area.

2. Materials and Methods

The sampling and genetic methodologies adopted by both projects were standardised as much as possible for all target species (Table 1). Samples were collected from the northern, middle and southern Adriatic Sea, mainly during the MEDITS 2001 trawl survey, except for *Solea vulgaris* and *Sardina pilchardus*, for which individuals were sampled *ad hoc* by commercial trawlers and the GRUND 1998 trawl survey, respectively. Temporal sample replicates were obtained for Adriatic *Sepia officinalis* and *Merluccius merluccius* in 2002 (Table 1). In *S. vulgaris*, sampling was extended to a wider area, covering the Adriatic Sea, Tyrrhenian and Ligurian Sea, south-western Ionian Sea, and the eastern Mediterranean to check for genetic structuring on a higher geographic scale.

Microsatellite loci were considered as the standard analytical molecular tools for population status and structure in the fishery stocks (Feral, 2002). Species-specific microsatellite loci were developed for most target resources by isolation from the DNA nuclear genome (Zane *et al.* 2002) and/or direct optimisation of PCR amplification parameters. New arrays of microsatellite loci were developed for *Loligo vulgaris*, *Lophius budegassa* and *Mullus barbatus* within the IPUAS and GENPOPADR projects. Mitochondrial DNA sequence markers were developed for *S. vulgaris* and *S. pilchardus* (Table 1).

Genetic features were used to infer relevant population biology and ecology issues indirectly (Feral, 2002). The microsatellite loci variation of samples was analysed in terms of genetic polymorphism (e.g. allelic diversity, heterozygosity, compatibility with Hardy-Weinberg equilibrium, haplotypic diversity) and of genetic differentiation (e.g. fixation indexes, gene flow rates), using specific statistical tests and software (some example are reported in

Guarniero *et al.* 2002; Garoia *et al.* 2004_a, 2004_b). Genetic diversity and differentiation of samples were used as estimators of stock status and structure, respectively (Feral, 2002).

Table 1. Sampling data and developed species-specific genetic markers for the analysis of the target fishery resources and stocks.

Species		Samples					Genetic markers	
Common name	Scientific name	Collecting areas	Country	N. samples	N. ind.	Years	Loci	Reference
Cuttlefish	<i>Sepia officinalis</i>	NAdr MAAdr SAdr	Italy	5	201	2001 2002	<u>Microsatellites</u> Sof1, Sof2, Sof4, Sof6, Sof7	Shaw & Perez-Losada 2000
European squid	<i>Loligo vulgaris</i>	NAdr MAAdr SAdr	Croatia, Italy	4	203	2001	<u>Microsatellites</u> LVchi21, LVD54, LVrip1, LVrip3, LVrip4, LVtleB22	Guarniero et al. 2003, Garoia et al. 2004b
Norway lobster	<i>Nephrops norvegicus</i>	NAdr MAAdr SAdr	Croatia, Italy, Slovenia	4	172	2001	<u>Microsatellites</u> Nnmic2-E4, Nnmic1-F2, Nnmic1-B11, Nnmic1-C12, NnmicT-G2	Streiff et al. 2001
Angler	<i>Lophius budegassa</i>	NAdr MAAdr SAdr	Albania, Croatia, Italy	3	203	2001	<u>Microsatellites</u> Lobu(CA)1 Lobu(CA)3 Lobu(CA)5 Lobu(CA)7 Lobu(CA)12 Lobu(CA)17	Garoia et al. 2003
Red mullet	<i>Mullus barbatus</i>	NAdr MAAdr SAdr	Albania, Italy	4	206	2001	<u>Microsatellites</u> Mb6, Mb7, Mb15, Mb26b, Mb39, Mb31	Garoia et al. 2004a
European hake	<i>Merluccius merluccius</i>	NAdr MAAdr SAdr	Albania, Italy	3	325	2001 2002	<u>Microsatellites</u> Hk3b, Hk20, Hk9, Hk29, Hk34b	Moran et al. 1999
Common sole	<i>Solea vulgaris</i>	Adriatic Sea, Tyrrhenian Sea, South Western Ionian, Eastern Mediterranean	Albania, Croatia, Italy, Turkey	9	260	1999 2002	<u>Microsatellites</u> F8-ICA9, F8 IGAA7, F8- ITG11, F8- IIGT15, F13- II8/4/7 <u>Mitochondrial sequence control region</u>	Iyengar et al. 2000, Guarniero et al. 2002
European piclhard	<i>Sardina pilchardus</i>	NAdr MAAdr SAdr South Western Ionian	Albania, Croatia, Italy	11	307	1998	<u>Mitochondrial sequence cytochrome b</u>	Tinti et al. 2002

3. Results and Discussion

A synthetic view of the genetic diversity and differentiation in the Adriatic demersal and pelagic fishery stocks is shown in Table 2. High levels of genetic polymorphism and the relevance of the Hardy-Weinberg equilibrium were scored in most species. This feature might suggest the current good status of Adriatic shared stocks. Similarly, high levels of genetic homogeneity were observed among geographical samples in most species, clearly suggesting the occurrence of single population units within the Adriatic shared stocks. Low genetic polymorphism and weak genetic divergences were occasionally found, which could easily be related to specific bio-ecological and behavioural features, and/or to methodological artefacts.

Table 2. Synthesis of the genetic results and of the bio-ecological and demographic issues for the fishery resources and stocks analysed.

Species	Relevant genetic results		population biology and ecology issues	Associated reference
	Genetic diversity	Genetic differentiation		
<i>Sepia officinalis</i>	Number of alleles: 2-12 (mean 6.4) Heterozygosity: 11%-89% (mean 47%) HWE fitting: 44% The Adriatic samples showed medium-low polymorphism and several significant departures from the HWE equilibrium	Fixation indices among Adriatic geographical samples are low and randomly significant Fixation indices of temporal sample replicate are significant The stock shows temporal rather than spatial genetic differentiation	A single population unit is probably present within the Adriatic stock. The seasonal migrations occurring for reproduction could determine admixture of different cohorts determining genetic disequilibrium and random genetic differentiation. Preliminary data show temporal genetic unstableness, suggest further analysis and recommend cautionary approach to the management	Garoia et al. 2004b
<i>Loligo vulgaris</i>	Number of alleles: 5-21 (mean 13.3) Heterozygosity: 53%-94% (mean 68%) HWE fitting: 77.5% The Adriatic samples stock showed high genetic variability and appear to be in equilibrium	Fixation indices among Adriatic samples were low and not significant The Adriatic samples resulted highly homogeneous for genetic allele structure	The high genetic diversity and homogeneity of Adriatic stock suggested the occurrence of a single highly panmictic population unit probably of large dimension The continental slope present in the southern part of Adriatic Sea does not act as a barrier against the migration for this highly mobile species	Garoia et al. 2004b
<i>Nephrops norvegicus</i>	Number of alleles: 9-40 (mean 24) Heterozygosity: 57%-94% (mean 76%) HWE fitting: > 80% The Adriatic samples showed high levels of genetic diversity and did not result at the HWE	Fixation indices among Adriatic samples were low and not significant, independently from geography and bathymetry The Adriatic samples proved genetically homogeneous without significant deviations from panmixia with high gene flow	No evidence of population sub-structuring, either along with a geographic cline or depth differences. A single panmictic population unit probably occurred in the Adriatic Sea High gene flow mediated by dispersal of larvae Lack of specific areas of larval retention Estimated effective population size (i.e. indirect genetic estimate based on genetic variation) increased from a northern-to- southern geographical cline	Guarniero 2004
<i>Lophius budegassa</i>	Number of alleles: 3-8 (mean) Heterozygosity: 12%-61% (mean 38%) HWE fitting: 16.7% The Adriatic samples showed low polymorphism and several cases of highly significant deviations from the HWE	Fixation indices among Adriatic samples were high and deviated significantly from the values expected under the panmixia The Adriatic samples were genetically heterogeneous and fragmented	The Adriatic stock appeared to be genetically heterogeneous The low level of genetic differentiation could be explained by the low dispersal ability of this species The genetic disequilibrium observed could be interpreted as the result of the admixture of genetically different samples (Wahlund effect) An alternative approach or maybe integrated (with morphology) is recommended to obtain reliable data on the population units and structure	unpublished data
<i>Mullus barbatus</i>	Number of alleles: 12-33 (mean 22.5) Heterozygosity: 60%-96% (mean 78%) HWE fitting: 80% The Adriatic samples showed high genetic diversity and respect of the HWE	Fixation indices among Adriatic samples were generally low but in some comparison they were medium-high and significant Subtle spatial genetic heterogeneity not related to a geographic cline	The randomness of genetic differences among samples indicated that the Adriatic red mullet stock probably belongs to a single population unit. However, individuals may group into local, genetically differentiated sub-populations Correlation between geographic distance and genetic differentiation was not detected The observed genetic fragmentation in the Adriatic stock may be generated by reproductive success, survival rates or fishing pressure.	Garoia et al. 2004a

The development of standard genetic tools and the genetic structure analyses is ongoing for the Adriatic stocks of *Eledone cirrhosa*, *E. moschata*, *Parapeneaus longirostris*, *Merlangius merlangus*, *Pagellus erythrinus*, *S. pilchardus* and *Engraulis encrasicolus*. Preliminary data, based on the microsatellite loci variation of a subgroup of Adriatic samples of most species

(data not presented in this paper) revealed a substantial genetic homogeneity within the Adriatic stocks.

Under concepts derived from studies in species and population genetics, the pattern of high genetic diversity and high genetic homogeneity found in analyses of in the Adriatic shared stocks of several species could probably be correlated to the existence of large-size and unfragmented population units. However, whether this pattern can be also associated with an undepleted status of fishery stocks is still a debated question at the fishery level of management. The genetic work carried out under the AdriaMed-FAO and MIPAF research projects undoubtedly represents the first example of a multi-species analysis of the population structure on internationally-relevant marine fishery resources, and it will constitute a referenced scientific milestone for improving stock assessment and sustainable management of the Adriatic shared stocks. The genetic and demographic features of Adriatic shared stocks shown by this analysis need to be confirmed by further genetic analyses at a finer scale, as well as being integrated with data obtained from other methodological approaches.

4. References

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