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An overview of genetic research in the Laboratory of Aquaculture at the Institute of Oceanography and Fisheries, Split, Croatia

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SUMMARY - This paper is an overview of marine aquaculture in Croatia and an in-depth coverage of works and achievements of scientists in the Laboratory of Marine Aquaculture at the Institute of Oceanography and Fisheries in Split, Croatia, with special regard to the field of fish and shellfish genetics. The Laboratory started its work by conducting research on reproductive biology of sea bass and gilthead bream. Soon afterwards experimental rearing of some potentially interesting species, like *Dentex dentex*, *Diplodus vulgaris* and *Diplodus sargus* was initiated. At the end of the 80's the first steps into the field of fish genetics were made by working on hybrids of *Sparus aurata* × *Diplodus puntazzo*, *Sparus aurata* × *Diplodus vulgaris* and finally *Dentex dentex* × *Diplodus sargus*; the last cross was found to be potentially interesting for marine aquaculture. Further research on chromosome manipulation was conducted to induce triploidy of *Sparus aurata* by temperature shocking freshly fertilized eggs. At the beginning of the 90's research on population genetics of mussel (*Mytilus galloprovincialis*) was conducted in the Laboratory in co-operation with French colleagues from IFREMER. The present work includes research on chromosome structure of Sparid species from the Eastern Adriatic as well as population genetics. These studies represent basic knowledge for our future work, which will include setting up a database on Adriatic fish, mainly with the goal to support a program of protection of wild populations of finfish.

Key words: Croatia, aquaculture, sparids, karyotypes, population genetics.

RESUME – *"Présentation de la recherche en génétique menée au Laboratoire d'Aquaculture Marine de l'Institut d'Océanographie et des Pêcheries de Split, Croatie". Cet article présente l'aquaculture marine en Croatie et passe longuement en revue les travaux et acquis des scientifiques du Laboratoire d'Aquaculture Marine de l'Institut d'Océanographie et des Pêcheries de Split, Croatie, en prêtant une attention spéciale au domaine de la génétique des poissons et coquillages. Le laboratoire avait démarré ses travaux par une recherche sur la biologie reproductive du bar et de la daurade royale. Quelque temps plus tard commençait l'élevage expérimental de certaines espèces potentiellement intéressantes, comme Dentex dentex, Diplodus vulgaris et Diplodus sargus. A la fin des années 80, les premiers pas furent faits dans le domaine de la génétique des poissons, en travaillant sur des hybrides de Sparus aurata x Diplodus puntazzo, Sparus aurata x Diplodus vulgaris et finalement Dentex dentex x Diplodus sargus. Les résultats ont montré que le dernier était potentiellement intéressant pour l'aquaculture marine. Des recherches ultérieures sur la manipulation des chromosomes ont été menées, pour essayer d'induire la triploïdie chez Sparus aurata par choc de température des oeufs juste après la fertilisation. Au début des années 90, les premières recherches sur la génétique des populations chez les moules (Mytilus galloprovincialis) ont été menées au Laboratoire en coopération avec des collègues français d'IFREMER. Les travaux actuels comportent des études de structure chromosomique chez les sparidés de l'Adriatique et ainsi que des études de génétique des populations. Toutes ces recherches représentent les connaissances fondamentales pour nos travaux futurs, qui incluront la constitution d'une base de données sur les poissons de l'Adriatique, principalement afin de servir d'appui à un programme de protection des populations de poissons sauvages.*

Mots-clés : *Croatie, aquaculture, sparidés, caryotypes, génétique des populations.*

Introduction

Modern aquaculture in Croatia started in early 1970s, with experimental rearing of some Sparid species. Following the first successful rearing of artificially produced fingerlings of sea bass in Palestrina rearing facility near Venice, Italy, at the end of 1970s, 50,000 fingerlings were successfully reared to market size in Novigrad Sea, Croatia. Soon afterwards, at the beginning of the 1980s, sea bass fingerlings were placed in the rearing facility in Limski kanal (Lima channel). These fingerlings were the first generation ever to be produced in the Laboratory of Aquaculture at the Institute of Oceanography and Fisheries in Split. Very soon two aquaculture companies CENMAR from Zadar and MARIMIRNA from Rovinj were started. These two companies together with a joint Italian-Croatian venture PORTO BUDAVA (near Pula, Croatia), today represent the most important Croatian enterprises in the field of marine aquaculture. A small percentage of finfish production comes from 21 other small companies, 30t capacity each, which are mostly small-scale family businesses.

Total production in 1996. was 1,500t of finfish. The dominant species in Croatian aquaculture is sea bass (*Dicentrarchus labrax*), which represents 90% of total finfish production. Gilthead sea bream (*Sparus aurata*) and some other marine fish, mostly sparids (i.e., red sea bream *Pagrus major*) reared today all together make up only 10% of total production. All rearing facilities in Croatia employ monoculture rearing technology in floating cages exclusively. Most of them work as in-shore facilities, while some new projects are being more off-shore oriented.

As opposed to finfish aquaculture, rearing of shellfish started in Croatia long ago, in the 17th century in the Republic of Dubrovnik. It is rather significant to note that in eastern Adriatic there still lives a healthy population of oysters (*Ostrea edulis*), which has not been infected with the fatal Bonamia disease that caused devastation of this species in the rest of Europe. Besides oyster, Croatia also produces mussels (*Mytilus galloprovincialis*). Total production of shellfish in 1996 was about 1000t of mussel and 700,000 specimen of oysters.

The laboratory of aquaculture at the Institute of Oceanography and Fisheries in Split

General data

The laboratory started working in 1978, guided by Dr. Ivan Katavic (Table 1). In 1980 the laboratory produced first ever reared fingerlings of sea bass in Croatia, which were later sent to Lima channel. At the same time new research on experimental rearing of sea bream in rotating cages was started. A more elaborate and intensive study of reproductive biology of sea bream followed. The most important research was on growth parameters (Glamuzina and Katavic, 1987), development of larvae (Glamuzina *et al.,* 1989; Jug-Dujakovic and Glamuzina, 1988), influence of feeding on larval mortality (Katavic, 1986), survival and larval growth in relation to temperature (Glamuzina and Jug-Dujakovic, 1988; Johnson and Katavic, 1986), salinity and feeding rhythm, cannibalism (Katavic *et al.,* 1989),

influence of starvation on the amount of ascorbic acid in tissues of fish (Marsic-Lucic *et al.,* 1992), and ecological parameters (Tudor *et al.,* 1994; Santic *et al.,* 1994), etc.

With the price of sea bass and sea bream decreasing on the European market the need to reduce culture costs and to domesticate new species increased. In 1987/88 a research on reproduction (maturation, induced spawning) and growth of larvae early development stages) of *Dentex dentex* was conducted (Jug-Dujakovic *et al.,* 1995). At the end of the 80's embryonic and larval development of *Diplodus vulgaris* was studied, which was followed by more elaborate research on the same topic in 1993/94. The genetic work described below may help improve the cost effectiveness of marine aquaculture.

Table 1. Present structure (by topic of research) of the Laboratory of Aquaculture at the IOF, Split

Topic of research	Research done
Diversification of marine aquaculture	Introduction of new species
	Hybridization
Environmental aspects of aquaculture	Aquaculture wastes
	Interaction of aquaculture and environment
Genetic research	Hybridization
	Isoenzymes
	Karyotype research
Biochemistry	Environmental chemistry
Consultancy	EAM Network

Genetic research in the Laboratory

(i) *Hybridization*

In the Laboratory for Aquaculture the first research on fish genetics started at the end of the 1980's. The most important work in this field was done on hybridization among sparid species (Table 2). Leaning on knowledge of reproductive biology of *Sparus aurata*, *Diplodus puntazzo* and *Diplodus vulgaris*, scientists from the Laboratory conducted the first intergeneric breeding among these species. Subsequent experiments followed the growth and development of *Sparus aurata*(f) × *Diplodus puntazzo*(m) hybrid and *Sparus aurata*(f) × *Diplodus vulgaris*(m) hybrid. Embryogenesis and yolk sack resorption of both hybrids was studied under the

temperature of 19.5°C and later compared to those of *Sparus aurata.* It was noted that there was no significant difference between growth rate and survival among the 3 species in the period from 6 to 30 days. The hybrid *Sparus aurata*(f) × *Diplodus puntazzo*(m) grew somewhat faster than the others (Jug-Dujakovic and Glamuzina 1990). The hybrids were not reared to their market size due to the technical problems that developed in the Institute, so we lack the further data on development and reproductive biology.

Additional work was done on hybridization of *Diplodus sargus* and *Dentex dentex* (Jug-Dujakovic and Glamuzina, 1993). The eggs of *D. sargus* were fertilized by *D. dentex* milt and the early stages were compared to those of the parental species. The percentage of fertilization and hatching did not differ significantly, but the time of incubation of hybrid eggs was significantly different from that of both parents. In the period from 6 to 30 days all larvae of *D. dentex* died, while growth rates of the hybrid and *D. sargus* did not differ significantly. This study indicated that our "monster" could possibly represent an interesting animal to be considered as a candidate for marine aquaculture.

Table 2. Species studied and genetical research conducted in the Laboratory

Species	Field	Comment
Dicentrarchus labrax		
Sparus aurata	K, P	P - temperature shock applied
Dentex dentex	K	
Diplodus puntazzo	K	
Diplodus vulgaris	K	
Diplodus sargus	K	
Dentex gibbosus	K	
Spondilosoma cantharus	K	
Ostrea edulis	Pg	Gel-electrophoretic isoenzymatic analysis
Hybrids		
Sparus aurata × *Diplodus puntazzo*	H	
Sparus aurata × *Diplodus vulgaris*	H	
Diplodus sargus × *Dentex dentex*	H	Most successful

H: hybridization; P: polyploidy; K: karyotypes; Pg: population genetics

(ii) *Polyploidy*

Genetic research in the Laboratory was also done on chromosome-set manipulations. *Sparus aurata* was studied to see the effect of temperature shock on inducing triploidy in this fish. Freshly fertilized eggs underwent the temperature shock treatment; the percentage of survival, growth rate, number of triploid and abnormal eggs were recorded. The temperatures applied were between 5°C and 10°C for the low shock treatment and 25°C and 40°C for the high temperature treatment. Exposure time was 10 minutes averagelly 30 minutes after fertilization. The experiment showed high mortality rate, revealing this species to have lower temperature tolerance than a number of freshwater species which were studied before.

(iii) *Population genetics*

In 1994 with the co-operation of French colleagues from IFREMER a study on population genetics of European flat oyster (*Ostrea edulis*) along the eastern Adriatic coast was initiated. The research focused on genetic structure and degree of migrations. Isoensymatic structure of populations of oysters were studied by gel electrophoresis. The conclusion of this work was that hatchery reared populations had lower levels of heterozigocy and polymorphism than natural populations (Marsic-Lucic, 1995). The difference in frequencies of allele of natural and reared populations were probably the result of founder effect on genetic drift. It was also shown that the number of allele per loci decreases from south to north in natural populations. This study was the start of present and future similar studies of both shellfish and finfish populations of the Adriatic.

(iv) *Present studies*

At the moment basic research on karyotypes of sparid species from eastern Adriatic is focusing particularly on species of potential interest in marine aquaculture. Also, new studies on population genetics of Sparid species have been initiated looking at allozyme variation.

Plans and projects

Taking into account all current needs of aquaculture, as well as the problems it faces, Laboratory plans to widen its scope of work in the field of fish and shellfish genetics. This idea was promoted by the lack of relevant biological data, especially in the area of genetics on many species potentially useful to aquaculture. The goal of our planned research is to widen basic knowledge of reproductive biology, growth, genetics and ecology of both finfish and shellfish species. Part of the research will be directed at genetic manipulation of the species reared and detailed study of biological and ecological characteristics of the Sparid hybrids. Parallel to this we shall start further research on population genetics of finfish and shellfish species of interest to aquaculture (Table 3). All data would be gathered and organized in a widely accessible data base.

Table 3. Plans and projects of the Laboratory

Field	Plans and projects
Karyotypes research	Planing to karyologically describe sparid species living in Adriatic, so the basis for the genetic data base on Adriatic fish could be configured
Isoenzymes	Apply the gel-electrophoresis technique on finfish, in order to compare wild and reared populations of commercially interesting species, and to preserve the biodiversity
Hybridization	Start an elaborate program of intergeneric hybridization which should hopefully point out species (hybrids) suitable for marine aquaculture

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