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Status of grouper (genus *Epinephelus*) investigations in Croatia

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SUMMARY – Research on the introduction of groupers from the genus *Epinephelus* to aquaculture in Croatia started in 1995. The four species researched are: the dusky grouper, *Epinephelus marginatus*, white grouper, *Epinephelus aeneus*, goldenblotch grouper, *Epinephelus costae* and dogtooth grouper, *Epinephelus caninus*. The recent achievements of experiments on these four species on broodstock formation and conditioning, artificial spawning and larval rearing, sex reversal and genetic studies are described.

Key words: Groupers, artificial spawning, rearing.

RESUME – *"Etat des recherches sur le mérou (genre* Epinephelus*) en Croatie". Les recherches sur l'introduction des mérous du genre* Epinephelus *dans l'aquaculture en Croatie ont commencé en 1995. Les quatre espèces objet de recherches sont : le mérou noir,* Epinephelus marginatus*, le mérou blanc,* Epinephelus aeneus*, la badèche,* Epinephelus costae*, et le mérou gris,* Epinephelus caninus*. Les réussites récentes des expériences faites sur ces quatre espèces sur la formation et la mise en condition des animaux destinés à la reproduction, la reproduction artificielle et l'élevage larvaire, le renversement sexuel et les études génétiques sont décrites.*

Mots-clés : *Mérous, reproduction artificielle, élevage.*

Introduction

Groupers are among the most highly priced and appreciated fish around the world. Due to high market values and the subjection to sport and spear diving fisheries, the populations of many species are under constant pressure. For this reason, groupers are also the most endangered fish species in many parts of the world, like the Nassau grouper, *Epinephelus striatus* in the Gulf of Mexico and the dusky grouper, *Epinephelus marginatus* in the Mediterranean (Sadovy, 1989; Zabala *et al.*, 1997).

Due to its high market value and the urgent need of producing fingerlings for restocking programs, groupers are the subject of intensive research on artificial spawning in many countries (Tucker, 1994; Pillay, 1995). The trend to research grouper started recently in the Mediterranean. Few species are subject to intensive research such as are the: dusky grouper, *Epinephelus marginatus* and white grouper, *Epinephelus aeneus*. A few others were listed as a potentially interesting species for aquaculture, such as the: goldenblotch grouper, *Epinephelus costae* and deepsea grouper, *Polyprion americanus*. In this paper, the recent advances and the status of research on the groupers in Croatia will be presented.

Investigated species

The subjects of our interest are four species of groupers from the genus *Epinephelus*. They are as follows:

(i) Dusky grouper, *Epinephelus marginatus*.
(ii) White grouper, *Epinephelus aeneus*.
(iii) Doogtooth grouper, *Epinephelus caninus*.
(iv) Goldenblotch grouper, *Epinephelus costae*.

At present, only the dusky grouper is a subject of research for its introduction to commercial rearing and for restocking purposes. The other three species are presently in stages of broodstock formation and acclimation, and are being used in genetic studies, mainly hybridisation.

Dusky grouper, *Epinephelus marginatus*

The dusky grouper is being researched in a few Mediterranean countries, mainly Italy (Marino *et al.*, 1999; Spedicato and Boglione, 1999) and Spain (Garcia and Castello-Orvay, 1995). After detailed ecological studies on dusky grouper populations in the south-eastern Adriatic in the late eighties (Skaramuca *et al.*, 1989), we initiated research on artificial spawning and early rearing stages (Glamuzina *et al.*, 1997). A few main problems appeared in the early stages of research, mainly the lack of males and the initiation of final oocyte maturation. The lack of males was solved by the hormonal treatment of juvenile and young females, through applying the male hormone 17α methyltestosterone either orally or by injection. The same approach and methodology in solving this problem were described by Italian researchers (Marino *et al.*, 1998; Spedicato *et al.*, 1998). We obtained fertile sperm with good motility in the dusky grouper, when the consumed quantity of 17α methyltestosterone reached a level of above 80 mg/kg, during four months of feeding (Glamuzina *et al.*, 1998a). However, an injected quantity of 20 mg/kg led to a sex change, but without active sperm, and we feel that it involved a hormone overdose (Glamuzina *et al.*, 1999a). Marino *et al.* (1998) observed that after the termination of hormone treatment, all males reversed back to female. So, such treatments are needed yearly or another approach to hormone application has to be developed.

The next problem was the final maturation of oocytes and the obtaining of ripe eggs. All researchers in the Mediterranean gave reports on no spontaneous spawning of the dusky grouper in captivity and researched different hormonal treatments in order to achieve successful spawning (Marino *et al.*, 1999; Spedicato and Boglione, 1999). We obtained better results when females where treated with two injections of HCG, 24 h apart, than with LHRH (a). Similar treatments were described by Spedicato *et al.* (1995) and Spedicato and Boglione (1999). However, the best results were reported by Marino *et al.* (1999) using GnRHa (triptoreline) in microspheres.

The main problem was the time of stripping after the second injection, as the eggs overripened very quickly. The model for stripping described by Watanabe *et al.* (1995) for Nassau groupers fit our dusky grouper broodstock well. The optimal stripping time for dusky grouper was 68-70 h at 18-19°C (Marino *et al.*, 1999), while we found 14-16 h as being optimal at 23°C. The difference is surely correlated to the different temperature holdings of broodstock.

The dusky grouper larvae obtained in our experiments were only 1.52 mm at hatching, with a functional mouth opening at first feeding between 100-120 μm. These two findings led to problems with the first feeding, inappropriate prey, high sensitivity and many deformities, and generally to very low survival. Up to now, in the whole Mediterranean, only 60 fingerlings were artificially produced in Cyprus (T. Atack, pers. comm.). Other researchers reported successful larval rearing up to the 15th day (Marino *et al.*, 1999) and up to the 50th day (Spedicato and Boglione, 1999), or in our experiments, up to the 10th day. Similar problems were reported for all groupers investigated in different parts of the world (Tucker, 1994; Watanabe *et al.*, 1995; and many others). This means that the low survival of grouper larvae could not be attributed only to a single factor (i.e. inappropriate first prey) and that we still need more biological, ecological and social data from wild populations in order to establish a successful protocol for artificial spawning and larval rearing.

White grouper, *Epinephelus aeneus*

The white grouper is a subject of research in Israel (Hassin *et al.*, 1997) and in a few other aquaculture enterprises (Abellán and Basurco, 1999). This species is presently an intruder in the Adriatic and could be used as biological evidence for the warming of Mediterranean waters (Glamuzina *et al.*, 1999d). A few fingerlings were acclimated to captivity and used in experiments with sex reversal. Two months after being injected with 2mg/kg of 17α methyltestosterone and with an accumulated dose of 12 mg/kg, one fish (300 g weight) started to spermiate (Glamuzina *et al.*, 1999a). This sperm was used to make a hybridisation with dusky grouper eggs. The hybrid embryogenesis and larval rearing did not differ from dusky grouper and this could be a sign of good potential for this hybrid in future research (Glamuzina *et al.*, 1999b).

Goldenblotch grouper, *Epinephelus costae*

This species is listed as a potentially interesting fish for introduction to scientific and commercial research (Abellán and Basurco, 1999). However, biological data and research on introduction to aquaculture is lacking. We formed a broodstock of ten females, ranging between 3 and 6 kg. Experiments on the sex reversal of juvenile fish (100-500 grams weight) with injections of 17α methyltestosterone were unsuccessful (Glamuzina *et al.*, 1999a), so experiments with female maturation and artificial spawning were not as yet performed.

Dogtooth grouper, *Epinephelus caninus*

This species is very rare in the Adriatic and it is difficult to form a broodstock with mature specimens. So, we started with the rearing of wild juveniles. A few of them were used in sex-reversal experiments with the injection of 17α methytestosterone, but without success. Due to broodstock formation difficulties, future research on the artificial spawning of this species is not bright, but it could be used in hybridisation experiments to obtain heterosis effects and better hybrid progeny than the parents themselves.

Discussion and conclusions

The main problem with the faster development of grouper aquaculture around the world is the bad results obtained with larviculture (Tucker, 1994; Pillay, 1995), evidenced by small larvae with a small mouth gape, and inappropriate feeding regimes. Although new preys, such as the oyster trochophores (fresh or cryopreserved), were introduced (Watanabe *et al.*, 1996; Duray *et al.*, 1997; Marino *et al.*, 1999), the results still remain unsatisfactory, if compared to other fish species. However, it seems that based on documented trochophore (Marino *et al.*, 1999) and rotifer ingestions (Glamuzina *et al.*, 1998b) in dusky grouper larviculture, other factors unrelated to feeding contribute to high larval mortality, such as the development of urinary calculosis (Glamuzina *et al.*, 1998c), high incidence of larval deformities (Glamuzina *et al.*, 1998b), low level of DHA fatty acids of rotifers (Marino *et al.*, 1999), and surely many other presently undetermined factors correlated with the complex ecological and social behaviour of wild dusky grouper.

Moreover, the major constraint in the commercial ongrowing of all groupers in the Mediterranean is and will be the unsatisfactory growth rate, compared to Asian and American relative species. Generally, the growth rate of the dusky grouper is similar or even lower than in sea bass, *Dicentrarchus labrax*, with daily growths of only 0.52 g at ambient conditions during the favourable summer season (Glamuzina, 1998). Artificially produced fingerlings reached around 500 grams during three years in Cyprus (T. Atack, pers. comm.). On the other hand, under ambient tropical conditions in Brazilian waters, dusky grouper reached 1 kg in two years (Benneti, pers. comm.). The growth rate at a constant 25°C is also promising, with a daily increment of around 1 g (Garcia and Castello-Orvay, 1995). So, it seems that the culture of grouper under the ambient conditions of most of the Mediterranean will not be economically feasible and that alternative rearing models need to be developed. A possible solution is rearing in recirculation systems at constant temperatures during the whole or part of the life cycle. Based upon grouper hiding behaviour and their living in shelters artificially made of tires (Chua and Theng, 1979; Glamuzina, unpublished data), with low motion activity and spending of energy, we could create special tanks to achieve a higher density and better biological and social conditions. The dusky grouper could probably qualify as one of the best candidates of marine fish for the fast development of fish rearing in modern recirculation systems.

The other use of artificially produced fingerlings will be in the restocking programs of parts of the Mediterranean where the species is endangered and in sea ranching programs in favourable locations. Due to many characteristics, such as good price, homing instinct, and territoriality, the dusky grouper is an excellent choice for sea ranching associated with the construction of artificial reefs. These facts are the main reasons for the continuation of research on the artificial production of these species in Croatia.

References

Abellán, E. and Basurco, B. (eds) (1999). Marine Finfish Species Diversification: Current Situation and Prospects in Mediterranean Aquaculture. *Options Méditerr., Series B*, 24.

Chua, T.-E. and Teng, S.-K. (1979). Relative growth and production of the estuary grouper, *Epinephelus salmoides* under different stocking densities in floating net-cages. *Mar. Biol.*, 54: 363-372.

Duray, M.N., Estudillo, C.B. and Alpasan, L.G. (1997). Larval rearing of the grouper, *Epinephelus suillus* under laboratory conditions. *Aquaculture*, 150: 63-76.

Garcia Lopez, V. and Castello-Orvay, F. (1995). Growth of *Epinephelus guaza* under different culture conditions. *Cah. Options Méditerr.*, 16: 149-155.

Glamuzina, B. (1998). *Artificial spawning and early stages characteristics of dusky grouper,* Epinephelus marginatus *(Lowe, 1834)*. PhD Thesis, University of Zagreb (in Croatian with English summary).

Glamuzina, B., Glavić, N., Kožul, V. and Skaramuca, B. (1998a). Induced sex reversal of the dusky grouper, *Epinephelus marginatus* (Lowe, 1834). *Aquac. Res.*, 29(8): 563-568.

Glamuzina, B., Glavić, N., Tutman, P., Kožul, V. and Skaramuca, B. (1999a). Hormone-induced sex reversal of fish case of dusky grouper (*Epinephelus marginatus*, Lowe 1834). In: *XXXV Croatian Symposium on Agriculture*, Opatija (Croatia), 22-25 February, p. 211.

Glamuzina, B., Kožul, V., Tutman, P. and Skaramuca, B. (1999b). Hybridization of Mediterranean groupers: *Epinephelus marginatus* female x *E. aeneus* male and early development. *Aquac. Res.*, 30: 625-628.

Glamuzina, B., Skaramuca, B., Glavić, N. and Kožul, V. (1997). First results of artificial spawning of dusky grouper, *Epinephelus marginatus* (Lowe, 1834). In: *Proceedings of Abstracts of the Paper of the Sixth Congress of Croatian Biologists*, Huber, Đ. (ed.), pp. 315-316.

Glamuzina, B., Skaramuca, B., Glavić, N. and Kožul, V. (1998b). Preliminary studies on reproduction and early stages rearing trial of dusky grouper, *Epinephelus marginatus* (Lowe, 1834). *Aquac. Res.*, 29: 769-771.

Glamuzina, B., Skaramuca, B., Glavić, N., Kožul, V., Dulčić, J. and Kraljević, M. (1998c). Egg and early larval development of laboratory reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834) (Pisces, Serranidae). *Sci. Mar.*, 62(4): 373-378.

Glamuzina, B., Skaramuca, B., Glavić, N., Tutman, P. and Kožul, V. (1999c). Female reproductive cycle of the dusky grouper, *Epinephelus marginatus* (Lowe, 1834) in captivity. *Sci. Mar.* (submitted).

Glamuzina, B., Skaramuca, B., Glavić, N., Tutman, P. and Kožul, V. (1999d). Adriatic groupers: Status of natural populations and perspectives of breeding. *Acta Adriatica* (in press).

Hassin, S., de Monbrison, D., Hanin, Y., Elizur, A., Zohar, Y. and Popper, D.M. (1997). Domestication of the white grouper, *Epinephelus aeneus.* 1. Growth and reproduction. *Aquaculture*, 156: 305-316.

Marino, G., Azzuro, E., Finoia, M.G., Messina, M.T., Massary, A. and Mandich, A. (1999). Recent advances in induced breeding of the dusky grouper *Epinephelus marginatus* (Lowe, 1834). In*:* Seminar on Mediterranean Marine Aquaculture Finfish Species Diversification, Zaragoza (Spain), 24-27 May 1999. *Cah. Options Méditerr.* (in press).

Marino, G., Massari, A., Di Marco, P., Azzuro, E., Finoia, M.G. and Mandich, A. (1998). Sex ratio, sexual maturity and sex change in *Epinephelus marginatus*. *Biol. Mar. Med*. (in press).

Pillay, T.V.R. (1995). *Aquaculture: Principles and Practices.* Fishing News Books, Oxford.

Sadovy, Y. (1989). Caribean fisheries. Problems and constraints. *Progress in Underwater Sci.*, 13: 169-184.

Skaramuca, B., Mušin, D., Onofri, V. and Carić, M. (1989). A contribution to the knowledge on the spawning time of the dusky grouper (*Epinephelus guaza* L.). *Ichthyologia*, 21(1): 79-85.

Spedicato, M.T. and Boglione, C. (1999). Main constraints in the artificial propagation of the dusky grouper *Epinephelus marginatus* (Lowe, 1834): Three years experimental trials on induced spawning and larval rearing. In: Seminar on Mediterranean Marine Aquaculture Finfish Species Diversification, Zaragoza (Spain), 24-27 May 1999. *Cah. Options Méditerr.* (in press).

Spedicato, M.T., Contegiacomo, P., Carbonara, C. and Lembo, G. (1998). Ovarian maturation and spawning in Epinephelus marginatus (Lowe, 1834) induced by hormone treatments. In: *Proceedings of the 33rd International Symposium on New Species for Mediterranean Aquaculture*, Alghero (Italy), 22-24 April 1998.

Spedicato, M.T., Lembo, G. and Di Marco, G. (1995). Preliminary results in the breeding of dusky grouper *Epinephelus marginatus* (Lowe, 1834). *Cah. Options Méditerr.*, 16: 131-148.

Tucker, J.W. Jr. (1994). Spawning by captive Serranid fishes: A Review. *J. World Aquac. Soc.*, 25: 345-354.

Watanabe, W.O., Ellis, S.C., Ellis, E.P., Kelley, C.D., Moriwake, A., Lee, C.S. and Bianfang, P.K. (1995). Progress in controlled breeding of Nassau grouper (*Epinephelus striatus*) broodstock by hormone induction. *Aquaculture*, 138: 205-221.

Watanabe, W.O., Ellis, S.C., Ellis, E.P. and Lopez, V.G. (1996). Evaluation of first-feeding regimes for larval Nassau grouper *Epinephelus striatus* and preliminary, pilot-scale culture through metamorphosis. *J. World Aquac. Soc.*, 27(3): 323-331.

Zabala, M., Garcia-Rubies, A., Louisy, P. and Sala, I. (1997). Spawning behaviour of the Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Pisces, Serranidae) in the Medes Islands Marine Reserve (NW Mediterranean, Spain). *Sci. Mar.*, 61: 65-77.