Long-Term Plan for Domestication of the White Grouper (*Epinephelus aeneus*) in Israel

Sergei Gorshkov*

Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel

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Abstract

The objective of this article is to highlight culture problems, bottlenecks to broodstock management, and possible genetic consequences to be taken into consideration when planning the domestication and captive breeding program for white grouper (*Epinephelus aeneus*) in Israel. Taking into account the biological features and genetic background of the white grouper, we suggest developing industrial cultivation in several stages. In stage 1, the establishment of broodstock and first steps towards domestication, emphasis should be placed on avoiding inbreeding in captive populations. Establishment of foundation population(s) is the most important step in broodstock management and determines the amount of genetic variation and culture potential of future domesticated generations. In stage 2, existing populations and strains will be tested under experimental and commercial growout conditions. In stage 3, traditional selective breeding methods including mass selection and crossbreeding will be implemented. In stage 4, development of marker-assisted selective breeding molecular techniques will enable assigning parentage to individual fish in mixed-progeny groups and design of an efficient genetic improvement program. Finally, in stage 5, organized and supervised domestication and selective breeding programs will provide Israeli farmers more control of broodstocks and sustainable development of white grouper aquaculture.

* Corresponding author. Fax: +972-8-6375761, e-mail: gorshkov_s@ocean.org.il
Prologue
"...Aquaculture, not Internet, represents the most promising investment opportunity of the 21st century." (Peter Drucker, economist and Nobel Laureate).

Introduction
Among the various warm water marine fish species, groupers (family Serranidae, sub-family Epinephelinae) are very attractive candidates for commercial mariculture because of their high prices on fish markets due to excellent taste, and characteristically rapid growth rates especially at high temperatures (Tucker, 1994; Hassin et al., 1997; Lupatsch and Kissil, 2005). Commercial rearing of groupers is being intensively developed in southern Asia. Since most groupers do not readily spawn in captivity, their culture and broodstock maintenance are based on wild-caught fish. Thus, this is an extensive capture-based aquaculture (Pierre et al., 2008) and its substantial negative impact is already being felt through the loss of adaptive genetic diversity of many species in the aquatic food chain, leading to a decrease of ecosystem stability (Halim, 2001).

The white grouper *Epinephelus aeneus* is a widely known, valuable, highly priced species in fish markets of the Mediterranean basin. In Israel, efforts to culture the white grouper in the mid-1990s showed its high aquaculture potential including a very fast growth rate in captivity (Hassin et al., 1997). In spite of this pioneering success, Mediterranean farming of grouper is still embryonic; it is based on wild populations captured from the natural environment and there are no domesticated strains resulting from breeding activity. In principle, this is equivalent to dairy farmers using wild buffalo for milk production, or wheat farmers using wild wheat seeds. Therefore, industrial culture of groupers in captivity poses numerous problems, tends to be inefficient and expensive, and is of variable quality and unreliable supply.

To maintain a competitive edge against the rapidly expanding European mariculture, the Israeli industry must adopt new species, develop more efficient growing practices, and domesticate superior strains of cultured species (Gordin, 2003). The development of an additional popular marine food species is necessary for Israeli mariculture, an important sector of the national economy, to broaden its industrial base and marketability. This is where the White Grouper Domestication Project in the aquaculture department of the Ministry of Agriculture comes in. The mission of the project is to develop innovative technologies aimed at domestication, breeding, and industrial rearing of the white grouper.

Commercial growers are not in a position to pay adequate attention to the domestication and breeding problems of the groupers they culture. Because grouper broodstocks are often in short supply, growers are forced to culture whatever stocks are available. The growers are businessmen, and because output assumes the highest priority in any commercial-level hatchery, they justifiably focus on providing fish to the market. Therefore, another important objective of the White Grouper Domestication Project is to provide Israeli fish
farmers with academically validated knowledge and biological and technical assistance in order to establish a long-term sustainable grouper farming industry. This project is the most extensive fish breeding and technical aquaculture effort in Israel, an original program intended to promote the economic development of a new fish farming industry. Below, we discuss the main biological and genetics bases of the project, which is organized in five stages.

**Stage 1: Broodstock establishment and first domestication steps**

For any cultured fish, establishment of domesticated captive broodstock starts with the following: (a) initial collection of wild-caught fish, (b) rearing and maintenance to sexual maturity of wild-caught populations in captivity, (c) establishment of breeding techniques, reproduction, and creation of captive broodstocks, (d) optimization of breeding management of captive populations across generations for production efficiency and fish welfare.

Fish breeders should carefully document the breeding history of all strains and wild-caught populations undergoing domestication. This documentation and its scientific analysis are of great importance because a number of domesticated populations or strains will be needed as base resources of genetic variation for future selective breeding programs. This documentation will also be important when it becomes desirable to regain genes that may have been lost in the domestication process.

Founder stocks should be collected from as wide a distribution as possible to ensure that domestication, at least in its initial stages, will not be associated with decreased genetic diversity but, rather, based on as broad a genetic base as possible. Loss of genetic diversity in the first steps of domestication may result in reduced reproductive success, higher mortality rates, and inbreeding depression (Kirpichnikov, 1981; Tave, 1999; Lutz, 2001). There are well known cases when hatchery broodstocks were started with a population of fish produced by a single mating of parents (Tave, 1986). It is not difficult to imagine that such populations will have little genetic variance, and that inbreeding will make it difficult to achieve breeding goals, even leading to loss of the breeding population.

In a broad sense, domestication is the adaptation of captive population(s) to husbandry under culture conditions. The modern genetic concept of domestication of cultured aquatic organisms considers this adaptation a genetic selection process mediated by reproductive isolation from wild counterparts, inbreeding, and small population sizes (Doyle, 1983; 1999). Although deliberate selection would not be applied, domestication itself implies unintentional selection of those individuals that adapt to specific environmental conditions including water quality and temperature, artificial feeds, crowding, human handling, etc. After one or two generations of such "domestication selection", captive population(s) are composed of individuals that are relatively tolerant to specific culture conditions, and survival and growth may improve. For example, domestication increased the growth rate
of channel catfish by an estimated 2-6% per generation (Dunham and Smitherman, 1983).

In addition to liberating farmers from a dependence on wild-caught broodstock or fingerlings, domesticated populations introduce a wider range of genetic improvement options due to variations in performance between populations (Knibb, 2000). Thus, the establishment of captive foundation population(s) of white grouper determines the initial amount of genetic variation and this variation will eventually specify the culture potential of future domesticated generations.

In 1990, the National Center for Mariculture (NCM) initiated the first collection of wild-caught white grouper for the first scientifically validated study of its reproductive biology and growth rates in captivity (Hassin et al., 1997). For the first time, experiments were carried out under controlled conditions in relatively big concrete tanks (16 m³, 0.8 m depth) supplied with sea water in a flow-through system. Broodstocks were fed pelleted dry food (40% protein), supplemented twice a week with frozen fish or squid. Sexual development in different wild-caught populations was monitored for several years and growth of different cohorts was compared. Under these rearing conditions, adult wild-caught females did not complete final oocyte maturation and natural spawning did not occur. Using hormonal injections, ovulated females and matured "running" males could be manually stripped and eggs were artificially fertilized with high rates of fertilization (Hassin et al., 1997).

Special indoor land-based facilities (50 m³, 2.5 m depth) at NCM allowed spontaneous spawning in wild-caught brooders (Gorshkova et al., 2002) and subsequent studies evaluated the reproductive potential of first generation progeny from wild-caught parents in May 2005 (Meiri-Ashkenazi et al., 2010). Thus, the full life cycle (including sexual maturation) of the 2005 generation was completed under the specific captive conditions at the NCM, representing the first step of domestication.

Since white grouper culture is an emerging aquaculture sector, the NCM has carried out different applied research studies on groupers so as to commercialize its culture. The NCM now has several genetically different white grouper broodstock with different origins and domestication histories. With the advent of techniques for inducing maturation, controlling reproduction, and managing broodstock and breeding, the white grouper has become an important concern of aquaculturists and fish geneticists.

Despite many efforts in Asia and the Mediterranean, there are no controlled captive-breeding and domestication programs for groupers (Pierre et al., 2008). It seems that groupers have very specific biological features and there are numerous unsolved problems connected with genetics, reproductive biology, larval rearing, and industrial culture. Generally-recognized bottlenecks include: (a) inconsistent survival of embryos and high larvae mortality; (b) sensitivity to diseases (among which viral nervous necrosis, VNN, is the most serious), particularly at the larval and early juvenile stages; (c) lack of experience in organizing domestication and broodstock management programs.
Domestication and broodstock management programs should take into account the following important intrinsic biological features of this species: (a) the white grouper is a protogynous hermaphrodite; individuals first develop into females and later turn into males, although there are indications that gonadal changes are not always stable (Hassin et al., 1997); (b) the species has very specific features of reproductive behavior and is, presumably, a size-assortative spawner (Zabala et al., 1997; our own observations); (c) the species has an enormous fecundity (4-7 kg adult females have up to several million eggs), thus millions of fertilized eggs can result from the mating of a single pair (Tucker, 1994); (d) the species (at least adult fish beyond spawning) has a solitary life-pattern (Zabala et al., 1997); (e) like most species of the genus Epinephelus, white grouper (juvenile and older) are benthic predators that feed on macro invertebrates and fishes near the bottom (Tucker, 1999); and (f) chromosomal disorders might affect survival during early embryonic stages (Gorshkova et al., 2002).

Stage 2: Testing of existing populations and strains

Variation in culture performance between fish populations is one of the main sources of species-specific genetic variability (Kirpichnikov, 1981; Tave, 1986). Differences in culture performance between wild domesticated populations and cultured marine species are well documented (Knibb et al., 1997; Lutz, 2001; Gorshkov et al., 2004). Populations exhibiting different culture performance can enable: (a) choosing populations for particular culture situations and (b) crossing populations to combine desirable traits into crossbred lines.

Most cultured fish, including carp, channel catfish, tilapia, sea bream, and sea bass, show variations between populations (strains) in growth, disease resistance, survival, sex ratio, and temperature tolerance. Such characteristics have been exploited in commercial farms (Kirpichnikov, 1981; Lutz, 2001). As shown for sea bream, crossbreds between lines can be used for commercial production of food fish, as well as for sales of eggs and fingerlings (Gorshkov, 2006). Maintaining different strains and populations allows breeders to adopt crossbreeding approaches that minimize future inbreeding problems (Hulata, 2001).

For practical applications, it is important to begin a new culture with a stock that meets industry performance objectives as closely as possible. Domestication of new cultured species should begin with the study, characterization, and assessment of phenotypic and genetic variations between populations or strains. Choosing populations with better performance in captive conditions, especially in intensive culture, could save years of breeding with genetically inferior or unsuitable populations (strains). Culture performance in captivity can be assessed only by experimental rearing trials and cannot be predicted a priori by assessment of genetic variation using molecular tools (Bentsen, 1994). Molecular genetic information can be used for aquaculture and fishery needs, but mostly to identify stocks, analyze population genetics and parentage, and for marker-assisted selection.
Variation in culture performance between captive populations of groupers remains unstudied. As the white grouper farming sector is starting to form captive broodstocks, it is important to study differences among existing populations. In addition, the magnitude of $G \times E$ (genetic by environment) interactions remains unknown and is of practical importance when choosing base population(s). Populations should be tested under controlled experimental and commercial growout conditions and chosen for economically important traits.

**Stage 3: Traditional selective breeding**

The productivity and profitability of grouper aquaculture can be enhanced by genetic improvement of cultured stocks. Later project phases will concentrate on selective breeding by exploiting the genetic variation within population(s) to choose the best individuals for creating the next generation. No single approach to selective breeding is universally best. Many breeding schemes and protocols require expensive and bulky facilities and are not suitable for the average farmer.

The initial stages of selective breeding in white grouper will involve mass selection and crossbreeding. In cultured marine fish, this is a relatively low-tech approach that uses information on measurable traits such as growth and makes reasonable assumptions about their genetic basis (Knibb, 2000, Gorshkov, 2006). Although it may seem simple to use the best performers as parents for the next generation, there is more to it than that. If the best growing groupers are close relatives, mating them with each other will result in inbreeding problems and, the closer the relationship, the greater the problems. Inbreeding depression occurs because relatives carry some of the same unfavorable alleles transmitted to them from common ancestors. Ironically, one of the characteristics that makes the white grouper so well suited to aquaculture – their huge fecundity and reproductive output – also increases the risks of inbreeding, because the entire production of a grouper hatchery can be the product of only a few parents. If the broodstock includes a large number of close relatives, all things can lead to inbreeding depression.

Considering $G \times E$ interactions, we expect that different genotypes will excel in distinct environmental conditions. For example, selected groupers that grow well in the clear, highly-saline, and relatively stable water temperature of the Red Sea would probably perform differently in the turbid and warmer industrial sea cages in the Ashdod port. Similarly, a superior genotype is no protection against poor husbandry and maintenance practices.

Choosing the best gene combinations is the basic idea of hybridization or crossbreeding. Plants geneticists have tried huge numbers of genetic combinations in order to create the best hybrid and produce hybrid seeds for culture. However, this form of genetics cannot be inherited since it is produced by combining specific pairs of parental strains or even specific parental individuals. In carp culture, hybridization produces a high-quality, uniform product that is widely used in Israeli fish farms (Wohlfarth, 1993;
Hulata, 1995). We do not yet know if the same thing can happen with groupers. The NCM carries out mass selection among different strains (lines) of gilthead sea bream and crossbreedings are conducted to exploit heterosis (Knibb, 2000; Gorshkov, 2006). It would be reasonable to implement similar procedures in breeding programs for the white grouper. The initial protocol will most likely require keeping at least two populations of broodstock (lines). All fish should be marked (pit-tagged) and, although different groups can be mixed in a common tank, it would be best to maintain populations separately. In further experiments, we would create all possible pair-wise mating combinations among the available strains or populations. The resulting genetic groups would be reared at different farms and tested for culture performance. If progeny of between-population matings outperform those of within-population matings, this would support the idea that specific crossbreds show heterosis and warrant investigating hybrid breeding strategies for further domestication and improvement.

**Stage 4: Marker assisted selective breeding**

Paradoxically, the main obstacle to grouper domestication and selective breeding is probably its huge fecundity. Selection requires immense physical resources (e.g., separate tanks and labor for each cohort), making this approach impractical for more than a few groups or families. The result would seem to be a rather modest selection intensity compared to what is theoretically attainable and, therefore, modest genetic improvement. However, the recent explosion of molecular techniques and development of affordable technologies for genetic fingerprinting can probably remove at least some of the technical and reproductive constraints for family selection in white grouper. It is now possible to assign parentage to cultured fish in mixed-progeny groups using microsatellite markers (Hulata, 2001; Dunham, 2004; Hallerman, 2006; Rexroad et al., 2006; Silverstein et al., 2006).

With information on relatedness and quantification of the contribution of each parent, a selective breeding program need not use physical tagging or separate rearing of genetically distinct groups. Knowledge of the linkage between DNA markers and quantitative trait loci (QTL) alleles may be used for marker-assisted selection (MAS), increasing the rate of genetic responses by affecting efficiency and accuracy of selection (Poompuang and Hallerman, 1997; Hallerman, 2006). Molecular genetic markers are not yet routinely used in commercial aquaculture due to the limited development of domesticated broodstocks and the high costs of genetic screening.

**Stage 5: Providing affordable genetic services**

NCM’s mission of domesticating and breeding the white grouper will be based on a strong mutual commitment between science and industry. It would be enormously helpful to Israeli mariculture if the NCM were to develop better performing grouper stocks. In a variety of aquaculture fish species, growth is the production trait most closely associated with profitability when genetic
improvement is mentioned. At the same time, disease resistance, food conversion ratio, body shape, and other economically important traits can be improved through traditional selective breeding approaches.

Production efficiency and culture performance can be positively impacted by integrating traditional selection and novel genetic technologies. These approaches, however, require the ability to completely control reproduction of the involved species. Further, many procedures related to genetic research and improvement (e.g., chromosome set manipulations, genetic markers and mapping, and complex mating designs) require advanced aquaculture knowledge and biological expertise such as knowing how to combine broodstock and predicting the timing of fertilization. When genetic improvement is based on traditional selective breeding and modern molecular approaches, broodstock culture may require little more than suitable nutrition, water quality, and environmental conditions.

The organization and supervision of domestication and selective breeding programs will eventually allow the NCM to offer farmers control over reproduction and affordable “genetic services.” Domesticated and genetically improved strains can be reared in a commercial production setting and farmers interested in genetic improvement would handle their selection strains or lines in the same manner as any other agricultural cohort, and measure the traits they wish to improve. Farmers could develop trademark features, such as unique morphological or taste patterns, that will serve as brand identification and add value to their product. However, it is necessary to clearly understand that the most important part of any domestication and selective breeding program is good organization of all supportive works and infrastructure as well as careful recording of phenotypes and pedigrees.

Modern technology complements traditional breeding but does not replace it. Nearly all progress in animal breeding has been obtained by traditional trait-based methodology (Weller, 2006). Eventually, the organizing and supervising of the domestication and selective breeding programs will allow the NCM to offer farmers more control over all aquaculture procedures, guaranteeing efficient progress and sustainable development of grouper aquaculture in Israel. The NCM is a unique scientific institution, in the position to solve complex biological problems associated with the white grouper domestication and breeding project: controlled reproduction, larval rearing, disease control, nutrition, and classical selective breeding.

References


Domestication of the white grouper in Israel


