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Practical Genetics in Israeli Mariculture: History and Present Status

Sergei Gorshkov*

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

Key words: mariculture, genetics, selective breeding, gilthead sea bream, Mediterranean sea bass

Abstract

The aim of this review is to show the progress and achievements made to date in the selective breeding of cultured marine fish in Israel. The National Center for Mariculture (NCM) in Eilat was the first scientific organization to determine that a long-term selective breeding program is the key strategy for genetic improvement of commercially important marine fish. The main objective of the program is the development of genetically improved strains. The immediate practical goal is to improve the profitability of national mariculture through growth improvement in the gilthead sea bream (*Sparus aurata*). In this species, due to reproductive constraints that limit the prospects for family selection, mass selection was found to be the most practical tool for growth improvement. Currently, the selective breeding program for sea bream consists of several selected lines. Four cycles of mass selection for growth have been completed in commercial production environments. Mass selection and crossbreeding are integrated in the program. In the Eilat hatchery sea bream strain, a recessive deleterious Mendelian mutation (named "ebony") was isolated by classical crossing experiments. A method was developed to use ebony for genetic protection of selected sea bream strains from unlicensed reproduction and propagation. Interspecific hybridization, chromosome set manipulation, and cytogenetic and sex control techniques were also applied; some were shown to be promising tools for short-term genetic improvement. In general, the NCM selective breeding program for sea bream showed that genetic gain for growth and faster economic return can be achieved within a "reasonable" time span of 10-12 years. The program is supervised by the Genetics and Physiology Department of the NCM. Presently, most Israeli commercial mariculture is using genetically improved strains of sea bream and newly domesticated strains of sea bass (*Dicentrarchus labrax*).

Introduction

In many countries bordering the Mediterranean Sea, mariculture is still based on wild fish stocks recently captured from the natural environment and no specific strains among cultured marine fish have been recognized as a result of any selective breeding activity. Therefore, sea farm production tends to be inefficient and produce tends to be

* Tel.: +972-8-6361432, fax: +972-8-6375761, e-mail: Gorshkov_s@ocean.org.il

expensive and of variable quality and unreliable supply. In aquaculture, the use of genetically improved fish strains is expected to be a major factor in increasing commercial competitiveness in the near future (for reviews see Dunham et al., 2001; Hulata, 2001; Lutz, 2001). However, genetic improvement studies are still hampered by short-term, scattered, and sporadic funding. In the area of practical genetics for mariculture, long-term genetic improvement efforts are especially neglected. Lack of genetically improved strains and expertise in organizing, developing, and managing practical selective breeding programs for marine fish greatly retard mariculture development. Except for Israel, there is to date no adequately developed and applied long-term selective breeding program for cultured marine fish. The present paper reviews the main steps of the Israeli selective breeding program for cultured marine fish since the early 1990s.

Establishment of the Selective Breeding Program at the NCM

The principal aim of the National Center for Mariculture (NCM) in Eilat is the development of modern and efficient marine farming technologies designed for the most commercially important marine organisms in the Mediterranean basin. During the last two decades, the main efforts were focused on fish species such as the gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*), gray mullet (*Mugil cephalus*), white grouper (*Epinephelus aeneus*), red sea bream (*Pagrus major*), doublebar bream (*Acanthopagrus bifasciatus*), and others.

The Genetics Department began operation in 1990 in close cooperation with the reproduction, larval rearing, nursery, and pathology research groups. In the late 1980s, the NCM already had a well-developed and workable technology for controlled reproduction of sea bream and sea bass. Various facilities, together with an advanced infrastructure, allowed studying fish under controlled environmental conditions. In addition, the sea bream stock had been domesticated for several generations and had undergone intensive research in a variety of biological disciplines (Gordin, 2003).

The NCM operates under the main concept that mariculture is an interdisciplinary science (Gordin, 2003). However, prior to the initiation of the Genetics Department, there was no sustained and applied selective breeding program for marine cultured fish elsewhere in the world. Thus, it was impossible to learn from previous experience how to develop and manage a program to suit the needs of Israeli mariculture. Nevertheless, we expected traditional selective breeding of cultured marine fish to be organized in a similar way as that which produced genetically improved strains in cultured freshwater and anadromous fish (Kirpichnikov, 1981; Dunham and Smitherman, 1983; Gjedrem, 1983; Gjerde, 1986; Gall, 1990). We also expected that, for a novel selective breeding program dealing with new marine cultured fish, it would not be possible to immediately choose the universally best genetic improvement approach even if it had been efficiently applied to other fish species. Depending on specific aspects of fish biology, broodstock management, and breeding objectives, it should be studied on a case-by-case basis.

In 1991, we determined that the best strategy for genetic improvement of commercially important marine fish should be a long-term selective breeding program. The main research objective of the program was the development of genetically improved cultured strains. The immediate practical goal was to improve the profitability of national mariculture. While major emphasis was placed on classical genetic selection, which was (and still is) one of the main genetic tools for genetic improvement in cultured fish (Kirpichnikov, 1981; Hulata, 2001), consideration was also given to other useful genetic techniques (so-called short-term genetic improvements) to be utilized in association with long-term selective breeding programs. Interspecific hybridization, chromosome set manipulation, and sex control were seen as promising tools for short-term genetic improvements.

The gilthead sea bream (hereafter sea bream) and the European sea bass (hereafter sea bass) were chosen as the first candidates for genetic improvement. The NCM selective breeding program consisted of the following key integrated elements: selective breeding objectives; choice, evaluation, and establishment of base population(s); genetic improvement strategies and selection methods; practical applications and commercialization of the program.

Selective Breeding Objectives

The ultimate selective breeding objective of any genetic improvement program is to increase productivity and profitability of the species of interest. For this purpose, the program must define performance traits that will be the targets of selection. In cultured fish, the main traits that contribute to productivity are growth rate, disease resistance, age at sexual maturity, feed conversion efficiency, flesh quality, etc. The traits should be chosen for each particular species of interest in accordance with specific aspects of its reproductive biology, time intervals between generations, husbandry practices, the production/marketing system, and budget resources available for the breeding program. Most selective breeding programs for fish begin with growth because this trait is of primary economic importance. For example, since the market prices of sea bream and sea bass are based on unprocessed whole body weight, selection for body weight should reduce the time required to grow the fish to market size. Our selective breeding research focused on growth improvement in the sea bream and sea bass.

Choice, Evaluation, and Establishment of Base Population(s)

For practical applications, it is important to begin the selection with a stock that meets the industry's performance objectives as closely as possible. Assessment of different strains should precede a long-term within-strain genetic selection program, as choice of the best strain could be equal to the genetic gains made by years of selection of inferior strains (Gunnes and Gjedrem, 1978). It is also important that evaluating and maintaining different strains allows the breeders to adopt a crossbreeding program that can minimize future inbreeding problems (Hulata, 2001).

During 1991-1994, we carried out pioneering studies aimed at testing culture performance in different strains of sea bream. Original broodstocks at the NCM were formed in 1972-1974 using juveniles transported from the Bardawil lagoon on the Sinai Mediterranean coast. Assessment included the Eilat hatchery strain, first generation offspring of wild-caught fish (from the Mediterranean Sea near Haifa), and strains obtained from crossbreeding. Even though few strains were compared, significant growth differences were detected. Most evident was the inferior performance of progeny resulting from parental wild fish in comparison with the progeny of the Eilat hatchery strain (Knibb et al., 1997a, 1998). We suggested that genetic differences among strains could have resulted from previously existing genetic variability in ancestral wild strains taken from different geographical origins. On the other hand, these differences possibly arose from "domestication selection" for growth performance in culture conditions. The overall experimental results suggested that considerable genetic variability for growth existed in the Eilat hatchery strain.

In 1998-2001, similar strain-testing experiments were also conducted with sea bass. We tested progeny of sea bass from three different strains (one originated from France and two from Egypt) and their crosses through the whole grow-out cycle to market size. We found that strains and crosses varied significantly in traits of economic interest such as growth, survival, body composition, sex ratio, sexual maturation patterns of males, and frequency of body shape abnormalities. Significant sexual dimorphism for length and weight was evident in all strains and crosses: at market size, the resulting weight advantages of females varied between strains and crosses, but the overall average weight advantage for females was about 39%. In addition, the results indicated that the proportion of males maturing in the second year of life (precocious males) varied significantly in different strains and crosses. Precocious maturation in males leads to considerable reduction in market value of the product because of the unpleasant appearance of such fish to consumers (Zanuy et al., 2001). Therefore, the results may be of significant importance for commercial mariculture. Lengths, weights, and growth rates of pit-tagged individual fish in all groups were significantly correlated during the different time intervals, indicating that the response to selection for size and weight at market time may be achieved by conducting mass (individual) selection of young fish. Generally, the results showed a high culture potential for sea bass strains originating from southeastern areas of the Mediterranean Sea (Egypt), suggesting that domesticated progeny of these strains ("marine" and "lake" strains) and their reciprocal hybrids could be exploited in commercial Israeli mariculture and used in future selective breeding programs (Gorshkov et al., 2004a).

Genetic Improvement Strategies and Selection Methods

Generally, any genetic improvement program for cultured fish can use long-term and/or short-term genetic improvement strategies. Long-term genetic improvement strategies usually include traditional selective breeding based on methods and techniques of classical genetic selection. Long-term improvement strategies require long-term data collection, record keeping, broodstock management, monitoring commercially important traits, and permanent scientific supervision. Short-term genetic improvement strategies may not require the same level of record keeping as long-term ones but they can generate significant genetic gains in a short period (Knibb, 2000; Dunham et al., 2001).

In the selective breeding of fish, the following classical genetic selection methods can be considered for obtaining genetic improvement: family (or selection for relatives) selection, mass (or individual) selection, and combined selection (for review see Kirpichnikov, 1981; Tave, 1986). Although crossbreeding is not really a genetic selection technique, this effective breeding approach has been frequently integrated into comprehensive selective breeding programs in freshwater fish (Kirpichnikov, 1981; Hulata, 2001). In addition, crossbreeding should be considered the reverse of inbreeding (Knibb, 2000).

During 1992-1994, in experiments with sea bream, we attempted the main family selection techniques that were widely used for cultured fresh water and anadromous fish. We used strip spawning, single-pair mating, and progeny testing to produce families from known (pit-tagged) sea bream parents. We found that the rates of producing family groups by artificial fertilization of stripped eggs and sperm from parental fish or the use of natural spawning of single-pair matings in a tank were low. The main failures were attributed to low egg production (many females failed to produce eggs in any quantity), poor egg quality, and low fertilization or hatching rates. Possible reasons for the low fertility included difficulties in synchronizing stripping with: (a) the exact daily spawning time of specific females, undergoing asynchronous daily development of oocytes, and (b) stress caused by keeping fish in single pairs rather than in a group. These problems, together with the protandrous hermaphroditic nature of sea bream, represented a specific reproductive constraint considerably limiting the prospects for conducting family selection with this species.

In spite of the low numbers of family groups produced for analysis, we were able to document substantial genetic influences on growth in sea bream. At slaughter weight, 300-500 g sires (males) accounted for approximately 14% of the total observed variance in body weight for half-sib groups produced through a progeny testing design and 29% of the total weight variance for full-sib family groups produced in the single-pair matings. From these experiments, we drew the following conclusions regarding sea bream: (a) specific reproductive constraints limit the prospects for conducting family selection with this species, (b) there is a substantial genetic variability for growth, and (c) mass selection offers a more practical option for genetic improvement (Gorshkov et al., 1997).

In selection experiments conducted in 1993-1995, we used a single-generation procedure of mass selection to estimate realized heritability (h^2) for weight in the Eilat hatchery strain of sea bream. Selected male groups (down-, control-, up-, and extreme up-"jumpers") were crossed with randomly chosen unselected groups of females. Growth of their progeny was evaluated under communal and separate tank rearing conditions. Heritability estimates for body weight were performed; their values varied between replicates and rearing conditions, but were, in general, positive with a moderate magnitude (0.34-0.45). Thus, the body weight of the sea bream responded to selection and we concluded that selection of phenotypically larger fish might result in genetic gain for growth in sea bream (Knibb et al., 1997b).

In subsequent cross-selection experiments, offspring of up-selected parents showed phenotypic differences in comparison with offspring obtained from unselected parents: potentially desirable changes included improved food conversion efficiency (Knibb et al., 1998; our unpublished data). Generally, the combined data on genetic variability of growth and low success rates for producing family groups in sea bream indicated that the Eilat hatchery strain was a suitable source for commencement of a commercial selective breeding program based on mass selection.

Interspecific hybridization. During 1992-1996, we carried out a number of interspecific hybridization experiments with the gilthead sea bream and the red sea bream (*Pagrus major*, Sparidae). We also produced several hundred viable triploid hybrids between sea bream and red sea bream, which were used in growth experiments. Culture performance in the diploid hybrids was compared with that of the parental species under the same controlled conditions. The reciprocal hybrids did not exhibit any significant growth advantage over the parental species. All adult hybrid fish were clearly immature and had only vestigial gonads; neither ovaries nor released milt was observed. Histological examination of the gonads showed that all the hybrids were completely sterile. Despite their sterility, neither the growth of the diploid nor the growth of the triploid hybrids displayed any advantage and, for this reason, profitable culture of these hybrids appeared to be questionable (Gorshkov et al., 2002).

Hybridization between European sea bass (*D. labrax*) females and American striped bass (*Morone saxatilis*) males was also attempted. Taking into account the differences in reproductive biology of striped bass (a species that spawns in fresh water) and sea bass (a species that occasionally spawns in brackishwater lagoons), fertilization was carried out in brackish water (8 ppt) to activate but not kill the sperm of the striped bass. Hybridization resulted in viable larvae but, according to cytological analysis, 28% were triploids and only the triploids appeared to survive up to six months. Possibly, the salinity changes (required for fertilization) resulted in an osmotic shock and triploidization of some embryos. At an age of eight months, surviving triploid hybrids showed poor growth compared to diploid sea bass (Knibb et al., 1998).

Another study conducted by our group with sturgeon hybrids (*Huso huso* x *Acipenser guldenstaedtii*, Acipenseridae), imported to Israel from Russia in 1992, should be mentioned. In 1993, we collected a sample of hybrid fish (Kibbutz Dan fish farm) for karyological analysis. Our results confirmed the intermediate origin of this hybrid, suggesting some uncertainty as to whether the adult hybrid would be completely sterile (Gorshkova et al., 1996a). Results could be useful in solving ecological and conservation problems in Lake Kinneret (Sea of Galilee), since several hybrid fish have escaped from the farm.

Chromosome set manipulations and cytogenetic analyses. During 1992-1995, we carried out pioneering chromosome set manipulation experiments with the gilthead sea bream. As a result, the optimal parameters for production of gynogenetic and triploid progenies of sea bream were established. The optimal heat shock temperature to induce diploidization of the maternal genome was 36.5-37.3°C. Time after fertilization for shock treatment and the duration of shock were 3 and 2.5 min, respectively. Untreated sea bream semen was used for triploid production and UV-irradiated sea bream or red sea bream semen was used for making gynogenetics. Similarly, meiotic gynogenetic sea bass progeny were produced using heat shocks to eggs fertilized with UV-irradiated sperm from sea bass males (Gorshkova et al., 1995; Knibb et al., 1998). The karyotypes of chromosomally manipulated forms were carefully studied and "marker" chromosomes, providing direct karyological confirmation regarding the hybrid nature and triploid origin of the offspring, were described (Gorshkov et al., 1998).

In 1999, we undertook cytogenetic examination of early embryonic development in the white grouper (*E. aeneus*). This species has very high commercial value and constant market demand in the Mediterranean basin. However, there are serious constraints for commercial culture of this species: the inconsistent and extraordinary low quality of spawned eggs, low survival rates of embryos, and extremely high mortality of larvae. Thus, understanding the possible genetic reasons for such phenomena was a major objective of this study. Eggs were obtained from different parents participating in natural spawnings at the NCM. Cytological analyses carried out on embryos at the blastula stage indicated that the progeny from different spawnings had various types of chromosomal aberrations. Proportions of such genetically abnormal embryos carrying different types of chromosomal aberrations varied significantly among parental fish and ranged 35.5-79%. We suggested that chromosomal disorders might be one of the detrimental genetic factors affecting survival during early developmental stages. It seems that cytogenetic monitoring of the early embryonic stages would be of immediate interest for the genetic broodstock management of the white grouper (Gorshkova et al., 2002).

Sex manipulation. In Mediterranean commercial mariculture, the European sea bass (a gonochoristic species) appears to be a major candidate for sex manipulation due to pronounced sexual growth dimorphism. Sea bass females reach market weight much earlier than males; at slaughter weight, females may weigh 40% more than males (Gorshkov et al., 1999; Zanuy et al., 2002). Since the production of an all-female population has obvious economic advantages, the ability to control sex is a very important goal in the practical genetics of this species. However, in the early 1990s, little information regarding this problem was available in Israel and elsewhere in the Mediterranean.

In 1992, we started a pioneering investigation of sex control in sea bass with the goal of producing all-female cohorts directly and/or indirectly through hormonal treatment and gynogenesis. First, we carried out large-scale recording and monitoring of sex ratios and culture performance in different untreated cohorts and progeny of sea bass reared at the NCM. We found that the overall sex ratio in different groups of sea bass (produced during 1992-1996) favored males: 78.6% males vs. 21.4% females. However, the sex ratios varied widely (98.1% to 44.2% males) among different cohorts and progeny produced at different times, indicating a high plasticity of sex determination in sea bass. At the same time, there were no deviations in sex ratio within the cohorts of progeny during ontogenesis and this fact clearly demonstrates that sex in *D. labrax*, once determined, remaines irreversible.

Sexual dimorphism in length and weight was evident for all cohorts and age-classes studied: females were significantly longer and heavier than males. The first significant features of size-weight divergence between females and males were found even before sexual maturity (at the age of 10-12 months). Histological examinations of gonads during ontogenesis showed that, at six months, some of the males (17%) had oogonia cells (so called intratesticular oocytes) imbedded in testicular tissue. However, the proportion of such males declined to 0 at 10 months, and from this age the typical histological and phenotypic patterns of gonads for males and females were well established. Treatment with high dosages of estrogens conducted on post-larvae indicated that the labile period for gonad differentiation occurs sometime prior to

115 days post-hatching (d.p.h.). Thus, these findings demonstrated that under NCM rearing conditions, a time scale of about four months could be considered the age of morphological sex differentiation (Gorshkov et al., 1999).

Gynogenetic experiments and karyological studies were carried out to study the presence of sex chromosomes in sea bass. Both the control and gynogenetic fish significantly deviated from a 1:1 sex ratio. Males predominated in the control groups, whereas females predominated in the gynogenetic groups. We found pronounced variation in the shape of subtelocentric (ST) chromosomes in the karyotype of *D. labrax* (2n = 48; 46 acrocentric and 2 subtelocentric chromosomes). Interestingly, males usually had a heteromorphic pair of ST chromosomes, while females usually had a homologous pair of ST chromosomes. This variability was significant, suggesting sex-associated differences in the shape of ST chromosomes (Gorshkova et al., 1996b).

During 2001-2002, our experiments with sea bass were aimed at studying the parental effects on sex ratios in progeny. The study was based on analyzing progeny resulting from a diallel crossing experiment (2 x 2 type or a complete bi-factorial mating design) reflecting both the maternal and paternal genetic relations in the progeny. The proportion of females varied significantly among different families (from 20.7 to 68.2%), indicating significant maternal and paternal effects on the proportion of females in the progeny. The effect of parental interactions on sex ratio was also significant. Thus, the results demonstrated that sex determination in the European sea bass is also influenced by genetic factors (Gorshkov et al., 2003).

Monosex fish populations may be produced by direct hormonal treatment (Piferrer, 2001). However, some countries (e.g., the European Union, the United States) prohibit such treatment when the fish are destined for human consumption,. Despite this fact, many European scientists working on sex control in sea bass are interested in optimizing the administration of relatively low dosages of estrogens to achieve complete feminization in cultured stocks. During 1998-2002, we carried out several experiments with the aim of testing the effects of low estrogen dosages on production of monosex female populations in sea bass. Based on our results, a simple practical protocol for complete feminization in sea bass was developed by feeding fry (80-100 d.p.h.) to satiation twice daily for 50-60 days, with a dry diet containing natural estrogen, estradiol-17β (E2), at a dosage of 12.5 mg/kg food (Gorshkov et al., 2004b).

Since the mid-1990s, Mediterranean sea bass mariculture has been recognized as a rapidly-growing industry (Ferlin and LaCroix, 2000). Considering the importance of sex control in sea bass, research activities in this area were focused on the influence of environmental and genetic factors on sex differentiation and sex determination in this species. We actively participated in international cooperative studies (a joint EC PROBSS project) that could provide insight into the nature and mechanisms of these processes. The overall results illustrated that sex determination in the European sea bass is a very flexible process that can be influenced by both environmental and genetic factors. Regarding practical applications, it was concluded that, in addition to temperature manipulations proposed to increase the proportion of females in sea bass (Mylonas et al., 2003), a desirable female to male ratio for the mariculture industry would be attained by directional selection of parents that produce the highest proportion of progeny females (Gorshkov et al., 2003).

Mendelian mutations in sea bream. Since 1992, the gilthead sea bream selective breeding program at the NCM has conducted research on color inheritance. As a result, a variety of color mutations were found, isolated, and studied. Using classical crossing experiments it was determined that the genes controlling the yellow, white, and ebony color phenotypes were each due to a single Mendelian recessive locus with complete dominance of the wild-type color gene (normally pigmented silver fish). These mutations have different pleiotropic effects on morphology, physiology, growth, survival, and fertility. Specifically, the yellow (golden body color) mutation has a pleiotropic effect which causes complete gonadal sterility in about 20-25% of the individuals. A white mutation (absence of body pigment) influences body morphology, causes fusion of vertebrae in about 70% of the fish, and is associated with a relatively slow growth rate compared to fish of the wild-type coloration. Using the Eilat hatchery strain (Knibb et al. 1997ab), we isolated a new and unique recessive Mendelian mutation for sea bream. This mutation (named ebony), in the homozygous condition, causes a distinctive darkening of the posterior region of the body (Fig. 1). The ebony gene has pleiotropic effects and causes abnormal development of the vertebral column and other severe deformities of body morphology in adult fish. Other pleiotropic effects are expressed by slower growth and lower viability when compared to normally colored fish. In addition, ebony phenotypes tend to be more sensitive to handling stress and anesthetics than normally pigmented fish. Interestingly, about 10% of the ebony males are fertile year-round, suggesting that preservation of this mutation in wild populations represents a definite selective advantage.

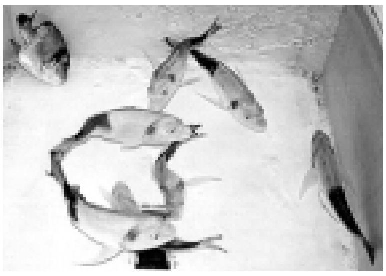

Fig. 1. The ebony (darkened posterior) sea bream represents a recessive homozygous Mendelian mutation with abnormal development of the vertebral column, retarded growth, and lower viability.

Mendelian mutations in marine cultured fish have great scientific importance and may be used as models for the study of genetic and physiological mechanisms of sterility, stress and disease resistance, heterosis, and genetic polymorphism in populations. They may also be used as useful genetic markers to identify a particular group in research with strain testing and chromosome set manipulations.

Production of transgenic fish. Genetic engineering attempts were made to introduce into the fish new genes or novel patterns of gene expression for commercial traits such as increased growth rate, improved food conversion efficiencies, or disease resistance. Since 1991, an initial step in our genetic engineering program was the isolation or cloning of suitable growth hormone genes, followed by their modification in a test tube so that these new recombinant genes, when reintroduced into the fish, would cause expression of the growth hormone at higher than normal levels. This work has been completed for a number of different growth hormone constructs. However, the genetic engineering technique was not successful in achieving any applied results with sea bream and all experiments were terminated in 1998. Currently, there is no commercial exploitation of genetically engineered marine fish in the world (Knibb, 2000) and growing public concern about the potential hazards of rearing transgenic fish (Dunham, 2004).

Practical Applications and Commercialization of the Selective Breeding Program

In the early 1990s, NCM had a large captive broodstock (total ~500 fish) of sea bream that resulted from several generations of domesticated selection of wild fish collected in the mid-1970s from the Bardawil lagoon on the Sinai Peninsula coast of the Mediterranean Sea. Experimental results suggested that large genetic variance existed in this domestic stock (Knibb et al., 1998). Consequently, it was used as a common founder population for the commercial selective breeding program. Since this broodstock had been divided into three independent reproductive groups (each consisting of ~150 brood-fish that were maintained and reproduced separately over many years), we could expect a definite genetic difference between the groups. Therefore, we conducted the first mass selection using progeny of each of the three broodstocks as if they were genetically different lines (strains). During 1991-1993, the first rounds of mass selection were carried out with progeny of these lines after growth in the NCM rearing facilities (Knibb et al., 1998).

The design and industrial development of a selective breeding program should involve farmers at the earliest possible stage. Since 1993, the local commercial fish farm Ardag Ltd. (Eilat) actively participated with the NCM in a long-term selective breeding program for sea bream by: (a) culturing large numbers of selected fish under commercial production environments to allow mass selection at commercial size and (b) maintaining the increasing number of genetically improved broodstocks resulting from the genetics program. This agreement freed resources and facilities of the Genetics Department and allowed us to concentrate our efforts on: (a) managing the overall program, (b) developing refinements to the selection procedures to increase the rate of response, (c) testing the rate of improvement due to the selection program, and (d) developing genetic methods for commercial protection of the improved strains. The general objective of the selection program was (and still is) to introduce innovative developments in selective breeding to the Israeli marine fish industry. During 1993-1994, three lines (strains) of sea bream were established and, by now, each line has undergone several cycles of mass selection in commercial production environments. In accordance with the reproductive biology of sea bream, each line undergoes mass selection in an industrial sea cage every three years, followed by industrial reproduction, nursery rearing, and grow-out in the industrial sea cages (Fig. 2). When necessary, a laboratory and/or commercial performance test is carried out.

Ardag is currently operating with five selected lines (strains) of sea bream that are being used both for continuation of the mass selection cycle and for grow-out. Crossbreds between different lines are being used for commercial production in Ardag's sea cages; in addition, sales of eggs and fingerlings

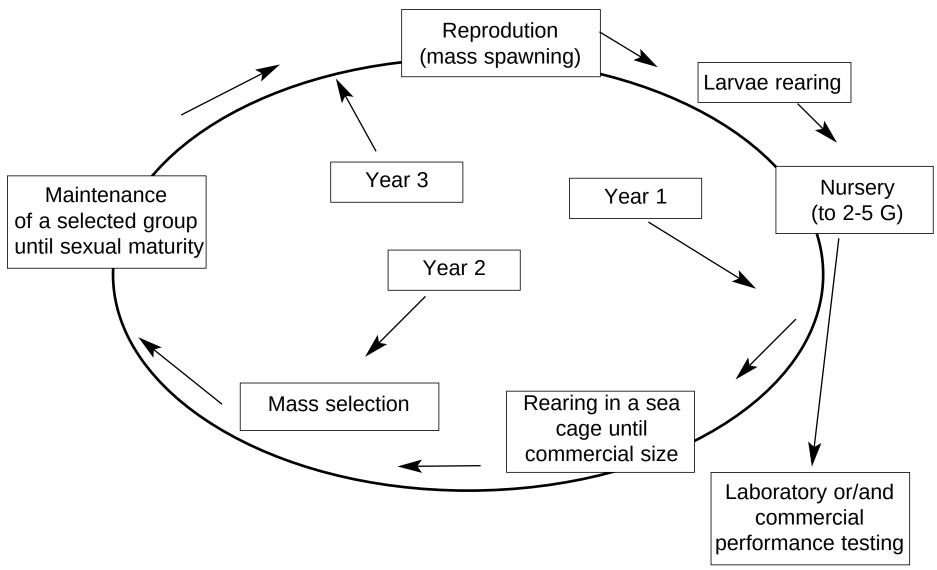

Fig. 2. The mass selection cycle at the ARDAG commercial fish farm in Eilat, Israel.

(resulting from crossbreeding) are intended for rearing by other fish farming companies.

Industry records are in agreement with our laboratory tests, indicating that the response to selection in terms of genetic gain of body weight is about 5-10% per generation (Knibb, 2000). We recently analyzed the culture performance of NCM-Ardag selected strains of sea bream and those originating from other Israeli commercial hatcheries. All strains were reared in the same industrial sea cages (raw data were kindly presented by Suf Fish Ltd., a private commercial fish farm in Eilat) and the same conventional farming techniques were used. The assessment of culture performance demonstrated that during 1998-2000, the NCM-Ardag selected strains significantly outperformed unselected sea bream strains purchased by Suf Fish from other fish farms (Fig. 3). Considering that the present production capacity of sea bream at Ardag is about 1500 tons per year, the estimated economic profit from our genetic improvement program is calculated to be about US$330-445 thousand per year. Faster-growing fish have a lower production cost than slower-growing fish and are therefore more profitable because the farmer has a faster turnover. The first two generations of sea bream mass selection have already reduced the time needed to reach a commercial size of 400 g in industrial sea cages by 100 days (Fig. 4).

To ensure long-term economic advantage from sales of genetically improved fish, the Israeli industry should keep control of the selected broodstocks. Therefore, without methods to protect improved strains from piracy (similar to protection of intellectual property), the research and development investment will be lost with the first sale of selected eggs or fingerlings. We have researched a number of genetic procedures for commercial protection. Options have been considered to create sea bream strains that would be suitable for grow-out but unsuitable for use as broodstock. One option involves production of sterile fish by hybridization and chromosome set manipulation (Gorshkov et al., 1998, 2002). The discovery of the ebony mutation in the Eilat hatchery strain of sea bream (Knibb et al., 1998) made it possible to develop and adopt, on an industrial level, a relatively simple but novel, original, and effective method of genetic protection of improved strains from

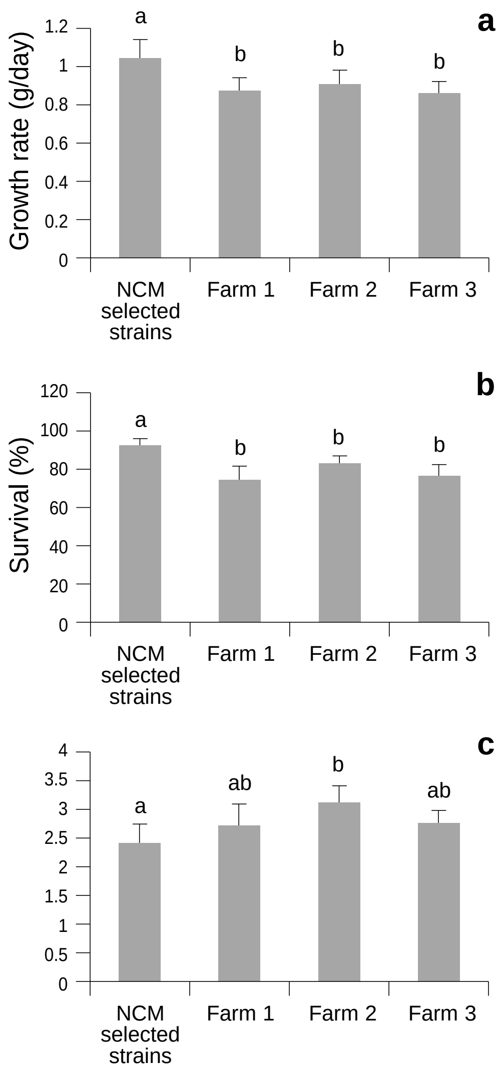

Fig. 3. Comparison of NCM-selected and unselected sea bream strains reared in industrial sea cages in the Gulf of Eilat (1997-2000): (a) growth rate (g/day), (b) survival (%), (c) food conversion ratio (FCR). Mean values are shown with SD. Values with different letters significantly differ (p<0.05).

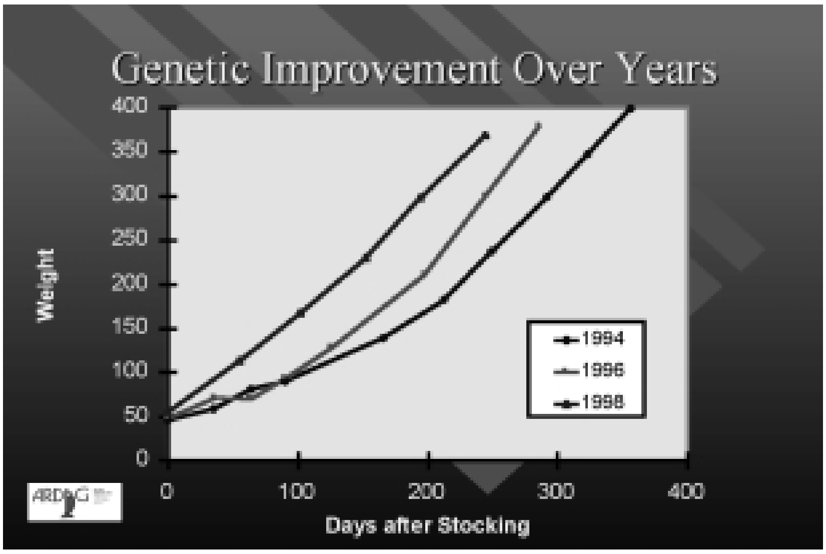

Fig. 4. Genetic improvement of growth in sea bream. (courtesy of G. Pagelson, ARDAG fish farm, Eilat, Israel).

unlicensed reproduction and industrial propagation. Since ebony is a pleiotropic mutation with severe deformation of the body shape, a higher mortality level, and lower growth, stocks with ebony fish are unsuitable for commercial production and sale. However, heterozygotes formed by crossing homozygotic ebony with any selected normal fish look normal phenotypically. That is, the fish appear normal and thus are suitable for food production but they are unsuitable for piratical use since heterozygous broodstock inevitably yield 25% homozygotic ebony that are unsuitable for commercial production and sale. Since 1998, this breeding protection technique has been periodically used by our local industry (Ardag) as an effective method of genetic protection of selected sea bream strains. As long as Israeli mariculture has no regulations regarding the authority on any selected marine fish product, our general policy remains genetic protection by the use of deleterious Mendelian mutations in sea bream.

Conclusion

The NCM selective sea bream breeding program demonstrated that genetic gain and economic return can be achieved within a reasonable time span (10-12 years). Presently, the local mariculture industry operates only with genetically improved sea bream strains. Faster-growing fish have lower production costs than slower-growing fish and, therefore, are more profitable because the farmer has a faster turnover of production cycles. Since the industrial marine fish farm, Ardag, is a major producer of sea bream in Israel, at least half of the Israeli production of sea bream is genetically improved. Implementation and commercialization of the selective breeding program can facilitate the establishment of an economically profitable and competitive mariculture industry in Israel. These all are reasons to believe that the gain from genetics programs in mariculture, expressed as economic return per year, compares favorably with other national agriculture developments.

Mariculture research in general and

genetic improvement research in particular are still hampered by short-term, scattered, and disjointed funding. It is necessary to realize that selective breeding programs for fish are expensive and labor intensive because they deal with generations, not growing seasons. The selective breeding program carried out at NCM is a notable example where the first substantial support for the research came from the government. However, the current funding situation is different despite the fact that production of sea bream and sea bass is becoming increasingly vital in the Mediterranean. Israeli sea bream and sea bass growers might consider allocating, say, 1-2% of their gross receipts from product sales to a direct fund dedicated to supporting research and development for long-term selective improvement programs. Because of governmental budgetary realities, it seems likely that the freshwater aquaculture industry might also have to support the development of sea bream and sea bass selective breeding research.

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