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PARENTAL EFFECTS ON SEX RATIOS IN PROGENY OF THE EUROPEAN SEA BASS *(DICENTRARCHUS LABRAX)*

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Abstract

In European sea bass *(Dicentrarchus labrax*), females grow 20-50% faster than males. Therefore, they are more in demand than males for commercial farming, generating much interest in the development of female monosex populations. Whereas most current research focuses on the influence of temperature on sex determination, the present experiments aimed at studying parental effects on sex ratios in progeny. The study analyzed progeny resulting from a diallel crossing (2 x 2 type or a complete bi-factorial mating design), reflecting both maternal and paternal genetic relatedness among progeny. The proportion of females varied significantly among families (20.7-68.2%). There were significant maternal and paternal effects on the proportion of females among the progeny. The effect of the parental interaction on the sex ratio in the progeny was also significant. Parents had a significant effect on total length and body weight. Sexual growth dimorphism, in favor of females, was evident in all the full-sib families and varied significantly between families. Among offspring at 9-9.5 months (68.9±23.7 g), females were 26.6% heavier than males. It is concluded that in addition to temperature manipulation in sea bass, as proposed in earlier studies, selection of parents will probably result in an improved ratio of female to male progeny.

Introduction

In European sea bass (*Dicentrarchus labrax*), females grow 20-50% faster than males. They are, therefore, in greater demand for commercial farming. This fact has generated much interest concerning the development of female monosex populations (for references see Zanuy et al., 2001). Most research activity is currently focused on the influence of tem-

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perature on sex determination in sea bass. The latest findings clearly reveal that the rearing temperature during the labile period of gonad development can be a significant factor influencing sex ratio in progeny (Blázquez et al., 1998; Pavlidis et al., 2000; Ben-Atia et al., 2002; Koumoundouros et al., 2002; Saillant et al., 2002). In many gonochoristic fish species, including sea bass, phenotypic sex is considered the outcome of environmental and genetic factors (Devlin and Nagahama, 2002). However, information regarding parental effects on sex ratio in progeny of sea bass is limited and only partially documented (Blázquez et al., 1999; Saillant et al., 2002).

Here we report the results of experiments aimed at studying the parental effects on sex ratio in progeny of sea bass.

Materials and Methods

The experiments were carried out during 2002 at the National Center for Mariculture (NCM), Eilat, Israel. The study analyzed the progeny of four full-sib families resulting from a diallel crossing experiment of the 2 x 2 type, reflecting both the maternal and paternal genetic relatedness among progeny (Kirpichnikov, 1981)

Broodstock. In 1995, approximately 60 wild-caught sea bass juveniles (30-50 g) were collected from the brackish Lake Edku located in the estuarine area of the Nile River (Egypt). The fish were transported to NCM and raised under controlled conditions (Gorshkov et al., 1999). In subsequent years, adult fish were used as broodstock for experiments and commercial production. For the present experiment, brood fish (5 females and 13 males with an average body weight [BW] of 2610±130 g and 1390±140 g, respectively) were held in a circular 5 m^3 water tanks, supplied with aeration. Ambient salinity of the Gulf of Eilat (40 ppt), water temperature of 14-15°C, and natural light conditions were maintained throughout the spawning season. Fish were individually tagged with implanted 11 x 2.1 mm passive integrated transponder (PIT) tags for identification. Females in the final stage of vitellogenesis (oocyte diameter 750-900 µm) were injected intramuscularly with 10 µg/kg BW of des-Gly^{10}[D-Ala^6]-LHRH ethylamide (Sigma; Kissil et al., 2000). Males were not treated because they almost invariably and regularly yielded milt. Two male/female pairs were simultaneously partitioned into 600-l tanks fitted with egg collectors. Two to three weeks after spawning, the same females were hormonally induced again and rotated between the same males. At the end of the experiment, four viable full-sib family groups were obtained, representing a complete bifactorial mating design (two dams x two sires).

We did not attempt to produce family groups through strip-spawning due to the usually high mortality observed in hatchery strains of sea bass at the NCM from wild-caught females resulting from stripping procedures and the need to repeatedly use them as broodstock for other experiments

Larval rearing. When available, 150 ml of developing eggs belonging to the same full-sib family groups were collected and stocked separately in duplicate at a density of 100 eggs/l into 1500 l larvae rearing tanks. Incubation of eggs and larvae rearing followed standard rearing protocols developed at NCM for sea bass (Kissil et al., 2000). Briefly, until hatching, the water temperature was maintained at 15°C. It was gradually increased to 17°C and maintained at this temperature until the end of larvae and post-larvae rearing. The tanks were supplied with continuously filtered seawater (40‰) at a flow rate resulting in two to four exchanges per day. The photoperiod regime was 14 light and 10 dark hours (Tandler, 1993). Freshly enriched live food (rotifers and *Artemia* nauplii) was supplied continuously during the first 50 days, afterwards a dry commercial diet (Koppens, Holland) was provided. Egg incubation, larvae and fry rearing lasted 105-115 days.

Rearing and sampling procedures. Weaning and growout were conducted in the nursery unit following routine sea bass husbandry procedures including growout of fish with well-developed swimbladders (Chatain and Corrao, 1992). Here, each full-sib family group was kept separately in duplicate 900 l conical circular tanks (~ 400 young fish/tank) for a period of four months after which they

were transferred to 5000 l circular duplicate tanks until the end of the experiment. During the growout period, fish were maintained at the ambient water temperature (22-26°C) and a natural photoperiod. Fish were fed a commercial feed (Koppens, Holland) according to their weight (Lupatsch et al., 2001). Fish were not size-graded at any time during the experiment.

Fish (99-100 fish per replicate) were sampled at the age of 9-9.5 months (270-285 days after hatching) and their total length (TL) and body weight (BW) were recorded to the nearest 0.5 cm and gram, respectively. Gonads of dissected fish were visually inspected, weighed to a precision of 0.001 g and sexed according to morphological structure and color as described by Gorshkov et al. (1999). The gonadosomatic index (GSI) was calculated as GSI = (Wg x 100)/BW, where Wg is the weight of the gonad. Sexual dimorphism for body weight was calculated using the formula: [(BW female - BW male)/BW male] x 100, where BW female and BW male are the respective mean body weights of females and males in each replicate tank of the four families.

Data analysis. The chi-squared (χ^2) test was applied to test sex ratios between replicates within full-sib families. As no significant ($p>0.05$) variation was found, the data from the replicates were pooled and the χ^2-test was used to examine sex ratios that deviated from 1:1 within each of the four full-sib families. To improve normality, the percentage of females (the unit of replication was a tank) was arc-sine square root transformed before being used in a one-way ANOVA. When F-statistics indicated significance ($p<0.05$), Duncan's multiple range test was used to compare mean percentages of females among families.

To account for variations in BW and TL between replicates, the tank was included as a nested factor in the ANOVA. As no significant variation in BW and TL between replicates was found, data from replicates were pooled for further statistical analysis. Data sets were checked for variance homogeneity and normality. A two-way ANOVA was used to assess the male (sire), female (dam) and sire x dam interaction effects (Sokal and Rohlf, 1981) on the proportion of females, the TL and the BW in full-sib progeny. As the data consisted of observations from many individuals, but few full-sib groups, we present variance components (Sokal and Rohlf, 1981) rather than heritability estimates in our tables. One-way ANOVA was used to determine differences in performance (TL, BW, GSI) between males and females within each full-sib family as well as differences in sexual dimorphism values among families. In all tests, a probability of $p<0.05$ was considered significant.

Results

Within each of the four full-sib families, the sex ratio deviated significantly ($p<0.05$) from a 1:1 proportion (Fig. 1). The average percent of females in all four full-sib families was 49.9% although the percent of females varied from 20.7% to 68.2% and differed significantly ($p = 0.0009$, df = 3, F = 60.3) among families. The ANOVA analysis showed that both parents significantly affected ($p<0.05$) the percentage of females among their progeny (Table 1). The dam and sire components of variance accounted for 27.4% and 30.8%, respectively, of the total phenotypic variance for the proportion of females. The dam x sire interaction was significant and accounted for 39.2% of the total variance.

The parents had a significant effect ($p<0.001$) on the total length (17.7-18.7 cm) and body weight (64.2-81.5 g) of the progeny, but accounted for a relatively small part of the total phenotypic variance (dam -0.3 and 3.6%, sire 3.1 and 1.2% for TL and BW, respectively, Tables 2 and 3). The dam x sire interaction was also significant ($p<0.01$), indicating the presence of non-additive genetic variance that depends on interactions between alleles.

Sex differences in total length, body weight and GSI were evident among all family groups. Overall, the offspring females were significantly ($p<0.0001$) longer and heavier than the males (Table 4) and had significantly ($p<0.0001$) higher GSI values. The sexual growth dimorphism, in favor of females, was

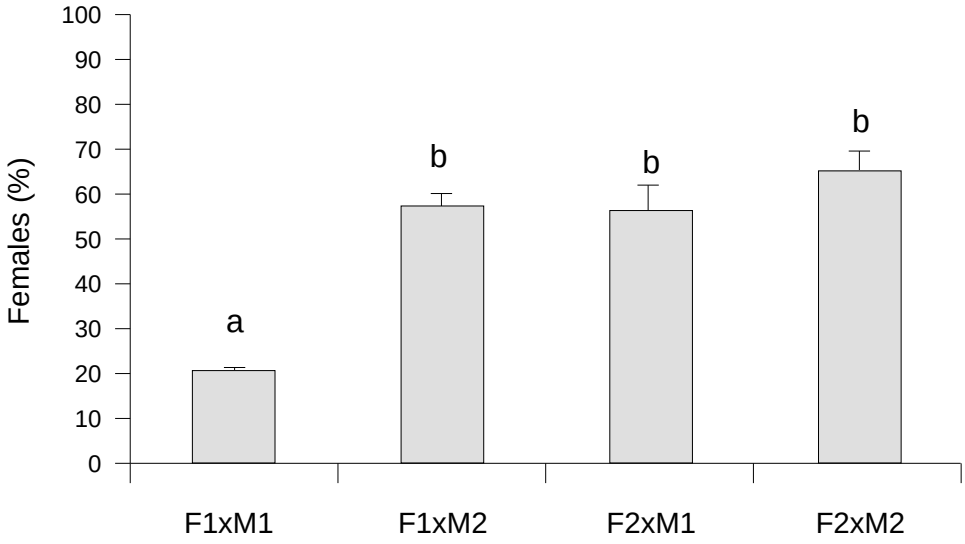

Fig. 1. Proportion of females (%) in four full-sib families of sea bass at age 9-9.5 months (378-406 fish per family were sexed). F1, F2, M1 and M2 are the two females and two males used in single-pair spawnings in the diallel crossing experiment of the 2 x 2 type. Mean values of two replicate tanks are shown; vertical lines are standard deviations. Families with a common letter do not differ significantly (p>0.05) using Duncan's multiple range test.

Table 1. Parental effects on the proportion of females among sea bass progeny derived from a diallel cross experiment of the 2 x 2 type.

Source of variation	*Degrees of freedom*	*Sum of squares (SS)*	*Mean squares (MS)*	*Variance components (% of total variance)*
Female (dam)	1	346.4	346.4 **	27.4
Male (sire)	1	370.5	370.5 **	30.8
Interaction (dam x sire)	1	147.2	147.2 *	39.2
Error	4	19.1	4.8	2.6
Total	7	883.2	126.2	

Note: Two females and two males were mated and cross-mated, producing four full-sib groups with paternal and maternal genetic relationships. ANOVA test was performed on arcsine square root transformed proportions of females in replicate tanks (182-214 fish per replicate were sexed) of the four families.
** p<0.001, * p<0.01

Table 2. Parental effects on the total length of sea bass progeny derived from a diallel cross experiment of the 2 x 2 type.

Source of variation	*Degrees of freedom*	*Sum of squares (SS)*	*Mean squares (MS)*	*Variance components (% of total variance)*
Female (dam)	1	35.5	35.5 **	-0.3
Male (sire)	1	78.6	78.6 **	3.1
Interaction (dam x sire)	1	38.9	38.9 *	2.8
Error	794	2372.9	3.0	94.3
Total	797	2536.2	3.2	

** $p<0.001$, * $p<0.01$

evident in all the full-sib families and varied (20.6-48.3%) significantly ($p = 0.049$, df = 3, F = 6.64) between them (Fig. 2). Among all offspring, the overall average weight advantage of the females was 26.6%.

Discussion

In the present experiment, the sex ratios of the progeny families significantly deviated from 1:1, indicating that Mendelian segregation of heteromorphic sex chromosomes is not responsible for sex determination in this species (Gorshkova et al., 1996). Further, this may implicate that polygenic or/and genotype-environmental interactions control the sex determination mechanism (Devlin and Nagahama, 2002). Despite the fact that female sea bass are more desirable for commercial rearing (due to their faster growth), the predominance of males in stocks is a common phenomenon on many fish farms in the Mediterranean area (Zanuy et al., 2001; our unpublished data). In the present study, the mean percent of females in all four families was about 50%. In earlier experiments with sea bass, we occasionally observed similar sex ratios in single-pair crossings (Gorshkov et al., 1999), though our data could not identify any particular genetic and/or environmental reason for the deviation in sex ratio.

The results of the present diallel crossing experiment demonstrate considerable parental effects on sex ratio in progeny of sea bass. Similar results, obtained from a more complex diallel crossing experiment, were recently reported by Saillant et al. (2002) for another strain of sea bass (the Western Mediterranean region). Additionally, these authors detected a significant dam-temperature and sire-temperature interaction on sex ratios in progeny. Thus, our findings as well as results obtained with temperature manipulations on sexual differentiation in sea bass (Blázquez et al., 1998; Pavlidis et al., 2000, Ben-Atia et al., 2002; Koumoundouros et al., 2002; Saillant et al., 2002) suggest that genotype-temperature interactions are the most likely factors responsible for sex determination in this species. However, this does not rule out the possibility that different strains of sea bass can display different responses (in terms of sex ratio) to the same temperature treatment.

In our experiment, sex had a significant effect on growth performance (total length

Table 3. Parental effects on the body weight of sea bass progeny derived from a diallel cross experiment of the 2 x 2 type.

Source of variation	*Degrees of freedom*	*Sum of squares (SS)*	*Mean squares (MS)*	*Variance components (% of total variance)*
Female (dam)	1	17693.2	17693.2 **	3.6
Male (sire)	1	12600.7	12600.7 **	1.2
Interaction (dam x sire)	1	10049.5	10049.5 **	4.5
Error	794	386952.4	487.3	90.8
Total	797	427381.9	536.27	

** $p<0.001$, * $p<0.01$

Table 4. Body weight, total length and gonadosomatic index (GSI) in female and male sea bass at age 9-9.5 months.

Sex	*No. of fish*	*Body weight (g)*	*Total length (cm)*	*GSI (%)*
Females	398	77.1±24.6[a]	18.6±1.7[a]	0.163±0.053[a]
Males	400	60.9±18.0[b]	17.3±1.6[b]	0.046±0.038[b]

Note: Data pooled from offspring of four full-sibs families. Values are means±standard deviation. Means in each column with different superscripts differ at the 0.0001 probability level (ANOVA).

and body weight in females were significantly higher than in males) even before sexual maturation. Similar results were reported in our previous study (Gorshkov et al., 1999). Recently, Saillant et al. (2001) reported similar results from an experiment performed on offspring born of a single mating pair. Interestingly, our results also show that the relative weight difference, representing sexual growth dimorphism within families, was parentally influenced, suggesting a more complex relationship between genotype, growth and phenotypic sex. It is likely that the distinguishing growth features among families also influence sexual growth dimorphism.

While relatively few families were tested in the present study, our analyses showed a substantial genetic contribution of parents to variation of sex ratio in their progeny. Variance components for the growth parameters in young sea bass progeny, reflecting the

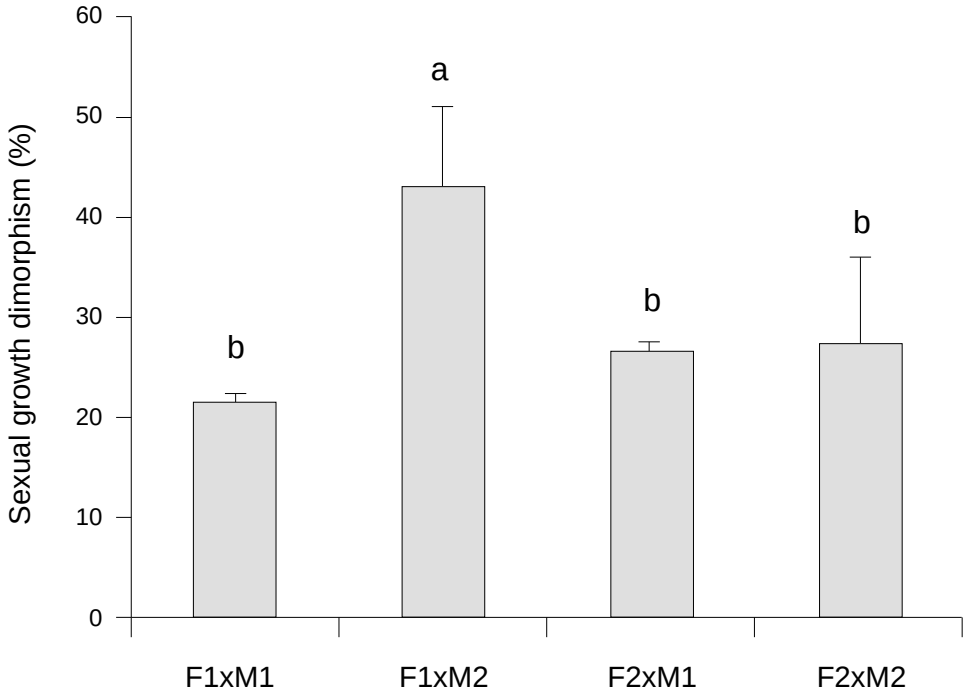

Fig. 2. Sexual growth dimorphism expressed as [(mean weight of females - mean weight of males)/(mean weight of males)] x 100 at age 9-9.5 months in four full-sib families of sea bass. F1, F2, M1 and M2 are the two females and two males used in single-pair spawnings of a diallel crossing experiment of the 2 x 2 type. Mean values of two replicate tanks are shown (99-100 fish per replicate). Vertical lines are standard deviations. Families with a common letter do not differ significantly (p>0.05) using Duncan's multiple range test.

genetic contribution of the parents, were significant but relatively low, similar to those we reported for sea bream (*Sparus aurata*) yearlings in progeny testing and single pair mating (Gorshkov et al., 1997). Relatively low parental contributions to growth variation of progeny were reported for juveniles of salmonid species (Linder et al., 1983; Bailey and Loudenslager, 1986) and sea bass (Garcia de Leon et al., 1998).

We suggest that, in addition to temperature manipulation, breeders whose progeny include a higher percentage of females should be selected to maximize the presence of females in cultured sea bass stocks. Use of DNA markers for parents and progeny produced by mass spawning may substantially facilitate this selection.

Future work aimed at the study of the basic mechanisms of sex determination in sea bass should focus on breeding experiments involving crosses of different factorial complexes to determine the distribution of genetic factors among parents. Chromosome set manipulations can enable examination of the sex determination mechanism by altering the balance of gene contribution that takes place in regular crosses.

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