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General and Comparative Endocrinology 142 (2005) 102–110
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Minireview

Genetic, endocrine, and environmental components of sex determination and differentiation in the European sea bass (*Dicentrarchus labrax* L.)

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Received 5 October 2004; revised 14 February 2005; accepted 14 February 2005
Available online 19 March 2005

Abstract

The European sea bass (*Dicentrarchus labrax* L.) is a differentiated gonochoristic marine teleost of the family Moronidae (closely related to the hermaphrodites of the family Serranidae), where many juvenile males exhibit intratesticular oocytes, suggesting a certain sexual lability. Like most fish, the sea bass does not have recognizable heterochromosomes or sex-linked markers but there are clear parental effects on the sex ratios. The data available so far indicate that the proportion of females resulting from individual crossings may range from as little as 1 to about 70%. Sex differentiation proceeds in a caudo-cranial fashion and starts when fish reach 8–9 cm standard length (usually about 200 days post-hatching, dph, under typical rearing conditions), with females differentiating first. Both forms of aromatase have been cloned in this species and their temporal expression has been studied. Brain aromatase is detectable already in the larval stages but its involvement in sex differentiation is not yet clear. The ovarian form increases after 100 dph before ovarian differentiation, with high levels in females and basal levels in males. Thus, ovarian aromatase seems to be involved in female differentiation. On the other hand, androgen receptor (AR) gene expression levels show the opposite pattern, with higher levels in males than in females. It is not yet known whether androgens are necessary for testicular differentiation or rather they are the result of it. Of the several environmental factors tested, temperature is the only one that has been shown to be able to clearly influence sex ratios. Larval and juvenile sea bass reared in captivity at high temperature usually develop as males. Recent research suggests that the high incidence of males under aquaculture conditions is due to the high water temperature used, and that the effects of temperature would be mediated by an inhibition of aromatase mRNA expression and activity in genotypic females. However, other effects of temperature mediated through alterations in developmental rates cannot be discarded. This paper reviews the current knowledge on sex determination and differentiation in the sea bass and suggests some directions for future research.

Keywords: Sex determination; Sex differentiation; Sea bass; Fish; *Dicentrarchus labrax*; Aquaculture; Aromatase; Temperature; Sex control

1. Sex determination

The sea bass (*Dicentrarchus labrax*) is a gonochoristic species without morphological secondary sex characters that, however, exhibits clear sex-related growth, with females growing faster and attaining larger size than males (Carrillo et al., 1995; Knibb et al., 1992; Saillant et al., 2001a). Larval and juvenile sea bass reared at 19–22 °C instead of the typical spawning temperature (~14 °C), as it is routinely practiced in the aquaculture of this species, develop as males, and the proportion of them can be as high as 100% (usually about 75%). Incidentally, males grow about 35% less than females. An update on sex determination and differentiation issues of relevance for the aquaculture of the sea bass has been published elsewhere (Piferrer et al., 2004).

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0016-6480/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2005.02.011

1.1. Cytogenetic evidence

The karyotype of the sea bass consists of 48 small chromosomes, most of which are acrocentric (Aref'yev, 1989). Nucleolar organizing regions (NORs) are located at the terminal or near-terminal sites on the short arms of the acrocentric chromosome pair 22 (Cano et al., 1996). Constitutive heterochromatin is also evident as a subcentromeric band on the long arms of a large chromosome pair (Sola et al., 1993). Based solely on morphology of karyotype preparations with conventionally stained slides, the sea bass has no readily distinguishable sex chromosomes, thus resembling the common situation in most teleosts (Devlin and Nagahama, 2002). However, chromosome variation was discovered in specimens from a wild population, involving the amount and the patterns of heterochromatin distribution in one of the two smallest chromosome pairs (pair 24) of the mitotic complement (Cano et al., 1996). In females, this pair comprised two chromosomes, which displayed roughly the same banding pattern. In males, in contrast, both homologs differed from one another as to the amount of heterochromatin and the pattern of distribution (C-bands). Thus, a large and intense C-band was evident in only one member of the pair. This band extended from the centromere to the middle of the arm. The other member of the pair had a weak C-band at the telomeric site. Thus, sex-related differences in C-banding pattern may be a reflection of minute differences in male vs female chromosomes that may be related to the existence of sex-determining regions, as found in *Oryzias latipes* (Kobayashi et al., 2004; Schartl, 2004).

The European sea bass used to be classified as a member of the family Serranidae, which is typically composed of hermaphrodites, with *Dicentrarchus* as a gonochoristic genus. Currently, it is classified as a member of the family Moronidae (Nelson, 1994). However, and regardless of classification issues, it is clear that *Dicentrarchus* is closely related to the Serranids. In a comparative study, the smallest chromosome pair in the closely related hermaphrodite *Serranus cabrilla* (Serranid) displayed a heteromorphism with respect to the size and the pattern of heterochromatin distribution. However, only males exhibited this heteromorphism, since the six fish in the study that displayed heteromorphism in pair 24 were acting as males and releasing sperm, and the three that did not were acting as females and releasing eggs (Cano et al., 1996). Therefore, based on this analogy, the variation in chromosome pair 24 found in *Dicentrarchus* was interpreted as a reflection of differences in gene activity in the absence of a strict sex chromosome differentiation, and it was concluded that the European sea bass was in the early stages of sex chromosome differentiation, with a probable sex determination mechanism of the XX/XY type, leading to gonochorism from the ancestral hermaphroditism of the Serranids (Cano et al., 1996). Such type of mechanism has not been experimentally demonstrated, and, in fact, based on other sources of evidence the WZ/ZZ system has also been proposed (Blázquez et al., 1999; Felip et al., 2002). In any case, the chromosomal basis for sex determination in the sea bass, if any, is far from clear and this is an area that certainly deserves more attention. Modern methods of chromosome analysis could be used to this end.

1.2. DNA and protein markers

No DNA markers are available to identify genetic sex in the sea bass so far. Probing its genome with non-coding sex-specific sequences, putative sex-determining sequences and sex-linked sequences operating in other systems (*ZFY*, *Sry*, etc.) does not allow the differentiation between males and females based on the resulting hybridization signals (Ollevier et al., 1998). Similarly, subtractive hybridization did not identify differences between males and females (Martínez et al., 1999). However, individual males can be distinguished from individual females based on sex-specific bands. Several polymorphic microsatellite markers are available for the sea bass (Castilho and McAndrew, 1998), and only two microsatellites were required for the unambiguous identification of parentage in a breeding program (García de León et al., 1998). Furthermore, a genetic analysis of quantitative characters relevant to production (survival, body weight and length, condition factor, and malformation incidence), performed on the resulting larvae of the above breeding program, identified significant differences among the tested families. Like the situation in other fish, the use of microsatellites will be a valuable tool in broodstock identification and family selection, but so far they have not been useful as an aid for discriminating sex.

Like other fish, sea bass mature females contain high levels of plasma vitellogenin (VTG) in their blood. Sea bass VTG has been purified, specific antibodies developed, and elevated plasma levels can be induced in males after injection with estradiol-17β (Mañanós et al., 1994). Vitellogenin content could be, in principle, used to determine sex in adult sea bass approaching the reproductive season. In this regard, plasma levels of sex steroids can also be used for the same purpose (Asturiano et al., 2000; Cerdá et al., 1995; Navas et al., 1998; Prat et al., 1990). However, other than that, so far no protein markers are available to identify sex in the sea bass at an early age. Given the absence of secondary sex characters, this impedes the determination of sex unless a biopsy of the gonads is performed, which for practical classification or selection is unrealistic.

Recently, clones representative of the HMG boxes of 12 *Sox* genes have been obtained in the sea bass (Galay-Burgos et al., 2004), which will allow to study their role in a variety of developmental processes including sex determination.

1.3. Sex ratios

The sex ratios of sea bass of wild origin have been studied in samples obtained from coastal lagoons. At ages 1–3 years, sex ratios were 34.5% males and 65.5% females (Barnabé, 1973) and 54.7% males and 45.3% females (Arias, 1980). At ages ranging between 4 and 7 years they were 19.1% males and 80.9% females (Barnabé, 1973) and 11.7% males and 88.3% females (Arias, 1980). The results suggest that in wild stocks, females predominate over males, especially at older ages. However, the sex ratio of the studied populations may actually be the breeding sex ratio and not necessarily correspond to the initial sex ratio of the whole population, which supposedly is representative of the sex determination system.

In contrast, the sex ratios of captive sea bass are repeatedly found to be highly skewed in favor of males, with an average 75% males and 25% females (Barbaro et al., 1998; Blázquez et al., 1995, 1998a, 1999, 2001; Bruslé and Roblin, 1984; Gorshkov et al., 1999; Saillant et al., 2001b, 2002, 2003b; see also Table 1). This suggests that some environmental conditions, i.e., those found in fish farms, could be modulating, and even overriding, the process of sex differentiation.

1.4. Parental influences

In the European sea bass, fertilization, survival during incubation, and hatching are highly correlated and strongly influenced by the female parent and, to a lesser extent, to the interaction between both parents (Saillant et al., 2001b, 2002). While the majority of parental effects on early performance in sea bass are due to the female, the effect of the male parent is only reflected on egg fertilization rate, egg survival, and on early development, i.e., on larvae growth during the endotrophic phase (Saillant et al., 2001b). Furthermore, the length of the larvae was found to be due to a significant interaction between both parents. Since gonadal development is highly dependent on size (Blázquez et al., 1999), these data suggest that gonadal development and thus the timing of sex differentiation may also be dependent on the progenitors or their interaction. Recently, direct evidence of parental influences on sex ratios in the sea bass has been provided by both dame and sire (Saillant et al., 2003a). Table 1 summarizes the range (min. – max.) and the average number of females obtained in experiments designed to test parental influences on sex ratios in the sea bass. The table average is 24.8% of females, i.e., the male:female sex ratio of 3:1 found under culture conditions. The sea bass, therefore, resembles the situation of some tilapias, where no sex chromosomes are identifiable but there are clear parental effects on sex ratios. Selection programs and the genetic management of the sea bass have been proposed (Volckaert et al., 1997). They could not only improve performance but perhaps could also avoid the high incidence of males or even favor female-biased sex ratios in cultured populations.

1.5. Crosses involving sex-reversed fish

The use of indirect methods has helped to elucidate the mechanisms of sex determination in several fish species (Solari, 1994). A group of sexually undifferentiated sea bass was treated with androgen resulting in a population of 100% males. Thirteen of these males were crossed with normal eggs generating 13 families that were reared separately until completion of sex differentiation. The results showed male-biased sex ratios in all but one family, with females ranging from 5 to 50%. Furthermore, only two families exhibited sex ratios that did not differ significantly from 1 male:1 female (Blázquez et al., 1999). It was concluded that the mechanism of sex determination in the sea bass was not of a XX /XY or ZW/ZZ type since no family exhibited a female-biased progeny, as would have been expected from both types. The study did not reveal the mechanism of sex differentiation in the sea bass since no sex proportions indicative of a particular genetic system were found. However, it raised the possibility that environmental factors could be modulating and even overriding the genetic basis of sex determination in this species (Blázquez et al., 1999).

1.6. Sex ratios of triploids and gynogenetics

Triploid sea bass, with one extra set of chromosomes of maternal origin, had 10% more females than control diploids but in both ploidies males predominated (Felip et al., 2001). Induced gynogenesis, i.e., the generation of

Table 1
Parental effects on the sex ratios in European sea bass

Temp. (°C)	Mean percent females	Min. percent females	Max. percent females	Reference
22.5	24.1	5.0	50.0	Blázquez et al. (1999)
18	21.4	0.9	50.0	Gorshkov et al. (1999)
17	49.9	20.7	68.2	Gorshkov et al. (2003)
17	16.7	2.1	40.7	Gorshkov et al. (2004a)
20	12.0	3.0	45.0	Saillant et al. (2003a)

Average number of females and the range (min. – max.) obtained in experiments designed to test parental influences on sex ratios in the sea bass. The water rearing temperature in each case is also indicated.

fish with exclusive maternal inheritance, showed little effect on gonadal development. Most meiogynogenetic sea bass, obtained by activating eggs with UV-irradiated sperm, followed by a cold shock of the activated eggs shortly after fertilization to block the extrusion of the second polar body and to restore diploidy, had normal gonads, indicating low occurrence of developmental imbalances (Felip et al., 2002). The sex ratios of gynogenetic sea bass did not deviate from 1:1 but were not statistically different from the controls, which in that particular study did not show the usual 3:1 male:female sex ratio. Even considering some environmental influence on sex differentiation (see below), the fact that the proportion of sexes was similar between gynogenetic and diploid controls is difficult to reconcile with the females being the homogametic sex and thus the ZW/ZZ type was suggested (Felip et al., 2002). However, in an independent study, only 39% of meiogynogenetic sea bass differentiated partly into males (Barbaro et al., 1998) and thus the proportion of females was higher than usual. As with the case of the sex ratios of sex-reversed individuals, the possible effect of the rearing conditions was not fully taken into account when evaluating the sex ratios of gynogenetic sea bass. Thus, it is possible that the sex ratios of gynogenetic sea bass in the studies of Barbaro et al. (1998) and Felip et al. (2002) reflect both the influences of gynogenesis and of the environment. Further, some parental variability has been detected regarding the degree of individual responses to ploidy manipulation in this species (Peruzzi and Chatain, 2000). This fact should be taken into account when drawing conclusions as to what sex determination mechanism may be operating, but in any case it would be worth to repeat the triploidy and gynogenesis experiments with fish reared at low temperature (see Section 4).

2. Sex differentiation

2.1. Development of germ cells

Under laboratory conditions, with rearing temperatures in the range of 17–22 °C, the sea bass has a histological sexual undifferentiated period that covers most of the first year of life, after which most individuals develop as males. At 18 °C, the first primordial germ cells (PGCs) can be detected before the end of the first month of life, and the undifferentiated gonad is formed by the third month (Roblin and Bruslé, 1983).

2.2. Gonadal development

Sex differentiation occurs along a caudo-cranial gradient, is of the direct type and more dependent on length than age (Blázquez et al., 1999). Ovarian and testicular differentiation occurred in fish 11–23 months old and from 80 to 187 mm SL. Intratesticular oocytes were frequently observed (Roblin and Bruslé, 1983). With higher temperatures, the first signs of morphological sex differentiation can be observed at about 4 months after hatching and sex, once determined, is irreversible (Gorshkov et al., 1999).

In a study on the chronology of sex differentiation in the sea bass and on the incidence of juvenile intersexuality, most fish were differentiated by 8 months and very few fish longer than 9 cm in standard length (SL) remained undifferentiated. Ovarian differentiation started at 168 days post-fertilization (dpf) (8.3 cm SL) and was completed by 419 dpf (12.3–18.5 cm SL). Male differentiation was more variable, with some males differentiating precociously at 168 dpf (8.3–9.5 cm SL) and others remaining undifferentiated until 250 dpf (11.8 cm SL). The presence of intratesticular oocytes is common in many males but their number does not increase but actually decreases after testicular differentiation is complete (Saillant et al., 2003b).

Cytochrome P450 aromatase is a key enzyme in the hormonal steroidogenic pathway that mediates the conversion of androgens into estrogens, determining the local balance between the two types of steroids and thus it has been implicated in the process of sex differentiation in fish (González and Piferrer, 2000). The gonadal form of sea bass aromatase has been cloned (Dalla Valle et al., 2002). The highest mRNA expression levels were found in the ovary, but it was also expressed, although at much lower levels, in testis and brain. Evidence of the existence of a second form (neural) of aromatase was obtained based on differences in kinetic properties of brain vs gonadal aromatase activity assays (González and Piferrer, 2002). Recently, the sea bass neural form of aromatase has been cloned and sequenced (Blázquez and Piferrer, 2004). Recent observations indicate that the neural form appears well before the ovarian form, suggesting a possible role of brain aromatase in sex differentiation. In addition, clear sex-related differences (females $\ggg$ males) in ovarian aromatase are also evident, starting at 100 dph, i.e., before sex differentiation is histologically observable, strongly suggesting an important function of ovarian aromatase in sex differentiation. The mRNA expression levels of the gonadal and the neural form of aromatase in this species are being currently studied during the first year of life (González, Blázquez, Mylonas, and Piferrer, unpublished observations).

The role of sex steroids and gonadotrophins has also been investigated as regards to sex differentiation. Plasma testosterone (T) levels were high in fish approaching the end of their first year of life but decreased over the next months, while levels of 11-ketotestosterone (11-KT) remained low and unchanged. During the first spawning season, at about 2 years, both steroids reached the highest annual levels (Rodríguez et al., 2000). Since the sex steroid binding globulin

(SHBG) regulates the bioavailability of androgens and estrogens during key reproductive events, the recent cloning of the sea bass SHBG (Miguel-Queralt et al., 2005) opens new possibilities for the study of sex steroid effects during sex differentiation and the reproductive cycle.

The effects of exogenous steroids on the process of sex differentiation in the sea bass have been reviewed elsewhere (Zanuy et al., 2001). Regardless of their potential applications to control sex ratios and precocious maturity for sea bass aquaculture, these studies have been useful to uncover the period of development during which the gonads are most sensitive to the action of steroids. In the sea bass, this is located around 110 (days post-hatch) dph (Table 2) and, like the situation with many fish (Piferrer, 2001), is thought to reflect critical periods for organizational effects of sex steroids. Here it is worth to recall that while the effects of temperature are more evident during the first 100 days, the effects of exogenous steroids are maximal around 100–120 dph, while a significant increase of ovarian aromatase gene expression and enzyme activity take place only after 100 dph (Fig. 1).

Regarding the relationship between size and sex, females predominate among the larger fish whereas males and undifferentiated fish predominate among the smaller ones already at the time of sex differentiation. Intersexes exhibit an intermediate size (Blázquez et al., 1998b). These results suggest that sex differentiation is more dependent on length than on age. Furthermore, females and feminized fish had the same growth, suggesting that growth is related to phenotypic sex (Saillant et al., 2001a). Precocious males, which abound in captive stocks and can be detected at 10 months of age, are smaller than the largest females but larger than some females, and also larger than non-precocious males and undifferentiated fish (Navarro, Blázquez, and Piferrer, unpublished observations). In contrast, in an independent study, Gorshkov et al. (1999) found that the effects of maturation on growth were not apparent or minor

Table 2
Effective treatment periods for the experimental manipulation of sex ratios in the European sea bass

Compound	Period (days)	Sex ratio male:female	Reference
MT	86–106	1:0	Blázquez et al. (2001)
MDHT	84–114	1:0	Chatain et al. (1999)
MT	65–140	1:0	Gorshkov et al. (1999)
E_2	65–140	0:1	Gorshkov et al. (1999)
E_2	88–148	0:1	Gorshkov et al. (2004b)
E_2	90–150	0:1	Saillant et al. (2001a)
AI	131–191	1:0	Navarro et al. (unpublished data)

Abbreviations: MT, 17α-methyltestosterone; MDHT, 17α-methyldihydrotestosterone; E_2, estradiol-17β; AI, Fadrozole (aromatase inhibitor). More than one successful treatment may have been found in a given referenced study.

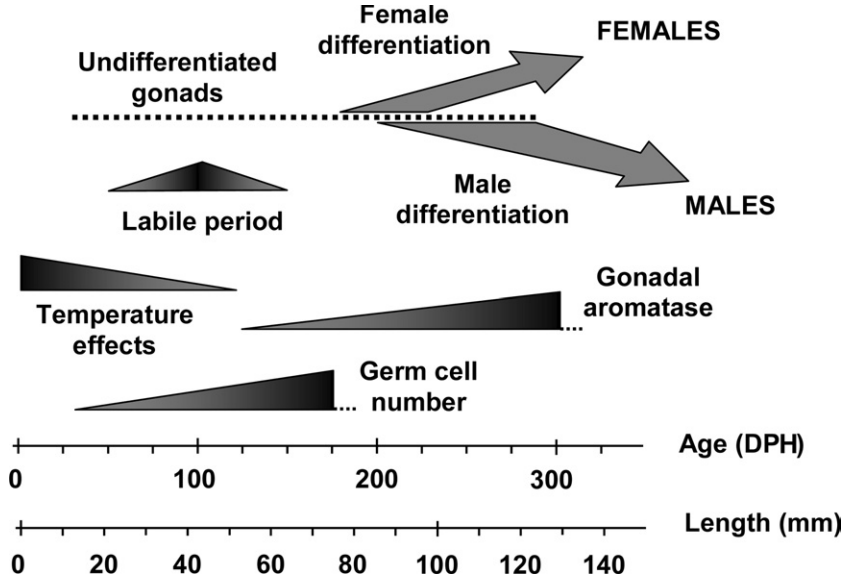

Fig. 1. Diagram showing the relationship between age, size, and gonadal sex differentiation in the sea bass, with indication of the relative importance of temperature on modifying this process, the localization of the labile period determined by the effects of exogenous steroids, and the increase in germ cell number and gonadal aromatase. Note that while sex differentiation is more dependent on size than age, the age–size relationship is also dependent on rearing temperature. Data obtained and averaged from different studies cited in the text as well as from our own unpublished material. DPH, days post-hatch.

under the culture conditions employed, since no significant weight differences were found between mature precocious males and immature fish. At age 6 months, some males had a few oogonia embedded among spermatogonia (intratesticular oocytes). The proportion of such males declined to 0% at age 10 months (Gorshkov et al., 1999).

Sexual dimorphism in length and weight can also be observed before sexual maturity, starting at age 10–12 months (Blázquez et al., 1999; Gorshkov et al., 1999; Saillant et al., 2003a). The evolution of sexual dimorphism as a function of the age and sexual maturity indicates that the growth advantage of females vs males ranges from 67% at 10 months of age (mean weight 27 g) to around 25% at 2 years and thereafter until to almost 4 years (1.2 kg) (Saillant et al., 2001a).

3. Neuroendocrine control

The presence of three forms of GnRH has been confirmed in the sea bass (González-Martínez et al., 2001; Zmora et al., 2002) and their distribution along the different brain areas has also been reported (González-Martínez et al., 2002a). Recently, a GnRH receptor has been cloned and its expression studied (González-Martínez et al., 2004). In addition, the ontogenic expression of the three GnRH systems in this species has recently been analyzed (González-Martínez et al., 2002b). In this regard, chicken GnRH-II (cGnRH-II) mRNA-expressing cells could be detected as early as 4 dph, reaching its final adult position by day 30. Salmon GnRH (sGnRH) cells became evident on day 7 while seabream GnRH (sbGnRH) mRNA-expressing cells were first detected at 30 days (González-Martínez et al., 2002b). These results indicate that the brain machinery responsible for the control of sex differentiation and reproduction is formed well before gonadal development. A study aimed at the measurement of the three GnRHs in the pituitary of sea bass males during sex differentiation and the first spawning (Rodríguez et al., 2000) showed that sbGnRH levels were consistently higher than cGnRH-II and sGnRH, with sGnRH always exhibiting the lowest levels. Interestingly, all GnRHs peaked in November, when fish were 9 months old, coinciding with the time sex differentiation can unambiguously be distinguished by conventional histological techniques. Thereafter, the levels of the three forms decreased and remained low during the first spawning season, with the exception of sbGnRH, which showed a significant increase the following November. Plasma LH levels were lowest in November, later increasing during the next months. These results suggest a possible role for all three GnRH forms in achieving gonadal differentiation, while sbGnRH may be the most relevant form in the regulation of the first spawning season in male sea bass (Rodríguez et al., 2000).

A clear relationship between aromatase activity in the brain and the reproductive season has been reported in adult sea bass (González and Piferrer, 2003). Males exhibited higher activities than females and the areas of the brain acknowledged regulating reproduction, showed the highest levels. This strongly suggests that the neural form of aromatase could be implicated in the regulation of reproduction, by changes in at least its activity, resulting in temporal and spatial modifications in neuroestrogen formation. In addition, a role for brain aromatase in neurogenesis has also been suggested (Blázquez and Piferrer, 2004).

4. Environmental effects on sex determination and differentiation

Phenotypic sex in several vertebrates including amphibians, reptiles, and some fish is a combination of genetic and environmental or social factors (Devlin and Nagahama, 2002). Like other fish, the sea bass exhibits a certain degree of ontogenetic plasticity which is amenable to temperature modifications (Koumoundouros et al., 2001). Thus, among the environmental factors, temperature is by far the most investigated one. It is known that temperature is able to modify the expression and activity of the enzyme aromatase, which, as stated above, is responsible for the balance between androgens and estrogens. These steroids are responsible for testicular and ovarian development, respectively, in lower vertebrates, including fish (Nakamura et al., 1998; Pieau et al., 1994; Wibbels et al., 1994).

Studies carried out with sexually undifferentiated sea bass subject to different rearing temperatures (Table 3) have shown that constant temperatures cannot induce ovarian differentiation (Blázquez et al., 1998b; Koumoundouros et al., 2002; Pavlidis et al., 2000; Saillant et al., 2002). Low temperature (13 °C) from day 0 until mid-

Table 3
Relationship between temperature, period of the thermal manipulation, and resulting sex ratios in some experiments with the European sea bass

Temp. (°C)	Period (dpf)[a]	Mean percent females	Reference
22	15–57	5.8	Blázquez et al. (1998b)
13	1–93	73.0	Pavlidis et al. (2000)
15	1–74	69.0	Pavlidis et al. (2000)
20	1–65	26.3	Pavlidis et al. (2000)
15	1–64	66.1	Koumoundouros et al. (2002)
15	1–38	47.1	Koumoundouros et al. (2002)
15	1–11	37.6	Koumoundouros et al. (2002)
20	1–56	18.1	Koumoundouros et al. (2002)
13	0–346	11.0	Saillant et al. (2002)
20	19–149	32.5	Saillant et al. (2002)
15	0–120	35.0	Navarro et al. (unpublished data)
21	15–120	12.0	Navarro et al. (unpublished data)

[a] dpf, Days post-fertilization.

metamorphosis (17–18 mm TL) resulted in 72–74% females (Pavlidis et al., 2000), the highest recorded proportion of females so far. In all groups, female fish were larger than males. These results showed that temperature during the very early developmental stages is capable of affecting the process of sex differentiation in this species. Further studies have provided clear evidence that the sea bass is sensitive to the effects of temperature during all different ontogenetic stages up to metamorphosis, and that sex ratio is correlated with the growth rate of the fish well before the differentiation and maturation of the gonads (Koumoundouros et al., 2002).

The combined effect of temperature and parental influence (Ben-Atia et al., 2002; Saillant et al., 2002) or strain influence (Mylonas et al., 2003) on sex ratios has been recently investigated. The parental effects on sex ratios may include differences in sensitivity to the effects of temperature. High temperatures resulted in an excess of males if given until fish reached 8.1 cm SL. However, male differentiation was even higher if fish were maintained at low temperature (13 °C), from fertilization to a mean length of 6.5 cm SL (346 dpf). In groups reared at high temperature, both parents had a significant additive effect on the percentage of females, and the interaction between the two parents was not significant (Saillant et al., 2002). Thus, the existence of genotype–temperature interactions in sea bass sex determination may reflect parental effects due to differential sensitivities to the environment, the same as in *Oreochromis niloticus* (Baroiller et al., 1995) and *Menidia menidia* (Conover and Heins, 1987). Genotype–temperature interactions suggest the interesting possibility of selecting genotypes less sensitive to the masculinizing effects of high temperatures in breeding programs (Saillant et al., 2002).

Environmental factors other than temperature have been less investigated in the sea bass. Constant salinities had no influences on sex ratio, although sudden changes on salinity are capable of inducing stress that may affect the process of sexual differentiation (Saillant et al., 2003c). On the other hand, it was shown that photoperiod did not affect the sex ratio (Blázquez et al., 1998b) and no effect of density could be detected either (Saillant et al., 2003a). However, the pH is known to influence sex differentiation of some fish species (Roemer and Beisenherz, 1996; Rubin, 1985), but so far effects of pH on sea bass sex ratios have not been found (Saillant et al., 2003a).

5. Conclusions and future prospects

In conclusion, the sea bass may have sex chromosomes as such although in a very primitive state of differentiation. However, despite several experimental approaches, including subtractive hybridization of male- and female-derived DNA, the study of sex ratios after the induction of triploidy and gynogenesis, and the crosses of sex-reversed fish with normal fish, the sex determination system of this species is still not known. Minute differences between the male and female genome are predicted which hinders the success of approaches such as subtractive hybridization. In addition, the lack of appropriate sex-linked markers makes it very difficult the isolation of candidate genes implicated in sex determination. In contrast, the process of sex differentiation is fairly well known at least from a descriptive point of view of its chronology, but molecular data providing a clear picture of the factors (receptors, steroids, etc.) and mechanisms implicated in this process are not yet available. When available, it should provide an improved picture of the mechanisms involved. It seems clear that temperature affects the sex ratios of the sea bass and is able to override part of the genetic component in sex determination but it remains unclear as to whether these effects have actual adaptive significance. Also, the relationship between sex differentiation and growth is not clear specially regarding the possible contribution of female sex hormones to this growth advantage, and thus deserves further research. Finally, the incidence of intratesticular oocytes could provide some insights in regard to sex determination.

Acknowledgments

M.B. was supported by a "Ramón y Cajal" contract from the Spanish Ministry of Education and Science (MEC). L.N. and A.G. were supported by a MEC predoctoral scholarship. Research supported by MEC Grants MAR96-1860 and AGL2002-02636 ("SEXRATIO"), and EU Grant Q5RS-2000-31365 ("PROBASS") to F.P. We thank Sílvia Joly and Elvira Martínez for technical assistance. Appreciation is extended to all other PROBASS participants for stimulating discussions, and to an anonymous reviewer for his/her encouraging words towards our research.

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