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The relationship between growth and sex differentiation in the European sea bass (*Dicentrarchus labrax*)

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ABSTRACT

The phenotype results from the interaction between the genotype and the environment but how a given environment drives the expression of a particular sexual phenotype is poorly understood. The European sea bass has a polygenic sex determining mechanism influenced by temperature and, as in any poikilotherm, temperature also affects growth rates. However, the exact relationship between growth and sex differentiation remains elusive. To analyze this relationship, two experiments involving the manipulation of fish growth with or without a previous size-grading were carried out. In the first experiment, sex ratios of non-size-graded fish were unaltered after disturbing growth rates at three different times during the sex differentiation period corresponding to fish of 8–12 cm standard length (SL). The groups with a reduction of their food intake (hence showing slow growth rates) exhibited catch-up growth after returning to normal feeding levels and also exhibited a degree of gonadal differentiation and maturation similar to that of the controls. In the second experiment, fish of ~4 cm SL, i.e., before the appearance of the first known differences in aromatase expression (5–6 cm SL), were size-graded and then subdivided into groups which were differentially fed to maximize differences in their resulting growth rates. Highly significant differences in sex ratios were found, with more females in the groups derived from the group with the largest fish at the time of grading irrespective of subsequent growth rates. Together, these results show that in the European sea bass sex-related differences in growth are established before the appearance of the currently known first molecular markers of sex and that growth rates during the sex differentiation period do not affect sex ratios. Based on these results, we propose the 3–4 cm as the size range where possible earlier differences between sexes should be looked for at the molecular level in the European sea bass. Moreover, from an applied point of view, we suggest that improvement of aquaculture protocols for this species might be achieved by combining size-grading to discard slow growing fish, and hence males, together with the control of the catch-up growth response in an attempt to reduce the amount of required food.

1. Introduction

Fish exhibit high phenotypic plasticity in response to environmental changes and this plasticity can affect gonadal development, since sex in fish can be the result of a combination of genetic, environmental and social factors (Devlin and Nagahama, 2002; Penman and Piferrer, 2008; Piferrer and Guiguen, 2008).

Sexual growth dimorphism is present in several aquacultured fish. In some popular species such as the rainbow trout, *Oncorhynchus mykiss* (Bonnet et al., 1999; Elvinson and Johansson, 1993), the Atlantic salmon, *Salmo salar* (Naevdal et al., 1983), the chinook salmon, *Oncorhynchus tshawytscha* (Silverstein et al., 1998), the black sea bass, *Centropristis striata* (Colburn et al., 2009), the Nile tilapia, *Oreochromis niloticus* (Lowe-McConnell, 1987; Toguyeni et al., 1997) or the silurids (Haffray et al., 1998), males can be larger than females. However, in fish, females are generally larger than males. In farmed species, this is the case of the European eel, *Anguilla anguilla* (Colombo et al., 1984; Panfili et al., 1994; Roncarati et al., 1997), the turbot, *Scophthalmus maximus* (Imsland et al., 1997), the Senegalese sole, *Solea senegalensis* (Sánchez et al., 2010), the southern flounder, *Paralichthys lethostigma* (Luckenbach et al., 2003) or the Eurasian perch, *Perca fluviatilis* L. (Fontaine et al., 1997) just to name a few. Females are usually larger due to two main reasons: fecundity in females directly increases with size (Wootton, 1998) and male-male competition is not strong in most species (Parker, 1992). The existence of sexual growth dimorphism in farmed fish can have undesirable consequences if the sex with the highest growth does not predominate in the cultured stocks (Piferrer, 2001).

In fish, the onset of sex differentiation is usually more related to size (length) than to age (Blázquez et al., 1999; Colombo et al., 1984; Grandi and Colombo, 1997; Malison et al., 1986; Paull et al., 2008). Furthermore, in many fish species there is an association between early growth rates and sex differentiation (Blázquez et al.,

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1999; Koumoundouros et al., 2002; Papadaki et al., 2005; Saillant et al., 2003a) usually with an abundance of females among the largest growing fish already at the time of sex differentiation (Lawrence et al., 2008; Paull et al., 2009; Roncarati et al., 1997). In mammals, on the other hand, there are sex-specific differences in both the size of the undifferentiated gonad (Baker et al., 1990) and the growth rate of the developing fetus (Mittwoch, 1989; Mittwoch et al., 1969). In gonochoristic vertebrates with genetic sex determination, phenotypic sex determines growth. A study of the protandrous hermaphrodite sea bream showed the presence of two Quantitative Traits Loci (QTLs) linking body weight and sex, suggesting also the existence of this growth–sex relationship in hermaphrodites (Loukovitis et al., 2011). A study carried out in 77 species of sex-changing animals (including fish, ectoderms, crustaceans and mollusks), found that sex change takes place when individuals attain on average 72% of their maximum size, being invariant across all animal species (Allsop and West, 2003).

Genetic elements aside, one of the most important factors determining growth rates in fish is food intake. In nature, after periods of food restriction, fish can experience an accelerated growth, converging in size with those fish that did not suffer from that restriction (Ali et al., 2003). Jobling (2010) proposed three terms: 1) compensatory growth: animals restore body composition and energy reserves by increasing growth rates, although their final size, when compared to individuals of the same age submitted to normal conditions, may be lower; 2) catch-up growth: animals exhibit full convergence in growth, without necessarily a recovery of body composition; 3) recovery growth: the combination of compensatory and catch-up growth.

The European sea bass (*Dicentrarchus labrax*) is a gonochoristic species that exhibits polygenic sex determination (Vandeputte et al., 2007) with environmental influences (Piferrer et al., 2005). Under natural conditions, low temperatures allow female sex differentiation. However, artificially elevated temperatures during early development, common under aquaculture conditions, increase the proportion of males, rendering cultured stocks with 75–100% males (Koumoundouros et al., 2002; Navarro-Martín et al., 2009b; Piferrer et al., 2005). Also, in the European sea bass it has been observed a positive relationship between weight and sex: not only females are heavier than males, but also fish — males or females — from batches with more females have higher mean weight than fish from batches with a lower number of females (Vandeputte et al., 2007). Sex-related growth starts to be visible before sex differentiation is complete (Blázquez et al., 1998, 1999; Carrillo et al., 1995; Saillant et al., 2001) and is present at the time of marketing when animals reach 300–400 g (Navarro-Martín et al., 2009b; Saillant et al., 2001). One plausible explanation for male-biased populations in farms (Chatain et al., 1999) in comparison to female predominant populations in the wild (Arias, 1980; Barnabé and Billard, 1984) is that aquaculture protocols modify the process of normal sex differentiation (Roblin and Buslé, 1983), changing the moment and the size/age relationship (Saillant et al., 2003a).

In the European sea bass the onset of sex differentiation is related to length rather than to age (Blázquez et al., 1999) and there is a correlation between early growth rates and sex differentiation (Blázquez et al., 1999; Papadaki et al., 2005; Saillant et al, 2003a) even before differentiation and maturation of the gonads (Koumoundouros et al., 2002). Early size-grading (84 dph; 3.6–4.5 cm) a sea bass population allowed isolating most of the females without further effect after additional gradings (Saillant et al., 2003b). Size-grading the population before and during sex differentiation, also produced female-dominant (faster growth) and male-dominant (slower growth) populations (Papadaki et al., 2005) increasing upon 91% the female percent at the large group just with two early size-gradings between 66 and 143, suggesting that sexual growth dimorphism may be already present before the first histological signs of sex differentiation at 150 dph. Thus, growth could influence sex differentiation and Kraak and de Looze (1993) hypothesized how this could happen. They postulated that an animal develops into a specific gender depending on whether its size has exceeded developmental and temporal thresholds or not, meaning that growth rate is exercising an influence on sex differentiation. However, other studies in the European sea bass suggest that growth depends on phenotypic sex since under similar culture conditions normal females and fish feminized by estradiol-17β (E_2) treatment presented similar growth (Saillant et al., 2001). Also, masculinized fish by Fadrozole (Fz, aromatase inhibitor) grew like normal males (Navarro-Martín et al., 2009a).

Thus, the relationship between growth and sex differentiation is still not clear. The aim of this study was to further investigate this relationship in fish using the European sea bass model. The so-called switch-once or switch-twice experimental designs (Valenzuela and Lance, 2004) were applied here to growth rates with or without previous size grading. Growth rates were manipulated during sex differentiation and thereafter the effects on sex ratios and catch-up growth were studied.

2. Materials and methods

2.1. Animals and rearing conditions

Two batches of European sea bass eggs obtained from a commercial hatchery (St. Pere Pescador, Girona, Spain) were collected at one day post fertilization (dpf) on April 2008, for experiment 1, and on March 2009, for experiment 2). Eggs were transported to the experimental aquarium facilities of the Institute of Marine Sciences in PVC bags filled with one quarter water and three quarters of pure oxygen. Eggs and newly hatched larvae were maintained in 19-liter PVC cylindrical containers placed inside 650-liter fiberglass tanks filled with seawater and fitted with a bottom nylon mesh of different pore diameters according to larval size. General procedures of egg incubation, larval and juvenile rearing were according to Moretti et al. (1999). Following Navarro-Martín et al. (2009b), after mid-metamorphosis (standard length; $SL > 18$ mm) juveniles were released in 650-liter fiberglass tanks and reared under natural conditions of photoperiod, pH (~7.9), salinity (~37.8 ppt), oxygen saturation (85–100%) and with a water renewal rate of 30% $\text{vol} \cdot \text{h}^{-1}$. In order to avoid temperature influences on the sex ratio, the following thermal regime was used (Navarro-Martín et al., 2009b): eggs were spawned in early spring at 13–14 °C. Larvae and postlarvae were reared at 16 ± 1 °C until 60 dpf. Then, temperature was increased to 21 °C at a rate of 0.5–1 $°\text{C} \cdot \text{day}^{-1}$ and maintained until the first fall, when temperature was let to follow the natural temperature in order to allow gonad maturation during the second year. The rearing density was also taken into account to avoid any possible distorting effect on sex ratios and therefore it was kept at safe ranges during all developmental stages: eggs (6000–10,000 $\text{eggs} \cdot \text{l}^{-1}$), larvae (200–350 $\text{larvae} \cdot \text{l}^{-1}$), juveniles (10–20 $\text{fish} \cdot \text{l}^{-1}$) and adults (11–12 $\text{kg} \cdot \text{m}^{-3}$). Artemia of the AF type first, and then amino acid-enriched Artemia of the EG type (INVE Aquaculture, Belgium) were used to manually feed larvae three times a day according to the manufacturers' instructions. Juveniles were fed *ad libitum* two times a day with dry food (ProAqua, S.A., Spain), unless stated otherwise. Fish were treated in agreement with the European Convention for the Protection of Animals used for Experimental and Scientific Purposes (ETS Nu 123, 01/01/91).

2.2. Experimental design

2.2.1. Experiment 1

The objective of this experiment was to study the effect of different growth rates, here achieved by controlling the amount of administered food at different times during the sex differentiation period, on the final sex ratio. In addition, the ability of growth compensation was assessed in the groups exposed to food restriction.