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The effects of an artificial and a natural diet on growth, survival and reproductive performance of wild caught and reared brill (*Scophthalmus rhombus*)

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ABSTRACT

During this research, the effects of two diets were tested on growth, survival and reproductive performance of wild caught and laboratory reared (F1) *Scophthalmus rhombus*. A total of 80 adult brill (502 ± 19 g) were randomly distributed in a flow-through system composed by 8 tanks of 4.2 m^2 (5.0 $m^3 \times 1.2$ m water depth). Four tanks were stocked with 10 animals each and fed *ad libitum* with frozen squid (*Loligo gahi*), while the remaining four tanks were stocked with 10 animals each and fed *ad libitum* with dry pellets (Vitalis repro®, from Skretting, Spain). Each replicate was stocked with 5 wild caught brill and 5 laboratory cultured brill. The experiment lasted for approximately one year (350 days). Origin of animals affected growth, with wild caught brill growing faster than F1. Sex also affected growth, with females attaining larger sizes than males. Diets only promoted differences in the months prior to reproduction, with animals fed dry pellets growing larger during these months. Only wild caught females matured. Among these, the ones fed with frozen squid showed higher reproductive potential, with more spawning females during a larger period of time. Neither diets or animal origin promoted differences on male reproductive potential. Different diets did promote differences in egg lipid composition.

1. Introduction

The great expansion of the European marine fish aquaculture industry can be attributed to the culture of only a few species, which markets begin to show signs of saturation. Therefore, aquaculture diversification is an important objective that must be enhanced for the sustainable development of marine finfish farming. Brill (*Scophthalmus rhombus*) is a promising species for aquaculture in the Southern Atlantic–Mediterranean coast, since it has high market price and growth rates. Large scale production of brill is limited by several aspects, such as the high larvae mortality (Hachero-Cruzado et al., 2009) and both poor and variable egg quality in captive broodstocks (Hachero-Cruzado et al., 2007).

Varying gametes quality is one of the limiting factors for successful mass production of fishes. Some factors influence spawning quality in fishes. Origin of breeders (wild caught and cultured) can influence the quality of the gametes. Presently, senegalese sole (*Solea senegalensis*) reproduction is obtained using breeders captured from the wild (Dinis et al., 1999), since reproduction of F1 broodstock has failed (Porta et al., 2006). On the other hand, some feed components greatly influence spawning quality in several species (Watanable, 1985; Verakunpiriya et al., 1997), and a deficiency of nutrients in broodstock diets decreases egg and larval quality (Watanable, 1985).

The limited energy available to animals has to be allocated among maintenance, somatic growth, and reproduction. Reproduction imposes considerable metabolic demands in fish, whatever the pattern of ovarian development and allocation of time and resources (Balon, 1975; Tyler and Sumpter, 1996). Synchronous fishes usually reduce their food intake during gonadal maturation, mobilizing nutrients from endogenous reserves in muscle, adipose tissue and liver (Iles, 1984; Lal and Singh, 1987; Nassour and Leger, 1989; Martín et al., 1993) to shed all the eggs over a short time interval (Wootton, 1990). In this way, reproduction success in these species is constrained by the allocation of energy prior to the elaboration of gonadal tissue (Henderson et al., 1996) and characterized by a lower dependence of food during spawning. Group-Synchronous fishes, such as brill (Caputo et al., 2001, Hachero-Cruzado et al., 2007), have a relatively short spawning season and yolk accumulation mostly depends on body reserves (Murua and Saborido-Rey, 2003). Conversely, females with asynchronous oocyte development, show a more regular periodicity in gonadal growth than synchronous spawners, by ovulating many times during the spawning season (Tyler and Sumpter, 1996). For these species, the number of spawning is affected by the food supply, temperature and other environmental factors (Wootton, 1990).

Lipids play a significant role in fish nutrition (Sargent et al., 1999). During embryonic development, lipid reserves are used by the

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embryo, both as substrates for energy metabolism and structural components in membrane biogenesis (Heming and Buddington, 1988; Rainuzzo, 1993; Finn, 1994; Sargent, 1995). The relationship between biochemical composition of the egg and embryo and larval quality has been determined (Rodríguez et al., 1993, 1997; Fiogbé, 1996; Abi-Ayad et al., 1997; Kestemont et al., 1999; Lund et al., 2008). These studies indicate that the biochemical composition of fish eggs is species-specific and varies both qualitatively and quantitatively, and that some lipid components could be related to differences in spawning quality. Thus, these lipid components could be considered as "descriptors" of differences in quality.

From 2002, the flatfish culture group at Centro IFAPA "Agua del Pino" (Cartaya, Huelva, Spain), in collaboration with other Institutions, is focusing on the study of brill reproductive biology in captivity among other aspects related to the rearing technique, as a necessary prerequisite for the development of a sustainable culture for this species. The aims of the present work are to gain comprehensive knowledge on the reproductive biology of brill in mariculture, and to determine the effects of two diets (one natural and one commercial pellet) and origin of brill (wild caught and cultured) on growth, survival, reproductive performance and egg lipid composition.

2. Materials and methods

Wild brill breeders were caught using trammel netting in the Gulf of Cádiz (SW Spain) between October 2006 and January 2007 and transported to the Centro IFAPA "Agua del Pino" (Cartaya, Huelva, Spain). Animals were acclimated to captivity until May 2007. Upon arrival, animals were treated with one daily preventive 100 ppm formalin bath during one week. A diet of fresh or frozen European pilchard (*Sardina pilchardus*), frozen squid (*Loligo gahi*), blue whiting (*Micromesistius poutassou*) and dry pellet (Vitalis repro®, from Skretting, Spain) were used to adapt breeders to feeding in captivity. Forty brill successfully adapted to captivity. Cultured brill used in the experiment were born in March 2005 from eggs laid by females in captivity, and raised in the Centro IFAPA — "Agua del Pino". All animals were tagged with an intramuscular passive integrated transponder tag (ID 100A; Trovan Ltd., United Kingdom). Fish were kept under natural conditions of photoperiod (37° 17′ N, 7° 9′ W) in 11.3 m^2 (13.6 m^3; 1.2 m water depth) circular fibreglass tanks, located indoors. No sand substrate was used. Acclimation tanks were supplied with running seawater (SW; 200% renovation per day) with 37–39 g L^{-1} salinity and 5–9 ppm oxygen. Water temperature fluctuated naturally between 12 °C and 20 °C.

At the start of the experiment, a total of 80 adult brill weighing 502 ± 19 g were randomly distributed in the experimental tanks, in a flow-through system composed by 8 tanks 4.2 m^2 (5.0 $m^3 \times 1.2$ m water depth). No differences ($p > 0.05$) were found in the weights of each replicate, at the start of the experiment. Four tanks were stocked with 10 animals each, and fed *ad libitum* with frozen squid (*L. gahi*), while the remaining four tanks were stocked with 10 animals each, and fed *ad libitum* with dry pellets (Vitalis repro®, from Skretting). Every replicate was stocked with 5 wild caught brill and 5 laboratory cultured brill. Each animal had an electronic tag that allowed individual identification. The experiment lasted for approximately one year (350 days). Experimental tanks were supplied with running seawater (SW; 200% renovation per day) with 37–39 g L^{-1} salinity and 5–9 ppm oxygen. Water temperature fluctuated naturally between 12 °C and 23 °C. During summer it was maintained below 23 °C, using a titanium plates heat exchanger (Alfa Laval, Sweden).

Brill were fed once a day, 14:00 h, and uneaten remains were removed the next morning, weighed and multiplied by a correction factor (0.586 for pellets and 0.779 for squid) to account for water absorption and leaching.

Every animal was weighed individually, on a monthly basis. Average growth rate (AGR) for the total length of the experiment, and feeding rates for every sampling period were calculated as: 1) Average growth rate (AGR) (% BWd^{-1}) = $((\ln W2 - \ln W1) / t) \times 100$, where $W2$ and $W1$ are the final and initial weight of the brill, respectively, ln the natural logarithm, t the number of days of the experiment, and 2) Feeding rate (FR) (%BWd^{-1}) = (FI / Average $W(t)$) × 100, where FI is the dry weight of food ingested and Average $W(t)$ is the average dry weight of the brill during that period. The cumulative mortality was accounted in the diets tested, and wild caught or cultured brill.

During spawning season (mid January to late April), all females were periodically checked for expression of hyaline eggs, which is an indication of egg viability (McEvoy, 1984; Fauvel et al., 1992). Ovulatory rhythms according to first ovulation date were determined using daily abdominal stripping at the start of the spawning season. This was done in order to assure that freshly-ovulated eggs were obtained, and avoid over-ripening (McElvoy, 1984). Before handling, fish were anaesthetized in 2-phenoxyethanol (500 ppm, maximum time of exposure: 5 min). Thereafter, eggs were hand-stripped being collected in a dry 2 L plastic beaker. The total number of eggs was calculated volumetrically under dry conditions, counting the eggs contained in one 500 µl aliquot and extrapolating it to the total volume of eggs collected, which was measured in a calibrated cylinder. The same aliquot was used to calculate the viability rate. Eggs were estimated to be viable when they showed a perfect spherical and translucent aspect (McEvoy, 1984; Fauvel et al., 1992).

Sperm motility and percentage of fluent males were determined with four sampling periods, during spawning season. For sperm collection, the urogenital pore was dried and sperm was collected with a syringe without needle, gently pressing the testis on the fish blind side. Samples were introduced into Eppendorf tubes and stored on ice until further analysis. Samples contaminated with urine or seawater were discarded. Sperm motility was measured activating 1 ml of sperm (pre-diluted 1:5 (v/v) in a Ringer solution) with 19 µl of seawater and scored immediately under light microscopy. The percentage of motile spermatozoa was scored according to Sánchez-Rodríguez (1975).

All egg batches (16) coming from 6 females were sampled for analysis of moisture, total lipids (TL), lipid classes (LC) and fatty acids (FA) in total lipid extracts. Moisture content was determined from 500 mg samples using the method of Horwitz (1980). Total lipid was extracted with chloroform:methanol (2:1 v/v) containing 0.01% of butylated hydroxytoluene (BHT) as antioxidant (Christie, 1982). The organic solvent was evaporated under a stream of nitrogen and the lipid content determined gravimetrically. Lipid classes were separated by one dimensional double development high performance thin layer chromatography (HPTLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (25: 25: 25: 10: 9 by vol.), as the polar solvent system and hexane/diethyl ether/glacial acetic acid (80: 20: 2 by vol.), as the neutral solvent system. Lipid classes were quantified by charring with a copper acetate reagent followed by calibrated scanning densitometry using a Shimadzu CS-9001PC dual wavelength flying spot scanner (Olsen and Henderson, 1989). Total lipid extracts were subjected to acid-catalyzed transmethylation for 16 h at 50 °C, using 1 ml of toluene and 2 ml of 1% sulphuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were purified by thin layer chromatography (TLC), and visualized by spraying with 1% (w/v) iodine in $CHCl_3$ (Christie, 1982). Prior to transmethylation, heneicosanoic acid (19:0) was added to the TL as internal standard. FAME were separated and quantified by using a Shimadzu GC 2010 gas chromatograph equipped with a flame ionization detector (250 °C) and a fused silica capillary column RTX–WAX™ (10 m × 0.1 mm I.D.). Helium was used as carrier gas and the oven initial temperature was 150 °C, followed by an increase at a rate of 90 °C min^{-1} to a final temperature of 250 °C for 3 min. Individual FAME were identified by reference to authentic standards and to a well-characterized fish oil.