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Fitness of early life stages in F1 interspecific hybrids between *Dicentrarchus labrax* and *D. punctatus*

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Abstract – Inter- and intraspecific crossbreeding experiments were conducted to evaluate the aquaculture potential of hybrids in the genus *Dicentrarchus*, focusing on fertilisation and hatching success. The experimental design consisted of 24 controlled crosses in which individual and pooled fertilisations were made between wild *D. labrax* individuals (8 dams and 5 sires originating from West Mediterranean and Atlantic populations) and wild *D. punctatus* (6 sires). Three experiments were successively performed: (1) dams from the Mediterranean population, individually considered to have good egg quality, (2) dams from Mediterranean population, individually considered to have poor egg quality and (3) crossbreeding using pooled eggs from the Atlantic population. In each case, batches of these eggs were fertilized by sperm from males of the two species. Sperm cell quality (concentration and motility) was verified before experimentation in all cases and equal numbers of sperm cells from each male were used to individually (without inter-sire competition) fertilise egg batches. Through the repeated artificial crosses between female common sea bass *Dicentrarchus labrax* (Linnaeus 1758) and male spotted sea bass *D. punctatus* (Block 1792), these experiments showed that no post-zygotic reproductive barriers exist to interspecific hybridisation between these two species when using *D. labrax* as dams and *D. punctatus* as sires. Phenotypically, the F1 hybrids were easily recognisable: they inherited the characteristic black spots of *D. punctatus*. Furthermore, embryo survival was significantly higher in interspecific crosses compared with intraspecific controls, showing increased fitness for this trait (increased performance at early life stage). Then, the experimental breeding design validates the observation that West Mediterranean and Atlantic common sea bass populations are different. Indeed, the inter-population crosses (between West Mediterranean dams and Atlantic sires) also revealed increased fitness at early life stages in comparison with the progeny of intra-Atlantic population crosses. However, these individuals were still smaller than their interspecific counterparts. The ability to produce viable F1 hybrids will have significant implications for the practical improvement of sea bass aquaculture.

Key words: Sea bass / Hybrid progeny / Offspring quality / Fitness / *Dicentrarchus labrax* / *Dicentrarchus punctatus*

1 Introduction

Interspecific hybridisation is an complementary approach that can used in breeding programs to improve desirable traits (such as growth rate, flesh quality, disease resistance and environmental tolerance) of cultured stocks via the exploitation of potential positive heterosis (hybrid vigour) in F1 crossbred offspring (Bartley et al. 2001). Indeed, in most cases heterosis in some reproductive fitness traits is expected when the parental lines are genetically distinct (Fairfull et al. 1987; Flock et al. 1991). Evidence of superior performance and interspecific hybrid vigour has been reported in a wide variety of fish species

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(Hulata 1995; Bartley et al. 2001). The success of interspecific hybridisation supposes that two levels of barrier to reproduction are overcome: (1) prezygotic barriers, which block various stages of reproduction from mating through fusion of the two pronuclei, and (2) postzygotic barriers, which are commonly stronger in fish than prezygotic ones and can induce the abortion of embryos (Campbell 1990). These reproductive barriers can be easily evaluated by assessing the development of fertilised eggs in interspecific crosses.

Three examples illustrate the interest of using hybrids of aquaculture fish. The striped bass (*Morone saxatilis*), the sister genus of *Dicentrarchus*, when exposed to a standardised confinement stress, had lower survival and resistance to infection than hybrid bass (*M. saxatilis* × *M. chrysops* and *M. saxatilis* × *M. americana*) (Noga et al. 1994). A significant proportion of farmed sturgeon is composed of interspecific hybrids (*Acipenser naccarii* × *A. transmontanus*) due to their high productive performance for caviar and flesh (Congiu et al. 2001). Finally, another well-known example of the semi-intensive culture of hybrid fish is the red Nile tilapia, which is a hybrid of *Oreochromis niloticus* and *O. mossambicus* (Medeiros et al. 2007).

Obtaining high quality gametes is one of the most important limiting factors in the modern aquaculture industry, for both freshwater and seawater species (Bromage 1995; Kjorsvik et al. 1990; Brooks et al. 1997; Bobe and Labbé 2010), as fertilisation rate (%) and hatching rate (%) are important production criteria. Egg quality can be defined as the potential of an egg to hatch into a viable larva. In some species, morphological aspects of larvae are used as indicators of gamete quality (Kjorsvik 1994; Kjorsvik et al. 2003). Egg quality is determined by several parameters that frequently change before and during the reproductive season. These include several extrinsic factors that can modify the female endocrine status during oogenesis, such as physicochemical parameters of water, photoperiod (Bromage et al. 1992, 1994), the amount and quality of ingested food (Watanabe and Kiron 1995), broodstock stress and the over-ripening of eggs after ovulation (Campbell et al. 1992, Christiansen and Torrisen 1997, Fauvel et al. 1992). The intrinsic properties of the egg, such as its genes, the nutrients in the yolk sac, and the contribution of the parental transcripts and proteins after fertilisation, can also determine the egg quality (Brooks et al. 1997; Aegerter et al. 2005; Bonnet et al. 2007a, 2007b; Crespel et al. 2008; Desvignes et al. 2011). In addition, the incubation environment also affects the success of production of viable offspring, both in the wild and in captivity (Lam 1994; Nagahama 1995).

The European (or common) sea bass, *Dicentrarchus labrax* (Linnaeus 1758) is efficiently bred in captivity and reared intensively in Mediterranean mariculture. Sea bass could be considered as a cultured species model, because its biology, physiology, behaviour, reproduction and phenotypic diversity have all been extensively studied. Previous genetic studies based on allozymes (Allegrucci et al. 1999), mitochondrial and random amplified polymorphic DNA (RAPD) markers (Caccone et al. 1997) have led to the identification of three genetically distinct populations: north-eastern Atlantic, western Mediterranean and eastern Mediterranean (Lemaire et al. 2005).

Sea bass shows a seasonal reproductive cycle with eggs produced when the environmental conditions are most conducive to survival. According to Mayer et al. (1990), wild sea bass females spawn discrete clutches of eggs at short intervals of time during the reproductive season (in winter). In captivity, they spawn spontaneously only 2 or 3 times over the reproductive season, i.e., once a month (C. Fauvel, pers. comm.). Short interval spawning by stimulation using slow release implants of an artificial hormone was tested by Fornies et al. (2001), but led to very low quality spawns; probably due to over-stimulation of under-ripe eggs. The species can be classified as a "group synchronous" spawner with long final maturation cycles (Wallace and Selman 1981).

Little is known about the spotted sea bass, *Dicentrarchus punctatus* (Bloch 1792), which is not reared at commercial scale due to its slow growth performance compared with the well-known *D. labrax* (Allegrucci et al. 1997). Although no spontaneous mating has been observed between these two species, this is probably due to differences between their sexual maturity times and behaviour in the wild (Bonhomme et al. 2002). The diploid chromosome number of both species is $2n = 48$ (Viturri et al. 1990), and studies at cytogenetic and molecular levels using three multi-gene families confirm that they are closely related (Merlo et al. 2010).

In the present study, we report the estimation of reproductive and early life characteristics, i.e., fertilisation and hatching rate, in interspecific hybrids between *D. labrax*, used as dams and *D. punctatus*, used as sires, in comparison with intraspecific crosses of *D. labrax*. Interspecific crosses were carried out by replicated artificial insemination. The feasibility of producing hybrids from these two species for practical use in sea bass aquaculture and their potential for future sea bass genetic improvement programs are discussed.

2 Materials and methods

2.1 Broodstock

D. labrax and *D. punctatus* were collected in the wild by fishermen specialising in live fish capture for genitor introduction to aquaculture farms and the public aquarium trade (http://www.poissons-vivants.com/index.htm). Fish were caught during spring and summer in 2002 to 2005 using nets and transported directly upon capture to the Ifremer aquaculture station at Palavas-les-flots (South of France). They were then maintained as broodstock for at least for 2 years in recirculating water systems in 15 m^3 tanks under natural conditions of photoperiod and temperature, which ranged from 10 °C in winter to 25 °C in summer. The fish were hand-fed: (1) daily with commercial pellets for marine fish (Le Gouessant) to apparent satiety and (2) once a week with frozen sardines. All fish were individually marked

Table 1. Broodstock used for interspecific hybridisation between *D. labrax* and *D. punctatus*: Eight dams from the West Mediterranean sea (WM) or Atlantic Ocean (AT), and eleven sires from the Atlantic were used. The genotype nomenclature is as follows: (1) West Mediterranean, *D. labrax* dams: F_{WM}, (2) Atlantic, *D. labrax* dams and sires: F_{AT} and M_{AT}, respectively, (3) *D. punctatus*, males: P. The last column indicates which experiments the genitors were used for.

Species	Sex	Geographic origin	Genotype	Experimental design
D. labrax	Female	WM	F_{WM-1}	1
	Female	WM	F_{WM-2}	1
	Female	WM	F_{WM-3}	1
	Female	WM	F_{WM-4}	2
	Female	WM	F_{WM-5}	2
	Female	AT	F_{AT-1}	3
	Female	AT	F_{AT-2}	3
	Female	AT	F_{AT-3}	3
	Male	AT	M_{AT-1}	1
	Male	AT	M_{AT-2}	2
	Male	AT	M_{AT-3}	2 & 3
	Male	AT	M_{AT-4}	3
	Male	AT	M_{AT-5}	3
D. punctatus	Male	AT	P_1	1
	Male	AT	P_2	1
	Male	AT	P_3	2 & 3
	Male	AT	P_4	2 & 3
	Male	AT	P_5	3
	Male	AT	P_6	3

using passively integrated transponder (PIT) tags. In these conditions the spawning season occurred from mid-January until the end of March, and spermiation season broadly covered the period.

Eight dams (all *D. labrax*) and 11 sires (6 *D. punctatus* and 5 *D. labrax*) were used for this study. *D. labrax* dams from the West Mediterranean populations will be referred to as F_{WM}; *D. labrax* dams and sires from the Atlantic population as F_{AT} and M_{AT}, respectively; and *D. punctatus* sires as P (Table 1). The selection criteria for fish used in the present experiment were that they should be healthy, with an adequate maturation stage (postvitellogenic stage C, as proposed by Fauvel and Suquet 1999). Females and males weighed 2 kg (± 200 g) and 1 kg (±200 g), respectively.

2.2 Female induction and precocious egg quality assessment

In order to choose the best females to use in crosses, ovarian biopsies were made. These were started shortly after the beginning of the reproductive season (indicated by the first spontaneous spawns) and were performed fortnightly on each female under anaesthesia, using a flexible plastic catheter designed for human endometrium sampling ("Pipelle de Cornier", laboratoire CCD, Paris, France). Egg maturation stage was assessed based on vitellus appearance and nucleus migration, according to Fauvel et al. (1999) and Fauvel and Suquet (1999).

Females with ovaries at stage C (initiation of lipid droplet formation and nucleus migration), were stimulated by a single injection of luteinising hormone releasing hormone analog (LHRHa) (Sigma) at 10 µg kg^{-1} body weight. In order to prevent spontaneous egg release, they were then transferred individually into 1 m^3 tanks at 11–13 °C with a recirculating water system. Ovulation occurred about 72 h after hormonal injection. The eggs were collected by stripping females and were assessed before fertilisation for their intrinsic quality according to four morphological features: perfect roundness, development of a perivitelline space, vitellus translucency and low number of lipid droplets. Only good and homogeneous egg batches were used for the experiment, except for F_{WM-4} and F_{WM-5} which produced poor quality eggs.

2.3 Collection and assessment of sperm quality

A random catch of sires was realised in the *D. labrax* or *D. punctatus* broodstocks. From these samples, sperm was obtained by applying slight pressure on the abdomen of a running male with milt. The external urogenital pore area was wiped dry with paper towel before collecting sperm. Urine and potentially urine-polluted semen were carefully discarded (identified by differences in colour and viscosity). To avoid sea water and faeces contamination, the initial male ejaculate was discarded. Sperm was drawn from males into a 2 ml syringe without a needle and kept refrigerated at 4 °C until needed.

Sperm cell quality was checked for concentration and motility in both species, according to the procedure of Fauvel et al. (1999):

- Concentration: Milt was first diluted in a 10-ml test tube by adding 10 µl milt to 9990 µl distilled well water (dilution 1:1 000) and then mixed using a vortex mixer. The sperm concentration was determined using a spectrophotometer at 260 nm wavelength to assess optical density, as a linear regression exists between this method and conventional sperm cell number assessment by haemocytometer counting (Fauvel et al. 1999).
- Motility: Sperm motility was checked at 40× magnification under a light microscope connected to a camera and video monitor. First, 10 µl sperm were diluted in 250 µl non activating medium, NAM (Fauvel et al. 1998). Then, 10 µl diluted sperm were activated by the addition of 1 ml seawater to give a final dilution of 1/2 500. Just at the point of activation, sperm were introduced into a Thoma counting cell already focused under the video-microscope (Axiolab Zeiss). This process took less than 10 s, enabling motility to be estimated visually by two observers,

according to Suquet et al. (1992). All the sire samples showing high initial motility (80 to 100% motile spermatozoa, spz) were choosen for experimentation.

After assessment of the concentrations of the different semens, they were individually adjusted to 10^{10} spz per ml by dilution in a non activating medium (Fauvel et al. 1998). Then the fertilization were performed using a similar volume of diluted sperm containing 200 000 spz per egg according to Fauvel et al. (1999).

2.4 Experimental mating design

Eggs were divided into equally-sized volumes of 50 ml, which were put in individual 200 ml beakers and fertilised with a mixture of 50 µl sperm and 25 ml seawater. Just after fertilization, the eggs were transferred into small cylindro-conical incubator-tanks (volume = 50 L) with recirculating sand-filtered water (at 13 °C and 38 g L^{-1} salinity) that was disinfected with UV light. Aeration from the bottom of the incubators provided gentle agitation of the eggs and surface seawater.

Three experiments were successively performed during the natural reproductive season, with experiments two weeks apart. All three experiments used a complete factorial design, i.e. all possible combinations of dam × sire (Lynch and Walsh 1998).

- Experiment 1: Dams with good egg quality.
  The factorial breeding scheme (3 × 3) used three dams, F_{WM-1}, F_{WM-2} , and F_{WM-3}, crossed with three sires: two *D.* punctatus, P_1 and P_2, and one *D. labrax*, M_{AT-1}.
- Experiment 2: Dams with poor egg quality.
  The factorial breeding scheme (2 × 4) used two dams, F_{WM-4} and F_{WM-5}, individually crossed with four sires: two D. punctatus P_3 and P_4, and two *D. labrax* sires M_{AT-2} and M_{AT-3}.
- Experiment 3: Crossbreeding using pooled eggs from the *D. labrax* Atlantic population.

Crosses used the pooled eggs from the 3 dams, F_{AT-1}, F_{AT-2}, and F_{AT-3}. Each dam was individually stripped and eggs were then equally mixed (300 ml of eggs per dam to obtain a total volume of 900 ml). The sperm of seven sires was individually collected: four D. punctatus (P_3, P_4, P_5 and P_6) and three D. labrax (M_{AT-3}; M_{AT-4} and M_{AT-5}).

2.5 Offspring quality parameters

Fertilisation rate (%) of the different crosses was individually evaluated 3 h post-fertilisation. Around 500 eggs were sampled from each incubator-tank/family under strong seawater agitation and placed in 200 ml containers. These eggs were then transferred to a Dolfuss counting cell in which eggs are randomly distributed between the small squares. The ratio between fertilised and non fertilised eggs was established by two different observers based on the first 100 eggs observed, starting from a randomly chosen square. For each incubator, four samples of 500 eggs were evaluated (2 trials per counter). Fertilised eggs were characterised by visibly dividing cells (2–4 cells stages, 3 h post fertilisation).

On the day of fertilisation, another 500 eggs were sampled from each incubator-tank and placed in a 200-ml Erlenmeyer. Offspring hatched approximately 5 days post-fertilisation under water conditions of 13 °C temperature and 20 g L^{-1} salinity. The hatched larvae and remaining eggs were then transferred to a Petri dish for counting. The first 100 larvae/eggs (hatching rate in %) were counted under a dissecting microscope (Stemi 2 000-C, Zeiss) by two different counters, beginning at a random starting point. For each incubator, three trials (n = 3) of 500 larvae/eggs were performed in duplicate (2 trials per counter).

2.6 Statistical analysis

The normality of the distributions were assessed using the Shapiro-Wilk test. Due to non normality of the distributions, median of the distributions are presented and non parametric Kruskal-Wallis tests were used to test for differences among the groups. This was followed by multiple comparison procedures to determine differences between groups, performed using Mann-Whitney tests. Corrections for multiplicity test were not used. A p-value of less than 0.05 was considered significant. Statistical analysis was performed using R software package (version 2.13.0).

3 Results

3.1 Phenotypic validation of hybrid status

The major phenotypic criterion that distinguished the F1 hybrids from commercial *D. labrax* offspring was pigmentation. At larval stages, F1 hybrids could be distinguished from *D. labrax* by their intense pigmentation (more spots in number and intensity) (Fig. 1a). Later, at the juvenile stage, the spots were easily observed in the hybrids but were lacking in the *D. labrax* commercial strain (Fig. 1b).

3.2 Assessment of fertilisation success

Fertilisation success was higher in all experimental designs when using *D. punctatus* sires than when using *D. labrax* sires in the intraspecific crosses.

In experiment 1 (Fig. 2a), which used individual West Mediterranean *D. labrax* dams with good egg quality, fertilisation success rates were significantly higher in interspecific crosses (min-max values from 60–100%) than in the intraspecific counterpart (min-max values from 7–47%). Indeed, p values for F_{WM-1} , F_{WM-2} , and F_{WM-3} dams were 10^{-7}, 7×10^{-8} and 10^{-6}, respectively. On average, fertilisation rates in the interspecific crosses were 2.7 fold higher than in the intraspecific one (80% vs. 29%).

In experiment 3 (Fig. 3a), which also used good quality eggs pooled from Atlantic *D. labrax* dams, fertilisation rates were also significantly higher in interspecific crosses (min-max values from 61 to 77%) than in the intraspecific one (min-max value from 10 to 22%) ($p = 4 \times 10^{-23}$). On average, fertilisation rates

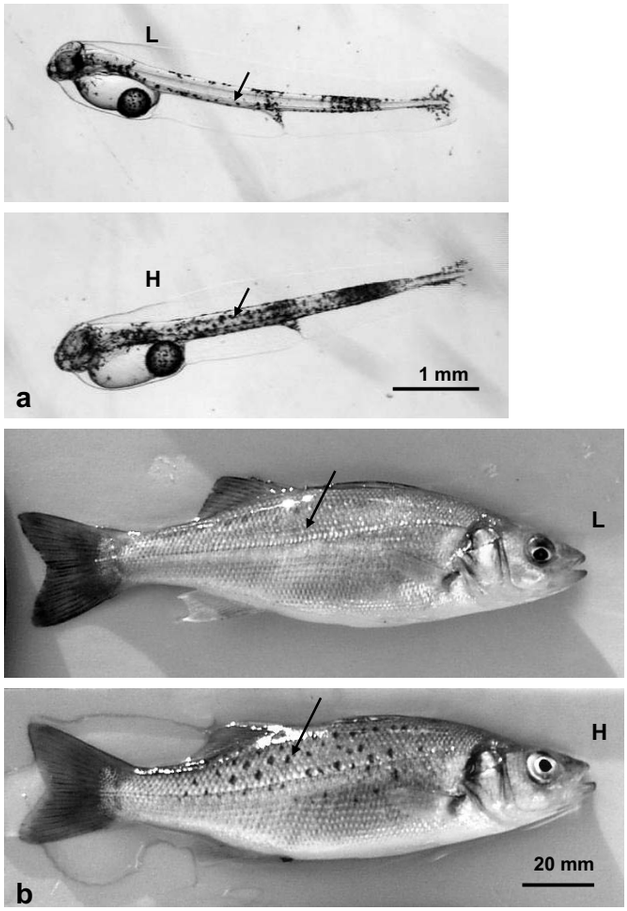

Fig. 1. External appearance of the common sea bass *D. labrax* (L) and F1 interspecific hybrid *D. labrax* × *D. puntatus* (H) at: larval stage, 2 days post-hatching (a), and juvenile stage, 250 days post-hatching (b). As shown by the arrows, the interspecific hybrids were spotted and phenotypically distinct from the farmed *D. labrax*.

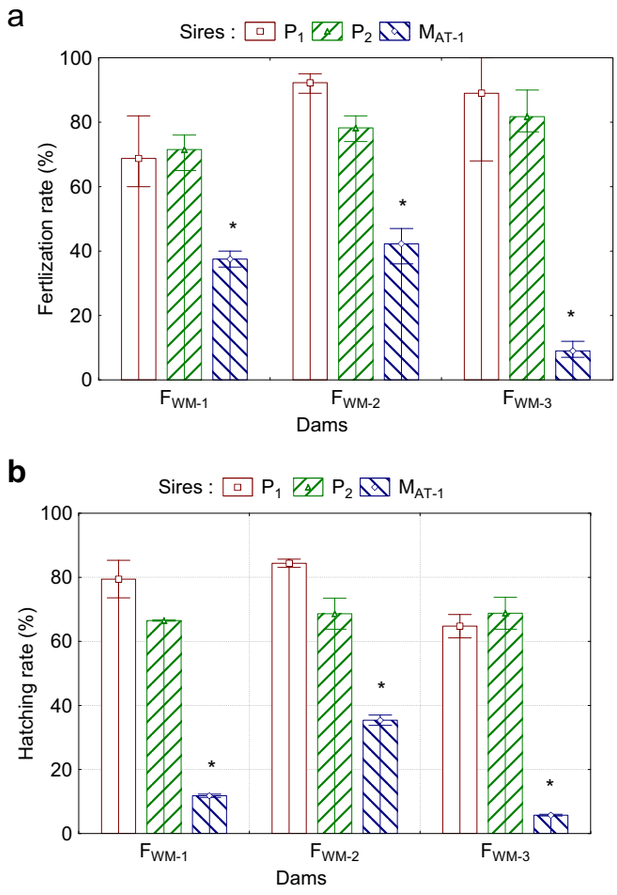

Fig. 2. Histograms showing median, minimum and maximum values of the fertilization rate (a) and hatching rate (b) in experiment 1: a complete factorial breeding scheme 3 × 3 (dams F_{WM-1}, F_{WM-2}, and F_{WM-3}, whose eggs were of good quality, individually mated with sires P_1, P_2, and M_{AT-1}). The data points significantly different at $p < 0.05$ are indicated with an asterisk (*).

in the interspecific crosses were 4.8 fold higher than the intraspecific ones (68% vs. 14%).

In experiment 2 (Fig. 4a), which used West Mediterranean *D. labrax* dams with poor egg quality, fertilisation rates were significantly different for F_{WM-4} and F_{WM-5} dams, with p values of 2×10^{-4} and 0.011, respectively. For dam F_{WM-4}, interspecific crosses led to a 1.9-fold higher fertilisation rate than the intraspecific counterparts (72% vs. 37%), with min-max values ranging from 65–80% vs. 26–43% (intraspecific crosses). For dam F_{WM-5}, interspecific crosses led to fertilisation rates 30% higher than the intraspecific counterparts.

Regarding the intraspecific *D. labrax* crosses used as the control in the good egg quality experiment (experiment 1), the fertilisation rate in inter-population crosses between West Mediterranean dams and Atlantic sires (3 crosses) was double that of the intra-Atlantic population crosses (experiment 3): 30% vs. 15%. For experiment 2 (poor egg quality), average fertilisation rate was 34% in inter-population crosses and, therefore, approximately equivalent to the value found in experiment 1.

3.3 Assessment of hatching rate

Hatching rate was significantly higher in experiments 1 and 3, where good quality eggs were used than in experiment 2 where egg quality was poor. In experiment 1 (Fig. 2b), p values for F_{WM-1}, F_{WM-2}, and F_{WM-3} dams were 2×10^{-3}, 3×10^{-3} and 2×10^{-3}, respectively. In experiment 3 (Fig. 3b), p value was 2×10^{-12} for the pooled females. On average, hatching rate was 4.0 and 4.6 fold higher using *D. punctatus* as sires than *D. labrax* in experiment 1 (Fig. 2b) and 3 (Fig. 3b) respectively.

In experiment 2 (poor egg quality), hatching rate was only significantly different with the F_{WM-5} dam ($p = 6 \times 10^{-5}$), but not with the F_{WM-4} dam ($p = 0.061$) (Fig. 4b).

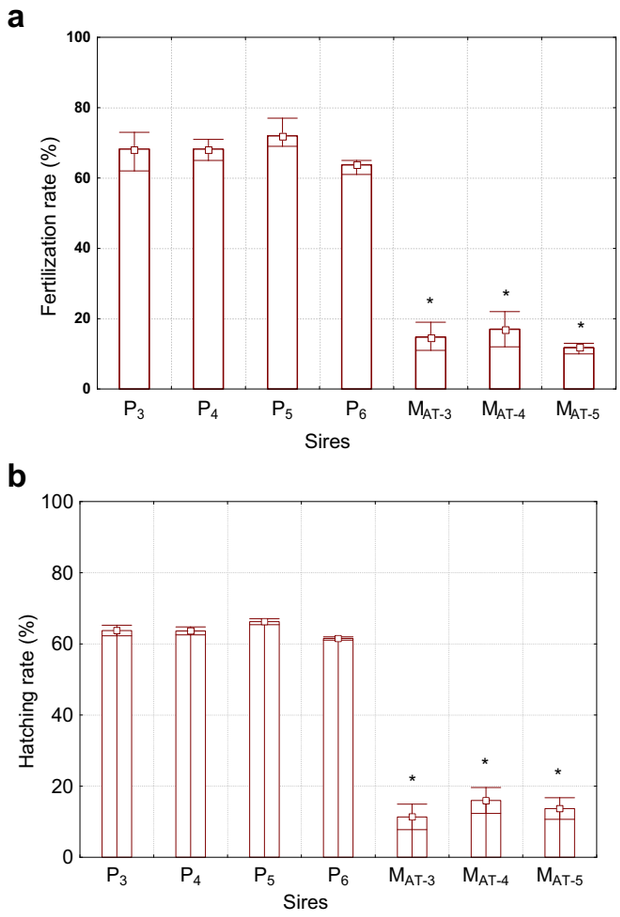

Fig. 3. Histograms showing median, minimum and maximum values of the fertilisation rate (a) and hatching rate (b) in experiment 3: cross-breeding using pooled eggs from three *D. labrax* dams F_{AT-1}, F_{AT-2}, and F_{AT-3} with four *D. punctatus* sires P_3, P_4, P_5 and P_6 and three *D. labrax* sires M_{AT-3}; M_{AT-4} and M_{AT-5}. The data points significantly different at $p < 0.05$ are indicated with an asterisk (*).

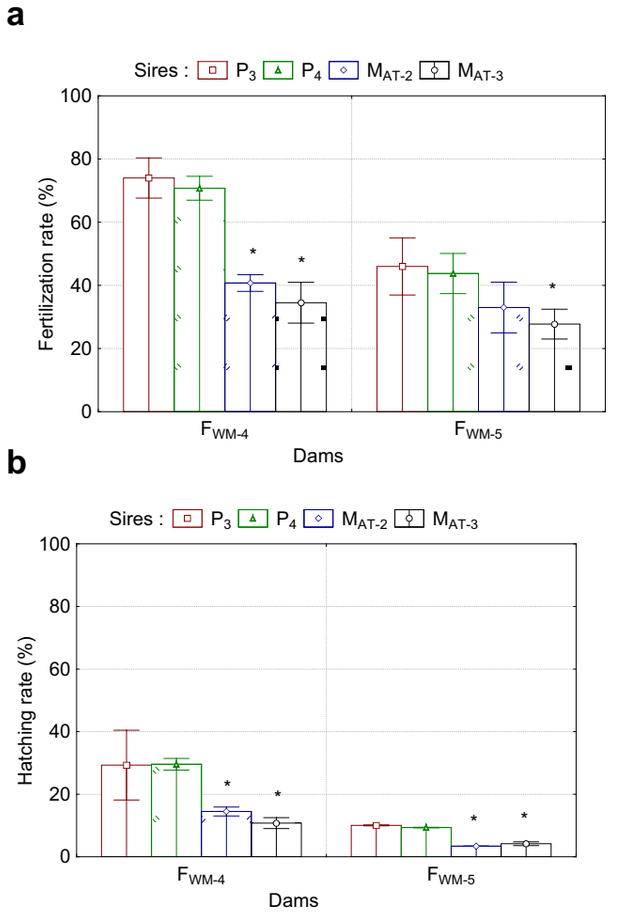

Fig. 4. Histograms showing median, minimum and maximum values of the fertilization rate (a) and hatching rate (b) in experiment 2: a complete breeding factorial scheme 2 × 4 (dams F_{WM-4} and F_{WM-5}, whose eggs were of poor quality, mated with sires P_3, P_4, and M_{AT-2}, M_{AT-3}). The data points significantly different at $p < 0.05$ are indicated with an asterisk (*).

4 Discussion

4.1 Fitness and parental species effects

The strong repeatability between our breeding experiments shows that interspecific crosses could be performed using *D. labrax* dams and *D. punctatus* sires, to produce viable diploid F1 hybrid progenies. The viability of such hybrids was also confirmed by Merlo et al. (2010), who analysed three multigene families as tools for the characterisation of *Dicentrarchus* hybrids. Our results clearly show that traits such as fertilisation and hatching rate were better in the interspecific cross between *D. labrax* × *D. punctatus* than in the intraspecific *D. labrax* cross for both an equivalent and limiting sperm concentration on eggs from the same female or pool. Such superior performance of hybrids in traits affecting fecundity would be expected to enhance fitness. Additionally, the advantage of F1 hybrids over *D. labrax* parental species at early developmental stages provides evidence of better early-life stage fitness in hybrids. This is not necessarily the case in other species of aquaculture interest. For example, interspecific hybridisation of shrimp had been successfully obtained for *Penaeus setiferus* × *Penaeus schmitti* (Bray et al. 1990) and reciprocal crosses of *Penaeus monodon* and *Penaeus penicillatus* (Lin et al. 1998), but spawning rate, hatching rate, and the survival of hybrid progeny to post-larval stages were low compared with intra-specific matings. This fitness reduction was attributed to the genetic mechanisms of outbreeding depression and/or underdominance (Miller et al. 2004).

It is commonly thought that a longer history of genetic isolation leads to a greater accumulation of genomic incompatibilities, related to genetic distance. Viability of hybrids

decreases with large genetic distances, such as those between genera. In this study, both *Dicentrarchus* species are closely related, they have a highly sympatric distribution, and they are not physically isolated in the wild (Bonhomme et al. 2002). It is interesting to consider whether the better fertilisation and hatching rates in the interspecific crosses, compared with their intraspecific counterparts can be explained from an evolutionary point of view. One response could be the influence of maternal and paternal effects, which largely govern these early life traits. Such effects have been found to affect early life history survival in salmonids (Nagler et al. 2000; Perry et al. 2005) and in sea bass (Saillant et al. 2001), and are well known to influence embryo development in general (Heath and Blouw 1998). Maternal and paternal effects could differ between the two *Dicentrarchus* species with regard to their physiological adaptation (temperature and oxygen) and tolerance of captive rearing conditions. Maternal effects, such as egg size and quality, may affect hatch timing and embryonic growth rate and consequently hatching success. These components have been estimated in salmonids (Wang et al. 2007), including whitefish (Rogers and Bernatchez 2006). In our experiments, interspecific and intraspecific crosses were made using the egg from the same female of pool of females (fixed maternal effect), meaning that the observed fitness differences can be linked to the sires. Paternal effect, i.e. spermatozoid quality and viability (beyond solely concentration and motility), may affect mortality or the ability of embryos to cope with the artificial environment. We know that *D. punctatus* has a more southerly geographic distribution and is frequently observed off the Senegal coast while, in contrast to *D. labrax*, it is never fished in Norway. Moreover, peak spawning of these two species occurs at different times in the nature and so gamete quality may be different between them at any given point in time. In our experiments, no bias due to differential gamete quality of *D. punctatus* and *D. labrax* affected the results. Indeed, these two species had been reared in our facilities, in the same environment (photoperiod, temperature, feeding), for two complete years before experimentation. Thus, synchronism in their spermiation periods had been achieved.

Increased fitness at an early life stage was also recorded in the intraspecific *D. labrax* crosses, with better performances in inter-population crosses between West Mediterranean dams and Atlantic sires (experiment 1), compared with the intra-Atlantic population crosses (experiment 3). This suggests that West Mediterranean and Atlantic dams are not similar and should be treated differently. However, this higher performance in the progeny of inter-population crosses is not as great as that found in interspecific crosses.

4.2 Potential impact for sea bass research programs and aquaculture

The possibility of producing F1 hybrids might provide a new direction for sea bass genetic research efforts. Fish are widely used in numerous fields of basic and applied research. Fish currently represent the third most common laboratory vertebrate group in terms of number of species studied, and are growing in importance. Although considerable progress has been made with regards to reproductive traits of *Dicentrarchus*, more research is required to provide a better understanding of its reproductive biology. It will be especially important to develop the means to predict and ensure the complete maturation of individual females chosen for spawning on the basis of their genetic value as broodstock. In our experience, for a given group of candidate sea bass spawners, only about a half of the females can be successfully reproduced using the best procedure available. The remainder fail to complete egg growth or yield eggs of low quality. Interspecific hybrid could facilitate mapping of large numbers of QTL (quantitative trait loci) of interest to the sea bass linkage map due to the great difference between the parental species for many phenotypic traits (Ky et al. 2000). This could possibly be achieved through an interspecific sea bass linkage map.

The genetic mechanism underlying the increased fitness at early life stages could be linked to heterosis. This could be examined in future studies by producing pure spotted sea bass families also. Other hybrid genetic combinations could be explored to test fitness or outbreeding depression though the study of F1 reciprocal crosses (*D. punctatus* dams and *D. labrax* sires), F2 hybrids and G2 (second generation) backcrosses. The potential sterility of some F1 progeny could be used to prevent accidental escape and gene impact on natural populations.

From a practical standpoint, commercial production of interspecific F1 hybrids between *D. labrax* and *D. punctatus* could represent a new potential for the farming industry if fitness advantages were also found for the main aquaculture traits of interest, including growth performance, hardiness and disease resistance. Production of new improved synthetic breeds with cytogenetic (ploidy level) manipulation of these F1 hybrids could be explored. In the future, this could lead to the maintenance of a new fish broodstock in commercial hatcheries or a long term cryopreservation unit for *D. punctatus* semen. In *Morone*, the sister genus of *Dicentrarchus*, interspecific striped bass hybrids (*Morone chrysops* × *M. saxatilis*) have been found to offer considerable potential, with regards to growth, hardiness and disease resistance (Kerby and Harrell 1990; Harrell 1997), for aquaculture throughout the United States (Hanfman 1993), as well as in other countries such as Israel and Taiwan (Garber and Sullivan 2006).

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