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The effects of salinity on reproductive development, plasma steroid levels, fertilisation and egg survival in black bream *Acanthopagrus butcheri* ☆

J.A. Haddy, N.W. Pankhurst *

School of Aquaculture and Co-operative Research Centre for Aquaculture, University of Tasmania, P.O. Box 1214, Launceston, Tasmania 7250, Australia

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Abstract

The effects of salinity of holding water of 5‰, 20‰ or 35‰ on seasonal reproductive development, plasma steroid levels, the efficacy of luteinizing hormone releasing hormone ethylamide (LHRHa) to stimulate ovulation, sperm motility, and egg fertility and development to hatching were investigated. Fish were captured from the wild from December to February, placed into salinity regimes in May and held until the normal times of spawning the following November. Blood samples were taken in August, September and November. Female fish were injected with saline or LHRHa (50 μg kg^{-1}) in November and bled and checked for ovulation for 5 days. Gonadal maturation was unaffected by salinity in both sexes. In females, seasonal plasma steroid levels were unaffected by salinity, whereas in males, plasma levels of 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) and 11-ketotestosterone were higher in fish held at 35‰ than in fish held at 5‰ in September, and in fish held at 5‰ and 20‰ in November, respectively. Plasma estradiol (E_2) and testosterone (T) levels in saline-injected fish, either remained low or were significantly suppressed. LHRHa treatment resulted in the short-term elevation of plasma E_2 and T levels at all salinities, whereas plasma 17,20βP levels were elevated over controls on days 1 and 2 post-injection in fish held in 20‰, but remained unchanged in fish held in 35‰ or 5‰. All but one fish ovulated in response to LHRHa, however, the number of ovulations and egg volumes was lowest in fish held at 5‰. Both fertilisation and sperm motility were significantly reduced at 5‰.

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* Corresponding author. Tel.: +61-3-6324-3801; fax: +61-3-6324-3804.
E-mail address: ned.pankhurst@utas.edu.au (N.W. Pankhurst).

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Naturally fertilised eggs (35‰; 2–8 cell stage) were incubated to hatching at salinities of 0‰, 5‰, 10‰, 15‰, 20‰, 25‰, 30‰ or 35‰. Eggs hatched in all salinities except 0‰, but with lowered survival at 5‰ and 10‰. Larvae showed high levels of deformity at salinities below 15‰. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Salinity; Reproduction; Gonadal steroids; Sparidae

1. Introduction

Black bream, *Acanthopagrus butcheri*, is an important recreational and commercial species endemic to southern Australia, and is currently being investigated as an aquaculture candidate for inland saline water culture. As black bream is an euryhaline species that spawns in the mid to upper reaches of estuaries at the interface between freshwater and the underlying salt wedge, it has been suggested that salinity may play a role in regulating reproductive activity (Sherwood and Backhouse, 1982; Haddy and Pankhurst, 1998). Current information indicates that the spawning of black bream occurs over a salinity range of 11‰–35‰ (Sherwood and Backhouse, 1982; Haddy and Pankhurst, 1998). Environmental factors such as salinity are known to directly affect fertilisation, survival and normal development of fish eggs in other species (reviewed in Holliday, 1969; Alderdice, 1998); however , to date there is no information on the effects of salinity on the early life stages of black bream.

Additional interest in black bream has been created by the problem of salination of agricultural land. Approximately 20% of Australia's inland aquifers are classified as being either brackish or saline with salinities ranging from 1.5‰ to 20.5‰ (Ingram et al., 1996). Rising saline groundwater is causing major loss of agricultural land, and current practice to reduce the height of the water table involves the pumping of saline groundwater into evaporation basins (Clayton, 1998; Blackwell, 1999). These saline ponds are currently not exploited and may be suitable for inland mariculture of a variety of species, including black bream (Ingram et al., 1996; Fielder et al., 1999). Techniques for the hormonal induction of ovulation have recently been developed for black bream (Haddy and Pankhurst, in preparation); however, information on the maturation of black bream in captivity and the effectiveness of hormonal treatment at differing salinities are yet to be investigated. Such information is critical to the selection of inland sites for the artificial propagation of black bream, and in determining suitable inland lakes for the development of new fisheries where natural recruitment can occur.

The aims of this study were to investigate the effect of holding water salinity on, reproductive development of wild-caught broodstock and subsequent fertilisation, egg development, and hatching. The effects of salinity on reproductive activity in adults were assessed by macroscopic gonadal condition, seasonal changes in plasma levels of cortisol, estradiol (E_2), testosterone (T), 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) and 11-ketotestosterone (11KT), and the efficacy of LHRHa treatment at inducing changes in plasma levels of gonadal steroids, and subsequent ovulation and egg production. The effects of salinity on gamete quality were assessed in terms of fertilisation, sperm activity, and survival and development of eggs to hatching.

2. Materials and methods

2.1. Fish capture and maintenance

Black bream were captured by rod and line from the Meredith (148°7′S, 42°4′E) and Swan Rivers (148°4′S, 42°4′E) at Swansea, Tasmania, at the end of the spawning season from December 1997 to February 1998. Fish were transported to the laboratory and placed in 1000-l temperature (20°C) controlled tanks supplied with recirculating sea water, under natural photoperiod. Fish were sexed by the presence of milt, and males and ''presumed females'' kept in separate tanks. At the beginning of May (when the GSI is low and the gonads are regressed in wild fish; Haddy and Pankhurst, 1998), fish were allocated to experimental tanks with recirculating sea water (37‰) and the salinity adjusted to 35‰, 20‰ (over 2 weeks) or 5‰ (over 4 weeks) by weekly water exchanges with fresh water. Salinities were held constant thereafter, and were checked weekly with a refractometer. Water exchanges and tank cleaning were conducted as required. Fish were fed to satiation on an in house marine fish pellet, and disturbances kept to a minimum. Temperatures were dropped from 20°C to 16°C at the end of April and thereafter dropped by 1°C every 15 days until August (minimum of 11°C) when temperatures were raised by 1°C every 15 days to simulate the natural temperature cycle in eastern Tasmania (Haddy and Pankhurst, 1998).

2.2. Seasonal sampling

On the 1st of August, fish were removed from tanks, placed into a 400-l holding tank and the time of first disturbance was recorded for each tank. Fish were anaesthetised in 0.05% 2-phenoxyethanol, blood sampled by caudal puncture using heparinized syringes and 22-g needles, dart-tagged for individual identification and males checked for spermiation. The presumed sexes were then equally distributed into two tanks at each salinity. Fish were blood sampled again on the 4th of September and 15th of November. Blood was stored on ice, plasma obtained by centrifugation, then frozen and stored at −18°C until required for assay.

2.3. Induced ovulation experiment

On the 15th of November, female fish were blood sampled as described above, biopsied for macroscopic gonad condition (Haddy and Pankhurst, 1998), weighed, and injected intraperitoneally with either 50 μg kg^{-1} body weight of 50 μg ml^{-1} des-Gly^{10} (D-Ala^{6})-luteinizing hormone releasing hormone ethylamide (LHRHa; pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-NHEt) or 1 ml kg^{-1} of teleost saline. Owing to logistical constraints there was only a single tank available for each treatment. This means that the possibility of tank effects cannot be discounted, however, this is viewed as unlikely due to the identical nature of the tank systems and conditions. Fish were bled daily and

checked for ovulation twice daily for a period of 5 days. Eggs released into the tanks were collected in egg collectors and the volumes recorded. Ovulated females were manually stripped of eggs, egg volumes recorded and the eggs from fish at each salinity (volumes over 10 ml) fertilised at 35‰, 20‰ and 5‰ salinity using fresh sperm pooled from three to four males held at 35‰ salinity. Fertilised eggs were viewed under a dissecting microscope and the viability (division to 2–8 cell stage) of the first 300 eggs encountered was recorded. Males were stripped by wiping dry the genital duct region and milt expressed using slight abdominal pressure. The first portion of stripped milt was not collected to ensure milt was not contaminated with urine. Milt was collected while being expressed into dry 5-ml syringes and placed on ice until use. Sperm motility was assessed by mixing a drop of freshly stripped milt with a drop of water at 5‰, 20‰ or 35‰ in a cavity slide. A coverslip was quickly placed over the cavity and sperm motility observed under a microscope. Motility was assessed within 10 s of activation and ratings assigned as: high, very active all sperm visibly progressing rapidly across the field of view; medium, less energetic movement, most with forward motion; low, slow movement, some spermatozoa progressing slowly or swimming in a spiral motion; or not activated, no swimming activity. Sperm was collected from three males held at 35‰ salinity, and sperm motility ranked at 1-min intervals over 5 min. The salinity at which sperm motility was first initiated was also determined for the three males by testing sperm motility at 1‰ intervals between 5‰ and 10‰.

2.4. Egg incubations

Fertilised eggs were collected from a group of naturally spawning bream held at 35 ‰ salinity. Between 5 and 11 eggs were pipetted into each well of a 24-well plastic tissue culture plate (Corning) using a modified pipette tip. The eggs were viewed under a dissecting microscope, and the number of fertile eggs (division to 2–8 cell stage) in each well was recorded. Eggs were then incubated in 1 ml water of salinity 0‰ (distilled water), 5‰, 10‰, 15‰, 20‰, 25‰, 30‰ or 35‰ (six replicates per salinity) at 20°C without light. The number of developing eggs, and hatched larvae was recorded on days 1 and 2, respectively, and the proportions of abnormal larvae were noted. Incubations were run from four separate spawnings. As we did not have unhandled naturally spawning fish held at 20‰ or 5‰, we could not conduct egg incubation experiments at these salinities.

2.5. Steroid measurement

Plasma steroid concentrations were measured by radioimmunoassay, using the reagents and protocols given in Pankhurst and Carragher (1992) for E_2, T, 17,20βP and cortisol, and Pankhurst and Kime (1991) for 11KT. Extraction efficiency was determined by recovery of [^{3}H]-labelled steroid extracted with plasma, and assay values were corrected accordingly. Assay detection limits in plasma were 0.15 ng ml^{-1} for E_2, T and 17,20βP, and 0.3 ng ml^{-1} for cortisol. Values that were below the detection limit

Table 1
Percentage of mature male (spermiated) and female (vitellogenic) fish maintained in water of 5‰, 20‰ or 35‰ salinity
nm = not measured.

Treatment	Aug	Sep	Nov
Males			
5‰	83	83	100
20‰	75	100	100
35‰	90	90	100
Females			
5‰	nm	nm	87.5
20‰	nm	nm	100
35‰	nm	nm	100

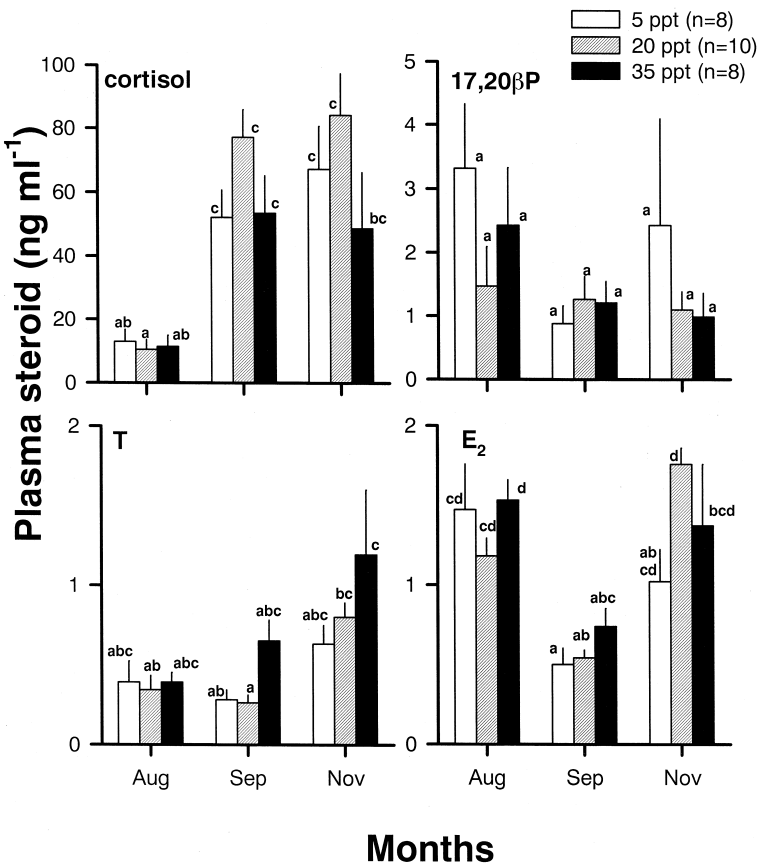

Fig. 1. The effect of salinity on plasma steroid levels (mean + se) in maturing captive female black bream. Values that are significantly different have different superscripts ($P < 0.05$).

were treated as being equal to the detection limit. Interassay variability (%CV) measured using aliquots of a pooled standard was as follows: $E_2 = 9.8\%$ ($n = 5$); T = 6.8% ($n = 6$); 17,20βP = 7.1% ($n = 6$), 11KT = 9.9 % ($n = 3$), and cortisol = 20.7% ($n = 6$).

2.6. Statistics

Kruskal–Wallis, one-way ANOVA and Tukey's multiple comparison of means tests were performed using the SPSS statistical package. Percentage data were arcsin transformed, and steroid data log transformed to satisfy homogeneity of variance requirements. As some data were in violation of the assumption of independence and, in some instances, homogeneity of variances, the significance level for one-way ANOVAs was set at 0.01 and the data were also assessed by Kruskal–Wallis one-way ANOVA. The statistical outcomes were the same regardless of whether parametric or non-parametric tests were applied. We chose to present the ANOVA results because of the utility of

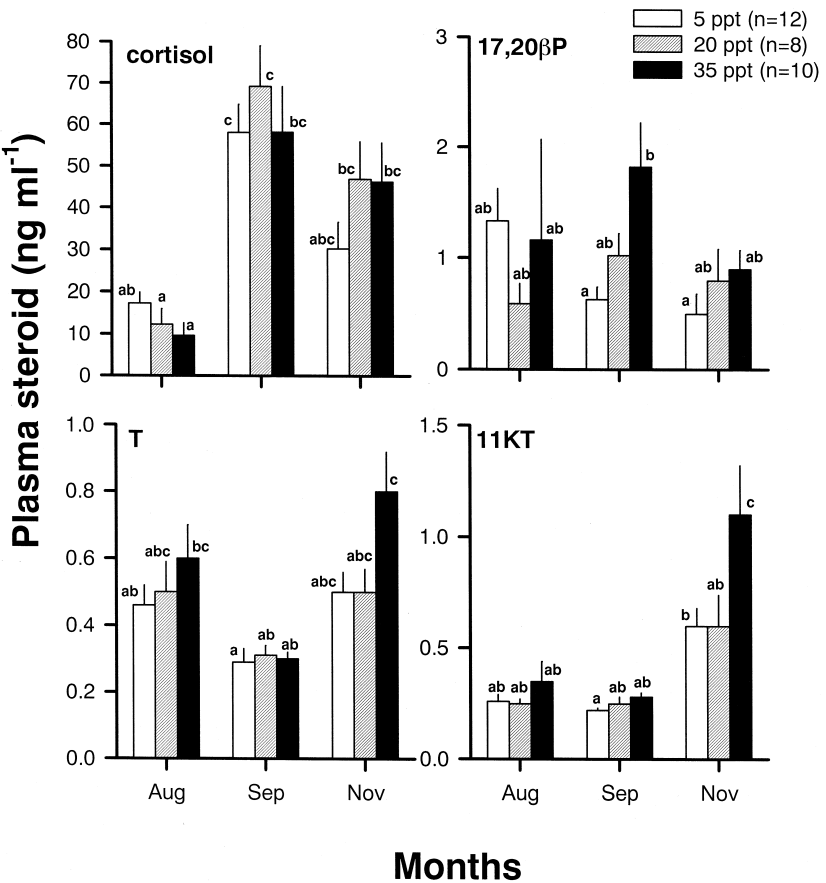

Fig. 2. The effect of salinity on plasma steroid levels in maturing captive male black bream. Other details as for Fig. 1.

Table 2
Summary of the proportions of fish ovulating and quantity of eggs produced from fish maintained in water of 5‰, 20‰ or 35‰ salinity and injected with LHRHa

Treatments*	Days (PI)						Totals
	0	1	2	3	4	5	
Ovulating fish (%)							
5‰ LHRHa	0	0	60	80	20	40	80
20‰ LHRHa	0	0	80	100	100	75	100
35‰ LHRHa	0	0	80	100	100	40	100
Total egg volumes (ml kg^{-1})							
5‰ LHRHa	0	0	23.2	72.1	35.0	5.9	136.2
20‰ LHRHa	0	0	36.9	373.4	237.8	44.9	693.1
35‰ LHRHa	0	0	113.2	283.3	274.9	88.3	759.7

* No saline-treated fish ovulated.

means comparison tests ($P < 0.05$), the ability of the tests to handle a decrease in sample numbers over time and the common use of these tests elsewhere.

3. Results

3.1. The effect of salinity on seasonal gonadal development and plasma steroid levels

Salinity did not affect the proportions of spermiated males, with a high percentage of males already spermiating by August and all males spermiating by November (Table 1.).

Table 3
The effect of salinity on egg fertilisation from fish maintained in water of 5‰, 20‰ or 35‰ salinity

Holding salinity	Fertilising salinity		
	35‰	20‰	5‰
5‰ ($n = 2$)	14.5 ± 8.2	14.8 ± 12.8	0 ± 0
20‰ ($n = 9$)	38.9 ± 10.7 ab	41.3 ± 10.8 b	7.4 ± 5.2 a
35‰ ($n = 13$)	65.8 ± 4.9 b	57.9 ± 7.8 b	19.7 ± 5.8 a

Table 4
Summary of sperm activity at 5‰, 20‰ and 35‰
H = high, M = medium, L = low and N = not activated.2

Salinity	Time (min)					
	0	1	2	3	4	5
5‰	N	N	N	N	N	N
20%	H	H	M	M	M–L	L
35‰	H	H–M	M	L	L	L

Similarly, ovarian recrudescence was not affected by salinity with only one fish failing to undergo vitellogenesis. Concentrations of plasma cortisol were lowest in August and high in September and November; however, in fish held at 35‰, cortisol levels in November were not significantly elevated over cortisol levels in August (Fig. 1). Mean plasma E_2 levels were high in August and November but low in September; however, plasma E_2 levels in fish held at 5‰ and 35‰ in November were not significantly elevated over plasma E_2 levels in September. Plasma T levels remained unchanged except in fish held at 20‰ where plasma T levels in November were significantly higher

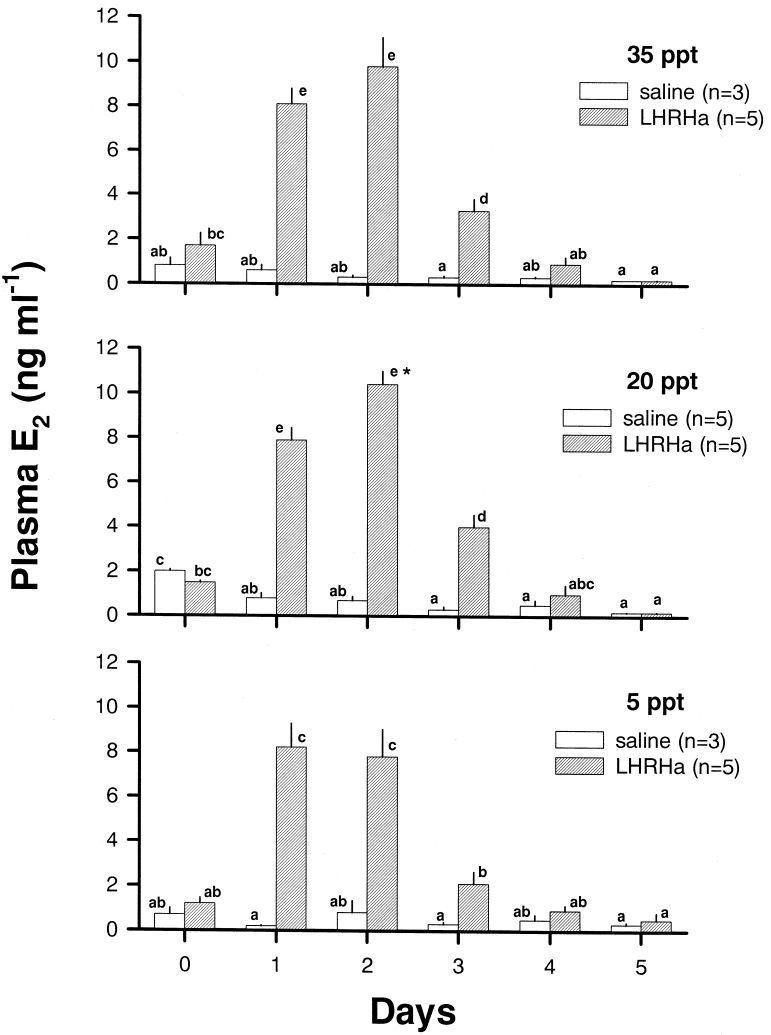

Fig. 3. Plasma E_2 concentrations in female black bream maintained at 5‰, 20‰ or 35‰ salinity and injected with either saline or 50 μg kg^{-1} LHRHa. * Indicates mortality, $n = 4$ thereafter. Other details as for Fig. 1.

than plasma T levels in September. Plasma 17,20βP levels remained unchanged throughout.

Concentrations of plasma cortisol in males were low in August and high in September and November; however, in fish held at 5‰, cortisol levels in November were not significantly elevated over cortisol levels in August (Fig. 2). Plasma T levels were not significantly different from August to November in fish held at 5‰ or 20‰; however, in fish held at 35‰, plasma T levels in November were significantly elevated over plasma T levels in September. Plasma 11KT levels were low in August and September and

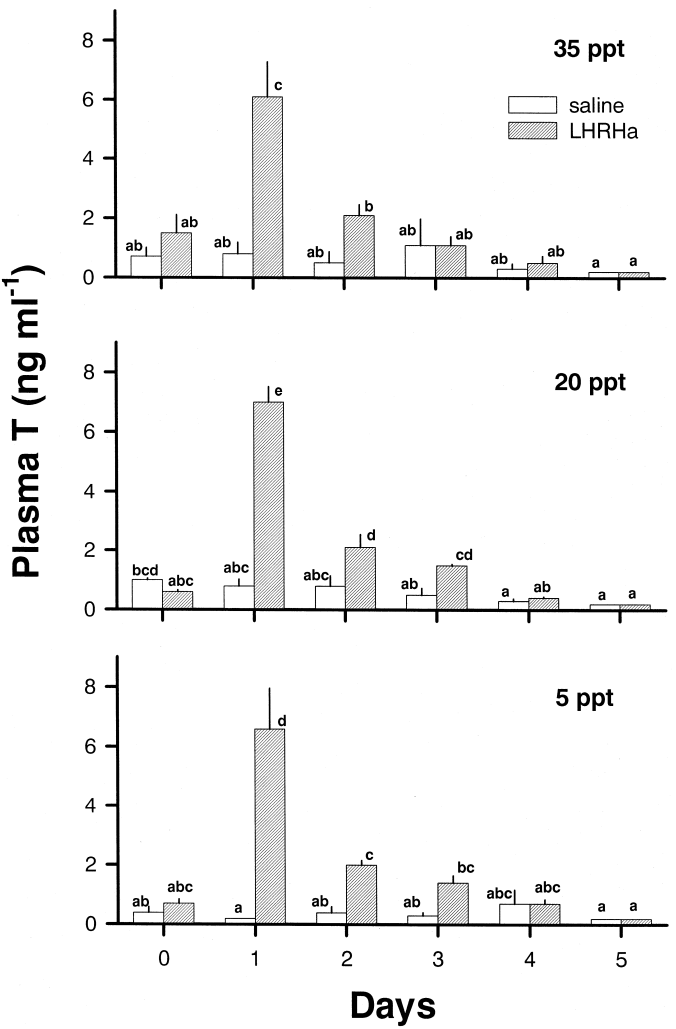

Fig. 4. Plasma T concentrations in female black bream maintained at 5‰, 20‰ or 35‰ salinity and injected with either saline or 50 μg kg^{-1} LHRHa. Other details as for Figs. 1 and 3.

peaked in November; however, in fish held at 20‰ this increase was not significant. Plasma 11KT levels in November were higher in fish held at 35‰ than in fish held at 5‰ or 20‰. Plasma 17,20βP levels were not affected by salinity in August or November, but were higher in September in fish held at 35‰ than in fish held at 5‰.

3.2. The effect of salinity on induced ovulation, plasma steroid levels, fertilisation and sperm motility

Ovulations first occurred 2 days post injection in all three salinities (Table 2). No saline injected fish ovulated. One fish, held at 5‰, failed to ovulate in response to

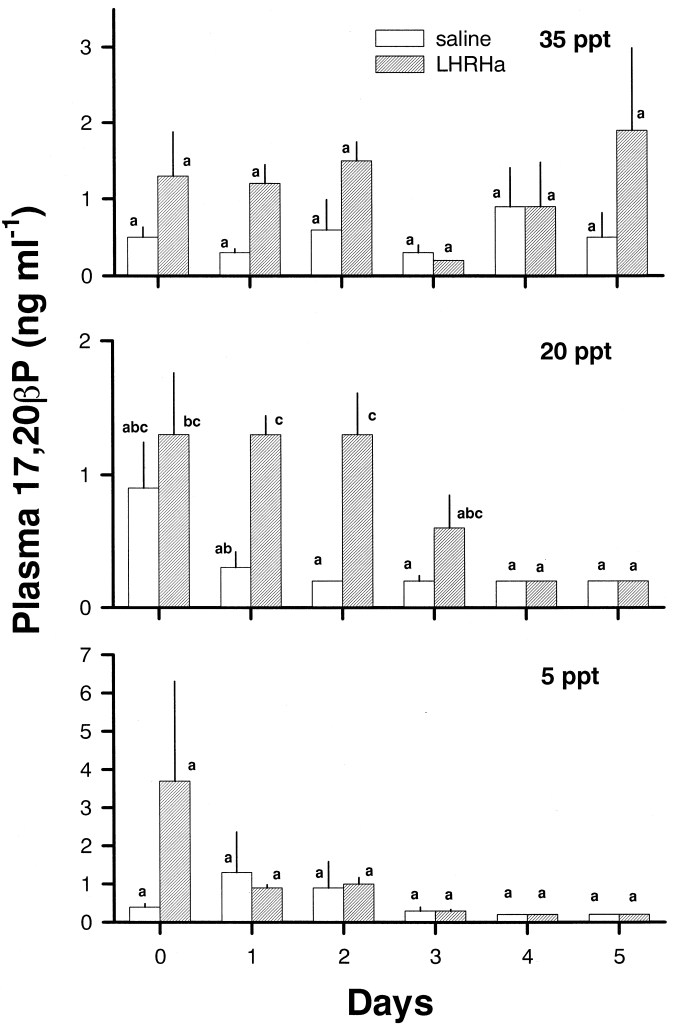

Fig. 5. Plasma 17,20βP concentrations in female black bream maintained at 5‰, 20‰ or 35 ‰ salinity and injected with either saline or 50 μg kg^{-1} LHRHa. Other details as for Figs. 1 and 3.

LHRHa injection. All fish injected with LHRHa and held in 35‰ and 20‰ serially ovulated with a mean of 3.2 and 3.5 ovulations per fish, respectively, whereas LHRHa treatment of fish in 5‰ resulted in three out of five fish serially ovulating with a mean of two ovulations per fish. In fish injected with LHRHa, total egg production was low in fish held at 5‰ and high in fish held in 20‰ or 35‰ (statistical comparison not made due to egg production being a mixture of spontaneously released eggs and manually stripped eggs). Egg fertility in fish held at 35‰ was significantly reduced when eggs were fertilised at 5‰ (Table 3). A similar trend was also evident in eggs from fish held at 5‰ (statistical comparison not made due to small sample size). In eggs from fish held at 20‰, fertility was higher at 20‰ than 5‰ but there was no difference between 35‰ and 20‰, or between 35‰ and 5‰. Initial sperm motility was high when sperm was activated by water of 20‰ or 35‰, with sperm activity decreasing within 1 to 2 min (Table 4). Sperms were not activated at a salinity of 5‰, with sperm motility being initiated at 6‰, 7‰ and 10‰ for the three males tested (data not shown).

Plasma cortisol concentrations were high (ranging from 29.1 to 96.8 ng ml^{-1}) at the time of injection and showed no change over time in all treatments (data not shown). At all three salinities, treatment with LHRHa significantly increased plasma estradiol levels over controls on days 1–3 post injection (Fig. 3). In fish held at 20‰ and injected with saline, plasma E_2 levels were significantly suppressed by day 1 and remained low thereafter; however, in saline-injected fish held at 5‰ or 35‰, plasma E_2 levels were

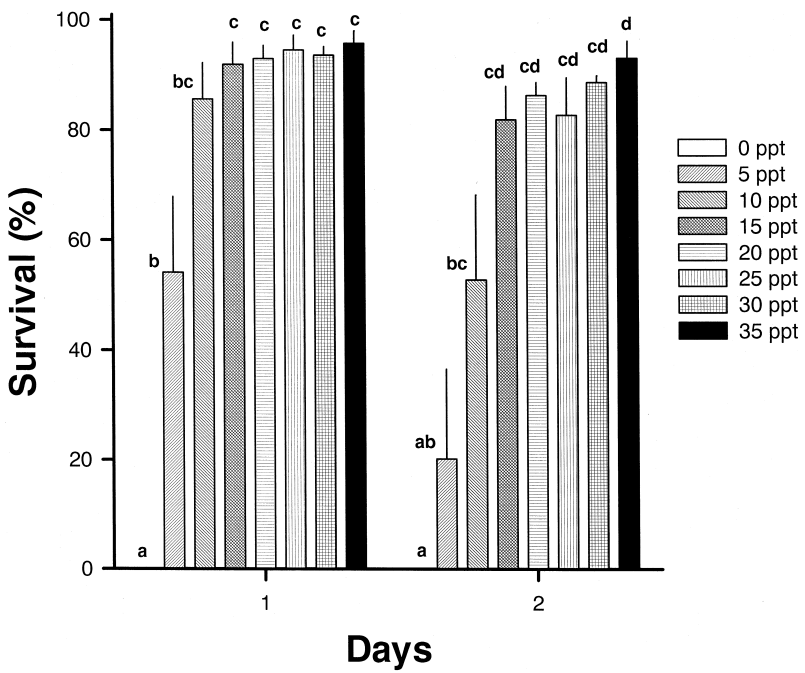

Fig. 6. The effect of salinity on black bream egg survival (day 1) and hatching (day 2). Values are mean + se ($n = 4$). Values with common superscripts are not different ($P > 0.05$).

low at the time of injection and remained unchanged. Plasma T levels in LHRHa injected fish peaked on day 1 at all salinities and were significantly elevated over controls on days 1 and 2, days 1–3, and day 1 in fish held at 5‰, 20‰ and 35‰, respectively (Fig. 4). In saline-injected fish, plasma T levels followed a similar pattern to E_2 except in fish held at 20‰ where plasma T levels were not significantly suppressed until days 4 and 5. Plasma 17,20βP levels were variable and not affected by LHRHa treatment in fish held at 35‰ or 5‰; however, in fish held at 20‰, plasma 17,20βP levels were elevated over controls on days 1 and 2 (Fig. 5).

3.3. The effect of salinity of egg development and hatching

The incubation salinity significantly affected both egg survival (day 1) and survival to hatch (day 2) (Fig. 6). Egg survival on day 1 was high in eggs incubated in water of 10‰–35‰, whereas eggs incubated in 5‰ had a significantly lower survival than at salinities above 10‰. No eggs survived when incubated in distilled water. Eggs hatched on day 2 in salinities from 5‰ to 35‰. Survival-to-hatch was high in eggs incubated from 15‰ to 35‰, whereas survival-to-hatch at lower salinities (10‰ and 5‰) was lower. Abnormalities of larvae at hatching were observed at all salinities where eggs hatched, and were characterised by curvature of the spine and tail flexure. All larvae that hatched at 5‰ were abnormal (Fig. 7). Normal larvae first appeared at 10‰ with the

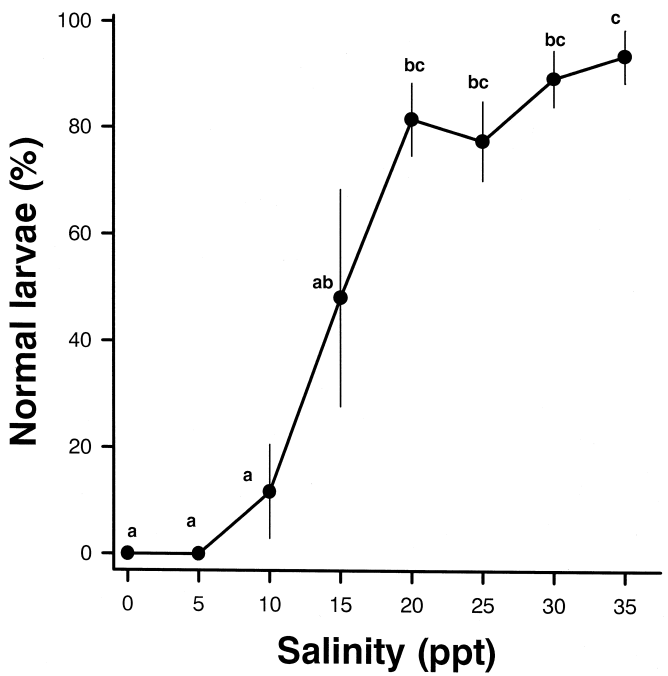

Fig. 7. Proportions of normal black bream larvae hatched from eggs incubated at salinities of 0‰–35‰. Other details as for Fig. 6.

proportions of normal larvae being highest at salinities from 20‰ to 35‰. Eggs incubated in 15‰ resulted in highly variable proportions of normal larvae with larval deformities ranging from 5‰ to 93%.

4. Discussion

Capture stress is known to rapidly inhibit reproductive activity and initiate ovarian atresia in many sparids including black bream (Carragher and Pankhurst, 1991; Cleary, 1998; Haddy and Pankhurst, 1999). The success of using wild caught fish as prospective broodstock is, therefore, dependent on the recovery from the stress of capture and captivity before the next cycle of gametogenesis (reviewed in Pankhurst, 1998). Cleary (1998) reported that wild snapper *Pagrus auratus* held for 5 years showed little evidence of acclimation to captivity. In contrast, results from the present study show that wild black bream will acclimatise to captivity, and undergo gonadal development within their first year of capture. Cortisol profiles in the present study indicate that captive black bream are still highly sensitive to stress, with handling resulting in the elevation of plasma cortisol and temporary suppression of gonadal steroids in both sexes. However, when fish were maintained with minimal disturbance (no handling from September to mid-November), plasma sex steroid levels either recovered or began to increase. This highlights that stress management is a key factor in the success of broodstock maintenance in stress-sensitive species such as black bream.

There is relatively little information on the effects of salinity on ovarian recrudescence and reproductive physiology in fish. Vitellogenin synthesis can be induced by estradiol administration in both freshwater and saltwater adapted eels *Anguilla anguilla* (Petersen and Korsgaard, 1989). Tamaru et al. (1994) showed that female striped mullet *Mugil cephalus* initiated vitellogenesis at salinities ranging from 0‰ to 35‰, but females maturing in freshwater exhibited a slower rate of oocyte growth, with a significantly lower number of females completing vitellogenesis. In contrast, Zanuy and Carrillo (1984) reported that changes in oocyte diameter and the time of gonadal recrudescence were similar in sea bass *Dicentrarchus labrax* reared at 3.5‰ or 37.8‰. In the present study, salinity did not apparently affect the plasma steroid levels or ovarian development in female black bream. Therefore, the initiation and maintenance of vitellogenesis in black bream appear to be more dependent on the classical cues of photoperiod and temperature rather than salinity.

Results from the present study demonstrate that black bream are responsive to LHRHa treatment at salinities ranging from 5‰ to 35‰. LHRHa treatment in black bream caused the short-term elevation of plasma E_2 and T and resulted in ovulations at all three salinities. In repeat spawning species, hormonal treatment aids the induction of multiple ovulations by the maintenance of development of successive clutches of vitellogenic oocytes (Morehead et al., 1998; Haddy and Pankhurst, in preparation). Therefore, our results are consistent with the established roles of plasma E_2 and T in ovarian recrudescence (Pankhurst, 1998), and demonstrate that reproductive endocrine processes remain intact in black bream over a wide range of salinity. In contrast to LHRHa-treated fish, saline-injected fish did not ovulate, and E_2 and T levels either

remained low or became significantly suppressed. The effects of daily handling on plasma cortisol levels and plasma E_2 and T levels in saline-injected fish are consistent with the effects of stress on reproduction in this and other species (Carragher and Pankhurst, 1991; Clearwater and Pankhurst, 1997; Cleary, 1998; Haddy and Pankhurst, under review).

Although 17,20βP levels in black bream show seasonal peaks during the spawning season and have been associated with final oocyte maturation (Haddy and Pankhurst, 1998), 17,20βP has not yet been conclusively identified as the maturational inducing steroid. In the present study, plasma levels of 17,20βP were unaffected by LHRHa treatment in fish held in 5‰ and 35‰, whereas 17,20βP levels in fish held in 20‰ were significantly elevated over controls on days 1 and 2. The physiological relevance of this finding is currently unclear, as the differences in 17,20βP levels were not associated with a marked difference in the proportions of fish ovulating or the volume of eggs produced. In stressed fish, 17,20βP levels are highly variable and are linked to the stress response, with stress-induced increases in 17,20βP levels being thought to be of interrenal origin (Haddy and Pankhurst, 1999). Therefore, in common with our previous work on induced ovulation in black bream (Haddy and Pankhurst, in preparation), 17,20βP levels in black bream appear to be an ambiguous marker of impending ovulation in hormone-treated fish.

Although there was no marked differences in the endocrine response to treatment with LHRHa treatment at differing salinity in female black bream, the proportions of ovulating fish, the number of serial ovulations and the volume of eggs produced were all lower in fish held at 5‰ than in fish held at higher salinities. Zanuy and Carrillo (1984) showed that although sea bass matured in low salinities, spawning did not take place unless the fish were transferred to sea water. Striped mullet can be induced to spawn over a salinity range of 0‰–35‰, however, no fertilised eggs were obtained in freshwater (Lee et al., 1992). Results from the present study show that the induction of final oocyte maturation and ovulation can be achieved over a wide salinity range in black bream; however, the efficacy of LHRHa treatment is reduced at low salinities (5‰). The cause of this effect is unknown.

In male black bream, T and 11KT are associated with spermatogenesis, whereas 17,20βP increases with spermiation (Haddy and Pankhurst, 1998). In the present study, plasma T levels were unaffected by salinity, whereas plasma levels of 17,20βP and 11KT varied with salinity in September and November, respectively. However, these differences had little apparent influence on the state of maturity of fish held at different salinities. This suggests that male black bream have the capacity to synthesise sufficient steroids for testicular development and milt production over a salinity range of 5‰–35‰.

The percentage of fertilised black bream eggs was significantly reduced at 5‰, suggesting that the viability of either the egg and/or the sperm is impaired at low salinity. Several studies have demonstrated a reduction in percentage of fertilised eggs at low salinities in euryhaline and stenohaline fish (Holliday, 1969; Lee et al., 1992; Hart and Purser, 1995). Results from the present study clearly demonstrate that sperm activity is impaired at low salinities, with sperm motility being lost between 6‰ and 10‰. Similar findings have also been reported for other teleosts including other *Acanthopagrus* species (Harris, 1986; Lee et al., 1992; Thorogood and Blackshaw, 1992; Palmer et

al., 1994; Litvak and Trippel, 1998). Palmer et al. (1994) showed that in pikey bream *Acanthopagrus berda*, sperm motility was most intense at salinities of 25‰–35‰ and the duration of activity was longest at salinities above 15‰. Results from the present study indicate that intense sperm motility is short lived, and drops within 5 min. This is consistent with studies on black porgy *Acanthopagrus schlegeli*, where sperm activated with artificial sea water exhausted their energy supply within 5 min (Gwo, 1995).

In the present study, naturally spawned eggs were removed from the spawning salinity of 35‰, and placed into the incubation salinity at the 2–8 cell stage. At this stage of development, the osmoregulatory capacity of the fertilised egg is one of resistive maintenance, achieved through a tight plasma membrane and limited transmembrane water and ion fluxes (Alderdice, 1998). Lee and Menu (1981) demonstrated that in striped mullet, the naturally spawned fertilised eggs transferred at the gastrula stage were more tolerant to salinity change than those transferred at the two-blastomere stage. In the present study, no eggs incubated at 0‰ survived and only 54% of eggs survived to day 1 (neurula stage) when incubated at 5‰, and egg survival was highly variable. By day 2, the percentage of hatched larvae was high ($>80\%$) and unaffected by salinity from 15‰ to 35‰. Similarly, fertilised eggs of Australian bass, *Macquaria novemaculeata*, cease developing within 2–3 h post transfer to fresh water, and at 5‰ and 10‰, only a small percentage of larvae hatch, but hatching success increases to above 75% when eggs are incubated at salinities of 15‰–35‰ (Van Der Wal, 1985). It has been suggested that failure to successfully hatch at low salinities results from poorly developed tail musculature and/or larvae finding it difficult to free themselves from the chorion (Holliday, 1969; Young and Dueñas, 1993). Results in the present study support this suggestion, as the hatching success of larvae dropped at low salinities and, in some cases, the larvae died in a partly emerged state.

In summary, this study has shown that black bream adapt well to captivity, but remain highly sensitive to stress and must be maintained with minimal disturbances. Under these conditions, reproductive development proceeds normally and is unchanged over a salinity range of 5‰–35‰. However, the ovulatory response to LHRHa is impaired at low salinity, with fish ovulating less frequently and producing smaller volumes of eggs in response to treatment. Fertilisation of eggs was significantly reduced at low salinity, most likely due to a reduction in sperm activity at 5‰. Finally, fertilised black bream eggs developed over a wide range of salinities with viable larvae being produced over a salinity range of 10‰–35‰; however, the proportion of viable larvae was highest at salinities above 20‰. These result suggest that some natural recruitment could occur in saline lakes with salinities above 10‰, and highlight that black bream is a potential candidate for the establishment of an inland mariculture industry.

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