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# The Effects of Oestradiol on the Prolactin and Growth Hormone Content of the Pituitary of the Tilapia, *Oreochromis mossambicus*, with Observations on the Incidence of Black Males

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**ABSTRACT**—In a preliminary experiment, male *Oreochromis mossambicus* which received silastic implants of oestradiol ($E_2$; 10, 50 or 125 μg/g body weight) had elevated serum $E_2$ levels 14 days later, compared with those receiving blank implants. In two subsequent experiments, groups of 15 males received either blank implants or one of these three doses of $E_2$, and were then transferred to 33% seawater; they were either maintained in this medium for the subsequent 10 days, or they were transferred back to freshwater on the day after implantation and maintained in the latter for the remaining nine days. There was evidence for a dose-independent increase in the proportion of black males in $E_2$-treated groups, regardless of salinity, implying increased territorial aggression. Subsequent to the 10-day exposure, polyacrylamide gel electrophoresis of their pituitaries was used to quantify levels of growth hormone (GH) and the large and small forms of prolactin (PRL). When comparing groups receiving blank pellets, levels of GH and both PRLs were greater in fish maintained for most of the post-operative period in freshwater. There was a dose-dependent increase in the pituitary content of all three hormones in fish receiving implants of $E_2$; this was most marked for the smaller of the two forms of PRL in fish adapted to freshwater.

## INTRODUCTION

Prolactin (PRL) and growth hormone (GH) belong to the same family of pituitary peptide hormones, and appear to be present in all gnathostome vertebrates (Kawauchi *et al.*, 1990). There is evidence for polymorphism of some members of this peptide family in certain species: for example, the teleost *Oreochromis mossambicus* has two distinct forms of PRL, both produced by the same pituitary cell-type (Specker *et al.*, 1985, 1993; Nishioka *et al.*, 1993). Various functions have been ascribed to members of the PRL-GH family in teleost fishes, especially in the context of growth and osmoregulation (e.g. Bern, 1983; Hirano, 1986; Madsen and Bern, 1992; Yada and Hirano, 1992; Wendelaar Bonga, 1993; Sakamoto *et al.*, 1993; Borski *et al.*, 1994; Yada *et al.*, 1994). In addition, there is evidence implicating these hormones in the stimulatory regulation of gonadal function in one or both sexes of various teleosts (e.g. Tan *et al.*, 1988; van der Kraak *et al.*, 1990; le Gac *et al.*, 1992; Rubin and Specker, 1992; Singh and Thomas, 1993; reviewed by le Gac *et al.*, 1993).

*O. mossambicus* has proved a fruitful model for the study of the hypothalamic and other regulatory mechanisms involved in the control of the synthesis and secretion of PRL(s) and GH by the teleost pituitary. *In vitro* studies with this species have identified a variety of different hypothalamic peptides and other modulators of synthesis and/or secretion, some of whose effects vary with medium osmolality (reviewed by Nishioka *et al.*, 1988; Grau and Helms, 1989).

In keeping with a role in reproduction, there are reports that the cell-types producing GH or PRL are subject to (positive) feedback control by gonadal steroids in various teleosts (Nishioka *et al.*, 1988; Wong *et al.*, 1993; Williams and Wigham, 1994; Huggard and Habibi, 1995; Peter and Yu, 1997). In the case of *O. mossambicus*, Wigham *et al.* (1977) reported that oestradiol ($E_2$) stimulated the *in vitro* synthesis, but not the release, of the smaller PRL (the only PRL identified then) in hyperosmotic medium (the only medium tested); this steroid may also modulate the response of these cells to TRH (Barry and Grau, 1986). Although plasma GH levels have been reported to be generally higher in female than in male *O. aureus* (Mol *et al.*, 1994), there is some evidence for the opposite in hybrids of this species with *O. niloticus*, where males show diurnal peaks not detected in females (Melamed *et al.*, 1995). Recently, Melamed *et al.* (1995) reported that prior treatment with methyltestosterone (MT) resulted in an increase in the plasma GH levels of male hybrids. In part, this may be through

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enhancing the sensitivity of the pituitary GH cells to hypothalamic stimulation: whilst an analogue of salmon gonadotrophin releasing hormone (sGnRHa) was able to stimulate GH secretion in mature male tilapia hybrids, it was ineffective in those with regressed testes unless they had been pretreated with MT (Melamed *et al.*, 1995). Similarly, *in vitro* pre-incubation with MT or $E_2$ enhanced the subsequent responsiveness to sGnRHa of the GH cells of pituitaries from regressed male hybrids (Melamed *et al.*, 1995).

The primary aim of the present short report was to determine the effects of elastomer implants of $E_2$ on the pituitary content of GH and both PRLs in *O. mossambicus*: such implants have proved to be a reliable means of inducing prolonged, stable elevations in plasma $E_2$ levels in other teleosts (Pankhurst *et al.*, 1986). This would thus extend the findings of Wigham *et al.* (1977), by determining whether $E_2$ also affects the production of the larger isoform of PRL; and those of Melamed *et al.* (1995), by investigating whether this steroid, like MT, also affects GH production *in vivo*. Since the experiments were done prior to the availability of radioimmunoassays for tilapia GH or the PRLs, we had to rely on measuring their pituitary contents using polyacrylamide gel electrophoresis (Barry and Grau, 1986; Borski *et al.*, 1992).

A secondary aim of the present experiments was to make a preliminary study of the behavioural effects of $E_2$-implants. As with other cichlid teleosts, *O. mossambicus* shows increased aggression associated with reproductive maturation; this is mainly restricted to males, which show evidence of lekking (Neil, 1964; Munro and Singh, 1986). Whilst studies on other cichlids suggest that steroid hormones may be partly responsible for this increase (reviewed by Liley and Stacey, 1983; Munro and Pitcher, 1983, 1985), Billy and Liley (1985) found no clear short-term effect of dietary MT on the behaviour of adult male *O. mossambicus*. Thus we also sought to determine whether elevated $E_2$ may also influence male aggressiveness, as assessed by the incidence of black (territorial) males in the late afternoon (Munro and Singh, 1986).

## MATERIALS AND METHODS

Mature male *Oreochromis mossambicus* were obtained from Jurong Bird Park and maintained in large fibre-glass holding tanks under ambient conditions.

Experimental fish were randomly distributed between the various treatments, with the proviso that each tank had approximately the same length distribution. Groups of similarly-treated males were housed in fibre-glass tanks (94 × 66 × 90 cm deep, containing 250 l water with aeration), set up in a quiet area under an open shelter. They were fed every morning.

### Steroid implants

This was based on Pankhurst *et al.* (1986). Oestradiol-17β ($E_2$; Sigma) was mixed with silastomer (Dow Corning; MDX4-4210) to give concentrations of 0, 10, 50 and 125 mg/g elastomer; accelerator (5 μl/g silastomer) was added, and the mixture placed in moulds (glass channels 72 × 2 × 2 mm deep) and left to harden. Thereafter, the surface excess was shaved away to give a standard square cross-section.

For implantation, fish were lightly anaesthetised in MS-222 (Sigma) and weighed. A slit was made in the left side of the body wall and the appropriate length of silastomer (1 mm/g body weight) was inserted; the wound was then closed with surgical silk. The fish were kept in ≈ 33% seawater (10 g/l hw-Marinemix; Wiegandt GmbH, Krefeld, FRG) for at least 24 hr to aid recovery.

### Experiment 1

This preliminary experiment aimed to determine the effects of implants on serum levels of $E_2$. Six fish were used for each dose, being kept in 33% seawater throughout. Fourteen days after implantation, the fish were bled from the caudal peduncle, and the blood processed for routine radio-immunoassay (Tan *et al.*, 1988).

### Experiment 2

Four groups of 15 fish (one for each dose of $E_2$) were maintained in 33% seawater for the duration of the experiment (10 days); a fifth group received blank implants and were maintained in 33% seawater for 30 hr before being transferred to freshwater. Four days before the end of the experiment, three-quarters of the top of each tank was covered with black polythene to minimise disturbance; for the following three days, the tanks were observed once daily, at about 5 p.m. (Munro and Singh, 1986), to count the number of black males.

### Experiment 3

All four groups in this experiment (15 fish for each dose of $E_2$) were kept in 33% seawater for only 24 hr; thereafter, this was replaced with freshwater and three-quarters of the top of each tank was screened off with black polythene. Each tank was observed once daily at about 5 p.m. for the duration of the ten day experiment.

### Analysis of pituitary hormones

At the end of experiments 2 and 3, the pituitaries were removed, pooled in groups of three and stored at −40°C. Extracts were prepared by sonication in 100 μl of alkaline physiological saline (pH 9.3) in an ice bath followed by centrifugation, as described by Barry and Grau (1986). The protein content of the pellet was measured after digestion with 0.1% SDS in 1 N NaOH, using a microassay (Bio-Rad) with BSA as a standard.

The electrophoretic separation of the extracts was done on gels (18 cm × 15 cm × 0.8 mm thick) using constant current of 40 mA with a discontinuous buffer system containing 0.1% SDS (Laemmli, 1970) at 4°C: the stacking gel was 4% polyacrylamide (pH 6.8), and the separating gel 15% (pH 8.8). Equal volumes of extract and sample buffer containing 5% mercaptoethanol (Sigma) were boiled for five minutes, with 30 μl being loaded onto the gel; one lane instead contained pre-stained low molecular weight markers (Pharmacia). At the end of the run, the gels were fixed in (25% isopropyl alcohol +10% glacial acetic acid) for 30 min, stained in 0.25% Coomassie blue R250 (freshly prepared from stock) for 15 min, and then destained for 2 hr in (50% methanol +10% glacial acetic acid). Fixation and subsequent steps were done on a rotary shaker, each being standardised to allow comparisons between gels. Destained gels were stored in 7% glacial acetic acid. Hormone levels in each lane were quantified using an Ultroscan XL enhanced laser densitometer (LKB) to measure peak area at 663 nm. Data were standardised by expressing them relative to the protein content of the pellet obtained when preparing the pituitary extract.

GH and the two PRLs were initially identified by Western blotting using extracts from other, trial fish run under electrophoretic conditions identical to the above with the exception of being at 20 mA constant current with one lane containing low molecular weight markers (Bio-Rad). At the end of the run, proteins from some gels were electroblotted onto nitrocellulose membranes for 2 hr at 40 V (18 mA/cm$^2$). After drying and washing, the membranes were incubated for 20 hr at 4°C with antisera against eel PRL, eel GH or bonito GH (diluted 1:5000); immunoreactive bands were detected by incubation with anti-rabbit IgG/peroxidase conjugate (1 hr at 25°C) followed by

reaction in the presence of 4-chloro-1-naphthol.

**Statistical analyses**

Since the data for serum and the pituitary hormone contents were parametric, they were analysed by ANOVA, followed by Duncan's multiple range tests. Data on the numbers of black males in experiment 2 were analysed by Kruskall-Wallis and Mann-Whitney U tests; ANOVA followed by Duncan's range test was used in the case of those for experiment 3. In each case, SPSS 4.0 was used.

## RESULTS

### The effects of elastomer implants on serum $E_2$ levels

Serum $E_2$ levels were significantly elevated in all groups receiving doped pellets in experiment 1; those receiving 50 or 125 μg/g pellets also had significantly higher serum $E_2$ levels than those receiving a 10 μg/g pellet (Fig. 1).

### Electrophoretic identification of GH and the two PRLs

The antiserum against bonito, but not that against eel, GH crossreacted with a single band of estimated molecular weight 23.5 kDa; this band was thus designated the GH band.

The antiserum against eel PRL crossreacted with two bands, corresponding to molecular weights of 22.5 and 25 kDa; immuno-staining was more intense for the 25 kDa band. These bands thus presumably correspond to the 20 and 24 kDa PRLs identified by Specker *et al.* (1985); they will be refered to as the "small" and "large" PRLs, respectively.

### Effects of $E_2$ on the pituitary content of GH and the two PRLs

#### Experiment 2

For fish receiving blank implants, pituitary GH content was higher in the fish maintained in freshwater, compared to those in 33% seawater ($P < 0.05$). Relative to their controls in 33% seawater, males treated with $E_2$ showed a dose-dependent increase in pituitary GH levels; the increase was significant in the case of treatment with 50 and 125 μg/g, where levels were comparable with freshwater controls (Fig. 2).

Both PRLs were present in the freshwater controls (with the smaller form tending to be more abundant), but neither was detectable in control males in 33% seawater (Fig. 2). Treatment with $E_2$ (50 and 125 μg/g) resulted in the appearance of measurable levels of the larger PRL in extracts in a dose-dependent fashion (Fig. 2). In addition, the highest dose of $E_2$ induced the accumulation of the smaller PRL to levels comparable with those of the larger (Fig. 2).

#### Experiment 3

The blank-implanted controls tended to have lower levels of each hormone than those transfered to freshwater in experiment 2 (Fig. 3). Treatment with $E_2$ resulted in a dose-dependent increase in pituitary contents of GH and both PRLs; the effect was most pronounced for the smaller PRL (Fig. 3).

### Effects of $E_2$ on the incidence of black males

Consistent with our previous observations, black males typically showed territorial behaviour and aggressive interactions with their tank-mates. When there were relatively more black males, some were semi-territorial (Chan and Ribbink, 1990).

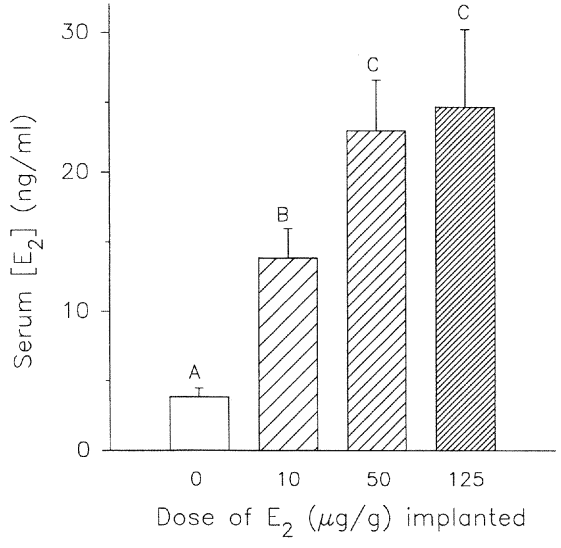


**Fig. 1.** The effects of different dosages of $E_2$ (0 - 125 μg/g) on serum $E_2$ levels of males maintained in 33% seawater for 14 days (mean + S. E.; N = 6 for each): *A*, *B* and *C* are significantly different from each other ($P < 0.05$).

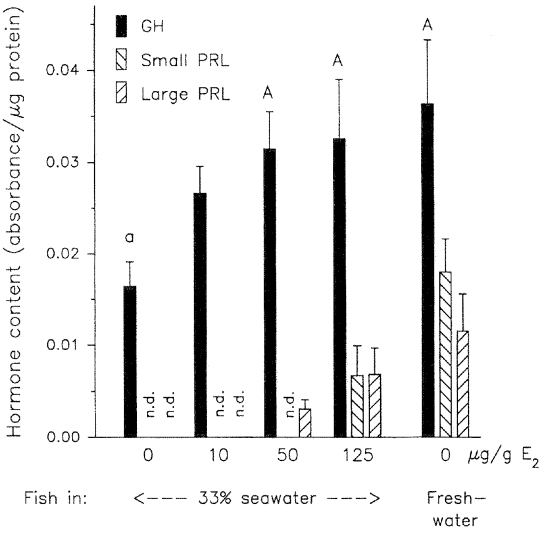


**Fig. 2.** Four groups were maintained in 33% seawater after receiving implants containing various doses of $E_2$; the fifth received blank implants, but were transfered back to freshwater the next day. Fish were sacrificed 10 days after implantation. There were five replicates for each treatment, each comprising three pituitaries. The graph shows the effects on pituitary hormone contents (n.d., not detectable); *A* and *a* are significantly different from each other ($P < 0.05$).

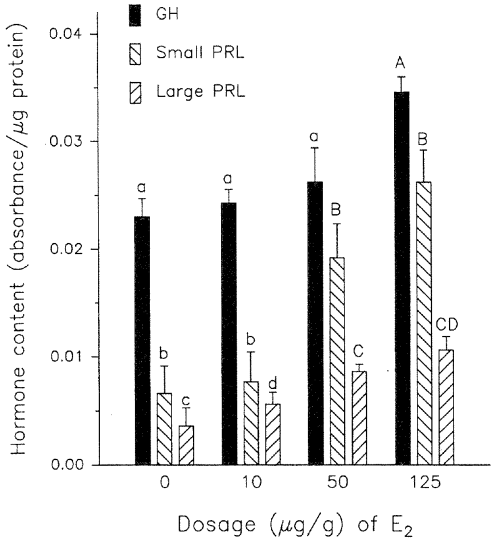


**Fig. 3.** Groups received varying doses of $E_2$, and were maintained in freshwater for all but the first day of the 10 day experimental period. There were five replicates for each treatment, each comprising three pituitaries. The graph shows the effects on pituitary hormone contents; columns surmounted by a capital letter are significantly different from those with a lower case label ($P < 0.05$).

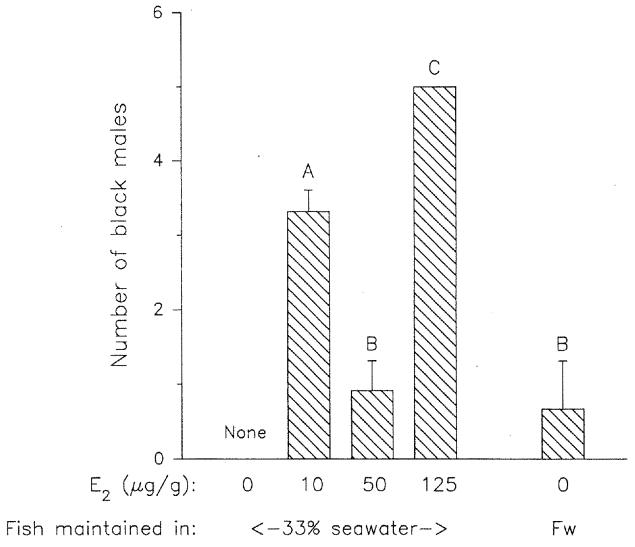


**Fig. 4.** Numbers of black males (mean ± S.E.) observed daily on days 7 - 9 after groups of 15 males were implanted with different dosages of $E_2$ and maintained in 33% seawater (experiment 2): an additional control group was transferred back to freshwater on the day after the implant operation. Groups with the same letter are not significantly different from each other ($P > 0.05$, Mann-Whitney U test).

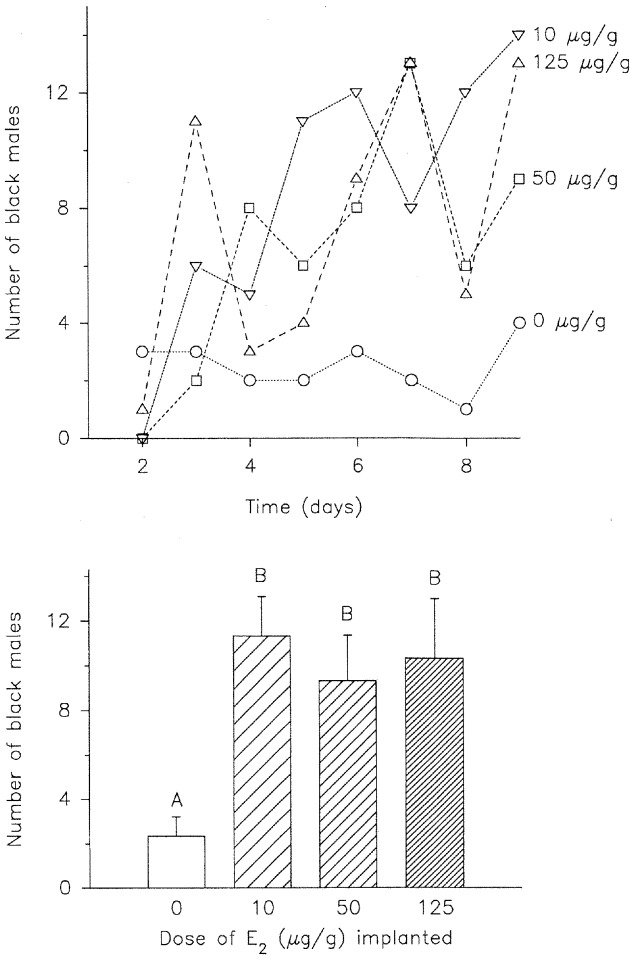


**Fig. 5.** (**top**) Daily fluctuations in the numbers of black males in groups of 15 males implanted with different dosages of $E_2$: all groups were transferred back to freshwater on the day after the implant operation (experiment 3). (**bottom**) Numbers of black males (mean ± S.E.) observed daily on days 7 - 9 for the same experiment. Groups with the same letter are not significantly different from each other ($P > 0.05$, Duncan's test).

## Experiment 2

There were significant differences between groups in the number of black males 7 - 9 days after the start of the experiment (Fig. 4). Amongst the controls (0 μg/g $E_2$), no black males were observed in the group maintained in 33% seawater, whereas an average of about 1 black male was observed in the freshwater tank. Implants of $E_2$ had an inconsistent effect, in that there was no clearcut dose dependence (Fig. 4): the groups treated with the lowest and highest dose of steroid showed a significant increase in the number of black males ($P < 0.05$), whereas that treated with the intermediate dose was comparable with the freshwater out-group (which received blank implants).

## Experiment 3

Throughout the eight days of observation, controls showed low, relatively stable numbers of black males (Fig. 5). Amongst the three groups receiving implants containing $E_2$, on the other hand, there was a general trend for a progressive, seemingly dose-independent increase in the number of black males during the course of the experiment (Fig. 5, top). Considering only the data for the last three days of observation (cf. experiment 2), all three $E_2$-treated groups showed significantly more black males than were recorded for blank

controls (Fig. 5, bottom).

## DISCUSSION

As reported by Pankhurst *et al.* (1986) for goldfish, elastomer implants resulted in elevated serum levels of $E_2$ in male tilapia. These values fell within the physiological range for female tilapias in two studies on *O. niloticus* (Rothbard *et al.*, 1991; Tacon *et al.*, 1995), where levels were reported to rise from a baseline of about 30 ng/ml to 60 - 100 ng/ml during the latter stages of courtship (Rothbard *et al.*, 1991). On the other hand, Smith and Haley (1988) measured levels of less than 10 ng/ml throughout the reproductive cycle of female *O. mossambicus*. In contrast to Rothbard *et al.* (1991), the latter found testosterone (the precursor of $E_2$) to be higher at all stages of the ovarian cycle, peaking at the end of vitellogenesis. Similarly, Mol *et al.* (1994) reported $E_2$ levels of about 10 ng/ml in mature female *O. aureus*, with higher testosterone levels; plasma concentrations of each steroid were higher than those for males.

In the present experiments, the estimated molecular weights for the two PRLs (22.5 and 25 kDa) and GH (23 kDa) are slightly higher than previous estimates (respectively 20, 24 and 21 kDa; Specker *et al.*, 1985). However Western blots, using antisera which also showed a specific crossreaction with the appropriate cell-types in the pituitary of *O. mossambicus* (Sampath-Kumar and Munro, unpublished), confirmed the identity of the polypeptides in question.

### Effects of salinity on PRL and GH content

Experiment 2 indicated that the pituitary content of both PRLs was reduced in fish maintained in 33% seawater. This can be compared with other studies on tilapia, where levels of both PRLs were reduced in full-strength seawater relative to freshwater (Borski *et al.*, 1992; Ayson *et al.*, 1993), with a parallel decrease in their plasma levels (Ayson *et al.*, 1993). Together, these data are consistent with the hypothesis that the synthesis and secretion of PRL are depressed in non-hypo-osmotic environments (e.g. Dharmamba and Nishioka; 1968; Wendelaar Bonga and van der Meij, 1981; Grau and Helms, 1989).

The smaller PRL tended to be more abundant than the larger form in males from freshwater in both experiments, although the difference was not significant. This is consistent with a radioimmunoassay study on *O. mossambicus* (Ayson *et al.*, 1993). These findings contrast with those of Borski *et al.* (1992) for the same species, employing a technique similar to that used here to measure levels of both PRLs in individual pituitaries; they reported that the larger PRL was in excess in freshwater-adapted males, with the opposite for those adapted to seawater. This may be the result of differences between stocks, whether genetic or epigenetic.

As with the PRLs, pituitary GH levels were reduced in males maintained in 33% seawater for 10 days. The significance of this effect of near iso-osmotic conditions on GH content is not clear. Other reports for this species, involving transfer to full-strength seawater, indicate a trend for pituitary GH levels to decrease in fish of unstated sex after 3 - 4 weeks (Ayson *et al.*, 1993); whereas a longer exposure (7 weeks) led to a significant increase in pituitary GH in males (Borski *et al.*, 1994). On the other hand, the time course for plasma GH levels showed only transient changes (mainly seen in males) over the first few days after transfer between salinities (Yada *et al.*, 1994).

### Effects of $E_2$ on PRL and GH content

Pituitary GH content was elevated by treatment with $E_2$. Other studies have also suggested a stimulatory effect of $E_2$ on overall GH cell activity in various other teleosts (reviewed by Nishioka *et al.*, 1988; Huggard and Habibi, 1995), with the apparent exception of salmonids (le Gac *et al.*, 1993).

Implants of $E_2$ also increased levels of both PRLs in the pituitary. This is consistent with an early report that $E_2$ stimulated the *in vitro* synthesis of the smaller form of PRL (Wigham *et al.*, 1977): the larger form was not investigated in that study. The present results indicate that the accumulation of the smaller form of PRL in response to $E_2$ may be greater than that of the larger form, at least in fish returned to freshwater (Fig. 3). Other than the possible effects of environmental salinity (Borski *et al.*, 1992; cf. Ayson *et al.*, 1993 and present study), this would appear to be the first treatment to have a differential effect on pituitary levels of the two PRLs in tilapia: other studies have found that the synthesis and secretion of both PRLs is under a common, uniform control (e.g. Specker *et al.*, 1985; Nishioka *et al.*, 1988; Planas *et al.*, 1990; Grau and Helms, 1989).

In affecting the activity of the PRL and GH cells, $E_2$ may be acting at one or more of three different levels. First, $E_2$ may have direct effects on the endocrine cells of the pituitary itself, as suggested by *in vitro* studies (Wigham *et al.*, 1977; Barry and Grau, 1986). A high aromatase activity has been reported in the GH cells of tilapia (Callard *et al.*, 1988) and another percomorph (Olivereau and Callard, 1985), suggesting that not only $E_2$ but also certain androgens may have actions on these cells. Although there was no histochemical evidence for steroid receptors in the GH cells of a poeciliid (Schreibman *et al.*, 1982), this may reflect species differences or technical limitations: as in goldfish (Wong *et al.*, 1993; Peter and Yu, 1997), there is evidence that sex steroids can increase the responsiveness of the GH cells to hypothalamic control factors *in vitro* in hybrid male tilapia (Melamed *et al.*, 1995).

A second possibility is that $E_2$ could be acting less directly, on one or more populations of hypothalamic neurones responsible for regulating pituitary activity. Consistent with this, there is ultrastructural evidence that preoptic implants of $E_2$ are associated with increased activity of the GH and PRL cells in *O. mossambicus* (Singh, 1990).

Finally, $E_2$ may be acting more indirectly. One possibility is that, by stimulating the hepatic synthesis and secretion of vitellogenin (e.g. Ding *et al.*, 1989; Chan *et al.*, 1991), $E_2$ disturbs the osmotic gradient between the plasma and the external medium; this might be expected to lead to a restorative

increase in PRL secretion, in freshwater teleosts at least. However, this would seem unlikely to be the sole cause, in view of *in vitro* and hypothalamic implant studies (Wigham *et al.*, 1977; Barry and Grau, 1986; Singh, 1990; Melamed *et al.*, 1995). Instead, it may be that the latter more direct actions could be pre-emptive, normally acting in anticipation of elevations in plasma osmolality resulting from the other, vitellogenic actions of $E_2$.

The physiological significance of the present data on pituitary GH and PRL levels must remain unclear in the absence of information about circulating hormone levels and the secretory dynamics of their respective cell-types. Data from other studies on *O. mossambicus* (Ayson *et al.*, 1993; Borski *et al.*, 1994) indicate that (in contrast to *O. aureus* × *O. niloticus* hybrids: Melamed *et al.*, 1995) there is generally a positive relationship between hormone levels in the pituitary and those in the plasma. Clearly further studies are required to determine the relevance of the observed effects of $E_2$ on PRL and GH cell-function, and the possibility of differences between the sexes. Of particular interest is the possibility that GH, at least, may play a role in the modulation of $E_2$-induced vitellogenesis (anguillids: Burzawa-Gerard and Dumas-Vidal, 1991; Kwon and Mugiya, 1994): a positive feedback by $E_2$ on GH-secretion (as described for goldfish: Wong *et al.*, 1993; Peter and Yu, 1997) may thus serve to ensure optimal hepatic responsiveness to $E_2$ (we have evidence that the pituitary is necessary for full $E_2$-induced vitellogenesis in male *O. mossambicus*: Munro *et al.*, unpublished). The feedback effects of sex steroids may also be related to a possible role for PRL in gonad hydration (Lal *et al.*, 1995).

### Effects of $E_2$ on the incidence of black males

The fish used in this study could not be subject to rigorous behavioural observations. Although the latter are clearly required in order to more fully characterise the effects of $E_2$, using a greater number of replicates, we nevertheless feel that the present experiments provide preliminary evidence that steroids may modulate aggression in male tilapia.

Although the fish were from the same original batch, $E_2$ had a lesser, more variable effect on the numbers of black males in experiment 2 than it had in experiment 3. The reason(s) for this is not clear. One possibility is that the differences between the two experiments reflect the effects of salinity: Watanabe *et al.* (1988) suggested that the improved growth rates of tilapia kept at higher salinities may be partly the result of reduced agonistic interactions, and it might be worth noting that there is some tentative evidence for this in the blank-implanted controls in the present experiments.

The present data are thus consistent with the hypothesis that gonadal steroids may play a positive feedback role in the dynamic interplay between the central nervous system and the gonads (see Cardwell and Liley, 1991) of *O. mossambicus*, in order to influence aggressiveness and thus potential reproductive performance (Oliveira *et al.*, 1996). The lack of any clear evidence for a dose-dependent effect of $E_2$ presumably indicates that the lowest dosage used (which was likely to result in an approximate tripling of circulating $E_2$ levels: Fig. 1) already had a maximal behavioural effect. The fact that female tilapia are generally less aggressive than males, despite having higher levels of $E_2$ and also testosterone (Mol *et al.*, 1994), suggests that the effects of $E_2$ detected here may be a consequence of prior organisational effect of steroids on the brain in fry (Billy and Liley, 1985).

At first sight, the present results would appear to contradict those for another, neotropical cichlid (*Aequidens pulcher*), where Munro and Pitcher (1985) concluded that the stimulatory effects of exogenous testosterone (dissolved in the aquarium water) were not mediated by its prior aromatisation to $E_2$. Apart from differences between species, another possibility is that the regime used to treat *A. pulcher* with $E_2$ resulted in circulating supra-physiological (anaesthetic?) elevations. In contrast, the present study suggests that, if testosterone is also behaviourally active in *O. mossambicus*, then this may be mediated by its aromatisation: MT, a non-aromatisable androgen, had no effect on agonistic behaviour in adult tilapia (Billy and Liley, 1985).

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