Steroid hormones in bluegill, a species with male alternative reproductive tactics including female mimicry

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The proximate mechanisms underlying the evolution and maintenance of within-sex variation in mating behaviour are still poorly understood. Species characterized by alternative reproductive tactics provide ideal opportunities to investigate such mechanisms. Bluegill (Lepomis macrochirus) are noteworthy in this regard because they exhibit two distinct cuckolder (parasitic) morphs (called sneaker and satellite) in addition to the parental males that court females. Here we confirm previous findings that spawning cuckolder and parental males have significantly different levels of testosterone and 11-ketotestosterone. We also report, for the first time, that oestradiol and cortisol levels are significantly different between cuckolder morphs. We hypothesized that oestradiol or cortisol may contribute to the profound differences in reproductive behaviour between cuckolder morphs, we hypothesized that oestradiol or cortisol may thus influence the energetic costs of the various tactics (Knapp 2003), and the possibility that glucocorticoids may thus shed light on whether male morphs in particular species represent evolutionarily stable strategies or are instead ‘making the best of a bad situation’ (Gross 1996; Moore et al. 1998).

In the present study, we determined circulating levels of four steroid hormones in spawning male bluegill that adopt one of three ART’s. Males from our study population are characterized by a discrete life history called ‘parental’ and ‘cuckolder’ (Gross & Charnov 1980). Parental males mature at the age of 7 years and compete with one another for nesting sites in colonies, court and spawn with females, and provide sole parental care for the offspring (Gross 1982). Cuckolder males mature precociously at approximately 2 years of age and use a parasitic tactic to steal fertilizations from parental males. When small (approx. 2–3 years of age), cuckolder males (‘sneakers’) use a sneaking tactic to sneak spawn, and when large (approx. 4–5 years of age), cuckolder males (‘satellites’) use female mimicry to gain access to nests (Dominey 1980; Gross 1982). Because a previous study (Kindler et al. 1989) found no difference in androgen levels between the two cuckolder morphs, we hypothesized that oestradiol or cortisol may contribute to the profound differences in spawning behaviour between sneaker and satellite males for the reasons outlined above.

2. MATERIAL AND METHODS

Detailed methods are provided in the electronic supplementary material. Briefly, fish were collected from a spawning colony on 14 June 2004. Males were assigned to reproductive tactic based on direct observations of spawning behaviour, then were collected by hand net and immediately brought to a boat where blood sampling occurred. Only data from samples collected within 180 s were used to avoid confounding baseline cortisol levels with a stress response. Fish were then measured for body size.

Plasma hormone levels were measured using radioimmunoassay (see Magee et al. 2006 for details). Each sample was assayed in duplicate. For oestradiol, a few samples were considered technically ‘non-detectable’ because they fell outside the steep portion of the sigmoid standard curve. However, the standard curve equation still allowed estimation of the low oestradiol levels for these samples. Data were analysed using SYSTAT v. 11 (San Jose, CA) as outlined in the electronic supplementary material.

3. RESULTS AND DISCUSSION

As documented previously by Kindler et al. (1989), courting parental males had significantly higher levels

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that cuckolder spawning behaviours are energetically more expensive or otherwise more stressful than courtship and spawning by parental males. However, reproduction by parental males is likely to be more energetically expensive overall when one considers the energetic costs of nest building and parental care. For example, we previously found that parental males’ cortisol levels are low on the day of spawning relative to that during nest construction and the week-long care of the young (Magee et al. 2006). Parental males can also lose 10% of their body mass over the course of parental care (Coleman & Fischer 1991; Magee et al. 2006). Our glucocorticoid data from free-living animals are the first from fishes with ARTs and add only to a few other studies of vertebrates with ARTs (e.g. Mendonça et al. 1985; Leary et al. 2004). Hormone manipulation studies on tree lizards (Urosaurus ornatus) and plainfin midshipman have shown that the parasitic morphs are more sensitive to corticosterone than are the displaying morphs (Knapp & Moore 1997; Remage-Healey & Bass 2007). Thus, although it is premature to draw firm conclusions, it appears that glucocorticoids may play a larger role in parasitic morphs than in displaying or territorial males and thereby could factor into the costs and benefits of the various alternative tactics. We also found that bluegill males’ oestradiol levels were low relative to those of females, but cuckolder males had higher levels than parental males (binomial test: \( p = 0.02 \); table 1). Oestradiol levels are currently known from only five other fish species with two ARTs (Oliveira 2006). In two species, the pattern matches what we report here; in three species, oestradiol levels did not differ significantly between the morphs. Oestradiol’s involvement in the expression of ARTs is also supported by neurological responses to oestradiol and morph differences in brain aromatase activity in plainfin midshipman (Schlinger et al. 1999; Forlano & Bass 2005; Remage-Healey & Bass 2007; but see Gonçalves et al. 2007).

We also explored the hormone dependency of behaviour. In this population of bluegill, as in many fishes, body length is highly correlated with age (Gross & Charnov 1980). We now report a significant negative relationship between body length and oestradiol for satellite males (figure 2). There was no other significant relationship with body length for any other hormones or male morphs (table 2). This finding of 11-ketotestosterone (11-KT) than cuckolder males and females (ANOVA: \( F_{3,45} = 73.28, p < 0.001 \); figure 1). This pattern of 11-KT levels is consistent with other fishes with ARTs (Brantley et al. 1993; Oliveira 2006). In contrast, cuckolder males had significantly higher testosterone levels than parental males and females (\( F_{3,45} = 7.63, p < 0.001 \); figure 1). This result differs from Kindler et al. (1989), who found no significant difference among the male morphs. The reason for the discrepancy between studies is unclear, but may be related to variation in social environment (see Oliveira 2004).

Cuckolder males also had significantly higher cortisol levels than parental males and females (\( F_{3,45} = 10.32, p < 0.001 \); figure 1). This result suggests that cuckolder spawning behaviours are energetically more stressful than parental spawning behaviours. For example, our glucocorticoid data from free-living animals are the first from fishes with ARTs and add only to a few other studies of vertebrates with ARTs (e.g. Mendonça et al. 1985; Leary et al. 2004). Hormone manipulation studies on tree lizards (Urosaurus ornatus) and plainfin midshipman have shown that the parasitic morphs are more sensitive to corticosterone than are the displaying morphs (Knapp & Moore 1997; Remage-Healey & Bass 2007). Thus, although it is premature to draw firm conclusions, it appears that glucocorticoids may play a larger role in parasitic morphs than in displaying or territorial males and thereby could factor into the costs and benefits of the various alternative tactics.

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**Table 1. Plasma oestradiol levels for the male ARTs and females in bluegill on the day of spawning.**

<table>
<thead>
<tr>
<th>ART</th>
<th>no. samples</th>
<th>detectable</th>
<th>non-detectable</th>
<th>median level (ng ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>sneaker males</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>0.18</td>
</tr>
<tr>
<td>satellite males</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>parental males</td>
<td>20</td>
<td>10</td>
<td>10(^a)</td>
<td>0.05</td>
</tr>
<tr>
<td>females</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>1.98</td>
</tr>
</tbody>
</table>

\(^a\) Significantly different from proportion of cuckolder (sneaker + satellite) samples with detectable hormone levels (one-tailed binomial test: \( p = 0.02 \)).

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**Figure 1.** Mean (± s.e.) plasma androgen (a) testosterone, (b) 11-KT and (c) cortisol levels for the three male ARTs and females in bluegill on the day of spawning. Groups with different letters differ significantly from each other (post hoc Tukey’s tests). Numbers in bars represent sample sizes.

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suggests that there is a diminished dependency of female mimicry behaviour on oestradiol with increased mating experience. We cannot, however, rule out that the negative relationship between oestradiol levels and body length (age) instead reflects an increase in oestradiol receptor concentrations and, hence, an increased sensitivity to oestradiol levels in older satellite males. Regardless, our data support that the endocrine mediation of mating behaviour in satellite males changes with age and experience. Because decreases in hormonal dependency of mating behaviour with experience have been reported for males from several mammalian species (Hull et al. 2002), our finding opens exciting avenues for future research into the interplay between endocrinology and mating experience in ARTs.

Figure 2. Relationships between body length and plasma hormone levels (ng ml$^{-1}$) for the male ARTs in bluegill: (a) testosterone, (b) oestradiol, (c) 11-KT and (d) cortisol. Sneakers are represented by squares, satellites are represented by circles and parentals are represented by triangles. The one line (and 95% CI) is from the only significant linear regression (table 2). Dotted data points for oestradiol represent estimates of hormone levels that were below the level of detectability of our assay (see table 2).

Table 2. Results for simple linear regression analyses of body length versus plasma hormone level for the male ARTs in bluegill. (n.d., not determined because half of the parental males had oestradiol levels below the level of detectability in the radioimmunoassay. We did not correct for multiple comparisons because several pairs of hormone levels are connected via steroidogenic enzymes. Thus, each correlation with body length does not represent an independent test as assumed by common corrections (see electronic supplementary material for additional information). Italics indicate relationship where $p<0.05.\)

<table>
<thead>
<tr>
<th>hormone</th>
<th>sneaker males ($n=9$)</th>
<th>satellite males ($n=9$)</th>
<th>parental males ($n=15$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$F_{1,7}$</td>
<td>$p$</td>
</tr>
<tr>
<td>testosterone</td>
<td>0.245</td>
<td>2.28</td>
<td>0.175</td>
</tr>
<tr>
<td>11-ketotestosterone</td>
<td>0.000</td>
<td>0.00</td>
<td>0.955</td>
</tr>
<tr>
<td>oestradiol</td>
<td>0.089</td>
<td>0.68</td>
<td>0.435</td>
</tr>
<tr>
<td>cortisol</td>
<td>0.042</td>
<td>0.31</td>
<td>0.596</td>
</tr>
</tbody>
</table>

The work conformed to guidelines of the Canadian Council on Animal Care and was approved by the University of Oklahoma Animal Care and Use Committee. We thank Jeff Stoltz, Tim Hain, Sunny Scobell, Elizabeth Adkins-Regan, Frank Phelan, Floyd Connor and Rich Broughton. This study was supported by funding from the Natural Sciences and Engineering Research Council of Canada and a Premier’s Research Excellence Award to B.D.N., and the National Science Foundation (IBN 0349449) and a University of Oklahoma Presidential International Travel Fellowship to R.K.


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NOTICE OF CORRECTION

Figure 2 is now correct.

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