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Paternal Mouthbrooding in the Black-Chinned Tilapia, *Sarotherodon melanotheron* (Pisces: Cichlidae): Changes in Gonadal Steroids and Potential for Vitellogenin Transfer to Larvae

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The black-chinned tilapia (*Sarotherodon melanotheron*) is a paternal mouthbrooder. Pairs of adult black-chinned tilapia were raised in freshwater and the males were sampled during the mouthbrooding cycle. Sampling also occurred 10 days after release of the free-swimming fry for comparison. During the first week of incubation of the eggs, total androgens and estradiol were low (<5 and <0.3 ng/ml, respectively). During the second week of brooding, when the eggs have hatched and they are called newly hatched embryos, plasma levels of gonadal steroids increased (13–38 ng androgen/ml and >0.6 ng estradiol/ml). The plasma concentrations of vitellogenin (VTG) in male parents changed during mouthbrooding, with decreases occurring between egg pickup and hatching of the embryo (Day 6 of mouthbrooding). The pattern of change in concentrations of VTG in surface mucus of male parents differed from the pattern in plasma, with peak concentrations occurring at the time of hatching. The amount of VTG in mucus was similar to that measured in the female *Oreochromis mossambicus* during mouthbrooding of embryos. The appearance of peak VTG levels in the mucus at the time of hatching when plasma levels have declined and the availability of comparable amounts of mucus VTG in both maternal and paternal mouthbrooding tilapia, despite unequivalent plasma levels, support the possibility that parental provisioning of the young occurs during mouthbrooding in tilapia. © 2000 Academic Press

Key Words: parental care; teleost; steroid hormones; vitellogenin; provisioning offspring.

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The black-chinned tilapia (*Sarotherodon melanotheron*) is a mouthbrooding cichlid fish in which the male picks up the fertilized eggs and incubates them until they are released as free-swimming fry (Trewavas, 1983). After about 5 days of incubation, the eggs hatch and the newly hatched embryos remain in his mouth for a little more than a week. The entire mouthbrooding behavior lasts about 2 weeks. Paternal care such as this is more common than maternal care among teleosts (Gross and Sargent, 1985). However, in extant cichlids, mouthbrooding by the female rather than the male is more common, although paternal mouthbrooding is considered the ancestral state (Keenleyside, 1991).

In our previous studies of the tilapia *Oreochromis mossambicus,* a maternal mouthbrooder, we discovered that intact vitellogenin is present in the surface mucus of brooding females (Kishida and Specker, 1994). Our working hypothesis was that nutrient-rich vitellogenin is transferred to newly hatched embryos. The young of many cichlids are known to contact their parent with a micronipping behavior that appears to result in the ingestion of mucus (Keenleyside, 1991). The nutritive value of this mucus has not to our knowledge been considered. Testing this hypothesis in a male mouthbrooder eliminated the confounding effect of vitellogenin's role as a yolk precursor during oocyte growth.

A further objective was to examine the relationships among mouthbrooding, vitellogenin, and gonadal steroids. Estradiol, in particular, induces vitellogenin production in oviparous vertebrates (Specker and Sullivan, 1994). Endocrine correlates of mouthbrooding in male fishes have not to our knowledge been consid-

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ered. In paternal fishes guarding eggs or embryos in a nest, androgen levels tend to be decreased (Kindler, Philipp, Gross, and Barr, 1989; Pankhurst, 1990; Sikkel, 1993; Knapp, Wingfield, and Bass, 1999). Consequently, we hypothesized that androgens would be low during mouthbrooding.

MATERIALS AND METHODS

Fish

Black-chinned tilapia were raised and kept in 60- or 200-liter tanks with flow-through or recirculated freshwater at 28°C under a long photoperiod (14L: 10D). Fish were fed with floating foodsticks (Tetra) once a day. Male and female pairs of tilapia were kept in individual 60-liter flow-through tanks. The front of the tank was covered with a piece of black plastic sheet as a blind. The pairs were checked every morning to record the brooding status. After they spawned, a plastic mesh screen was inserted in the tank to separate the pair to avoid the loss of the brood primarily due to the aggression between the pair (Oppenheimer and Barlow, 1968). The tilapia were treated using standards approved by our Institutional Animal Care and Use Committee.

Sample Collection from Brooding Males

Mucus from the body surface and blood were collected after the male was anesthetized in 0.2% 2-phenoxyethanol (Sigma). Mucus was collected from the side of the body by gentle stroking with a spatula and aspirating into a pasteur pipette, and blood was drawn from the caudal vein using a heparinized needle and a syringe. Blood was centrifuged to separate plasma. Plasma and mucus mixed with aprotinin (4%, v/v) were stored at −80°C until use. VTG was analyzed using mucus extracts. Thawed mucus was weighed and mixed with an equal volume of 0.1 M phosphate-buffered saline, pH 7.4, containing Tween 20. After centrifugation at 3000 rpm for 15 min at 4°C, supernatant was collected and used for enzyme-linked immunosorbent assays (ELISAs). Samples were collected from the males at only one phase of a particular brooding cycle, males were sampled multiple times, and no male was used more than once for each phase. The collection days were the day of spawning (Day 1), 5 or 10 days after spawning (Day 6 and Day 11), or the day when fry were released from the mouth (Release day, Days 14–18). For comparison with brooding, samples were also collected from individually housed, nonbrooding males 10 days after the fry were released (after release day). Eggs hatch about 5 days after spawning (Day 6). After males release fry, they will not mouthbrood them again. When the samples were collected, the brood was taken away from the parents, and the same males were allowed to reproduce repeatedly.

Extraction of Steroids from Plasma

Plasma samples (35 μl) were extracted twice with 1.5 ml ethyl ether. The ether layer containing steroids was air-dried under the hood overnight. The extracts were reconstituted with the assay buffer (0.01 M phosphate-buffered saline, pH 7.4, containing 1% BSA and 0.1% NaN_3) and used in the radioimmunoassays (RIAs) for testosterone and estradiol. The recoveries of unlabeled testosterone and estradiol added to the plasma before extraction and vortexing were 84 and 92%, respectively. The coefficients of variation due to the extraction procedure were 6.5% for testosterone ($n = 6$) and 5.5% for estradiol ($n = 6$).

RIAs for Testosterone and Estradiol

Testosterone and estradiol used for standards were purchased from Sigma, [^{3}H]testosterone and [^{3}H]estradiol from New England Nuclear, and antisera to testosterone and estradiol from Endocrine Science. The method was adapted with modifications from Young (1986). The estradiol antiserum showed <0.01% cross-reactivity with testosterone and 11-ketotestosterone. The testosterone antiserum showed <0.01% cross-reactivity with estradiol and <25% cross-reactivity with 11-ketotestosterone. The displacement by 11-ketotestosterone in the testosterone RIA was not parallel to the standard curve. Serial dilutions of female and male plasma showed displacement curves parallel to the standard curves of testosterone and estradiol (Fig. 1), suggesting little interference by 11-ketotestosterone in the testosterone RIA. Nevertheless we report on total androgens. The lower limit of detection in both RIAs was 0.1 ng/ml when the equivalent of 10 μl plasma was assayed. However, plasma samples that approached the RIA limits were rerun using a larger volume. Unknowns thus fell well within the limits of the standard curves. The interassay coefficients of variation were 18.8% ($n = 10$) for the testosterone RIA and 10.4% ($n = 8$) for the estradiol RIA. The intraassay coefficient variation was 4.5%

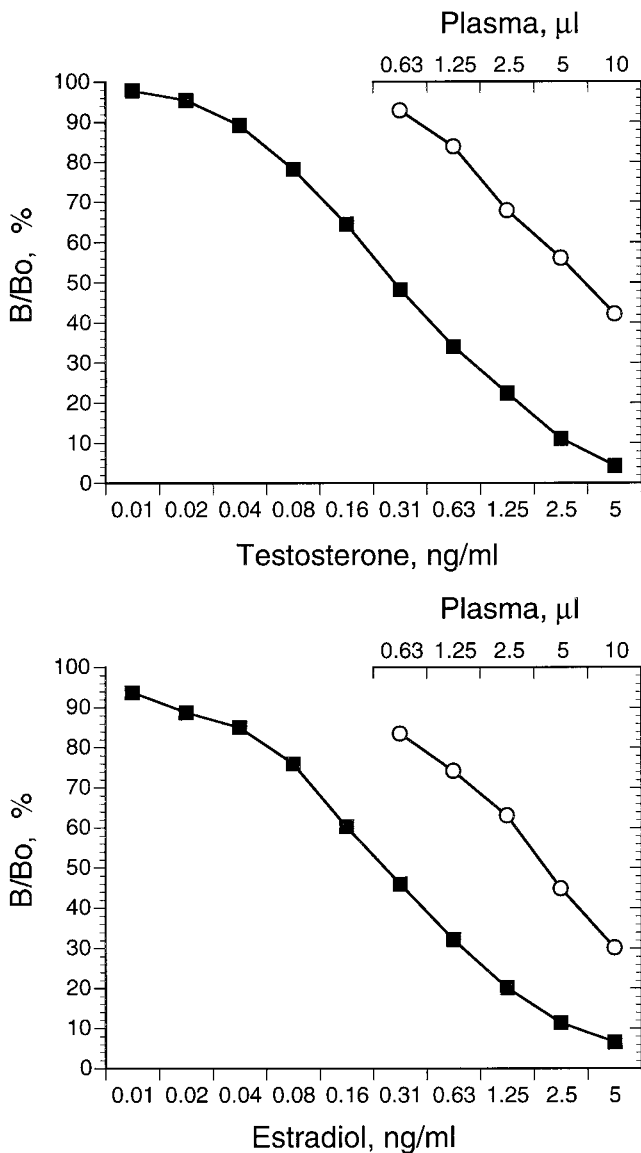

FIG. 1. Radioimmunoassays for testosterone (upper panel) and estradiol (lower panel) showing parallelism of serially diluted plasma from male black-chinned tilapia.

(n = 12) for the testosterone RIA and 2.2% (n = 12) for the estradiol RIA.

Enzyme-Linked Immunosorbent Assay for VTGs

VTG concentrations in plasma and mucus extracts were measured by competitive ELISAs described by Kishida and Specker (1993) using *O. mossambicus* VTGs as coating antigens and standards and the antisera to these VTGs. Protein concentrations in the mucus extracts were measured by the Bio-Rad protein assay (Bio-Rad) (Bradford, 1976) to normalize VTG concentrations in mucus extracts. Volumes of the unknowns were adjusted so that their displacement fell mid-range (20–80% binding) on the standard curves. Validation of the ELISAs for VTG from black-chinned tilapia was conducted by measuring displacement by serially diluted plasma and mucus extracts from female and male black-chinned tilapia.

Statistical Analyses

The slopes of serially diluted plasma and mucus and standard curves were linearized by taking the logit of the percentage bound (logit $\%B = \log[\%B/(1 - \%B)]$) and log of the standard dose. Deviation from parallelism (heterogeneity of slopes) was then detected by testing for an interaction effect (analysis of covariance; Cody and Smith, 1997; model is log hormone concentration = logit $\%B$ + group + group∗logit $\%B$). The concentrations of androgen, estradiol, and VTGs in plasma over the brooding cycle were compared using ANOVA. The data were log-transformed before analysis to decrease heterogeneity of variances; the antilog of the mean of the transformed data is reported in the text and the y-ordinate of the graphs is a logarithmic scale. If overall ANOVAs were significant, appropriate Tukey-type multiple comparisons were conducted to determine which phases of the cycle differed. Changes in the concentrations of VTGs in the mucus during the brooding cycle were analyzed using Kruskal-Wallace nonparametric ANOVA, followed by nonparametric multiple comparisons with unequal sample sizes (Zar, 1996, p. 228). The median values of the data are represented graphically and the 25 and 75% quartiles (Q_{1-3}) are reported in the text. All analyses were conducted using SAS (version 6.12), except for the nonparametric multiple comparison. Significance was accepted when $P < 0.05$.

RESULTS

Gonadal Steroids

Plasma androgen and estradiol concentrations changed during the brooding cycle (Fig. 2). Plasma androgen concentrations were lowest on Days 1 and 6 (4.1 and 2.5 ng/ml) and increased thereafter to 13.1 ng/ml on Day 11 and to a high of 37.5 ng/ml on Release day (for overall ANOVA, P = 0.0001). After

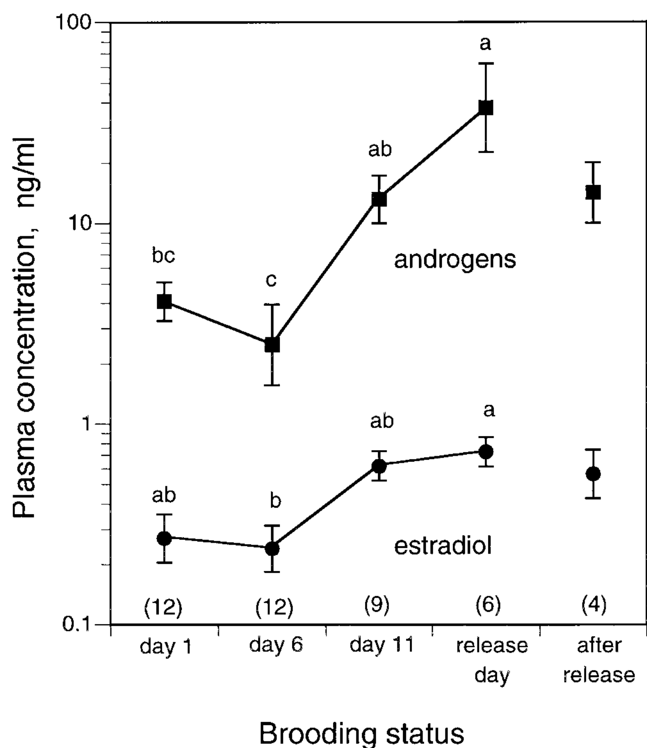

FIG. 2. Changes in androgens and estradiol concentrations in plasma from male black-chinned tilapia during the brooding cycle (mean ± SE). The numbers in parentheses indicate sample size for both assays. For each hormone, symbols with the same letter are not statistically different. The concentrations 10 days after release in nonbrooding males are shown for comparison.

the brooding cycle at 10 days after release of the fry, plasma androgen averaged 14.0 ng/ml. Plasma estradiol concentrations were lowest on Days 1 and 6 (0.27 and 0.24 ng/ml) and increased to 0.62 and 0.72 ng/ml on Day 11 and Release day (for overall ANOVA, P = 0.01). After the brooding cycle, plasma estradiol averaged 0.55 ng/ml.

Quantification of Vitellogenins in Black-Chinned Tilapia

ELISAs using *O. mossambicus* VTGs and their antisera were appropriate for the measurement of VTGs in *S. melanotheron.* The injection of estradiol into male *S. melanotheron* induced two proteins of molecular masses at 200 and 130 kDa in plasma (data not shown). These estradiol-inducible proteins have apparent molecular masses similar to those of the VTGs previously purified from *O. mossambicus,* tVTG-200 and tVTG-130 (Kishida and Specker, 1993). Serial dilutions of plasma from a female and male exhibited displacement curves parallel to the standard curve in ELISAs for tVTG-200 and tVTG-130 (Fig. 3). Serial dilutions of mucus extracts from the female and male fish showed displacement curves parallel to the standard curve in both assays (Fig. 4). These results, and the detection of intact tVTG-130 and tVTG-200 in the mucus by Western blot (data not shown), indicate that VTGs present in plasma and mucus of *S. melanotheron* can be quantified by the ELISAs.

Plasma and mucus VTG concentrations also changed during the brooding cycle (Fig. 5). In the plasma, the patterns of change for tVTG-200 and tVTG-130 were similar and the overall tests showed statistically significant changes in both (P = 0.01 and P = 0.02, respectively). Plasma tVTG-200 concentrations decreased from Day 1 (70.8 μg/ml) to Day 6 (24.0

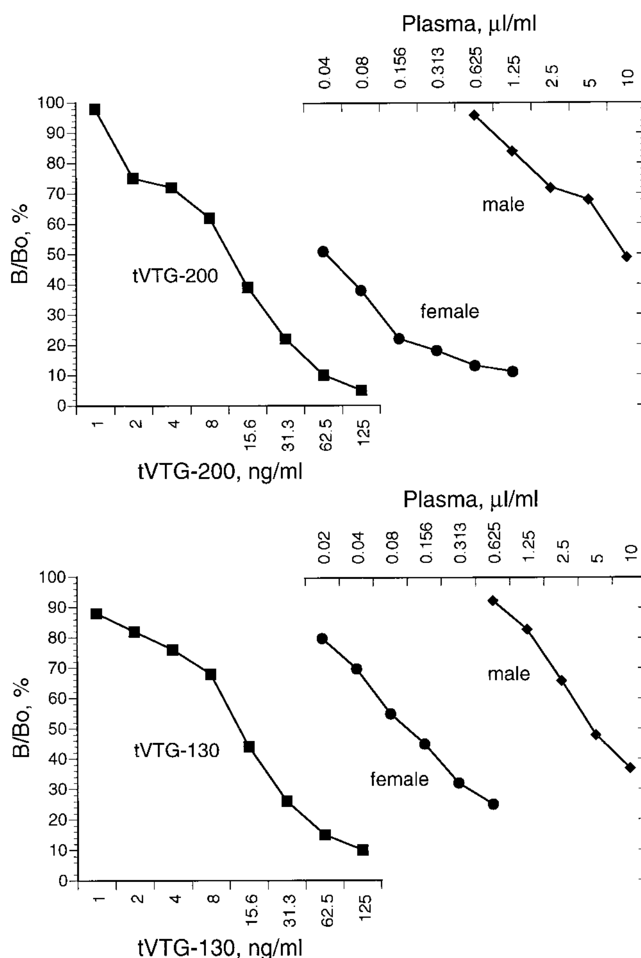

FIG. 3. ELISAs for tVTG-200 (upper panel) and tVTG-130 (lower panel) in plasma of black-chinned tilapia. Serial dilutions of plasma from female and male fish were parallel to the standard curves ($P < 0.05$). B/B_0 = bound/maximum binding.

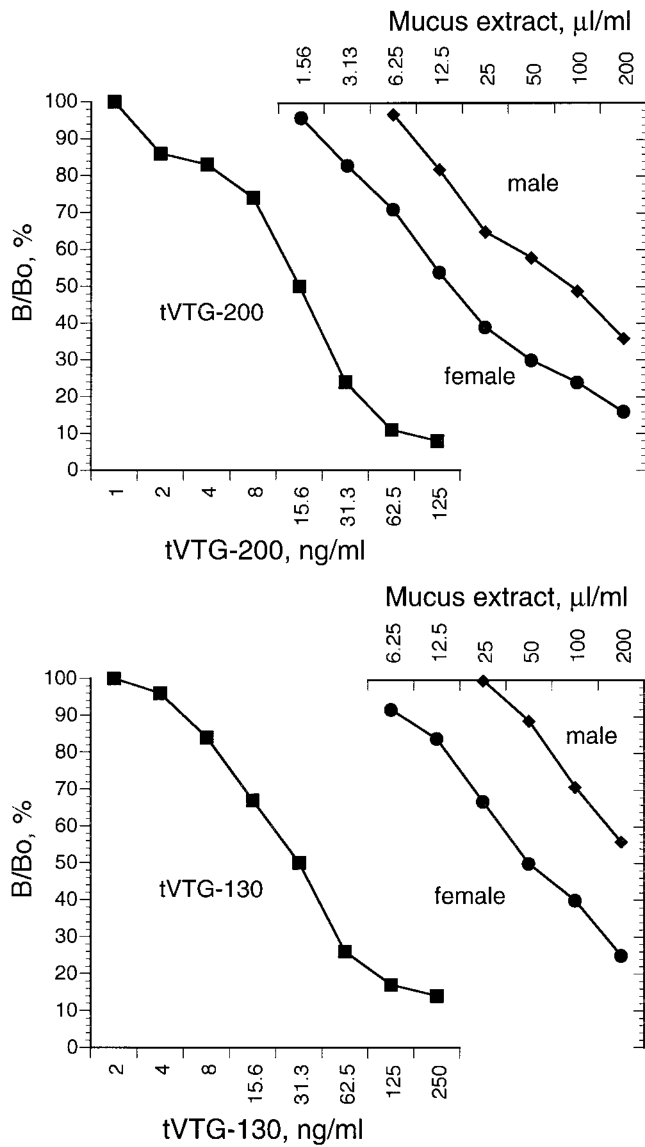

FIG. 4. ELISAs for tVTG-200 (upper panel) and tVTG-130 (lower panel) in mucus of black-chinned tilapia. Serial dilutions of mucus extract from female and male fish were parallel to the standard curves ($P < 0.05$). B/B_0 = bound/maximum binding.

μg/ml) and stayed low through Day 11 (20.4 μg/ml). On Release day levels were 61.7 μg/ml. In nonbrooding males, plasma tVTG-200 was 59.5 μg/ml which was similar to Day 1 and Release day. The pattern of plasma tVTG-130 concentrations was similar to that of tVTG-200, with concentrations of 35.5, 13.5, 12.9, and 35.5 μg/ml on Days 1, 6, and 11 and Release day during the brooding cycle and 46.7 μg/ml 10 days after release. No pairwise comparison of plasma tVTG-130 showed a significant difference.

The patterns of change of tVTG-200 and tVTG-130 in the mucus differed from the patterns of change in plasma, although again both VTGs changed similarly (Fig. 5). Mucus levels of tVTG-200 changed during brooding ($P = 0.04$), starting on Day 1 with 0.47 μg/mg protein ($Q_{1\text{-}3}$ = 0.22–0.86 μg/mg protein),

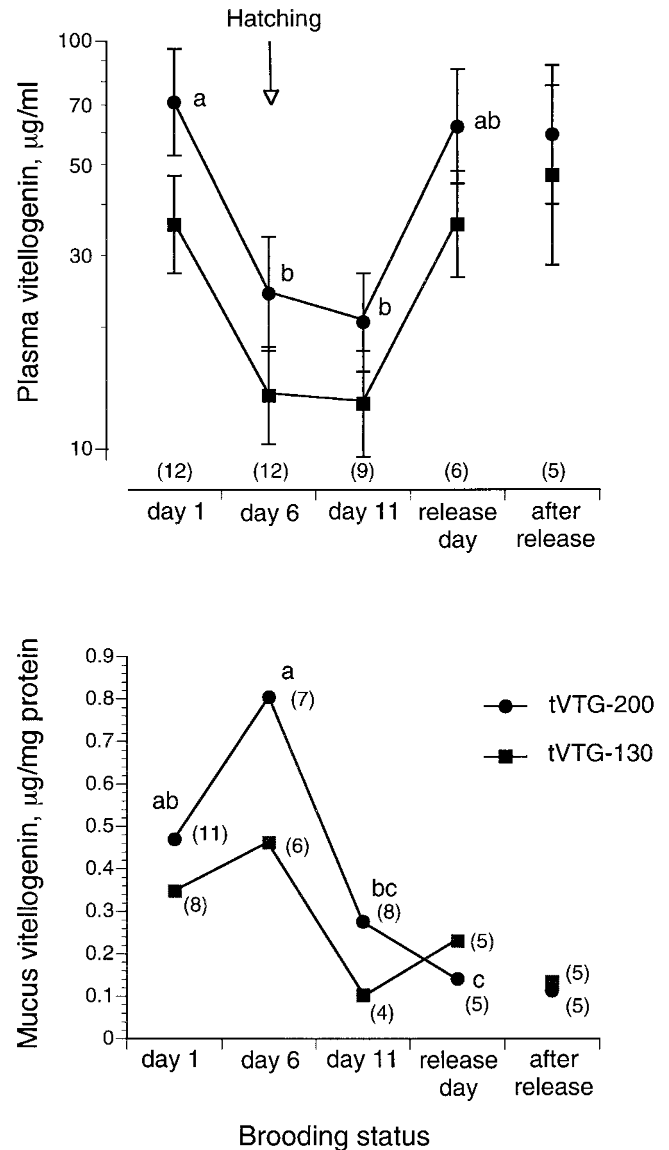

FIG. 5. Changes in tVTGs in plasma (upper panel, mean ± SE) and body surface mucus (lower panel, medians) from male black-chinned tilapia during the brooding cycle (mean ± SE). Symbols with the same letter are not statistically different. Concentrations of the VTGs in nonbrooding males 10 days after release of the fry are shown for comparison. Sample sizes for both assays of plasma are shown in parentheses at the base of the upper panel and in parentheses near the median of each assay of mucus in the lower panel. The interquartile values for VTG in mucus are supplied in the text.

with a peak on Day 6 of 0.80 μg/mg protein (Q_{1-3} = 0.35–1.36 μg/mg protein), a significant decline by Day 11 to 0.28 μg/mg protein (Q_{1-3} = 0.12–0.34 μg/mg protein), and on Release day mucus tVTG-200 was 0.14 μg/mg protein (Q_{1-3} = 0.12–0.18 μg/mg protein). There was no statistically significant change in mucus tVTG-130 concentration during the brooding cycle; however, the medians (Q_{1-3} in parentheses) for Days 1, 6, 11, and Release were 0.35 (0.10–0.46), 0.46 (0.17–1.10), 0.10 (0.06–0.25), and 0.23 (0.23–0.28) μg/mg protein. In nonbrooding males 10 days after release of fry, median levels of mucus tVTG-200 and tVTG-130 were 0.11 (0.09–0.21) μg/mg protein and 0.13 (0.10–0.23) μg/mg protein.

DISCUSSION

This is the first study documenting endocrine correlates of paternal mouthbrooding in a teleost fish. The mouthbrooding black-chinned tilapia resembles other paternal caretakers in that gonadal steroids are suppressed during some phase of parental care. In this case, plasma androgens and estradiol are low in the first week of mouthbrooding relative to the second week and after release. Our findings on changes in vitellogenin during the brooding cycle are consistent with the hypothesis that nutrient transfer from parent to offspring is occurring. The significance of changes in steroids and vitellogenin to mouthbrooding is discussed below.

Gonadal Steroids and Mouthbrooding

Plasma androgen levels in male black-chinned tilapia were about 4-fold lower during mouthbrooding of eggs than they were during mouthbrooding of hatched embryos. The levels on the day the free-swimming fry were released were 10-fold higher than during the first week of mouthbrooding and 2.5-fold higher than they were 10 days after release in nonbrooding males. The increase in androgens from about 4 to 40 ng/ml is interestingly similar to the increase from <5 to about 20 ng/ml observed at the beginning and end of mouthbrooding in female *O. mossambicus* (Smith and Haley, 1988).

The pattern of androgens during mouthbrooding in black-chinned tilapia is consistent with the hypothesis that plasma androgens would be depressed during parental care, but additionally interesting in that levels appear elevated on Release day. Sikkel (1993) observed a similar pattern in wild male garibaldi (*Hypsypops rubicundus*) in which both testosterone and 11-ketotestosterone were lowest early in the nest brooding cycle and slightly elevated late in the cycle. In male demoiselles (*Chromis dispilus*) tending their nests in the wild, plasma testosterone levels were about 1 ng/ml during early brooding, increased significantly by about 50% at the end of brooding, and during spawning were as high as 4–5 ng/ml (Pankhurst, 1990). Both testosterone and 11-ketotestosterone were low during the brooding phase of the bluegill sunfish (*Lepomis macrochirus*) (Kindler, Phillip, Gross, and Bahr, 1989). In birds and amphibians, the male can also exhibit decreased plasma concentrations of androgens during a period of parental care (Townsend and Moger, 1987; Wingfield, Hegner, Dufty, and Ball, 1990). The increase in plasma androgens at the end of the brooding cycle in black-chinned tilapia occurs in the absence of incoming males and thus cannot be explained by the challenge hypothesis (see Wingfield *et al.,* 1990). Elevated androgens on the day of release in black-chinned tilapia could reflect a very quick resumption of testicular activity.

The identity of androgens in the black-chinned tilapia probably includes both testosterone and 11-ketotestosterone. Among tilapia, 11-ketotestosterone has been reported in the urine of *O. mossambicus* (Oliveira, Almada, and Canario, 1996). There is also a report that the testis of the nile tilapia (*Oreochromis niloticus*) does not produce 11-ketotestosterone (Hines, Wasson, and Watts, 1995), even though it is present in early gonadal development (Hines, Boots, Wibbels, and Watts, 1999). 11-Ketotestosterone showed nonparallel displacement in the testosterone RIA. However, serially diluted male plasma was parallel to the testosterone standard curve, indicating the RIA is quantitative and that 11-ketotestosterone was not present in sufficient quantity to interfere.

Estradiol levels were 100-fold less than the testosterone levels in male black-chinned tilapia and estradiol increased from 0.3 on Day 1 to 0.8 ng/ml at the time of release. In female *O. mossambicus,* estradiol concentrations increased from <1 to about 4 ng/ml at the corresponding times (Smith and Haley, 1988). The significance, if any, of circulating estradiol in male fish is relatively unexplored.

Vitellogenins and Mouthbrooding

The plasma concentrations of tVTG-200 and tVTG-130 changed in parallel with the highest levels present at the beginning and end of mouthbrooding and comparable to those after release. The average plasma

vitellogenin concentrations in male black-chinned tilapia during and after mouthbrooding ranged from 20 to 71 μg tVTG-200/ml and from 13 to 47 μg tVTG-130/ml. This compares with a range of about 200 to 5000 μg/ml in female *O. mossambicus* during mouthbrooding (Kishida and Specker, 1994). Although these differ by one or two orders of magnitude, the amount of vitellogenin in the male is not insignificant. The pattern showed depressed plasma vitellogenin levels in the middle of the mouthbrooding cycle. The decrease in plasma levels of the vitellogenins might be explained by (1) a delayed response to low estradiol levels in the first half of the mouthbrooding cycle, (2) the inhibition of vitellogenin production by some factor other than testosterone which is low at this time, and/or (3) the more rapid removal of vitellogenin from the plasma.

The pattern of change in the amount of the vitellogenins in surface mucus did not correspond to the pattern observed in plasma. Instead, the highest and not the lowest levels were measured at the time of hatching and levels were lower during the second half of the brood cycle. At the time of hatching around Day 6, the surface mucus of the male mouthbrooder contained 0.80 μg tVTG-200/mg protein and 0.46 μg tVTG-130/mg protein. These amounts are very similar to those found in surface mucus of female *O. mossambicus* at the time free-swimming embryos appeared (1.4 μg tVTG-200/mg protein and 0.6 μg tVTG-130/mg protein) (Kishida and Specker, 1994). Given the difference in plasma levels of vitellogenins between the mouthbrooding parent of the two species, the similarity in mucus vitellogenin content is intriguing. Whether this amount corresponds to what might be exuded into the mouth and whether the amount could be energetically significant are not known. We have detected vitellogenin in the mouth mucus of *O. mossambicus* (not published); however, routinely sampling from the mouth is more damaging and difficult than sampling from the body.

Our study extends the previous observation of vitellogenin in the surface mucus of a female mouthbrooder to comparable availability of vitellogenin in a male mouthbrooder, making more enticing the possibility of transfer of parental (maternal or paternal) vitellogenin to the young to serve nutritional and/or transport protein role(s). In support of this hypothesis, mouthbrooded fry of the tilapia *O. aureus* are known to acquire more protective immunity from the parent than nonbrooded fry (Sin, Ling, and Lam, 1994), indicating that immunoglobulin was transferred from parent to offspring during mouthbrooding. It has been noted that in some species that exhibit developed parental behavior the young nip at the parent's surface mucus (see Noakes, 1979). We previously showed that yolk protein-like substances are present in the intestine of mouthbrooded embryos of *O. mossambicus;* however, whether these were maternally derived or endogenously produced has been difficult to solve (Kishida and Specker, 1994).

The two vitellogenins in *S. melanotheron* appear to be very similar to the vitellogenins in *O. mossambicus* in their apparent molecular masses, their immunoreactivity, and their parallelism in displacement curves in the *O. mossambicus* ELISAs for tVTG-200 and tVTG-130 (Kishida and Specker, 1993). Two forms of vitellogenins have been reported in other tilapia species, *O. aureus* (Ding, Hee, and Lam, 1989) and *O. niloticus* (Buerano, Inaba, Natividad, and Morisawa, 1995). Whether both forms are products of the vitellogenin gene family remains unknown. However, we have shown that antisera raised against the two estradiol-inducible proteins in *O. mossambicus* stain the major protein bands in yolk protein extract that is devoid of chorion proteins, which are also estradiol-inducible (Kishida and Specker, 1993). Thus both proteins serve functionally as vitellogenins as originally defined (Pan, Bell, and Telfer, 1969). In *S. melanotheron,* the two proteins elute separately from a DEAE column (data not shown), indicating they are distinct proteins rather than subunits of a single molecule. In competitive conditions used for ELISA, displacement curves for plasma and for mucus from *S. melanotheron* were parallel to the standard curve, indicating the assays could be used quantitatively.

Role for Estradiol in Male Fish

The presence of estradiol in male fishes is more generally known than the presence of vitellogenin in some male fishes. Estradiol levels in males from several families of teleost fishes have been reported as being in the range of 0.1 to 3 ng/ml (see Fostier, Jalabert, Billard, Breton, and Zohar, 1983; Kadmon, Yaron, and Gordin, 1985; Rosenblum, Pudney, and Callard, 1987). The presence of vitellogenin in untreated male fish has been reported in some species (So, Idler, and Hwang, 1985; Sumpter, 1985; Copeland, Sumpter, Walker, and Croft, 1986; Copeland and Thomas, 1988; Pelissero, Cuisset, and Le Menn, 1989; Goodwin, Grizzle, Bradley, and Estridge, 1992), including tilapia (Ding, Hee, and Lam, 1989; Kishida and Specker, 1993). In some cases the authors claim that the presence of vitellogenin is due

to estrogens contained in the feed (Sumpter, 1985; Pelissero *et al.,* 1989; Goodwin *et al.,* 1992). This may well be true, because the levels of vitellogenin in males do not seem to correlate with the gonadal cycle in some fish examined (Sumpter, 1985; Goodwin *et al.,* 1992). However, the data presented in this study show that there is a significant change in plasma estradiol concentration over the brooding cycle, the change in estradiol parallels the change in testosterone, and changes in plasma vitellogenins follow by a few days the changes in estradiol. Taken together, these observations indicate that production of the vitellogenins in this male fish could be regulated by endogenous estradiol.

Conclusions

In sum, we have shown for the first time changes in plasma androgen and estradiol concentrations in a paternal mouthbrooder. These changes are similar in pattern to those seen in a closely related female mouthbrooder (Smith and Haley, 1988). The suppression in androgens during the early phases of mouthbrooding resembles the pattern observed in male egg guarders. We also show significant changes in plasma vitellogenin levels during mouthbrooding that could be most simply explained by a recent suppression of estradiol levels at the onset of mouthbrooding. Both tVTG-200 and tVTG-130 are present in the surface mucus of male *S. melanotheron* during and after mouthbrooding in amounts strikingly similar to the amounts measured in mucus of female *O. mossambicus* (Kishida and Specker, 1994), supporting the hypothesis of a trophic relationship between parent and offspring. An alternative hypothesis is that the presence of vitellogenin in surface mucus of mouthbrooding parents may be the result of unavoidable leakiness of the epithelium. Or, mucus vitellogenin may be evolutionarily significant but currently vestigial. Nevertheless, consistent with the hypothesis that mucus vitellogenin could serve as a nutrient for the larvae are (1) the comparable amounts of mucus vitellogenin in both maternal and paternal mouthbrooders, despite unequivalent plasma levels, (2) the appearance of peak vitellogenin levels in the mucus at the time of hatching, and (3) the apparently estradiol-regulated presence of significant amounts of vitellogenin in a male mouthbrooder. Whether parentally derived vitellogenin is taken up by hatched embryos and serves a nutritive function is an open question.

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REFERENCES

Buerano, C. C., Inaba, K., Natividad, F. F., and Morisawa, M. (1995). Vitellogenins of *Oreochromis niloticus:* Identification, isolation, and biochemical and immunochemical characterization. *J. Exp. Zool.* **273,** 59–69.

Cody, R. P., and Smith, J. K. (1997). *Applied Statistics and the SAS Programming Language.* Prentice Hall, Upper Saddle River, NJ.

Copeland P. A., Sumpter, J. P., Walker, T. K., and Croft, M. (1986). Vitellogenin levels in male and female rainbow trout (*Salmo gairdneri* Richardson) at various stages of the reproductive cycle. *Comp. Biochem. Physiol. B* **83,** 487–493.

Copeland, P. A., and Thomas, P. (1988). The measurement of plasma vitellogenin levels in a marine teleost, the spotted seatrout (*Cynoscion nebulosus*) by homologous radioimmunoassay. *Comp. Biochem. Physiol. B* **91,** 17–23.

Ding, J. L., Hee, P. L., and Lam, T. J. (1989). Two forms of vitellogenin in the plasma and gonads of male *Oreochromis aureus. Comp. Biochem. Physiol. B* **93,** 363–370.

Fostier, A., Jalabert, B., Billard, R., Breton B., and Zohar, Y. (1983). The gonadal steroids. In W. S. Hoar, D. J. Randall, and E. M. Donaldson (Eds.), "Fish Physiology," Vol. 9, Part A, pp. 277–372. Academic Press, New York.

Goodwin, A. E., Grizzle, J. M., Bradley, J. T., and Estridge, B. H. (1992). Monoclonal antibody-based immunoassay of vitellogenin in the blood of male channel catfish (*Ictalurus punctatus*). *Comp. Biochem. Physiol. B* **101,** 441–446.

Gross, M. R., and Sargent, R. C. (1985). The evolution of male and female parental care in fishes. *Am. Zool.* **25,** 807–822.

Hildemann, W. H. (1959). A cichlid fish, *Symphysodon discus,* with unique nurture habits. *Am. Nat.* **868,** 27–34.

Hines, G. A., Boots, L. R., Wibbels, T., and Watts, S. A. (1999). Steroid levels and steroid metabolism in relation to early gonadal development in the tilapia *Oreochromis niloticus* (Teleostei: Cyprinoidei). *Gen. Comp. Endocrinol.* **114,** 235–248.

Hines, G. A., Wasson, K. M., and Watts, S. A. (1995). Testicular steroidogenesis in relation to social interactions in the nile tilapia. In F. Goetz and P. Thomas (Eds.), *Proc. Fifth Int. Symp. Repro. Physiol. Fish,* p. 232. Univ. of Texas, Austin, TX.

Kadmon, G., Yaron, Z., and Gordin, H. (1985). Sequence of gonadal events and oestradiol levels in *Sparus aurata* (L.) under two photoperiod regimes. *J. Fish Biol.* **26,** 609–620.

Keenleyside, M. H. A. (1991). Parental care. In M. H. A. Keenleyside (Ed.), *Cichlid Fishes: Behaviour, Ecology and Evolution,* pp. 191–208. Chapman & Hall, University Press, Cambridge.

Kindler, P. M., Philipp, D. P., Gross, M. R., and Bahr, J. M. (1989). Serum 11-ketotestosterone and testosterone concentrations associated with reproduction in male bluegill (*Lepomis macrochirus:* Centrarchidae). *Gen. Comp. Endocrinol.* **75,** 446–453.

Kishida, M., and Specker, J. L. (1993). Vitellogenin in tilapia (*Oreochromis mossambicus*): Induction of two forms by estradiol, quan-

tification in the plasma and characterization in oocyte extract. *Fish Physiol. Biochem.* **12,** 171–182.

Kishida, M., and Specker, J. L. (1994). Vitellogenin in the surface mucus of tilapia (*Oreochromis mossambicus*): Possibility for uptake by the free-swimming embryos. *J. Exp. Zool.* **268,** 259–268.

Knapp, R., Wingfield, J. C., and Bass, A. H. (1999). Steroid hormones and paternal care in the plainfin midshipman fish (*Porichthys notatus*). *Horm. Behav.* **35,** 81–89.

Noakes, D. L. G. (1979). Parent-touching behavior by young fishes: Incidence, function and causation. *Environ. Biol. Fish.* **4,** 389–400.

Oliveira, R. F., Almada, V. C., and Canario, A. V. M. (1996). Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus. Horm. Behav.* **30,** 2–12.

Oppenheimer, J. R., and Barlow, G. W. (1968). Dynamics of parental behavior in the black-chinned mouthbreeder, *Tilapia melanotheron* (Pisces: Cichlidae). *Z. Tierpsychol, Bd.* **24** (Heft 8), 889–914.

Pan, M. L., Bell, W. J., and Telfer, W. H. (1969). Vitellogenic blood protein synthesis by insect fat body. *Science* **165,** 393–394.

Pelissero, C., Cuisset, B., and Le Menn, F. (1989). The influence of sex steroids in commercial fish meals and fish diets on plasma concentration of estrogens and vitellogenin in cultured Siberian sturgeon *Acipenser baeri. Aquat. Living Resour.* **2,** 161–168.

Pankhurst, N. W. (1990). Changes in plasma levels of gonadal steroids during spawning behavior in territorial male demoiselles *Chromis dispilus* (Pisces: Pomacentridae) sampled underwater. *Gen. Comp. Endocrinol.* **79,** 215–225.

Rosenblum, P. M., Pudney, J., and Callard, I. P. (1987). Gonadal morphology, enzyme histochemistry and plasma steroid levels during the annual reproductive cycle of male and female brown bullhead catfish, *Ictalurus nebulosus* Lesueur. *J. Fish Biol.* **31,** 325–341.

Sikkel, P. C. (1993). Changes in plasma androgen levels associated with changes in male reproductive behavior in a brood cycling marine fish. *Gen. Comp. Endocrinol.* **89,** 229–237.

Sin, Y. M., Ling, K. H., and Lam, T. J. (1994). Passive transfer of protective immunity against ichthyophthiriasis from vaccinated mother to fry in tilapias, *Oreochromis aureus. Aquaculture* **120,** 229–237.

Smith, C. J., and Haley, S. R. (1988). Steroid profiles of the female tilapia, *Oreochromis mossambicus,* and correlation with oocyte growth and mouthbrooding behavior. *Gen. Comp. Endocrinol.* **69,** 88–98.

So, Y. P., Idler, D. R., and Hwang, S. J. (1985). Plasma vitellogenin in landlocked Atlantic salmon (*Salmo salar* Ouananiche): Isolation, homologous radioimmunoassay and immunological cross-reactivity with vitellogenin from other teleosts. *Comp. Biochem. Physiol. B* **81,** 63–71.

Specker, J. L., and Sullivan, C. V. (1994). Vitellogenesis in fishes: Status and perspectives. In K. G. Davey, R. E. Peter, and S. S. Tobe (Eds.), "Perspectives in Comparative Endocrinology," pp. 304–315. Natl. Res. Council, Ottawa.

Sumpter, J. P. (1985). The purification, radioimmunoassay and plasma levels of vitellogenin from the rainbow trout, *Salmo gairdneri.* In B. Lofts and W. N. Holmes (Eds.), "Current Trends in Comparative Endocrinology," pp. 355–357. Hong Kong University Press, Hong Kong.

Takemura, A., and Takano, K. (1997). Transfer of maternally derived immunoglobulin (IgM) to larvae in tilapia, Oreochromis mossambicus. *Fish Shellfish Immunol.* **7,** 417–427.

Thistlethwaite, G. E. (1974). The oral epithelium in the brooding male catfish, *Galeicthys felis. Proc. Louisiana Acad. Sci.* **10,** 195–196.

Townsend, D. S., and Moger, W. H. (1987). Plasma androgen levels during male parental care in a tropical frog (*Eleutherodactylus*). *Horm. Behav.* **21,** 93–99.

Trewavas, E. (1983). *Tilapine Fishes of the Genera Sarotherodon, Oreochromis and Danakilia.* Cornell Univ. Press, New York.

Wingfield, J. C., Hegner, R. E., Dufty, A. M., Jr., and Ball, G. F. (1990). The "challenge hypothesis": Theoretical implications for patterns of testosterone secretion, mating system, and breeding strategies. *Am. Nat.* **136,** 829–846.

Young, G. (1986). Cortisol secretion in vitro by the interrenal of coho salmon (*Oncorhynchus kisutch*) during smoltification: Relationship with plasma thyroxine and plasma cortisol. *Gen. Comp. Endocrinol.* **63,** 191–200.

Zar, J. H. (1996). *Biostatistical Analysis,* 3rd ed. Prentice-Hall, Upper Saddle River, NJ.