

## BRIEF COMMUNICATION

### Androgen levels of reproductive competitors in a co-operatively breeding cichlid

R. F. OLIVEIRA\*†, K. HIRSCHENHAUSER\*, A. V. M. CANÁRIO‡  
AND M. TABORSKY§

\**Unidade de Investigação em Eco-Etologia, Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco 34, 1149-041 Lisboa, Portugal*, ‡*Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 800-117 Faro, Portugal* and §*Department of Behavioural Ecology, Zoological Institute, University of Bern, Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland*

(Received 7 February 2003, Accepted 10 September 2003)

Androgen levels of family groups of *Neolamprologus pulcher* were assessed using non-invasive methods. There were no significant differences in the excretion rates neither of testosterone nor of 11-ketotestosterone between territorial and helper males. © 2003 The Fisheries Society of the British Isles

Co-operative breeding has been described in many birds and mammal species, in which young individuals, usually genetically related to the breeding pair, help to raise the offspring produced in a group (Emlen, 1991). In teleosts co-operative breeding has been described only for a few species (Taborsky & Limberger, 1981; Taborsky, 1984, 1994, 2001). It can take the form of associations between a bourgeois and a satellite male that trade help in defending the territory, courting females and building nests for easier access to parasitic fertilizations (Taborsky *et al.*, 1987; Taborsky, 1994, 1999, 2001; Martin & Taborsky, 1997; Oliveira *et al.*, 2002). Co-operative brood care has also been documented in teleosts and an extreme specialization of this pattern is the occurrence of extended families in which younger individuals stay with the breeding pair and actively participate in parental care (Taborsky & Limberger, 1981; Taborsky, 1984, 1994; Balshine-Earn *et al.*, 1998, 2001).

Helpers of co-operatively breeding birds and mammals have, generally, lower androgen levels than breeding males, which have been interpreted as a psychological castration of helpers by the breeding dominant male of the family group (Reyer *et al.*, 1986; Schmidt *et al.*, 1991; Schoech *et al.*, 1991; Wingfield *et al.*, 1991; Poiani & Fletcher, 1994; Vleck & Brown, 1999; Peters *et al.*, 2001). This pattern, however, has not been found in species in which helpers have

†Author to whom correspondence should be addressed. Tel.: +351 21 8811708; fax: +351 21 8860954; email: [ruiol@ispa.pt](mailto:ruiol@ispa.pt)

substantial mating opportunities and may share paternity of the offspring (e.g. beta males in co-operatively breeding packs of African wild dogs *Lycaon pictus*, Creel *et al.*, 1997).

*Neolamprologus pulcher* (Trewavas & Poll) is a teleost species with extended families, among which males compete for reproduction (Taborsky & Limberger, 1981; Taborsky, 1984, 1985), and male helpers are able to share in paternity (Dierkes *et al.*, 1999). Thus differences in androgen levels between breeding and helper males are not expected in this species.

In the present study the androgen levels (testosterone, T; 11-ketotestosterone, KT) of the different members of family groups of *N. pulcher* were investigated (*i.e.* breeding male, breeding female, male helpers and female helpers). The dominant male breeder, the dominant female breeder, the largest male helper and the largest female helper of each family group were sampled in a controlled laboratory situation. Only families were sampled in which the standard length ( $L_S$ ) of the largest male helper was  $>3.5$  cm since this is considered the size at maturity for male helpers (Taborsky, 1984). The composition of each family group is summarized in Table I. Sampling occurred always between 1700 and 1900 hours to avoid effects of the daily variations in androgen levels that are known to occur in cichlid fishes (Oliveira *et al.*, 2001a).

Since individuals were too small to allow blood sampling without sacrificing the fish androgen excretion was assayed. Steroid excretion rates of goldfish *Carassius auratus* (L.) measured in holding water have been shown (Scott & Sorensen, 1994) to match well blood plasma levels in fish receiving similar treatments (Moriwaki *et al.*, 1991). The injection of males of *Oreochromis mossambicus* L. with luteinizing hormone-releasing hormone (LHRH) leads to subsequent increase in excretion of KT and T measured in holding water (Hirschenhauser *et al.*, 2002). Individual *N. pulcher* were caught with a hand

TABLE I. Group composition of the studied families of *Neolamprologus pulcher*

Family	Number of helpers in the group	Presence of fry or eggs	Mass of territorial male (g)	Mass of breeding female (g)	Mass of male helper (g)	Mass of female helper (g)
A	15	No	19.7	15.1	n.a.	n.a.
B	3	Yes	11.2	8.7	8.6	n.a.
C	4	Yes	11.5	11.5	5.4	n.a.
D	5	Yes	7.5	10.5	2.5	1.7
E	3	No	25.9	12.6	9.3	n.a.
F	5	Yes	16.0	9.6	n.a.	2.5
G	18	Yes	18.3	16.8	2.0	10.4
H	9	No	25.3	16.7	5.3	8.9
I	3	Yes	14.5	8.7	4.3	n.a.
J	11	Yes	15.2	17.2	1.3	2.2
K	4	Yes	17.7	13.4	7.4	8.3
L	11	Yes	13.2	10.4	3.8	3.9
M	13	Yes	20.1	7.6	3.4	7.9

n.a., not available in this family group.

net and placed in a small aquarium (beaker diameter = 15 cm), which contained 500 ml of water and were left for 1 h. Free, sulphated and glucuronidated steroids were solid phase extracted (Sep Pak RP-18, Merck) following procedures previously described (Scott & Sorensen, 1994; Greenwood *et al.*, 2001) and stored at  $-20^{\circ}\text{C}$  until the radioimmunoassays (RIA) for T (Scott *et al.*, 1984) and KT (Kime & Manning, 1982) were performed. A pool of water extracts were separated on thin-layer chromatography and fractions assayed to confirm the specificity of the antisera for the samples. Only one immunoreactive peak was detected for each of the RIAs matching the elution position of the respective androgens (R.F. Oliveira, K. Hirschenhauser, T. Oliveira & A.V.M. Canário, unpubl. data). Since excretion rates are expected to be related to body mass and body mass varied significantly among the four types of individuals (Kruskal–Wallis ANOVA, d.f. = 3 and 52,  $P=0.01$ ), androgen values were related to individual body mass ( $\text{ng g}^{-1}$  body mass). Since only one breeding female and three female helpers had androgen levels above the limit of reliable measurements of the assays used ( $0.4 \text{ ng sample}^{-1}$ ), females were not included in the statistical analysis (androgens levels in the breeding female: T excretion rate =  $0.40 \text{ ng h}^{-1} \text{ g}^{-1}$  body mass, KT excretion rate =  $0.29 \text{ ng h}^{-1} \text{ g}^{-1}$  body mass; female helpers: average T excretion rate =  $0.57 \text{ ng h}^{-1} \text{ g}^{-1}$  body mass, average KT excretion rate =  $0.60 \text{ ng h}^{-1} \text{ g}^{-1}$  body mass).

Non-parametric statistics were applied using the statistical package Statistica V.5.0A (©Statsoft Inc., U.S.A.).

There were no significant differences in the excretion rates of T or KT between territorial ( $n=9$ ) and helper males ( $n=11$ ) (Mann–Whitney  $U$  test: KT,  $P=0.34$  and T,  $P=0.24$ ; Fig. 1). There were no effects of family group size on androgen levels (either KT or T) neither among territorial males ( $n=9$ ; Spearman rank correlation, KT,  $P=0.80$  and T,  $P=0.87$ ) nor among male helpers ( $n=11$ ; Spearman rank correlation, KT,  $P=0.27$  and T,  $P=0.54$ ). Again, with the sample size used in this study, the variation in male androgen levels was not explained by the presence of eggs and fry (0/1) in the family group, neither among territorial males ( $n_{\text{without fry}}=5$ ,  $n_{\text{with fry}}=4$ ; Mann–Whitney  $U$  test, KT,  $P=0.22$  and T,  $P=0.22$ ) nor among male helpers ( $n_{\text{without fry}}=6$ ,  $n_{\text{with fry}}=5$ ; Mann–Whitney  $U$  test, KT,  $P=0.36$  and T,  $P=0.27$ ).

*Neolamprologus pulcher* male helpers also share paternity and their helping behaviour is therefore part of an alternative reproductive tactic. In all teleost species with alternative reproductive tactics for which androgen levels are known, the bourgeois courting morph has higher KT levels than the parasitic non-courting morph (Brantley *et al.*, 1993; Oliveira *et al.*, 2001b). Therefore, the present results may be seen as the first exception to this rule. In *N. pulcher*, however, there is no qualitative differentiation of helpers as an alternative morphotype. Helpers are subordinate to breeders but they display all reproductive behavioural patterns present in breeders, including territorial defence and brood care (Taborsky & Limberger, 1981; Limberger, 1983; Taborsky, 1984). Moreover, in this species helper males do not have higher gonado-somatic indices ( $I_G$ ) than territorial males (F. Neat, M. Taborsky & S. Balshine, unpubl. data), which do not conform to the pattern found in teleost species with alternative reproductive tactics, in which parasitic spawners (*e.g.* sneakers) generally have higher  $I_G$  values than bourgeois males (Taborsky, 1994, 1999, 2001).

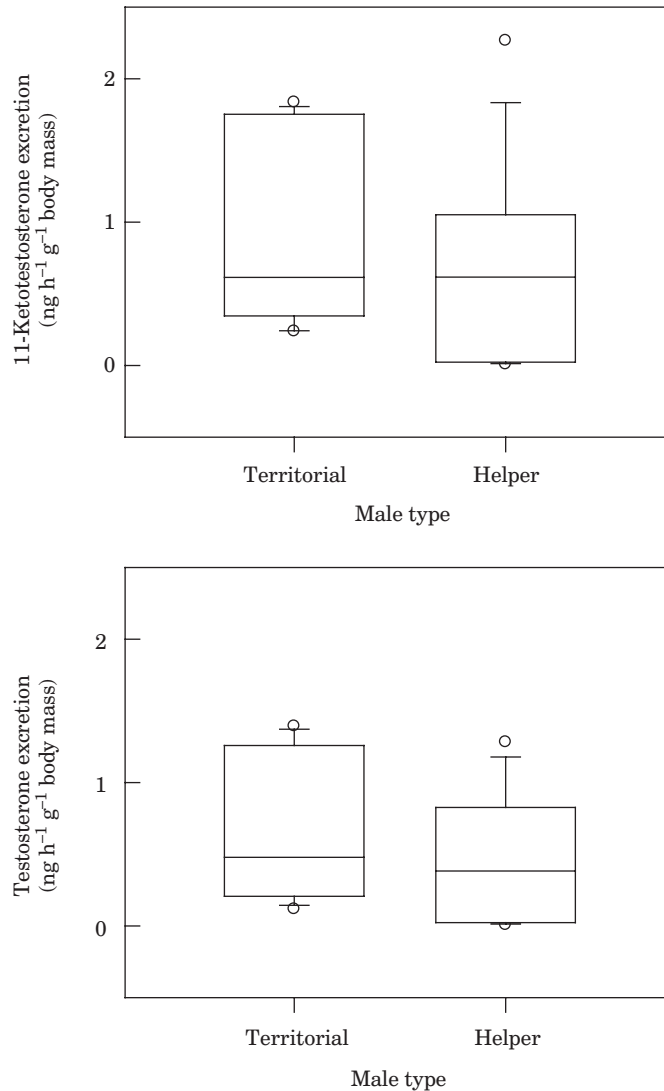


FIG. 1. Androgen levels in territorial males ( $n=9$ ) and male helpers ( $n=11$ ); bar = median; box = quartiles; whiskers = min–max values; open circles = outliers.

In conclusion, the results of this study confirm for the first time in a teleost the prediction derived from observations in higher vertebrates that in co-operatively breeding species in which helpers' reproduction is not suppressed, helpers and breeding males have similar androgen levels.

The authors acknowledge E. Couto's technical assistance in the steroid measurements and the comments provided by A. Ros and the late L. Carneiro that contributed to improve the manuscript. This study is part of a larger research project of RFO funded by Fundação para a Ciência e a Tecnologia (FCT, Praxis XXI/P/BIA/10251/1998). RFO research is supported by a Plurianual Research Grant (FCT R&D research unit 331/94) and MT by a SFN Grant (31-64396.01).

## References

- Balshine-Earn, S., Neat, F. C., Reid, H. & Taborsky, M. (1998). Paying to stay or paying to breed? Field evidence for direct benefits of helping behaviour in a cooperative breeding cichlid fish, *Neolamprologus pulcher*. *Behavioral Ecology* **9**, 432–438.
- Balshine-Earn, S., Leach, B., Neat, F., Reid, H., Taborsky, M. & Werner, N. (2001). Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioural Ecology and Sociobiology* **50**, 134–140.
- Brantley, R. K., Wingfield, J. C. & Bass, A. H. (1993). Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes. *Hormones and Behavior* **27**, 332–347.
- Creel, S., Creel, N. M., Mills, M. G. L. & Monfort, S. L. (1997). Rank and reproduction in cooperatively breeding African wild dogs: behavioural and endocrine correlates. *Behavioural Ecology* **8**, 298–306.
- Dierkes, P., Taborsky, M. & Kohler, U. (1999). Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behavioural Ecology* **10**, 510–515.
- Emlen, S. T. (1991). The evolution of cooperative breeding in birds and mammals. In *Behavioural Ecology: an Evolutionary Approach* (Krebs, J. R. & Davies, N. B., eds.), pp. 301–337. Oxford: Blackwell.
- Greenwood, L. N., Scott, A. P., Vermeirssen, E. L. M., Mylonas, C. C. & Pavlidis, M. (2001). Plasma steroids in mature common dentex (*Dentex dentex*) stimulated with a gonadotropin-releasing hormone agonist. *General and Comparative Endocrinology* **123**, 1–12.
- Hirschenhauser, K., Ros, A. F. H., Carneiro, L. A., Oliveira, T., Silva, A., Canario, A. V. M. & Oliveira, R. F. (2002). Non-invasive hormone assessment from fish holding water. *Advances in Ethology* **37**, 139.
- Kime, D. E. & Manning, N. J. (1982). Seasonal patterns of free and conjugated androgens in the brown trout *Salmo trutta*. *General and Comparative Endocrinology* **48**, 222–231.
- Limberger, D. (1983). Pairs and harems in a cichlid fish *Lamprologus brichardi*. *Zeitschrift für Tierpsychologie* **62**, 115–144.
- Martin, E. & Taborsky, M. (1997). Alternative male mating tactics in a cichlid *Pelvicchromis pulcher*: a comparison of reproductive effort and success. *Behavioral Ecology and Sociobiology* **41**, 311–319.
- Moriwaki, T., Kobayashi, M., Aida, K. & Hanyu, I. (1991). Changes in plasma gonadotropin and steroid hormone levels during ovulation induced by HCG treatment in female goldfish. *Nippon Suisan Gakkaishi* **57**, 41–43.
- Oliveira, R. F., Lopes, M., Carneiro, L. A. & Canário, A. V. M. (2001a). Watching fights raises fish hormone levels. *Nature* **409**, 475.
- Oliveira, R. F., Canário, A. V. M. & Grober, M. S. (2001b). Male sexual polymorphism, alternative reproductive tactics and androgens in combtooth blennies (Pisces: Blenniidae). *Hormones and Behavior* **40**, 266–275.
- Oliveira, R. F., Miranda, J. A., Carvalho, N., Gonçalves, E. J., Grober, M. S. & Santos, R. S. (2002). The relationship between the presence of satellite males and nest-holders' mating success in the Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis*. *Ethology* **108**, 223–235.
- Peters, A., Astheimer, L. B. & Cockburn, A. (2001). The annual testosterone profile in cooperatively breeding superb fairy-wrens, *Malurus cyaneus*, reflects their extreme infidelity. *Behavioral Ecology and Sociobiology* **50**, 519–527.
- Poiani, A. & Fletcher, T. (1994). Plasma levels of androgens and gonadal development of breeders and helpers in the bell miner (*Manorina melanophrys*). *Behavioral Ecology and Sociobiology* **34**, 31–41.
- Reyer, H. U., Dittami, J. P. & Hall, M. R. (1986). Avian helpers at the nest: are they psychologically castrated? *Ethology* **71**, 216–228.
- Schmidt, L. G., Bradshaw, S. D. & Follett, B. K. (1991). Plasma levels of luteinizing hormone and androgens in relation to age and breeding status among cooperatively breeding magpies (*Gymnorhina tibicen* Latham). *General and Comparative Endocrinology* **83**, 48–55.

- Schoech, S. J., Mumme, R. L. & Moore, M. C. (1991). Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). *Condor* **93**, 354–364.
- Scott, A. P. & Sorensen, P. W. (1994). Time course of release of pheromonally active gonadal steroids and their conjugates by ovulatory goldfish. *General and Comparative Endocrinology* **96**, 309–323.
- Scott, A. P., MacKenzie, D. S. & Stacey, N. E. (1984). Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni*. II. Steroid hormones. *General and Comparative Endocrinology* **56**, 349–359.
- Taborsky, M. (1984). Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. *Animal Behaviour* **32**, 1236–1252.
- Taborsky, M. (1985). Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* **95**, 45–75.
- Taborsky, M. (1994). Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. *Advances in the Study of Behavior* **23**, 1–100.
- Taborsky, M. (1999). Conflict or cooperation: what determines optimal solutions to competition in fish reproduction? In *Behaviour and Conservation of Littoral Fishes* (Almada, V. C., Oliveira, R. F. & Gonçalves, E. J., eds), pp. 301–349. Lisboa: I.S.P.A.
- Taborsky, M. (2001). The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *Journal of Heredity* **92**, 100–110.
- Taborsky, M. & Limberger, D. (1981). Helpers in fish. *Behavioral Ecology and Sociobiology* **8**, 143–145.
- Taborsky, M., Hudde, B. & Wirtz, P. (1987). Reproductive behaviour and ecology of *Symphodus (crenilabrus) ocellatus*, a European wrasse with four types of male behaviour. *Behaviour* **102**, 82–118.
- Vleck, C. M. & Brown, J. L. (1999). Testosterone and social and reproductive behaviour in *Aphelocoma* jays. *Animal Behaviour* **58**, 943–951.
- Wingfield, J. C., Hegner, R. H. & Lewis, D. M. (1991). Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *Journal of Zoology* **225**, 43–58.

# MARKED PROOF

## Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

<i>Instruction to printer</i>	<i>Textual mark</i>	<i>Marginal mark</i>
Leave unchanged	... under matter to remain	Stet
Insert in text the matter indicated in the margin	⤴	New matter followed by ⤴
Delete	⤵ through matter to be deleted	⤵
Delete and close up	⤵ through matter to be deleted	⤵
Substitute character or substitute part of one or more word(s)	/ through letter or ⤵ through word	New letter or new word
Change to italics	— under matter to be changed	≡
Change to capitals	≡ under matter to be changed	≡
Change to small capitals	= under matter to be changed	=
Change to bold type	~ under matter to be changed	~
Change to bold italic	≡ under matter to be changed	≡
Change to lower case	Encircle matter to be changed	⊖
Change italic to upright type	(As above)	⊖
Insert 'superior' character	/ through character or ⤴ where required	⤴ under character e.g. ⤴
Insert 'inferior' character	(As above)	⤵ over character e.g. ⤵
Insert full stop	(As above)	⦿
Insert comma	(As above)	,
Insert single quotation marks	(As above)	⤴ and/or ⤵
Insert double quotation marks	(As above)	⤴ and/or ⤵
Insert hyphen	(As above)	⊖
Start new paragraph	⤴	⤴
No new paragraph	~	~
Transpose	⤴	⤴
Close up	linking ⦿ letters	⦿
Insert space between letters	⤴ between letters affected	#
Insert space between words	⤴ between words affected	#
Reduce space between letters	⤴ between letters affected	⤴
Reduce space between words	⤴ between words affected	⤴