Bulletin of Fish Biology	Volume 11	Nos. 1/2	31.12.2009	15-20

The expression of sexual dimorphisms in the weakly electric fish *Mormyrus rume proboscirostris* (Teleostei, Mormyridae) following exposure of larvae to 17α-methyldihydrotestosterone*

Die Ausprägung von Sexualdimorphismen bei dem schwach elektrischen Fisch *Mormyrus rume proboscirostris* (Teleostei, Mormyridae) nach Behandlung der Larven mit 17α-Methyldihydrotestosteron

Peter Moller[1], Cristian Schugardt[2], Bryan Dowling[1], & Frank Kirschbaum[3]

[1] Department of Psychology, Hunter College and The Graduate School of the City University of New York, 695 Park Avenue, New York, NY 10065, USA; pmoller@hunter.cuny.edu
[2]Institute of Animal Sciences, Humboldt Universität zu Berlin, D 10115 Berlin, Germany,
[3]Humboldt University Berlin, Faculty of Agriculture and Horticulture, Unter den Linden 6, D-10099 Berlin, Germany

Zusammenfassung. Larven von *Mormyrus rume proboscirostris* wurden im Alter von 18, 21, 24 und 27 Tagen für jeweils 3 h mit 3,0 mg/l 17α-Methyldihydrotestosteron (17α-MDHT) behandelt. In einer Gruppe von 70 überlebenden Fischen wurde im Alter von 2 Jahren der Gonadenstatus der Fische histologisch bestimmt, Röntgenaufnahmen der Analflosse angefertigt und die elektrische Entladung aufgezeichnet. Das genotypische Geschlecht der Fische war nicht bekannt. 35 der 70 Fische zeigten einen normal entwickelten Eierstock, ein Fisch einen normalen Hoden und 25 Fische hermaphroditische Gonaden. Bei neun Fischen fand sich keine entwickelte Gonade. Männchentypische Vergrößerungen der Flossenstrahlbasis in der Analflosse fanden sich bei einem Teil der Weibchen und bei fast allen Tieren mit hermaphroditischen Gonaden. Alle behandelten Fische zeigten eine männchentypische Entladung.

Schlüsselwörter: Sexualdimorphismus, *Mormyrus rume proboscirostris*, 17α-Methyldihydrotestosteron

Summary. Larvae of *Mormyrus rume proboscirostris* were treated for 3 hours with 3.0 mg/l 17α-methyldihydrotestosterone at the age of 18, 21, 24 and 27 days. In a group of 70 surviving fish, at the age of 2 years, their electric organ discharge was recorded, the gonadal status histologically determined, and x-rays of their anal fin were taken. The genotypic sex of the fish was not known. 35 fish had developed a normal ovary, only one fish a normal testis, and 25 fish hermaphroditic (intersex) gonads. Gonads were not developed in 9 fish. Male-typical enlargements of the anal fin ray base were found in some females and in almost all animals with hermaphroditic gonads. All treated fish exhibited the elongated, male-typical electric organ discharge.

Key words: Sexual dimorphism, *Mormyrus rume proboscirostris*, 17α-Methyldihydrotestosterone

1. Introduction

African freshwater Mormyridae are a family of teleost fishes that generate weak electric organ discharges (EODs) that in concert with a unique electrosense serve in social communication and orientation (Hopkins 1986, Kramer 1990, Moller 1995, 2006, von der Emde 1998, 2002). As do most mormyrids, *Mormyrus rume proboscirostris*, the subjects of this study, possess

* This is an excerpted version of a talk P.M. presented on 28. January 2008 at the I.G.B. honoring Professor Frank Kirschbaum.

sexually dimorphic characters including the waveform of their EOD, with males emitting longer EODs than females; characteristic basal bone expansions that only affect the male's anal-fin, differences in anal-fin morphology, and a male-typical flexing of the anal fin during courtship. By assessing the mature phenotype, we have investigated the organizational effects of early life exposure to a non-aromatizable androgen, 17α-methyldihydrotestosterone (17α-MDHT), on three of these characters, EOD and bone expansion, as well as on gonadal development. Based on the reported organizational effects of this androgen on embryonic stages in other teleosts, resulting in complete masculinizing of the adult phenotype (Guerrero 1975, Fitzpatrick et al. 1997; Kitano et al. 2000), we hypothesized that exposure to 17α-MDHT would have the same effect on the adult *M. rume proboscirostris* phenotype.

2. Material and methods

Fish were bred in our laboratory at Humboldt University following Kirschbaum (1987) and Schugardt & Kirschbaum (1998, 2004). Larvae were exposed for 3 h to water-dissolved 3.0 mg/l 17α-MDHT on days 18, 21, 24, and 27 days post fertilization (DPF) and raised to maturity. For a cohort of 70 surviving fish at 24 months of age, we monitored EODs with a pair of stainless steel electrodes from individual fish at rest in a recording chamber filled with water from their respective home aquaria. EODs were pre-amplified and displayed on digital storage oscilloscopes (Tektronix, TDS 3100 or Tektronix 3012). The duration of the two phases of the fish's EOD (P1, P2) was read off the oscilloscope screen and stored for further analysis. To assess changes in the fish's fin morphology, fish were sacrificed and then x-rayed under low-intensity radiation (30-40 KVP) for two minutes (Hewlett-Packard, Faxitron 43807 N). The radiographs were scanned into computer and screened for the occurrence of expanded anal-fin ray bases using commercial imaging software (SigmaScanTM Pro). Gonads were fixated in Bouin (24 h) and transferred into 70% ethanol. Gonadal tissue was embedded in paraffin, sectioned (5 μm), and stained following Domagk (Romeis 1989).

3. Results and Discussion

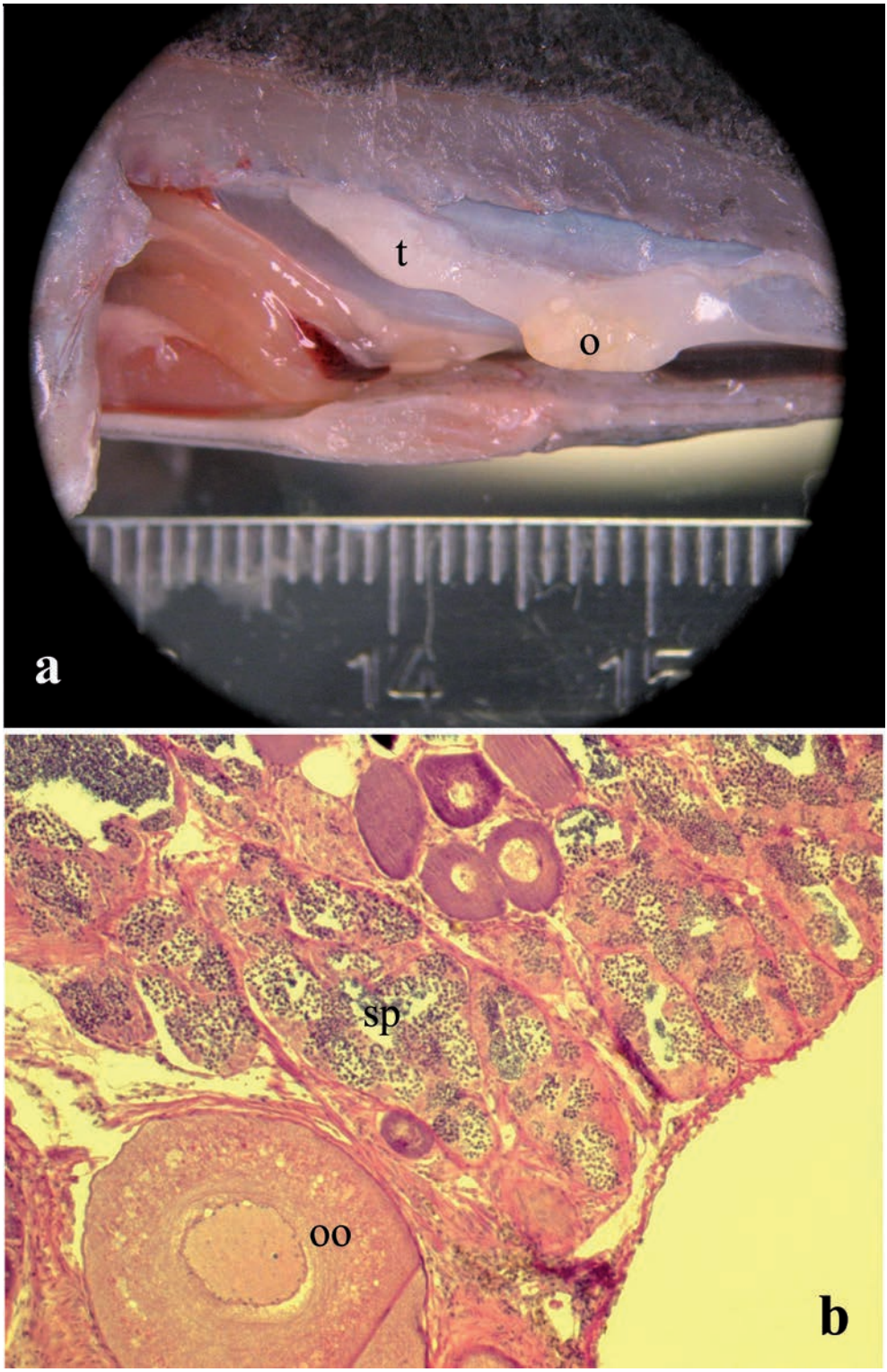
The genotypic sex of our fish was unknown. Therefore, we are reporting here for all fish, independent of genetic sex, the effects of early larval exposure to 17α-MDHT on the electric organ discharge, as well as on anal-fin ray and gonad morphology. In contrast to reported data on other teleosts (see above), our findings lent only partial support to our hypothesis.

3.1. Gonadal status

The gonadal phenotype showed both normally developed gonads and severe gonadal abnormalities. There were 35 fish with a normally developed ovary and only one fish with a normally developed testis. (Note: mormyrids possess only one developed gonad.) Twenty-five fish exhibited different stages of hermaphroditic gonadal development as illustrated in figures 1 and 2. In nine fish a gonadal assignment to sex could not be made.

Although the majority of teleost fishes are undifferentiated gonochorists (Yamamoto 1969), there are no data on the mode of sexual differentiation in Mormyridae. Notwithstanding a plausible caveat, namely that environmental variables such as temperature can affect the sex ratio in teleosts (Strussmann 2002), based on data by Moller et al. (2004) and Stell (2006), we assumed a sex ratio of 1:1 in our species, and suggest that our cohort of 70 fish consisted of 35 genetic males and 35 genetic females.

Figs. 1a and b: Example of an intersex phenotype. **a** Macroscopic structure of ovotestis (t, o). **b** Section shows ovotestis with spermatozoa (sp) and oocytes (oo).
Abb. 1a und b: Intersex-Phänotyp. **a** Makroskopische Ansicht des Ovotestis. **b** Histologischer Schnitt eines Ovotestis mit Spermien (sp) und Oozyten (oo).


t
o
a
14
15
sp
oo
b

An undifferentiated mode of sexual differentiation, i.e. the existence of an initial 'female stage' followed by later differentiation into the male stage would imply two critical organizational periods, first the organization of the ovary, followed later by the organization of the testis. We assume that the complete masculinization of androgen-treated teleosts reported by GUERRERO (1975), DULKA & MALER (1994), WASSERMAN & ALFONSO (2003), and EZAZ et al. (2004), and the incomplete gonadal masculinization in our fish, was due to differences in the timing of our treatment schedule. We propose that by the time we started treatment at 18-27 DPF, the normal developmental path for the ovary was already organized, thus half the fish developed normal ovaries as our data have shown. The 'organizational window' for the differentiation of testis, however, was still 'open', and 17α-MDHT-treatment interfered with the normal expression of the male phenotype safe in one case, resulting in incomplete basal anal-fin bone expansion, and various forms of gonadal intersex. The exact nature of this interference is not known. A deficit in aromatizable testosterone, and thus estradiol, which contributes to normal testicular development and spermatogenesis, could have affected the observed gonadal abnormalities.

3.2. Electric organ discharge

The EOD of *M. rume proboscirostris* consists of two phases P1 and P2. A comparison with untreated fish showed that the mean duration of P2 in all treated fish was male-typical in all hormone treated fish. The structural organization of the electric organ retains a high degree of plasticity even in adulthood, and exposure to androgens will result in an increase in the androgen-sensitive phase duration (BASS & HOPKINS 1983, 1985, HERFELD & MOLLER 1998; LANDSMAN et al. 1990). Our data suggest that early larval exposure to androgens both organized and activated the development of a male-typical adult electric organ, and thus also a male-typical EOD (DOWLING 2008). This leaves a currently unresolved puzzle: How can 17α-MDHT treatment of a functional larval electric organ at 17-27 DPF affect the much later developing adult organ (about 45-90 DPF)? At this time, it is not known how 17α-MDHT affects the fish's larval electric organ. Future studies should therefore

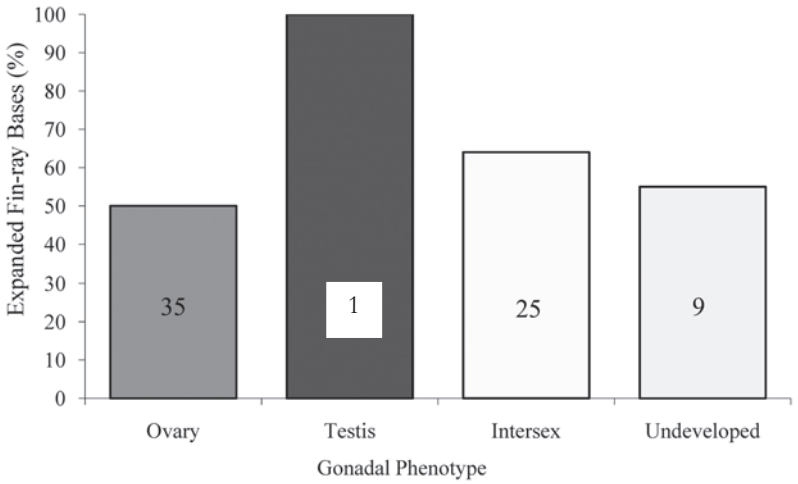


Fig. 2: Percentage (with standard deviation) of fish with expanded anal-fin ray-bases as a function of gonadal phenotype. Adapted from DOWLING(2008).
Abb. 2: Prozentualer Anteil (mit Standardabweichung) von Fischen mit einer vergrößerten Basis der Analflosse als Funktion des gonadalen Phänotyps. Nach DOWLING (2008).

assess and follow the developmental status of the electric organ from the time of treatment to the emergence of the adult organ.

3.3. Anal-fin ray morphology

At 24 months (sexual maturity), more than half of the hormone-treated fish exhibited the male-typical base expansion (57%). In a control group consisting of sexually mature males (n=7) and females (n=8) in breeding conditions, all of the males showed basal fin ray expansions while none of the females did. Having inferred the genetic sex of *M. r. proboscirostris* in this study, we can correlate the gonadal genotype with corresponding results from EOD phases and basal fin-ray expansion (fig. 2). While the EOD remained masculinized in nearly 100% of fish, basal fin-ray expansions were clearly affected by the inferred genotypic sex. The only male with a normally developed testis exhibited expanded fin ray bases; about half the females showed this trait, which we attributed to insufficient levels of androgens 24 months after treatment, and 60% of the intersex phenotypes (genetic males) had developed the male-typical fin-ray expansion.

Acknowledgements

We thank Ms. WIECZOREK for her assistance with histology. We acknowledge the use of the radiographic facility at the American Museum of Natural History (Division of Vertebrate Zoology, Ichthyology). This study was supported by PSC-CUNY grants, a CUNY-Hunter College Equipment grant to PM, and a grant from the National Center for Research Resources (RR03037-NCRR–NIH). The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of NCRR or NIH.

Literature

BASS, A.H., & C.D. HOPKINS. 1983. Hormonal control of sexual differentiation: Changes in electric organ discharge waveform. Science 220, 971-974.

BASS, A.H., & C.D. HOPKINS. 1985. Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fish. Journal of Comparative Physiology 156A, 587-604.

DOWLING, B. 2008. Endocrine modulators and the development of sexually dimorphic characters in weakly electric fish, *Mormyrus rume proboscirostris*. PhD thesis, City University of New York.

DULKA, J.G., & L. MALER. 1994. Testosterone modulates female chirping behavior in the weakly electric fish, *Apteronotus leptorhynchus*. Journal of Comparative Physiology 174A, 1432-1440.

EZAZ, M.T., J.M. MYERS, S.F. POWELL, B.J. MCANDREW, & D.J. PENMAN. 2004. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia, *Oreochromis niloticus*. Aquaculture 232, 205-214.

FITZPATRICK, M.S., C.B. SCHRECK, & W.L. GALE. 1997. Masculinization of tilapia through immersion in 17α-methyltestosterone or 17α-methyldihydrotestosterone. Pond Dynamics/Aquaculture. Collaborative Research Support Program. 14th Annual Technical Report, 93-98.

GUERRERO, R.D. 1975. Use of androgens for the production of all-male tilapia. Transactions of the American Fisheries Society 104, 342-348.

HERFELD, S., & P. MOLLER. 1998. Effects of 17α-methyltestosterone on sexually dimorphic characters in the weakly discharging electric fish, *Brienomyrus niger* (GÜNTHER, 1866) (Mormyridae): electric organ discharge, ventral body wall indentation, and anal-fin ray bone expansion. Hormones and Behavior 34, 303-319.

HOPKINS, C.D. 1986. Behavior of Mormyridae, pp. 527-576. In: Electroreception (BULLOCK, T.H., & W. HEILIGENBERG, eds). Wiley, New York.

KIRSCHBAUM, F. 1987. Reproduction and development of the weakly electric fish, *Pollimyrus isidori* (Mormyridae, Teleostei) in captivity. Environmental Biology of Fishes 20, 11-31.

KITANO, T., K. TAKAMUNE, Y. NAGAHAMA, & S. ABE. 2000. Aromatase inhibitor and 17α-methyltestosterone cause sex-reversal from genetic females to phenotypic males and suppression of P450 aromatase gene expression in Japanese flounder (*Paralichthys olivaceus*). Reproduction and Development 56, 1-5.

KRAMER, B. 1990. Electrocommunication in teleost fishes. Behavior and experiments. Zoophysiology, 29. Springer, Berlin.

LANDSMAN, R.E., C.F. HARDING, P. MOLLER, & P. THOMAS. 1990. The effects of androgens and estrogen on the external morphology and electric organ discharge waveform of *Gnathonemus petersii*

(Mormyridae, Teleostei). Hormones and Behavior 24, 532-553.

Moller, P. 1995. Electric Fishes: History and Behavior. Chapman and Hall, London.

Moller, P. 2006. Electrocommunication: history, insights, and new questions, pp. 579-598. In: Communication in Fishes (Ladich, F., S.P. Collin, P. Moller, & B.G. Kapoor, eds). Science Publishers, Enfield, New Hampshire.

Moller, P., C. Schugardt, & F. Kirschbaum. 2004. Permanent and seasonal expressions of sexual dimorphisms in a weakly electric fish, *Mormyrus rume proboscirostris,* Boulenger 1898 (Mormyridae, Teleostei). Environmental Biology of Fishes 70, 175-184.

Romeis, B. 1989. Mikroskopische Technik. 17. Aufl. (Bock, P., ed.) Urban und Schwarzenberg Verlag, 16. Aufl. Oldenbourg Verlag , München, Wien.

Schugardt, C., & F. Kirschbaum. 1998. Sozial- und Fortpflanzungsverhalten von Mormyriden, pp. 73-84. In: Verhalten von Aquarienfischen (Greven, H., & R. Riehl, eds), pp. 73-84. Birgit Schmettkamp Verlag, Bornheim.

Schugardt, C., & F. Kirschbaum. 2004. Control of gonadal maturation and regression by experimental variation of environmental factors in the mormyrid fish, *Mormyrus rume proboscirostris*. Environmental Biology of Fishes 70, 227-233.

Stell, S. 2006. Effects of gonadal steroids on sexual dimorphic characteristics in the weakly discharging electric fish, *Brienomyrus niger* (Günther, 1866) (Mormyridae): Solving a paradox. Ph.D. Dissertation, City University of New York.

Strussmann, C.A. 2002. Morphology, endocrinology, and environmental modulation of sex differentiation in teleost fishes. Fish Physiology and Biochemistry 26, 13-29.

von der Emde, G. 1998. Electroreception, pp. 313-343. In: The Physiology of Fishes (2nd ed.) (Evans, D.H., ed.). CrC Press, Boca Raton.

Wasserman, G.J., & L.O. Alfonso. 2003. Sex reversal in Nile tilapia (*Oreochromis niloticus*) by androgen immersion. Aquaculture Research 34, 65-71.

Yamamoto, T.O. 1969. Sex differentiation, pp. 117-175. In: Fish Physiology, Vol. 3 (Hoar, W.S., & D.J. Randall, eds). Academic Press, New York.

Received: 15.10.2009
Accepted: 21.12.2009