A WEAKLY DISCHARGING ELECTRIC FISH, *GNATHONEMUS PETERSII* (MORMYRIDAE, TELEOSTEI), AS A MODEL OF INTEGRATED ANDROGEN EFFECTS ON STRUCTURE AND BEHAVIOR

By

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

2003

UMI Number: 3074690

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This manuscript has been read and accepted for the Graduate Faculty
in Psychology in satisfaction of the dissertation requirement
for the degree of Doctor of Philosophy.

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Abstract

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Advisor: Professor Peter Moller

This research was designed to examine the plasticity of structural and behavioral male-specific characteristics in a weakly discharging electric fish, *Gnathonemus petersii* (Günther, 1862) (Mormyridae, Teleostei), and the sexual hormones contributing to the development of these characteristics. Adult males are distinguished by massive bone expansion of the bases of a select number of anal-fin rays, a dorsally directed indentation of the posterior ventral body wall (possibly, a sign of muscular hypertrophy), and a characteristic electric organ discharge (EOD) with a longer duration of phases 2 and 3 and lower associated peak power spectral frequency (PPSF) of the Fourier transform. An anal-fin reflex, a characteristic mating display, was reported in adults of both sexes. In Experiment 1a, juvenile subjects were gonadectomized and implanted with silastic tubing containing either testosterone (T), dihydrotestosterone (DHT), 17β-estradiol (E) or nothing (blanks) for four weeks. DHT was most effective in inducing morphological changes: bone expansion in the bases of the anal-fin rays and alteration of the anal-fin

topography. Histological data also suggested DHT-induced hypertrophy of the anal-fin musculature. T was most effective in producing behavioral changes (EOD). Both androgens induced the anal-fin reflex. E was ineffective. Experiment 1b confirmed the ineffectiveness of E: no morphological or behavioral changes were found in gonadectomized animals implanted with E for eight weeks. Experiment 2 tested the reversibility of androgen-induced changes and compared the effects of T and 11-ketotestosterone (11-KT). Gonadectomized subjects were implanted with either T or 11-KT for seven weeks, at which point the original implants were recovered and replaced with blanks, and the fish were observed for an additional six weeks. Both androgens produced male-specific changes in anal-fin morphology, characteristics of the EOD, and resulted in the appearance of the reflex. Following androgen withdrawal, behavioral characteristics reverted to their baseline level and the anal-fin reflex disappeared, whereas masculine morphological characteristics persisted. No significant differences were found between the effects of T and 11-KT. Together, these studies suggest a functional separation of androgen effects in the masculinization process in *G. petersii*: organizational effects of DHT on anal-fin morphology and activational effects of T on reproductive behavior.

ACKNOWLEDGEMENTS

In thanks to God.

To my parents, Elvira and Vladimir Voustianiouk, without whom this work would never have been started, and to Natalia Chebalina, without whose unconditional help and support it would never have been finished.

This is to set forth my heartfelt gratitude to all those who by their knowledge, expertise, work and simple kindness made this research possible:

Dr. Peter Moller, my thesis advisor and Mentor in the best and truest sense of this word

Drs. Cheryl Harding and Barbara Brown, the core members of the supervisory committee who generously shared their knowledge and provided invaluable comments on the earlier versions of this manuscript

Drs. James Gordon and Vanya Quinones-Jenab, the outside readers, who helped make this work better

Dr. Robert Landsman, who trained me in fish surgery

Ms. Judy Choi, who was an expert anesthesiologist and assistant during many of those surgeries and an expert fish nurse after them

Mr. Radford Arrindell, who trained me in fish radiography

Ms. Damaris Rodriguez, for her fantastic preparations of cleared and stained specimens

Ms. Sonja Herfeld, for expert histological preparations

Mr. Tom Terleph, for invaluable help during video recording sessions

Dr. Linda Perrotti, for her help and encouragement during initial stages of this work

Ms. Ellen Breheny, for her unique ability to resolve any administrative problem in the fastest, simplest and best manner

My sincere thanks also go to the faculty and staff of the Psychology Department of Hunter College and to many fellow students of the Biopsychology doctoral subprogram whose help at different stages of this research is deeply appreciated.

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GLOSSARY

11-KT	11-ketotestosterone
17-MT	17α-methyltestosterone
A/D ratio	ratio obtained by dividing the measured thickness of the muscle tissue in the anal-fin area by the maximal thickness of the muscle tissue in the dorsal fin area
AFL	length of anal fin
AFR	anal-fin reflex
DHT	dihydrotestosterone
E	17β-estradiol
EOD	electric organ discharge
PPSF	peak power spectral frequency
r-AFL	length of anal fin measured from the radiograph
RD	relative differential – the amount of change from the baseline level to the moment of measurement in reference to the baseline level [RD = (M_{W4} – M_{BL}) / M_{BL} where M_{BL} – the value measured at the baseline (prior to implant surgery), and M_{W4} – the value measured at the end of postimplant week 4]
SL	standard length
T	testosterone

INTRODUCTION

Hormones are involved in the regulation of a vast variety of biological processes: from sexual differentiation and development to growth, maturation, reproduction, and death. Regardless of the target of hormonal influence – a morphological element or a behavioral sequence – all effects of sex hormones can be classified into two main categories: organizational and activational, as proposed by Charles Phoenix and colleagues (Phoenix, Goy, Gerall and Young, 1959). The classic distinction lies in that organizational effects are permanent and take place during early development, typically within some rather narrow time interval called critical or sensitive period, whereas activational effects are transient and observed in the mature organism, often in a cyclic fashion and triggered by environmental or social cues. Instances of both types of effects have been conclusively demonstrated in many avian and mammalian species, with sexually dimorphic behaviors being attributed to specific sex differences in the brain. Development and execution of a bird song and the copulatory postures in the rat are probably the best-known examples (Nottebohm, 1981; Nottebohm and Arnold, 1976; Dörner, 1974; recent review: Gorski, 2000).

Mormyrid fish, among other Teleosts, provide excellent research subjects to study complex effects of endocrine changes on structure and behavior for a number of reasons. (1) They have been extensively studied both in the field and in captivity (review: Moller, 1995). Being seasonal breeders, these fish follow an established pattern of cyclic fluctuations in their hormonal levels throughout the year, making it easy to predict upcoming changes or to control them by varying environmental conditions in the

laboratory (Kirschbaum, 1979, 1987, 1995). **(2)** Mormyrid fish exhibit easily observable, quantifiable characteristics directly affected by endocrine changes, which include, depending on the species, some or all of the following: amount of ossification of the bases of the anal-fin rays and magnitude of the indentation of the posterior ventral body wall (Brown, Benveniste and Moller, 1996; Herfeld et al., 1997; Herfeld and Moller, 1998; Landsman and Moller, 1988; Landsman, Harding, Moller and Thomas, 1990; Pezzanite and Moller, 1998), an anal-fin reflex (AFR) (Kirschbaum, 1987), properties of the electric organ discharge (EOD) (review in Landsman, 1995), and, in several species of the genus *Pollymirus*, vocalization (Crawford, Hagedorn and Hopkins, 1986; Bratton and Kramer, 1989). **(3)** These characteristics appear to show inter- and intra-specific differences (Brown et al., 1996; Herfeld et al., 1997; Pezzanite and Moller, 1998) making this group of fish valuable for taxonomic studies (Sullivan, Lavoue and Hopkins, 2002). **(4)** Mormyrid species possess osteocytic bone that appears to be plesiomorphic in relation to bone of tetrapods (Meunier and Huysseune, 1992; Parenti, 1986). At the same time, quite unlike birds and mammals, at least some of the mormyrids do not have a clear sensitive window in their development: for instance, androgen treatment can elicit structural and behavioral masculinization in both juveniles and adult females of *G. petersii* (Landsman et al, 1990) and *B. niger* (Herfeld and Moller, 1998). Thus, a better understanding of hormonal mechanisms underlying the dynamics of bone metabolism in these species may advance our understanding of structural and behavioral plasticity, and ontogenetic and phylogenetic relationships among various groups of vertebrates. **(5)** Finally, such integrative research approach with simultaneous inclusion of structural and behavioral characteristics among the studied variables has already been successfully

applied in studies of organismic plasticity (Rosa-Molinar, Hendricks, Rodriguez-Sierra and Fritzsch, 1994; Herfeld and Moller, 1998).

HORMONAL EFFECTS ON BONE METABOLISM

Information on hormonal effects on bone metabolism in mormyrids is extremely limited, which makes it necessary to extrapolate from the studies conducted on other teleost fishes and other vertebrates. It is difficult to interpret the data, however, since species in the family Mormyridae together with many other lower teleost and all non-teleost fishes possess cellular bone and are very similar to birds and mammals in terms of bone cell types and structure as well as osteogenesis (Lopez, 1970a, 1970b; Lopez, Peignoux-Deville, Lallier, Colston and Macintyre, 1977; Lopez, Peignoux-Deville, Lallier, Martelly and Millet, 1976; Meunier and Huysseune, 1992; Moss, 1961; Weiss and Watabe, 1979). Yet, osteocytic fish bone lacks osteones, typical of mammalian species, – structural elements resulting from the associations of central vascular canals with the concentric sheets of bone matrix around them (Moss, 1965). Thus, on the one hand, it is unclear to what extent data on androgen-controlled osteogenesis obtained from poeciliid (recent review: Rosa-Molinar, Fritzsch and Hendricks, 1996), cyprinodontid or cyprinid species (e.g. Mugiya and Watabe, 1977), all of which have acellular bone (Parenti, 1986), are applicable to mormyrids. On the other hand, extrapolation of results from the studies on avian and mammalian species should be done with caution due to substantial endocrine differences between higher and lower vertebrates. For instance, fish lack the parathyroid gland (Pang, 1971; Parenti, 1986) and do not respond significantly to

exogenous parathyroid hormone (Lopez et al., 1977), which plays a prominent role in bone metabolism in mammals (review: Smith, 1990).

In mammals, both androgens and estrogen appear to facilitate bone formation and maintenance in a marked dose-related fashion (Williams et al., 1992). Estrogen inhibits production and/or action of cytokines (e.g., IL-6) which promote development of osteoclasts, bone-absorbing cells (Jilka et al., 1992). Estrogen also upregulates calcitonin (Wimalawansa, 1990), a hormone found to participate in bone metabolism in the eel, *Anguilla anguilla*, (Lopez, 1970a; Lopez and Deville, 1973; Lopez et al., 1976), a teleost with a cellular bone (Parenti, 1986), which in turn suppresses osteoclastic activity (Liu and Howard, 1991). There is also an abundance of data suggesting that both T and estrogen can exhibit anabolic effects on bone through a direct stimulation of genomic receptors in osteoblasts (Eriksen, Berg et al., 1987; Ericksen, Colvard et al., 1988; Komm, Sheetz et al., 1987; Komm, Terpening et al. 1988; Malone and Wiren, 1992; Takano-Yamamoto and Rodan, 1990). Estrogen can also contribute to bone formation by directly increasing Ca^{2+} uptake by duodenal cells (Arimandi and Kalu, 1992). A number of recent *in vivo* studies, however, raised questions regarding separate "ultimate" bone-regulatory functions of androgens and estrogen at the organismic level. The results of these studies suggest upregulation of bone turnover (resorption and formation) and subsequent bone preservation and growth by androgens, especially dehydroepiandrosterone and dihydrotestosterone (DHT), and downregulation of bone turnover by estrogen leading only to preservation of already existing bone, without additional growth (Chamoux et al., 1997; Coxam et al., 1996; Gill, Turner, Wronski and Bell, 1998; Martel et al., 1998; Mason and Morris, 1997; Tobias, Gallagher and

Chambers, 1994; Wronski, Cintron, Dohetry and Dann, 1988; but see also Koh, Yeh, Bourdeau, Chen and Om, 1996, who found that external estrogen prevented osteoporosis on gonadectomized rats whereas external testosterone could not).

In mormyrid fish, external application of 17-MT, a synthetic androgen, triggered male-typical osteogenesis in the bases of the anal-fin rays in juvenile and adult female *Gnathonemus petersii* (Günther, 1862) (Voustianiouk, unpublished) and *Brienomyrus niger* (Günther, 1866) (Herfeld and Moller, 1998), whereas *G. petersii* and *B. brachyistius* (Gill, 1863) specimens previously implanted with 17β-estradiol (E_2) did not show any bone expansion in the anal fin area (Gannon, specimens were treated by R.E. Landsman; cited in Herfeld and Moller, 1998).

Many studies focusing on morphology employed 17-MT (e.g., Herfeld and Moller, 1998; Rosa-Molinar, Hendricks, Rodriguez-Sierra and Fritzsch, 1994). This synthetic androgen hormone is water-soluble, making it convenient to work with, its metabolism is well-known and the resulting metabolites are functionally similar to those of T. For example, Cravedi et al. (1993) described conversions of 17-MT to 11-keto-17-MT and methyl-DHT similar to the way T is converted to 11-KT and DHT (review: Borg, 1994). Some investigators (e.g., Landsman [1995]) criticized the use of 17-MT in behavioral research because of its synthetic origin and highly hypertrophied results reported in a number of studies. For instance, Grobstein (1944) described gross structural hypertrophies in female *Platypoecilus maculatus* treated with 17-MT in order to induce regeneration of the anal fin. Unfortunately, it is impossible to compare Grobstein's results with those of later studies because he did not report on the used dosages of the hormones.

THE REPRODUCTIVE STRATEGY OF MORMYRID FISH: MORPHO-ETHOLOGICAL IMPLEMENTATIONS AND HORMONAL EFFECTS

Mormyrid fish inhabit murky turbid waters of African rivers and lakes (Moller, Serrier, Belbenoit and Push, 1979; Hopkins, 1981). Given environmental limitations on the use of vision, these fish appear to rely primarily on their electric sense for navigation as they migrate from resting sites to feeding grounds and back, for object location during locomotion and feeding, and for social communication and sexual identification (review: Moller, 1995; see also von der Emde, 1998; Rojas and Moller, 2002).

In many mormyrid species, the number of eggs does not exceed several dozen, spermatozoa are immobile (Jamieson, 1991; Mattei, 1991), and the gametes might be viable only for a limited time following their contact with water (Iles, 1960; Kirschbaum, 1987, 1995). Therefore, precise coordination of mating behavior is required from both partners to ensure reproductive success. The individual EOD properties and rate of discharging contain information about sex, reproductive maturity and hierarchical status of the sender (Figure 1), thus enabling the receiver to act accordingly by approaching or avoiding the former (review: Moller, 1995; see also Herfeld and Moller, 1998; Carlson, Hopkins and Thomas, 2000).

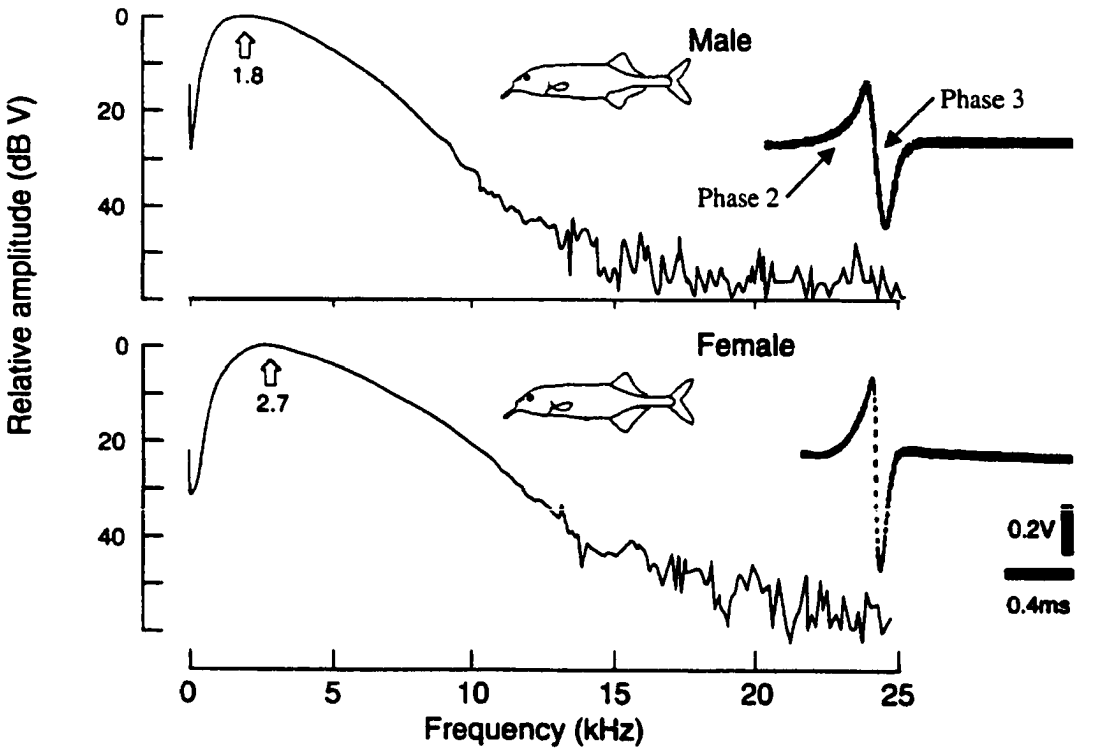


Figure 1. Electric organ discharge in mature male and female *G. petersii*. Male EOD is characterized by longer duration of phases 2 and 3 (solid arrows) and lower value of the associated peak power spectrum frequency (open arrows). Modified after Moller, 1995.

A sense of touch also appears to contribute to reproductive behavior of mormyrids (Iles, 1960; Kirschbaum, 1987, 1995; Kramer, 1990). A peculiar ability to display a concave flexure of the anal fin during courtship and in response to tactile stimulation (AFR) has been documented by Kirschbaum (1987) for male and female *G. petersii* and male *Pollimyrus adspersus* (Günther, 1866) (initially misidentified as *P. isidori* (Valenciennes, 1846)), and observed by J. Choi (personal communication, 1998) in male *B. niger*. An anal-fin reflex is assumed to exist also in several other species (e.g., *Mormyrus kannume* Forskål, 1775) (Iles, 1960). Iles suggested that during oviposition this behavior facilitates formation of a spawning pouch which acts as a fertilization

chamber where immobile gametes are placed in very close proximity to increase the likelihood of fertilization.

Mature males of many mormyrid species have a conspicuous indentation in the posterior ventral body wall (at least, during the reproductive season), while in females this wall is nearly straight at all times (Kirschbaum, 1995; Pezzanite and Moller, 1998). This sex-specific difference was not observed in immature *G. petersii* (Pezzanite and Moller, 1998). Iles noted that in male *M. kannume* this modification appears concomitantly with gonadal maturation, and after breeding "the testis regresses and the anal fin gradually assumes its original form." Okedi (1969b), on the other hand, observed this indentation in males of *Mormyrus* and *Petrocephalus* during and outside their breeding season, and found that the size of the indentation correlates with the size (and, therefore, the age) of the fish, and that, once developed, this characteristic is permanent. Herfeld and Moller (1998) suggested a permanent nature of the indent in 17α-methyl-testosterone-treated *B. niger*. Permanence of indentation in *G. petersii* remains an open question: Landsman (1990) described this characteristic as permanent and due to the exposure to high levels of circulating androgens (Landsman et al., 1990). On the other hand, Moller (personal communication, 1998) radiographed several dozens of clearly mature *G. petersii*, captured outside their breeding season, and found bone expansion in subjects with no externally noticeable indentation. Subsequent gonadal inspection confirmed that these "paradoxical" specimens were males.

Brown et al. (1996) noted that in young male *B. brachyistius* the anal-fin rays were expanded to a lesser degree than in larger, older males (thus confirming Okedi's (1969b) data for *Mormyrus* and *Petrocephalus*) and proposed that the "indenting of the

body seems to be related to an increase in bone at the bases of the anal-fin rays." Pezzanite and Moller (1998) made a similar observation of a size-related degree of bone expansion and body wall indentation in male *G. petersii*. When these fish reach the standard length (SL) of 120 mm, sexual dimorphisms in the degree of bone expansion and body wall indentation become apparent.

Reproductive priority among males is typically a function of a male's overall social status, which is linked to the level of androgens circulating in the blood. Cardwell and Liley (1991a, 1991b) described a correlation between plasma levels of testosterone (T) and 11-ketotestosterone (11-KT) (both aromatizable, naturally occurring androgens) and social status (expressed in an ability to acquire and defend a high-quality breeding territory) in males of stoplight parrotfish, *Sparisoma viride*. Brantley, Wingfield and Bass (1993) compared levels of T and 11-KT in a number of teleost fishes and reported an interesting correlation between the level of 11-KT and male reproductive tactics: individuals with higher levels of 11-KT tended to be more active displayers during courtship. Carlson, Hopkins and Thomas (2000) extended these findings to mormyrids by showing a positive correlation between the duration of EOD, plasma level of 11-KT and social status of males *B. brachyistius*: more dominant males have higher levels of 11-KT, but not T, and emitted EODs of longer duration. Similar relationships are known in bird species where dominant males are often chosen for mating based on their loudest and most persistent singing (Sokolov, 1987).

The reviewed information suggests a possibility that at least some mormyrid species may be characterized by a system of male-typical, androgen-driven, coordinated and temporally synchronized changes in internal and external morphology and behavior,

through which masculinization of developing and breeding males takes place. Masculinization may include, depending on the species, some or all of the following components: expansion of the anal-fin ray bases, increase in size and/or the number of electrocytes and myocytes (body wall indentation), male-typical (longer) EOD, AFR, and vocalization.

These androgens-driven changes in development may unfold as follows: as a result of age- and/or season-dependent increases in exposure to androgens **(1)** the bases of anal-fin rays expand providing additional surfaces for muscle attachment; **(2)** the myocytes proliferate and/or become thicker and attach to newly available surfaces enabling males to flex their anal fin more efficiently; **(3)** the myogenic electrocytes proliferate and become thicker and more convoluted resulting in longer EODs (Bass, 1986; Freedman et al., 1989); **(4)** longer EODs, because of their higher energy content, are presumably more prominent and easier to detect during the breeding season (Dunlap, Thomas and Zakon, 1998).

Concurrent changes in adjacent bone and muscle tissues are common in many vertebrate species, with bone usually leading the way to provide additional surfaces for future muscle attachment (e.g., comparative studies of bird species reveal a clear correlation between the size of wing musculature and the size of the keel where these muscles are attached (Sokolov, 1987)). In mammals, treatments that trigger bone growth (e.g., exposure to androgens) also tend to raise body weight (Coxam et al., 1996), which is indicative of the increased muscle volume.

There is a significant body of evidence that various derivatives of T act as active stimulators of anabolic processes in mammalian muscle (review: Florini, 1987). The

thickening of myocytes together with expansion of the bases of the anal-fin rays may be responsible for the androgen-induced indentation in the dorsal margin of the anal fin or the ventral body wall, the appearance of which correlates well with "masculinization" of EOD characteristics in androgen-treated juvenile and adult female fish (Landsman and Moller, 1988; Landsman et al., 1990; review: Landsman, 1995; see also Herfeld and Moller, 1998). Only reproduction-related structures exhibit any sexual dimorphisms or are affected by androgen treatment. For instance, the margin of the dorsal fin (which, unlike the anal fin, does not play a specific part in spawning) is identical in males and females all the time (Brown et al., 1996; Pezzanite and Moller, 1998) and does not respond to hormonal manipulation (Herfeld and Moller, 1998).

Hypertrophied muscle tissue around the anal fin might enable the male to curve its fin around that of the female more efficiently, improving the shape of the spawning pouch. This would explain a more prominent indentation in older males (Pezzanite and Moller, 1998), who have gone through a greater number of breeding cycles, or in males during the reproductive period, when investments of energy and nutrients into building this muscle structure are necessitated by the expected breeding advantages.

The site of the peripheral hormonal action in mormyrid species is likely to be on the electrocyte membrane (Bass and Hopkins, 1983). Following androgen treatment, electrocytes become thicker and more convoluted, resulting in the male-typical increase in EOD duration (Bass and Hopkins, 1983; Bass, Denizot and Marchaterre, 1986; Freedman et al., 1989). Except for the gymnotiform family *Apteronotidae*, whose electric organ is neurogenic (Bennett, 1971), electrocytes in all electric fishes have evolved from muscle tissue including, among others, modified portions of caudal (*Mormyridae*),

branchial (*Torpedinidae*), pectoral (*Malapteruridae*), or extraocular (*Uranoscopidae*) musculature (reviews: Bennett, 1971; Bass, 1986; Moller, 1995). Therefore, it is reasonable to assume that the impact of sex steroids on myocytes and myogenic electrocytes should be similar, i.e. both types of cells in the androgen-treated fish should become thicker, with changes in electrocytes reflected in EOD.

Sex differences in EODs are readily affected by steroid hormone administration. In a number of mormyrid fish androgen treatment results in an increase of the duration of EOD phases (particularly phases 2 and 3) and a decrease in the associated peak power spectrum frequency (PPSF) (review: Landsman, 1995; Herfeld and Moller, 1998). Since the male-type EOD is distinctively different from the female's, is of seasonal nature, and subject to hormonal and environmental influences (review: Landsman, 1995), this elongated EOD can be considered a functional equivalent of male vocalization in many avian species, as it assists in an identification of the sex and social status of a conspecific (Nottebohm, 1980, 1981).

The temporal synchronization of all these processes may ensure the optimal timing of the EOD-based "announcement" of reproductive readiness. Considering the degree of interrelation among the individual elements, both functionally (breeding success) and in terms of underlying physiological mechanisms (androgen control), a strong correlation can be expected among them, with some features being of predictive value with respect to others. Females may choose their partners on the basis of their EOD properties: a longer EOD would reflect thicker electrocytes and, by inference, anal-fin myocytes, that, in turn, indicate the ability to form a more efficient fertilization pouch. A similar model of a female choice based on sexual dimorphism in frequency of

discharging (sexual advertisement) was proposed for *Sternopygus macrurus*, a wave-type gymnotiform (Fleishman, 1992; Fleishman et al., 1992; Hagedorn, 1986; Wilczynski, 1986).

The same action of sex hormones may be responsible for sexual dimorphisms in several mormyrid species. External administration of 17α-methyltestosterone (17-MT) masculinizes juvenile and adult female *G. petersii* (Voustianiouk, unpubl.), *B. niger* (Herfeld and Moller, 1998), *Mormyrus rume proboscirostris* (P. Moller, personal communication, 2000): following several weeks of treatment, the EOD assumed male-typical phase durations, the prominent ventral body wall indentation developed, and the bases of the anal-fin rays expanded. Landsman et al. (1990) described a more prominent masculinizing effect on the EOD in *G. petersii* by T than either DHT (non-aromatizable androgen) alone or in combination with E (the latter two did not differ), and observed a feminizing effect of E on PPSF of adult females, but not that of juveniles (the latter finding is in contradiction with the previous reports regarding masculinizing effects of estrogen on the EOD in juvenile *Brienomyrus* (Bass, 1986; Bass and Hopkins 1983)). Landsman et al. also reported that both T and DHT, though not E, produced an indentation of the posterior ventral body wall in *G. petersii*, but did not provide quantitative data.

On the basis of information reviewed thus far, the following **specific hypotheses** can be proposed, with *G. petersii* as a test subject:

1. Administration of exogenous androgens to should elicit osteogenesis (expansion of the bases of the anal-fin rays), myogenesis (changes in the anal-fin geometry),

masculinization of EOD (increase in duration of phases 2 and 3), and development of AFR.

2. Following removal of exogenous androgens, behavioral changes should revert (demasculinization of EOD, disappearance of AFR) while structural changes (expanded bases of the anal-fin rays and altered anal-fin geometry) should persist.
3. DHT should be more effective in inducing structural changes, while T in inducing behavioral changes. Effects of 11-KT are expected to be similar to those of T.

To test these hypotheses a sequence of two experiments was designed:

Experiment 1a - to evaluate the expected temporal and functional differences between the effects of naturally occurring androgens (T and DHT) and estrogen and to investigate interrelationships between hormonal effects on the structural and behavioral targets.

Experiment 1b - based on the results of Experiment 1a to clarify the role of estrogen by replicating Experiment 1a for the estrogen group on a sample of larger size.

Experiment 2 - to test the reversibility of behavioral and structural androgen effects. It was hypothesized that following the drop in androgen plasma levels (i.e., at the end of the breeding season) the behavioral male-specific characteristics (EOD and AFR) revert to the pre-breeding condition: EOD phases become shorter and the reflex disappears. The structural characteristics, on the other hand, were expected to remain. The second goal of Experiment 2 was to investigate the morphological and behavioral effects of 11-ketotestosterone (another naturally circulating fish androgen), and to compare them with those of testosterone.

All animal treatment procedures were approved by the Institutional Animal Care and Use Committee at Hunter College of The City University of New York, protocols PM/AV 12/97-T1, PM/AV 12/98-T1, PM/AV 12/98-T2.

EXPERIMENT 1a

Subjects

Twenty juvenile (standard length = 112-138 mm, weight = 14-24 g, see Table 1 for details on treatment groups) *G. petersii* imported in February (the middle of the non-breeding season) 1997 from Nigeria were obtained through Quality Tropicals (Wallington, NJ) and housed in individual 20 l aquaria (actual water volume 15 l). Juvenile vs. adult classification was based on Pezzanite and Moller (1998), i.e. all specimens with standard length of 180 mm or less were considered juveniles. Each aquarium was equipped with a ceramic shelter and standard aeration and filtration devices. Water temperature was kept at 25° ± 2°C and water conductivity at 140 ± 25 μS/cm. To maintain water conductivity, 1/3 of the water was exchanged twice a week. Fish were kept on an L:D = 12:12 cycle (with lights on at 9:00 h), and fed tubifex (ad-lib). Two weeks of adaptation were provided before data collection began.

Since the sex of immature *G. petersii* cannot be identified by either external observation, EOD characteristics, or radiography, it was not possible to ensure an even representation of males and females in the treatment groups. However, since the gonads, removed during surgery, were reliably identifiable as testes or ovaries, sex of each subject was recorded at that point. One fish (male, SL = 120 mm) possessed male-typical spur-bearing anal-fin rays at baseline. Taking into account that in *G. petersii* both sexes

respond to androgen treatment in a similar way (review: Landsman, 1995), data for males in females were pooled.

Surgical Procedures

The surgical procedure was adapted from Landsman et al. (1990). Fish were anesthetized with buffered tricaine methane sulfonate (MS-222, Sigma): 1 g MS-222 and 1 g sodium bicarbonate in 5 l of aerated aquarium water. Gonadectomy and insertion of the silastic implants were performed through a 1.5-cm incision on the left ventral surface, approximately 0.5 cm posterolateral of the ventral fins. Silastic implants were inserted into the musculature over the gut cavity, anterior to the incision. Following gonadectomy and implantation, the incision was sutured (6.0 silk, Ethicon) and fish were revived by flowing conditioned aquarium water through their mouths and gills. Fish then were returned to their individual tanks and treated with chloramphenicol (Sigma), an antimicrobial agent. Mild fungal disease that developed in some subjects was controlled by administration of Furan-2 (an antifungal agent). Following sacrifice at the end of the study, fish were examined for completeness of the gonadectomy.

Hormone Manipulations

T, DHT and 17β-estradiol (E) were obtained from Sigma Chemical Co. (St. Louis). Implants were prepared by packing crystalline hormone into silastic tubing (Dow-Corning 0.065 in. o.d. and 0.03 in. i.d.) at the dose of 1 mm of packed silastic per 2.5 g fish body weight. The ends of the implants were sealed using Silicone A glue (Factor II). Control subjects received matching empty (blank) implants (B). Dosages of T, DHT, and E

in this study matched the "low dose" quantities used by Landsman et al. (1990). Landsman (1995) showed that this dosage produces blood levels of T comparable to those of males during breeding season. Thus, it is possible to rule out any pharmacological effects.

Radiographic Procedures

Radiography was performed in the photolaboratory of the Department of Ichthyology at the American Museum of Natural History (AMNH). Fish were anesthetized and immobilized with sodium bicarbonate buffered MS-222, as described above, then placed inside the X-ray apparatus (Hewlett-Packard, 43807 N, Faxitron series; Kodak ReadyPack II Industrex M film, routine development) and exposed to low-intensity radiation: 30-40 KVP for 30-45 s, depending on the size of the specimen. Following the completion of radiography, subjects were revived in a tank containing highly aerated aquarium water. This procedure was shown to be harmless to the animals and not to interfere with their normal behavior or change their EOD characteristics (Voustianiouk, personal observation). All experimental and control subjects were radiographed upon arrival (baseline), and two and four weeks after surgery.

EOD Monitoring

Electric organ discharges (EODs) were monitored from the fish's home tank with a pair of Ag/AgCl electrodes, placed inside plastic tubes glued to the opposite walls of the tank at the ends of its long axis. EODs were differentially preamplified (custom-modified AD625) and fed directly into a digital oscilloscope (Hitachi, VC 6023) and spectrum analyzer (Hewlett-Packard, HP3582A, range: 0-25 kHz, resolution: 100 Hz) to

obtain the waveform parameters (duration of the four phases as well as the amplitude of phases 2 and 3) and the associated peak power spectrum frequency (PPSF of the Fast Fourier transform). The oscilloscope allowed storage and subsequent inspection of pre-trigger events so that all phases of the EOD could be analyzed. The duration of the four phases was determined following Herfeld and Moller (1998). Two measurements were taken for each subject during a given recording session and then averaged. All measures were corrected to water temperature of 25°C. Correction coefficients were calculated based on previous data (Voustianiouk, personal observation) as follows: -7.1 μs (Phase 1), -7.7 μs (Phase 2), -9.3 μs (Phase 3), 21.4 μs (Phase 4), 278.5 Hz (PPSF) (all values are per 1°C of temperature increase). All EOD recordings were made between 10:00 and 19:00 h (EST). There are no reported circadian rhythms in the EOD waveform of *G. petersii.*

Analysis of External Morphology and Bone Expansion

Anal fin areas of the radiographic images were digitized (UMAX Astra 1200S scanner with transparency adapter, 600 dpi resolution, 200% magnification at 1:1 aspect ratio) and analyzed with SigmaScan Pro image analysis software (version 4.01; SPSS, Inc.). The extent of body wall indentation was quantified using total area of the indentation and maximal indentation. The extent of expansion of the bases of the anal-fin rays was determined by measuring the area and perimeter of the bases of the first 20 rays. Additionally, each of the first 20 rays was classified as being of "male" or "female" type based on the presence of characteristic male-specific "spurs" – bony processes protruding in rostral-caudal direction from the base of the ray (in *G. petersii* the sexual dimorphism in the size and shape of the anal-fin ray bases is limited to rays 1-20 [Pezzanite and

Moller, 1998]). Figure 2 illustrates the elements of the morphometric analysis. The same measures were also taken for the dorsal fin areas as controls for potential hormonal action.

Measuring the perimeter of the base, in addition to its area, is important because the side view of the base, provided by a two-dimensional radiographic projection, does not allow a direct evaluation of the lateral expansion. Under these circumstances, an increase in the perimeter of the part of the base, visible in the lateral view, suggests increase in the base lateral surface, even if the magnitude of lateral expansion is presumed unchanged.

Morphometric and meristic characteristics including SL, the distance between the base of the first and last anal-fin rays (anal fin length, measured from the radiographs [r-AFL]), the total number of anal-fin rays, total number of the spur-bearing rays, number of the first and last rays with spurs, and number of the ray in the location of maximal indentation were also recorded.

The maximal indentation and total area of the indentation were transformed into the indentation index and indentation area index by dividing raw measurements by r-AFL or its square, respectively, to adjust for variations in subject size. The position number of the ray in the location of maximal indentation was expressed as a number of rays preceding the point of maximal indentation (including the point-marking ray itself). Taking into account a considerable variability in size among anal-fin ray bases, depending on the ray number, the relative differential values were computed for the area and perimeter of the ray bases, rather than using the raw data. The relative differential

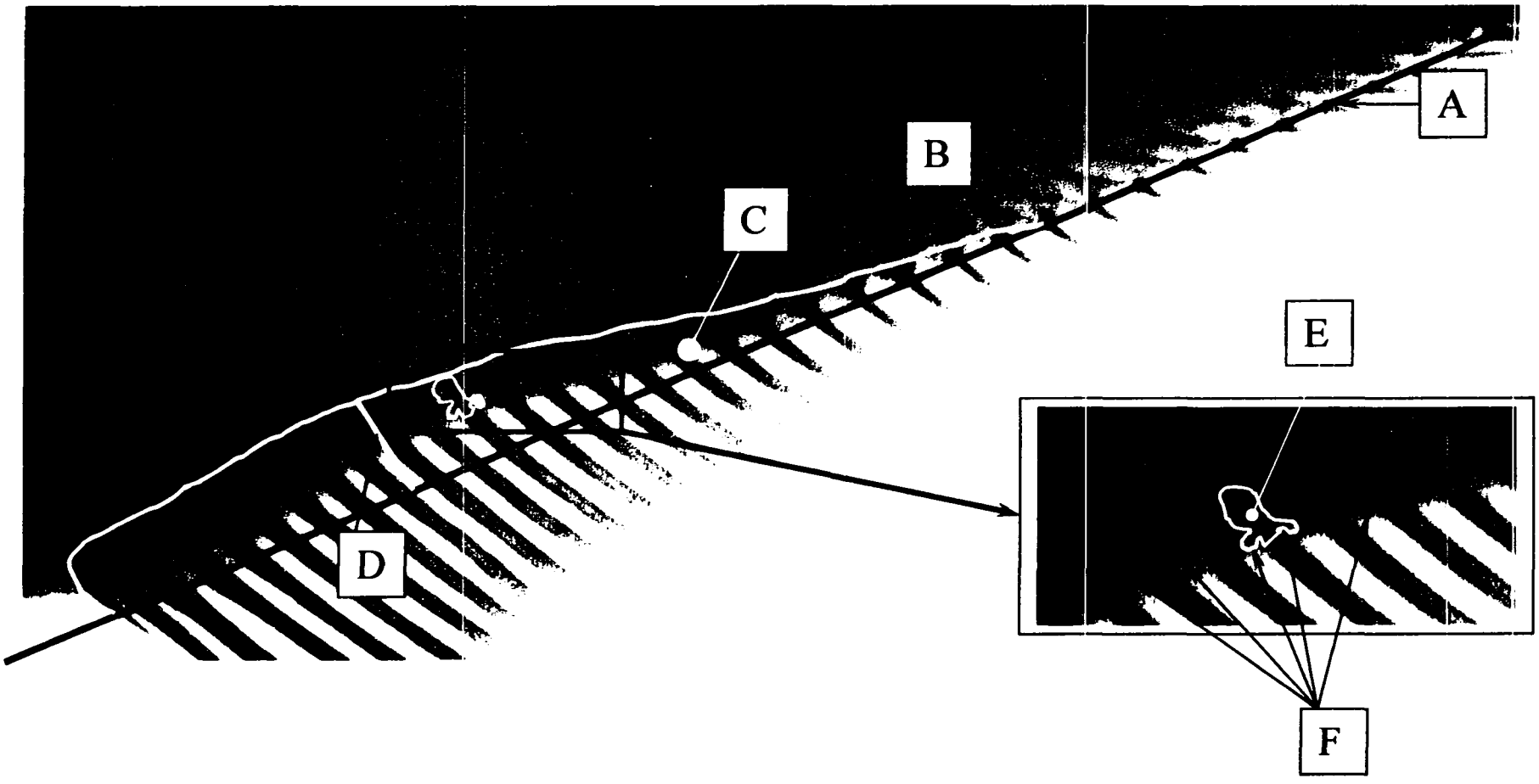


Figure 2. A typical scanned radiograph of the anal fin of *G. petersii* showing elements of the morphological analysis. Insert: enlarged segment illustrating "spurs" – bony processes protruding longitudinally from the base of the ray. A: A reference line, connecting the base of the last ray and the most anterior-ventral external point of the body wall indentation. B: An outline of the anal-fin ray bases. C: Measured area of the body wall indentation (enclosed by A and B). D: Projection of the point of maximal dorsal indentation onto the reference line, defined as maximal indentation. E: Measured area of the ray base. F: Spurs at the bases of the fin rays.

represents the amount of change from the baseline level to the end of the fourth postimplant week in reference to the baseline level, and was calculated as follows:

$$RD = (MW4 - MBL) / MBL$$

where RD – the relative differential, MBL – the value measured at the baseline (prior to implant surgery), and MW4 – the value measured at the end of postimplant week 4.

One of the T-treated females produced an atypical osteological response: the ray bases did not develop spurs, but showed massive non-structured expansions with most rays split. In the light of such an atypical reaction, this subject's data were removed from further osteological analyses. At the present time, no definite explanation for such reaction is available.

Two DHT-treated and two control fishes were selected for a detailed representative anatomical evaluation. One animal from each group was subjected to clearing/staining treatment with subsequent microscopic examination of the anal-fin ray bases. The anal fin of the two remaining subjects was cut on a freezing microtome (thickness setting 12 μm). Following the standard procedure (Humason, 1997), every third cut was stained by Milligan's Trichrome Stain and subjected to microscopic examination. The slides were scanned (see details above) and the thickness of muscle tissue in the anal-fin area was measured. To adjust for the differences in the size of the animals, the index of the thickness of musculature (A/D ratio) was computed by dividing the measured thickness of the muscle tissue in the anal-fin area by the maximal thickness of the muscle tissue in the dorsal fin.

Statistical Data Analysis

One-way ANOVAs and independent-measures t-tests were used to assess initial differences among the treatment groups and between the sexes, respectively. Two-way ANOVAs (treatment [between groups] x time block [within groups], with repeated measures on the time block factor) were used on EOD data (duration of the entire pulse and individual phases, PPSF, ratio of amplitude of phases 2 and 3), as well as indentation and indentation area indexes, and numbers of the preceding and spur-bearing rays. Two-way ANOVAs were also used to compare the initial sizes of the anal-fin ray bases (sex [between groups] x ray [within groups]) and the relative differentials for the area, and perimeter of the ray bases (treatment [between groups] x ray [within groups]). Significant findings were followed by tests for simple main effects and by Student-Newman-Keuls tests for multiple comparisons ($\alpha = 0.05$). A bivariate correlation analysis was performed to investigate relationship between the morphological and behavioral (EOD) measurements, and resulting Pearson product-moment coefficients were tested for significance. All analyses were performed using SPSS for Windows (release 6.1.4, 1996; SPSS, Inc.).

Results

There were no pre-treatment differences in weight [$F(3,16) = 0.88$, $p = 0.47$] and standard length [$F(3,16) = 0.68$, $p = 0.58$] among treatment groups (Table 1). Males and females did not differ in their EOD characteristics and total number of the anal-fin rays. However, they were significantly different in the size of the indentation area, maximal indentation, and the number of anal-fin rays preceding the point of maximal indentation

(Table 2). The total anal-fin ray count ranged from 33 to 36, which agrees with Boulenger (1909).

Table 1. Pre-treatment weight and standard length for different treatment groups.

		Weight (g)		Standard Length (mm)	
Treatment	n	Mean	SD	Mean	SD
Testosterone:					
Males	3	18.3	5.1	129.0	7.8
Females	3	18.5	3.0	123.7	7.4
All	6	18.4	3.8	126.3	7.4
DHT:					
Males	4	17.3	1.7	123.0	7.4
Females	2	17.0	0.0	121.0	5.7
All	6	17.2	1.3	122.3	6.4
17β-estradiol:					
Males	3	18.3	1.0	120.7	1.2
Females	2	20.8	1.1	123.5	4.9
All	5	19.3	1.6	121.8	3.0
Blank:					
Males	0	–	–	–	–
Females	3	19.5	2.5	125.3	7.0
All	3	19.5	2.5	125.3	7.0

Table 2. Pretreatment indentation data by sex of subjects and results of independent-measures t-test for mean difference (degrees of freedom in parentheses).

	Males (n = 10)		Females (n = 10)			
Characteristic	Mean	SD	Mean	SD	t (18)	p
Indentation area index	0.020	0.004	0.015	0.004	3.33	0.004
Indentation index	0.046	0.006	0.040	0.004	3.03	0.007
Number of preceding rays	7.9	1.612	4.0	1.130	6.26	0.000006

Morphology

Treatment with either T or DHT resulted in masculinization of morphological features of the anal fin. No changes were observed in subjects treated with E or in blank controls (Figure 3). The morphology of the dorsal fin was not affected in either control or any of the experimental groups (data not presented) indicating that androgen action was limited to reproduction-related targets.

Sex-related Differences in Morphological Changes

With the exception of the number of rays preceding the point of maximal indentation in T-treated fish (see below), no statistically significant sex x time block interaction effect was found. This finding indicates similarity of response to hormonal

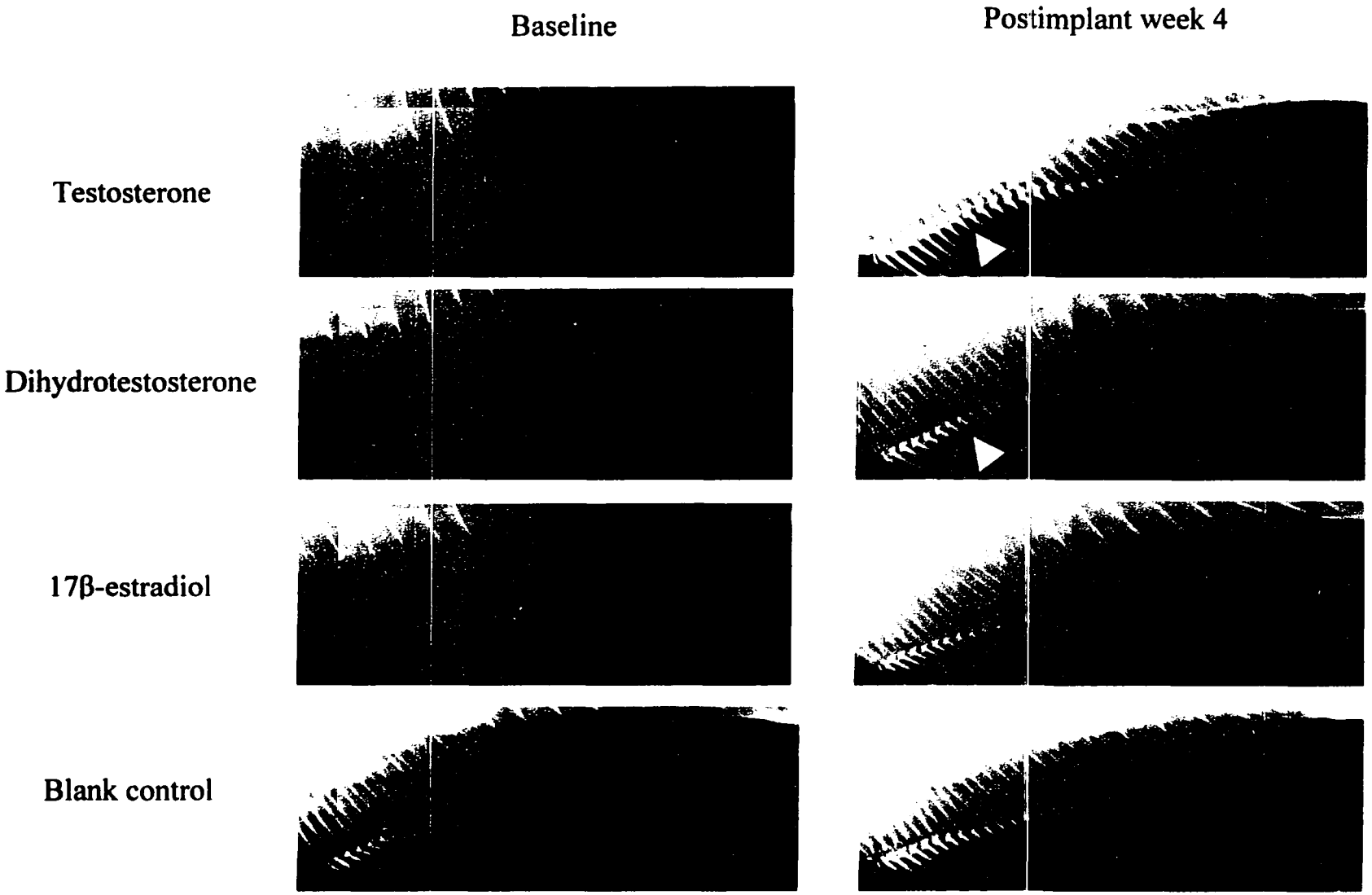


Figure 3. Radiographic representation of the anal-fin morphology in representative subjects at pre-implant baseline and at the end of the fourth postimplant week. Treatments: testosterone, dihydrotestosterone, 17β-estradiol, and control with a blank silastic implant. A prominent indentation (white arrows) developed in fish implanted with androgens, but not in estrogen-treated or control subjects.

treatment by males and females and justifies pooling data from male and female subjects within each treatment group. However, the graphical presentations of morphological data, separately for each sex, are provided for descriptive purposes (Appendix A, Figures A1-A3).

Indentation Area Index

The magnitude of the indentation area index was affected only by androgens. A significant treatment x 2-week block interaction effect [$F(6,48) = 2.64$, $p < 0.03$] (Figure 4) revealed that by the end of the first postimplant 2-week block to the end of the study, the mean area index in T- and DHT-treated groups was significantly higher than in E-treated and B groups, as well as at their respective baseline levels. Neither the E-treated nor the B group deviated significantly from their respective baseline levels.

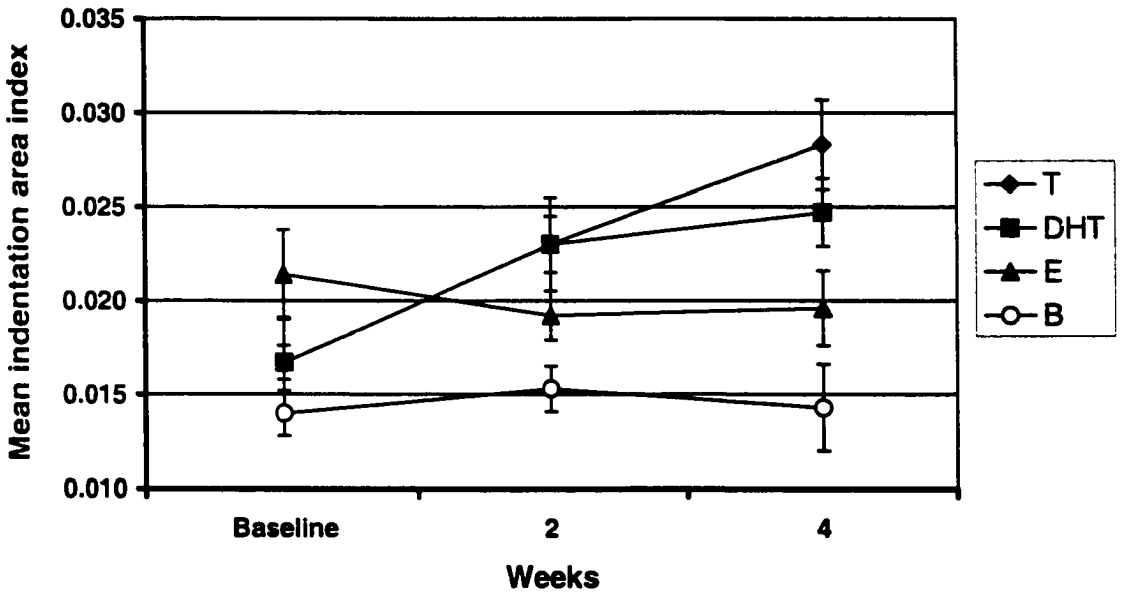


Figure 4. Mean (±SE) indentation area indexes for pre-implant baseline and at the end of subsequent 2-week postimplant blocks. Treatments: testosterone (T, n = 6), dihydro-testosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Indentation Index

The treatment x 2-week block interaction effect was significant [$F(6,48) = 3.17$, $p < 0.011$] (Figure 5), indicating that by the end of the second postimplant 2-week block, both androgen-treated groups reached mean indentation index values significantly higher than B and E-treated groups, as well as their respective pre-implant baseline values. Mean indexes in the B and E-treated groups were not significantly different from each other or their respective baseline values.

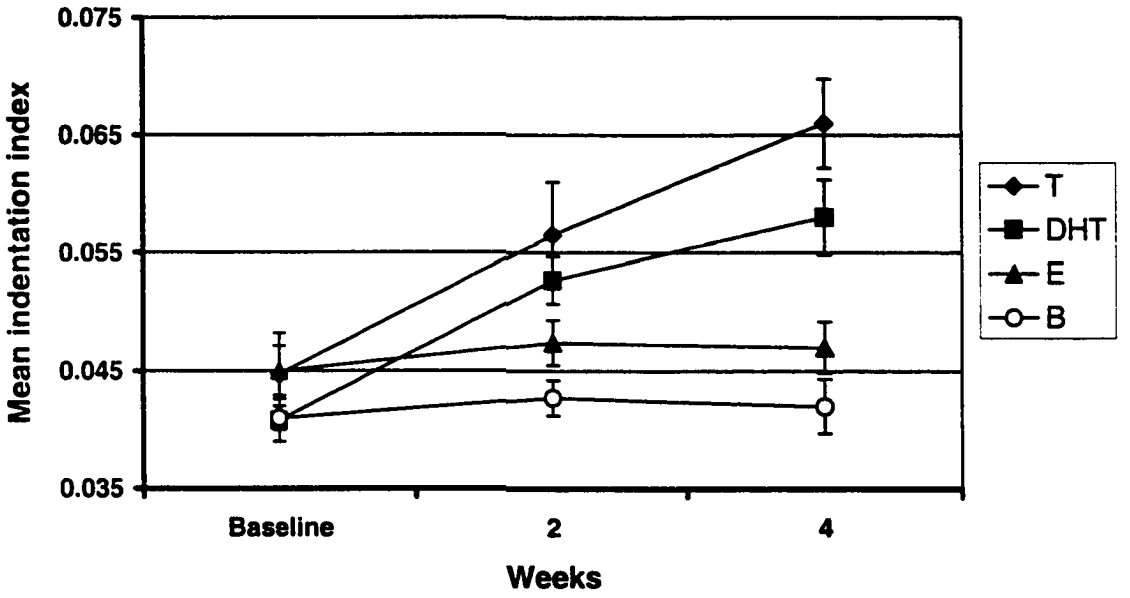


Figure 5. Mean (±SE) indentation indexes for pre-implant baseline and at the end of subsequent 2-week postimplant blocks. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Location of Maximal Indentation

Due to uncovered *post hoc* sex-linked differences in T-induced changes of this character, male and female data could not be pooled. That precluded a meaningful

statistical analysis within all treatment groups except for the T-treated one. In the T-treated group, there was a significant sex x 2-week block interaction effect on the number of rays preceding the point of maximal indentation [$F(2,12) = 9.02, p < 0.004$] (Figure 6), indicating significant changes from the baseline levels in the T-treated females but not in males that exhibited indentation prior to treatment. As a result, the difference between sexes, significant at the pre-implant baseline, lost its significance by the end of the second postimplant 2-week block. A similar trend is seen in the DHT-treated subjects (Appendix A, Figure A4, *middle*).

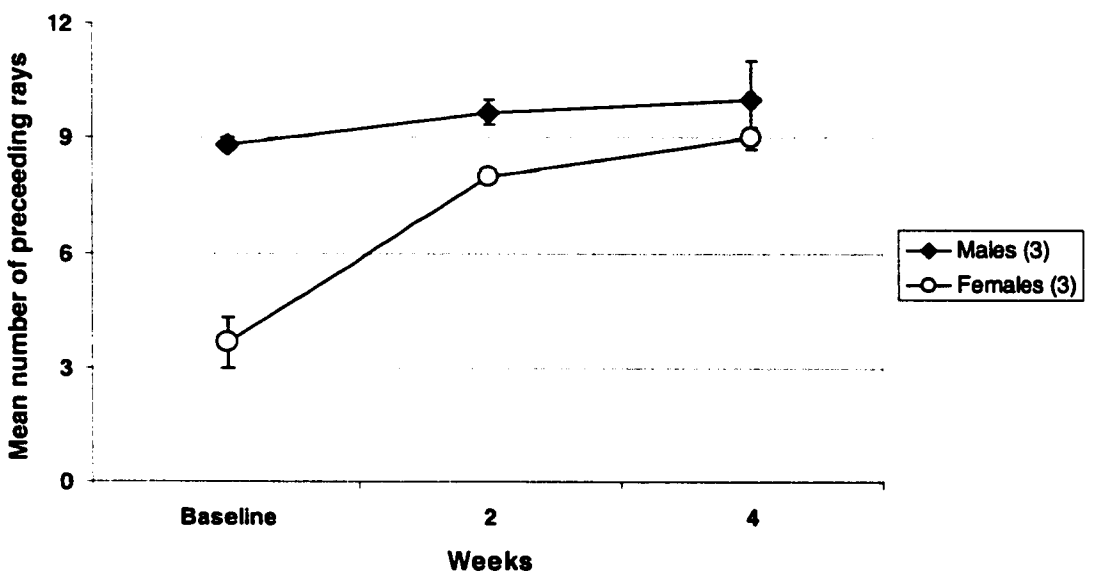


Figure 6. Mean (±SE) numbers of anal-fin rays preceding the point of maximal indentation for pre-implant baseline and at the end of subsequent 2-week postimplant blocks in testosterone-treated fish. Numbers in the legend refer to sample sizes.

Spur-bearing Anal-fin Rays

There was a significant treatment x 2-week block interaction effect on the number of spur-bearing anal-fin rays [$F(6,45) = 7.21, p < 0.001$] (Figure 7). DHT- and T-treated

groups reached significantly greater mean ray counts compared to B or E-treated groups by the end of the first and second postimplant 2-week block, respectively. Both DHT- and T-treated subjects were also significantly different from their baseline levels by the end of the postimplant block 2. The E-treated group showed no change from its baseline or from blank-implanted controls. While spurs were observed on rays 4-18 in DHT- and 5-20 in T-treated fish, the range of rays bearing double spurs (extending both rostrally and caudally) was limited to 9-15 in DHT- and 9-17 in T-treated animals.

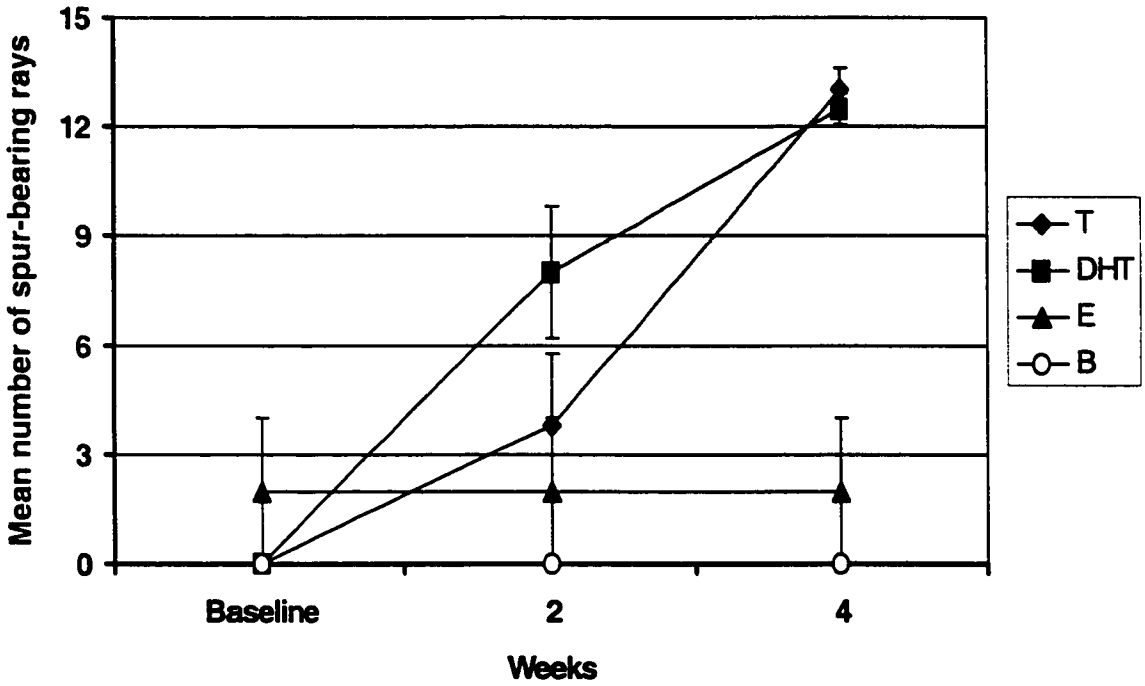


Figure 7. Mean (±SE) numbers of spur-bearing anal-fin rays for pre-implant baseline and at the end of subsequent 2-week postimplant blocks. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Area of the Ray Bases at Baseline

There was no significant sex x ray interaction effect on the size of the ray bases at baseline [F(19,340) = .20, p > 0.999]. The ray main effect, however, was significant [F(19,340) = 38.54, p < 0.001] (see Figure 8). Multiple comparisons identified rays 3 through 7 as the most expanded and rays 16 through 20, as well as ray 1, as the least expanded.

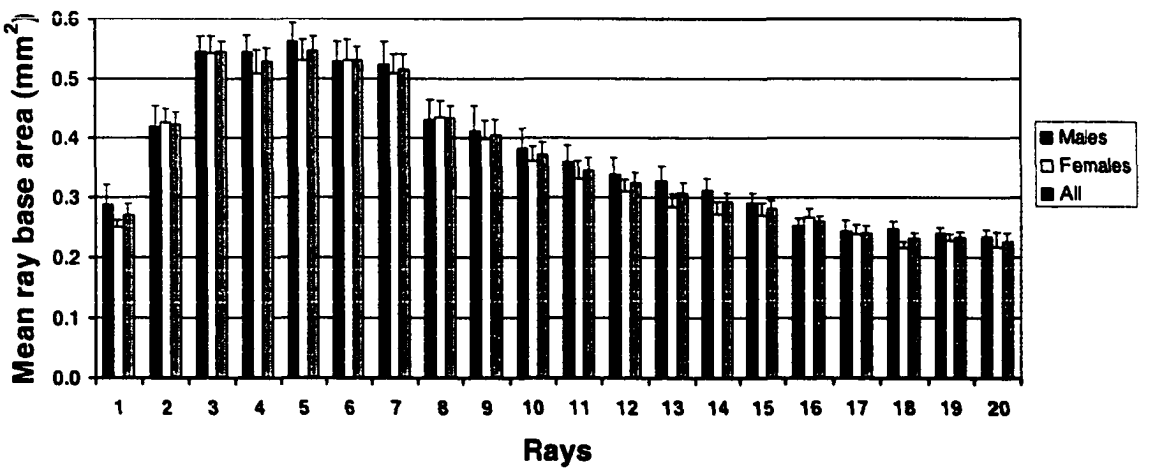


Figure 8. Mean (+SE) ray base areas for the first 20 anal fin rays at pre-implant baseline for male, female, and all subjects combined (n = 20).

Relative Differential of the Ray Base Area

Significant treatment x ray interaction effect was observed on the relative differential of the ray base area [$F(57,300) = 1.59$, $p < 0.008$] (Figure 9). DHT-treated fish showed a significantly greater relative increase in ray base area than animals in either E-treated or B group in all rays but 5 and 18-20, whereas T-treated fish differed significantly from those in E-treated and B groups only in rays 12-17. DHT was significantly more potent than T in rays 7, 9, 10, 13, and 14. E-treated fish did not differ

from blank controls. There were no differences in ray base area in either E-treated or B groups, while in the T-treated fish rays 16-17 were significantly more affected than first 7 rays, and in DHT-treated subjects rays 11-15 were significantly more affected than rays 1-6 and 18-20.

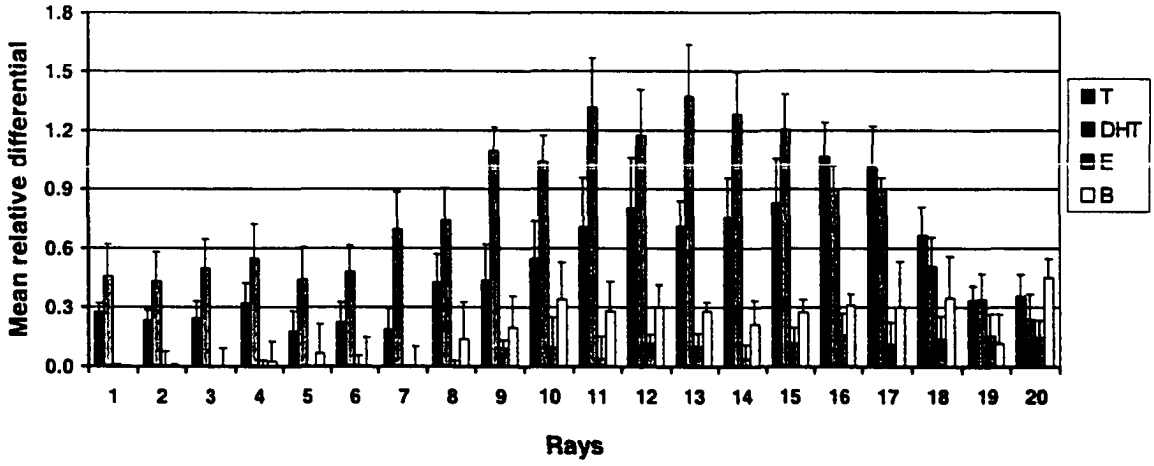


Figure 9. Mean (±SE) relative area differential for the first 20 anal fin rays. See text for explanations on relative differential computations. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Relative Differential of the Ray Base Perimeter

There was a significant treatment x ray interaction effect on the relative differential of the ray base perimeter [$F(57,300) = 3.47, p < 0.001$] (see Figure 10). Again, DHT was significantly more potent than either E or B in all rays but ray 20, and more potent than T in rays 7, 9-14. T caused a significantly greater relative increase in the ray base perimeter than either E or B in rays 1, 3, 4, and 8-18. There were no differences between the effect of B and E, or among rays, in fish implanted with E or blanks. DHT-treated subjects showed a significantly greater increase in relative perimeter in rays 9-15

as compared to rays 1-8 and 16-20. In the T-treated group, rays 11-17 were affected significantly greater than rays 1-7 and 19-20.

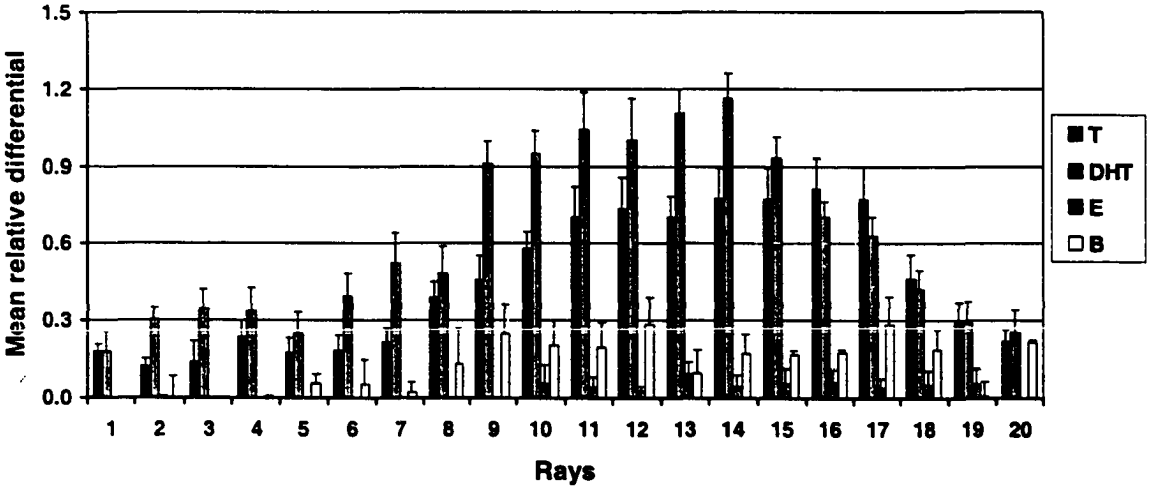


Figure 10. Mean (±SE) relative perimeter differential for the first 20 anal fin rays. See text for explanations on relative differential computations. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Analysis of the EOD

Sex-related Differences in EOD Changes

There were no sex-related differences in changes of EOD characteristics. This finding justified pooling data of male and female subjects within each treatment group. However, the graphical presentations of EOD data, separately for each sex, are provided in the Appendix (Figures A4 – A10).

Total Pulse Duration

Total EOD duration is the sum of the durations of the four individual phases. A significant treatment x weekly block interaction [$F(12,80) = 6.17, p < 0.001$] revealed that both androgens increased total pulse durations, with the T-treated group achieving a significant difference within one week after implant surgery, and the DHT-treated group – within two weeks (Figure 11). At the end of the second week, mean total EOD duration was also significantly higher in E-treated fish than in controls, although this was more likely due to the substantial shortening of phases 1 and 4 in B-implanted subjects than to an estrogen effect, for this difference disappeared by the end of the third week. Both androgen- and estrogen-treated fish showed a total EOD duration significantly longer than their respective baseline levels within seven days past surgery and maintained it throughout the study, whereas fish with blank implants showed no such stable pattern (see discussion below).

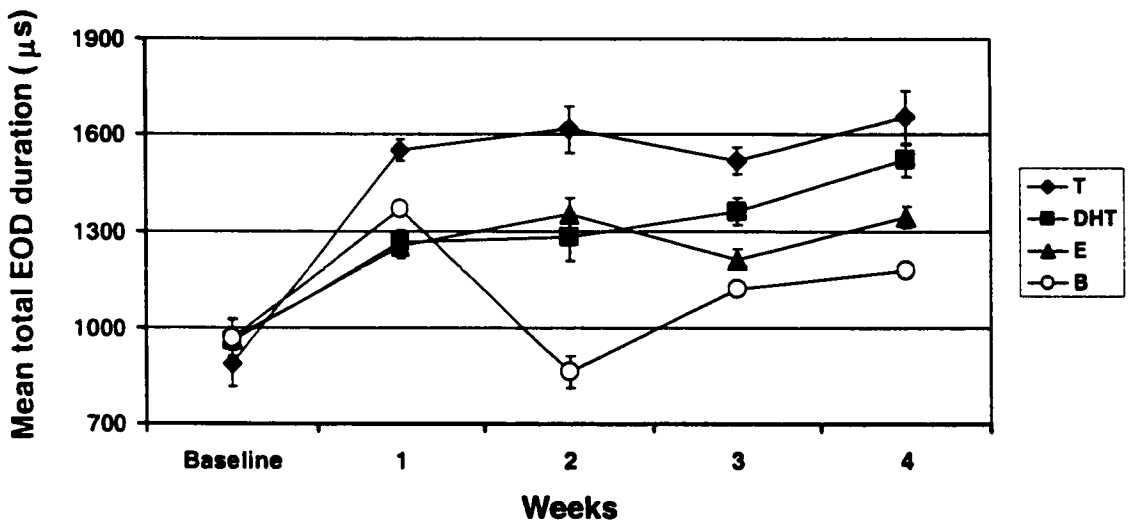


Figure 11. Mean (±SE) total EOD duration for pre-implant baseline and subsequent weekly postimplant blocks. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Phases 2 and 3

Treatment x weekly block interaction effect was significant on phase 2 [$F(12,80) = 6.83, p < 0.001$] (Figure 12) and phase 3 [$F(12,80) = 3.95, p < 0.001$] (Figure 13), with T-treated subjects significantly increasing phase durations by the end of the first week after implantation and maintaining this effect throughout the study. By the end of postimplant week 3, DHT-treated fish showed a significant difference from B-implanted controls, but not from E-treated animals. Compared to baseline levels, phases 2 and 3 significantly increased in duration only in the androgen-treated groups. The effect took place by the end of postimplant week 1 and remained until the end of the observation.

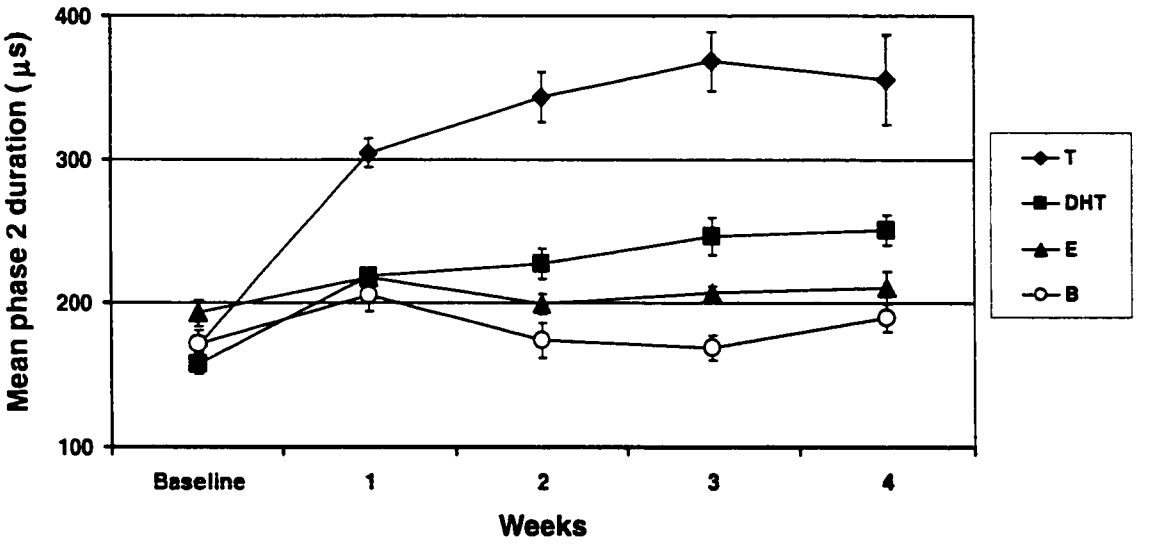


Figure 12. Mean (±SE) phase 2 duration for pre-implant baseline and subsequent weekly postimplant blocks. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

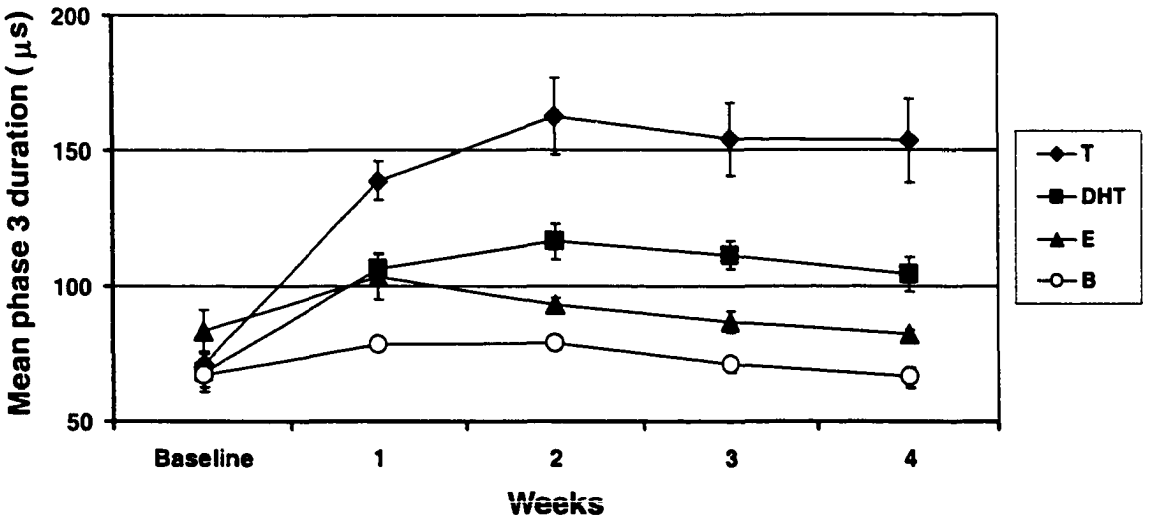


Figure 13. Mean (±SE) phase 3 duration for pre-implant baseline and subsequent weekly postimplant blocks. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Phases 1 and 4

No significant interaction effect was observed on the duration of phase 1 [$F(12,80) = 1.73, p = 0.075$] (Figure 14). Both main effects (hormonal treatment and weekly observation block) were significant, however [$F(3,80) = 9.61, p < 0.001$ and $F(4,80) = 9.03, p < 0.001$, respectively]. Compared to baseline level, only T-implanted fish significantly increased phase 1 duration by the end of the first postimplant week and maintained it throughout the study. Phase 1 was significantly longer in all hormone-treated groups than in B-implanted subjects at the end of postimplant week 2; this effect, however, was temporary and disappeared by the end of week 3. It is interesting to note the simultaneous increase in phase 1 duration in all groups within the first postimplant week and corresponding decrease by the end of second week (see discussion below).

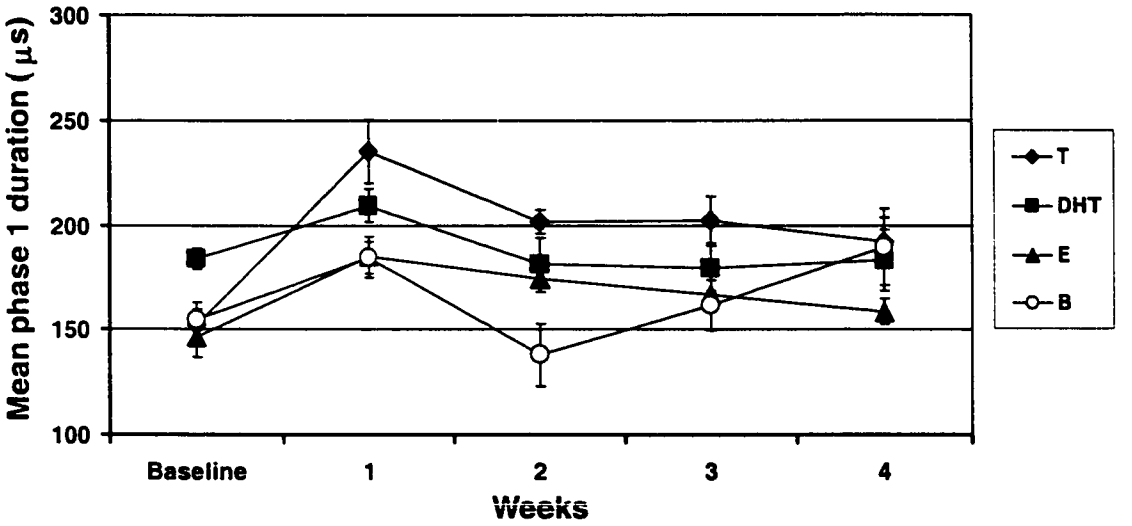


Figure 14. Mean (±SE) phase 1 duration for pre-implant baseline and subsequent weekly postimplant blocks. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

A significant treatment x weekly block interaction [$F(12,80) = 5.15, p < 0.001$] (Figure 15) revealed a simultaneous significant increase in phase 4 duration by the end of postimplant week 1 in all treatment groups. Only hormone-implanted subjects maintained their levels above baseline at the end of week 2. Phase 4 in control fish significantly decreased during the second week, but rose again to the level of the other groups by the end of week 3. Over the fourth postimplant week, the duration of phase 4 significantly increased in both androgen- and estrogen-treated groups, while controls maintained their week-3 level, and at the end of the fourth week their phase 4 was again significantly shorter than in T-, DHT- and E-treated groups.

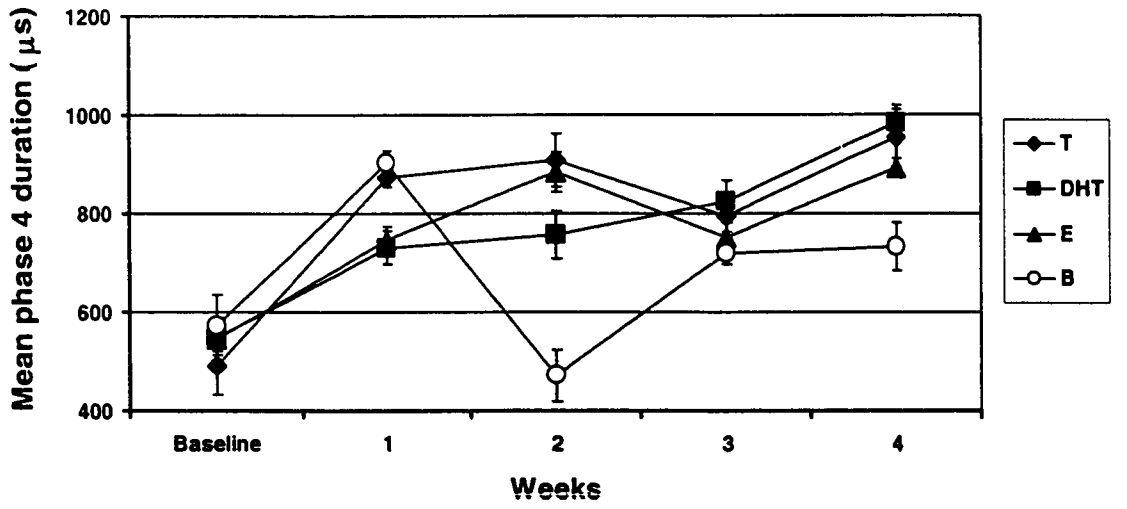


Figure 15. Mean (±SE) phase 4 duration for pre-implant baseline and subsequent weekly postimplant blocks. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Fourier Spectra of the EOD

At the end of postimplant week 4, only T-implanted fish still showed a significant decrease in mean PPSF, which differed significantly from other groups and from the T group's baseline level, as indicated by the significant treatment x weekly block interaction [$F(12,80) = 6.56, p < 0.001$] (Figure 16). DHT-treated subjects achieved PPSFs significantly lower than the B group by the end of postimplant week 1 and lower than the E-treated group by the end of postimplant week 2. This effect did not last, however, and DHT, E, and B groups did not differ significantly at the end of the fourth week. The mean PPSF in the E-treated group was also significantly lower than in controls at the end of the first week although this difference was no longer significant by the end of the second week. Only the T- and DHT-treated groups were significantly different

from baseline levels; this effect set in by the end of postimplant week 1 and remained throughout the study.

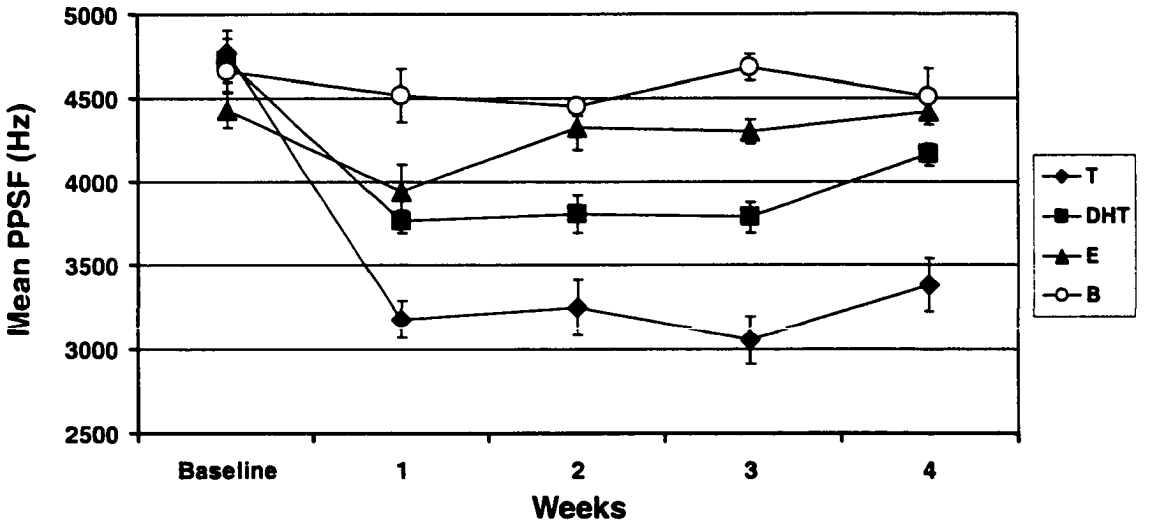


Figure 16. Mean (±SE) PPSF for pre-implant baseline and subsequent weekly postimplant blocks. Treatments: testosterone (T, n = 6), dihydrotestosterone (DHT, n = 6), 17β-estradiol (E, n = 5), and controls with blank silastic implants (B, n = 3).

Correlation between Morphology and EOD

Table 3 summarizes the results of a bivariate correlation analysis and presents the Pearson product-moment coefficients computed for pairs of behavioral and morphological measures and their respective levels of significance. Note: *r*-values were evaluated based on the sample size of 20, rather than 60, because triplets of morphological and behavioral data obtained for each subject at baseline and 2 and 4 weeks after the surgery cannot be considered independent observations. Phase 2 and total EOD duration correlated most strongly with morphological measurements. The indentation and area indexes correlated most strongly with EOD characteristics. No

significant correlation was found between the duration of phases 1 and 3 and any of the morphological characters.

Table 3. Bivariate correlation analysis of behavioral (rows) and morphological (columns) characteristics in *G. petersii*. See text for comments on evaluation of significance.

	Indentation area index	**Indentation index**	Number of preceding rays	Number of spur-bearing rays
Phase 1	0.2837	**0.2802**	0.3938	0.2312
Phase 2	**0.5805*** **	**0.7005*** **	**0.4570***	**0.5185***
Phase 3	0.3402	**0.4395**	0.3238	0.2732
Phase 4	0.4567*	**0.5316***	0.4121	0.4915*
EOD total	0.5247*	**0.6175*** **	0.4725*	0.5207*
PPSF	-0.4991*	**-0.5916*** **	-0.4894*	-0.3849

Note: * $p < .05$, *** $p < .01$.

EXPERIMENT 1b

Subjects and Procedures

Thirteen juvenile and subadult *G. petersii* (standard length = 97-131 mm, weight = 11-16 g) imported in January 1998 from Nigeria (the middle of the non-breeding season) were obtained through Quality Tropicals (Wallington, NJ) and housed in individual 20 l aquaria, as described above. All experimental procedures were the same as described for Experiment 1a, **except**: 1) the duration of the experiment was set at eight weeks with radiography to be performed at baseline, and four and eight weeks after

surgery; 2) analysis of behavioral data was limited to durations of phases 2 and 3, and the PPSF; 3) each week, fish were lightly anesthetized with buffered tricaine methane sulfonate (MS-222, Sigma), as described above, and tested for the anal-fin reflex by gently touching their anal fin with the fingertip.

Following baseline data collection, fish were randomly assigned to either experimental (n = 8) or control group (n = 5). After gonadectomy, experimental subjects were implanted with E, while controls received matching "blanks" (see Experiment 1a on details of implant preparation and surgical procedures). All data collection and analysis routines were performed as described for Experiment 1a.

Results

There were no pre-treatment differences in weight [$t(11) = 2.01, p = 0.17$] and standard length [$t(11) = 0.44, p = 0.68$] among treatment groups. Males (n = 6) and females (n = 7) did not differ in either behavioral (EOD) or morphological characteristics. E-treated subjects did not differ from blank-implanted controls in any of the observed anatomical or behavioral characters throughout the entire study. Neither group differed significantly from its respective baseline level in any of the studied characteristics. The AFR was not observed in any subject prior to surgery and was not induced by either E or B implants. An investigation of a sex-related sensitivity to E or B treatment was not possible in this study because of an unfavorable sex ratio distribution (males-to-females ratios were 2:6 in the E-treated group and 4:1 in the B group).

EXPERIMENT 2

Subjects and Procedures

Nineteen juvenile *G. petersii* (standard length = 95-112 mm, weight = 7-15 g) imported in August (shortly after the end of the breeding season) 1998 from Nigeria were obtained through Quality Tropicals (Wallington, NJ) and housed in individual 20 l aquaria, as described above. All experimental procedures were followed exactly as described for Experiment 1a, **except**: 1) following the initial surgery (gonadectomy + insertion of hormonal implants), the subjects were observed for seven weeks (radiography was performed at baseline, and three and seven weeks after the initial surgery), at which point the initial implants were recovered and replaced with blanks, and the fish were observed for six more weeks and radiographed three and six weeks after the second surgery; 2) analysis of behavioral data was limited to durations of phases 2 and 3, and the PPSF; 3) each week, fish were lightly anesthetized with buffered tricaine methane sulfonate (MS-222, Sigma), as described above, and tested for the anal-fin reflex by gently touching their anal fin with the fingertip.

Following baseline data collection, fish were randomly assigned into either T (n = 9) or 11-KT group (n = 10), gonadectomized and implanted with testosterone or 11-ketotestosterone, respectively (see Experiment 1a on details of implant preparation and surgical procedures). All data collection and analysis routines were performed as described for Experiment 1a.

Results

Double surgery had an adverse effect on several animals. Removal of these subjects from the study decreased the effective sample size of T- and 11-KT-treated groups to 6 (4 males, 2 females) and 7 (3 males, 4 females) fish, respectively. Subsequent statistical analyses utilized data from these subjects only. No pre-treatment differences were found in weight [$t(11) = 0.18, p = 0.86$] and standard length [$t(11) = 0.29, p = 0.78$] among treatment groups. Males (n = 7) and females (n = 6) did not differ in either behavioral (EOD) or morphological characteristics.

Morphology

Treatment with either T or 11-KT resulted in masculinization of morphological features of the anal fin: a significant time block main effect indicated that both T and 11-KT treatments produced a significant increase in the indentation area index [$F(13,168) = 3.44, p < 0.001$] (Figure 17) and indentation index [$F(13,168) = 3.80, p < 0.001$] (Figure 18) by the end of the third week after initial surgery. Recovery of the initial androgen-containing implants did not reverse changes in morphology throughout the six weeks of observation. No differences between the magnitudes of effects of the two androgens were found. There was no significant interaction effect in any of the morphological variables.

Both androgen groups showed a significant increase in the number of anal-fin rays preceding the point of maximal indentation [time block main effect, $F(13, 168) = 6.23, p < 0.001$] (Figure 19) as well as the number of spur-bearing rays [time block main effect, $F(13, 168) = 8.02, p < 0.001$] (Figure 20) by the end of the third week following initial surgery. Again, recovery of the T- and 11-KT-containing implants did not restore

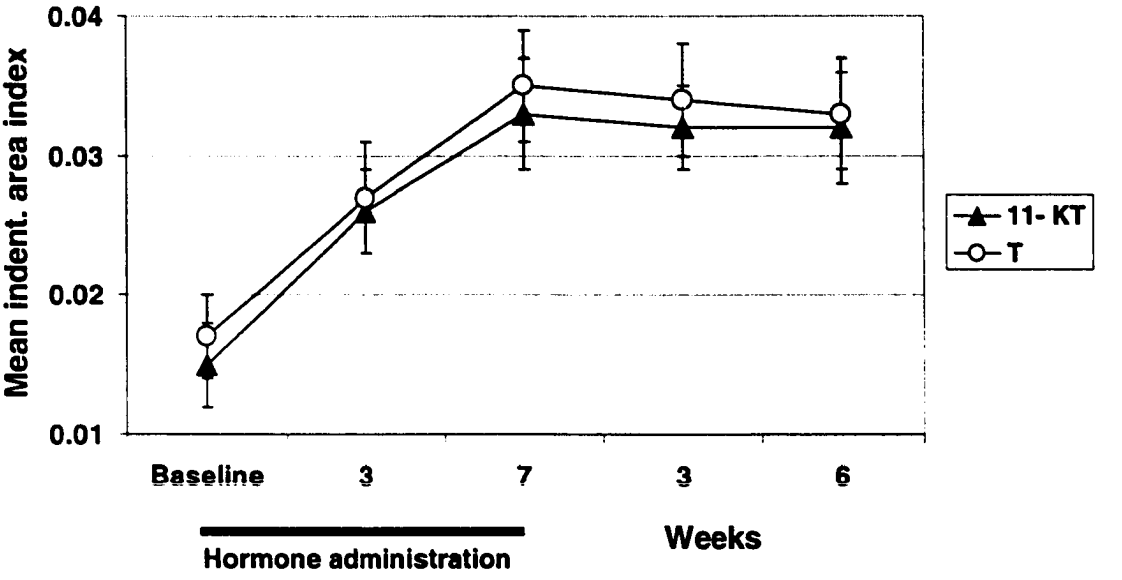


Figure 17. Mean (±SE) indentation area indexes for pre-implant baseline, at the end of the third and seventh week of hormonal administration, and at the end of the third and sixth week following implant removal. Treatments: testosterone (T, n = 6), 11-keto-testosterone (11-KT, n = 7).

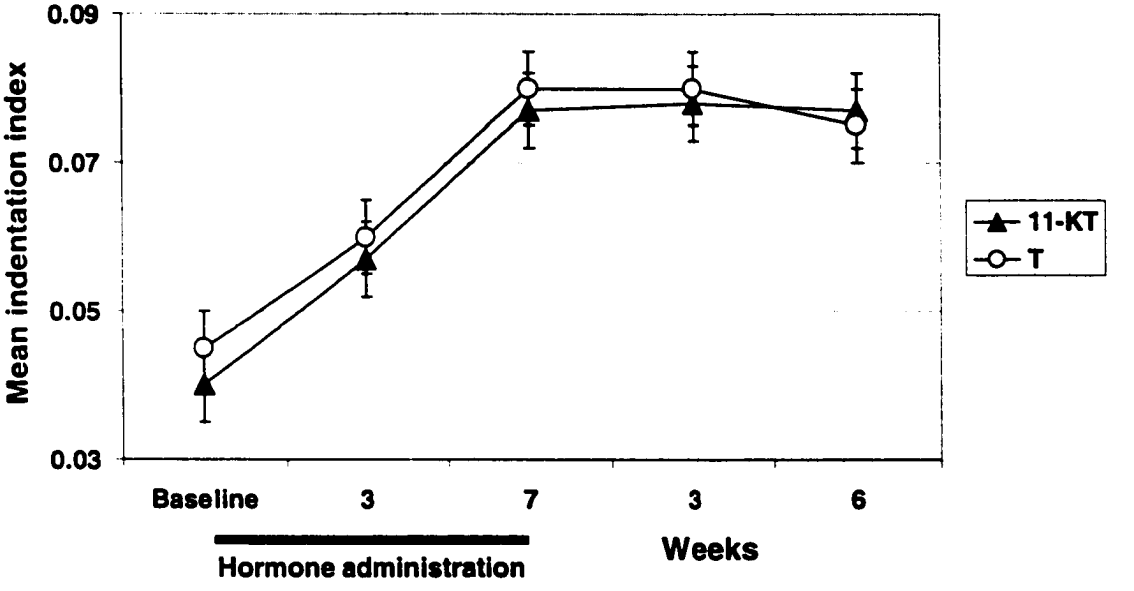


Figure 18. Mean (±SE) indentation indexes for pre-implant baseline, at the end of the third and seventh week of hormonal administration, and at the end of the third and sixth week following implant removal. Treatments: testosterone (T, n = 6), 11-keto-testosterone (11-KT, n = 7).

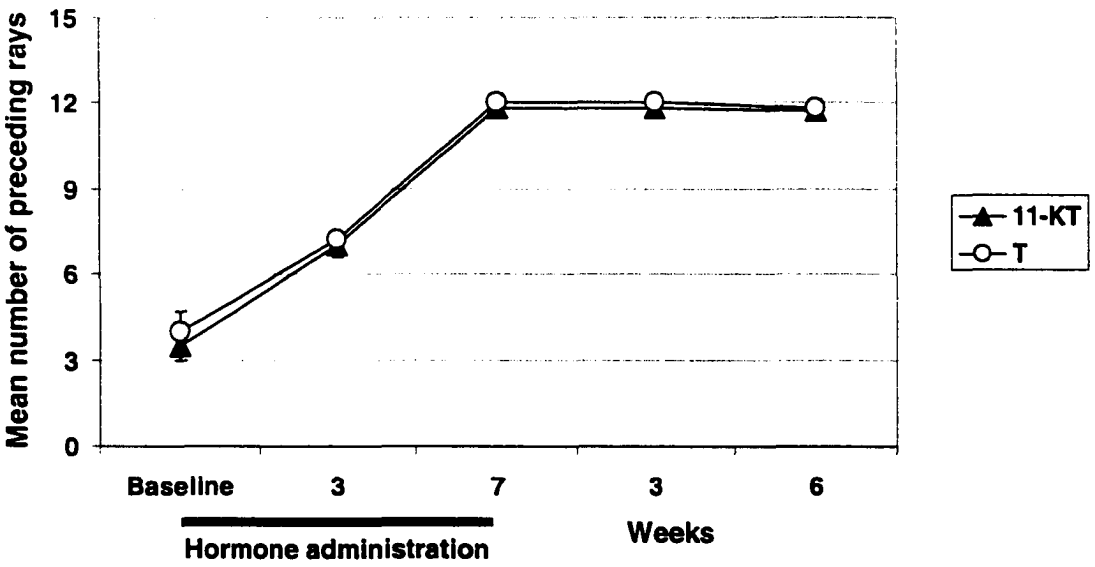


Figure 19. Mean (±SE) number of anal fin rays preceding the point of maximal indentation for pre-implant baseline, at the end of the third and seventh week of hormonal administration, and at the end of the third and sixth week following implant removal. Treatments: testosterone (T, n = 6), 11-ketotestosterone (11-KT, n = 7).

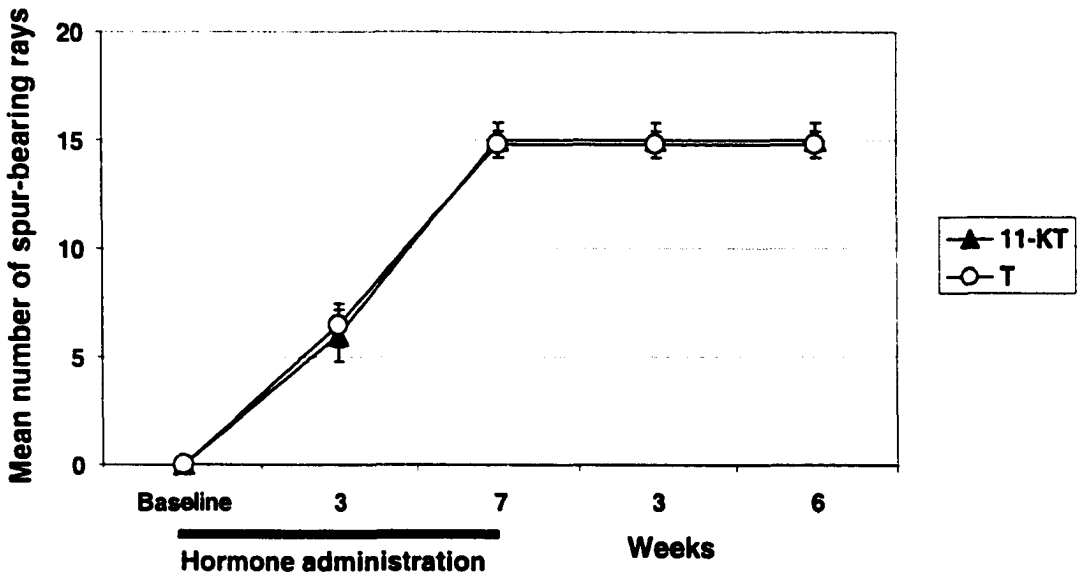


Figure 20. Mean (±SE) number of spur-bearing anal fin rays for pre-implant baseline, at the end of the third and seventh week of hormonal administration, and at the end of the third and sixth week following implant removal. Treatments: testosterone (T, n = 6), 11-ketotestosterone (11-KT, n = 7).

these two characteristics to their baseline levels within the six-week post- recovery observation period. EOD

There was no significant interaction effect on either duration of phases 2 or 3, or PPSF. However, the main effect of the weekly observation block was significant in all three variables [$F(13,168) = 9.61, p < 0.001$; $F(13,168) = 9.03, p < 0.001$; and $F(13,168) = 11.51, p < 0.001$, respectively] (Figures 21-23). Fish in both androgen groups significantly increased the duration of phases 2 and 3 and decreased the associated PPSF values by the end of the first postimplant week. Correspondingly, both groups reversed to their baseline levels by the end of the fourth week following recovery of androgen implants. No differences were found between groups.

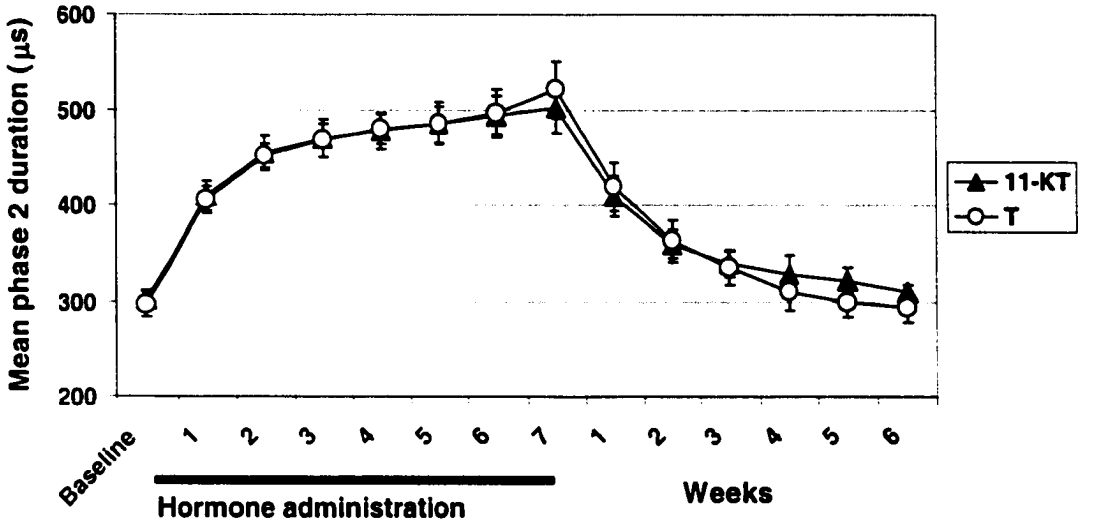


Figure 21. Mean (±SE) phase 2 duration for pre-implant baseline, at the end of the third and seventh week of hormonal administration, and at the end of the third and sixth week following implant removal. Treatments: testosterone (T, n = 6), 11-ketotestosterone (11-KT, n = 7).

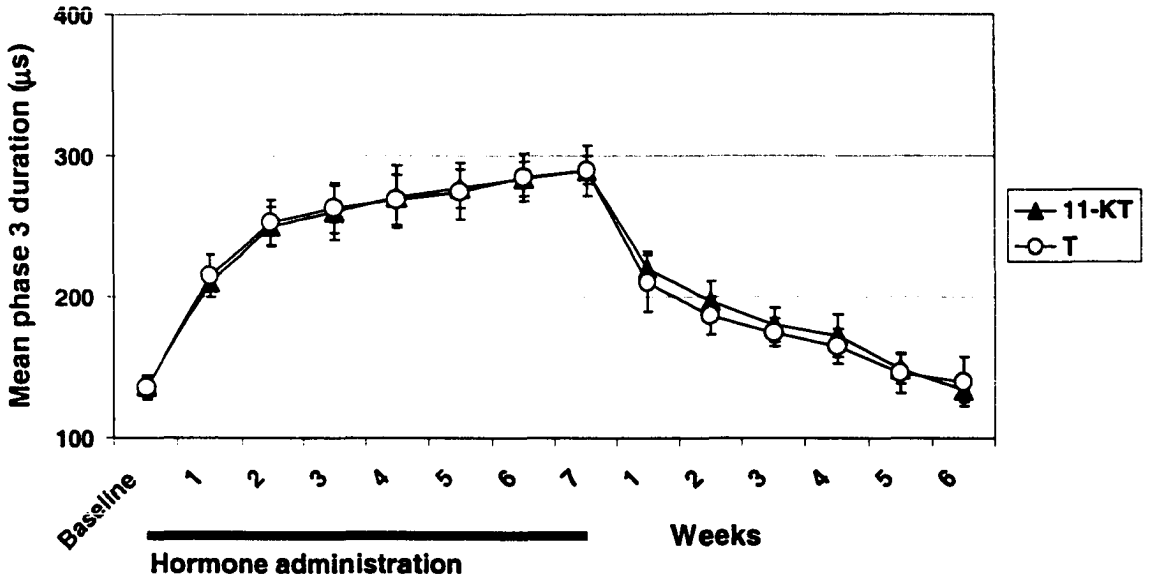


Figure 22. Mean (±SE) phase 3 duration for pre-implant baseline, at the end of the third and seventh week of hormonal administration, and at the end of the third and sixth week following implant removal. Treatments: testosterone (T, n = 6), 11-ketotestosterone (11-KT, n = 7).

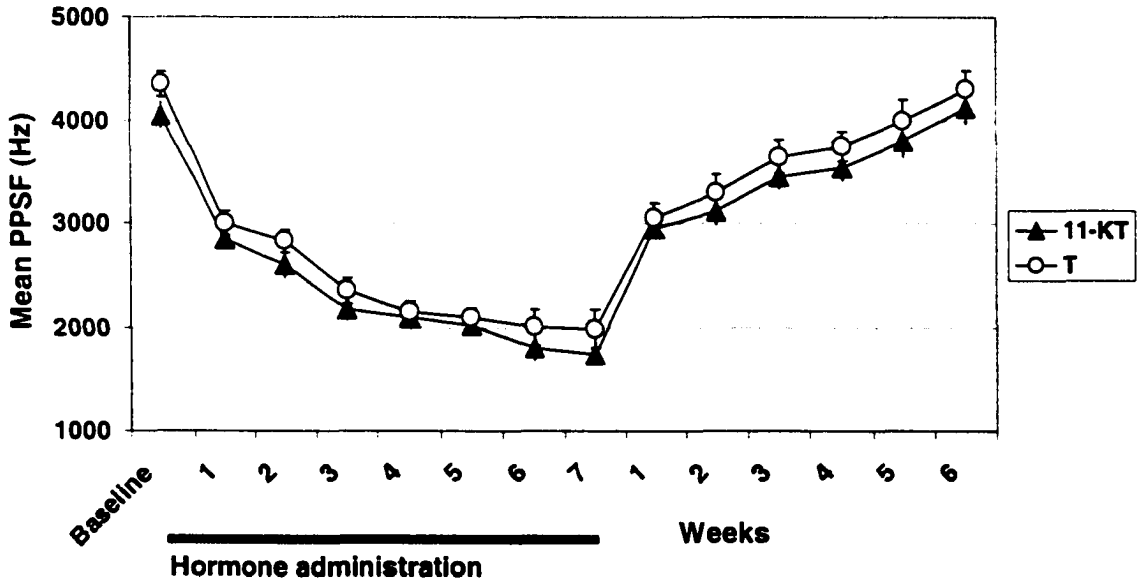


Figure 23. Mean (±SE) PPSF for pre-implant baseline, at the end of the third and seventh week of hormonal administration, and at the end of the third and sixth week following implant removal. Treatments: testosterone (T, n = 6), 11-ketotestosterone (11-KT, n = 7).

Anal-fin reflex

All subjects in both T and 11-KT groups developed a prominent AFR by the end of the third week following initial surgery. The AFR is a highly stereotypical behavioral display that consists of a wave-like flexing of the anal fin (Figure 24) with each bout lasting on average 0.20 seconds. This behavior was dramatically affected by the recovery of androgen-containing implants. The AFR completely disappeared in all subjects by the end of the fourth week following implant recovery.

A B C D E

Figure 24. Anal-fin reflex sequence in a T-treated female, postimplant week 4, video-captured at 0.05-sec. intervals. White arrow marks location of ray 12; white line traces the distal margin of the anal fin. As the flexion wave travels from anterior to posterior end of the fin (B-D), a muscle ripple in the location of ray 12 is clearly visible.

DISCUSSION

The present study for the first time reports on differential effects and timing of the action of natural androgen hormones (T, DHT, and 11-KT) on three secondary sexual characteristics affecting the anal fin morphology, EOD and the AFR in a mormyrid fish, *G. petersii*. It also provides evidence for the plasticity and reversibility of behavioral changes, and supports the hypotheses developed to understand the timing of androgen-driven sexual differentiation (masculinization) in these fish.

Ventral body wall indentation and expansion of anal-fin ray bases

Sex differences in the shape and/or size of the ventral body wall indentation are well documented in a number of mormyrid species, e.g., *Mormyrus kannume* (Iles, 1960), *Pollimyrus isidori* (Lücker and Kramer, 1981), mormyrids from the Nile (Nawar, 1960) and Lake Victoria Basin (Okedi, 1969a, 1969b). The first successful systematic attempts to quantify the size of the ventral body wall indentation and changes in anal-fin osteology were made by Pezzanite and Moller (1998) in *G. petersii* and by Herfeld and Moller (1998) in *B. niger*.

The present data clearly indicate that both the overall size (indentation area) and the "height" (maximal indentation) of the ventral body wall in *G. petersii* are androgen-dependent. Further, these effects are androgen-specific and not attributable to aromatization, since DHT was just as effective as T, while E had no effect.

One of the most interesting findings in the dynamics of the ventral body wall indentation was the androgen-induced change in the number of the anal-fin ray corresponding to the point of maximal indentation. Treatment with all three androgens (T, DHT, and 11-KT) caused the point of maximal indentation to "move" caudally. In juvenile and female *G. petersii* the point of maximal indentation usually coincides with the third or fourth ray, whereas in mature males it is located above ray 12 (Pezzanite and Moller, 1998). Thus, androgen treatment effectively masculinized the anal-fin geometry. The significance of this dimorphism in mating is not clear. It is possible that a more symmetrical anal fin enables males to flex their anal fin more efficiently when forming a fertilization pouch. Furthermore, as some form of AFR in female *G. petersii* might be present during breeding season, it would be advantageous not to have points of maximal

flexion of two overlapping structures directly against each other, as this would result in a collision rather than wrapping of one around the other. Having points of maximal flexion in different locations would resolve this potential problem.

Fish used in Experiment 1a were subadults and measured about 120 mm. This size corresponds to the beginning of sexual differentiation in the anal-fin morphology in *G. petersii* (Pezzanite and Moller, 1998). Prior to treatment, the point of maximal indentation coincided with ray 4 in females, and rays 7-9 in males. Following 4 weeks of androgen treatment, the number of the maximal indentation ray was 10 in males ($n = 7$) and 9 in females ($n = 5$). There is every reason to believe that longer exposure to androgen would have "moved" the point of maximal indentation to ray 12. Experiment 2 confirmed this assumption: 7 weeks of juvenile *G. petersii* exposure to T or 11-KT shifted the point of maximal indentation from ray 4 to ray 12. Thus, the development of the anal fin geometry in *G. petersii* is clearly under androgen control, and the apparent "movement" of the point of maximal indentation is part of the developmental process in males and associated with the anal-fin function during spawning. A similar process was described by Rosa-Molinar et al. (1996) in the Western mosquitofish, *Gambusia affinis affinis*, where administration of 17-MT induced male-typical changes in the anal fin. In mosquitofish, however, and in contrast to mormyrids, while anatomical masculinization can be achieved at any stage of development, the establishment of the functional male "genital area" is possible only during a specific "sensitive period" during embryonic development (Rosa-Molinar et al., 1994, 1996; Turner, 1941). The existence of such period or developmental window was also shown in poeciliid species *Platypoecilus maculatus* (Grobstein, 1940, 1948) and *Xiphophorus helleri* (Regnier, 1938), where

treatment of adult females with various synthetic androgens (including 17-MT) masculinized the anal fin but failed to result in normal male gonopodia. By contrast, Landsman, David and Drew (1987) reported development of functional gonopodia and normal secondary male-typical sexual characteristics in adult female *Poecilia reticulata* treated with 17-MT (mixed with food) or T and DHT implants, suggesting the absence of a sensitive period in this species.

The existence of a critical period in the development of the AFR in mormyrid fish remains an open question: Landsman et al. (1990) reported an external masculinization of the anal fin in adult female *G. petersii* treated with T and DHT implants, and Herfeld and Moller (1998) reported hypertrophied masculinization in 17-MT-treated adult female *B. niger*, but no tests for the AFR were performed in either study.

On the other hand, all subjects used in the present research were juveniles or subadults, not mature animals. Occasional testing for AFR in Experiment 1a and systematic testing for it in Experiment 1b revealed presence of AFR by the end of the third postimplant week in T- and 11-KT-treated animals and the forth postimplant week in DHT-treated fish, but never in the E-treated or B group (for up to 90 days following surgery). Furthermore, data from Experiment 2 allow us to conclude that the AFR is both androgen-dependent and transient, thus probably displayed only during the breeding season. It has been shown that in many teleosts plasma levels of T and 11-KT peak during the breading season and that T levels during that time are as high in females as they are in males (review: Borg, 1994). These findings are in good agreement with our observations of T- and 11-KT- induced AFR in both sexes of *G. petersii* and with the

report by Kirschbaum (1987) of AFR performed by both males and females of breeding *G. petersii*.

Brown et al. (1996) and Pezzanite and Moller (1998) provided systematic descriptions of the anatomy of the anal-fin ray bases in three mormyrid species, emphasized qualitative aspect of the existing sexual dimorphism, and suggested its androgen dependence. Such dimorphism was also documented in auchenipterid (Ferraris, 1988, 1991) and poeciliid species (Rosa-Molinar et al., 1996 and references cited therein) and in a more primitive genus *Polypterus* (Bartsch and Britz, 1996). A considerable number of previous studies dealt with the effects of androgen on male-typical osteological changes in the anal fin of juveniles and/or adult females of poeciliid species (Grobstein, 1940, 1944, 1948; Regnier, 1938; Rosa-Molinar et al., 1994, 1996; Turner, 1941, 1942a, 1942b). In all these studies, however, the fish were exposed to synthetic androgens and all but one (Turner, 1942b) provided a quantitative analysis of these effects. Arai (1967) compared the effects of T and 11-KT on secondary sexual characteristics, including papillary processes on the anal-fin rays in females of medaka, *Oryzias latipes*. The hormones in this study were dissolved and added to aquarium water and the only quantitative osteological measure was the count of the processes. Herfeld and Moller (1998) reported androgen-induced bone expansion in the anal-fin rays of adult female *B. niger*. The authors, too, used the synthetic androgen, 17-MT and described and quantified its effects on the fish's body wall indentation and the number of individual fin rays responding to hormone treatment.

Prior to treatment, the ray base areas in subadult *G. petersii* were not sexually dimorphic. The base area of the individual rays within a fin, however, differed in size

from one another. The areas of rays 3-7 were the largest, a finding that correlates well with the location of the point of maximal body wall indentation in juveniles. The most parsimonious interpretation, considering the size of the fish (mean SL just over 120 mm), is that masculinization of the ray bases has not yet become apparent. Treatment with T, 11-KT or DHT resulted in a significant increase of the base area and perimeter, relative to the pretreatment values. These changes, including the appearance of "spurs" suggest an increase of the ray base surface – exactly what would be expected if the expansion is needed to produce additional surface for muscle attachment.

The location of the most profound changes in the described characteristics in androgen-treated *G. petersii* is remarkable. The greatest relative increases in ray base area and perimeter were observed in rays 9-15, that is around ray 12, the location of the maximal indentation of the ventral body wall in mature males (Pezzanite and Moller, 1998). Adrogen treatment induces bone expansion around anal-fin ray 12, and as new bone surface becomes available for muscle attachment, muscle tissue itself begins to grow, thus changing the fin geometry and "moving" the point of maximal indentation. A remarkable increase in the number of spur-bearing rays, triggered by androgen treatment, provides further support for this developmental program. The histological evaluation of the anal-fin complex provides additional support. The greatest degree of muscular hypertrophy in the DHT-treated fish was observed around rays 9 and 10. This, again, represents a "shift" from the point of ray 4, where the muscle tissue was most prominently developed in a control female subject. Furthermore, it strengthens the validity of results obtained from radiographic images: both methods point at ray 9 as the

location of greatest muscular hypertrophy in the DHT-treated animal at the time of testing.

One obvious methodological peculiarity of the present research consists in the use of two-dimensional radiographic images to draw conclusions about three-dimensional structures. As a way of verifying validity of this methodological approach, two animals (one DHT-treated and one control) we cleared, stained and examined under the microscope. The examination of the anal-fin ray bases revealed a three-dimensional expansion of the base structures in the affected rays (Figure 25). This renders support to the data, obtained from the two-dimensional radiographic images. The most prominent expansion was observed in ray 9, whereas rays 16-20 showed no sign of expansion at all, confirming findings obtained from the radiographs.

Another interesting question worth investigation would be determining whether the observed increase in muscle volume was due to an increase in the number of muscle fibers (true myogenesis) or merely to increase in the thickness of the myocyte. Although this question was not the aim of the present study, two muscle-stained histological preparations of the anal-fin (one from the DHT-treated fish and one from a control subject) were made and examined. The examination confirmed muscular hypertrophy observed in the treated animal (Figure 26). The evaluation of the relative extent of this hypertrophy at various points along the anal fin revealed two distinct patterns: the point of maximal muscle thickness (maximum of the corresponding index) was observed around anal ray 4 in a control female and shifted towards rays 9-12 in a female that has been implanted with DHT for 4 weeks (Figure 27), in good agreement with the data obtained from the radiographic images.

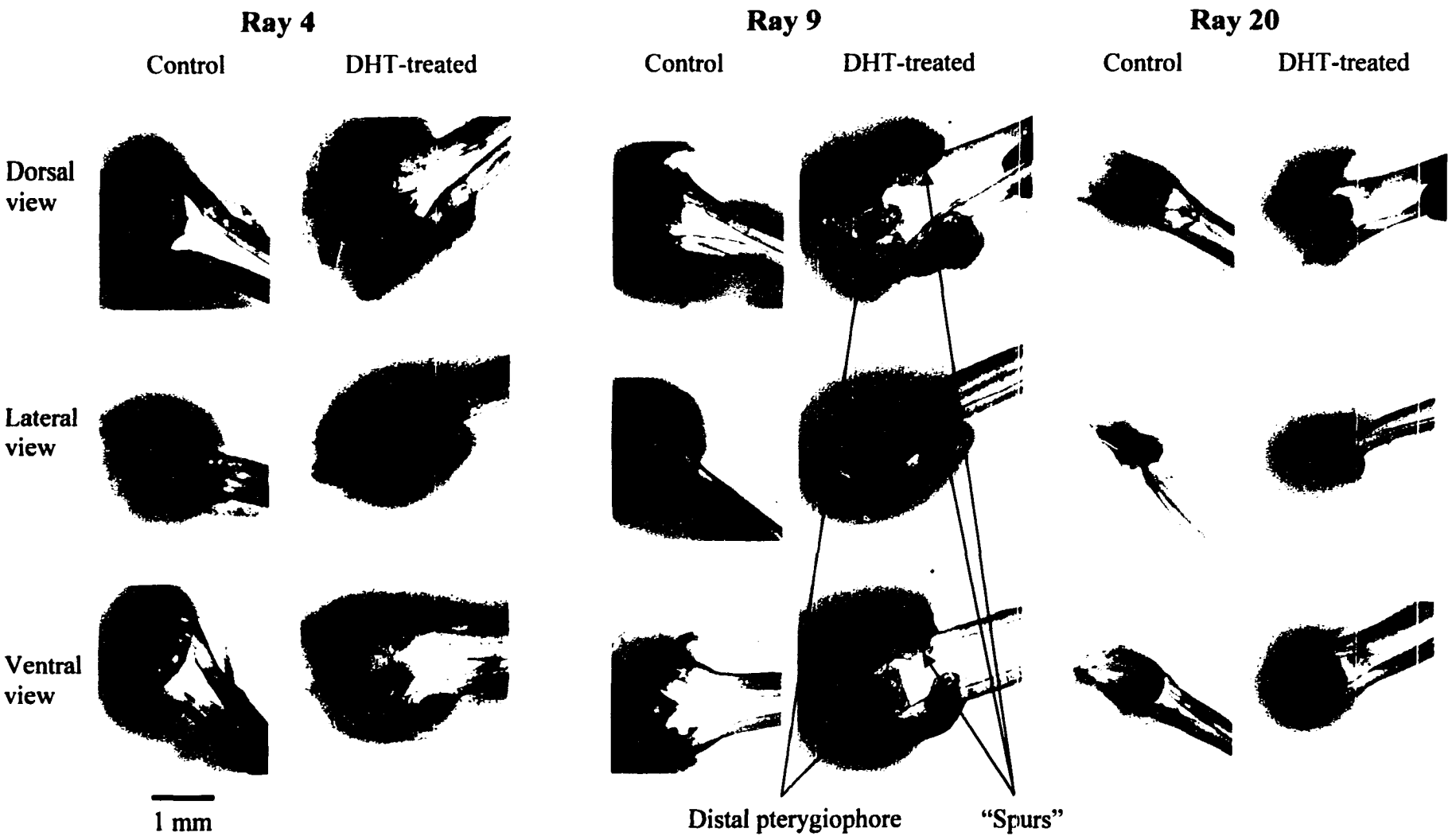


Figure 25. Microphotographs of the bases of three representative anal-fin rays from a control and a DHT-treated subjects (cleared and stained preparations). Magnification: X10. Distal pterygiophore is visible between split elements of the fin ray in the dorsal and ventral views.

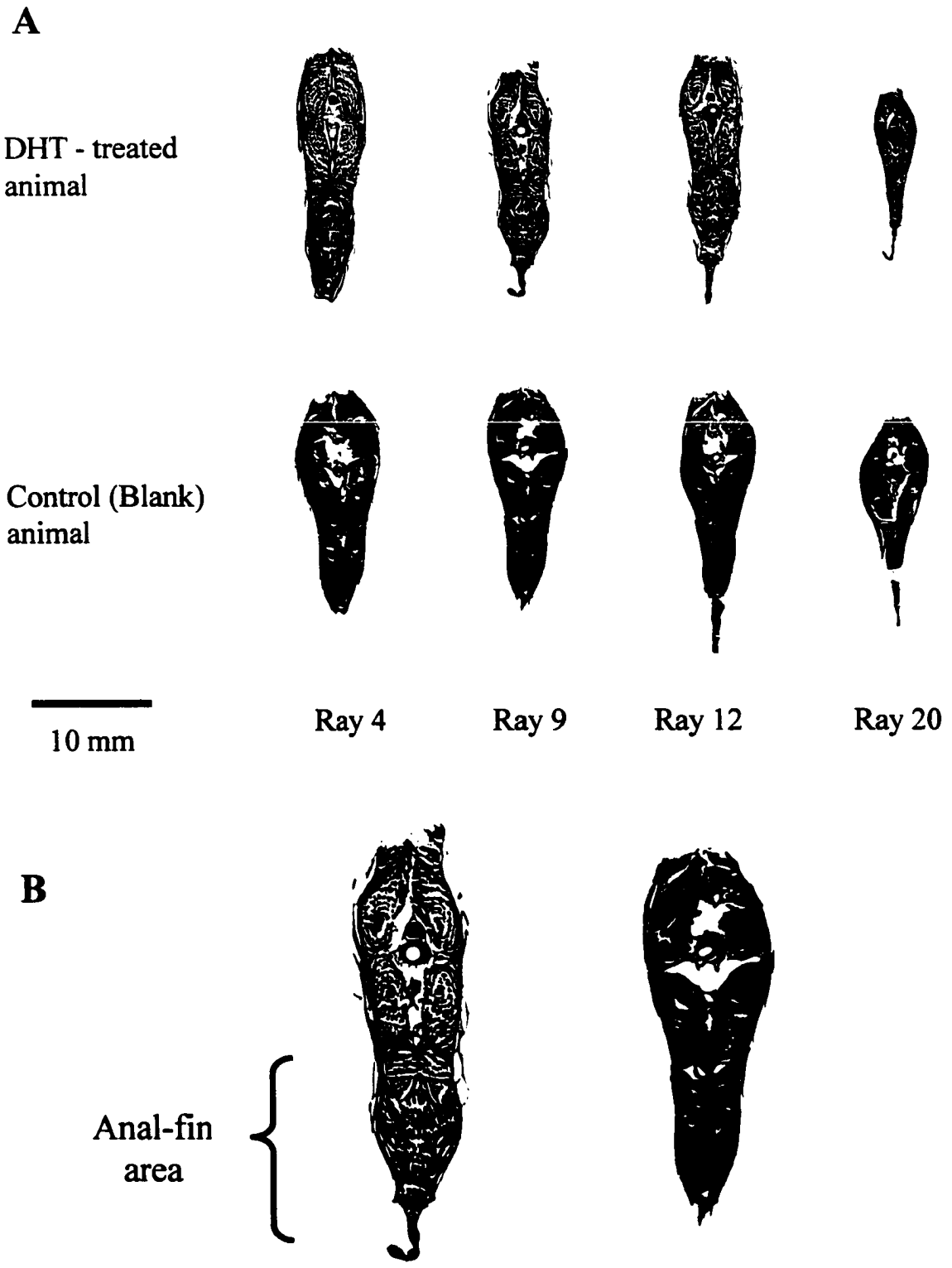


Figure 26. A: Microphotographs of the crossections through four representative anal-fin rays from a control and a DHT-treated subjects (freezing microtome, thickness = 12 μm, Milligan's Trichrome Stain). Magnification: X2. B: Enlarged crossections in the area of ray 9.

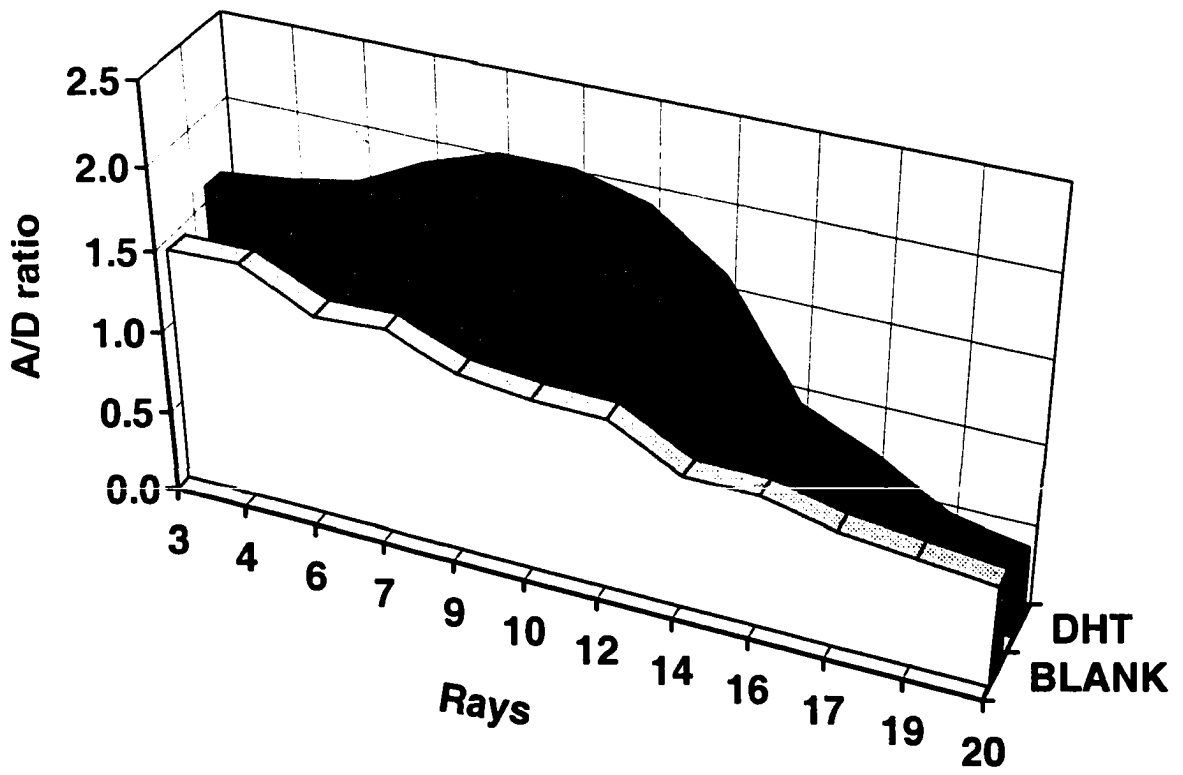


Figure 27. Musculature thickness index (A/D ratio) computed for selected anal-fin ray locations in a female implanted with DHT for 4 weeks and a control female with a blank silastic implant. Measurements were made from the muscle-stained histological preparations.

Differential Androgen Effects

Differential bone expansion caused by the three androgens is of the great interest. DHT was consistently more effective than T, particularly in terms of relative perimeter increase in ray bases around ray 12. *In-vivo* studies using a rat model of bone metabolism showed that DHT stimulates both the longitudinal bone growth and formation of periosteal bone, the principal site of muscle and tendon attachment (Coxam et al., 1996; Tobias et al., 1994). Given anatomical and physiological similarities between the bone of

mammals and mormyrids (Lopez, 1970a, 1970b; Lopez et al., 1976, 1977; Meunier and Huysseune, 1992; Moss, 1961; Weiss and Watabe, 1979), there is every reason to expect a similar action of DHT in mormyrid bone. To be sure, T alone is sufficient for normal bone metabolism in a rat, even when its reduction to DHT is prevented (Rosen et al., 1995), but DHT is well known to be biologically more potent. In most bioassay systems DHT is about twice as potent as T, which points at DHT as a major cellular mediator of androgen action, particularly in peripheral tissues (Griffin and Ojeda, 1992). This would explain greater effects of DHT, compared to T, on the number of spur-bearing rays at the end of postimplant week 2, but not postimplant week 4: the onset of T effects is delayed by the need to reduce it to DHT in the peripheral tissue. Considering similarity of the magnitude of bone effects exhibited by T and 11-KT, and the fact that the latter was shown to promote courtship behavior in a number of teleost fishes to a greater extent than T (Brantley et al., 1993), it would be of interest to replicate our Experiment 2, with inclusion of DHT as the third androgen.

Electric Organ Discharge

The results support the principal findings by Landsman et al. (1990) on hormonal sensitivity of the EOD waveform in *G. petersii*. The clearest effect was observed on the duration of phases 2 and 3, which were lengthened by androgens, but not estrogen. In both cases T induced a more powerful change than DHT.

Mean duration of phase 1 increased during the first and decreased during the second postimplant week in all treatment groups, including the controls. Landsman et al. (1990) reported fluctuations in the duration of phase 1 and attributed it to temperature

variations. This explanation can be ruled out here, since all phase duration data in the present study were temperature-adjusted. The effect of postsurgical stress seems a likely explanation, especially considering a similar change shown by all subjects regardless of the treatment.

There was no consistent pattern in changes of the duration of phase 4. This phase reflects residual electric current flowing through the electrocytes after both membranes have been depolarized (review: Moller, 1995) and its duration is extremely variable. As this phase is the longest and contributes 60-75% of the total EOD duration, it is not surprising to see changes in the total EOD duration follow closely those of phase 4. Given very low amplitude of phase 4, it is likely to be detected only under low water conductivity conditions, which corresponds to conditions during rainy (breeding) season. However, Landsman (1990, 1993, 1995) found no sexual dimorphisms in the duration of phases 1 and 4 and the total EOD in *G. petersii* imported during breeding season. Thus, the duration of these phases and of the total EOD is unlikely to play a role in this fish's social communication, and future endocrine studies involving this species might exclude measurements and analysis of phases 1, 4 and of the total pulse duration as markers of sexual identity and maturity.

The sex differences in the EOD in many mormyrid species are manifested in the PPSF: a male's signal is longer in duration and, therefore, its associated PPSF is lower (review: Landsman, 1995). The main finding of this study supports a conclusion by Landsman et al. (1990): androgen treatment decreases PPSF in subadult *G. petersii*. Unlike Landsman et al., however, the present data indicate a significant DHT effect on PPSF in response to "the low dose" treatment (Landsman et al.), although the potency of

DHT was lower than that of T. There is less agreement in the estrogen effects on PPSF reported by different authors. Bass and Hopkins (1983, 1985) reported lowering of the PPSF (masculinization) in juvenile *B. brachyistius* implanted or injected with E, but this work was criticized for lack of proper control (Landsman, 1995). Landsman et al. (1990) observed no change of PPSF in juveniles and an increase of the PPSF (feminization) in adult *G. petersii*, treated with E implants. That report, however, was based on only three subjects, with two males and a female mixed together. In Experiment 1a, using the same dosage of E as Landsman et al., we observed significant, although short-lasting (1 week), masculinization in five E-treated subadult *G. petersii*. However, when the experiment was repeated (Experiment 1b), no E effects were found on either PPSF or any other EOD characteristics. Thus, considering the small size (n = 3) and mixed nature (2 males, 1 female) of the E-treated group, the effect observed by Landsman et al. might be an artifact. While among gymnotiforms, androgens and estrogen have been shown to act in the opposite directions, respectively masculinizing or feminizing both EOD frequency and duration (Dunlap, McAnelly and Zakon, 1997; Dunlap, Thomas and Zakon, 1998), the situation in mormyrids at present remains unresolved.

Morphology – Behavior Interaction

The results of a correlation analysis support the hypothesized androgen effects on structure and behavior. Based on the proposed hypotheses, properties of the EOD are expected to correlate more closely with changes in size and shape of the muscle tissue rather than bone, since mormyrid electrocytes are of myogenic origin (Bass et al., 1986; Freedman et al., 1989; reviews: Bass, 1986; Bennett, 1971; Moller, 1995). Androgen

action leading to a thickening of the myocytes should have similar effects on electrocytes, thus, masculinizing the EOD. Furthermore, the closest relationship to muscular morphology would be expected for those EOD properties that are most influenced by the increase in the electrocyte size and thickness of the electrocyte membrane (Freedman et al., 1989). The duration of phases 2 and 3 are directly affected by the geometry of the electrocytes (Bennett, 1971) and should be considered such properties. The results, however, indicate a strong correlation between muscle morphology and phase 2, but not phase 3. The following explanation is possible: electrocytes in the electric organ of *G. petersii* have a single penetrating stalk (Bass, 1986; Bennett, 1971; Moller, 1995). The duration of the second (head-positive) phase (phase 2) of the EOD is determined by a delay between the onsets of depolarization of the caudal (initial depolarization) and rostral (second depolarization) membranes of the electrocyte (Westby, 1984). Changes in electrocyte geometry (e.g., thickness) s that increase such a delay would also increase the duration of phase 2. As phase 3 is not affected by the electrocyte shape, a strong correlation between these two characteristics should not be expected. Steroid hormones were also shown to influence duration of phase 3 by affecting ion channels in the electrocyte's membrane (Bass et al., 1986; Freedman et al., 1989). Such hormonal effects cannot be seen in the results of the morpho-behavioral correlation analysis.

Finally, although the hypothesized correlations were found to be significant, simple cause-effect relationships concerning the mechanisms of androgen actions cannot be inferred from this type of analysis, as 1) teleosts possess a number of other controlling circuits (e.g., the hypothalamus-pituitary-gonad axis) utilizing the same androgens that were used in our study or their metabolites; and 2) despite verified completeness of

gonadectomy, metabolism of implanted androgens most likely took place not only in the peripheral target loci (myocytes and electrocytes) but also in the liver and interrenal tissues (review: Borg, 1994).

Sexually Dimorphic EOD: Adaptive Advantages and Costs

The production of longer discharges requires additional energy. Utilization of behavior with higher energetic cost may indicate its possible adaptive advantage. Outside the breeding season, both male and female *G. petersii* emit shorter ("feminine") EODs (Landsman, 1995), which are more energy efficient. The emission of longer, more energy-demanding EODs during the breeding season does not only serve as advertisement of reproductive readiness, but may also provide the basis for mate selection. Females may prefer males that emit longer EOD, as those are likely to be healthy mature males. Thus, the duration of EOD may act as an indicator of the sender's health and even his reserve holding potential (Krebs and Davis, 1993). Commenting on vocalizing in the mormyrid *Pollimyrus adspersus*, Crawford and Huang (1999) argued that acoustic signaling is more energetically demanding and, therefore, compared to electric signaling, makes a more honest advertisement of the sender's physical condition. This argument is likely to be correct. *P. adspersus* is considerably smaller than *G. petersii* and males of this species maintain sexually dimorphic (longer) EODs all year, suggesting that energy expenses involved in their production are not too taxing. Furthermore, such a relatively low cost of long EODs implies the possibility for males to cheat in signaling their physical condition and social status, if those were assessed by the conspecifics on the basis of the EOD alone. The more than 10-fold longer EOD in the

much larger *G. petersii* raises the cost of producing these signals. This should make male cheating on the basis of the electric signaling considerably less likely.

Masculinization in *G. petersii*

It appears that the juvenile developmental condition in *G. petersii* is feminine-like. Juveniles of both sexes resemble females both anatomically (anal-fin morphology) and behaviorally (EOD, absence of the AFR). The natural masculinization process begins when males reach the standard length of about 120 mm (Pezzanite and Moller, 1998). Artificial masculinization of secondary sexual characteristics can be induced by androgen administration in juveniles as well as in adult females, suggesting the absence of a rigid developmental window. Considering that DHT and 11-KT, the non-aromatizable androgens, are just as effective in inducing masculine characteristics as the aromatizable T, and that estrogen is ineffective, androgen aromatization does not seem to be part of the process. Furthermore, since DHT elicits morphological transformations faster than T, α-reduction (a process by which T is converted to DHT) is likely to be involved as an intermediate step in inducing structural masculinization. In controlling behavioral characteristics, T is more potent than DHT, E or the combination of the two (Landsman et al., 1990) suggesting specific receptor affinity as the factor determining behavioral potency of these androgens. Virtually identical results obtained with T and 11-KT are not surprising as both are dominant androgens in teleosts fishes and in most species their plasma levels rise and fall in synchrony (Borg, 1994). Whether both hormones in the course of their metabolism in mormyrid species bind to the same receptors or to the separate ones remains to be determined.

The hypothesized androgen-dependent masculinization in *G. petersii* may progress as follows:

(1) When males reach in size to about 120 mm their testes increases production and release of androgens.

(2) Extended exposure to circulating androgens (specifically, DHT) triggers osteogenesis (primarily of the periosteal tissue) at the bases of the anal-fin rays, followed by muscle development (through muscle fiber growth and/or myogenesis) along the ventral body wall and resulting from it, changes in the anal-fin geometry.

(3) Enlarged/new muscle fibers attach to newly formed bone surface on the ray bases creating an efficient mechanism to execute the AFR.

Steps (1–3) represent organizational effects and require relatively low levels of plasma androgens. These processes are slow, gradual, permanent, and mediated primarily by DHT.

(4) With the onset of the breeding (rainy) season, in response to environmental cues (review: Kirschbaum, 1995), the testis sharply increases production and release of T.

(5) In response to higher T levels, the AFR develops and the size and thickness of electrocytes increase resulting in a longer duration of the EOD.

(6) Males are ready to spawn and announce their sexual identity and maturity by emitting longer (and, therefore, possibly more easily detectable) EODs.

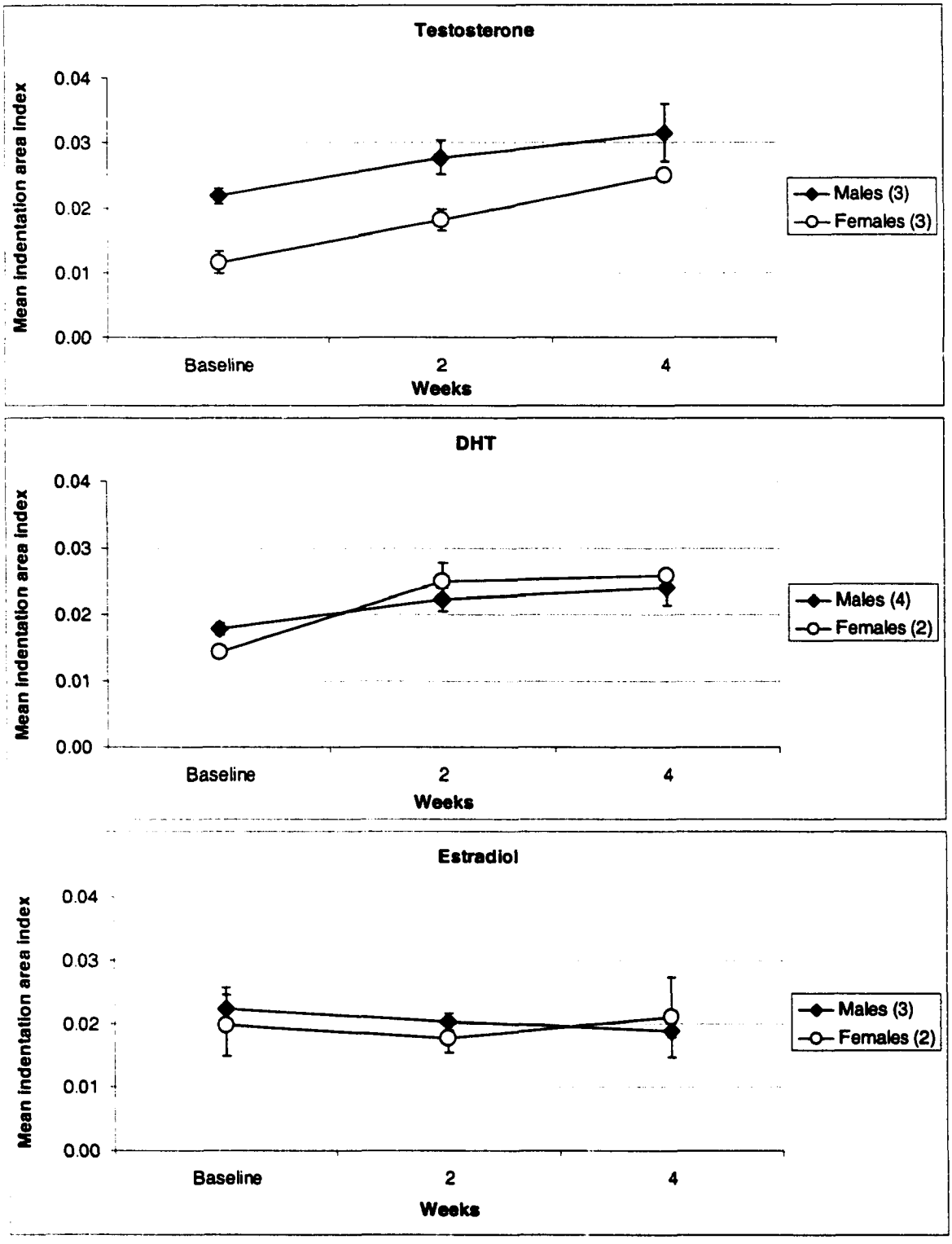
(7) At the moment of spawning, the male wraps its anal fin around that of the female to form a spawning pouch (AFR). This increases the probability of fertilization, and thus, reproductive success.

(8) At the end of the breeding period, T levels decrease, followed by reduction in the size of the electrocytes and termination of the AFR (hence, no EOD dimorphism outside the breeding season [Landsman, 1990, 1993, 1995]).

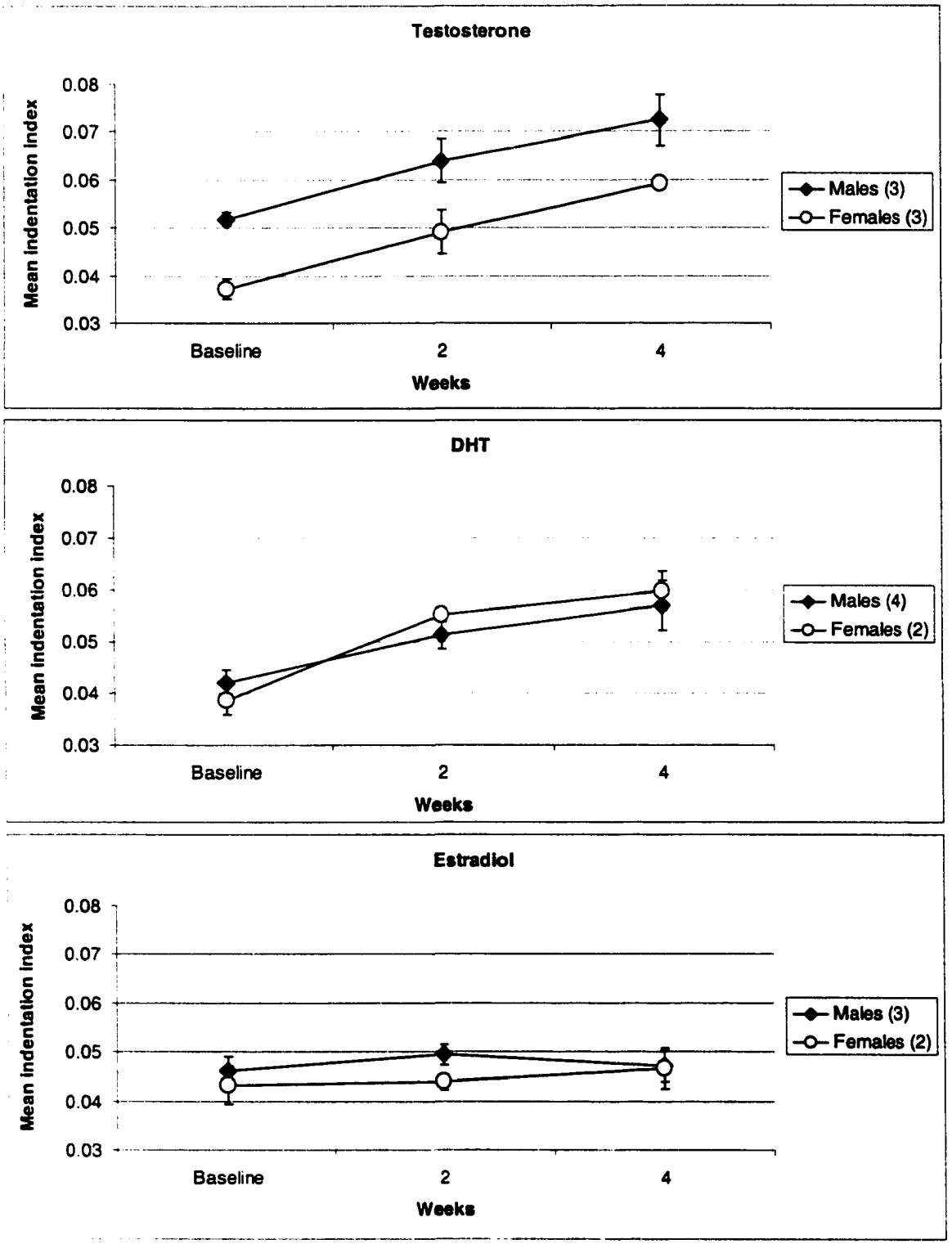
Steps (4–8) represent activational effects and require relatively high androgen plasma levels. These are fast, transient and mediated primarily by T.

The reversal of the male EOD characteristics to a female-like status at the end of the breeding season appears to have a purely "economical" reason: maintaining a longer, more energy-consuming EOD all year-round might be too costly when it offers no adaptive advantage.

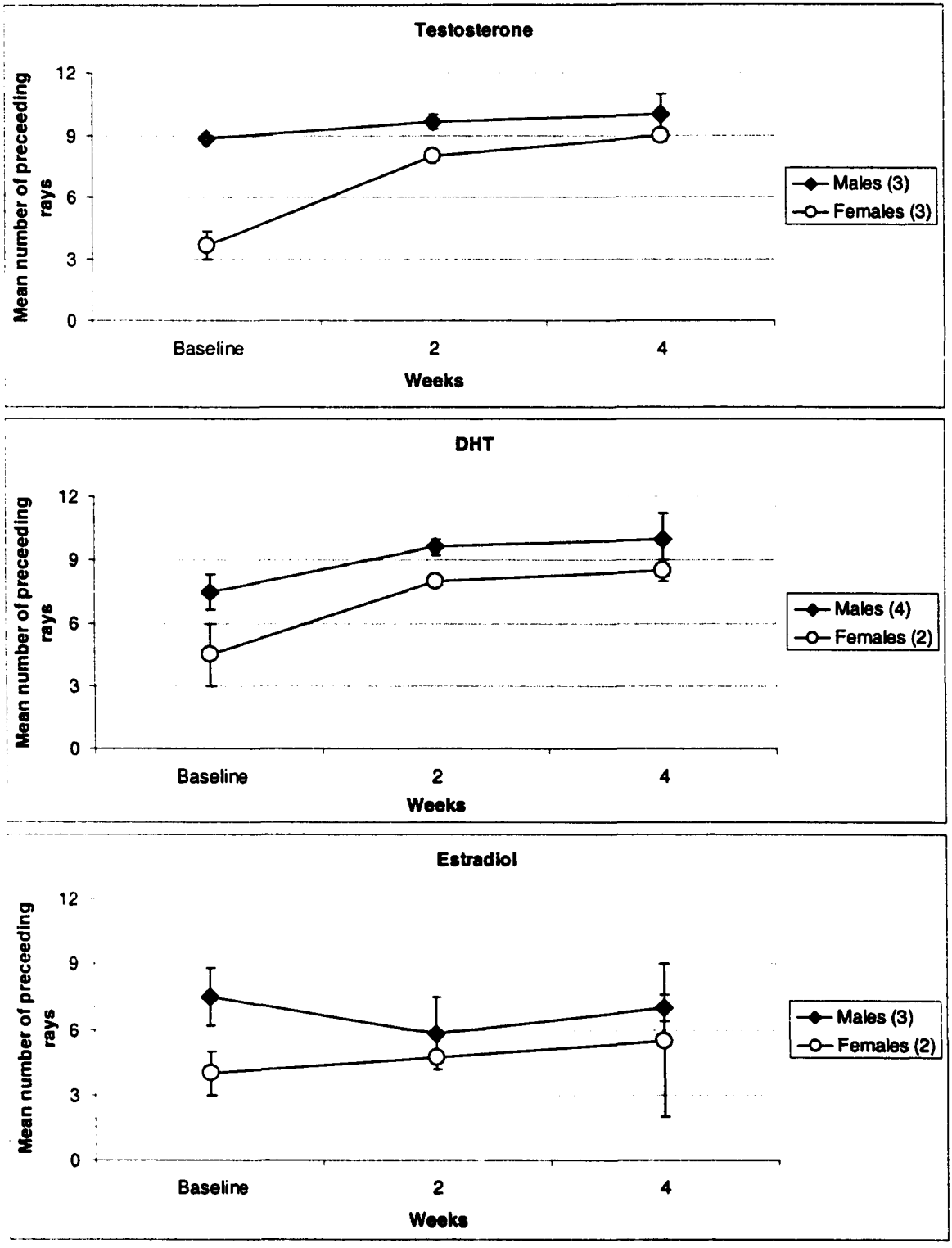
Appendix A1. Experiment 1a. Indentation area index.



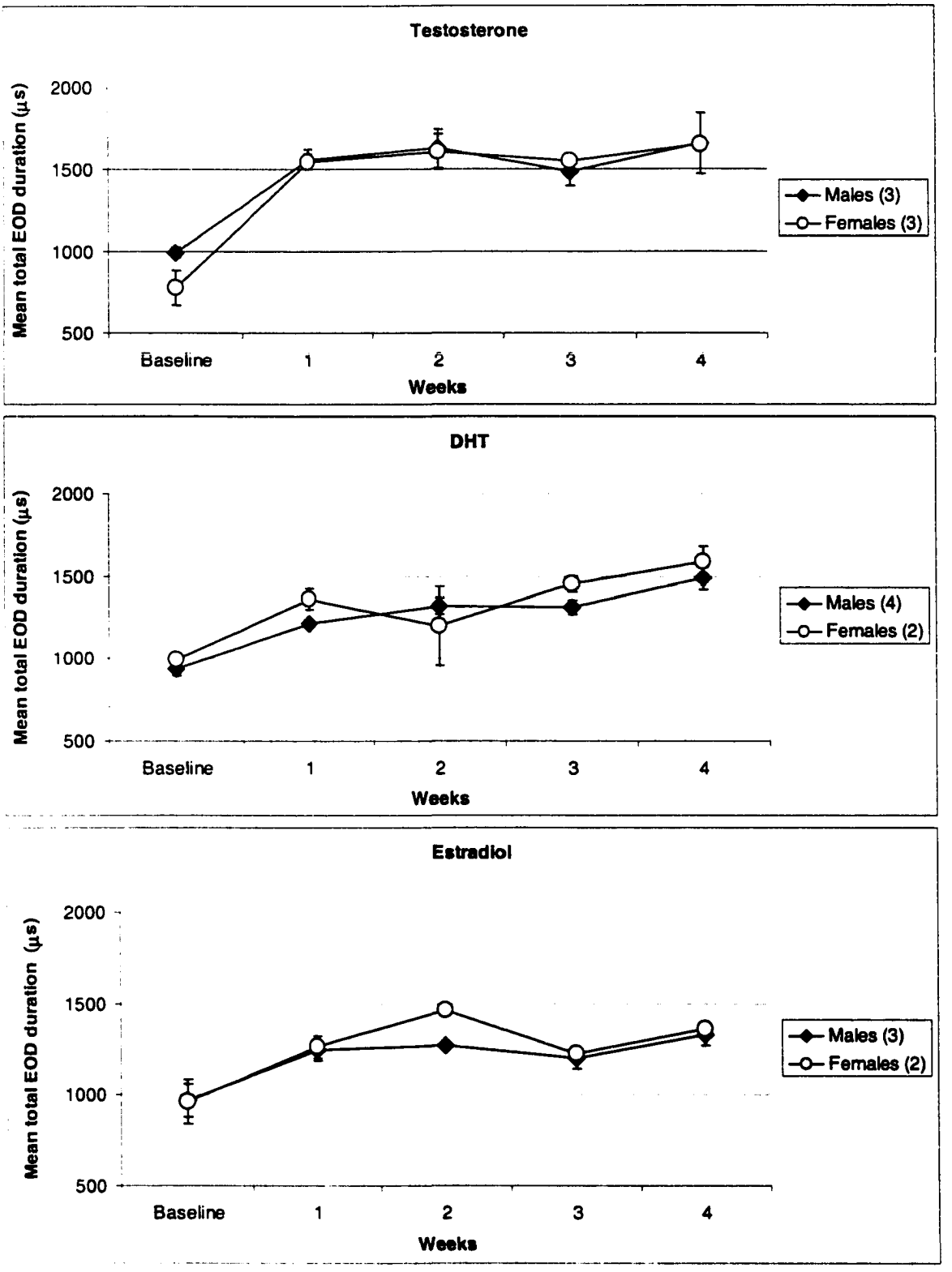
Appendix A2. Experiment 1a. Indentation index.



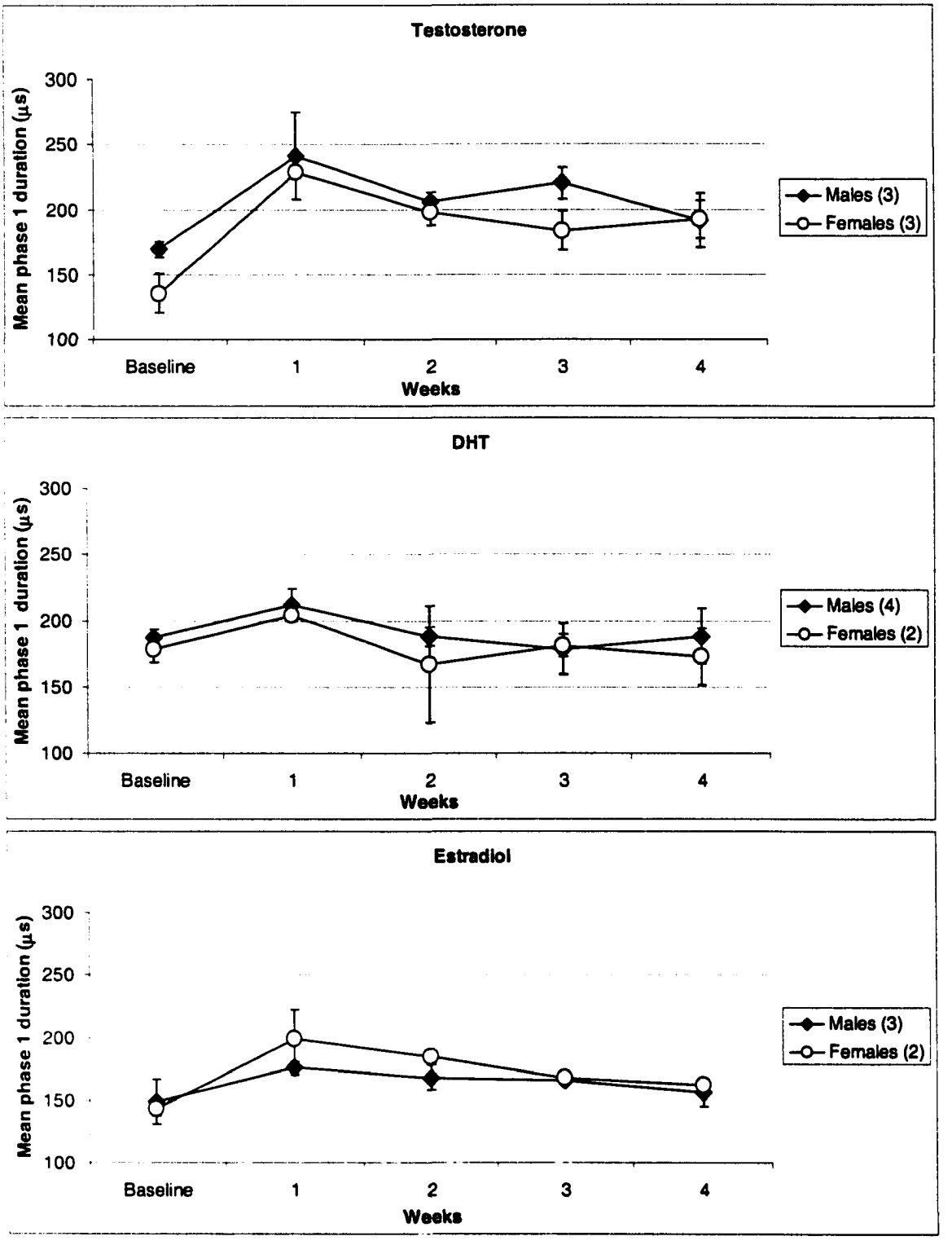
Appendix A3. Experiment 1a. Number of preceding rays.



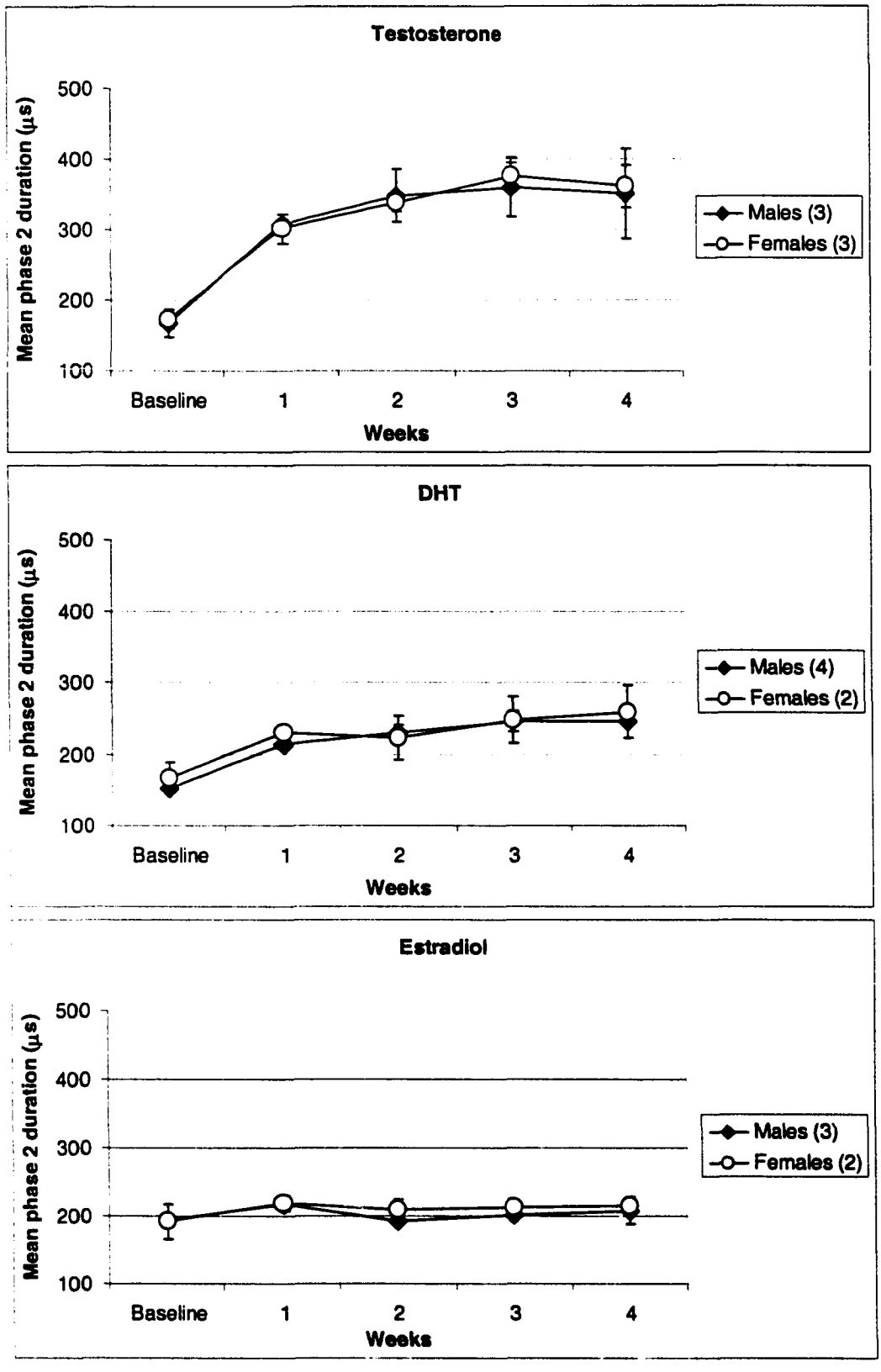
Appendix A4. Experiment 1a. Total EOD duration.



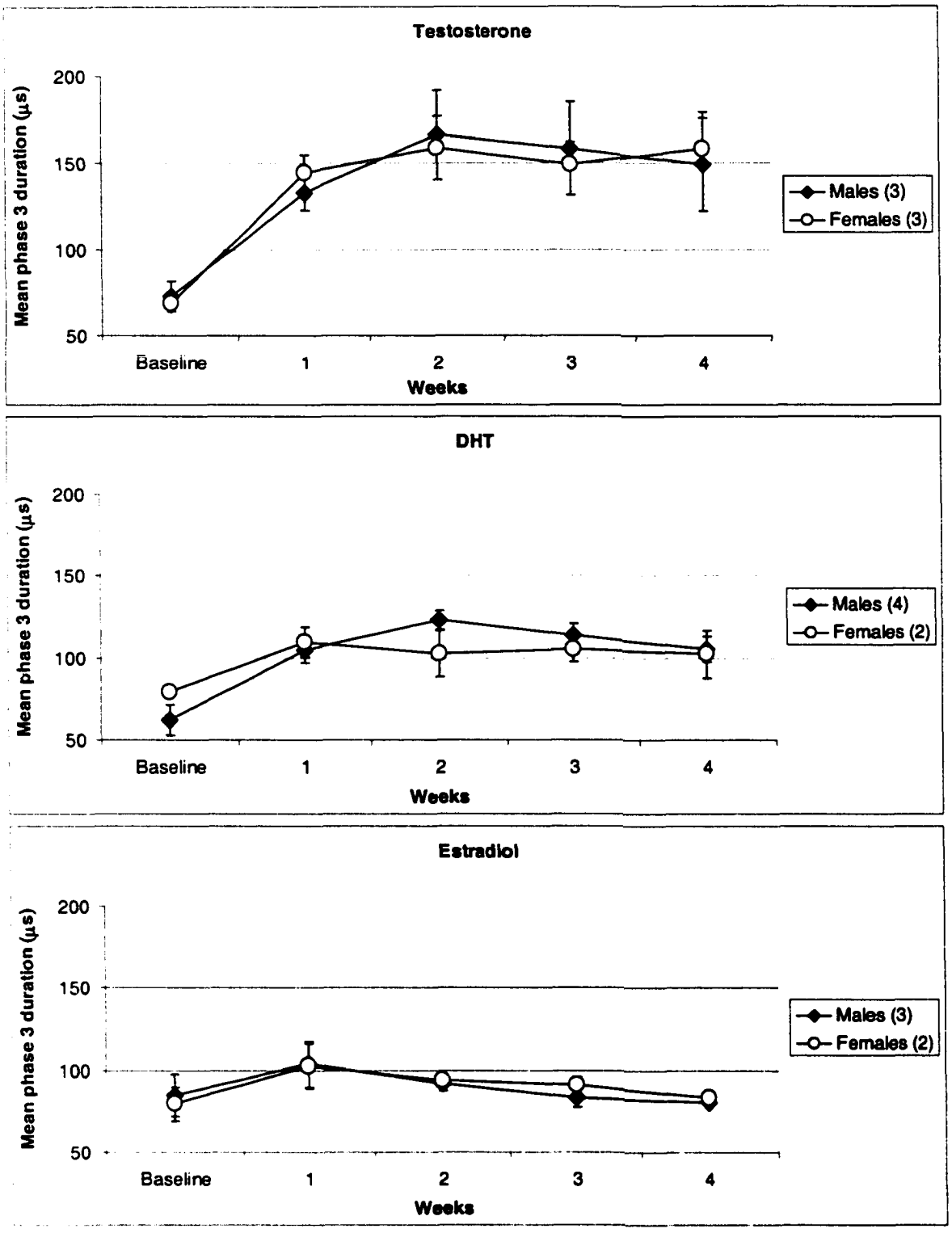
Appendix A5. Experiment 1a. Phase 1 duration.



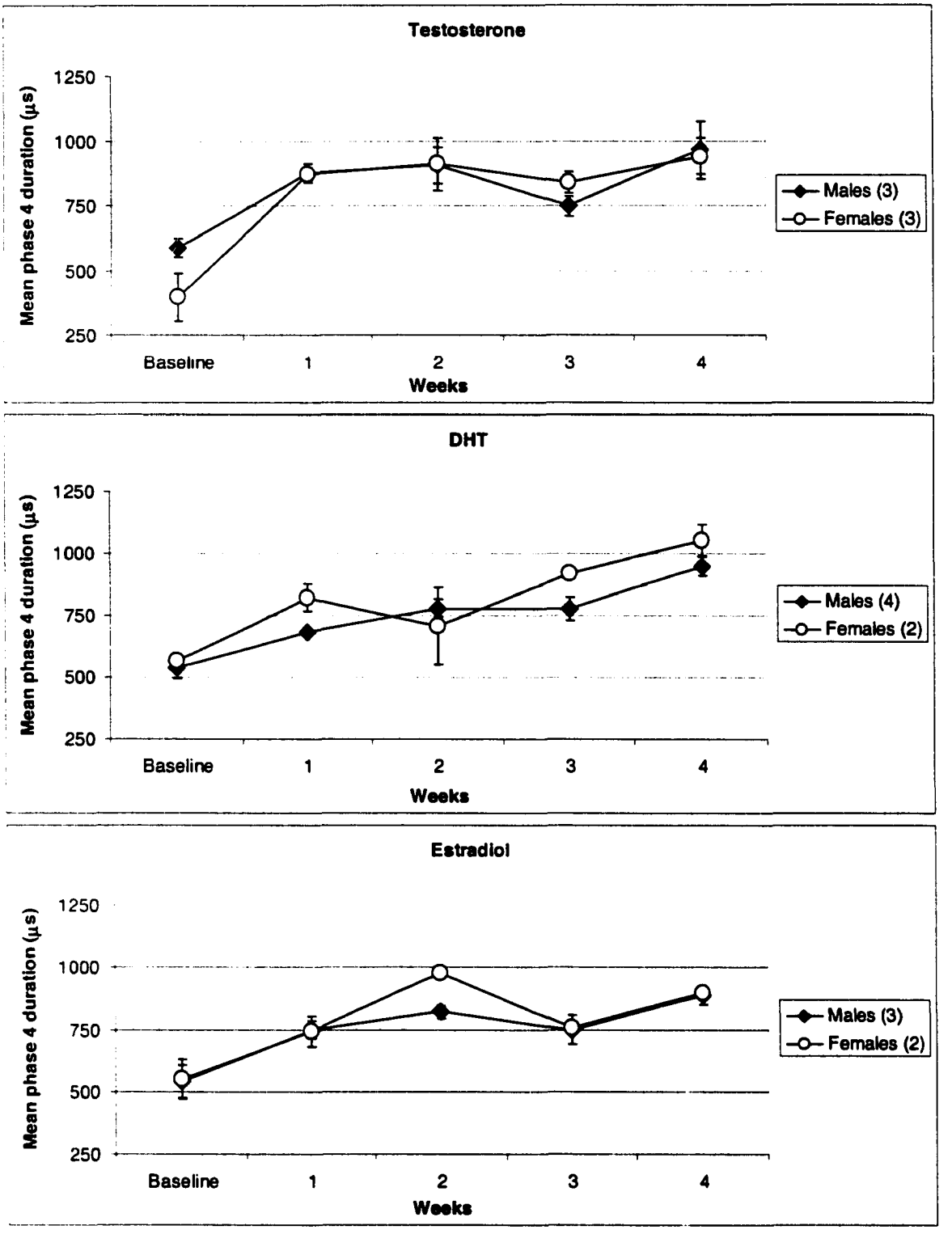
Appendix A6. Experiment 1a. Phase 2 duration.



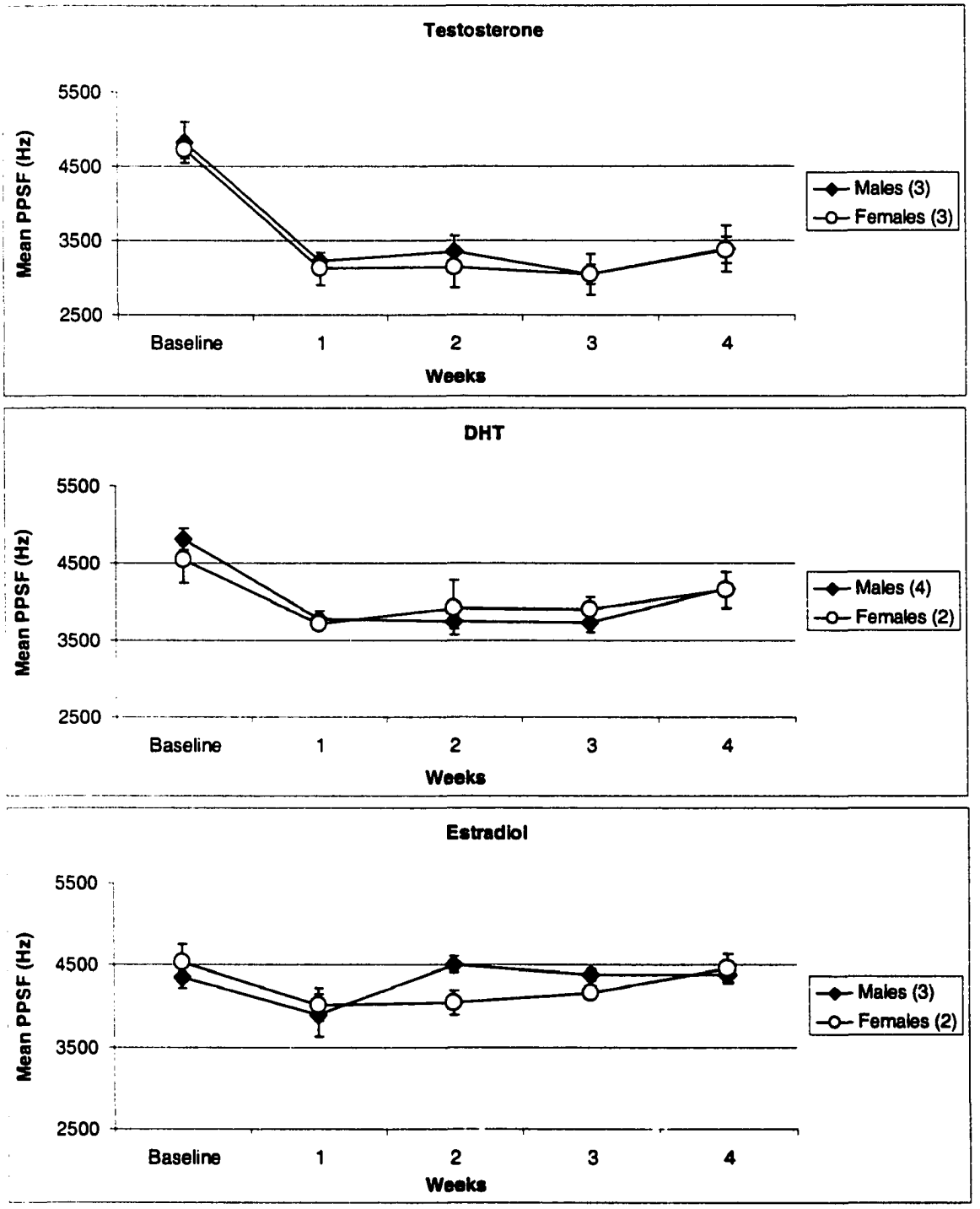
Appendix A7. Experiment 1a. Phase 3 duration.



Appendix A8. Experiment 1a. Phase 4 duration.



Appendix A9. Experiment 1a. PPSF.



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