



## Full length article

# Can estrogens be considered as key elements of the challenge hypothesis? The case of intrasexual aggression in a cichlid fish

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## ABSTRACT

Territorial aggression has been widely studied in males and it has been historically suggested that androgens are key mediators of this behavior. However, more recent evidence suggests that it is the aromatization to estrogens, rather than androgens themselves, that is key to regulating this behavior. Females also display aggressive behaviors, but the physiological regulation of female aggression is still understudied when compared to males. In this context, the challenge hypothesis postulates that male-male aggressive interactions stimulate the production of androgens in males in periods of social instability. Here we determine plasma sex steroid levels in *Cichlasoma dimerus* to assess whether estrogens are related to aggressive behavior and to test the challenge hypothesis in both males and females. We set-up challenge trials as intrasexual dyadic encounters and determined androgen and estrogen levels before and after the trial in both winners and losers. Even though there were no differences in initial estradiol-17 $\beta$  plasma levels between male winners and losers, initial levels were higher ( $p = .046$ ) in female winners than in losers, while there were no differences in testosterone or 11-ketotestosterone levels. After trials, both males and females showed elevated levels of estradiol-17 $\beta$  and both androgens, but only males exhibited a significant 1.45, 5.42 and 3.2-fold increase in estradiol-17 $\beta$ , testosterone and 11-ketotestosterone, respectively ( $p = .023$ ,  $p = .016$ ,  $p = .018$ ). Moreover, changes in circulating levels of estradiol-17 $\beta$  in females after the trials do not depend on their reproductive status or on the outcome of the contest. We suggest that female aggression is associated with initial levels of estradiol-17 $\beta$ , and that the challenge hypothesis, originally defined for androgens, could also be extended to estrogens.

## 1. Introduction

Patterns of aggression have direct and indirect fitness consequences, and mechanisms involved in regulating aggression are complex and include physiological, genetic, social and environmental factors [1]. Agonistic behavior is highly conserved across species, and there is growing evidence suggesting that at least some of the mechanisms regulating aggression in fish, amphibians, birds and mammals are phylogenetically conserved as well [2–6,73]. In this context, comparative studies and the use of non-traditional species as biological models will significantly advance our knowledge of the control of aggressive behaviors.

Aggression has been mostly studied in a social context or during agonistic encounters among males competing for resources such as a mate, territory and food. In marked contrast, female aggression is surprisingly understudied. It has been suggested that reproductive conflict among females may be more frequently resolved peacefully rather than with overt aggression when compared to conflict among males, because offspring are more costly to produce for females and reproduction is more difficult to conceal [7]. However, females of several species also show aggressive behavior [8–10]. Social behavior and aggressiveness are strongly linked to sex steroids, glucocorticoids and neuropeptides [4, 11, 12], and hormone levels usually vary among individuals from species with hierarchical social systems [13–16]. In particular,

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androgenic regulation is one of the most studied physiological mechanisms involved in male aggression in fish, reptiles, birds and mammals [11, 17, 18]. Dominant males, which usually maintain their social status with aggressive displays, show elevated androgen levels [10, 15, 19–23].

The challenge hypothesis suggests that behavioral interactions increase testosterone (T) levels above the breeding baseline which, in turn, stimulates male-male aggression and, as a consequence of a “positive” feedback, T levels subsequently increase even more [24]. According to this original study, T seems to be involved in aggression associated exclusively with reproduction, such as mate-guarding behavior and defense of a breeding territory rather than with other forms of aggression [24]. According to this hypothesis, androgen levels should increase in response to social instability, and this prediction has been confirmed in several vertebrate taxa, including fish, birds and mammals [25–29]. A revision of the challenge hypothesis suggests that an increase in androgen baseline concentrations can be caused not only by aggressive interactions, but also in response to seasonal fluctuations, male-male competition, presence of receptive females, and to non-social environmental cues [30]. Nevertheless, this framework has focused mainly on males and not on females.

Even though T modulates male aggression, aggressive behavior can be also observed when circulating T levels are low [31–33]. As a consequence, the hypothesis that high T levels increase aggression has limited explanatory power, and there is evidence suggesting a possible role of estradiol-17 $\beta$  (E<sub>2</sub>) [6]. Estrogens have been historically associated with female-typical behaviors, but several authors suggest they are key factors regulating male aggression as well [34–36]. This way, when studying aggression it is interesting to take into account the enzymatic regulation of aromatase, which converts testosterone into estradiol [34], the relationship between androgens and estrogens, and also the indexes of conversion of testosterone into estradiol and other androgens such as 11-ketotestosterone [22].

Cichlid fish are amenable models to study physiological mechanisms underlying aggression, since they present hierarchical social systems in which dominant individuals defend their status by aggressive displays towards other submissive, low-ranked animals [37–39]. This is particularly the case of *Cichlasoma dimerus*, a Neotropical cichlid locally known as chanchita, which is a serially monogamous species with biparental behavior (see [40, 41] for review). This species shows a peculiarity that differs from social behavior in other cichlids, since both males and females in this species are equally aggressive in dyadic encounters, they show the same aggressive displays and have similar motivation towards aggression [42]. Marked aggression in males and females, together with the fact that both sexes have measurable levels of estrogens and androgens [22, 43], suggests that *C. dimerus* is a very suitable species to test the challenge hypothesis in both sexes and to analyze the role of sex steroids regulating aggression.

As a consequence, this evidence suggests that estrogens rather than androgens regulate aggression, that estrogens are also the dominant sex steroid hormone for females and that females show high levels of aggression in several species. In this context, an interesting question could be whether male and female aggression is similarly regulated, and how different levels of estrogens affect the regulation of aggression. The first aim of this study was to determine whether there is a relationship between circulating sex steroids and aggression in both sexes of *C. dimerus* and whether initial plasma concentrations can predict winner or loser status. The second aim was to evaluate if our data supports the challenge hypothesis and to determine if estrogens, in addition to androgens, also increase as a consequence of social instability. In this regard, it was necessary to disentangle whether this individual increase in hormonal levels could be related to size differences, and to social or reproductive status. Challenge trials consisted in placing two animals in neutral aquaria to evaluate territorial aggression in dyadic intrasexual agonistic encounters and to compare sex steroid levels before and after encounters, in both winners and losers. These dyadic interactions

constitute socially unstable encounters because territorial competition is evident in *C. dimerus* and a clear territorial division emerges as a result of winning or losing these trials [42]. As a consequence, if the challenge hypothesis is present, an increase of plasma androgens is expected during challenge trials. We expected that plasma levels of sex steroids can predict winner and loser status in both males and females, with higher levels of plasma sex steroids in winners. Furthermore, regarding our second aim, we expected that both males and females would exhibit an increase in T, 11-ketotestosterone (11-KT) and E<sub>2</sub> after the challenge trial.

## 2. Materials and methods

### 2.1. Animals and holding conditions

*Cichlasoma dimerus* adults were caught in Esteros del Riachuelo (27°35'S; 58°45'W; Corrientes, Argentina). Animals were transferred to our laboratory in Buenos Aires and housed in aquaria for acclimation in filtered tap water (8–10 fish per tank, 150 L, pH 7.8, conductivity 250  $\mu$ S/cm, total alkalinity 44.1 mg/L, O<sub>2</sub> 8 mg/L) with artificial aquarium plants and stones for at least one month before experiments. Animals were fed daily one hour before the behavioral test with cichlid pellets (Koi Vibrance Color Enhancer Fish Food, Tetra Brand [44].) and were maintained under conditions similar to their natural habitat during reproductive season (25  $\pm$  2 °C, 14 h:10 h light:dark [45].). Holding conditions and experimental design were performed in accordance with international standards on animal welfare, minimizing pain or discomfort of the animals. All procedures were compliant with the Guide for Care and Use of Laboratory Animals [46] and with institutional (Comisión Institucional para el Cuidado y Uso de Animales de Laboratorio, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires) and national (Comité Nacional de Ética en la Ciencia y la Tecnología) regulations.

In order to study aggressive behavior in males and females of *C. dimerus*, for each trial two animals of the same sex were isolated from different aquaria, thus excluding possible previous interactions between them. Both animals were simultaneously isolated in an individual aquarium (isolation tank, 21 L; 25  $\pm$  2 °C; 14:10 light:dark) during seven days to minimize any effect of prior social status while in the social tank and to control the winner effect [26, 47, 48]. After this period, approximately 100  $\mu$ L blood samples were obtained with heparinized syringes (needle: 27 gauge x 1/2 in.) by puncture of the caudal vein to measure initial steroid hormone levels. All samples were collected in heparin-coated tubes between 12 and 13 h to control for potential daily variation in behaviors and hormonal levels. Animals were then returned to isolation aquaria for four days to allow recovery, and after this period they were exposed to the challenge trial.

### 2.2. Challenge trial

A total of 9 female-challenge trials and 8 male challenge trials were analyzed for this study. Each encounter was performed in an experimental tank without gravel or plants (experimental tank, 21 L; 25  $\pm$  2 °C). The challenge trial involved intrasexual agonistic encounters in neutral aquaria and observing the ensuing interaction for 60 min. Territorial competition and social instability in *C. dimerus* is evident when placing both animals simultaneously in a neutral aquaria, and a clear territorial division emerges as a consequence of winning or losing these encounters, eventually resulting in dominant and subordinate status [42]. As a consequence, in these challenge trials both fish were simultaneously placed in the experimental tank and even though there was no flat slab where this species tends to lay its eggs (reproductive territory), territorial aggression in this context has already been described by [42]. In each trial, both fish were allowed to swim freely and all interactions were videotaped. Filming was performed between 12 and 13 h with a JVC HD Everio camera. All

aggressive (bites, tail hits, mouth holdings, chases, approaches and frontal displays) and submissive (passive coping, escapes and tremors) interactions were later recognized and quantified by the same observer according to the ethogram recently described in intrasexual dyadic agonistic encounters in *C. dimerus* [42]. The status of the “winner” of the contest was clearly determined by the spatial dominance over the central territory of the aquarium, while “losers” typically adopted a peripheral location in the lower corners or in the surface of the aquarium, and showed signs of trauma [42]. Winner and loser status was assigned at the end of each 60-min interaction.

### 2.3. Specimen processing, plasma and tissue collection

At the end of each challenge trial blood samples were obtained as described above. Fish were then anesthetized with 0.1% benzocaine until opercula movement ceased, and weight and total and standard length of each animal were recorded. Animals were euthanized by decapitation and sex was corroborated by gonadal inspection. Gonads were dissected, weighted and used for the calculation of the gonadosomatic index (GSI%; gonad weight/body weight  $\times$  100).

### 2.4. Hormone assay

Blood samples were centrifuged at 3000 rpm for 15 min, after which plasma was frozen at  $-20^{\circ}\text{C}$  for later measurement of T, 11-KT and  $\text{E}_2$  [49] using commercial ELISA kits following manufacturer instructions (for T: DRG Instruments GmbH, Germany; for 11-KT: Cayman Chemical Company, MI, USA; for  $\text{E}_2$ : DRG Instruments GmbH, Germany). Working plasma dilutions for males and females were usually 1:1.5 to 1:10 for T and  $\text{E}_2$ , respectively, depending on the GSI% in females and on whether concentration was over the upper curve limit, and were 1:6 and 1:2 for 11KT in males and females, respectively. Samples were assayed in duplicate. Some samples did not have enough volume for measurement of all hormones, resulting in a lower number of samples analyzed. Parallelism between 4 serially diluted samples and hormone concentrations was analyzed, and correlation coefficients were 0.99 for all three steroids after log transformation.

### 2.5. Video analysis

All agonistic interactions performed by both animals during the challenge trial were recorded and measures of total aggression and submission of each animal were calculated by summing the total number of aggressive and submissive acts, respectively. These behavioral measures were used to calculate individual winning indices, which is the sum of all aggressive behaviors minus the number of all submissive behaviors observed per animal. This index was defined previously by Renn et al. [10], where the dominance index for *Astatotilapia burtoni* was calculated in social context. Here, since we refer only to dyadic agonistic encounters, the dominance index was renamed as a winning index (WI). The WI was used to assign winner or loser status of each animal within the dyadic encounter. Considering that in winners, aggressive displays are more frequent than submissive ones, positive WI values correspond to winners, while negative ones indicate losers.

### 2.6. Statistical analyses

Plasma sex steroid levels, hormone ratios and GSI% were expressed as mean  $\pm$  standard error (SE). The GSI% was used to measure relative gonad size. Two-tailed tests were used throughout and data from hormone plasma levels were square root transformed to meet homoscedasticity and normality when required. Potential differences between winners and losers were compared by Student's *t*-test, and changes in hormonal levels in each animal were compared by paired *t*-test. To determine if sex steroids increased or decreased during female challenge trial, we calculated a delta value for T, 11-KT and  $\text{E}_2$  plasma levels

in each animal, subtracting initial (pre-contest) hormone levels to the final (post-contest) plasma levels. In those cases in which there were no statistically significant changes in plasma sex steroids after the challenge trial, it was necessary to analyze contest variability in more detail and calculate individual delta values (subtracting final hormonal levels to initial ones). Moreover, considering that challenge trials were not size-matched because this study accounts for morphometric variability in the population, it was necessary to exclude the possibility that, if there is no increase in hormonal concentrations, it was not due to differences in size between opponents. Taking into account that in female dyads of *C. dimerus*, usually winners are larger than losers [42], in order to analyze whether size differences between opponents could mask a potential increase in hormone levels in females, hormonal levels were divided by each morphometric variable and values before and after the contest were compared by paired *t*-test. Also, we analyzed whether the increase or decrease in hormone levels was related to the relative size of both opponents (e.g., hormone levels increasing in the smallest female and decreasing in the larger female). Hormone ratios were also compared, since plasma  $\text{E}_2/(\text{E}_2 + \text{T})$  ratio is indicative of the physiological rate at which T is converted to  $\text{E}_2$ , while  $11\text{KT}/(11\text{KT} + \text{T})$  ratio indicates T that is converted to 11KT [22]. Finally, in order to disentangle whether the increase in hormonal levels after the challenge trial could be related to reproductive status, GSI% in winners and losers were compared with paired *t*-test and Pearson's correlation coefficient was used to examine the relationship between changes in  $\text{E}_2$  levels and GSI% in females.

## 3. Results

### 3.1. Can initial sex-steroid levels predict winner or loser status?

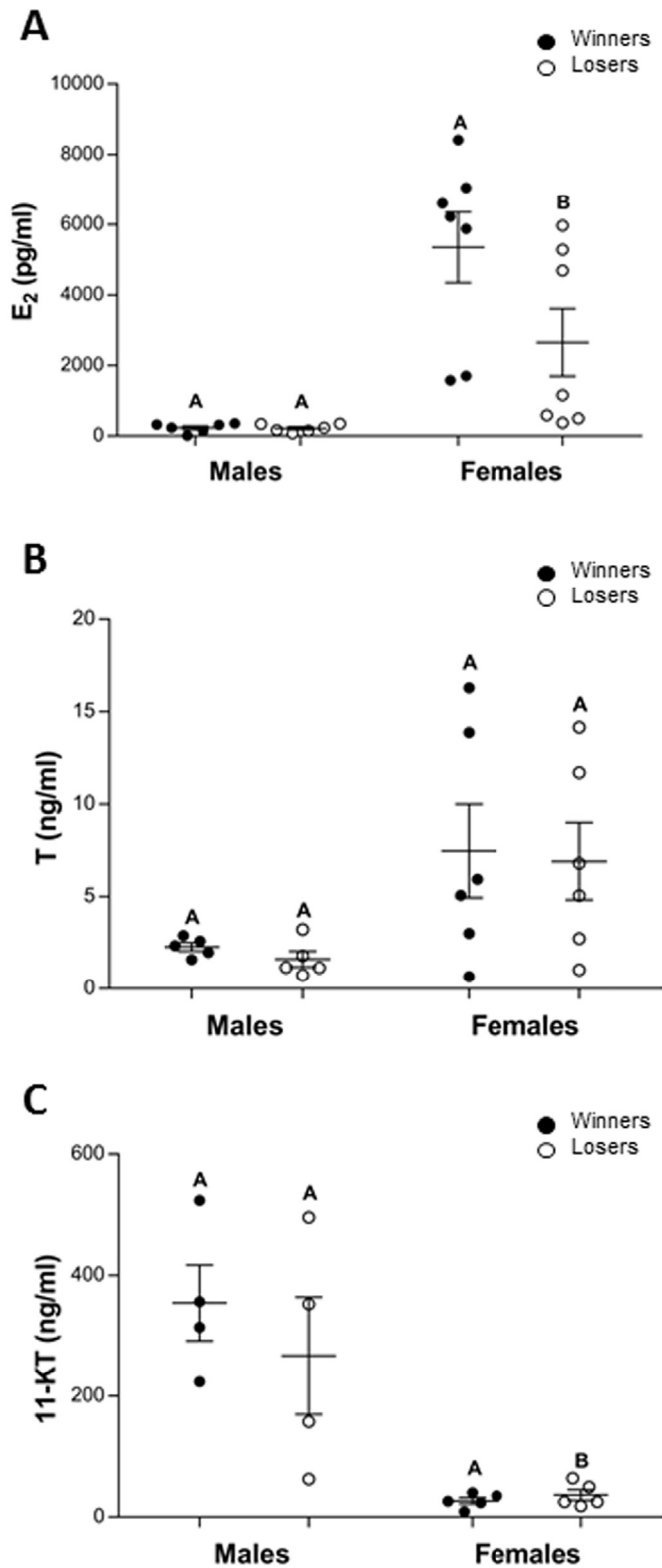
Females that won the challenge trial had higher plasma  $\text{E}_2$  levels before the contest than animals that lost ( $p = .046$ , Fig. 1A, Table 1), but there were no differences in male winners and losers ( $p = .855$ , Fig. 1A, Table 1). Regarding plasma T levels, no differences were detected either between winner and loser females ( $p = .845$ , Fig. 1B, Table 1) or between winner and loser males ( $p = .253$ , Fig. 1B, Table 1). Similar results were observed for plasma 11-KT levels, since no differences were detected in females ( $p = .245$ , Fig. 1C, Table 1) or in males ( $p = .149$ , Fig. 1C, Table 1). Plasma  $\text{E}_2$ , T and 11-KT levels before trials showed no significant correlation with individual levels of aggression (data not shown).

### 3.2. Do sex steroids increase in response to challenge trials?

When analyzing male winners and losers together, during the challenge trial there was a significant increase in plasma levels of  $\text{E}_2$ , T and 11-KT by 1.5, 5.4 and 3.2-fold ( $p = .023$ ,  $p = .016$ ,  $p = .018$ , respectively, Fig. 2, Table 2). However, in females, sex steroids did not increase in response to the challenge trial ( $p = .678$  for  $\text{E}_2$ ,  $p = .189$  for T, and  $p = .918$  for 11-KT, Fig. 2, Table 2).

Considering there are no statistically significant changes in plasma sex steroid levels in females, when analyzing variability in more detail, delta values for hormonal changes are similar for all three hormones: in some animals hormonal levels increased during the contest and resulted in a positive delta value, while in others hormonal levels decreased, resulting in a negative delta value, irrespective of whether the animal won or lost the challenge trial (Fig. 3).

Hormonal levels were also corrected by all morphometric variables measured (e.g., total length, standard length and body weight), and were compared before and after the contest. Results showed that independent of size differences, there was no increase in any of the plasma steroid levels as a consequence of the contest, either in winner or in loser females (Fig. 1 Supp Mat). Furthermore, we analyzed whether the increase or decrease in hormone levels was related to the relative size of both opponents, but changes in sex steroid levels did not



**Fig. 1.** Initial plasma levels of sex steroids before sex-matched challenge trials. Black dots represent winners, while white dots represent losers. A. Plasma E<sub>2</sub> levels were higher in female winners than losers (*n* = 7 for both winners and losers), while there were no differences in males (*n* = 6 for both winners and losers). B. Plasma testosterone levels did not differ between winners and losers in either sex (*n* = 6 for both female winners and losers; *n* = 5 for both male winners and losers). C. Plasma 11-KT levels did not differ between winners and losers in either sex (*n* = 5 for both female winners and losers; *n* = 4 for both male winners and losers). Individual values are represented by dots, bars express means ± SE and data was compared by paired *t*-test. Different letters indicate statistical difference between winners and losers from each sex.

**Table 1**  
Statistical analysis comparing hormonal initial levels of sex steroids in winners and losers.

	95% I CI	95% S CI	<i>T</i>	<i>p</i>	Cohen's <i>d</i>	<i>df</i>
<b>Estradiol</b>						
Males	-142.98	169.3	0.19	0.855	0.11	10
Females	64.06	5710.84	2.21	0.046	1.14	13
<b>Testosterone</b>						
Males	-0.48	1.6	1.22	0.253	0.76	9
Females	-6.11	7.35	0.2	0.845	0.11	12
<b>11-ketotestosterone</b>						
Males	-120.43	661.63	1.60	0.149	1.01	8
Females	-2.85	0.81	-1.23	0.245	0.74	11

depend on female size (data not shown).

**3.3. Is the estimated conversion of testosterone to estradiol-17β and to 11-ketotestosterone different in winners and losers?**

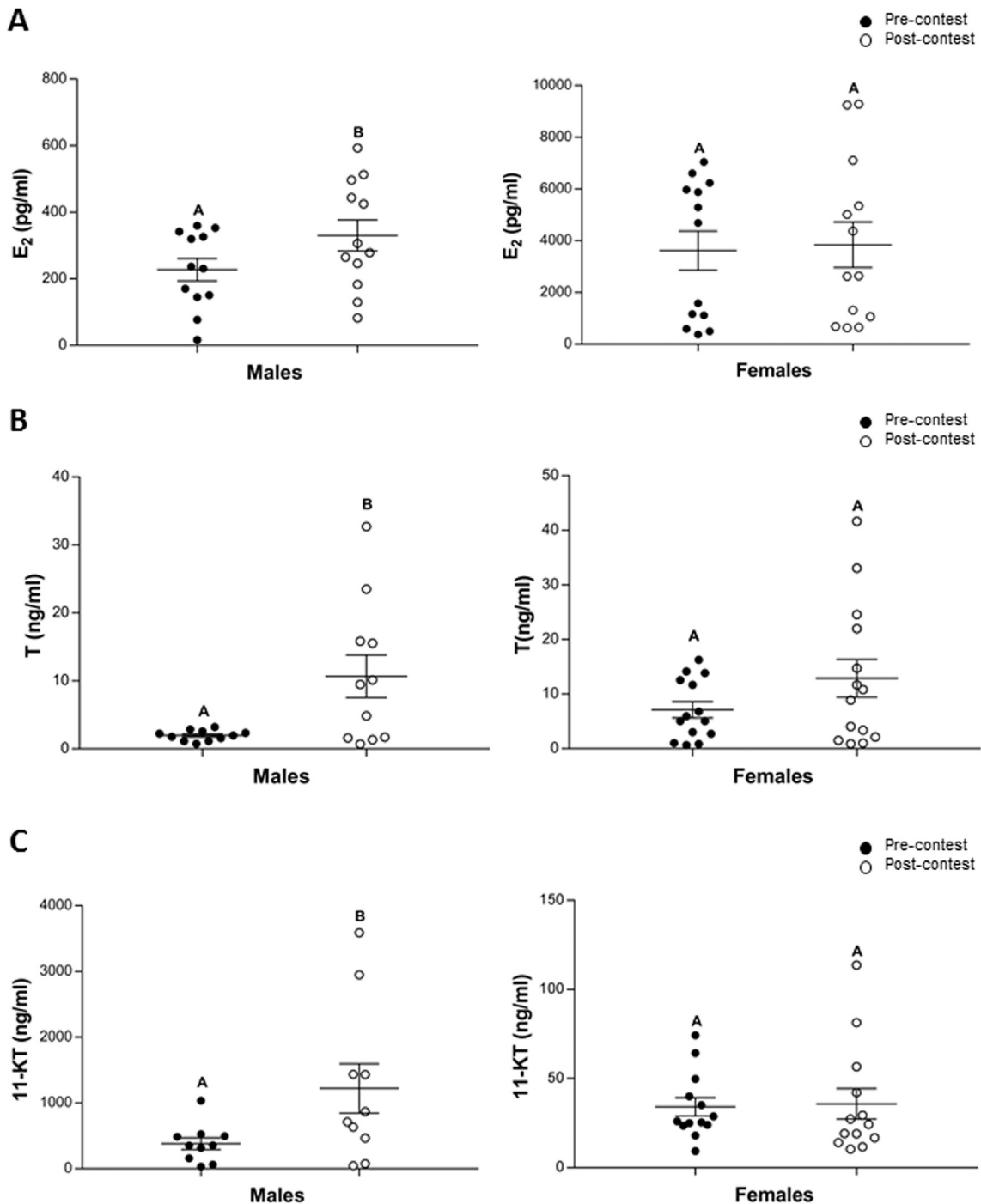
The plasma E<sub>2</sub>/(E<sub>2</sub> + T) ratio is indicative of the physiological rate at which T is converted to E<sub>2</sub> [22]. Results showed that when considering initial hormone levels (pre-contest), there were no differences in conversion to E<sub>2</sub> between winner and loser females (*p* = .190) or males (*p* = .334, Fig. 4A, Table 3). Moreover, there were also no differences in this conversion between winners and losers when considering hormone levels after the contest in both females (*p* = .884) and males (*p* = .142, Fig. 4B, Table 3). Similarly, plasma 11-KT/(11-KT + T) ratio indicates the physiological rate at which T is converted to 11-KT [22]. When comparing initial hormone levels between winners and losers, there were no differences in females (*p* = .645) nor in males (*p* = .175, Fig. 4C, Table 3). There were no differences between winners and losers when considering final hormone levels in females (*p* = .892) nor in males (*p* = .096, Fig. 4D, Table 3).

**3.4. Is social status in females related to their GSI?**

Female winners and losers did not differ in their GSI% (*p* = .460, 95% I CI = -2.8, 95% S CI = 1.62, *T* = -0.57, Cohen's *d* = 0.8, *df* = 16, Fig. 5A). Moreover, there were no significant correlation between delta E<sub>2</sub> and GSI% for both winners and losers (*p* = .55, *r* = -0.18, *n* = 13, Fig. 5B).

**4. Discussion**

Aggressive behavior has been historically related to androgens, and the ‘challenge hypothesis’ postulates that male aggressive interactions stimulate the production of androgens in periods of social instability. In this context, the aims of this study were to determine whether there is a relationship between circulating sex steroids and aggression, and to analyze if estrogens, in addition to androgens, increase as a consequence of social instability in males and females of *C. dimerus*. Our results show that initial plasma E<sub>2</sub> levels before contests were higher in female winners than in female losers but there were no differences in their androgen levels, suggesting that female aggression can be predicted by initial levels of E<sub>2</sub> (Fig. 1). These results are of particular interest because female aggression is surprisingly understudied when compared to males, and because this behavior is mostly studied in relation to androgens. Moreover, our data does not support the challenge hypothesis for females (Fig. 2), and changes in hormonal levels do not depend on their reproductive or social status (Figs. 3 and 5). Also, winner and loser status in females are not related to the reproductive status. Furthermore, our data supports the challenge hypothesis for males, who exhibited an increase in both plasma androgens and E<sub>2</sub> after the trials (Fig. 2). The notion that not only androgens, but also estrogens, increase after a period of social instability can have an impact on



**Fig. 2.** Plasma levels of sex steroids in winners and losers before and after sex-matched challenge trials. Black dots represent pre-contest, while white dots represent post-contest. A. Plasma E<sub>2</sub> levels increased after the contest in males ( $n = 12$ ) but not in females ( $n = 13$ ). B. Plasma testosterone levels increased after the contest in males ( $n = 11$ ) but not in females ( $n = 14$ ). C. Plasma 11-KT levels increased after the contest in males ( $n = 10$ ) but not in females ( $n = 13$ ). Individual values are represented by dots, bars express means  $\pm$  SE and data was compared by paired  $t$ -test. Different letters indicate statistical difference between winners and losers from each sex.

the way in which the challenge hypothesis is interpreted.

Aggression has been historically analyzed and described in males rather than in females, and for many years androgens have been suggested as the main mediators of this behavior [50–52]. However, in several species evidence suggests that it is the aromatization of T to E<sub>2</sub>, rather than androgens themselves, the key step which regulates aggression (reviewed by [6]). In birds, for example in pied flycatchers and in song sparrows, aromatase activity in the diencephalon correlates with aggressive behavior, and treatment with an aromatase inhibitor

decreases aggressive singing and chasing behavior in males [53, 54]. Moreover in mammals, studies using knock-out male and female mice suggest that estrogens increase aggressive behavior through estrogen receptor  $\alpha$  (ER $\alpha$ ), modulating the formation of neuronal circuitry during development and acting in adulthood as well [55, 56]. Several lines of evidence suggest that estrogens also increase aggressive behavior in fish. For example, in zebrafish (*Danio rerio*) brain aromatase (*cyp19a1b*) or estrogen receptor (*er1*, *er2a*, *er2b*) gene expression is associated with aggressiveness in dominant males and females [57].

**Table 2**  
Statistical analysis comparing plasma hormone levels before and after challenge trials.

	95% I CI	95% S CI	T	p	Cohen's d	df
<b>Estradiol</b>						
Males	−222.19	16.62	−1.79	0.023	0.73	22
Females	−1230.67	2423.26	0.13	0.678	0.05	27
<b>Testosterone</b>						
Males	−14.79	−2.05	−2.91	0.016	1.19	11
Females	−13.66	2.13	−1.53	0.189	0.58	18
<b>11-ketotestosterone</b>						
Males	−1699.73	21.94	2.17	0.018	0.97	10
Females	−22.35	18.96	−0.17	0.918	0.07	24

Moreover, in dominant *A. burtoni* males, brain aromatase levels were higher than in subordinate males, and pharmacological inhibition of aromatase with fadrozole reduced aggressive behavior [34, 58].

*Cichlasoma dimerus* is a South American bi-parental and serially monogamous Neotropical cichlid with a hierarchical social system in which dominant females aggressively defend their territory together with males [40, 41, 59]. Despite the fact that there is no evidence on natural environment, the frequency of aggressiveness of territorial females did not differ from that of territorial males in social aquaria resembling natural conditions, in which there is competition for food and for the reproductive territory (a flat slab where this species tends to lay its eggs) [22]. Moreover, it has been recently suggested that in this species both sexes are equally aggressive and have similar motivation towards aggression in neutral aquaria [42]. In this sense, when studying female aggression, it is important to bear in mind that this species differs from *Astatotilapia burtoni*, a polygamous and a maternal mouth-brooder cichlid in which females naturally do not present social hierarchies and they only adopt aggressive behavior in the absence of males [10, 15]. In *C. dimerus*, it is worth mentioning that despite the fact that both males and females of this species are aggressive, there are sex differences regarding aggression and size in dyadic encounters in neutral aquaria. Briefly, in female dyads, winners were larger than losers, but this does not apply for males. Also, when analyzing individual aggressiveness, there is no correlation with individual morphological variables (standard and total length, and body weight). However, significant correlations arise when analyzing the morphometrical differences between opponents and total aggressiveness of the encounter: more similar females showed higher levels of total aggressiveness, but these correlations were not found in males [42]. Considering that dyads were not size-matched because these studies account for size variability, it was necessary to include morphometric variables in this analysis in order to rule out the possibility that size differences between opponents did not mask potential differences in hormone levels (Fig. 1) Supp Mat.

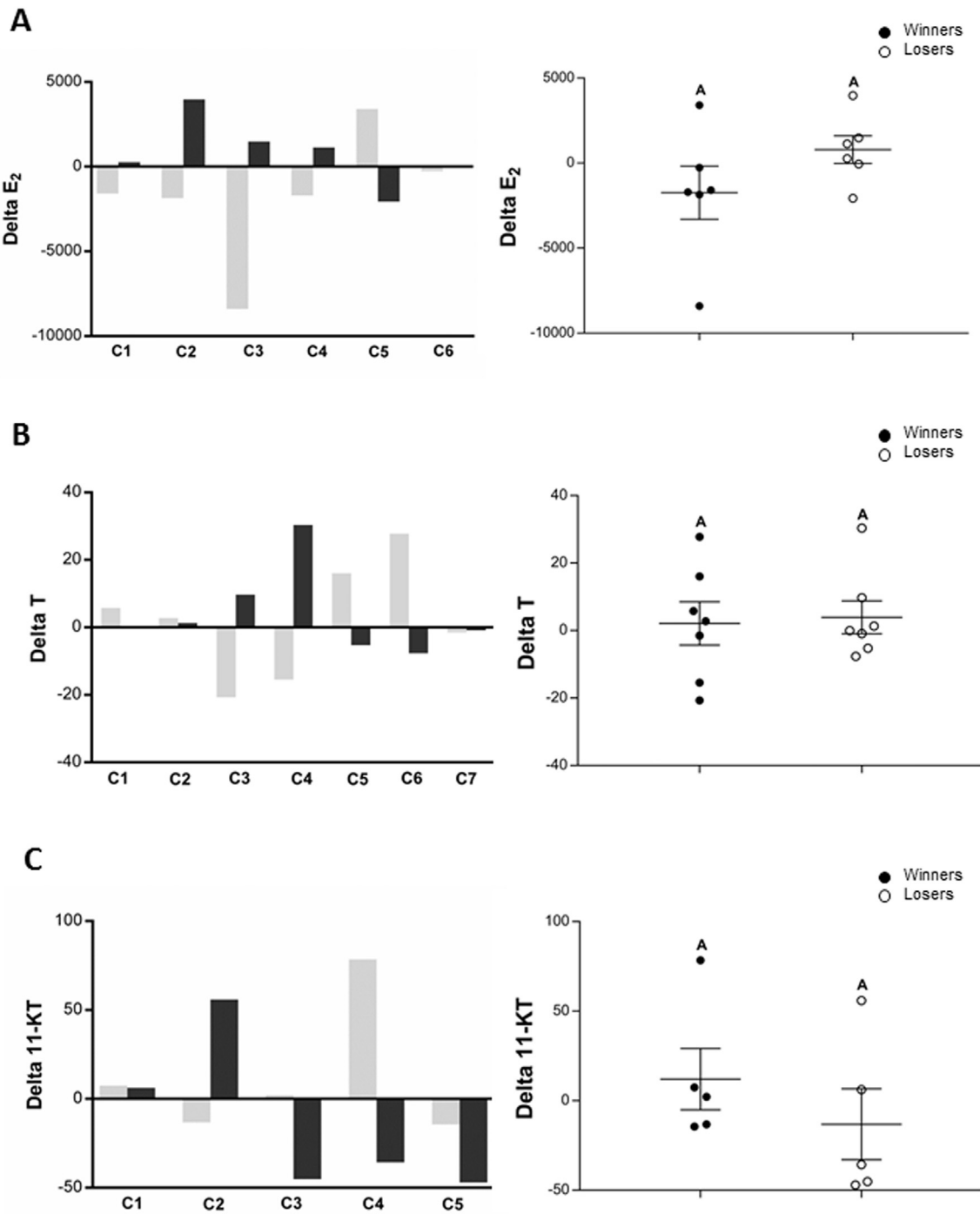
Aggressive displays are fundamental for the outcome of fights, and they are intrinsically related to the establishment of social hierarchies. Most studies on female aggression focus on the physiological mechanisms related to maternal care and reproductive stages [43, 44, 60]. Our results in the context of neutral arena suggest that high initial estrogen levels can predict aggression in females, but not in males (Fig. 1). Previous evidence in other cichlids suggests that androgen levels measured before a territorial intrusion were good predictors of aggression, but unfortunately authors did not determined plasma estradiol levels [25]. Several studies analyzed the regulation of social hierarchies by sex steroids in cichlids (reviewed by [13]). In males of *A. burtoni* there is a positive association between dominance and androgen and estrogen levels [15, 61, 62]. In *C. dimerus* even though circulating androgen levels are higher in territorial dominant males, plasma E<sub>2</sub> levels show an opposite tendency [22]. The fact that dominant males show

higher androgen levels than subordinates in this previous study in *C. dimerus* seems to contrast with results presented in this manuscript in winners and losers (Fig. 1). These differences could be explained by the fact that both studies refer to different ethological contexts: most previous evidence on cichlids are related to dominant and subordinate animals in a stable social context, while in this study we refer to the immediate outcome of dyadic challenge trials. As a consequence, circulating hormone levels in an established hierarchy can differ from hormonal levels in winners and losers in a neutral arena. In reference to challenge trials, hormone levels in winners and losers after sex-matched contests have already been compared in the cichlid fish *Neolamprologus pulcher*. In this species, which has a cooperative breeding behavior, winner females had similar 11-KT but higher T than losers, while males showed the opposite trend [23]. Unfortunately, in that study hormonal levels refer only to values after the contest and to a newly acquired status. Here, since plasma was also sampled before the contest, we were able to compare initial physiological states of future winners and losers. This way, the present results suggest that estrogens and not androgens could predict aggressive behavior in female *C. dimerus*.

It is worth emphasizing that the fact that there were no differences in circulating levels of estrogens in males does not rule out a possible central role of estrogens as mediators of aggression and further studies analyzing local brain aromatization could disentangle this issue. Regarding the mechanism of action of estradiol regulating this behavior, recent evidence in mice suggest that activation of neurons expressing estrogen receptor 1 in the ventromedial hypothalamus are enough to initiate attack and to maintain ongoing aggression, while neurons not expressing this receptor are not [63]. This suggests that aggression can be regulated by estradiol with a direct mechanism of action, but it does not rule out the possibility that other factors could also regulate social behavior, such as arginine vasotocin expression [64].

The increase of androgens as a consequence of social instability, predicted by the challenge hypothesis, has been tested in several fish species [25, 29, 60, 65]. Taking into account that the challenge hypothesis was initially proposed in males, very few studies have focused on females despite the fact that they can also be aggressive [66–68]. In *N. pulcher*, circulating levels of 11-KT and T increased in females after simulated territorial intrusion [26]. Surprisingly, results in this study suggest that there was no increase of androgens in females after sex-matched challenge trials after correcting for morphometric variables and regardless of social status. Our results do not support the challenge hypothesis for females of *C. dimerus*, since in some animals hormonal levels increased after the contest and in others they decreased, regardless of their winner/loser status, their morphometric variables and their reproductive stage (Figs. 3 and 5, Fig. 1 Supp Mat). Differences with *N. pulcher* could be explained considering that *C. dimerus* is a monogamous and biparental species while *N. pulcher* has a cooperative breeding behavior, and that predictions of the challenge hypothesis can differ according to the parental care behavior [24]. Moreover, this lack of increase in androgen levels is similar to evidence in female song sparrows (*Melospiza melodia*) and in California mice (*Peromyscus californicus*), and it could suggest the existence of unknown physiological mechanisms in females of these species that could buffer the expected increase in androgens as a consequence of social instability.

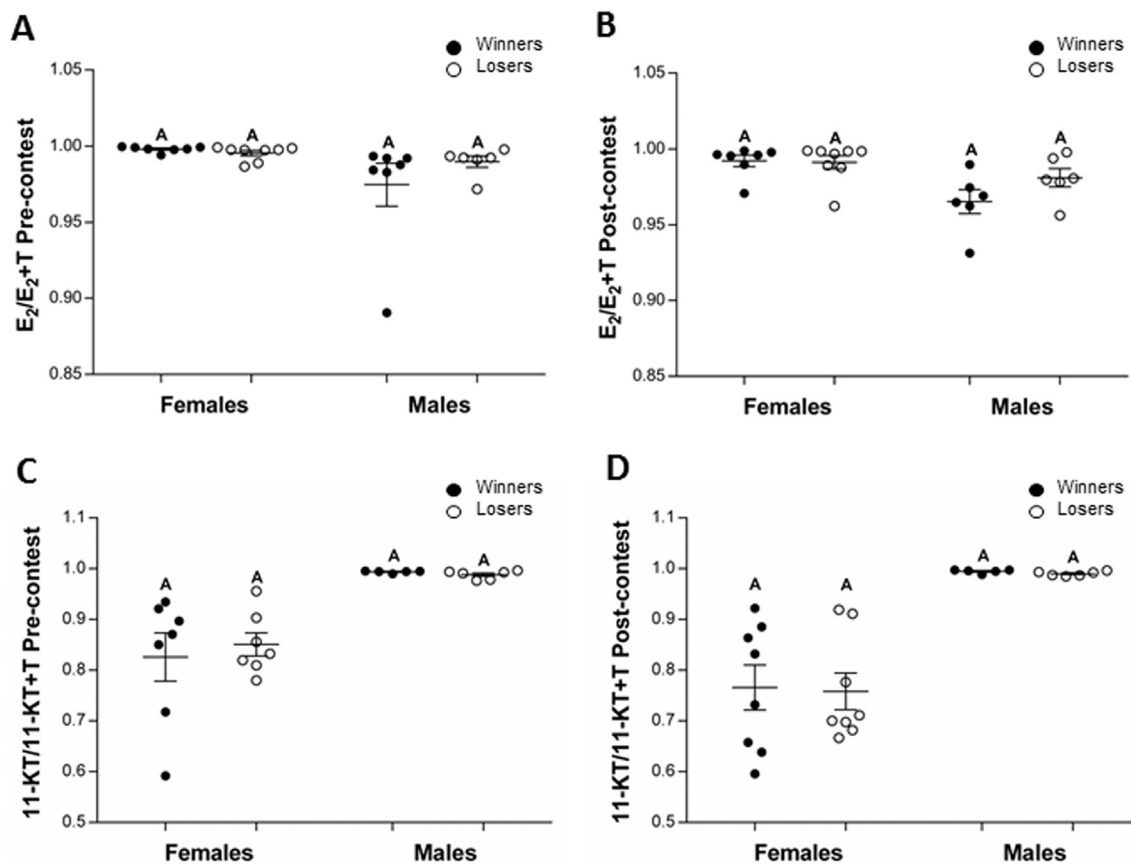
Regarding males, our results are consistent with the increase in androgen levels after social instability (Fig. 1). In this regard, a recent revision on the challenge hypothesis suggests that an increase in androgen levels does not necessarily reflect a hormonal response to social interaction between males, and that other social and environmental factors could also lead to androgen increase, such as seasonality, inter-male competition, presence of receptive females and non-social cues [30]. However, it is worth mentioning that most of these factors have limited explanatory power in this experimental design, since challenge



**Fig. 3.** Variation in intra-individual steroid changes in female winners and losers. Changes in plasma hormonal levels are expressed as *Delta* and were calculated subtracting initial (pre contest) hormonal levels to the final plasma levels (post contest). Each contest is referred to as C in the x-axis. In left panels, grey bars represent each female winner, while black bars represent each female loser. In right panels, black dots represent individual data of winners and white dots represent losers, and bars express means  $\pm$  SE. A. Changes in plasma E<sub>2</sub> levels ( $n = 6$  for losers and winners). B. Changes in plasma T levels ( $n = 7$  for losers and winners). C. Changes in plasma 11-KT levels ( $n = 5$  for losers and winners). Values are expressed as mean  $\pm$  SE.

trials were performed in controlled conditions to avoid seasonal fluctuations and in neutral aquaria in the absence of receptive females and resources such as food or shelter. Moreover, in spite of growing evidence suggesting that estrogens are important mediators of aggression in different taxonomical groups, the challenge hypothesis has been only referred to androgens. Our results suggesting that E<sub>2</sub> also increases as a

consequence of challenge trials in males (Fig. 2), differ from *N. pulcher* in which, as a consequence of social instability, males show higher levels of circulating 11-KT, but not T or E<sub>2</sub> [26]. Despite species differences, which could be related to different reproductive and parental behavior, this is the first evidence suggesting that the challenge hypothesis could also be extended to estrogens.



**Fig. 4.** Testosterone conversion to E<sub>2</sub> and to 11-KT before and after sex-matched challenge trials. Black dots represent winners, while white dots represent losers. A. Testosterone conversion to E<sub>2</sub> before trials (Pre-Contest) did not differ between winners and losers in either sex (*n* for female winners and losers were 7 and 8, respectively; *n* for male winners and losers were 7 and 6, respectively). B. Testosterone conversion to E<sub>2</sub> after trials (Post-Contest) did not differ between winners and losers in either sex (*n* for female winners and losers were 7 and 8, respectively; *n* for both male winners and losers were 6). C. Testosterone conversion to 11-KT before trials (Pre-Contest) did not differ between winners and losers in either sex (*n* = 7 for both female winners and losers; *n* for both male winners and losers were 5 and 6, respectively). D. Testosterone conversion to 11-KT after trials (Post-Contest) did not differ between winners and losers in either sex (*n* = 8 for both female winners and losers; *n* for both male winners and losers were 5 and 6, respectively). Bars express means ± SE and were compared by *t*-test. Different letters indicate statistical difference.

**Table 3**

Statistical analysis comparing the conversion of initial and final levels of testosterone to products in winners and losers.

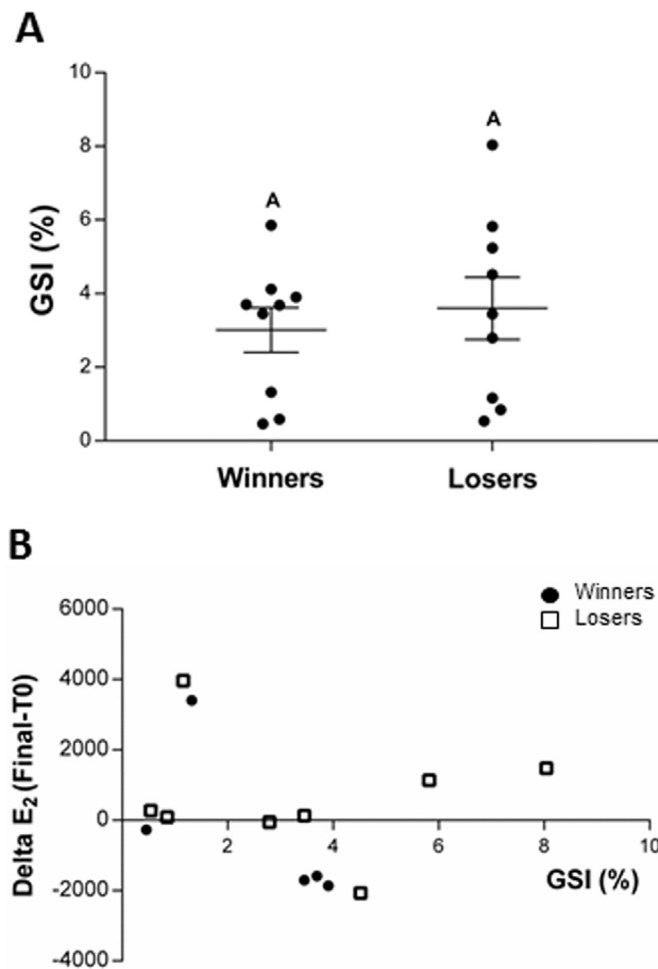
	95% I CI	95% S CI	<i>T</i>	<i>p</i>	Cohen's <i>d</i>	<i>df</i>
To estradiol						
Pre-contest in males	-0.05	0.02	-1.04	0.334	1.48	7
Post-contest in males	-0.04	0.01	-1.59	0.142	2.22	10
Pre-contest in females	-0.002	0.01	1.42	0.190	1.89	9
Post-contest in females	-0.01	0.01	0.15	0.884	0.25	13
To 11-ketotestosterone						
Pre-contest in males	-0.003	0.01	1.54	0.175	2.18	6
Post-contest in males	-0.001	0.01	1.86	0.096	2.68	9
Pre-contest in females	-0.14	0.09	-0.47	0.645	0.67	12
Post-contest in females	-0.11	0.13	0.14	0.892	0.19	14

As a first approach to estimate E<sub>2</sub> and 11-KT biosynthesis, the index of conversion of T to 11-KT and E<sub>2</sub> were also compared in male and female winners and losers. Our results suggest highest levels of conversion of T to E<sub>2</sub> in females and to 11-KT in males, suggesting an expected predominance of estrogen and androgen production, respectively (Fig. 4). Moreover, each conversion index showed no differences between male and female winners and losers. This way, our data differs from previous results in males of the same species, in which territorial males had lower conversion to E<sub>2</sub> than non-territorial males [22].

However, both studies refer to different ethological contexts, and steroid biosynthesis in dominant territorial males in an established social hierarchy may drastically differ from winner males analyzed immediately after social instability. It is worth noting that this ratio is a first-level approach to study sex steroid conversion and it does not directly assess aromatase enzyme activity. Taking into account that there is evidence suggesting brain aromatization and a complete active biosynthetic machinery [69, 70], presence of estrogen and androgen receptors, aromatase and detectable levels of E<sub>2</sub> and 11-KT in teleost brain [15, 60, 71, 72], our results do not exclude the possibility that local aromatization, or local conversion to 11-KT in the brain, might be involved in the aggressive behavior of winners.

In summary, here we show that circulating E<sub>2</sub> could predict female aggression and our results represent the first evidence suggesting that the challenge hypothesis in males could be also extended to estrogens. Finally, there is a need to analyze local brain aromatization after social instability, to measure brain steroid levels and to determine other less studied androgens and estrogens, such as dehydrotestosterone, androstenedione and estrone, which could also modulate aggressive behavior. Taking these results into account, future field studies will help us understand the ecological context and the relevance of male and female aggression in the natural environment of *C. dimerus*, thus contributing to our knowledge on social behavior of South American neotropical cichlids, which still remains understudied.





**Fig. 5.** Social status and gonadal investment in females. **A.** Gonadosomatic index (GSI%) did not differ between female winners and losers ( $n = 9$  for both winners and losers). Dots represent individual values, bars express means  $\pm$  SE and data were compared by paired  $t$ -test. **B.** Relationship between changes in plasma  $E_2$  levels and GSI% in females.  $\Delta$  was calculated subtracting initial (Pre-contest)  $E_2$  levels to the final (Post-contest) plasma levels. Black dots indicate winners, while white squares indicate losers. There was no significant correlation between changes in plasma  $E_2$  and GSI% ( $n = 13$ ).

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.physbeh.2018.06.028>.

#### References

- R.J. Davidson, K.M. Putnam, C.L. Larson, Dysfunction in the neural circuitry of emotion regulation—a possible prelude to violence, *Science* 289 (2000) 591–594.
- J.L. Goodson, The vertebrate social behavior network: evolutionary themes and variations, *Horm. Behav.* 48 (2005) 11–22.
- S.W. Newman, The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network, *Ann. N. Y. Acad. Sci.* 877 (1999) 242–257.
- I.S. Parhar, S. Ogawa, T. Ubuka, Reproductive neuroendocrine pathways of social behavior, *Front. Endocrinol. (Lausanne)* 7 (2016) 28.
- J.P. Scott, *Aggression*, University of Chicago Press, Chicago, 1975.
- B.C. Trainor, H.H. Kyomen, C.A. Marler, Estrogenic encounters: how interactions between aromatase and the environment modulate aggression, *Front. Neuroendocrinol.* 27 (2006) 170–179.
- M.A. Cant, A.J. Young, Resolving social conflict among females without overt aggression, *Philos. Trans. R. Soc. B* 368 (1631) (2013) 20130076.
- Å.A. Borg, T. Åsmul, G.H. Bolstad, Å. Viken, A. Berglund, G. Rosenqvist, Interactions among female guppies (*Poecilia reticulata*) affect growth and reproduction, *Ethology* 118 (2012) 752–765.
- R.F. Oliveira, V.C. Almada, Dominance hierarchies and social structure in captive groups of the Mozambique tilapia *Oreochromis mossambicus* (Teleostei, Cichlidae), *Ethol. Ecol. Evol.* 8 (1996) 39–55.
- S.C.P. Renn, E.J. Fraser, N. Aubin-Horth, B.C. Trainor, H.A. Hofmann, Females of an African cichlid fish display male-typical social dominance behavior and elevated androgens in the absence of males, *Horm. Behav.* 61 (2012) 496–503.
- E. Adkins-Regan, *Hormones and animal social behavior*, Princeton University Press, 2005, pp. 49–52.
- N. Kagawa, S. Hirose, K. Fujimoto, C. Nomura, Y. Fujita, A. Honda, M. Komori, Social rank-dependent expression of gonadotropin-releasing hormones and kisspeptin in the medaka brain, *Gen. Comp. Endocrinol.* 249 (2017) 48–54.
- K.P. Maruska, Social regulation of reproduction in male cichlid fishes, *Gen. Comp. Endocrinol.* 207 (2014) 2–12.
- O. Mendonça-Furtado, M. Edaes, R. Palme, A. Rodrigues, J. Siqueira, P. Izar, Does hierarchy stability influence testosterone and cortisol levels of bearded capuchin monkeys (*Sapajus libidinosus*) adult males? A comparison between two wild groups, *Behav. Process. Pt. A* (2014) 79–88.
- L.A. O'Connell, J.H. Ding, H.A. Hofmann, Sex differences and similarities in the neuroendocrine regulation of social behavior in an African cichlid fish, *Horm. Behav.* 64 (2013) 468–476.
- J.M. Setchell, T. Smith, E.J. Wickings, L.A. Knapp, Stress, social behavior, and secondary sexual traits in a male primate, *Horm. Behav.* 58 (2010) 720–728.
- N.G. Simon, S. Lu, Androgens and aggression, in: R.J. Nelson (Ed.), *Biology of Aggression*, Oxford University Press, New York, 2005, pp. 211–230.
- J.C. Wingfield, A continuing saga: The role of testosterone in aggression, *Horm. Behav.* 48 (2005) 253–255.
- D.J. Albert, M.L. Walsh, B.B. Gorzalka, Y. Siemens, H. Louie, Testosterone removal in rats results in a decrease in social aggression and a loss of social dominance, *Physiol. Behav.* 36 (1986) 401–407.
- R.A. Duckworth, M.T. Mendonça, G.E. Hill, Condition-dependent sexual traits and social dominance in the house finch, *Behav. Ecol.* 15 (2004) 779–784.
- R.F. Oliveira, K. Hirschenhauser, L.A. Carneiro, A.V. Canario, Social modulation of androgen levels in male teleost fish, *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 132 (2002) 203–215.
- M.R. Ramallo, A. Birba, R.M. Honji, L. Morandini, R.G. Moreira, G.M. Somoza, M. Pandolfi, A multidisciplinary study on social status and the relationship between interindividual variation in hormone levels and agonistic behavior in a Neotropical cichlid fish, *Horm. Behav.* 69 (2015) 139–151.
- M.D. Taves, J.K. Desjardins, S. Mishra, S. Balshine, Androgens and dominance: sex-specific patterns in a highly social fish (*Neolamprologus pulcher*), *Gen. Comp. Endocrinol.* 161 (2009) 202–207.
- J.C. Wingfield, R.E. Hegner, A.M. Dufty Jr., G.F. Ball, The ‘challenge hypothesis’: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies, *Am. Nat.* 136 (1990) 829–846.
- O. Almeida, E. Gonçalves-De-Freitas, J.S. Lopes, R.F. Oliveira, Social instability promotes hormone-behavior associated patterns in a cichlid fish, *Horm. Behav.* 66 (2014) 369–382.
- J.K. Desjardins, M.R. Hazelden, G.J. Van der Kraak, S. Balshine, Male and female cooperatively breeding fish provide support for the “challenge hypothesis”, *Behav. Ecol.* 17 (2005) 149–154.
- R.V. Lacava, L. Brasileiro, R. Maia, R.F. Oliveira, R.H. Macedo, Social environment affects testosterone level in captive male blue-black grassquits, *Horm. Behav.* 59 (2011) 51–55.
- A.V. Rincon, L. Maréchal, S. Semple, B. Majolo, A. MacLarnon, Correlates of androgens in wild male Barbary macaques: testing the challenge hypothesis, *Am. J. Primatol.* 79 (2017) e22689, <http://dx.doi.org/10.1002/ajp.22689>.
- M.C. Teles, R.F. Oliveira, Androgen response to social competition in a shoaling fish, *Horm. Behav.* 78 (2016) 8–12.
- W. Goymann, M.M. Landys, J.C. Wingfield, Distinguishing seasonal androgen responses from male-male androgen responsiveness – revising the challenge hypothesis, *Horm. Behav.* 51 (2007) 463–476.
- G.S. Caldwell, S.E. Glickman, E.R. Smith, Seasonal aggression independent of seasonal testosterone in wood rats, *Proc. Natl. Acad. Sci. U. S. A.* 81 (1984) 5255–5257.
- G.E. Demas, C.A. Moffatt, D.L. Drazen, R.J. Nelson, Castration does not inhibit aggressive behavior in adult male prairie voles (*Microtus ochrogaster*), *Physiol. Behav.* 66 (1999) 59–62.
- R. Pinxten, E. De Ridder, M. De Cock, M. Eens, Castration does not decrease non-reproductive aggression in yearling male European starlings (*Sturnus vulgaris*), *Horm. Behav.* 43 (2003) 394–401.
- L.S. Huffman, L.A. O'Connell, H.A. Hofmann, Aromatase regulates aggression in the African cichlid fish *Astatotilapia burtoni*, *Physiol. Behav.* 112–113 (2013) 77–83.
- B.A. Schlinger, G.V. Callard, Aromatization mediates aggressive behavior in quail, *Gen. Comp. Endocrinol.* 79 (1990) 39–53.
- K.K. Soma, K.A. Sullivan, A.D. Tramontin, C.J. Saldanha, B.A. Schlinger, J.C. Wingfield, *J. Comp. Physiol. A.* 186 (2000) 759–769.
- G.P. Baerends, J.M. Baerends-Van Roon, An introduction to the study of the ethology of cichlid fishes, *Behav. Suppl.* 1 (1950) 1–235.
- G.W. Barlow, *The Cichlid Fishes: Nature's Grand Experiment in Evolution*, Perseus

- Publishing, New York, 2000.
- [39] K.M. Sefc, Mating and parental care in Lake Tanganyika's cichlids, *Int. J. Evol. Biol.* 470 (875) (2011).
- [40] M. Pandolfi, M.M. Cánepa, F.J. Meijide, F. Alonso, G.R. Vázquez, M.C. Maggese, P.G. Vissio, Studies on the reproductive and developmental biology of *Cichlasoma dimerus* (Perciformes, Cichlidae), *Biocell* 33 (2009) 1–18.
- [41] M.R. Ramallo, L. Morandini, F. Alonso, A. Birba, C. Tubert, A. Fiszbein, M. Pandolfi, The endocrine regulation of cichlids social and reproductive behavior through the eyes of the chanchita, *Cichlasoma dimerus* (Percomorpha; Cichlidae), *J. Physiol. Paris* 108 (2014) 194–202.
- [42] M.F. Scaia, L. Morandini, C.A. Noguera, M.R. Ramallo, G.M. Somoza, M. Pandolfi, Fighting cichlids: dynamic of intrasexual aggression in dyadic agonistic encounters, *Behav. Process.* 147 (2018) 61–69, <http://dx.doi.org/10.1016/j.beproc.2017.12.015>.
- [43] C. Tubert, F. Lo Nostro, V. Villafañe, M. Pandolfi, Aggressive behavior and reproductive physiology in females of the social cichlid fish *Cichlasoma dimerus*, *Physiol. Behav.* 106 (2012) 193–200.
- [44] M.R. Ramallo, R.M. Honji, A. Birba, L. Morandini, M.L. Varela, G. Genovese, R.G. Moreira, G.M. Somoza, M. Pandolfi, A game of two? Gene expression analysis of brain (*cyp19a1b*) and gonadal (*cyp19a1a*) aromatase in females of a neotropical cichlid fish through the parental care period and removal of the offspring, *Gen. Comp. Endocrinol.* 252 (2017) 119–129.
- [45] J.R. Casciotta, A.E. Almirón, J. Bechara, Peces del Iberá – Hábitat y Diversidad, UNDP, Fundación Ecos, UNLP y UNNE, Grafikar, La Plata, Argentina, 2002 987-05-0375-6.
- [46] National Research Council, Guide for the Care and Use of Laboratory Animals, Eighth ed., (2011) Washington, DC.
- [47] R.F. Oliveira, V.C. Almada, A.V.M. Canario, Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus*, *Horm. Behav.* 30 (1996) 2–12.
- [48] R.R. Oliveira, A. Silva, A.V. Canário, Why do winners keep winning? Androgen mediation of winner but not loser effects in cichlid fish, *Proc. R. Soc. B Biol. Sci.* 276 (2009) 2249–2256.
- [49] L. Morandini, R.M. Honji, M.R. Ramallo, R.G. Moreira, M. Pandolfi, The interrenal gland in males of the cichlid fish *Cichlasoma dimerus*: relationship with stress and the establishment of social hierarchies, *Gen. Comp. Endocrinol.* 195 (2014) 88–98.
- [50] A.P. Arnold, The effects of castration on song development in zebra finches (*Poephila guttata*), *J. Exp. Zool.* 191 (1975) 261–277.
- [51] J. Balthazart, Hormonal correlates of behavior, *Avian Biol.* 7 (1983) 221–365.
- [52] D.A. Edwards, Effects of cyproterone acetate on aggressive behavior and the seminal vesicles of male mice, *J. Endocrinol.* 46 (1970) 477–481.
- [53] B. Silverin, M. Baillien, J. Balthazart, Territorial aggression, circulating levels of testosterone and brain aromatase in free-living pied flycatchers, *Horm. Behav.* 45 (2004) 225–234.
- [54] K.K. Soma, A.D. Tramontin, J.C. Wingfield, Oestrogen regulates male aggression in the non-breeding season, *Proc. R. Soc. B Biol. Sci.* 267 (2000) 1089–1096.
- [55] S. Ogawa, V. Eng, J. Taylor, D.B. Lubahn, K.S. Korach, D.W. Pfaff, Roles of estrogen receptor- $\alpha$  gene expression in reproduction-related behaviors in female mice, *Endocrinology* 139 (1998) 5070–5081.
- [56] K. Toda, T. Saibara, T. Okada, S. Onishi, Y. Shizuta, A loss of aggressive behavior and its reinstatement by estrogen in mice lacking the aromatase gene (*Cyp19*), *J. Endocrinol.* (2001) 2217–2220.
- [57] A.L. Filby, G.C. Paull, T.F. Hickmore, C.R. Tyler, Unravelling the neurophysiological basis of aggression in a fish model, *BMC Genomics* 11 (2010) 498.
- [58] S.C. Renn, N. Aubin-Horth, H.A. Hofmann, Fish and chips: functional genomics of social plasticity in an African cichlid fish, *J. Exp. Biol.* 211 (2008) 3041–3056.
- [59] F. Alonso, M. Cánepa, R.G. Moreira, M. Pandolfi, Social and reproductive physiology and behavior of the neotropical cichlid fish *Cichlasoma dimerus* under laboratory conditions, *Neotrop. Ichthyol.* 9 (2011) 559–570.
- [60] K.P. Maruska, R.D. Fernald, Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish, *Horm. Behav.* 58 (2010) 230–240.
- [61] K.P. Maruska, L. Becker, A. Neboori, R.D. Fernald, Social descent with territory loss causes rapid behavioral endocrine and transcriptional changes in the brain, *J. Exp. Biol.* 216 (2013) 3656–3666.
- [62] V.N. Parikh, T.S. Clement, R.D. Fernald, Androgen level and male social status in the African cichlid, *Astatotilapia burtoni*, *Behav. Brain Res.* 166 (2006) 291–295.
- [63] K. Lee, D.W. Kim, R. Remedios, T.E. Anthony, A. Chang, L. Madisen, H. Zen, D.J. Anderson, Scalable control of mounting and attack by *Esr1+* neurons in the ventromedial hypothalamus, *Nature* 509 (2014) 627.
- [64] A.K. Greenwood, A.R. Wark, R.D. Fernald, H.A. Hofmann, Expression of arginine vasotocin in distinct preoptic regions associated with dominant and subordinate behaviour in an African cichlid fish, *Proc. Biol. Sci.* 275 (2008) 2393–2402.
- [65] E. Gonçalves-de-Freitas, T.B. Carvalho, R.F. Oliveira, Photoperiod modulation of aggressive behavior is independent of androgens in a tropical cichlid fish, *Gen. Comp. Endocrinol.* 207 (2014) 41–49.
- [66] E.S. Davis, C.A. Marler, The progesterone challenge: steroid hormone changes following a simulated territorial intrusion in female *Peromyscus californicus*, *Horm. Behav.* 44 (2003) 185–198.
- [67] M.M. Elekonich, J.C. Wingfield, Seasonality and hormonal control of territorial aggression in female song sparrows (Passeriformes: Emberizidae: *Melospiza melodia*), *Ethology* 106 (2000) 493–510.
- [68] N.E. Langmore, J.F. Cockrem, E.J. Candy, Competition for male reproductive investment elevates testosterone levels in female dunnocks, *Prunella modularis*, *Proc. R. Soc. Lond. B* 269 (2002) 2473–2478.
- [69] P. Coumailleau, E. Pellegrini, F. Adrio, N. Diotel, J. Cano-Nicolau, A. Nasri, C. Vaillant, O. Kah, Aromatase, estrogen receptors and brain development in fish and amphibians, *Biochim. Biophys. Acta* 1849 (2015) 152–162.
- [70] N. Diotel, J.L. Do Rego, I. Anglade, C. Vaillant, E. Pellegrini, M.M. Gueguen, S. Mironov, H. Vaudry, O. Kah, Activity and expression of steroidogenic enzymes in the brain of adult zebrafish, *Eur. J. Neurosci.* 34 (2011) 45–56.
- [71] G.T. Ankley, K.M. Jensen, E.J. Durhan, E.A. Makynen, B.C. Butterworth, M.D. Kahl, D.L. Villeneuve, A. Linnum, L.E. Gray, M. Cardon, V.S. Wilson, Effects of two fungicides with multiple modes of action on reproductive endocrine function in the fathead minnow (*Pimephales promelas*), *Toxicol. Sci.* 86 (2005) 300–308.
- [72] A. Pradhan, P.E. Olsson, Germ cell depletion in zebrafish leads to incomplete masculinization of the brain, *Gen. Comp. Endocrinol.* (2018), <http://dx.doi.org/10.1016/j.ygcen.2018.02.001>.
- [73] L.A. O'Connell, H.A. Hofmann, Evolution of a vertebrate social decision-making network, *Science* 336 (2012) 1154–1157.