

## **Building the case for a novel teleost model of non-breeding aggression and its neuroendocrine control**

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### **Key words**

Agonistic behavior, territorial aggression, submission signals, estradiol, aromatase, gonadal steroids, female aggression, electric fish, *Gymnotus omarorum*

### **Abstract**

In vertebrates, aggression has been traditionally associated with high levels of circulating androgens in breeding males. Nevertheless, the centrality of androgens as primary modulators of aggression is being reconsidered in at least in two particular cases: 1) territorial aggression outside the breeding season, and 2) aggression by females. We are developing the weakly electric fish, *Gymnotus omarorum*, as a novel, advantageous model system to address these two alternative forms of aggression. This species displays a short, escalated contest, after which a clear hierarchical status emerges. Subordination of individuals involves three sequential decisions: interruptions of their electric discharges, retreats, and chirps. These decisions are influenced by both size asymmetry between contenders and aggression levels of dominants. Both females and males are aggressive, and do not differ in fighting ability nor in the value placed on the resource. Aggression is completely independent of gonadal hormones: dominance status is unrelated to circulating androgen and estrogen levels, and gonadectomy in males does not affect aggression. Nevertheless, estrogenic pathways participate in the modulation of this non-breeding aggression. Our results parallel those put forth in other taxa, heightening the value of *G. omarorum* as a model to identify commonalities in neuroendocrine strategies of vertebrate aggression control.

## 1. Introduction

Agonistic behavior is the social behavior related to animal conflict and it has shaped sociality across evolution (King, 1973; Lorenz, 1963). It arises in the confrontation between conspecific individuals over limited resources (mates, food, shelter, territory), and it is resolved when one individual keeps the resource (dominant) and the other loses it (subordinate). Despite the universal occurrence of agonistic behavior, it always follows the same stages (evaluation, contest, and post-resolution) and usually includes aggressive displays during the contest phase to settle the conflict (Nelson, 2006; Summers and Winberg, 2006). Animals make a balance of costs and benefits in search of maximal fitness payoffs to make their decision of fighting or giving up. These decisions are the output of the profound evaluation process inherent to agonistic behavior and arise from the assessment of differences in the fighting ability and resource value between contenders (Maynard Smith and Parker, 1976; Morris *et al.*, 1995; Parker and Rubenstein, 1981).

Understanding the control of aggression has been a longstanding aim of biology. The strong foundation of current knowledge is mostly built on studies on reproductive male aggression, which often requires gonadal androgens. Correlation between aggression and circulating androgens has been supported by castration–hormone replacement experiments (Balthazart, 1983; Borg, 1994; Demas *et al.*, 2007; Huffman *et al.*, 2012; Nelson, 2005; O'Connell *et al.*, 2013; Urich, 1938; Wingfield and Hahn, 1994; Wingfield *et al.*, 1990). Testosterone (T) partially affects aggression directly and also through its aromatization into estradiol (E) (Borg, 1994; Huffman *et al.*, 2013; Matsumoto *et al.*, 2003; O'Connell *et al.*, 2013; Schlinger and Callard, 1990; Silverin *et al.*, 2004; Toda *et al.*, 2001). In addition to the changes of plasma hormone levels throughout the annual cycle, acute modifications in brain hormone levels can be produced by social behavior in a region-specific manner and with a rapid time course (Ramage-Healey *et al.*, 2008). Steroids can exert their neuromodulatory effects through nuclear hormone receptors and thus affect gene transcription on the scale of hours-days producing enduring effects associated with the breeding cycle (Etgen and Pfaff, 2010); or steroids can rapidly affect behavior in seconds-minutes by modifying neuronal excitability through non-genomic effects (Hayden-Hixson and Ferris, 1991; Lambert *et al.*, 2003; Woolley, 2007).

Although androgens are clearly crucial modulators of male aggression in many vertebrates, the centrality of androgens as the main modulators of aggression needs to be revisited in at least two particular cases: 1) territorial aggression dissociated from the breeding season, and 2) aggression by females. Investigating aggression across sexes and seasons may lead to a more comprehensive understanding of its underlying neuroendocrine mechanisms.

Territorial aggression is generally associated with high androgen levels, but it may also occur when gonads are regressed and circulating androgen levels are low. This has been reported in birds (Gwinner *et al.*, 1994; Logan and Wingfield, 1990; Soma *et al.*, 1999; Wingfield, 1994; Wingfield *et al.*, 1997), mammals (Caldwell *et al.*, 1984; Demas *et al.*, 2007; Jasnow *et al.*, 2000), reptiles (Moore and Marler, 1987), and fish (Batista *et al.*, 2012; Vullioud *et al.*, 2013). Moreover, aggression can persist after castration (Christenson *et al.*, 1973; Davis, 1957; Demas *et al.*, 1999; Garrett and Campbell, 1980; Tiefer, 1970). Research on aggression outside breeding conditions, has brought forth the roles of non gonadal steroids in the control of this behavior. In the well-studied Siberian hamsters (*Phodopus sungorus*) adrenocortical steroids are key modulators. Circulating dehydroepiandrosterone (DHEA), an adrenal androgen which can be converted into active sex steroids in the rodent brain (Tsutsui, *et al.* 2000; Agis Balboa *et al.* 2007), is elevated under short day treatment (Demas and Jasnow, 2004). It is proposed that DHEA converts to an active steroid influencing aggression (Scotti *et al.* 2009). This alternative hormonal control of aggression has also been reported in birds. Song sparrows (*Melospiza melodia*) also display non-breeding aggression that correlates to high circulating DHEA (Soma and Wingfield, 2001; reviewed in Soma *et al.* 2015) which can be converted into T and E in the brain (Pradham *et al.* 2010). In particular, estrogens have been reported to have a pivotal role in the regulation of non-breeding aggression in the song sparrow (Soma *et al.*, 2000b). These brain derived estrogens most probably act mainly through non-genomic mechanisms (Hau *et al.*, 2000; Schlinger *et al.*, 1992; Soma and Wingfield, 1999; Soma *et al.*, 2000a; Soma *et al.*, 2000b; Trainor and Nelson, 2012).

Females, just like their male counterparts, sometimes use aggression to defend territories or other resources (Floody, 1983; Jaeger *et al.*, 1982; LaPrade and Graves, 1982; Woodley and Moore, 1999; Yasukawa and Searcy, 1982). Aside from maternal aggression (Lonstein and Gammie, 2002; Rosenblatt *et al.*, 1963), female aggressive behavior has received much less attention, with relatively little emphasis on its hormonal control. The few attempts to link circulating sex steroid hormones with female aggression have led to conflicting results: plasma T and/or E concentrations in females are associated with aggression in some species of birds (Gill *et al.*, 2007; Langmore *et al.*, 2002; Rosvall, 2013), mammals (Albert *et al.*, 1992; Razzoli *et al.*, 2003), reptiles (Rubenstein and Wikelski, 2005; Woodley and Moore, 1999), and fish (Desjardins *et al.*, 2006); but not in other birds (Elekonich and Wingfield, 2000), mammals (Davis and Marler, 2003), and fish (Hay and Pankhurst, 2005). These results do not rule out a role of T and/or E in specific brain regions influencing aggression, as circulating precursors may be involved. Studies in the highly territorial female Siberian hamsters have shown that they display increased aggression outside of breeding (Scotti *et al.* 2007) and that DHEA is a key candidate for the regulation of aggression (Rendon *et al.* 2016). Females show increased aggression in short-day context, and interestingly, melatonin has been shown to coordinate a seasonal switch from gonadal to adrenal regulation of aggression (Rendon *et al.* 2015).

South American weakly electric fish (Order Gymnotiformes), are an important model to understand the neural basis of behavior. They produce and perceive electric organ discharges (EOD), which serve two critical functions: to sense objects in the environment, as the EOD is a self-generated carrier signal (active electrolocation) and to interact socially (electrocommunication) (reviewed in Caputi *et al.*, 2005). The circuits generating the EOD have key elements in common with other vertebrate communication systems in that they are basically modified motor control systems: the organ that produces the signal, just like those in the birdsong and fish vocalization systems, is muscle-derived; it is innervated by spinal motor nuclei which, in turn, receive input from the hindbrain (reviewed in Bass, 1986). A key component of the system, the medullary pacemaker nucleus, fires spontaneously and controls the timing of each EOD, in a one-to-one fashion, in all weakly electric fish (reviewed in Caputi *et al.* 2005).

Non-breeding territorial aggression has been examined in only two teleost species: *Stegastes nigricans* (Ros *et al.*, 2014; Vulllioud *et al.*, 2013) and *Gymnotus omarorum*. We have developed *G. omarorum* as a valuable model species to study the hormonal control of this form of aggression (Batista *et al.*, 2012; Jalabert *et al.*, 2015; Silva *et al.*, 2013; Zubizarreta *et al.*, 2012; Zubizarreta *et al.*, 2015).. This species has several strong advantages: its non-breeding territorial aggression is well characterized, males and females display equal levels of aggression and this aggression depends upon non-gonadal estrogenic pathways rather than gonadal hormones.

## **2. Behavioral characterization of non-breeding aggression in *Gymnotus omarorum***

*Gymnotus omarorum* displays a clear-cut example of pure territorial aggression (Batista *et al.*, 2012; Silva *et al.*, 2013; Zubizarreta *et al.*, 2015). During the non-breeding season, when gonads are regressed and no reproductive motivation is expected to drive competence, males and females of this sexually monomorphic species fiercely defend territories in intrasexual and intersexual encounters. We have developed a careful behavioral protocol to describe this non-breeding territorial aggression.

We staged dyadic agonistic encounters between non-breeding adults *Gymnotus omarorum* in a behavioral arena in which space is the only resource animals fight for (Batista *et al.*, 2012). Fish with the same previous experience and residence time were placed in equal-sized compartments separated by a removable glass gate. When the gate was lifted, all fish (in same-sex and opposite sex dyads) engaged in rapid agonistic encounters that were usually resolved in < 3 min with the establishment of a clear dominant-subordinate status (Fig. 1A). During conflicts, contenders evaluate the costs and benefits of continuing the fight or retreating, although it is not always possible to have clear evidence of this decision-making process. Taking advantage of the electric channel of communication, subordinates of *G. omarorum* make three distinct and consecutive decisions to signal their submission across phases (Fig. 1A): 1) subordinates first interrupt their EOD to hide from the dominant, 2) they then stop attacking and retreat, and 3) finally, they emit transient high-rate electric signals termed chirps. This sequence of behaviors enable the subordinate to be more explicit in signaling its surrender.

After the foundational work of Black-Cleworth (1970) on the agonistic behavior of *Gymnotus carapo*, more recent studies have reported distinctive agonistic electric displays, either produced by dominants or subordinates, in several species of South American electric fish (Fugère *et al.*, 2011; Hagedorn and Zelick, 1989; Hupé and Lewis, 2008; Perrone *et al.*, 2009; Triefenbach and Zakon, 2008; Westby, 1975a; Westby, 1975b). Some electric signals have been interpreted as threats, such as abrupt rate changes (*G. carapo*, Black-Cleworth, 1970), and chirps (*Apteronotus leptorhynchus*, Triefenbach and Zakon, 2008; *Brachyhypopomus pinnicaudatus*, Perrone *et al.*, 2009). On the other hand, cessations in the emission of electric signals (offs) have been recognized as general submissive displays in several species (Batista *et al.*, 2012; Black-Cleworth, 1970; Westby, 1975a; Zubizarreta *et al.*, 2012). In *G. omarorum*, only transient subordination electric signals, offs and chirps, have been identified (Batista *et al.*, 2012). These signals are emitted in a sequential pattern of subordination that provides one of the best understood examples of the electric dialogue in electric fish. As expected for a graded signal of intention, offs are first emitted before contest resolution and gradually increase in rate and duration during the post-resolution phase (Fig. 1B). After resolution, the pattern of electric signaling changes; the subordinate continues to emit more frequent and longer duration offs and adds the emission of chirps as more unambiguous submission signals (Fig. 1B). As a continuous signal electrical subordination, the EOD rate of subordinates decreases after resolution (Silva *et al.*, 2013) yielding a hierarchical EOD rate rank between dominants and subordinates as observed in other gymnotiform species (Fugère *et al.*, 2011; Hagedorn and Heiligenberg, 1985; Hagedorn and Zelick, 1989; Silva *et al.*, 2013; Westby, 1975a). In summary, *G. omarorum* employs different electric submission displays that decrease ambiguity in subordination signaling, and evince a complex decision-making process.

Aggression within agonistic encounters is usually a ritualized behavior, intended to demonstrate the power of one contender over the other and provide information to determine which one will win the contest rather than to cause injury or death. As part of the mutual evaluation among contenders during the agonistic encounter, aggression leads to displacing, or dominating another individual (Nelson, 2006). Contenders begin by displaying a relatively low cost aggressive behavior and only increase the intensity of interaction if they cannot settle (Jennings and Gammell, 2013). Once in contest,

animals usually use progressively greater aggression to challenge the decision of their contender to escalate the fight and to drive them to subordination (Enquist and Leimar, 1983). As the contest escalates, variation in the performance of aggressive behaviors influences the decisions made by contenders to solve the conflict, thus influencing contest dynamics. In many agonistic interactions, non-aggressive displays are enough to evaluate relative fighting ability between contenders without the costs of engaging in injurious fights (Enquist *et al.*, 1990; Koops and Grant, 1993; Pratt *et al.*, 2003).

In *Gymnotus omarorum*, individuals immediately perform direct evaluation of fighting ability through aggression rather than remotely evaluating the contender through electrocommunication signals (Zubizarreta *et al.*, 2015). This is remarkable and unexpected since a) passive and active electric images of *G. omarorum* differ between contenders; so this information could be used remotely to evaluate the fighting ability of the adversary (Pedraja *et al.*, 2016), and b) electric fish encode body size in their EOD amplitudes (Gavassa *et al.*, 2013). After the onset of this staged contest, both individuals perform aggressive displays (bites, nudges, nips and jaw locks) until the subordinate fish stops attacking and retreats. During this short phase of contest, dominants display higher levels of aggression than subordinates (Fig. 2A). On the other hand, the attack rates of both contenders are strongly correlated, suggesting an escalation in the conflict (Fig. 2B). In the experimental tank, in which subordinates cannot flee, the dominant fish persists in attacking the defeated contender after the contest resolution, forcing the emission of more unambiguous signals of surrender.

Theoretical models predict that the evaluation of fighting ability is important in defining different characteristics of agonistic encounters (Maynard Smith and Parker, 1976). Body size is the most common predictor of fighting ability across taxa, influencing the outcome (Jennions and Backwell, 1996; Umers *et al.*, 2012), timing (Enquist *et al.*, 1990; Junior and Peixoto, 2013), and intensity of fights (Morris *et al.*, 1995). If resource value is symmetric among contestants, contest outcome is expected to depend only on fighting ability asymmetries. Indeed, in *Gymnotus omarorum*, body size is the most important proxy of fighting ability, and hence, body mass difference between contenders is the only predictor of contest outcome; *i.e.*, the heavier fish wins (Batista *et al.*, 2012). Interestingly, size asymmetry also influences the three decisions of the subordinate: 1) electrical hiding (offs): body mass asymmetry is correlated negatively

with first off latency; 2) retreat: body mass asymmetry is correlated negatively with contest duration; and 3) post-resolution chirping: body mass asymmetry is marginally correlated negatively with first chirp latency (Table 4 in Batista *et al.* 2012). In addition to body size, the intensity of aggression is also an indicator of fighting ability in *G. omarorum* (Zubizarreta *et al.*, 2015). First, since aggression levels correlate with body mass, overt aggression is an indirect indicator of fighting ability (Zubizarreta *et al.*, 2015). Secondly, the intensity of aggressive encounters may also be evaluated directly by contenders in decision-making. When subordinates are subjected to more intense aggression during the contest phase, they more quickly signal submission with shorter latencies in producing offs (first decision), retreats (second decision), and chirps (third decision) (Fig 3). Furthermore, the fact that the dominant attack rate is correlated negatively with contest duration (Zubizarreta *et al.*, 2015), indicates that the intensity of aggression is evaluated directly between contenders, and that subordinates assess how hard they are attacked when signaling submission.

### **3. Aggression displayed by both males and females**

Female contests, although less studied, follow the same general phases of agonistic encounters. However, sex differences in fighting ability, or life history (*e.g.*, energy dedicated to reproduction), may cause males and females to differentially value contested resources or to employ sex-specific decision-making strategies during contests (Wofford *et al.*, 2015). For example, in Texas cichlids *Herichthys cyanoguttatum* (Draud *et al.*, 2004) males and females both show intrasexual aggression with the same behavioral repertoire. However, contest outcome is related to body size in males but not in females, suggesting female contests may be decided more often by the value of the contested resource than by asymmetries in their fighting ability. On the other hand, in Convict cichlids *Amatitlania nigrofasciata*, behavioral repertoires differed between sexes, but the outcome was influenced by body size in contests of either sex (Arnott and Elwood, 2009). The non-breeding territorial aggression of the sexually monomorphic *Gymnotus omarorum* provides a very clean example to test predictions of sex influence on contest outcome. *G. omarorum* is not expected to show dimorphic fighting ability as the sexes do not differ in body size. Indeed, Batista *et al.* (2012) demonstrated that contest outcome in *G. omarorum* is only influenced by body weight asymmetry, and that the individual sex and the sex-composition of the dyads



(same or opposite sex) had no significant influence. Thus, we conclude that sexual asymmetries in resource value, if any, do not influence the outcome of agonistic contests in non-breeding conditions in this species.

During the breeding season, the asymmetry between sexes in resource value is undeniable even for territorial species with sexually monomorphic fighting abilities (Alexander, 1974). On the other hand, the fact that animals are in the non-breeding season does not itself imply that both sexes give equal value to territory. Sexual differences in the time structure and/or the intensity of fights may reflect sexual differences in decision-making, even in the absence of reproductive motivation. To further evaluate sex differences, we compared the dynamics of agonistic behavior in male-male and female-female dyads, focusing on the decisions made by subordinates, and the intensity of both aggression and submission. Males and females make the same behavioral decisions: potential subordinates produce offs in their electric signals (first decision), retreat repeatedly (second decision, Table 1) and chirp (third decision). Moreover, there were no sex differences in fight intensity, (i.e., attack rates of dominant and subordinate fish were not significantly different between sexes), or submission levels, (i.e., the rate of off and chirps were not significantly different between sexes). (Table 1). Therefore, both male and female non-breeding intrasexual contests in *Gymnotus omarorum* shared the same behavioral repertoire including the same sequential electric signaling of submission in which both males and females make the same decisions. Overall, we present strong evidence that both sexes not only have symmetric fighting abilities, but also give the same value to the resource they are fighting for during the non-breeding season.

Although male and female *Gymnotus omarorum* display indistinguishable non-breeding intrasexual aggression, they may differ in the underlying mechanisms, even if the same modulators are involved. In our studies on neuroendocrine mechanisms underlying non-breeding aggression, we must work with previously identified males and females, which is difficult to determine non-invasively as this species presents no morphological or electrophysiological sexual dimorphism (Richer-de-Forges *et al.*, 2009). We validated a method of sex identification by visual inspection of gonads through a surgical incision, and then compared agonistic encounters between fish that were not identified prior to the experiment (intact) and those that were sexually identified (ID). There was no

significant difference between the behavior of ID fish and intact fish in any of the variables studied, be it between males or between females. Moreover, ID fish showed no differences in aggressive behavior between sexes (Table 1). This validation allowed us to improve our experimental design and to focus on the hormonal control mechanisms of intrasexual aggression. Given the scarcity of studies on female aggression, we believe *G. omarorum* will be an advantageous model system to contribute to the understanding of the control of vertebrate aggression.

#### **4. Non breeding aggression is independent of gonadal hormones**

Behaviors that are expressed similarly in different phases of the annual reproductive cycle probably do not have the same hormone control mechanisms. Aggression, which is correlated to high circulating androgens during the breeding context, may be sustained by novel mechanisms after gonadal regression, ensuring robust aggressive behavior but avoiding the costs associated with prolonged high androgen levels (reviewed in Wingfield et al, 1990; Nelson, 2005). In this sense, the dependence of non breeding territorial aggression on estrogens raises the question the source of the steroids involved.

To evaluate commonalities in the underlying mechanisms of non-breeding aggression, we analyzed aggression in relation to gonadal hormones in *Gymnotus omarorum* by measuring the steroid response to dominance and by comparing agonistic behavior of castrated and intact males. Specifically, we measured circulating steroids in adults with no social stimuli and in those which had recently won an agonistic same-sex encounter. Plasma 11KT (the main gonadal androgen in teleosts) levels were similar in dominant and isolated males (Fig 4A, Batista, 2011; Jalabert *et al.*, 2015). Furthermore, plasma estrogen levels in dominant females, although variable, did not differ from those in isolated females (Fig 4B, Zubizarreta *et al.*, 2016).

Across vertebrates, the relationship between dominance and androgen levels in males is under constant revision. The Challenge Hypothesis predicts that agonistic encounters transiently increase circulating androgens, from breeding levels to a maximum physiological level (Wingfield *et al.*, 1990). More recent studies have found that androgen levels can be transiently increased even in absence of social challenge, in

response to the perceived outcome of an interaction, and that this increase likely modulates behavioral expression in subsequent social interactions (Oliveira and Oliveira, 2014; Oliveira, 2009). Our results are expected for animals in the non-breeding season, which have overall low levels of gonadal hormones. Studies in aggressive non-breeding males report an absence of circulating androgenic response to social challenge in many teleost species (Batista, 2011; Jalabert *et al.*, 2015; Landys *et al.*, 2007; Ros *et al.*, 2014; Vullioud *et al.*, 2013). Moreover, reports in non-breeding females also show no changes in plasma androgens (Vullioud *et al.*, 2013) or estrogens (Hau *et al.*, 2004) in response to social challenge. On the whole, aggression in the non-breeding season does not correlate with plasma concentrations of sex steroids.

To rule out the participation of all gonadal hormones in the control of aggression, we compared dyads of castrated males with those of intact males (Jalabert *et al.*, 2015). Both gonadectomized (GDX) and sham gonadectomized (SH) dyads engaged in short, aggressive contests, typical for the species. The subordinate fish displayed the electric (EOD interruptions) and locomotor (retreats) signals of submission with no difference in timing between treatment groups (Fig. 5A). The intensity of displays was also equally expressed: attack rate of dominant GDX fish showed no significant difference in comparison to SH (Fig 5B), while the off rate displayed by subordinate fish was also indistinguishable between GDX and SH individuals (Fig 5C). This proves that non-breeding aggression in males is independent of all gonadal hormones (Jalabert *et al.*, 2015).

## **5. First report on the role of estradiol in teleost non-breeding aggression**

Aggression has been shown to be estrogen-dependent in non-breeding birds and aromatase, which converts androgen to estradiol, is highly expressed in brain regions underlying social behavior (Soma *et al.*, 2003; Wacker *et al.*, 2010). The source of androgen substrate for brain aromatase could be adrenal DHEA, shown to have a key role in regulation of aggression in the aforementioned well-studied Siberian hamster and song sparrow models (reviewed in Soma *et al.* 2015). Alternatively, *de novo* brain synthesis of steroids may also regulate non-breeding aggression, as key enzymes have been identified in the brains of birds (Tsutsui *et al.*, 2003; Tsutsui and Yamazaki, 1995; Tsutsui *et al.*, 2006) and mammals (Corpechot *et al.*, 1981).

In our studies of *Gymnotus omarorum* we showed that aggression depends on normal aromatase activity. We analyzed agonistic behavior in dyads in which the potentially dominant contender, the one with the heaviest body weight, was injected with the inhibitor of aromatase, Fadrozole (FAD) (Jalabert *et al.*, 2015). After FAD administration, agonistic behavior was distorted, and dominance could no longer be predicted by body size asymmetry (prediction that can be made for the control group injected with the vehicle solution). In one third of the cases, dominant–subordinate ranks did not emerge, as fights were not resolved, and in other cases the outcome was reversed. In all, large males became dominant in only one fourth of the contests. Aromatase inhibition also significantly decreased the aggression levels of potential dominants. The fast effect of the inhibition on the aggression suggests that the underlying mechanisms involve rapid estrogenic non-genomic signaling mechanisms (Cornil and Charlier, 2010), as has been reported in non-breeding aggressive behavior in mammals and birds (reviewed in Trainor and Nelson, 2012). There is not yet data on the source of estrogens regulating non-breeding aggression in this species. Teleosts have the capability of producing DHEA (reviewed in Torkarz *et al* 2015) and low circulating levels of DHEA have been reported in the eel *Anguila japonica* (Matsubara *et al* 2004). Moreover, zebrafish (*Danio rerio*) has been reported to have brain steroidogenic enzymes which enable not only the possibility of converting circulating precursors to active sex steroids, but also *de novo* synthesis from cholesterol (Diotel *et al* 2011).

Steroid hormones in the breeding season have multiple roles. They coordinate physiological and behavior functions and their levels are, in turn, modulated by social performance. The adaptive function of the social modulation of androgen levels may be to fine tune the expression of androgen-dependent behavior according to the perceived social environment (Oliveira and Oliveira, 2014). In the non-breeding season, animals do not display reproductive behavior and face less diversity of social information to process and integrate into their decision making, their repertoire of social behaviors is narrower, and their reproductive axis is at rest. In this scenario, localized brain derived estrogen may take a lead role in the control of aggression. *Gymnotus omaromum*, as the Siberian hamster and the song sparrow, displays aggression all year long, independent of gonadal hormones (revised in Soma *et al* 2015). This behavior is strongly dependent

on the estrogenic pathway, as has been shown in the sparrow (Soma et al 2000b), and the source of these key hormones is currently under study. It is a species with exciting prospects for valuable contributions to understanding the hormonal control of aggression. Moreover, it adds a teleost species to the current mammal and bird models which will allow a better comprehension of the commonalities of this regulation across taxa.

## **Acknowledgments**

All research procedures complied with ASAP/ABS Guidelines for the Use of Animals in Research and were approved by the Universidad de la República Institutional Ethical Committee (Comisión Bioética, Instituto Clemente Estable, MEC, 07-28-2008 and 007/02/2010). This research was supported by Agencia Nacional de Investigación e Innovación (projects: FCE2007\_569 , FCE2009\_2472 , FCE2009\_2657, FCE 104272 and FCE 100485) and Programa de Desarrollo de las Ciencias Básicas (PEDECIBA).

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Dyad	Latency Off	Contest duration	Attack rate Dominant	Attack rate Subordinate	Off rate	Chirp rate
<i>Male-male</i>	<b>38</b> (20) 7	<b>78</b> (21) 9	<b>0.21</b> (0.16) 9	<b>0.11</b> (0.05) 9	<b>0.02</b> (0.02) 9	<b>0.05</b> (0.043) 9
<i>Female-female</i>	<b>78</b> (49) 6	<b>152</b> (97) 7	<b>0.11</b> (0.025) 7	<b>0.07</b> (0.046) 7	<b>0.01</b> (0.01) 7	<b>0.003</b> (0.003) 7
<i>ID male-male</i>	<b>175</b> (35) 6	<b>114</b> (52) 6	<b>0.12</b> (0.08) 6	<b>0.06</b> (0.039) 6	<b>0.014</b> (0.007) 6	<b>0</b> (0) 6
<i>ID female-female</i>	<b>45</b> (16) 6	<b>172</b> (133) 5	<b>0.15</b> (0.07) 5	<b>0.08</b> (0.04) 5	<b>0.017</b> (0.017) 5	<b>0.06</b> (0.06) 5
Overall comparison (p)	0.1	0.79	0.7	0.49	0.9	0.6

### Table legend

Comparison of male and female intrasexual dyads in both intact and surgically identified (ID) contenders in their behavioral decisions, intensity of aggression and submission levels. Shaded rows: male-male and female-female dyadic agonistic encounters. Sexual identification of these fish was performed after the behavioral experiment (intact fish). White rows: male-male and female-female dyadic agonistic encounters of fish with a previous gonadal inspection by surgical incision (ID fish).

Values are expressed as medians, errors are expressed as median absolute deviation (MAD) between brackets, n values are shown below the MADs. Statistical comparison shows there is no significant difference between any of the groups, (overall comparison, Kruskal-Wallis test, p values in bottom row).

Attack rate was calculated dividing the number of attacks by contest duration time in seconds, and is expressed in attacks per second. Off and chirp rate were calculated dividing the number of offs and chirps produced in post-resolution phase by the duration of this phase in seconds. Latencies to first off and first chirp, and contest duration, are expressed in seconds. Absence of values is due to insufficient n.

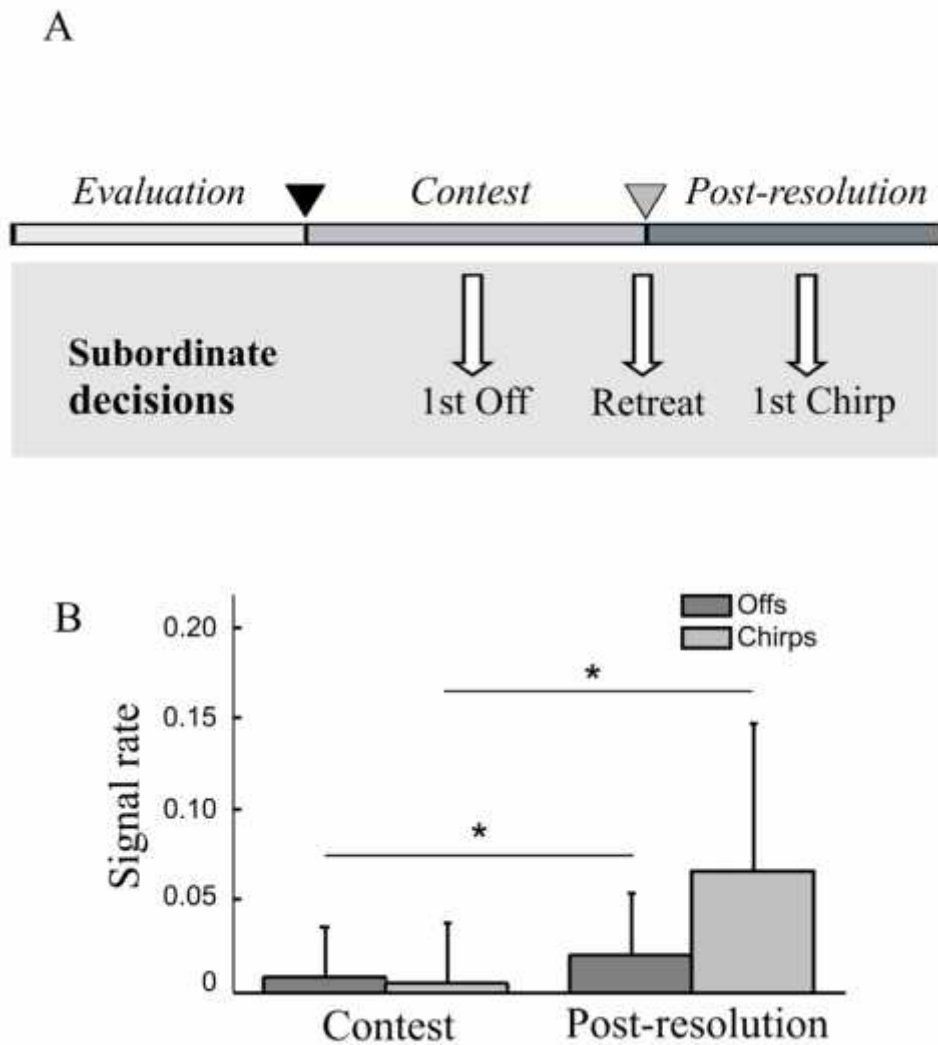


Figure 1: Characteristics of *Gymnotus omarorum* agonistic behavior, and decisions of the subordinate fish. A: Temporal structure of agonistic encounters. Agonistic behavior can be divided into three different stages: evaluation phase, from gate removal (time 0) to the occurrence of the first attack (black triangle); contest phase, from the occurrence of the first attack to conflict resolution (gray triangle); and post-resolution phase, 10 min. after conflict resolution. In the shaded area, the decisions made by the subordinate fish are illustrated by arrows, in order of appearance: first, the EOD interruption, second the retreat, and third the emission of the first chirp in the post resolution phase. B: Distribution of electric submission signals in different stages of agonistic behavior. Off rate and chirp rate increased from the contest to the post-resolution phase. Off and chirp rates were calculated dividing by the number of offs and chirps produced in each phase by the duration of the phase in seconds. Values are expressed as medians, error bars represent interquartile range (IQR). IQR. \* $p < 0.05$ , Wilcoxon test,  $N = 25$  and  $N = 21$  respectively. Modified from Batista *et al.*, 2012.

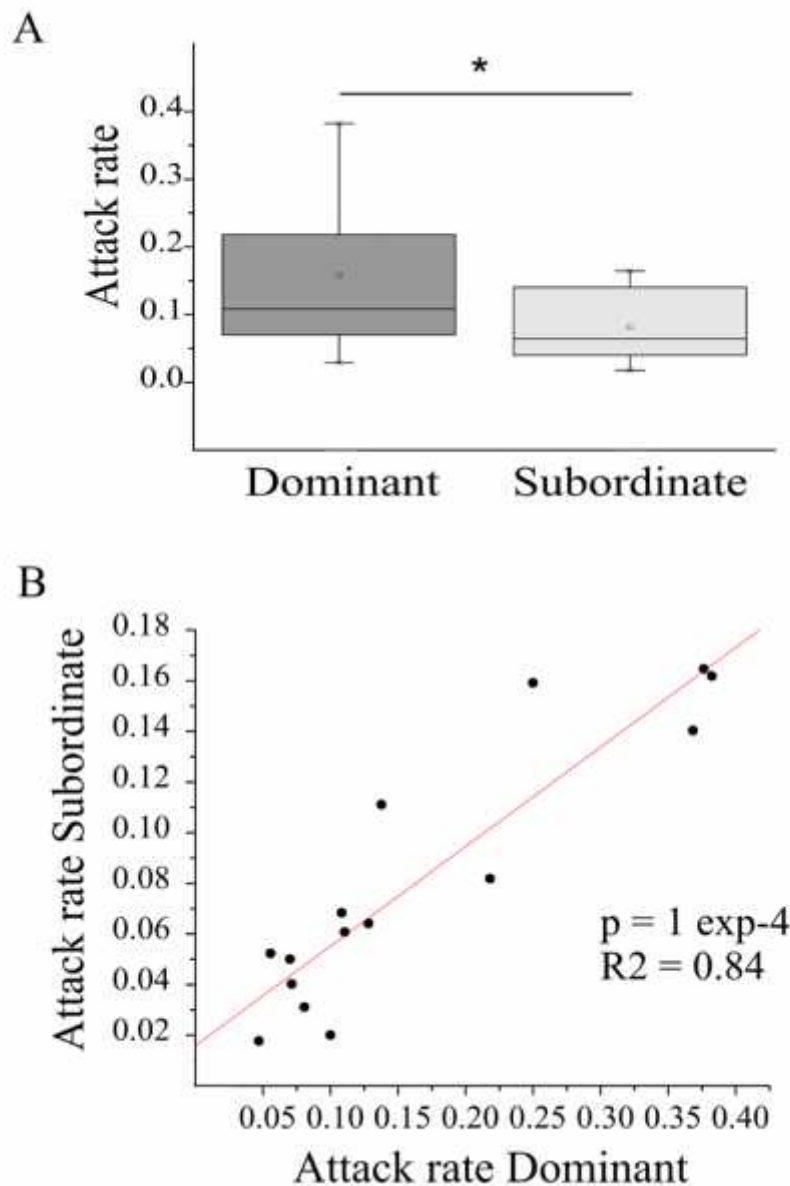


Figure 2: Aggression levels of dominants and subordinates.

A: Box plot representation of differences in the attack rate between dominant and subordinate fish. Dominant fish displayed significantly higher aggression compared to subordinates. \* $p = 3 \text{ exp-}5$ , Wilcoxon matched pairs test,  $N = 15$ . Modified from Zubizarreta *et al.* 2015.

B: During the contest phase, there was a significant positive correlation between the attack rate of dominants and subordinates.  $R^2 = 0.84$ ,  $p = 1 \text{ exp-}4$ ,  $N = 15$ . Modified from Zubizarreta *et al.* 2015.

In both A and B, attack rate was calculated dividing the number of attacks by contest duration time in seconds and is expressed in attacks/s. Box plot in this figure and throughout: horizontal line represents median value; length of the box represents interquartile range, small square represents the mean value, crosses show minimum and maximum values.



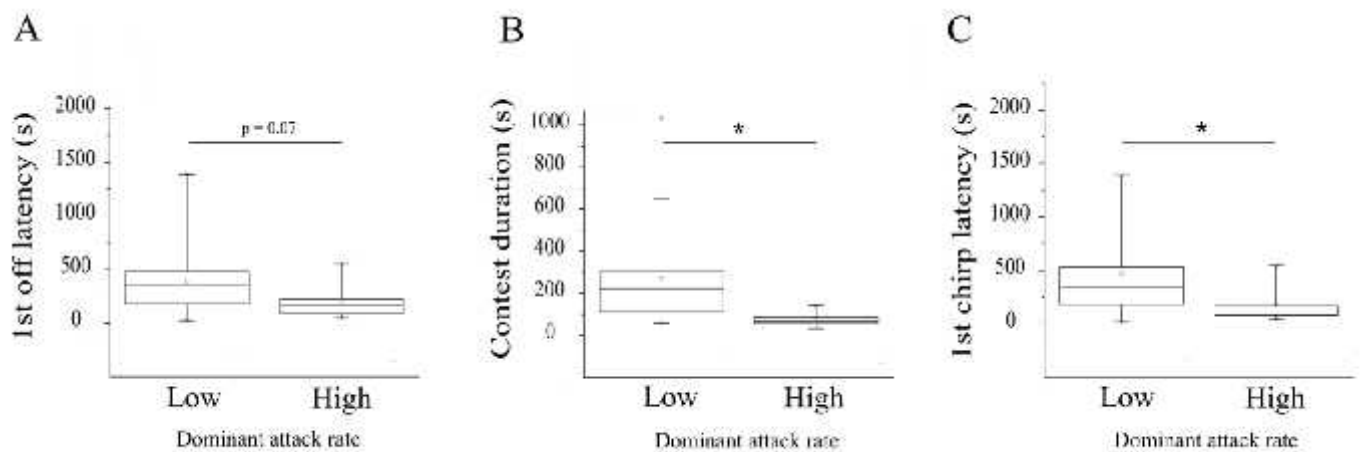


Figure 3: Influence of dominant aggression levels on the decisions made by the subordinate fish.

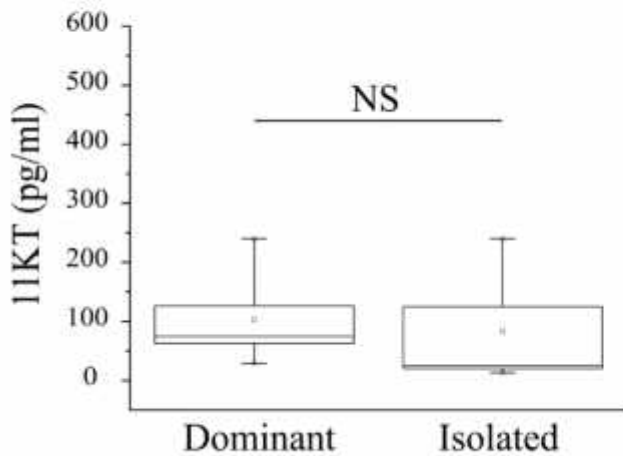
A: Box plot representations of the subordinate's first decision (off emission) in two different conditions of dominant attack rate. When the subordinate fish was confronted with a higher dominant attack rate it decreased its latency to the first off,  $p = 0.075$ , Mann Whitney U test, Low  $N = 17$ , High  $N = 7$ . Modified from Zubizarreta *et al.*, 2015.

B: Box plot representation of the subordinate's second decision (retreat) in two different conditions of dominant attack rate. When the subordinate fish was confronted with a higher dominant attack rate it decreased the contest duration,  $p = 0.001$ , Mann Whitney U test, Low  $N = 23$ , High  $N = 9$ . Modified from Zubizarreta *et al.*, 2015.

C: Box plot representation of the subordinate's third decision (chirp emission) in two different conditions of dominant attack rate. When the subordinate fish was confronted with a higher dominant attack rate it decreased its latency to the first chirp latency,  $p = 0.048$ , Mann Whitney U test, Low  $N = 18$ , High  $N = 7$ . Modified from Batista *et al.*, 2012.

In A, B and C, attack rate was calculated dividing the number of attacks by contest duration time in seconds. In this representation, low aggression levels are attack rates  $< 0.2$  attacks/s; and high aggression levels are attack rates from 0.2 to 0.4 attacks/s. Box plot description in legend of Fig 2.

### A Males



### B Females

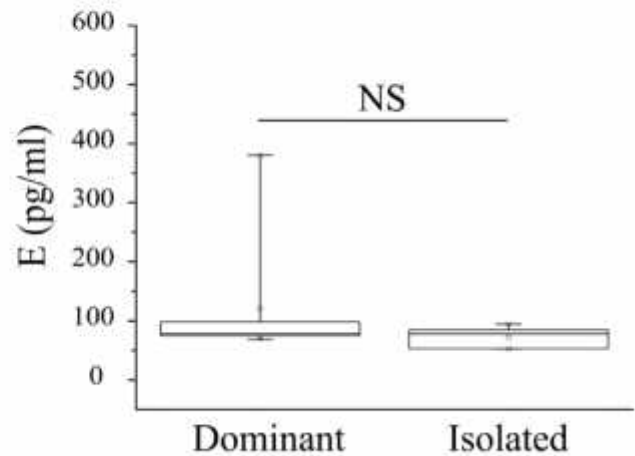


Figure 4: Levels of circulating sex steroids hormones and dominance in males and females.

A: Box plot representation of circulating levels of 11-Ketotestosterone levels (pg/ml). Dominant 11KT levels did not differ from control (isolated) in males.  $p = 0.4$ , Mann Whitney U test,  $N = 6$  in dominants and controls. Modified from Jalabert *et al.*, 2015.

B: Box plot representation of circulating levels of 17-B Estradiol levels (pg/ml). Dominant E levels did not differ from control (isolated) in females.  $p = 0.19$ , Mann Whitney U test, dominants  $N = 7$ , controls  $N = 7$ . In A and B, dominants animals were bled 10 minutes after conflict resolution and isolated animals were subjected to the same manipulations as dominants. Plasmatic levels of 11KT and E were measured using enzyme linked immunoassay kits (Cayman Chemical Co. for 11KT, and IBL International for E). Box plot description in legend of Fig 2.

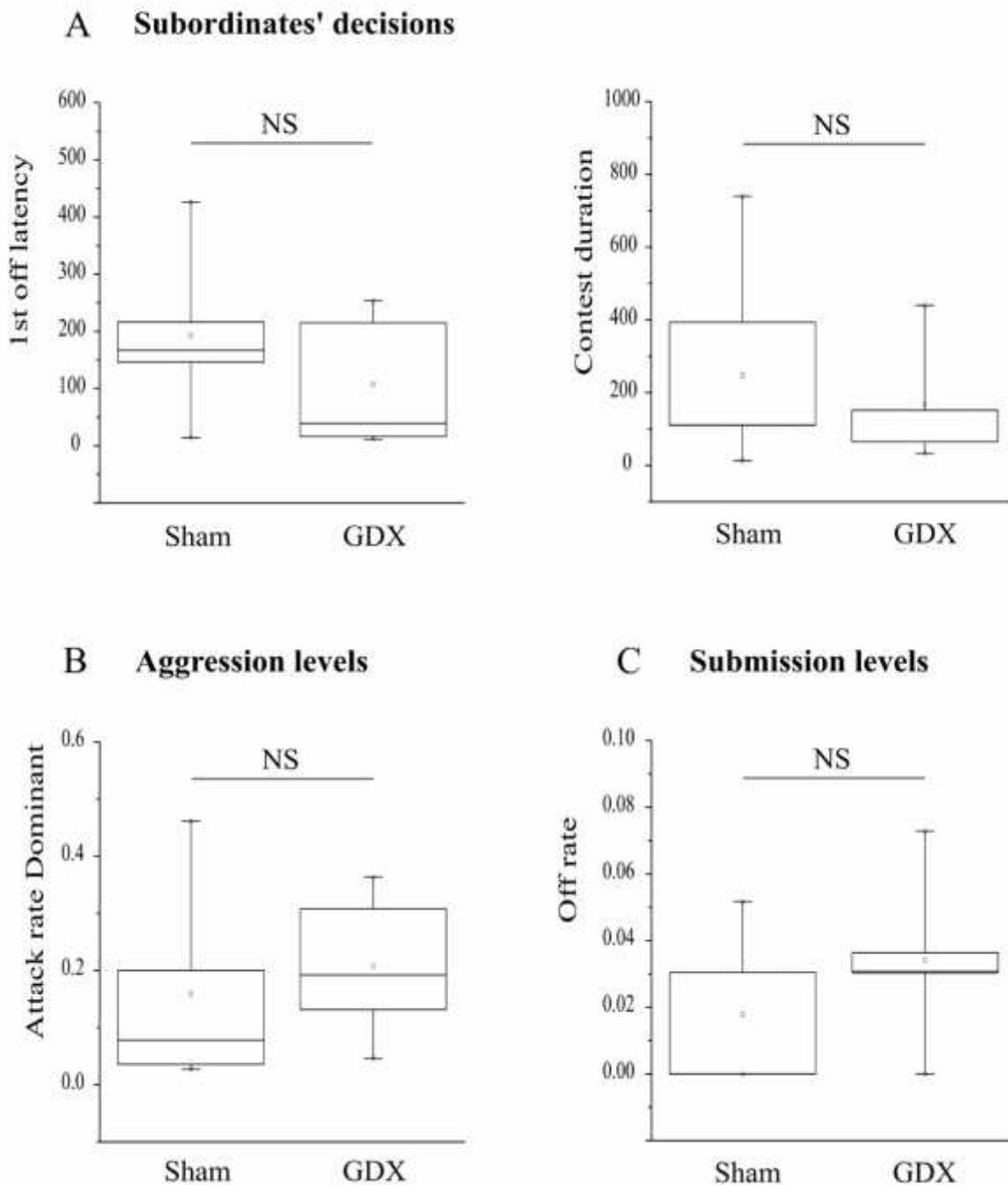


Figure 5: Effects of gonadectomy on male-male agonistic behavior.

A: Box plot representation of the decisions made by the subordinate fish. Gonadectomized fish (GDX) showed no significant difference in comparison to control sham-gonadectomized (Sham) fish in their latency to the first off,  $p = 0.54$  Mann Whitney U test, nor in their contest duration,  $p = 0.97$ , Mann Whitney U test, Sham  $N = 6$ , GDX  $N = 5$ .

B: Box plot representation of aggression levels. Gonadectomy had no effect on dominant attack rate,  $p = 0.53$  Mann Whitney U test, Sham  $N = 6$ , GDX  $N = 5$ .

C: Box plot representation of submission levels. Gonadectomy does not affect off rate,  $p = 0.27$  Mann Whitney U test, Sham  $N = 6$ , GDX  $N = 5$ . Modified from Jalabert *et al.*, 2015. Box plot description in legend of Fig 2.