



Review Paper

The endocrine regulation of cichlids social and reproductive behavior through the eyes of the chanchita, *Cichlasoma dimerus* (Percomorpha; Cichlidae)



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ABSTRACT

Sociobiology, the study of social behavior, calls for a laboratory model with specific requirements. Among the most obvious is the execution of social interactions that need to be readily observable, quantifiable and analyzable. If, in turn, one focuses on the neuroendocrinological basis of social behavior, restrictions grow even tighter. A good laboratory model should then allow easy access to its neurological and endocrine components and processes. During the last years, we have been studying the physiological foundation of social behavior on what we believe fits all the aforementioned requirements: the so called “chanchita”, *Cichlasoma dimerus*. This Neotropical cichlid fish exhibits biparental care of the eggs and larvae and presents a hierarchical social system, established and sustained through agonistic interactions. The aim of the current article is to review new evidence on chanchita's social and reproductive behavior.

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Abbreviations: 11-KT, 11-ketotestosterone; AVT, arginine vasotocin; DI, dominance index; FSH, follicle stimulating hormone; GH, growth hormone; GnRH, Gonadotropin-releasing hormone; HPG, hypothalamic-pituitary-gonadal; LH, luteinizing hormone; MCH, melanin-concentrating hormone; NT, non-territorial; PRL, prolactin; SL, somatolactin; T, territorial.

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1. Introduction

Social interactions that determine a position within a hierarchical system have profound and diverse effects over animals' reproductive behavior and physiology (Maruska and Fernald, 2013). This social control of reproduction is present in many animal species to a higher or lesser extent and, as cichlid fish exhibit many of those attributes, they have been extensively studied in the last decades. The African cichlids, *Astatotilapia burtoni* from Lake Tanganika (Davis and Fernald, 1990; Hofmann et al., 1999; Wapler-Leong

and Reinboth, 1974; reviewed by Fernald and Maruska, 2012; Fernald, 2003) and *Oreochromis mossambicus*, inhabitant of the Limpopo and Zambese Rivers (Neat et al., 1998; Oliveira, 2009; Overmier and Gross, 1974; Ros et al., 2006), are the most investigated species to date.

Our laboratory model is the Neotropical cichlid *Cichlasoma dimerus*, locally known as “chanchita”. This substrate breeding fish shows biparental care of the eggs and larvae (Meijide and Guerrero, 2000), and presents a dominance hierarchy that determines access to breeding territories among males and females. In view of its highly social behavior and effortless maintenance under laboratory conditions, chanchita has emerged as a suitable biological model to investigate the intertwined relationship between hormones, social context, and behavior.

In recent years, we have expounded fundamental aspects of chanchita's reproductive and developmental biology (reviewed in Pandolfi et al., 2009b). For instance, the immunolocalization of pituitary hormones involved in reproduction and background color control was established in both adult and developing individuals (Pandolfi et al., 2001a,b), as well as the effect of FSH on ovarian differentiation (Pandolfi et al., 2006). These findings have been accompanied by the localization of some of their brain-derived controlling hormones, such as GnRH and MCH (Pandolfi et al., 2003, 2005). For example, brain derived gonadotropins were shown – *in vitro* – to control pituitary hormones secretion (Pandolfi et al., 2009a). In parallel to these characterizations, we have developed histological atlases for the brain, gonads and diverse endocrine glands (pineal complex and interrenal gland), elaborated ethograms and carried out descriptions of genes involved in the regulation of behavior, stress and reproduction.

The emerging picture shows *C. dimerus* as an interesting biological model with a solid background for comparative studies, correlating neurohormones, pituitary hormones and behavior throughout all the organism's levels. Presently, we are trying to understand the ever going dialogue between the brain and behavior. The brain affects behavior by the release of neurotransmitters and neuromodulators within specific brain regions, and neurohormones that act on distant target organs. Yet, behavior and social context also dictate brain functioning (Desjardins and Fernald, 2008). To analyze these interactions we mainly focus on perhaps the most important process in an animal's life: reproduction. In this framework, we aspire to elucidate how fish control their access to reproduction and acquire relevant information to improve the culture of economically relevant and potentially edible regional cichlid fish.

The present review aims to summarize the new evidence about the social regulation of reproduction in chanchita with emphasis on its behavior and reproductive and stress physiology, while comparing it with other established cichlid fish model systems. We begin by describing the general aspects of chanchita's social structure; then, we will focus on some of the endocrinological correlates of social and reproductive status and behavior, for both male and female *C. dimerus*. Finally we will analyze the effect of the non-social environment (ie: photoperiod) on aggressiveness and mate selection. At the end, we mention forthcoming directions of our projects.

2. Social life: chanchita's social structure

Fish display a vast repertoire of mating systems, and cichlid species do not lag behind, with monogamy, polygynandry, polygyny and polyandry, among the many represented systems (Barlow, 2000). Mating systems are extremely related to the type of parental care showcased, as the latter may interfere with future mating opportunities (Kuwanura, 1986). Chanchita is an example of a monogamous and substrate breeding species, where both parents

guard eggs and larvae (Meijide and Guerrero, 2000), therefore differing from the more established models *A. burtoni* and *O. mossambicus*, species displaying maternal mouth-brooding in a leklike system (Fernald and Hirata, 1977; Oliveira, 2009).

As social dynamics are clearly enmeshed with the aforementioned parental and mating attributes, distinct and varied patterns of social behavior are expected between cichlid species. For instance, when a group of adult chanchitas is placed in an aquarium under a natural long photoperiod and adequate temperature (25–28 °C), fish begin to display a set of aggressive interactions between them, such as bites, chases, fin erections, opercula openings and submissive displays, like escapes and fin retractions (Alonso et al., 2011). These interactions derive in the establishment and maintenance of a social hierarchy that seems to be linear. Chanchitas' male position within the hierarchy is strongly determined by body size and weight, usually resulting in the largest male emerging as the most dominant individual within the group (Alonso et al., 2011). Frequently, this male forms a pair with a female in the top ranked

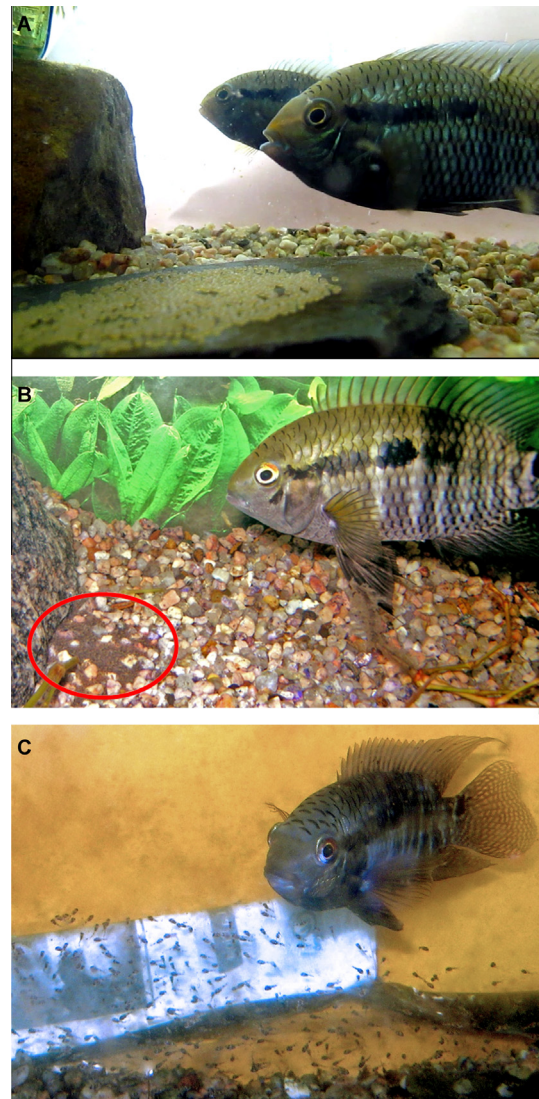


Fig. 1. *Cichlasoma dimerus* parental care. (A) Photograph of a territorial pair taking care of their eggs laid over a flat slab. (B) Photograph of a member of the territorial pair guarding the larvae held within a previously dug pit (red circle). (C) Photograph of a member of the territorial pair protecting the swimming larvae. Although a single parent is seen on the last two photographs, both parents protect the offspring. Generally one of the parents remains guarding the fry while the other feeds or defends the territory.

Table 1
Subordinate fish physiology and social rank.

Correlated variables	Spearman's rank correlation coefficient (r)
♂ Body mass vs. DI	0.84**
♂ STD length vs. DI	0.87**
♂ GSI vs. DI	0.91**
♂ β-LH pituitary content vs. DI	0.91**
♂ Plasma cortisol vs. DI	−0.61*
♀ Plasma cortisol vs. DI	−0.8*

Relationship between different physiological parameters of subordinate male and female chanchita and social rank. DI: dominance index; STD length: standard length; GSI: gonadosomatic index. Asterisk indicates a statistical difference **p* < 0.05; ***p* < 0.01.

positions, and both individuals aggressively defend and expand their territory, where they will spawn and guard the eggs and larvae (Fig. 1). As in chanchita, *A. burtoni* dominant males hold territories that they defend and use plenty of their time trying to court and spawn with females (Fernald and Hirata, 1977).

In chanchita males, size positively correlates with their DI (briefly, the DI takes into account aggressive and submissive displays, with greater values related to a dominant phenotype; Lenher, 1996) (Table 1). In this sense, although bigger males seem to occupy higher ranks, other traits could be implicated in this social arrangement; for example, a recent study performed by Alcazar et al. (2014) in *A. burtoni* males, showed that experience may overcome a size disadvantage. Contrary to males, *C. dimerus* female rank does not appear to be determined by body or ovarian size, suggesting a preponderance of intrinsic features such as aggressiveness, personality or experience.

Chanchitas' T and NT individuals differ in many physiological and behavioral attributes within an established social hierarchy. For instance, they display distinct body color patterns with blotches and stripes that “turn on and off” in specific situations that seem to be related with social status (Alonso et al., 2011). In general, NT individuals exhibit dark background body coloration whereas T ones are brighter and present a dark ventral pigmentation, remarks consistent with observations in other cichlids (Rican et al., 2011). Fast changes in body color patterns may act as intraspecific signals that help fish gather information from social context (O'Connor et al., 1999). Aggressive displays, mostly exhibited by higher ranked individuals, and submissive ones, may be acting as visual cues (Chen and Fernald, 2011).

Hierarchical arrangements are not, however, static; in *A. burtoni*, for example, social phenotypes are plastic and quickly reversible, males may switch between dominant and subordinate statuses multiple times during their lifetime (Maruska and Fernald, 2013). Animals within a social hierarchy engage in energetically costly agonistic encounters to acquire and maintain their position. In this regard, the existence of hierarchical social systems should implicate some kind of benefit to the members of a species, as it's been positively selected (Chase et al., 2002).

Top ranked NT chanchita (higher DI; more aggressive displays) of both sexes ascended after the removal of the T individual from the aquarium. Remarkably, males did not differ in their gonadosomatic indexes (Alonso et al., 2012), which suggests that, although being socially inhibited, NT individuals may not be reproductively incompetent (Maruska and Fernald, 2011).

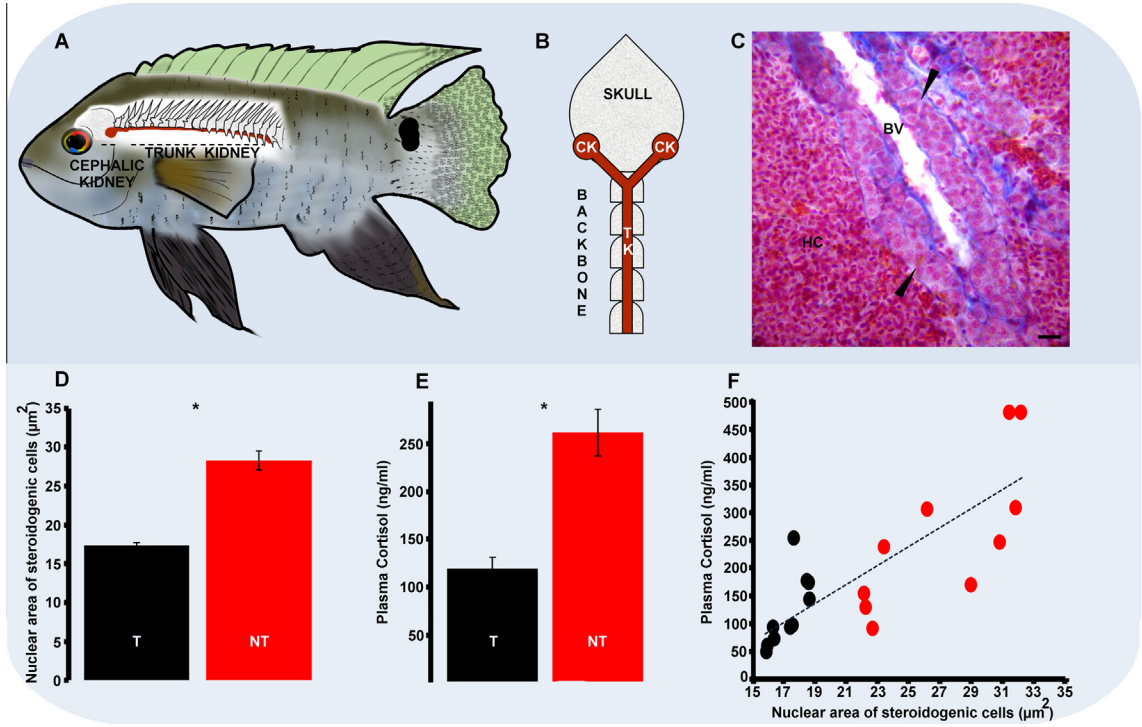


Fig. 2. *C. dimerus* interrenal gland: cortisol synthesis. (A) Drawing highlighting the relative position of the trunk and cephalic kidneys in chanchita. (B) Schematic representation of the ventral view of *C. dimerus* kidney. The trunk kidney (TK) is in intimate contact with the ventral surface of the vertebral column and the ribs. The cephalic kidney (CK) is bilateral and lodged within the head region. Drawings not to scale. (C) Photomicrograph of the interrenal gland in the posterior portion of the cephalic kidney. Cells were arranged in chords (arrowheads) at the margins of the post-cardinal vein and its tributaries (collectively: blood vessels: bv) surrounded by hematopoietic components (hc). These chords were separated from the rest of the parenchyma by a thin layer of connective tissue. Scale bar = 10 μm. (D) Mean nuclear area of steroidogenic cells of territorial (T) vs. non-territorial (NT) males of lowest social rank. (E) Mean cortisol plasma levels of territorial (T) pre-spawning males and non-territorial (NT) males of lowest social rank. For (D) and (E) Values are expressed as means ± SEM. Asterisk indicates a statistical difference (*p* < 0.01). (F) Relationship between nuclear area of steroidogenic cells and plasma cortisol levels. When pooling territorial males (black dots) and non-territorial males (red dots) all together, plasma cortisol levels and steroidogenic cell nuclear area were positively correlated (*r* = 0.80; *p* < 0.0001). Figure modified from Morandini et al. (2014).

Thus, chanchita males and females exhibit a presumable linear social hierarchy, where each individual occupies a delimited position established and sustained by aggressive and submissive interactions. Top ranked NT will be the first to ascend in case an opportunity arises, accompanied by a greater reproductive preparedness. This adjustment may be achieved through their perceived social rank, suggesting that in chanchita males, the reproductive capacity is fixedly coupled with social status.

3. Hormonal landscape: social status, rank and hormones

The outcome of social and reproductive behaviors and their related physiological processes are strikingly dependent on social information from conspecifics (Golan and Levavi-Sivan, 2013). For example, different social contexts modulate hormonal responses in cichlid species (e.g., Maruska et al., 2013; Sessa et al., 2013). On the other hand, inherited factors, previous physiological states or differential experience, modulate behavior (see for example Galhardo and Oliveira, 2014; Taborsky et al., 2012). This push and pull between physiology, behavior and social contexts is abundantly present in chanchita's social life.

3.1. Stress hormones: cortisol

In teleosts, as in mammals, steroidogenic cells are the unique source of cortisol, the main corticosteroid in fish (Mommensen et al., 1999) and, together with chromaffin cells, constitute the fish interrenal gland (Youson, 2007). This gland has been reported to be diffusely located in the trunk and cephalic sections of teleost's kidney (Fig. 2A and B) (Grassi Milano et al., 1997), in close association with the walls of the posterior cardinal vein, its tributaries and sinusoids. In *C. dimerus*, however, it exhibits a more specific location as it is restricted to the posterior portion of both cephalic kidneys (Morandini et al., 2014). In particular, steroidogenic cells can be recognized by their histological characteristics (e.g., spongy cytoplasm), and are arranged in chords of one or more cells, surrounding sinusoids (Fig. 2C) (Morandini et al., 2014).

Physiological and behavioral correlates of stress in social species have been extensively studied in cichlids (e.g., Fox et al., 1997; Galhardo and Oliveira, 2014; Wood et al., 2011) and non-cichlid species (e.g., Backström and Winberg, 2013; Jeffrey et al., 2012, 2014). As cortisol is the main corticosteroid related to stress, and exploiting the fact that chanchita's interrenal gland exhibits a

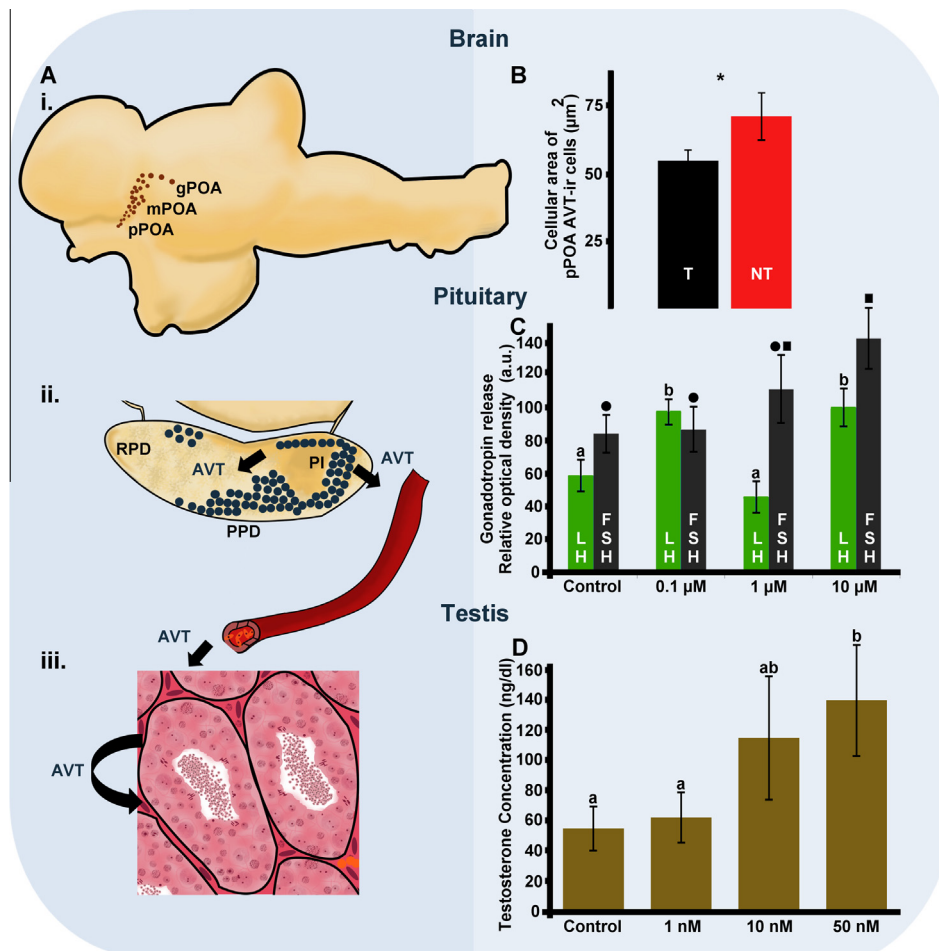


Fig. 3. Chanchita's vasotocinergic system. (A) Schematic representation of the vasotocinergic system in *C. dimerus* across the hypothalamus–pituitary–gonad axis. (A. i) At the level of the brain AVT-immunoreactive cells were found exclusively within the preoptic area (POA), scattered among the parvo- (pPOA), magno- (mPOA), and gigantocellular (gPOA) subpopulations. (B) Non-territorial (NT) males had pPOA cells 23% larger than territorial (T) males ($p < 0.05$). (A. ii.) At the pituitary level, AVT-immunoreactive fibers (blue dots) were observed at the proximal pars distalis (PPD) and mainly at the pars intermedia (PI), while the rostral pars distalis (RPD) showed scarce immunolabeling. (C) Cultured whole pituitary glands exposed to different AVT concentrations prompted the release of LH and FSH analyzed by Western Blot. β -LH (green bars) showed a triphasic response to the presence of AVT in the culture media. Different letters indicate significant differences ($p < 0.01$). β -FSH (gray bars) showed a dose-dependent response. Different symbols indicate significant differences ($p < 0.01$). Values are expressed in arbitrary units (a.u.) as means \pm SEM. (A. iii.) At the testis AVT was locally synthesized by scarce, tapered shaped cells in the interstitial compartment, which might exert a local paracrine action. (D) Incubation of testicular fragments with different AVT concentrations stimulated androgen synthesis in a dose dependent manner. Different symbols indicate significant differences ($p < 0.01$). Values are expressed as means \pm SEM. Figure modified from Ramallo et al. (2012).

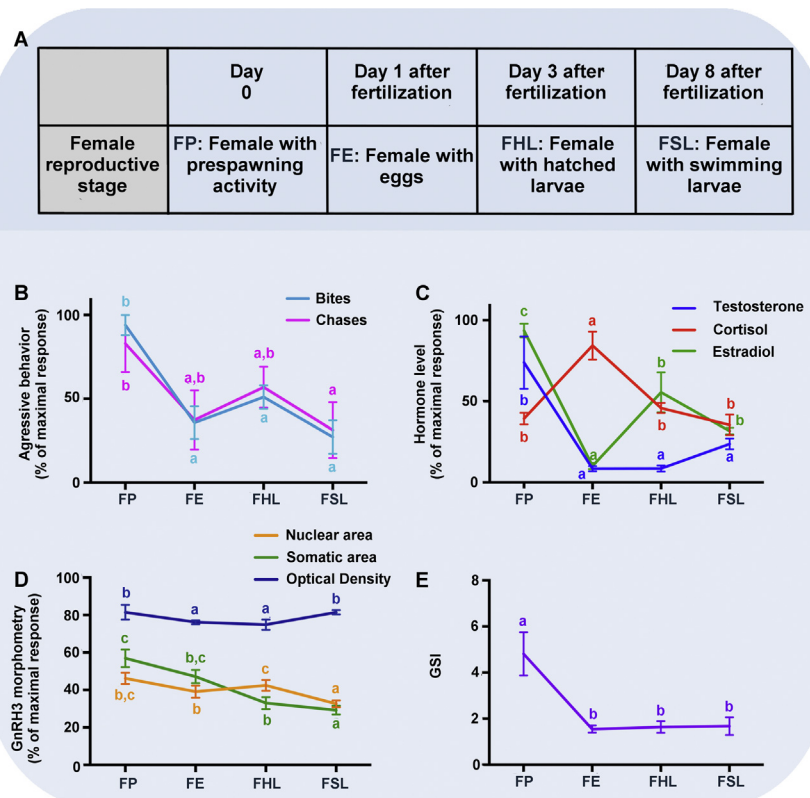


Fig. 4. (A) Female reproductive stage classification according to the spawning event. (B) Aggressive behavior of females in different reproductive stages quantified as the number of bites and chases against the intruder female. Results are presented as the percentage of the maximal response for each parameter. (C) Hormone levels of females in different reproductive stages. Testosterone, estradiol and cortisol were measured. Results are presented as the percentage of the maximal response for each hormone. (D) GnRH3 morphometry of females in different reproductive stages represented by the nuclear and somatic area, and by the optical density of GnRH3 neurons. Results are presented as the percentage of the maximal response for each parameter. (E) Gonadosomatic index of females in different reproductive stages. In all cases data are represented as mean \pm SEM. Different letters indicate significant differences ($\alpha = 0.05$). Figure modified from Tubert et al. (2012).

concrete location, we wondered if T and NT chanchita males' interrenal glands could differ in some of its attributes (Morandini et al., 2014). Nuclear steroidogenic cell area and, therefore, synthetic activity of steroidogenic cells, was greater in NT males of lowest social rank (Fig. 2D), a fact that positively correlated with plasma cortisol levels (Fig. 2F). Similar results regarding the morphometry of interrenal gland cells have been reported only in non-cichlid species (Noakes and Leatherland, 1977; Scott and Currie, 1980). Previously, we had already reported that chanchita NT males and females presented higher plasma cortisol levels than T individuals of their same sex (Alonso et al., 2011), and these plasma levels were negatively correlated with DI in both sexes (Table 1).

In highly social fish species, hierarchies are a foremost source of psychological and physical stress (Gilmour et al., 2005), and this seems to be the case in chanchita. Broad evidence indicates that individuals occupying lower ranked positions are chronically stressed. As found in chanchita, Fox et al. (1997) showed that NT males of *A. burtoni* had significantly higher cortisol levels than T ones. However, an opposite pattern seems to be present in a cooperatively cichlid breeding species, *Neolamprologus pulcher*, where dominants exhibited higher cortisol levels than subordinates (Mileva et al., 2009). Interviewing even more the picture with respect to cortisol levels, in the territorial cichlid Nile tilapia (*Oreochromis niloticus*), similar levels were present in T and NT males (Correa et al., 2003), suggesting that distinct social systems elicit divergent stress responses.

As steroidogenic cell nuclear area varied between two distinct social ranks and positively correlated with the major hormone involved in stress response in fish, the morphometry of interrenal gland cells may be considered as an alternative indicator of chronic

stress. This would be particularly useful in those species in which, by a matter of size, it is difficult to obtain blood samples in order to assess cortisol plasma levels. On the other hand, the punctual localization of chanchitas' interrenal gland makes this species an excellent model to determine the effect of different metabolites and hormones upon cultured interrenal gland cells (e.g., Lim et al., 2013; Medeiros and McDonald, 2012).

3.2. Sex hormones: androgens and gonadotropins

Chanchita T and NT males exhibit distinct androgen and gonadotropin profiles. When analyzing pituitary hormone content through western blot analysis, T males exhibited higher quantities of FSH and SL than NT, probably associated with territory defense, reproduction, body color and parental care, but no differences appeared in LH content (Alonso et al., 2011). Lessened 11-KT plasma levels were present in NT chanchita males of lowest social rank (Morandini et al., 2014) when compared with T ones. With respect to females, no differences appeared in pituitary content of none of the three analyzed hormones (β -FSH, β -LH and SL) (Alonso et al., 2011).

Subordinate fish physiological parameters related to reproduction have been assessed in both established and changing social hierarchies in cichlid fish (Burmeister et al., 2005; Maruska et al., 2011; Maruska and Fernald, 2010, 2011). In chanchita, and once the dynamic of social ascent was characterized, we investigated whether subordinates' physiological and morphometric variables related to reproduction, stress, and body color could be correlated with social rank (Alonso et al., 2012). Although no correlation appeared between DI and testosterone or 11-KT plasma levels,

NT male's social rank was found to be positively correlated with FSH pituitary content and gonadosomatic index (Table 1). In contrast with chanchita males, no correlation was detected between NT females' social rank and the selected morphological and physiological variables related to reproduction (testosterone and 11-KT plasma levels, β -FSH, β -LH and SL pituitary content and spleen-, hepato- and gonadosomatic index) suggesting no reproductive inhibition in this sex at a physiological level.

All together, these results suggest an adaptive mechanism within NT males, consistent with an adjustment of their reproductive investment related to their perceived likelihood of social ascent opportunity. In highly social species males respond to social challenges with a rise in androgen levels ("challenge hypothesis") adjusting their agonistic motivation to changes in the social environment (Antunes and Oliveira, 2009; Oliveira, 2004). As already exposed, retention of some reproductive competence is present beyond the social reproductive suppression (Kustan et al., 2011).

3.3. Neuropeptides: arginine vasotocin

The nonapeptide AVT, homologous to mammalian arginine vasopressin (Urano and Ando, 2011), is mainly known for its osmoregulatory actions. Nonetheless, in the last two decades there has been ever-growing evidence of its involvement in the regulation of social behavior and reproductive physiology (see Godwin and Thompson, 2012, for review). This small peptide acts as a neuro-modulator within the central nervous system, and as a neurohormone secreted by the posterior pituitary. This dual role can be exerted by single neurons, as individual AVT neurons can project to both the neurohypophysis and various brain regions (Goodson and Bass, 2000; Saito et al., 2004). Thus AVT, as we showed in *C. dimerus*, may modulate social and reproductive behavior all across the HPG axis (Fig. 3A) (Ramallo et al., 2012).

In spite of AVT strong linkage to social behavior, there are only a few studies on cichlid fish species (Almeida et al., 2012; Aubin-Horth et al., 2007; Bordieri and Cioni, 2004; Greenwood et al., 2008; Huffman et al., 2012, 2014; Oldfield and Hofmann, 2011; Oldfield et al., 2013; Ramallo et al., 2012). In chanchita, AVT neurons are located exclusively within the preoptic area, distributed among the parvo-, magno-, and gigantocellular subpopulations (homologous to the supraoptic and paraventricular nucleus in tetrapods), lining the border of the third ventricle (Ramallo et al., 2012). This pattern of distribution is consistent with all vertebrate species studied to date, including hagfish, lampreys and gnathostomes (Goodson and Bass, 2001). Different AVT neuronal subpopulations regulate distinct aspects of social behavior. The parvocellular pathways are involved in the activation/modulation of submissive neural circuits or inhibition of aggressive/dominance neural networks, while gigantocellular pathways are hypothesized to be associated with an up regulation of aggressive and courtship behaviors (Greenwood et al., 2008).

After immediate hierarchy establishment, NT *C. dimerus* males showed increased soma area of the parvocellular AVT subpopulation, compared to T ones (Fig. 3B). This may reflect changes in the synthesis, accumulation or AVT release that would be necessary for the maintenance or modulation of social behaviors. Similarly, in *A. burtoni*, AVT expression within the parvocellular nucleus was significantly higher in stable NT males (Greenwood et al., 2008). Moreover, this region has proven to be important in mediating stress responses in fishes, prompting the release of cortisol through the action of AVT on the hypothalamus–pituitary–adrenal axis (Baker et al., 1996; Pierson et al., 1996). In the Mozambique tilapia, NT males had higher AVT pituitary content, which suggest an increased AVT secretion; though no correlation was observed with cortisol plasma levels (Almeida et al., 2012).

Unlike tetrapods, teleost lack a hypophyseal portal system, and brain fibers are in direct contact with cells of the pituitary. Such is the case for vasotocinergic fibers, which in *C. dimerus* are mainly associated with the *pars intermedia*, to a lesser extent with the proximal *pars distalis* and an even lesser degree of association is observed with pituitary cells of the rostral *pars distalis*. The relationship between AVT nerve terminals and the proximal *pars distalis*, where gonadotropes are located (Pandolfi et al., 2006), suggested a possible role of AVT as a modulating factor of the release of gonadotropins. As a consequence, we performed cultures of whole pituitaries, and analyzed the effect of exogenous AVT on β -FSH and β -LH release (Ramallo et al., 2012). AVT stimulated the release of both gonadotropins in a dose-dependent manner (Fig. 3C).

As a neurohormone, AVT synthesized in the brain is released into the bloodstream through the neurohypophysis. Many organs present AVT receptors, and are thus putative targets for AVT action (Lema, 2010). Among those are the testes, which in *C. dimerus* respond by increasing testosterone synthesis (Fig. 3D). Accordingly, *in vitro*, AVT shows an indirect effect (through gonadotropins) and a direct effect on testicular steroideogenesis. In addition, we detected the expression and synthesis of local AVT on testicular interstitial tissue, suggesting a paracrine mode of action (Ramallo et al., 2012). Hence, in chanchita, the AVT system appears to be involved not only in the establishment of social hierarchies but also in the reproductive physiology of the species in a highly complex fashion, with multiple putative sites of action along the HPG axis.

4. It is not all about the males: female aggressiveness

Deep understanding of the social and reproductive behavior of a species requires for the comprehension of the intertwined, sometimes opposing, strategies and counterstrategies of both composing sexes (Hrdy and Williams, 1983). However, with regards to aggressiveness, female behavior has been largely overlooked. Historically, there has been a supported notion that males are more aggressive than females, and that male to male territorial aggression is mainly regulated by androgens (see Chichinadze et al., 2011 for a review on aggression). However, in many species, females can also be aggressive and evidence has been accumulating for its ecological, evolutionary and physiological role (e.g., Borg et al., 2012; Oliveira and Almada, 1996; Renn et al., 2012; Taves et al., 2009; Tubert et al., 2012; Vulliamd et al., 2013).

Chanchita pair members cooperate with the guarding of a spawning site and later take care of the eggs and larvae. In 2012, Tubert et al., classified the reproductive and parental care stages of *C. dimerus*, as being composed of four continuous phases, according to the degree of development of the offspring: pair with pre-spawning activity, pair with eggs, pair with hatched larvae (3 days after fertilization), and pair with swimming larvae (8 days after fertilization) (Fig. 4A). Chanchita females aggressively defend their territory and progeny throughout the four phases, and thus we wondered whether female aggressiveness could be changing qualitatively and quantitatively along them.

Under a resident-intruder paradigm, females from the four phases were exposed to size-matched pre-spawning intruder females. We quantified the frequency of bites, chasings and mouth holdings performed by the local fish. The analysis revealed that pre-spawning females were the most aggressive ones, as they displayed the maximal number of bites (Fig. 4B). This differential investment may result from the fact that fish with eggs and larvae must divide their time between parental displays and defense of their young and territory, whereas pre-spawning individual's main investment is securing a reproductive site. Besides aggressive behavior, testosterone and 17 β -estradiol plasma levels also peaked

in pre-spawning females (Fig. 4C). A highly similar hormonal pattern was observed in *A. burtoni* females, where testosterone peaked four days prior to spawning, while 17 β -estradiol plasma levels reached its maximum two days earlier (Kidd et al., 2013). In teleost fish, estrogens are involved in oocyte growth and maturation, and together with testosterone regulate final oocyte maturation (Peter and Yu, 1997).

In chanchita, GnRH1 and GnRH3 neuronal populations are located in the ventral forebrain with projections that contribute to the pituitary innervations, while GnRH2 is restricted to the mid-brain tegmentum (Pandolfi et al., 2005). The GnRH3 system is believed to act as a neuromodulator within broad areas of the brain, integrating visual, olfactory and somatosensory information (Ramakrishnan and Wayne, 2009; Yamamoto and Ito, 2000), and affecting the outcome of reproductive behaviors (Ogawa et al., 2006). The pre-spawning phase in chanchita females seems to be accompanied by a higher expression, synthesis, and peptide accumulation of GnRH3 than females with eggs or larvae (evidenced through the analysis of nuclear and soma area, and GnRH3-immunoreactive optic density; Fig. 4D). Interestingly, optical density, as an indicator of peptide accumulation, was maximal in pre-spawning females, but it decreased abruptly in females with eggs, which might point for a possible role of GnRH3 in spawning (Tubert et al., 2012).

5. Non-social environment: photoperiod, aggression and mate selection

Timing of breeding to an adequate season is a requirement for a favorable reproductive outcome in seasonally breeding species (Goldman, 2001), such as chanchita. Photoperiodic signals, among other external information, are capable of modifying reproductive behavior and physiology in fish (Andersson et al., 2013; Bulger et al., 2002) and other vertebrate species (Trainor et al., 2006; Walton et al., 2011).

To date, it is not reliably known how photoperiodic cues are endogenously transduced into outputs of the brain–pituitary–gonad axis (Badruzzaman et al., 2013). Nevertheless, recent studies had shed light on the role played by melatonergic and gonadotropin systems (e.g., Servili et al., 2013), with a predominant effect of GnRH3 on reproduction (Karigo and Oka, 2013).

Within this context, we performed some studies to evaluate how different photoperiods could modulate reproductive behaviors in *C. dimerus*, such as males' territorial aggressiveness and pair formation (Fiszbein et al., 2010). With this purpose, we exposed physically isolated individuals to either a short (8L:16D; SP) or a long photoperiod (14L:10D; LP), which mimicked the natural breeding condition for this species) for 4 weeks under constant temperature. When LP males were joined with SP ones (size-matched), the former became dominant in less than 1 h. These observations were supported by quantitative analysis of aggressive interactions and by the fact that LP males initiated the attacks in all the cases. With respect to pair establishment, SP females, contrary to LP ones, never formed a pair with LP males. Moreover, SP females were attacked at the beginning by LP males, and by both LP individuals after the LP pair was established.

Besides behavioral variables, we also characterized some reproductive physiological parameters, such as pituitary hormonal content, GnRH3 cell morphometry and optical density. GnRH3 immunoreactive neurons optical density was reduced in chanchitas' SP males and females (Table 2; Fiszbein et al., 2010). These results provide evidence for a potent link of GnRH3 neurons as neuromodulators of reproductive behavior, taking into account the behavioral outcomes addressed above. Along the same line, in the monogamous New World convict cichlid *Amatitlania nigrofasciatus*, soma size of GnRH1 expressing neurons in the POA varied

Table 2

Effect on photoperiod on male reproductive physiology.

Physiological parameter	LP male	SP male
Optical density of GnRH3-ir neurons (a.u.)	152 \pm 4.5	117 \pm 4.8*
β -LH pituitary content (a.u.)	385 \pm 34	98 \pm 11.3*
PRL pituitary content (a.u.)	305 \pm 131	113.5 \pm 24*
GH pituitary content (a.u.)	427 \pm 66.5	109 \pm 25*
Hepatosomatic index (%)	2.7	1.5*

Mean values (\pm SEM). LP male: long photoperiod exposed male; SP male: short photoperiod exposed male. GnRH3-ir neurons: GnRH-immunoreactive neurons. β -LH: β subunit of luteinizing hormone; PRL: prolactin; GH: growth hormone; a.u.: arbitrary units. Asterisk indicates a statistical difference * p < 0.05.

within social status in males and breeding state in females (Chee et al., 2013).

At the pituitary, chanchita SP males showed lower levels of β -LH, PRL and GH than LP ones (Table 2). These lower GH levels were consistent with lessened feeding activity and reduced hepatosomatic index. On the other hand, as LH and PRL regulate crucial aspects of reproduction in many lineages including fish (Whittington and Wilson, 2013; Yaron and Levavi-Sivan, 2011), their diminished pituitary content in SP males may be related to impaired or altered reproductive performance. Taken together, these results suggest that in chanchita photoperiodic signals are relevant environmental cues for reproductive behavior and physiology.

6. Concluding remarks and future studies

Chanchita *C. dimerus* arises as a suitable model to investigate the intertwined relationship between hormones, social context, and behavior. Several characteristics support the latter consideration: (1) *C. dimerus* is easy to sustain and reproduce under laboratory conditions; (2) its brain, pituitary, gonadal, and interrenal anatomy are well known; (3) relevant reproductive events (sexual differentiation, maturity and annual reproductive cycle) have been extensively described; and (4) it exhibits complex and abounding behavioral repertoires, dissimilarly displayed by individuals of distinct social ranks. Unlike its more studied relatives (*A. burtoni* and *O. mossambicus*), chanchita is a monogamous species with biparental care, which opens a door for the study of parental behavior and associated physiology on both male and female simultaneously, on an accessible and well characterized model system.

Our actual areas of research aim to shed some light on the following inquiries: (a) Is adult neurogenesis modulated by social rank? (b) Which are the signals involved in the social communication between males, females, and individuals from distinct social ranks? (c) What role do serotonergic and dopaminergic systems play in the social control of reproduction? Is it feasible to manipulate those systems to improve the farming of edible and ornamental species?

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