



# Dominance hierarchies and social status ascent opportunity: Anticipatory behavioral and physiological adjustments in a Neotropical cichlid fish

Felipe Alonso <sup>a</sup>, Renato Massaaki Honji <sup>b</sup>, Renata Guimarães Moreira <sup>b</sup>, Matías Pandolfi <sup>a,\*</sup>

<sup>a</sup> Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, (C1428EHA) Buenos Aires, Argentina

<sup>b</sup> Departamento de Fisiologia, Instituto de Biociências-USP, Rua do Matão, travessa 14,n.321, sala 220 Cidade Universitária, São Paulo, SP, Brazil

## ARTICLE INFO

### Article history:

Received 8 August 2011

Received in revised form 8 February 2012

Accepted 3 April 2012

Available online 12 April 2012

### Keywords:

*Cichlasoma dimerus*

Aggressive behavior

Females social hierarchy

## ABSTRACT

In this work we characterized the social hierarchy of non-reproductive individuals of *Cichlasoma dimerus* (Heckel, 1840), independently for both sexes, and its relationship to the opportunity for social status ascent. Female and male individuals who were located on the top rank of the social hierarchy, ascended in social status when the opportunity arose, therefore indicating that dominance is directly correlated with social ascent likelihood. Dominance was positively correlated with size in males but not in females, suggesting for the latter a relationship with intrinsic features such as aggressiveness or personality rather than to body and/or ovarian size. Physiological and morphometrical variables related to reproduction, stress and body color were measured in non-reproductive fish and correlated with dominance and social ascent likelihood. Dominance was negatively correlated with plasma cortisol levels for both sexes. No correlation with dominance was found for androgen plasma levels (testosterone and 11-ketotestosterone). No correlation was detected between dominance and the selected morphological and physiological variables measured in females, suggesting no reproductive inhibition in this sex at a physiological level and that all females seem to be ready for reproduction. In contrast, social hierarchy of non-reproductive males was found to be positively correlated with follicle stimulating hormone (FSH) pituitary content levels and gonadosomatic indexes. This suggests an adaptive mechanism of non reproductive males, adjusting their reproductive investment in relation to their likelihood for social status ascent, as perceived by their position in the social hierarchy. This likelihood is translated into a physiological signal through plasma cortisol levels that inhibit gonad investment through pituitary inhibition of FSH, representing an anticipatory response to the opportunity for social status ascent.

© 2012 Elsevier Inc. All rights reserved.

## 1. Introduction

During the reproductive season, some species establish a social dominance hierarchy which determines access to resources and reproduction for individuals of the highest rank [1]. Predation or environmental changes may generate an opportunity for social ascent of lower ranked individuals, allowing them to reproduce. Cichlid fish (Cichlidae) are a widespread family of Teleost fish that comprises three major monophyletic clades – Malagasy/Indian, African and Neotropical [2]. Cichlids have been widely studied in the field of social control of reproduction since they exhibit complex social behaviors, territoriality and parental care of the fry [3]; however most studied species are of African origin: *Astatotilapia burtoni* (Günther, 1894) and *Oreochromis mossambicus* (Peters, 1858) [4–9]. In contrast,

Neotropical cichlids behavior and physiology is poorly understood although they represent a separate clade of more than 60 genera and 600 species inhabiting a great diversity of environments and presenting particular ecological and behavioral traits as a consequence of their independent evolution from African cichlids what suggest that probably there are considerable differences at the physiological and behavioral levels between these groups. Rapid physiological and genomic changes occur during social status ascent in *A. burtoni* [10–13]. Non-territorial males and animals ascending in social rank showed an increased growth rate, whereas territorial males and animals descending in social rank slowed their growth rate or even shrank [14]; changes in the size of neurons involved in the control of growth (e.g. somatostatin-expressing neurons) were also observed [15]. Differences in reproductive physiology were also found between territorial and non-territorial fish in this species, probably mediated by the hormone cortisol [4]. However, subordinate individuals of *A. burtoni* retain reproductive competence during social suppression [9]. Concordantly, in *Cichlasoma dimerus* (Heckel, 1840), no reproductive inhibition at the gonadal level was observed in non-reproductive individuals [16].

\* Corresponding author at: Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, C1428EHA, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina. Tel.: +54 11 4576 3348; fax: +54 11 4576 3384.

E-mail address: [pandolfi@bg.fcen.uba.ar](mailto:pandolfi@bg.fcen.uba.ar) (M. Pandolfi).

The possibility of an anticipatory physiological response prior to social status ascent has not yet been explored. Is it possible that non-reproductive individuals can adjust their reproductive investment, as an anticipatory physiological response, in relation to the likelihood of social status ascent? Can fish sense this likelihood through its social rank and translate it into a physiological anticipatory response? The aim of this work was to characterize hierarchy of non-reproductive animals and explore these questions using the highly social Neotropical Cichlid fish *C. dimerus* (Heckel, 1840) as a model organism. If social hierarchy predicts the likelihood of social ascent, then it may be of a positive adaptive biological value that rank could be translated into a physiological signal regulating reproductive investment.

In the biparental, substrate brooder, South American cichlid fish *C. dimerus*, the largest male is always dominant and reproductively active. Non-reproductive individuals maintain a linear hierarchy following establishment of the reproductive territories and are reproductively inhibited by behavioral interactions with dominant fish. Compared with reproductive individuals, non-reproductive fish presented higher plasma cortisol levels, lower pituitary content of follicle stimulating hormone (FSH) and somatolactin (SL), hormone involved in body color changes and background adaptation [17]. No inhibition at the gonadal level was observed [16].

The first goal of this study was to characterize social hierarchy and predict which of the non-reproductive individuals would ascend in social status by manipulating social environment. Secondly, we explored the association between hierarchy of non-reproductive individuals and some physiological and morphological variables related to reproduction, body color and stress. Three hypothesis were tested: a) individuals of higher rank in the social hierarchy ascend in social status and form a reproductive pair when an opportunity for social ascent arises; b) Hierarchical rank of individuals is determined by size and body mass; and c) Hierarchical differences between non-reproductive individuals correlate with profiles of different hormones related to reproduction and dominance, in particular, with pituitary content of FSH, SL and luteinizing hormone (LH), and plasma levels of cortisol, testosterone and 11-keto-testosterone. Since reproductive strategies and physiology may differ between sexes, we decided to test these hypotheses independently for males and females.

## 2. Materials and methods

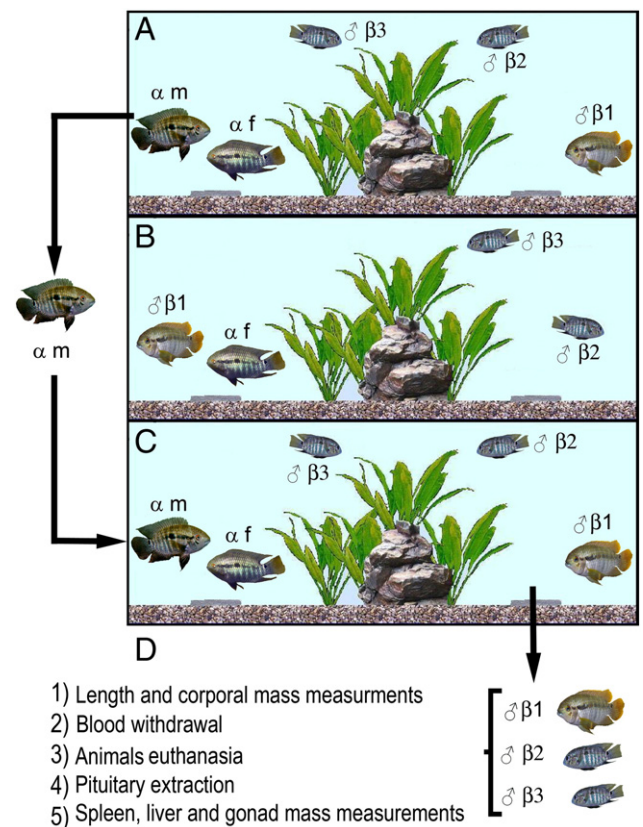
### 2.1. Animals

*C. dimerus* is a substrate breeding Neotropical cichlid fish, endemic to the Paraná and Paraguay River basins in South America. It displays biparental care of the fry and a dominance hierarchy that determines access to breeding territories among males, and to males with territories among females. Prior to the start of the reproductive season, fish exhibit gregarious behavior associated with a pale body color. With the onset of reproductive behaviors, a dominance hierarchy is established through aggressive interactions. Territorial individuals have bright body color patterns and non-territorial fish an opaque gray color. Black ventral coloration is associated with reproductive individuals [16].

The individuals used in this work were captured in Esteros del Riachuelo: 27°35'S; 58°45'W (Corrientes, Argentina) and housed in aquaria under conditions mimicking their natural habitat [16,18] for at least one month before the start of the experiments. Conditions were  $(25 \pm 1)^\circ\text{C}$  and a 14:10 light:dark cycle with full spectrum illumination. A layer of gravel (~4 cm) covered the bottom of the aquaria and natural aquatic plants and stones were placed within it to delimit territories and provide substrate for egg deposition. Animals were fed *ad libitum* every morning with commercial cichlid pellets. Appropriate actions were taken to minimize pain or discomfort of fish, and experiments were conducted in accordance with international standards on animal welfare, as well as being compliant with national regulations and the *Comité Nacional de Ética en la Ciencia y la Tecnología*.

### 2.2. Experimental design

Two sets of experiments were performed, under controlled conditions of temperature and photoperiod, to test dominance hierarchy independently for both sexes. A schematic representation of the experimental design is presented in Fig. 1. For studying male social structure and its related physiology, 1 female and 4 size-matched males were kept in a single aquarium (90 L; density = 27 individuals/square meter). Standard length and mass of fish (expressed as mean  $\pm$  standard error) were  $(9.1 \pm 0.2)$  cm and  $(40.9 \pm 3.0)$  g for males, and  $(9.2 \pm 0.3)$  cm and  $(20.2 \pm 0.9)$  g for females. The aquarium was divided into two territories by a barrier of rocks and plants placed in the middle; each side had a flat dark stone over the gravel, as *C. dimerus* showed preference for this kind of surface to lay their eggs. For the duration of the experiment, fish were observed twice daily for at least 15 min, once in the morning and once in the afternoon, to verify social status, body color patterns and general behavior. The establishment of territories, social status, and reproductive behavior were observed and registered following Alonso et al. [16]. When a reproductive pair was formed and started to defend a territory, social hierarchy was determined by an interaction matrix; a dominance index (DI) [19] was calculated for each individual from this matrix as:  $DI = \sum(W_i/T_i)/N$ , where  $W_i$  is the number of interactions won to a particular individual  $i$ , defined as biting or chasing  $i$ ,  $T_i$  is the number of interactions with the individual  $i$ , and  $N$  is the total number of opponents. To assess which of the



**Fig. 1.** Schematic representation of the experimental design used for males. An analogous design was used for females. A) One female and four males were placed in an aquarium and, when a reproductive pair was formed, the dominance hierarchy was registered and the reproductive male was removed and placed in a separate aquarium. B) Which of the non-reproductive males formed a pair with the female was registered. C) All fish were transferred to a new aquarium along with the previously removed reproductive male. When a reproductive pair was established once again, hierarchy was registered. D) Non-reproductive animals were anesthetized, length and body mass were measured and blood samples were taken. Fish were sacrificed for pituitary extraction and organ dissection. Spleen, liver and gonads were weighed. Abbreviations: α = reproductive male and female, respectively. β1, β2, β3 = non-reproductive males. Dominance hierarchy = ♂ α > β1 > β2 > β3.

non-reproductive males would ascend in social status when the opportunity was given, the reproductive male was removed and kept isolated in a separate aquarium. The non-reproductive male who ascended in social status, formed a pair with the female, what never took more than a day, after what, all the fish were transferred to a new aquarium along with the original dominant male. After two weeks, when social hierarchies and a reproductive pair were established once again and remained stable, a new dominance matrix was recorded to test whether any behavioral differences had occurred compared to the first matrix, before removal of the reproductive male. The aim of this was to control a possible effect of simply removing and replacing the original reproductive male. Removal of the reproductive male for one day only and allowance for the re-establishment of the social hierarchy for two weeks after transfer had the purpose of minimizing this effect. Fish were then anesthetized, length and body mass were measured, and blood samples were taken to determine plasma concentrations of cortisol, testosterone (T) and 11-ketotestosterone (11-KT). Animals were sacrificed by decapitation and gonads, liver and spleen were weighed in order to determine their respective organosomatic indexes (OI) calculated as  $OI = \text{organ mass} / \text{total body mass}$ . Pituitaries were processed as described below for Western blot techniques, in order to semiquantify  $\beta$ -follicle stimulating hormone ( $\beta$ -FSH), somatolactin (SL) and  $\beta$ -luteinizing hormone ( $\beta$ -LH) content. The experiment was repeated 5 times.

For females, an analogous experiment was performed following the same procedure as for males. Briefly, 4 size-matched females and 1 male (standard length and mass, expressed as mean  $\pm$  standard error,  $(7.3 \pm 0.2)$  cm and  $(21.3 \pm 1.6)$  g for females,  $(8.4 \pm 0.1)$  cm and  $(31.4 \pm 2.4)$  g for males, respectively) were placed in an aquarium till establishment of social hierarchies and a reproductive pair. Social hierarchy was studied as described before and the reproductive female was removed and placed in a separate aquarium, and which of the non-reproductive females in the social hierarchy formed a pair with the male was observed. Afterwards, fish were transferred to a new aquarium together with the original dominant female. After two weeks, social hierarchy was registered once again and animals were processed as described above. The experiment was repeated 3 times.

All samples were taken and all physiological and behavioral variables were measured at the same time of day, between 11.30 am and 12.30 pm.

### 2.3. Plasma cortisol, 11-keto-testosterone and testosterone measurement

Blood samples were taken from fish immediately after netting (less than 4 min) by caudal vein puncture in heparinized tubes and allowed to clot. Serum (200  $\mu$ l/animal) was separated by centrifuging the samples at 3000 rpm for 20 min and stored at  $-20^\circ\text{C}$  until assayed. Cortisol (C) levels were measured by ECLIA (electrochemiluminescence immunoassay) using a Cobas analyzer (Roche). Plasma levels of testosterone (T) and 11-ketotestosterone (11-KT) were quantified by hormone enzyme-linked immunoassorbent assays (ELISA) using commercial kits (Interbeck, Virginia, USA for T and Cayman Chemical Company, MI, USA for KT). Analyses were carried out according to the manufacturer's instructions and a standard curve was run for each ELISA plate. Pilot assays using three different dilutions of ten samples (five samples per sex) were run to establish the appropriate working dilution and all samples were assayed in duplicate. The assays were validated with standards provided in the kit indicating that each kit effectively detects *C. dimerus* T, 11-KT and C. The slope of this dilution series was parallel to the standard curve for all kits. Intra e inter-assay tests were also performed for all kits.

### 2.4. Semiquantification of $\beta$ -FSH, $\beta$ -LH, and SL pituitary content

In order to semi-quantify pituitary content of hormones related to reproduction ( $\beta$ -FSH,  $\beta$ -LH) and body color background adaptation (SL), a 15% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blot was performed. Proteins were

immunodetected using heterologous antisera, the specificity of which had been previously tested in this species [20,21]. After dissection, each pituitary was homogenized in 100  $\mu$ l of Tris-HCl buffer 50 mM, pH 7.4, with 1  $\mu$ l of protease inhibitor cocktail (Sigma, St Louis, Mo.). Equal amounts of protein (15  $\mu$ g) were mixed with loading buffer (120 mM Tris-HCl pH 6.8, 3% dodecylsulfate, 10% glycerol, 1%  $\beta$ -mercaptoethanol), heated at  $100^\circ\text{C}$  for 5 min and loaded onto the gel. Following electrophoresis, proteins and molecular markers (See-Blue Plus2 PreStained Standard; Invitrogen) were transferred to a nitrocellulose membrane (Amersham Biosciences) for 60 min at  $4^\circ\text{C}$  and 75 V. Membranes were washed in TBST pH 7.5, blocked overnight with TBST containing 3% non-fat dry milk and incubated for 3 h at room temperature (RT) with the different primary antisera (Table 1). Afterwards, membranes were washed in TBST, incubated with a biotinylated anti-rabbit IgG (Sigma-Aldrich) (1:1000) for 1 h at RT, washed again and finally incubated with a streptavidin complex conjugated to alkaline phosphatase (Sigma-Aldrich) (1:2000) for 45 min at RT. After a final rinse, positive reaction was visualized using an alkaline phosphatase developing kit (BCIP/NBT, Vector Blue, Dako). Developed membranes were dried and digitalized. Pituitary proteins content was semi-quantified by densitometric analysis; optical density of immunoreactive bands was obtained using Image Gauge software version 3.12 (Fuji Photo Film). Hormones optical density was normalized to the optical density obtained for  $\alpha$ -tubuline in order to control possible loading errors in the SDS-PAGE.

### 2.5. Statistical analysis

In each experiment, absolute values of variables were transformed into an ordinal scale to test for correlation between order and dominance. Spearman's ranks correlation coefficients and tests of null correlation were performed to test correlation between order in body mass, length, organosomatic indexes, pituitary content of  $\beta$ -FSH,  $\beta$ -LH and SL, and plasma cortisol and androgen concentrations of each individual with their dominance indexes. Statistical significance was established at  $\alpha = 0.05$  for all tests. In the correlations of body mass and standard length with dominance indexes, reproductive individuals of the corresponding sex were also considered in the analysis, as the relationship between these variables and the dominance hierarchy in general was explored. For the remaining variables, only non-reproductive individuals were considered. Data is presented as mean  $\pm$  SEM. Infostat 2010 software (FCA, Universidad Nacional de Córdoba, Argentina) was used for analyses.

## 3. Results

### 3.1. Reproductive pair establishment, dominance hierarchy and social ascent opportunity

Under experimental conditions, in all replicates of the experiments, both for males and females, the individuals that formed the reproductive pair in the aquarium were the highest in rank of the social hierarchy (those with the higher DI = 1). When one of those individuals was removed, the second in rank (the one with higher DI of the remaining

**Table 1**

Antisera used for the Western blot analysis and molecular mass estimate of *Cichlasoma dimerus* pituitary hormones.

Antisera	Dilution	Source	Donated by	Detected ir-band/s
Anti-SL	1:2000	<i>Sparus aurata</i>	Dr Antonio Astola <sup>a</sup>	32 & 28 kDa
Anti- $\beta$ FSH	1:1000	<i>Fundulus heteroclitus</i>	Dr Akio Shimizu <sup>b</sup>	19 & 15 kDa
Anti- $\beta$ LH	1:2000	<i>Fundulus heteroclitus</i>	Dr Akio Shimizu <sup>b</sup>	24 kDa

<sup>a</sup> Dr. Antonio Astola (Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Cádiz, Spain).

<sup>b</sup> Dr. Akio Shimizu (National Research Institute of Fisheries Science, Fisheries Research Agency, Kanazawa, Yokohama, Japan).



individuals;  $DI=0.66$ ) of the same sex within the social hierarchy ascended in social status to a reproductive state and formed a pair with the remaining member of the original reproductive pair. When the reproductive individual who was removed from the aquarium was reintroduced, in a new aquarium, it rapidly regained its hierarchical position (highest  $DI=1$ ) by aggressive interactions and reconstituted a pair with its original mate displacing the individual which had previously ascended in social status ( $DI=0.66$ ). The reproductive male ( $DI=1$ ) was always the largest one (in terms of standard length and body mass), whereas the second in the social hierarchy ( $DI=0.66$ ), which ascended in social status when the opportunity was given, was the following in size (Figs. 2 and 3).

In contrast, females which formed the original reproductive pair ( $DI=1$ ), as well as the ones which ascended in social status ( $DI=0.66$ ), were not the largest. Females which ascended in social status were always those in the higher hierarchical position ( $DI=0.66$ ), even though, no other correlations were found with any of the morphometrical variables measured.

Males were much more aggressive than females, and also reproductive individuals were more aggressive than non-reproductive ones, especially towards those individuals lowest in the social hierarchy.

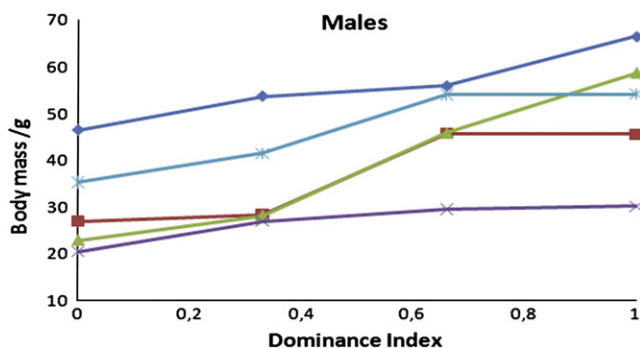
A qualitative correlation between color and dominance was observed, with those animals higher in the social hierarchy being brighter and more colorful, especially males, than those in lower positions, which presented a darker body color pattern. In addition, lower ranked animals generally remained near the water surface while higher ranked fish remained at the bottom of the aquarium.

### 3.2. Size and dominance hierarchy

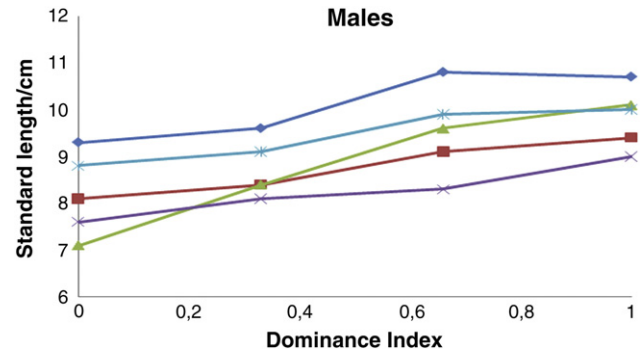
For males, a positive correlation between dominance and body mass ( $r=0.84$ ;  $p=0.00024$ ; Fig. 2) and between dominance and standard length ( $r=0.87$ ;  $p=0.00014$ ; Fig. 3) was found. Males of higher social rank had greater body mass and standard length values than those in lower positions. No similar correlations were found for females (for standard length:  $r=-0.45$ ,  $p=0.14$ ; for body mass:  $r=-0.38$ ,  $p=0.2$ ).

### 3.3. Spleen, liver and gonadosomatic indexes and dominance hierarchy

A positive correlation was found between dominance and gonadosomatic index for males ( $r=0.91$ ;  $p=0.00066$ ; Fig. 4). Males of higher rank in the social hierarchy had larger gonadosomatic indexes than those in a lower position. The other correlations tested, between liver and spleen somatic indexes and dominance hierarchy, were not



**Fig. 2.** Relation between body mass and dominance indexes in males. Reproductive individuals (dominance index = 1) were included in the graph and in the analysis. Data was transformed into an ordinal scale and analyzed together with a Spearman ranks correlation coefficient and test of null correlation. A positive correlation was found between the order of body mass values of the individuals and their dominance indexes for each experiment ( $r=0.84$ ;  $p=0.00024$ ;  $\alpha=0.05$ ). Values of individuals of the same experiment are connected by lines and share the same symbols.



**Fig. 3.** Relation between standard length and dominance indexes in males. Reproductive individuals (dominance index = 1) were included in the graph and in the analysis. Data was transformed into an ordinal scale and analyzed together with a Spearman ranks correlation coefficient and test of null correlation. A positive correlation was found between the order of standard length values of the individuals and their dominance indexes for each experiment ( $r=0.87$ ,  $p=0.00014$ ;  $\alpha=0.05$ ). Values of individuals of the same experiment are connected by lines and share the same symbols.

statistically significant, indicating that no differences were found at this level in the metabolic and immune state of fish (Table 2).

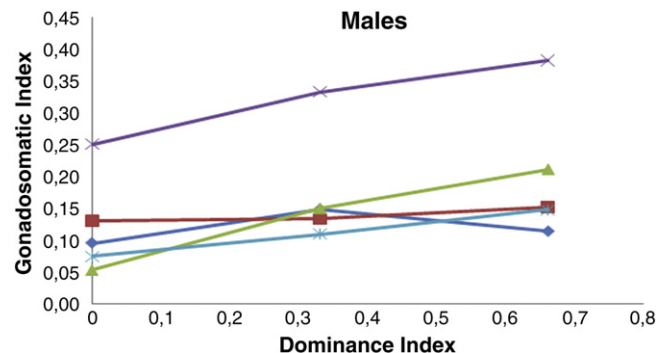
Females of higher social rank and those which ascended in social hierarchy were not the ones with the largest GSI, nor the ones with the largest absolute gonadal mass (results not shown). No correlation was found between liver and spleen somatic indexes and dominance indexes for females.

### 3.4. Plasma cortisol levels and dominance hierarchy

A negative correlation was found between plasma cortisol levels and dominance for both females ( $r=-0.8$ ;  $p=0.02$ ; Fig. 5.A) and males ( $r=-0.61$ ;  $p=0.02$ ; Fig. 5.B). For both sexes, fish of lower rank in the social hierarchy had higher plasma cortisol levels than those in higher ranks.

### 3.5. Testosterone and 11-keto-testosterone plasma levels and dominance hierarchy

No significant correlation was found between 11-keto-testosterone plasma levels and dominance, neither for males ( $p=0.32$ ) nor females ( $p=0.26$ ). Testosterone plasma levels were likewise not significantly correlated with dominance (males  $p=0.94$  and females  $p=0.26$ ).



**Fig. 4.** Relation between gonadosomatic and dominance indexes in males. Only non-reproductive individuals were included in the graph and in the analysis. Data was transformed into an ordinal scale and analyzed together with a Spearman ranks correlation coefficient and test of null correlation. A positive correlation was found between the order of gonadosomatic indexes values of the individuals and their dominance indexes for each experiment ( $r=0.91$ ;  $p=0.00066$ ;  $\alpha=0.05$ ). Values of individuals of the same experiment are connected by lines and share the same symbols.

**Table 2**

Correlation between organosomatic indexes and dominance indexes. Data was transformed into an ordinal scale and analyzed together with a Spearman ranks correlation coefficient ( $r$ ) and test of null correlation ( $p$ ).

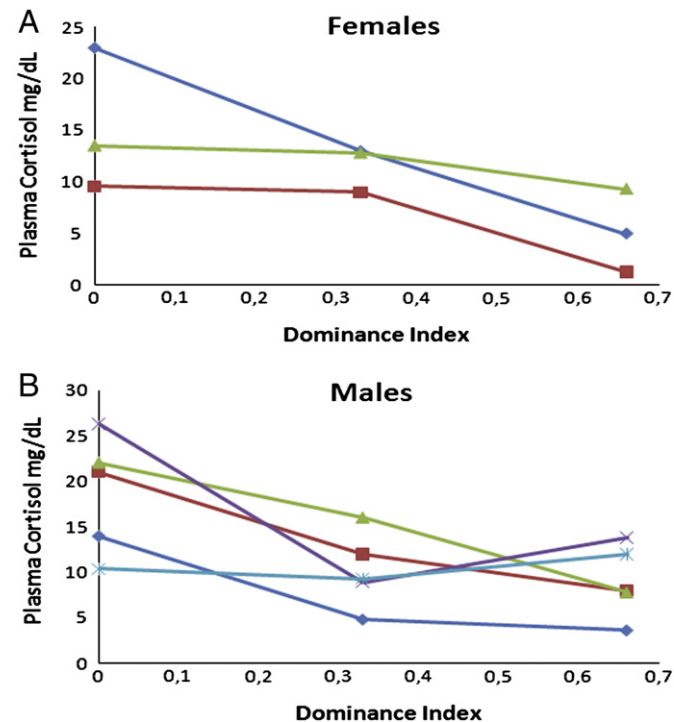
Variables correlated with dominance index				
Variable	Males		Females	
	$r$	$p$	$r$	$p$
Spleen-somatic index	0.22	0.4	0.33	0.38
Hepatosomatic index	0.11	0.69	−0.05	0.89
Gonadosomatic index	0.91	$6.6 \times 10^{-4}$	0.25	0.48

### 3.6. $\beta$ -FSH, $\beta$ -LH, and SL pituitary content and dominance hierarchy

For males, a positive correlation was found between dominance and  $\beta$ -FSH pituitary content ( $r = 0.91$ ;  $p = 0.00066$ ; Fig. 6). Males of higher social rank had greater  $\beta$ -FSH pituitary content levels than those in a lower position. No correlations were detected for the other pituitary hormones measured and dominance (Table 3).

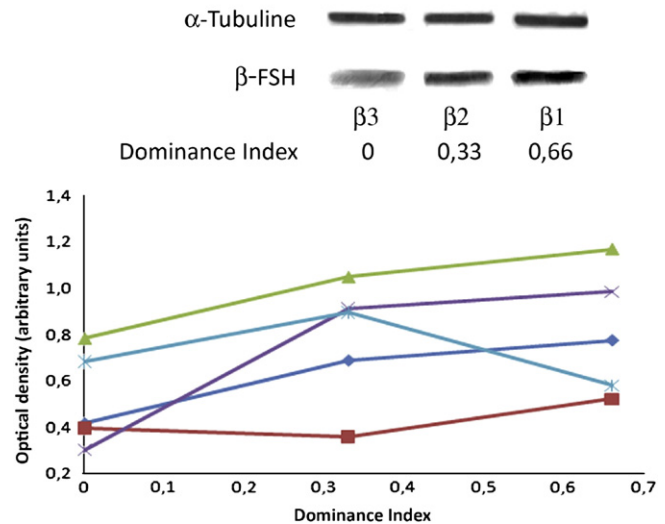
## 4. Discussion

In the present study, social hierarchy of *C. dimerus* among non-reproductive females and males was characterized independently under laboratory conditions. Social hierarchy was also correlated with morphometrical variables and hormones related to color change (SL) [17] and reproductive physiology. The relationship of these variables with dominance and the opportunity for social status ascent was explored. It is important to remark that differences between reproductive and non-reproductive fish regarding general behavior and reproductive physiology have been previously characterized in this species [16]. We found that social hierarchies were linear for both sexes. Social rank



**Fig. 5.** Relation between plasma cortisol levels and dominance indexes in females (A) and males (B). Only non-reproductive individuals were included in the graph and in the analysis. Data was transformed into an ordinal scale and analyzed together with a Spearman ranks correlation coefficient and test of null correlation. A negative correlation was found between the order of plasma cortisol levels of the individuals and their dominance indexes for each experiment (for females  $r = -0.8$ ;  $p = 0.02$ ; and for males  $r = -0.61$ ;  $p = 0.02$ ;  $\alpha = 0.05$ ). Values of individuals of the same experiment are connected by lines and share the same symbols.

### Males $\beta$ -FSH pituitary content



**Fig. 6.** Relation between  $\beta$ -FSH pituitary content and dominance indexes in males. Only non-reproductive individuals were included in the graph and in the analysis. Data was transformed into an ordinal scale and analyzed together with a Spearman ranks correlation coefficient and test of null correlation. A positive correlation was found between the order of  $\beta$ -FSH pituitary content of the individuals and their dominance indexes for each experiment ( $r = 0.91$ ;  $p = 0.00066$ ;  $\alpha = 0.05$ ). Values of individuals of the same experiment are connected by lines and share the same symbols.

was size dependent in males, but not in females, in which there were no correlations between standard length and body mass with dominance indexes. Female social hierarchy in *C. dimerus* appears therefore to be dependent on other variables not measured in this study, such as motivation or personality, as it was described for males of the bluefin killifish, *Lucania goodei*, in which neither behavioral type nor dominance rank was associated with body size or body condition [22]. The repeatability of behavioral types and stability in the outcome of aggressive interactions suggest that behavioral phenotypes are inherent characteristics of individuals rather than short-term responses to recent social experience or daily levels of food or stress, and may potentially have an early environmental and/or genetic basis [22].

We also studied the relationship between dominance hierarchy of non-reproductive individuals and the likelihood of social status ascent (related to a reproductive opportunity). We found that non-reproductive individuals with the highest social rank (a higher dominance index) were always the ones to ascend to a reproductive status when the opportunity for social ascent was given by removing the reproductive individual of the same sex. The maintenance of hierarchies among non-reproductive individuals seems to be directly related to the likelihood of social ascent. Nevertheless, it can also be related with food and shelter acquisition, as fish of a lower rank remained near the water surface, a position exposing them to predation by birds in natural habitats.

**Table 3**

Correlation between pituitary content of the selected hormones and dominance indexes. Data was transformed into an ordinal scale and analyzed together with a Spearman ranks correlation coefficient ( $r$ ) and test of null correlation ( $p$ ).

Variables correlated with dominance index				
Variable	Males		Females	
	$r$	$p$	$r$	$p$
$\beta$ -FSH	0.91	$6.6 \times 10^{-4}$	−0.05	0.88
$\beta$ -LH	0.46	0.08	−0.20	0.57
SL	0.46	0.08	−0.65	0.06

We also found a negative correlation between plasma cortisol levels and dominance of non-reproductive individuals for both sexes. Cortisol could be acting as an endogenous signal of social stress due to the aggressive interactions perceived by the individuals, what could represent different levels of chronic stress. This may be indirectly mediating dark body color of subordinate individuals as a submissive display. Also, we have observed in a previous work that reproductive individuals had lower cortisol levels than non-reproductive ones, which perceived greater levels of aggressions [16].

Remarkably, no significant correlation was found between androgens (Testosterone and 11-keto-testosterone) and dominance for either sex. Although the existence of a possible effect between these factors cannot be discarded due to the low number of replicates assayed ( $n = 5$  for males,  $n = 3$  for females), these results are in agreement with the 'challenge hypothesis' which states that during periods of social stability male androgen levels are lower than during socially unstable times [23]. Changes in androgen levels are expected to occur after a social ascent opportunity and not before. There seems to be a tighter connection with transient increases in androgens with aggression compared to dominance status. In the African cichlid *A. burtoni*, circulating 11-ketotestosterone (11-KT) levels were elevated within 30 min following social opportunity, coincident with increased aggressive behavior. However, territorial behaviors and serum 11-KT levels were then dissociated by 72 h after social ascent, suggesting either rapid social stability and/or increased physiological potential for androgen production [11]. Taken together, these results suggest that before an opportunity for social ascent presents itself androgens may not differ between non-reproductive animals of different hierarchical levels, however an increase in androgen levels would be triggered when social opportunity is perceived. Short time studies should be performed in *C. dimerus* to address this issue. Also, in *Neolamprologus pulcher* (Trewavas & Poll, 1952), relative levels of 11-KT and testosterone are implicated in female dominance behaviour [24].

In a previous work, we found that reproductive *C. dimerus* males had higher pituitary levels of FSH and SL than non-reproductive males, while no differences were found among females [16]. In the present study, dominance was positively correlated with FSH pituitary content and gonadosomatic indexes for non-reproductive males. This could be the result of gonadal inhibition of lower ranked males through lower FSH levels, in turn induced by an increase in plasma cortisol levels due to aggressive interactions. Inhibition would be stronger in lower ranked individuals, making them less likely to ascend in social status. This regulatory mechanism would be of positive adaptive value for those individuals since they would be able to invest more energy on growth and less energy on territory defense, aggressive interactions, and gonadal investment, being therefore more likely to secure a reproductive opportunity in the future by ascending in hierarchy. In accordance with this hypothesis, in the African Cichlid *Neolamprologus pulcher*, fish with low aggressiveness grew faster than aggressive fish [25]. In addition, in *Haplochromis burtoni*, non-territorial males and animals ascending in social rank showed an increased growth rate whereas territorial males and animals descending in social rank slowed their growth rate or even shrank [14]. No inhibition of the reproductive axis was detected in females, suggesting a different reproductive strategy, although the existence of differences cannot be discarded due to the low number of replicates ( $n = 3$ ). In the gregarious African cichlid *Neolamprologus pulcher*, subordinate females are reproductively capable, but apparently egg laying is behaviorally suppressed [26].

No correlation was found between SL pituitary content and dominance indexes (males  $r = 0.46$ ,  $p = 0.08$ ; females  $r = -0.65$ ,  $p = 0.06$ ). However, the lack of statistical significance could be the result of low statistical power related to the relatively low sample size ( $n = 5$  in males,  $n = 3$  in females) rather than to a true absence of correlation, since differences in the intensity of body color patterns could be qualitatively observed between fish of different hierarchical ranks.

Males of the Neotropical convict cichlid *Amatitlania nigrofasciata* (Günther, 1867) prefer gravid over non gravid females, and the largest between two gravid females [27]. A similar preference was not found for *C. dimerus*, as the female which ascended in social status to a reproductive state was neither the largest nor the one with the greatest gonadosomatic index or absolute gonadal mass. The only observable predictor of which female would ascend in social status was their position in the social hierarchy. Males do not appear to be choosing the reproductive female or, if they are, choice is not based on the possible number of eggs laid. Alternately, males could be choosing dominant females since they would be better at defending the offspring, behavior favored under these experimental conditions with a high density of animals. If a decrease in predator pressure was perceived by males, choice could shift from fry defense (more aggressive female) to maximizing clutch size (females with larger gonads that could lay more eggs). In our experiments, females could not exercise choice since there was only one male with a reproductive territory. Higher ranked females could also be establishing a reproductive pair by preventing males from choosing, not allowing contact between the male and lower ranked females.

In *A. burtoni*, disruption of the habitat of a colony caused males to switch social status more frequently than animals kept in a stable environment [14]. In *C. dimerus*, males in natural populations would normally establish a hierarchy determined by size granting access to reproductive territories. Females would also establish a social hierarchy giving priority in choosing males with territories. When an opportunity for social ascent is generated by predation or habitat modifications (i.e.: floodings), individuals immediately below in the social hierarchy would access a reproductive territory, and hence a chance to form a reproductive pair with a female, in the case of males, or a male with an established reproductive territory in the case of females. In this scenario, males brighter colors and size may be traits selected by females. Although intrasexual selection related to territory defense could also account for this trait, it may have been reinforced by intersexual selection (female's choice).

A possible effect of simply removing the reproductive male (Fig. 1.B) on the physiological variables measured could not be completely discarded, although this effect was minimized and controlled by the experimental design. A dominance matrix was registered and general behavior was observed in fish during all three stages (Fig. 1.A, .B and .C), and no behavioral differences were found between the stages represented in Fig. 1.A and .C. The stage corresponding to Fig. 1.B never lasted more than one day, since the non-reproductive individual quickly ascended in social status and was then replaced by the "original" reproductive individual (Fig. 1.C). This resulted in a very brief period during which the fish ascending in social status was completely dominant before returning to its original social environment. After reintroducing the reproductive individual with the remaining fish in a new aquarium, fish were allowed two weeks to reconstitute social hierarchies and stabilize their physiology according to their new social status before sample collection for physiological variables measurements. We consider that this design minimizes the effect of removal of the dominant fish, which was corroborated by behavioral observations.

In this work we showed that non-reproductive *C. dimerus* fish exhibit a social hierarchy that determines social status ascent when given an opportunity. In males, this was related to size and mass of the individuals and with physiological changes at the pituitary-gonadal axis (FSH and GSI) suggesting that males make physiological adjustments previous to an opportunity for social ascent, related to the likelihood of social ascent as a result of dominance hierarchy.

## Acknowledgments

We would like to dedicate this work to our former president Dr. Nestor Kirchner (1950–2010) for the support given to all scientists

in Argentina. We also would like to thank three anonymous reviewers for their pertinent comments and suggestions that helped to improve the present manuscript. This work was supported by the following grants: PICT 75 (Agencia de Promoción Científica y Técnica), UBACyT X-053 (Universidad de Buenos Aires) and PIP 0020 (CONICET).

## References

- [1] Dewsbury DA. Dominance rank, copulatory behavior, and differential reproduction. *Q Rev Biol* 1982;57:135–59.
- [2] Farias IP, Ortí G, Meyer A. Total evidence: molecules, morphology, and the phylogenetics of cichlid fishes. *J Exp Zool* 2000;288:76–92.
- [3] Keenleyside MHA. Cichlid fishes—behavior, ecology, and evolution. London: Chapman and Hall; 1991.
- [4] Fox HE, White AW, Kao MH, Fernald RD. Stress and dominance in a social fish. *J Neurosci* 1997;17:6463–9.
- [5] Ogawa S, Akiyama G, Kato S, Soga T, Sakuma Y, Parhar IS. Immunoneutralization of gonadotropin-releasing hormone type-III suppresses male reproductive behavior of cichlids. *Neurosci Lett* 2006;403:201–5.
- [6] Grosenick L, Clement TS, Fernald RD. Fish can infer social rank by observation alone. *Nature* 2007;445:429–32.
- [7] Antunes RA, Oliveira RF. Hormonal anticipation of territorial challenges in cichlid fish. *Proc Natl Acad Sci U S A* 2009;106:15985–9.
- [8] Oliveira RF, Silva A, Canário AVM. Why do winners keep winning? Androgen mediation of winner but not loser effects in cichlid fish. *Proc R Soc B* 2009;276:2249–56.
- [9] Kustan JM, Maruska KP, Fernald RD. Subordinate male cichlids retain reproductive competence during social suppression. *Proc R Soc B* 2011, <http://dx.doi.org/10.1098/rspb.2011.0997> rspb.2011.0997v1-rspb20110997.
- [10] Burmeister SS, Jarvis ED, Fernald RD. Rapid behavioral and genomic responses to social opportunity. *PLoS Biol* 2005;3:e363.
- [11] Maruska KP, Fernald RD. Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish. *Horm Behav* 2010;58:230–40.
- [12] Maruska KP, Fernald RD. Plasticity of the reproductive axis caused by social status change in an African cichlid fish: II. Testicular gene expression and spermatogenesis. *Endocrinology* 2011;152:291–302.
- [13] Maruska KP, Levavi-Sivan B, Biran J, Fernald RD. Plasticity of the reproductive axis caused by social status change in an African cichlid fish: I. Pituitary gonadotropins. *Endocrinology* 2011;152:281–90.
- [14] Hofmann HA, Benson ME, Fernald RD. Social status regulates growth rate: consequences for life-history strategies. *Proc Natl Acad Sci U S A* 1999;96:14171–6.
- [15] Hofmann HA, Fernald RD. Social status controls somatostatin neuron size and growth. *J Neurosci* 2000;20:4740–4.
- [16] Alonso F, Cánepa M, Guimarães Moreira R, Pandolfi M. Social and reproductive physiology and behavior of the social cichlid fish *Cichlasoma dimerus* under laboratory conditions. *Neotrop Ichthyol* 2011;9:559–70.
- [17] Cánepa MM, Pandolfi M, Maggese MC, Vissio PG. Involvement of Somatolactin in background adaptation of the cichlid fish *Cichlasoma dimerus*. *J Exp Zool* 2006;305:410–9.
- [18] Casciotta JR, Almirón AE, Bechara J. Peces del Iberá. Hábitat y Diversidad. La Plata, Argentina: UNDP, Fundación Ecos, UNLP, UNNE; 2005. Ed Glafikar.
- [19] Lenher PN. Handbook of ethological methods. 2° ed. Cambridge University Press; 1996.
- [20] Pandolfi M, Paz DA, Maggese MC, Ravaglia MA, Vissio PG. Ontogeny of immunoreactive somatolactin, prolactin and growth hormone secretory cells in the developing pituitary gland of *Cichlasoma dimerus* (Teleostei, Cichlidae). *Anat Embryol (Berl)* 2001;203:461–8.
- [21] Pandolfi M, Lo Nostro FL, Shimizu A, Pozzi AG, Meijide FJ, Rey Vazquez G, et al. Identification of immunoreactive FSH and LH cells in the cichlid fish *Cichlasoma dimerus* during the ontogeny and sexual differentiation. *Anat Embryol (Berl)* 2006;211:355–65.
- [22] McGhee KE, Travis J. Repeatable behavioural type and stable dominance rank in the bluefin killifish. *Anim Behav* 2010;79:497–507.
- [23] Wingfield JC, Hegner RE, Dufty AM, Ball GF. The “Challenge Hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat* 1990;136:829–46.
- [24] Taves MD, Desjardins JK, Mishra S, Balshine S. Androgens and dominance: sex-specific patterns in a highly social fish (*Neolamprologus pulcher*). *Gen Comp Endocrinol* 2009;161:202–7.
- [25] Riebli T, Avgan B, Bottini AM, Duc C, Taborsky M, Heg D. Behavioral type affects dominance and growth in staged encounters of cooperatively breeding cichlids. *Anim Behav* 2011;81:313–23.
- [26] Heg D. Reproductive suppression in female cooperatively breeding cichlids. *Biol Lett* 2008;4:606–9.
- [27] Nuttal DB, Keenleyside MHA. Mate choice by the male convict cichlid (*Cichlasoma nigrofasciatum*; Pisces, Cichlidae). *Ethology* 1993;95:247–56.