

Fighting cichlids: Dynamic of intrasexual aggression in dyadic agonistic encounters



María Florencia Scaia^{a,b,*}, Leonel Morandini^{a,b}, Cristobal Alejandro Noguera^{a,b},
Martín Roberto Ramallo^{a,b}, Gustavo Manuel Somoza^c, Matías Pandolfi^{a,b,*}

^a Instituto de Biodiversidad y Biología Experimental y Aplicada – CONICET, Ciudad Autónoma de Buenos Aires, Argentina

^b Laboratorio de Neuroendocrinología y Comportamiento, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

^c Laboratorio de Ictiofisiología y Acuicultura, Instituto de Investigaciones Biotecnológicas– Instituto Tecnológico de Chascomús (IIB-INTECH. CONICET-UNSAM), Chascomús, Argentina

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ABSTRACT

Aggression is an extremely complex behaviour and female aggression is understudied when compared to males. Despite the fact that it has been suggested that conflict among females may be more frequently resolved peacefully, in many species females show high levels of aggression. We used *Cichlasoma dimerus* to describe dynamics and conflict outcome in intrasexual agonistic encounters. We performed encounters of two sex-matched animals in a neutral arena and we recorded agonistic interactions during one hour. All aggressive and submissive behaviours were described and quantified to perform the ethogram. Encounters followed three phases: pre-contest, contest and post-resolution. Latency, time of resolution and frequency of aggressive displays did not differ between sexes. Relative variations in size between female opponents better explained aggression outcome in each contest, since higher levels of aggression occurred in dyads of more similar fish. However, this was not observed in males, suggesting that probably morphological characteristics could be less relevant in male conflict resolution. Altogether these results suggest that in this ethological context, *C. dimerus* females are as aggressive as males and that they have similar motivation towards territorial aggression, emphasizing the need of deepening the study of aggression in females and not only in males.

1. Introduction

Social behaviours such as courtship, mating and aggression, involve competition for limited resources as food, mating opportunities, shelter and/or social status. This competition is based on a conflict of interest between competing animals, which can be resolved peacefully or with overt aggressive behaviour if the benefits are larger than the risks or than energetic costs during the interaction (Cant and Young, 2013; Johnsson et al., 2006). Aggression can be defined as an overt behaviour that leads to displacing, dominating or harming other individual (Nelson, 2006). Depending on the context, different types of aggression can be defined, such as territorial aggression, aggression over food, aggression over dominance status, sexual aggression (mate acquisition and mate guarding) and parental aggression (Wingfield et al., 2006).

Aggression is an extremely complex behaviour, and can be influenced by social, environmental, genetic and physiological factors. Mechanisms involved in regulating aggression allow or constraint

phenotypic plasticity and generate inter-individual variation which is, in turn, shaped by evolution (Adkins-Regan 2008, 2012; Ketterson and Nolan, 1999). Even though there are many species differences, recent studies suggest that at least some of the molecular and physiological mechanisms regulating aggression are phylogenetically conserved among vertebrates (Goodson 2005; Thomas et al., 2015). This allows us to select species as biological models based on their behaviour and not necessarily on taxonomic criteria, and to broaden insights which can potentially enlighten knowledge on all vertebrates (Goodson, 2005).

Aggression has been historically analyzed and described in male intrasexual competitions, rather than in female encounters. For example, one of the first classifications defining types of aggression referred to intermale aggression and, instead of defining interfemale aggression *per se*, female aggressive behaviour was related to parental activity and defined as *maternal aggression* (Moyer, 1968). More recent definitions refer to parental aggression as both maternal and paternal (Wingfield et al., 2006) and research on female aggression can be

* Corresponding authors at: Ciudad Universitaria, Intendente Güiraldes 2160, Pabellón 2, Piso 4°, Lab. 26. Ciudad Autónoma de Buenos Aires, Buenos Aires, (CP:C1428EGA), Argentina.

E-mail addresses: mflorenciascaia@bg.fcen.uba.ar (M.F. Scaia), pandolfi@bg.fcen.uba.ar (M. Pandolfi).

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addressed not necessarily related to maternal behaviour (Klass and Cords, 2015; O'Connell et al., 2013; Rendon et al., 2015; Sterck et al., 1997). However, many authors have analyzed female aggression and its physiological mechanisms intrinsically related to maternal care and reproductive stages (Maruska and Fernald 2010; Tubert et al., 2012).

When analyzing the evolution of aggression and social behaviour, some authors have developed a game-theoretic model to examine the conflict between players (Cant, 2012; Smith, 1974; Wyse et al., 2017). Based on these theoretical models, it has been suggested that reproductive conflict among females may be more frequently resolved peacefully rather than with overt aggression when compared to conflict among males, because offspring is more costly to produce for females and reproduction is more difficult to conceal (Cant and Young, 2013). In spite of this, and even though males have been historically considered as more aggressive than females, in many species females show high levels of aggressive behaviour, and yet female aggression is an understudied topic (Borg et al., 2012; Oliveira and Almada, 1996; Renn et al., 2012).

Cichlid fish are very suitable models to study male and female aggression, since they have hierarchical social systems in which dominant individuals defend their status by aggressive displays towards other submissive, lower-ranked animals (Baerends and Baerends-van Roon, 1950; Barlow, 2000; Maruska, 2014; Sefc, 2011). In particular, our model species *Cichlasoma dimerus* is a Neotropical cichlid locally known as *Chanchita*. It is a serially monogamous species with a bi-parental behaviour in which both dominant territorial reproductive male and female aggressively defend their territory from subordinate, lower-ranked animals (see Ramallo et al., 2014 for review). However, most studies refer to social context (Ramallo et al., 2015, 2017) or to a resident-intruder paradigm (Tubert et al., 2012) instead of analyzing dyadic contests in neutral arena, which would then allow testing several physiological mechanisms involved in aggression, such as the challenge hypothesis (Almeida et al., 2014; Archer, 2006), endocrine regulation in winners and losers (Baird et al., 2014; Earley et al., 2013; Oliveira et al., 2009a, 2009b) and androgen or estrogen regulation of aggression (reviewed by Rosvall, 2013; Trainor et al., 2006).

Considering that the mechanisms involved in regulating aggression are key elements to understand evolutionary constraints of this social behaviour, we analyzed *C. dimerus* aggressive and submissive behaviour as a first step to deepen our knowledge on physiological and neuroendocrine mechanisms regulating aggression. The main purpose of this study is to describe the dynamics of intrasexual dyadic encounters in this species, to analyze and compare male and female aggressive and submissive displays and to assess to what extent aggressive behaviour can be explained by morphological variables of animals involved in each encounter.

2. Materials & methods

2.1. Animals and holding conditions

C. dimerus adults were caught in Esteros del Riachuelo (27°35'S; 58°45'W; Corrientes, Argentina) and a total of 34 animals were used for experiments. Animals were transferred and housed in acclimation aquaria (150 L, 8–10 fish per tank) containing artificial aquarium plants and stones for at least one month before experiments. Specimens were fed daily with food pellets (Koi Vibrance Color Enhancer Fish Food, Tetra Brand) and were maintained under conditions similar to their natural reproductive habitat (25 ± 2 °C; 14:10 light:dark, Casciotta et al., 2002). Holding conditions and the experimental design were performed in accordance with international standards on animal welfare, minimizing pain or discomfort to the animals. All procedures were compliant with the Guidelines for the Use of Animals in Research (Animal Behaviour, 1991), with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) which are in accordance and with local regulations.

Six randomly selected *C. dimerus* adults were placed in social tanks (53 L; 25 ± 2 °C; 14:10 light:dark) to allow the establishment of dominant pair as previously described (Alonso et al., 2011; Ramallo et al., 2015). Each aquarium contained artificial aquarium plants, a layer of gravel (~2 cm) and a flat slab, which fish use to delimit their territory and to lay their eggs on. In order to study intrasexual aggressive behaviour in dyadic agonistic encounters, two sex-matched animals were simultaneously and randomly selected from different aquaria, thus excluding possible recent interactions, and were immediately isolated in individual aquarium (isolation aquaria, 21 L; 25 ± 2 °C; 14:10 light:dark) during seven days. Fish were fed daily with food pellets. Both animals were unable to see each other but were able to see a same isolated juvenile, which was placed in a small aquarium in front of both isolation aquaria. It is worth mentioning that the same juvenile was maintained throughout all encounters. Considering that *C. dimerus* is a social species, this arrangement minimized possible stress due to isolation and, since the juvenile fish was smaller than the adults, its presence did not represent a threat to the experimental fish.

2.2. Agonistic encounters

A total of 9 female–female and 8 male–male dyadic agonistic encounters were analyzed for this study. Each encounter was performed in an experimental tank with no gravel nor plants (experimental tank, 21 L; 25 ± 2 °C). In each encounter, both fish were simultaneously placed in the experimental tank and they were allowed to swim freely. All interactions were videotaped and aggressive and submissive

Table 1

Ethogram outlining the aggressive and submissive behavioural repertoire during sex-matched dyadic agonistic encounters of *C. dimerus*.

Aggressive displays	Physical contact	Bite	Mouth of focal fish hits the body (fins or sides) of its opponent.
		Tail hit	Focal fish performs an antero-posterior waving of the body with its tail, resulting in physical contact with its opponent.
	No physical contact	Mouth holding	Both fish bite each other's mouth simultaneously for up to three minutes.
		Chase	Focal fish swims towards or after the opponent at high speed with no physical contact. If it culminates with focal fish hitting the opponent with its mouth, then this outcome involves also a Bite.
		Approach	Short distance and quick chasing that never culminates in physical contact.
Submissive displays	Passive coping	Frontal display	Focal fish stays in its place when bitten or approached by its opponent; it does not swim away from attacks.
		Passive coping	Focal fish faces its opponent and performs very subtle movements towards the other, without swimming any distance. Unusual behaviour.
		Escape	Focal fish swims away from its opponent, which is chasing, biting or approaching it.
		Tremor	Rapid trembling of focal fish during several seconds, usually after being attacked by its opponent. Unusual behaviour.

Ethogram for in intrasexual dyadic encounters in *Cichlasoma dimerus*.

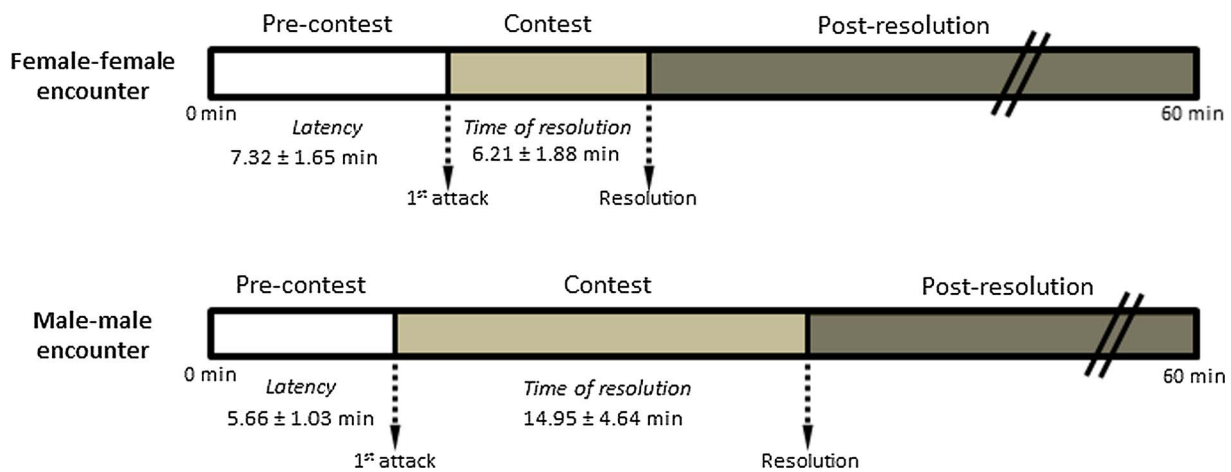


Fig. 1. Time variables in female–female and male–male encounters. Three phases were defined in each female–female and male–male agonistic encounter ($n = 9$ and $n = 8$, respectively). Values are expressed as Mean \pm SE and were compared by t -test.

behaviours were later quantified by the same observer according to the ethogram in Table 1. This way, the ethogram was modified from the original ethogram for *C. dimerus* in social context (Alonso et al., 2011) and it is defined for sex-matched dyadic encounters in neutral arena. Trials lasted one hour and were always conducted between 11 and 12 h to control for possible circadian variation in behaviours.

2.3. Specimen processing and tissue collection

At the end of each encounter fish were anesthetized by immersion in 0.1% benzocaine until opercula movement ceased. The weight (W), total and standard length (TL and SL, respectively) of each animal were registered. Animals were euthanized by decapitation and sex was confirmed by gonadal inspection. Gonads were dissected, weighted and used for the calculation of their gonadosomatic indexes (i.e., $GSI = [\text{gonad weight}/\text{total body weight}] \times 100$). Pituitaries, brains, gills and dorsal muscle were also dissected, processed and stored for future research.

2.4. Video analysis

All agonistic interactions performed by both animals during the encounter were recorded and these observations are summarized in the ethogram in Table 1. In each encounter, three phases were distinguished: pre-contest (both animals explore the experimental tank), contest (both animals display aggressive and/or submissive behaviours, resulting in a winner and a loser), post-resolution (the winner maintains its status with aggressive displays towards the loser). Conflict resolution is defined when one animal (loser) stops displaying aggressive behaviour towards the other one (winner). Moreover, latency is defined as the period of time before the beginning of the conflict (i.e. before any of both opponents performs aggressive displays towards the other), while time of resolution is the duration of the conflict; this is, since one of the animals attacks the other until the conflict resolution.

Latency and time of resolution were determined in each case, and measures of total aggression and submission per contest were assessed as the frequency of all aggressive or submissive displays performed by both animals in each encounter (number of aggressive displays performed by both opponents, divided by the duration of contest and post-resolution). In order to analyze the dynamics of the contests, behaviours were analyzed and quantified in time intervals of 10 min. Individual behaviour was calculated as the frequency of all aggressive or submissive displays performed by each animal during contest and post-resolution, summing the total number of either aggressive or submissive displays per animal, respectively, and dividing them by the duration time in minutes.

2.5. Statistical analysis

Differences in latency, time of resolution and frequencies of aggressive and submissive displays in males and females were expressed as means \pm SE and compared by t -test. Two-tailed tests were used throughout the analysis. Body weight, gonadosomatic index and total and standard length in winners and losers were expressed as means \pm SE and then compared intrasexually by paired t -test. Percentages of aggressive and submissive displays in each sex were compared by ANOVA and t -test, respectively. Pearson's correlation coefficient was used to examine the relationship between: individual frequency of aggressive displays and individual morphological variables (SL, TL, W and GSI); total frequency of aggressive displays per contest and coefficient of variation in morphological variables of both animals per contest; total frequency of aggressive displays per contest and latency; and total frequency of aggressive displays per contest and time of resolution. Data were tested for normality and homoscedasticity. In the correlation analysis of standard length CV% and total aggressiveness in females, we found one outlier (studentized residuals $N \pm 2$), and it was excluded from the analyses. To correct for multiple hypothesis testing we applied the false discovery rate (FDR) two-stage sharpened method correction (Benjamini et al., 2006), and thus all presented p -values are modified accordingly using the spreadsheet-based software provided in Pike (2011). Effect size was calculated using CohenCohes d . A p -value ≤ 0.05 was used as the threshold for significant difference.

3. Results

3.1. Do male and female agonistic encounters have a similar contest dynamic?

A total of 17 h of video from 17 sex-paired dyadic agonistic encounters were analyzed to build the ethogram of *C. dimerus* (Table 1). In each encounter in both sexes, all three phases were clearly distinguished and winner and loser emerged as a result of the conflict resolution (Fig. 1). There were no significant differences in latency or time of resolution between sexes ($p = 0.4213$ for latency, $p = 0.1190$, for time of resolution, Table 2), suggesting that males and females have similar contest dynamics.

3.2. Do females show reduced levels of territorial aggression when compared to males?

No differences were found in the frequency of aggressive and submissive displays between sexes ($p = 0.3115$, $p = 0.4743$, respectively)

Table 2
Statistical analysis comparing latency and time of resolution in female–female and male–male encounters.

	95% I CI	95% S CI	T	p	Cohen's d	df
Latency	–2.62	5.94	0.83	0.4213	0.4085	15
Time of resolution	–20.28	2.8	–1.75	0.119	0.9159	8

Latency and time of resolution in intrasexual dyadic encounters.

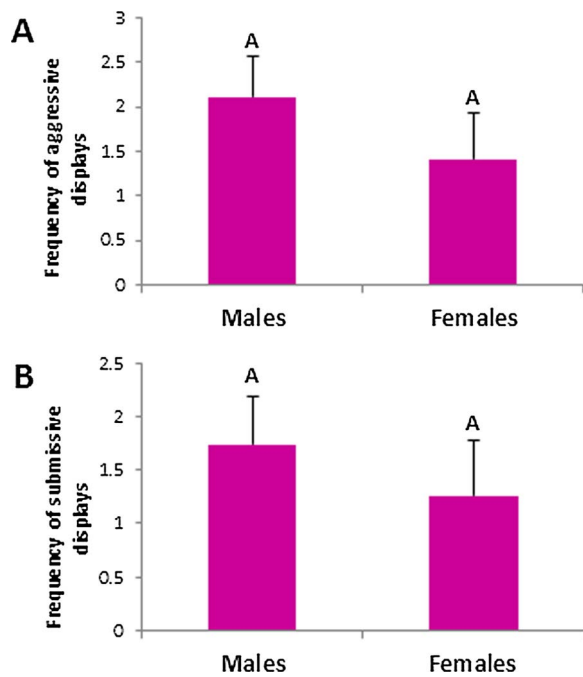


Fig. 2. Comparison between male and female aggressive and submissive behaviour. A. Frequency of aggressive displays. B. Frequency of submissive displays. Values are expressed as number of total displays per minute, as Mean ± SE. Potential differences between males and females were compared by *t*-test and same letters indicate no statistical difference.

Table 3
Statistical analysis comparing frequency of aggressive and submissive displays in female–female and male–male encounters.

	95% I CI	95% S CI	T	p	Cohen's d	df
Frequency of Aggressive displays	–2.11	0.69	–1.03	0.3115	0.3524	32
Frequency of Submissive displays	–1.84	0.87	–0.72	0.4743	0.2481	32

Aggressive and submissive displays in intrasexual dyadic encounters.

(Fig. 2, Table 3).

3.3. Is it possible to describe a general pattern for dyadic intrasexual agonistic encounters?

As a general pattern in both sexes aggressive displays during contest included chases, bites, tail hits, approaches, mouth holdings and frontal displays, while submissive behaviour included tremors, escapes and passive coping. However, there was a great variability in the dynamics of aggressive displays in female–female and male–male encounters, since each encounter presented individual characteristics. For example, in some female–female encounters, animals performed bites throughout the whole contest and approaches mostly towards the end (Fig. 3A), while others showed the opposite pattern (Fig. 3B). Moreover, some

male–male encounters presented only few displays, such as approaches and bites (Fig. 3C), while others included all the aggressive displays (Fig. 3D). However, despite the fact that there was great variability among encounters, there is a general pattern in males and females since in most encounters the contest was resumed with the outcome of mouth holdings, which generally occurred during the first half of the contest (Fig. 3A, B and D). As a consequence, animals dominating mouth holding displays became winners of the whole encounter. Also in both sexes during the post-resolution period, the winner maintained its status by keeping the central position in the aquarium and by performing chases, bites and approaches (Fig. 3A, B and D). Furthermore, in most contests usually the number of aggressive displays in the late encounter and post-resolution is higher than during the early encounter (Fig. 3A–C). On the other hand, the loser performed either passive coping or escaped from aggression, adopting a peripheral location in the lower corners or in the surface of the aquarium. In most encounters, losers showed a marked darkening of their body and eyes, and fin erections (dorsal, pelvic and/or pectoral). Altogether, these signs allow us to define trauma as the sum of morphological and behavioural characteristics experienced by the loser as a consequence of being persistently attacked during post-resolution. It is worth mentioning that from a total of 17 encounters, 16 resulted in a clear winner/loser status and only one male–male encounter resulted in no resolution of the contest.

3.4. Do males and females exhibit qualitative differences in their agonistic repertoire?

When comparing intrasexual aggressive behaviour, males and females show certain differences in their aggressive and submissive displays. For example, in female–female agonistic encounters the majority of the aggressive displays performed were approaches ($p = 0.0005$, Fig. 4A, Table 4) while in male–male dyads the most common aggressive displays observed were mouth holdings, bites and approaches ($p = 0.0001$, Fig. 4B, Table 4). It is worth mentioning that aggressive displays in female–female encounters involved both physical and non-physical contact ($p = 0.398$, Fig. 4B, Table 4), while in male–male encounters most displays involved physical contact ($p = 0.0176$, Fig. 4C, Table 4). In male–male dyads most submissive displays were escapes ($p = 0.0307$, Fig. 4F, Table 4) while there were no statistical differences for female–female encounters ($p = 0.5571$, Fig. 4E, Table 4).

3.5. Do winners and losers differ in their morphological attributes?

In order to analyze whether winner or loser status are related to morphological attributes of each animal, we compared TL, SL, W and GSI in both animals of each contest. TL, SL and W were higher in winners of both sexes, but these differences were only significant in female–female encounters (for females: $p = 0.0283$, $p = 0.0146$, $p = 0.0125$, respectively; for males: $p = 0.0737$, $p = 0.0516$, $p = 0.0737$, respectively, Fig. 5A–C, Table 5). Moreover, GSI of winners and losers did not differ in either sex ($p = 0.2416$ for females, $p = 0.3623$ for males, Fig. 5D, Table 5).

3.6. Is it possible to explain part of the variability in individual aggressiveness by individual morphological variables?

We tested whether individual aggressiveness correlates with morphological variables (TL, SL, W and GSI). Since there were no significant correlations between individual aggressiveness and any morphological variable for both sexes (Table 6), variability in individual aggressiveness is not explained by the individual morphological variables analyzed.

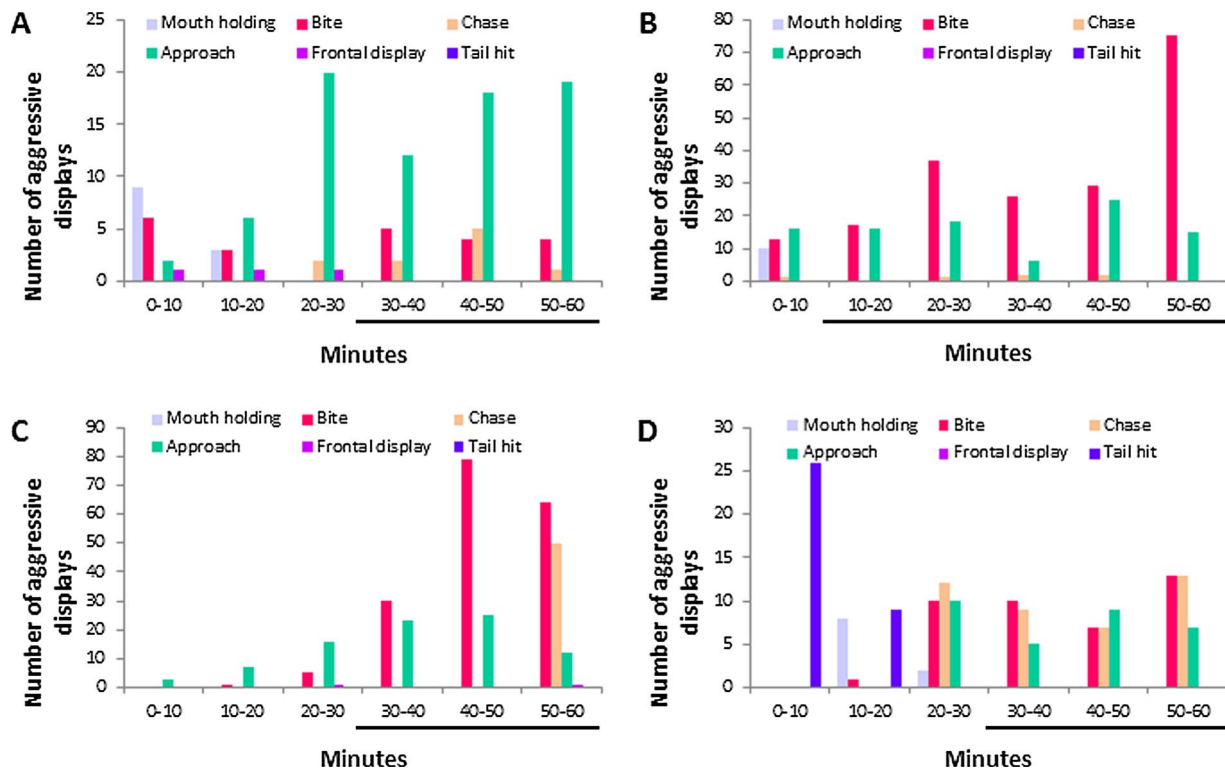


Fig. 3. Dynamics of aggressive displays in dyadic encounters. Each agonistic encounter lasted 60 min and behaviours were analyzed and quantified in time intervals of 10 min. A, B. Examples of female–female encounter. C, D. Examples of male–male encounter. Black horizontal line indicates the post-resolution period in each encounter.

3.7. Is total aggressiveness in each contest related to inter-individual variations in morphological attributes?

In female–female encounters, there was a significant negative correlation between total aggressiveness and the coefficient of variation of standard lengths ($p = 0.0458$, $r = -0.7588$, Table 7), total length ($p = 0.0481$, $r = -0.6756$, Table 7) and body weight ($p = 0.0364$, $r = -0.7885$, Table 7), but no correlation was detected with GSI's coefficient of variation ($p = 0.1674$, $r = 0.4602$, Table 7). In male–male encounters, there were no significant correlations between total aggressiveness and the coefficient of variation of neither of the morphological variables (Table 7). As a consequence, total aggressiveness in each contest can be explained by inter-individual variations in the morphological variables only in female–female encounters. Moreover regarding time variables, while total aggressiveness did not correlate with time of resolution nor latency in female–female encounters, there was a significant negative correlation between total aggressiveness and latency in male–male dyads ($p = 0.0048$, $r = -0.951$, Table 7).

4. Discussion

Aggression has been historically analyzed in male competitions and, despite the fact that females show high levels of aggressive behaviour, female aggression is still an understudied topic. In this study we used the South American cichlid fish *C. dimerus* as a model to describe and compare conflict outcome in sex-matched agonistic encounters in a neutral arena. The ethogram presented is similar to others described for related species (Baudy et al., 2017; Taves et al., 2009), and it is based on the ethogram defined for the same species in a social context (Alonso et al., 2011). Even though the dynamics and duration of encounters have been previously analyzed in male cichlids (Barlow et al., 1986; Copeland et al., 2011), to our knowledge this is the first study describing phases and temporal differences in behavioural displays in intrasexual agonistic encounters. Phases during real opponent contests have already been described in other fish species such as electric fish

but not in cichlids (Batista et al., 2012; Zubizarreta et al., 2012). However, here we define the conflict resolution when one animal (loser) stops displaying aggressive behaviour towards the other one (winner), while for electric fish resolution was defined as the moment when observers register the third consecutive retreat of the one fish without attacking back (Batista et al., 2012). The results presented in this paper suggest that encounters in both sexes followed the three phases, pre-contest, contest and post-resolution, as described for electric fish by Silva et al. (2013). Surprisingly, neither latency nor time of resolution differed between sexes, suggesting that both males and females have similar motivation towards territorial aggression. Interestingly, no sex difference in the frequency of aggressive displays was observed, suggesting that in *C. dimerus* neither sex resolves territorial conflicts peacefully and that females are as aggressive as males. Female aggression has been reported in dyadic agonistic encounters in the cooperatively breeding cichlid fish *Neolamprologus pulcher* (Taves et al., 2009), also suggesting similar levels of aggression between both sexes. Female aggression has been also reported in the convict cichlid *Amantitlania siquia* (Bloch et al., 2016) and altogether these evidences suggest that females of different cichlid species are as aggressive as males and that they should also be taken into account as biological model to study physiological mechanisms involving aggression. This topic has been addressed in *Astatotilapia burtoni*, which lives in a lek-like social system (O'Connell et al., 2013). In this species, females are usually not aggressive and social hierarchies are only presented by males. However, when they are placed in female-exclusive communities in the absence of males, they develop social hierarchies, aggression and masculinized behaviour. In this paper we show that non-masculinized females of *C. dimerus* are as aggressive as males and, even though *A. burtoni* is an extremely interesting model to study neuroendocrine regulation of aggressive behaviour, our results suggest that monogamous cichlid species and species without lek-like system could be more suitable models to study neuroendocrine mechanisms involved in female territorial aggression.

Even though sex-matched encounters showed great variability in

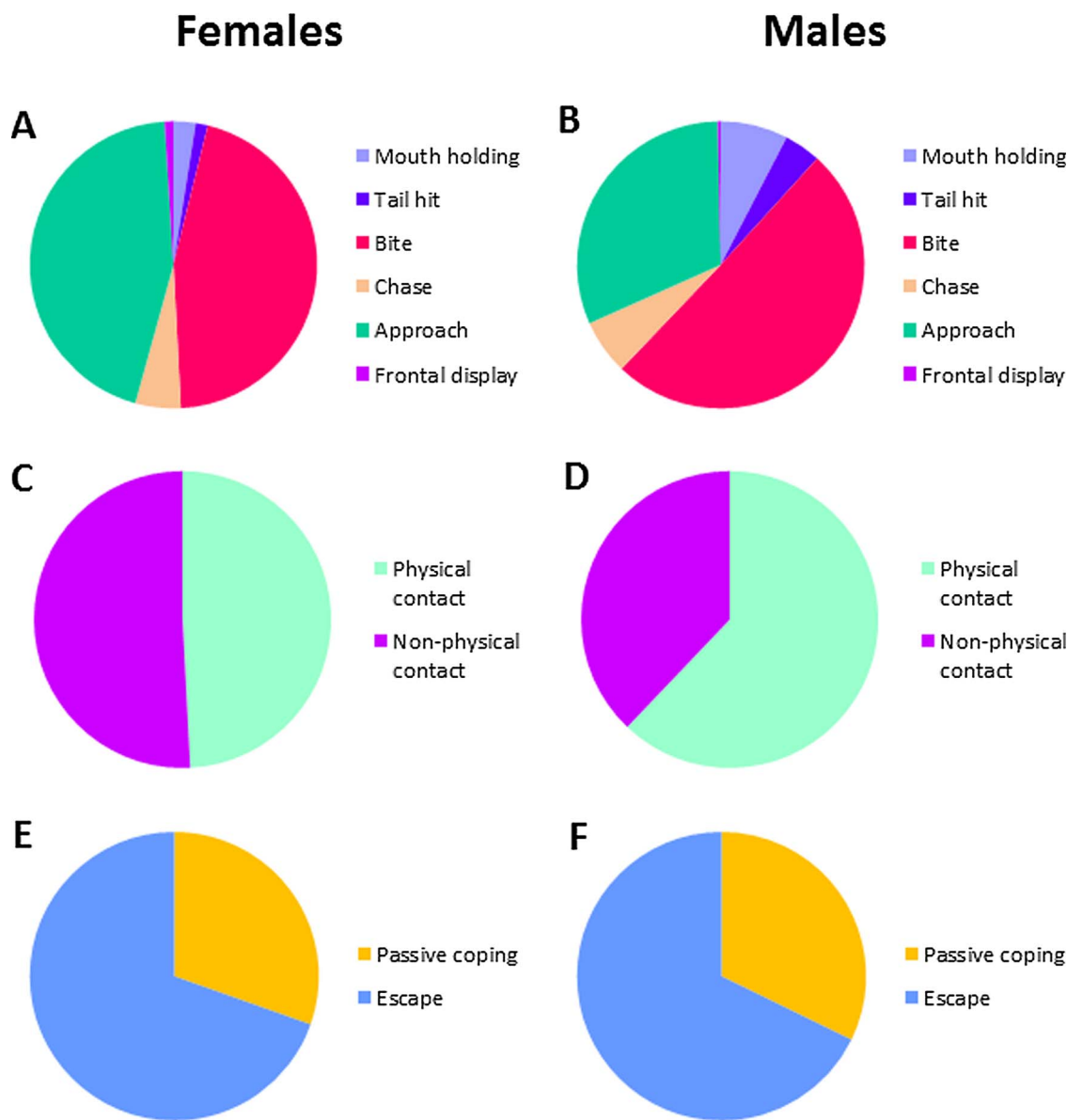


Fig. 4. Aggressive and submissive displays in dyadic agonistic encounters. A, B. Aggressive displays. C, D. Aggressive displays with or without physical contact. E, F. Submissive displays. A, C, E. Female-female encounters. B, D, F. Male-male encounters.

Table 4
Statistical analysis comparing behavioural displays in female–female and male–male encounters.

Aggressive displays	F	p	df				
Females	4.86	0.0005	107				
Males	7.72	0.0001	95				
Submissive displays	95% I CI	95% S CI	T	p	Cohen's d	df	
Females	–19.53	35.61	0.59	0.5571	0.1976	34	
Males	2.77	52.85	2.27	0.0307	0.8017	30	
Displays with/without contact	95% I CI	95% S CI	T	p	Cohen's d	df	
Females	33.25	13.54	–0.86	0.398	0.2855	34	
Males	4.67	45.22	2.51	0.0176	0.8864	30	

Behavioural displays in intrasexual dyadic encounters.

their dynamics, they share some common features. For example, some aggressive and submissive displays were more characteristic of a specific phase: while chases, bites and approaches occurred during the whole encounter, tail hits, frontal displays and mouth holdings were

only registered during contest and not in post-resolution. In most cases, the contest was resolved with the outcome of one or several mouth holdings, after which the winner maintained its status by chases, bites and approaches during post-resolution. During this phase, winners defended the central territory in the aquaria and losers were limited to a peripheral location. Similarly, in gymnotiforms dominants also maintain their winning condition after contest resolution, with higher electric organ discharge (EOD) rates than subordinates (Perrone et al., 2010; Silva et al., 2013).

As a result of being persistently attacked during post-resolution, in most contest losers showed several symptoms such as erected dorsal and pectoral fins and a marked darkening of their bodies and eyes. We define the sum of these characteristics as trauma. Notably, fin erection has been described as a threatening display of aggressive behaviour in this same species in social context (Alonso et al., 2011). These observations could seem contradictory, since the same display is reported for aggressive dominants and also for submissive losers. However, it is important to recall that they refer to two different paradigms: Alonso et al. (2011) described aggressive behaviour in dominants in a social context, while in this work losers emerged as a result of dyadic sex-

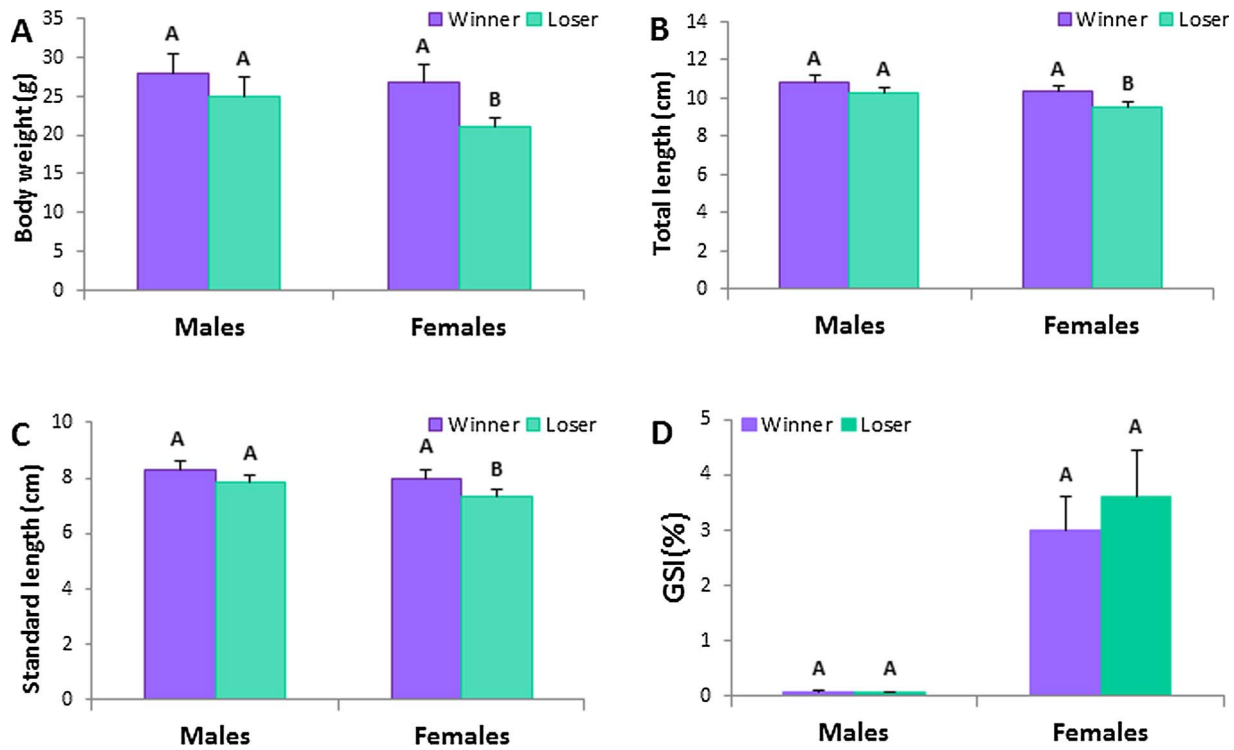


Fig. 5. Comparisons of morphological variables between winners and losers of intrasexual agonistic encounters. A total of 9 female–female and 8 male–male dyadic agonistic encounters were analyzed. A. Body weight was higher in female winners than losers, while no significant difference was found in males. B. Total length was higher in female winners than losers, while no significant difference was found in males. C. Standard length was higher in female winners than losers, while no significant difference was found in males. D. Gonadosomatic Index (GSI%) did not differ between winners and losers of either sex. Values are expressed as Mean ± SE and were compared by paired t-test. Different letters indicate statistical difference.

Table 5

Statistical analysis comparing morphometrical variables in winners and losers from female–female and male–male encounters.

	95% I CI	95% S CI	T	p	Cohen's d	df
Body weight						
Males	-0.04	5.77	2.53	0.0737	0.433	10
Females	0.31	10.89	2.44	0.0125	0.998	16
Total length						
Males	0.15	0.75	3.83	0.0737	0.564	10
Females	0.22	1.43	3.13	0.0283	0.938	16
Standard Length						
Males	-0.01	0.71	2.53	0.0516	0.489	10
Females	0.24	1.03	3.71	0.0146	0.769	16
GSI(%)						
Males	-0.04	0.08	1.07	0.3623	0.836	8
Females	-2.35	1.17	-0.78	0.2416	0.267	16

Morphometrical variables in winners and losers in intrasexual dyadic encounters.

matched encounters. Alternatively, fin erection could also indicate stress, since constantly defending territory from all subordinates in a social context could be as stressing as losing a contest and being steadily attacked during post-resolution phase. In this way, fin erection could indicate other individual physiological and context-dependent situations such as stress, rather than aggressive or submissive behaviours *per se*. Moreover, regarding body and eye darkening in losers during trauma, previous studies have already addressed eye darkening after dyadic encounters in other cichlid species. For example, in *Oreochromis niloticus* and *Geophagus brasiliensis* subordination has been associated to dark eye colors (Volpato et al., 2003; Miyai et al., 2011). Interestingly, results presented here could broaden evidence linking eye-darkening and a reduction in aggressive behaviour in cichlids.

As regards to aggressive and submissive displays performed by

Table 6

Pearson correlation between individual aggressiveness and morphological variables of each animal, in female–female and male–male encounters. Individual aggressiveness was assessed as the frequency of all aggressive displays performed by each fish.

	95% I CI	95% S CI	p	r	n
Females					
W	-0.4103	0.5200	0.819	0.07	18
GSI	-0.7178	0.1089	0.504	-0.38	18
SL	-0.3742	0.5505	0.819	0.11	18
TL	-0.2990	0.6068	0.819	0.2	18
Males					
W	-0.4700	0.5524	0.9135	0.06	15
GSI	-0.3200	0.7510	0.9135	0.32	12
SL	-0.4780	0.5452	0.9135	0.05	15
TL	-0.4549	0.5656	0.9135	0.07	15

Correlations between individual aggressiveness and morphometrical variables in intrasexual dyadic encounters.

males, our results show that the most frequent aggressive displays from their agonistic repertoire consisted on bites, approaches and mouth holdings, similarly to results in social context (Ramallo et al., 2015). However, the aggressive repertoire of females mostly consisted on approaches. The fact that females show equal proportion of contact and non-contact displays, while male's behavioural repertoire mainly included contact displays, differs from results in *N. pulcher* in dyadic encounters, where males performed mostly non-contact displays and females showed the opposite (Taves et al., 2009). In that species, sex differences are presumably due to the fact that females only control a single territory and some males often control several territories (Desjardins et al., 2008). As a consequence, the loss of territory would not have drastic consequences for males but it would represent a drastic reproductive disadvantage for females, which are considered to perform more risky displays (Hurd and Enquist, 2001) and being more

Table 7

Pearson correlation between total aggressiveness per encounter and the coefficient of variation of morphological variables between both animals (CV%); and time variables in (i) female–female and (ii) male–male encounters. Total aggressiveness was assessed as the frequency of all aggressive displays performed by both animals in each experiment.

	95% I CI	95% Sup CI	p	r	n
Females					
W CV%	−0.9534	0.2609	0.0364	−0.79	9
GSI CV%	−0.2939	0.8612	0.1674	0.46	9
SL CV%	−0.9029	0.1124	0.0458	−0.76	8
TL CV%	−0.9248	0.02047	0.0481	−0.68	9
Latency	−0.9147	0.04485	0.1349	−0.63	9
Time of Resolution	−0.5088	0.7777	0.5703	0.23	9
Males					
W CV%	−0.6881	0.8062	0.7261	0.14	7
GSI CV%	−0.8218	0.9232	0.7261	0.22	5
SL CV%	−0.9107	0.4046	0.4399	−0.50	7
TL CV%	−0.7939	0.7057	0.7261	−0.10	7
Latency	−0.9932	0.7051	0.0048	−0.95	7
Time of Resolution	−0.9594	0.3143	0.3938	−0.67	6

Correlations between total aggressiveness and variation on morphometrical variables in intrasexual dyadic encounters.

“willing to take greater risks to establish or defend territory” (Taves et al., 2009). In the opposite way, the fact that *C. dimerus* males performed more contact-displays could imply that in this species males would be more willing to engage in riskier contact aggression than females, suggesting that territorial loss could incur a greater cost for males than for females. This difference between the two species could be explained because, unlike *N. pulcher*, *C. dimerus* is a monogamous species in which both dominant males and females defend a single territory (see Pandolfi et al., 2009 and Ramallo et al., 2014 for review).

Size is one of the main cues for the establishment of a hierarchical position in many animals including fish (Beacham, 1987; Chase et al., 2002), since it increases the probability of winning contests and acquiring a dominant status. In the goby cichlid *Eretmodus anostictus*, habitat quality correlates with body size, and smaller fish occupy vacant territories of worse quality than larger fish (Taborsky et al., 2014). Moreover, in *N. pulcher*, social hierarchies are based on size, with small helpers at the bottom of the hierarchy, and body mass is usually included as a covariate when analyzing social behaviour and physiological regulation (Bender et al., 2008). Similarly, previous studies in social context suggest that in *C. dimerus* dominance positively correlates with size in males but not in females (Alonso et al., 2012; Alonso et al., 2011; Ramallo et al., 2014). Surprisingly, when comparing the outcome of the contests in this paper, results suggest that only female winners, and not males, are larger than losers. Similarly, dyadic contests in *N. pulcher* showed a different tendency than in social context, since heightened aggressiveness was observed among small individuals (Reddon et al., 2013). It is worth mentioning that in our study the range of size analyzed is small (e.g. 6.5–10.7 cm for standard length), which could have an impact on statistical significance and strength of correlations. However, in this regard, the coefficient of variation of the morphometrical variables analyzed is near 30%, which is similar to other studies comparing length or size in cichlid opponents (Reddon et al., 2013). Furthermore, this discrepancy between dyadic and social context could be probably explained by differences in paradigm, since morphological variables and visual cues could have different relative importance in a social context and in an agonistic dyadic encounter. Moreover, the fact that male losers are not smaller than winners could also be explained by the desperado hypothesis, which postulates that weak or “poor-quality” individuals may have nothing to lose from escalating conflicts over resources they are not likely to secure otherwise (Grafen, 1987). This hypothesis has been taken into account to explain unexpected heightened aggression in small individuals in *N. pulcher* (Reddon et al., 2013). Taking into account this hypothesis, it is also

possible that small individuals aggressively motivated could be part of winner and loser groups regardless of their opponents size, masking probable size difference predicted by previous studies in a social context in *C. dimerus*.

Moreover, our results suggest that aggressiveness in dyadic encounters could be better explained by morphological variations between opponents, suggesting that aggressiveness could depend on the opponents presence and its characteristics rather than on individual characteristics. Non-significant correlations between individual aggression and individual morphological variables suggest that larger animals are not more aggressive than smaller ones. Instead, the aggression outcome in each contest can be better explained by relative variations in size between opponents, since higher levels of aggression occurred in contests with smaller variations in weight or length (e.g. more similar fish). Surprisingly, significant negative correlations between total aggressiveness and coefficient of variations were only observed in female contests and not in males. These sex differences could be explained if morphometrical characteristics are important in conflict resolution in *C. dimerus* females, but probably this is not the case for males. Following this explanation, visual cues and morphometrical attributes could be less relevant in male contests of this species, and conflict resolution could be better explained by different or multiple sensory modalities (Fernald, 2014). However, this explanation goes against recent evidence on males of *Oreochromis mossambicus* suggesting that, in fact, visual cues are most important in mirror-image fights and that chemical cues (e.g. male urine) modulate behaviour by reducing aggression in conspecifics (Keller-Costa et al., 2016). As a consequence, an alternative explanation to our results is that visual cues may also be important for males of *C. dimerus*, but animals could take into account morphological characteristics other than the morphometric variables analyzed in this study. Finally, it is worth mentioning that the fact that morphometrical differences between females could help understand variations in levels of aggression among female contests, does not mean that other cues (e.g. chemosensory, physiological, mechanosensory) could be equally or even more important to explain variability in female aggression.

5. Conclusions

In conclusion, this study analyses contest dynamics and describes the temporal sequence and aggressive displays in sex-matched agonistic encounters of *C. dimerus*. Results here suggest that both males and females of this species have similar motivation towards territorial aggression and that females are as aggressive as males, at least in intrasexual dyadic encounters. Moreover, the fact that morphometric differences between both opponents could explain high aggression and the winning status in females, but not in males, highlights the importance of addressing the study of aggression from a perspective involving multiple sensory modalities. Finally, sex differences in aggressive repertoire and in the relationship between morphometric variables and aggression, emphasizes the need of deepening the study of this behaviour in female cichlids. Future research should explore physiological and neuroendocrine mechanisms regulating aggressive behaviour in females and not only in males.

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