

Personality and physiological traits predict contest interactions in *Kryptolebias marmoratus*



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ABSTRACT

Personality and physiological traits often have close relationships with dominance status, but the significance and/or direction of the relationships vary between studies. This study examines whether two personality traits (aggressiveness and boldness) and three physiological traits (testosterone and cortisol levels and oxygen consumption rates) are associated with contest decisions/performance using a mangrove killifish *Kryptolebias marmoratus*. The results show that individuals that attacked their own mirror images (an aggressiveness index) at higher rates or had higher levels of testosterone were more likely to attack their opponent and win non-escalated contests, while individuals that had higher levels of cortisol were more likely to lose. After the contests, (1) individuals that had attacked their opponents or won had higher post-contest oxygen consumption rates, and (2) individuals that had attacked their opponents also had higher post-contest levels of cortisol. Although no significant correlations were detected among pre-contest physiological traits, post-contest levels of cortisol were positively correlated with oxygen consumption rates. Overall, personality and physiological traits provide useful predictors for the fish's contest decisions/performance. Contest interactions subsequently modified post-contest physiological traits and potentially also promoted associations between them. Nevertheless, the fish's physiological traits remained rather consistent over the entire study period.

1. Introduction

To compete for limited and important resources, individuals sometimes fight other conspecifics. Behaviour and performance when fighting a potential competitor are affected by various intrinsic and environmental factors. One of the most prominent and studied of these is body size; in general, larger and/or heavier individuals are more able to win in competitive interactions (Arnott and Elwood, 2009; Hsu et al., 2006). Although body size is important, other factors such as an individual's personality (Bell and Sih, 2007; Colléter and Brown, 2011; Réale et al., 2007; Rudin et al., 2017; Sih et al., 2004; Sih and Bell, 2008; Smith and Blumstein, 2008; Stamps and Groothuis, 2010; van Oers et al., 2005) and physiological traits (Haller et al., 1998; Koolhaas et al., 1999; Oliveira, 2009; Øverli et al., 2007; Summers et al., 2005; Wingfield, 2005) also play important roles in its motivation and ability to compete. Similarly sized individuals can differ in their behaviour and performance in contests because of the differences in their personality

and physiological traits.

Animal personality is generally defined as 'individual differences in behaviour that are consistent across time and/or across contexts' (Stamps and Groothuis, 2010). Differences in personality traits can be heritable (Réale et al., 2007; van Oers et al., 2005) and have consequences for fitness (Réale et al., 2007; Smith and Blumstein, 2008). Furthermore, different behavioural traits are sometimes correlated and form behavioural syndromes (Sih et al., 2004). These correlations could arise because the behavioural traits share the same proximate mechanisms (e.g. pleiotropic gene effects) and are difficult to decouple (Sih et al., 2004). Or, they could arise because the combination of the related behavioural traits results in higher fitness (Sih et al., 2004). Aggressiveness and boldness (the tendency to take risks) are two of the most studied personality traits and have been observed to be correlated in many animal species (Sih and Bell, 2008), although the relationship might be plastic and dependent on the environment (Bell and Sih, 2007). Individuals that differ in dominance statuses often also differ in

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these two traits. In rainbowfish (*Melanotaenia duboulayi*), for example, dominant individuals are larger, more aggressive and bolder than subordinates (Colléter and Brown, 2011). In male Australian field crickets (*Teleogryllus oceanicus*), dominants are bolder than subordinates despite the two groups of individuals not differing significantly in body size (Rudin et al., 2017). Interestingly, such associations between dominance status and behavioural traits could have arisen from (1) aggressiveness and boldness predicting contest performance and/or (2) dominance status promoting boldness and aggressiveness.

Contest decisions and performance are closely linked to hormones. Androgens and glucocorticoids play important roles in social interactions and stress responses (Haller et al., 1998; Koolhaas et al., 1999; Oliveira, 2009; Øverli et al., 2007; Summers et al., 2005; Wingfield, 2005). Testosterone affects an individual's motivation and ability to compete and also responds to the individual's social interactions. In the California mouse (*Peromyscus californicus*), for example, elevated testosterone was found in males that received multiple winning experiences (Oyegbile and Marler, 2005) and was essential for them to behave more aggressively after wins (Trainor et al., 2004). In Mozambique tilapia (*Oreochromis mossambicus*), a winning experience increases an individual's probability of winning the next fight, and this effect is probably mediated by transient changes in androgen levels (Oliveira et al., 2009). The relationships between testosterone and aggression and dominance status are, however, complex, because they are modulated by season, mating system and sex (Wingfield, 2005) and can differ between vertebrate taxa (Hirschenhauser and Oliveira, 2006). Glucocorticoids are associated with stress and also have complex relationships with behaviour and dominance status (Creel, 2001, 2005; Koolhaas et al., 1999). For example, individuals of rainbow trout (*Oncorhynchus mykiss*) selected for high and low post-stress cortisol levels differ not only in reactivity to stress, but also in several behavioural traits. Individuals with low post-stress cortisol levels resume feeding sooner after stress and show aggression and social dominance more frequently than individuals with high post-stress cortisol levels (Øverli et al., 2005; Pottinger and Carrick, 2001). As with testosterone, the influence of dominance relationship on glucocorticoids could also depend on the social system of the study animals (Creel, 2001; Goymann and Wingfield, 2004).

Fighting is an energetically costly process (Grantner and Taborsky, 1998; Hack, 1997), especially when it involves overt aggression (Ros et al., 2006). Whether and how individuals of different dominance statuses differ in their energy consumption are, however, not entirely clear. In male crickets (*Acheta domesticus*), contest winners performed more agonistic acts and thus had higher cumulative net oxygen consumption than losers (Hack, 1997). In juvenile Atlantic salmon (*Salmo salar*) that were raised in a group, a positive association between dominance scores and oxygen consumption (measured as standard metabolic rate) was detected (Metcalfe et al., 1995). Conversely, in juvenile Ambon damselfishes (*Pomacentrus amboinensis*), dominants and subordinates did not differ in pre-contest oxygen consumption rates (measured as routine metabolic rate), but subordinates showed an increase in post-contest oxygen consumption while dominants showed no change, despite the fact that they performed more aggressive acts than subordinates (Killen et al., 2014). In rainbow trout, the most dominant individuals in groups with stable dominance hierarchies appeared to have lower oxygen consumption rates than the subordinates, even though the dominant individuals exhibited more aggressive acts than the subordinates during morning feedings (Grobler and Wood, 2013). The relationship between oxygen consumption rates and contest performance thus appears to differ between different species/studies.

As summarised above, personality and physiological traits often have close relationships with dominance status, but the significance and/or direction of the relationships are not always consistent between different studies. Moreover, the ability of different traits to predict dominance status in the same individual is not entirely clear because

most studies examined one or a small subset of the traits. This study used an androdioecious mangrove killifish *Kryptolebias marmoratus* to examine, simultaneously, the influence of behavioural traits, hormones and oxygen consumption rate on contest decisions and contest performance.

Previous studies of *K. marmoratus* have shown that aggressiveness and boldness are repeatable in the fish (Edenbrow and Croft, 2012), and bolder individuals behave more aggressively (Chang et al., 2012). Chang et al. (2012) also showed that the positive relationship between aggressiveness and boldness in the fish is robust and not influenced by previous contest experiences. Moreover, the fish's boldness and aggressiveness both have a positive relationship with levels of testosterone and cortisol, lending support to hypothetical common underlying physiological mechanisms (Chang et al., 2012). And, although the fish's aggressiveness was found to be affected by prior winning/losing experiences in the study (Chang et al., 2012), the levels of testosterone or cortisol were not. Consistent with these findings, by staging contests between individuals of different body sizes, Earley and Hsu (2008) showed that contest winners and losers did not differ in either pre- or post-contest levels of testosterone or cortisol. The study, however, revealed the importance of contest interactions to the fish's post-contest physiology. Contest losers that did or did not escalate contests into physical fights with the winners differed in their post-contest physiology; losers of escalated contests had higher post-contest levels of testosterone and cortisol than the loser of non-escalated contests (Earley and Hsu, 2008).

Given the previous findings about the fish's behaviour and associated physiology, what remained unclear included (1) whether personality traits predict the fish's behaviours in contests with conspecifics, (2) whether the inability of previous studies to detect any differences between hormone levels in contest winners and losers was due to differences in the body size of the winners and losers and (3) whether oxygen consumption rate plays an important role in the fish's contest behaviours and outcomes. To address these issues, we tested the hypothesis that both personality and physiological traits are closely associated with the fish's contest decisions and performance by staging contests between size-matched individuals to minimize the influence of body size on behaviours and physiology. We examined (1) whether pre-contest personality (aggressiveness and boldness) and physiological traits (levels of testosterone and cortisol and oxygen consumption rate) predict the fish's contest behaviours (attacking the opponent or not) and contest performance (winning or losing) in size-matched contests and (2) how contest interactions (contestants attacked their opponents or not, contests were escalated into physical interactions or not) and outcomes affect post-contest physiological traits.

2. Materials and methods

2.1. Study organism

Natural populations of *K. marmoratus* consist mainly of isogenic homozygous hermaphrodites with < 1 % males (Mackiewicz et al., 2006). For detailed descriptions of the fish's ecology, see Taylor (2012). This fish usually hides under cover and behaves aggressively in both the field and the laboratory in competition for shelters (Huehner et al., 1985; Taylor, 1990). In the field, the chasing of a smaller individual by a larger individual frequently results in the emersion of the smaller individual (Taylor, 1990). In the laboratory, when provided with small pieces of cover, one fish usually occupies one refuge and the larger fish tend to be territorial (Huehner et al., 1985). Also, in the laboratory, adding food to a fish tank is observed to cause dominant fish to chase and attack subordinate fish (personal observations).

This study used the hermaphroditic descendants (> 8 months old) of five isogenic lineages of *K. marmoratus* originally collected by Dr. D. Scott Taylor from various locations (DAN2K: Dangriga, Belize, collected in 2000; HON9: Utila, Honduras, 1996; RHL: San Salvador, Bahamas,

1997; SLC8E: St. Lucie, FL, USA, 1995; VOL: Volusia, FL, USA, 1995). Within a month of hatching fish were placed in individual translucent polypropylene maintenance containers ($13 \times 13 \times 9$ cm) filled with 550 ml 25 ppt synthetic sea water (Instant Ocean™ powder) and given unique identification codes. Fish were kept at 25°C ($\pm 2^\circ\text{C}$) on a 14L:10D photoperiod and fed newly hatched brine shrimp (*Artemia*) nauplii at 10:00 h every day. Maintenance containers were cleaned, and water replaced every two weeks.

2.2. Experimental design and procedures

On Day 0, we fed the test individuals newly hatched brine shrimp in the morning and then in the afternoon (approximately 3 h after feeding) measured them and divided them into size matched (difference in standard length, SL, ≤ 1 mm) pairs of the same lineages. The mean (\pm SE) SL of the fish used for the study was 31.69 ± 0.03 mm and the mean difference in SL of the 58 contest pairs was 0.27 ± 0.03 mm. We then marked the two test individuals of a pair by using a needle to break the non-vascular thin membrane between the two soft rays in the upper or lower margins (randomly assigned) of the caudal fin for individual identification. The test individuals were returned to their maintenance containers after marking. Marking did not cause bleeding or observable adverse effects upon the fishes' health or behaviour. The membrane usually grows back completely in 3 d.

On Day 1, at approximately 09:30 h, we removed test individuals from their maintenance containers to collect water samples for pre-contest hormone levels (See 2.3. *Hormone collection, extraction and assay* for procedures). Immediately after the completion of the procedures, at approximately 10:30 h, the test individuals were set up for the measurement of their pre-contest oxygen consumption rates (See 2.4. *Measurement of oxygen consumption rates* for procedures). After the measurements, the test individuals were returned to their maintenance containers and fed.

On Day 2, at approximately 09:30 h, the test individuals were removed from their maintenance containers for the two personality tests, aggressiveness and boldness (See 2.5. *Aggressiveness and boldness* for procedures). The order the two tests was assigned randomly. Each behavioural test was preceded by 1 h of acclimatisation in the test aquarium. Each test was terminated after 30 min, whether or not the fish had displayed the behaviour concerned, and the fish was moved on to acclimatise for the next test. The aggressiveness and boldness tests were both recorded with camcorders. After the behavioural tests were complete, the test individuals were replaced in their maintenance containers and fed. At 17:00 h, the two test individuals of a designated pair were placed one in each of the two compartments (randomly chosen) of a contest aquarium ($12 \times 8 \times 20$ cm, containing water 12 cm deep and 2 cm of gravel) separated by a black partition and were left in the aquarium overnight to acclimatise.

On Day 3 at approximately 08:30 h, we removed the black partition in the contest aquarium to start the size-matched contest (See 2.6. *Size-matched dyadic contests* for procedures). All contests were recorded with camcorders. After the contest was completed, the partition was replaced to separate the two test individuals. The two test individuals were then removed from the aquarium to two separate beaker sets to collect water samples (individually) for post-contest hormones (See 2.3. *Hormone collection, extraction and assay* for procedures). Immediately after the completion of the water sample collection procedures, the two test individuals' post-contest oxygen consumption rates were individually measured (See 2.4. *Measurement of oxygen consumption rates* for procedures). After the measurements of the oxygen consumption rates were completed, we measured the fishes' body weight.

2.3. Hormone collection, extraction and assay

Three-piece beaker sets were used to collect water samples for hormone analyses; a cylindrical glass sieve (height: 10.5 cm; diameter:

8.0 cm tapered into 6.0 cm) placed inside a 500 ml glass beaker which was then housed inside a translucent plastic container. Each of the test individuals was placed for 1 h in its beaker set with 400 ml clean 25 ppt synthetic sea water. We then removed the fish from the water by lifting the glass sieve. We quickly placed the glass sieve (with the test individual in it) inside another translucent plastic container with clean 25 ppt synthetic sea water to transport the fish to the apparatus for the measurement of its oxygen consumption rate which was less than 5 m away.

Hormone extraction and assay followed Earley and Hsu (2008). Water was removed from the beaker with a vacuum pump and passed through C-18 solid phase columns (Lichrolut RP-18, 500 mg, 3.0 ml; Merck) fitted to a 24-port manifold to extract hormones. Before use, the columns first were primed with 2 consecutive washes with 2 ml HPLC grade methanol (MeOH) followed by 2 consecutive washes with 2 ml distilled water. After use, the columns were purged of sea salt with 2 consecutive 2 ml washes of distilled water and stored at -80°C until further processing. Freeze storage of water samples and columns has been determined not to influence steroid concentrations (Ellis et al., 2004).

To prepare for hormone extraction, columns were thawed and purged with two consecutive washes with 2 ml of distilled water. Hormones were then eluted from the columns by two consecutive washes with 2 ml ethyl acetate. The eluted solvent was evaporated at 40°C with nitrogen gas ($\sim 0.7\text{ n/m}^2$). The resulting hormone pellet was re-suspended in 800 μl of enzyme-immunoassay EIA buffer supplied with the Cayman Chemicals Inc. EIA kits and the samples stored at -20°C until assay. Testosterone and cortisol were assayed using Cayman Chemicals Inc. EIA kits catalogue numbers 582701 and 500360, following the manufacturer's recommended procedures. Plates were read at 405 nm on a BioTek microplate reader. Assay of cortisol and testosterone in mangrove killifish using Cayman Chemicals Inc. EIA kits has previously been validated by Earley and Hsu (2008). Intra-assay coefficients of variation were (assay plate 1–7) 1.6, 2.0, 1.1, 2.2, 0.1, 2.5 and 2.4 % for testosterone and 3.0, 2.5, 6.3, 4.4, 3.8, 9.4 and 1.2 % for cortisol. The inter-assay coefficient of variation was 5.0 % for testosterone and 7.5 % for cortisol. All hormone data are presented as picogrammes per millilitre (pg/ml).

2.4. Measurement of oxygen consumption rates

Pre- and post-contest oxygen consumption rates were measured between 10:30 and 11:30 h, approximately 24 and 22 h respectively after the previous feeding, using procedures modified from Hu et al. (2014a, 2014b). Each test individual was gently placed in a 155 ml glass respiration chamber containing clean 25 ppt synthetic seawater at 25°C . The respiration chambers were closed and housed in opaque containers to reduce environmental disturbance. Oxygen concentration was monitored every 5 s for 1 h using fibre-optic oxygen sensors (PreSens sensor spots, type PSt3) which had previously been calibrated with oxygen saturated sea water and water with zero oxygen (10 % sodium bisulphate solution). Each sensor was attached to the bottom of the chamber and connected to an OXY-4 mini multichannel fibre-optic oxygen meter (PreSens GmbH, Regensburg, Germany). Oxygen concentration decreased linearly over the hour, but remained above 75 % (Mean = 83.51 %; range 78.97 %–95.32 %) (Hu et al., 2014a, 2014b). This decrease was used to calculate oxygen consumption expressed as $\mu\text{mol O}_2/\text{g}/\text{h}$. In tests on 30 glass respiration chambers with clean 25 ppt synthetic seawater but no fish, mean (\pm SE) bacterial respiration was $-0.01 \pm 0.05 \mu\text{mol O}_2/\text{h}$ (not significantly different from 0, $t_{29} = 0.21$, $p = 0.834$), while mean (\pm SE) pre- and post-contest oxygen consumption by test fish were 8.66 ± 0.19 and $9.23 \pm 0.19 \mu\text{mol O}_2/\text{g}/\text{h}$.

Despite a growing perception that the intermittent flow method is superior to the long used closed circuit method, a recent study that used a paired design on red drum (*Sciaenops ocellatus*) found no differences in

the critical oxygen threshold measured from the two methods (Negrete and Esbaugh, 2019). Furthermore, a study that used goldfish (*Carassius auratus auratus*) as the study animal also concluded that the choice of respirometric technique (closed vs. intermittent) does not affect the critical oxygen tension or the rate of oxygen uptake (Regan and Richards, 2017).

2.5. Aggressiveness and boldness

Procedures for quantifying aggressiveness and boldness followed Chang et al. (2012). The order of the two tests was randomised. Aggressiveness and boldness tests were recorded with camcorders.

Aggressiveness was quantified by a mirror test in a standard aquarium (12 × 16 × 20 cm, containing water 12 cm deep and 2 cm of gravel). The mirror was attached to one of the narrow sides of the tank (randomly assigned), and the fish acclimatised for 1 h behind a black partition 10 cm from the mirror. After acclimatisation, the partition was removed for 30 min. Both (1) the latency (in s) to the first mirror attack and (2) the number of attacks per minute over the following 10 min (or the remaining time, if shorter) were used as aggressiveness indices, more aggressive individuals attacking more quickly and more frequently. All physical contacts of an individual with the mirror were counted as attacks, including biting and pushing and hitting against the mirror image. The video recording of one fish's aggressiveness tests was missing and thus the sample size for aggressiveness test was 115. Three of the 115 remaining fish did not attack their mirror images in the 30 min; they were assigned attack latencies of 1800s and rates of 0 attacks/min.

The latency to emerge from a shelter was used as the boldness index, bolder individuals emerging more quickly (Brown et al., 2005). A standard aquarium (12 × 16 × 20 cm, containing water 12 cm deep and 2 cm of gravel) was fitted with a 6 × 6 × 8 cm black plastic shelter with a door. The test fish acclimatised in the shelter for 1 h with the door closed. When the door was opened, the time (in s) for the entire fish to emerge from the shelter for the first time was recorded. Seven of the 116 test individuals did not emerge from their shelters before the tests were terminated in 30 min and were assigned a latency to emerge of 1800s.

2.6. Size-matched dyadic contests

After the aggressiveness/boldness tests, the fish of a designated pair were fed and placed one in each compartment (randomly chosen) of a contest aquarium (12 × 8 × 20 cm, containing water 12 cm deep and 2 cm of gravel) separated by a black partition and were left there overnight to acclimatise. The next day (Day 3), we removed the black partition to start the contest. After the partition was lifted, one of the contestants usually initiates interactions by orienting and swimming towards its opponent. After a few bouts of mutual displays, if neither contestant has retreated, one of them usually launches a first attack by swimming rapidly towards and pushing against or biting its opponent. The individual that has received the first attack either retreats or responds with attacks. The individual that persistently backed away and retreated from its opponent's approaches/displays/attacks for 5 min without retaliating was considered the loser of the contest. A loser's opponent was the winner. A contest was classified as escalated if resolved only after mutual attacks between the contestants and as non-escalated if the loser retreated after mutual displays or one initial attack from the winner. In some cases the two contestants did not interact with each other but appeared to be swimming around in the aquarium individually. If this swimming around behaviour lasted for 1 h with no clear winner and loser to be assigned, the contest was terminated and classified as 'unresolved'. After the contest was resolved or terminated, the partition was replaced to separate the two test contestants. All contests were recorded with camcorders.

2.7. Ethical approval

The Animal Care and Use Committee of National Taiwan Normal University approved *K. marmoratus* as the study organism and the procedures for the use of the fish permit #102027.

2.8. Statistical analyses

A total of 58 size-matched contests (116 individuals) were staged (11 pairs of DAN2K, 11 pairs of HON9, 11 pairs of RHL, 11 pairs of SLC8E and 14 pairs of VOL). All these contests were used for the analyses of the relationships between attacking/not attacking opponents and personality and physiological traits. As noted earlier, one video recording for an aggressiveness test was missing which reduced the sample size for aggressiveness to 115. Fourteen of the 58 contests were resolved with clear winners and losers after mutual attacks ("escalated"), 24 were resolved without mutual attacks ("non-escalated") and 20 were terminated after an hour with no clear winner ("unresolved"). Only the 38 contests that were resolved with a clear winner and loser in 1 h were used for the analyses of the relationships between winning/losing outcome and personality and physiological traits.

We used JMP 8 and SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA) for statistical analyses. Different types of transformations were applied to hormone levels ($\sqrt[3]{\text{Ln}(\text{value})}$), oxygen consumption rates ($\text{Ln}(\text{value})$), the latency to the first mirror attack ($\text{Ln}(\text{value} + 1)$), the frequency of mirror attacks ($\sqrt{\text{value}}$) and the latency to emerge from the shelter ($\text{Ln}(\text{value} + 10)$) to improve their distributions.

We used Pearson's correlation coefficients (r) to examine the pairwise correlations among personality traits and among physiological traits. We used linear mixed-effects models (Proc MIXED) with restricted maximum likelihood (REML) parameter estimation and Kenward-Roger denominator degree of freedom calculation to examine whether individuals that (1) attacked or did not attack their opponents and (2) won or lost in dyadic contests differed in their personality (aggressiveness and boldness) or physiological traits (pre- and post-contest testosterone, cortisol and oxygen consumption rates). For (2) we evaluated the importance of contests being escalated into mutual attacks because a previous study showed this to affect post-contest hormone levels (Earley and Hsu, 2008). All MIXED analyses included "pair" nested within isogenic lineage as a random variable to account for the possible interdependence of the two individuals in the same contest. SL was included in all linear models to account for its influence.

3. Results

3.1. Relationships between personality traits and contest interactions

Significant relationships were detected among the three personality indices. The rate of mirror attacks related negatively with the latency to first mirror attack ($r = -0.362$, 95 % CIs: -0.511 to -0.191 , $n = 115$, $p < 0.001$) and the latency to first emergence from shelter ($r = -0.210$, 95 % CIs: -0.378 to -0.026 , $n = 115$, $p = 0.025$). The latency to first mirror attack related positively with the latency to first emergence from shelter ($r = 0.373$, 95 % CIs: 0.203 – 0.520 , $n = 115$, $p < 0.001$).

Contestants that did or did not attack their opponents in dyadic contests did not differ in their latency to the first mirror attack ($p = 0.299$) (Table 1a-I, Fig. 1a-I; also see Supplementary Fig. S1a-I). Nor did contest winners and losers differ in their latency to the first mirror attack ($p = 0.523$) and the trends were not dependent on whether or not the contests were escalated into mutual attacks (Win/Lose × Escalate, $p = 0.663$) (Table 1a-II, Fig. 1a-II; also see Supplementary Fig. S1a-II).

Contestants that attacked their opponents in dyadic contests attacked their own mirror images at higher rates than those that did not attack their opponent ($p = 0.004$) (Table 1b-I, Fig. 1b-I; also see

Table 1

Linear mixed effects models evaluating whether test individuals that (I) did or did not attack their opponents and (II) won or lost contests differed in (a) latency to first mirror attack, (b) rate of mirror attacks or (c) latency to first emergence from the shelter. For (II), we also evaluated the importance of whether contests were escalated (Win/Lose \times Escalate) and examined the differences between winners and losers in escalated (Esc: Win/Lose) and non-escalated (Non-esc: Win/Lose) contests. All models included the test individuals' standard length (SL), with pair (nested within lineage) as a random variable. Significant effects are in bold.

Variable	df	b \pm SE	F	p
(a) Latency to 1st mirror attack				
(I) Launching attacks	1,111		1.1	0.299
SL	1,58.6	-0.02 \pm 0.10	0.0	0.851
(II) Win/Lose	1,35.8		0.4	0.523
Escalate	1,34.9		2.1	0.155
Win/Lose \times Escalate	1,35.8		0.2	0.663
Esc:Win/Lose	1,35.5		0.0	0.897
Non-esc:Win/Lose	1,36.2		0.8	0.381
SL	1,36.4	0.05 \pm 0.11	0.2	0.672
(b) Rate of mirror attacks				
(I) Launching attacks	1,109		8.8	0.004
SL	1,58.1	-0.05 \pm 0.07	0.5	0.463
(II) Win/Lose	1,36		2.9	0.098
Escalate	1,35		2.0	0.171
Win/Lose \times Escalate	1,36		2.6	0.115
Esc:Win/Lose	1,35.8		0.0	0.959
Non-esc:Win/Lose	1,36.5		7.3	0.010
SL	1,36.3	-0.05 \pm 0.07	0.4	0.539
(c) Latency to 1st emergence from the shelter				
(I) Launching attacks	1,113		0.1	0.715
SL	1,58.9	0.05 \pm 0.09	0.3	0.599
(II) Win/Lose	1,36.1		0.2	0.662
Escalate	1,35.1		0.1	0.730
Win/Lose \times Escalate	1,36.1		0.6	0.455
Esc:Win/Lose	1,36.1		0.6	0.457
Non-esc:Win/Lose	1,36		0.1	0.797
SL	1,37.1	-0.06 \pm 0.12	0.3	0.587

Supplementary Fig. S1b-I). Contest winners and losers did not differ significantly in the rate of mirror attacks ($p = 0.098$), and the trends were independent of whether the contests were escalated (Win/Lose \times Escalate, $p = 0.115$). However, post-hoc analyses revealed that the losers of non-escalated ($p = 0.010$) but not escalated ($p = 0.959$) contests had lower rates of mirror attacks than the winners (Table 1b-II, Fig. 1b-II; also see Supplementary Fig. S1b-II).

Contestants that did or did not attack their opponents in dyadic contests did not differ in the latency to first emergence from the shelter ($p = 0.715$) (Table 1c-I, Fig. 1c-I; also see Supplementary Fig. S1c-I). Nor did contest winners and losers differ in this latency ($p = 0.662$), and the trends were not dependent on whether the contests were escalated (Win/Lose \times Escalate, $p = 0.455$) (Table 1c-II, Fig. 1c-II; also see Supplementary Fig. S1c-II).

Overall, the rate of mirror attacks was the only personality index that predicted the fish's behaviour in size-matched dyadic contests. Fish that attacked their own mirror images at higher rates were more likely to attack their opponent and win non-escalated size-matched contests.

3.2. Relationships between pre-contest physiological traits and contest interactions

There were no significant correlations among pre-contest testosterone levels, cortisol levels and oxygen consumption rates ($-0.041 \leq r \leq 0.099$, $n = 116$, $0.291 \leq p \leq 0.665$).

Contestants that attacked their opponents in contests had higher pre-contest testosterone than those that did not ($p = 0.003$) (Table 2a-I, Fig. 2a-I; also see Supplementary Fig. S2a-I). Contestants that escalated contests into mutual attacks had higher pre-contest testosterone than those that did not ($p = 0.017$) (Table 2a-II, Fig. 2a-II;

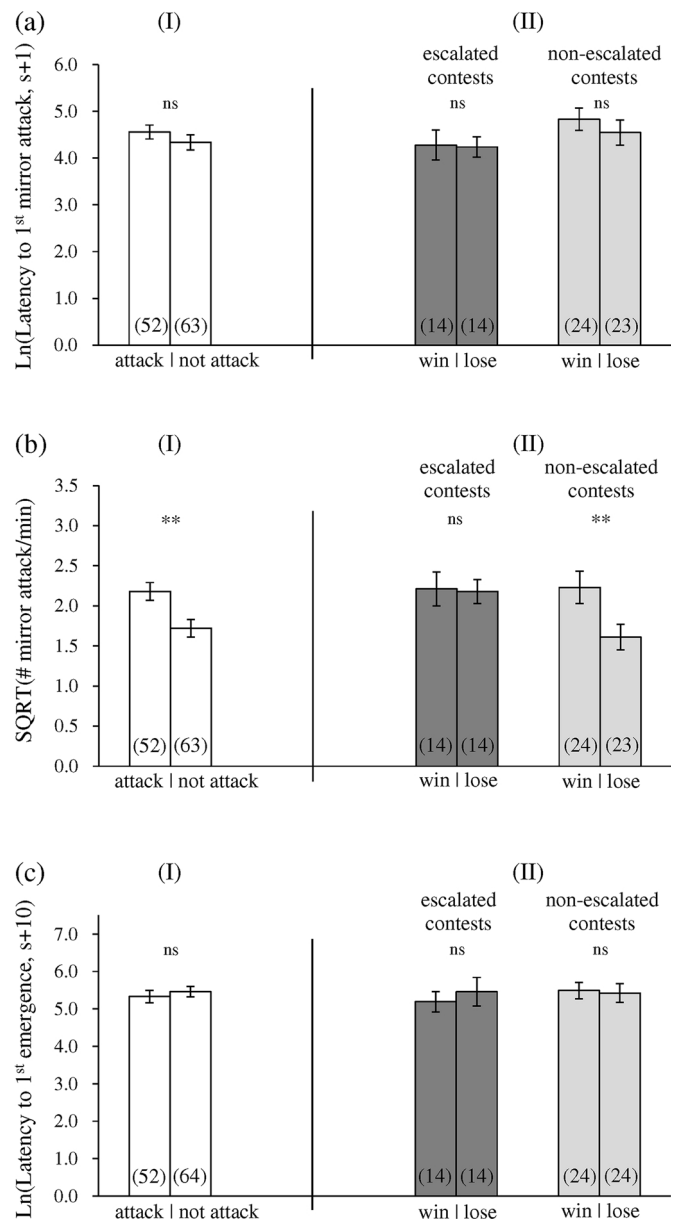


Fig. 1. Pre-contest measurements of (a) the latency (in s) to the first mirror attack, (b) the rate of mirror attacks and (c) the latency to the first emergence from the shelter (mean \pm SE) for test individuals that (I) did or did not attack their opponents in dyadic contests (clear bars) and (II) won or lost escalated (darker grey bars) or non-escalated contests (lighter grey bars). The sample size for each bar is presented in brackets. The indications of significant differences are taken from the linear mixed models presented in Table 1 which considered the influences of other factors. ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$.

also see Supplementary Fig. S2a-II). Contest winners and losers, however, did not differ significantly in their pre-contest testosterone ($p = 0.202$), and the trends were not dependent on whether the contests were escalated (Win/Lose \times Escalate, $p = 0.175$). However, post-hoc analyses showed that losers of non-escalated ($p = 0.034$) but not escalated ($p = 0.958$) contests had lower pre-contest testosterone than winners (Table 2a-II, Fig. 2a-II; also see Supplementary Fig. S2a-II).

Contestants that did or did not attack their opponents in dyadic contests did not differ in their pre-contest cortisol ($p = 0.587$) (Table 2b-I, Fig. 2b-I; also see Supplementary Fig. S2b-I). Contest losers had higher pre-contest cortisol than winners ($p = 0.012$), and the trends were not dependent on whether the contests were escalated (Win/Lose \times Escalate, $p = 0.133$); however, when analysed separately,

Table 2

Linear mixed-effects models evaluating whether test individuals that (I) did or did not attack their opponents or (II) won or lost contests differed in their pre-contest (a) testosterone (T), (b) cortisol (F) or (c) oxygen consumption rate (O_2). For (II), we also evaluated the importance of whether contests were escalated (Win/Lose \times Escalate) and examined the differences between winners and losers in escalated (Esc: Win/Lose) and non-escalated (Non-esc: Win/Lose) contests. All models included the test individuals' standard length (SL), with pair (nested within lineage) as a random variable. Significant effects are in bold.

Variable	df	b \pm SE	F	p
(a) Pre-contest T				
(I) Launching attacks	1,99		9.3	0.003
SL	1,63	0.004 \pm 0.002	3.1	0.085
(II) Win/Lose	1,36.1		1.7	0.202
Escalate	1,35.1		6.4	0.017
Win/Lose \times Escalate	1,36.1		1.9	0.175
Esc:Win/Lose	1,36.2		0.0	0.958
Non-esc:Win/Lose	1,36		4.9	0.034
SL	1,38.8	0.000 \pm 0.003	0.0	0.870
(b) Pre-contest F				
(I) Launching attacks	1,113		0.3	0.587
SL	1,113	-0.006 \pm 0.006	1.1	0.303
(II) Win/Lose	1,71		6.7	0.012
Escalate	1,71		2.1	0.149
Win/Lose \times Escalate	1,71		2.3	0.133
Esc:Win/Lose	1,71		6.7	0.012
Non-esc:Win/Lose	1,71		0.8	0.381
SL	1,71	-0.006 \pm 0.007	0.7	0.392
(c) Pre-contest O_2				
(I) Launching attacks	1,112		2.1	0.153
SL	1,58.6	-0.086 \pm 0.016	28.2	< 0.001
(II) Win/Lose	1,36.1		0.1	0.831
Escalate	1,35.1		0.1	0.759
Win/Lose \times Escalate	1,36.1		0.2	0.643
Esc:Win/Lose	1,36.1		0.2	0.670
Non-esc:Win/Lose	1,36		0.0	0.836
SL	1,36.6	-0.090 \pm 0.018	24.0	< 0.001

losers of escalated ($p = 0.012$) but not non-escalated ($p = 0.381$) contests had higher pre-contest cortisol than winners (Table 2b-II, Fig. 2b-II; also see Supplementary Fig. S2b-II).

Contestants that did or did not attack their opponents in contests did not differ in pre-contest oxygen consumption ($p = 0.153$) (Table 2c-I, Fig. 2c-I; also see Supplementary Fig. S2c-I). Nor did contest winners and losers ($p = 0.831$), and the trends were not dependent on whether the contests were escalated (Win/Lose \times Escalate, $p = 0.643$) (Table 2c-II, Fig. 2c-II; also see Supplementary Fig. S2c-II).

To summarise, contestants with higher levels of pre-contest testosterone behaved more aggressively and were more likely to attack their opponents, escalate contests into mutual attacks and win non-escalated contests. Conversely, contestants with higher levels of pre-contest cortisol were more likely to lose the contests.

3.3. Influence of contest interactions on post-contest physiological traits

Post-contest cortisol showed a positive relationship with post-contest oxygen consumption ($r = 0.279$, 95 % CIs: 0.102–0.439, $n = 116$, $p = 0.002$). There was no significant relationship between post-contest testosterone and either post-contest cortisol ($r = -0.023$, 95 % CIs: -0.204–0.160, $n = 0.116$, $p = 0.809$) or post-contest oxygen consumption ($r = -0.110$, 95 % CIs: -0.287–0.074, $n = 116$, $p = 0.239$).

Contestants that did or did not attack their opponents in dyadic contests did not differ in post-contest testosterone ($p = 0.255$) (Table 3a-I, Fig. 2d-I; also see Supplementary Fig. S2d-I). Nor did contest winners and losers ($p = 0.173$), and the trends were independent of whether the contests were escalated (Win/Lose \times Escalate, $p = 0.747$) (Table 3a-II, Fig. 2d-II; also see Supplementary Fig. S2d-II). Both linear models showed post-contest testosterone to be positively related with pre-contest levels ($p < 0.001$) (Table 3a-I, a-

II).

Contestants that attacked their opponents in contests had higher post-contest cortisol ($p = 0.035$) (Table 3b-I, Fig. 2e-I; also see Supplementary Fig. S2e-I). The difference between contest winners and losers in the levels of post-contest cortisol depended on whether the contests were escalated (Win/Lose \times Escalate, $p = 0.020$); losers of non-escalated ($p = 0.020$) but not escalated ($p = 0.245$) contests had lower post-contest cortisol than winners (Table 3b-II, Fig. 2e-II; also see Supplementary Fig. S2e-II). Both linear models showed post-contest cortisol to be positively related with pre-contest levels ($p \leq 0.002$) (Table 3b-I, b-II).

Contestants that attacked their opponents in contests had higher post-contest oxygen consumption ($p < 0.001$) (Table 3c-I, Fig. 2f-I; also see supplementary Fig. S2f-I). Contest winners had higher post-contest oxygen consumption than losers ($p < 0.001$), and the trends were independent of whether the contests were escalated (Win/Lose \times Escalate, $p = 0.411$) (Table 3c-II, Fig. 2f-II; also see Supplementary Fig. S2f-II). Both linear models showed post-contest oxygen consumption to be positively related with pre-contest rates ($p \leq 0.001$) (Table 3c-I, c-II).

Generally, higher post-contest oxygen consumption was the most observable physiological trait in attackers and winners after contest interactions. Attackers also had higher post-contest cortisol. The fish's physiological traits remained rather consistent over the entire study period as the post-contest measurements of testosterone, cortisol and oxygen consumption rate were positively related with their corresponding pre-contest measurements.

4. Discussion

This study examines whether, and if so how, personality (aggressiveness and boldness) and physiological traits (testosterone and cortisol levels and oxygen consumption rates) are associated with contest decisions/performance using a mangrove killifish *Kryptolebias marmoratus*. The results of the study show that the rate of mirror attacks predicted the fish's behaviour in size-matched dyadic contests and that physiological traits have bidirectional relationships with contest interactions and outcomes. We discuss these results below.

4.1. Relationships between contest interactions/outcomes and personality traits

In this study, individuals of *K. marmoratus* that attacked their own mirror images at higher rates were more likely than others to attack their opponent and win non-escalated contests. This result shows that the fish's intrinsic aggressiveness affects its contest decisions and performance. In a previous study of size-matched contests in *K. marmoratus*, individuals that initiated attacks in the contests had a higher tendency to win, suggesting that aggressive individuals are better fighters (Hsu et al., 2009). However, the present result shows that intrinsic aggressiveness only confers an advantage in non-escalated contests (when non-aggressive opponents retreat) and not in physical fights. Together the results of the two studies suggest that, while aggressive individuals do not necessarily have better physical strength, being aggressive increases an individual's chance of winning especially when encountering non-aggressive opponents.

Our study showed that the responses of individuals of *K. marmoratus* towards their mirror images predicted their responses toward unfamiliar conspecifics. Behaviours towards a mirror image were also found to correlate positively with aggressive behaviours in live agonistic trials in male rainbow kribis (*Pelvicachromis pulcher*) (Scherer et al., 2016). The validity of mirror tests in assessing individual aggressiveness, however, should not be assumed in all species of fish (Balzarini et al., 2014; Earley et al., 2000; Elwood et al., 2014). Balzarini et al. (2014) evaluated the usefulness of mirror tests by comparing an individual's responses towards its own mirror image and

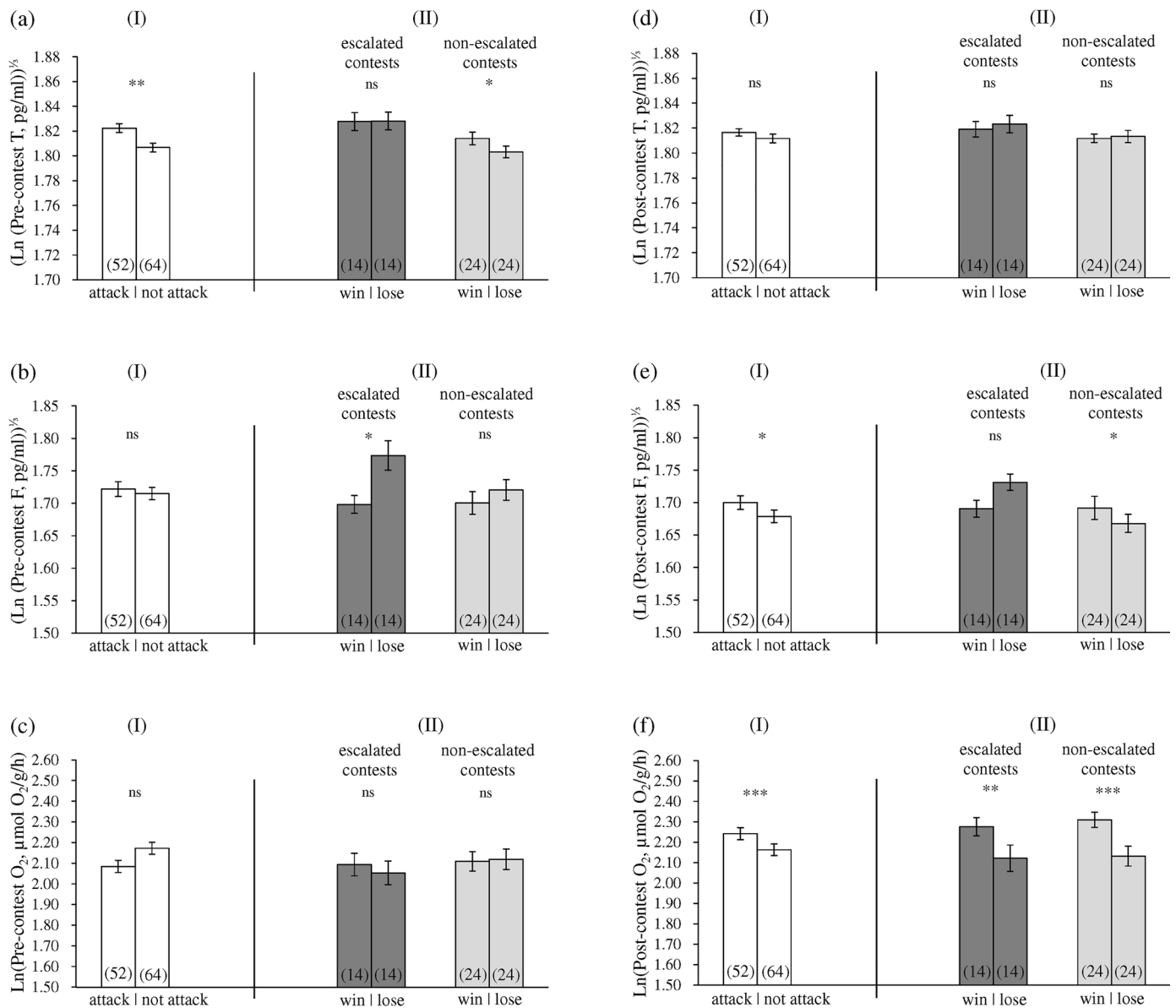


Fig. 2. (a–c) Pre-contest and (d–f) post-contest levels of testosterone (T), levels of cortisol (F) and oxygen consumption rate (O₂) (mean ± SE) for test individuals that (I) did or did not attack their opponents in dyadic contests (clear bars) and (II) won or lost escalated (darker grey bars) or non-escalated contests (lighter grey bars). The sample size for each bar is presented in brackets. The indications of significant differences are taken from the linear mixed models presented in Table 2(a–c) and 3 (d–f) which considered the influences of other factors. ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

towards a size-matched opponent using three species of sympatric cichlids (*Neolamprologus pulcher*, *Telmatochromis vittatus*, and *Lepidolamprologus elongatus*) from Lake Tanganyika. The results showed that the behavioural responses toward mirror images and toward conspecifics were correlated only in *Neolamprologus pulcher*. Overall, the validity of mirror tests in estimating individual aggressiveness might vary between species and should be evaluated before using mirror tests to quantify individual aggressiveness.

The two aggressiveness indices (the latency to the first mirror attack, the rate of mirror attacks) in the study provided consistent measurements; individuals that were quicker to attack their own mirror images attacked their own mirror images at higher rates. Moreover, aggressive individuals were also bolder (shorter latency to first emergence from a shelter), consistent with the results of a previous study (Chang et al., 2012). The boldness-aggressiveness syndrome thus appears to be a relatively stable behavioural characteristic in this fish. Despite the fact that the three behavioural measurements were significantly correlated in this study, only the rate of mirror attacks

predicted the fish's behavioural decisions in the size-matched contests. The two latency measurements did not correspond to the contest interactions/outcomes in this fish. In rainbowfish (*Melanotaenia duboulayi*), however, dominant individuals emerged more quickly from the smaller compartment of a test aquarium (i.e. were bolder) than lower ranked individuals (Colléter and Brown, 2011). We should note that we measured personality traits before dyadic contests while the rainbowfish study measured boldness after the contests. We therefore do not know whether winning and/or losing size-matched contests would affect post-contest latency to leave a shelter in this fish. A previous study of this fish, however, showed that forcing a winning or losing experience on the fish by pitching it against a smaller/weaker or larger/stronger opponent, respectively, did not significantly affect the fish's latency to leave a shelter (Chang et al., 2012). Boldness, therefore, does not appear to be associated with dominance status or the ability to win in this fish.

Table 3

Linear mixed-effects models evaluating whether test individuals that (I) did or did not attack their opponents or (II) won or lost in contests differed in their post-contest (a) testosterone (T) (b) cortisol (F) or (c) oxygen consumption rate (O_2). For (II), we also evaluated the importance of whether contests were escalated (Win/Lose \times Escalate) and examined the differences between winners and losers in escalated (Esc: Win/Lose) and non-escalated (Non-esc: Win/Lose) contests. All models included the test individuals' corresponding pre-contest physiological measure and standard length (SL), with pair (nested within lineage) as a random variable. Significant effects are in bold.

Variable	df	b \pm SE	F	p
(a) Post-contest T				
(I) Launching attacks	1,108		1.3	0.255
SL	1,60	0.002 \pm 0.002	1.3	0.256
Pre-contest T	1,107	0.474 \pm 0.076	39.3	< 0.001
(II) Win/Lose	1,35.8		1.9	0.173
Escalate	1,36.7		0.0	0.965
Win/Lose \times Escalate	1,35.9		0.1	0.747
Esc:Win/Lose	1,35		0.5	0.503
Non-esc:Win/Lose	1,37.2		1.9	0.174
SL	1,36.3	0.002 \pm 0.002	0.9	0.346
Pre-contest T	1,67.4	0.406 \pm 0.094	18.5	< 0.001
(b) Post-contest F				
(I) Launching attacks	1,102		4.6	0.035
SL	1,61.7	-0.011 \pm 0.006	2.8	0.102
Pre-contest F	1,78.6	0.223 \pm 0.070	10.1	0.002
(II) Win/Lose	1,37		0.2	0.650
Escalate	1,35.5		1.9	0.178
Win/Lose \times Escalate	1,35.8		6.0	0.020
Esc:Win/Lose	1,37		1.4	0.245
Non-esc:Win/Lose	1,35.3		6.0	0.020
SL	1,38.8	-0.009 \pm 0.007	1.7	0.204
Pre-contest F	1,51.9	0.291 \pm 0.080	13.3	< 0.001
(c) Post-contest O_2				
(I) Launching attacks	1,102		17.8	< 0.001
SL	1,72	-0.050 \pm 0.019	7.1	0.010
Pre-contest O_2	1,107	0.313 \pm 0.090	12.0	< 0.001
(II) Win/Lose	1,35		32.7	< 0.001
Escalate	1,34.5		0.0	0.985
Win/Lose \times Escalate	1,35.1		0.7	0.411
Esc:Win/Lose	1,35.1		9.4	0.004
Non-esc:Win/Lose	1,34.9		29.2	< 0.001
SL	1,47	-0.034 \pm 0.021	2.7	0.110
Pre-contest O_2	1,59.6	0.348 \pm 0.097	12.9	< 0.001

4.2. Bidirectional relationships between contest interactions/outcomes and physiological traits

In the present study, contestants with higher pre-contest testosterone behaved more aggressively than others in size-matched contests. These individuals were more likely to attack and to escalate contests into mutual attacks, and won more non-escalated contests. Contestants with higher pre-contest cortisol, however, were more likely to lose contests. After the contests, (1) contestants that had attacked their opponents or won had higher oxygen consumption rates than non-attackers and losers and (2) contestants that had attacked their opponents also had higher levels of cortisol. Although the three physiological traits did not correlate with each other prior to the contests, a positive correlation between cortisol levels and oxygen consumption rates arose after the contests.

Testosterone has long been linked to increases in aggressive behaviour, and our result that individuals of *K. marmoratus* with higher levels of pre-contest testosterone behaved more aggressively in size-matched contests is consistent with this notion. The result that winners had higher pre-contest testosterone than losers only in non-escalated contests, and not in escalated contests, indicates that individuals with higher testosterone behave more aggressively but are not better at winning physical fights. These individuals had an advantage in winning only when encountering submissive opponents that retreated immediately when challenged.

Interestingly, no difference in post-contest testosterone was detected between winners and losers in the study despite the facts (1) that individuals with higher levels of pre-contest testosterone behaved more aggressively in the study and (2) that this fish has been shown to exhibit winner-loser effects and become more and less aggressive after recent wins and losses, respectively, in many studies (Earley et al., 2013; Huang et al., 2011; Lan and Hsu, 2011). A previous study of the fish also reported no differences between the winners and losers of size-mismatched contests in post-contest levels of testosterone or 11-ketosterone (Earley and Hsu, 2008). The behavioural changes in this fish after wins or losses are, therefore, probably not mediated by changes in the androgen levels as in some other species (e.g., Mozambique tilapia *Oreochromis mossambicus*, Oliveira et al., 2009). In *K. marmoratus* it has been shown that winning and losing experiences affect the fish's brain androgen receptor: repeated losses decreased, whereas repeated wins increased androgen receptor gene expression in individuals with low testosterone levels (Li et al., 2014). Thus, it is possible that, in order to maintain its unique hermaphroditic reproductive system, the mangrove killifish responds to contest experience and mediates aggressiveness toward conspecifics by altering androgen receptor gene expression levels without changing plasma testosterone levels (Li et al., 2018).

Cortisol had complex relationships with contest interactions in this study. Individuals with higher levels of pre-contest cortisol were more likely than others to lose size-matched contests, consistent with much of the existing literature (Summers et al., 2005). Furthermore, despite the fact that no overall difference in the level of post-contest cortisol was detected between eventual winners and losers, (1) contestants that had attacked their opponents had higher post-contest cortisol than those that had not, and (2) contestants that had won the contests without escalation had higher levels of post-contest cortisol than those that had lost them. In fact, because the losers of non-escalated contests were also individuals that retreated without retaliation when attacked (the non-attackers), the difference in the level of post-contest cortisol between winners and losers of non-escalated contests probably merely echoes the difference in post-contest cortisol between attackers and non-attackers. (Attackers had higher levels of post-contest cortisol.) The result that winners and losers did not differ in post-contest cortisol is consistent with the finding of a previous study (Earley and Hsu, 2008) of *K. marmoratus*. Although higher levels of cortisol are usually expected of subordinate individuals (Schuett and Grober, 2000; Summers et al., 2005), no difference in post-contest cortisol between contest winners and losers was also reported for convict cichlids (*Archocentrus nigrofasciatus*) (Earley et al., 2006) and Nile tilapia (*Oreochromis niloticus*) (Corrêa et al., 2003). In rainbow trout (*Oncorhynchus mykiss*), a difference in post-fight cortisol between the winners and losers was not detected 5 min after contest resolution but was detected 3 h after (the winner and loser were allowed to interact for the intervening period) because winners' cortisol returned to the control level quickly (in 3 h) while the losers' cortisol remained elevated (Øverli et al., 1999). We collected water samples for post-contest hormones immediately after contest resolution and there was no evidence to indicate that contest interactions caused different levels of stress for winners and losers. The results of (1) this study that attackers had higher levels of post-contest cortisol than non-attackers and (2) a previous study of the fish that winners that delivered more attacks to losers had higher levels of post-contest cortisol (Earley and Hsu, 2008), however, suggest that attacking behaviour is stressful and/or energetically costly in this fish (Goymann and Wingfield, 2004).

Our results also showed that, although pre-contest oxygen consumption was not associated with contest interactions or outcomes, post-contest oxygen consumption was greatly influenced by contest interactions and outcomes such that attackers and winners had higher post-contest oxygen consumption rates than non-attackers and losers. Because we measured post-contest metabolic rates shortly after the contests, these results suggest that physical attacks incur high metabolic costs and that contest interactions were more metabolically costly to

winners than to losers for both escalated and non-escalated contests. Contest interactions being more metabolically costly to winners than to losers has been demonstrated in other species. In male crickets (*Acheta domesticus*), contest winners performed more agonistic acts and thus had higher cumulative net oxygen consumption than losers (Hack, 1997). Factors other than winners' higher levels of physical activity might also contribute to their higher metabolic costs. In juvenile Ambon damselfishes (*Pomacentrus amboinensis*), for example, although the dominants performed more aggressive acts than the subordinates, dominants had lower increases in oxygen uptake rate than subordinates (Killen et al., 2014). Nonetheless, when the dominants and subordinates were exposed to each other again two hours after the contests, the dominants showed higher increases in oxygen uptake than the subordinates, and the changes in metabolic rate did not correlate with the activity or aggression displayed, which suggested that an autonomic stress response might be involved in hierarchy maintenance and impose a higher metabolic cost on dominants (Killen et al., 2014). The contest pairs in our study were size matched; it is therefore conceivable that winners had to exert greater force than losers in contest interactions in order to secure victory. Furthermore, contest pairs were allowed to interact for five minutes after the loser's first retreat to confirm the winner-loser status, during which time winners continued to harass and chase the losers. The post-contest interactions could also have contributed to the difference between the winners' and the losers' post-contest metabolic rates if active harassment is more energy demanding than passive avoidance. Overall, these results suggest that, in *K. marmoratus*, the capability and/or willingness of contestants to pay high metabolic costs has an important influence on contest decisions and outcomes.

Studies that have investigated the relationships between hormones, metabolic rates and behaviour have produced mixed results (Moore and Hopkins, 2009), despite the role of hormones regulating both behaviour and energy metabolism. In adult male common lizards (*Zootoca vivipara*), sociability and activity were negatively associated with resting metabolic rate but were not associated with corticosterone levels (Mell et al., 2016). In juvenile Senegalese soles (*Solea senegalensis*), cortisol correlated negatively with the number of escape attempts, resting metabolic rates correlated positively with latency to escape and there was no significant association between cortisol and metabolic rate (Martins et al., 2011). In the Mozambique tilapia (*Oreochromis mossambicus*), resting metabolic rates were positively correlated with levels of 11-ketotestosterone but not with testosterone (Ros et al., 2004). In our study, oxygen consumption rates did not have a detectable relationship with pre-contest levels of either testosterone or cortisol. However, after contest interactions, a significant positive correlation between post-contest oxygen consumption rates and cortisol emerged. This positive correlation therefore probably arose as a result of an individual's behavioural performance (e.g. activity levels) during contests affecting both cortisol levels and oxygen consumption rates. We did not evaluate how long this positive correlation lasted for; these results, however, suggest (1) that social interactions and/or status could promote associations between different physiological traits and (2) that the relationships between different physiological traits change rather quickly in response to social interactions.

The results that the fish's post-contest physiological measurements (hormone levels and oxygen consumption rates) significantly and positively related with the corresponding pre-contest measurements indicate that these physiological measurements remained consistent over time (about two days). These relationships were robust such that the aggressiveness test, boldness test and size-matched contest in between the two measurements did not cause the relationships to become undetectable. The consistency of these mechanisms over longer periods of time, however, remains to be investigated.

5. Conclusions

Pre-contest personality (rate of mirror attacks) and physiological traits (testosterone and cortisol levels) predicted the fish's behavioural decisions in size-matched contests. Contest interactions subsequently modified post-contest physiological traits (cortisol level and oxygen consumption rate) and potentially also promoted associations between them. The differences between contest winners and losers in pre- and post-contest physiological traits depended on whether or not the contests were escalated into physical interactions. Nevertheless, the fish still exhibited individual physiological consistency, since pre- and post-contest physiological traits were highly correlated with each other.

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ORCID iD authorship contribution statement

Cheng-Yu Li: Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision. **Yung-Che Tseng:** Methodology, Resources, Writing - original draft, Writing - review & editing, Funding acquisition. **Yu-Ju Chen:** Investigation, Data curation, Writing - original draft. **Yusan Yang:** Investigation, Data curation, Writing - original draft. **Yuying Hsu:** Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.beproc.2020.104079>.

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