



Metabolic costs of fighting are driven by contest performance in male convict cichlid fish

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One of the most important decisions that an animal faces is whether to persist or surrender in an aggressive contest. It pays to win these encounters, but it might be just as economical to evaluate fighting costs and adjust behavioural decisions accordingly. Relatively few studies have explored the physiological basis of contest costs and fewer have determined the internal signals that animals might attend to during combat. We explored relationships between physiological parameters, including energy substrates, anaerobic metabolites and steroid hormones, and both contest performance and contest success in male convict cichlids, *Amatitlania nigrofasciata*. Size-matched pairs were allowed to fight until one member of the pair submitted, and tissues were subsequently harvested for analysis. There were no significant status differences (winner, loser, control) in mean postfight physiological measures. Dominance reinforcement during a short postsettlement period buffered the winners' stress response. In both winners and losers, muscle lactate concentrations correlated positively with contest intensity, while only losers showed a significant increase in plasma glucose concentrations as contests intensified. Larger cichlids used more costly fighting tactics than smaller conspecifics, and size asymmetries affected contest outcomes between larger animals significantly more than they did those between smaller animals. These findings suggest that fighting is metabolically costly and that the ramifications of these costs may differ in a size-dependent fashion. We use a recent literature on the scaling of anaerobic tolerance to evaluate the possibility that larger cichlids may be more tolerant of lactate accumulation, and thus able to both engage in more intense fighting tactics and persist longer in escalated fights than marginally smaller opponents. Contest intensity varied independently of mass asymmetries but varied positively with the absolute size of both the winner and loser, suggesting that convict cichlids use self-assessment strategies during contests with similarly sized opponents.

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The factors that influence fight outcomes among animals are complex and multifaceted. Classic views centre on fighting ability, termed resource-holding potential (RHP), and its role in moderating animal contest dynamics (Parker 1974; Briffa & Sneddon 2010). A major component of RHP is the animal's body size, and there is generally a positive correlation between relative body size/mass and contest success (Arnott & Elwood 2009a). The specific mechanisms through which size may confer greater RHP (e.g. strength, hormone titres, or mass-specific physiological tolerances) have not been thoroughly investigated in most animals. However, it is reasonable to suspect that, during fights, animals use specific

mechanisms to assess their own RHP, that of their opponent, or both. Selection should favour quick resolution of aggressive interactions between highly asymmetrical pairs, particularly for interactions that are energetically costly and/or tend to result in injury. Therefore, animals must decide whether to engage or escalate in contests based on, for example, visual or chemical cues indicative of opponent fighting ability, or internal cues reflecting accumulation of energetic costs or injury (Briffa & Elwood 2001; Arnott & Elwood 2009a). Animals may decide whether and for how long they will fight by using self-assessment and/or mutual assessment (Hsu et al. 2008; Arnott & Elwood 2009a).

Mutual assessment (MA) posits that contestants can gauge each other's fighting ability and thus forgo futile escalation once they realize their relative RHP (Enquist & Leimar 1983). Models of self-assessment (SA) state that contestants assess only their own ability to perform in a contest and while a positive relationship should exist between the loser's RHP and contest duration, no

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relationship is expected for the winner's RHP. This expectation is based on the reasoning that, regardless of MA or SA, losers ultimately dictate contest duration but, under SA, the loser's willingness to persist in a contest is based on its own 'absolute' RHP rather than relative RHP (i.e. its own RHP compared to that of its opponent). Under SA, the contestant with the greater RHP only needs to match the other contestant's intensity and by self-assessing should therefore be able to outlast its opponent; this often results in a shallow, nonsignificant relationship between winner RHP and contest duration. However, it has been recognized that even within contests involving SA, a contestant may be influenced by the actions of the other contestant (Payne 1998). This model, termed 'cumulative assessment' (CAM), accounts for the effects of, for example, injury infliction on the decision to give up. So, in addition to evaluating their energetic state, a contestant may use SA to evaluate injury cost and submit once a threshold level of injury has been incurred. For a recent review of assessment models, including cumulative assessment, see Arnott & Elwood (2009a) and Briffa & Sneddon (2010). Given the appreciable costs of fighting, including energy depletion, increased predation risk and injurious fighting (Austad 1983; Neat et al. 1998a, b; Brick 1999; Briffa & Elwood 2001), selection should favour assessment strategies such as MA and SA that provide the most reliable information about whether and for how long to persist in a fight. MA would prevent fight escalation between highly asymmetrical contestants because RHP differences would be communicated with less costly behaviours such as threat displays. However, because body size is a major component of fighting ability, MA would be difficult to use effectively when contestants are similarly sized. Moreover, studies have highlighted the fact that traditional tests for MA, such as regressing body size asymmetries with contest duration, are not sufficient to distinguish it from SA (Taylor & Elwood 2003; Arnott & Elwood 2009a; see Briffa & Elwood 2009 for additional perspectives on distinguishing MA and SA, as well as various SA models such as energetic wars of attrition and cumulative assessment).

While MA should depend critically on information exchanged during the fight, the decision to initiate or persist under SA scenarios is likely to be driven by another source of information; changes in physiology. Indeed, endocrine and energetic profiles are sensitive to both the dynamics and outcome of aggressive encounters, and can influence a contestant's willingness and ability to fight (Hsu et al. 2006; Briffa & Sneddon 2007). Fighting requires mobilization of glucose and glycogen, and in searching for a relationship between energy expenditure and contest dynamics many studies have found the metabolites glucose and lactate to be the most informative (Neat et al. 1998b; Sneddon et al. 1999; Briffa & Elwood 2001, 2004; Brandt 2003; Matsumasa & Murai 2005; Prenter et al. 2006). Many agonistic behaviours have high energy demands and consequently production of ATP may become heavily anaerobic, with lactic acid (lactate) manufactured as a by-product. Studies in hermit crabs, *Pagurus bernhardus*, have revealed that attackers that fail to evict an opponent from its shell (losers) show increased haemolymph lactate relative to their opponents, while those that successfully evict the defending crab (winners) show similar lactate concentrations as their opponents (Briffa & Elwood 2001). This suggests that lactate accumulation represents a potentially serious cost and may negatively influence the decision to continue fighting (i.e. success may depend on lactate accumulation rates or tolerance to lactate). Moreover, defenders that successfully resist eviction have higher glucose concentrations than evicted defenders, suggesting that carbohydrate-derived energy reserves are important in dictating fight persistence (Briffa & Elwood 2001). In teleost fishes such as *Betta splendens*, opercular displays used in aggressive interactions decrease in hypoxic environments, a behavioural mechanism that perhaps combats the accumulation

of metabolites of anaerobic respiration such as lactate (Abrahams et al. 2005). Although these authors did not measure lactate, it is reasonable to suggest that the reduction in oxygen uptake owing to impaired gill ventilation during opercular display imposes a greater demand for anaerobic production of ATP (Abrahams et al. 2005). In *Tilapia zillii*, losers have higher muscle lactate and lower hepatic glucose/glycogen than both winners and controls (Neat et al. 1998a). Additionally, Briffa (2008) found that house crickets that had won a contest had higher glucose levels than losers. Collectively, these studies suggest that fighting is costly in terms of energy depletion and that greater lactate accumulation and/or less energy mobilization during a fight may contribute to the decision to submit.

From an endocrine perspective, steroid hormones such as cortisol and androgens (e.g. testosterone; 11-ketotestosterone) are associated in many ways with the expression of aggressive behaviour and with both contest dynamics and eventual social status. Cortisol, the primary stress hormone in fish, influences both contest behaviour and outcome (Øverli et al. 2002; DiBattista et al. 2005). Rainbow trout, *Oncorhynchus mykiss*, that have been selected for high and low stress responsiveness (defined by differential cortisol responses to a stressor), differ in their dominance ability (low-responsive fish dominated in 43 of 46 paired encounters; Pottinger & Carrick 2001) and, when treated with exogenous cortisol, become less aggressive and subordinate (DiBattista et al. 2005). Similarly, mangrove rivulus, *Kryptolebias marmoratus*, with lower endogenous prefight cortisol levels initiate and win contests more often (Earley & Hsu 2008). In addition to being a good predictor of contest dynamics and outcome, cortisol also appears to respond in a graded fashion to contest intensity; convict cichlids, *Amatitlania nigrofasciata*, that engage in more intense fights show elevated stress hormone levels (Earley et al. 2006). Androgens similarly predict and respond to aggressive social encounters. Earley & Hsu (2008) found a positive relationship between endogenous prefight testosterone levels and both contest initiation and success in the mangrove rivulus. Moreover, a number of studies have shown that newly dominant animals of both sexes show elevated concentrations of testosterone or 11-ketotestosterone (Oliveira et al. 1996; Elofsson et al. 2000; Desjardins et al. 2006). Androgens may also function to reinforce social status, highlighted by the observation that blocking androgen receptors significantly reduces the chances of winning future contests in Mozambique tilapia, *Oreochromis mossambicus*, that are established winners (Oliveira et al. 2009).

In this study we investigated the physiological responses of convict cichlids, *A. nigrofasciata*, to fight dynamics and outcome, with particular emphasis on energetic substrates, metabolites and steroid hormones. This New World cichlid is known for being highly aggressive and territorial, and has well-defined fighting behaviours (Koops & Grant 1993; Leiser & Itzkowitz 1999; Leiser et al. 2004). One behaviour of particular interest is mouth wrestling (defined in Methods section), which is believed to be highly anaerobic since it impedes proper opercular beating (Neat et al. 1998a). We tested the hypothesis that status-dependent differences in tissue energy substrates/metabolites and plasma hormone concentrations would emerge following a contest. We also hypothesized that contestants gauge these physiological measures during a fight to guide decisions to persist or flee, particularly when pitted against a size-matched opponent. Internal cues can guide self-assessment only if they grade incrementally with some measure of contest performance. Thus, we expected energy substrates, anaerobic metabolites and hormone titres to be tightly correlated with contest dynamics, and that scaling relationships between the most physiologically informative behavioural parameters and winner/loser RHP would allow us to discern whether cichlids use MA or SA strategies during contests.

METHODS

Staging Fights

Fish were purchased from a commercial distributor (Pet Solutions, Inc., Beaver Creek, OH, U.S.A.) and were maintained in two artificial ponds with dimensions 137 × 200 × 61 cm filled with 550 litres of water. The ponds were aerated, chemically and biologically filtered, and shelter was provided with gravel substrate and broken terra cotta pots. Fish were fed once daily to satiation between 0900 and 1100 hours on a diet of brine shrimp, blood worms and cichlid flakes under a 12:12 h light:dark cycle at 25 °C (±2 °C). Water changes were conducted biweekly coupled with the addition of API freshwater salt (Mars Fishcare, Inc., Chalfont, PA, U.S.A.), Nutrafin® Cycle (denitrifying bacteria; R. C. Hagen, Mansfield, MA, U.S.A.) and Amquel® (Kordon LLC, Hayward, CA, U.S.A.) to condition the water. Stress Coat® (API) was added after water changes and any time the fish were handled (e.g. netting) to replace the slime coating and maintain proper health.

Cichlids used in this study varied in body mass from 3 to 16 g, and fighting pairs were distinguished with a single cut at the top or bottom half of the caudal fin made through the keratinous matrix between the fin rays. Body measurements were taken (Table 1) and contestants and controls (defined below) were isolated 3 days prior to fighting in order to reduce the effects of previous social experience obtained in the communal housing tanks; winner and loser effects in fishes are known to persist for at least 1 day (Hsu et al. 2006). All isolated fish were kept under housing and feeding regimes identical to the communal fish stocks (with the exception of being isolated). Twenty-four hours before the fight, two size-matched fish (on average within 5% body mass) were placed in a three-chambered tank (60 × 20 × 30 cm) with a gravel substrate of 3 cm and two black removable partitions such that the two peripheral chambers housed the contestants and the middle chamber housed an artificial territory (terra cotta pot), heater and filter.

Fights began when the heater and filter were removed from the central compartment and the black partitions were simultaneously removed. Contests were considered settled when one contestant retreated and no longer reciprocated aggressive acts; contests were given a period of 5 min following the first retreat to confirm settlement (behaviours were still observed and quantified during this time). In this study, status was never reversed following the first retreat. Two fights were staged per day from 0930 to 1200 hours; mass-representative controls for the two fights were housed together in a tank with the exact specifications as those of the fight tanks described above. Control fish remained behind the black partitions and were not permitted to engage with the other control fish in the tank.

Contestants were removed from the tank following the 5 min settlement confirmation period, and both opponents of a given fight, plus a time- and size-matched control animal, were

processed simultaneously according to the following procedure: blood sampling, rapid decapitation, liver and caudal fin muscle excision. Blood sampling was achieved using caudal venipuncture with heparinized syringes and 29-gauge needles; average ± SE time for blood withdrawal was 152.2 ± 6.8 s; we failed to obtain adequate volumes of blood from nine fighters and 13 controls. Blood was kept on ice for no more than 10 min before centrifuging at 10 000×g for 5 min to isolate plasma. The liver was used to measure glucose/glycogen stores and caudal fin muscle was chosen for lactate measurement because the caudal fin is used extensively during most fighting behaviours (e.g. during mouth wrestling, the fish typically beat their caudal fins rapidly in attempts to push their opponent backward). Tissue samples were fast-frozen in liquid nitrogen (average ± SE time to freezing: liver = 314.7 ± 1.7 s; muscle = 302.9 ± 8.8 s) as were plasma samples, and were stored at –80 °C until assays were performed. Two fights were discarded from the analysis because one of the contestants was confirmed to be female (gonad inspection) and three fights were discarded because dominance status was unclear; this left a total of 27 contests ($N = 54$ opponents, $N = 32$ control animals).

Quantifying Behaviour

Fights were videorecorded (JVC GR-D850 MiniDV Camcorder with 35X Optical Zoom) using miniDV cassette tapes, which were later converted to digital format (Windows Media Player). Fights were subsequently viewed and behaviours quantified by one author (C.B.F.) who was naïve to the hypotheses being tested. Contestants were distinguishable by a small slit on the top or bottom half of the caudal fin. We tallied the frequency of lateral displays, a unilateral or reciprocal act characterized by intense undulating across the lateral surface of the opponent; mouth wrestling, where both contestants lock mouths and attempt to push their opponent with vigorous caudal fin thrusting; circling, which is tallied when both fish complete one head-to-tail revolution in pursuit of each other; attacks, which is identified when a contestant rapidly approaches the opponent and is frequently followed by biting (mouth contact with an opponent). We also measured the time spent mouth wrestling and total duration of the contest.

Biochemical Analyses

Reagents for glucose/glycogen assays were supplied by Glucose (HK) assay kits from Sigma–Aldrich (catalogue number: GAHK-20), and by Trinity Biotech for lactate assays (Lactate Reagent, catalogue number: 735-10; Lactate Standard Solution, catalogue number: 735-11). Hepatic glucose and glycogen concentrations were determined according to Lankford et al. (2005) with some modifications. Briefly, we homogenized liver samples (46.65 ± 0.88 mg) in 500 µl of ice-cold 0.1 M citrate buffer at room temperature with a motor

Table 1
Mean ± SE body size measurements for opponents in contests between male convict cichlids

Contestant	Total length (mm)	Standard length (mm)	Body depth (mm)	Body mass (g)
Smaller	68.10±2.37 (55.6–93.7)	52.56±1.72 (43.3–52.6)	23.48±0.90 (18.1–32.9)	6.41±0.70 (2.95–15.9)
Larger	70.16±2.40 (55.9–95.0)	54.15±1.73 (43.8–71.7)	24.30±0.90 (18.8–33.7)	6.69±0.72 (3.1–16.1)
Eventual loser	68.84±2.23 (55.6–93.7)	53.61±1.70 (43.3–72.5)	23.51±0.84 (18.1–32.9)	6.43±0.67 (3.0–15.9)
Eventual winner	69.83±2.38 (55.7–95.0)	54.33±1.76 (43.6–72.5)	23.74±0.87 (18.6–33.7)	6.57±0.69 (3.1–16.1)
Asymmetry	1.98±0.35 (0–7.9)	1.42±0.26 (0–5.4)	0.77±0.10 (0–2.3)	0.27±0.05 (0–0.95)
%Asymmetry	2.89±0.51 (0–10.8)	2.72±0.51 (0–11.7)	3.29±0.47 (0–10.7)	4.07±0.60 (0–13.69)
Controls	70.19±2.01 (56.0–91.6)	54.83±1.58 (43.6–70.9)	23.96±0.72 (18.432.6)	6.70±0.59 (3.1–14.6)

Body sizes of small and large individuals of the pair and the eventual winner and loser of the pair are shown. Mean absolute and percentage asymmetries in all body size parameters also are shown. $N = 27$ opponent pairs; $N = 32$ controls. Means for 'smaller' and 'larger' opponents were derived from fewer samples because opponents of a pair had the same total length ($N = 1$ pair), standard length ($N = 3$ pairs), body depth ($N = 2$ pairs) or mass ($N = 1$ pair). Ranges of all body size measurements are shown in parentheses.

homogenizer (Fisher Scientific PowerGen Model 125, catalogue number: 14-261-02), followed by 10 s of vortexing and centrifugation at $3000\times g$ for 5 min. We transferred 50 μl of supernatant containing both free glucose and glycogen into each of two 12×75 mm borosilicate vials (on ice). To one tube, we added 50 μl of amyloglucosidase enzyme (Sigma–Aldrich, catalogue number: A7095) and 500 μl of 5% trichloroacetic acid (TCA) to the supernatant, followed by 10 s of vortexing and centrifugation at $3000\times g$ for 2 min; adding TCA immediately after amyloglucosidase prevents enzyme activity and the liberation of glucose from glycogen stores (this sample thus measures free glucose). To the second tube, we added 50 μl of amyloglucosidase enzyme to the supernatant and allowed it to incubate for 2 h at room temperature in an oscillating water bath, after which time we added 500 μl of 5% TCA, followed by centrifugation at $3000\times g$ for 2 min. Glucose assayed in these samples represent the sum of free glucose and glucose liberated from glycogen stores; liver glycogen content was estimated by subtracting the values of the first supernatant (free) from the second (sum of free and liberated). All samples were stored at -80°C until assay. Hepatic glucose/glycogen content (mg/ml) was quantified using a hexokinase assay that results in the production of NADH, which is measured spectrophotometrically at 340 nm (BioTek ELx800 absorbance microplate reader). We obtained liver glucose and glycogen readings for 53 fighters and 29 controls. Plasma glucose (10 μl) was measured by the same spectrophotometric hexokinase assay but without the homogenization and processing steps described for the liver; because we failed to obtain blood for some animals, we assayed 45 fighters and 19 controls for plasma glucose.

Muscle L-lactate was measured according to Bergmeyer (1983) with some modifications. Briefly, we placed muscle samples (51.41 ± 1.09 mg) in 1 ml of ice-cold 8% perchloric acid (with 1 mM EDTA), homogenized the samples (as described above for the liver), then vortexed each sample for 10 s and set it on ice for 30 min. We centrifuged the samples at $9000\times g$ for 5 min at 4°C and neutralized the supernatant aliquots with 290 μl of 2 M KOH containing 0.4 M imidazole and 0.4 M KCl. Following neutralization, the mixture was centrifuged at $9000\times g$ for 30 s at 4°C and the resulting supernatant was stored at -80°C until assay. To assay lactate, we added 450 μl of hydrazine-glycine buffer (to promote unidirectional conversion of lactate to pyruvate) to all test wells of a 96-well plate, followed by addition of 50 μl of diphosphopyridine nucleotide (5×10^{-2} M β -DPN) solution, which was the source of NAD^+ . We added 400 μl of ultrapure water to all sample wells, followed by 100 μl of standards or samples. The contents of each well were mixed by shaking slowly for 10 min. We took two background spectrophotometric readings (340 nm) at 3 min intervals, then added 10 μl of lactate dehydrogenase and mixed the samples for 20 min by slow shaking. We took a final reading and subtracted this value from the background values to determine lactate concentration (mg/dl) against a standard curve. We obtained muscle lactate readings for 53 fighters and 31 controls.

Steroid Hormone Extraction and Assay

For those animals with enough plasma volume remaining from the glucose assays ($N = 38$ fighters, $N = 14$ controls), we conducted steroid hormone extractions. We diluted 2–5 μl of plasma in 16 ml of ultrapure water in 18×150 mm borosilicate vials. Sep-Pak C18 columns (3 ml, 500 mg, Waters Corporation, Milford, MA, U.S.A.) were fitted to a vacuum manifold and primed with two consecutive 2 ml washes of methanol (MeOH) followed by two consecutive 2 ml washes of distilled water; approximately 0.5 ml of the second distilled water wash remained in the column to keep it moist. Tygon[®] tubing (Saint-Gobain, formulation 2275) was fitted to the

columns and placed into the borosilicate vial containing the diluted plasma sample. The vacuum was engaged and the steroids were trapped on the C18 column. Any salts were removed from the columns with two consecutive 2 ml distilled water washes followed by elution of the steroid into 13×100 mm borosilicate vials with two consecutive 2 ml MeOH elutions. MeOH was evaporated with a stream of ultrapure nitrogen at 37°C in a water bath. Samples were reconstituted with 340–500 μl of enzyme-immunoassay buffer (Cayman Chemicals, Inc., Ann Arbor, MI, U.S.A.; reconstitution volume depends on initial plasma volume); dilution factors ranged from 1:100 to 1:250 and were accounted for in calculating final steroid hormone concentrations. Cortisol, 11-ketotestosterone (KT) and testosterone (T) were assayed with 96-well enzyme immunoassays (Cayman Chemicals, Inc.), and we followed kit instructions precisely. All samples were assayed in duplicate on two plates, and absorbance was read with a BioTek ELx800 absorbance microplate reader at 450 nm. A convict cichlid plasma pool was generated by combining 100 μl of the reconstituted hormone from all individuals that were assayed. This pool was used as an assay control (assayed in duplicate at the beginning and end of each plate), and for kit validations (see next paragraph). Intra-assay coefficients of variation for plates 1 and 2, respectively, were 1.1% and 1.0% for cortisol, 15.7% and 8.6% for KT, and 4.3% and 10.7% for T. Interassay coefficients of variation were 3.1% for cortisol, 11.8% for KT and 10.4% for T.

To validate the Cayman Chemicals EIA kits, we conducted serial dilutions of the cichlid plasma pool (lowest dilutions were 1:128 for cortisol, 1:32 for KT and 1:16 for T). The serial dilution curves were parallel to the standard curves (comparison of slopes; Zar 1996, page 355; cortisol: $t_{12} = 0.29$, $P = 0.78$, KT: $t_{10} = 0.03$, $P = 0.98$, T: $t_9 = 0.16$, $P = 0.87$). To evaluate recovery, 75 μl of cichlid plasma pool was aliquoted to eight microcentrifuge tubes and spiked with an equal volume of each kit standard. Slopes (β) from the regressions of expected against observed steroid hormone concentrations were 0.84 for cortisol, 1.09 for KT and 1.09 for T, indicating adequate recovery (a slope of 1.0 would indicate perfect recovery). Kit sensitivities were 7.81 pg/ml for cortisol, 0.78 pg/ml for KT and 3.91 pg/ml for T.

Statistical Analysis

We tested absolute status differences in plasma glucose, hepatic glucose, hepatic glycogen, muscle lactate and steroid hormones (T, 11KT and cortisol) with a repeated measures ANCOVA using status as the 'within-experimental unit' factor and body mass as a covariate; the analyses were performed using the MIXED procedure in SAS version 9.1.3 (SAS Institute, Cary, NC, U.S.A.) with unstructured covariance, and physiological measures were transformed to achieve normality (Table 2). The repeated measures approach to analysing contest data in which the winners, losers and controls constitute 'repeated' measures within the experimental unit (i.e. fighting pair plus size- and time-matched controls) has recently been forwarded as a more powerful, and statistically appropriate, alternative to traditional ANOVA (Briffa & Elwood 2010). Paired t tests (two-tailed) comparing winners, losers and controls revealed identical patterns as the repeated measures ANCOVA. We used principal components analysis (PCA) to distill 16 measures of contest performance down to four components; PCA statistics and component interpretations can be found in Table 3. We used simple regressions to evaluate relationships between contest dynamics (principal component scores) and physiological measures. Slope comparisons (Zar 1996, page 355) were used to determine differences between winners and losers (or smaller and larger contestants) in the rate of physiological change during

a contest. We used linear regression to test for relationships between body size measures and contest intensity and duration.

Ethical Note

This study was approved by the Institutional Animal Care and Use Committee at California State University, Fresno (File No. 127). All fighting behaviour was voluntary and refuges of escape were available for the loser. Individual marking entailed placing the fish on a moist towel, folding the towel over the head to calm and immobilize the fish, and quickly (less than 5 s) cutting the keratinous matrix between caudal fin rays with scissors; no bleeding or infection was ever observed and the fish were returned to their tanks and treated with Stress Coat™. Fish were handled without the use of anaesthetics because commonly used chemicals (e.g. MS-222, clove oil) can significantly alter glucose and lactate measures in fishes (e.g. Iversen et al. 2003; Wagner et al. 2003; Velíšek et al. 2009), both of which were essential for this study. Thus, we obtained blood by gently placing the animal on a moist towel, as described above, and performing caudal venipuncture, followed by rapid decapitation, an approved method of euthanasia in fishes (AVMA 2007). Brain removal and transfer to a preservative (RNA later) immediately followed decapitation and served as a method of pithing; we could not destroy the brain with a probe because the tissue was to be used in further analyses.

RESULTS

Effects of Status and Contest Dynamics on Physiology

All differences in metabolic/endocrine state were measured after the fight had settled. There were no significant differences between status classes (winner, loser, control) in any of the measured physiological parameters (Table 2). These analyses, however, considered only absolute differences between the status classes, not physiological responses to variation in fight dynamics. Muscle lactate concentrations of both winners and losers increased significantly with contest intensity (PC1: losers: $F_{1,25} = 8.5$, $P = 0.008$, $R^2 = 0.25$; winners: $F_{1,26} = 6.9$, $P = 0.014$, $R^2 = 0.21$; Fig. 1a; Table 3). Slopes did not differ significantly between winners and losers, suggesting that rates of lactate accumulation as

a function of contest dynamics are not status dependent (comparison of slopes; Zar 1996, page 355; contest intensity (PC1): $t_{49} = 1.42$, $P = 0.16$).

Despite the minor asymmetry (<5% on average; Table 1), larger animals won significantly more often than smaller animals (standard length, larger won 16 of 25 where an asymmetry existed: $\chi^2_1 = 3.97$, $P = 0.046$; body depth, larger won 17 of 26: $\chi^2_1 = 5.00$, $P = 0.027$; mass, larger won 17 of 27: $\chi^2_1 = 3.67$, $P = 0.055$). There were no absolute differences in physiology between the smaller and larger animals of a pair (all parameters $P > 0.05$; same statistical tests as in Table 2). Similar relationships between contest dynamics and physiological parameters were evident when analysed for the smaller versus larger animal of a contest pair (compared to winner versus loser). Muscle lactate concentrations of both the smaller and larger contestant increased significantly with contest intensity (PC1: smaller: $F_{1,23} = 7.1$, $P = 0.014$, $R^2 = 0.24$; larger: $F_{1,24} = 11.23$, $P = 0.003$, $R^2 = 0.32$), and slopes did not differ between smaller and larger contestants (PC1: $t_{47} = 1.9$, $P = 0.06$).

Plasma glucose increased significantly with contest intensity (PC1) in losers ($F_{1,22} = 10.27$, $P = 0.004$, $R^2 = 0.32$; Fig. 1b) and in smaller contestants of a pair ($F_{1,20} = 10.87$, $P = 0.004$, $R^2 = 0.35$) but not in winners ($F_{1,19} = 0.17$, $P = 0.68$, $R^2 = 0.01$; Fig. 1b) or larger animals of a pair ($F_{1,20} = 0.06$, $P = 0.81$, $R^2 = 0.003$). Also, there was a significant negative relationship between postsettlement, low-intensity aggression (PC4) and plasma cortisol for winners ($F_{1,16} = 5.4$, $P = 0.03$, $R^2 = 0.25$) but not for losers ($F_{1,21} = 0.10$, $P = 0.75$, $R^2 = 0.005$; Fig. 1c). There were no other significant relationships between contest dynamics and physiological measures.

Body Size, Contest Dynamics and Contest Outcome

As described above, larger contestants won a significant proportion of the contests despite minimal size asymmetries (Table 1). This result suggests either that convict cichlids are capable of determining small differences in RHP via mutual assessment, or that persistence thresholds of closely matched opponents differ and are monitored through self-assessment. Both mutual- and self-assessment predict a negative relationship between size asymmetry and contest intensity or duration, provided there is adequate variation in asymmetries to test the relationship. Using body mass as a measure of RHP, we found no

Table 2
Mean \pm SE physiological parameters for winners, losers and controls in paired contests between male convict cichlids

Physiological parameter	Transformation	Status class			F	df	P
		Winner	Loser	Control			
Plasma glucose (mg glucose/ml per mg tissue)	ln	0.530 \pm 0.11 (N=21)	0.471 \pm 0.08 (N=24)	0.415 \pm 0.06 (N=15)	0.70	2, 26	0.50
Hepatic glucose (mg glucose/ml per mg tissue)	ln	0.0013 \pm 0.00 (N=25)	0.0015 \pm 0.00 (N=26)	0.0012 \pm 0.00 (N=23)	2.28	2, 26	0.12
Hepatic glycogen (mg glycogen/ml per mg tissue)	SQRT	0.015 \pm 0.00 (N=26)	0.014 \pm 0.00 (N=27)	0.016 \pm 0.00 (N=23)	0.78	2, 26	0.47
Muscle lactate (mg lactate/dl per mg tissue)	ln	0.1052 \pm 0.02 (N=26)	0.0996 \pm 0.01 (N=25)	0.0976 \pm 0.01 (N=26)	0.24	2, 26	0.79
Plasma cortisol (ng/ml)	ln	52.38 \pm 4.52 (N=15)	63.20 \pm 9.26 (N=16)	61.58 \pm 11.16 (N=9)	1.38	2, 15	0.28
Plasma testosterone (ng/ml)	ln	6.42 \pm 1.23 (N=15)	6.42 \pm 1.75 (N=15)	7.92 \pm 3.44 (N=9)	1.98	2, 15	0.17
Plasma 11-KT (ng/ml)	ln	0.78 \pm 0.08 (N=15)	1.49 \pm 0.38 (N=16)	0.85 \pm 0.11 (N=8)	2.63	2, 15	0.10

True means are shown, but analyses were conducted on ln-transformed or square-root transformed (SQRT) data. Descriptive statistics for 'status' effect are from repeated measures ANCOVA (see Statistical Analysis). For hormone analysis, only fights where the steroid was measured in both winner and loser were included in these analyses. Mass (covariate) and the 'status \times mass' interaction were not significant (glucose, glycogen and lactate analyses: mass: $F_{1,25-26} < 2.50$, $P > 0.12$; status \times mass: $F_{2,25-26} < 2.32$, $P > 0.12$; hormones: mass: $F_{1,15} < 0.90$, $P > 0.36$; status \times mass: $F_{2,15} < 2.92$, $P > 0.09$). Plasma glucose scaled significantly positively with mass (ANCOVA: mass: $F_{1,26} = 8.29$, $P = 0.008$; linear regression: $R^2 = 0.11$, $F_{1,58} = 7.49$, $P = 0.008$, $\beta = 0.036$); despite the lack of a status \times mass interaction, this effect was driven primarily by losers (linear regression; losers: $R^2 = 0.32$, $F_{1,22} = 10.14$, $P = 0.004$, $\beta = 0.059$; winners: $R^2 = 0.01$, $F_{1,19} = 0.35$, $P = 0.56$, $\beta = 0.017$; controls: $R^2 = 0.25$, $F_{1,13} = 4.32$, $P = 0.06$, $\beta = 0.028$).

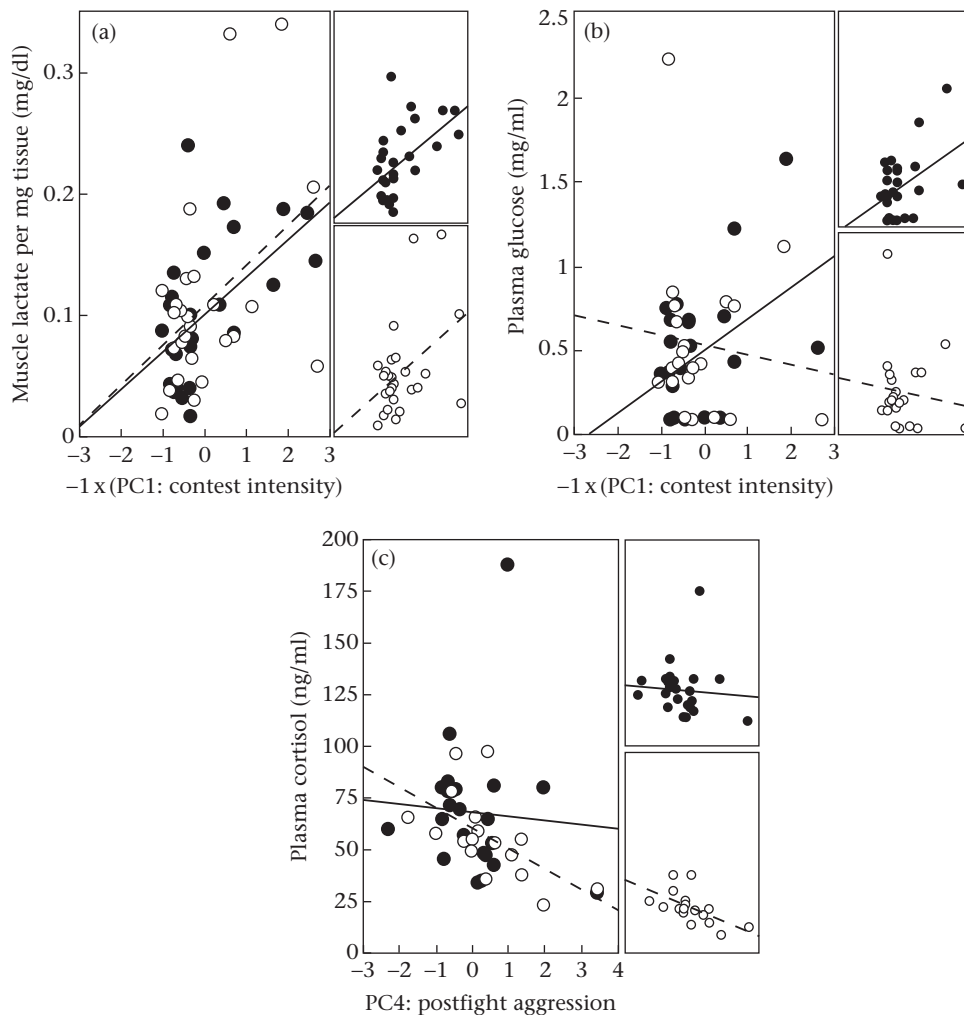


Figure 1. Contest dynamics and physiological parameters in male convict cichlids. Relations between composite contest intensity score (principal component 1, PC1) and (a) muscle lactate and (b) plasma glucose levels in winners and losers. (c) Relation between dominance reinforcement (low-intensity postsettlement aggression, PC4) and plasma cortisol levels in winners and losers. Negative PC1 values correspond to more intense contests (Table 3); for clarity, we have multiplied PC1 by -1 . Filled circles, solid lines: losers; open circles, dashed lines: winners. Side panels show data for winners and losers separately. See Statistical Analysis and Table 3 for more information on the principal components, and see Results for statistical details on these relationships.

significant relationship between mass asymmetries and measures of contest intensity (e.g. number of mouth-wrestling bouts; mass asymmetry: $F_{1,25} = 0.03$, $P = 0.86$, $R^2 = 0.001$; Fig. 2a, b) or contest duration (mass asymmetry: $F_{1,25} = 0.13$, $P = 0.73$, $R^2 = 0.07$). This is not surprising given that contestants were size-matched with asymmetries of, on average, less than 5%. Following Taylor & Elwood (2003), we examined the relationship between absolute body mass (RHP) of both contestants and measures of contest duration or intensity. Mutual assessment predicts that the body mass of the smaller/larger contestant should positively/negatively correlate with contest duration or intensity; self-assessment, however, predicts a positive relationship between contest duration or intensity and the body size of both the smaller and larger contestants. Body mass of both the larger ($F_{1,24} = 12.34$, $P = 0.0018$, $R^2 = 0.34$) and smaller ($F_{1,24} = 12.52$, $P = 0.0017$, $R^2 = 0.34$) contestant were significantly positively correlated with the number of mouth-wrestling bouts; animals with greater RHP mouth-wrestled more frequently regardless of size class (Fig. 2c, d, e). Trends for time spent mouth wrestling and contest duration were in the same direction but were weaker and nonsignificant (larger contestant: time spent mouth wrestling: $F_{1,24} = 2.62$, $P = 0.12$, $R^2 = 0.10$; duration: $F_{1,24} = 2.80$, $P = 0.11$, $R^2 = 0.10$; smaller

contestant: time spent mouth wrestling: $F_{1,24} = 2.50$, $P = 0.13$, $R^2 = 0.09$; duration: $F_{1,24} = 2.58$, $P = 0.12$, $R^2 = 0.10$).

DISCUSSION

We evaluated the relationship between two important characteristics of animal contests, intensity and outcome, and potential physiological costs of fighting. Our results reveal that physiological systems in the convict cichlid are far less sensitive to fight outcome than to variation in fight dynamics (see also Earley et al. 2006). Thus, it was not the outcome of a contest but rather how that outcome was achieved that differentiated the postfight physiological profiles of male convict cichlids. We first address the potential for postsettlement aggression to act as a mechanism used by winners to buffer the stress response. We then turn to the crux of our findings, which implicate lactate as an important internal cue that, through self-assessment, might mediate decisions to persist in aggressive contests. We draw from a recent review (Nilsson & Östlund-Nilsson 2008) to provide some new perspectives on the associations of absolute size, size asymmetries, accumulation of physiological costs and decisions to fight or flee.

Table 3

Summary of principal components analysis (PCA) on behavioural measures of convict cichlids

Behaviour	Loadings			
	PC1	PC2	PC3	PC4
Presettlement				
Approaches	-0.029	-0.063	-0.812	0.034
Threat displays	-0.424	0.164	-0.602	-0.368
Attacks	-0.358	0.124	-0.626	-0.377
Bites	-0.641	0.070	-0.547	-0.194
Mouth wrestling	-0.941	0.024	-0.031	-0.108
Time spent mouth wrestling	-0.561	0.160	-0.318	0.109
Circling bouts	-0.899	-0.011	-0.046	-0.046
Avoids	-0.129	0.630	-0.179	-0.102
Retreats	0.020	0.794	0.271	-0.027
Contest duration	-0.914	0.030	-0.082	-0.121
Postsettlement				
Approaches	0.039	-0.583	-0.216	0.486
Threat displays	0.104	-0.059	-0.013	0.776
Attacks	0.016	-0.463	-0.614	0.274
Bites	-0.138	-0.172	-0.812	0.137
Avoids	0.024	0.768	0.110	0.406
Retreats	-0.058	0.562	-0.026	-0.121
Eigenvalue	4.798	3.142	1.591	1.188
% Variation	29.99%	19.64%	9.94%	7.42%
Cumulative % variation	29.99%	49.63%	59.57%	66.99%

Heavy loadings on each component are shown in bold. Interpretations of the components are as follows: PC1: contest intensity and reciprocal aggression; (-) values indicate more intense contests. PC2: submission; (+) values indicate more submission. PC3: unidirectional aggression; (-) values indicate more unidirectional aggression. PC4: low-intensity aggression; (+) values indicate more aggressive reinforcement postsettlement. % Variation: percentage of variation in the observed behavioural variables explained by each principal component (PC). Cumulative % variation: sum total percentage of variation in the observed behavioural variables explained by a given PC and its preceding PCs.

Contest Outcome and Endocrine Profiles

Postfight cortisol concentrations did not differ significantly between winners and losers, a result in accord with what has

previously been observed in this species (Earley et al. 2006). Our finding that neither winners nor losers differed from controls in postfight cortisol levels suggests that fighting, and the attainment of dominant or subordinate status, is not particularly stressful for convict cichlids over the short term. However, we cannot rule out the possibility that these fish respond to social subordination with elevated cortisol (Winberg & Lepage 1998; Sloman et al. 2001), perhaps over a longer time course than was allowed in our study. A delayed cortisol response has been observed in rainbow trout, where plasma cortisol levels of losers progressively increased and those of winners progressively decreased up to 3 h after contest settlement (Øverli et al. 1998). Additionally, elevated plasma cortisol was found in subordinate Arctic charr, *Salvelinus alpinus*, after 4 days of interaction with another male (Elofsson et al. 2000). These studies indicate that the endocrine profiles of winners and losers diverge markedly in situations where there is an extended period of postfight dominance reinforcement.

In our study, only 5 min of interaction was permitted after contest settlement to firmly establish that the fight had been settled. In every fight there was continued aggression from the winner, even though the loser swam to the periphery of the tank and never reciprocated aggressive acts. Plasma cortisol concentrations of winners decreased linearly and significantly with increases in postsettlement aggression directed towards the loser. This may be a sort of 'stress relief' for the winner whereby the stress response is attenuated as status is reinforced by unreciprocated aggression from the loser. Studies have shown winners to have lower postfight cortisol levels when prolonged cohabitation with the loser is allowed (Hannes et al. 1984; Øverli et al. 1998; Winberg & Lepage 1998; Sloman et al. 2001), but this is the first report of a significant, progressive attenuation in winners' cortisol in relation to short periods of postfight aggression; forthcoming studies from our laboratory have replicated these results (S. C. Wong, B. Sivaraman, H. Stephenson, K. Miller & R. L. Earley, unpublished data).

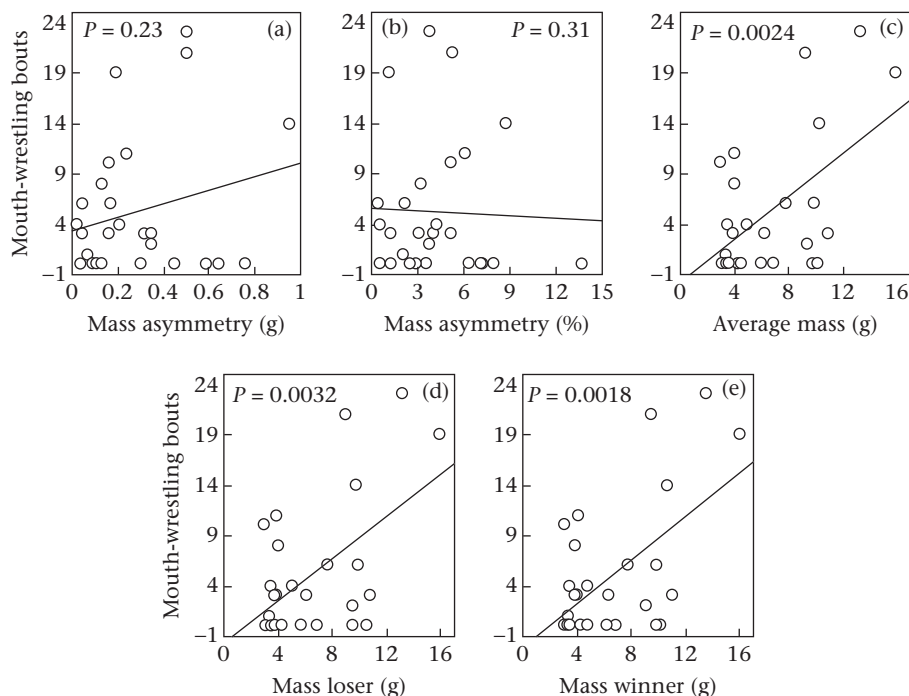


Figure 2. Determination of potential assessment strategies by male convict cichlids of similar size. Relations between number of mouth-wrestling bouts and (a) absolute mass asymmetries (g), (b) percentage mass asymmetry, (c) average mass, (d) mass of loser and (e) mass of winner.

Metabolic Profiles and Contest Outcome

Overall, there were no significant status-dependent (winner, loser, or control) differences in plasma glucose, hepatic glucose/glycogen, or muscle lactate. Additionally, no differences were observed between contestants (winners and losers) in the rate of lactate accumulation as a function of contest dynamics, suggesting that losing is not the result of asymmetrical accrualment of costs associated with anaerobic metabolism (i.e. that losers do not have proportionally greater lactate accumulation than winners). This contrasts with findings in African cichlids (*T. zillii*), where it was observed that losers had significantly higher levels of muscle lactate (Neat et al. 1998a). The specific location of muscle sampled for lactate was not mentioned by Neat et al. (1998a), which could contribute to the difference between their results and ours. Studies in invertebrates identify lactate, or the sugars from which lactate is derived, as a strong predictor of contest persistence with, for instance, hermit crabs withdrawing from a fight as a result of over-accumulation of lactate (Briffa & Elwood 2001, 2004; Briffa & Sneddon 2007). While perseverance in a fight does not always predict outcome, it may be regarded as a reliable predictor in war-of-attrition style fighting, where persistence is the primary means by which the victor is determined. Moreover, the lack of status differences in the measured metabolites and hormones should not be particularly surprising. Cichlid contests lasted 2–90 min, with some fights involving frequent mouth wrestling and others none at all. We would thus expect energy depletion and lactate accumulation to vary much more markedly with contest dynamics than with status alone.

Plasma glucose was significantly positively related to contest intensity (PC1) only in convict cichlids that lost, suggesting that rates of energy depletion may indeed be different for eventual winners and losers. Several studies on invertebrates indicate that whole body, haemolymph or muscle glucose concentrations are elevated in fighters relative to nonfighters, often to a greater degree in winners than losers, and that glucose concentrations increase as a function of contest duration (e.g. Briffa & Elwood 2001; Briffa 2008). In cichlid fishes, muscle sugars are depleted in both winners and losers relative to controls, while liver reserves decrease at a higher rate in losers (Neat et al. 1998a, b). These studies clearly demonstrate that energy mobilization (and depletion) is an important component of fighting costs, albeit reflected differently in invertebrates and vertebrates, and among tissues. Our results show that losers mobilize energy stores more rapidly than winners, perhaps from the liver (Neat et al. 1998a, b) to the plasma to meet the demands of fighting. More rapid energy depletion therefore may forecast a losing outcome for these cichlids.

Muscle lactate concentrations were strongly associated with contest dynamics, evident by a significant positive correlation with contest intensity (PC1) in both winners and losers. Fighting among fishes creates a strong demand for anaerobic metabolism (Abrahams et al. 2005; Campbell et al. 2007) and mouth wrestling in particular is likely to contribute considerably to lactate accumulation. Mouth wrestling requires fish to interlock their mouths and propel themselves forward at their opponent with strong caudal fin beats. The locked jaw appears to impede effective respiration because the operculum cannot fully articulate to pass oxygenated water over the gills. In our study, it was often observed during intense fights that contestants would rest, a behaviour characterized by a cessation of jerking and propulsive movements but maintenance of interlocked mouths. It was expected that if lactate accumulation influenced contest outcome, significant differences would exist in muscle lactate concentrations of winners and losers, and in their rate of accumulation. However, contestants accumulated lactate at the same rate, which would argue against

there being a universal threshold concentration of muscle lactate that, once reached, causes contestants to submit. The possibility remains, however, that slight differences in body mass may confer a physiological advantage, enough to drive differences in the contestants' willingness to persist and their winning probabilities despite accruing metabolic costs at similar rates. This is discussed further in the following section.

Contest Dynamics and Body Size

Larger fish, regardless of status or relative body size to opponents, engaged more frequently in mouth wrestling than small fish. In other words, as absolute size increased, so too did fighting intensity in contests between matched opponents. Previous studies have noted that larger individuals fight longer and more intensely than smaller conspecifics (Dixon & Cade 1986; Jennions & Backwell 1996; Whitehouse 1997). However, the majority of these studies examined insects or crustaceans, so it is possible that the relationships may not translate well to vertebrates, given the marked metabolic disparities between the two groups. When looking at more closely related animals, our results appear to conflict with those obtained in a similar study using size-matched and size-grouped (e.g. large versus small group) convict cichlids. Leiser et al. (2004) found no size-related differences in contest dynamics of male convict cichlids except that larger pairs were slower to escalate and spent more time engaging in less escalated behaviours such as lateral displays. The source of discrepancy between Leiser et al.'s study and our own may have to do with the size distributions used. The fish in our study ranged from 2.95 to 16.08 g, while Leiser et al. (2004) used small and large size classes that ranged between 5–12 g and 15–30 g, respectively. Fusing the findings of Leiser et al. (2004) with our own suggests that the propensity for male convict cichlids to escalate might be defined by an inverted U-shaped curve where small (e.g. 2–8 g) and large (>15 g) fish escalate much less frequently than mid-sized fish (8–15 g; see Fig. 2). These findings support a recent model by Logue et al. (2011) that predicted adaptive shifts in aggressive behaviour with changes in body size, with selection favouring high aggressiveness at intermediate body sizes.

Our finding that larger fish mouth-wrestled more frequently creates the possibility for a mass-specific physiological tolerance and thus a mechanism for why larger cichlids (within the size range of our study) not only fight harder, but also win over marginally smaller opponents (see below). There is mounting evidence that body size of fishes is associated positively with anaerobic tolerance (survival time). Nilsson & Östlund-Nilsson (2008) provide an excellent review of the scaling relationships in hypoxia tolerance of fishes with the general trend that anaerobic capacity increases exponentially with body mass (see also Ferguson et al. 1993; Baldwin et al. 1995; Kieffer & Tufts 1998). Using anaerobic tolerance as a proxy for lactate tolerance, we suggest that such a scaling relationship may exist within the context of fighting in the convict cichlid such that relatively larger individuals can withstand higher concentrations of lactate and persist longer in a fight.

In light of this information, we performed a post hoc analysis to examine differences in contest success as a function of body mass of the fighting pairs. If lactate tolerance scales exponentially with body mass and also influences contest success, we might expect differences in the likelihood of success for the slightly larger animal as body mass moves along the curve of lactate tolerance (i.e. physiological consequences of size asymmetries are amplified because of the exponential nature of lactate tolerance; Fig. 3). Contestant pairs were divided into either large (mean \pm SE: 9.41 ± 0.59 g, range 5.24–16.08 g) or small (3.81 ± 0.11 g, range

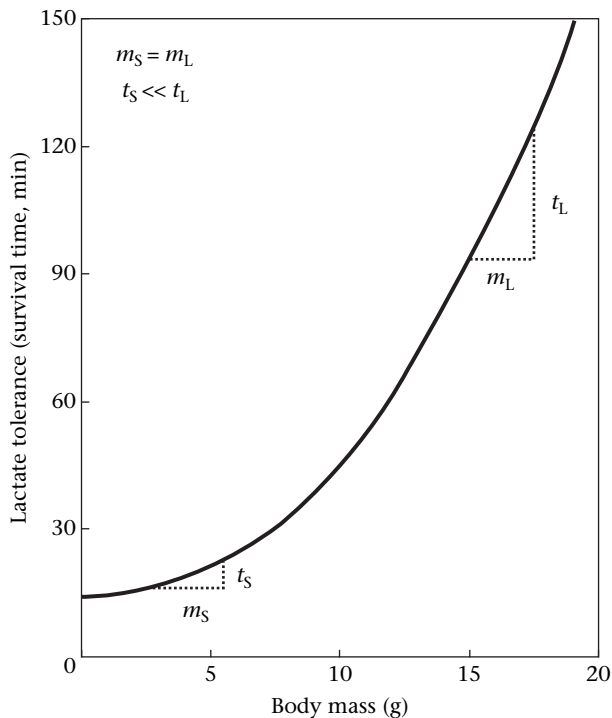


Figure 3. Exponential scaling between body mass and lactate tolerance (curved line; see Nilsson & Östlund-Nilsson 2008). The graph shows the contest persistence duration for a male cichlid of any size, within the range of our study, for a given lactate load. For a specified mass asymmetry (m), the asymmetry in lactate tolerance (persistence time; t) is much greater for pairs of large (L) opponents than for pairs of small (S) opponents. The graph highlights that larger pairs might fight for longer but also that size asymmetries will influence contest outcome to a much greater extent in fights between larger opponents than in fights between smaller opponents because of differences in lactate tolerance.

2.95–5.07 g) body mass categories. Being the larger fish in the large category conferred a significantly greater chance of winning (larger animals won 9 of 12 fights; Fisher's exact test: two-tailed $P = 0.039$; one-tailed $P = 0.0196$), but the larger fish in the small category did not (larger animals won 8 of 14 fights; Fisher's exact test: two-tailed $P = 0.71$; one-tailed $P = 0.35$). This result fits well with the predictions of Nilsson & Östlund-Nilsson (2008) when applied to contest persistence instead of survival time. Proportionally equal differences in body mass will have dramatically different consequences depending on the size category in which the paired contestants fall (Fig. 3). In our study it is possible that mass asymmetries in smaller fish reflect very little about physiological asymmetries that could bear on contest outcome but, that the same proportional mass asymmetry in larger pairs amplified size-dependent physiological asymmetries to the point of significantly affecting contest outcome.

In summary, we found no evidence of a universal lactate threshold, whereby contest duration might be dictated by one contestant's predetermined muscle lactate concentration beyond which further fighting would be perilous. Contestants did not differ in their muscle lactate load according to status (winners versus losers or larger versus smaller contestants), and they did not accrue lactate at different rates. We suggest that winners may win because, for a given level of contest escalation, the slightly larger fish is more tolerant to the associated lactate load and can thus persist; even if absolute lactate concentrations are indistinguishable between the larger (winner) and smaller (loser) fishes, scaling relationships between body size and lactate tolerance drive contest outcome. This is not to say that lactate is the lone factor governing

the decision to submit or continue fighting; our results also demonstrate an important role for glucose mobilization. But, for convict cichlids, lactate appears to be central and certainly worthy of further investigation.

Assessment Strategies and Summary

Regarding the assessment models discussed earlier, we show that convict cichlids may use self-assessment to decide how long and how intensely to fight. This is evidenced by the positive relationship between body size and contest intensity in both the winner and loser (or smaller and larger opponents) (Taylor & Elwood 2003). We found no evidence of mutual assessment, for which there is some support in the convict cichlid (Keeley & Grant 1993; Koops & Grant 1993). Note, however, that contests between size-matched opponents decrease the resolution with which the predicted relationships for mutual assessment can be observed; in particular, that contest duration should increase with the size of the eventual loser and decrease with the size of the eventual winner. No study has reevaluated assessment strategies in convict cichlids in light of more contemporary treatments (Taylor & Elwood 2003; Arnott & Elwood 2009a), but experiments that use a range of asymmetries, more detailed comparisons (e.g. absolute and relative body size parameters), more sophisticated statistical techniques that can accommodate unresolved fights (e.g. Cox regression; Hsu et al. 2008) and perhaps both sex- (Arnott & Elwood 2009b) and size-based differences will prove enlightening. Briffa & Elwood (2009) also remind us that mutual assessment and cumulative assessment models make essentially the same predictions about RHP versus contest duration relationships. Although our results support an energetic war-of-attrition style of assessment in size-matched cichlid pairs, distinguishing various assessment models in asymmetric pairs, where the opportunity for mutual assessment is greater, will require detailed analyses of the degree of behavioural matching between contestants and the bout structure (e.g. escalation/de-escalation between or within phases) of fights (e.g. Jennings et al. 2005a, b; Briffa & Elwood 2009). Developing methods to quantify injury costs in fishes also will be useful for evaluating the underlying assumptions of CAM.

We have provided correlational evidence that convict cichlids use self-assessment to determine contest persistence and that mass-specific differences in lactate tolerance may account for the greater likelihood of victory for marginally larger contestants. Additionally, we provide evidence that increasing absolute size can amplify asymmetries in physiological tolerances between contestants such that a larger fish is increasingly likely to defeat a smaller fish as absolute body mass increases; even if there is no proportional change in body mass between the contestants. This suggests, for example, that a 10% difference in body mass will be more advantageous in a fighting pair averaging 15 g compared to a fighting pair averaging 5 g. We recognize that some of the proposed mechanisms were not explicitly tested in our study; however, additional studies can address these hypotheses to determine whether support is warranted. Manipulations of plasma lactate concentrations and greater sample sizes with multiple distinctive size groups will provide the resolution to better decipher the relationship between body size and lactate tolerance as it pertains to fighting.

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