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Aggressive behaviour and energy metabolism in a cichlid fish, *Oreochromis mossambicus*

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Abstract

We have investigated the effect of mirror-elicited agonistic behaviour on oxygen consumption in the Mozambique tilapia, *Oreochromis mossambicus* (Cichlidae). Males exposed to their mirror image showed higher frequencies of both lateral display and tail-beating and escalated aggression more frequently than males exposed to a transparent glass that was used as a control for the presence of a novel object in the tank. This aggressive response was correlated with an increase in oxygen consumption. Overt aggression was highly correlated with display behaviour and with locomotor activity. Bivariate analyses showed high correlation (explaining about 64% of variation) between overt aggression, locomotor activity and metabolic rates. Weakly positive bivariate correlations between displays and metabolic rates turned spurious after partialling out aggression. The data suggest that energetic costs only emerge late during the conflict, when animals escalate their aggressive behaviour.

Keywords: Mirror-elicited aggression; Agonistic display; Metabolic rate; Tilapia; *Oreochromis mossambicus*

1. Introduction

Competing males use a ritualized sequence of visual, acoustic, and tactile signals that may escalate to overt (physical) aggression during agonistic interactions [1–4]. This sequence has been interpreted as a way to sort out winners and losers at an early stage of the conflict, thereby preventing the escalation of the fights during which both parties incur physical damage ([5–10], but see [11]). In order to prevent cheating at the early stage of conflicts, it is expected that the expressed agonistic signals (e.g. aggression and displays) convey honest information on the relative fighting ability of the opponents. This may occur when their expression has some significant intrinsic cost associated [12,13]. In this paper we investigate the potential energetic costs of such displays.

The metabolic consequences of fighting have been described in detail in *Betta splendens* by Haller [14]. This species directly escalates in staged dyads, and amino acid and glycogen content of the muscles significantly decreases already after 10 min of fighting. Neat et al. [8] showed that fighting *Tilapia zillii* males depleted sugar reserves and accumulated lactate in their muscles. In both these studies, losers were reported to incur higher costs than winners [8,14]. Grantner and Taborsky [15] measured direct oxygen consumption using respirometry in males of another cichlid species, *Neolamprologus pulcher*. In these males, agonistic behaviour increased approximately five times the energy expenditure relative to the basal metabolic rate. These authors used a mirror to elicit aggression in their experiment which has the advantage that during fighting no damage is inflicted on the focal animal and data can be collected on an individual basis [16]. Both studies on cichlids suggest that the high energy expenditure of fighting is restricted to the escalation part of the interaction, which is likely to be a consequence of the increased motor activity associated with the expression of overt aggression [8,15].

The objective of this study was to investigate energetic costs of agonistic behaviour in Mozambique tilapia (*Oreochromis mossambicus*) males. This species was chosen because males show escalated fighting towards their own mirror image [17], and also because individuals of this species readily adapt to staying in the metabolic chambers used in respirometry [18–20]. The experiment was part of a study on the effects of the androgen

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11-ketotestosterone on metabolic rate. Males treated with the androgen had higher basal metabolism [21]. Here we test whether this treatment showed any interaction with the relationship between behaviour and metabolism.

2. Materials and methods

2.1. Animals and housing conditions

Adult *O. mossambicus* males of 2 to 4 years old were kept in mixed sex groups, typically holding two males and three females, in 750–800 l aquaria at the animal housing facilities of the Instituto Superior de Psicologia Aplicada, Lisbon, Portugal [22]. Only males were selected that were reproductively active (see below), and all males had experienced multiple spawnings in the stock aquaria (males may reach sexual maturity at about 9 months of age [23]). Water was continuously aerated and kept at a temperature of 26 °C (±1 °C), and the photoperiod regime was 13 L:11 D.

In half of the animals ($n=14$) the levels of the androgen 11-ketotestosterone (KT) were experimentally elevated by implanting them with a silastic KT implant. The other half ($n=13$) received a silastic implant with castor oil only (for details about surgery see Ros et al. [21]). The levels of circulating KT in both groups were 1.34±1.31 (avg±SD) ng/ml in control males and 2.45±0.83 (avg±SD) ng/ml in KT treated males [21].

At day one of the study, dominant males were identified in stock tanks and caught. These males were individually housed in 12 l aquaria during seven days, visually and chemically isolated from other males. To standardize body condition, during the first six days of isolation they were fed proportionally to their metabolic body mass: 9 g food pellets per $kg^{0.8}$ fish per day (pellets were made at the Department of Aquaculture Systems and Animal Nutrition in the Tropics and Subtropics, University of Hohenheim: 42.0% crude protein, 9.9% crude lipid, 11.3% crude ash, 20.3 kJ gross energy). This was followed by 1 day of fasting to prevent interference of heat increment of feeding on oxygen consumption measurements [24]. Fasting was continued for the three days during which the males were in respirometry.

At experimental day 8, body mass (average 75.8±4.3 g) was measured and they were individually placed in the sealed respirometer chambers that were visually isolated from each other.

At experimental day 11 mirror and control tests were carried out. Two adjacent chambers were sampled for oxygen consumption and fish behaviour was recorded on video for later analyses. Oxygen consumption was sampled once every 3 min per chamber. A reference oxygen value was taken from a chamber without fish before the start of the experiment. To calculate base-line oxygen consumption, two measurements were taken before the start of the mirror or control stimulation. Stimulation started by placement of a mirror or a similar sized glass window on one of the sides of the chambers and ended 36 min later by taking it away. Three hours later the experiment was repeated but the chamber that previously had a mirror treatment now received a glass treatment and vice versa, so that the order of presentation of mirror vs. glass was balanced among all fish. During stimulation, oxygen was measured once every 3 min. Once every 9 min, a reference value was obtained from the chamber without fish. Three consecutive oxygen consumption values were averaged resulting in eight values per individual (four periods of 9 min for mirror and control stimulation). At the end of the experiment (day 12), all males were returned to their original stock tanks.

2.2. Measurements of activity and behaviour

Video recordings were analysed using the software package JWatcher (v. 0.9, Animal Behaviour Laboratory, Macquarie University, Sydney, Australia). The following behaviours were scored: Motor *Activity*: the percentage of the total time in which the animal was not immobile and lying on the bottom of the chamber; Overt *Aggression*: buts and bites which in this set-up were directed towards the side of the chamber where the mirror/glass was placed; *Tail-beating*: a sudden slap of the tail. *Frontal display*: the male is in a swimming position and oriented frontally towards the side of the chamber where the mirror/glass was placed with dorsal fin extended. Observations from video recordings limited observation of extension of the opercula and therefore this aspect of the display was left out of analyses; *Lateral display*: as Frontal display but shown in a lateral orientation in respect to the side of the chamber where the mirror/glass was placed; Other social behaviours such as circling were performed in very low frequency and thus were left out of analyses.

2.2.1. Calorimetric system and oxygen consumption measurements

Energy metabolism was measured using an open flow-through respirometry system [25], designed to record oxygen concentrations sampled from eight different respiration chambers at constant intervals [21]. Water was kept at a constant temperature of 26 °C (±1 °C), filtered over charcoal (Eheim filter, Germany), and oxygenated with an air stone. Each chamber of the respirometer was made from flat, optically clear 12 mm thick acrylic plastic (Perspex) (outside dimensions 154×154×262 mm). A chamber containing no fish was used as control to correct for possible consumption of oxygen by algae and bacteria present in the water.

Automated continuous flow sampling allowed to measure oxygen consumption of several chambers over a single sensor (CellOx® fitted with stirrer type R2 300 in a through flow cell type D201; WTW GmbH, Germany). The oxygen meter (Oxi 197; WTW GmbH, Germany) was logged to a computer that allowed for automated online acquisition of the data for later analyses.

Metabolic rates were calculated using the method of Niimi [26] which corrects for a time-lag due to washout delays caused by the volume of water in the respiration chamber [25,27]. Oxygen consumption is strongly influenced by the amount of metabolically active tissue in the organism, and therefore scales with body mass with an allometric factor of 0.8 [18,28]. Therefore we report oxygen consumption rates (r) corrected for body mass (M) using this allometric factor ($r \cdot M^{-0.8}$ mmol O_2 h^{-1} $kg^{-0.8}$). After correction no significant correlation of body mass (range 33