Ventilation rates indicate stress-coping styles in Nile tilapia

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Behavioural responses to stress can form distinct profiles in a wide range of animals: proactive and reactive profiles or coping styles. Stress responsiveness can also differentiate between the behavioural profiles. The tendency to regain feed intake following transfer to a novel social-isolation tank (the speed of acclimation) can discriminate between proactive or reactive profiles. Consequently, differential stress responsiveness can be linked to this feeding behaviour trait. This study shows that ventilation rates of Nile tilapia, *Oreochromis niloticus* (L.), correlate with the rate of feeding resumption, following transfer to a novel social-isolation aquarium. Therefore, ventilation rate (VR) indicates coping styles; consequently, VR is a proxy for the way fish will deal with environmental challenges.

1. Introduction

Behavioural responses to stress can form distinct profiles in a wide range of animals (Koolhaas *et al.* 1999; Carere and van Oers 2004; Schjolden *et al.* 2005a; Øverli *et al.* 2007; Fucikova *et al.* 2009). On the extreme sides of these profiles, a distinction can be made between proactive and reactive profiles or coping styles. Proactive animals display a slight cortisol increase and act in an active (e.g. high-level aggression or avoidance) manner in response to stress. Reactive animals have large cortisol increases and respond in a passive (e.g. low-level aggression and immobility) manner to remedy the stressful condition. These profiles were initially characterized in mammals (Koolhaas *et al.* 1999), but this paradigm was extended to other vertebrates, including fish and birds (Carere and van Oers 2004; Schjolden *et al.* 2005a; Øverli *et al.* 2007; Fucikova *et al.* 2009). Only the extremes of these profiles are described in most studies, but the behavioural variation is continuous. Thus, to characterize these behavioural profiles, in a sense, considering the continuous axes between the coping styles and to seek for indicators of those, is an important goal that contributes to our understanding about the way animals deal with environmental challenges.

For fish, feeding behaviour is an indicator of coping style (Øverli *et al.* 2007; Silva *et al.* 2010). The tendency to regain feed intake following transfer to a novel social-isolation tank (the speed of acclimation) can differentiate between proactive or reactive profiles (Schjolden *et al.* 2005a). A gradient of feeding behaviour is expected between the two profiles; however, proactive fish tend to acclimate faster than reactive fish. Since these profiles are associated with reactions to stress (Øverli *et al.* 2004, 2006), the distinct behaviours of the two profiles have been used to characterise stress responsiveness. Likewise, stress responsiveness can differentiate between the behavioural profiles. However, the continuous axes between extremes have not been well characterized.

A sensitive indicator of fish physiological responses to stress is ventilation rate (VR). VR is particularly useful for measuring stress levels because it is easily quantifiable and can be measured noninvasively (Barreto and Volpato 2004; Bell *et al.* 2010). VR is a sympathetic response that quickly increases in response to stressors (Priede 1985), such as confinement (Barreto and Volpato 2004; Brown *et al.* 2005), or to predator cues, possibly in preparation for an escape response (Barreto *et al.* 2003a; Hawkins *et al.* 2004; Gibson and Mathis 2006; Barreto *et al.* 2010). On the other hand, VR can be linked to metabolic rates (Alvarenga and Volpato 1995). Stress responses can induce catabolic responses and consequently modulate metabolism (Mommsen

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et al. 1999), therefore causing variation in the VR. Individual differences in metabolic rates have recently been proposed to be a physiological background of coping styles (Careau *et al.* 2008). Since some behaviours can be used to characterize stress responsiveness (Carere and van Oers 2004; Øverli *et al.* 2004, 2006, 2007; Fucikova *et al.* 2009; Schjolden *et al.* 2005a) and since VR can indicate stress (Barreto and Volpato 2004; Brown *et al.* 2005; Bell *et al.* 2010), VR could be used to identify individual profiles.

Based on the above statements, VRs could be linked to different tendencies in feeding behaviour. This hypothesis was tested by evaluating the correlation between VR and feeding behaviour in Nile tilapia, *Oreochromis niloticus* (L). This species was chosen as the occurrence of several distinct aggressive profiles has been shown to predict stress response, including VR baselines (Barreto *et al.* 2009). This trait also fits to previous findings for rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), wherein aggressive behaviour and stress responses were identified as distinctive coping styles (Øverli *et al.* 2004, 2006; Schjolden *et al.* 2005a,b; Pottinger and Carrick 2001).

2.   Materials and methods

2.1   *Fish and holding conditions*

The stock population consisted of hatchery-grown, mixed-sex, sexually immature Nile tilapia (*O. niloticus*). Three indoor stock tanks were maintained at a density of 30 fish/80 L for a 7 day acclimation period. The tank water was maintained at room temperature (approximately 24°C) and was systemically re-circulated through a biological-mechanical filter. Continuous aeration was provided by an air pump connected by plastic tubing to an air stone. The photoperiod was set at 12 h light/12 h dark. The fish were fed commercial dry pellets (22% protein, Purina® Ltd, Campinas, SP, Brazil). Leftover food was removed periodically, and approximately 20 L of de-chlorinated water was gradually changed three times during the acclimation period.

2.2   *Experimental protocol and procedures*

Correlations between the VR baseline and feeding behaviours were evaluated while fish were introduced to a novel environment. Twelve fish (mean±SD) were randomly selected from the stock tank and individually placed (weight=12.8±0.5 g and standard length=7.6±0.4 cm) in isolation in glass aquaria (40×24×20 cm). Observation started after 1 h and lasted for 8 consecutive days. On the first day, VR was measured, and 1 h later, fish were provided food at which time feeding behaviour was assessed. Feeding behaviour was recorded daily during the following 7 days. These procedures were conducted twice (12 fish each trial) for

a total of 24 fish. During the 8 days of the trials, an air stone connected to an air pump via plastic tubing provided continuous aeration. The water temperature was maintained at approximately 24°C, pH 7.0, and <0.1 mg/L ammonia.

2.3   *Behavioural-physiological measurements*

VR was visually estimated by counting the time necessary for 20 successive opercular or buccal movements (adapted from Alvarenga and Volpato 1995). VR was measured three times per fish in a period of 3 consecutive minutes, was averaged for each fish and used in subsequent analyses.

Feeding behaviour was measured as (1) the day of feeding onset, (2) the number of days that the fish fed and (3) mean food ingestion during the days that fish fed. Fish were fed by hand once a day. We offered >10% of the fish's body mass of food per day. Fish were unable to eat all of the food offered, but this technique guaranteed fish were able to eat until satiation. This prevented an upper limit based on an arbitrary quantity of food. The leftover food was removed after 10 min.

An important factor of coping style and personality research is that individuals consistently differ from each other. As there are quality differences between the individuals, we calculated the body Fulton's condition factor (K = weight/length^3) for each fish (Froese 2006) to check whether there is association between the measures and condition, assessing if the potential differences were due to quality differences instead of coping styles.

2.4   *Data analyses*

Data were normally distributed according to the Kolmogorov and Smirnov test. Therefore, Pearson's correlation test was used to identify possible correlations between VR, condition factor and feeding behaviour.

3.   Results

The mean VR baseline (mean±SE) was 97.6±6.75 opercular beats per minute, a rate consistent with previously published values of 96–109 beats per min in tilapia of similar sizes (Barreto and Volpato 2004). Additionally, no correlation between body size and VR was found (R=0.03; R^2=0.0009; P=0.89) as all fish were similar in size (weight coefficient of variation=4.3%). Fish with high baseline VRs began feeding later than fish with low baseline VRs but ate more food each time they did eat. Therefore, VR was significantly and positively correlated with the day of feeding onset (R=0.86; R^2=0.75; P<0.0001; figure 1A) and with the mean food ingestion during the days that the fish fed (R=0.66; R^2=0.44; P=0.0004; figure 1B). Consequently, VR was also significantly and negatively
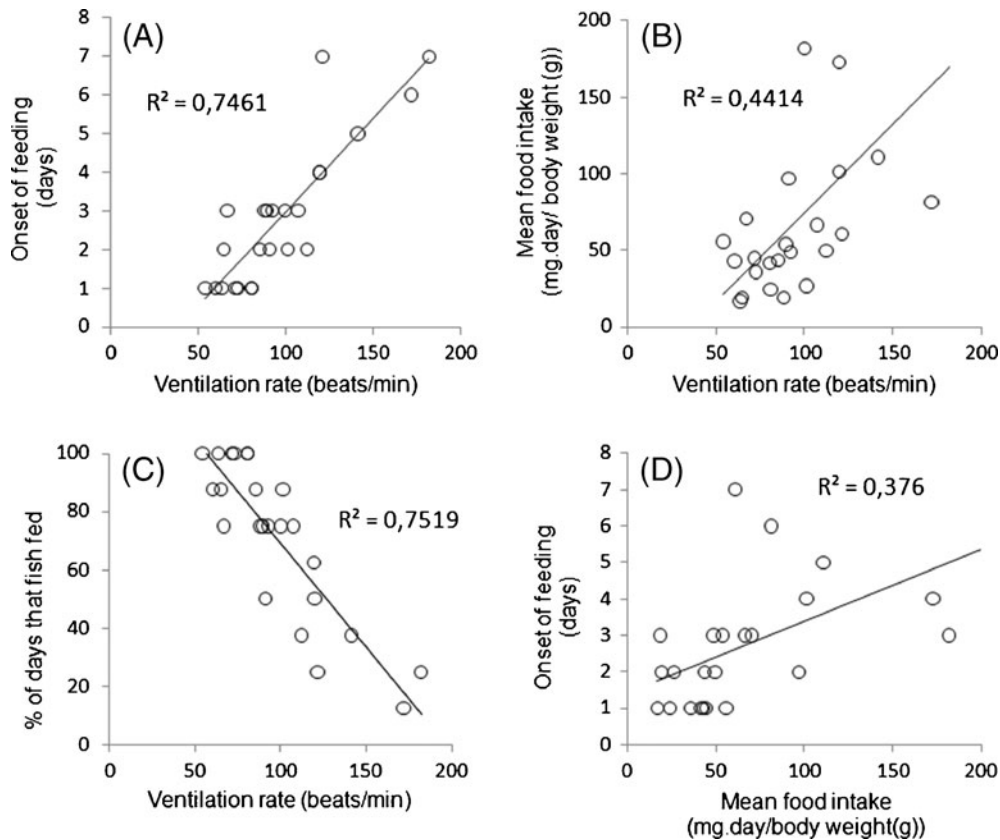
Figure 1. Pearson's correlations between VRs and feeding behaviours and between feeding onset and mean food ingestion during the days that the Nile tilapia ate.

correlated with the number of days that fish fed ($R=-0.87$; $R^2=0.75$; $P<0.0001$; figure 1C). An additional correlation between the delay of feeding onset and the mean food ingestion on the days that fish fed was calculated. These variables in feeding behaviour were significantly and positively correlated ($R=0.61$; $R^2=0.38$; $P=0.0014$; figure 1D), indicating that the longer the fish went without feeding, the more they ate at each feeding once their eating resumed.

The condition factor (mean ± SD; 0.03 ± 0.0035 g/cm^3) was not significantly correlated with VR ($R=-0.028$; $R^2=0.0008$; $P=0.90$), feeding onset ($R=-0.184$; $R^2=0.0034$; $P=0.39$) or the number of days that fish fed ($R=0.039$; $R^2=0.0015$; $P=0.86$). These correlations indicate that the association between VR and feeding behaviour are not due to individual quality differences, but are due to differences in coping styles. In the case of food ingestion, it was significantly and negatively correlated with condition factor ($R=-0.47$; $R^2=0.22$; $P=0.022$). In this analysis, individual food ingestion was not pondered by fish individual weight, as the weight is used for calculation of condition factor. Despite this correlation, it reinforces that fish in a better feeding state did not present compensatory feeding behaviour.

4. Discussion

This study indicated that VR is correlated with several aspects of feeding behaviour in Nile tilapia upon their introduction to a novel social-isolation tank. The variation in the way fish feed in a novel environment can indicate fish behavioural coping styles. Thus, variation in baseline resting VRs is linked to behavioural coping styles in fish. To our knowledge, this is the first report of such correlations for fish.

Nile tilapia specimens were transferred from stock tanks to experimental social-isolation tanks and their baseline VRs were measured 1 h after the transfer. Theory predicts that a negative correlation between VR and body size should occur (Schmidt-Nielsen 2002). Since we used fish of similar body sizes, the differences in VR were not likely due to body size variation. In fact, no correlation was found between VR and body size. Therefore, the variability of VR among fish of similar sizes is assumed herein as variation of the individual's ability to adjust to novelty (social isolation in a new tank).

Variation in feeding onset when introduced to a novel tank is a commonly observed trait in fish (Silva *et al.* 2010).

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The tendency to resume feeding following transport to a new rearing facility in social isolation depends upon the coping styles of the fish (Schjolden *et al.* 2005a; Øverli *et al.* 2006). The proactive animals are characterized by behaving in an active manner to counteract the aversive condition, while reactive animals do the contrary (Øverli *et al.* 2007). The proactive fish had lower VRs and started to feed sooner than the reactive fish. Although the proactive fish ingested less food per day, they fed more days within the observation period than did the reactive fish with higher VRs. Since the reactive fish took longer to start feeding, they compensated for the period of food deprivation by exhibiting hyperphagia during the days in which they ingested food. The differences between the behavioural profiles are clearly depicted by the positive correlation between feeding onset and food ingestion and because the total amount of food eaten throughout the experiment was similar among the fish. However, comparison of the amount of food eaten by each fish to the number of days that each fish fed, indicated that the reactive individuals that ate on fewer days ate much more on those days than did the proactive fish. As seen previously, hyperphagia is an expected response in unfed Nile tilapia (Barreto *et al.* 2003b, 2008), a reaction likely mediated by an increased neuropeptide Y level in fish (Campos *et al.* 2010). Together these correlations indicate that baseline VRs are linked to the proactive or reactive Nile tilapia profiles.

VR is a good tool for assessing both alertness and stress levels in fish. VRs are rapidly altered in response to alert stimuli, such as a gentle 4-s introduction of a partition into aquaria (Barreto and Volpato 2004) and to predation risks signalled by alarm substances (Gibson and Mathis 2006; Barreto *et al.* 2010), visual predator cues (Barreto *et al.* 2003a; Queiroz and Magurran 2005) or a predator's odour (Hawkins *et al.* 2004, 2007; Gibson and Mathis 2006). VRs are also altered in response to more extended stressors, such as social stress (Alvarenga and Volpato 1995) and confinement (Barreto and Volpato 2004; Brown *et al.* 2005). VRs can be measured by counting ventilations through direct observation of the fish; thus, sophisticated equipment is not required and invasive, painful or stress-inducing techniques are avoided. We extended the paradigm of using VR as an indicator of behavioural-physiological processes by showing that VR is a good tool for predicting feeding behaviour in the same context.

Acknowledgements

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