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Effects of acute stress on aggression and the cortisol response in the African sharptooth catfish *Clarias gariepinus*: differences between day and night

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African sharptooth catfish *Clarias gariepinus* were housed under continuous dim light (1 lx) or 12L:12D (350–0 lx) cycles. The number of skin lesions, as indicator of aggressive acts, and plasma cortisol levels, as indicator of stress-axis activity, were measured at baseline as well as following a stressor (given in the light or dark phase). Results showed that (1) baseline plasma cortisol levels were not different between photoperiods, (2) the number of baseline skin lesions was highest for *C. gariepinus* housed under continuous dim light, (3) stressor-induced peak levels of plasma cortisol were highest in the light phase and (4) the number of skin lesions following a stressor was highest in the dark phase. The higher number of stressor-related skin lesions in the dark (active) phase suggests increased stressor-induced aggression while in the active phase. In addition, the data suggest that housing under continuous dim light does not result in higher stress-axis activity, as measured by baseline levels of cortisol, but does result in more stressor-induced aggression, as measured by the higher number of skin lesions. The latter may be related to the fact that the continuous dim light photoperiod has twice the number of dark-phase (active) hours in which stressor-induced aggression is stronger compared to the 12L:12D photoperiod, which has a light phase in which stressor-induced aggression is lower.

Key words: behaviour; *Clarias gariepinus*; photoperiod; skin lesions.

INTRODUCTION

In the Netherlands, the African sharptooth catfish *Clarias gariepinus* (Burchill 1822) is an important species in aquaculture and is grown in recirculating aquaculture systems (RAS). Sustainability is becoming more and more important in aquaculture practices, of which one aspect is animal welfare (Broom, 2010). Although animal welfare has been defined in different ways (Spruijt, *et al.*, 2001; Korte *et al.*, 2007; Broom, 2011; Ohl & van der Staay, 2012), key to all definitions is that poor welfare is associated with overtaxing the coping capacity of animals [allostatic overload (McEwen & Wingfield,

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2003)], which may result in chronic stress-related physiology, behaviour, pathology and increased mortality.

Recently, it was shown that a 3 h overland transport of marketable *C. gariepinus* might be considered a strong stressor as indicated by increased levels of plasma cortisol at 24–48 h following transport (Manuel *et al.*, 2014). In addition, the number of skin lesions was increased in control and transported fish compared to unhandled fish (Manuel *et al.*, 2014). The number of skin lesions on the bodies of fishes is often used as an indicator of aggression by conspecifics (Britz & Pienaar, 1992; Kaiser *et al.*, 1995; Hossain *et al.*, 1998; van de Nieuwegiessen *et al.*, 2008) and are associated with poor welfare. Acts of aggression spike after exposure to stressors such as handling (Manuel *et al.*, 2014) and netting (van de Nieuwegiessen *et al.*, 2008). Performing aggressive behaviour may serve to reduce stress, *i.e.* it is a form of redirected behaviour (mammals: Levine *et al.*, 1989; fishes: Winberg *et al.*, 1996; Øverli *et al.*, 2004). Hence, it may be hypothesized that by reducing the stress-load on *C. gariepinus*, stressor-induced aggressive behaviour is also lowered.

In aquaculture, housing conditions are primed towards production and it is a common practice to house fish species under artificial photoperiods to optimize growth and delay maturation (Almazán-Rueda *et al.*, 2004; Mustapha *et al.*, 2012). Farming of *C. gariepinus* in recirculating aquaculture systems is often done under a constant photoperiod (*i.e.* 24 h low light) to improve production, but doing so removes an important *zeitgeber* the day–night cycle. *Clarias gariepinus* is a nocturnal species, active at night and resting during the day (Babiker, 1979). The onset of light initiates the synchronization of many biological processes that follow a circadian rhythm (Roenneberg & Merrow, 2005), among them the stress-axis, also known as the hypothalamus–pituitary–interrenal (HPI) axis (Nader *et al.*, 2010). Changes in the circadian rhythm of cortisol, the corticosteroid produced by the HPI-axis, due to changing the day–night cycle, can result in inadequate responses to challenges (Weibel *et al.*, 2002; Kassi & Chrousos, 2013), and may lead to compensatory hyperactivity of other mediators (McEwen, 2000). Farmed fishes are potentially exposed to a multitude of challenges, such as handling (Manuel *et al.*, 2014), poor water quality (Roques, 2013), inappropriate stocking densities (Kaiser *et al.*, 1995; van de Nieuwegiessen *et al.*, 2008) and live transport (Iversen *et al.*, 2005; Nomura *et al.*, 2009; Brinn *et al.*, 2012; Nikoo & Falahatkar, 2012; Manuel *et al.*, 2014; Boerrigter *et al.*, 2015*a*). When fishes are unable to properly cope with challenges, they may experience allostatic overload (McEwen, 2000).

It, therefore, follows that providing a natural day–night cycle may be beneficial for the biology, physiology and welfare of *C. gariepinus* by reducing allostatic load, enabling *C. gariepinus* to cope better with and recover from challenges, and potentially lowering aggressive behaviour. To test this hypothesis in a laboratory setting, in experiment 1, *C. gariepinus* were housed under 12 h light and 12 h completely dark (12L:12D) and 24 h dim light (0L:24D) photoperiods. Before and during the recovery period after a 15 min forced crowding and air-exposure stressor, plasma cortisol levels were measured and the level of aggression was indirectly assessed by counting the number of skin lesions (Almazán-Rueda *et al.*, 2004). Under 0L:24D, the peak plasma cortisol response was lower, but the number of baseline and stressor-induced skin lesions was higher, compared to the 12L:12D photoperiod. To assess whether these differences were related to the moment that the stressor was given, *i.e.* dark *v.* light phase, or to housing without a light–dark cycle *per se,* experiment 2 was performed.

Clarias gariepinus were housed under similar 12L:12D and 0L:24D photoperiods, but this time the stressor was given in either the dark and active phase (12L:12D and 0L:24D groups) or the light and resting phase (12L:12D).

MATERIALS AND METHODS

ETHICAL APPROVAL

Experiments were approved by the Animal Ethics Committee of Wageningen UR (#2012045.b) and the Radboud University (#RU-DEC2010-092) and were conducted in agreement with Dutch law ('Wet op de Dierproeven', 1996) and European regulations (Directive 86/609/EEC).

ANIMALS AND HOUSING

Clarias gariepinus (experiment 1: average individual body mass: 40 g at a stocking density of *c.* 18·50 kg m^{-3}; experiment 2: 10 g at *c.* 6·67 kg m^{-3}) were obtained from Fleuren and Nooijen (www.fleuren-nooijen.nl) and randomly assigned to the experimental tanks. At the end of the experiment, the average body mass of *C. gariepinus* was 150 g (at *c.* 60 kg m^{-3}) for experiment 1 and between 217 g (at *c.* 100 kg m^3) at sampling point 1 and 332 g (at *c.* 75 kg m^{-3}) at sampling point 2 in experiment 2.

In both experiments, *C. gariepinus* were kept in custom-made glass aquaria (length × width × height: 100 cm × 50 cm × 60 cm). The water level was 30 cm, giving a volume of 150 l. Each tank had its own recirculation system and biological filter, increasing the total water volume of the system to 450 l. Water quality was assessed by daily measurements of ammonia, nitrate and nitrite concentrations using commercially available tester kits (Sera; www.sera.de/en/home.html). Electronic sensors inside the tanks measured temperature and pH continuously. All experiments were carried out at 28° C.

EXPERIMENTAL DESIGN

Experiment 1

Two groups of *C. gariepinus* were used in this experiment, the first housed under a 12L:12D photoperiod (0700–1900 hours lights on; 350 lx at the surface of the water during the light phase and 0 lx during the dark phase) and the second under a constant photoperiod of 0L:24D (0000–2400 hours dim light; 1 lx at the surface of the water) [Fig. 1(a)]. Both groups were given a fixed amount of food three times daily (0700, 1500 and 2300 hours) *via* an automatic feeder (Velda; www.velda.nl).

Clarias gariepinus were housed and monitored under these conditions for 4 weeks after which they were exposed to the stress protocol [see stress protocol; Fig. 1(c)]. Only one group was sampled per day and sampling was done once every 2 days to reduce the possible side effects of sampling activity of the previous day. Both groups were stressed at the same time: 0900 hours [Fig. 1(a)].

Experiment 2

Three groups (two replicate tanks per group) were housed under different photoperiods [Fig. 1(b)]: 12L:12D (0700–1900 hours lights on; 350 lx at the surface of the water and 0 lx during the dark phase), 12D:12L (1200–2400 hours lights on; 350 lx at the surface of the water during the light phase and 0 lx during the dark phase) and 0L:24D (0000–2400 hours dim light; 1 lx at the surface of the water). *Clarias gariepinus* were fed three times daily (12L:12D and 0L:24D at 0700, 1500 and 2300 hours; 12D:12L at 0400, 1200 and 2000 hours) with a fixed amount of food *via* an automatic feed dispenser (Velda). One tank of *Clarias gariepinus* from each photoperiod group was exposed to the stress protocol [see stress protocol; Fig. 1(c)] after

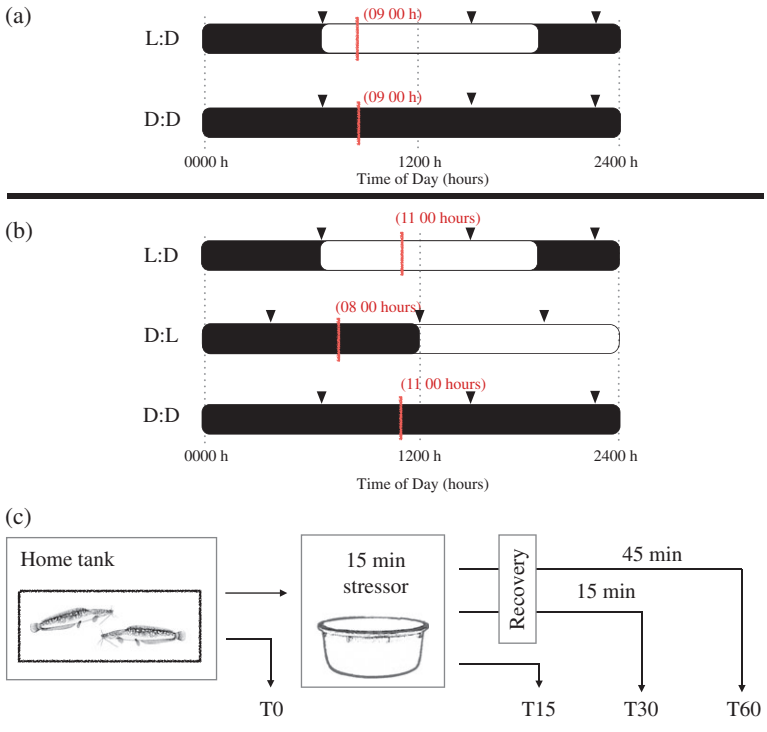

FIG. 1. A schematic overview of (a) experiment 1 and (b) experiment 2 where different experimental groups of *Clarias gariepinus* are shown as: light–dark (12L:12D), dark–light (12D:12L)and dark–dark (0L:24D). ▬ indicate periods of lights off and ▭ indicate periods of lights on. Each full bar represents 24 h as is noted at the bottom of the figures and ▼ indicate the fixed feeding moments. Time of stressor exposure is indicated as |. (c) The stress protocol: *C. gariepinus* were either sampled for basal conditions directly from their home tank (T0) or were exposed to a 15 min forced air exposure and crowding stressor. *Clarias gariepinus* were then directly sampled (T15) or give a recovery time of either 15 min (T30) or 45 min (T60) before being sampled.

3 months of housing, and the second tank after 4 months. One group was sampled per day and sampling was done every other day to reduce the possible side effects of sampling activity of the previous day. Groups were stressed at different times of the day to correct for shifts in feeding times: 12L:12D and 0L:24D were stressed at 1100 hours and 12D:12L was stressed at 0800 hours [Fig. 1(b)]. The duplicate experiments revealed a similar outcome in results and were merged into a single read-out for ease of reading and data interpretation.

STRESS PROTOCOL

One day before the stressor was applied, *C. gariepinus* were fasted to prevent adverse water conditions during the stress protocol and to mimic aquaculture practices, where fish are fasted before handling and sorting (Manuel *et al.*, 2014). In both experiments, *C. gariepinus* were netted from their home tank and placed together in a dry plastic mortar tub (65 cm diameter × 37 cm height) for 15 min to evoke a stress response (forced air exposure and crowding). To assess the stress response, between seven and 10 *C. gariepinus* from each group were sampled at each of the following time points: directly before the stressor to function as baseline levels (T0) and at three time points after the stress protocol, directly after (T15) and after a recovery period of 15 or 45 min (T30 and T60). During the recovery period (similar stocking density and lighting

condition for all experimental groups), the *C. gariepinus* for T30 and T60 were housed into two separate tanks (each time point one tank), filled with water from their home tank. A schematic overview can be seen in Fig. 1(c).

FISH HANDLING, EUTHANASIA AND BLOOD COLLECTION

Clarias gariepinus were collected by netting and placed within a large mortar tub filled with water containing 0·1% (v/v) 2-phenoxyethanol (Sigma; www.sigmaaldrich.com). Once deeply anaesthetized (within 1 min), blood was drawn and *C. gariepinus* were immediately killed by transecting the spinal cord behind the skull.

Blood (1 ml) was drawn from the caudal vein, using heparinized syringes, collected in 1·5 ml reaction vials (Eppendorf; www.eppendorf.com) and immediately put on ice. Subsequently, reaction vials were centrifuged (18 620 rcf, 4° C, 10 min) and blood plasma was separated from blood cells and stored at −20° C until cortisol analysis.

PLASMA CORTISOL

Plasma cortisol was measured as previously described (Gorissen *et al.*, 2012). Briefly, 96 well microtitre plates were coated with mouse cortisol-antibodies in coating buffer. Plates were cleared of coating buffer and washed with a wash buffer before blocking possible non-specific binding sites with blocking buffer. Wells were cleared of blocking buffer and 10 µl of standard or sample, together with 90 µl of ^{3}H-cortisol, was added to the wells. After an incubation period of 4 h at room temperature, wells were cleared and washed before scintillation liquid was added. Activity was measured with a liquid scintillation counter (Wallac 1450 Microbeta Plus; www.perkinelmer.com; detection limit: 4 ng ml^{-1}; interassay VC: 12·5% and intra-assay VC: 2·5%).

SKIN LESIONS SCORING

Skin lesions were scored as previously described (Manuel *et al.*, 2014). Briefly, at each of the sampling intervals all *C. gariepinus* in an experimental group were checked for lesions. The number per individual (including those on fins and barbels) was derived from the average of two separate counts performed by two researchers. For experiment 1, only the total number was recorded. In experiment 2, the old (*i.e.* non-bloody scars) and the new (*i.e.* bloody wounds) lesions were counted separately. In this experiment, the old lesions were used as indicator for aggression during housing (baseline) and the new lesions as indicator for stressor-induced aggression.

STATISTICS

Statistical analyses were performed with GraphPad Prism 5.0 for Mac (GraphPad Software Inc.; www.graphpad.com). Differences in plasma cortisol between groups were analysed with a two-way ANOVA (factors: 'photoperiod' and 'time'). In case of significant interaction or main effects, *post hoc* analysis was performed. For the factor photoperiod, a Bonferroni post-test was used to assess significant differences between groups at a given sampling point. For the factor time, a one-way ANOVA across the different sampling times (T0, T15, T30 and T60) was used for each photoperiod, followed by a Dunn's *post hoc* analysis to compare each group to the T0 group (*i.e.* basal conditions).

Differences in skin lesions between groups were assessed with a two-way ANOVA (similar as plasma cortisol) for experiment 1. For experiment 2, differences in the number of skin lesions were assessed by a Kruskal–Wallis test, as a D'Agostino & Pearson omnibus normality test revealed that groups did not conform to a Gaussian distribution. In case of a significant outcome of the Kruskal–Wallis test, Dunn's *post hoc* analysis was used to compare each group to every other group. For ease of reading, statistics are included in the captions of the figures. In all cases, significance was accepted at $P \leq 0{\cdot}05$ unless otherwise indicated.

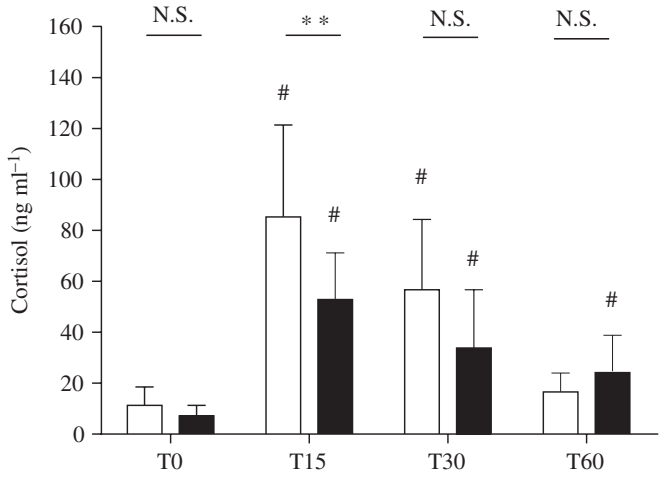

FIG. 2. Plasma cortisol levels of *Clarias gariepinus* housed under a 12L:12D (■) and 0L:24D (□) photoperiod before (T0) and after (T15, T30 and T60) exposure to an acute 15 min stressor. Each bar shows the group mean + S.D.; $n = 14$ (T0) and $n = 7$ (T15, T30 and T60). A significant interaction effect in a two-way ANOVA ($F_{3,62} = 3{\cdot}745$, $P \leq 0{\cdot}05$) was followed by a *post hoc* analysis. Significant differences ($P \leq 0{\cdot}05$) compared to basal (T0) within a photoperiod are indicated above each bar (#); significant differences between photoperiods at a given sampling time are indicated with an asterisk (** $P \leq 0{\cdot}01$; N.S., not significant).

RESULTS

EXPERIMENT 1

Plasma cortisol

Compared to baseline plasma cortisol (T0), plasma cortisol levels for 12L:12D were significantly higher directly following the stressor (T15) and after 15 min recovery (T30). No significant difference was observed after 45 min recovery (T60). For the 0L:24D group, plasma cortisol levels were significantly higher at all sampled time points (T15, T30 and T60) compared to baseline (T0) (Fig. 2).

In addition to the difference in recovery time between 12L:12D and 0L:24D, plasma cortisol levels were significantly higher for the 12L:12D group than the 0L:24D group directly following the stressor (T15). No significant differences, however, were observed between photoperiods at baseline (T0) or the recovery period (T30 and T60) (Fig. 2).

Skin lesions

Compared to the baseline number of skin lesions (T0), no significant difference was observed for the number of skin lesions directly following the stressor (T15) nor during the recovery period (T30 and T60) in the 12L:12D group (Fig. 3). In contrast, in the 0L:24D group, an increasing number of skin lesions following the stressor was observed, with a significant difference compared to baseline (T0) at T60.

This time-dependent increase of stressor-induced skin lesions in the 0L:24D group was associated with an overall higher number of skin lesions in the 0L:24D group

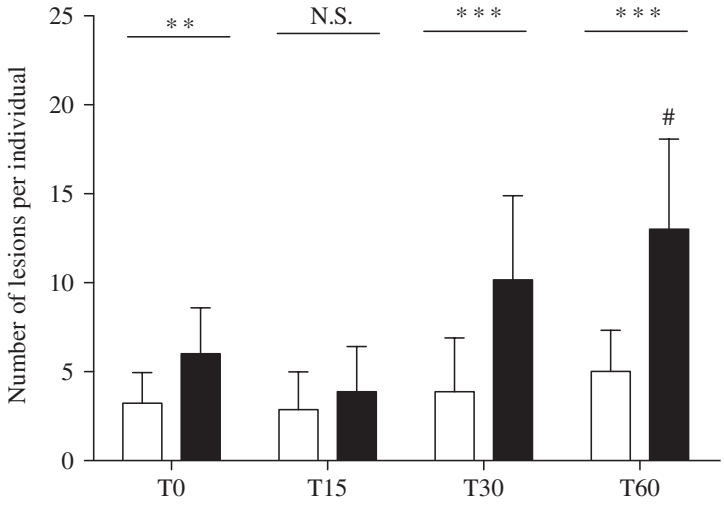

FIG. 3. Number of skin lesions in *Clarias gariepinus* housed under a 12L:12D (■) and 0L:24D (□) photoperiod before (T0) and after (T15, T30 and T60) exposure to an acute 15 min stressor. Each bar shows the group mean + S.D.; $n = 14$ (T0) and $n = 7$ (T15, T30 and T60). A significant interaction effect in a two-way ANOVA ($F_{3,62} = 4{\cdot}222$, $P \leq 0{\cdot}01$) was followed by a *post hoc* analysis. Significant differences ($P \leq 0{\cdot}05$) compared to basal (T0) within a photoperiod are indicated above each bar (#); significant differences between photoperiods at a given sampling time are indicated with an asterisk (**$P \leq 0{\cdot}01$; ***$P \leq 0{\cdot}001$; N.S., not significant).

compared to the 12L:12D group during the recovery period (T30 and T60) (Fig. 3). In addition, the number of skin lesions at baseline (T0) was higher in the 0L:24D group than in the 12L:12D group.

EXPERIMENT 2

Plasma cortisol

There were no significant differences in baseline (T0) plasma cortisol among photoperiods (12L:12D, 12D:12L and 0L:24D) (Fig. 4). Regardless of whether the stressor was applied during the light and resting phase (12L:12D) or the dark and active phase (12D:12L and 0L:24D), plasma cortisol levels increased significantly directly afterwards (T15) compared to baseline (T0). Peak levels of plasma cortisol, however, were significantly higher in *C. gariepinus* stressed in the light and resting phase (12L:12D) than in the dark and active phase (12D:12L and 0L:24D). No difference in peak levels of plasma cortisol was observed between 12D:12L and 0L:24D. Plasma cortisol levels returned to baseline values at T30 for 12L:12D and 0L:24D and at T60 for 12D:12L.

Skin lesions

Clarias gariepinus housed under 12L:12D had a significantly lower number of non-bloody skin lesions compared to *C. gariepinus* housed under 12D:12L and 0L:24D [Fig. 5(a)]. The average number of bloody skin lesions following the stressor (T15, T30 and T60) was significantly lower in *C. gariepinus* stressed during the light and resting phase (12L:12D) than during the dark and active phase (12D:12L and 0L:24D) [Fig. 5(b)].

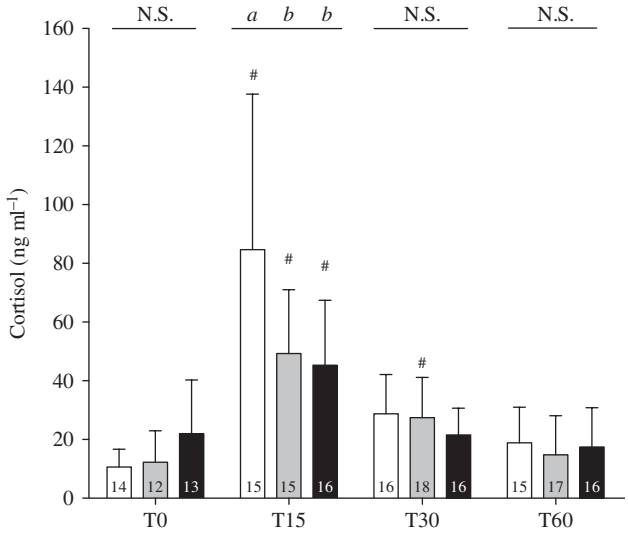

FIG. 4. Plasma cortisol levels of *Clarias gariepinus* housed under a 12L:12D (□), 12D:12L (▨) and 0L:24D (■) photoperiod. Each bar shows the group mean + S.D. The number of *C. gariepinus* per group is shown at the base of each bar. A significant interaction effect in a two-way ANOVA ($F_{6,171} = 4{\cdot}679$, $P \leq 0{\cdot}0001$) was followed by a *post hoc* analysis. Significant differences ($P \leq 0{\cdot}05$) compared to basal (T0) within a given photoperiod are indicated (#); significant differences between photoperiods at a given sampling time are indicated with different letters ($P \leq 0{\cdot}05$; bars that share the same letter are not significantly different from each other).

DISCUSSION

PLASMA CORTISOL

The overall absence of any differences in baseline cortisol suggests no difference in baseline stress-axis (HPI-axis) activity due to different photoperiods. As samples were collected only at one time point across a 24 h cycle, it is not possible to unequivocally conclude that photoperiod did not affect baseline activity of the HPI-axis as differences at other times may be possible. It should also be noted that, previously it has been reported that the highest levels of cortisol following stress were at 1 h post stressor exposure (van de Nieuwegiessen *et al.*, 2008), but here, however, the highest levels were directly following the stressor. Similar observations were made after applying the same stressor (15 min forced air exposure and crowding) in another series of experiments (Boerrigter *et al.*, 2015*b*) on *C. gariepinus*. The comparatively rapid occurrence of the peak as well as the swift recovery may have masked day and night differences in the present experiments. Yet, a slightly longer recovery time was observed in groups housed under 0L:24D compared to 12L:12D (*i.e.* significantly higher compared to basal) in one of the two experiments. Thus, these data do not as yet provide evidence that continuous dim light affects the coping capacity of *C. gariepinus*.

Groups that were stressed during the dark and active phase (12D:12L and 0L:24D) had similar peak concentrations of plasma cortisol (50 ng ml^{-1}) to those previously reported for farmed *C. gariepinus* following transport (50 ng ml^{-1}) (Manuel *et al.*, 2014). In contrast, groups that were exposed to the stressor during the light and

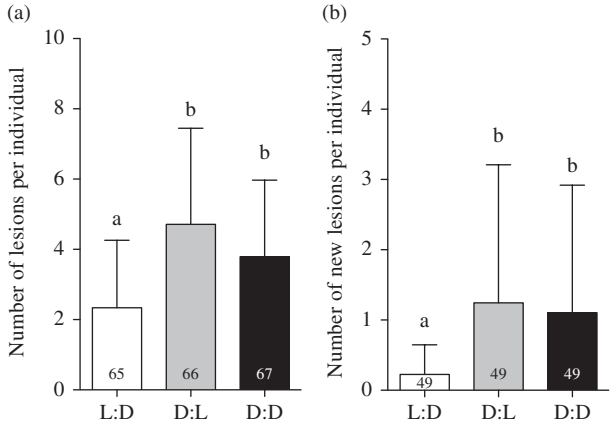

FIG. 5. (a) The number of skin lesions under basal housing conditions (old lesions) and (b) newly inflicted skin lesions during the recovery period (fresh lesions) in *Clarias gariepinus* housed under a 12L:12D (□), 12D:12L (▨) and 0L:24D (■) photoperiod. Each bar shows the group mean + S.D. The number of *C. gariepinus* per group is shown at the base of each bar. A significant Kruskal–Wallis [(a) $H = 30{\cdot}71$, $P \leq 0{\cdot}001$; (b) $H = 10{\cdot}43$, $P \leq 0{\cdot}001$] was followed by a *post hoc* analysis. Significant differences between photoperiods are indicated with different letters ($P \leq 0{\cdot}05$; bars that share the same letter are not significantly different from each other).

resting phase (12L:12D) had significantly higher peak levels of plasma cortisol (80–200 ng ml^{-1}). An effect on the plasma cortisol response to a stressor that is linked to time of day is not uncommon and has been reported previously in a wide range of other species, such as rats *Rattus norvegicus* (Atkinson *et al.*, 2006), Galápagos marine iguanas *Amblyrhynchus cristatus* (Romero & Wikelski, 2006), white-crowned sparrows *Zonotrichia leucophrys* (Breuner *et al.*, 1999), green sturgeon *Acipenser medirostris* Ayres 1854 (Lankford *et al.*, 2003) and Senegalese sole *Solea senegalensis* Kaup 1858 (López-Olmeda *et al.*, 2013).

One function of cortisol is to increase the amount of energy (glucose) available, which is needed to mount an adequate response to challenges (Flik *et al.*, 2006; Gorissen & Flik, 2016). During the light phase, *C. gariepinus* is normally inactive (Babiker, 1979) which is associated with reduced oxygen uptake, indicative of lower metabolism (Babiker, 1979). Thus it may be hypothesized that, compared to a nightly challenge, *C. gariepinus* challenged during the day require higher levels of cortisol to recruit the amount of energy needed to cope with the situation.

Another possible explanation could be that plasma cortisol levels follow a circadian rhythm, characterized by a cortisol awakening response (CAR) in which plasma cortisol levels rise several hours before waking. In nocturnal species, such as *R. norvegicus* (Djordjevic *et al.*, 2008) and *S. senegalensis* (Oliveira *et al.*, 2012) the CAR occurs during the day (light), while for diurnal species, such as the common carp *Cyprinus carpio* L. 1758 (Redgate, 1974) and human *Homo sapiens* (Clow *et al.*, 2010), this occurs during the night (dark). When cortisol levels rise during CAR, the sensitivity of the adrenal glands (or interrenal glands in fish) is increased (Clow *et al.*, 2010). Similarly, a reduction in the sensitivity of the adrenals is observed when cortisol levels decline during CAR. It follows that an individual exposed to a stressor has the potential for a stronger cortisol response during the rising phase of CAR than in the declining phase. Based

on the data presented in this study and the ecology of *C. gariepinus* (*i.e.* a nocturnal species), it is hypothesized that the CAR in *C. gariepinus* occurs during the light phase, with rising cortisol levels during the light phase and a decline during the dark phase.

SKIN LESIONS

In experiment 1, a lower baseline number of skin lesions was observed for *C. gariepinus* housed under 12L:12D compared to those housed under 0L:24D, confirmed in experiment 2. The lower number of basal skin lesions under 12L:12D might be related to the resting behaviour observed during the light phase of 12L:12D (Video S1, Supporting Information). This resting behaviour was not observed under 0L:24D, suggesting that this behaviour is typical for the light and resting phase. These observations are in line with previous studies, where *C. gariepinus* displayed more activity during the dark phase and more resting during the light phase (Kaiser *et al.*, 1995; Almazán-Rueda *et al.*, 2004). Although some studies have reported less activity during the dark phase (Almazán-Rueda *et al.*, 2005), all studies share the observation that the phase in which activity was lowest was associated with the least aggression and skin lesions. Together, this suggests that the number of skin lesions relates to the active and resting phase of *C. gariepinus* and not to the light or dark phase *per se*.

Surprisingly, the baseline number of skin lesions was higher in *C. gariepinus* housed under a 12D:12L photoperiod (1200–2400 hours lights on) compared to those housed under a 12L:12D photoperiod (0700–1900 hours lights on), as both groups were housed under a similar light regime. These contrasting data may, however, be reconciled when the effects of stressors on the number of skin lesions are considered. Previous studies (van de Nieuwegiessen *et al.*, 2008; Manuel *et al.*, 2014) have reported an increase in the number of skin lesions following a stressor: similar to the results in the current study. Interestingly, a greater increase in fresh wounds was observed for *C. gariepinus* challenged in the dark and active phase compared to the light and rest phase. This observation might explain the higher number of skin lesions found at baseline for the 12D:12L group. This group was housed under an artificial photoperiod that did not mirror the natural light hours (*i.e.* 2400–1200 hours lights out). Work activity in the aquarium facilities in which the fish were housed starts at 0800 hours and it is not unlikely that this activity was associated with spikes in background noise or vibrations. These disturbances may act as stressors (Johansen *et al.*, 2006) and *C. gariepinus* under 12D:12L would show a stronger behavioural response (*i.e.* aggression) to these disturbances compared to *C. gariepinus* housed under 12L:12D, as these disturbances happen during part of their dark and active phase (*i.e.* between 0800 and 1200 hours).

It should be noted that the number of lesions was not increased immediately after the stressor but only during the recovery phase: 15 and 45 min following the challenge. This suggests that aggressive acts may strongly increase following a stressor and not necessarily during the stressor.

CORTISOL AND SKIN LESIONS

In general, a stronger physiological stress response (*i.e.* higher plasma cortisol levels) was related to lower aggression (*i.e.* fewer skin lesions). Although not quantified by van de Nieuwegiessen *et al.* (2008), their data suggest a similar observation, *i.e.*

groups of fish that displayed a stronger cortisol response had a lower number of skin lesions following a stressor. It is interesting to note that the plasma cortisol peak levels were observed directly following the stressor, whereas the increase in skin lesions was seen during the recovery phase. This may suggest that the behavioural response (*i.e.* aggression) is more associated with the recovery phase to cope with or recover from the stressor, *e.g.* redirected aggression to reduce stress (Levine *et al.*, 1989; Winberg *et al.*, 1996; Øverli *et al.*, 2004). There also seems to be a relation between the activity level of *C. gariepinus*, the number of stressor-induced skin lesions and the release of cortisol to the bloodstream (peak levels and duration): the more active a fish is prior to exposure to a stressor, the lower the cortisol release, but the higher its aggressive behaviour. This offers a starting point for future studies to focus on reducing aggressive behaviour in *C. gariepinus* aquaculture. For example, whether the increase in skin lesions observed during the active phase is simply due to a higher rate of encountering other subjects during the active phase, as it is relatively easy to perform (redirected) aggression towards active conspecifics, or if it may relate to physiological, hormonal and behavioural mechanisms associated with the active phase (*i.e.* circadian rhythm), which result in a higher level of aggression when provoked or stressed.

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Supporting Information

Supporting Information may be found in the online version of this paper:
Video S1. Typical resting behaviour of *C. garipeinus* observed during the light phase. At the end of the video fish respond to food falling on the water surface..

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