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Stressful Daylight: Differences in Diel Rhythmicity Between Albino and Pigmented Fish

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In laboratory experiments, variously colored strains of animals, including those with albino phenotypes, are commonly used. The melanocortin theory suggests, however, that coloration phenotypes alter animal physiology and behavior. Animals with the albino phenotype show photoreceptor degradation associated with lowered visual accuracy, escape reactions, etc., presumably accompanied by prevailing nocturnal activity and lowered aggressiveness. This assumption was tested in small groups of albino and pigmented European catfish, *Silurus glanis*, during the diel cycle. The frequency of agonistic interactions was observed during mutual contests for shelters, and subsequently, blood plasma, brain, gill, and liver samples were collected to evaluate stress parameters. In an experimental arena with shelters, the light/dark rhythmicity of locomotor activity and aggressiveness of the two phenotypes were comparable; the peak was observed at night, and a lower peak was observed at dawn. In an experimental stream without shelters, the peak of locomotor activity occurred at night for only the pigmented phenotype. In the evaluation of 4 antioxidants and 1 oxidative stress indicator, representing a total of 15 indices, albino fish showed significant rhythmicity for 8 indices, whereas pigmented catfish showed significant rhythmicity for 5 indices. The production of blood stress parameters with the peak during the day occurred only in albino fish. A complex model was fitted with the aim of evaluating the links between behavioral and biochemical indices. Time periodicity was modeled using a sine wave and confirmed parallel courses of agonistic interactions in the catfish groups; the peak at dawn was associated with a 4.08-fold (conf. int. 3.53–4.7) increase in such interactions. The changes in glucose and superoxide dismutase concentrations varied with phenotype, while the effects of cortisol, lactate and catalase did not. In summary, the rhythmicity of locomotor activity and changes in the aggressiveness of catfish were influenced by shelter availability, and the effect of light-induced stress was more apparent in albino fish than in pigmented conspecific fish. The results suggested that laboratory-raised animals with pigmentation patterns naturally occurring in the wild show more reasonable values during experiments than those with an albino phenotype.

Keywords: albinism, melanocortin theory, locomotor activity, aggressiveness, oxidative stress

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INTRODUCTION

In laboratory experiments, variously colored strains of animals, including those with albino phenotypes, are commonly used. The melanocortin theory suggests, however, that coloration phenotypes alter animal physiology and behavior (Ducrest et al., 2008; Santostefano et al., 2019). Albino animals exhibit physiological limitations that separate their behavior during the diurnal cycle from that of pigmented conspecifics. Ocular anomalies related to retinal degradation are most frequently mentioned (Oetting, 2000; Prusky et al., 2002). A lack of melanin can lead to an underdeveloped central part of the retina adapted for high-acuity vision (fovea centralis) and a reduction in rod number (Jeffery, 1997). Physiological changes are followed by reduced visual acuity (Balkema and Dräger, 1991; Buhusi et al., 2005), impaired perception of movement (Hupfeld and Hoffmann, 2006) and higher sensitivity to light (van Abeelen and Kroes, 1967; Kehas et al., 2005). The eyes of albino rodents show reduced adaptation to light, often leading to photoreceptor degradation (Prusky et al., 2002; Refinetti, 2007; Marc et al., 2008), which in turn can cause loss of vision (Buhusi et al., 2005) and, eventually, photophobia and acrophobia (van Abeelen and Kroes, 1967; Owen et al., 1970). Albino animals are less active (Defries, 1969) with prevailing nocturnal activity (Stryjek et al., 2013), and deteriorated spatial orientation is probably the reason why, e.g., albino fish, show a reduced escape response (Ren et al., 2002).

Light is an important zeitgeber that controls diurnal rhythms through the melanocortin system (Sánchez-Vázquez et al., 1997; Oliveira et al., 2013). Light information passes through the retina to the pineal organ and initiates the secretion of melatonin hormone, which is released to blood in low concentrations during the day and high concentrations during the night, thereby controlling the daily patterns of behavior (López-Olmeda et al., 2009; Oliveira et al., 2009; Sánchez-Vázquez et al., 2019). Fish specifically respond to light alternation by showing diurnal, nocturnal or crepuscular daily patterns of behavior (Madrid et al., 2001). The locomotor activity associated with light oscillation may be altered, however, due to food intake (Sánchez-Vázquez et al., 1995, 1997), the number of individuals (Boujard, 1995; Vera et al., 2011) or social relationships (Alanärä et al., 2001; Brännäs, 2008), and individuals can switch their activity from diurnal to nocturnal and vice versa or show dual activity (Alanärä and Brännäs, 1997; Vera et al., 2009; Slavík and Horký, 2012).

Light conditions are associated with the level of aggressiveness (Britz and Pienaar, 1992; Almazán-Rueda et al., 2004). Increasing melatonin secretion during low light intensities attenuates the activity and aggressiveness of organisms (Almazán-Rueda et al., 2004; Carvalho et al., 2012). Biological interpretation of a decrease in aggressiveness during the dark phase suggests that, e.g., fish, do not see each other, as well as prey, and hence, stimuli to be aggressive are waned (Valdimarsson and Metcalfe, 2001; Lopes et al., 2018). Agonistic interactions are accompanied by stress that can be observed as changes in blood plasma parameters (Pottinger and Carrick, 2001; Sloman et al., 2001), e.g., cortisol hormone concentration (Pickering, 1992; Øverli et al., 2004) and brain antioxidant activity (Costantini et al., 2011; Chowdhury and Saikia, 2020). For example, a lower level of cortisol is reported in dominant and more aggressive fish than in subordinate and less aggressive fish (Koolhaas et al., 1999; Sloman et al., 2001; Øverli et al., 2004). Agonistic behavior is a metabolically demanding process (Haller, 1995; Copeland et al., 2011) requiring mobilization of glucose from storage glycogen, leading to its increase in the blood (Fernandez et al., 1994); hence, it is accompanied by hepatic glycogen reduction and an increase in the glucose level in the plasma (Haller, 1991; Neat et al., 1998) that is higher for subdominant individuals and losers (Elofsson et al., 2000; Copeland et al., 2011). The cortisol level also corresponds with the light/dark (L/D) cycle and feeding schedule, while the glucose level response is weaker (Polakof et al., 2007; López-Olmeda et al., 2009; Oliveira et al., 2013). The activity of antioxidants in fish brains is reported as an oxidative stress, which is caused by an imbalance between harmful free radicals and antioxidants neutralizing these radicals (Chowdhury and Saikia, 2020). Oxidative stress can occur after acute stress as a response to increased glucocorticoids, which may induce oxidative stress by increasing the metabolic rate and thus free radicals (Costantini et al., 2011). The rise in antioxidant activity is associated with changes in light (Sreejith et al., 2007; Kim et al., 2016) and the level of aggressiveness (Almeida et al., 2009). In summary, the L/D cycle influences fish behavior, which can be recorded as changes in locomotor activity and aggressiveness accompanied by changes in physiological parameters in blood plasma and antioxidant activity.

Animals, including fish, with a higher level of melanin are more aggressive and dominant (Horth, 2003; Kittilsen et al., 2009) and vice versa; i.e., less pigmented individuals are more sensitive to stress and are often subdominant (Höglund et al., 2000; Kittilsen et al., 2012). In fish, individuals with an albino phenotype show lower aggressiveness, often being ostracized by pigmented conspecifics (Slavík et al., 2015, 2016b), and display higher sensitivity to stress (Slavík et al., 2022) than those with the pigmented phenotype. Data on the behavioral response of albino animals to light alterations during the L/D cycle are rare, and specific information about albino fish is not available.

In our study, we observed the locomotor activity and aggressiveness of albino and pigmented European catfish, *Silurus glanis* (Linnaeus, 1758), during the L/D cycle. We assumed that the locomotor activity of the albino phenotype would occur predominantly during the dark phase of a day and the frequency of aggressiveness would be higher during the light phase, corresponding to increasing avoidance of light and shelter availability. Blood plasma, brain, gill, and liver samples were collected to evaluate stress parameters accompanying aggressive behavior. Our study aimed to compare the behaviors of albino and pigmented phenotypes of catfish in terms of expected differences in aggressiveness during the L/D cycle. We issue a word of caution regarding the use of experimental albino strains of animals for the simulation of behavioral responses of animals in the wild.

MATERIALS AND METHODS

Ethics Statements

All the experimental laboratory procedures complied with valid legislative regulations (Law no. 246/1992, §19, art. 1, letter c), which was derived from the Directive 2010/63/EU; the permit (no. CZ02233) was awarded to O. Slavík, qualified according to Law no. 246/1992, §17, art. 1. All laboratory sampling, including passive integrated transponder (PIT) implantation, was carried out with relevant permission (63479/2016-MZE-17214). The maintenance staff were trained by law in animal care to maintain the high quality of the experiment. The number of experimental animals and all methods used complied with the reduction, replacement, and refinement of animal experimentation. The study did not involve endangered species.

Experimental Animals

The European catfish is a large predator currently widely distributed outside its native range. Albino individuals are observed in the wild, and juveniles are commonly used for laboratory experiments (Dingerkus et al., 1991; Lechner and Ladich, 2011). Two groups of 7-month-old juvenile catfish from the Znojmo hatchery, Czechia, were transported to the laboratory 1 month prior to the start of the experiments. Both groups, the group of pigmented individuals (160 inds) and the group of albino individuals (160 inds), were from different pigmented parents. In the laboratory, experimental animals were further divided into 8 groups (4 albino and 4 pigmented groups), with 40 individuals each, that were kept in separate tanks (380 L each) for 1 month. Fish were placed under general anesthesia (2-phenoxyethanol, water bath concentration 0.2 ml × l^{-1}), tagged with PITs with a unique code and kept under standard conditions. Water was purified using biological filters with an integrated UV sterilizer (Pressure-Flo 5000, Rolf C. Hagen Inc.[1]). Water temperature was controlled automatically using external air conditioning and held at an average of 20°C. Light was controlled to a 12-h day/12-h night regime. Fish were fed *ad libitum* with BioMar pellets (BioMar Group, Denmark[2]) once per day. Once per day, the tank was manually cleaned of unconsumed feed and other solid residuals. Each individual was used only once during the experiment.

Experimental Design

Experiment I: Locomotor Activity in an Experimental Stream Without Shelters

A total of 36 albino (mean weight 41.4 g, range 23–30 g) and 36 pigmented (mean weight 42.8 g, range 28–93 g) randomly selected juvenile catfish were used in this experiment. No significant size differences between the pigmented and albino catfish ($P > 0.4$) were detected. Locomotor activity was measured in a straight artificial stream (260 cm long, 70 cm wide, and 40 cm deep) equipped with a PIT system according to previous measures of catfish activity (Slavík et al., 2016a). The stream was divided into five subunits by using four equidistantly distributed PIT antennae. The antennae (inner area 49 cm × 25 cm) were designed as frames capable of detecting an individual swimming through them. There was no space between the antenna and the wall of the stream as antennas were inserted in a specific partition with a gap corresponding to the size of the antenna in the middle. The antennae were connected to a recorder to store collected data (PIT tag code, date, time and antenna number) in the internal memory. A group of four same-colored fish from the holding tank were released at the same end of the artificial stream and left there to habituate for 24 h. Their locomotor activity, considered throughout the manuscript as a spontaneous movement in the safe environment (Réale et al., 2007; Conrad et al., 2011) was continuously observed for the next 24 h, resulting in nine separate trials for albino catfish and nine separate trials for pigmented catfish (albino and pigmented catfish trials were regularly rotated). The whole experimental stream was cleaned and rinsed thoroughly after each trial. Fish were kept under a photoperiod of 12 h of light/12 h of darkness during the experiment, allowing us to distinguish between the day and night.

Experiment II: Locomotor Activity, Aggressiveness, and Shelter Reassignment in the Experimental Arena With a Shelter

A total of 56 albino (mean weight 28.2 g, range 20–43 g) and 56 pigmented (mean weight 28.3 g, range 12–49 g) randomly selected juvenile catfish were used in this experiment. No significant size differences between the pigmented and albino catfish ($P > 0.85$) were detected. The behavioral features (locomotor activity, aggressiveness, and shelter reassignment) were observed in a square experimental arena (footprint 30 cm × 30 cm) with one tube shelter (5 cm × 15 cm) placed in the middle of the arena. Fish were exposed to the same shelters in holding tanks, and the provision of only one shelter was chosen to stimulate competition for limited resources. Catfish behavior was recorded with a Bosch Dinion IP Starlight automatic digital camera (Bosch Sicherheitssysteme, GmbH, Germany) that is designed to provide clear images at night or under low-light conditions. A group of four same-colored fish from the same holding tank were released into the experimental arena and left there to habituate for 24 h. Their behavioral features were recorded for the next 24 h, resulting in 14 separate trials for albino catfish and 14 separate trials for pigmented catfish (albino and pigmented catfish trials were regularly rotated). The whole experimental arena was cleaned and rinsed thoroughly after each trial. Fish were kept under a specific photoperiod using custom-made programmable lights allowing simulation of 11.5 h of daylight, followed by a 0.5 h dusk, 11.5 h of darkness, and a 0.5 h dawn. Dawn and dusk were simulated as a stepwise increase/decline in light intensity during six 5-min intervals. Fish behavior was automatically recorded during each of these sampling intervals. Another set of equidistantly distributed evenly long 5-min records were collected during the day as well as during darkness, covering the whole 24 h period. As a result, there was an equal number of sampling intervals during every light interval (i.e., day, dusk, darkness,

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[1] www.lagunaponds.com

[2] www.biomar.com

and dawn) to assure better comparability of the light intervals in further analyses.

Experiment III: Physiological Patterns in the Experimental Arena With a Shelter

The experiment followed the same design and used the same conditions as Experiment II, with the aim of observing physiological parameters. During the experiment, we collected blood and tissue (brain, gill, and liver) samples from tested individuals. In total, 32 albino (mean weight 47.2 g, range 25–97 g) and 32 pigmented (mean weight 53.1 g, range 29–86 g) randomly selected juvenile catfish were used in this experiment. No significant size differences were detected between the pigmented and albino catfish ($P > 0.15$). A group of four same-colored fish from the same holding tank were released into the experimental arena and left there to habituate for 24 h. Blood and tissues were sampled during the following 24 h in eight equidistantly distributed sampling intervals (three during daytime, one at dusk, three during darkness, and one at dawn). All four individuals in a group were sampled at the same time within each trial and the trial was terminated after sampling, resulting in eight trials/intervals of four individuals for albino catfish and eight trials/intervals of four individuals for pigmented catfish (albino and pigmented catfish trials were regularly rotated). The whole experimental arena was cleaned and rinsed thoroughly after every trial. Catfish blood was sampled from the *vena caudalis* using a heparinized syringe (heparin inj., Léčiva, Czechia) according to the unified methods for the hematological examination of fish (Borges et al., 2007; Sudová et al., 2009). For biochemical plasma analysis, blood was separated *via* centrifugation at 12,000 × *g* for 10 min at 4°C, and plasma samples were stored at −80°C until analysis. Cortisol, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium (Ca), phosphorus (P), glucose (GLU), magnesium (Mg), triglyceride (TRIG), creatine kinase (CK), lactate (LAC), amylase (AMYL), lipase (LIPA), albumin (ALB) and globulin (GLOB) concentrations were measured using a VETTEST 8008 analyzer (IDEXX Laboratories Inc., Westbrook, ME, United States; Gallardo et al., 2010; Li et al., 2011). After blood sampling, fish were euthanized by severing the spinal cord, and samples of the brain, gills and liver were taken for an evaluation of oxidative stress. Samples for evaluation of oxidative stress biomarkers and antioxidant enzymes were homogenized and prepared for analysis according to Stara et al. (2013). Thiobarbituric acid reactive substances (TBARSs) were estimated according to the method of Lushchak et al. (2005), while total superoxide dismutase (SOD) activity was estimated by the method of Marklund and Marklund (1974). Catalase (CAT) activity was assayed using the method of Beers and Sizer (1952). Reduced glutathione (GSH) levels were assayed using the methods of Sedlak and Lindsay (1968) with the modification reported by Ferrari et al. (2007). The total catalytic concentration of glutathione-*S*-transferase (GST) was determined according to Habig et al. (1974). Protein levels were estimated spectrophotometrically by the method of Bradford (1976), using bovine serum albumin as a standard.

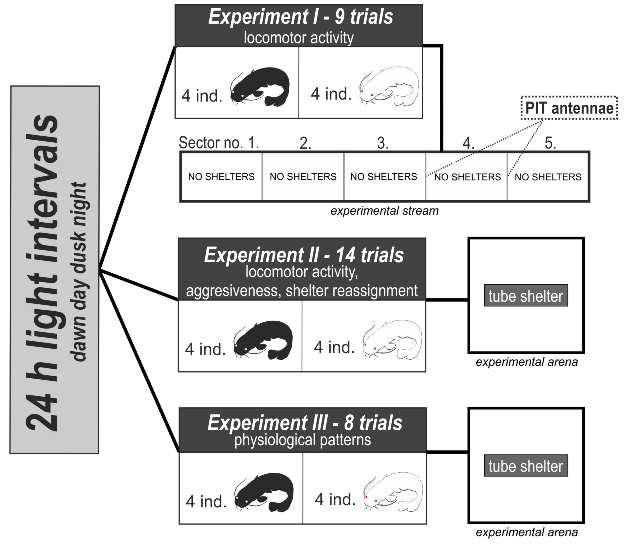

FIGURE 1 | Illustrative figure of the experimental design.

Data Analyses

Data from all experiments (**Figure 1**) were analyzed using the light intervals approach, distinguishing for ecologically relevant light levels across 24 h (e.g., Slavík et al., 2007), to facilitate comparability and mutual interpretation of various datasets. The datasets generated for this study can be found in the Dryad repository (Valchářová et al., 2022).

Experiment I

Over 160,000 raw data points were obtained during the artificial stream experiment. The "locomotor activity" of each fish was calculated as the number of artificial stream units passed within the 1-min time grid according to the activity measures of juvenile chub (Horký et al., 2014; Hubená et al., 2020) or catfish (Slavík et al., 2016a). All the values were subsequently grouped into four equidistantly distributed sampling intervals (two during the day and two during night) with the mean individual locomotor activity values. One pigmented catfish trial was not included in the final dataset due to technical difficulties, resulting in a total of eight pigmented and nine albino catfish trials.

Experiment II

Aggressive interactions that were accounted for included biting, chasing and frontal and lateral displays as described for catfish by Slavík et al. (2016b). The sum of all aggressive interactions within each group of four individuals, referred to as "aggressiveness," was used in further analyses. Altogether, 724 aggressive interactions were detected. Locomotor activity was defined as the total number of all movements of the four individuals in a group. Altogether, 7246 movements were detected. "Shelter reassignment" was defined as the total number of reassignments of shelters within a group. Altogether, 131 shelter reassignments were recorded. Diel behavioral patterns were analyzed across four light intervals (dawn, day, dusk, and night). One albino catfish trial was not included in the final dataset due to technical difficulties, resulting in a total of 14 pigmented and 13 albino catfish trials.

Experiment III

Each physiological sample was successfully analyzed, resulting in 64 data points in the final dataset used to analyze diel physiological patterns across four light intervals (dawn, day, dusk, and night). Data from Experiments II and III were consequently merged to evaluate the links between behavioral and biochemical indices. For the purpose of this complex analysis, the "aggressiveness" and "shelter reassignment" variables were summed to form a single variable, "agonistic interactions," including the total number of all recorded agonistic interactions per minute. The mean values of biochemical indices obtained during Experiment III were assigned corresponding values of agonistic interactions from Experiment II.

Statistical Analysis

The statistical analyses were performed using SAS software (SAS Institute Inc., version 9.4[3]). The data from Experiments I to III were analyzed using separate mixed models with appropriate distributions (continuous variables – normal distribution; count variables – Poisson distribution) and random factors. The data were transformed for normality (logarithmic or square-root transformation) prior to analyses when needed. The random factors were used to account for the repeated measures collected for the same experimental units throughout the experiment. The significance of the explanatory variables was assessed using *F* tests. The differences between classes were examined with *t* tests, and Tukey–Kramer adjustment was used for multiple comparisons. The degrees of freedom were calculated using the Kenward–Roger method (Kenward and Roger, 1997).

The links between behavioral and biochemical indices were modeled using R software (R Core Team, 2019). Several different modeling approaches were applied, and a sine profile was found to have the best fit for the overall diel rhythmicity of agonistic interactions. The generalized linear model (GLM) regression framework was then applied, and a model for Poisson data with a logarithmic link was employed. The random effects were included to properly account for the dependence within the experiments and the variability between them. The sine baseline profile was included in the model at first, and the other covariates were added in a forward stepwise manner.

RESULTS

The diel rhythms of the behavioral and biochemical indices generally varied in pigmented and albino catfish (**Tables 1**, **2**). The locomotor activity in the artificial stream without shelters showed no rhythmicity in albino catfish, while pigmented catfish showed a peak during the nocturnal phase of the day (**Figure 2**). When subjected to limited shelter availability in the experimental arena, pigmented and albino catfish showed similar diel rhythmicities in locomotor activity (**Figures 3A,B**), aggressiveness (**Figures 3C,D**), and shelter reassignment (**Figures 3E,F**). The peak of locomotor activity occurred at night. High-level locomotor activity was also maintained during dawn together with the peak of aggressiveness and shelter reassignment. A nocturnal peak in locomotor activity indicates natural activity patterns, while a dawn peak reflects competition for limited resources (shelter) with the aim of avoiding daylight conditions. The avoidance of daylight was more pronounced in albino catfish, as the presence of a shelter induced their switch to diel rhythmicity, and their stress from daylight was apparent from the peaks in blood cortisol (**Figure 4A**), glucose (**Figure 4B**), and lactate (**Figure 4C**) levels. In contrast, pigmented catfish showed no diel rhythmicity in blood cortisol, glucose, or lactate level. Analogously, significant diel rhythmicity was detected in 11 of the 15 measured blood biochemical indices in albino catfish, while pigmented catfish showed rhythmicity in none of them (**Table 1**). Among the 4 antioxidants and 1 oxidative stress indicator detected in 3 tissues (brain, gill, and liver), with 15 indices in all, albino catfish showed significant diel rhythmicity in 8 of them, whereas pigmented catfish showed rhythmicity in 5 of them (**Table 2**). Peaks of antioxidants in the brain (SOD – **Figure 5A**; CAT – **Figure 5B**) or liver (SOD, GST, and GSH) of albino catfish were detected during the day, suggesting their relation to daylight stress. On the other hand, antioxidants (GST and GSH) in the gills of albino catfish peaked during dawn, suggesting their relation to the increase of aggressiveness and shelter reassignment. The antioxidants in the liver (SOD) and brain (CAT – **Figure 5C**) of pigmented catfish peaked at night, suggesting their relation to increased activity, while the antioxidants in gills peaked during the day (SOD) or at dusk (CAT). The oxidative stress indicator TBARS peaked during dusk in both catfish groups; however, in albino catfish, a significant trend was found in the liver, while in pigmented catfish, a significant trend was found in the gills.

A complex model was fitted with the aim of evaluating the links between behavioral and biochemical indices. Time rhythmicity was modeled using a sine wave and confirmed a parallel course of agonistic interactions in both catfish groups ($P < 0.001$; **Figure 6**), with the peak during dawn associated with a 4.08-fold (conf. int. 3.53–4.7) increase. The effect of SOD concentration in the brain was significant only in pigmented catfish ($P < 0.01$), in which a one unit increase in SOD (i.e., 10^{-2} nmol NBT/min/mg protein) was related to a 7% decrease in agonistic interactions. The effect of glucose was significant, but opposite effects were observed in pigmented ($P < 0.001$) and albino ($P < 0.001$) catfish. An increase in glucose by one unit (i.e., 10^{-1} mmol/L) was associated with a 14% decrease in agonistic interactions in albino catfish, while in pigmented catfish, agonistic interactions increased by 342%. The effects of blood cortisol, lactate, and catalase in the brain were significant ($P < 0.001$) but not influenced by catfish pigmentation. Thus, an increase in cortisol by one unit (i.e., 10^{-1} ng/ml) caused an increase in agonistic interactions of 177%, while an increase in lactate or catalase in the brain by one unit (i.e., 10^{-1} ng/ml; resp. 10^{-2} μmol H_2O_2/min/mg protein) was related to a 16% (LAC) or 39% (CAT) decrease in agonistic interactions in both catfish groups.

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[3] www.sas.com

TABLE 1 | Diel rhythms of behavioral and biochemical indices.

Variable	Pigment	Significance	Dawn	Day	Dusk	Night
Activity	Albino	NS				
	Pigmented	$F_{1,56} = 18.92$, $P < 0.0001$	NA	*	NA	Peak*
Activity-shelter	**Albino**	$F_{3,518} = 45.21$, $P < 0.0001$		*	*	Peak*
	Pigmented	$F_{3,501} = 67.26$, $P < 0.0001$		*	*	Peak*
Aggressiveness	**Albino**	$F_{3,518} = 16.46$, $P < 0.0001$	Peak*	*	*	*
	Pigmented	$F_{3,501} = 21.03$, $P < 0.0001$	Peak*	*	*	*
Shelter reassignment	**Albino**	$F_{3,518} = 10.90$, $P < 0.0001$	Peak*	*	*	*
	Pigmented	$F_{3,501} = 28.24$, $P < 0.0001$	Peak*	*	*	*
Cortisol	**Albino**	$F_{3,32} = 82.06$, $P < 0.0001$	*	Peak*		*
	Pigmented	NS				
ALP	**Albino**	$F_{3,32} = 3.94$, $P < 0.0169$	Peak*			*
	Pigmented	NS				
ALT	Albino	NS				
	Pigmented	NS				
AST	**Albino**	$F_{3,32} = 3.38$, $P < 0.03$		*		Peak*
	Pigmented	NS				
Ca	**Albino**	$F_{3,32} = 20.41$, $P < 0.0001$	*	Peak*		*
	Pigmented	NS				
P	**Albino**	$F_{3,32} = 6.69$, $P < 0.0012$		*		Peak*
	Pigmented	NS				
GLU	**Albino**	$F_{3,32} = 50.89$, $P < 0.0001$	*	Peak*	*	*
	Pigmented	NS				
Mg	Albino	NS				
	Pigmented	NS				
TRIG	**Albino**	$F_{3,32} = 25.00$, $P < 0.0001$	*	Peak*	*	*
	Pigmented	NS				
CK	Albino	NS				
	Pigmented	NS				
LAC	**Albino**	$F_{3,32} = 35.07$, $P < 0.0001$	*	Peak*		*
	Pigmented	NS				
AMYL	**Albino**	$F_{3,32} = 28.82$, $P < 0.0001$	*	Peak*		*
	Pigmented	NS				
LIPA	**Albino**	$F_{3,32} = 16.17$, $P < 0.0001$		Peak*		*
	Pigmented	NS				
ALB	Albino	NS				
	Pigmented	NS				
GLOB	**Albino**	$F_{3,9.62} = 8.09$, $P < 0.0055$		Peak*		*
	Pigmented	NS				

*Variability of variables across the light intervals in pigmented and albino catfish: significant variability (highlighted as bold; statistics provided) non-significant variability (noted as NS). Peaks of variables and significant differences among intervals (marked as *; adj. $P < 0.05$).*

DISCUSSION

Albinism is associated with higher light sensitivity and photoreceptor degradation (Prusky et al., 2002; Vihtelic et al., 2006) with behavioral repercussions (Ren et al., 2002). For example, increased sensitivity to sunlight was reported for albino Caribbean Sea urchins, *Tripneustes ventricosus* (Lamarck, 1816), which tend to cover themselves with plants more than pigmented individuals (Kehas et al., 2005). In albino rodents, avoidance of light and lower locomotor activity during daylight are generally known behavioral characteristics (Fuller, 1967; Defries, 1969; Stryjek et al., 2013), but information about the physiological processes underlying photophobia is rare, e.g., in the albino cave tetra *Astyanax mexicanus* (De Filippi, 1853), higher sensitivity to stress is associated with mutation of the oca2 gene and catecholamine synthesis (Kvetnansky et al., 2009; Bilandžija et al., 2013). In our study, we observed differences in behavior between albino and pigmented European catfish, *S. glanis*, during the L/D cycle influenced by the complexity of the environment. These differences were accompanied by changes in physiological parameters in blood plasma and the brain, gill, and liver. In nature, the locomotor activity of pigmented *S. glanis* follows the L/D cycle, being mostly nocturnal; however, it is highly variable across seasons (Slavík et al., 2007). Moreover, the

TABLE 2 | Diel rhythms of antioxidants and oxidative stress indicators detected in fish tissues (brain, gill, and liver).

Variable	Pigment	Significance	Dawn	Day	Dusk	Night
SOD – brain	**Albino**	$F_{3,32} = 7.11, P < 0.0009$		Peak*		*
	Pigmented	NS				
SOD – gill	Albino	NS				
	Pigmented	$F_{3,32} = 13.08, P < 0.0001$	*	Peak*	*	*
SOD – liver	**Albino**	$F_{3,32} = 4.27, P < 0.0001$		Peak*	*	
	Pigmented	$F_{3,32} = 6.69, P < 0.0012$		*		Peak*
CAT – brain	**Albino**	$F_{3,32} = 8.27, P < 0.0003$		Peak*	*	*
	Pigmented	$F_{3,32} = 4.82, P < 0.0070$		*	*	Peak*
CAT – gill	Albino	NS				
	Pigmented	$F_{3,32} = 3.66, P < 0.0226$		*	Peak*	
CAT – liver	Albino	NS				
	Pigmented	NS				
GST – brain	Albino	NS				
	Pigmented	NS				
GST – gill	**Albino**	$F_{3,32} = 6.75, P < 0.0012$	Peak*		*	*
	Pigmented	NS				
GST – liver	**Albino**	$F_{3,32} = 3.14, P < 0.0387$		Peak*		*
	Pigmented	NS				
GSH – brain	Albino	NS				
	Pigmented	NS				
GSH – gill	**Albino**	$F_{3,32} = 7.23, P < 0.0008$	Peak*	*	*	*
	Pigmented	NS				
GSH – liver	**Albino**	$F_{3,32} = 7.02, P < 0.0009$		Peak*	*	*
	Pigmented	NS				
TBARS – brain	Albino	NS				
	Pigmented	NS				
TBARS – gill	Albino	NS				
	Pigmented	$F_{3,32} = 4.73, P < 0.0076$			Peak*	*
TBARS – liver	**Albino**	$F_{3,32} = 4.76, P < 0.0075$	*		Peak*	
	Pigmented	NS				

*Variability of variables across the light intervals in pigmented and albino catfish: significant variability (highlighted as bold; statistics provided), non-significant variability (noted as NS). Peaks of variables and significant differences among intervals (marked as *; adj. $P < 0.05$).*

activity of *S. glanis* is highly individualistic; some individuals show nocturnal activity, some show daylight activity, and some do not prefer any phase of the L/D cycle (Slavík and Horký, 2012), as commonly reported for other fish species (Madrid et al., 2001; Vera et al., 2009; Sánchez-Vázquez et al., 2019). Under experimental conditions, the locomotor activity and food intake of *S. glanis* during the L/D cycle are affected by the social environment; for example, individually bred fish are arrhythmic, while grouped fish are rhythmic (Boujard, 1995).

Rhythmicity of Locomotor Activity in the Experimental Stream Without Shelters

In our study, the locomotor activity of albino catfish in the environment without shelters was arrhythmic, while the activity of pigmented catfish was rhythmic. Animals use shelters to avoid risks from predators or adverse conditions (Álvarez and Nicieza, 2003; Weiss et al., 2008; Larranaga and Steingrímsson, 2015), and under laboratory conditions, *S. glanis* prefers an environment with shelters (Slavík et al., 2012). Behavioral rhythmicity during the diel cycle can be influenced by stressors (Sánchez-Vázquez et al., 2019), and the absence of shelters during our experiment represents such a stressor for the albino phenotype. For example, the lack of availability of shelters stressed the freshwater nocturnal predator burbot, *Lota lota* (Linnaeus, 1758), during daylight even when its predators were not present (Fischer, 2000). Hence, the explanation for why the pigmented phenotype displayed rhythmicity in locomotor activity while the albino phenotype did not is presumably related to their different sensitivities to stress induced by the lack of availability of shelters.

Rhythmicity of Locomotor Activity and Stress Parameters in the Experimental Arena With a Shelter

In the environment with a shelter, the locomotor activity of both the pigmented and albino phenotypes was rhythmic, with the peak during the dark, as observed for aggressiveness and shelter reassignment. Correspondingly, higher locomotor activity is associated with an increase in aggressiveness (Huntingford et al., 2012; Manuel et al., 2016), manifesting as an increasing

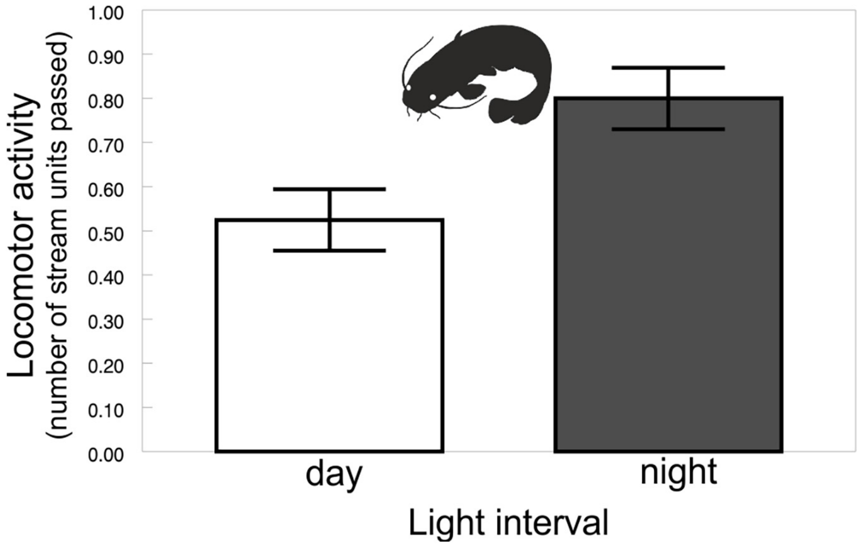

FIGURE 2 | Pigmented catfish locomotor activity in the experimental stream without shelters across light intervals. Values (±SEs) are predicted from the mixed model (i.e., presented data are after the adjustment by the statistical model).

frequency of changes in shelter use by *S. glanis* in our study. Analyses of biochemical indices from blood plasma, however, showed rhythmicity of stress parameters only in albino catfish. Circadian oscillation of cortisol levels in fish is species-specific and variable during the L/D cycle (Pickering and Pottinger, 1983; Lankford et al., 2003; López-Olmeda et al., 2009). Nocturnal species, e.g., the Senegalese sole *Solea senegalensis* (Kaup, 1858), show higher values at the end of the day (Oliveira et al., 2013). Conversely, a species with daylight activity, the green sturgeon *Acipenser medirostris* (Ayres, 1854), exposed to stressful conditions displayed an increased level of plasma cortisol with peak at night (Lankford et al., 2003). Stressed individuals of the sea bream *Sparus aurata* (Linnaeus, 1758) showed diurnal rhythmicity of cortisol with an increase at night, while unstressed conspecifics showed no rhythmicity (Vera et al., 2014), which corresponds with the differences between albino and pigmented *S. glanis* found in our study. We recorded rhythmicity, e.g., in cortisol, glucose and lactate, only in albino catfish with the peak at night and dawn. In fish, the diel rhythmicity of lactate is apparent (Lankford et al., 2003; Polakof et al., 2007), while that of glucose is not necessarily obvious (Lankford et al., 2003; López-Olmeda et al., 2009; Oliveira et al., 2013). Cortisol pronouncedly contributes to an increase in glucose via gluconeogenesis (Pankhurst, 2011), and anaerobic glycolysis during intense exercise leads to the increased production of lactate and its secretion into plasma (Begg and Pankhurst, 2004; Pankhurst, 2011). All three parameters have been used to determine stress (Øverli et al., 1999; Sloman et al., 2001; Begg and Pankhurst, 2004; Costas et al., 2011) in fish. Locomotor activity during the L/D cycle, production of hormones, e.g., cortisol, and expression of genes for biological rhythmicity are linked and can be assigned to fish personality, showing high rhythmicity in proactive individuals and no/low rhythmicity in reactive conspecifics (Tudorache et al., 2018). Albino individuals can be considered reactive, displaying lower aggressiveness (Slavík et al., 2016b), higher stress in response to variability in the physical and social environments (Slavík et al., 2022) and higher light-induced stress, including associated rhythmicity in stress parameters, as observed during this study. The higher stress induced by allocation and defense of resources, e.g., shelters, can be considered a key reason for the rarity of albino individuals in nature, also taking into account the previously considered significant influence of predation on their abundance (Ellegren et al., 1997), which does not seem to be as apparent (Stephenson et al., 2021) as previously expected.

Rhythmicity of Oxidative Stress in the Experimental Arena With a Shelter

Oxidative stress is associated with hormonal production during the stress response. Increased production of free radicals occurs with an increase in catecholamine (Smythies and Galzigna, 1998) and/or cortisol and glucose correspond to higher energy production (McIntosh et al., 1998; Liu and Mori, 1999). The variability in values of some antioxidants can be used as an indicator of oxidative stress. For example, Costantini et al. (2008) observed an increased antioxidant serum capacity in a group of mice characterized as reactive, showing lower levels of aggressiveness and shyness and higher sensitivity to stress, while in a group of proactive individuals usually displaying more aggression and a lower stress response, the antioxidative capacity was lower. The increased antioxidant capacity of reactive individuals can be considered a strategy for managing high levels of stress. For example, Almeida et al. (2009) reported higher CAT and SOD activities in dominant individuals of the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) than in submissive conspecifics, indicating the influence of the social environment on the oxidative stress level. In our study, the evaluation of

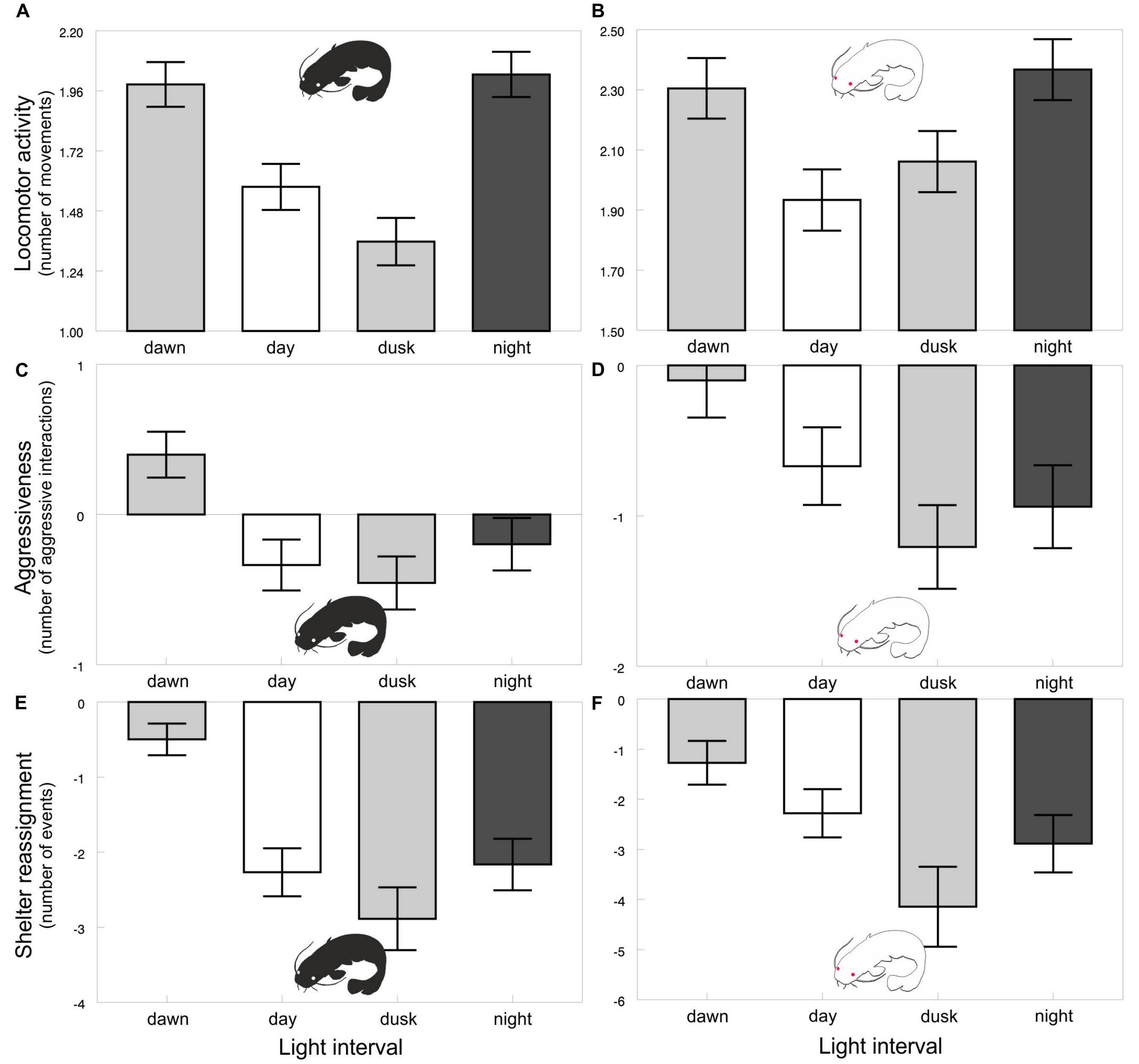

FIGURE 3 | Locomotor activity of pigmented **(A)** and albino **(B)** catfish, aggressiveness of pigmented **(C)** and albino **(D)** catfish and shelter reassignment of pigmented **(E)** and albino **(F)** catfish in the arena with a shelter across light intervals. Values (±SEs) of behavioral indices are predicted from the mixed models (i.e., presented data are after the adjustment by the statistical model).

4 antioxidants and 1 oxidative stress indicator in the tissues (brain, gill, and liver), representing a total of 15 indices, revealed significant rhythmicity for 8 indices in albino catfish, whereas pigmented catfish showed significant rhythmicity for only 5 indices. In albino catfish, the maximal level of antioxidants in the brain and liver was detected during daylight, while for gills, it was detected during dawn. This suggests that the light-induced stress from daylight was accompanied by an increase in fish aggressiveness during the effort of conspecifics to find shelters. In contrast, in the pigmented phenotype, the antioxidant levels in the brain and liver reached a maximum during dusk and at night, indicating their nocturnal activity. An increase in oxidative stress in fish with increasing light intensity (Giannetto et al., 2014; Kim et al., 2016) has been reported. For example, Sreejith et al. (2007) reported an increase in CAT and SOD activities in climbing perch, *Anabas testudineus* (Bloch, 1792), exposed to a continuous illumination photoperiod and a decrease in activity and glutathione content after melatonin treatment. These results suggested higher stress in albino individuals than in pigmented conspecifics during the light phase of the day.

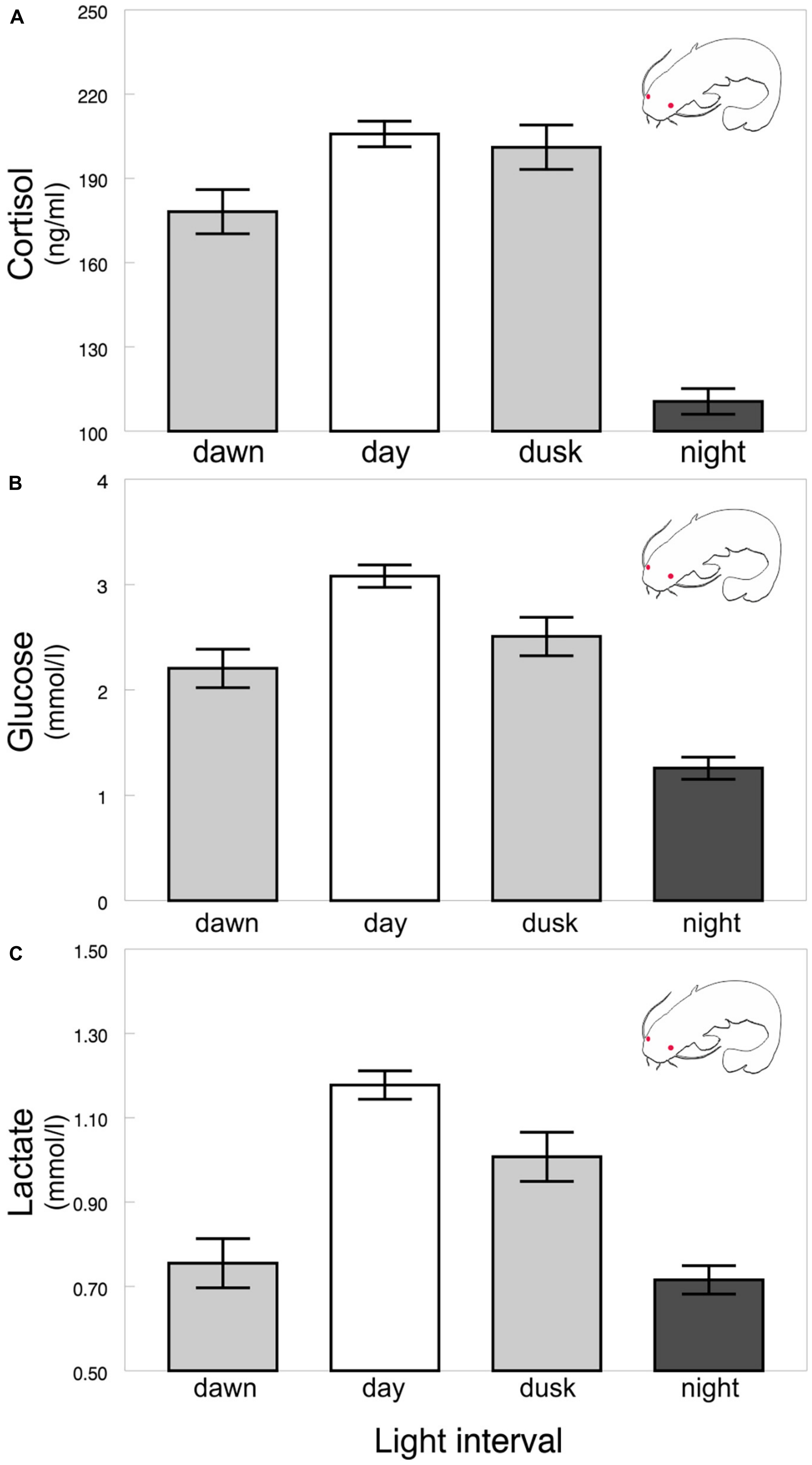

FIGURE 4 | Albino catfish blood cortisol **(A)**, glucose **(B)**, and lactate **(C)** levels across light intervals. Values (±SEs) of biochemical indices are predicted from the mixed models (i.e., presented data are after the adjustment by the statistical model).

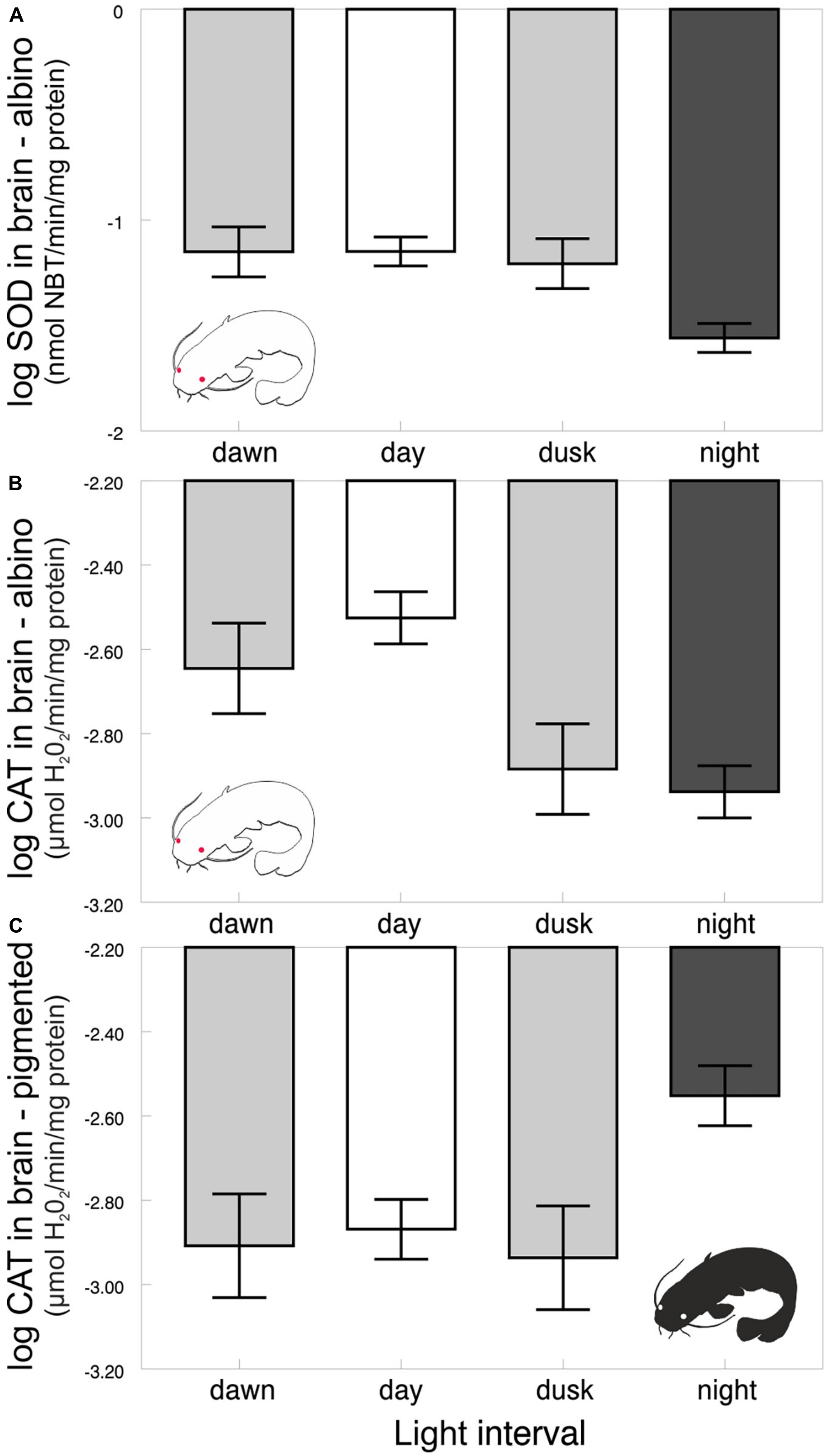

FIGURE 5 | Levels of antioxidants in brain of albino (SOD – **A**; CAT – **B**) and pigmented (CAT – **C**) catfish across light intervals. Values (±SEs) of biochemical indices are predicted from the mixed models (i.e., presented data are after the adjustment by the statistical model).

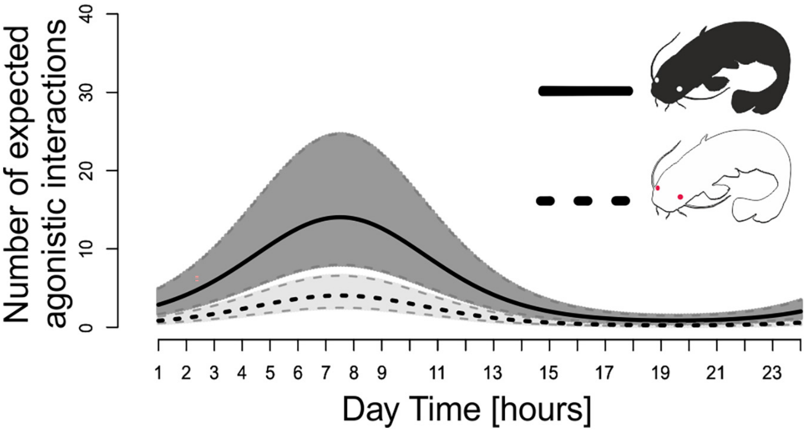

FIGURE 6 | Number of expected agonistic interactions related to sampling interval of albino (dashed black line) and pigmented (solid black line) catfish throughout the diel cycle. Predicted lines are displayed with gray 95% pointwise confidence bands.

Complex Model for Light/Dark Rhythmicity of Aggressiveness and Stress Parameters

Aggressiveness is influenced by L/D rhythmicity during a diel cycle (Hood and Amir, 2018). Hence, in our study, a complex statistical model was prepared to analyze aggressiveness, the biochemical indices from blood plasma, the level of oxidative stress and L/D rhythmicity. For the analyses of oxidative stress, brain samples with the closest relation to behavioral and stress patterns were used. For both phenotypes, the model displayed a sinusoid course with the peak of agonistic interactions during dawn. Both phenotypes showed predominantly nocturnal activity; hence, the importance of shelters increased during dawn. Sufficient shelter availability reduces overall aggressiveness (Höjesjö et al., 2004; Kobler et al., 2011), while a limited number of shelters encourages aggressive behavior (Mikheev et al., 2005). The effects of glucose and SOD varied with phenotype, while the effects of cortisol, lactate and catalase did not. An increase in SOD for only the pigmented phenotype manifested as a small decrease in aggressiveness, shows that oxidative stress accompanies the behavioral response of social interactions (Costantini et al., 2008; Almeida et al., 2009). Interestingly, a one-unit increase in glucose in the albino phenotype was associated with a 14% decrease in aggressiveness, while in the pigmented phenotype, a one-unit increase in glucose accompanied a 342% increase in aggressiveness. These apparent differences demonstrate physiological and behavioral variation between albino and pigmented phenotypes. The correlation between glucose level and aggressiveness (McIntosh et al., 1998; Liu and Mori, 1999) suggests that agonistic interactions are more exhausting for albino individuals than for pigmented conspecifics, indicating different behavioral strategies between the phenotypes characterized by an extreme increase in aggressiveness in the pigmented phenotype in response to glucose increase. This difference can be considered the reason why aggressive interactions are rarely observed in individuals with the albino phenotype (Slavík et al., 2016b). Hence, we can assume that light intensity was the key factor influencing the different levels of aggressiveness and stress indices between the two phenotypes.

CONCLUSION

Our results suggested that melanin absence is associated with the typical behavioral performance of the albino phenotype during the L/D cycle and showed more stress in the albino phenotype under light conditions than in pigmented conspecifics. Rare albino individuals observed in nature display different behavioral and physiological responses under experimental laboratory conditions compared to those of pigmented individuals, which show a lower stress response and behavioral patterns approaching those observed during ecological studies in natural conditions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in Dryad repository (Valchářová et al., 2022).

ETHICS STATEMENT

The animal study was reviewed and approved by the Ministry of Agriculture of the Czech Republic (63479/2016-MZE-17214).

AUTHOR CONTRIBUTIONS

OS and PH conceptualized the research. PH, JV, MM, and TV managed the data curation. JV and AS conducted the biochemical analyses. PH, IH, MM, and MP conducted the statistical analyses. OS, JV, AS, PH, and TV conducted the investigation and sampling in the laboratory. OS, PH, JV, and AS developed the methodological approach. TV, OS, and PH wrote the manuscript with input from all authors.

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