Phototactic behaviour of subterranean Copionodontinae Pinna, 1992 catfishes (Siluriformes, Trichomycteridae) from Chapada Diamantina, central Bahia, northeastern Brazil

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Abstract: The phototactic behaviour of three Copionodontinae (Trichomycteridae) catfish species (two troglobites and one epigean) from Chapada Diamantina was studied in order to detect modifications related to isolation in the subterranean environment. Differences in response under different luminosities were detected and, unlike other cavefish, Copionodontinae cave species have shown to be more photophobic than the epigean syntopic to them. The troglobitic Glaphyropoma spinosum is the most photophobic, presenting this behaviour under all light intensities, and more homogeneous regarding morphological characters. It suggests that this population is probably isolated for a longer time in the subterranean environment compared to Copionodon sp. n., the other cave species, which is only photophobic under low light intensities. The indifference to light exhibited by the epigean species C. pecten could be an answer to a recent predation pressure, an ecological aspect, and perhaps this character-state is under fixation in this population. There are also evidences that the skin has a relevant role in the perception of light for the Copionodontinae species.

Keywords: reaction to light; photophobia; cave catfish; Glaphyropoma; Copionodon

Received 10 April 2012; Revised 7 August 2012; Accepted 26 November 2012

INTRODUCTION

Most troglobite specializations involve character regression, frequently assigned to the loss of function or to the relaxing of stabilizing selective pressure (Wilkens, 1992). Besides the classic troglomorphisms (changes in morphological characters), like eye and melanin pigmentation regressions until total loss of these characters, there are also regressions of some behaviour patterns (Parzefall & Trajano, 2010). Therefore, subterranean organisms are good models for studies of ecology/behaviour and evolution (Trajano & Bockmann, 1999).

Reaction to light represents the best studied behaviour on subterranean organisms and, considering the fishes, at least ten families were studied regarding this aspect (Characidae - genus Astyanax Baird & Girard 1854, Cyprinidae, Balitoridae, Heptapteridae, Trichomycteridae, Loricariidae, Claridae, Amblyopsidae, Bythitidae, and Poeciliidae), with no taxonomic evidence in the obtained results (e.g., Trajano & Gerhard, 1997; Parzefall, 1998; Trajano & Borowsky, 2003; Parzefall & Trajano, 2010). According to Trajano & Gerhard (1997) the phototactic behaviour displayed by some troglobite catfishes may vary in the same species according to the stimulus context, i.e., between controlled tests with choice chamber in the laboratory and sudden illumination in the natural environment. Therefore, the responses are part of different behavioural traits, displayed in different functional contexts and possibly following independent evolutionary paths (Trajano & Gerhard, 1997).

There are around 170 species of subterranean fishes in the world and 25 of them occur in Brazil (15% of the total richness) (Proudlove, 2010; G. Proudlove, pers. comm.). Most of Brazilian subterranean fishes belong to the Order Siluriformes: catfishes (Trichomycteridae, Heptapteridae and Calllichthyidae families) and armoured catfishes (Family Loricariidae) (Proudlove, 2010). Considering the Trichomycteridae catfishes, the subfamily Copionodontinae Pinna, 1992 is endemic of Chapada Diamantina, central Bahia, and nowadays there are records of two genera.
and five species in the region, two of them occurring and coexisting (syntopic) in the sandstone/quartzitic caves of Chapada Diamantina, showing variability in the trinomorphic characters (regression of eyes and melanic pigmentation): *Glaphyropoma spinosum* Bichuette, Pinna & Trajano, 2008 and *Copionodon* sp., this latter represents a new species, under description (M.E. Bichuette, in prep.).

Compared to other trichomicterids, copionodontines show plesiomorphic condition of many morphological character-states, shared with its possible sister group *Trichogenes* Britski & Ortega 1983 (for instance, the complete infraorbital latero-sensory canal), and a unique primitive condition that suggests its basal position within Trichomycteridae (the more anteriorly located dorsal fin) (Pinna, 1992). Many of these conditions are relevant to understand the relationships among other families of Siluriformes and also within the Loricarioidea group (Pinna, 1997).


A huge variation is observed in the phototactic behaviour of Brazilian subterranean fishes: many species exhibit from a photophobia to a tendency to photofilia, with cases of indifference to light [e.g., *Taunayia* sp. (= *Rhamdiopsis* sp. 2 - Trajano & Gerhard, 1997)]. Moreover, a variation in the response to light is observed for intraspecific and interspecific contexts (populational and individual) and the variability of this behaviour is related to variation of morphological characters, for example, eyes and melanic pigmentation (e.g., for the armoured catfishes *Ancistrus* spp. and *Ituglanis* catfishes - Bessa & Trajano, 2001; Bichuette, 2003); and to the developing life cycle (Secutti & Trajano, 2009). When compared to the epigean relatives (in the case of known and/or existing species), some tendencies are observed, for example, the epigean fish show marked photophobic habits compared to the troglobitic ones (Langecker, 1992; Trajano, 2003).

Langecker (1992) proposed that the maintenance of photophobia in the troglobitic species could be a relictual characteristic, being a plesiomorphic character-state. However, to polarize this character, comparing the troglobitic species with at least two other epigean taxa is necessary (Maddison et al., 1984).

Following this thought, Poulson (1964) and Romero (1985) consider that the maintenance of photophobia in troglobitic fishes is a relict of the epigean existence and not an adaptation to the cave life.

In this study the reaction to light was analyzed in laboratory, and eventual observations were made in the natural environment, in order to detect modifications related to the isolation in the subterranean environment. Copionodontinae catfishes probably represent a basal group in the phylogeny of trichomycterid (Pinna, 1992) and the study of behaviour traits can provide valuable information to corroborate the relationships within Trichomycteridae. Thus, we compare the response to light in three copionodontine species from the same region: *Glaphyropoma spinosum* and *Copionodon* new species (both troglobites) and the epigean species *Copionodon pecten*.

**MATERIALS AND METHODS**

**Study area**

Chapada Diamantina region (Fig. 1) is an extension of Serra do Espinhaço and its relief is composed of hills, mountains and subterranean galleries formed.
by metasedimentary rocks from the Paraguaçu and Chapada Diamantina groups. These rocks were deposited one billion years ago and the dominant lithologies are sandstone, siltstones, argillites, conglomerates and pelites (Schobbenhaus et al., 1999). The altitudes vary from 1,000 to 1,700 m, representing a watershed between the São Francisco and the East Basins. The climate is tropical semi-arid, with distinct wet/dry periods, Aw according to Köppen’s (1948) classification. Medium annual temperature is around 20°C (Nimer, 1989).

**Studied species**

The troglobitic copionodontines (G. spinosum and Copionodon new species) occur in the following sandstone/quartzite caves: Cobras cave, Paredes Vermelha cave, Morro de Alvo System, Rio dos Pombos cave, Torras System (Torras I e II caves), Criminoso cave and Lobo cave. These caves are located inside the Chapada Diamantina National Park. The epigean species, C. pecten, occurs in the rivers Coisa Boa, Xavier and Paraguaçu, in the boundaries of the cave localities. This epigean species was also recorded inside some of the caves where the troglobites occur, showing an interesting case of syntopy.

In natural environment, G. spinosum presents eyes and pigmentation regression (showing iridescence), with little variability in these characters, configuring a homogeneous population (Bichuette et al., 2008 – Fig. 2). Considering Copionodon new species, a higher morphological variability is observed, with not so pronounced regression of eyes (from reduced to normal eyes) and a mosaic of characters concerning pigmentation, varying from pale yellow to light gray, in its natural habitat (Fig. 3). The epigean species C. pecten is homogeneous, showing developed black eyes and typically light brown coloration, with dark and iridescent lines in the body sides (Fig. 4).

**Specimens collecting and laboratory maintenance**

Copionodontinae catfishes were captured with hand nets and transported in three liter gallons, conditioned in thermal boxes, with low temperature achieved with addition of recycled hipergel.

Intraspecific groups up to 10 individuals are maintained in 50-liter aquaria with continuous filtration, aeration and available shelters. The aquaria are installed at the Laboratório de Estudos Subterrâneos (LES) at the Departamento de Ecologia e Biologia Evolutiva of Universidade Federal de São Carlos (UFSCar), in permanent darkness (except during maintenance activities). The epigean Copionodontinae are also maintained in the LES, but in a separate compartment, covered with a black shield, where a lamp attached to a timer simulates the day phase (12 hours per day). The catfishes have been fed once a week with commercial food for carnivorous fishes (dry or frozen Artemia salina) at no fixed days and times.

**Reaction to light tests**

The choice-chamber method was used in the tests of light reaction, as in the study of other troglobitic catfishes (Langecker, 1992; Trajano & Souza, 1994; Trajano & Gerhard, 1997; Bichuette, 2003; Timmermann & Plath, 2009), which simulates the interface between epigean and subterranean environments (Trajano & Gerhard, 1997). The tests were performed one month after the catfishes arrived in the laboratory.

The test aquarium consisted in a 50-liter tank, divided in two sectors by a black plastic screen with openings in the middle and at the sides. One of these sectors was illuminated by a white light bulb and the other remained dark, covered with a black cardboard. The plastic screen prevented the entrance of light, but did not block the movements of the catfishes across the aquarium floor and through the walls. We studied the fishes reaction to light under 40 lux (twilight), 170 lux (beginning of twilight) and 1,700 lux (cloudy day), using a white fluorescent bulb. Twelve specimens of...
each species (G. spinosum, Copionodon new species and C. pecten) were tested individually. Only the individuals 13 and 25 were juveniles, the remaining being adults.

In order to determine the acclimation time, we made previous observations, which demonstrated that an acclimation time of less than one hour before the beginning of tests was enough, since the individuals showed low and calm swimming and opercular beating.

At the beginning of the tests, a focal fish was taken from a stock tank and gently introduced into the test-aquarium, where it had one hour to acclimate to the new environment and to the light intensity. After this period, the control test was initiated, with also one hour duration, and we counted the total time that the individual spent in the left sector of the aquarium (the side that would be the lighted one during the actual tests). During acclimation period and control, the whole aquarium remained illuminated.

After the control, the right sector of the aquarium was covered with black cardboard (dark chamber), and the total time spent in the lighted sector of the aquarium was recorded during one hour. The same procedure was carried on for the other luminosities. Between one test and the other, that is, before changing light intensity, the light was turned off for at least 20 minutes. The order of the light intensities was always random, but never in an increasing or decreasing order, to prevent biases in responses. The phototactic tests were carried on in the same LES room, between 8 am and 7 pm.

After tests, standard-length and eye diameter of each specimen were measured with a digital caliper, 0.001 millimeters of precision. The eyes were considered as reduced when less than 0.1 millimeter of diameter, and as developed when between 0.1 and 1.0 millimeter. Also, the pigmentation was observed under white light and the fishes were divided in four classes of coloration: light gray with iridescence, medium gray, dark gray and purple.

Responses to light were divided in four photophobia degrees: photophilia (more than 31 minutes in lighted chamber), photophobia (between 29 and 11 minutes in lighted chamber), marked photophobia (10 minutes or less in lighted chamber) and indifference to light (30 minutes in each chamber).

Statistical analysis

Statistically significant differences between the total time spent in the illuminated sector in tests and in control were detected by One-way ANOVA Repeated measures with $a=5\%$ (Zar, 1996).

RESULTS

The troglobitic species G. spinosum is photophobic under the three light intensities, confirmed by statistically significant differences between control and tests (40 lux, $p=0.03073$; 170 lux, $p=0.00014$; 1,700 lux, $p=0.00495$ – Fig. 5). Copionodon sp. n., the other troglobitic species, is only photophobic under the lower light intensities (40 lux, $p=0.03732$; 170 lux, $p=0.01941$ – Fig. 6). The epigean species, C. pecten, showed indifference under all light intensities, corroborated by the statistical results of the One-way ANOVA with repeated measures tests, which did not show significance (40 lux, $p=0.9935$; 170 lux, $p=0.4403$; 1,700 lux, $p=0.7907$ – Fig. 7).

Thus, there is a photophobia degree exhibited by the three Copionodontinae species: the troglobite G. spinosum is the most photophobic one, followed by the new species of Copionodon and finally the epigean species C. pecten, which reacted indifferently under the three intensities.

Comparing light reaction to morphologic characters, we note that G. spinosum, which has regressed eyes size and pigmentation, exhibited strong avoidance under the three light intensities. Copionodon sp. n. only showed response to light in the lower intensities (40 and 170 lux), and also presents variability for eyes and pigmentation. The epigean species C. pecten, clearly homogeneous in relation to eyes and pigmentation, showed indifference in the response to light in all intensities.

DISCUSSION

Most troglobitic catfishes studied concerning phototactic behaviour present epigean relatives displaying photophobia (Parzefall & Trajano, 2010). However, for the epigean Copionodontinae, C. pecten, we observed an inverse pattern: the troglobitic species, G. spinosum and Copionodon sp. n. displayed more photophobic habits than the epigean one, which is indifferent to light. This inverted pattern was also observed for Balitoridae (Eurasian Cypriniformes fishes) from Thailand: while the epigean is generally diurnal and non-cryptobiotic/benthonic, the troglobitic species Schistura oedipus Kottelat 1988 shows indifference to light and a weak photophobia to lower light intensities plus strong photophobia to higher light intensities (Trajano et al., 2002). This inverted pattern can raise the hypothesis that the cave species probably originated from an ancestral which showed twilight or nocturnal activities.

Furthermore, some ecological hypotheses can be considered. Additional data from observation and collecting in the same locality of occurrence of the epigean species indicated the existence of two major nocturnal predators coexisting with C. pecten: the trarihias Hoplerythrinus unitaeniatus Spix & Agassiz, 1829 and the catfish Trachelyopterus galeatus Linnaeus, 1766. Thus, a predation pressure, probably recent (Hoplerythrinus unitaeniatus is an exotic fish and was introduced about 180 years ago – R. C. Santos, pers. comm.), must be the principal triggering process for this indifference to light behaviour in C. pecten. Indeed, groups of C. pecten gather either in exposed and hidden places during the day phase. Thus, it is possible that, if C. pecten has once had photophobic behaviour, it is in process of inversion, due to this predation pression at night, which would force them to forage during the day.
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It is also important to point out that we observed changes in pigmentation pattern in all cave copionodontine individuals that remained in captivity: all specimens of *G. spinosum* changed from pale yellow to purple 10-15 days after arrival from the field, indicating that this species may perceive light by the skin, a typical extraocular photoreception (EOP), very common in vertebrates (Langecker, 1992). Indeed, these pigmentation changes cannot be related to food, since the food varies during maintenance in laboratory, nor to changes related to aging, since pigmentation manifested in both juvenile and adult specimens. Several authors have suggested that the skin of some cave vertebrates may mediate light reactions, for instance, the characidae *Astyanax fasciatus* Cuvier, 1819 (Kähling, 1961), the amblyopsidae *Amblyopsis spelaea* DeKay, 1842 (Payne, 1907) and the salamander *Proteus anguineus* Laurenti, 1819 (Hawes, 1946). For *Copionodon* sp. n., however, the pigmentation changes were subtle, changing from light gray to medium or dark gray, suggesting that the eyes must still be the main light receptor in this species.
Moreover, this study reinforces the idea of different reactions to light according to the stimuli context (gradation in laboratory and sudden stimuli in natural habitat) since Copionodontinae catfishes did not show any reactions under lantern light in the natural environment. The same was observed for trichomycterid catfishes I. bambui and T. itacarambiensis, heptapterid catfishes P. kronei, and armoured loricariid catfishes A. formoso (Bichuette, 2003; Trajano & Gerhard, 1997), besides the balitorid cave fishes from Thailand S. oedipus (Trajano et al., 2002).

Finally, there is, apparently, some correlation between morphological traits and reaction to light regarding G. spinosum. The fact that this species, morphologically homogeneous, reacts similarly under the three light intensities suggests that the response is related to its low variation degree of eyes and pigmentation. On the other hand, Copionodon sp. n., a heterogeneous population, reacted differently under the three lights intensities, which could be due to individual differences. It suggests that this latter species has been isolated in the hypogean realm for a longer time when compared to the more homogenous (regarding both morphological and behavioural aspects) G. spinosum.

In fact, for some Brazilian cave catfishes the type of response could be related to some individual characteristics: depigmented specimens of P. kronei, for instance, were photophilic, whereas semipigmented and pigmented ones were photophobic (Trajano & Gerhard, 1997). On the contrary, albino T. itacarambiensis individuals were more photonegative than the pigmented ones (Trajano & Gerhard, 1997). However, for the vast majority of Brazilian cavefishes, variability in phototactic response is usually related to variation in morphological traits, such as in the troglobitic catfish P. kronei, which shows a mosaic of eye size and melanic pigmentation and a weak photophobia, with intrapopulation variability (Trajano & Gerhard, 1997); the armoured catfish Ancistrus formoso shows a mosaic for behaviour and morphology, varying from indifference to light to photophobia (Bessa & Trajano, 2001). For the Trichomycteridae catfishes, Ituglanis and Trichomycterus genera, the same pattern was observed: photophobia is variable among populations and correlated to eye size and pigmentation for the former (Ituglanis spp.) and weak photophobia for the latter (T. itacarambiensis), which is variable in many morphological levels, including true albinism (Bichuette, 2003; Trajano & Gerhard, 1997). Nevertheless, for a correlation between phototactic behaviour and morphological data regarding cave Copionodontinae, new tests with focal-groups and other statistical analysis, focusing categorical variables, are required.

ACKNOWLEDGEMENTS

We are grateful to several colleagues and friends who helped us during the field and laboratory work, specially to Raimundo C. dos Santos (“Xiquinho”), our guide in Igatu, J. E. Gallão, L. B. Simões and T. L. C. Scatolini for help in the fieldwork. We also thank P. Dodonov for help in statistical analysis. This work is part of the Master degree of Bianca Rantin and part of a major research project of Maria E. Bichuette, both were financially supported by the Fundação de Amparo à Pesquisa no Estado de São Paulo (FAPESP, processes numbers 2008/08910-8 and 2008/05678-7). Thanks also to the environmental governmental agency ICMBIO (Instituto Chico Mendes de Biodiversidade) for the collecting permission document (license number 20165-1) and to PGERN/UFSCar (Programa de Pós Graduação em Ecologia e Recursos Naturais da Universidade Federal de São Carlos/ Brasil), for the infrastructure to develop this work. Eleonora Trajano (IBUSP), Nivaldo Nordi (UFSCar), Andréa Peripato (UFSCar) and Lilian Casatti (UNESP) contributed with suggestions and critical considerations for the manuscript.

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International Journal of Speleology, 42 (1), 57-63. Tampa, FL (USA) January 2013