

Air-breathing Behavior of the Jeju Fish *Hoplerythrinus unitaeniatus* in Amazonian Streams

João Alves de Lima Filho¹, Jhany Martins¹, Rafael Arruda^{2,3}, and Lucélia Nobre Carvalho²

¹ Programa de Pós-Graduação em Ecologia e Conservação da Biodiversidade, Instituto de Biociências, Universidade Federal de Mato Grosso, *Campus* de Cuiabá, 78060-900, Cuiabá, Mato Grosso, Brazil

² Núcleo de Estudos da Biodiversidade da Amazônia Mato-Grossense (NEBAM), Instituto de Ciências Naturais, Humanas e Sociais, Universidade Federal de Mato Grosso, *Campus* Universitário de Sinop, 78557-267, Sinop, Mato Grosso, Brazil

ABSTRACT

The breathing behavior of the jeju fish *Hoplerythrinus unitaeniatus* has been the focus of several studies in recent decades. Few of these studies, however, have described how the fish's air breathing functions in natural environments. We examined changes in the behavior of *H. unitaeniatus* during daily variations in the dissolved-oxygen (DO) content of the water in Amazonian streams. We recorded the mean time intervals between instances when the fish breached the water surface to take in atmospheric air and the variation in the DO concentrations. We also observed the number of individual fish that breached the surface together as well as their territory-forming behavior. We recorded the individual and collective anti-predator strategies in the presence of a potential predator, experimentally testing the capacity of *H. unitaeniatus* to recognize the shape of a predator. The results indicated that as the DO concentration decreased, the individuals of *H. unitaeniatus* tended to breach the water surface at shorter time intervals, which increased their exposure to predators. To minimize the risks from this longer exposure time, the species tends to adopt anti-predator strategies individually or as a group. In well-lit locations, the fish recognized the predator's silhouette, left their territory and took a longer time to return. We provide an original contribution to the understanding of the anti-predatory tactics of the species by describing the behavior of group formation and territory definition as a tool to maximize efficiency in the defense against predators, a behavior that until now was an unknown strategy for this species.

Abstract in Portuguese is available in the online version of this article.

Key words: Erythrinidae; interspecific interaction; intraspecific relationship; *terra firme* streams; tropical wet forest.

THE *HOPLERYTHRINUS UNITAEINIATUS* FISH, COMMONLY KNOWN IN BRAZIL AS JEJU, is found in a variety of habitats, including lakes, ponds, streams, and large rivers (Oyakawa 2003). It belongs to the family Erythrinidae, of which two genera, *Hoplerythrinus* and *Erythrinus*, are facultative air-breathers; these genera are restricted to South America, mainly the Amazon basin (Oyakawa 2003). *Hoplerythrinus unitaeniatus* is omnivorous and is abundant in temporary, oxygen-poor waters (Lowe-McConnell 1987). Both genera have the interesting ability to diffuse atmospheric oxygen into their bloodstream through a vascularized swim bladder, capturing the gas during sequential rises to the surface of the water body and therefore requiring an effective tactic to minimize predation. Their strategy of air breathing has been studied by several authors (Boker 1933, Godoy 1975, Stevens & Holeyton 1978, Smith 1979, Piper 1989, Jucá-Chagas 2004). These authors observed a greater efficiency of atmospheric oxygen uptake and gas exchange in oxygen-poor locations.

Laboratory experiments have demonstrated that in *Hoplerythrinus unitaeniatus*, the length of time that the fish remain submerged varies with the local oxygen concentration and the efficiency of oxygen absorption (Kramer 1978). This laboratory study, however, did not consider stochastic factors of the natural

environment that can significantly influence the submersion time. Predation, for example, is a decisive factor affecting the patterns of spatial-temporal distribution of prey species. Minimizing predator efficiency is highly important for *H. unitaeniatus*, because this species must rise to the surface periodically to obtain atmospheric oxygen, increasing its susceptibility to predation (Kramer *et al.* 1983, Kramer 1987, 1988).

Studies of strategies to minimize predation rates are important for understanding the distribution and permanence of species in certain habitats (Carvalho & Del-Claro 2004, Sazima *et al.* 2006, Zuanon *et al.* 2006). The formation of groups, for example, is widely cited as an important anti-predatory mechanism because the synchronized movement can confuse the predator (Partridge 1982, Carvalho *et al.* 2006). In addition, group formation increases the chances of prey escaping in various directions in the presence of a potential predator, allowing efficient evasion (Milinski 1986). Group formation has been described for breeding migrations in Amazonian streams (Lowe-McConnell 1987, Lima *et al.* 2005) and for groups of juveniles that form small shoals of about 12 individuals (Lowe-McConnell 1987). Galvis *et al.* (2006) described Amazonian erythrinids as predatory and territorial and described members of the genera *Hoplerythrinus* as gregarious and traveling in shoals. Territoriality and group behavior related to air breathing in the natural environment, however, were not mentioned.

Received 7 January 2011; revision accepted 31 August 2011.

³Corresponding author; e-mail: rafael.arruda@pq.cnpq.br

Here we show, using a combination of direct observations and field experiments, that changes in the submersion duration of *H. unitaeniatus* throughout the day are related to the concentration of dissolved oxygen (DO) in the small forest stream habitat. We found that individuals of this species that share the same habitat demonstrate behavioral strategies that help to minimize the efficiency of predation at the time of atmospheric air consumption, such as the formation of groups and the establishment of a constant group territory.

METHODS

STUDY AREA.—The study was carried out at the São Nicolau farm, located in the municipality of Cotriguaçu in northwest Mato Grosso, Brazil. This farm contains a large natural reserve of Amazon rain forest, reforestation areas, and several small streams in *terra firme* forests that are locally called *igarapés*. The study was conducted in two streams (9°51'40" S, 58°58'08"W and 9°51'20" S, 58°15'10" W) in the drainage basin of the Juruena River (see a map of the study area in Rodrigues *et al.* 2011). The study was conducted from October through December 2009, which is the end of the dry season in the region. The climate is tropical, hot and humid, with annual mean temperatures of 23°C to 26°C and two well-defined seasons, a rainy summer from December through May and a dry winter from June through November. The vegetation is classified as tropical rain forest and tropical dense forest (Loureiro *et al.* 1980). During the study, the stream pH ranged from 5.2 to 6.8 with an average of 6.3 during the day. The dissolved-oxygen concentration ranged from 1.4 to 4.8 mg/L, with an average of 2.7 mg/L, and the conductivity was 15–24 µS/cm. The water temperature averaged 25°C, ranging from 24.7 to 28.5°C. At some points, the streams form pools where the high water transparency often allows direct observation of several individuals of *H. unitaeniatus*. The choice of observation points was based on the location of this species in the streams. We selected six observation points, four in one stream (points 1–4) and two in the other (points 5 and 6). The sampling points had moderate water flow with no rapids, but the water residence time was short (approximately 10 min). In these locations, the substrate alternates between leaf litter and sand, with decomposing leaves predominating. The surrounding vegetation, typically gallery forest, has been conserved. Some individuals were collected and preserved to confirm their identification and were subsequently deposited in the fish collection of the National Institute for Amazon Research.

DATA COLLECTION ON TERRITORIAL BEHAVIOR AND GROUP FORMATION.—To estimate the size of the *Hoplerthrinus unitaeniatus* territories observed in this study at the six observation points, we used an adaptation of the convex polygon method described by Odum and Kuenzler (1955). This method aims to locate individuals in a site at different intervals during a specified period to visually establish the boundaries. The final size of the territory is estimated when the individual movements are restricted to a visually established area. After the observations, the previously

determined limits are measured, and the final area (in square meters) of the territory is determined from the distance between the points of the polygon formed. We performed this calculation with R software using the ‘function’ available in the ‘stats’ package. We made preliminary observations to estimate the observation time needed to calculate the territory size of *H. unitaeniatus*. For this study, based on the preliminary observations, the minimum time was set at 30 min. The number of fish per territory was determined by counting the individuals that ascended to the surface to breathe air. For the determination of group-formation behavior, we made observations to establish the time during which individuals remain together within a demarcated territory. These observations were made concurrently with the observation of air breathing. We considered a cluster of individuals of *H. unitaeniatus* at each study site to be a group if they stayed together in the same sampling point throughout the study period. The six groups observed in the different pools were numbered 1 to 6 because each group was found in a distinct location. In addition, during all observation periods, no individual of *H. unitaeniatus* shifted from its original group to another group.

OBSERVATIONS OF AIR-BREATHING BEHAVIOR.—Individuals of *H. unitaeniatus* measuring approximately 10–25 cm were observed using the ‘focal animal’ and ‘all occurrences’ sampling methods (Lehner 1996) for a total of 36 h of *in situ* observation. We recorded their movements during the air-breathing cycle, and we also counted the number of individuals that rose to the surface together at each emersion event. Each group of fish was observed for 360 min, in twelve 30-min observation sessions that started at 0600 h and ended at 1800 h.

THE RELATIONSHIP BETWEEN BREATHING BEHAVIOR AND DISSOLVED-OXYGEN CONCENTRATION.—The variations in respiratory behavior resulting from changes in the dissolved-oxygen concentration were observed by recording the time each individual spent submerged at different oxygen concentrations throughout the day. The DO concentration was measured at 1-h intervals between 0600 and 1800 h with a portable oxygen meter. The measurements were made inside the territory occupied by the fish, and care was taken to avoid affecting their behavior. The submersion time was measured using a digital timer, starting the count when the individual(s) submerged and finishing with the next emersion.

EXPERIMENTAL TEST OF THE EFFECT OF A PREDATOR.—We performed an experiment to test whether individuals of *Hoplerthrinus unitaeniatus* recognize the presence and shape of a potential predator and if the fish change their behavior when they perceive the predator. We used only groups 1, 2, and 3 because these groups were in locations that are separated by small physical barriers, making it difficult for the groups to join in the event of escaping the predator used in the experiment. Another group was excluded due to the presence of several bushes near the stream that affected the positioning of the predator models.

Just prior to the start of the experiment, we spent 10 min recording the times that each group of *H. unitaeniatus* remained submerged after rising to the surface to breathe air. Next, we simulated the arrival of a predator using a manufactured model with the shape, color, and size of a Great White Egret *Ardea alba* (Aves: Ardeidae). Similar methodology was used by Carvalho and Del-Claro (2004) in laboratory experiments with the serpa tetra fish, *Hyphessobrycon eques*, and a taxidermy specimen of the rufescent tiger-heron, *Trigrisoma lineatum* (Aves: Ardeidae). The predator model was left standing for 10 min in the territory of each group. We monitored each territory to determine if the groups remained in place or left the territory, timing the frequency of air-breathing intervals and counting the individuals who were in the area while the predator was present. Then we removed the predator model, and during the next 10 min, we observed if the individual fish returned to the same territory, the time taken to return, the frequency of air-breathing intervals of these individuals, and the number of individuals that surfaced for air breathing. One hour after the experiment with the predator heron, the same experiment was performed with a control predator model for which we used a cubical brown object, approximately 45 cm wide by 30 cm long by 40 cm deep, that did not resemble any kind of animal. All of the territories of the three groups were characterized according to the following variables: substrate composition (%), water flow velocity (cm/s), depth (cm), and canopy cover (%). These variables were selected to provide information about the structural complexity of the location where the experiment was performed.

DATA ANALYSIS.—To determine the distribution pattern of the numbers of individuals that breach the surface together, we applied a comparative method of randomization (Equation 1). The method consists of generating random data, following the same algorithmic characteristics of the sample observed in the field, through the following model:

$$AL = (N_{\max, x} - N_{\min, x} \times Ta) \times At \quad (1)$$

where AL stands for randomizations generated for the comparisons, N_{\max} is the maximum number of individuals that emerge from the surface together, N_{\min} is the minimum number of individuals that emerge from the surface together, Ta represents the size of the sample, and At refers to the amount of randomization set.

This model was created virtually using the ‘function’ function in the ‘stats’ package available in the library of the statistical program R. After applying the model, the averages are calculated for each set of randomized data and subsequently compared with the observed data. This comparison is done using the t -test embedded in the created function, which is available in the ‘stats’ package. An ANOVA was conducted to compare the simulated data sets with the observed data in the field. For the ANOVA, the model was created using the ‘aov’ function in the ‘stats’ package. The results obtained from the t -test and the ANOVA were assessed based on their probability values (P), thereby generating

a frequency of significant or non-significant values. If the means of the observed data were different from the randomized data at a frequency less than or equal to five percent, the data were considered random. Above that level, the data were considered to be biased.

The possible relationships between the submergence time and dissolved oxygen concentration were evaluated on a local scale for each observation point using simple linear regression and on a regional scale by correlating all of the points using multivariate multiple regression. For this analysis, we compared two models in which the mechanics represented the best relationship between the variables. The first model (Equation 2) assigned greater weight among the *H. unitaeniatus* groups for the relationship between the submergence time and dissolved-oxygen concentrations:

$$Y_i + Y_2 + (Y_i : Y_2) \dots Y_\alpha = B_0 + B_1 X_{1i} + B_2 X_{2i} \dots B_p X_{pi} + e_i \quad (2)$$

where Y represents the surface response, α is the number of response variables, N is the number of observations, and p is the number of predictors.

The second model (Equation 3) assigns greater weight to differences between the daytime hours for the possible relationship between the submergence time and DO concentration.

$$Y_i + Y_2 \dots Y_\alpha = B_0 + B_1 X_{1i} + B_2 X_{2i} \dots B_p X_{pi} + e_i \quad (3)$$

The best-fitting model was selected using the Akaike Information Criterion (AIC).

We used t -tests to determine the true differences in the mean submergence time observed between the groups. All statistical analyses were performed using R software, v. 2.11.0 (R Development Core Team 2009).

RESULTS

TERRITORIAL BEHAVIOR AND GROUP FORMATION.—We observed aggressive behavior on the part of individuals of *Hoplerythrynus unitaeniatus* belonging to the same group. Generally, this behavior was evidenced when individuals of other species passed the boundary of the area occupied by the group, and we suggest the behavior is a territorial defense strategy by *H. unitaeniatus*. The area of the territory selected for occupation by *H. unitaeniatus* is associated with the pools, *i.e.*, is deeper and wider than other parts of the water body. This study, however, found two exceptions, where groups 3 and 4 established themselves in a shallow location. The size of the six territories examined ranged from 1.68 to 3.83 m² (mean = 2.79 m², SD = 0.76 m²). The maximum number of individuals per group ranged from 14 to 18 (mean = 16.1, SD = 1.6). At all sampling points during the observation sessions, *H. unitaeniatus* individuals remained grouped together. These results indicate that these individuals form groups and establish territories with well-defined spatial boundaries. With

respect to displacement, we observed that the fish did not move individually, but instead moved over short distances in subgroups of 3–12 and rarely passed beyond the defined territory.

RESPIRATORY BEHAVIOR.—Air breathing in *Hoplerythrinus unitaeniatus* is a standard surface-breaching behavior. First, individuals of *H. unitaeniatus* generally move from deeper areas to areas near the water surface, where they remain stationary for up to 10 s. Then with one strong thrust of the caudal fin, one individual breaches the surface and captures atmospheric oxygen. Immediately after the first individual emerges, the others follow it, breaching the surface together in groups of 3–18 individuals. They then rapidly return to the deeper part of the pool, releasing the excess gas in small bubbles from the operculum as they move downwards (Fig. 1).

We observed that during air breathing, the subjects that rose to the surface did not show a sequential pattern; that is, the members of the group did not follow a pattern with regard to the number of individuals that emerged together, even with different oxygen concentrations or longer submersion times. Comparing the data on the number of breaching fish with the data generated by a random distribution, we found no difference in 97.3 percent of the comparisons performed (*t*-test, $P > 0.05$). This suggests that the number of individuals that emerge from the surface is random (Fig. S1).

RELATIONSHIP BETWEEN AIR-BREATHING BEHAVIOR AND DISSOLVED-OXYGEN CONCENTRATION.—We observed variations in the duration of submergence between air-intake events. Groups 3 and 6

showed a positive relationship between the DO concentration and the duration of submersion during the day ($R^2 = 0.29$, $P = 0.05$ and $R^2 = 0.34$, $P = 0.03$, respectively). Groups 1 ($R^2 = 0.02$, $P = 0.58$), 2 ($R^2 = 0.04$, $P = 0.48$), 4 ($R^2 = 0.002$, $P = 0.88$), and 5 ($R^2 = 0.23$, $P = 0.09$), however, showed no such relationship, although group 5 showed a trend toward an increasing duration of submergence as the DO concentration increased (Fig. 2).

The second model (Equation 3) better represented the relationship between the DO concentration and the submersion duration of *H. unitaeniatus* in the groups studied (AIC = 59.87, $F_{6, 6} = 5.7$, $P = 0.025$). Therefore, we can infer that as the DO levels in the stream decrease, the fish remain submerged for shorter periods. In general, the oxygen concentration tended to decrease between 1200 and 1700 h in some parts of the stream, reaching critical values of 1.4 mg/L (Fig. 3).

EXPERIMENTAL TEST ON THE EFFECT OF A PREDATOR.—The territories were similar only with respect to the speed of water flow, with lentic characteristics. The areas differed somewhat with respect to the remaining variables (Table S1).

The responses of the three groups of *Hoplerythrinus unitaeniatus* to the presence of the heron and control model predators differed. In group 1, the control predator did not cause a mass displacement of individuals, and smaller groups remained in place in the presence of the predator (Martins *et al.* 2011). When the heron model was inserted into group 1's territory, most of the fish left their territory and breached less often, with only one air-breathing event from a small subgroup during this period. When

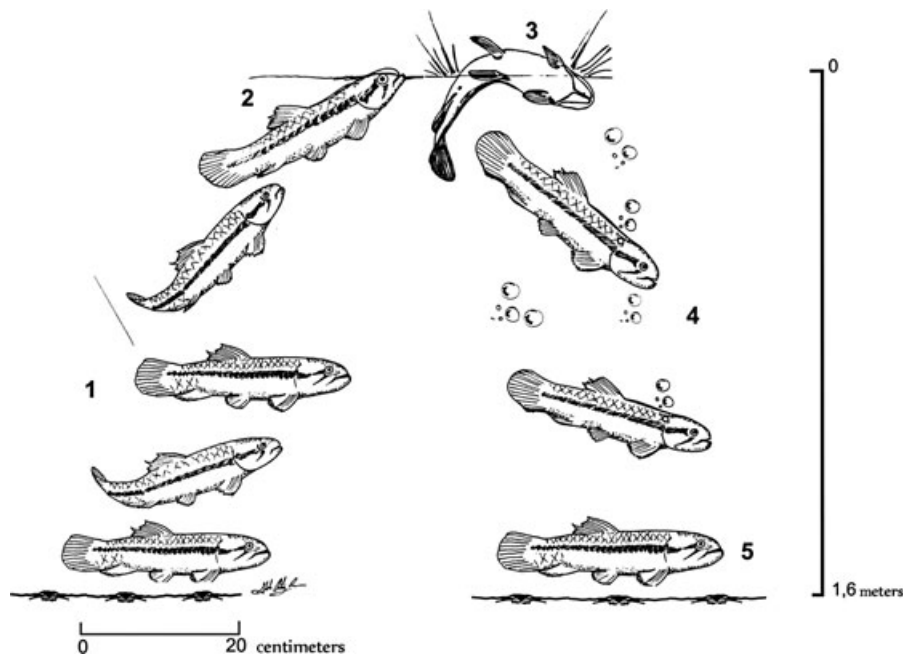


FIGURE 1. Air-breathing cycle of *Hoplerythrinus unitaeniatus*. (1) an individual rising to the surface; (2) taking in air; (3) breaching, by thrusting the caudal fin with the head directed downwards followed by rotating the body and splashing water; (4) returning to the bottom of the stream with excess gas bubbles escaping from the operculum; and (5) completing the cycle and remaining on the stream bottom.

both predator models were removed from the site, the group returned to the territory and resumed respiration (Martins *et al.* 2011).

In group 2, individuals of *Hoplerythrinus unitaeniatus* immediately abandoned their territory and moved to another area in response to the presence of the predator, and we did not observe any air-breathing events during this period. The same response occurred when the heron model was inserted into that territory. In both experiments, once the predators were removed, the group returned to the previous territory (Martins *et al.* 2011). Similar to group 2, group 3 totally abandoned the territory when the control and heron model predators were present, and this group also returned to the site after the models were removed (Martins *et al.* 2011).

After the removal of the heron and control model predators, the individuals in group 1 returned to the territory after about 1.5 min (Fig. S2). The ascents for air breathing were more frequent in the absence of the control predator, although the individuals continued to seek external oxygen even in its presence (Martins *et al.* 2011). After the removal of the heron and control models, the subjects in group 2 returned to the territory after about 1 min (Fig. S2). The frequency of air breathing and the number of individuals per group increased after the removal of the heron model (Martins *et al.* 2011). The individuals in group 3 returned to the territory more quickly after the withdrawal of the control predator (about 1 min) than after the removal of the heron predator (about 5 min) (Fig. S2). The frequency of air

breathing was also lower after removal of the heron model (Martins *et al.* 2011).

DISCUSSION

TERRITORIAL BEHAVIOR AND GROUP FORMATION.—Territory formation is seldom mentioned in the literature on *Hoplerythrinus unitaeniatus*, and Galvis *et al.* (2006) first recorded its territorial habits. According to our observations, this species tends to form territories where the individuals remain together in groups. Territory formation is observed mainly in marine fishes, and observations for freshwater fishes have been made mostly in cichlids (see Keenleyside 1991, Barlow 1993) and a few other species (*e.g.*, Gerking 1953, Sazima 1980, 1988, Titus 1990, Silva *et al.* 2009). Territory formation is an adaptive response to environments with limited resources (Robertson & Gaines 1986) and is also an anti-predator strategy (Brown 1964, Schoener 1971, Davies & Houston 1984). By definition, the occupied territory is aggressively defended by its inhabitants against invaders (Gerking 1953). In this study, however, we observed few agonistic encounters that resulted in aggressive defense action. Those encounters that occurred were between *H. unitaeniatus* and *Hoplias malabaricus* and lasted only a short time.

According to the hypothesis proposed by Balshine *et al.* (2001), high-quality territories can support larger groups of individuals and facilitate cooperation among the groups. This hypothesis could explain the aggregation of *H. unitaeniatus* in relatively

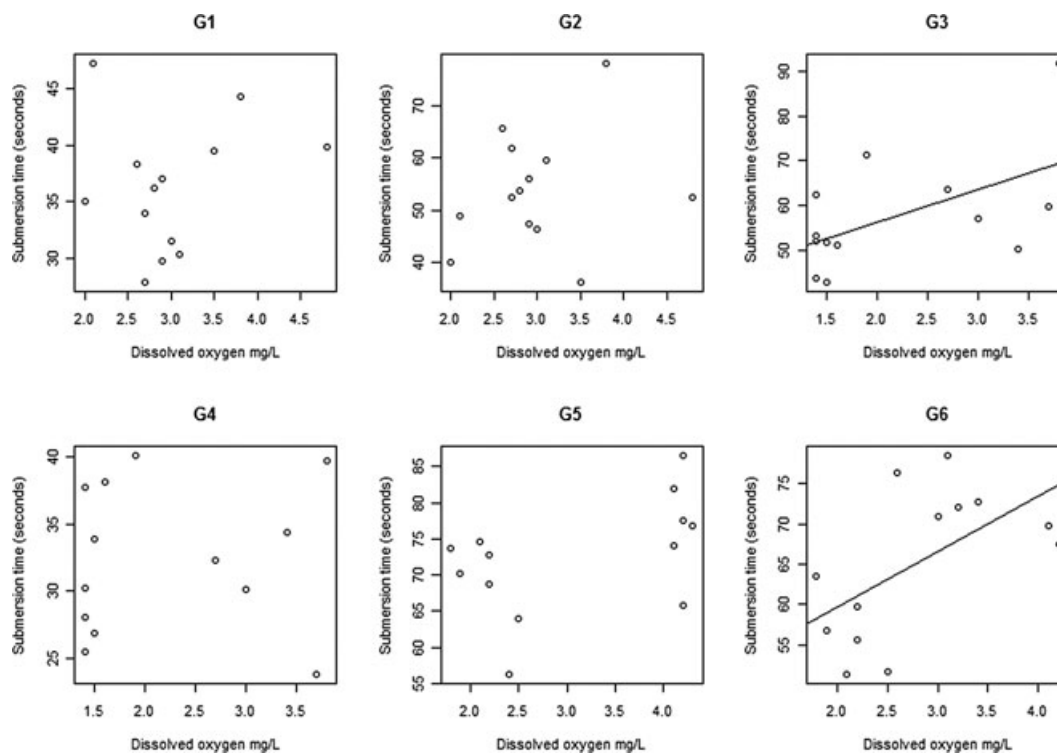


FIGURE 2. Variation in the submersion duration of *Hoplerythrinus unitaeniatus* in water with different dissolved-oxygen concentrations (DO, in mg/L) observed during the daytime at different points along the streams.

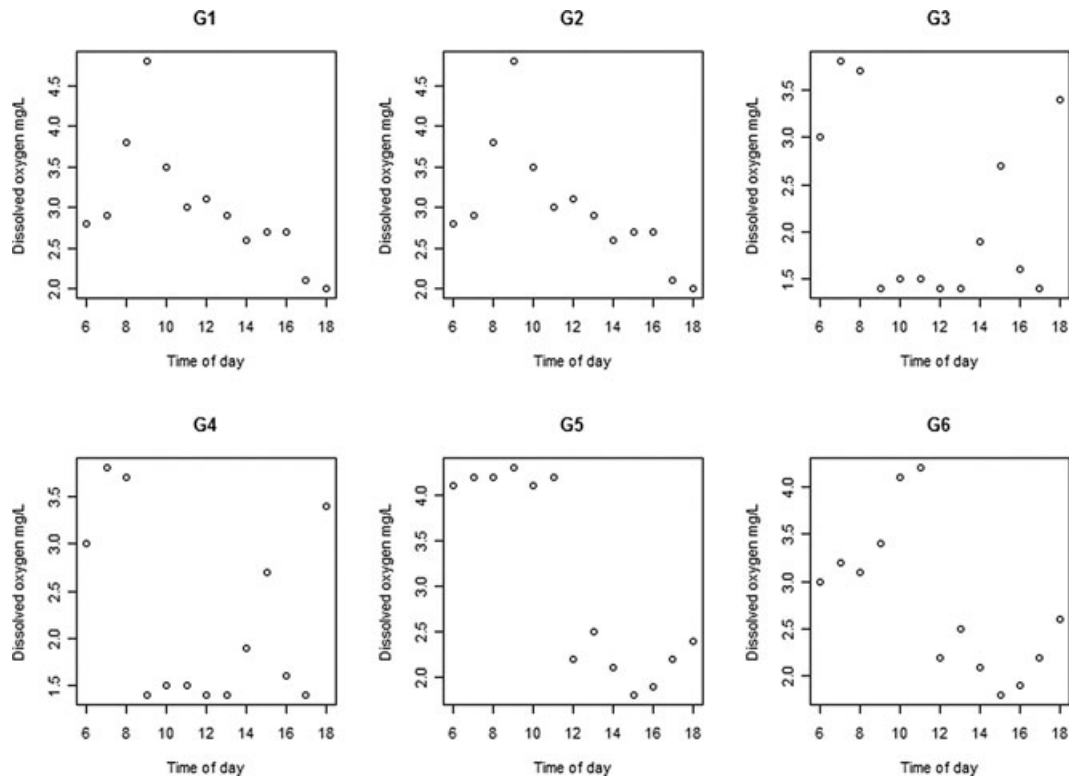


FIGURE 3. Variation in the dissolved-oxygen concentration (DO, in mg/L) during the period from 0600 to 1800 h in six groups (G) of *Hoplerythrinus unitaeniatus* in the two streams.

small areas. It is likely that these areas provide some resources, leading the fish to prefer specific territories within the range of microhabitats available in the stream (e.g., Bravo *et al.* 2008). Another advantage of group formation that is widely discussed in the literature, and has been demonstrated for *H. unitaeniatus*, is an increased protection from predators (Hamilton 1971, Krebs & Davies 1993). The consequences of group formation are so positive that approximately 10,000 species of fishes show this behavior (Shaw 1978). In this study, we observed that group formation was highly advantageous for territory defense because it increased the efficiency of expelling intruders and reduced the time spent on this activity, as observed in the interactions with *H. malabaricus* ($N = 4$). It is possible that several other factors are associated with group life in *H. unitaeniatus* and that group formation relies on complex interactions.

RESPIRATORY BEHAVIOR.—On the basis of our observations, we suggest that the breaching behavior of *Hoplerythrinus unitaeniatus* makes these fish more vulnerable to predation due to the increased visual and noise exposure caused by the water movement. It is possible that evasive strategies, which are unusual for other species, minimize the internal and external pressure from predators.

Our observations suggest that each phase of *Hoplerythrinus unitaeniatus*'s air-breathing cycle consists of small behavioral adaptations that occur on the individual and group levels and that

noticeably decrease the predation risk. A key feature of an individual's adaptation is the strategy of remaining motionless for a period of time in the middle of the water column during the breathing cycle. This behavior enables the individual to see the predators' silhouette or movements outside the water and therefore to take immediate evasive action if necessary. This behavior was observed during the experiment when the potential predator was presented. The rapid emergence from the surface to take in air along with the strong tail movement disrupts the water surface, confusing the predator during its final attack; this tactic is especially effective against those predators that focus visually on their prey. Furthermore, the more individuals emerge together on the surface, the greater the water disturbance and the visual distortion effect (Guthrie & Muntz 1993). The breaching of the surface, though usually performed by several individuals, needs to be initiated by a single individual. This initiation is performed by a single individual that rises rapidly to the surface and is then followed by other group members (Chapman & Chapman 1994). In environments where the predation pressure is intense, this strategy might be effective for allowing escape from the predator's attacks. The attack centered on the first breaching individual may allow the others to escape and may aid them in finding another location to breach safely. Studies suggest that the selective factor for breathing synchrony in different animals is predation pressure, which drives the species to acquire coordinated behaviors to minimize the predator's attack efficiency, as observed in this study

(Kramer & Graham 1976, Gee 1980, Baird 1983). Kramer (1978) described the respiratory behavior of *H. unitaeniatus* using a physiologic interpretation of the air-breathing cycle. He reported that air bubbles appeared soon after the capture of atmospheric oxygen, a finding that is in accordance with our results. The observations *in loco*, however, enabled us to gauge a possible anti-predatory strategy. The air bubbles exhaled while the fish descends to deeper parts of the pool can interfere with the predator's visual tracking. Therefore, a predator that follows the bubbles would be far enough from the prey to facilitate the fish's escape.

The confusion effect is a behavioral strategy acquired by species that live in groups to minimize predation pressure. This effect is created in a coordinated manner, where prey individuals positioned in the middle of the group are safer than individuals positioned at the edge of the group (Hixon & Carr 1997). In the case of *H. unitaeniatus*, this confusion effect is observed when the fish rise to the surface for air breathing: most of the fish are positioned within the group, and only a few individuals breach at the edge of the group or farther away. There is evidence that the predator suffers a kind of confusion when it strikes dense prey groups (Neill & Cullen 1974). This confusion effect would explain the fact that predators prefer to attack at the edges of prey groups. Therefore, it would be expected that the individuals of *H. unitaeniatus* present at the edges of the groups show individual defense strategies to minimize predation.

In general, the ecologic cost of losing individuals from a group can be very high because the group's stability depends on interactions among its members and with individuals outside the group, and this dependence is more evident in groups with fewer members (Higashi & Yamamura 1993). Based on this general rule, it is expected that defensive-cooperative behavior will be observed within groups of *H. unitaeniatus*. The numbers of individuals that rise to the surface together supports this observation. As the demand for oxygen differs among individuals depending on their body mass or activity (Oliveira *et al.* 2004), individual fish commonly rise to the surface at different time intervals; the number of individuals that emerge together is almost always different, but the fish always rise in the company of other individuals. Therefore, we concluded that the number of individuals that surface together follows a random distribution. This distribution inhibits learning by the predators in both the short and long term and impedes coevolution between the predator and prey. This peculiar behavior appears to be an important anti-predatory cooperative tool and is only secondarily related to air breathing *per se*.

RELATIONSHIP BETWEEN RESPIRATION AND DISSOLVED-OXYGEN CONCENTRATION.—The dissolved oxygen concentrations observed in the streams were at critical levels, according to the conditions described for most fish species in small streams (Uieda & Castro 1999). In such conditions, it is usual to find a larger number of species that have compensatory morphologic and physiologic adaptations to low oxygen levels (Graham *et al.* 1977, Soares *et al.* 2006). The increased frequency of breaching

that we observed in this study is evidence of this compensatory activity and concords with the laboratory observations conducted by Oliveira *et al.* (2004). The behavior is also linked to offsetting a fall in the DO levels (Kramer 1987). The increase in the frequency of breaching is physiologically related to the oxygen retention capacity by the swim bladder (Kramer 1978, Mariano *et al.* 2009). This organ is highly vascularized and adapted for this function (Kramer 1978), but its storage capacity and the diffusion rate of gas into the bloodstream are limited (Oliveira *et al.* 2004). Increased demand increases the oxygen diffusion rate into the bloodstream, and consequently, the fish must rise to the surface more often (Oliveira *et al.* 2004). The study by Kramer (1978) suggests that the amount of absorbed oxygen tends to be similar in all air-intake events. In low-oxygen conditions, the fish must rise to the surface more often to compensate (Kramer 1987). Although accessory air breathing is useful for the species, increasing the breaching frequency also increases the vulnerability to predation.

EFFECT OF A POTENTIAL PREDATOR ON THE RESPIRATORY BEHAVIOR OF *HOPLERYTHRYNUS UNITAENIATUS*.—As previously mentioned, individuals of *Hoplerythrynus unitaeniatus* tend to form groups for air breathing, probably as a defense from predators (Martins *et al.* 2011). Unlike group 1, groups 2 and 3 were intimidated and left the air-breathing territory in the presence of both predator models (heron and control). This probably occurred because there was a higher percentage of shade (80%) in the territories of groups 2 and 3 that may have prevented individuals from discriminating the silhouette of the heron from that of the control (Martins *et al.* 2011). Some fish have adapted to low-light conditions, whereas others are totally dependent on light for their foraging activities and anti-predator defense in different environments (Lowe-McConnell 1987, Rodríguez & Lewis 1997). These results indicate that in better light conditions, the fish apparently recognize the silhouette of a true predator, as noted in group 1's behavior. In dim light, the fish perceive that there is a potential predator, but do not differentiate its silhouette and therefore choose to leave the territory, albeit temporarily, as observed in the behavior of groups 2 and 3. The ability to distinguish between true and false predators probably depends on the light conditions, but if they are unable to perceive the difference, the fish seem to opt for temporary escape, returning to the territories once the predators are removed. Although the escape and subsequent return to the territory require some energy expenditure, their benefit (protection from predation) outweighs their cost and may explain in part why territoriality is favored by natural selection (Adams 2001, Begon *et al.* 2006). Perhaps the presence of a predator can cause a non-lethal effect (*sensu* Lima 1998) on the population of *H. unitaeniatus*, as observed by Carvalho and Del-Claro (2004) for *H. eques*. We suggest that studies should be undertaken to demonstrate the complex inter- and intraspecific relationships of *H. unitaeniatus* and to identify the main resources in the environment that influence the territorial behavior of this species.

ACKNOWLEDGMENTS

Our thanks to Thiago Izzo and Jansen Zuanon for advice and help with the fieldwork. We thank Thiago Izzo, Rubens Honda and two anonymous reviewers for helpful comments on earlier drafts of this manuscript. Donald Kramer kindly gave us a special and inspiring review of the manuscript that opened our minds to various aspects of the behavior of *H. unitaeniatus*. We thank Aldo Ortiz for composing Figure 1. We thank the Federal University of Mato Grosso, São Nicolau Farm and ONF Brasil for logistical and financial support for the fieldwork. We are grateful to CNPq for providing graduate fellowships to J. A. Lima Filho and J. Martins and to CNPq/FAPEMAT for providing a postdoctoral fellowship to R. Arruda. This is publication 09 in the NEBAM technical series and contribution 24 of the Projeto Igarapés.

SUPPORTING INFORMATION

[The supplementary material for this article has been corrected after publication on 25 November 2011.]

Additional Supporting Information may be found in the online version of this article:

TABLE S1. *Composition of substrate, depth, canopy cover and water velocity in stream territories occupied by three different groups of Hoplerythrinus unitaeniatus.*

FIGURE S1. Relationship between of numbers of individuals of *Hoplerythrinus unitaeniatus* that breach the surface with randomized data, and observed data, based in variation of dissolved oxygen concentrations at two sampled streams.

FIGURE S2. Time elapsed to return to the territory by individuals of *Hoplerythrinus unitaeniatus* after the placement of a heron model or a control model predator in both groups.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

LITERATURE CITED

- ADAMS, E. S. 2001. Approaches to the study of territory size and shape. *Annu. Rev. Ecol. Syst.* 32: 277–303.
- BAIRD, T. A. 1983. Influence of social and predatory stimuli on the air-breathing of the African clawed frog, *Xenopus laevis*. *Copeia* 1983: 411–420.
- BALSHINE, S., B. LEACH, F. NEAT, H. REID, M. TABORSKY, AND N. WERNER. 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behav. Ecol. Sociobiol.* 50: 134–140.
- BARLOW, G. W. 1993. The puzzling paucity of feeding territories among freshwater fishes. *Mar. Freshw. Behav. Physiol.* 23: 155–174.
- BEGON, M., C. R. TOWNSEND, AND J. L. HARPER. 2006. *Ecology: From Individuals to Ecosystems*. Blackwell Publishing, Oxford, UK.
- BOKER, H. 1933. Über einige neue Organe bei luftatmenden Fischen und im Uterus der Anakonda. *Anat. Anz.* 76: 148–155.
- BRAVO, A., K. E. HARMS, R. D. STEVENS, AND L. H. EMMONS. 2008. *Collpas*: Activity hotspots for frugivorous bats (Phyllostomidae) in the Peruvian Amazon. *Biotropica* 40: 203–210.
- BROWN, J. L. 1964. The evolution of diversity in avian territorial systems. *Wilson Bull.* 76: 160–169.
- CARVALHO, L. N., AND K. DEL-CLARO. 2004. Effects of predation pressure on the feeding behaviour of the serpa tetra *Hyphessobrycon eques* (Ostariophysi, Characidae). *Acta Ethol.* 7: 89–93.
- CARVALHO, L. N., J. ZUANON, AND I. SAZIMA. 2006. The almost invisible league: Crypsis and association between minute fishes and shrimps as a possible defence against visually hunting predators. *Neotrop. Ichthyol.* 4: 107–118.
- CHAPMAN, L. F., AND C. A. CHAPMAN. 1994. Observations on synchronous air breathing in *Clarias liocephalus*. *Copeia* 1994: 246–249.
- DAVIES, N. B., AND A. I. HOUSTON. 1984. Territory economics. In J. R. Krebs, and N. B. Davies (Eds.). *Behavioural Ecology* (2nd edition), pp. 148–169. Blackwell Scientific, Oxford, UK.
- GALVIS, G., J. I. MOJICA, S. R. DUQUE, C. CASTELLANOS, P. SANCHEZ-DUARTE, M. ARCE, A. GUTIÉRREZ, J. F. JIMÉNEZ, M. SANTOS, S. VEJARANO-RIVADENEIRA, F. ARBELÁEZ, E. PRIETO, AND M. LEIVA. 2006. Peces del medio Amazonas, Región de Leticia. Série de guías tropicales de campo No. 5. Conservation Internacional, Editorial Panamericana, Bogotá, Colombia.
- GEE, J. H. 1980. Respiratory patterns and antipredator responses in the central mudminnow, *Umbra limi*, a continuous, facultative air-breathing fish. *Can. J. Zool.* 58: 819–827.
- GERKING, S. D. 1953. Evidence for the concept of home range and territory in stream fish. *Ecology* 34: 347–365.
- GODOY, M. P. 1975. Família Erythrinidae. In M. P. Godoy (Ed.). *Peixes do Brasil, subordem Characoidei: Bacia do rio Mogi-Guaçu*, pp. 440–444. Franciscana, Piracicaba, Brazil.
- GRAHAM, J. B., D. L. KRAMER, AND E. PINEDA. 1977. Respiration of the air breathing fish *Piabucina festae*. *J. Comp. Physiol. B.* 122: 295–310.
- GUTHRIE, D. M., AND W. R. A. MUNTZ. 1993. Role of vision in fish behavior. In T. J. Pitcher (Ed.). *The Behavior of Teleost Fishes* (2nd edition), pp. 89–124. Chapman & Hall, New York.
- HAMILTON, W. D. 1971. Geometry for the selfish herd. *J. Theor. Biol.* 31: 295–311.
- HIGASHI, M., AND N. YAMAMURA. 1993. What determines animal group size? Insider-outsider conflict and its resolution. *Am. Nat.* 142: 553–563.
- HIXON, M. A., AND M. CARR. 1997. Synergistic predation, density dependence, and population regulation in marine fish. *Science* 277: 946–949.
- JUCÁ-CHAGAS, R. 2004. Air breathing of the neotropical fishes *Lepidosiren paradoxa*, *Hoplerythrinus unitaeniatus* and *Hoplosternum littorale* during aquatic hypoxia. *Comp. Biochem. Physiol. A* 139: 49–53.
- KEENLEYSIDE, M. H. A. 1991. *Cichlid Fishes: Behaviour, Ecology and Evolution*. University Press, Cambridge, UK.
- KRAMER, D. L. 1978. Ventilation of the respiratory gas bladder in *Hoplerythrinus unitaeniatus* (Pisces, Characoidei, Erythrinidae). *Can. J. Zool.* 56: 931–938.
- KRAMER, D. L. 1987. Dissolved oxygen and fish behavior. *Envir. Biol. Fishes* 18: 81–92.
- KRAMER, D. L. 1988. The behavioral ecology of air breathing by aquatic animals. *Can. J. Zool.* 66: 89–94.
- KRAMER, D. L., AND J. B. GRAHAM. 1976. Synchronous air breathing, a social component of respiration in fishes. *Copeia* 1976: 689–697.
- KRAMER, D. L., D. MANLEY, AND R. BOURGEOIS. 1983. The effect of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. *Can. J. Zool.* 61: 653–665.
- KREBS, J. R., AND N. B. DAVIES. 1993. *An Introduction to Behavioural Ecology* (3rd edition). Wiley-Blackwell, New Jersey.
- LEHNER, P. N. 1996. *Handbook of Ethological Methods*. Cambridge University Press, Cambridge, U.K.
- LIMA, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions. *Bioscience* 48: 25–34.
- LIMA, F. C. T., L. RAMOS, T. BARRETO, A. CABALZAR, G. TENÓRIO, A. BARBOSA, F. TENÓRIO, AND A. S. RESENDE. 2005. Peixes do Alto Tiquié. In A. Cabalzar (Ed.). *Peixe e gente no Alto Rio Tiquié: Conhecimentos*

- Tukano e Tuyuka, ictiologia e etnologia, pp. 111–282. Instituto Socioambiental, São Paulo, Brazil.
- LOUREIRO, R. L., A. A. DIAS, AND H. MAGNAGO. 1980. Vegetação. In RADAMBRASIL (Ed.) – Levantamento de Recursos Naturais, Folha Sc. 21 Juruena, pp. 325–376. MME, Rio de Janeiro, Brazil.
- LOWE-McCONNELL, R. H. 1987. *Ecological Studies in Tropical Fish Communities*. Cambridge University Press, Cambridge, U.K.
- MARIANO, W. S., E. T. OBA, L. R. B. SANTOS, AND M. N. FERNANDES. 2009. Respostas fisiológicas do jeju (*Hoplerthrinus unitaeniatus*) expostas ao ar atmosférico. *Rev. Bras. Saúde Prod. An.* 10: 210–223.
- MARTINS, J., J. A. LIMA FILHO, R. ARRUDA, AND L. N. CARVALHO. 2011. Comportamento territorialista e táticas anti-predatórias do peixe jeju, *Hoplerthrinus unitaeniatus*, em um igarapé da bacia do rio Juruena, Amazônia Meridional. In D. J. Rodrigues, T. J. Izzo, and L. D. Battistola (Org). *Descobrendo a Amazônia Meridional: Biodiversidade da Fazenda São Nicolau*, pp. 259–272. Pau e Prosa Comunicação Ltda, Cuiabá, Brazil.
- MILINSKI, M. 1986. Constraints placed by predators on feeding behaviour. In T. J. Pitcher (Ed.). *The Behaviour of Teleost Fishes*, pp. 236–252. Croom Helm, Australia.
- NEILL, S. R. J., AND J. M. CULLEN. 1974. Experiments on whether schooling by their prey affects the hunting behaviour of cephalopods and fish predators. *J. Zool.* 172: 549–569.
- ODUM, E. P., AND E. J. KUENZLER. 1955. Measurement of territory size and home range size in birds. *Auk* 72: 128–137.
- OLIVEIRA, R. D., J. M. LOPES, J. R. SANCHES, A. L. KALINI, M. L. GLASS, AND F. T. RANTIN. 2004. Cardiorespiratory responses of the facultative air-breathing fish jeju, *Hoplerthrinus unitaeniatus* (Teleostei, Erythrinidae), exposed to graded ambient hypoxia. *Comp. Biochem. Physiol. A* 139: 479–485.
- OYAKAWA, O. T. 2003. Erythrinidae (Trahiras). In R. E. Reis, S. O. Kullander, and C. J. Ferraris Jr (Eds.). *Checklist of the Freshwater Fishes of South and Central America*, pp. 238–240. EDIPUCRS, Porto Alegre, Brazil.
- PARTRIDGE, B. L. 1982. The structure and function of fish schools. *Sci. Amer.* 246: 114–123.
- PIPER, J. 1989. Factors affecting gas transfer in respiratory organs of vertebrates. *Can. J. Zool.* 67: 2956–2960.
- R DEVELOPMENT CORE TEAM. 2009. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- ROBERTSON, D. R., AND S. D. GAINES. 1986. Interference competition structures habitat use in a local assemblage of coral reef surgeonfishes. *Ecology* 67: 1372–1383.
- RODRIGUES, D. J., T. J. IZZO, AND L. D. BATTIROLA. 2011. *Descobrendo a Amazônia Meridional: Biodiversidade da Fazenda São Nicolau*. Pau e Prosa Comunicação Ltda, Cuiabá, Brazil.
- RODRÍGUEZ, M. A., AND W. M. J. LEWIS. 1997. Structure of fish assemblages along environmental gradients in floodplain lakes of the Orinoco River. *Ecol. Monogr.* 67: 109–128.
- SAZIMA, I. 1980. Behavior of two Brazilian species of parodontid fishes, *Apareiodon piracicabae* and *A. ibitiensis*. *Copeia* 1980: 166–169.
- SAZIMA, I. 1988. Territorial behaviour in a scale-eating and a herbivorous neotropical characiform fish. *Rev. Brasil. Biol.* 48: 189–194.
- SAZIMA, I., L. N. CARVALHO, F. P. MENDONÇA, AND J. ZUANON. 2006. Fallen leaves on the water-bed: diurnal camouflage of three night active fish species in an Amazonian streamlet. *Neotrop. Ichthyol.* 4: 119–122.
- SCHOENER, T. W. 1971. Theory of feeding strategies. *Annu. Rev. Ecol. Syst.* 2: 369–404.
- SHAW, E. 1978. Schooling fish. *Amer. Sci.* 66: 166–175.
- SILVA, S. E., W. R. C. ASSUNÇÃO, C. DUCA, AND J. PENHA. 2009. Cost of territorial maintenance by *Parodon nasus* (Osteichthyes: Parodontidae) in a Neotropical stream. *Neotrop. Ichthyol.* 7: 677–682.
- SMITH, N. J. H. 1979. A pesca no Rio Amazonas. Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil.
- SOARES, M. G. M., N. MENEZES, AND W. J. JUNK. 2006. Adaptations of fish species to oxygen depletion in a central Amazonian floodplain lake. *Hydrobiologia* 568: 353–367.
- STEVENS, E. D., AND G. F. HOLETON. 1978. The partitioning of oxygen uptake from air and from water by erythrinids. *Can. J. Zool.* 56: 965–969.
- TITUS, R. G. 1990. Territorial behavior and its role in population regulation of young brown trout (*Salmo trutta*): New perspectives. *Ann. Zool. Fenn.* 27: 119–130.
- UIEDA, V. S., AND R. M. C. CASTRO. 1999. Coleta e fixação de peixes de riacho. In E. P. Caramachi, R. Mazzoni, and P. R. Peres-Neto (Eds.). *Oecologia Brasiliensis: Ecologia de peixes de riacho*, pp. 1–22. UFRJ, Rio de Janeiro, Brazil.
- ZUANON, J., L. N. CARVALHO, AND I. SAZIMA. 2006. A chamaeleon characin: the plant-clinging and colour-changing *Ammocryptocharax elegans* (Characidiinae: Crenuchidae). *Ichthyol. Explor. Freshwaters* 17: 225–232.