Notes on feeding and reproduction of the threatened phreatic fish *Stygichthys typhlops* Brittan & Böhlke, 1965 (Characiformes: Characidae) from eastern Brazil

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Stygichthys typhlops Brittan and Böhlke, 1965 is endemic to phreatic waters within the Córrego Escuro drainage located in Jaíba County, Minas Gerais, eastern Brazil. This drainage is in a large karst area of the Middle São Francisco River Basin belonging to the Bambui Geomorphological Unit. The first specimen was collected in 1962 from a well used by local inhabitants (Brittan and Bölkhe 1965), but was not observed for 40 years until it was rediscovered and re-described in 2002. Moreover, some information about its life history also has been elucidated (Moreira et al. 2010). *Stygichthys typhlops* (**Figure 1**) is threatened with extinction, primarily because its habitat (phreatic waters) is highly impacted and exploited as a water source and for irrigation for banana plantations. Because of these threats, new studies on the conservation status and aspects of its biology are urgently needed to develop conservation and management programs.

We present new data on the feeding (diet and foraging behavior in laboratory) and reproduction of *S. typhlops*. We examined the stomach contents and reproductive condition of seven individuals collected during the rainy season (November 2010) at “Mina do Mandioqueu,” Jaíba County, Minas Gerais, using hand-nets and minnow-traps baited with commercial fish and dry cat food. A previous attempt to collect specimens during the dry season (July 2010) was unsuccessful due to the low level of the water table. These specimens were euthanized and fixed in 10% formalin immediately after capture. Standard length (SL) and weight were measured in the field with a millimeter precision caliper and dynamometer (precision of 0.01 g), respectively.

The sex and reproductive condition of each individual was determined by examination of gonad stages (mature, starting maturation, or juvenile with no gonad development) in the laboratory. We determined the degree of gonad maturation by the number and size of oocytes. Mature females showed enlarged ovaries with spherical and translucent oocytes (Vazzoler 1982), occupying almost the entire abdominal cavity. Six individuals were classified as females and one as a juvenile (sex undetermined). Of the six females, four presented mature gonads, apparently in an intermediate level of maturation and two showed gonads that were starting to mature (**Table 1**). The sampled data indicate that *S. typhlops* females reach sexual maturity at 22.5 mm SL (53.7% of maximum length recorded) (**Table 1**).

Figure 1. *Stygichthys typhlops* in life (51.0 mm SL). Photo by Dante B. Fenolio.

Although we sampled few individuals, there was a trend toward a female bias in sex ratio (6:0 female to male). This bias toward mature or maturing females may be related to the time of sampling (beginning of the rainy season in November), which also has been documented for other Brazilian subterranean fishes (Bichuette and Trajano 2003). The rainy season represents a time of increased food availability flushed into the hypogean environment, providing the best seasonal conditions to begin reproduction (Hüppop 2000). Females may be actively foraging during this time to meet the high-energy demands of reproduction.

We found significant variation in body mass among individuals with similar standard lengths. This may be related to their general nutritional state, as heavier individuals were those with higher volume of stomach contents, but not necessarily the larger fishes (in SL).

Table 1. Stomach contents for seven specimens of *Stygichthys typhlops* sampled in November 2010. Standard length (SL) in mm, mass in g, life stage, and stomach contents of seven *S. typhlops*. Life stage (degree of gonad maturation): JU = juvenile, SM = gonad starting to mature, and MA = mature gonad. All specimens were female but the juvenile whose sex could not be determined. Stomach contents: TW = presence of termite (Termitidae) wings, *plus one termite head; A = presence of unidentified arthropod fragments, HH = number of unidentified hemipteran heads, CL = number of unidentified coleopteran larvae, and UI = number of unidentified insect heads.

					Stomach Contents				
Specimen	**SL (mm)**	**Mass (g)**	**Life stage**	**Volume (mm^3)**	**TW**	**AF**	**HH**	**CL**	**UI**
1	20.9	0.15	JU	2	+				
2	22.5	0.15	SM	3	+				
3	22.9	0.3	SM	5		+	1	1	
4	33.2	0.6	MA	2		+			
5	33.3	1.0	MA	25	+*	+			
6	35.0	1.2	MA	37	+	+			
7	41.9	1.6	MA	62	+	+			2

To estimate stomach content volumes, samples were measured against a 1x1 mm background grid. We obtained volume by using two 1 mm high plastic plaques bordering the contents and covering the items with a glass slides. We then categorized all items to estimate their frequency of occurrence (Bennemann et al. 2006). Unidentified arthropods fragments and insect larvae comprised the majority of stomach content volume (**Table 1**). We could positively identify some fragments of adults Isoptera (Termitidae) and Hemiptera (**Figure 2**).

Intestine length is often an indicator of diet for many taxa and is also used to compare species habits (Kramer and Bryant 1995). Indeed, it is related directly to diet and food digestibility (Kapoor et al. 1975; Bowen 1983; Sturmbauer et al. 1992; Lobon-Cervia and Rincón 1994). There was a positive relationship between the quantity of detritus in the diet and intestine length. We measured intestine relative length (IRL) as the ratio of the length of the intestine to the body length of the animal (SL). The results showed that the diet of *S. typhlops* is typical of a carnivorous fish (**Table 2**). In general, the IRL of carnivorous fish ranges 0.2–2.5 (0.6 on average), 0.6–8.0 (commonly higher than 0.8) for omnivores and 0.8–15.0 (commonly higher than 0.9) for herbivores (Zavala-Camin 1996; Mazzoni and Soares da Costa 2007). We compared the data obtained for *S. typhlops* with other characids from epigean rivers in Brazil (**Table 2**) that were also deposited at scientific collection of Laboratório de Estudos Subterrâneos–UFSCar. We obtained similar values of IRL for all studied fishes (0.31–0.59, **Table 2**), indicating a consistence with IRLs for carnivorous fish (Sabino and Castro 1990; Kramer and Bryant 1995).

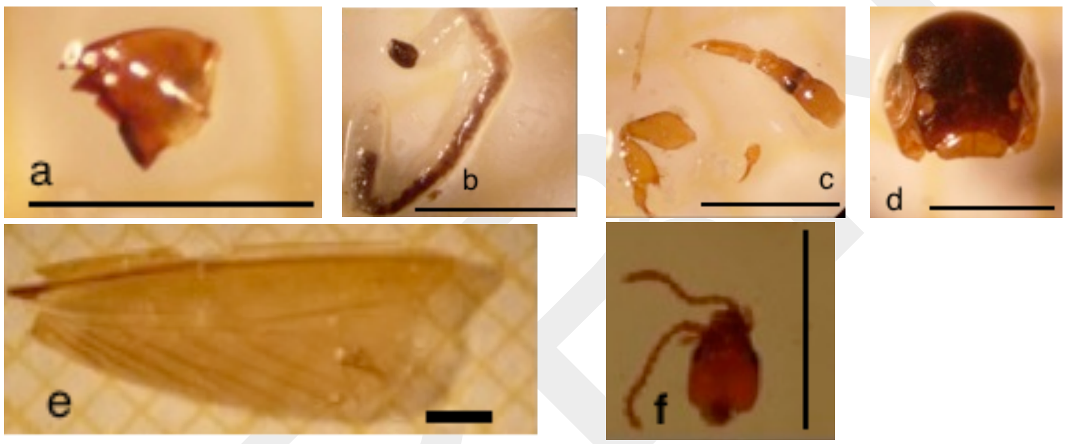

Figure 2. Food items recorded for *Stygichthys typhlops*: (a) mandibular fragment of undetermined arthropod; (b) aquatic Coleoptera larva; (c) head and appendages of Hemiptera; (d) head of undetermined larva; (e) wings of Termitidae and (f) head of Termitidae. Scale bars = 1 mm.

In contrast to Sampaio et al. (2012), we found no evidence of herbivory in any of the seven stomachs analyzed or by direct observations in the field. These authors based their results in the morphology of teeth, which should be reanalyzed considering our results. They also observed *S. typhlops* taking plant matter in laboratory. However, the ingestion of plant matter by *Stygichthys* in the wild is probably accidental. This behavior is common in carnivorous fishes, which may ingest sand and/or organic matter when foraging but not necessarily those items that are part of the diet (Gerking 1994; Bichuette 2003). It is also important to highlight that Moreira et al. (2010) previously observed ostracods in radiographs of the stomachs of *S. typhlops* (5 of 16 stomachs). Both the IRL and the high frequency of occurrence of arthropod fragments (mainly Termitidae insects; **Figure 2**) strongly suggest a carnivorous habit for *S. typhlops*. The source of Termitidae prey recorded in the stomachs of *S. typhlops* is likely associated with the local construction (rotten wood) covering the entrance of the well. All seven cavefish were observed foraging close to this entrance, probably because food is more available at the opening of this pool.

We observed the feeding behavior of five specimens of *S. typhlops* in captivity. These individuals were collected from the same locality in 2008 by R.L. Ferreira and donated to M.E. Bichuette in 2009. The fish were maintained in the same 40 L aquarium and were fed once per week. We observed their reaction to three different processed commercial fish food: *Artemia salina*, dry Chironomidae larvae (floating item) and pellet for general carnivorous fishes. We conducted observations of the feeding behavior response of all five individuals collectively once a week for four consecutive weeks. These observations were made in 2010, so the fish had been acclimated to laboratory conditions for at least one year. The three food items (of same volume) were gently placed at water surface at the same time. We continued observations until all fish had enlarged stomachs, which could easily be visualized through their skin.

Table 2. Standard length (SL), intestine length (INT) and intestine relative length (IRL) measured for other Characiformes species (families Crenuchidae and Characidae) and compared to *Stygichthys typhlops*. All lengths in mm. *Characidium* sp. collected 15 June 2008 from Chapéu Mirim River, Iporanga, SP; *Astyanax* sp. 1 collected 6 September 2009 from Tesouro Cave, São Roque de Minas, MG; *Astyanax* sp. 2 collected 30 March 2009 from Apiai, SP; and *Stygichthys typhlops* collected 7 November 2010 from Mandioqueu, Jaiba, MG.

Family/Species	Specimen	SL	INT	IRL
Crenuchidae/*Characidium* sp.	1	41.9	17.6	0.42
	2	44.3	15.2	0.34
	3	40.4	18.3	0.45
Characidae/*Astyanax* sp. 1	1	39.8	12.9	0.32
	2	34.7	10.7	0.31
	3	30.8	8.5	0.28
Characidae/*Astyanax* sp. 2	1	41.6	15.8	0.38
	2	41.6	18.2	0.44
Characidae/*Stygichthys typhlops*	1	38.0	18.6	0.49
	2	39.5	23.5	0.59
	3	38.4	12.0	0.31
	4	30.4	13.5	0.44

All five fish displayed the same feeding behavior. The moment food items made contact with the water surface we noted an increase in both swimming speed and movements (such as turning around), which was relatively constant and slow in general. Then, fish began moving toward a food item, and often ingested it at the surface. *Stygichthys typhlops* quickly accepted all food items presented. Fish were observed foraging at the bottom, midwater and also at the surface, often biting or shaking the food item in an attempt to tear it apart. Some individuals tried to ingest a large piece of food and would spend several seconds (30 seconds on average) trying to swallow it. On some occasions, they foraged at an angle of 45° in relation to the bottom spending several seconds exploring for food, occasionally touching the substrate with their snouts.

We also fed fish individually. First, we placed a single specimen into a 3 L aquarium. After 24 h of acclimatization, the three food items were all offered in a sequence. We repeated these observations once per week for a month. Fish never accepted any food when fed in isolation. They remained indifferent to all food items even if the item was very close to or even made contact with their heads.

Finally, we also made *ad libitum* observations in the field for three hours in 2010. We observed seven specimens that exhibited midwater and surface swimming as well as bottom exploratory behavior (i.e., foraging behavior). The seven individuals formed a tight aggregation often swimming in close proximity to each other (around 2–3 cm). These observations yield an interesting hypothesis that *S. typhlops* may primarily feed in aggregations that warrants further study.

Conservation status

Stygichthys typhlops is threatened due to intensive use of the subterranean aquifer, in which it lives for local agriculture, mainly banana plantations. Local people rely heavily on underground water, which has been dropping considerably since the 1980s (Silva 1984). The pumping station is operated once every ten days during the rainy season. However, it operates for several hours every day during the dry season, which has a significant impact on the local groundwater, availability of habitat, and consequently to its inhabitants. The increase of human activity in the region is causing an over exploitation of groundwater resources. The stream had a permanent surface water flow until at least 1984 (Silva 1984). During our fieldwork, we noticed that some wells that harbor the species have also begun to or are already dry. Consequently, some windows into the subterranean aquifer are now inaccessible to humans, making accurate assessments about the status of *S. typhlops* uncertain.

We also noticed how aggressive the pump suction system could be at Mandioqueu. Once started, the pumping machinery immediately flushed all the upwelling water from the pool with such force that it even displaced our traps that were attached to the floor. The pumps were shut down after several minutes, and water that contained a great amount of mud and sediment flowed intensively back to the entrance of the well. It is highly likely that this sudden suspended sediment as well as repeated changes in the water level are stressors to *S. typhlops*. Older rural people from the surrounding area claim, “these white little fish use to come along with tap water.” Such anecdotal information helps reinforce how great a threat water pumping represents to the groundwater aquifer and to this rare cavefish population. Unless some conservation policies are established, the long-term survival of *S. typhlops* will remain threatened and uncertain.

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