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COMPARATIVE FIELD ECOLOGY AND MORPHOLOGY OF TWO POPULATIONS OF THE TROGLOBITIC CATFISH *PIMELODELLA KRONEI* (SILURIFORMES: PIMELODIDAE) FROM SOUTHEASTERN BRAZIL

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ABSTRACT

This study focused on characterizing the Bombas resurgence population of troglobitic catfishes Pimelodella kronei *from ecological and morphological points of view, and on making comparisons with the previously studied population of* P. kronei *catfishes from Areias cave. Bombas population ended to be more numerous and with a higher density than Areias population. Considering morphological data, Bombas fishes were smaller than Areias fishes in all parameters measured. On the other hand, values of condition factor for Bombas fishes were higher than those for Areias fishes. Thus, although smaller, they were proportionally heavier. The depigmentation rate of Bombas catfishes was more homogeneous than that of Areias catfishes, being Bombas fishes lighter than Areias fishes in average. Considering ecological and behavioural data, there are some autapomorphies for each population and some similarities which could be either synapomorphies or convergences. Thus, either the populations evolved from the same ancestral and nowadays there is a large reduction of genetic flow (and they may have even speciated), or the two populations had different origins. Anyway, there are many evidences to consider them as different species, at least using the phylogenetic species concept, which should be tested.*

Keywords: *Pimelodella kronei*, cave fishes, population ecology, Brazilian caves.

INTRODUCTION

Troglobites, cave restricted animals, usually present morphological and physiological modifications related to the subterranean life (Culver, 1982; Parzefall, 1986; Holsinger & Culver, 1988). An important feature of troglobitic populations is related to their size. There has been generally accepted that the populations are small because of the isolation, which leads to physical restriction, and because of food scarcity (Culver, 1982). However, there are only a few studies with precise estimations, using for instance capture-recapture methods. One of these studies estimated one especially numerous population of characids *Astyanax* sp. as having 8500 ± 7000 individuals (Mitchell *et al.*, 1977). Some previous visual estimates of the same population suggested very small numbers, between 200 and 500 individuals (Avise & Selander, 1972). Other visual estimates were those for the amblyopsids *Amblyopsis rosae*, with about 150 individuals (Willis & Brown, 1985), and *Typhlichthys subterraneus* and *Amblyopsis spelaea*, with similar values (Poulson, 1963). Populational densities for these two latter were estimated as 0.03 ind/m^2 and 0.05 ind/m^2.

Troglobitic populations are said to be K-selected, i.e., with increased longevity, delayed reproduction, low fertility and low growth rate (Barr, 1968; Culver, 1982; Hüppop & Wilkens, 1991). Among the amblyopsids, Poulson (1963) showed that troglobitic species had increased life cycle, longer time of development and increased lifespan than the troglophilic *Chologaster agassizi*.

The troglobitic pimelodid catfish *Pimelodella kronei* was so far recorded in five caves of the upper Ribeira River Valley, southeastern Brazil (Trajano & Britski, 1992): Areias cave, Bombas resurgence, Corrego Seco cave, Alambari de Cima cave and Gurutuva shaft. The streams of Areias cave and Bombas resurgence are both right tributaries of the Betari River. The populations of these caves may either have been originated from the same evolutionary lineage (which inhabited a single cave system including the two localities) or a result of independent colonizations by the same ancestral and, thus, two different evolutionary lineages (Trajano & Britski, 1992). These are the only two populations studied in some detail so far: Pavan (1945) made morphological and behavioural observations of both; and Trajano (1987) studied the ecology, biology and morphology of the Areias population, including a comparison with the eyed catfish *Pimelodella transitoria*, the probable epigean sister species of *P. kronei*.

The ecological studies of *P. kronei* done by Trajano (1991) in a 800 m long sample of the streams from Areias cave estimated population size around 150-200 individuals and population density of 0.04 ind/m^2. The great number of recaptures in the same site where they were marked suggested a sedentary life

and possible territoriality. Growth rates less than 1mm/month and lifespan of 10-15 years, together with evidences of infrequent reproduction, indicated a K-selected life cycle.

In order to help the comprehension of the evolution of the Brazilian cave fishes, I have surveyed all localities where *P. kronei* was recorded before (see Hoenen, 1997); and, with more details, I have made field and laboratory observations of the Bombas troglobitic catfishes, making comparisons with the available data from the Areias population. The results focusing on behavioural studies are presented elsewhere (Hoenen, 1996). I present herein the ecological and morphological results.

METHODOLOGY

Between November 1993 and January 1995 I visited Bombas resurgence monthly, capturing fishes by fishing them, since hand traps and funnel-type minnow traps were not efficient. Fieldwork was done for two successive nights each trip: in the first night I captured, marked and released the fishes, and in the second I observed their behaviour.

It should be stressed that it is impossible to go further inside Bombas cave because large boulders intercept our way through the low ceiling galleries (see figure 3 in Hoenen, 1997). Thus, troglobitic catfishes are only seen and captured while wandering outside the cave by night, when they probably came out for feeding. Yet, the cave area explored by the fishes is not known.

I mostly followed the methodology of Trajano (1991). All marks and measurements were done with fishes anaesthetized in a MS-222 solution. The standard and maxillary barbel length were measured using a millimeter scale and the body weight was measured using a spring scale with 1.0 g precision. Standardization of body length measurements was achieved measuring from nose tip to end of the body, except caudal fin, since breakage of those fins usually occurs resulting in accidental measurement variation. Measurements of barbel length were always made for the right barbel, even if it seemed to be broken and regenerating.

The depigmentation rate was estimated following four categories: I, highly pigmented catfishes (dark-grey colour), but not as dark as epigean catfishes; II, semi-pigmented catfishes (light-grey colour), with dorsal region darker than ventral; III, semi-pigmented catfishes with dorsal region as light as ventral; IV, depigmented catfishes (pink-yellowish colour).

The fishes were marked by subcutaneous injection, making no more than three spots between dorsal and adipose fins. Blue and green acrylic dyes were used, following methodology described by Trajano (1991). Position of

marks, colours combinations and other individual characteristics allowed individual recognition of all marked fishes.

In order to evaluate feeding condition of Bombas population, I estimated the condition factor from standard length and weight using methodology described by Vazzoler (1981) and Trajano (1991). Although this parameter vary with sexual maturity, I was not able to determine the degree of gonadal development, because I handled only living fishes. However, from data from Areias population, it is known that the smallest fishes among the mature ones had standard length between 7.5 and 8.5 cm (Trajano, 1991). Therefore, in addition to fishes longer than 10.0 cm, as did Trajano (*op cit.*), I also considered fishes longer than 7.5 cm in my analysis, because of the large number of fishes within this range of length.

To estimate population size, I used the Fisher-Ford method, described in Begon (1979). Because of the low rate of recapture, other methods for estimating population size (e.g., Jolly and Manly-Parr - as in Begon, *op cit.*) did not work.

RESULTS AND DISCUSSION

POPULATION SIZE AND DENSITY

By December 1994 I had marked 116 fishes from Bombas resurgence, from which 11 were recaptured once and 3 were recaptured twice. This determines a recapture rate (number of recaptured fishes in relation to total number of marked ones) of 12.07%. Table 1 summarizes the data used for estimating the population sizes, and Table 2 presents the resulting monthly estimates of the *P. kronei* population size using Fisher-Ford method.

From Table 2, in one year of observations a large monthly variation was detected, between 135 and 1552 individuals, without any seasonal tendency. The resulting high value of population size in October could be explained by the fact that in this month occurred the highest capture rate within a phase of lower captures, with no recapture. This caused a mathematical overestimation. Moreover, the impossibility of going inside Bombas cave (which causes variation in capture rates because of different climatic conditions) together with errors associated with the estimation method could explain the large variations observed. However, when the extreme values are not considered, a variation from 200 to 300 individuals seems to be an acceptable average for the population size along the year.

The reduced recapture rates suggested either that the Bombas population was more numerous or that the fishes had a larger life area when compared with

Areias population data. As it is not possible to establish the total hypogean area colonized by Bombas population, the fish density in the cave can only be estimated indirectly, by comparing life area of individuals. Life areas were supposed to be similar for both cave populations by considering similar locomotor rates, since these fishes had several behavioural similarities (Hoenen, 1996). For Areias population, the life area was estimated as 200 m (Trajano, 1991). By assuming this value, the density for the Bombas population would be 0.75 ind/m^2, which is much higher than that for Areias catfishes, 0.04 ind/m^2 (Trajano, 1991). Nevertheless, this is acceptable when the concentration of fishes near resurgence, profiting from a higher food supply is considered. Once Bombas fishes are less aggressive than Areias fishes (Hoenen, 1996), a higher density of fishes in Bombas cave may be acceptable, or even expected.

On the other hand, if one expects to find the same recapture rate in both populations because the fishes would have the same behaviour and hence the same chance of being captured/recaptured, the life area and density could be reestimated. Because the recapture rate of 12% in Bombas population was about four times smaller than that of Areias cave, we could correct the Bombas density value by equally dividing it by four. The resulting Bombas density would be of 0.19 ind/m^2, which is still much higher than that of Areias cave.

In order to statistically detect if there is any difference between population size estimated for Bombas and Areias fishes, the Mann-Whitney test was applied (Siegel, 1975). The resulting statistically significant difference (n_1=11; n_2=11; U=14; p=0.002) indicated that Bombas population is larger than Areias population.

EXTERNAL MORPHOLOGY

The percentile distribution of standard length measured in Bombas catfishes is shown in Fig. 1. The resulting mode of standard length is 10.5-10.99 cm. Pavan's (1945) data for this population resulted in a mode of 13.0-14.0 cm, with a second peak between 11.0-12.0 cm, which is bigger than the present result. The values for Bombas catfishes are also smaller than those for Areias catfishes, which had a mode of 13.0-13.5 cm (Trajano & Britski, 1992). Even considering only the fishes from Areias entrance, which would be ecologically comparable to Bombas fishes, raw data from that cave (Trajano, unpublished data) showed fishes bigger than 11.0 cm and hence bigger than Bombas fishes.

Using the morphometric data of both populations presented by Trajano (1987), I have done Hotelling's multivariate statistical analysis (Kendall & Stuart, 1968) to detect any morphological difference between them. This analysis showed

a statistically significant difference (p=0.002), with Bombas fishes being smaller in all parameters analyzed. Therefore, based on collections made both recently and about 20 years ago, Bombas fishes are significatively smaller than Areias fishes.

The percentile distribution of weight measured for 116 individuals are shown in Fig 2. From the figure, the mode of 8-11.9 g, was smaller than that of Areias catfishes - 18-22 g (Trajano & Britski, 1992), which agrees with the fact that Bombas fishes are mostly smaller.

The measurements of barbel length were always made for the right barbel, even if it seemed to be broken and regenerating. Considering all measurements smaller than 3.5 cm, which correspond to broken or regenerating barbels, together with other five fishes with clear signals of barbel regeneration, 13.5% of the captured fishes had broken barbels. Barbels breakage was often detected among Areias fishes (almost 55% of the captured fishes) and regeneration occurred in a few months (Trajano, 1987). In captivity, accidental breakage of barbels of the latter fishes was unusual and the few cases were related to agonistic encounters. Probably, this was the main reason for barbel breakage also in caves thus, the less frequent barbels breakage among Bombas fishes might reinforce the hypothesis of fewer agonistic interactions and less aggressiveness in comparison with Areias fishes (Hoenen, 1996).

In relation to depigmentation rate, from the 116 captured fishes, 74.8% were classified as semi-pigmented with dark dorsal region (II); 12.6% as semi-pigmented with light dorsal region (III); 7.2% as depigmented (IV) and only 5.4% as pigmented (I). When compared with data from Areias fishes (Trajano & Britski, 1992), Bombas fishes of categories IV and III combined (19.8%) (which corresponds to "depigmented" category of Trajano & Britski) were relatively less numerous than those of Areias cave (33.5%). The percentage of pigmented fishes was also smaller in Bombas population (5.4%) than in Areias population (13%). The intermediate pigmented fishes of category II were the most abundant in both caves, but especially in Bombas population (74.8% against 53.5% in Areias).

I have never found any troglobitic catfish with vestigial eyes from Bombas resurgence - all 116 captured fishes were completely eyeless. Previous data of this population (Pavan, 1945) recorded 2 fishes with vestigial eyes among 132 fishes collected. In Areias cave, 16 among 170 fishes had visible vestigial eyes (Trajano & Britski, 1992). These data agreed with Trajano & Britski's (*op cit.*) hypothesis that ocular regression in *Pimelodella kronei* occurred faster than pigmentation regression. This has also been seen among other troglobitic fishes, such as the characid *Astyanax mexicanus* (Poulson, 1985), the clariid *Uegitglanis zammaranoi* and the cyprinid *Phreatichthys andruzzi* (Ercolini *et al.*, 1982), in which total regression of ocular structures always occurred, including optical nerves.

CONDITION FACTOR

The mean values for the condition factor at each month are plotted in figure 3, both for individuals larger than 10.0 cm and larger than 7.5 cm (which includes those larger than 10.0 cm too). Only the results for individuals larger than 10.0 cm were considered in order to compare with Trajano's data (Trajano, 1991). The general tendencies of both curves are very similar, suggesting that considering values for fishes as small as 7.5 cm does not affect results greatly.

Monthly values obtained in this work for catfishes more than 10 cm long were higher than those presented by Trajano (1991) for Areias population, except for September. This result indicates that Bombas population consisted of better nourished fishes, probably because they are exploiting a habitat with higher food supply. This is true at least for the sampled Bombas fishes, which live near the entrance. If fishes exist which only dwell inside Bombas cave, they were not included in this study because of the impossibility of going inside (even trying to dive throughout the boulders was not possible so far).

Coherently, values recorded for individuals captured near Areias entrance (1.5×10^{-2}) and for individuals maintained in captivity and regularly fed (1.2×10^{-2}) (Trajano, *op cit.*) are included in the range detected herein (1.1×10^{-2} - 1.6×10^{-2}). Therefore, it would be very interesting if breaking into Bombas cave becomes possible because it would allow the collection and study of "deep-dwelling" fishes.

In turn, when considering diet data from gut contents analysis (Trajano, 1989), the percentage of empty guts among Bombas fishes was higher than that of Areias fishes, which led that author to suppose that the former were subjected to scarce food supply or were inefficient in food uptaking. However, according to the collectors of the fishes analyzed by Trajano (H.A. Britski, pers.comm.), the animals were captured during the day. If so, it is possible that the great number of empty guts could be explained by the fact that the fishes had just come out to feed still during daytime and did not have time enough to eat anything before being captured. In contrast, because all the present collections were made during the night, the fishes were heavier probably because they had time enough to feed. Furthermore, we can not discard the possibility that Bombas fishes are actually larger nowadays than they have been years ago, hypothetically due to either changes in food availability in the environment or enhanced food detection and capture by the fishes.

Among all 116 fishes captured, only seven had standard length smaller than 7.5 cm. In addition to not showing any seasonal tendency, the range of values of condition factor estimated for the small fishes (0.7×10^{-2} - $1,7 \times 10^{-2}$) includes those ranges obtained for fishes larger than 7.5 cm (1.2×10^{-2} - 1.6×10^{-2}). This result shows that small fishes were also well nourished, and among them there were even several proportionally heavier than the larger fishes.

GENERAL DISCUSSION AND CONCLUSIONS

The population of Bombas resurgence ended to be more numerous and showed a higher density than the Areias population. This probably occurred due to a concentration of fishes near the resurgence, profiting from the higher food supply.

Considering morphological data, Bombas fishes were smaller than Areias fishes in all parameters measured, including standard length and weight measured in the field, even though values of condition factor were higher for Bombas fishes. Thus, although smaller, they were proportionally heavier. Again, this result could be explained by the higher food supply on the resurgence. In Bombas population, most fishes were semi-pigmented. Thus, Bombas population seemed to be more homogeneous than Areias population, which had a higher variation of fish pigmentation.

Morphological similarities between the two cave populations (lack of eyes and pigmentation) may be due to modifications related to cave life and may represent convergences, since they differ from those of epigean eyed catfishes and even from the probable epigean sister species. On the other hand, morphological differences may be related to ecologically different habitats.

As for the phylogenetic relationships, two hypothesis can be considered: 1) similarities may represent sinapomorphies, considering one single event of colonization, either within a single range including both caves, or inside one cave with later dispersion to the other; or 2) similarities represent convergences, considering independent colonizations from the same ancestor. It should be noted that those similarities in the reduction processes are common among very distantly related troglobitic species (such as different arthropods and vertebrates), and are thus commonly related to convergent evolution. Moreover, the caves are located far from each other and, unfortunately, the test of colouring the waters was not done yet - thus, it is not known if the rivers of the two caves connect. If they do, this would favour the first hypothesis; if not, the second.

From the ecological point of view, both populations show autapomorphic behavioural characteristics: attenuation of aggressiveness for Bombas population, and reduction of photophoby (although politypic) for Areias population (see Hoenen, 1996).

Therefore, although it is not possible, so far, to assure that morphological and behavioural similarities are synapomorphies or convergences, there are many evidences (especially the fact that each population has its autapomorphies) to consider them as different lineages and, at least using the phylogenetic concept, different species. However, because this is not a taxonomic paper and because all data concerning cave catfishes populations should be further analyzed, the establishment of two species will not be done herein.

Table 1. Number of marked *Pimelodella kronei* recaptured in Bombas resurgence by marking date. CAPT.: number of fishes captured. COLL.: number of fishes collected alive for laboratory observations (*: fishes fixed). REL.: number of fishes released.

DATE	DAY	CAPT.	COLL.	REL.	RECAPTURES FROM EACH MARKING DATE									
					1	38	65	94	120	151	178	215	248	283
02.94	1	22	-	22										
03.94	38	13	-	13	0									
04.94	65	14	4	10	1	0								
05.94	94	2	-	2	0	0	0							
06.94	120	32	-	32	1	1	3	1						
07.94	151	7	-	7	0	1	1	0	1					
08.94	178	8	-	8	2	0	0	0	0	0				
09.94	215	3	-	3	0	0	0	0	0	0	0			
10.94	248	15	1*	14	0	0	0	0	0	0	0	0		
11.94	283	6	1*	5	1	0	0	0	0	0	0	0	0	
12.94	306	4	3	1	0	0	0	0	1	0	0	0	0	0

Table 2. *Pimelodella kronei* population size in Bombas resurgence, estimated using the Fisher-Ford method. POP. : number of individuals estimated in the population, for each date. Resulting day-to-day survival rate = 1.0.

DATE	DAY	POP.
02.94	1	0
03.94	38	308
04.94	65	263
05.94	94	135
06.94	120	222
07.94	151	158
08.94	178	258
09.94	215	376
10.94	248	1552
11.94	283	389
12.94	306	290
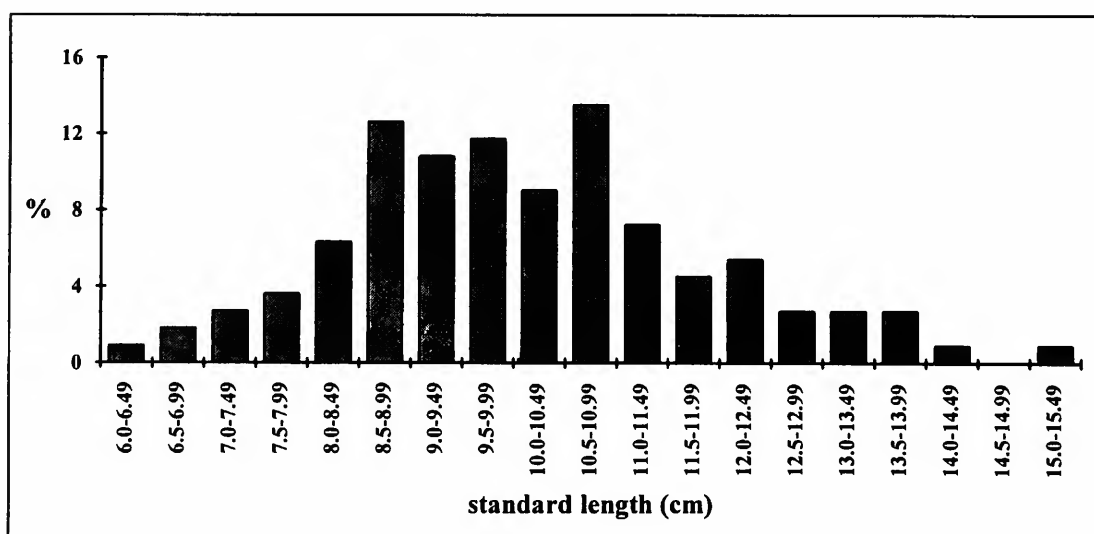
Figure 1. Percentile distribution of standard length measured in *Pimelodella kronei* fishes from Bombas resurgence.
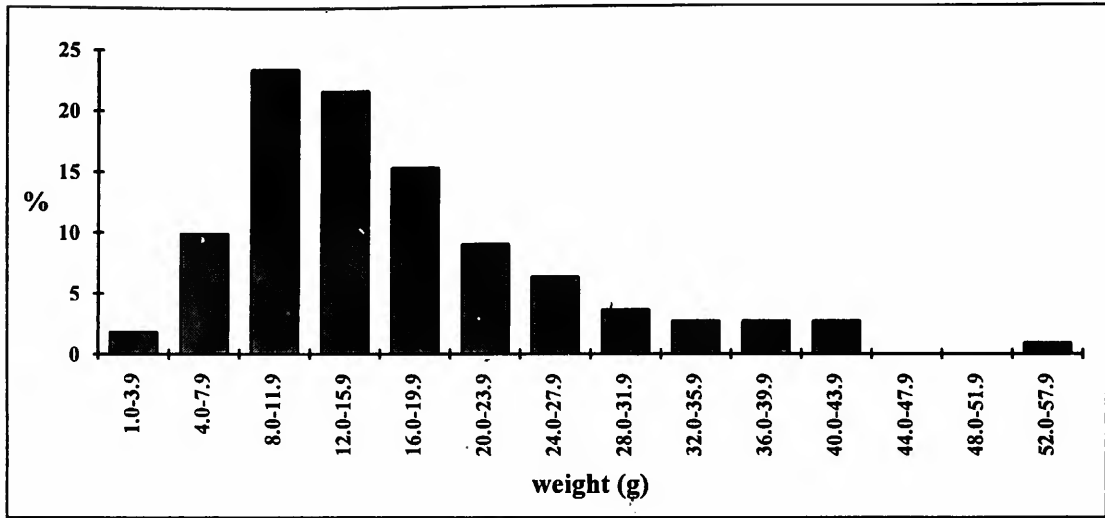
Figure 2. Percentile distribution of weight measured in *Pimelodella kronei* fishes from Bombas resurgence.
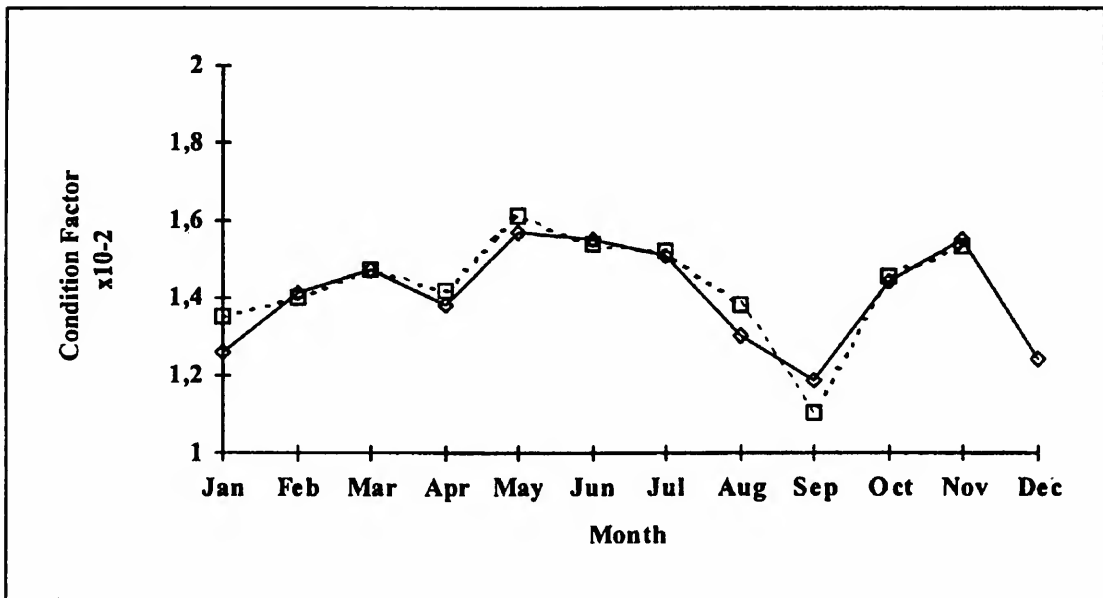

Figure 3. Mean condition factor values of *Pimelodella kronei* from Bombas resurgence for individuals larger than 10.0 cm (◆) and larger than 7.5 cm (■) (hence including those larger than 10.0 cm).

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