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# Influence of environmental integrity on feeding, condition and reproduction of *Phalloceros harpagos* Lucinda, 2008 in the Tarumã stream micro-basin

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**ABSTRACT.** With the objective of evaluating the influence of the environmental integrity on feeding, condition factor and reproduction of *Phalloceros harpagos*, the species biology was analyzed in the Tarumã Stream micro-basin, Naviraí, Mato Grosso do Sul State (upper Paraná river). Samples were collected from 2007 to 2010, and biometric data of specimens was registered. Stomach and gonad were taken for the analysis of feeding and reproduction, respectively. The species is detritivore, showing a high flexibility in the diet. Debris and sediment, followed by plant and algae were the most ingested items. As for the condition factor, no definite pattern was observed in the values for both sexes, probably because the species has a long reproductive period. The frequency of occurrence distribution of gonad maturity stages indicated a greater number of immature females in the dry season at one of the least impacted sites. On the other hand, in the most affected site females were observed with increased fecundity. From the results obtained, it was found that the different levels of environmental degradation have no significant influence on feeding and condition factor, but rather probably exert influence on the reproduction of the species.

**Keywords:** Poeciliidae, guppy, environmental degradation.

## Influência da integridade ambiental na alimentação, condição e reprodução de *Phalloceros harpagos* Lucinda, 2008 na microbacia do córrego Tarumã

**RESUMO.** Com o objetivo de avaliar a influência da integridade ambiental na alimentação, fator de condição e reprodução de *Phalloceros harpagos* a biologia da espécie foi analisada na microbacia do córrego Tarumã, Alto Rio Paraná. As coletas foram realizadas de 2007 a 2010 e os exemplares tiveram as suas informações biométricas anotadas. Foram retirados os estômagos e as gônadas para as análises da alimentação e reprodução, respectivamente. A espécie é detritívora e apresentou alta flexibilidade na dieta, sendo detritos/sedimento, seguido por vegetais e algas, os itens ingeridos em maior quantidade. Quanto ao fator de condição, não foi observado um padrão sazonal ou espacial nos valores encontrados para ambos os sexos, provavelmente devido ao fato da espécie apresentar um prolongado período reprodutivo. A distribuição de frequência de ocorrência de estágios de maturação gonadal indicou maior quantidade de fêmeas imaturas durante a estação seca em um dos locais menos impactados. Por outro lado, no local mais impactado foram observadas fêmeas com maior fecundidade. A partir dos resultados obtidos, foi verificado que os diferentes estados de degradação ambiental não possuem influência significativa sobre a alimentação e fator de condição, mas, provavelmente, exerceram influência sobre a reprodução da espécie.

**Palavras-chave:** Poeciliidae, barrigudinho, degradação ambiental.

## Introduction

The use of fish as bioindicators of environmental conditions is justified by their biological and socioeconomic importance, and indeed indices based on fish species have been developed worldwide to assess the ecological status of rivers where they live (ROSET et al., 2007). Besides that, Cetra and Petrere (2006) stated that fish provide an integrated view of the aquatic environment by the availability of information, wide range of foods, habitats and by their representativeness in aquatic food webs. The study of fish diets in streams under the influence of different environmental disturbances is useful to assess the biotic integrity of streams, providing important information on the adaptability of species tolerant to such environmental conditions and the actual needs to support conservation and restoration actions of degraded areas (BONATO et al., 2012).

Following the same reasoning, the condition factor is widely used in studies on fish biology, as it provides important information about the physiological status of the animals from the assumption that heavier individuals at a given length are in a better condition. Thus, changes in this index can be used as additional data to the study of seasonal cycles of feeding and reproduction and also to understand how the physiological state of these animals is conditioned by the interaction of other factors, like environmental integrity (LIMA-JUNIOR et al., 2002; SANTOS et al., 2006; VAZZOLER, 1996).

In the same way that food habits and condition factor of fish may change, reproduction can also be modified by the environmental integrity. According to Vazzoler (1996), the beginning and end of the breeding season of fish depend on good environmental conditions, availability of dissolved oxygen and nutrients in the early periods of growth and minimal predation risk to offspring. In agreement with Gomiero et al. (2007), the knowledge of reproductive strategies is of utmost importance to guide measures for the management and conservation of fish fauna, given the impacts caused by human activities. Also importantly is the knowledge of feeding interactions between fish and riparian systems for the rehabilitation of degraded environments and to prevent depletion of fish stocks.

Tarumã and Touro streams, objects of this study, are small water bodies that cross the urban area of the municipality of Naviraí, Mato Grosso do Sul State (Amambai river Basin, upper Paraná river), and as a consequence are under impacts related to human activities, such as the dumping of domestic and industrial sewage, removal of riparian vegetation, and many other impacts that lead to changes in the aquatic environment and biodiversity thereof. Since 2005, the Public Ministry of Mato Grosso do Sul, State have been working to promote the restoration of riparian vegetation in the micro-basin of the Tarumã Stream and prevent irregular discharge of sewage into the waters of the streams of this micro-basin, as well as providing, through Terms of Adjustment of Conduct applied to companies in illegal situation, funding research projects for monitoring of biotic and abiotic factors in the micro-basin. This is the background of the present work.

*Phalloceros harpagos*, popularly known as guppy, belongs to the family Poeciliidae. In general, species of this family exhibit marked sexual dimorphism, with females larger than males. They are considered omnivorous, feeding mainly on aquatic and terrestrial invertebrates, detritus, algae and plants, but have a tendency to eat insect larvae, are present in many different habitats, from tropical to temperate zones, and show a high adaptability and tolerance to thermal and salinity variations. Besides, they are most commonly found in lentic habitats, especially along their banks (NASCIMENTO; GURGEL, 2000).

Based on this context, this study aimed at evaluating the influence of the environmental integrity - considering organic and inorganic water pollution and degradation of riparian vegetation - on feeding, condition factor and reproduction of *Phalloceros harpagos* in the micro-basin of the Tarumã Stream, Naviraí, Mato Grosso do Sul State.

## Material and methods

Samplings were conducted from 2007 to 2010, twice a year during the dry (August) and rainy (February) seasons, at four different sites distributed in the micro-basin of Tarumã and Touro streams (23°00' - 23°10'S and 54°15' - 54°20'W) (Figure1).

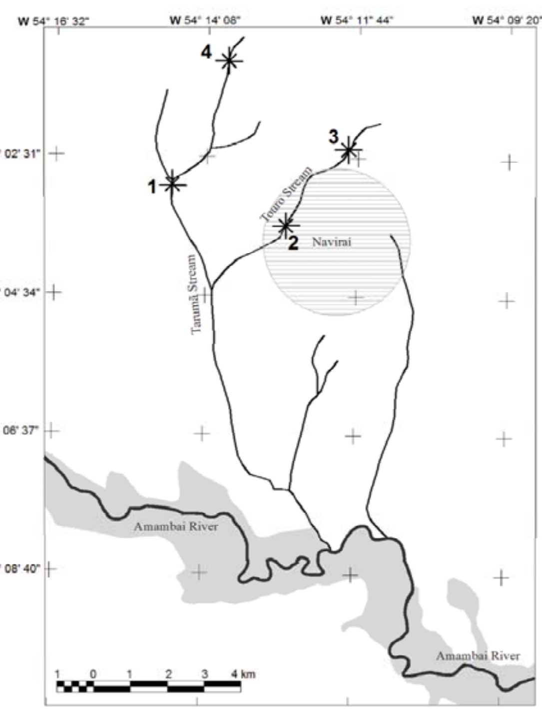


**Figure 1.** Location of the Tarumã Stream micro-basin, identifying the sampled sites numbered from 1 to 4.

Site 1 - located in the Tarumã Stream, has no waste on its banks or water, with pasture as the dominant vegetation on its banks; site 2 - located in the Touro Stream, passes through the city of Naviraí, Mato Grosso do Sul State, and consequently receives domestic sewage and waste, which were present in large amounts on its banks or water, with shrubs as the dominant vegetation on its margins; site 3 - corresponds to the headwaters of the Touro Stream, has no waste on its banks or water, with pasture as the dominant vegetation on its banks; site 4 - is the headwaters of the Tarumã Stream, shows little amount of waste on its banks or water, with pasture as the dominant vegetation on its banks.

For data analysis, sampled sites were divided into "least impacted" (sites 1, 3 and 4) and "most impacted" (site 2). The following environmental variables were measured in each site: stream width (m) and depth (cm) – with a rigid measuring tape –, and altitude (m), pH, water temperature (°C), conductivity (μS $cm^{-1}$), total dissolved solids (ppm) and oxidation-reduction potential (mV), with a multiparameter analyzer. Table 1 lists the characteristics of the sites from the mean values observed for each of the eight environmental variables studied.

**Table 1.** Characterization of the sampling sites by the mean values observed for each of the eight environmental variables studied.

| | Sampled sites | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Width (m) | 3.00 | 2.00 | 1.00 | 1.50 |
| Depth (cm) | 50.00 | 20.00 | 30.00 | 25.00 |
| Altitude (m) | 292.00 | 301.00 | 329.00 | 341.00 |
| pH | 6.95 | 6.69 | 6.28 | 5.84 |
| Water temperature (°C) | 23.35 | 24.56 | 21.96 | 23.12 |
| Conductivity (μS $cm^{-1}$) | 11.71 | 95.71 | 30.57 | 10.86 |
| Total dissolved solids (ppm) | 6.14 | 48.14 | 15.71 | 5.57 |
| Oxidation-reduction potential (mV) | 153.73 | 138.41 | 153.74 | 205.00 |

The least impacted sites showed lower values for conductivity and TDS. The site rated as the most impacted (site 2) has the highest values of conductivity and TDS (Table 1).

The collection of the individuals was performed in the afternoon using a rectangular sieve (80 x 120 cm), with a mesh of about 2 mm, repeating 20 times at each location in each collection. Specimens of *P. harpagos* were fixed in the field and biometric data was subsequently registered, including total and standard length (mm), total weight (g), sex and gonad maturity stage.

For the diet analysis, the contents of the stomachs with food (n = 461) were weighed (g), inspected (using a stereo-microscope and a microscope), and each food item was given a value proportional to its abundance. The reference used for these values was the standard weight (SW), which is the approximate arithmetic mean of the stomach content weight of the sample, as proposed by Lima-Junior and Goitein (2001). Assuming that the SW is equivalent to 4 points, the stomach contents were initially assigned a total value according to the proportion of its mass to the SW. Based on a simple visual inspection, this total value was then divided among the food items, according to their relative volume.

Each site, at each season, was then considered as a separate sample, and the Importance index of the food items was calculated as follows, according to the method described by Lima-Junior and Goitein (2001): the sum of points attributed to each food item, divided by the number of stomachs with food in the size class, resulted in the mean of the ascribed values of each food item. This mean was multiplied by 25 to be transformed into a percent value, the so-called Volumetric analysis index (Vi). The result obtained through the multiplication of Vi by the frequency of occurrence (HYSLOP, 1980) of each item corresponds to the Importance index of the food item in the sample. These results were then statistically analyzed applying the method described by Fritz (1974), in which the food items are ranked in each sample and compared using the Spearman rank correlation coefficients. The correlation was considered statistically significant when $p < 0.05$.

The analysis of covariance (SOKAL; ROHLF, 1995) was employed to determine the condition factor. For this analysis, data of length and weight were first converted into natural logarithms to obtain a linear relationship between these variables. Males and females were examined separately. For cases in which the spatial factor had a significant influence on the adjusted weight of individuals, we used the Tukey-Kramer *a posteriori* test, in order to perform multiple comparisons between sites. The significance level was set at 0.05.

For the analysis of reproduction, it was performed the classification and distribution of the frequency of occurrence of maturity stages. As it is a species with internal embryonic development, gonads were macroscopically classified into five different stages, based on the study of Machado et al. (2001), with some adaptations: A-immature: reduced size and translucent ovaries, whitish, B-mature oocytes and/or fertilized eggs: with much yolk, yellowish; C- early embryo: early spinal cord and eyes, with little or no dorsal pigmentation; D-intermediate embryo: with large eyes and accentuated dorsal pigmentation, moderate yolk; E-late embryos: little or no yolk, formed body, almost ready to hatch out.

In order to check for significant differences in the frequency of occurrence distribution of gonad maturity stages between sites and seasons analyzed, a chi-square contingency table was applied.

The absolute fecundity was estimated by counting the oocytes/embryos in the samples. To check for differences in fecundity between sampling sites and seasons, separately, a one-way analysis of variance (ANOVA) was employed, followed by *a posteriori* Tukey's test in order to draw comparisons between sites.

## Results

After analyzing 461 specimens with some content in the stomach, it was possible to observe that the most important food items consumed in both seasons were debris/sediment, followed by plant remains (dry season) and algae (rainy season). Adult insect and larvae were also found, chironomids was the most abundant item within this group. Arachnida, microcrustaceans (Cladocera and Copepoda) and fish remains (scales and bones) were also registered in smaller proportions. Tables 2 and 3 list the AI found in the diet of the species, at each site and season.

**Table 2.** Values of Importance index of food items (AIi) found in the diet of *P. harpagos* in the sites sampled in the dry season (n = 269).

| Food items | Sampled sites | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | n = 38 | n = 41 | n = 61 | n = 129 |
| Detritus/sediment | 19.633 | 30.207 | 85.123 | 25.713 |
| Plants remains | 6.752 | 7.830 | 20.194 | 1.581 |
| Algae | 4.176 | - | 32.746 | - |
| Insect remains | | | | |
| Formicidae | - | 0.030 | - | - |
| Insect larvae | | | | |
| Coleoptera | - | - | 0.003 | - |
| Elmidae | - | 0.006 | - | - |
| Chaoboridae | - | 0.097 | - | - |
| Simuliidae | 0.074 | - | - | - |
| Chironomidae | 0.070 | 6.273 | 0.413 | 0.026 |
| Trichoptera | 0.002 | - | - | - |
| Fish remains (scales and bones) | - | - | 0.134 | - |

The comparison of the AI at each site in both seasons and between sites by the Spearman rank correlation coefficient pointed out a significant correlation ($p < 0.05$), i.e. absence of significant difference, in most comparisons, except between sites 1 x 2 ($p = 0.058$) and 1 x 4 ($p = 0.061$) during the dry season, and in the seasonal comparison of the site 1 ($p = 0.110$), which indicate differences in the order of importance of the food items for the species in these three comparisons.

The ANCOVA results for the condition factor showed that males presented higher condition factor in the site 2 in the dry season, when compared with the site 4, and during the rainy season between sites 1 x 3, 1 x 4 and 2 x 3 ($p < 0.05$). For females, in the dry season, the site 4 differed from the other sites and in the rainy season, significant differences were detected ($p < 0.05$) between sites 1 x 2, 1 x 3, 1 x 4, 2 x 3 and 3 x 4 (Table 4 and Figure 2).

**Table 3.** Values of Importance index of food items (AIi) found in the diet of *P. harpagos* in the sites sampled in the rainy season (n = 192).

| Food items | Sampled sites | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | n = 23 | n = 21 | n = 61 | n = 87 |
| Detritus/sediment | 18.157 | 26.281 | 45.289 | 52.707 |
| Plants remains | 6.493 | 2.245 | 14.844 | 4.257 |
| Algae | 18.093 | 2.358 | 16.436 | 21.745 |
| Insect remains | | | | |
| Ephemeroptera | 0.379 | - | 0.024 | 0.001 |
| Formicidae | - | - | 0.005 | - |
| Odonata | - | - | - | 0.007 |
| Insect larvae | | | | |
| Dytiscidae | - | - | 0.020 | - |
| Chironomidae | 0.359 | 2.041 | 0.399 | 0.035 |
| Chaoboridae | 0.000 | 0.057 | 0.040 | - |
| Diptera Pupa | 0.118 | - | 0.013 | - |
| Plecoptera | - | - | 0.007 | - |
| Odonata | 0.047 | - | - | - |
| Class Arachnida | | | | |
| Acari | - | - | - | 0.002 |
| Araneae | - | - | 0.017 | 0.002 |
| Microcrustaceans (Cladocera and Copepoda) | - | - | 0.010 | 0.010 |
| Fish remains (scales and bones) | 0.014 | - | - | - |

**Table 4.** ANCOVA results for the condition factor analysis, separating individuals by sex and seasons for site comparisons. Differences are significant when $p < 0.05$. NS: non-significant difference.

| Site comparisons | Male | | Female | |
|---|---|---|---|---|
| | dry season | rainy season | dry season | rainy season |
| 1 x 2 | NS | NS | NS | p < 0.05 |
| 1 x 3 | NS | p < 0.05 | NS | p < 0.05 |
| 1 x 4 | NS | p < 0.05 | p < 0.05 | p < 0.05 |
| 2 x 3 | NS | p < 0.05 | NS | p < 0.05 |
| 2 x 4 | p < 0.05 | NS | p < 0.05 | NS |
| 3 x 4 | NS | NS | p < 0.05 | p < 0.05 |

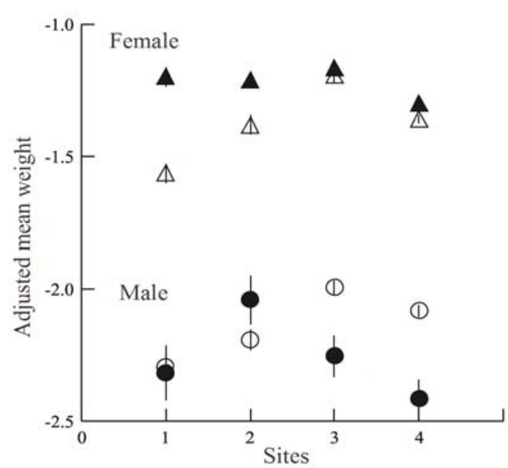


**Figure 2.** Mean values and standard deviation of the condition factors for males and females of *Phalloceros harpagos*, in the sites and seasons analyzed (black circle=dry season, empty circle=rainy season).

The chi-square contingency table test applied to the frequency of occurrence distribution of maturity stages in the sites and seasons considered (Figure 3) evidenced significant spatial differences in the dry season, in which the site 1 was different from the others, especially due to the higher occurrence of the stage A (41.2%) and absence of the stage C

($X^2 = 28.74$; $p = 0.004$). The same analysis, performed for each pair of data from the same site in the comparison between seasons, showed no significant seasonal differences ($p > 0.05$) in the distribution of gonad maturity stages.

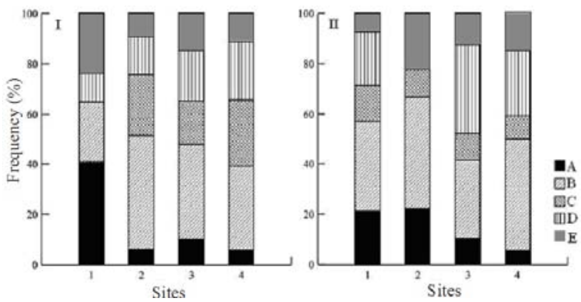


**Figure 3.** Frequency of occurrence distribution of the maturity stages in the sites and seasons analyzed, where I: dry season, II: rainy season, A: immature, B: mature oocytes and/or fertilized eggs, C: early embryo, D: intermediate embryo and E: late embryos.

Females of the site 2 presented higher fecundity in both seasons. In the other sites, the dry season had the highest reproductive activity (Figure 4). The ANOVA results evidenced a significant difference in the fecundity during the dry season ($F = 5.878$; $p = 0.001$), with differences between sites 3 x 2 ($p < 0.001$) and 4 x 2 ($p = 0.004$). In the rainy season it was also verified a difference between sites ($F = 20.214$; $p < 0.001$), and the site 2 was different from the other sites examined ($p < 0.05$).

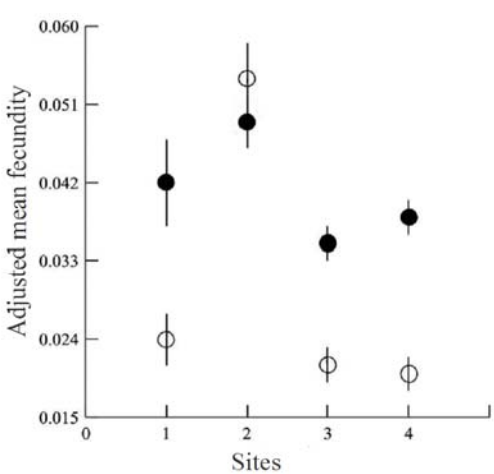


**Figure 4.** Mean values and standard deviation of the fecundity for females of *P. harpagos* observed in both seasons analyzed (black circle=dry season, empty circle=rainy season).

## Discussion

In the present study, the most important food item consumed by the species in both seasons and in all sites examined was detritus/sediment. Significant amounts of plant and algae were also found, indicating that, in the location studied, the species behaves as detritivorous. Although Cyprinodontiformes are traditionally considered as insectivorous (ARANHA; CARAMACHI, 1999), Oliveira and Bennemann (2005) described *Phalloceros caudimaculatus* as a detritivorous fish. On the other hand, Fogaça et al. (2003) classified this species as algivorous. There are also studies where it was classified as omnivorous (GOMIERO; BRAGA, 2008), omnivorous with tendency to insectivory (CASTRO; CASSATTI, 1997), omnivorous with tendency to herbivory (CASATTI, 2002; SABINO; CASTRO, 1990), insectivorous (UIEDA et al., 1997) and herbivorous (ARANHA; CARAMASCHI, 1999). It is also noteworthy that Deus and Petrere-Junior (2003) evaluated seasonal changes in the diet of *P. harpagos*, and observed a detritivorous behavior in the summer and algivorous behavior in the winter, besides finding some few invertebrates in the stomach content.

Among insect larvae registered in stomachs, those belonging to the family Chironomidae were the most abundant in the site 2, which is explained by the higher level of environmental degradation at this site (SOUZA; LIMA-JUNIOR, 2013), and because these larvae are considered r-strategist organisms, with high fecundity and hemoglobin to withstand low oxygen concentrations (BAXTER, 1977). These organisms may be used in studies of environmental assessment and biomonitoring, as they are widely distributed in most aquatic ecosystems with high density and richness (HIRABAYASHI; WOOTON, 1998).

All these authors confirm the results obtained herein, since the species has high flexibility in its diet, as probably the most exploited resources were those available at the time. In Touro and Tarumã streams it is a typically detritivorous fish, but also consumes plant material, algae and insects.

In agreement with Vazzoler (1996), the condition factor also indicates conditions of feeding and varies with the sexual maturation cycle, which coupled with other evidence and information, may indicate the breeding period of most fish. In the present study, it was not observed a spatial or temporal pattern in the values of the condition factors for both sexes, which can be associated with the extended reproductive period (ARANHA; CARAMASCHI, 1999) and because reproduction is an activity that plays a great influence on the condition factor.

Furthermore, Machado et al. (2001) stated that the reproduction of the guppy in southeastern Brazil is extremely seasonal, different from observed in our results. According to these authors, eggs begin to mature during the cold-dry season, with the first mating probably occurring in June-July. The total incubation time is about three months, and the first juvenile recruitment occurs in October-November, in the early warm-rainy season. Gurgel et al. (1991) suggest that the reduction in the condition factor may be related to the use of body reserves for gonadal development. Additionally, Gurgel et al. (1997) point out that the condition factor may be changed depending on intrinsic factors (organic reserves, gonad development and size of the specimens) and extrinsic factors (food availability, temperature and photoperiod).

Araujo et al. (2009) analyzed *P. reticulata* and *P. caudimaculatus* and observed that in general the condition factor has not changed among sites studied, except for *P. reticulata* males, which presented higher condition in a location with higher availability of food due to the organic input from urban effluents. Environments where fish undergo major and frequent erratic physical and chemical changes may cause stressful or lethal conditions for the biota (MARTIN-SMITH, 1998, MEADOR; GOLDESTEIN, 2003), but *P. harpagos* does not act in such a way, because the species has high adaptability, and is able to survive in both environments with riparian vegetation as deforested ones (CASTRO; CASATTI, 1997).

The reproductive cycle of freshwater fish can be influenced by several factors, including photoperiod, temperature, water flow, availability of food and nest sites (LOWE-MCCONNELL, 1979). Machado et al. (2001) examined *P. caudimaculatus* in a stream in São Paulo State and observed a positive correlation between the day length and the frequency of females with offspring, indicating that the breeding season of this species can be triggered by photoperiod. In the present study, a significant spatial difference was detected in the frequency distribution of maturity stages only in the dry season, in which the maturity stages observed in the site 1 differed from those observed in other sites, with a higher frequency of immature females. Considering that Poeciliidae are non-migratory fish (AGOSTINHO et al., 2007), spatial differences can be assigned to different local characteristics. Despite the site 1 shows larger volume of water and contains no waste on its banks, probably in the dry season the food availability was lower when compared with sites 2 and 3 (evidenced by the lower values of importance index of food items), leading to a lower development of females, which were smaller at this location. On the other hand, the lack of difference between the frequency distribution of maturity stages in the rainy season may be related to increased volume of water and hence of food availability, creating thus more favorable conditions for a better reproductive development.

Similarly to the feeding habits of fish, reproduction can also be modified by environmental quality. Environmental factors cause different effects depending on the stage of the reproductive cycle, such as long-term effects on the growth of gonadal tissue and, at short-term, maturation and release of oocytes.

As observed by Machado et al. (2001) for *P. caudimaculatus* in a stream of São Paulo State, the number of embryos is positively correlated to the size of females. In this way, the reproductive success varies with investment of each female, but also according to environmental conditions such as temperature and availability of resources. Females of the site 2, in both seasons, presented higher fecundity, which can be related to the higher consumption of insect larvae a food item energetically more advantageous than plant material (JOBLING, 1994). The site 2 is the most affected, and its pollution largely derives from wastewater, which leads to a higher input of organic matter to the environment, favoring the proliferation of Chironomidae, which as above discussed, are highly adaptable to impacted environments (BAXTER, 1977). Jobling (1995) and Vazzoler (1996) point a direct relationship between food availability and fecundity, whereas Jobling (1994) states that given a decline in the food supply for fish, only few individuals present gonadal development.

## Conclusion

The different levels of environmental degradation had no major influence on the feeding of *P. harpagos*, which showed detritivorous habits. Also, had no great influence on the condition factor, probably because the species has a long reproductive period and therefore do not present a clear pattern of variation in this index. Nevertheless, the different states of environmental degradation probably influence the reproduction, once the least impacted sites presented a greater number of immature females during the dry season. And, in the most impacted site, females had higher fecundity, possibly because they had available more energetic food.

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