Life cycle of *Cyclestheria hislopi* (Baird, 1859) (Crustacea, Branchiopoda)

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(With 7 figures)

Abstract

The Conchostraca (clam shrimps) are a group of microcrustaceans found in freshwater habitats. They inhabit the benthos, yet many can swim actively and are often associated with macrophytes. They are filter-feeders, deriving their food from suspended particles or solids stirred up from the bottom. In Brazil, five species have been recorded and the life cycle of one of these, *Cyclestheria hislopi* (Baird, 1859) was investigated in this study. Specimens were collected from fish-farming tanks in upstate São Paulo and from the floodplain of the Miranda River, in the Mato Grosso swamplands of center-west, Brazil. We determined the following variables: post-embryonic development, duration of life cycle (longevity), mean period between broods, duration of instars and individual growth. Taxonomic features of the *Cyclestheria* sp. specimens from Mato Grosso were also assessed to check the possibility that they belonged to another species of the same genus or a separate ecotype of *C. hislopi*.

Keywords: Cyclestheria hislopi, Conchostraca, life cycle.

Ciclo de vida de *Cyclestheria hislopi* (Baird, 1859) (Crustacea, Branchiopoda)

Resumo

Os Conchostraca são microcrustáceos de água doce, de hábito bentônico, embora muitos nadem ativamente e se associem a macrófitas. São filtradores, alimentando-se de partículas suspensas ou ressuspensas do substrato. No Brasil há registros de ocorrência de cinco espécies. No presente trabalho, estudou-se o ciclo de vida da espécie *Cyclestheria hislopi*, coletada em tanques de piscicultura do CEPTA – Centro de Pesquisa e Treinamento em Aquicultura, em Pirassununga, e também indivíduos coletados em lagoas de inundação às margens do Rio Miranda, na localidade do Passo do Lontra, no Pantanal mato-grossense. As seguintes variáveis do seu ciclo de vida foram avaliadas: desenvolvimento pós-embrionário, duração do ciclo de vida ou longevidade, período médio entre as ninhadas, duração dos instares e crescimento individual. A taxonomia de indivíduos de *Cyclestheria* sp. oriundos do Pantanal também foi analisada, devido à possibilidade de que se tratasse de uma nova espécie deste gênero ou de um ecótipo.

Palavras-chave: Cyclestheria hislopi, Conchostraca, ciclo de vida.

1. Introduction

The class Branchiopoda, a primitive group typified by clam shrimps and water fleas, within the subphylum Crustacea, is subdivided into three orders: Anostraca, Notostraca and Diplostraca. This last is further divided into four suborders: Laevicaudata, Spinicaudata, Cyclestherida and Cladocera (Martin and Davis, 2001). Three of these suborders, Laevicaudata, Spinicaudata and Cyclestherida can be grouped together loosely under the name Conchostraca, a now discontinued order.

The Conchostraca are found on all the continents except Antarctica and are characterized by the body being almost entirely enclosed by a carapace that resembles a bivalve shell. This shell is held on the body by a strong adductor muscle which passes through the dorso-anterior portion of the body. The latter consists of two parts, the head and the post-cephalic trunk (Olesen, 1998; Olesen et al., 1996).

Most of the group is benthic and, while the typical habitats differ from species to species, the various species may be found in any temporary pond or similar habitat and their mode of distribution – by means of long-lasting eggs that are carried by water currents or wind – means that some species are very widespread (Martin and Boyce, 2004). They use both the trunk appendages and the second pair of antennae for locomotion and feed on suspended particles.

In Brazil, five species of conchostracans have been recorded so far, belonging to the Cyzicidae, Cyclestheriidae and Limnadiidae families (Young, 1998). In the state of São Paulo, only one species has been recorded to date, namely the *Cyclestheria hislopi* (Baird, 1859) (Figure 1) in the family Cyclestheriidae. This is the only conchostracan that does not appear in the initial phase of temporary water bodies, but develops its population later on (Roessler, 1995).

According to Roessler (1995) in Colombia, wild *C. hislopi* specimens prefer to stay at a specific depth that depends on water temperature, amount of sunlight and type of vegetation; when macrophytes are absent, at the well-oxygenated edges of pools, these animals hide in the soft mud of the bottom, where they begin to secrete sheets of mucus over the outer surface of the "valves" of the carapace. After a time, the individual becomes wrapped in a capsule of mucus, with two openings to allow water to flow continuously through the capsule, impelled by the beating of the appendages used for filter-feeding.

In this study, we recorded some of the features of the lifecycle of laboratory cultured *C. hislopi* from specimens collected from fish-farming tanks in São Paulo state and from flooded river banks in the Mato Grosso swamplands. Small differences in size and development times noticed in cultures raised the possibility that the two sets of specimens might belong to different sub-species (implying the existence of an ongoing speciation process for *Cyclestheria*). Therefore, it is worth analysing individuals from each population, as well as making a detailed analysis of their life cycle.

2. Material and Methods

2.1. Organisms

Two sets of specimens were used in the life cycle and taxonomic studies. In the first set, specimens of *Cyclestheria hislopi* were collected in fish-farming tanks maintained by the Aquaculture Research and Training Center (CEPTA) of the environmental authority IBAMA, on the banks of Mogi-Guaçu River, in the rural district of Cachoeira das Emas in the city of Pirassununga (SP, Brazil). In the other set, *Cyclestheria* sp. specimens were taken from flooded areas beside the Miranda River at a spot known as Passo do Lontra, located at longitude 57° 00' 42'' W and latitude 19° 34' 37'' S in the extended swamplands of Mato Grosso in center-west Brazil. Specimens were collected with a zooplankton net with 68 μm aperture.

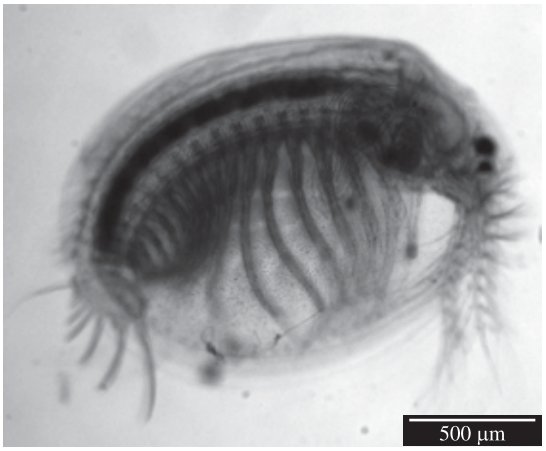

Figure 1. General view of adult *Cyclestheria hislopi* carrying embryos in the brood pouch, in a culture of specimens collected from fish-farming tanks at CEPTA/ IBAMA, Pirassununga, SP, Brazil.

2.2. Methods

Adult females of *Cyclestheria* sp. were individually placed in 100 mL atoxic plastic beakers, Copasa trade, with 50 mL of reconstituted fresh water and maintained at 25 ° ± 2 °C under a 12:12 photoperiod, with 8 replicates in each experiment. These animals were fed twice a week with a suspension (final density 5.10^6 cells/mL) of the microscopic chlorophycean *Pseudokirchneriella subcapitata*.

The number of days from the birth of the neonate to its first production of eggs was recorded. From these data, the mean time in days taken by a neonate to become an adult female was calculated. In addition, the longevity was recorded as the mean number of days between the neonate birth and adult death. During this experiment, further data were noted: the interval of time (ΔT) from one brood of neonates to the next (from which the developmental period when the reproductive rate was highest could be found) and the duration of each instar (showing the period in which the animal grows fastest).

Individual growth was also assessed. This is expressed as the variation in a particular dimension, usually the total length or weight, as a function of age (Margalef, 1974). Using a stereoscopic microscope, this growth was followed in terms of the height and length of the replicate females, the size of the neonates, the maximum size of each instar and the size of the primipara. Growth curves were adjusted by the von Bertallanffy equation (with initial parameters of the curve obtained by the Ford-Walford transformation (Centeno, 1999) (Equation 1):

$$L_t = L\infty\left[1 - e^{-K(t-t_0)}\right] \quad (1)$$

where: L_t is the size at the time interval t, expressed in mm; $L\infty$ = maximum size also in mm; K = a constant related to the growth rate; e = basis of neperian logarithms; t_0 = parameter related to the size of the individuals at birth (L_0), expressed in days.

In order to test if there were significant differences in the life cycle parameters between the two populations, the non-parametric Mann-Whitney (U) test was used at the $\alpha = 0{,}05$ significance level. The program XLStat 2008 was used.

Taxonomic analysis was carried out with the help of identification keys (Dumont and Negrea, 2002; Martin and Boyce, 2004; Richter and Timms, 2005; Timms, 2009) and by comparing anatomical features of specimens of *Cyclestheria* sp., collected from the flooded area in the swamplands, with those of *Cyclestheria hislopi*, obtained from the CEPTA tanks.

Taxonomically relevant features (Figures 2 and 3), unique to this species, were used to compare specimens of *Cyclestheria* sp., collected from the Miranda River floodplain, and *C. hislopi*, from the CEPTA fish tanks, with the keys. The female body parts used were: serrated anterior rostral border, first pair of antennae tubular, with setation restricted to distal tip (Martin and Boyce, 2004), dorsal margin of the telson with large spines similarly sized, carapace circular and flattened, with growth lines (Timms, 2009).

3. Results

3.1. Life cycle

3.1.1. Duration of post-embryonic development of Cyclestheria hislopi (CEPTA).

Daily measurements of individual growth were plotted in order to analyze the species growth. The growth of *Cyclestheria hislopi* individuals (Figures 4 and 5) was found to follow the logistic curve, with an exponential growth up to the 21st day, followed by a fall in the growth rate as the curve approached an upper limit asymptotically.

The main parameters for the life cycle of *C. hislopi* individuals are presented in Table 1. The cultured conchostracans attained a maximum mean length of 3.0 ± 0.1 mm and maximum mean height of 2.6 ± 0.1 mm, under the conditions used. The first three ecdyses occurred, on average, up to the tenth day of free life, with an interval varying from 3.0 to 4.14 days between each one. From then on, ecdyses occurred less frequently and the intervening period was progressively longer at each instar. The longevity of individual animals varied between 52 and 77 days, with a mean of 66 ± 8.94 days. The first reproduction of neonates occurred at 21-26 days of life and the average age was 22 ± 1.8 days. At this point, the mean length of the adults was 2.7 ± 0.1 mm and the mean height was 2.2 ± 0.1 mm,

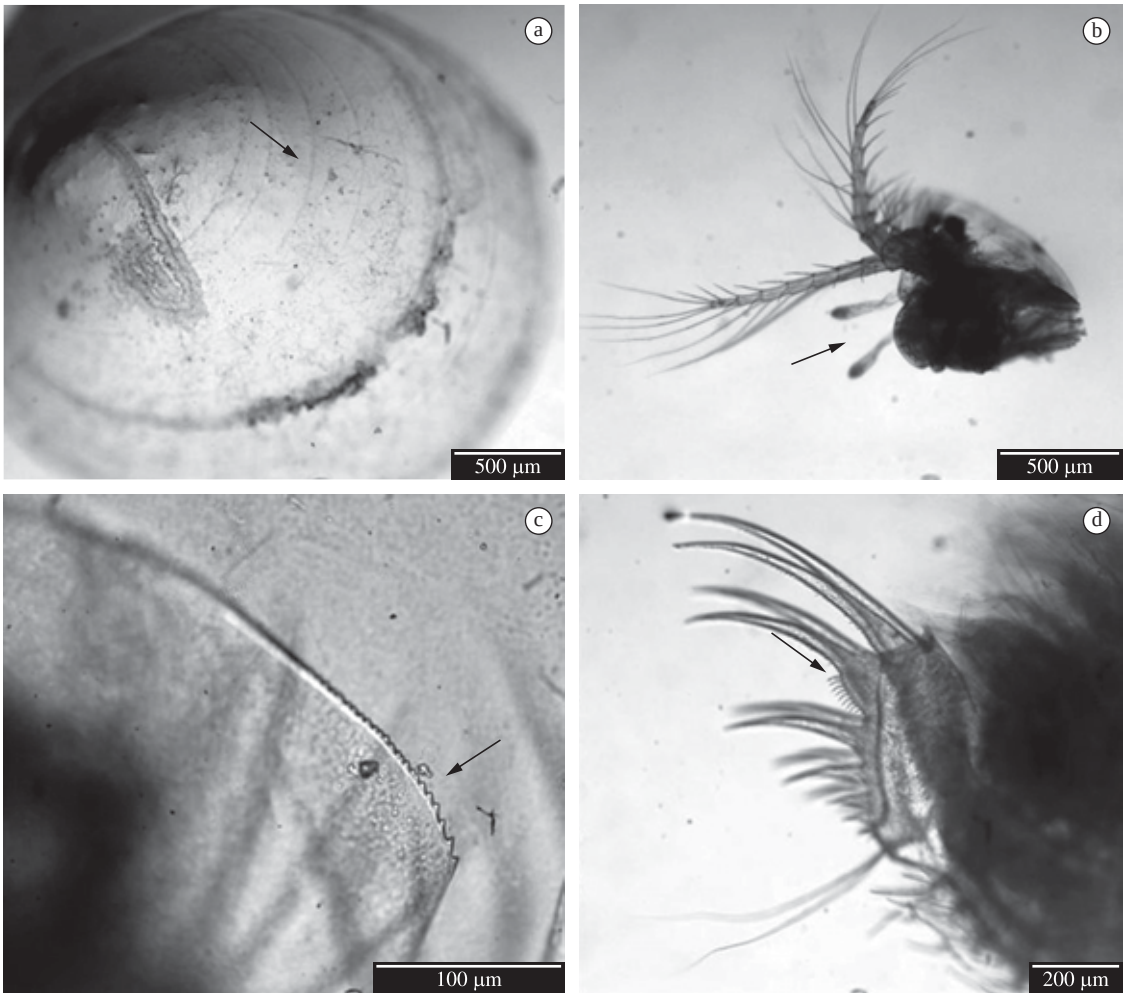

Figure 2. Relevant anatomic features of *Cyclestheria hislopi* from fish tanks at CEPTA. a) Carapace circular and flattened, with growth lines; b) first pair of antennae tubular, with setation restricted to distal tip; c) serrated anterior rostral border; and d) dorsal margin of the telson with large spines similarly sized.

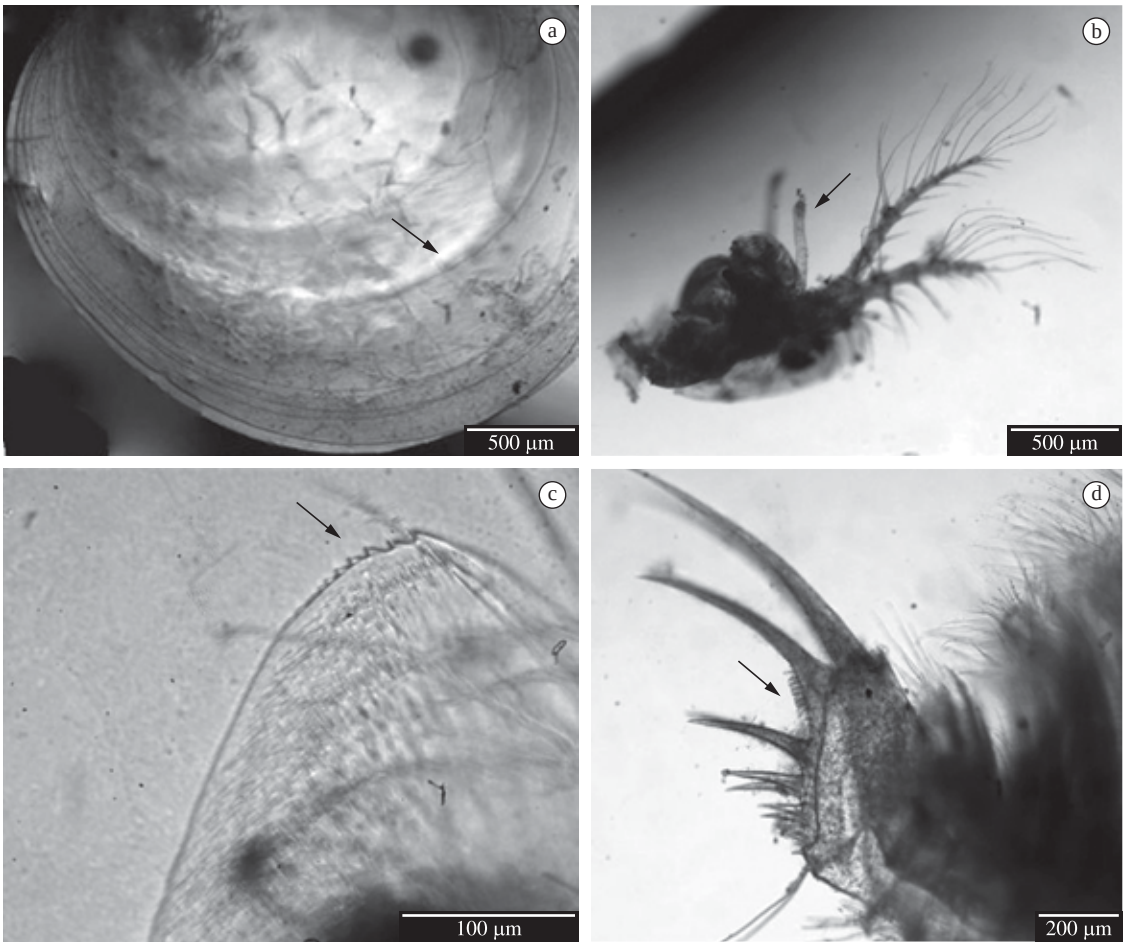

Figure 3. Relevant anatomic features of *Cyclestheria hislopi* from the floodplain of the Miranda river in the central Brazilian swamplands. a) Carapace circular and flattened, with growth lines; b) first pair of antennae tubular, with setation restricted to distal tip; c) serrated anterior rostral border; and d) dorsal margin of the telson with large spines similarly sized.

implying that, on average, they acquired 90 and 84.61% of their final length and height, respectively, during the pre-reproductive phase. The number of neonates produced at the first reproduction varied from two to five, with an average of 3.6 ± 1.1 neonates per female in the first brood. Average brood sizes varied from 2.75 to 5 neonates for each female, with an overall mean value of 3.9 ± 0.9 per brood per individual. Females produced from one to four broods each, averaging 2.7 ± 0.9 broods per female. The majority of the females had three broods. After the first brood (at 22 ± 1.8 days), the second took a further 9.3 ± 0.8 days to occur (around day 30 ± 0.8). The third brood occurred 27.0 ± 7.6 days after the second (around day 58 ± 7.9). Thus it could be observed that the interval of time between the production of a new brood increased throughout the life cycle.

3.1.2. Duration of post-embryonic development of Cyclestheria sp. from Pantanal floodplain

In the case of the strain collected in a swampland habitat, the mean individual growth followed a logistic curve (Figures 6 and 7), in which the exponential growth phase was logarithmic and continued up to about day 20, when the rate slowed as the dimensions approached their asymptotic values.

The maximum mean length and height of the cultured *Cyclestheria* specimens from the floodplain were 2.7 ± 0.2 and 2.2 ± 0.1 mm, respectively. The first five ecdyses occurred in the first 20 days of life and the interval between each ecdysis varied from 3.0 to 5.0 days. After that, intervals became progressively longer, at each instar. The mean longevity was 64 ± 14.0 days. The first reproduction was observed after 15 to 22 days of free life (19.2 ± 3.4 days). The mean length of the females at this point was 2.4 ± 0.1 mm and the mean height was 1.87 ± 0.1 mm. Thus, the animals grew to 88.9 and 85.0% of this full length and height, respectively, during the pre-reproductive phase. The number of neonates in the first brood varied from two to three (mean value 2.5 ± 0.6 neonates), while the mean number per brood per female varied from 2.5 to 4 (mean value 3.4 ± 0.6 neonates). The number of broods produced by one female varied from

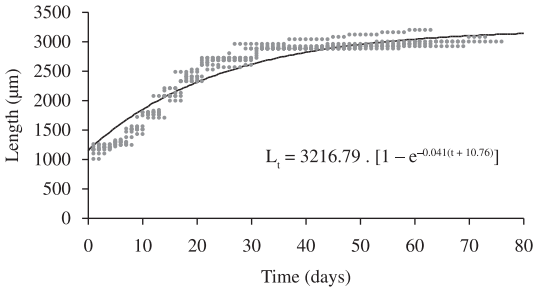

Figure 4. Growth curve of mean length of *Cyclestheria hislopi* in culture originating from specimens taken from fish-farming tanks at CEPTA/IBAMA (Pirassununga, SP, Brazil), throughout post-embryonic development. The conchostracans were cultured in reconstituted water at 25 ° ± 2 °C, with specimens of the aquatic fern *Salvinia auriculata*, and fed on the chlorophycean *Pseudokirchneriella subcapitata* at a density of 5.10^6 cells/mL.

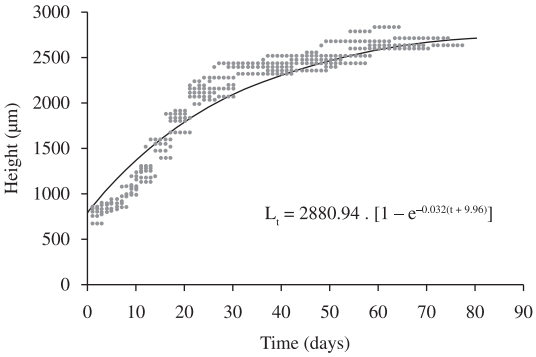

Figure 5. Growth curve of mean height of *C. hislopi* in culture originating from specimens taken from fish-farming tanks at CEPTA/IBAMA (Pirassununga, SP, Brazil), throughout post-embryonic development. See Figure 2 for culture conditions.

Table 1. Parameters of main life cycle variables for individual specimens of *Cyclestheria hislopi*, collected from CEPTA fish tanks and from a floodplain in the Mato Grosso swamplands, cultured in reconstituted water at 25 ± 2 °C, in the presence of several water ferns (*Salvinia auriculata*), fed on a suspension of the chlorophycean *Pseudokirchneriella subcapitata* at a final density of 5.10^6 cells/mL.

Variables	CEPTA Fish tanks	Pantanal floodplain	Mann-Whitney Test	
	Mean ± SD (N = 7)	Mean ± SD (N = 5)	U	P value
Maximum length (mm)	3.01 ± 0.10	2.77 ± 0.15	24.5	0.048*
Maximum height(mm)	2.65 ± 0.13	2.22 ± 0.15	27.5	0.012*
Number of instars	7.85 ± 0.70	7.29 ± 1.49	15.5	0.903
Longevity (days)	65.7 ± 8.94	64.0 ± 14.0	16	0.788
Age at first reproduction (days)	22.0 ± 1.82	19.2 ± 3.40	18	0.54
Length at first reproduction (mm)	2.66 ± 0.09	2.45 ± 0.09	27	< 0.0001*
Height at first reproduction (mm)	2.16 ± 0.12	1.87 ± 0.08	28	0.010*
Fertility (total number of neonates)	10.14 ± 3.39	8 ± 1.22	19	0.39
Mean number of neonates/Brood	3.89 ± 0.89	3.37 ± 0.63	17	0.63
Number of broods	2.71 ± 0.95	2.5 ± 1.0	17	0.54
Growth rate (K) in lenght	0.0413	0.0513	-	-
Growth rate (K) in height	0.0325	0.046	-	-

*statistically significant difference

two to four (mean value 2.5 ± 1.0 broods). Most of the females had just two broods and the mean time interval between them was 7.5 ± 1.3 days. The first reproduction occurred around the 19th day of life (SD ± 3.4 days), the second around the 27th day (SD ± 4.4 days). Despite this small number of broods per adult, there is a perceptible increase in the duration of embryonic development of the neonates at each new brood produced.

The Mann-Whitney test showed that there are no significant differences between the two populations regarding most of the life cycle parameters. The only parameters showing a statistically significant difference between the two populations were: maximum length and height of adults and length and height at first reproduction (Table 1).

3.2. Taxonomic analysis

The comparison between the specimens of *Cyclestheria* sp., collected from the Miranda River floodplain, and those collected from the fish tanks in the CEPTA revealed that there are no morphological differences of taxonomical importance between the individuals from both populations. All individuals presented: a serrated anterior rostral border with larger serrate protuberances in the apical region, diminishing in size backwards (Figures 2a and 3a); The first pair of antennae are tubular with setation restricted to distal tip (Figures 2b and 3b); dorsal margin of the telson with large spines similarly sized (Figures 2c and 3c); carapace circular and flattened, with growth lines (Figures 2d and 3d); All characteristics checked corresponded to those described for *Cyclestheria hislopi* diagnosis.

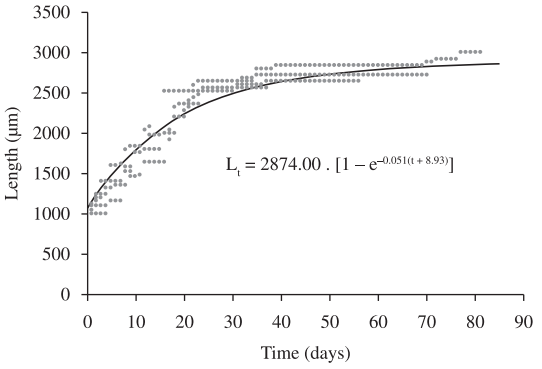

Figure 6. Growth in length of *Cyclestheria* sp. in culture originating from specimens collected from flooded area beside the Miranda River at Passo do Lontra in the Mato Grosso swamplands, throughout post-embryonic development. See Figure 2 for culture conditions.

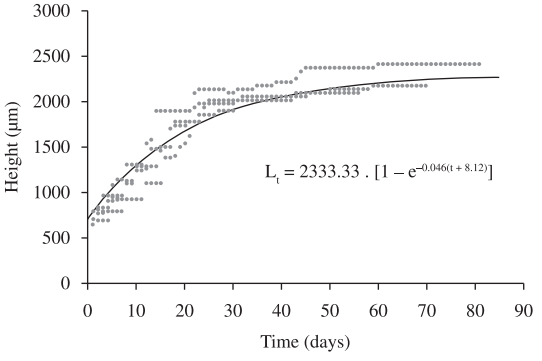

Figure 7. Growth in height of *Cyclestheria* sp. in culture originating from specimens collected from flooded area beside the Miranda River at Passo do Lontra in the Mato Grosso swamplands, throughout post-embryonic development. See Figure 2 for culture conditions.

4. Discussion and Conclusion

The conchostracan *Cyclestheria hislopi* is readily cultured in the laboratory and isolated populations remain stable for long periods (more than two years).

Based on the taxonomic analysis of specimens from the two populations, we can conclude that they belonged to a single species, *C. hislopi*. The differences observed in the life cycle of the individuals from Pantanal and those from CEPTA fish-farming ponds, namely the slightly lower performance of the floodplain specimens regarding maximum size and size at first reproduction (length and height) may result from physiological responses to differences between the culture conditions they were acclimated at the laboratory and the natural habitat swampland in Mato Grosso.

A large variation in life cycle parameters can also be observed within the same population. Paul and Nayar (1977) observed size variation among the populations of *C. hislopi* found in a shallow and seasonal pond in Irinjalakuda, Kerala, India, throughout the year. They reported a large variation in the mean size of all individuals in the population (1.11 to 2.19 mm) on a monthly basis and that *C. hislopi* attained maturity around 3.0 mm, a size slightly higher than those found for the populations in this study. Nair (1968) recorded the mean size of 2.6 mm at maturity for *C. hislopi* from laboratory cultures, a value similar to those obtained in the present study. These findings corroborate with the fact that individuals in populations from different regions or at different culture conditions do show large variations in size and other parameters of the life cycle.

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