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ECOSYSTEMS

Chironomus columbiensis (Diptera: Chironomidae) as test organism for aquatic bioassays: Mass rearing and biological traits

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Abstract: Chironomidae are aquatic insects that have become a model in laboratory tests to evaluate toxic effect of different pollutants. The use of chironomids as test organisms depends on standard protocols for the rearing under laboratory conditions and expanding knowledge on its biology. We standardize a culture of *C. columbiensis* and analyze its life cycle under laboratory conditions. The experiments were carried out with different treatments for water, temperature, and food. As a result, a protocol was proposed for the rearing of *C. columbiensis* under the following conditions: semi-soft and reconstituted water, shredded paper towels as a substrate, soft aeration, temperature of 22 ± 2°C, fed with 0.3 g TetraMin® twice a week and weekly renewal of 50% of the water. Under these conditions, the duration of the life cycle was 17-31 days, with a continuous growth of 1.16 to 14.05 mm in the larval stage and an average of 530 eggs per oviposition. In comparison with other species in the family, this study indicated that *C. columbiensis* is a species with a short life cycle, sensible to changing conditions (e.g., temperature and food), wide distribution, so is a good organism for being used in aquatic bioassays.

Key words: *Chironomus columbiensis*, laboratory rearing, life cycle, test organism.

INTRODUCTION

Chironomidae (Diptera) comprises one of the more diverse and abundant groups of aquatic insects in river systems (Ferrington 2008). They are known mainly for their broad geographical distribution and different types of aquatic environments, from limnetic to brackish (Delettre 2000). Armitage (1995) estimated that in this family there are about 15,000 grouped species, according to Ashe et al. (1987), in 339 genera, 22 tribes, and 11 subfamilies, of which 4,147 species are aquatic in their immature stages. In the Neotropical region, 109 genera and about 1,500 species have been reported (Coffman et al. 2008, Ferrington 2008, Trivinho-Strixino 2011).

Chironomids are an important trophic resource for fish, birds and other invertebrates and participate actively in detritivory and organic matter recycling processes (Rieradevall et al. 1995). In recent decades, this group has been used in toxicity studies (Arambourou et al. 2012, 2014, Salmelin et al. 2015, Odume et al. 2016) because of its short life cycle and physiological tolerance to pollution. These insects are of great interest in this type of study since most of their life cycle is in direct contact with the sediment, which acts as a final deposit site of chemical residues in water resources (Nebeker et al. 1984, Warwick 1985, Armitage 1995, Bechard et al. 2008). In addition, some species have been reared under laboratory conditions using procedures standardized by APHA (American Public Health Association), ASTM (American Society for Testing and Materials) and FAO (Food and Agriculture Organization) for carrying out acute and chronic bioassays in ecotoxicological

testing for evaluating water quality (USEPA 1994, Reynoldson & Day 1993).

The genera *Chironomus* and *Goeldichironomus* have been one of the most used groups as test organism for aquatic bioassays, the specimens are generally collected through strainer or aquatic nets and in the egg stage by means of plastic pipettes (Corbi & Trivinho-Strixino 2006, Murrieta-Morey et al. 2016, Zanotto-Arpellino et al. 2016). The duration of the life cycle of the species generally varies from 14 to 34 days, in laboratory conditions, with controlled temperature (21 - 26 ° C). For chironomid cultures, the constant circulation or aeration of water has frequently been used and feeding is carried out with TetraMin®, Avemycin-Purina® and micro algae, the first two ones correspond to fish and poultry feed, respectively (Corbi & Trivinho-Strixino 2006, Murrieta-Morey et al. 2016, Zanotto-Arpellino et al. 2016). The life cycle of chironomids includes egg, larva (four instars), pupa and adult (Nolte 1993). They can reproduce several times a year in natural conditions, it depends on the local climate (Tokeshi 1995, Ristola 2000). According to Choi & Roche (2004), Faria et al. (2006) and Roongruangwongse et al. (2005) knowledge on the life cycle, taxonomy and ecology of bioindicator organisms makes water quality biomonitoring more accurate because these species can have different levels of tolerance.

Chironomus columbiensis is one of the few species of this group identified in Colombia, described by Wülker et al. (1989) and has been reported in Colombia, Brazil, Guatemala and the United States (ZipcodeZoo 2015), but its biology and ecology are still little known (Montaño-Campaz et al. 2019, Ospina-Pérez et al. 2019). Therefore, the objective of this study was to know the effects of temperature, availability food and type of water in the laboratory rearing of the species *Chironomus columbiensis*. Besides, to establish the rearing conditions and deepen knowledge on the biology of *C. columbiensis* under controlled conditions since it may be promising as a test organism for aquatic bioassays.

MATERIALS AND METHODS

Collection of individuals in the field

The *Chironomus* specimens were collected in the Chinchiná River (Km 4, routed from Manizales to -Chinchiná, Colombia) and in an artificial pond in the Botanical Garden of the Universidad de Caldas in Manizales (Colombia). The sampling sites had a reduced depth, standing water and muddy substrate. The specimens were collected using a Surber sampler (0.09 m^2, 300 µm mesh size), deposited on white trays with water from the site and separated with Pasteur pipettes to maintain the integrity of the specimens. Each sample was placed in 500 ml plastic containers with water from the sampling site and transferred to the laboratory for reared (Manizales, Colombia). Additionally, 6 L of water were collected from each sampling site for the acclimation of the samples in the laboratory.

Acclimation of the specimens

The *Chironomus columbiensis* specimens were separated and placed in glass aquariums of 35 cm long x 30 cm wide x 15 cm high in the laboratory. Two sheets of shredded paper towels were used per aquarium as substrate. Each culture aquarium was covered with tulle fabric, approximately 35 x 30 x 30 cm, to prevent adults from escaping. Initially, in the acclimatization process, 5 L of water was used from the collection site per aquarium, which was gradually replaced by reconstituted water Type I (Table I). Semi-soft and reconstituted water was used, with a pH of 7.3 to 7.8, a hardness of 80 to 100 mg/L $CaCO_3$ and dissolved oxygen greater than 4.0 mg/L.

Identification of the species

To confirm the identification of the specimens, the cephalic capsules, and the genitalia was mounted, following the Pinder protocol (1983), and a photographic record of the larvae and adults was made with Stereomicroscope Leica M205-C with coupled camera. Taxonomic keys by Trivinho-Strixino (2011) and Prat et al. (2012) were used. Moreover, specialists from the Universidade Federal de São Carlos in Brazil confirmed the species. Tests were carried out to determine the type of water, food, photoperiod and the suitable temperature to guarantee reproduction, emergency and low mortality of the larva and pupa.

Rearing with different types of water

Four treatments were carried out in triplicate with the different types of water, each containing 60 larvae in their first instar (less than 72 h old). The treatments were: 1) Water from the dechlorinated aqueduct, 2) Distilled water, 3) Reconstituted semi-soft water Type I, and 4) Reconstituted semi-soft water Type II (Table II). In this experiment the same procedure explained for the reared and identification of the specimens was followed, and the survival of larvae, pupae, and adults and the number of spawning in each replication were recorded.

Adult emergency with different types of diet

Three diet treatments were evaluated, one with fish food (TetraMin®), another with bread yeast, and the third with dog food (Pedigree®). For the experiment was used reconstituted water Type II and shredded disposable towels on the bottom as substrate were placed 60 *C. columbiensis* larvae with less than 72 h hatch. The specimens

Table I. Preparation of semi-soft, reconstituted water Type I for reared of *Chironomus columbiensis* under controlled conditions.

Compounds		SLN Stock	Preparation
Name	Nomenclature	Prepared in 20 ml	Quantity per liter
Sodium bicarbonate	$NaHCO_3$	—	0.01 g
Sodium chloride	NaCl	—	0.05 g
Calcium chloride	$CaCl_2$	—	0.01 g
Monopotassium phosphate	KH_2PO_4	—	0.002 g
Magnesium sulphate	$MgSO_4$	—	0.01 g
Magnesium chloride	$MgCl_2$	—	0.018 g
Thiamin		0.12 g	0.25 ml
Iron chloride	$FeCl_3$	0.20 g	0.20 ml

Table II. Preparation of reconstituted, semi-soft water Type II for the reared of *Chironomus columbiensis* under controlled conditions.

Compounds		**SLN Stock**	**Preparation**
Name	**Nomenclature**	**Prepared in 500 ml**	**Quantity per liter**
Potassium chloride	KCl	3.6 g	0.56 ml
Magnesium sulphate	$MgSO_4$	13.5 g	2.22 ml
Sodium bicarbonate	$NaHCO_3$	43,2 g	1.11 ml
Thiamin		0.3 g	0.25 ml
Hydrated calcium sulfate	$CaSO_4{\cdot}2H_2O$		0.06 g

were fed twice a week and 50% of the water was changed once a week. The data recorded were the emergency of males and females.

Adult emergency with different photoperiod

Three photoperiod regimes (16 h L (Light): 8 h D (Darkness); 12 h L:12 h D and natural light) were evaluated in 60 *C. columbiensis* larvae with less than 72 h hatch. In the experiment was used Type II reconstituted water and the organisms were fed with TetraMin® twice a week. The data recorded were the emergency of males and females.

Duration of the larval stage at different temperatures

In the glass aquariums with 5 L of reconstituted Type II water and a shredded disposable towel on the bottom as a substrate, 60 *C. columbiensis* larvae less than 72 h old were deposited, fed with 0.3 g of TetraMin® twice a week, at 16, 21, 24 and 26 °C with 50% of the water changed once a week.

Longevity of adults and food

To evaluate the longevity of the individuals in the adult stage, 50 organisms were deposited per aquarium at a 1:1 ratio of males and females with three replicates per treatment. The treatment I consisted of exposing 50 specimens in the aquariums to water-soaked towels with sugar on top of the tulle fabric, and treatment II consisted of aquariums without towels. In each experiment, the longevity and the number of average spawnings deposited by the *C. columbiensis* populations were recorded.

Evaluation of the life cycle under controlled conditions

After the standardization of the rearing protocol, the egg masses were removed and placed in glass aquariums (35cm x 30cm x 15cm) covered with tulle fabric (35cm x 30cm x 35cm) for retention of the adults. In each aquarium, 5 L of permanently aerated reconstituted water were added at 22 ± 2°C and 50% were replaced once a week. These specimens were fed with 0.3 g of commercial fish food (TetraMin®) twice a week. During all experiment, in each aquarium brought out 10 larvae daily. This process continued until the specimens reached their pupal stage, and it was deposited in 96% alcohol. Each specimen was observed and photographed with a Leica M205C stereomicroscope with coupled camera, and the total length and ventral length of the cephalic capsule were measured.

Data analysis

To analyze mortality of larvae and pupae, the number of spawning according to the type of water, it was used generalized linear models (GLMs) with the "glm" function through the distribution of "Poisson" by means of the fitdistrplus library (Delignette-Muller et al. 2019). In this model, the response variable was the number of larvae and pupae deaths and the number of spawning, while the type of water was included as explanatory variables. For the case of the number of spawning according to the type of water, it was used a generalized linear model (GLM) with the "glm" function through the distribution "Gamma". In this model, the response variable was the duration time of the specimens to complete the larval stage in "days", and temperature was included as an explanatory variable. The mortality of larvae and pupae, the number of spawning according to the type of water, and the duration of the larval stage duration at different temperatures were analyzed using a generalized linear model (GLM). The model was fitted using error Poisson and Gamma by means of the fitdistrplus library (Delignette-Muller et al. 2019). Type II analysis of variance tables were used to evaluate the

importance of the terms in GLM using the ANOVA function in the car library (Fox & Weisberg 2019). All statistical analyzes were performed in R 3.6.2 (R Core Team 2019) using RStudio (RStudio Team 2020) and the "lme4" package (Bates et al. 2015).

RESULTS

Rearing with different types of waters

The mortality of larvae and pupae, number of females and number of spawnings in each of the experiments with the different types of waters showed significant differences, except for the number of males (Table III). The mortality of the larvae and pupae of *C. columbiensis* fluctuated between 0 and 13.3%, with higher mortality in the treatment with dechlorinated water and lower mortality in the treatment with reconstituted water Type II. During the experiments, greater *C. columbiensis* mortality was observed in the pupal stage than in the larval stage (Figure 1). The adult emergence of *C. columbiensis* was high for all treatments, between 80 and 98.3%. The treatment with dechlorinated water presented the lowest emergence value, unlike the Type I and II reconstituted water, which showed the highest value (Figure 2). Additionally, it was observed that in the treatments with dechlorinated water, distilled water and reconstituted Type I water, the emergence of males was greater than that of females. In contrast, in Type II reconstituted water, a slightly larger number of females emerged than males (Figure 2). Reconstituted water Type II presented the highest number of spawning (32%).

Adult emergency with different types of diet

The emergence in *C. columbiensis* fed with different diets varied between 0% and 96.7%, where TetraMin® treatment was the best diet (96.7%), unlike the specimens fed with bread yeast (31.7%) and Pedigree® (0%). Regarding the ratio in emergence, it was 1:1 (males and females) in the yeast and TetraMin® treatments, respectively.

Adult emergency with different photoperiod

The emergence of *C. columbiensis* specimens varied between 60% and 98.3% with different photoperiods. The 12 h L: 12 h D photoperiod was the best treatment with an emergency rate of 98.3%, followed by the 16 h L: 8 h D treatment (91.7%), and finally the photoperiod with natural light (60%). All treatments were found to show a 1:1 emergence sex ratio.

Table III. Average mortality, survival and reproduction of *Chironomus columbiensis* with different types of water.

Parameter	Dechlorinated	Distilled	Reconstituted Type I	Reconstituted Type II	$\chi 2$	p-value
Dead larvae	8	4	0	0	66.00	< 0.001
Dead pupae	4	2	2	1	15.08	0.002
Adult females	21	25	26	30	19.82	< 0.001
Adult males	27	29	32	29	5.94	0.114
Spawn	11	16	18	43	118.32	< 0.001

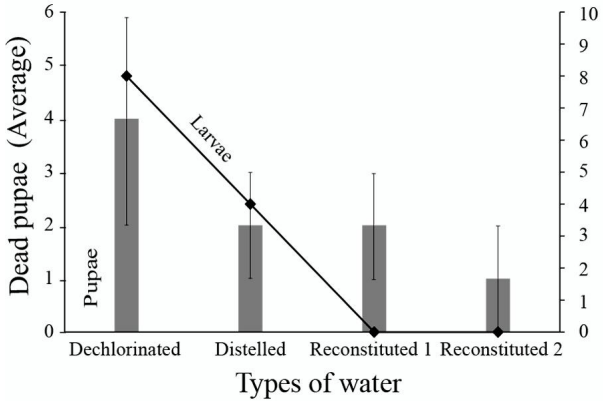

Figure 1. Average mortality (%) in the immature stages of *Chironomus columbiensis* under controlled conditions. Variation is expressed as standard deviation.

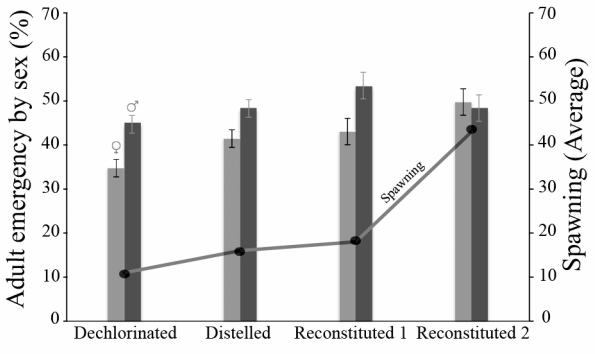

Figure 2. Emergence of males and females (%) and number of spawns of *Chironomus columbiensis* under controlled conditions. Variation expressed as standard deviation.

Duration of the larval stage at different temperatures

The larval stage decreased its development time as the temperature increased (χ^2 = 54, p <0.001). The generalized linear model by treatment showed significant differences between 16°C and 24°C (χ^2 = 18, p <0.001) and 16°C and 26°C (χ^2 = 40.5, p <0.001), but not between 16°C and 21°C (χ^2 = 0.5, p = 0.479). The development of the larvae at 16°C lasted 13.67 ± 0.33 days ($\bar{x}$ ± 95% CI), while the 24°C and 26°C the treatments lasted 11.67 ± 0.47 days ($\bar{x}$ ± 95% CI) and 10.67 ± 0.47 days ($\bar{x}$ ± 95% CI; Figure 3).

Longevity of adults with and without food

Adults of *C. columbiensis* without food had average longevity of 9 days (for females and males), while adults fed with sucrose survived on average 19.7 days for females and 20.7 for males, that is, the duration of the adult stage doubled (Figure 4).

Life cycle

The *C. columbiensis* females presented an elongated and globular oviposition, consisting of a gelatinous mass, which confers protection to eggs when these are in contact with water. This gelatinous mass has an elongated tubular shape with several rows of eggs, with an average of 530 eggs per laying. *C. columbiensis* larvae begin to hatch after 48 h of oviposition.

The life cycle duration of *C. columbiensis* presented an average of 24 days and fluctuated between 17 to 31 days. The egg stage lasted for 2 to 3 days, the larva for 9 to 13 days, the pupa stage for one day and the adult stage for 5 to 14 days (Figure 5). There was a continuous growth in the larval stage in an average period of 11 days, with initial sizes of 1.16 mm in its first larval instar and 14.05 mm in the fourth larval stage (Table IV and Figure 6). In addition, the ventral length of the cephalic capsule increased

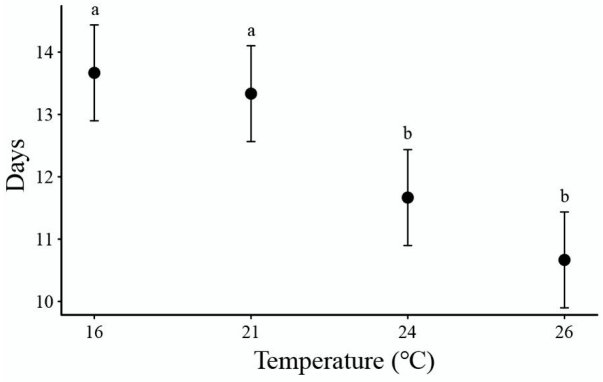

Figure 3. Average duration (days) of the larval stage of *Chironomus columbiensis* subjected to different temperature ranges. Bars represent mean ± 95% confidence intervals. The different letters on the bars represent a significant difference. Variation expressed as standard deviation.

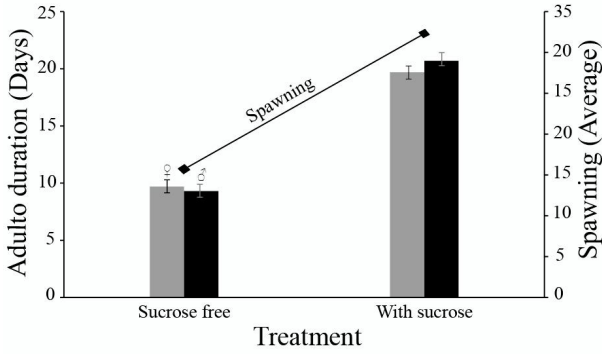

Figure 4. Duration (days) of the adult stage and number of spawning of *Chironomus columbiensis* with or without sucrose feedings. Variation expressed as standard deviation.

continuously during the larval stage. By relating the body growth and the ventral capsule of the *C. columbiensis*, the four larval stages were clearly differentiated (Figures 7 and 8), with a longer duration in the last stage.

DISCUSSION

In general, larvae and pupae mortality were low in all treatments, which reflects the fact that *Chironomus columbiensis* is sensitive in both the larval phase and the pupal stage. Evaluating type of water in culture is very important to verify if the environmental conditions are optimal for the survival and reproduction of the specimens. According to OECD (2011), the type of water (natural, distilled, reconstituted or dechlorinated) in the reared of chironomids is considered suitable when the specimens survive acclimatization without showing signs of stress. Accordingly, the Type II reconstituted water used in this experiment was the most optimal for the reared of *C. columbiensis* since, in this medium, the specimens showed fewer signs of stress (mortality of larvae and pupae). Additionally, the greater mortality of pupae concerning larvae may have been since, in this state, Diptera reduce food consumption and are more sensitive to environmental stress, as some authors have proposed (Kambysellis & Heed 1971, Benoit et al. 1997). In respect of the high percentage of emergency and low mortality of *C. columbiensis* observed in this study may also be related to the use of shredded disposable towels as a substrate since they can be used by the organisms for the construction of shelters, which provide protection and food.

The higher percentage of spawning obtained with the Type II reconstituted water may have been related to the proportion of males:females that emerged per population since, in this treatment, the emergence of females was 2% higher. In contrast, in the other three treatments, the emergence of males was 9.2% greater than

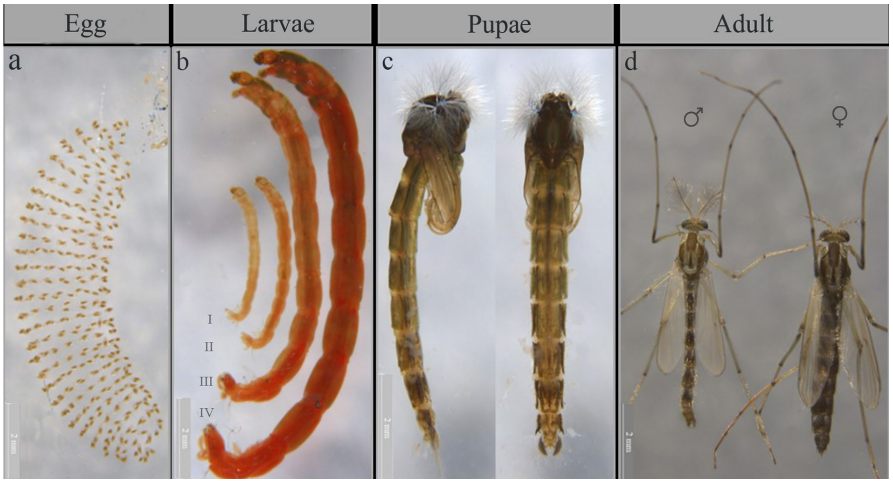

Figure 5. Life cycle of *Chironomus columbiensis*. a) Egg. b) Larvae of the four stages. c) Pupae. d) Male and female adults.

that of females. According to the study carried out by Thornhill (1976), the reproductive success of males depends on the number of fecundated females.

The results found with respect to the duration of the larval stage were similar to those recorded by Strixino & Trivinho-Strixino (1985) in the study with *Chironomus xanthus*, who observed that the duration of the larval stage was reduced from 40 days at a temperature of 15°C to 15 days when the temperature was increased to 25°C. Although the duration of the larval stage of Chironomidae depends on the species, temperature constitutes one of the main factors controlling the larval development period. Menzie (1981 in Tokeshi 1995), found that, under laboratory conditions, *Cricotopus sylvestris* completed its larval cycle in 28 days at 15°C, but, when subjected to a temperature between 22 and 29°C, the cycle decreased to 10 days. According to Konstantinov (1958 in Tokeshi 1995), the larval stage of this same species extended to 21 days at 18°C, and at 22°C it reduced to 14 days (Tokeshi 1995). In the studies by Peck et al. (2002) and Fonseca & Rocha (2004), it was found that the life cycle of *C. crassiforceps* extended 11 days at 27°C and that the life cycle of *C. xanthus* lasted 13 days at 23°C.

The results of the present study show that the life cycle duration of *C. columbiensis* can vary with the temperature in the larval stage and with the supply of food in the adult stage. It was also observed that the females increased the number of spawning when they had food (Figure 4). While in the present study the males and females were fed sucrose, Oliver (1971) observed that chironomid adults did not feed. The food supply in these organisms increases reproductive effectiveness since they obtain the necessary energy to complete their life cycle successfully.

The photoperiod has been shown to be a fundamental physical factor influencing biological cycles (Lawrence & Soame 2004, Pérez-Valdés & Contreras-Guzmán 2016), is essential in the development of organisms since it can significantly affect their feeding, growth, and survival (Hart et al. 1996, Gorla 2018). The greatest percentage of emergency of *C. columbiensis* in the 12h L: 12 h D photoperiod probably is due to this experimental condition resemble the natural conditions of this species, since a reduction in photoperiod inhibits emergence by preventing pupation of final instar larvae (Danks 1978).

Trivinho-Strixino & Strixino (1989) found that some species can perform up to three oviposition per female according to the number of oocytes in each ovary, but Fonseca & Rocha (2004) found that 36% of females lay a single spawning and only 6% achieve a third. In this study, the egg mass found for *C. columbiensis* was similar to that recorded by Fittkau (1965), Trivinho-Strixino & Strixino (1998), and Corbi & Trivinho-Strixino (2006) for other genera and species of Chironomidae. In a review of the

Table IV. Body length, width and length of the cephalic capsule (mm), and duration of each larval instar of *Chironomus columbiensis* reared under controlled conditions at 22 ± 2°C.

Instar larval	Body length (mm)	Ventral width of the cephalic capsule (mm)	Ventral length of the cephalic capsule (mm)	Duration in days
Instar I	1.16-2.79	0.07-0.12	0.07-0.13	3
Instar II	2.90-7.00	0.18-0.23	0.19-0.25	2
Instar III	5.57-9.85	0.26-0.32	0.32-0.39	2
Instar IV	10.20-14.05	0.39-0.61	0.42-0.57	4

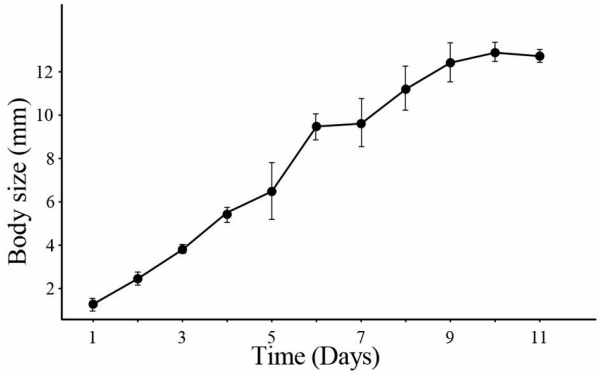

Figure 6. Average growth curve of *Chironomus columbiensis* larvae with a temperature range between 20 and 24°C. Variation expressed as standard deviation.

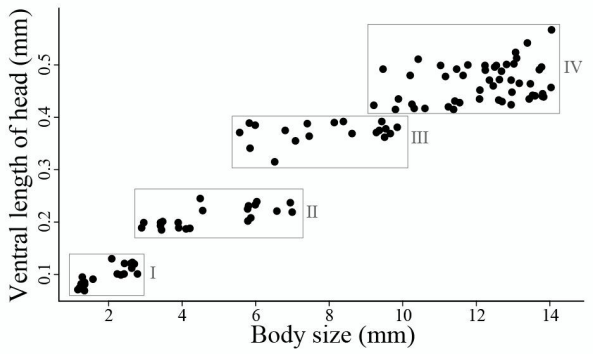

Figure 7. Relationship between the ventral length of the cephalic capsule (mm) and body (mm) in the larval stage of *Chironomus columbiensis*.

family, Nolte (1993) indicated that the gelatinous mass is a general characteristic of certain groups of chironomids. In addition, the average number of eggs found for *C. columbiensis* was like that reported for *C. sancticaroli* by Fonseca (1997), with an average in the first laying of 550 eggs per spawn, but Strixino (1980) recorded an average of 745 eggs for this species.

Studies on the life cycles of the Chironomidae can provide important knowledge on the bionomy of the species of this family, which has been very incipiently studied in the Neotropical region (Strixino & Strixino 1982). In addition, they can also reflect temporary changes in the environment in the monitoring programs (Corbi & Trivinho-Strixino 2006). These traits make them good bioassay organisms for ecotoxicological evaluations. For this group of insects, obtaining the energy accumulated in the larval phase is important for successfully overcoming the metamorphosis, mainly the change from pupa to adult, taking into account that these insects do not feed at the pupal stage (Kambysellis & Heed 1971). The different values for the width of the cephalic capsule of *C. columbiensis* obtained in the present study were compared with those of other *Chironomus* species (Table V), finding that the cephalic capsules of this species are among the largest, after *C. tentans* (ASTM 2000), and leaving *C. xanthus* as the smallest (Fonseca & Rocha 2004). Fonseca & Rocha (2004) found that the width of the cephalic capsule is the best variable to define the stage of chironomid larvae, but in the present study, it was possible to demonstrate that the length of the cephalic capsule and the length of the body are also good variables to determine larval instars in *C. columbiensis*.

In conclusion, the relevant requirements for the reared protocol of *C. columbiensis* included the use of reconstituted semi-soft water Type II, shredded disposable towels as a substrate and feeding with 0.3 g of TetraMin® twice a week. The laboratory temperature and photoperiod should be constant (22 ± 2°C; 12 h L: 12 h D,

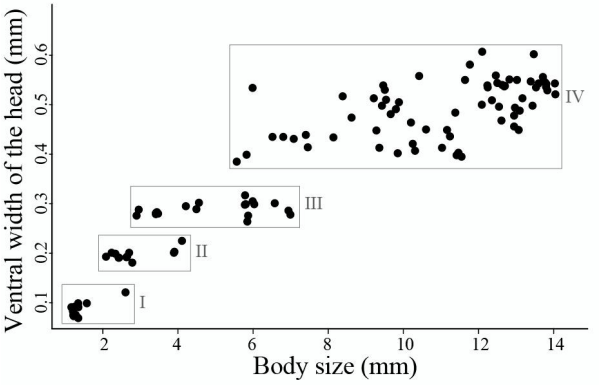

Figure 8. Relationship between the ventral width of the cephalic capsule (mm) and the body (mm) in the larval stage of *Chironomus columbiensis*.

Table V. Cephalic capsule width (mm) of *Chironomus columbiensis* and other *Chironomus* species under controlled conditions.

Species	Culture conditions	Width of the cephalic capsule (mm)				Reference
		Instar I	Instar II	Instar III	Instar IV	
C. tentans	TetraMin® - 23°C	0.10	0.20	0.38	0.67	ASTM 2000
		(0.09 a 0.13)	(0.18 a 0.23)	(0.33 a 0.45)	(0.63 a 0.71)	
C. crassiforceps	Aqua-lab – 27°C	0.09	0.16	0.9	0.47	Peck et al. 2002
		(0.08 a 0.10)	(0.14 a 0.18)	(0.26 a 0.32)	(0.42 a 0.52)	
C. sancticaroli	Avemicina, chicken ration 19-26°C				0,49	Trivinho-Strixino & Strixino 1981
					(0. 43-0.53)	
C. xanthus	TetraMin® - 23°C	0.09	0.15	0.26	0.45	Fonseca & Rocha 2004
		(0.08 a 0.10)	(0.15 a 0.17)	(0.24 a 0.28)	(0.38 a 0.49)	
C. columbiensis	TetraMin® 21-25 °C	0.10	0.20	0.29	0.50	Present study
		(0.07 a 0.12)	0.18 a 0.22	0.26 a 0.32	0.39 a 0.61	

respectively). The reconstituted water should be renewed weekly in 50%, and eliminate all residue, exuviae, and dead specimens. Each month spawns should be exchanged between the different aquariums in order to reduce inbreeding among the populations. The protocol of mass rearing standardized in laboratory, added to the high number of generations (14) per year and the large number of spawning eggs that *C. columbiensis* presents make this species an optimal test organism for aquatic toxicity bioassays.

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