# Laboratory cultures of the native species *Chironomus xanthus* Rempel, 1939 (Diptera-Chironomidae).

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**ABSTRACT: Laboratory Cultures of the native species *Chironomus xanthus* Rempel, 1939 (Diptera-Chironomidae).** Previous works have indicated that the midge *Chironomus xanthus* Rempel is easily maintained in laboratory. The purpose of this study was to develop a toxicity test procedure with a benthic endemic species, to be used with sediment samples. This research was thought to be relevant considering that there is a lack of pre-established procedures available. The methodology adopted for the maintenance was different from that already described in the literature, differing in three aspects that were: the adoption of a sand layer at the bottom of the tray as the substrate; the addition of TETRAMIN goldfish food ration and the use of green algae suspension for feeding the first larval instar. After egg hatching, a total of 100 larvae were transferred to plastic trays. Trays contained a 3cm thick sterilized layer of sand and 4 liters of maintenance water. To feed those larvae $10^5$cels.mL$^{-1}$ of green algae were added plus 0.04 mg.mL$^{-1}$ SSV/day of the TETRAMIN goldfish food. The system was maintained under continuous aeration at 24± 1$^o$C and a light regime of 12 hours light and 12 hours dark. The cage, which consisted of a screened wooden support (± 100μ) for the adult retention was covered by a cloth. Approximately two days after emerging, adults started copulating. Next, pregnant females deposited their grouped eggs involved by a gelatin-like mass on the tray wall. Each female laid 500 to 600 eggs, arranged in a spiral shaped mass. After approximately 44 to 48 hours, the larvae hatched. The first larval instar head capsule measured 0.09±0.01 mm width. The *C. xanthus* larvae started to build their cases on the second developmental stage. The life cycle lasted approximately 13 days and a large fraction of individuals survived in all larval stages. The high survival rates, the short duration of life cycle and the easy of culturing make them an adequate species as test-organism.

**Key-words:** Chironomidae, *Chironomus xanthus,* development duration, life cycle, laboratory culture.

**RESUMO: Cultivo em laboratório da espécie nativa *Chironomus xanthus* Rempel, 1939 (Diptera-Chironomidae).** Trabalhos desenvolvidos anteriormente indicaram que a espécie *Chironomus xanthus* Rempel é de fácil manutenção em laboratório. Visando a utilização deste organismo, em testes de toxicidade com sedimento, o presente trabalho foi realizado, visto a carência de metodologias pré-estabelecidas para ensaios com espécies nativas, tipicamente bentônicas. A metodologia de manutenção dos organismos foi modificada com base naquela descrita na literatura. As modificações introduzidas incluem a utilização de uma camada de areia no fundo das bandejas, da ração TETRAMIN e de uma suspensão de algas clorofíceas como alimento para o primeiro ínstar. Um total de 100 larvas recém-eclodidas foram transferidas para bandejas plásticas contendo uma camada de areia esterilizada de 3cm de espessura e 4 litros de água de manutenção. Como alimento foram adicionadas $10^5$ céls.mL$^{-1}$ de uma mistura de algas e a ração de peixes TETRAMIN na proporção de 0,04 mg.mL$^{-1}$ SSV/dia. O sistema foi mantido sob aeração contínua à temperatura de 24±1 $^o$C, fotoperíodo de 12 horas, coberto por gaiolas de nylon para retenção dos adultos. Aproximadamente dois dias após a emergência, os adultos copulam e as fêmeas grávidas depositam os ovos reunidos numa massa gelatinosa na parede da bandeja. Cada fêmea deposita de 500 a 600 ovos dispostos em forma de espiral.

Após um período aproximado de 44 a 48 horas, ocorre a eclosão das larvas que medem 0,09 ± 0,01 mm de largura da cápsula cefálica. As larvas de *C. xanthus* iniciam a construção dos casulos a partir do 2º estágio de vida. A duração de todo o ciclo de vida é de 13 dias, com grande sobrevivência de organismos em todos os estágios larvais, o que possibilita a sua utilização como organismo - teste.
**Palavras-chaves:** Chironomidae, *Chironomus xanthus*, ciclo de vida, cultivo em laboratório.

## Introduction

Toxicity testing with benthic macroinvertebrates is an effective tool among various methodologies used to assess the sediment toxicity in contaminated sites.

Since they are considered good indicators, species from the Chironomidae and Oligochaeta groups have been widely used, especially because they are the most abundant organisms in the benthic community.

They are extremely important in the food chain and because they live and feed themselves from sediment particles, they are directly exposed to toxic compounds by body contact and indirectly, through contaminated food ingestion.

Some Chironomidae species, for instance *Chironomus tentans* (USA) and *Chironomus riparius* (Europe), have been used in sediment toxicity bioassays because besides the fact that they perform an important role in the food chain, they are highly sensitive to persistent toxic compounds, such as heavy metals and pesticides (Giesy & Hoke 1989).

Many factors have contributed to the use of these organisms in water quality monitoring programs, such as the easy maintenance and manipulation in laboratory (the size and red color of these organisms ease the screening after test) (Nebeker et al, 1984), the short life cycle (25 to 30 days, at 23ºC, Benoit et al, 1997) and a large background of information resulting from the many research studies that have been done with these organisms. For these species there are standard procedures by APHA, ASTM (American Society for Testing and Materials) and FAO (Food and Agriculture Organization) for acute and chronic toxicity tests with sediment samples (USEPA, 1994; Reynoldson & Day, 1995).

In Brazil, the Chironomidae have not yet been used in sediment toxicity assessments due to the shortage of detailed biology studies with native species.

*Chironomus xanthus* was originally described by Rempel, 1939 and is a senior synonymous to *Chironomus domizzi* Paggi, 1977 and *Chironomus sancticaroli* Trivinho-Strixino & Strixino, 1981. At the moment the geographic distribution is restricted to Brazil and Argentina. The holotype was found in Campina Grande, Paraíba - Brazil (Spies & Reiss, 1996). Trivinho-Strixino & Strixino (1982) report the occurrence of this species in the São Carlos region, São Paulo State, and show it as a specie easy to be maintained in laboratory.

The present work aimed to improve the culture method since the previous study included the use of antibiotics, what cannot be adopted in ecotoxicological protocols. Also aimed the study of *C. xanthus* life cycle under well-defined conditions, a step required for the standardization of this species as a test-organism. The use of native species avoids the accidental introduction of exotic species, which is a serious problem all around the world. Also, much effort has been made with the intention to find native species of different levels of the food chain (Fonseca, 1991; Bohrer, 1995), which can be used as test organisms not only for sediment toxicity assessment but also for water toxicity assessment, due to both, the easy obtaining of inoculum and the consequent advances in the ecological knowledge on the native species.

## Material and methods

The first *C.xanthus* egg masses were collected from fish pools (CEPTA-IBAMA, Centro de Pesquisa e Treinamento em Aquicultura, Pirassununga) according to tests done by Fregadolli (1996).

A 350m² fish farm pond was fertilized with chicken manure and filled up with water up to a 30 to 40 cm level. 60 cm long dry bamboo sticks were placed in, with part of them in contact with the water. These segments worked as a breeding substrate to the *C. xanthus* female egg (Fig.1). This operation was carried out in the afternoon. In the following morning, segments with egg masses were taken out and brought to the laboratory.

After laboratory hatching, the larvae were transferred to plastic trays with sterilized sediment from the field. The egg masses were collected and maintained in a room at 24±1°C temperature and 12L:12D photoperiod.

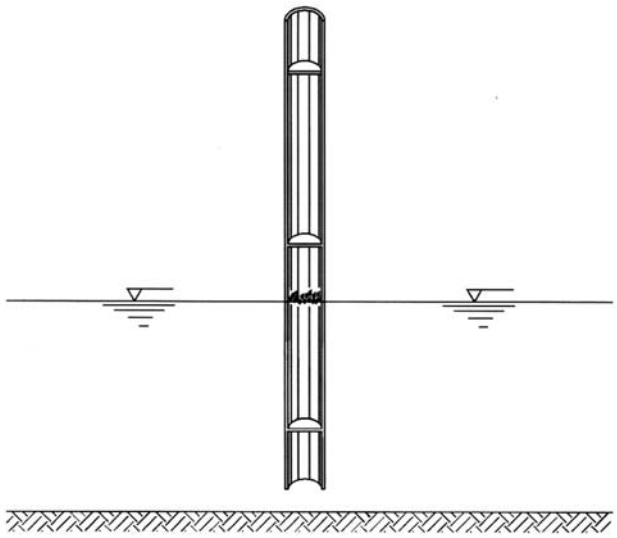

Figure 1: General view of the device used for collecting *Chironomus xanthus* in the field.

The organisms were fed with fermented chicken manure up to the moment the first adults emerged. The adults mated and new egg masses were produced. These laboratory egg masses were isolated and observed until the emergence of adults that were collected for taxonomic identification.

From this step on the maintenance methodology was based on studies done by Strixino (1980) and Trivinho-Strixino & Strixino (1982). From their studies some information was obtained; such as tray length, adult collection procedures, the number of organisms per tray, the volume of culture water and aeration system.

The modifications used in the present research include the introduction of a sand layer at the bottom of the tray and the adoption of the TETRAMIN goldfish food and algae as food to the first instar larvae. This type of food was adopted based on experimental results. Two kinds of food were tested: a chicken powdered food (Purina) (Trivinho-Strixino & Strixino, 1982), and fish food (TETRAMIN), which is usually used for feeding *C. tentans* by the EPA laboratories (USEPA, 1994).

One hundred recently ecloded larvae in triplicate, were separated from a single egg mass and maintained in trays with 4 L of algal suspension at $10^5$ cels.mL$^{-1}$, plus 1.0g of the chicken food and 22.0mL$^{-1}$ of Tetramin suspension (5.0 g.L$^{-1}$, corresponding to 0.04g.mL$^{-1}$ SSV). From the second instar on the algal suspension was no longer used. Daily, three specimens were taken from each tray and preserved on 70% alcohol for posterior measurements of total body length and head capsules length and width. The experiment was carried during approximately 15 days or until the adult emergence.

Egg masses were subsequently placed on glass dishes with culture water until egg -hatching and larvae abandoned the gelatin-like mass. One hundred larvae were transferred to 45x35x6 cm plastic trays, which contained a sterilized sand layer and 4 liter culture water.

The trays were continuously aerated and covered by a nylon screen to retain adults emerged. Room temperature was maintained at 24±1°C, and a 12L:12D photoperiod was used (Fig. 2)

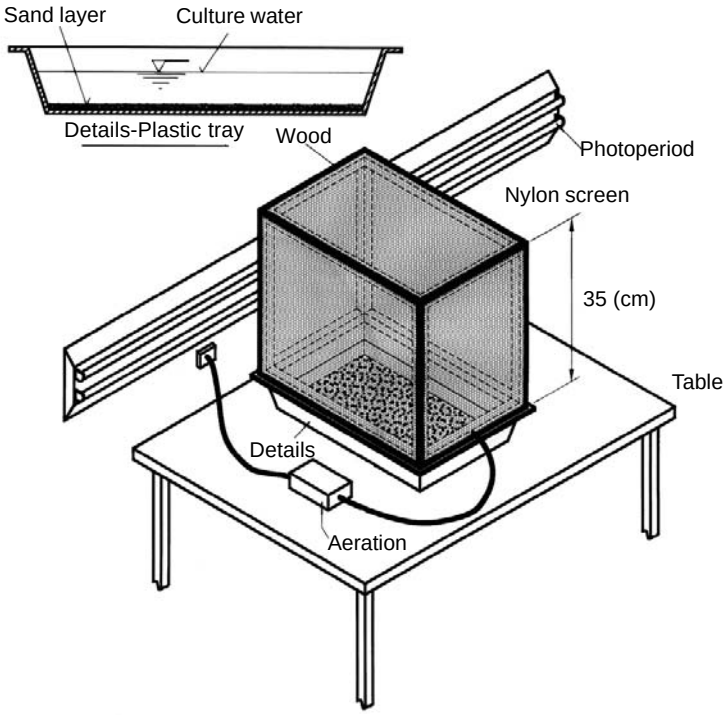


Figure 2: General view of the tray used for maintaining *Chironomus xanthus* cultures in the laboratory.

## Results

Two days after emergence, *C. xanthus* adults mate and fertilized females deposit their eggs into a gelatin-like mass on the tray wall. The gelatinous egg-mass is hung by a pendunculus just bellow the water surface. The ovigerous masses remain fixed by feet at the wall of the tray. Each female produce an average of 500 to 600 eggs in a spiral arrangement. Oviposition usually took place early in the morning.

The gelatin-like mass involving the eggs expands when in contact with the water thus giving the egg-mass a curved tube aspect. After approximately 44 to 48 hours, at an incubation temperature of 24±1°C the larvae begin hatching.

The first instar had a head capsule width of 0.09± 0.01 mm (N=12).

The *C. xanthus* larvae start the construction of their cases at the second instar, since during the first instar they just remain among the substrate particles, feeding on bacteria. This stage is basically planktonic.

The life cycle lasts approximately 13 days (12 to 15), and a large fraction of the larval stages survive.

The results obtained have shown that there are no differences in the development time of *C.xanthus*, as evidenced by the length and width of head capsules of larvae grown with both feeding regimes (Tab. I). It can be observed that the duration of instar II was 1 day longer for larvae grown on chicken ration than on TETRAMIN fish food. In relation to body size, the larvae grown on TETRAMIN ration had larger body size for all the instars tested. However, this variable does not precisely reflects the development duration as the head capsule size.

Table I: *Chironomus xanthus* total body length (mm), head capsule width and length and the maximum development duration of each instar cultured with types of food rations, at 24±1°C.

| | Chicken food | | | | TETRAMIN® | | | |
|---|---|---|---|---|---|---|---|---|
| **Instars** | **Lt** | **W** | **L** | **days** | **Lt** | **W** | **L** | **days** |
| **Instar I** | 0.82-1.47 | 0.085-0.097 | 0.088-0.096 | 4 | 0.82-1.57 | 0.085-0.10 | 0.088-0.094 | 4 |
| **Instar II** | 2.58-3.57 | 0.15-0.156 | 0.160-0.166 | 3 | 2.87-4.31 | 0.150-0.161 | 0.157-0.159 | 2 |
| **Instar III** | 4.33-6.18 | 0.25-0.26 | 0.26-0.27 | 2 | 5.12-6.60 | 0.258-0.266 | 0.26-0.27 | 2 |
| **Instar IV** | 10.18-12.7 | 0.43-0.46 | 0.43-0.45 | 5 | 9.37-15.1 | 0.42-0.46 | 0.43-0.46 | 5 |

## Discussion

Chironomids are widely distributed. A great richness of species is found in many freshwaters. Some species are tolerant to environmental stressors and therefore occur in a variety of habitats, from ponds to large lakes and reservoirs or from rapid streams to great rivers. There are species typical of cold or warm waters, occurring in poor or well oxygenated habitats (Lindegaard, 1995).

Chironomidae life cycle is divided into four stages: egg, larva, pupae and adults. The last two stages are a very short time period. The adult stage only requires a mating shelter, egg maturing and oviposition. On the other hand, a large part of the organism life cycle occurs at the larval period, since it is in this stage that the development energy necessary is acquired, once the adults don't feed themselves (Oliver, 1971).

The *Chironomus xanthus* egg mass is deposited either in the morning or late in the afternoon. During the laying process, the female stands still next to the water, and her abdomen is lightly curved down. As the egg packet is eliminated she moves her body sideways. After the egg packet extrusion there is a short fly, which is followed by the deposition into the walls of the container. This deposition is still in contact with water. The eggs in the egg mass are helicoidally arranged in parallel rows (Trivinho-Strixino & Strixino, 1989). The same authors report that, according to the number of ovocytes in each ovariole, it is possible to admit that the *C. xanthus* presents 3 postures. However, in laboratory cultures, only 36% of the analyzed females did only one posture and only 6% achieve the admitted potential (third posture). This behavior was also observed in this study, especially in what it refers to the posture schedule (morning and dawn) as well as the arising of two or more postures laid by the same females. Only in the first posture a large number of eggs (500 to 600) was produced while in the other two a much lower number (around 100 fewer eggs) was obtained.

How long the embryo stage lasts is not something usually described in the literature for field data because it is obtained in laboratory, since it is directly related to the temperature (Sweeney, 1984).

The embryonic development of *C. xanthus* lasted 3.75 days at 15°C; 2 days at 19 to 22°C and 1.25 days at 30°C according to Trivinho-Strixino & Strixino (1989). In the present study the embryonic development lasted 40 to 48 hours at a temperature of 23°C.

Larvae cultured in the laboratory frequently fed themselves on the gelatin surrounding the eggs. According to Hinton (1980 apud Pinder, 1995) the gelatin, composed mainly of carbohydrates is a very important support to the larvae during the dispersion phase. The larvae leave the gelatin and remain planktonic until they find an appropriate habitat and the energy required for swimming is obtained by eating algae and scraps (Oliver, 1971).

Chironomids have different feeding habits consuming a large variety of food. Five categories can be considered: algae, scraps and associated microorganisms, macrogametes, wood scraps and invertebrates. Usually the food consumed can only be determined by analyzing digestive tract content (Berg, 1995).

The green algae is an important food source in the chironomids diet, particularly for

the filter-feeding. The analysis of *Chironomus crassicaudatus* intestine revealed that 68 % of the contents were the cyanophyceans *Gloeocapsa, Lyngbya*, and *Merismopedia* (Ali, 1990, apud Berg, 1995).

Although several studies reported the importance of scraps on chironomid diet, the non-living components nutritional value has been disputed since much of the consumed material is not digestible (cellulose, lignin and ashes) and quickly pass through the intestine. On the other hand, microbes organisms have a high nutritional value and can be important in the synthesis of essential vitamins which are used by chironomids. Microorganisms such as bacteria, fungi and protozoans can be used as food by chironomids or can perform the role of transforming residues into more nutritional and eatable forms (Mclachlan et al., 1979; Ward & Williams, 1986, apud Berg, 1995).

In this work the use of green algae for the first instar and the TETRAMIN fish food for later instars was an efficient method, considering that the survival rate in all larval phases were high (90 - 95% ). Although no analysis of intestine content were performed, bacteria and fungi originating from the aerobic fermentation of ration can possibly be a plenty source of food.

Credland (1993) has also used the TETRAMIN ration together with algae in *Chironomus riparius* permanent laboratory culture. He obtained successful larval development as they were kept under continuum aeration at a temperature of 25°C. Standard cultures of *C. tentans* larvae used in toxicity testing are also fed with this kind of ration and algae (USEPA, 1994).

All Chironomidae build a case with the substrate in which they live. This case consists of particles of the own substrate gathered by a secretion similar to silk threads, which is liberated by the salivary glands. The larvae usually occupies a few centimeters in the substrate, usually down to 10 cm (Oliver, 1971).

The use of sand with grain size between 0.25 and 0.10 mm, as adopted for substrate in *C. xanthus* cultures in the present work seemed to be favorable to the organisms attachment and later case building. This type of substrate was probably responsible for the high adult survival rates.

Head capsule width is the best variable to define the instar of chironomid larvae. The different values of head capsule width of *C. xanthus* obtained in the present work are compared with those of other species of *Chironomus* as obtained by different authors (Tab.II). *C. xanthus* head capsules are the smallest among the three species compared, and *C. tentans* the largest.

Table II: Head capsule dimensions (mm) for *C. xanthus* and others species of *Chironomus*.

| Species | Culturing conditions | Head capsule width (mm) | | | | Authors |
|---|---|---|---|---|---|---|
| | | instar I | instar II | instar III | instar IV | |
| *C. tentans* | Tetramin – 23°C | 0.10 (0.09 to 0.13) n - | 0.20 (0.18 to 0.23) n - | 0.38 (0.33 to 0.45) n - | 0.67 (0.63 to 0.71) n - | ASTM, 2000 |
| *C. crassiforceps* | Aqua-lab – 27°C | 0.09 (0.08 to 0.10) n - | 0.16 (0.14 to 0.18) n- | 0.9 (0.26 to 0.32) n - | 0.47 (0.42 to 0.52) n - | Peck et al, 2002 |
| *C.sanctícaroli* | Avemicina, chicken ration, 19 – 26°C | | | | 0.49 (0.43-0.53) n = 15 | Trivinho-Strixino & Strixino 1981 |
| *C. xanthus* | Tetramin, 23°C | 0.09 (0.08 to 0.10) n = 12 | 0.15 (0.15 to 0.17) n = 12 | 0.26 (0.24 to 0.28) n = 9 | 045 (0.38 to 0.49) n = 9 | Present work |

Instars have different durations and this also varies from species to species. The 4th instar has the longest duration. Species that take a year or more to complete the life cycle usually spend most time in the 4th instar. That happens, for example, with *Chironomus anthracinus* (occurring in the North of Ireland). It develops from the 1st to the 3rd instar between May and July and spends the last 9 months in the 4th instar (Carter, 1980 apud Tokeshi, 1995). The longest duration of the 4th instar is regarded to the geometric growth rate in body size compared to that of the other instars (Tokeshi, 1995). This fact was also observed in the present study although *C. xanthus* 4th instar lasted a 5-day period while the remaining three instares lasted only two days (Tab. III).

Also Peck et al. (2002) observed a longer duration for the fourth instar (5- 8 days) compared with two days for the other instars of *Chironomus crassiforceps,* a species occurring in Australian wetlands, cultured at 27 °C and used in toxicity tests for evaluating metal availability in sediments (Tab.III).

Table III: Time at instar (day) for *C.tentans, C. sancticaroli* , *C.crassiforceps* and *C. xanthus* from laboratory cultures.

| Species | Culturing conditions | Time at instar (day) | | | | Authors |
|---|---|---|---|---|---|---|
| | | instar I | instar II | instar III | instar IV | |
| *C.tentans* | Tetramin – 23°C | 1 to 4.4 | 4.4 to 8.5 | 8.5 to 12.5 | 12.5 - | ASTM, 2000 |
| *C.crassiforceps* | Aqua-lab – fishes ration, 27°C | 0 to 2 | 2 to 3 | 3 to 5 | 5-8 | Peck et al, 2002 |
| *C.sancticaroli* | Avemicina-chicken ration, 19 – 26°C | 0 to 4 | 4 to 6 | 6 to 8 | 8 to 14 | Trivinho-Strixino & Strixino, 1982 |
| *C. xanthus* | Tetramin – fishes ration, 23°C | 0 to 4 | 4 to 6 | 6 to 8 | 8 to 12 | Present Study |

Strixino (1980) points out that the success of cultures comprehend several factors such as:

a) Supply of appropriate quantities of food together with enough water oxigenation, so as to avoid intense fermentation.

b) Raising container dimensioning, according to the amount of larvae.

c) Cage appropriate dimensioning, so as to allow the swarm to be formed and the adults to mate. This will, then, make ovoposition possible.

The size of the cage seems to be an important factor since, according to Hilsenhoff & Narf (1967, apud Strixino, 1980) some species such as *Chironomus plumosus* usually reproduce in large swarms and, therefore, need a lot of space. The cage dimension proposed by Strixino (1980) and used in the present work was satisfactory. They allowed *C. xanthus* adults to be captured as well as the pair mating and the laying of new egg masses.

As it is known, temperature constitutes the main controlling factor of larval development times. For instance, Menzie (1981 apud Tokeshi, 1995) reported that in laboratory culture *Cricotopus sylvestris* has completed its larval development in 28 days at a 15°C temperature and in 10 days at a temperature of 22 to 29°C. Meanwhile Konstantinov (1958 apud Tokeshi, 1995) documented for the same species a development time of 21 days at 18°C and 14 days at 22°C (Tokeshi, 1995).

Trivinho-Strixino & Strixino (1985) found that *C. xanthus* in culture kept at the temperature of 15°C the life cycle lasted 40 days and for the ones kept at 25°C the life

cycle was 15 days long. Peck et al. (2002) found a life cycle duration of 11 days for *Chironomus crassiforceps*, cultured at 27°C. The *C. xanthus* life cycle duration obtained in the present research was in average 13 days long, when they were kept at a temperature of 23°C, a relatively fast development, what might be also related to its small size. That indicates an elevated number of generations per year and implies that laboratory culture is totally viable. It can, therefore, be used as test-organism in toxicity evaluation of contaminated sediments.

## References


American Society For Testing Materials. 2000. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. E 1706. Philadelphia.(PA.P.30-32)

Benoit, D.A., Sibley, P.K., Juenemann, J.L & Ankley, G.T. 1997. *Chironomus tentans* life-cycle test; design and evaluation for use in assessing toxicity of contaminated sediments. Environ. Toxicol. Chem., 16:1165-1176.

Berg, M.B. 1995. Larval food and feeding behaviour. In: Armitage,P., Cranston, P.S. & Pinder, L.C.V (eds.). The Chironomidae : the biology and ecology of non-biting midges. Chapman & Hall, London. cap.7, p.136-168.

Bohrer, M.B.C. 1995. Biomonitoramento das lagoas de tratamento terciário do Sistema de Tratamentos dos Efluentes Líquidos Industriais (SITEL) do Pólo Petroquímico do Sul, Triunfo, RS, através da comunidade zooplanctônica. São Carlos, Universidade Federal de São Carlos, 469p. (Ph. D).

Credland, P.F. 1993. A new method for establishing a permanent laboratory culture of *Chironomus riparius* Meigen (Diptera: Chironomidae). Freshwater Biol., 3:45-51.

Fonseca, A.L. 1991. A biologia das espécies *Daphnia laevis*, *Ceriodaphnia silvestrii* (Crustacea, Cladocera) e *Poecilia reticulata* (Pisces, Poecilidae) e o comportamento destes em testes de toxicidade aquática com efluentes industriais. São Carlos, Escola de Engenharia de São Carlos, Universidade de São Paulo, 210p (Master Thesis).

Fregadolli, C.H. 1996. Efeito da disponibilidade de alimento e da predação por ninfas de Odonata no crescimento e sobrevivência de larvas de tambaqui *Colossoma macropomum* Cuvier 1818). São Carlos, Universidade Federal de São Carlos, 152p (Ph.D).

Giesy,J.P. & Hoke,R.A. 1989 Freshwater sediment toxicity bioassessment rationale for species selection and test design. J. Great Lakes Res., 15:539-569.

Lindegaard, C. 1995. Classification of water-bodies and pollution. In: Armitage, P., Cranston,P.S. & Pinder, L.C.V (eds). The Chironomidae: The biology and ecology of non-biting midges. Chapman & Hall, London. cap. 15, p.385-404.

Nebeker, A.V., Cairns., M.A. & Wise, C .M. 1984 Relative sensitivity of *Chironomus tentans* life stages to copper. Environ. Toxicol. Chem., 3:151-158.

Oliver, D.R. 1971. Life history of the Chironomidae. Ann. Rev. Entomol., 16:211-230.

Peck, M.R., Klessa, D.A. & Baird, D.J. 2002. A tropical sediment toxicity test using the dipteran *Chironomus crassiforceps* to test metal bioavailability with sediment pH change in tropical acid-sulfate sediments. Environ. Toxicol. Chem., 21:720-728.

Pinder, L.C.V. 1995. The habitats of chironomid larvae. In: Armitage, P., Cranston, P.S. & Pinder, L.C.V. (eds). The Chironomidae: the biology and ecology of non-biting midges. Chapman & Hall., London. cap.6, p.107-135.

Reynoldson, T.B. & Day, K.E 1995. Freshwater sediments, In: Calow, P. (ed). Handbook of Ecotoxicoloy. Oxford , Blackwell Scientific Publications. p.83-100.

Rempel, J.G. 1939. Neue Chironomiden aus Nordostbrasilien. Zool. Anz., 127:209-216.

Strixino, S.T. 1980. Estudos sobre a fecundidade de *Chironomus sancticaroli sp. n.* (Diptera: Chironomidae) . Instituto de Biociências, USP,157p (Ph.D).

Strixino, G. & Trivinho-Strixino, S. 1985. A temperatura e o desenvolvimento larval de *Chironomus sancticaroli* (Diptera: Chironomidae). Rev. Bras. Zool., 3(4):177-180.

Trivinho-Strixino, S. & Strixino, G. 1981. Nova espécie do gênero *Chironomus* Meigen do Sul do Brasil (Díptera: Chironomidae). Rev. Bras. Entomol., 25:333-340.

Trivinho-Strixino, S. & Strixino, G. 1982. Ciclo de vida de *Chironomus sancticaroli* Strixino & Strixino, (Diptera, Chironomidae). Rev. Bras. Entomol., 26:183-189.

Trivinho-Strixino, S. & Strixino, G. 1989. Observações sobre a biologia da reprodução de um quironomídeo da região neotropical (Diptera, Chironomidae). Rev. Bras. Entomol., 33:207-216.

Sweeney, B.W. 1984. Factors influencing life-history patterns of aquatic insects. In: Resh, V.H. & Rosenberg, P.M. (eds). The ecology of aquatic insects. Chapman & Hall, New York. chap. 4, p.56-99.

Spies, M. & Reiss, F. 1996. Catalog and bibliography of neotropical and mexican Chironomidae(Insecta, Diptera). Spixiana Suppl., 22:61-119.

Tokeshi, M. 1995. Life cycles and population dynamics. In: Armitage, P., Cranston, P.S. & Pinder, L.C.V (eds). The Chironomidae: the biology and ecology of non-biting midges. Chapman & Hall, London. chap.10, p.225-268.

USEPA. United States Enviromental Protection Agency. 1994. USEPA/600/R -94/024. Methods for measuring the toxicity and bioaccumulation of sediment associated contaminants with freshwater invertebrates. Washington(DC). 133p.

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