

## REARING TANYPODINAE, TELMATOGETONINAE AND ORTHOCLADIINAE IN BRAZIL – AN EMPIRICAL APPROACH.

**By Humberto Fonseca Mendes**

Departamento de Biologia da FFCLRP/USP

Av. Bandeirantes, 3900 Ribeirão Preto – SP

CEP 14040-901

E-mail: hfmendes@usp.br

This text reflects my experience in rearing chironomids in Brazil.

Just after sorting, the larvae were isolated in small vials in order to be sure about the associations. The vials stayed open and as soon as the larvae became pupae, the larval exuviae was fixed and the pupa transferred to a larger container with a lower superficial tension (provided by the superficial area) so they could emerge. The vials suggested by EPLER (1995; 2001) and by MERRIT, RESH & CUMMINS (1996) weren't efficient for most of the chironomids I've tried unsuccessfully to rear.

### **Transport from field to laboratory:**

I've got good results with isolated larvae in ice coolers to keep the temperature low. The water level in the vials must be low so it facilitates gas exchange and prevent mechanical shock, and a substratum should be provided for the larvae (avoiding extra stress).

### **Temperature in the laboratory:**

Most chironomids live well at room temperatures, even the ones collected in streams. The main problem is not the temperature, but the water level which is related to oxygen. I've reared some fast-flowing chironomids with very low water levels in the vials.

### **Association of the larvae with the environment:**

The chironomids associated with soft substrata, as a whole, need at least a fine layer of substratum where they can move and build their tubes. Coelotanypodini and Procladiini don't build tubes, but they arrange the sediment into "paths" in which they can hide.

The chironomids associated with submerged vegetation and macrophytes need an appropriate substratum to live on. An easy way to solve this problems is to incorporate some small leaf pieces to serve as substratum for the larvae.

Most of orthoclads live in streams, springs and other fast flowing waters. One easy way to rear them is by keeping them in small vials with

shallow water, another way is by placing them in a flowing water system with enough oxygen. Many orthoclads live in "non-aquatic" environments (some are semi-aquatic, semi-terrestrial, terrestrial or marine) in these special environments, rearing methods must be as similar as possible to the environments where they were collected.

In streams and fast flowing waters the larvae must be sorted as soon as possible, these environments are generally very rich in oxygen and the larvae are very demanding in this parameter. The vials must be with little water, generally when full of it, the relationship of depth to surface area is such that there isn't enough oxygen provided. Another way to do this is keeping the animals in flowing water.

Larvae of slow flowing to standing waters are, generally, more resistant to oxygen depletion, some can survive in very low concentrations of oxygen, like *Chironomus*. Most of these animals require a fine layer of sediment in the bottom of the vial. Some animals from standing waters and pools live on macrophytes, stones, dead leaves and submerged trunks, for them the best was to take a piece of leaf to set in the vials with the larvae.

Phytotelmata are generally good sources for chironomids. I've collected some chironomids in the leaf axils of bromeliads, and the best results were obtained by washing the substratum with a sieve as soon as it was taken from the leaf. Washing can be conducted with filtered water. Also, the animals might be placed in a white tray, sorted and isolated in the field. For more about phytotelmata chironomids see FRANK (1983).

The mining chironomids, like *Stenochironomus*, should never be taken out of their places; most of them aren't able to continue mining after they have been taken out. One way to solve this problem is to keep some submerged trunks and leaves in the laboratory (emergence trails) and wait till the animals emerge. More information on the Chironomidae associated with submerged

trunks in Brazil have been provided by TRIVINHO-STRIXINO & STRIXINO (1998).

### **To feed or not to feed? That is the question**

If one wants to rear animals from the early instars, one must feed the larvae. But, on the other hand, if all one wants to do is to rear some adults, fourth instar larvae isolated in small containers will generally pupate and some of these will emerge without having been fed. But some comments are required: Even some prepupae larvae of tanypods and orthoclads aren't able to pupate without feeding, or they aren't able to emerge, and I prefer to feed the larvae to get better results.

### **Terrestrial Environments:**

Part of the environment must be sampled as a whole, with part of the substratum. When working with mosses on rocks and trunks, the moss must be taken off without damaging the animals, knives help sometimes.

Once sampled, the material must be handled very carefully in order not to kill the larvae. The mosses must be cut into pieces so they fit well in Petri dishes, paying attention to the height of the sample, with scissors one may cut off the top and bottom of the moss.

Dead leaves may be very good for chironomids, so collecting them must be a good choice. They can be used to collect live material to rear, so the entire sample must be placed in Petri dishes to rear. As soon as the adults dry their wings, they are killed and the pupal and larval exuviae found. The more time one leaves before looking for the exuviae, the more difficult it is to find them, since the exuviae might sink.

Terrestrial chironomids don't need to be replaced after pupating, they can emerge in small dishes. When using closed vials don't forget to open it daily!

One clever method that helps sorting the pupal exuviae is to fill up the Petri dishes with water. Some pupal exuviae will float, but not all of them, so that's an alternative to be used after trying to find the exuviae under a stereomicroscope. This method does not damage the larvae nor the pupae, they can survive in water up to 3 hours: if not found within that time after flooding the sample, it is not worth continuing the search. Sometimes the larvae must be sorted and isolated to be sure about the associations with pupa and adults, especially when working with more than one species in the same genera. In *Bryophaeocladius*, *Gymnometriocnemus* and *Antillocladius* the larval exuviae can easily be

found near the pupal exuviae or the pupa itself. My own experience with these genera shows that one can only be sure about the associations just when working with isolated material, because very often I found two or more species in the same sample.

### **How to obtain a sterile terrestrial sediment:**

Most of the terrestrial orthoclads I've reared lived among mosses and tree trunk lichens, which can be easily sterilised of insects by putting some water and letting it dry for a week and repeat the dehydration twice more. This will provide a sterile substratum on which the larvae might be reared. But pay attention to drought tolerant larvae! Most of the drought tolerant larvae don't die with this method, then I sorted the substratum under a stereomicroscope to be sure there was only one larva in each vial.

### **Marine environment:**

The only marine larvae I found and tried to rear was *Thalassomyia*, but none was successfully reared. The water dried out too fast so no larvae could survive. I haven't tested the filter-aquarium suggested by BAY, 1967.

### **Supporting cultures:**

Algae. Algae are needed to feed many larvae, specially when one has third instar larvae and has to rear them till adult. Many algae can be good sources for chironomids. One must choose the algae according to the aims. I've chosen to cultivate three different species: *Ankistrodesmus*, *Scenedesmus* and *Chlamydomonas*.

Rotifera. Some rotifers might be collected with the substratum, and they can be cultivated adding some organic nutrients, such as dried leaves and rice grains, to filtered water. Sometimes benthic colonial species are better to feed the larvae.

Oligochaeta. These animals might be easily cultivated. Those associated with leaves might be cultivated with some detritus and leaves from the place where they were collected. This is a good source of food for chironomids since one single specimen might be enough food for about 7 tanypods. The animals must be cut into pieces before being given to the chironomids and must be offered in pots. This method both protects the chironomids from the Oligochaeta mucus, which attaches to the mouth and kills the larvae; and prevents water pollution since the extra food is taken out immediately.

*Chironomus* spp. Some egg masses can be got in lakes. Some species have been cultivated as

laboratory insects, and the first and second instars are very good food sources for third and fourth instar tanypods.

#### **Importance of Isolated material:**

Quite often there are more than one species of the same genus living in the same habitat, so rearings are from isolated larvae; this method will ensure correct associations of the adults with the larval and pupal exuviae. I've already found seven species of the same genus in the littoral zone of one lake (*Labrundinia*).

Another way to ensure associations without isolating, is to get larvae from egg masses or pregnant females, for those, the methods described by BRANCH (1923), CREDLAND (1973), EDWARD (1963) BIEVER (1965) and DOWNE & CASPARY (1973) work very well.

#### **Notes on the reared material:**

##### **Orthocladiinae**

*Antillocladius*, *Bryophaenocladus*, *Gymnometriocnemus* and Orthocladiinae new Genus (being described by Morrøye & Sæther). No additional food is required for these larvae. They feed on the sediment and substratum. All species I've already reared fed on sediment and decomposed lichens and bryophytes. Very often there are more than one species of *Bryophaenocladus* in the same sample, so be sure there is only one larva in the vial.

*Corynoneura*, *Onconeura* (Andersen & Sæther, in press) and *Thienemanniella*. This are very easy to rear as the larvae feed on flavoured fish food. One must pay attention to how much is required, and be sure it won't decompose and waste the water oxygen (specially by *Thienemanniella*). The *Corynoneura*-group and some other genera build transparent cocoons for the pupa. Taking the pupa out of these cocoons can be very difficult without damaging the pupa, so the entire dish where the larva became pupa must go into the bigger container. Some animals of this group emerge in the vials suggested by EPLER (1995), but many *Thienemanniella* don't.

*Cricotopus*. The larvae become adult if fed with periphyton attached to roots and submerged leaves, which must be taken with the collection of the larvae.

*Ichthyocladus*. These animals live on catfish and must stay there till the adults emerge. Each fish may have only one larva on it (to prevent wrong associations) and must stay in an isolated aquarium with a net covering. The fish must be fed with periphyton and one doesn't

have to be worried about feeding the orthoclads. As soon as the pupae emerge, the adults must be killed, the pupal exuviae will remain on the water surface and the larval exuviae will remain in the cocoon attached to the fish.

##### **Tanypodinae**

*Ablabesmyia*. These larvae may be fed with dead chironomid larvae, small living larvae and pieces of Oligochaeta. I've already reared larvae from second instar till adults feeding them only with Oligochaeta.

*Coelotanypus* and *Clinotanypus*. Most of the larvae were fed with sediment detritus and first instar of Chironomini.

*Conchapelopia* and *Pentaneura*. The larvae were fed with Oligochaeta only.

*Fittkauimyia*. These animals generally won't finish the development without being fed with other chironomids or Oligochaeta.

*Labrundinia*. The only way to rear these animals was with feeding them with algae and colonial benthic rotifers. I've got some animals from eggs to adults feeding them this way.

*Larsia*. Many species of this genus can be fed only with parts of Oligochaeta.

*Monopelopia*. This is a difficult genus that can feed on live animals or on detritus. All species I've reared from Phytotelmata fed on detritus and some drops of detritus were sufficient to get adults from second instar larvae.

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- [*Editorial comment: I have been very successfully using the techniques described here for a quarter of a century. Although I described the technique in my PhD thesis, embarrassingly I never got around to publishing them. Humberto has done us a service by recording these techniques in print. PHL*]