THE EFFECT OF DIET QUALITY ON GROWTH AND DEVELOPMENT OF RECENTLY HATCHED LARVAE OF Chironomus gr. plumosus

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Palabras clave: Chironomus gr. plumosus, crecimiento y desarrollo, calidad de la dieta.
Key words: Chironomus gr. plumosus, growth and development, diet quality.

ABSTRACT
Recently hatched larvae of two egg-masses of Chironomus gr. plumosus were used in feeding experiments lasting for 12 days. Three diets were selected to test the effect of food quality and feeding mechanism on growth and development of larvae: detached benthic algae and leaf debris in the form of leaf disks of 1.5 cm diameter (CPOM) and leaf particles of size less than 250 μm (FPOM). Significant effects of diet were observed for growth and development of larvae of both egg-masses. Benthic algae, more properly algae derived detritus, proved to be the food of higher quality, producing a higher rate of growth than did the CPOM diet. In the FPOM diet, no significant growth was obtained over the experiment, and after 12 days no larvae were found alive on this diet. On the CPOM diet the larvae were observed to ingest mostly fungal biomass, scraping the surface of leaf disks. However, on the FPOM diet the leaf tissue was ingested in higher proportion possibly because of the impossibility of microbial selection due to the small particle size. These results suggest that leaf tissue itself was of poor nutritional value for larvae, unlike fungal biomass.

INTRODUCTION
The recognition of the energetic importance of chironomids in freshwater benthos (BENKE et al., 1984; BERG & HELLENTHAL, 1993) highlights the importance of addressing studies on the trophic ecology of lotic and lentic midges (e.g. WARD & CUMMINS, 1979; BAKER & McLACHLAN, 1979; TITMUS & BADCOCK, 1981; JOHANNSON and BEAVER, 1983; BALL & BAKER, 1995).

Although feeding takes place in adults and may improve survival rates, this energy supply is unlikely to have much effect on the fitness of the individuals, since mating often takes place almost immediately after adult eclosion (ARMITAGE, 1995). The life cycle strategy of the vast majority of chironomid species seems to lie in the improvement of reproductive output by maximizing the energy acquisition over the four larval phases and minimizing the duration of the adult stage (TOKESHI, 1995). Most laboratory and field studies concerned with larval chironomid feeding ecology, growth and development have focused on second-, third- and especially fourth-instar larvae. This emphasis may be justified because the last-instar larva often occupies most of the larval phase and the formation of reproductive structures occurs within it (BALL & BAKER, 1995). First instar larvae are usually considered neither for field nor laboratory studies (TOKESHI, 1995), partially due to their presumed very short duration but mainly because of difficulties of identification and sorting from the natural substrata, in spite of the energetic importance of the smallest instar larvae demonstrated by production studies (BERG & HELLENTHAL, 1993).

Several factors have been shown to affect chironomid larval growth: temperature (e.g. WARD & CUMMINS, 1979; LADLE et al., 1984), quality and quantity of food (e.g. WARD & CUMMINS, 1979; MATTINGLY et al., 1981; BALL & BAKER, 1995), intra- and interspecific competition for food resources (e.g. KAJAK, 1963; IWAKUMA, 1986) and the risk of predation (BALL & BAKER, 1995). Under field conditions, larval growth and development can reportedly be influenced by the combination of the above factors and others such as
The cages were placed in linesville Creek (2 cm height with a 7 cm bottom of 50 pm mesh) and were incubated at 21 ± 1°C, i.e. approximately 200 degree-days, a reportedly adequate period for a good microbial conditioning of the leaves (ANDERSON & CUMMINS, 1979). After retrieval from the stream the leaves were carefully rinsed with tap water in order to remove unwanted fauna. A portion of the conditioned leaves was cut into disks of 1 cm diameter and 15 disks per cage were supplied to the larvae on the diet referred to as CPOM (coarse particulate organic matter). Another part of the conditioned leaves was ground with filtered (50 pm) stream water and sieved; the fraction of particles lower than 12 μm was used for the experiment of feeding. Before the beginning of the experiment and after the rearing of the larvae, 10 animals per cage were randomly collected for measurements. This treatment was repeated in each of the different diets. Head capsule and total body length of the larvae were measured under a dissecting microscope at 100x or 40x magnification, by positioning the larvae on a white filter paper where the larvae remained immobile. Once measured, the larvae were returned alive to, their corresponding cages, except for three, which were dissected to examine the type of food ingested. These larvae were cleared with lactic acid (1 h, 60°C), mounted in Hoyer solution and the gut content was examined at 400x magnification. After 12 days on FPOM, no larvae remained from the two egg-masses and the experiment was terminated. Over the experiment, notes were kept on the external aspect and behaviour of the larvae on the different diets, based on 1 h observation per diet every two days, under a dissecting microscope (40x) in the rearing cage. The mortality on each diet was not recorded because of the difficulty of perceiving minuscule larvae, especially in the diets with fine particles.

**Assay organisms**

Two egg-masses of Chironomus gr. plumosus were collected in Linesville Creek (Crawford County, Pennsylvania), a small non-polluted woodland stream (COFFMAN et al., 1971), by placing a floating cotton tied to the stream banks on the water surface. The two egg-masses, adhering to the string about 10 cm apart, differed in the number of eggs but presented other characteristics in common (Table 1). When the larvae from both egg-masses reached the fourth instar, they were similar in morphological characters, suggesting a single specific identity. The taxonomic diagnosis followed the work of LINDEBERG & WIEDERHOLM (1979). The egg-masses were incubated at 21 °C in Petri dishes, and in two days almost all the first instar larvae had emerged. These recently hatched larvae were used for the feeding experiments. Each feeding treatment was assayed with a different number of floating cages of 2 cm height with a 7 x 7 cm bottom of 50 pm mesh size, containing 30 larvae (see below). The cages were placed in an artificial stream filled with stream water with forced aeration. The temperature over the experiment ranged between 22 and 19°C (mean of 20°C), range similar to that observed in Linesville Creek (21-18°C) over the same period of time.

**Materials and Methods**

**Assay organisms**

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**Diets**

Three diets differing in nutritional quality and particle size were selected to test the effect of food quality and mode of feeding on the growth and development of the larvae. Alder leaves were made into packs and incubated in Linesville Creek for ten days at 18-21°C, i.e. approximately 200 degree-days, a reportedly adequate period for a good microbial conditioning of the leaves (ANDERSON & CUMMINS, 1979). After retrieval from the stream the leaves were carefully rinsed with tap water in order to remove unwanted fauna. A portion of the conditioned leaves was cut into disks of 1 cm diameter and 15 disks per cage were supplied to the larvae on the diet referred to as CPOM (coarse particulate organic matter). Another part of the conditioned leaves was ground with filtered (50 pm) stream water and sieved; the fraction of particles lower than

### Table 1. Biometry of eggs and first instar larvae and differentiation of larval instars of larvae from the two egg-masses used for the experiment of feeding.

<table>
<thead>
<tr>
<th>Egg-mass</th>
<th>No of eggs</th>
<th>Egg length (μm)</th>
<th>Egg width (μm)</th>
<th>Egg length: width ratio</th>
<th>Body length (μm)</th>
<th>Head length (μm)</th>
<th>Head width (μm)</th>
<th>Menton width (μm)</th>
<th>Instar differentiation (Head length μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>993</td>
<td>304±22</td>
<td>108±6</td>
<td>2.88±0.26</td>
<td>859±105</td>
<td>119±4</td>
<td>109±2</td>
<td>34±1</td>
<td>150-260, 260-420, &gt;450</td>
</tr>
<tr>
<td>#2</td>
<td>676</td>
<td>305±18</td>
<td>111±37</td>
<td>2.88±0.45</td>
<td>846±130</td>
<td>126±3</td>
<td>105±5</td>
<td>34±2</td>
<td>150-260, 260-420, &gt;450</td>
</tr>
</tbody>
</table>

Oxygen, pH, toxic substances, discharge, etc. (TOKESHI, 1995). However, it is also widely recognized that the outcome of food acquisition depends substantially on morpho-behavioural adaptations coupled with the type of food available (MERRITT & CUMMINS, 1984; MERRITT et al., 1992). Flexibility in the mode of feeding has been demonstrated for several chironomid species, involving changes in the mode of feeding according to changes in sediment composition (for instance in Chironomus plumosus, reported by HODKINSON & WILLIAMS, 1980).

The aim of the present work was to study the effects of food, different particle size and nutritional quality, and mode of feeding on the growth and development of recently hatched larval Chironomidae (Chironomus gr. plumosus).
250 μm was given to the larvae (10 ml wet volume per cage). This diet is referred to as FPOM (fine particulate organic matter). On the other hand, plastic sheets were incubated for 10 days in a riffle reach of Linesville Creek. After retrieval, the sheets were carefully rinsed with tap water and scraped to detach the periphytic algae, which were then supplied to the larvae (10 ml wet volume per cage) on the diet referred to as FPOM. Microscopic inspection of the plastic sheets revealed that the periphytic algae assemblage was composed mostly of diatoms.

Over the experiment, new supplies of food material were added once every two days to each cage to avoid food limitation or competition between the larvae.

Depending on the number of larvae available from each egg-mass the treatment combinations were divided or not into blocks (cages): egg-mass #1 = 3 cages algae, 3 cages FPOM, 3 cages CPOM; egg-mass #2 = 2 cages algae, 1 cage FPOM, 1 cage CPOM; each cage with 30 larvae.

Statistical treatment of the data

Comparisons of growth rates between diets and between larvae of the different egg-masses for the same diet were performed by ANCOVAs on ln(x+1) transformed data of body length. One-way ANOVAs were used to test for differences in body length (on ln(x+1) transformed data) between dates on FPOM diet.

Two-way ANOVAs were used for comparing the percentages of instars between diets and time for larvae of egg-mass #1, using cages as replicates. Data of percentage of instars were arcsin square-root transformed to satisfy the requirement of normality and homocedasticity. Tukey’s test (HSD) for unequal sample size (ZAR, 1984) was selected for post-hoc multiple comparison between diets and dates. All statistical analyses were performed with the software STATGRAPHICS (1994).

RESULTS

Natural history of egg-masses and recently hatched larvae

Except for the number of eggs, both egg-masses had quite similar characteristics (Table 1), both being globular clear gelatinous masses with an irregular disposition of the eggs. The eggs of both masses were almost identical in size (Table 1), had a yellowish colour and shape, and were reniform to deltoid. The length:width ratio of both egg types fell within the normal range for the Chironomidae, which according to NOLTE (1993) is 2.5 to 3.0.

Recently hatched larvae of both egg-masses were also highly similar morphometrically (Table 1) and behaviourally.

Over the process of ecdision the larvae moved their mandibles and anterior parapods with compulsive extensions, alternating periods of activity with inactivity. Finally, the cover of the egg became tattered and the larvae went free. In recently hatched larvae the abdomen was flattened dorsoventrally but in two or three hours it became cylindrical and more expanded caudally. The digestive tract had an intense yellowish colour, that disappeared about 24 h after hatching. The larvae were vigorously active immediately after emergence searching for food and moving their mandibles, some of them feeding on the gelatin and others ingesting particles of organic matter adhering to the mucopoly saccharide. This behaviour appears to be common among recently hatched chironomid larvae. The gelatin provides nutrition important sustaining the young larvae during the dispersal phase at least under laboratory conditions (PINDER, 1995). The small larvae, once provided the diet, began building cases from the beginning of the experiment, indicating that this is an activity also common among the first-instar larvae.

The instar differentiation, by mean head-capsule length of the larvae, was the same for the larvae of both egg-masses (Fig. 1, Table 1).

![Figure 1: Head-capsule length of the larval instars from the two egg-masses of Chironomus plumosus used in the experiment of feeding.](image-url)
Effects of food quality on growth and development of the larvae

The growth pattern was best described by exponential fitting (Table 2) in algae and CPOM diets. On the FPOM diet, larvae of both egg-masses did not grow significantly over the experiment (egg-mass #1: F = 2.82, p > 0.05; egg-mass #2: F = 1.36, p > 0.05), except between 10 and 12 days for larvae of egg-mass #1 (Tukey's test HSD, p < 0.05).

Although the characters of egg-masses, eggs and the larvae were very similar, possibly belonging to the same species, significant differences appeared in larval growth on the algae diet between the two egg-masses (Table 3), which might be caused, at least partially, by the different number of replicates. Therefore, the results of growth and development for the two egg-masses were treated separately.

No significant differences were noted for growth between replicated cages of larvae belonging to egg-mass #1 and on the same diet. (ANOVA, F = 2.70, P > 0.05). Therefore, the cage block effect was not taken into account in the statistical treatment of growth data.

For larvae of both egg-masses the diet of algae was clearly the best food, producing a significantly higher growth rate than that on the CPOM diet, and this latter diet proved significantly higher than that obtained on FPOM diet (Fig. 2; Table 2 and 3).

Diet significantly affected development of larvae belonging to egg-mass #1 (Fig. 3, Table 4). The overall percentage of first-instar larvae was significantly higher on the FPOM than on the CPOM and algae diets. Overall percentages of third and

Table 2. Results of the regression of body length data versus time for larvae of the two egg-masses of *Chironomus plumosus* and for the different dietary treatments. *Linear regression on raw data of body length. **Linear regression on ln(x+1) transformed data of body length. ***Exponential regression (L = a + e^t; L = body length, t = time) on raw data of body length. SE of the parameters of the exponential regression is the asymptotic standard error. R^2 is the percentage of variance explained by the regression models.

<table>
<thead>
<tr>
<th>Regression</th>
<th>Algae</th>
<th>CPOM</th>
<th>FPOM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>slope (b) ±SE</td>
<td>slope (b) ±SE</td>
<td>slope (b) ±SE</td>
</tr>
<tr>
<td>Linear*</td>
<td>0.4333 ± 0.2187</td>
<td>0.3359 ± 0.0248</td>
<td>0.3350 ± 0.1471</td>
</tr>
<tr>
<td>Linear**</td>
<td>0.6514 ± 0.0493</td>
<td>0.0899 ± 0.0053</td>
<td>0.4930 ± 0.0609</td>
</tr>
<tr>
<td>Exponential***</td>
<td>0.2931 ± 0.1415</td>
<td>0.0123 ± 0.0046</td>
<td>0.0123 ± 0.1176</td>
</tr>
</tbody>
</table>

FIGURE 2. Growth patterns (mean body length ± 1 SE) of larval instars of *Chironomus plumosus* from egg-mass in the three dietary treatments.
fourth instars were significantly higher on algae diet than on the other two diets. The significant interaction effect "diet x time" for instars I, III and IV (Table 4) can be interpreted as a consequence of different rates of development. On the diet of algae, after two days, the larvae already showed a reddish colour, becoming intensely red after about four days. On the CPOM diet the larvae started to develop the reddish colour later (after about 6 days of incubation) whereas on the FPOM diet the larvae remained colourless over the course of the experiment.

The inspection of the gut contents of the larvae revealed that on the FPOM and algae diets the ingestion of particles was in agreement with the corresponding diet. However, on the CPOM diet most of the ingested material was composed of fungal hyphae, while leaf tissue was seldom ingested.

**DISCUSSION**

One presumed feature of the development of chironomids is a brief first-instar larvae period (BERG, 1995; TOKIEH, 1995). In the present experiment, even on the diet of highest quality for growth and development, some first-instar larvae lasted for 8 days, which is similar to the duration of second-instar larvae. However, as the experiment did not cover the development of all instar larvae, data were not compiled on the relative duration of the different instars. Furthermore, from the initial inflexion of growth curves (in algae and CPOM diets) (Fig. 2) can be concluded that growth rate of first-instar larvae was slower than that of second- and third-instar larvae.

The present results indicate that the type of food clearly influences the growth and development of *Chironomus gr. plumosus* larvae. Certainly, periphytic benthic algae (mainly diatoms) proved to be a food of higher quality when compared with leaf debris, CPOM and FPOM. According to BRUNDIN (in BERG, 1995) the importance of algae for chironomids, particularly diatoms, is not surprising, given that the primitive chironomid habitat is thought to have been rich in diatoms (high mountain streams). Most field studies concerned with gut contents of *Chironomus* species have revealed the importance of algae, mainly diatoms, as a major source of food intake (KAJAK & WARDA, 1968; JOHANSSON & BEAVER, 1983; SMIT et al., 1993), although this may be partially as a consequence of the long-lasting nature and easy recognition of the diatom valves into the gut compared to other ingested materials. Reports on assimilation efficiency, however yield conflicting results. On the one hand, for instance, JOHANSSON & BEAVER (1983) concluded that diatoms contributed little to *Chironomus plumosus* f. semireductus energetics. On the other hand, JOHNSON et al. (1989) found that algal carbon, especially from the diatom Melosira, was nutritionally important source for *Chironomus plumosus*, contributing up to 84% of the larva's average daily carbon requirement. Similarly, KAJAK & WARDA (1968)
and MARKER et al. (1986) reported that diatoms are readily assimilated by several chironomid species. This latter set of studies agrees with the results presented here. Nevertheless, the benthic algae used in the present experiment were not pure cultures. Presumably, this diet contained a proportion of microbial biomass that was directly attached to the plastic sheets incubated in the stream and/or developed in the cages over the course of the experiment. Therefore, the diet algae should be called more accurately algae-derived detritus. This detritus, made of higher proportions of labile compounds, has been frequently recognized to be a higher quality food source than leaf-litter detritus, because of its higher microbial biomass and rapid decomposition rates (BERG, 1995). Whether algae had more or less nutritional value than associated bacteria and/or fungi was not investigated in the present experiment. Under natural conditions, both items are intimately associated in benthic biofilms of erosional and depositional habitats.

The growth and development on the diet CPOM was significantly higher than on the diet FPOM. On the latter diet, larvae did not grow significantly over the experiment, except larvae belonging to egg-mass #1 between 10 and 12 days of incubation; after 12 days of incubation, no larvae of either egg-masses were found alive on this diet. Such results might be considered contradictory, since the larvae of species in the genus Chironomus are widely recognized to be collector-gatherers or collector-filterers (COFFMAN & FERRINGTON, 1984). In fact, the feeding behaviour of the larvae on the diets with fine particles (algae and FPOM) was typical of a collector-gatherer in all instar larvae. The tube-dwelling larvae feed by extending from their tubes and collecting detritus from a roughly semicircular area at both ends of the tube. However, on the CPOM diet, larvae were observed mostly scraping on the surface of leaf disks, frequently abandoning the tubes. About halfway through the experiment, a substantial amount of faeces appeared in the cages with this diet, though the larvae were not visibly ingest faeces, which, however, were extensively used to build tubes. A scraping behaviour has been recorded by BAKER & BALL (1995) for larvae of Chironomus tentans inhabiting aquarium sand-beds. Intraspecific flexibility in the mode of feeding has been recognized for several Chironomus species and other chironomid species. For instance, Chironomus plumosus, typically considered to filter food in the profundal zone of lakes, exhibited high flexibility in its mode of feeding in an experimental woodland pond; that is, in the presence of leaf-litter accumulation, larvae fed by deposit-feeding because their living under the leaves prevented them from filter feeding (HODKINSON & WILLIAMS, 1980). In the present experiment, the shift to a scraper mode of feeding allowed the larvae to ingest microbial biomass of the biofilm on leaf disks (fungi hyphae observed in the gut contents). However, on the FPOM diet the small particle size probably determined the ingestion by the larvae of much more leaf debris than on the CPOM diet and thereby a higher proportion of refractory material than the more readily assimilable associated microbial biomass. The lack of significant growth on the FPOM diet is somewhat surprising, considering the results of WARD & CUMMINS (1979) on a deposit-feeding chironomid, Paratendipes albimanus. These authors reported significant higher instantaneous growth rates using diets of FPOM made of hickory and oak leaves than in other types of fine particulate detritus (Tipula faeces and natural stream detritus). Possibly, the incubation time of alder leaves for the present experiment was insufficient to produce microbial conditioning enough to sustain the growth of the larvae. WARD & CUMMINS (1979) incubated the leaves for 6 weeks in aquariums with stream water, and artificially stimulated the microbial growth with mineral salts.

Many studies have reported that deposit-feeding invertebrates appear to digest primarily the microflora associated with detrital particles (e.g. FENCHEL, 1970; ANDERSON & CUMMINS, 1979). This includes deposit-collector chironomid species (CUMMINS & KLUG, 1979; WARD & CUMMINS, 1979; LAMBERTI & MOORE, 1984). This seems especially true when dealing with leaf detritus because of the higher content of undigestible components (cellulose, lignin and ash) which rapidly passes through the gut (BERG, 1995).

The present results for FPOM and CPOM indicate that leaf tissue itself was of poor nutritional value for the larvae and that the microbial conditioning of FPOM was not enough to allow the larvae to reach the third instar -- only a small proportion of larvae of egg-mass #1 reached the second instar. Furthermore, the larvae were apparently unable to discriminate between microbial biomass and leaf tissue due to the small particle size in the FPOM diet, unlike in CPOM diet where, thanks to a flexible feeding behaviour, larvae scraped fungal mycelia from the leaf surface, action resembling the feeding mechanism of several leaf-eating invertebrates, such as Asellus aquaticus (GRAÇA et al., 1993).

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