Dietary effects on growth and fecundity of *Chironomus riparius*: The role of fatty acids

Master´s thesis, 20 credits.

by

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1. ABSTRACT

Laboratory experiments were conducted to study the importance of polyunsaturated fatty acids (PUFA), in particular eicosapentaenoic acid (EPA, 20:5ω3), for growth, development, and fecundity of the detrivorous midge *Chironomus riparius*. Larvae were fed Tetraphyll®, a commercial fish food, oatflakes, and the cyanobacteria *Spirulina* sp. Tetraphyll® is rich in the essential fatty acids of the ω3- and ω6- families and contains modest concentrations of EPA (PUFA content 36%). Oatflakes are rich in linoleic acid (18:2ω6) and relatively poor in α-linolenic acid (18:3ω3) (PUFA 44%) and lack long-chain (>20 C) PUFAs. *Spirulina,* in contrast, contains γ-linolenic acid (18:3ω6) and low concentrations of EPA (PUFA 21%). Additionally, feeding experiments were conducted with the green alga *Scenedesmus obliquus* (rich in 18:3ω3, poor in 18:3ω6, but lacking EPA, PUFA 47%), and with artificially EPA-supplemented *Scenedesmus*. Experimental endpoints were larval and adult size, survival, fatty acid composition of larvae, development rate, and fecundity.

Growth rates of larvae were affected both by food type and food concentration for Tetraphyll®, oatflakes and *Spirulina*, but only by food concentration for *Scenedesmus* and EPA-supplemented *Scenedesmus*. Highest growth rates were reached in the Tetraphyll®, oatflakes and *Spirulina* treatments. Growth was not improved with supplementation of *Scenedesmus* with EPA. In larval bodies, palmitic acid (16:0), palmitoleic acid (16:1ω7) and linoleic acid (18:2ω6) were the dominant fatty acids. Adult midge size showed strong positive relationship with food concentration in treatments with Tetraphyll® (r=0.97), oatflakes (r=0.98) and *Spirulina* (r=0.93) whereas no relationship was found between fecundity and food type.

The FA analysis of larval bodies showed that *Chironomus* is able to synthesise long-chain PUFA from shorter precursor FA and that the conversion capacities apparently meet their physiological demands. The results also showed that even at low concentrations *Chironomus* growth is apparently not limited by the absence of long-chain PUFA, thereby contradicting results from zooplankton studies.

2. INTRODUCTION

2.1. Lipids

[If not marked, all information is taken from Lehninger et al. (1993)]

Biological lipids are a chemically diverse group of compounds and their biological functions are equally diverse. As fats and oils they are the principal stored forms of energy in many organisms (Stryer, 1995). Depending on their polarity, lipids can be grouped into neutral and polar lipids. Triacylglycerols and wax esters are neutral lipids and abundant storage lipids that provide metabolic energy through oxidative catabolism (Olsen, 1999). Phospholipids represent the polar lipids. They are ubiquitous constituents of cell membranes and are therefore both structurally and functionally important.

The most simple lipids constructed from fatty acids are the triacylglycerols (TAG). TAG are composed of three fatty acids, each in ester linkage with a single glycerol. The TAG molecule is a dominant energy and carbon storage product in animals and is ubiquitous in terrestrial animals (Olsen, 1999). As stored fuels, TAG have two significant advantages over polysaccharides such as glycogen and starch. Because TAG are hydrophobic and therefore unhydrated, the energy is stored without the concomitant deposition of large quantities of water (Vance & Vance, 1996). Carbohydrates, in contrast, are much more polar and hydrated to a higher degree. Their ready solubility in water makes carbohydrates, such as glucose and glycogen, easily available sources of metabolic energy.

Phospholipids are assembled from a backbone molecule, usually a glycerol, a fatty acid attached to the backbone and a hydrophilic head group, joined to the backbone through a phosphodiester linkage. The phospholipids of the cell membranes are synthesized by enzyme systems that exhibit higher affinity for polyunsaturated fatty acids than for other fatty acids (Olsen, 1999). Biological membranes play a crucial role in almost all cellular phenomena. Singer and Nicolson (1972) postulated that the central architectural feature of biological membranes must be a fluid double layer of lipids. The ability of lipids to assume the basic bilayer organisation is dictated by a unifying characteristic of membrane lipids, namely their amphipathic character, which is indicated by the presence of a polar or hydrophylitic head group region and non-polar of hydrophobic region (Vance & Vance, 1996). The lipid bilayer constitutes a barrier to the

passage of polar molecules and ions and forms impermeable barriers that separates cellular compartments. The structure of the lipid bilayer itself is stable, and the degree of fluidity depends on lipid composition and temperature (which are membrane-specific). Most lipid species can undergo a transition from a very viscous gel/paracrystalline array at low temperatures to the fluid (melted) liquid-crystalline state as the temperature is increased (Vance & Vance, 1996). Saturated fatty acids in a bilayer pack well into a paracrystalline array, but the kinks in unsaturated fatty acids interfere with this packing. Thus, high amounts of unsaturated fatty acids in the membrane prevent the formation of a paracrystalline solid state. Unsaturated fatty acids have very low melting points compared to other biolipids.

Singer and Nicholson (1972) point out the characteristic response of poikilotherm cell membranes to reductions in ambient temperatures, which results in an increase in the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SAFA) in the lipid bilayer. Adjusting cell membrane fluidity is advantageous for organisms which remain active at low temperatures and has been well established for many aquatic poikilothermes as well as freshwater cladocerans and marine and freshwater copepodes (Farkas, 1979). Sargent et al. (1995), however question this conventional view that the abundance of long-chain ω3 PUFA in aquatic organisms is an adaption to life at low temperatures. They believe that the abundance of docosahexaenoic acid (DHA, 22:6ω3), can be explained by the light-transducing membranes of the visual apparatus in vertebrates which are particularly highly evolved in fish. DHA displays a unique conformation, arising from the basic methylene-interrupted *cis*-dienoic structure (Ghioni et al.,1996). These di-DHA phosphoglycerides maintain a highly structured but fluid membrane bilayer able to accommodate very rapid protein conformational changes, initiated by the *cis-trans* conversion undergone by the retinal chromophore of rhodopsin.

2.2. Fatty acids

Fatty acids are carboxylic acids with hydrocarbon chains of 4 to 36 carbons. The most commonly occurring fatty acids in living organisms have even numbers of carbon atoms in an unbranched chain of 12 to 24 carbons. SAFA lack double bonds in the chain;

otherwise the position of double bonds is regular; in most monounsaturated fatty acids (MUFA) the double bond is between C9/C10. In PUFA, which have two or more unsaturated bonds, the double bonds are generally between C12/C13 and C15/C16. Highly unsaturated fatty acids (HUFA) have four or more unsaturated bonds. All bonds introduced by oxidative desaturation in animals are in the *cis* geometric configuration (Vance & Vance, 1996). Due to these regularities fatty acids can be characterised by the formula $x : y\omega z$, where x designates the total number of carbon atoms, y is the number of unsaturated double bonds and ωz signifies the position of the first double bond from the methyl end of the molecule (Vance & Vance, 1996).

Since fatty acids play a variety of cellular roles, the ability to synthesize a variety of fatty acids is therefore essential to all organisms. The fundamental reaction sequence by which the long chains of carbon atoms in fatty acids are assembled are conducted by a multi-enzyme complex, the fatty acid synthase. The principle product of this enzyme in animal cells is palmitic acid (16:0), which then may be elongated to form stearic acid (18:0). As well palmitic as stearic acid serve as precursors of the two most common monounsaturated fatty acids of animal tissues: palmitoleic acid (16:1ω7) and oleic acid (18:1ω9). The double bond is introduced into the saturated fatty acid by an enzyme, called desaturase. The position of double bond introduction is determined by the type of desaturase. In the case of desaturation of stearic acid (18:0) to oleic acid (18:1ω9) the double bond is introduced between C9 and C10 by the Δ9-desaturase.

Linoleic acid (LA; 18:2ω6) and α-linolenic acid (ALA; 18:3ω3) are almost exclusively synthesized by plants (Parrish, 1999). The Δ15 desaturase, which is only found in organisms with chlorophyll, introduces a double bond at C12 and C15 position. Mammals for example can readily introduce double bonds at the Δ9 position of saturated fatty acids but cannot introduce additional double bonds in the chain between C10 and the methyl-terminal end. Both mammals (Hanson et al., 1985) and marine fish and crustaceans studied (Xu et al., 1993) lack the ability to synthesize LA and ALA. Since these fatty acids are necessary precursors for the synthesis of other products, they are essential for animals and must be obtained from plant material in the diet. However, the *de novo* synthesis of LA from oleic acid (18:1ω9) has also been reported in some terrestrial insects (Blomquist et al., 1982).

The elongation and desaturation of ALA to eicosapentaenoic acid (EPA, 20:5ω3) and DHA (22:6ω3) can be done by many organisms (Olsen, 1999). It is known that some insect species can convert C18 PUFA to their C20 products and that the elongation and desaturation pathways involved are not different from those occurring in mammals (Ghioni et al. 1996). Watanabe (1982) found that marine fish are unable to convert ALA to EPA and DHA, while freshwater fish can. Recent studies revealed that this is a generalisation that cannot be hold. Ahlgren et al. (1999) pointed out that strictly carnivorous freshwater fish either do not show Δ5 desaturase activity. They argued, that it is more likely that the difference in transformation capacities depends on the abundance and fatty acid composition of food available in these two very different environments. However, conversion rates in animals seem to be inefficient and there is reason to suspect that most organisms, whether aquatic or terrestrial, invertebrate or vertebrate, will grow better when provided with direct sources of EPA and DHA.

Larval organisms may even be more dependent on dietary HUFA than adults because their high somatic growth rates cannot be satisfied by their fatty acid conversion capacities, which probably vary considerably during their life cycle (Brett & Müller-Navarra, 1997). It is well appreciated that oils from marine organisms, until recently nearly always teleost fish, are the only readily available commercial sources of EPA and DHA (Sargent et al., 1995a).

The derivatives of the 20-carbon PUFA arachidonic acid (ARA; 20:4ω6) and EPA are termed eicosanoids. Eicosanoids exert a variety of extremely potent hormonelike actions on various tissues of animals. Their significance relates to two broad areas in animal biology, as mediators of crucial cellular events and of certain ecological interactions (Stanley-Samuelson, 1994). There are three classes of eicosanoids: prostaglandins, thromboxanes and leukotrienes. Eicosanoids exert analogous physiological actions in many invertebrates and lower vertebrates, as well as mammals. They modulate epithelial salt and water transport in species that represent mammals, frogs, toads, fishes, molluscs, and insects (Stanley-Samuelson, 1994). Eicosanoids are also involved with the reproductive and egg-laying behaviour in several insect species (Howard & Stanley-Samuelson, 1996).

2.3. Nutrition and food quality

The nutritional requirements of heterotrophic organisms in regard to nutrition can roughly be divided into two classes. Firstly, the organism needs energy for activity and internal maintenance. The need for energy can be satisfied by a variety of compounds (e.g. carbohydrates, glycogen, starch etc.) that are oxidised and is therefore called a non-specific need. Secondly, heterotrophic organisms need a supply of specific substances for synthesis of new tissue. Such specific needs can only be satisfied by a limited suite of organic compounds: the essential nutrients (Vos, 2001). Requirements for some essential nutrients (e.g. PUFA, amino acids and vitamins) are fairly consistent among vertebrates and invertebrates whereas the requirements for other nutrients vary, depending on the particular taxonomic or physiological group or developmental stage of the animal (Downer, 1981).

The determination of food quality is a problem of increasing interest in aquatic science because of its importance to the understanding of how food limitation can affect the structure of consumer populations (Müller-Navarra, 1995). Since autotrophic organisms are at the basis of aquatic food webs, they are generally thought to fuel secondary production. It is generally understood that in freshwater and marine foodwebs, phytoplankton production is limited by the availability of inorganic nutrients, mainly phosphorus, nitrogen, silica and iron (in open oceans) (Horne & Goldman, 1994). A row of experiments have shown that not only the biochemical composition of phytoplankton is sensitive to environmental stress but also the quality of lipids, including fatty acids (Ahlgren et al., 1998). Environmental stress, expressed as elemental limitations, induces increased production of TAG, composed mainly of SAFA and MUFA, but leads to decreased production of phospholipids, comprised mainly of PUFA (Ahlgren et al., 1992). Large disparities are found in the nutritional quality of the most common phytoplankton taxa, with some groups, such as diatoms and cryptophytes, promoting high zooplankton growth rates (Brett & Müller-Navarra, 1997). In general, cyanobacteria and green algae lack or have only traces of EPA and DHA, whereas diatoms are rich in palmitoleic acid (16:1ω7) and EPA, and flagellates are rich in both EPA and DHA. Fatty acid analysis of different freshwater algae, conducted by Ahlgren et al. (1992), displayed a lack of substantial amounts of PUFA in green algae, modest

amounts of SAFA (16:0), whereas ALA generally had the highest abundance. The ratio of ω3/ω6, which is a good indicator of food quality, was ≥ 2 for nearly all analysed taxa of chlorophyceae (Goedkoop et al., 1998). The varying fatty acid composition within species can be explained due to different growth phases. Ahlgren et al. (1992) found that total lipid content usually increased in the static growth phase compared to exponential growth. Two tested *Scenedesmus* species (Chlorophyceae) showed more or less unchanged patterns of ω3 fatty acids in both growth phases, but ω6 fatty acids decreased in the exponential growth phase, resulting in an increase in the ω3/ω6 ratio.

Lipid quality is not only influenced by environmental stress but also by seasonal variations. An investigation of plankton net samples and sediment traps from a mesotrophic Swedish lake was conducted by Ahlgren et al. (1997) to determine differences and seasonal changes in the quality of food available to pelagic zooplankton and benthic invertebrates. It was assumed that food quality is related to the content of PUFA including the ω3 FA. Their results showed consistently higher values in net plankton than in trap samples for SAFA, MUFA, and PUFA of both the ω3 and ω6 type. This result suggests that benthic fauna had access to high-quality food in spring and autumn, due to the dominance of sedimenting diatoms whereas food quality was low during late summer. The study of Goedkoop et al. (2000) on seasonal trends in FA concentrations of major food sources of invertebrates supports the findings of this study. They found that the ω3/ω6 ratio steadily declined in sedimenting matter during summer stratification and increased after the autumn turnover.

The primary food of most zooplankton is algae. The known essential fatty acids for zooplankton and other metazoans appear to be LA and ALA and perhaps EPA (Goulden et al., 1999). Sargent et al. (1995b) state that there is little or no evidence bearing on whether zooplankton can elongate and further desaturate shorter-chain ω3 PUFA to their longer-chain homologues and, in particular, convert EPA to DHA. However, given the luxus of EPA in their natural diets, it is unlikely that zooplankton have any requirement to form this fatty acid from ALA. Several attempts have been made (see Müller-Navarra, 1995; DeMott & Müller-Navarra, 1997; Sundbom & Vrede, 1997; Weers & Gulati, 1997; Gulati & DeMott, 1997) to quantify the effect of PUFA in phytoplankton on growth of zooplankton, especially daphnids. Müller-Navarra (1995) investigated whether sestonic essential ω3 fatty acid scarcity contributes to *Daphnia* growth limitation in

nature. She found a weak correlation between phosphorus and *Daphnia* growth and slightly stronger relationships with carbon and nitrogen. Strong relationships were found within the ω3 fatty acids. Within the ω3 FA family DHA showed a weaker correlation with growth than with EPA. She concluded that *Daphnia* was limited by EPA during the investigation season and especially during summer. Ahlgren et al. (1990) supposed ω3-HUFAs in general to be important in determining food quality for cladocerans, but Müller-Navarras experiment highlights the importance of just EPA. In a laboratory study DeMott and Müller-Navarra (1997) investigated the role of ω3 HUFA in zooplankton nutrition. Supplementing food algae with emulsions rich in ω3 HUFA markedly improved growth of the used *Daphnia* species. Further specification on which ω3 HUFA was the most important FA in nutrition could not be made, since fish oil emulsions include both EPA and DHA.

Sundbom & Vrede (1997) also stressed the importance of EPA for cladoceran growth while DHA seems to be less important because of low incorporation and conversion rates of DHA, at least in *Daphnia galeata*. Weers & Gulati (1997) pointed out the importance of EPA as an essential FA, but its presence in food does not seem to be indispensable for growth and reproduction in *Daphnia*. Their investigation showed that *Daphnia* developed normally and without timelag on a diet lacking EPA. Thus, daphnids have the ability to elongate and desaturate EPA precursors (esp. linolenic acid) which are found in high concentrations in many green algae species, although this conversion activity is very low. Goulden et al. (1999) thus concluded that a low rate of conversion indicates that daphnids are quite dependent on the fatty acid composition of their food. This finding is consistent with the general opinion of low conversion capacities of unsaturated fatty acids for other aquatic invertebrates (Kanazawa et al., 1979).

The role of food composition and quality has been more intensely studied for zooplankton, especially cladocerans. Most of this research has focused on elemental C, N, P and fatty acids (e.g. Brett & Müller-Navarra, 1997, DeMott & Müller-Navarra, 1997, Gulati & DeMott, 1997) but these parameters may not be suitable as indicators of food quality for benthic invertebrates. Processes such as chemical oxidation and bacterial decomposition have partly taken place before food settles on the sediment as detritus

(Ahlgren et al., 1997). Thus, food composition may be a limiting factor in the life history of detritus-feeding invertebrates.

Both phosphorus and polyunsaturated fatty acids are positively correlated with food quality for pelagic grazers and good correlations have sometimes been detected between the EPA (20:5ω3) and DHA (22:6ω3) content of phytoplankton and the growth rate of zooplankton, both in laboratory and nature (Ahlgren et al., 1990; Müller-Navarra, 1995). The benthos of temperate lakes often experience a seasonal variation of food supply. For instance, the diatom bloom, that eventually settles to the profundal zone of the lake, may be the main source of high-quality food for the benthos during the year. To survive periods when high-quality food is not reaching the benthic community, cold stenothermic benthic invertebrates often store energy as lipids (i.e. TAG) or waxes when food is abundant (Cavaletto & Gardner, 1999). Waxes are the chief storage form of metabolic fuel in some aquatic organisms (e.g. insects, larvae and copepods) (Olsen, 1999).

Since decomposition of phyto-detritus during the sedimentation process as well as dilution with refractory or inorganic resuspended matter decreases the food quality for profundal invertebrates, they are rather limited by food quality than by food quantity. Focus is laid here on the family of *Chironomidae*, a representant of the group of profundal invertebrates. Chironomids are selective feeders, feeding on detrital particles, bacteria and algae. Algae (especially diatoms) can be an important part of their diet. (Johnson et al., 1989). A study on quality of food items for *Chironomus riparius* was conducted by Vos (2001). Her results demonstrated an interaction between food quantity and food quality of larval growth, i.e., different food components were the limiting factors for growth at different food levels. High N, P, and lipid contents stimulated growth at high food levels, whereas the amount of carbohydrates appeared to be important in controlling growth at low food levels. Carbohydrates have a high energy content and are usually used as an energy source. Lipids and proteins can be used as energy source as well but are used preferentially to deliver essential components necessary for growth. However, at low food concentrations, it is more probable that energy is the limiting factor since an increasing proportion of food consumed is utilised for basal metabolism and is therefore not sufficient for growth (Brett & Müller-Navarra, 1997).

2.4. The biology of chironomids

[if not marked, all information is taken from „The Chironomidae" edited by P. ARMITAGE, P.S. CRANSTON and L.C.V. PINDER]

The non-biting midge *Chironomus riparius* belongs to the family of the Chironomidae, which makes it a member of the order of true flies (Diptera). There are estimated to be as many as 15 000 species of chironomids world-wide, who have adapted to lentic, lotic and terrestrial environments. Geographically seen, they are the most widely distributed free-living holometabolous insects. They are the most abundant insects in freshwater. Larvae of the ubiquitous *Chironomus* group, commonly known as bloodworms, often dominate the profundal zoomacrobenthos community in numbers and biomass (Johnson et al. 1989). The sediment-ingesting, haemoglobin-bearing larvae live in U-shaped tubes, through which an intermittent irrigation current is maintained (Walshe, 1947). Undulating movements of their body in the tube permits replenishing oxygen and flushing out metabolites and carbondioxide. In contrast to vertebrate haemoglobin, chironomid haemoglobins have a high affinity for oxygen. A *Chironomus* specific pigment enables the larvae to pick up and unload oxygen at low concentrations. Most kinds of haemoglobin can load oxygen only at high concentrations and unload at low concentrations. Since sediment-living species often experience very low oxygen levels this special mechanism in *Chironomus* makes it possible to maintain constant activity (Horne & Goldman; 1994). Due to the predominance of the genus *Chironomus* in eutrophic lakes, Naumann and Thienemann used these indicator species for the development of the first classification of lakes (Lampert & Sommer, 1999).

Chironomids can be grouped into several functional feeding groups, based on larval feeding modes. The genus *Chironomus* is classified as a collector-gatherer or a collector-suspension feeder, i.e. animals that feed on surficial sediments or suspended matter. This functional feeding group predominates where fine particulate organic matter accumulates. Chiromomides can ingest food either non-selectively on the basis of availability or selectively on the basis of size, nutritional value or food type. The occurrence of selective or non-selective feeding can vary considerably within a single species. Larvae may also exhibit ontogenetic changes from selective to non-selective feeding as they mature. Collector-gatherers feed by extending the head and anterior part of the body outside the tube while using the posterior prolegs to maintain contact with

the inner surface of the tube. Foraging areas are therefore restricted to a region immediately surrounding the tube.

As insects belonging to the Holometabola, the life cycle of *Chironomus* is divided into four distinct stages. Figure 1 displays the life cycle of *Chironomus*.

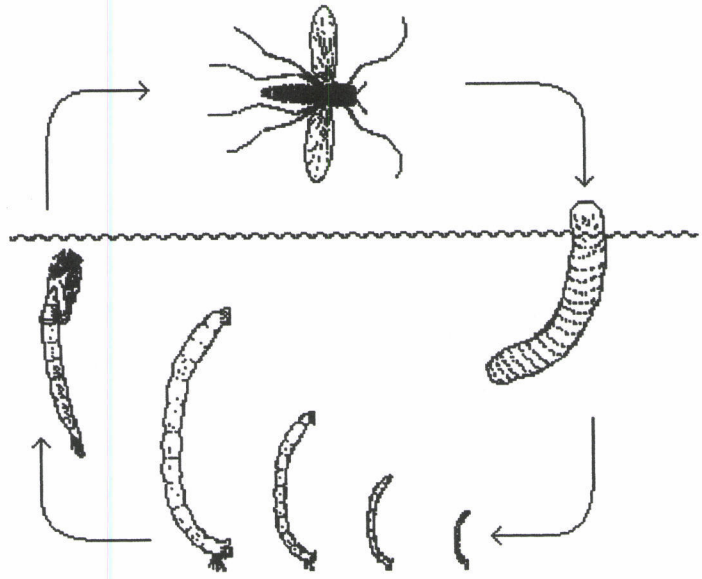


Figure 1. Life cycle of Chironomidae displaying the egg stage, the four larval instars, the pupal stage and the terrestrial imago (modified from Gullan & Cranston, 1996).

Typical egg masses of freshwater chironomids contain from 20 or 30 eggs up to 2000. In general, a single egg mass is produced and deposited on firm substrata close to the water's edge at dusk or dawn. The eggs are normally encased in a clear gelatinous mass, being commonly elliptical in shape. The time taken from egg deposition to the point of hatching lies within three days at 20 °C. After having left the egg, larvae in laboratories often spend several hours within the egg mass, during which time they feed on the gelatin. The gelatin is considered to consist mainly of carbohydrates and seems to be an important nutrition source for young larvae. The first larval instar differs distinctly from the other instar stages. 1st instars are planktonic whereas the following stages reside within the upper layer of the sediment in tubes constructed from sedimentary materials attached by mucous secretions (Benoit et al., 1997). The pupal stage is short-lived, particularly compared to the larval stage. This stage involves major changes in morphology as the larvae metamorphoses to the adult. The final stadium of chironomids is short-lived and mainly concerned with reproduction. It is common view that the adults do not feed, although contrasting observations have been made. Adults often emerge

simultaneously and form huge mating clouds. Males can be distinguished from females by their plumose antenna. Both males and females typically die within 7 days of emergence (Benoit et al., 1997). The whole life-cycle of *Chironomus riparius* can be carried out within 25–30 days under favourable conditions (23°C).

2.5. Study objectives

Chironomids represent an abundant group of benthic insects in freshwater ecosystems. However, the role of food composition for benthic in vertebrates has been studied very little in contrast to zooplankton, especially cladocerans (e.g. Müller-Navarra, 1995; Brett & Müller-Navarra, 1997; DeMott & Müller-Navarra, 1997, Weers & Gulati, 1997). It has been found that cladocerans showed increased growth rates feeding on algae supplemented with eicosapentaenoic acid (EPA). Food composition might be a limiting factor in the life history of detritus-feeding invertebrates since partial decomposition and oxidation have taken place before organic material reaches the sediment as detritus.

The objective of this study was to determine the importance of the biochemical food composition for growth and fecundity of the benthic invertebrate *Chironomus riparius*. Several tests were conducted to assess whether the content of EPA in food is a good indicator of food quality for these detrivorous animals, and if *Chironomus* can complete its life-cycle only on short FA, i.e. if conversion rates from precursor FA are sufficient to meet their physiological demands.

3. METHODS

3.1. Culturing of algae

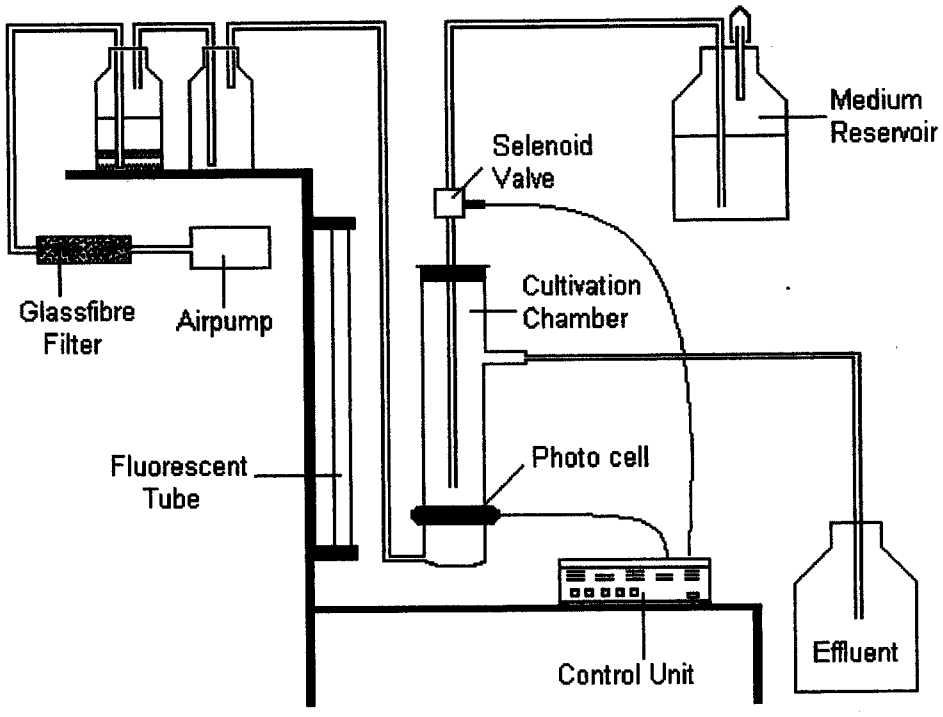


Figure 2. Diagrammatic sketch of the turbidostat (after Kebede & Ahlgren, 1996).

The turbidostat chamber (Figure 2) is a type of continuous culture which is controlled by the turbidity of the culture. In a continuous culture the growth of the organism is exponential and the volume is maintained constant by an overflow device (Ahlgren, 1977). The turbidostat chamber (Volume 0.5 l) is continuously illuminated by fluorescent tubes (Philips TL 20W/33). Optimal light conditions are achieved by variation of the number of tubes and/or by moving the cultivation chamber closer to or farther from the source of light. Air is provided constantly from an aquarium pump, going by a glass fibre filter and is moistened by passing through a flask of deionised water before entering the cultivation chamber of the turbidostat. The temperature in the cultivation room is kept constant to 20°C.

The density of the algal suspension inside the cultivation chamber is measured automatically by an optical head, consisting of a photodetector and a lamp, clamped onto the cultivation chamber. The photodetector is connected to an electronic control unit

(ACC-50, Techtum Instrument; Umeå, Sweden) which is, in turn, adjusted to a fixed density level. If the algal density level is exceeded this control unit, a selenoid valve opens and algal medium from the medium reservoir flows into the culture vessel and dilutes the culture medium to the pre-set value. Via an effluent the excess suspension is harvested. At steady state, i.e. when algal biomass and flow rate of excess volume are constant, the specific growth rate (μ) per day is equal to the dilution rate (D) which is defined as

$$D = \mu = \frac{F}{V} * \frac{24}{t}$$

where F is the flow rate of excess volume (ml d^{-1}), measured over t (in hours), and V is the culture volume (ml).

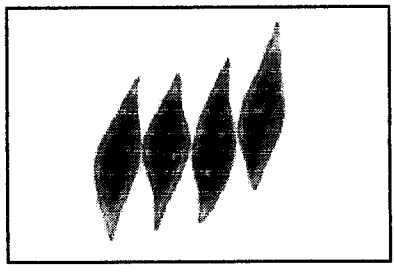


Figure 3. *Scenedesmus obliquus* (after Tikkanen & Willén, 1992)

Scenedesmus obliquus (Turpin) Kützing (Figure 3 and 15) originated from E. van Donk, Nieuwersluis, Netherlands. An inoculum was taken from a batch culture and grown in fresh Z8'"medium 100% (Ahlgren, 1977) for one week at 20 °C and a light intensity of 33.2 µMol $m^{-2}s^{-1}$. From this culture 15-20 ml served as inoculum for the turbidostat cultures. Before inoculating the two cultivation chambers were filled with 0.5 litres Z8'medium, respectively. Initially the culture was subjected to a high light intensity and the cultivation chamber was therefore shaded with a thin layer of tissue during day one and two of cultivation. This reduced the light intensity inside the cultivation chamber by 50% to 66.4 µMol $m^{-2}s^{-1}$ measured with an immersible spherical sensor in the centre of the cultivation chamber (QSI-100, Biospherical Instruments Inc.). Removal of tissue after two days led to a light intensity of 125 µMol $m^{-2}s^{-1}$ inside the cultivation chamber.

Scenedesmus was harvested at steady state. The excess suspension was filtrated over a polycarbonate filter 3 µm Millipore. Algae were removed from the filter and stored in small containers in the refrigerator until they were used for the growth experiments with *Chironomus* larvae. *Scenedesmus* is generally colonial in lake plankton but was mainly single celled in the monocultures used. Single cells were approximately 8 µm long and 2-3 µm wide. The preparation of Z8'-medium is done according to Table 1. Deionised water was ventilated for at least 30 minutes with carbon dioxide. From the stock

solutions a suspension is prepared. To avoid precipitation of $CaCO_3$, the $CaCl_2$ stock solution is added after addition of CO_2 saturated water to the suspension. The suspension was then autoclaved for one hour. The pH value after autoclaving was 6.5-7.7.

Table 1. Stock solutions for nutrient medium Z8' according to Ahlgren (1977).

Stock solution	mg l^{-1}	Element	mg l^{-1}
CO_2 saturated H_2O	100 ml l^{-1}	N	77
$NaNO_3$	467	Ca	10
$CaCl_2 * 2H_2O$	36.7	P	5.5
K_2HPO_4	31.0	K	14
$MgSO_4 * 7H_2O$	25.0	Mg	2.4
Na_2CO_3	21.1	Na	135
Fe-EDTA-complex solution *)	10 ml l^{-1}	Fe	0.575
Gaffrons's Trace Elements**)	0.08 ml l^{-1}	Cl	19

*) Fe-EDTA-complex solution: 10.5 ml 0.1 N $FeCl_3 * 6H2O$ in 0.1 N HCl is mixed with 10 ml 0.1 N NaOH and diluted to 1000 ml.

**) see Appendix

3.2. Culturing of larvae

Larvae and adult midges of *Chironomus riparius* (Meigen) were cultured in an aquarium (60 cm x 27 cm x 24 cm) at the Department of Environmental Assessment, Uppsala. The animals were grown at room temperature (20°C) and under a light:dark cycle of 18:6 hours. Larvae received a tea-spoon of food every second day. The sediment in the aquarium was of natural origin (Lake Erken, Sweden) and was approximately 3 cm thick. The overlying water column was approximately 14 cm deep and was a mixture of 50% tap-water and 50% deionised water. This mixture was used to decrease the high conductivity of Uppsala tap water. The culture was constantly aerated by two aquarium pumps (Sacem, SR 300). The culture aquarium was surrounded by a mating cage (76 cm x 40 cm x 110 cm) with the front side covered by a screen (1000 µm) to prevent hatching midges from escaping. Adult midges mated and oviposited in the cage preferable on the inside of the aquarium walls at the water-air interface. The egg masses were collected with a spoon and a spatula and transferred to small Petri dishes containing 15 ml of aerated 50% tap-water and 50% deionised water. Usually the eggs were kept under room temperature in the laboratory and experienced a natural light and day cycle

until hatching. At some instances the egg masses were stored in the fridge (5°C) for one or two days to slow down and/or to synchronise development with other eggs. These egg masses experienced 24 hours of darkness.

The preparation of M7-medium followed the protocol given in Streloke & Kopp (1995) (Table 2). Each stock solution I was prepared individually and from these solutions a combined stock solution II was prepared. 50 ml from the combined stock solution II and the given volumes of each macro nutrient stock solution (Table 3) were added and diluted with deionised water to prepare the M7-medium. A vitamin stock solution was prepared by dissolving three vitamins in deionised water (Table 3). 0.1 ml of the combined vitamin stock was added to the final M7-medium shortly before use. The medium is aerated and stored cold (2-3°C).

Table 2. Stock solutions of trace elements for medium M7.

stock solutions I	amount (g) made up to 1 litre deionised water	to prepare the combined stock solution II mix the following volumes (µl) of stock solution (I) and dilute to 1 litre with deionised water	final concentrations (mg/l)
H_3BO_3	57.19	0.25	0.715
$MnCl_2$	7.21	0.25	0.090
LiCl	6.12	0.25	0.077
RbCl	1.42	0.25	0.018
$SrCl_2 * 6 H_2O$	3.04	0.25	0.038
NaBr	0.32	0.25	0.004
$Na_2MoO_4 * 2 H_2O$	1.26	0.25	0.016
$CuCl_2 * 2 H_2O$	0.335	0.25	0.004
$ZnCl_2$	0.260	1.0	0.013
$CoCl_2 * 6 H_2O$	0.200	1.0	0.010
KJ	0.065	1.0	0.0033
Na_2SeO_3	0.0438	1.0	0.0022
NH_4VO_3	0.0115	1.0	0.00058
$Na_2EDTA * 2 H_2O$*)	5		0.625
$FeSO_4 * 7 H_2O$*)	1.991	5.0	0.249

*) These solutions are prepared individually, then poured together and autoclaved immediately.

Table 3. Macro nutrient stock solutions for M7 medium.

Stock solution	amount (g) made up to 1 litre deionised water	amount of macro nutrient stock solutions added to prepare medium M7 (ml/l)	final concentrations (mg/l)
$CaCl_2 * 2 H_2O$	293.8	1.0	293.8
$MgSO_4 * 7 H_2O$	246.6	0.5	123.3
KCl	58.0	0.1	5.8
$NaHCO_3$	64.8	1.0	64.8
$NaSiO_3 * 9 H_2O$	50.0	0.2	10.0
$NaNO_3$	2.74	0.1	0.274
KH_2PO_4	1.43	0.1	0.143
K_2HPO_4	1.84	0.1	0.184

Table 4. Vitamin stock solution for medium M7.

	amount made up to 1 l of deionised water (g)	amount of vitamin stock solution added to prepare medium M7 (ml/l)	final concentration in test solution M7 (mg/l)
Thiaminehydrochloride	0.075	0.1	0.075
Cyanocobalamin (B_{12})	0.010		0.0010
Biotine	0.0075		0.00075

3.3. Chemical composition of food types

The food types used in the experiment were Tetraphyll®, a fish food generally used in laboratory studies (TetraWerke, Germany), oatflakes (AXA Havregryn, Cerealia Breakfast Cereals AB, Järna, Sweden), *Spirulina* sp. (Lindroos Hälsoagenturer, Örebro, Sweden) and cultured *Scenedesmus obliquus.*

The fatty acid composition of these food types and of the *Chironomus* larvae was determined with gas–chromatography at the Department of Geriatrics, Uppsala. The analysis follows the procedure described in detail in Ahlgren et al. (1994). Pre-weighed samples were analysed for their fatty acid pattern. In the printouts from the analysis individual fatty acids were identified by comparing the retention times with several mixtures of commercially available external standards (Sigma, USA). The FA were quantified (mg/g dry weight (dw)) by injecting fixed amounts of the dissolved, pre-weighed samples and comparing the area of the peaks with the peak of an internal

standard (2.5 mg ml^{-1} 23:0). Total carbon and nitrogen content of food types were measured with a Carlo Erba Analyser (Carlo Erba Instruments NA1500). Total phosphorus contents were analysed according to Murphy & Riley (1962).

Table 5. Elemental concentrations and ratios of food types expressed in mg/g dw (n=3).

Elemental Concentrations	Tetraphyll®	Oatflakes	*Scenedesmus*	*Spirulina*
C	43.7 ± 0.9	43.8 ± 1.6	48.6 ± 1.2	43.0 ± 0.6
N	7.9 ± 0.3	2.1 ± 0.5	7.8 ± 1.8	9.6 ± 0.1
P	1.1 ± 0.5	0.3 ± 0.2	1.2 ± 0.7	1.2 ± 0.0
Elemental Ratios				
C/N	5.5 ± 0.1	21.9 ± 6.3	6.54 ± 1.9	4.5 ± 0.0
C/P	40.8 ± 1.2	132.7 ± 6.9	41.2 ± 1.4	37.4 ± 0.7
N/P	7.4 ± 0.4	6.4 ± 2.1	6.6 ± 2.2	8.3 ± 0.1

The food types used in the experiments (Tetraphyll®, oatflakes, *Scenedesmus*, *Spirulina*) were more or less similar in their carbon concentrations, ranging between 43.0% and 48.6% (Table 5). The N concentration, however, differed more than a factor four among the different food types. Lowest in N were oatflakes (2.1 ± 0.5 mg/g dw) while the other food types had N concentrations ranging between 7.8% and 9.6%. Oatflakes were also the lowest in P (0.33 ± 0.21 mg/g dw), which was a third of the P-concentration of the two algae and Tetraphyll®. Due to this low P concentration oatflakes displayed the highest C/P ratio with 132.7 ± 6.86. The N/P ratios of the food types I used in the experiments ranged from 6.4 ± 2.14 to 8.3 ± 0.12.

Table 6. Fatty acid composition (mg/g dw) of the food types used in the experiments.

Fatty acid	Acid name	Tetraphyll®	Oatflakes	*Scenedesmus*	*Spirulina*
14:0		1.71 ± 0.72	0.10 ± 0.01	1.32 ± 0.00	0.58 ± 0.01
16:0		9.45 ± 3.72	7.52 ± 1.17	2.36 ± 0.03	10.90 ± 0.43
16:1ω7		2.21 ± 0.92	0.12 ± 0.02	1.10 ± 0.02	1.32 ± 0.04
18:0		3.35 ± 0.97	0.75 ± 0.06	0.07 ± 0.01	9.23 ± 1.21
18:1ω9		8.57 ± 2.92	17.06 ± 2.81	1.30 ± 0.03	1.13 ± 0.00
18:2ω6	LA	12.94 ± 5.61	19.49 ± 3.66	1.90 ± 0.03	2.15 ± 0.04
18:3ω6	GLA	–	–	0.61 ± 0.01	4.28 ± 0.13
18:3ω3	ALA	1.42 ± 0.62	0.63 ± 0.16	12.37 ± 0.19	0.04 ± 0.0
18:4ω3	SA	–	–	2.89 ± 0.04	–
20:0		1.72 ± 0.27	0.06 ± 0.0	–	0.08 ± 0.00
20:4ω6	ARA	0.21 ± 0.05	–	–	0.10 ± 0.00
20:5ω3	EPA	1.45 ± 0.70	–	–	0.10 ± 0.02
22:6ω3	DHA	2.53 ± 0.00	–	–	–
Σ SAFA		16.17 ± 5.74	8.61 ± 1.45	11.63 ± 0.55	20.98 ± 1.66
Σ MUFA		13.83 ± 6.67	17.44 ± 2.54	4.08 ± 0.06	1.49 ± 0.01
Σ PUFA		17.65 ± 9.15	20.12 ± 3.82	18.08 ± 0.14	6.58 ± 0.07
Σ FA		49.70 ± 24.46	46.27 ± 7.94	38.37 ± 1.60	29.32 ± 1.74

* Σ FA includes other FA and, consequently, is larger than Σ SAFA+Σ MUFA+Σ PUFA.

However, the fatty acid pattern of the food types differed markedly in contrast to the more or less homogeneous composition of elements (Table 6). Tetraphyll®, oatflakes, *Scenedesmus* and *Spirulina* contain linoleic acid (LA, 18:2ω6), albeit in varying concentrations. Concentrations of LA were highest in oatflakes and Tetraphyll®, 19.49 ± 3.66 mg/g dw and 12.94 ± 5.61 mg/g dw, respectively, and lowest in *Spirulina* (2.15 ± 0.04 mg/g dw) and *Scenedesmus* (1.90 ± 0.03 mg/g dw) (Table 6). High concentrations of α-linolenic acid (ALA, 18:3ω3), in contrast, were found in *Scenedesmus* (12.37 ± 0.19 mg/g dw), whereas in Tetraphyll® the concentration of ALA was eight times lower (1.42 mg/g dw) and in oatflakes even 16 times lower (0.63 ± 0.16 mg/g dw). In *Spirulina*, ALA was nearly not detectable (0.04 ± 0.00 mg/g dw) The polyunsaturated fatty acids (PUFA) arachidonic acid (ARA, 20:4ω6) and eicosapentaenoic acid (EPA, 20:5ω3) were present in Tetraphyll® and in traces in *Spirulina*, but not in oatflakes and *Scenedesmus*. Docosahexaenoic acid (DHA, 22:6ω3) was only found in Tetraphyll®.

Concentration of total fatty acids (ΣFA) was highest in Tetraphyll® (49.70 ± 24.46 mg/g dw) and lowest in *Scenedesmus* (38.37 ± 1.60 mg/g dw) and *Spirulina* (30.74 ± 1.80 mg/g dw). PUFA came to a fifth of total FA in *Spirulina*, a third of total FA in Tetraphyll® and a half in oatflakes and *Scenedesmus*.

3.4. Data Analysis

It was presupposed that the data followed a Gaussian distribution, but to achieve an even better approach to a normal distribution, the data were logarithmically transformed for the statistical analyses (Lozan & Kausch, 1998). The statistical tests were conducted with an α-value of 5%. The statistic program StatView 5.0 was used to apply two-way ANOVA, one-way ANOVA, and *t*-Tests to the experiments. Scheffe's posthoc tests were run for pairwise comparisons. All values are given as means ± standard deviation (SD).

3.5. Pilot Studies

Three different pilot studies were conducted to test parameters influencing growth and survival of *Chironomus riparius* larvae. The food-quality test (3.5.1.) was conducted to inspect how food types, which differ in their fatty acid (FA) pattern, affect growth of *Chironomus riparius* larvae. This test comprised treatments with three food types which were added in a concentration series of five different concentrations. In the life-cycle test (3.5.2.) I investigated whether *Chironomus* larvae could accomplish the transformation from the larvae to the adult with only pulverised oat as food. Since oatflakes only contain traces of ω3-fatty acids (Table 6), the larvae must have a functioning Δ5 desaturase to produce the highly unsaturated fatty acid EPA. The elongation of ALA occurs via stearidonic acid (18:4ω3) to 20:4ω3. The Δ5 desaturase then introduces one more double bond to create EPA (see also Appendix). This test was conducted on different types of sediment than the usual sea-sand and can therefore be seen in context with the sediment

test. In the sediment test (3.5.3.) I tried to overcome the problem, encountered in the food quality test, of *Scenedesmus* disappearing from the sediment surface after one or two days. The alga was thus less available to the surface feeding *Chironomus* larvae. A sediment with a smaller mean grain size than sea-sand was used.

Since the pilot studies were quite similar in their experimental set-up a general description is given first. If not specifically mentioned this general set-up is valid for all three pilot studies.

The pilot studies were conducted in glass microcosms (bottom area=80 cm^2, 1000 ml). Each microcosm contained 50 g of acid-purified sea-sand (0.2-0.5 mm) and 100 ml of rearing solution (M7 medium) respectively, before ten newly-hatched (<24 hours), randomly selected larvae were added. Larvae were selected from at least three egg masses. Lids on the containers prevented evaporation of the medium. Microcosms were aerated with capillary tubing (inner diameter 0.76 mm) and aquarium pumps (ELITE 802). Duration of the tests was restricted to ten days to avoid that larvae would reach the pupal stage. Last-instar larvae of several Diptera species have a different chemical composition than earlier instars, particularly a pronounced increase of lipid content, which may imply a difference in growth response to food composition compared to earlier instar-larvae (Vos et al., 2001). Each treatment consisted of three replicates and the replicates were started on different days. The microcosms were kept at 20°C with a 16:8 hours light:dark cycle. Light intensity was about 5 $\mu Mol\ m^{-2}s^{-1}$.

After termination of the tests, the whole content (sand, medium, larvae) of each microcosm was transferred to a white plastic tray. The larvae were selected from the sand and anaesthetised with a few drops of carbonated water in a small Petri dish. Each larva was placed on millimetre paper straightened and its length was measured to the nearest half millimetre. Individual fresh weight of larvae was determined on an analytical balance (Mettler Toledo, 1801 Sartorius) which records to the nearest 0.1 mg. If the biomass of the single larva was below the detection limit of the analytical balance, the pooled biomass of all larvae from one microcosm was determined. A few pooled samples were even below the detection limit and therefore no fresh weight was recorded. Larvae of each microcosm were preserved in 2% formaldehyde solution and stored.

Tetraphyll® and oatflakes were grounded to a fine grained powder with a kitchen blender. The powder was dried for 4 hours at 60 °C and stored in small plastic containers

in a dessicator until use. *Scenedesmus* was cultured in a turbidostat chamber and harvested over a polycarbonate filter 3µm Millipore at exponential growth phase (3.1.). All food items were weighed on the day before feeding on an analytical balance (Mettler Toledo, Sartorius MC1 RC210D) which records to the nearest 0.01 mg.

3.5.1. Food quality test

For all food items the carbon additions of 0.8, 2.2, 5.8, 11.4 and 22.8 mg per microcosm per ten days were run. Food was added twice during the test phase at day 0 and day 5, respectively. Pulverised food items Tetraphyll® and oatflakes were added to the overlying medium in the microcosms. *Scenedesmus* was resuspended with a little M7-medium from the microcosm before adding. All food items were allowed to settle to the sand. To facilitate settlement, the aeration was switched off for two hours after feeding.

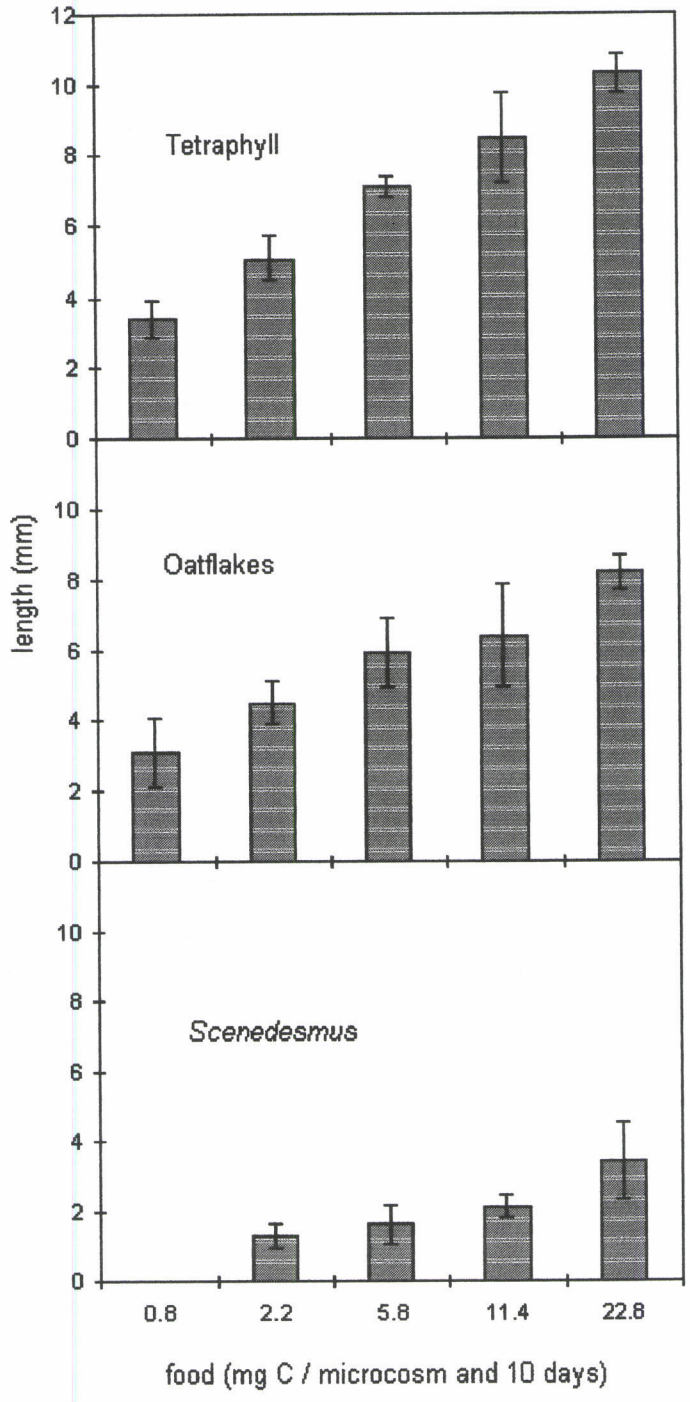


Figure 4. Mean length of *Chironomus riparius* larvae after 10 days of growth on different food types. Error bars indicate standard deviation (n=3).

Food type as well as food concentration had an effect on growth and survival of *Chironomus* larvae (Table 7). Growth was not affected by a combined effect of food type and food concentration whereas survival was affected

Table 7. Two-way ANOVA for mean length (mm) and survival (%) of *Chironomus* fed with different food types (n=3).

	Length			Survival		
	df	F-value	*P*-value	df	F-value	*P*-value
food type	2	150.591	< 0.0001	2	46.510	< 0.0001
food concentration	3	22.160	< 0.0001	4	6.370	0.0008
food type x food concentration	6	0.836	0.5548	8	7.351	< 0.0001

Pairwise comparisons of food concentrations within each treatment showed that there was no significant difference in mean length between adjacent food concentrations, except between the concentrations 0.8 and 2.2 mg C/10d in the Tetraphyll® treatment (*P*=0.174). Pairwise comparisons of concentrations among food types showed that between Tetraphyll® and oatflakes no difference was found at any concentration, but differences at each corresponding concentration were found between the Tetraphyll® and *Scenedesmus* treatment and between the oatflakes and *Scenedesmus* treatment. Mean length of larvae in the Tetraphyll® treatment increased from 3.4 ± 0.5 mm at the lowest to 10.3 ± 0.5 mm at the highest concentration (Figure 4). With oatflakes as food type, larval length ranged from 3.1 ± 1.0 mm to 8.2 ± 0.5 mm and from 1.3 mm ± 0.4 mm to 3.4 ± 1.1 mm in the *Scenedesmus* treatment.

Larval survival exceeded 90% in the Tetraphyll® treatment and was 93% in the oatflakes treatment (Table 8). With *Scenedesmus* as food, survival ranged from 0% in the lowest concentration (0.8 mg C/10d) to 87% at the highest food concentration (22.8 mg C/10d) (Table 8). Overall mean survival on *Scenedesmus* was 45%.

Table 8. Survival (%) ±SD of *Chironomus* larvae on different food types and concentrations. (n=3)

food type	0.8 mg C/10d	2.2 mg C/10d	5.8 mg c/10d	11.4 mg C/10d	11.8 mg C/10d
Tetraphyll®	93 ± 6	96 ± 12	90 ± 0	90 ± 10	80 ± 20
Oatflakes	80 ± 17	93 ± 12	103 ± 15	87 ± 15	97 ± 15
Scenedesmus	0	10 ± 10	50 ± 36	77 ± 15	87 ± 6

3.5.2. Lifecycle test

The prior aim of this test was to investigate whether the detrivore *Chironomus riparius* could complete its lifecycle with oatflakes as sole food item. Since oatflakes contain only low amounts of α-linolenic acid (ALA) they must have a well functioning Δ5 desaturase to meet their demands of highly unsaturated fatty acids (HUFA). 21.0 mg carbon as pulverised oatflakes were added per microcosm per five days. Food was added every fifth day to the overlying medium in the microcosms until the emergence of the first adults. After ten days the lids on the microcosms were replaced with emergence traps (Figure 5) as described in Benoit et al. (1997). Since after 10 days larvae have reached the fourth larval instar and moult from pupa to adult will soon appear, the lids were replaced in advance to avoid escape of adults. Male/female emergence observations were scored from day 10 and double checked by counting the exuviae inside the microcosms. Emerging adults were collected with an aspirator. Emerged female and male midges from the same microcosm were transferred to the reproduction chamber (Figure 5). Pairs were only formed if sufficient males respectively females had hatched. The test was ended after 27 days.

Due to the use of two different sediments in this test, I was also able to determine the availability of a pulverised food type sediments with a deviating mean grain size than sea-sand. Limestone powder (Kalkstenmjöl, Nordkalk KÖ 500, Partek) had a mean grain size smaller than 0.2 mm, whereas bird sand had a mean grain size >0.3 mm.

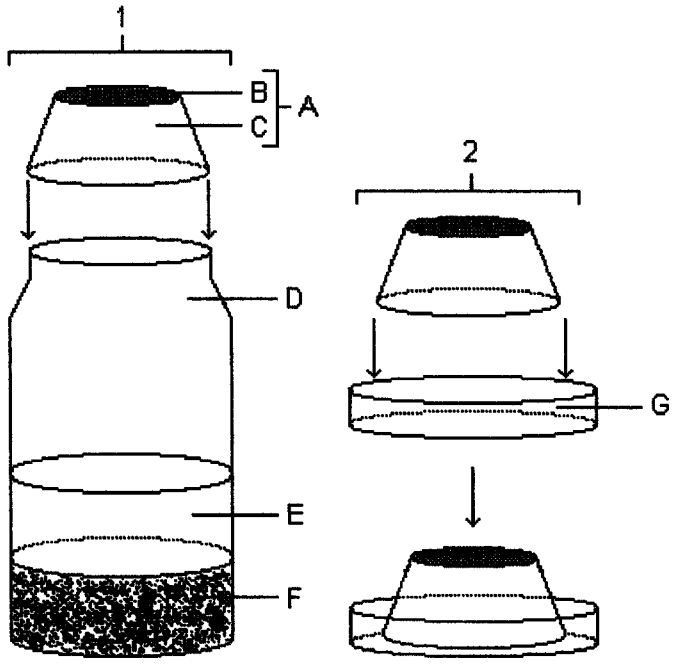


Figure 5. Left panel (1) demonstrates emergence trap design. A, Emergence trap; B, 1.2 mm mesh screen; C, inverted 75 ml plastic cup; D, microcosm; E, M7 medium; F, sediment. Right panel (2) demonstrates reproduction/oviposition chamber design. G, 100x20 mm Petri dish containing M7 medium.

This test showed that *Chironomus* seemed to be able of elongation of essential fatty acids to HUFA such as EPA. The elongation capacities of the animals seemed to meet their physiological demands of HUFA. However, the different substrates had an effect on emergence, since emergence occurred four days earlier in microcosm with bird sand than in those with limestone powder. Mean time until emergence was 19 ± 1 days in bird sand series compared to 23 ± 1 days in limestone series. Sediment type, thus had a significant effect on emergence (One-way ANOVA, df=1, P=0.0006). The first animals to emerge were mostly males, females appeared at least one day later than males. However, whether the differences in emergence are due to availability of food particles or other factors, such as higher pH in limestone treatments, could not be answered. Since not all animals had emerged at the end of the experiment, the determination of survival takes also pupae or larvae still living in the sediment into account. Survival of animals in the bird sand series was 100 ± 0% and in the limestone powder series 97 ± 6%.

3.5.3. Sediment test

The observation that *Scenedesmus* sank between the sand grains to the bottom of the microcosm after one or two days, led to the choice of an alternative sediment. The limestone powder, already mentioned in 3.4.2., with its mean grain size smaller than 0.2 mm should prevent disappearance of *Scenedesmus* cells. The test comprised a series of five different concentrations of *Scenedesmus.* Carbon additions of 0.8, 2.2, 5.8, 11.4 and 22.8 mg per microcosm per ten days were run. Food was added twice during the test phase on day 0 and day 5, respectively. *Scenedesmus* was resuspended with M7-medium from the microcosm before adding. Settling of *Scenedesmus* was facilitated by switching off the aeration for two hours after feeding.

This experiment showed than limestone powder was not an appropriate sediment despite of its smaller mean grain size. Mean mortality of *Chironomus* larvae on limestone powder was very high. Overall mean mortality was 87 ± 23%. Mean length of larvae was only 3 ± 0 mm. This result was surprising since availability of *Scenedesmus* seemed higher than with sea-sand as sediment. With limestone powder as sediment I could observe that the algae did not disappear between the sediment particles but formed a green carpet on the surface. Thus, other reasons must be responsible for the low growth and survival rates of *Chironomus* on stone powder, e.g. high pH or low oxygen concentration.

3.6. Main experiments

The main experiments comprised two times six treatments with the following food types:

a) pulverised food

- •Tetraphyll®, (n=3)
- •Oatflakes, (n=3)
- •*Spirulina*, (n=3)

b) cultured *Scenedesmus obliquus*

- •*Scenedesmus obliquus* (n=3/n=2))
- •*Scenedesmus obliquus* (control) (n=3/n=2)
- •*Scenedesmus obliquus* + EPA (n=3/n=2)

The above treatments were run in concentration series of 1.0, 3.0, 9.0, 27.0, and 54.0 mg C per microcosm and per 10 days. The experiments with pulverised food types were run separately from the ones with *Scenedesmus*. Microcosms consisted of 1000-ml glass containers with a bottom area of 80 cm^2. Each microcosm contained 100 g of acid-purified sea-sand (0.2-0.5 mm) (Merck) and 200 ml of M7 medium (Streloke & Kopp, 1995). Lids on the microcosms prevented evaporation of the medium.

Two days before the start of the experiment the microcosms were acclimatised to experimental conditions and to increase oxygen content in the medium. At the start of the experiment the oxygen concentration was determined with an oxygen probe (Dissolved Oxygen Meter, YSI Model 51 B) and 10 newly hatched larvae (< 24 hours), randomly selected from five egg packages (ca. 1000 larvae), were added to each microcosm. To assure that exactly 10 larvae were added to each microcosm, the larvae were selected with a Pasteur pipette and transferred to a small Petri dish. Here the number of the larvae was checked (and if necessary corrected) before they were transferred to the microcosms. Larvae were released under the surface of the medium to avoid that they would get trapped in the surface tension at the air–water interface. Aeration was provided via capillary tubing (inner diametre=0.76 mm) from an aquarium pump (ELITE 802). The experiment was performed at 20°C and with a 16:8 hours light:dark cycle. Light intensity was about 5 µMol $m^{-2}s^{-1}$ which was far below the

compensation point (based on growth rates of *Spirulina platensis* (Kebede & Ahlgren, 1996) and thus a change in carbon concentrations, for example by algal growth, can be excluded.

The pulverised food types (Tetraphyll® and oatflakes dried at 60 °C for 4 hours), were weighed on an analytical balance (Mettler Toledo, Sartorius MC1 RC210D), which records to the nearest 0.01 mg, and stored in Eppendorf tubes. One hour before feeding, one millilitre deionised water was added to each tube to soak the food particles. Soaking of food facilitated settlement and provided an even distribution of food particles in the microcosm. The food suspension was added to each microcosm with a Pasteur pipette. Rinsing of Eppendorf tubes and pipettes assured transfer of the whole amount of food. Food was added to the microcosms every fifth day but concentrations are always given as mg C/10 days.

After 10 days three (respectively two) replicates of each treatment were sacrificed and survival and individual length of the larvae was determined. Larval length was taken as an indicator of larval growth. To determine length, larvae were anaesthetised in carbonated water and stretched out on millimetre paper under a stereo-microscope, using 20 times magnification. A measuring ocular made it possible to make length readings to the nearest 0.03 mm. The larvae were then stored in small glass vials under N_2 in the refrigerator until freeze-drying. From all the treatments with concentrations of 54.0 mg C/10d freeze-dried larvae were analysed for their fatty acid concentrations.

In the remaining two replicates of the treatments with Tetraphyll®, oatflakes and *Spirulina*, *Chironomus* larvae were allowed to complete their life-cycle, i.e. until the emergence of adults. These two replicates were sacrificed after 61 days. Daily checks for adults were made from day 15. Emerged males were removed from the microcosms and stored in 70% ethanol, while each emerged female was transferred individually to a reproduction/oviposition chamber (Figure 4). Subsequently three males from the *Chironomus* laboratory-culture were added to each reproduction chamber. After oviposition the eggs were transferred to small Petri dishes and females were stored in 70% ethanol. Eggs were stored at room temperature until the hatching of larvae. For each female midge the number of viable offspring and non-viable eggs were scored. Egg packages were kept under room temperature for at least 6 days to see if they were fertilised and larvae hatched. Unfertilised eggs were transferred to Eppendorf tubes, each containing approximately 1 ml 2 M H_2SO_4, and left overnight to dissolve the gelatinous

matrix surrounding the egg mass and thus facilitating egg counting (Benoite et al., 1997). After digestion, the eggs were collected with a Pasteur pipette, spread across a microscope slide and counted at 90 times magnification.

Mean development rates were calculated from emergence data according to OECD Guideline 218 (OECD, 2001). The mean development time represents the mean time span between the introduction of larvae (day 0 of the test) and the emergence of the experimental cohort of midges.

The mean development rate per microcosm ($\bar{x}$) was calculated according to:

$$\bar{x} = \sum_{i=1}^{m} \frac{f_i x_i}{n_e}$$

where: $\bar{x}$: mean development rate per microcosm

i : index of inspection interval

m : maximum number of inspection intervals

f_i : total number of midges emerged in the inspection interval i

n_e : total number of midges emerged at the end of experiment $(=\sum f_i)$

x_i : development rate of the midges emerged in interval i

$$x_i = \frac{1}{\left(day_i - \frac{l_i}{2}\right)}$$

where: day: inspection days (days since application of larvae)

l_i : length of inspection interval i (days, usually 1 day).

Wing length of preserved emerged adults was determined as a measure of size of males and females (Armitage, 1995). For that, animals were turned dorsally and the wing was spread under an angle of approximately 90° to see its basis.

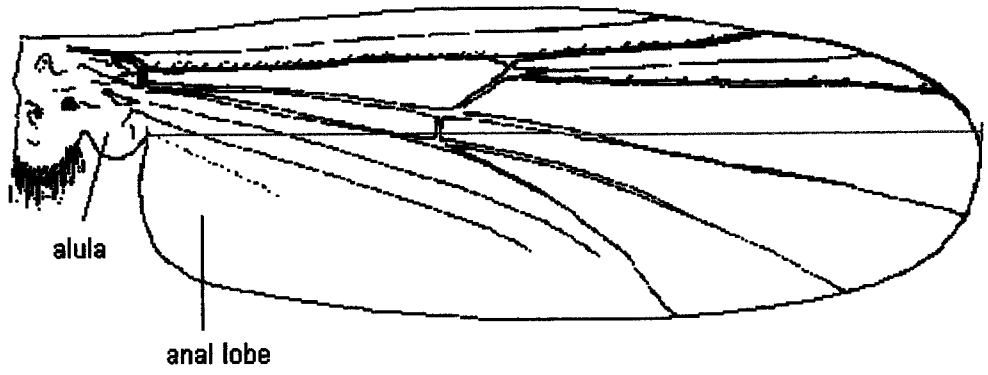


Figure 6. Chironomid wing. The red line indicates how wing length was determined (after Armitage et al., 1995).

Wing length was measured from the cleft, where the alula turns into the anal lobe, to the tip of the wing at 20 times magnification in a stereo-microscope (Figure 6).

Scenedesmus supplementation with EPA was done using a method described by von Elert (by courtesy of von Elert, University of Konstanz, unpublished). Cultured algae were harvested over a polycarbonate filter 3 µm Millipore, weighed on an analytical balance (Mettler Toledo, Sartorius MC1 RC210D), recording to the nearest 0.01 mg, and stored in small plastic vials. In order to test von Elerts method to supplement algae with an artificial, single fatty acid I applied this method to 4 mg C of fresh *Scenedesmus* cells.

Table 9. FA concentration (mg/g dw) of *Scenedesmus* (n=3) and of *Scenedesmus* after supplementation with EPA (n=1).

Acid code	Acid name	*S. obliquus*	*S. obliquus* + EPA
14:0		1.32 ± 0.00	0.33
16:0		2.36 ± 0.03	3.04
16:1ω7		1.10 ± 0.02	3.54
18:0		0.07 ± 0.01	2.10
18:1ω9		1.30 ± 0.03	0.42
18:2ω6	LA	1.90 ± 0.03	2.34
18:3ω3	ALA	12.37 ± 0.19	4.06
20:5ω3	EPA	–	2.67
Σ SAFA		11.63 ± 0.55	5.46
Σ MUFA		4.08 ± 0.06	7.39
Σ PUFA		18.08 ± 0.14	9.07
Σ FA		38.37 ± 1.60	21.92

The analysis showed that the incubation of a *Scenedesmus* suspension with the highly unsaturated eicosapentaenoic acid (EPA, 20:5ω3) resulted in an obvious increase of EPA (Table 9).

On feeding day, algae were filtered and supplemented with EPA, before a resuspension of algae with Z8 medium was added to the microcosms. Since algal cultures did not provide the necessary food amounts, *Scenedesmus* treatments with the concentrations corresponding to 1.0; 3.0; 9.0 mg C/10d and those with 27.0 and 54.0 mg C/10d were run separately. The treatments with food concentrations of 27.0 and 54.0 mg C/10d were run 10 days after the termination of those with 1.0, 3.0, 9.0 mg C/10d. Due to the slow growth rates of *Scenedesmus* only two replicates for the concentrations 27.0 and 54.0 mg C/10d were run. In the data analyses of the *Scenedesmus* treatments only the treatments *Scenedesmus* and *Scenedesmus*+EPA were included, since the *Scenedesmus* control-treatment was excluded from the analysis since a two-way ANOVA found no differences in food types in the *Scenedesmus* treatments (Table 12). The ratio of freshly filtrated *Scenedesmus* (fresh-weight = fw) to freeze-dried *Scenedesmus* (dry-weight = dw) was 4.34 ± 0.45. To assure constancy of conditions under which the divided experiment with *Scenedesmus* was run, temperature was recorded daily during the experiments. Mean temperature was 20°C, and temperature ranged from 19 to 21°C. Constancy of temperature was tested with a *t*-test (df=9, t=1.54).

4. RESULTS

4.1. Growth and survival of *Chironomus riparius*

Mean larval length increased across the food quantity gradient for all pulverised food items (Table 10). Effects of food concentration were found in treatments for Tetraphyll®, oatflakes, and *Spirulina*, respectively (Table 10). Pairwise comparisons in both the Tetraphyll® and *Spirulina* treatment showed significant effects of food concentration on larval length between all adjacent food concentrations. In the oatflakes treatment

pairwise comparisons showed no differences in larval length for consecutive food concentrations. Effects of food type, in contrast, were only found at the concentrations 1.0 mg C/10d and 3.0 mg C/10d (Table 10), but not for the remaining concentrations. A pairwise comparison showed that growth at the lowest concentration of 1.0 mg C/10d differed among all three food types (*Spirulina*, Tetraphyll® P=0.0003, *Spirulina*, oatflakes P=0.009, Tetraphyll®, oatflakes P=0.0168). At 3.0 mg C/10d pairwise comparisons showed differences between *Spirulina* and Tetraphyll® (P=0.0138), and Tetraphyll® and oatflakes (P=0.0292), but no longer for *Spirulina* and oatflakes (P=0.7973).

Table 10. One-way ANOVA a) for the effect of food type and b) for the effect of food concentration (mg C/10d) on mean length of *Chironomus* larvae within treatments with different food types.

a) food type	df	F-value	*P*-value
Tetraphyll®	4	207.074	<0.0001
Oatflakes	4	17.400	0.0002
Spirulina	4	509.868	<0.0001

b) food concentration	df	F-value	*P*-value
1.0	2	40.146	0.0003
3.0	2	10.978	0.0099
9.0	2	1.133	0.3825
27.0	2	1.934	0.2248
54.0	2	3.789	0.0863

Highest growth at all food concentrations was reached on Tetraphyll® where larval mean length increased from 4.4 ± 0.2 mm at 1 mg/10d to 12.9 ± 0.8 mm at the highest food concentration (Figure 7). At the concentrations 1.0, 3.0, and 9.0 mg C/10d larvae grown on oatflakes were bigger than larvae grown on *Spirulina* but at concentrations 27.0 and 54.0 mg C/10d this pattern was reversed and larvae grown on *Spirulina* became bigger than those fed oatflakes.

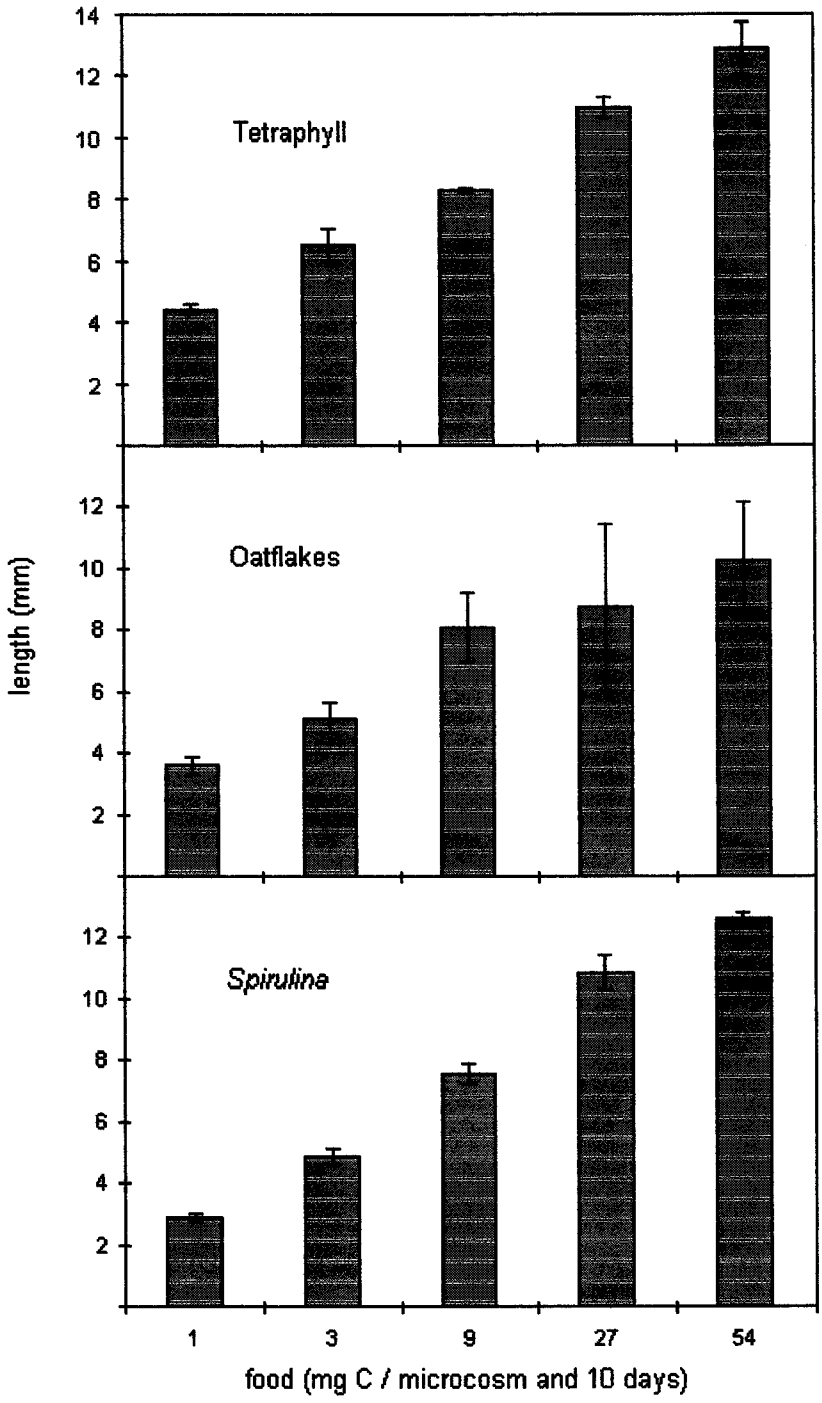


Figure 7. Mean length of *Chironomus* larvae after 10 days on different food types. Error bars indicate standard deviation (n=3).

Larval length was affected by both food type and food concentration in treatments with the three pulverised food types (Tetraphyll®, oatflakes, and *Spirulina*) (Table 11). The interaction term between food concentration and food type was significant for length of *Chironomus* larvae.

Table 11. Two-way ANOVA for effects of pulverised food types and food concentrations on mean length of *Chironomus*.

	df	F-value	*P*-value
food type	2	13.801	<0.0001
food concentration	4	174.833	<0.0001
food type x food concentration	8	3.398	0.0068

Survival of *Chironomus* larvae was generally very high (≥83%) in all treatments with pulverised food types (Table 12). Thus, more than 8 out of 10 larvae were recovered from the microcosms.

Table 12. Survival (%) of *Chironomus* on different food types at different food concentrations (mg C/10d) (n=3).

	food concentration				
food type	1.0	3.0	9.0	27.0	54.0
Tetraphyll®	93 ± 6	97 ± 6	97 ± 6	97 ± 6	97 ± 6
Oatflakes	90 ± 17	100 ± 0	83 ± 6	86 ± 6	90 ± 17
Spirulina	100 ± 0	87 ± 6	87 ± 15	97 ± 6	83 ± 29

In feeding experiments with *Scenedesmus* larval growth was much lower than in the treatments with pulverised food types. On a *Scenedesmus* diet mean larval length increased from 1.9 ± 0.36 mm at the lowest concentration to 6.7 ± 1.70 mm a concentration of 54.0 mg C/10d, and from 2.3 ± 0.48 mm at 1.0 mg C/10d to 5.9 ± 1.45 mm at 54.0 mg C/10d in the *Scenedesmus*+EPA treatment (Figure 8). Larval length was affected by food concentration but not by food type (Table 13). However, the interaction term between food concentration and food type was not significant for growth (Table 13).

Table 13. Two-way ANOVA for effects of *Scenedesmus* treatments on mean length of *Chironomus*.

	df	F-value	*P*-value
food type	1	1.442	0.2473
food concentration	4	55.181	<0.0001
food type x food concentration	4	0.348	0.8416

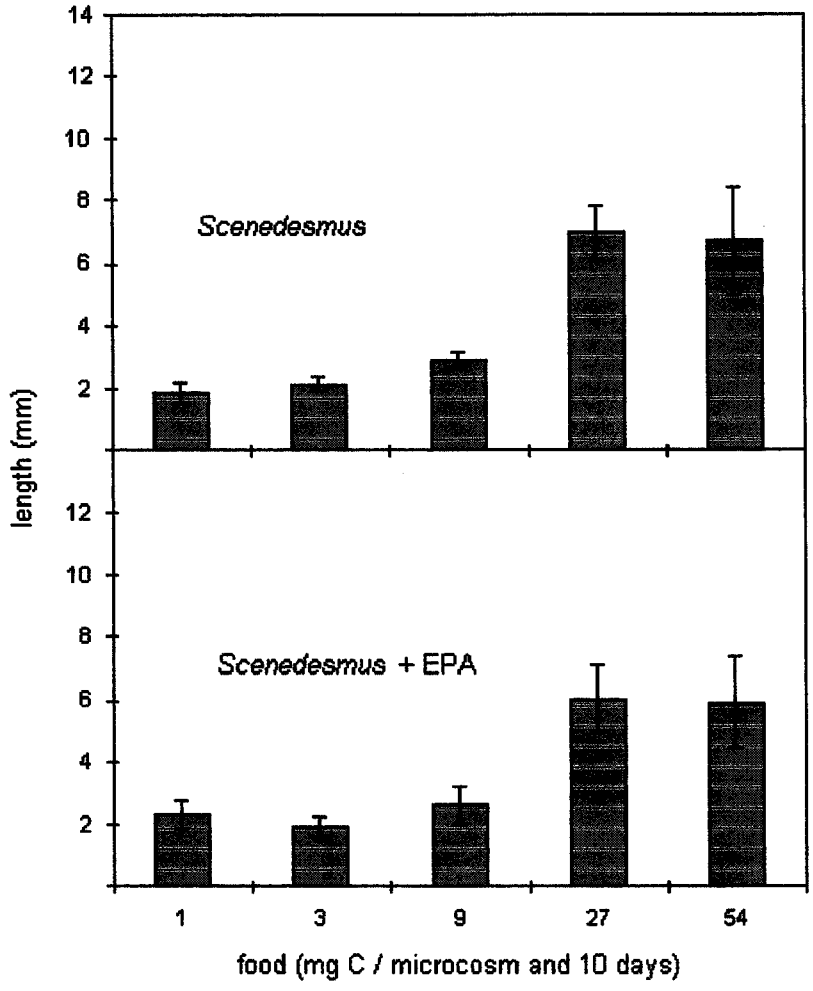


Figure 8. Mean length of *Chironomus* larvae after 10 days of growth on *Scenedesmus* and EPA-supplemented *Scenedesmus*. Error bars indicate standard deviation. (n=3 for concentrations 1-9, n=2 for concentrations 27 and 54)

Effects of food concentration on larval length within treatments were significant between treatments with *Scenedesmus* (One-way ANOVA, $P<0.0001$) and *Scenedesmus* + EPA (One-way ANOVA, $P=0.0004$). Pairwise comparisons showed that supplementation of *Scenedesmus* with EPA had no effect on growth for *Chironomus* at any food concentration.

Table 14. Survival (%) of *Chironomus* larvae on *Scenedesmus* and *Scenedesmus*+EPA at different food concentrations (mg C/10 days). (n=3 for food concentrations 1.0-9.0 mg C/10d, n=2 for food concentrations 27.0 and 54.0 mg C/10d).

	food concentration				
food type	1.0	3.0	9.0	27.0	54.0
Scenedesmus	63 ± 25	87 ± 15	80 ± 0	45 ± 7	70 ± 0
Scenedesmus+EPA	57 ± 35	57 ± 15	73 ± 12	60 ± 28	60 ± 0

Survival in the *Scenedesmus* and *Scenedesmus*+EPA treatment was consistently lower (≥46%) (Table14) than in treatments with pulverised food types. Pairwise comparisons for all six food types showed that mean larval length in both *Scenedesmus* treatments was lower than mean length in the treatments with pulverised food types at nearly all corresponding food concentrations. No significant differences in mean length were found between *Scenedesmus* and *Spirulina* at a concentration of 1.0 mg C/10d and at 27.0 mg C/10d and between *Scenedesmus*+EPA and *Spirulina* at a concentration of 1.0 mg C/10d. No differences can neither be found between the *Scenedesmus* and oatflakes treatment at concentration of 27.0 mg C/10d and 54.0 mg C/10d and the *Scenedesmus* and Tetraphyll® treatment at 27.0 mg C/10d (Table15).

Table 15. Pairwise comparisons (*P*-values, Scheffe's posthoc test) of mean length of *Chironomus* grown on pulverised food types and cultured *Scenedesmus*/EPA-supplemented *Scenedesmus*.

	Food concentrations (mg C/10d)				
food types	1.0	3.0	9.0	27.0	54.0
Spirulina/*Scenedesmus*	0.0993	<0.0001	<0.0001	0.1125	0.0119
Spirulina/*Scenedesmus*+EPA	0.1925	<0.0001	<0.0001	0.0375	0.0048
Tetraphyll®/*Scenedesmus*	0.0014	<0.0001	<0.0001	0.1034	0.0092
Tetraphyll®/*Scenedesmus*+EPA	0.0026	<0.0001	<0.0001	0.0345	0.0038
Oatflakes/*Scenedesmus*	0.0094	<0.0001	<0.0001	0.6646	0.0880
Oatflakes/*Scenedesmus*+EPA	0.0186	<0.0001	<0.0001	0.2832	0.0323

4.2. Fatty acid composition of *Chironomus riparius*

The FA composition of the whole body of *Chironomus* larvae (Table 16) mirrored the FA composition of the food types they were grown on (Table 6). Concentrations are expressed as mg/g dw.

Table 16: Fatty acid composition (mg/g dw) of the whole body of *C. riparius* grown on different food types. All Larvae were incubated 10 days. (n=3 for Tetraphyll®, oatflakes, and *Spirulina*; n=2 for *Scenedesmus* and, *Scenedesmus*+EPA. Larvae from both replicates in these treatments had to be pooled to be over the detection limit of the FA analysis.)

		Larvae grown on:				
Fatty acid	Acid name	Tetraphyll®	Oatflakes	*Spirulina*	*Scen*	*Scen* +EPA
14:0		1.89 ± 0.18	1.70 ± 0.03	1.44 ±0.56	0.99	0.69
16:0		9.49 ± 0.99	14.23 ± 2.28	11.90 ± 2.88	6.24	4.96
16:1ω7		13.22 ± 2.27	5.18 ± 0.74	12.38 ± 2.63	6.52	4.61
18:0		4.19 ± 0.06	7.49 ± 2.14	6.78 ± 0.93	3.75	3.58
18:1ω9		3.91 ± 0.44	9.79 ± 3.23	4.21 ± 1.37	4.64	5.76
18:2ω6	LA	10.59 ± 0.49	17.65 ± 3.58	9.08 ± 2.99	6.41	6.82
18:3ω6	GLA	0.15 ± 0.01	0.18 ± 0.10	3.45 ± 0.93	0.30	0.31
18:3ω3	ALA	0.55 ± 0.08	0.43 ± 0.10	0.11 ± 0.02	2.41	1.82
18:4ω3	SA	–	–	–	–	–
20:0		0.91 ± 0.09	0.57 ± 0.09	0.40 ± 0.02	0.60	0.57
20:4ω6	ARA	0.54 ± 0.11	0.36 ± 0.25	0.46 ± 0.14	1.12	1.13
20:5ω3	EPA	1.66 ± 0.55	0.32 ± 0.20	0.37 ± 0.18	0.84	1.26
22:6ω3	DHA	–	–	–	–	–
Σ SAFA		16.48 ± 1.17	23.99 ± 4.53	20.53 ± 2.66	11.57	9.80
Σ MUFA		20.0 ± 3.11	17.75 ± 2.57	18.42 ± 2.45	16.49	14.37
Σ PUFA		13.50 ± 1.17	18.58 ± 3.37	12.29 ± 4.22	11.08	11.33
Σ FA		49.89 ± 4.62	60.32 ± 9.47	51.24 ± 9.10	39.14	35.50
Σω3		2.21 ± 0.61	0.64 ± 0.28	0.43 ± 0.19	3.25	3.07
Σω6		11.29 ± 0.56	17.95 ± 3.59	11.87 ± 4.04	7.83	8.25
Σω3/Σω6		0.19 ± 0.04	0.04 ± 0.01	0.04 ± 0.00	0.42	0.37

* Σ FA includes other FA and, consequently, is larger than Σ SAFA+Σ MUFA+Σ PUFA.

The dominating FA in the larvae were palmitic acid (16:0), palmitoleic acid (16:1ω7) and linoleic acid (LA, 18:2ω6). Among the HUFA, EPA was the dominating FA in larvae grown with Tetraphyll® and *Scenedesmus*+EPA, whereas ARA dominated in larvae from *Scenedesmus* and *Spirulina* treatments. Larvae grown with *Scenedesmus* and *Scenedesmus*+EPA displayed relatively low concentrations of ALA (2.41 mg/g dw) although this was the most abundant fatty acid in *Scenedesmus* and *Scenedesmus*+EPA (12.37 mg/g dw; Table 9). Conversely, a markedly increased concentration of LA (6.41 mg/g dw) was found in the larvae fed with *Scenedesmus and Scenedesmus*+EPA, whereas the food itself contained only 1.90 ± 0.03 mg/g dw (Table 9). All larvae contained ARA and EPA, even when their food resource was devoid of these FA. ARA concentrations were about two times higher in larvae fed with *Scenedesmus and Scenedesmus*+EPA (1.12/1.13 mg/g dw) than in larvae fed with Tetraphyll® (0.54 ± 0.11 mg/g dw), oatflakes (0.36 ± 0.25 mg/g dw) or *Spirulina* (0.46 ± 0.14 mg/g dw). EPA concentrations were highest in larvae raised on Tetraphyll® (1.66 ± 0.55 mg/g dw) and *Scenedesmus*+EPA (1.26 mg/g dw) and ranged from 0.32 ± 0.20 mg/g dw in the oatflakes treatment to 0.84 mg/g dw in the *Scenedesmus* treatment. DHA was not found in any of the larval bodies though it was present in Tetraphyll®. Larvae grown on oatflakes were fattest (60.32 ± 9.47 mg/g dw), displaying also the highest concentration of SAFA (23.99 ± 4.53 mg/g dw), consisting mainly of 16:0 (14.23 ± 2.28 mg/g dw). Total FA concentrations in larvae ranged from 35.5 mg/g dw in the *Scenedesmus*+EPA treatment to 51.24 ± 9.10 mg/g dw in the *Spirulina* treatment.

4.3. Emergence of adults

After 61 days, i.e. after the termination of the experiment, only 140 adults had emerged from 30 microcosms, that had contained 10 larvae each. Adult emergence appeared first in the microcosms that had received the highest food concentration (54.0 mg C/10d).

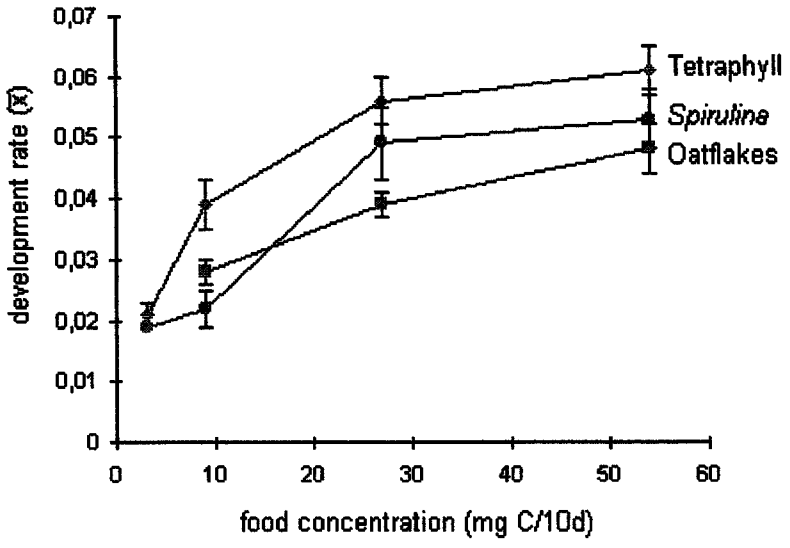


Figure 9. Mean development rate ($\bar{x}$) [d^{-1}] of *Chironomus* for different food types and concentrations after 61 days (n=2).

Food quantity had a positive effect on the larval development rate (Figure 9). First emergence in the Tetraphyll® treatment was after 15, 16, 21 and 40 days at the food concentrations 54, 27, 9, and 3 mg C/10d, respectively. Mean development rate ($\bar{x}$) in Tetraphyll® treatments ranged from 0.061 ± 0.004 d^{-1} at a concentration of 54.0 mg C/10d to 0.021 ± 0.002 d^{-1} at a concentration of 3.0 mg C/10d (Figure 9). Adults emerged in the *Spirulina* treatment after 17, 20, 36, and 52 days at decreasing food concentrations. In this treatment mean development rate ranged from 0.053 ± 0.005 d^{-1} at 54.0 mg C/10 to 0.019 d^{-1} at 3.0 mg C/10d. Development time was lowest in the treatment with oatflakes. First emergence was after 17, 20, and 26 days at food concentrations 54, 27, and 9 mg C/10. Mean development time ranged from 0.048 ± 0.004 d^{-1} to 0.028 ± 0.002 d^{-1} along the food concentration gradient. In this treatment no adults emerged at a concentration of 3.0 mg C/10d. No adults emerged in any treatments with food concentrations of 1.0 mg C/10d. Food concentration had an effect on the development rate whereas food type did not (Table 17).

Table17. Two-way ANOVA for development rate ($\bar{x}$) of adults, grown on different food types and food concentrations (n=3).

	df	F-value	p-value
food type	2	0.129	0.8795
food concentration	4	5.166	0.0110
food type x food concentration	8	0.690	0.6038

Pairwise comparisons of concentrations revealed no differences in development rate within each treatment. Development rate between males and females did not differ in any treatment (*t*-test, t_{Tetra}=2.64, df=3; t_{oat}=2.36, df=2; $t_{Spirulina}$=2.64, df=3) though female development rate tended to be lower than male development rate. Male:female ratios ranged from 0.9 ± 0.1 to 2.5 2.1 (Table 18) with most ratios deviating from an even distribution between males and females.

Table 18. Total number of emerged adults and male:female ratio of emerged adults, grown on different food types and concentrations (mg C/10d).

	Tetraphyll®		Oatflakes		*Spirulina*	
food concentration	adults	male:female	adults	male:female	adults	male:female
54.0	12	1.0 ± 0	8	2.3 ± 2.5	15	1.3 ± 1.1
27.0	20	0.9 ± 0.2	20	1.3 ± 0.4	11	2.5 ± 2.1
9.0	17	0.9 ± 0.1	12	1.7 ± 0.5	14	1.3 ± 1.0
3.0	8	1.4 ± 0.9	0	-	3	1.0 ± 0
	Σ=57		Σ=40		Σ=43	

4.4. Adult size

Adult size, determined as wing length, differed not significantly between males and females (*t*-test, *P*>0.05) though females tended to have slightly longer wings. Wing length decreased from 4.1 ± 0.2 mm at a concentration of 54.0 mg C/10d to 3.2 ± 0.1 mm at 3.0 mg C/10d in the Tetraphyll® treatment. Midges grown on oatflakes had wing lengths ranging from 4.0 ± 0.5 mm at 54.0 mg C/10d to 3.1 ± 0.3 mm at a food

concentration of 9.0 mg C/10d. In the *Spirulina* treatment wing length ranged from 3.7 ± 0.2 mm at the highest food concentration to 3.0 mm at a concentration of 9.0 mg C/10d (Figure 10).

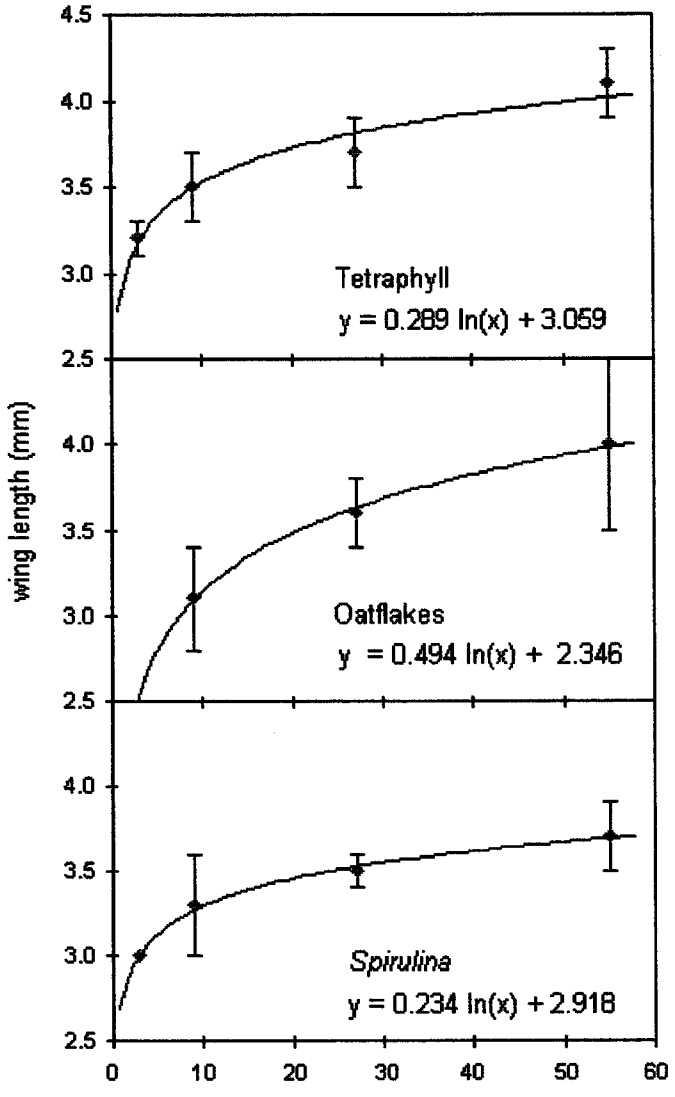


Figure 10. Relationship between food concentration (mg C/10 d) and adult wing length (mm) (n=2).

Table 19. Two-way ANOVA for wing length of adult midges, grown on different food types and food concentrations (n=3).

	df	F-value	*P*-value
food type	2	6.300	0.0195
food concentration	2	17.247	0.0008
food type x food concentration	4	0.543	0.7146

Food type and food concentration had a significant effect on adult wing length (Table 19). Wing length was longer in the Tetraphyll® and the *Spirulina* treatment at a concentration of 27.0 mg C/10d (One-way ANOVA, P=0.0385). Within each food type no differences in wing length between consecutive food concentrations occurred. However, food concentration had an effect on wing length in the Tetraphyll® treatment (One-way ANOVA, P=0.0125).

4.5. Fecundity

Fecundity was determined as the number of eggs per female and showed large variation. Egg packages were found in the reproduction chambers approximately two days after the transfer of females to the chambers and addition of males. In total, egg packages were found only from 35 out of 64 emerged females. 56% of the females in the Tetraphyll® treatment laid eggs, 63% of the females grown on *Spirulina*, and 40% in the oatflakes treatment. All egg packages contained unfertilised eggs. Fecundity varied strongly within each treatment and concentration (Table 20) and a positive trend between food concentration or food type and fecundity was not found. Neither food type (Two-way ANOVA, P=0.1531) nor food concentration (Two-way ANOVA, P=0.4186) had an effect on fecundity.

Table 20. Mean fecundity of females given as number of eggs/female ± sd. Females were grown on different food types and food concentrations.

	Tetraphyll®		Oatflakes		*Spirulina*	
mg C/10d	egg-packages	eggs/female	egg packages	eggs/female	egg packages	eggs/female
54.0	4	292 ± 153	3	359 ± 75	3	291 ± 103
27.0	5	280 ± 64	1	332	4	190 ± 79
9.0	6	308 ± 107	2	276 ± 74	4	277 ± 95
3.0	2	182 ± 0	0	–	1	145

The highest (447) and the lowest number (90) of eggs were found in the Tetraphyll® treatment at a food concentration of 54.0 mg C/10d. Numbers of eggs ranged from 208 to 376 at a concentration of 27.0 mg C/10d , from 433 to 136 at 9.0 mg C/10d, and from 312 to 232 at 3.0 mg C/10d. Within the oatflakes treatment, numbers of eggs varied with a factor 1.5 at all concentrations. In the *Spirulina* treatment variation of numbers of eggs was largest at a concentration of 27.0 mg C/10d (119 to 295), and lowest at a concentration of 9.0 mg C/10d (279 to 297). Numbers of eggs varied with a factor 2 (from 178 to 317) at the concentration of 54.0 mg C/10d.

5. DISCUSSION

The results of my experiments show that the fatty acid pattern of *Chironomus riparius* is strongly influenced by food quality, as indicated by the concentrations of PUFA of the ω3-family. Maximum larval length was reached in treatments with the highest food concentration of the fish food Tetraphyll®, which is naturally rich in EPA. However, larval length at this concentration was not significantly higher than that in corresponding treatments with oatflakes or *Spirulina* (Figure 7). The supplementation of *Scenedesmus* with EPA did not increase growth of *Chironomus* (Figure 8) and even inhibited growth in experiments with supplementation of oatflakes with EPA (see below). Thus, EPA is apparently not as significant for this detrivorous invertebrate as has been shown for zooplankton (Müller-Navarra, 1995, DeMott & Müller-Navarra, 1997).

Larval concentrations of FA reflected in most cases the FA composition of the food types they were grown on. Only larvae grown on *Scenedesmus*, respectively Scenedesmus+EPA, displayed a deviating ALA and LA pattern. The dominating FA in *Scenedesmus* cells was ALA (12.37 mg/g dw), but in the larvae concentrations of LA dominated over ALA. The relationships between the FA composition of food types and larvae (Figure 11) indicate which FA were accumulated in the larval body. The majority of accumulated FA were the saturated FA as 14:0, 16:0, 18:0 and 20:0, as well as the mono-unsaturated FA 16:1ω7 and 18:1ω9 (Figure 11). These FA are needed for energy storage and internal maintenance. The PUFA, which were not accumulated, can be considered as biologically active, and, for example, may be needed for the synthesis of new tissues. Surprisingly, larvae fed with both species of algae, accumulated LA, additionally to the above named FA. Since both *Scenedesmus* and *Spirulina* itself had relatively low concentrations of LA, compared to Tetraphyll® and oatflakes, this indicated a surplus of this FA relative to the animals needs. However, larvae grown with *Scenedesmus* or EPA-supplemented *Scenedesmus* still had the lowest concentrations of LA compared to larvae grown with the other food types. Highest concentrations of ALA, in contrast, were found in larvae receiving *Scenedesmus*/*Scenedesmus*+EPA, being approximately four times higher than concentrations in larvae grown on other food types. This was due to the fact that *Scenedesmus* had the highest concentrations of ALA (12.37 mg/g dw) among all food types. The absence of 18:4ω3 (SA) in all larval bodies seems to be inconclusive since *Scenedesmus* contained this FA (Table 16). However, SA is a substrate for the Δ5 desaturase and one can assume that all SA is elongated/desaturated to EPA. The detection of ARA and EPA in larvae grown on oatflakes and *Scenedesmus* proved that *Chironomus* is capable of elongation and desaturation of LA, respectively ALA, to their higher homologues, i.e. ARA and EPA, respectively. Larvae grown on *Scenedesmus* and EPA-supplemented *Scenedesmus* had the highest concentrations of ARA (1.12 and 1.13 mg/g dw) though the algae itself contained, relatively to the other food types, the lowest concentration of the precursor LA. The highest concentrations of ARA should be expected in larvae from the oatflakes treatment, since LA was the most abundant FA in oatflakes. However, conversion rates within the ω3-family (from ALA to EPA) seem to be well adjusted to the physiological demands of *Chironomus*, since at low concentrations of oatflakes conversions of ALA to EPA seem to provide more EPA

than *Spirulina*. It is not possible, though, to prove from these data that at low concentrations larvae can produce more EPA from oatflakes than they ingest with Spirulina, since only larvae fed with 54.0 mg C/10d were analysed for their FA composition.

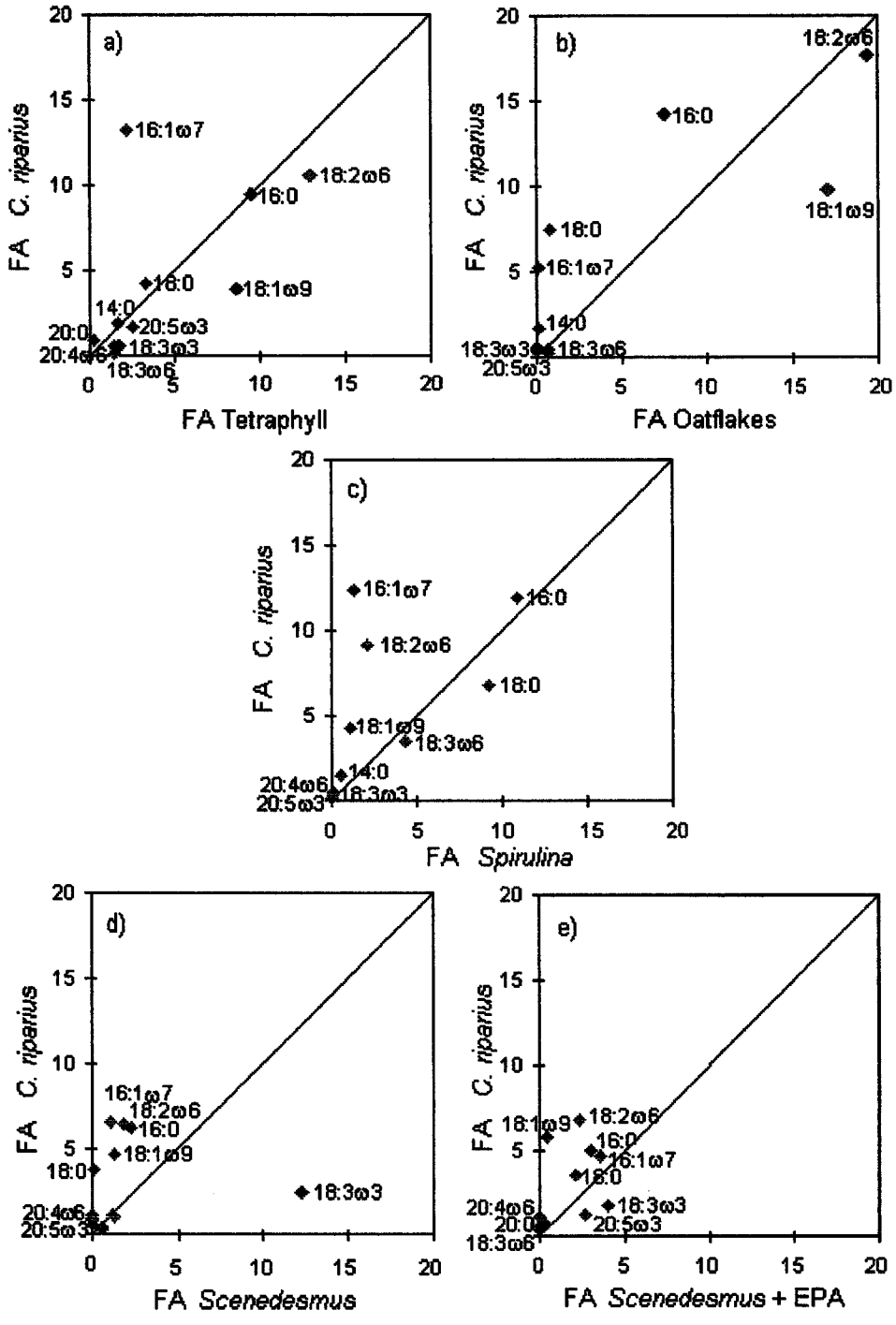


Figure 11. Relationship between FA composition of food types (mg/g dw) and FA composition of *Chironomus* larvae (mg/g dw). Larvae were fed with 54 mgC/10d and analysed after 10 days of growth. FA above the black line are accumulated in the body.

The ratio between ω3 and ω6 FA is a good indicator of food quality (Goedkoop et al., 2000) and the concentrations of LA and ALA have a strong influence on this ratio. An ω3/ω6 ratio of 0.19 was found in larvae fed with Tetraphyll®, and this ratio seemed to be physiologically optimal for *Chironomus* since it supported maximum length of larvae. A twice as high ω3/ω6 ratio of 0.4 was found in larvae fed *Scenedesmus* and *Scenedesmus*+EPA which might be a reason for their low growth rate. The ω3/ω6 ratio of 0.04 for oatflakes and *Spirulina* seemed to be very low, but the larvae seemed to handle this lower ratio better than the ten times higher ratio. Negative effects of elevated ω3/ω6 ratios have been shown for fish (Ahlgren et al., 1999). The ω3/ω6 ratio for *Chironomus plumosus* and *Chironomus anthracinus* under field conditions though displayed much higher values in their body tissues (*C. plumosus*: 1.1; *C. anthracinus*: 0.7) as has been shown by Goedkoop et al. (2000). The observed ω3/ω6 ratio of 0.19 indicates the importance of ω6 FA for *Chironomus*. Usually, terrestrial animals are dominated by ω6 FA, whereas in aquatic animals ω3 FA dominate over ω6 FA (Olsen, 1999).

Among insects, aquatic species appear to have far higher proportions of C20 PUFAs in their tissue lipids than do terrestrial insects, which often have no more than traces of these compounds (Hanson et al., 1985). EPA was the major PUFA in Plecoptera, Chironomidae and the Ephemeroptera (Ghioni, et al., 1996). One reason for the presumed ubiquity of C20 PUFA in insects is their role as precursors of eicosanoids (Stanley-Samuelson & Pedibhotla, 1996). The three C20 PUFA, 20:3ω6, 20:4ω6 and 20:5ω3 are all eicosanoid precursors in vertebrates (Ghioni et al., 1996) and are assumed to have the same function in invertebrates. The presence of large amounts of these acids cannot be attributed solely to dietary intake as, for example, late instar *Clistoronia magnifica* (Trichoptera) showed an order of magnitude increase in tissue levels of ARA and EPA on a diet with no detectable amounts of either FA (Beenakkers et al., 1985). Though their specific role in aquatic insects is unknown, is has been hypothesised that, apart from a function as precursors for prostaglandins, high levels of ARA and EPA may be essential for proper membrane function in aquatic insects adapted to low environmental temperatures (Beenakkers et al., 1985).

Since food quality had obviously an effect on growth and FA composition of the consumer it is necessary to define food quality. Food quality can only sensibly determined in terms of the effect of the food on the animal that eats it. Food quality can be defined as the amount of biomass produced per unit of ingested food. Food sources, promoting higher maximum growth rates, can be assigned higher quality than those which allow lower maximum growth rates (Vos, 2001). Long-chain (>20 C) polyunsaturated fatty acids (PUFA) or phosphorus (P), nitrogen (N), and carbon (C) are often used as indicators of food quality (Müller-Navarra, 1995). The elemental ratios of C/N, C/P, and N/P are good indicators of the nutrient status of food. A common N/P ratio for algae is 10/1, with higher ratios indicating a deficiency of phosphorus (Horne & Goldman, 1994). The N/P ratios of the algae used in my experiments were lower than 8, meaning that no phosphorus limitation occurred.

Since food quality cannot be the sole factor affecting growth, other factors such as food quantity, size or shape of the food particles have also be taken into consideration. Lampert (1977), for example, defined the threshold food concentration, or starvation threshold, as the amount of food that an animal needs to balance its metabolic losses, resulting in no change in mass (Figure 12).

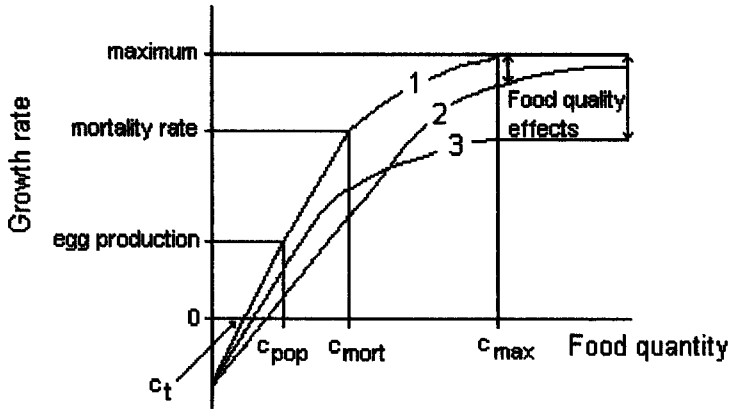


Figure 12. Hypothetical relationship between food quantity (carbon units) and specific growth rate of zooplankton (units of carbon gained per individual per time) on three food types. Type 1 is "ideal" or standard food, while types 2 and 3 are suboptimal foods. The zooplankter is able to compensate for the low nutritional value of food type 2, and can achieve maximal growth rate at high food concentrations. Food type 3, however, can never support maximal growth. Food quality is measured as the *difference* between growth on the standard food and growth on another food type *at any given quantity*. Note that the shapes of the actual curves may vary. C_t is the threshold food concentration at which growth rate is exactly zero; C_{pop} is the population threshold food concentration at which egg production becomes possible (after Sterner, 1998).

By using increasing concentrations of food I tested whether *Chironomus* was able to compensate for lower food quality at high food concentrations. In the pilot studies it was not possible to detect if larval growth reached a maximum level at the food concentrations between 0.8 and 22.8 mg C/10d) (Figure 4). Hence, food quantity was increased to 54.0 mg C/10d in the main experiment. Mean larval lengths recorded in the main experiments have been displayed earlier and are transformed to growth rates in Figure 13 by using the following equation:

$$r = \frac{\ln c_t - \ln c_0}{t_2 - t_1}$$

where c_t: mean length of larvae after 10 days

c_0: mean length of larvae at day 0

t_2-t_1: duration of the experiment

Figure 13 is aligned with the representation of Figure 12 to reveal the differences in food quality of the food types used in my experiments.

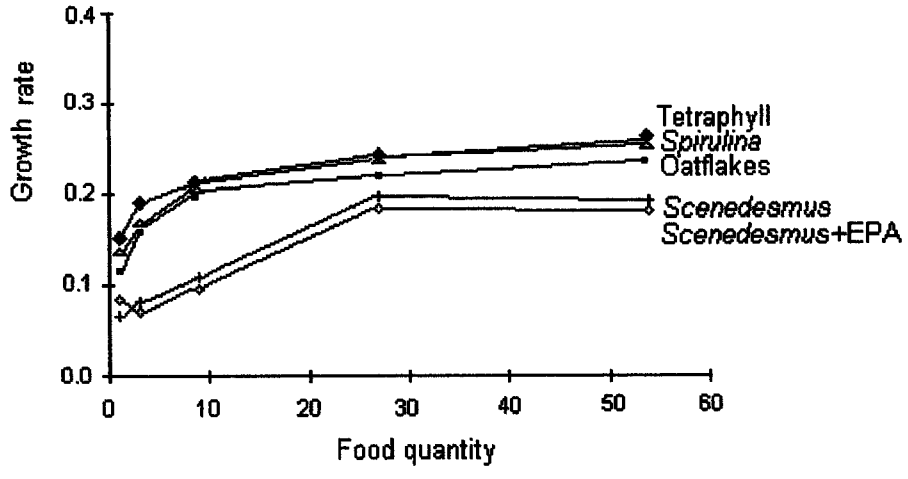


Figure 13: Relationship between food quantity (mg C/10d) and mean growth rate (d^{-1}) of *Chironomus* larvae.

Growth did not fully reach a maximum level at food quantities of >40-50 mg C/5d for the food types Tetraphyll® and *Spirulina*. The growth curves of *Chironomus* with Tetraphyll® and *Spirulina* as food differed only at food quantities of 1.0 and 3.0 mg

C/10d and were similar at higher food concentrations. Since Tetraphyll® contained relatively high concentrations of EPA (20:5ω3), one can expect conversion rates from ALA (18:3ω3) to EPA to be low, even at low food quantities. *Spirulina*, in contrast, provides *Chironomus* only with low concentrations of EPA at low food quantities. Though containing traces of EPA, *Spirulina* can be considered as a suboptimal food at low food quantities but as an ideal food at high quantities. At low quantities conversion from ALA to EPA could possibly meet any physiological demands of EPA during somatic growth. At high food concentrations *Spirulina* provides enough EPA so that conversions from ALA to EPA might no longer be limiting. *Spirulina* is the only food type containing γ-linolenic acid (GLA) in considerable amounts and this FA can be elongated/desaturated to ARA, an important precursor for eicosanoids (Stanley-Samuelson & Pedibhotla, 1996).

The growth curve for larvae fed on oatflakes, though, levelled out unlike in the Tetraphyll® and *Spirulina* treatments but mean length of larvae was not significantly lower than in the other two treatments. Thus, *Chironomus* seemed to be able to compensate for the lower nutritional value of oatflakes. The plateau reached on oatflakes indicates that larval growth supposedly was limited by another important element, maybe P or N. Regarding the FA composition oatflakes should be a food type of higher quality than *Spirulina* at lower food concentrations because oatflakes contained approximately 16 times more ALA than *Spirulina* (Table 6). Thus, the conversion from ALA to EPA at low concentrations of oatflakes should provide the larvae with sufficient EPA. *Spirulina*, in contrast, contained too low concentrations of ALA and EPA at low food quantities of 1.0 -3.0 mg C/10d. Thus, only low conversion rates from ALA to EPA can be expected. Hence, *Spirulina* is a worse food than oatflakes for *Chironomus* at these quantities.

The growth curve of larvae fed on oatflakes was indeed higher than the growth curve on *Spirulina* at a concentration of 1 mg C/10d. But already at concentrations of 3.0 mg C/10d *Spirulina* supported higher growth, though not significantly, than oatflakes. This was interpreted as EPA contents in *Spirulina* now were sufficient to meet conversion rates from ALA to EPA in larvae fed on oatflakes. Since oatflakes contain much more LA than ALA and the Δ6 desaturase is responsible for elongation and desaturation of both ω3 and ω6-FA, one could expect conversion rates from LA to ARA to be higher than in the ω3 family. However, conversions from ALA to EPA are

favoured since the ω3-family has a higher affinity for the fatty acid desaturase and probably also for the Δ5 desaturase than the ω6 families (Sargent et al.,1995). The fact that oatflakes at higher concentrations supported lower growth, though not significantly, than *Spirulina* can also be explained by the low N and P content of oatflakes. N and P contents were approximately 4 times lower in oatflakes than in the cyanobacteria and thus might be the limiting factor in growth of *Chironomus*.

Scenedesmus, whether supplemented with EPA or not, never supported such high growth as Tetraphyll®, *Spirulina* or oatflakes. *Scenedesmus* was the only food type rich in ALA and should thus be a higher quality food than oatflakes. However, it seemed that FA composition of *Scenedesmus* was not a good indicator of food quality for *Chironomus*. Since the alga contained all the for animals essential FA and *Chironomus* was supposed to be able to elongate ALA to EPA, it seemed more likely that the low availability of the algal cells made this food type a low quality food. Not even supplementation of *Scenedesmus* with EPA increased growth of *Chironomus*, which made it impossible to assess the importance of EPA for this detrivorous invertebrate. Hence, a further experiment was conducted where oatflakes were supplemented with EPA. Growth of larvae increased from concentration 1.0 mg C/10d to the next concentration level, but decreased again at the following concentrations. Larval length did not differ at concentrations of 1.0 and 3.0 mg C/10 between oatflakes and EPA-supplemented oatflakes treatments ($P>0.8443$). But larvae grown on oatflakes became larger at the remaining concentrations ($P<0.0218$). Decreasing length at high concentrations of oatflakes + EPA indicated that EPA seemed to have a toxic effect on larvae. PUFA are sensitive to oxidation, which can lead to increased amounts of free radical production (Ahlgren et al., 1999).

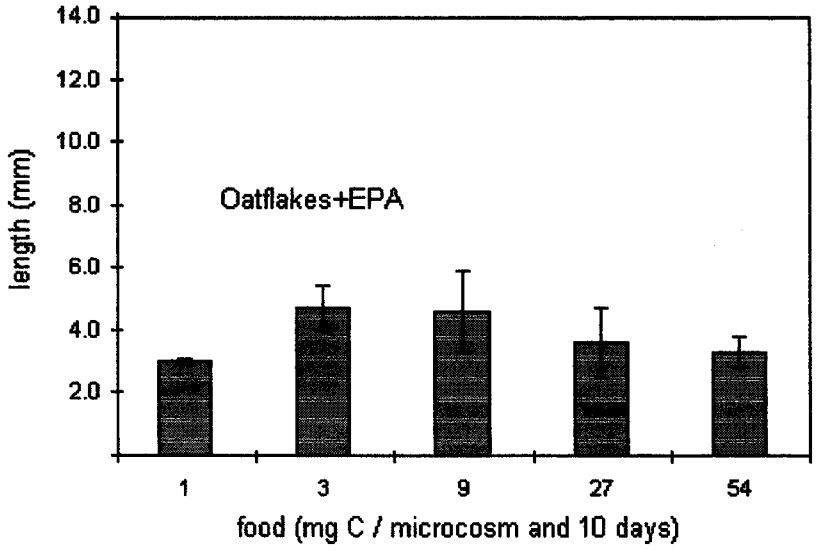


Figure 12. Mean length of *Chironomus* larvae after 10 days of growth on oatflakes supplemented with EPA. Error bars indicate standard deviation (n=2).

Survival of larvae was lowest at the concentrations 13.5 and 27.0 mg C/5d (50 and 65%), compared to survival >85% at the lower concentrations, supporting the assumption of a negative impact of EPA. Additionally, the supplementation treatment seems to impair food quality of oatflakes, since larval length was at concentrations 9.0 to 54.0 mg C/10d significantly lower than in the oatflakes treatment. A toxic effect of EPA on larvae fed with *Scenedesmus*+EPA, though, was not observed. This might again be due to the low availability of the algal cell, thus *Chironomus* larvae simply did not ingest as much EPA with algal cells as they did with oatflakes. I am aware of the fact that I, against better knowledge, statistically compared *Scenedesmus* treatments, consisting of fresh algal cells, with treatments of pulverised food types, and the oatflakes treatment with the EPA-supplemented oatflakes, though these experiments were not conducted at the same time.

Consequently, food quality cannot be solely determined by its (bio)chemical composition, but availability (seasonal or spatial) and digestibility have to be taken into consideration as well. The attempt to increase the availability of algal cells to *Chironomus* by using a sediment with a smaller mean grain size failed. A low feeding rate of *Chironomus* on *Scenedesmus* cells can be excluded since analysis of the guts of larvae fed on *Scenedesmus* (Figure 27) revealed that the degree of fullness of the guts was not different to fullness of larval guts fed on *Spirulina* (Figure 28), or Tetraphyll®(Figure

29). Since 3rd or 4th instar larvae were analysed for their gut contents, it still could be possible that the algal cells could not be ingested by 1st instar larvae, used in the experiments, due to a limited mentum width. The algal cells used in the experiment were though mainly single celled and measured 8 µm x 2µm and were thus within the particle size 1st instar larvae can ingest (Vos, 2001). Thus, low growth must be due to low digestibility of algal cells, for example thick cell walls. Microscopical observations of larval guts revealed that most algal cell walls were not broken down. Kajak & Warda (1968) found that the only well digested and assimilated group of algae by *Chironomus* were diatoms, whereas green algae, in contrast, were only very slightly digested. Though often cited as an important food resource, green algae usually constitute less than 4% of gut content in *Chironomus* (Johnson, 1987).

It is common knowledge that cellulose catabolism requires cellulase enzymes. There are three potential sources of cellulose-degrading enzymes associated with animals: tissue-level synthesis, microbial symbionts, and acquired enzymes from the diet (Sinsabaugh et al., 1985). Cellulase analysis on among others two *Chironomus* species (*C. anthracinus* and *C. plumosus*) revealed that neither *C. anthracinus* nor *C. plumosus* were able to degrade long-chain cellulose on their own (Bjarnov, 1972). Tissue level synthesis of cellulases among insects have been reported only in certain termites and cockroaches (Sinsabaugh et al., 1985). Lacking cellulase activity, however, does not preclude the possibility of cellulose digestion. From mammalian ruminants and certain arthropods, lacking their own cellulases, it is known that they rely on the production of cellulases by associated microflora (Begon et al., 1996). However, microbial symbionts can be excluded since *Chironomus* has a simple, tubular gut that lack distinctive fermentation chambers found in most animals harbouring cellulolytic endosymbionts. A substantial number of studies report on the presence of gut microbes in aquatic invertebrates (Bjarnov, 1972, Sinsabaugh et al., 1985, Chamier, 1991, Martin et al., 1980, McGrath & Matthews, 2000, Harris, 1993) and acquired microbial cellulases, have been reported in the amphipod *Gammarus* spec. (Chamier, 1991, Chamier & Willoughby, 1986). The most commonly reported association between aquatic invertebrates and gut microbes is that of ingestion of bacteria. Lysed microbes may, however, contribute enzymes that remain active in the gut, i.e., acquired bacterial enzymes, and these may provide the host with additional digestive abilities (Harris, 1993).

The model organism is a detrivore of the collector-gatherer type (Armitage et al., 1995). They feed on particles that vary throughout time in abundance and in state of decomposition. The intensity of feeding is dependent on the value of the food (Kajak & Warda, 1968). Food resources available to detrivores differ in nutritional quality during the course of year (Ahlgren et al., 1997). *Chironomus* seems on the one hand well adapted to low food quality, being able to synthesise long chain PUFA from their precursors, which are found in organic matter. This may be an adaption that enables *Chironomus* to grow on low quality food. However, the animal is not able to perform intensive digestion processes what explains short passing times of sediments in the gut (Bjarnov, 1972). *Chironomus* seems to be thus dependent on conditioning of organic matter with bacteria. It is also likely that detrivores only use the most easily absorbed components present in the ingested sediment (Vos, 2001). On the other hand, Chironomus seems to be an opportunistic feeder, being able to respond to inputs of PUFA with rapid growth or sexual maturation. Their strong response to seasonal inputs of newly produced organic matter suggests that the food availability of high-quality food is a limiting factor during most of the year (Goedkoop & Johnson, 1996). Earlier work has shown that benthic invertebrates are highest during spring and autumn, due to the dominance of diatoms (Ahlgren et al., 1997). It has been shown for different profundal chironomid taxa that their FA content responded to the seasonal variation of their food (Goedkoop et al., 1998).

Conclusions

This study contributes insight in the food requirements of a detrivorous benthic invertebrate. The results illustrate the importance of food quality and food quantity for growth of *Chironomus riparius* and raise the unsolved discussion about definition of food quality. Food quality cannot solely be described by its FA content but a complete description of food quality requirements should also include other important substances such as essential amino acids, vitamins and trace elements. High contents of the long-chained fatty acid EPA are apparently not important for detrivorous animals. Instead, *Chironomus riparius* seems to prefer a very low ω3/ω6 ratio, with an optimum of

approximately 0.2. With respect to this ω3/ω6 ratio it can be suggested that this *Chironomus species* is more related to terrestrial invertebrates than to aquatic animals.

The finding that *Chironomus* is able to synthesise long-chain PUFA from shorter precursor FA and that the conversion capacities apparently meet their physiological demands, opposes the general opinion that *Chironomus* is supposed to have higher nutritional demands than some other invertebrate detrivores. Therefore more attention should be paid to biochemical composition and quantity of natural food sources to understand growth of detrivorous sediment feeding chironomids under natural conditions.

6. REFERENCES

AHLGREN, G. 1977. Growth of *Oscillatoria agardhii* in chemostat culture. 1. Nitrogen and phosphorus requirements. Oikos 29: 209-224

AHLGREN, G., LUNDSTEDT, L:, BRETT, M., FORSBERG, C. 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. Journal of Plankton Research 12:809-818

AHLGREN G., GUSTAFSSON I.-B., BOBERG M. 1992. Fatty acid content and chemical composition of freshwater microalgae. J. Phycol. 28: 37-50

AHLGREN, G., BLOMQVIST, P., BOBERG, M., GUSTAFSSON, I.-B. 1994. Fatty acid content of the dorsal muscle - an indicator of fat quality in freshwater fish. Journal of Fish Biology 45: 131-157

AHLGREN, G., GOEDKOOP, W., MARKENSTEN, H., SONESTEN, L., BOBERG, M. 1997. Seasonal variations in food quality for pelagic and benthic invertebrates in Lake Erken - the role of fatty acids. Freshwater Biology 38: 555-570

AHLGREN, G., ZEIPEL, K., GUSTAFSSON, I.-B. 1998. Phosphorus limitation effects on the fatty acid content and nutritional quality of greeen alga and a diatom. Verh. Internat. Verein. Limnol. 26: 1659-1664

AHLGREN, G.,CARLSTEIN, M. , GUSTAFSSON, I.-B. 1999. Effects of natural and commercial diets on the fatty acid content of the European grayling. Journal of Fish Biology 55: 1142-1155

ARMITAGE, P. D. 1995 Behaviour and ecology of adults. pp 194-224 in: ARMITAGE, P., CRANSTON, P.S., PINDER, L.C.V. (eds.) 1995. The Chironomidae. The biology and ecology of non-biting midges. Chapman&Hall

ARMITAGE, P., CRANSTON, P.S., PINDER, L.C.V. (eds.) 1995. The Chironomidae. The biology and ecology of non-biting midges. Chapman&Hall

ARTS, M.T.; WAINMAN, B.C. (eds.) 1999. Lipids in Freshwater Ecosystems. Springer Verlag

BEENAKKERS, M.TH., VAN DER HORST, D.J., VAN MARREWIJK, W.J.A. 1985. Insect lipids and Lipoproteins, and their role in physiological processes. Prog. Lipid Res. 24:19-67

BEGON, M., HARPER, J.L., TOWNSEND, C.R. 1996. Ecology. Blackwell Science

BENOITE, D., SIBLEY, P., JUNEMANN, J.L., ANKLEY, G. T. 1997. *Chironomus tentans* life-cycle test: design and evaluation for use in assessing toxicity of contaminated sediments. Environmental Toxicology and Chemistry Vol 16; No 6 1165-1176

BJARNOV, N. 1972. Carbohydrases in Chironomus, Gammarus and some Trichoptera larvae. Oikos 23: 261-263

BLOMQUIST, G.J., DWYER, L.A., CHU, A.J., RYAN, R.O., DE RENOBALES, M. 1982. Biosynthesis of linoleic acid in a termite, cockroach and cricket. Insect Biochem. 12: 349-353

BRENNER R.R. 1989. Factors influencing fatty acid chain elongation and desaturation. pp 45-79 in: The role of fats in Human Nutrition (Vergroesen, A.J. & Crawford, M. (eds.), London, Academic Press

BRETT, M.T., MÜLLER-NAVARRA, D.C. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38: 483-499

CAVALETTO, J. F., GARDNER, W.S. 1999. Seasonal Dynamics of Lipids in Freshwater Benthic Invertebrates. pp 109-131 in: M.T. ARTS and B.C. WAINMAN (eds.). 1999. Lipids in freshwater ecosystems. Springer Verlag

CHAMIER, A.C. 1991. Cellulose digestion and metabolism in the freshwater amphipod *Gammarus pseudolimnaeus* Boussfield. Freshwater Biology 25: 33-40

CHAMIER, A.C., WILLOUGHBY, L.G. 1986. The role of fungi in the diet of the amphipod Gammarus pulex (L.): an enzymatic study. Freshwater Biology 16: 197-208

DEMOTT, W. R., MÜLLER-NAVARRA, D.C. 1997. The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsion. Freshwater Biology 38: 649-664

DOWNER, R.G.H. 1981. Physiological and environmental considerations in insect bioenergetics. in: DOWNER, R.G.H. (ed.) Energy metabolism in insects. Plenum Press.

FARKAS, T. 1979. Adaption of fatty acid compositions to temperature - a study on planktonic crustaceans. Comp. Biochem. Physiol. 64 B: 71-76

GHIONI, C., BELL, J.G., SARGENT, J.R. 1996. Polyunstaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. Comp. Biochem. Physiol. 114 B No. 2: 161-170

GOEDKOOP, W., JOHNSON, R.K. 1996. Pelagic benthic coupling: profundal benthic community response to spring diatom deposition in mesotrophic Lake Erken. Limnol. Oceanogr. 41: 636-647

GOEDKOOP, W., SONESTEN, L., MARKENSTEN, H., AHLGREN, G. 1998. Fatty acid biomarkers show dietary differences between dominant chironomid taxa in Lake Erken. Freswater Biology 40:135-143

GOEDKOOP, W., SONESTEN, L., AHLGREN, G., BOBERG, M. 2000. Fatty acids in profundal benthic invertebrates and their major food resources in Lake Erken, Sweden:seasonal variation and trophic indications. Can.J. Fish. Aquat. Sci. 57: 2267-2279

GOULDEN, C.E., MOELLER, R.E., MCNAIR, J.N., PLACE, A.R. 1999. Lipid Dietary Dependencies in Zooplankton. pp 91-108 in: M.T. ARTS and B.C. WAINMAN (eds.). 1999. Lipids in freshwater ecosystems. Springer Verlag

GULATI, R. D., DEMOTT, W.R. 1997. The role of food quality for zooplankton: remarks on the state-of-the-art, perspectives and priorities. Freshwater Biology 38: 753-768

GULLAN, P.J., CRANSTON, P.S. 1996. The insects. An Outline of Entomology.

HANSON, B., CUMMINS, K.,CARGILL, A.S., LOWRY, R.R. 1985. Lipid content, fatty acid compostion and the effect of diet on fats of aquatic insects. Comp. Biochem. Physiol. 80B No 2: 257-276

HARRIS, J.M. 1993. The Presence, Nature, and Role of Gut Microflora in Aquatic Invertebrates: A Synthesis. Microbial Ecology 25: 195-231

HORNE, A.J., GOLDMAN, C.R. 1994. Limnology. McGraw-Hill, Inc.

HOWARD R., STANLEY-SAMUELSON DAVID W. 1996. Fatty acid composition of fat body and Malpighian tubules of the Tenebrionid Beetle, *Zophobas atratus*: Significance in eicosanoid-mediated physiology. Comp. Biochem.Physiol. 115B: No 4 429-437

JOHNSON, R. 1987. Seasonal variation in diet of *Chironomus plumosus* (L.) and *C. antracinus* Zett. (Diptera: Chironomidae) in mesotrophic Lake Erken. Freshwater Biology 17: 525-532

JOHNSON, R., BOSTRÖM, B., VAN DEN BUND, W. 1989. Interactions between *Chironomus plumosus* and the microbial community in surficial sediments of a shallow, eutrophic lake. Limnol. Oceanogr. 34(6): 992-1003

KAJAK, Z., WARDA, J. 1968. Feeding of benthic non-predatory Chironomidae in lakes. Ann. Zool. Fenn. 5: 57-64

KANAZAWA, A., TESHIMA, S.I., ONO, K. 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comp. Biochem. Physiol. 63 (B) 295-298

KEBEDE, E., AHLGREN, G. 1996. Optimum growth conditions and light utilization efficiency of Spirulina platensis (=Arthrospira fusiformis) (Cyanophyta) from Lake Chitu, Ethiopia. Hydrobiologica 332:99-109

LAMPERT, W. Studies on the carbon balance of *Daphnia pulex* as related to environmental conditions. 1977. Arch. Hydrobiol. Beih. 48: 336-360

LAMPERT, W., SOMMER, U. 1999. Limnoökologie. Thieme Verlag

LEHNINGER, A.L., NELSON, D.L., COX, M.M. 1993. Principles of Biochemistry. Worth Publishers, New York

LOZAN, J.L., KAUSCH, H. 1998 Angewandte Statistik für Naturwissenschaftler. Pareys Studientexte

MARTIN, M.M., MARTIN, J.S., KUKOR, J.J., MERRITT, R.W. 1980. The digestion of Protein and Carbohydrate by the Stream detrivore, Tipula abdominalis (Diptera, Tipulidae). Oecologia 46: 360-364

MCGRATH, C.C., MATTHEWS, R.A. 2000. Cellulase activity in the freshwater amphipod *Gammarus lacustris.* J. N. Am. Benthol. Soc. 19(2): 298-307

MÜLLER-NAVARRA, D.C. 1995. Evidence that highly unsaturated fatty acid limits *Daphnia* growth in nature. Arch. Hydrobiol 132: 297-307

MURPHY, J., RILEY, J.P. 1962. A modified single-solution method for the determination of phosphate in natural waters. Analyt. Chim. Acta 27:31-36

OECD Guideline for the testing of chemicals. 2001 Proposal for a new guideline 218.

OLSEN, Y. 1999. Lipids and Essential Fatty Acids in Aquatic Food Webs: What can Freshwater Ecologists Learn from Mariculture? pp 161-202 in: M.T. ARTS and B.C. WAINMAN (eds.). 1999. Lipids in freshwater ecosystems. Springer Verlag

PARRISH, C.C. 1999 Determination of Total Lipid, Lipid Classes, and Fatty Acids in Aquatic Samples. pp 4-20 in: M.T. ARTS and B.C. WAINMAN (eds.). 1999. Lipids in freshwater ecosystems. Springer Verlag

SARGENT, J.R., BELL, M.V., BELL, J.G., HENDERSON, R.J., TOCHER, D.R. 1995a. Origins and Functions of n-3 Polyunsaturated Fatty Acids in Marine Organisms. pp 248-259 in: CEVC G., PALTAUF F. (eds.) Phospholipids: Characterization, Metabolism and Novel Biological Applications. Amer. Oil Chem. Soc. Press, Champaign, III, USA

SARGENT, J.R., BELL, M.V., BELL, J.G., HENDERSON, R.J., TOCHER, D.R. 1995b. Requirement criteria for essential fatty acids. J Appl. Ichthyol. 11:183-198

SINGER, S.J., NICOLSON, G.L. 1972. The fluid Mosaic Model of the Structure of Cell Membranes. Science 175: 720-731

SINSABAUGH, R.L., LINKINS, A.E., BENFIELD, E.F. 1985. Cellulose digestion and assimilation by three leaf-shredding aquatic insects. Ecology 66(5): 1464-1471

STANLEY-SAMUELSON, D.W. 1994. The biological significance of prostaglandines and related eicosanoids in invertebrates. Amer. Zool. 34: 589-598

STANLEY-SAMUELSON, D.W., PEDIBHOTLA, V.K. 1996. What can we learn from prostaglandins and related eicosanoids in insects? Insect. Biochem. Molec. Biol. 26 (3): 223-234

STERNER, R.W., SCHULZ, K.L. 1998. Zooplankton nutrition: recent progress and reality check. Aquatic Ecology 0:1-19

STRELOKE, M., KOPP, H. (eds.) 1995. Long term toxicity test with *Chironomus riparius*: Development and validation of a new test system. Mitteilungen aus der biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin Dahlem. Heft 315. Biologische Bundesanstalt für Land und Forstwirtschaft, Abteilung für Pflanzenschutzmittel und Anwendungtechnik, Braunschweig

STRYER, L. 1996. Biochemistry. Spektrum Akademischer Verlag GmbH Heidelberg, Berlin, Oxford

SUNDBOM, M., VREDE, T. 1997. Effects of fatty acid and phosphorus content of food on the growth, survival and reproduction of *Daphnia*. Freshwater Biology 38: 665- 674

TIKKANEN, T., WILLÉN, T. 1992. Växtplanktonflora. Naturvårdsverket

VANCE, D.E., VANCE J.E. (eds.) 1996. Biochemistry of Lipids, Lipoproteins and Membranes. Elsevier

VOS, J.H. 2001. Feeding of Detrivores in Freshwater Sediments. Ph.D. thesis, University of Amsterdam, Netherlands

WATANABE, T. 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. 73(B):3-15

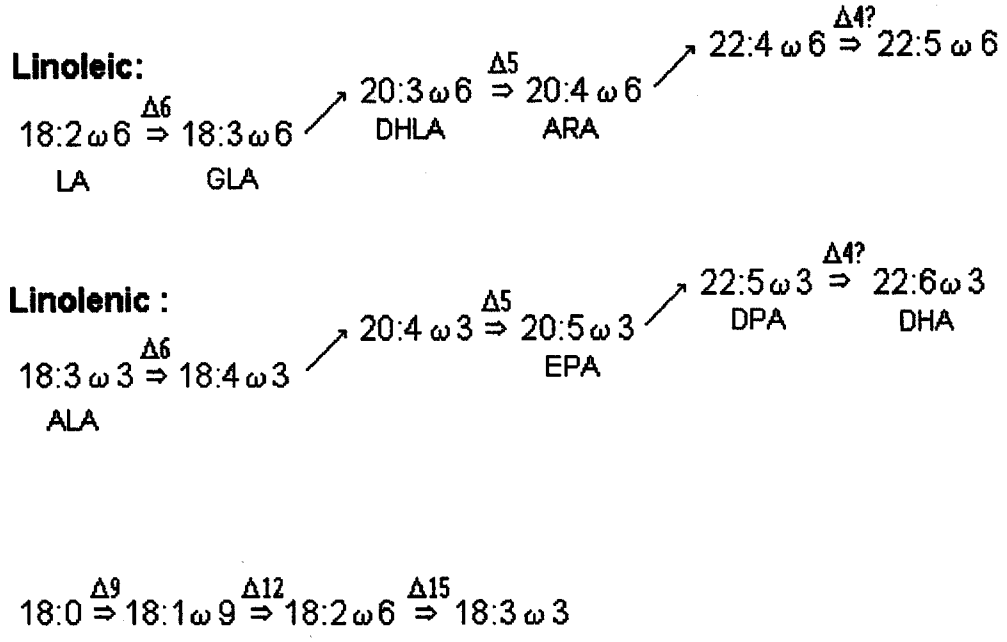
WALSHE, B.M. 1947. Feeding Mechanisms of *Chironomus* Larvae; Nature 160: 474

WEERS, P.M.M., GULATI, R.D. 1997. Effect of the addition of polyunsaturated fatty acids to the diet on the growth and fecundity of *Daphnia galeata*. Freshwater Biology 38: 721-729

XU, X.L., JI, W.J., CASTELL, J.D., O'DOR, R.K. 1993. The nutritional value of dietary n-3 and n-6 fatty acids for the Chinese prawn (*Panaeus chinensis)*. Aquaculture 118: 277-285

7. Appendix

Desaturation and elongation routes of unsaturated fatty acids:
(modified from Brenner, 1989)



Abbreviations, names and code of fatty acids:

LA	= linoleic acid	18:2ω6
GLA	= γ-linolenic acid	18:3ω6
DHLA	= dihomo-γ-linolenic acid	20:3ω6
ARA	= arachidonic acid	20:4ω6
ALA	= α-linolenic acid	18:3ω3
SA	= stearidonic acid	18:4ω3
EPA	= eicosapentaenoic acid	20:5ω3
DPA	= docosapentaenoic acid	22:5ω3
DHA	= docosahexaenoic acid	22:6ω3
Δx	= desaturation enzymes	
Δ4?	= enzyme not yet found (probably does not exist)	

TAG	= triacylglycerols
PUFA	= polyunsaturated fatty acid
SAFA	= saturated fatty acid
MUFA	= monounsaturated fatty acid
HUFA	= highly-unsaturated fatty acid

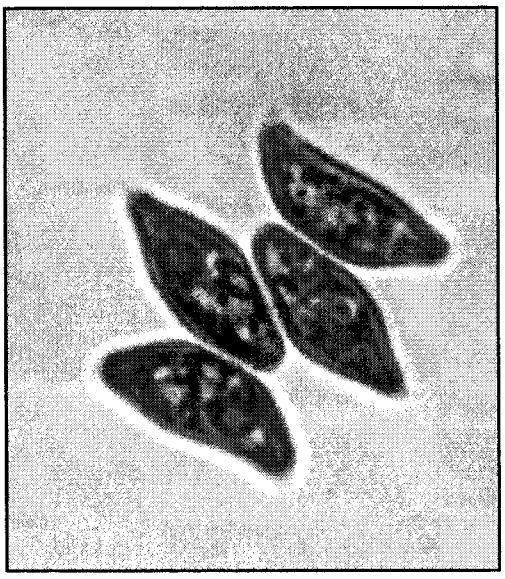


Figure15. Microscopical image of *Scenedesmus obliquus* at 400x magnification. Length of single cells=8.2µm; width of single cells=2.5µm. The alga was cultured in two turbidostat chambers and harvested at exponential growth phase, since the ω3/ω6 ratio appears to be highest in algae in this phase (Ahlegren et al., 1992). The specific growth rate μ differed in the two cultivation chambers (A, B) nearly by a factor two (μ_A=0.59 ± 0.66; μ_B=1.09 ± 0.88). These differnt specific growth rates could be due to different biomasses in the two cultures. A more turbid culture (A) grows slower due to less light per algal cell. Since the excess volumes of both cultures were used in the experiments, weighing of the proportions of excess volume of culture A and B was necessary. Approximately 1/3 of the excess volume of culture A and 2/3 of the excess volume of culture B were used, thus the specefic growth rate μ can be calculated to 0.47 d^{-1}.

Gaffron's trace elements

Table 21: Concentration of salts and elements in Gaffron's trace elements.

Concentration of salts	µg/l	Concentration of elements	µg/l
$MnSO_4$ x 4 H_2O	223	Mn	81.0
H_3BO_3	310	B	54.0
KBr	12.0	Br	8.00
KJ	8.30	J	6.35
$ZnSO_4$ x 7 H_2O	28.7	Zn(II)	6.55
$Cd(NO_3)_2$ x 4 H_2O	15.4	Cd	5.60
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	8.80	Mo(VI)	4.85
$CuSO_4$ x 5 H_2O	12.5	Cu	3.20
$Co(NO_3)_2$ x 6 H_2O	14.6	Co	2.95
$NiSO_4(NH_4)_2SO_4$ x 6 H_2O	19.8	Ni	2.95
$Al_2(SO_4)_3K_2SO_4$ x 24 H_2O	47.4	Al	2.70
Na_2WO_4 x 2 H_2O	3.30	W(VI)	1.85
$Cr(NO_3)_3$ x 9 H_2O	4.10	Cr	0.530
V_2O_5	0.890	V	0.500

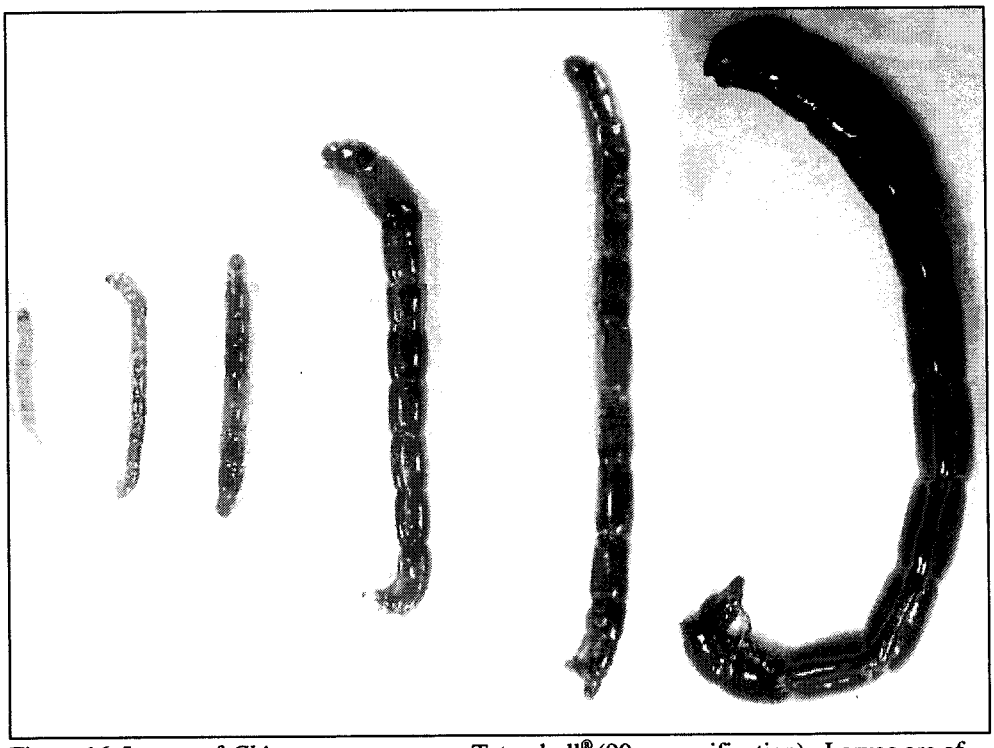


Figure 16. Larvae of *Chironomus* grown on Tetraphyll® (90x magnification). Larvae are of different sizes and thus of different instars.

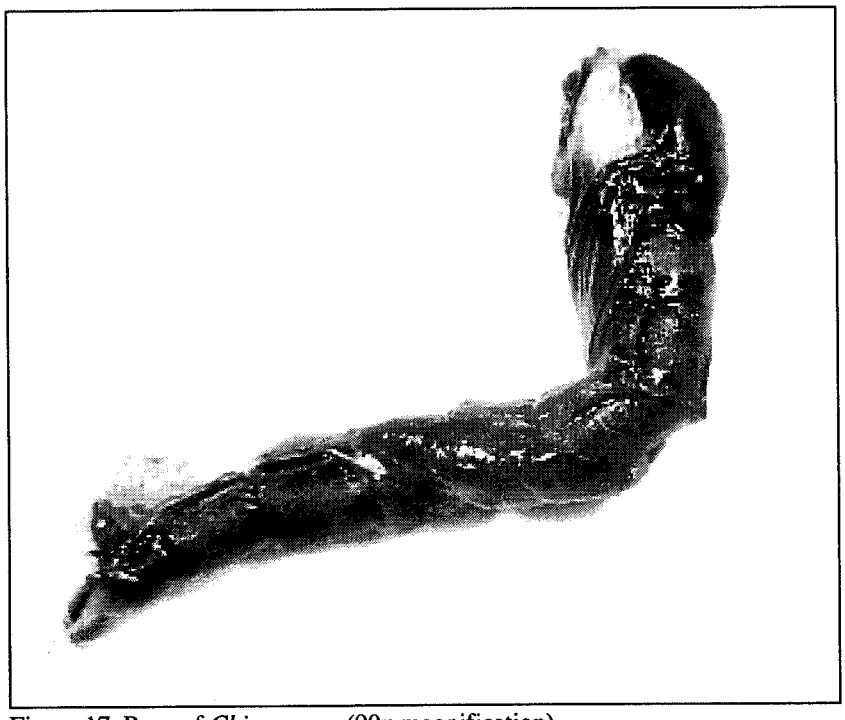


Figure 17. Pupa of *Chironomus* (90x magnification).

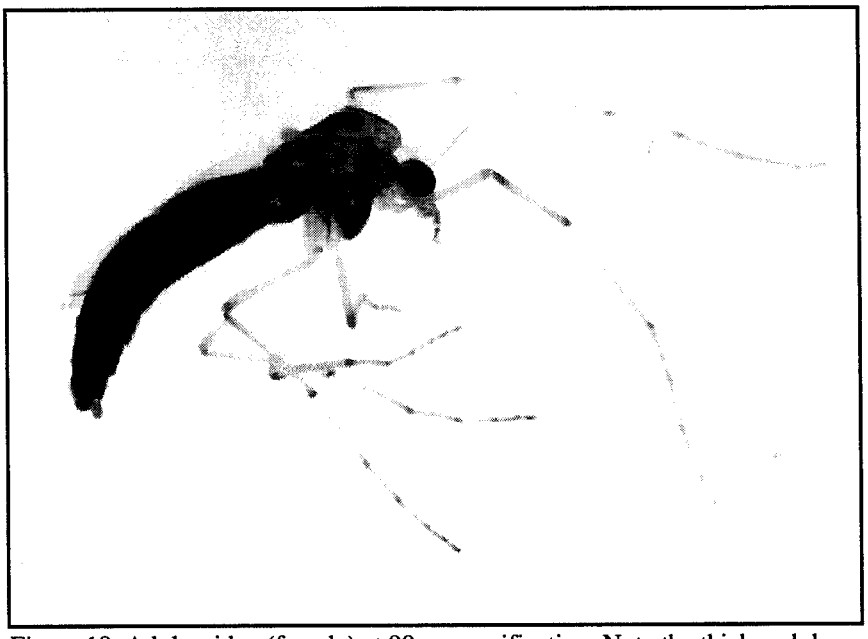


Figure 18. Adult midge (female) at 90 x magnification. Note the thicker abdomen in contrast to the male.

Figure 19. Details of the adult female (120 x magnification).
Note the antenna in contrast to the plumosed antenna of the males.

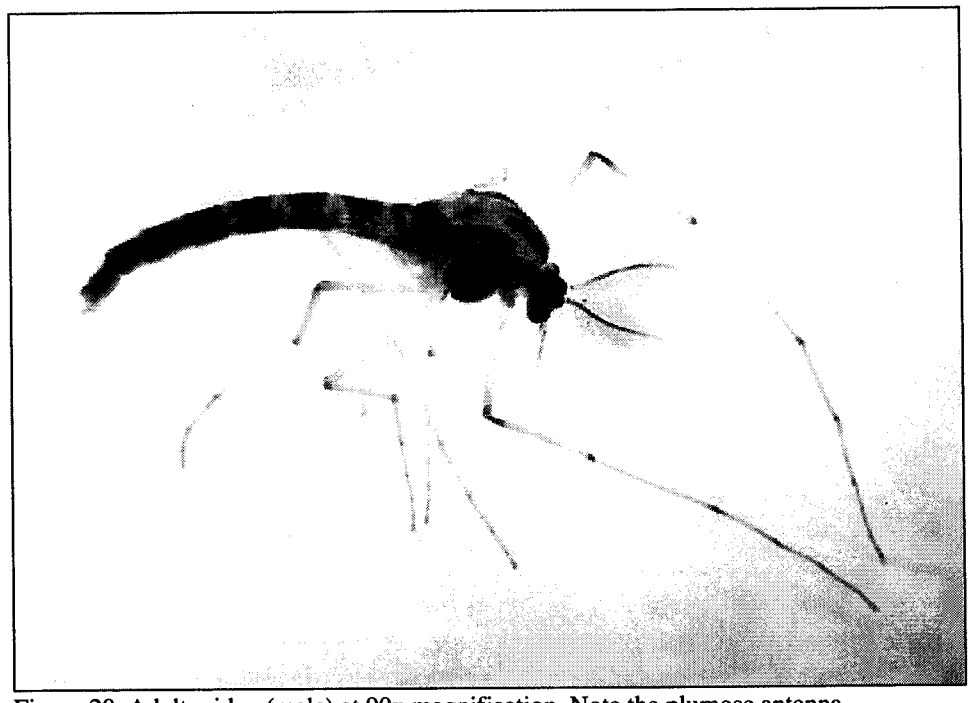


Figure 20. Adult midge (male) at 90x magnification. Note the plumose antenna.

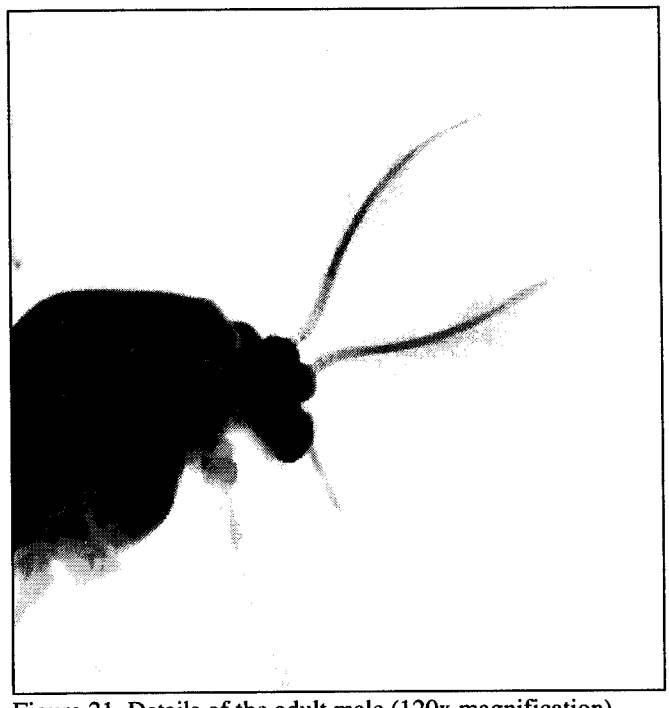


Figure 21. Details of the adult male (120x magnification).

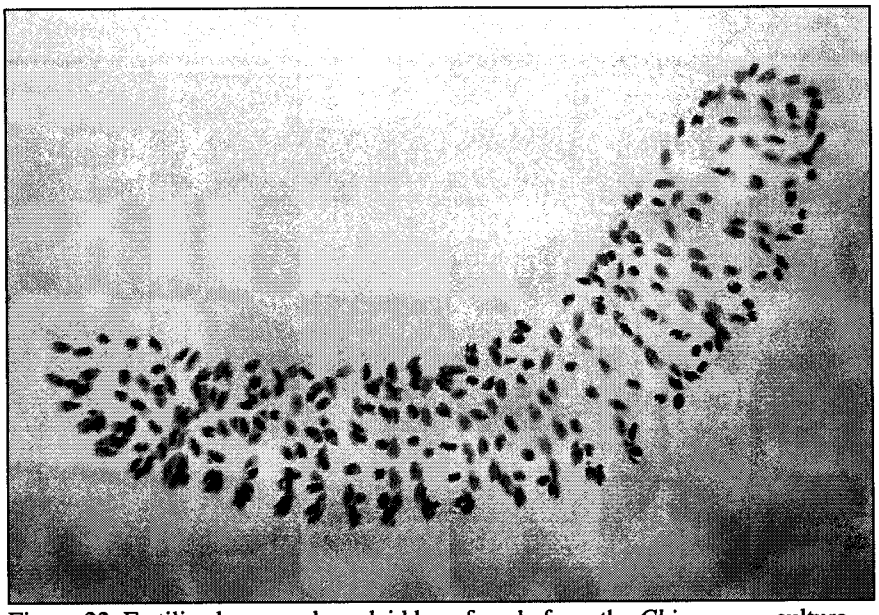


Figure 22. Fertilized egg-package laid by a female from the *Chironomus* culture.

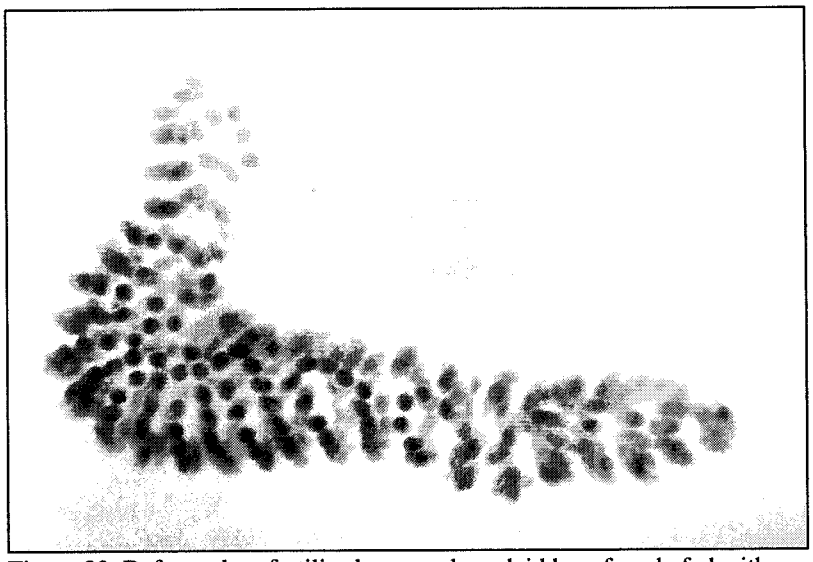


Figure 23. Deformed, unfertilised egg-package laid by a female fed with Tetraphyll®.

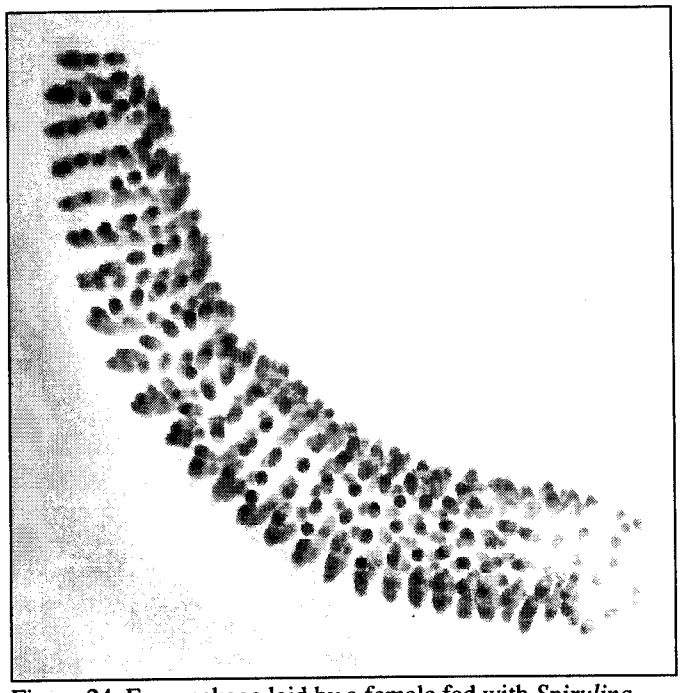


Figure 24. Egg-package laid by a female fed with *Spirulina* (54.0 mg C/10d).

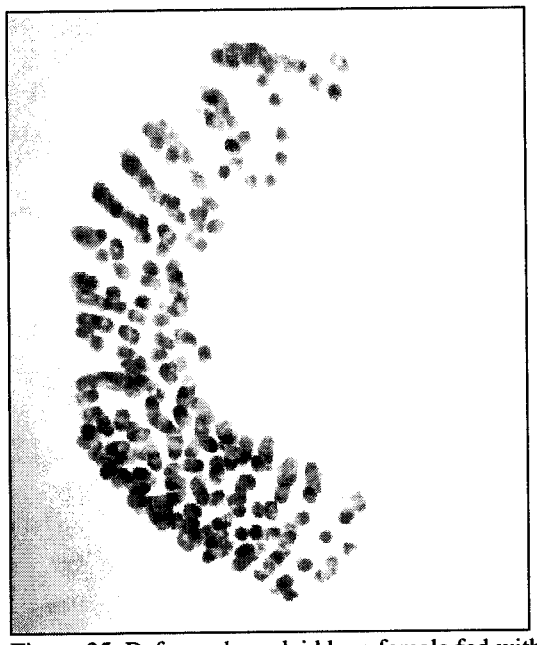


Figure 25. Deformed egg laid by a female fed with *Spirulina* (27.0 mg C/10d).

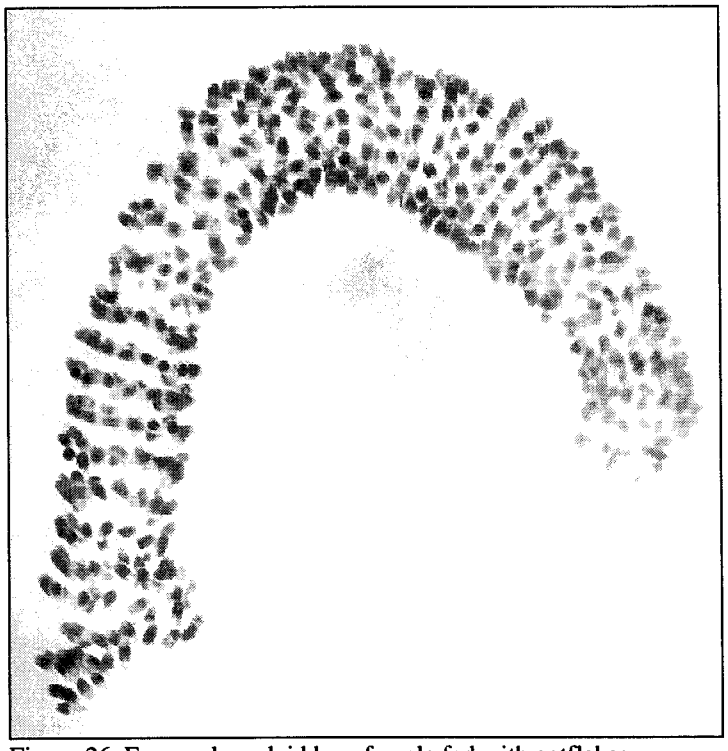


Figure 26. Egg-package laid by a female fed with oatflakes (54.0 mg C/10d).

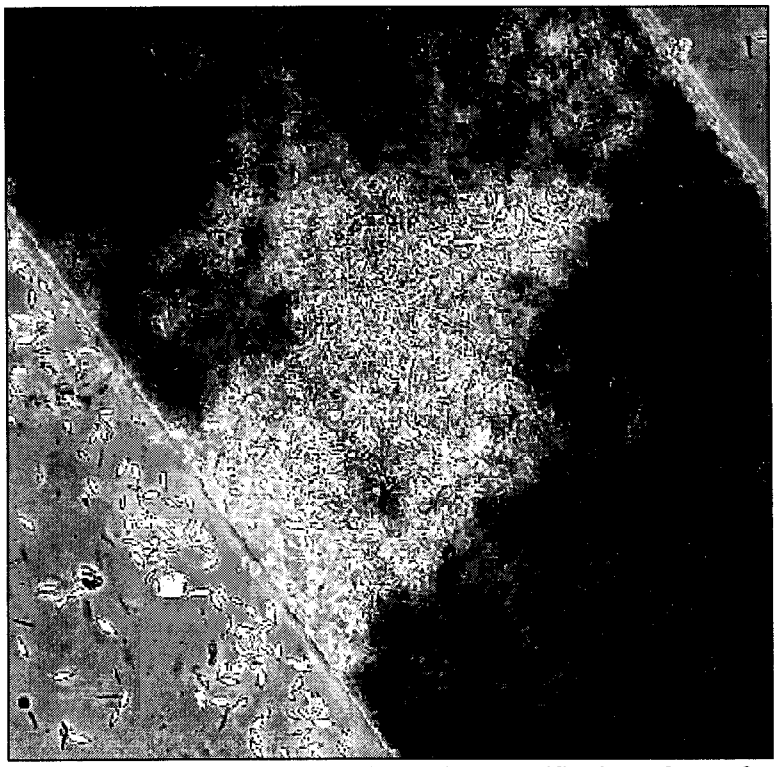


Figure 27. Microscopical observations (200x magnification) of guts of *Chironomus* fed with *Scenedesmus*.

Figure 28. Microscopical observations (200x magnification) of guts of *Chironomus* fed with *Spirulina*.

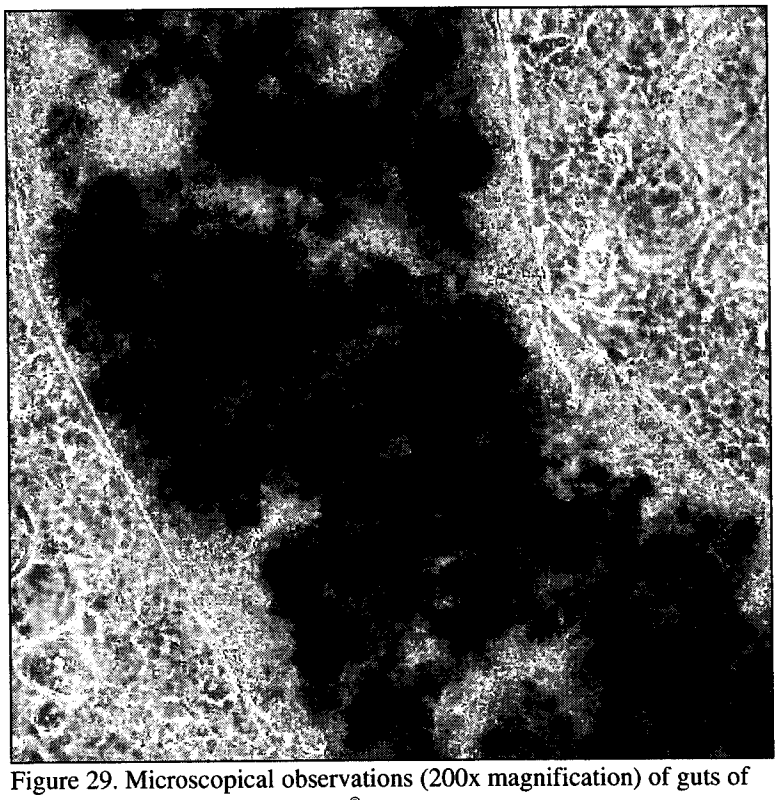


Figure 29. Microscopical observations (200x magnification) of guts of *Chironomus* fed with Tetraphyll®.