Growth and development of Chironomus thummi (Kieffer) on various algal diets.

GROWTH AND DEVELOPMENT OF *CHIRONOMUS THUMMI* (KIEFFER) ON VARIOUS ALGAL DIETS

A Thesis Presented

By

Daniel Weissman

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C H A P T E R I

INTRODUCTION

The aquatic larvae of the non-biting midges (Chironomidae) have been advocated by several fish culturists and entomologists as a dietary supplement for cultured fish. Most intensively studied in this connection are the hemoglobin-containing larvae of the genus Chironomus. Several observations on the biology and ecology of Chironomus larvae suggest their suitability for mass rearing as an adjunct to fish culture:

1. Larvae of the family Chironomidae often predominate by weight in the diet of bottom-feeding fish in eutrophic lakes (Johnson 1929, Gerking 1962, Charles et al. 1974), implying a good nutritive value for the type of fish commonly raised in fish ponds. Normally, the benthic macrofauna of fish ponds consist chiefly of chironomids by numbers and by weight (Kalugina 1971, Dvořák 1978).

2. Chironomus can subsist on plant matter and detritus, an important factor in making the artificial rearing of the larvae economically feasible.

3. Their ability to thrive at high densities (Jónasson 1972) and their multivoltinism enable chironomids

to rank among the most productive of benthic invertebrates (Waters 1977).

4. Members of the genus Chironomus tend to be tolerant of the high organic content and seasonally low oxygen tension of eutrophic lakes (Mundie 1957, Jónasson 1972), and they even abound in waters which are too polluted for most other benthos (Gaufin and Tarzwell 1952, Paine and Gaufin 1956, Grodhaus 1967). This tolerance makes Chironomus amenable to intensive rearing in ponds fertilized with inexpensive organic waste.

5. The high reproductive potential of a single female midge (500-2000 eggs depending on species), the short larval existence (2-3 weeks under favorable conditions), the ability of many species to breed in cages, and the apparent lack of obligatory adult feeding, are biological factors which make the large scale rearing of Chironomus feasible (Sadler 1935, Konstantinov 1958, Biever 1965, Yount 1966, Yashouv 1970, McLarney et al. 1974).

Fish culturists around the world add a wide variety of supplementary foods to their ponds, and the cheapest fish foods are those which are locally available, e.g., grains, agricultural waste, and trash sea fish (Hickling 1971). The conversion rate of food into fish is also an important consideration. In this respect chironomids are

one of the best supplementary foods (ibid., Table VII). Sturgeon fed only chironomids gain 1 g for each 2 g wet wt. of larvae (during the first two weeks of life), and these fish are healthier than sturgeon fed only oligochaetes or crustaceans (Konstantinov 1958).

In the U.S.S.R. rearing of chironomids has moved beyond the experimental stage, and "factory-produced" larvae are used to feed salmon and sturgeon. (It is not clear whether the fish receive other types of food as well.) The larvae are reared in shallow pans and fed yeast. Other chironomid rearing methods have shown promise in the experimental stage, e.g., the use of plastic-lined pools fertilized with manure or sewage sludge. A major challenge is to determine the overhead and labor costs of the various rearing procedures. Obviously one can permit these costs to be higher when cultivating the more valuable kinds of fish, such as trout, salmon, and sturgeon.

In view of the major ecological position of the Chironomidae as aquatic primary consumers, and in particular their importance as food for fish, it would be useful to know more about the feeding preferences of the non-predaceous chironomids. Algae often form a large share of the gut contents of collected specimens. This observation has led to speculation on the nutritive value of various taxonomic groups of algae, with the diatoms believed to be

the most important. However, gut analyses yield limited information, since generally several species of alga are present in the gut of a collected specimen, along with other non-algal food items. By rearing chironomids in the laboratory on a single species of alga, one could learn whether that species supports the development of the insect from eclosion to imago. Likewise, one could estimate the efficiency with which algae is converted to larval biomass. With these objectives in mind, the author proceeded to rear Chironomus thummi (Kieffer)[1] on monocultures of three common freshwater algae.

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[1]A European lake species (Pause 1918) familiar to students of chromosome puffing (Keyl and Strenzke 1956). It was given to the author by Hans Laufer of the University of Connecticut.

C H A P T E R I I

LITERATURE REVIEW

Feeding Habits of Chironomidae

In his review of trophic relations of aquatic insects, Cummins (1973) recognized four categories of insect by feeding mechanism (shredders, collectors, scrapers, predators) and four categories by food (herbivores, carnivores, detritivores, herbivore-detritivores[2]). Larval Chironomidae can be found in all of these categories. Among the shredders is Cricotopus, which mines the leaves of a common pond weed (Berg 1950). Rheotanytarsus spins a plankton net across the upstream end of its case, and so it is a collector (Walshe 1951). The marine tribe Clunionini of the rocky intertidal zone are scrapers, and the subfamily Tanypodinae contains many predators (Coffman 1978).

Most chironomid larvae have combined methods of feeding. For example, some predatory species are known to ingest algae and detritus (Roback 1969, Oliver 1971), while the filter-feeding Endochironomus will leave its tube to hunt

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[2]Detritus is decaying organic matter (see Oliver 1971).

down small invertebrates if phytoplankton is scarce (Izvekova 1971). Also, from personal observations, the author would classify C. thummi as a herbivore-detritivore; and yet several larvae have been observed to attack a pupa when little algal food was available.

Larvae of the subfamily Chironominae build a case from any available particles of the proper size (Oliver 1971). The case is held together with threads secreted by the salivary glands. In its usual habitat of lake mud, C. thummi makes a vertical U-shaped tube, the openings projecting conelike above the mud surface (Pause 1918). The author has observed late first instars in cases constructed of algae, when no other substrate was offered. In the latter circumstance, the cases are straight and lie horizontally. Larvae can be observed to undulate inside their algae cases, and this behavior draws a current through the case. No filtering net has been observed by the author. Walshe (1951) held that Chironomus is not a net-spinner, with the single exception of C. plumosus. However, Konstantinov (1958, pg. 35) wrote that Walshe was in error concerning C. plumosus, and that no member of the genus Chironomus is known to construct a net.

Briefly, the feeding mechanism of C. thummi may be described as collecting-scraping. For a more detailed description of the feeding mechanism, see Sadler (1935),

whose observations on C. tentans agree in full with this author's view of feeding in C. thummi.

Importance of Algae in the Diet of Chironomus

It seems that every investigator of the feeding habits of Chironomus has emphasized the presence of algae in ingested material (Pause 1918, Sadler 1935, Konstantinov 1958, Oliver 1971). Konstantinov (1958) examined the gut contents of three species of Chironomus collected from a fish culture pond during the summer. The average proportions by weight were: sand and detritus, 31-66%; Chlorophyta[3], 5-15%; and diatoms, 7-12%. Of the diatoms, Navicula, Synedra, and Pinnularia were most frequently encountered.

Armitage (1968) found that Pagastiella filled its gut completely with diatoms in spring and autumn, while Cavanaugh and Tilden (1930) observed Tanytarsus feeding exclusively on algae in a glass aquarium. (These genera belond to the Chironominae.) In the latter study, diatoms predominted over other forms of algae in the gut.

Questions naturally arise as to the degree of selectivity exercised by larvae in their ingestion of algae. Can they sort out organic from inorganic material, and can they preferentially ingest certain algae from a

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[3]Green algae.

mixture of algae? Davies (1975) found that the gut contents of larvae always had a higher proportion of organic material than experimental substrate. In a similar study, McLachlan et al. (1978) found that the organic content of the gut equaled that of the substrate, implying that larvae are unable to select particles of higher nutritive value. They suggested that the selectivity seen by Davies was actually due to a difference in the size of organic and inorganic particles, the former being smaller and hence easier to ingest. Moore (1977a) has cautioned that selectivity in the feeding of benthic herbivores may only be apparent, because microhabitat selection by algae makes certain algae more available; also gut retention times vary for different species of algae. Variable retention times may give a biased picture when gut contents are analyzed.

Diets Restricted to One Species of Alga

The relative nutritive value of algae vs. detritus is not ascertained with surety by gut analyses, and so Armitage (1968) has suggested rearing larvae solely on one or the other to resolve this problem. Even more obscure is the nutritive role of single species of alga. Brock (1960) observed a mutalistic symbiosis between the colonial

cyanophyte[4] Nostoc parmelioides and the miner Cricotopus in a California stream. The larva fed only on the gelatinous filaments; however, bacteria must have played an unknown nutritional role, since bacteria are intimately associated with gelatinous cyanophytes.

Algal monocultures have been used in many feeding studies on freshwater and marine invertebrates (Richman 1958, Arnold 1971, Edmondson and Winberg 1971, Monakov 1972; see Epifanio and Ewart 1977, for a marine organism). Typically, the research animal is a filter feeder such as Daphnia. Fredeen (1964) reared blackfly larvae to the imago on a bacterified culture of Chlamydomonas, but with poor results. Heavy bacterial cultures without algae worked many times better. Zack (1976) showed that two species of shore fly (Ephydridae) could complete their life cycles on monocultures of Chlorophyta, Cyanophyta, Chrysophyta[5], or Euglenophyta. One chrysophyte species even permitted 100% survival to emergence. Recently, McCullough et al. (1979) studied the energy budget of a mayfly nymph, using diatoms and cyanophytes in monoculture.

Laboratory investigations on the ability of

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[4]Blue-green alga.

[5]A taxon of algae which includes the diatoms.

Chironomidae to utilize algal monocultures have been conducted exclusively in the U.S.S.R. (Sorokin and Meshkov 1959, Rodova and Sorokin 1965, Izvekova and Sorokin 1969, Izvekova 1971, Monokov 1972). By marking algae with C^{14}, it was possible to ascertain the rate of assimilation of algae into larval tissue. The general picture presented by these studies is a high nutritive value for diatoms and cyanophytes, but a poor nutritive value for chlorophytes. Interestingly, the chlorophytes gave better results if killed or decayed. However, the data do not permit a calculation of assimilation or growth efficiencies; nor was it indicated whether larvae could survive to pupation or emergence when reared on monocultures.

An animal uses food for growth and to provide metabolic energy. If one views the food ingested, animal biomass, and excreted material in caloric terms, then the animal's energy budget is:

Ingestion = Growth + Respiration + Egestion

(The quantity "growth" can be refined to consider reproduction, secretion, or exuviae.) For any particular food:

$$\text{Growth efficiency} = \frac{\text{growth}}{\text{ingestion}} \times 100$$

$$\text{Assimilation efficiency} = \frac{\text{growth + respiration}}{\text{ingestion}} \times 100.$$

Because the energy used for respiration is technically difficult to determine, many authors simply express the energy budget in terms of mass, i.e. dry weight. While the formula for growth efficiency remains as above, assimilation efficiency is now the percent of ingested material which is digested. The latter can be determined by using C^{14}-labeled food (Sorokin and Meshkov 1959, Monakov 1972). Efficiencies calculated in terms of mass are generally lower than those obtained from caloric determinations on account of the ash content of the food.

In a review of the literature on aquatic consumers, Welch (1968) found that assimilation efficiencies ranged from 20 to 90%, and growth efficiencies from 15 to 35%. The efficiencies presented by Monakov (1972) for aquatic invertebrates and by Waldbauer (1968) for terrestrial insects also generally fall within the same bounds.[6] Some of the available data for aquatic insects are given in Table 1.

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[6]The literature sometimes refers to growth efficiency as "gross growth efficiency" or "eff. of conversion of ingested food."

Table 1. Efficiencies of assimilation and growth of a few aquatic insects. (Expressed as percent.)

Insect	A.E.	G.E.	Units	Food
Tricorythodes[a]	57		calorie	*Nitzschia* (diatom)
	35		"	*Anabaena* (cyanophyte)
	27	16	"	mixed diatoms
Stenonema[a]	45	14	"	"
Lestes[b]	37	23	"	*Daphnia*, tubificids
Chironomus[c]	≈66	≈33	dry wt	detritus

[a]Ephemeroptera. McCullough et al. 1979.

[b]Odonata. Welch 1968.

[c]Izvekova 1971.

Role of Bacteria in Nutrition

Most investigators assume that the nutritional contribution of bacteria in algal cultures is negligible. However, it is known that freshwater oligochaetes live off the bacterial film associated with detritus (Moore 1978), while *Chironomus* has been reared to the imago on bacteria alone (Rodina 1949). The rate at which chironomids assimilate bacteria compares well with rates for algae (Rodova and Sorokin 1965) and *C. thummi* digests at least half the bacteria it ingests (Baker and Bradnam 1976). Thus feeding experiments which use non-axenic cultures of algae ought to take into account the nutritional role of bacteria.

In summary, it can still be said that "very little is known about the digestive capabilities or efficiencies of aquatic insects" (Cummins 1973). Where data exist, they should be viewed with caution unless attempts have been made to rear the insect in the long term on the food being tested. Thus, in several of the studies cited above, cyanophytes proved highly assimilable in tracer experiments of a few hours duration. Yet when Arnold (1971) compiled life tables for Daphnia raised on Cyanophyta, the rates of natural increase were in some instances zero, even though the algae were readily assimilated (> 70%) in the short term.

C H A P T E R I I I

GROWTH AND DEVELOPMENT OF *CHIRONOMUS THUMMI* (KIEFFER)[7,8] ON VARIOUS ALGAL DIETS

Aquatic larvae of non-biting midges are often an important component of the diet of bottom-feeding lake fish (Gerking 1962, Charles et al. 1974). Hence several pisciculturists and entomologists have advocated rearing chironomids as a dietary supplement for cultured fish (Sadler 1935, Konstantinov 1958, Yashouv 1970, McLarney et al. 1974). In this connection the genus *Chironomus* seems especially suitable because it feeds on plant matter and detritus (Coffman 1978), can live at high densities (Jónasson 1972), tolerates water with considerable organic content (Mundie 1957), and the larvae tend to be larger than those in other genera. Moreover, the high natural productivity recorded for some Chironomidae (Waters 1977) indicates the potential of controlled mass-rearing.

In addition to their value as fish food, the Chironomidae are abundant primary consumers in eutrophic

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[7]Diptera: Chironomidae.

[8]This research was supported by Hatch Project 320.

lakes, so it would be instructive to know more about the feeding preferences of their larvae. Investigators have noted the presence of algae in the guts of collected specimens, sometimes stressing the importance of diatoms (Cavanaugh and Tilden 1930, Armitage 1968, Jónasson 1972). Since ingested material usually contains detritus and a diverse mixture of algae, gut analyses do not reveal the relative nutritive value of each food component. Armitage (1968) has suggested feeding larvae solely detritus or algae to resolve this problem.

In the present study, Chironomus thummi (Kieffer), an inhabitant of European lakes (Pause 1917, Keyl and Strenzke 1956), was reared on monocultures of three common freshwater algae. The object was to determine whether a single species of alga could support the development of the insect from eclosion to imago. (Rearing multiple generations of insects on algae was beyond the scope of this research.) A secondary objective was to estimate the efficiency with which a nutritionally suitable alga is converted to larval biomass.

Materials and Methods

Midge colony. A constant supply of 1st instars for experimental purposes was provided by maintaining a laboratory colony of C. thummi[9] at 22.0±0.5°C, 80-90% R.H. and a 15:9 h L:D photoperiod. Larvae were grown in enamel pans (base 400-600 cm^2) filled with 20-mesh sand to a depth of 1 cm and then 1 cm of distilled water. Each pan received 100-200 ml air per min from an aquarium air pump by means of a tube fixed to a corner of the pan. Dog Kisses® (Biever 1965) and Tetra Conditioning Food® (aquarium food made by Tetra Werke) were equally suitable diets in terms of adult body size and egg production. The Dog Kisses were sifted through 40-mesh screen, while Tetra was simply ground with a mortar and pestle; food was then shaken into a water suspension and added to rearing pans at the rate 0.5-1.0 mg/larva/day, irrespective of larval age. Changing old water for fresh was found to be unneccessary. It was best not to exceed a stocking rate of one egg per 3 cm^2; because at higher densities the adults were small, reluctant to swarm, and produced few egg masses. Each day adults emerging from several pans were transferred by aspirator to a screen

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[9]Eggs were obtained from a longstanding colony through the courtesy of Hans Laufer, Department of Biology, University of Connecticut, Storrs 06268.

and plexiglass cage (40 x 30 cm base, 25 cm height) for mating and oviposition. Swarming was initiated by turning on, about 5 min after the end of the photophase, a 75 W light bulb placed 1.5 m to the side of the cage. A 30 x 20 cm enamel pan, filled with water and a thin layer of sand, functioned as an oviposition site and swarm marker. The cylindrical egg masses (averaging 500 eggs/mass) could be stored at 3°C up to 10 days, but eggs for experiments were never stored more than 2 days.

Although adults did not require a carbohydrate source, this author found that both sexes fed readily on dry table sugar, as confirmed by the anthrone test (Van Handel 1972). Konstantinov (1958) held the widely accepted view that adult chironomids do not feed, but Oliver (1971) noted a few exceptions.

Algae cultures. The siliceous diatoms Synedra sp. and Navicula pelliculosa, and the green alga Scenedesmus quadricauda were cultured as described by Guillard (1975). (Navicula was obtained from the Culture Collection of Algae, Dept. of Botany, Univ. of Texas, Austin, cat. no. 668; Synedra and Scenedesmus came from the Carolina Biological Supply Co., cat. nos. 15-3095 and 15-2510.) Algae were harvested in the early stationary phase by centrifugation at 4000 g for 5 min. The bacterial density in algae

cultures was monitored by counting the colonies on triplicated pour plates after 3 days incubation at 37°C. Bacterial growth medium for the latter consisted of 1% Trypticase Soy Broth (BBL) and 1% agar dissolved in Guillard's WC (Guillard and Lorenzen 1972) algal growth medium.

Feeding experiments. Larvae for survival and developmental studies were reared in lidded hard plastic freezer crispers (base 70-80 cm^2), henceforth called "units." Each unit, containing approximately 100 ml of distilled water and algae, was immersed in a 24±1°C water bath and subject to a 15:9 h L:D photoperiod. Aeration was provided to the water in each unit through thin Tygon tubing at the rate 100 ml air/min. Units were initiated with newly eclosed larvae from 3 or more pooled egg masses. Only actively swimming larvae were selected, and these were pipetted individually onto a glass slide and then shaken from the slide into the rearing unit.

A measured quantity of concentrated algae or Tetra was added to each unit at half-weekly intervals. To prevent fouling, the water was changed with every second feeding. Algal growth in the units was assumed to be negligible, due to the dilution of inorganic salts and the use of dim lighting. For Synedra, at least, this

this assumption was checked as follows. Vials of 2-ml capacity were filled with fresh Synedra, capped securely with dialysis membrane, and laid in units containing Synedra and growing larvae. Thus, larval metabolites and other molecules of less than 12,000 mol. wt. were free to diffuse into the vials, but algae in the vials were not exposed to predation. Hemacytometer counts of the "viable" cells (i.e., containing the normal amount of golden-brown pigment) in triplicate vials after 0, 2, 3, and 4 days immersion revealed a gradual decline in numbers. Thus, 74% were still viable after 3 days.

Growth efficiency, defined here as the efficiency of conversion of available food to larval biomass, was ascertained as follows. Larvae reared on Tetra were placed singly in upright vials (17 mm ID) together with a known quantity of test food. The water column in each vial was kept less than 1 cm high to facilitate gas exchange. To obtain a range of initial weights, animals were taken from several cohorts, the fastest growing individuals being chosen from each cohort. A Mettler balance was used to obtain the initial weight and the weight after 3 days feeding for each animal to the nearest 0.01 mg. This delicate operation was performed by pipetting a larva onto absorbent filter paper and then quickly transferring the dry larva onto a preweighed square of Glad Wrap®.

After the square was folded on itself so as to enclose the larva, the whole was weighed. This technique avoided dessication while the animal was being weighed and appeared to cause no injury. By means of duplicate weighings on 10 individuals, the standard deviation of the error was estimated to be 0.04 mg for live weights, or 0.008 mg for the quantity "dry weight gain." Larvae were weighed with their digestive tracts full since no weight loss was statistically detectable when larvae were reweighed after a few hours starvation. Environmental parameters were as for the midge colony, except that the vials were shaded from direct light to avoid exciting the animals.

Biological constants. Various conversion factors were needed to compute the dry weight, nitrogen, and phosphorus content of biological material. The percent dry wt. of larvae was determined for two weight classes, 1-2 and 4-7 mg wet wt. Animals were weighed in groups of 4 and 2, respectively, then oven-dried for 24 h at 80°C, and reweighed. Since the t-test ($P \leq 0.05$) revealed no significant difference between light and heavy larvae (13.3±1.0 vs. 13.4±0.5 S.D.), the figure 13.4% dry wt. was used for all larvae.

The dry wt. per cell for Scenedesmus and Synedra was obtained by regression of dry wt. on cell number. Each regression contained 5 observations from 3 different

stationary phase batch cultures. Cells were counted by hemacytometer, collected and washed on preweighed filters (Whatman GF/A glass microfibre), and then dried as for midge larvae. This gave 95% confidence intervals of $7.0 \pm 1.4 \times 10^{-10}$ g/*Scenedesmus* cell and $3.7 \pm 0.3 \times 10^{-9}$ g/*Synedra* cell. To quantify algae used in feeding experiments the above constants were simply multiplied by hemacytometer counts. Clumping made *Navicula* difficult to count, so the dry wt. of aliquot samples was found directly.

Total N and P analyses of algae and larvae were done at the Microanalytical Laboratory of the University of Massachusetts.

Results

Development and survival. At 22°C the embryonic stage lasted 3 days. Development of colony larvae was asynchronous (Fig. 1), taking 15-27 days from eclosion to mature 4th instar. Larvae attained a maximum wet wt. of 10 mg.

Units were initiated with 25 larvae and kept at approximately constant feeding levels for up to 4 weeks (Table 2). Within one day, 1st instars had constructed cases of algae on the bottom of the unit, irrespective of the type of algae being tested. It was possible to see the larvae through their thin cases during the first week of growth--a factor which facilitated making a census of

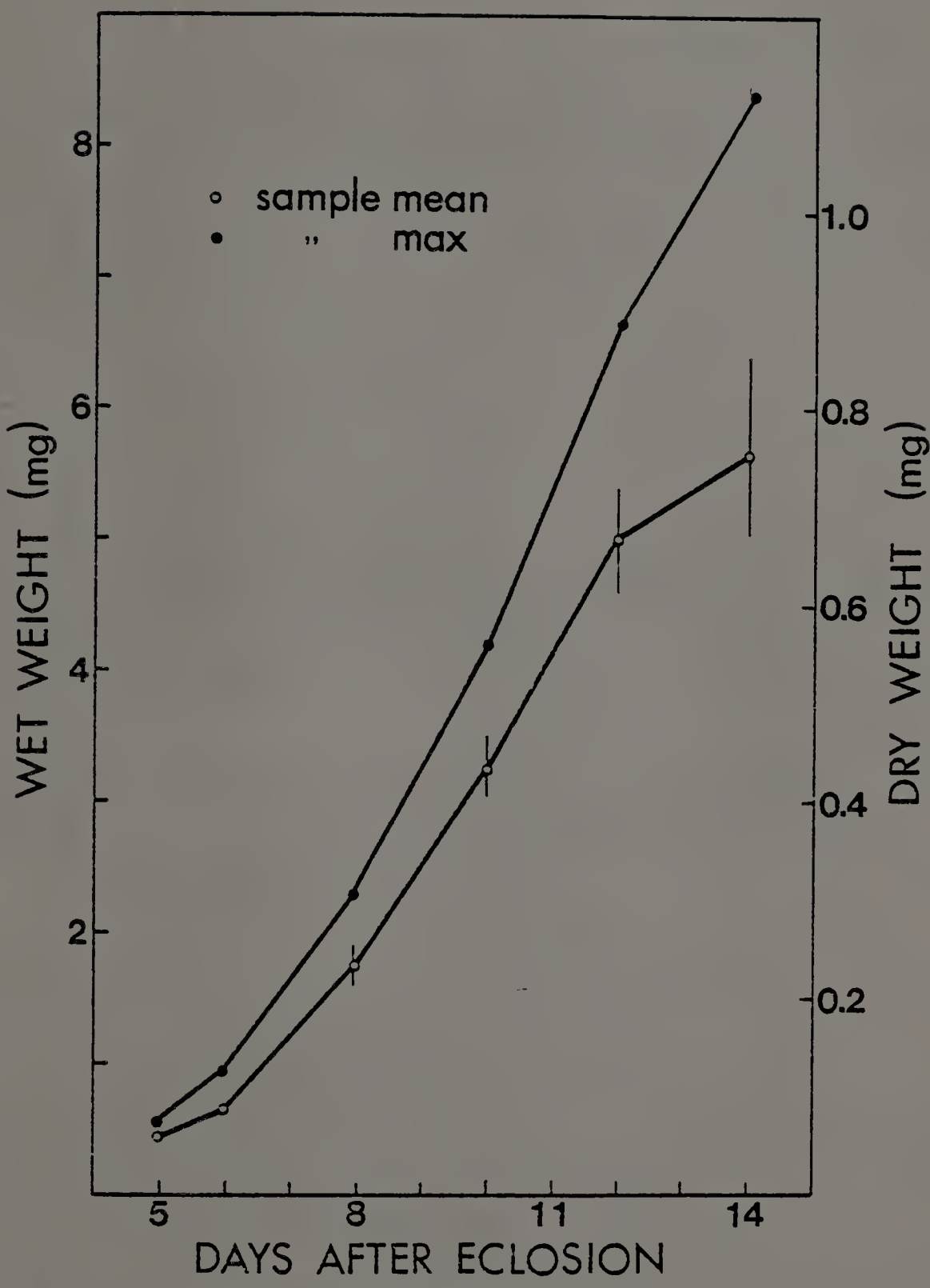

Fig. 1. Growth of C. thummi larvae reared on Tetra at 22°C. Points on lower curve are log transformed means ± S.D. for samples of at least 40 individuals. Upper curve shows maximum weight in each day's sample. Growth was exponential from days 5 to 10, and linear regression on log weights gave: WET WT = 0.062exp(0.404 DAYS), r=0.99.

survivors. As can be seen from Table 2, the diatom Synedra was roughly comparable to Tetra in terms of one-week survival. Scenedesmus larvae showed less than half the survival of Synedra larvae, while survival on the diatom Navicula was practically nil.

Not all of the mortality in the rearing units could be attributed to the quality of the food, and the survival figures for Tetra-fed larvae would have been higher if the units had been initiated with eggs rather than 1st instars. If an egg mass is allowed to hatch in an enamel pan without sand, then one-week survival on Tetra approaches 100%. It seems that some important feeding takes place before eclosed larvae have wriggled free of their gelatinous egg mass. Particles of food readily adhere to the egg mass, and larvae can be observed to feed on these particles. This knowledge was used in an attempt to improve survival on Navicula (see 40 mg level, Table 2). About 100 eggs were allowed to hatch in 75 mg of Navicula; at 5 days, survival was well over 50%. Then 25 of these larvae were counted into a unit containing 40 mg Navicula, while a control group of 25 were put into a unit with the same quantity of Tetra. Two days later (i.e., at 1 week) all control group larvae were still alive and had molted to the 2nd instar. Only 7 of the Navicula group

Table 2. Survival and development of C. thummi larvae reared on algae or Tetra.

Food	Feeding rate (mg/unit/wk)	No. units	One-week Survival (%)	Comments
Synedra	25	2	48	Lowest feeding level continued 4 weeks: 22% emergence, 1st adult at 13 days.
	50	4	70	
	100	2	42	
Navicula	25	2	4	All died within 2 weeks as 1st or 2nd instars.
	85	1	0	
	40	1	28[a]	
Scenedesmus	37	2	18	One pupa obtained on killed algae but failed to emerge.
	33[b]	2	22	
Tetra	25	4	62	Greatest emergence (38%) at 100 mg level. 1st adult at 12 days.
	50	4	60	
	100	4	60	

[a]Represents survival from 5th to 7th day. (See text for details.)

[b]Algae killed by heating 5 minutes in boiling water bath.

survived and these were still 1st instars. Although one 2nd instar was subsequently obtained, all larvae fed solely on this diatom died within 2 weeks.

Of the three algae, only Synedra supported development to the imago. Adults from the lowest Synedra feeding level were approximately the same size as those reared on Tetra in enamel pans (Table 3). Although the wing lengths were 7% smaller for Synedra adults (significant by rank sum test, $P \leq 0.05$), the abdominal lengths did not differ significantly.

Table 3. Size comparison of 1 day old C. thummi adults reared on Synedra or Tetra at 24°C.

	Mean length±S.D. (mm)			
	Males		Females	
Food	Wing[a]	Abdomen[b]	Wing	Abdomen
Synedra[c]	2.70±0.09	3.43±0.14	2.74±0.22	2.86±0.14
Tetra[d]	2.89±0.08	3.32±0.16	2.93±0.09	2.89±0.20
Ratio Synedra:Tetra	0.93 : 1	1.03 : 1	0.94 : 1	0.99 : 1

[a]From wing tip to junction of alula and anal lobe.

[b]From posterior edge of 1st segment to posterior edge of 7th segment (males) or 8th segment (females).

[c]Means of 7 observations.

[d]Means of 25 observations.

The green alga Scenedesmus supported development to the pupal stage, but mortality was high. Scenedesmus units were not replicated at higher feeding levels, because even at the moderate rates shown in Table 2, the water was clouded to a thick green. (Unlike diatoms, this alga stays in suspension.) Using radiolabeled Scenedesmus, Rodova and Sorokin (1965; see also Monakov 1972) obtained an assimilation rate of 2.6% body weight per day for the midge Cricotopus sylvestris. When these workers killed the algae by boiling, the figure jumped to 22.1% per day--quite a high rate in view of the 50% growth rate of C. thummi during the exponential phase of growth (Fig. 1). Accordingly, this author tried heat-killed Scenedesmus, but with no better results than for live algae. Dead cells sink to the bottom of the rearing dish, making them more available as food, perhaps explaining the higher assimilation rate seen by Rodova and Sorokin. Their experiment lasted only 6 hours and so it is possible that this high rate would not have continued in the long term.

Gut analyses. For each type of alga, two vigorous 4th instars were placed in a beaker filled with 1 cm of algal monoculture and allowed to feed for 2 days. Then the intestinal tracts, which were completely packed with algae, were pulled out, and the hind contents of each midgut were squeezed into separate drops of growth

medium on microscope slides. The proportion of viable cells in the hind midgut was estimated by counting enough microscope fields to sample ≈ 100 cells. It can be seen from Table 4 that only Synedra appeared to be digested.

Bacteria. Between 10^8 and 10^9 live bacteria were introduced into Synedra units with each new batch of algae, according to plate counts. This may have amounted to 0.1 mg (assuming 1 bacterium = 10^{-13}g), far less than the quantity of algae present in the unit. Nevertheless, there was cause for concern because of the unknown amount of dead or unassayable bacteria. Chironomus has been

Table 4. Ability of C. thummi larvae to digest algae as determined by percentage of pigmented cells in rear midgut.[a]

	% pigmented cells	
Alga	Larval environment	Rear midgut
Synedra	≥95	≤35
Navicula	≥95	≥90
Scenedesmus	≥90	≥90

[a]Two larvae dissected for each alga and about 100 cells sampled from each midgut.

reared to the imago on bacteria alone (Rodina 1949). To

assess the contribution of bacterial biomass to larval growth and development, two units were fed a Synedra harvest with the diatoms removed. This was accomplished by centrifuging 25 mg of diatoms for 2 min in a clinical centrifuge, a procedure which precipitated all intact diatoms while leaving half the total viable counts of bacteria in the supernatant. Two other units were fed in the normal manner from the same harvest of diatoms. Survival on the supernatant was better than expected (Table 5), but growth was miniscule. It is likely that the larvae were feeding on broken diatom debris, which appeared as a diffuse band above the band of intact cells after centrifugation. This band of debris was created by the vigorous aeration of the diatom cultures, and it could be reproduced by grinding intact Synedra cells in a tissue macerator--an insignificant percentage of broken cells would produce a broad band. Since the length of a Synedra cell equaled the width of the 1st instar head capsule (100μm), it seems likely that 1st instars fed on cell debris rather than on whole cells.

Table 5. Bacteria and diatom debris as food for C. thummi.[a]

Food/unit/week	No. larvae	One-week survival (%)	10-day larval biomass mg/unit	10-day larval biomass no./unit
Supernatant of 50 mg Synedra	50	36	0.16	7.5
25 mg Synedra	25	56	1.45	6
50 mg Synedra	25	68	3.88	14

[a]Treatments in first two rows estimated to contain same quantity of bacteria as determined by plate counts.

Growth efficiency. As Synedra appeared to be a suitable food for C. thummi, more precise tests were performed to determine growth efficiency on this diatom. It was not possible to estimate by hemacytometer counts the number of cells consumed, since larvae incorporated the cells into their cases. Hence efficiency was calculated on the basis of food available:

$$E = \frac{\text{dry wt. gain}}{\text{dry wt. of food available}} \times 100.$$

Larvae ranging from late 3rd to late 4th instar were given either 1 or 2 (±10%) mg Synedra and allowed to feed for 3 days. (A few of the older larvae pupated and were discarded.) This feeding period was chosen because it was known to

be sufficient time for the smaller larvae to use up 2 mg of Tetra, assuming the growth efficiency did not exceed 20% (see upper curve of Fig. 2). When dry weight gains were plotted against initial larval weight, it was seen that E did in fact approximate 20% for the smaller larvae at the 1 mg feeding level (Fig. 2). There was a significant negative correlation between weight gain and initial weight at both feeding levels (Kendall's coefficient of rank correlation, $P \leq 0.05$). At the 2 mg level, more diatoms were diverted into case building, so E was lower.

The same tests were performed with Tetra as food. It can be seen from Table 6 that the mean E was 15-16% for both Synedra and Tetra at the 1 mg feeding level. However, animals fared poorly on 2 mg Tetra due to fouling.

Table 6. Growth efficiency E of larvae given 1 or 2 mg food.

Food	Initial dry wt (mg)	1 mg food No.	1 mg food E (%)	2 mg food No.	2 mg food E (%)
Synedra	0.15 - 0.40	30	18.5	18	14.7
	0.40 - 0.90	27	11.8	21	11.4
	Total	57	15.3±1.3[a]	39	12.9±1.0[a]
Tetra	0.20 - 0.40	20	16.5	21	6.4
	0.40 - 0.70	18	14.9	14	4.7
	Total	38	15.8±1.6[a]	35	5.7±1.1[a]

[a]95% confidence interval for mean. The log initial weight was uniformly distributed over the interval sampled.

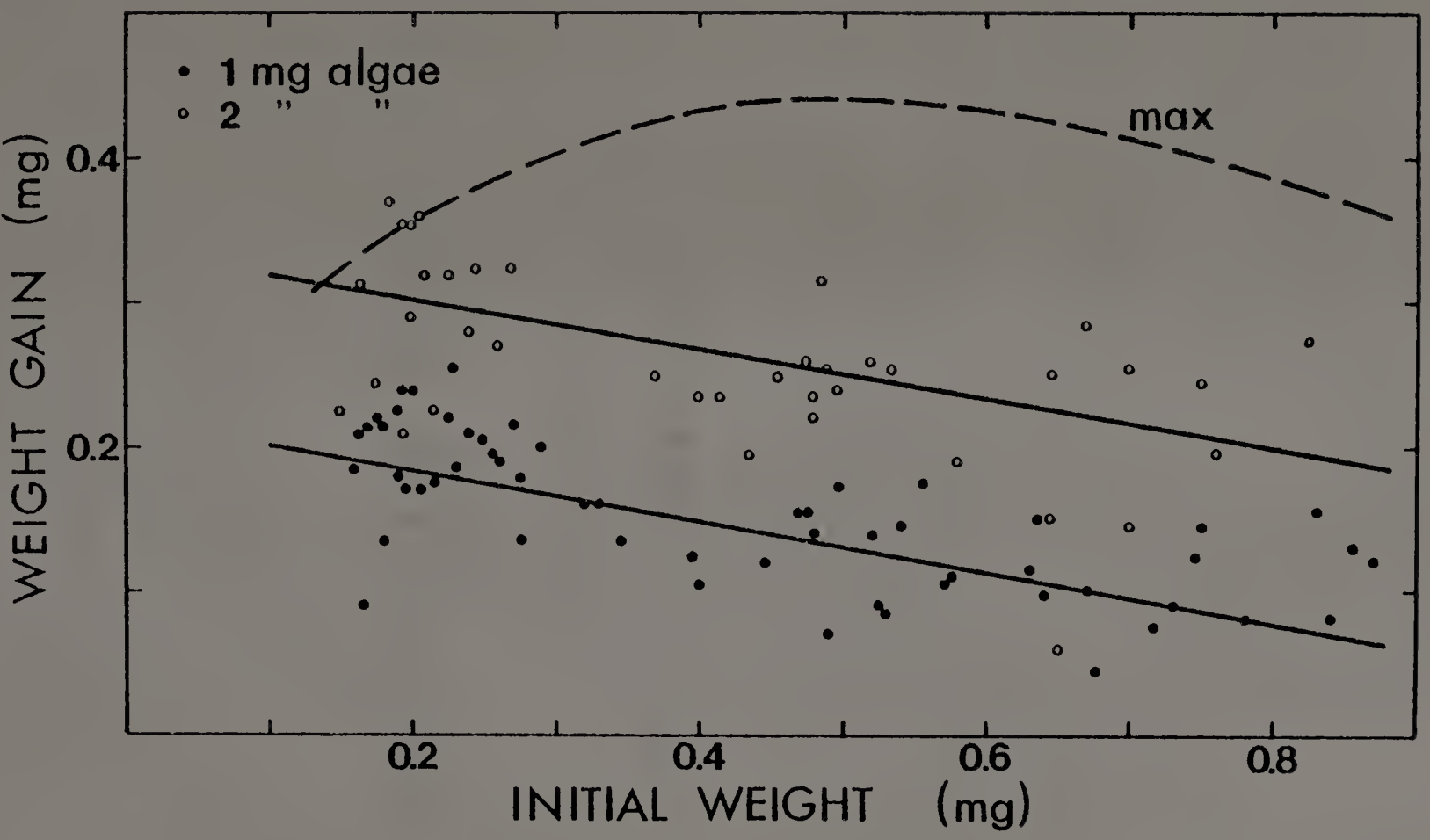

Fig. 2. Dry weight gain of C. thummi larvae on 1 or 2 mg Synedra. The least squares approximation to each set of points (straight lines) indicates a negative correlation between gain and initial weight, which was significant by Kendall's coefficient of rank correlation ($P \leq 0.05$). The broken line is the theoretical upper limit to weight gain over 3 days, calculated from the upper curve of Fig. 1.

Insects typically show an efficiency of conversion of ingested food (E.C.I.) in the range 15-35% (Waldbauer 1968). Among the few E.C.I. values available for aquatic insects are 14% and 16% for the mayfly nymphs Stenonema and Tricorythodes feeding on diatoms (McCullough et al. 1979). These figures were calculated in terms of the caloric content of the food and the insects, and so they might have been lower if calculated in terms of mass, due to the ash content of the diatoms. (Synedra had an ash content of 43%, which is within the normal range for Bacillariophyceae, i.e., 20-50%. See Nalewajko 1966.) Furthermore, this author's E would tend to underestimate the true E.C.I. The values given for the mayfly nymphs still agree well with results obtained for C. thummi.

Nitrogen and phosphorous. The role of the elements N and P in aquatic food chains has interested limnologists because either one can be a limiting factor in primary production. In situations where they are the major benthic macrofauna, chironomids must be important secondary consumers of N and P. The efficiency of conversion of N or P was calculated according to Waldbauer's formula:

$$E.C.I.(X) = \frac{\text{amount of X in body}}{\text{amount of X in food ingested}} \times 100.$$

If it is assumed that 1 mg of Synedra is converted to 0.15 mg of body weight, then it can be seen that N in particular is sequestered very efficiently (Table 7). Under natural conditions the E.C.I. for N and P must vary, depending on the type of food; moreover, the N content of larvae fluctuates seasonally between 8 and 11% of dry weight (Jónasson 1972).

Table 7. Larval utilization of nitrogen and phosphorous.

	N (%)	P (%)
Synedra, dry wt	2.25	0.27
Larvae, dry wt[a]	10.01	0.68
E.C.I.	67	38

[a]Pooled 3rd and 4th instars reared on Synedra.

Discussion

The results indicate that some diatoms (Bacillariophyceae) are suitable for chironomid larvae while others are not. McLachlan et al (1978) found that among several species of field-collected chironomid larvae, the smaller individuals ingested proportionally more small particles, than did large individuals. If the size of an alga were an indication of its suitability for 1st instars, then one would expect Navicula (8 x 5 µm) to be better nutritionally than Synedra (100 x 10µm). The reverse proved

to be true; and as noted above, 1st instars were probably able to survive on Synedra due to the presence of broken cells. It is interesting that Zack and Foote (1978) found N. pelliculosa to be a suitable food for larval shore flies (Ephydridae), while two other diatoms were unsuitable.

The possiblity lingers that chironomids could complete their life cycle on Scenedesmus, but the results for C. thummi at least are not encouraging. Fecal pellets of larvae fed live Scenedesmus were masses of apparently normal cells. It is noteworthy that other aquatic primary consumers such as larval black flies (Moore 1977b) and oligochaetes (Moore 1978) also are unable to digest Scenedesmus and other Chlorophyta. Perhaps the digestive enzymes of Chironomus cannot penetrate this alga's cell wall, which contains cellulose. In nature, decomposed and decaying Scenedesmus might nevertheless add a nutritious component to lake sediment.

The fact that larvae were able to utilize two-thirds of the nitrogen present in Synedra indicates that chironomids are an efficient link in the aquatic food chain from algae to fish. Nitrogen utilization in terrestrial insects tends to be lower, around 40% (Waldbauer 1968, Schroeder 1976). Figures for phosphorus utilization in other insects

were not available. However, it is noteworthy that the P component of the diatom was utilized about twice as efficiently as the diatom as a whole.

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APPENDIX A

Notes on Algae Cultures

Algae cultures were maintained as 1-l volumes in 2-l Erlenmeyer flasks or as 0.5-l volumes in 1-l Erlenmeyer flasks, and aerated. However, aeration could have been omitted if cultures were kept shallow, e.g., 0.5 l in a 2-l flask.

Soil extract was added to growth medium for algae stock cultures at the rate 10 ml per liter. Soil extract was not used for large batch cultures, and for Synedra at least, soil extract made no difference in the yield.

The silicate concentration of WC medium was found to be limiting for Synedra. By tripling the silicate concentration, the yield could be tripled (to 2×10^7 cells/liter).

Harvesting of Synedra or Navicula can be simplified by letting the culture to be harvested sit for 24 h in a graduated cylinder at 5°C or less. The diatoms settle to the bottom and the supernatant can be siphoned off.

APPENDIX B

Determination of Algae Dry Weights

A linear regression of dry weight on cell number was calculated for each of the algae Synedra and Scenedesmus. The average dry wt. of a single cell was given by the slope of the regression line. In Figs. 3 and 4 solid circles, open circles, and triangles represent observations from three different stationary phase batch cultures.

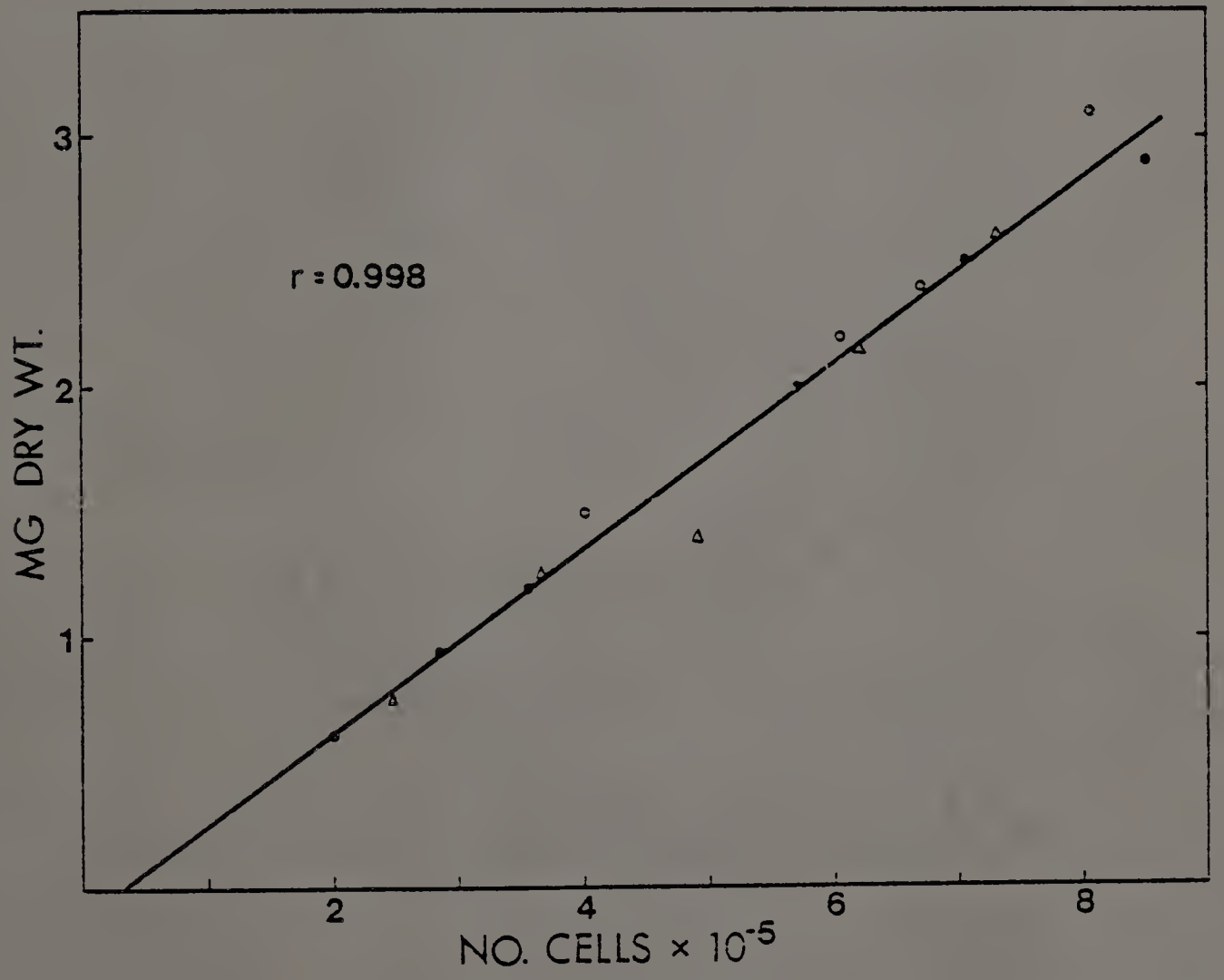

Fig. 3. Linear regression of dry wt. on cell number for Synedra sp. Slope = 3.7 ± 0.3 x 10^{-9} g/cell, $P \leq 0.05$.

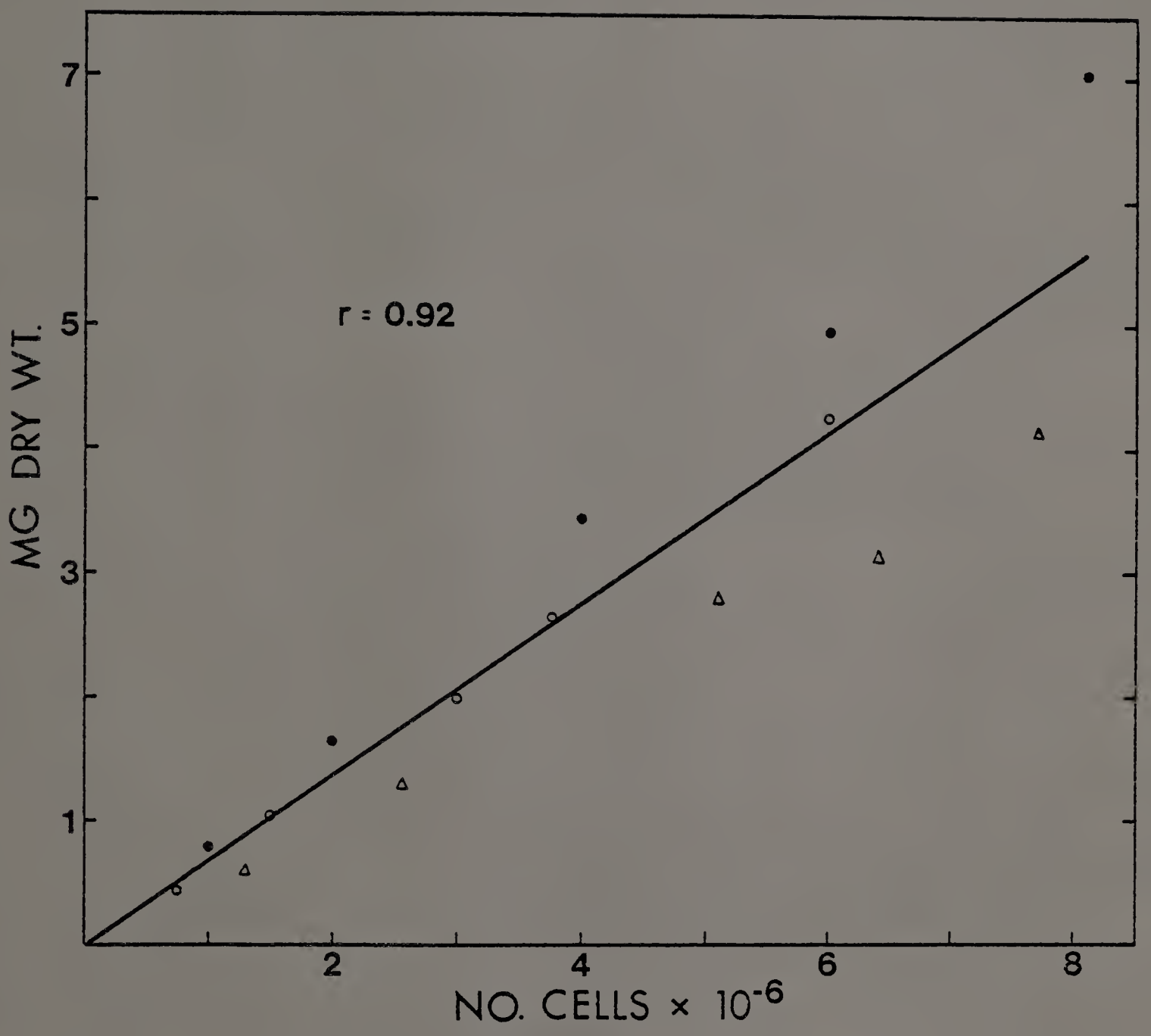

Fig. 4. Linear regression of dry wt. on cell no. for Scenedesmus quadricauda. Slope = 7.0 ± 1.4 x 10^{-10} g/cell, $P \leq 0.05$. (In reality, this alga consists of colonies of four cells, but for simplicity each colony has been called a "cell.")

APPENDIX C

Larval Head Width and Weight by Instar

Table 8. Head capsule width and live weight of C. thummi larvae by instar. Larvae reared on Tetra at 22.0 ± 0.5°C.

Instar	Width	SD (mm)	n	Weight range (mg) min.	max.
1	0.105	± 0.003	9		
2	0.185	± 0.008	11		
3	0.323	± 0.007	7	0.23	1.56
4	0.573	± 0.021	19	1.16	10.12

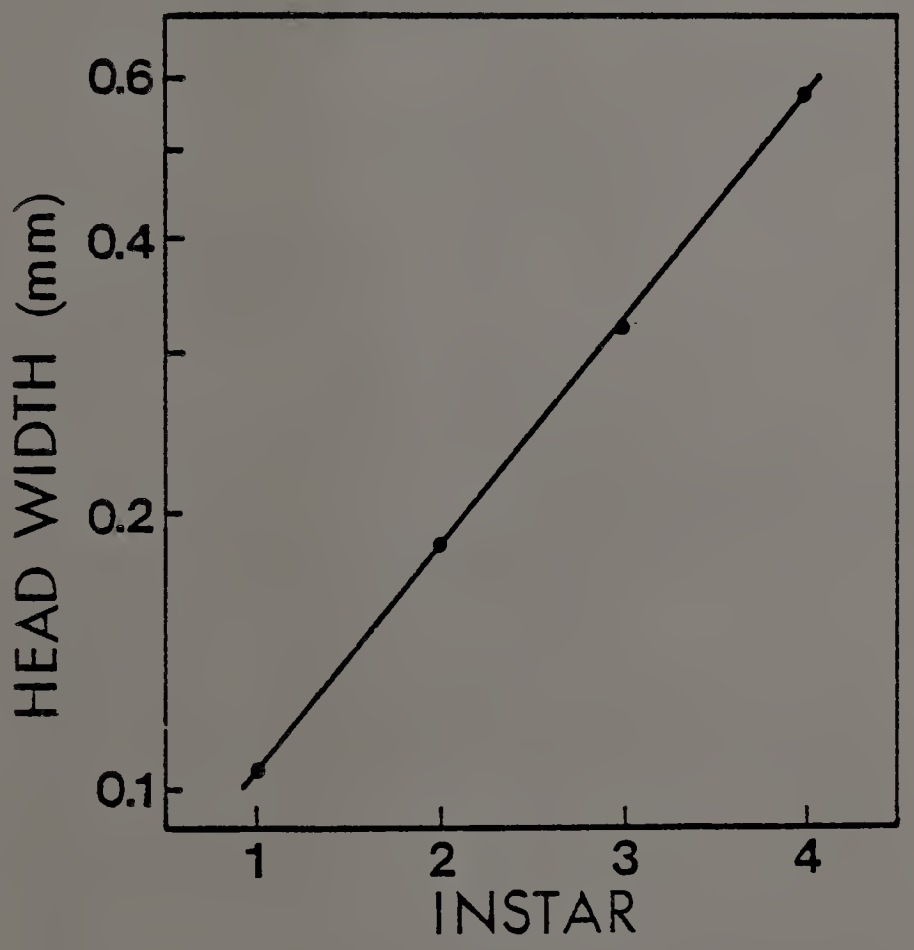

Fig. 5. Semilogarithmic plot of head capsule width vs. instar for C. thummi larvae. See Table 8.

APPENDIX D

Statistical Analyses of Weight Gains

A one-way analysis of covariance (Table 9) showed a highly significant difference between 1 and 2 mg *Synedra* fed larvae with respect to mean weight gain. A second analysis (Table 10) showed no significant difference ($P \leq 0.05$) between the mean weight gains of larvae fed 1 mg Tetra or 1 mg *Synedra*.

Table 9. ANCOVA for 1 mg *Synedra* vs. 2 mg *Synedra*.

Group	N	Group Mean	Adj. Group Mean	Slope Within Group
1 mg	57	0.15300	0.15345	-0.1563
2 mg	39	0.25759	0.25693	-0.1689

Source of variance	DF	Sum Sq.	F Value	Tail Value
Equality of Adj. means	1	0.2479	128.2583	0.0000
Zero slope	1	0.1129	58.4000	0.0000
Error	93	0.1798		
Equality of slopes	1	0.0002	0.0819	0.7754
Error	92	0.1796		

Table 10. ANCOVA for 1 mg Tetra vs. 1 mg *Synedra*.

Group	N	Group Mean	Adj. Group Mean	Slope Within Group
Tetra	38	0.15770	0.15668	-0.0653
Syned.	57	0.15300	0.15367	-0.1563

Source of variance	DF	Sum Sq.	F Value	Tail Value
Equality of adj. means	1	0.0002	0.1122	0.7384
Zero slope	1	0.0664	36.1210	0.0000
Error	92	0.1691		
Equality of slopes	1	0.0048	2.6782	0.1052
Error	91	0.1643		