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INTRODUCTION:

Our work with midge (Chironomid) larvae in 1974 concentrated on two areas: further improvement of culture methods and tests of the effectiveness of the larvae as a growth-promoting supplement in fish diets.

For details of the technique used in our low labor midge culture system, as developed in 1973, see McLarney, Henderson and Sherman (1974) and McLarney (1974). A major change in the culture system in 1974 was the adoption of a two stage culture system utilizing nursery ponds and growth ponds. Burlap culture substrates were first laid horizontally in small pools for natural inoculation by wild adult midges. These ponds were fertilized with a mixture of Milorganite (R), soy meal, pond mud and fine sand which settled onto the burlap to provide an optimal substrate for larval attachment and growth. After a culture of early stage larvae had developed, the burlap substrates with attached larvae were transferred to deeper ponds where they were hung vertically until the larvae grew to optimum size for fish food. Using this two stage method it appears that some improvement was made over 1973 yield rates. However, due to circulation problems in the high volume

growth pools and significantly increased labor in the two stage system, we are currently doing further research with simpler methods before publishing details of an optimum cost and labor-effective midge larvae culture technique.

The feeding trials (McLarney, Levine and Sherman, in preparation) were very successful and will be reported here in some detail.

Our research has been predicated on the "hunch" of some aquaculturists that *Chironomid* larvae are not merely good fish food, but have unusual growth-promoting qualities, even when fed in very small quantities. This assumption was tested, on a pilot scale, by Yashouv (1956) and Yashouv and Ben Shachar (1967), but their samples were not large enough to provide definitive information. Our studies represent the first statistically meaningful test of the food value of midge larvae.

We tested our cultured midge larvae (Chironomus sp., a member of the C. tentans Fabricius group) on Tilapia aurea (Steindachner) and Israeli carp (Cyprinus carpio var. specularis Lacépède), the two major fish varieties cultured at New Alchemy East. Concurrent-

ly, Joseph Levine of the Boston University Marine Program tested our larvae as a food for juvenile American lobsters (Homarus americanus Milne-Edwards). T. aurea is generally considered to be highly herbivorous, but it has been shown that the young feed extensively on invertebrates (McBay, 1961). Israeli carp are omnivorous at all life stages, while lobsters are largely carnivorous.

FISH FEEDING TRIALS:

Methods: Both species of fish used in the experiments were housed in a series of twelve fifty-five gallon aquaria kept in a plastic greenhouse. The tanks were aerated, but filtration was not provided. Cleaning was effected by siphoning off twenty-five per cent of the water weekly and replacing it with fresh tap water; most fecal matter and other detritus was removed in this process.

Each group of fish received a standard diet composed of seventy-five per cent rolled oats and twenty-five per cent roasted soy meal. The standard diet was fed at the rate of two per cent of the total weight of fish, six days a week. As the tanks all soon developed dense green algae blooms, the fish were able to augment their diet by filter feeding. In four control tanks, the fish received no additional food. In a second group of four tanks, the fish received a supplement of midge larvae (Chironomous sp., a member of the tentans group) comprising two percent by (wet) weight of the grain diet. The final four tanks received midge larvae at the rate of ten per cent of the grain diet. Each group of fish was weighed three times at the start of the experiment, two weeks later, and four weeks later. All fish were fin-clipped so that individual, as well as group, growth rates could be determined. Data from the full four-week period of the tilapia trials and the first two-week period of the carp trials are presented here.

Test groups of fish were chosen to have approximately the same total weight of fish in each tank at the start of the experiment. In the first experiment

with *T. aurea*, six fish were stocked per tank and weights of individual fish varied from 0.7 to 18.0 g; group weights were 31.1 to 48.0 g. In the second *T. aurea* experiment, only five tilapia were stocked per tank, and these fish were chosen to be more nearly uniform in size than those in the first experiment. Individual weights ranged from 1.0 to 7.3 g; group weights from 16.1 to 23.5 g. The carp trials involved six fish per tank. Total weight of groups ranged from 58.3 to 72.3 g and weight of individuals from 2.3 to 21.2 g.

Water temperatures were 22 to 33°C during the first *T. aurea* experiment, 27 to 33°C during the *T. aurea* experiment and 20 to 32°C during the carp experiment.

Results: In the first T. aurea experiment, there was a slight increment in growth rate with the amount of midge larvae fed, but the difference was not significant and certainly would not justify any effort to provide midge larvae for young T. aurea. However, if the fish are broken down into two size groups, the differences in growth rate are more striking. Since it is well known that younger fish generally have a greater need for animal food, the data for all fish weighing less than 5 g at the start of the experiment were considered separately. Among these fish, those receiving a two per cent midge larvae supplement increased their weight considerably more than those receiving no midges. Those receiving a ten per cent midge larvae supplement grew faster than those receiving a two per cent supplement, but the difference was not as great as between the fish receiving a two per cent supplement and those receiving no larvae.

It was decided to repeat the experiment using more uniform sized, smaller fish. The results are similar to those obtained with the small fish in the first experiment. Results of all the *T. aurea* trials are shown in Table 1.

For purposes of statistical analysis, growth data from the smaller fish in the first trial were combined with those from the second trial. Each set of three aquaria (those receiving 0, 2% and 10% midge supple-

TABLE 1
Feeding trials with *Tilapia aurea*

	First Trial June 1-28			First Trial June 1-28*			Second Trial July 5 - August 2		
	No Midges	2% Midges	10% Midges	No Midges	2% Midges	10% Midges	No Midges	2% Midges	10% Midges
No. of Fish Final Weight	24	23	24	11	9	12	20	20	20
(grams)	236.1	241.8	235.1	53.1	43.6	69.2	144.8	149.5	151.1
Initial Weight	163.5	162.9	153.9	32.2	21.9	32.2	88.5	80.0	74.2
Gain in Four Weeks	72.6	78.9	81.2	20.9	21.7	37.0	56.3	69.5	76.9
Per Cent Gain	44.4	48.4	52.8	64.9	99.1	114.9	63.6	86.9	103.9

^{*}Fish weighing five grams or more at start of experiment excluded.

TABLE 2

Per cent weight increments of *Tilapia aurea* in eight sets of experimental aquaria and their rank within sets.

	Nọ Mi	dges	2% Mi	dges	10% Midges	
Set No.	% Gain	Rank	% Gain	Rank	% Gain	Rank
1	55.4	3	79.0	2	112.2	1
2	72.0	3	116.4	2	126.0	1
3	51.8	2	43.9	3	57.3	1
4	100.0	3	151.2	2	184.5	1
5	43.5	3	58.0	2	63.6	1
6	69.2	3	73.1	2	107.0	1
7	95.8	2	84.7	3	141.6	1
8	104.6	3	145.2	1	115.2	2
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Sum	of Ranks	22		17		9

ments) was considered separately and the total gain in weight of the fish in the three members of the set was ranked (Table 2). Applying the Kendall Coefficient of Concordance (Siegel, 1956) to the ranked data, s = 86, $x^2 = 10.752$ and the differences in the weight increments of the three experimental lots of fish are significant at the 1% level.

TABLE 3
Feeding trials with Israeli carp, August 12-30

	No Midges	2% Midges	10% Midges
No. of Fish	24	24	23
Final Weight	290.5	292.5	279.9
Initial Weight	260.8	252.5	241.4
Gain in Two Weeks	29.7	40.0	38.5
% Gain	11.4	15.8	15.9

Growth rate of Israeli carp in the experiment was markedly less than that of *T. aurea* (Table 3). This can probably be ascribed to the fact that the carp were very nervous and did not adapt to aquarium life as readily or as well as the tilapia. The mean weight increments for the three experimental lots of carp differed in the same manner as for the tilapia, but the difference was not significant.

The difference in growth rate between carp receiving midge larvae and the controls was greater after two weeks than at the conclusion of the experiment. The decline in growth during the latter half of the experiment may have been due to an infestation of anchor worm during that time. About half the fish were affected, and four individuals lost weight during this period.

LOBSTER TRIALS:

Methods: The lobster trials will not be described in as much detail as the fish trials, on the assumption that lobster culture is of less interest to our readers than culture of fish which are potential staple protein sources. However, the results further support the hypothesis that midge larvae are an excellent growth-promoting food. Full details of our procedures can be found in McLarney, Levine and Sherman (in preparation).

The test lobsters were juveniles, 6.0 to 6.5 mm in carapace length, and were fed a standard diet of commercially available frozen brine shrimp (Artemia) at the rate of 0.018 g dry weight/lobster/day. This constituted the entire diet of the controls; test animals received Chironomus larvae in amounts equivalent to two per cent and ten per cent by dry weight of the brine shrimp diet. Experimental feeding was continued until the animals had molted twice, and the growth increment was calculated by comparing intermolt period length, carapace length and total weight measured immediately after each molt.

Results: Lobster results are summarized in Table 4. As expected from previous work, there was no significant difference in the lengths of the intermolt periods due to large sample variance.

Increase in carapace length was noticeably higher in both experimental groups than in the control. The mean increase shows 0.5 mm increments between the experimental groups (Table 4). Weight gain and percentage weight gain, on the other hand, indicate significant differences between both groups given midges and the controls, but not between the midgefed groups themselves.

DISCUSSION:

The results of these experiments argue for the feasibility of culturing midge larvae, using the hanging substrate method (McLarney, Henderson and Sherman, 1974; McLarney and Sherman, in preparation), as a dietary supplement for food animals. In the fish experiments, not only were *Chironomus* larvae an effective growth promoter, they appeared to be more effective with the smaller fish tested.

TABLE 4

Feeding trials with American lobsters, including significance values determined by T-test for independent samples

	Average Intermolt (days)	Increase in Carapace Length (mm)	Absolute Weight Gain (gms)	Per cent Weight Gain	
Control (Artemia only)	18.6±3.2¬ NS	1.0±0.4	0.05±0.0 ¬	27%±4 - 7 .01	
Artemia + 2% Larvae	J NS 19.3±1.5 7 NS	1.5±0.3 7 .05	0.18±0.10 NS	77%±33 7 NS	
Artemia + 10% Larvae	16.0±7.4	2.0+0.4	0.19+0.06	76% <u>+</u> 25-	

The early life stages are at once the most critical period for the fish culturist, and the time when it is easiest to provide a relatively high percentage portion of larvae.

The difference in growth between fish receiving the ten per cent midge supplement and those receiving the two per cent supplement was in all instances less than the difference between those receiving the smaller supplement and the controls. In interpreting this data, it should be kept in mind that while rolled oats and roasted soy meal are essentially dry, eighty-six per cent of the weight of a live C. tentans larva is water. On a dry weight to dry weight basis, then, the rates of dietary supplementation with midge larvae in the fish experiments were 0.28 per cent and 1.40 per cent. Such a pronounced effect on growth rate from such small weights of midges suggests that we are dealing, not with the effect of increased quantity of protein, but with a vitamin or amino acid effect.

In the lobster trials, both absolute and percentage weight gain showed the same effects observed in the tilapia. The carapace length data, however, show significant differences not only between the controls and the experimental groups, but also between the two experimental groups. This apparent discrepancy can be explained by observing that carapace length alone, though a standard measurement in the literature, does not reflect possible differences in claw size and length of abdomen.

While the weights of midge larvae and frozen brine shrimp used in the lobster trials were reckoned on a dry weight - dry weight basis, the differences in growth rates of midge-fed and control lobsters are greater than one might expect. To postulate a protein or amino acid effect here does not seem satisfactory. Chemical analysis of larvae of the midge *Chironomus plumosus* and various other invertebrates cultured for use as fish foods in the U. S. S. R. (Ivleva, 1969) did not indicate that *C. plumosus* larvae differ notably from the rest, except in that *Artemia salina* do not contain

Vitamin A. It should also be noted that Chironomids are unusual among invertebrates in containing large amounts of hemoglobin.

Artemia are a standard component in the diet of many cultured aquatic animals. In some cases, including some lobster cultures, they are the sole food. It has been shown recently that in such cultures, live Artemia are superior to frozen (Schleser and Gallagher, in preparation). No technical explanation has been advanced for this phenomenon, but it has historical precedent in the "live food mystique" of aquarists. It is possible that some nutrients are lost in the freezing process. If this were true, the addition of a small amount of live food, e. g., the midge larvae in our experiments, might provide a factor critical to the growth of cultured animals.

In the present instance the picture is further complicated by the results of studies in which lobsters were reared in the same system used in these experiments and fed on one hundred per cent live food diets. Percentage weight increment of our two experimental groups reared on frozen Artemia and small amounts of live midges (seventy-seven per cent ± thirty-three; seventy-six per cent ± twenty-five) did not differ significantly from that of lobsters reared on a Ceramium - Jassa - Mytilus association (eighty-one per cent ± twenty) (Levine, in preparation) and on high density Capitella capitata cultures (seventy-one per cent ± fourteen) (Mencher, in preparation).

From the results of work done to date, we cannot say whether or not there is a unique growth-promoting component or combination of components in midge larvae, or what that component or combination of components might be. We can say that midge larvae added in small quantities to standard fish and lobster diets resulted in significant enhancement of growth and that the ease of their cultivation and utilization renders them desirable for use in many forms of aquaculture.



We do not recommend midge larvae for culture as the principal food for any type of fish. There are many other good foods which can be provided more easily in bulk. As can be seen from our work, the effectiveness per weight of midge larvae is greatest when they constitute only a small proportion of the total diet. We do recommend their inclusion as a supplement in the diets of cultured fresh water and marine animals. If we assume a larval production rate of 100 g/m² of water surface/week (which we have attained in our best pools), then 10 m² of ponds could provide a two per cent supplement continually for eighty thousand young fish averaging 5 g each. If the increment in growth of the fish were comparable to that achieved in our experiments, a midge culture system would certainly be a worthwhile expenditure of time and space.

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William O. McLarney
 Joseph S. Levine
 Marcus M. Sherman

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