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Division of Biomedical and Environmental Research
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TROPHIC STRUCTURE MODIFICATIONS BY PLANKTIVOROUS FISH IN AQUATIC MICROCOSMS

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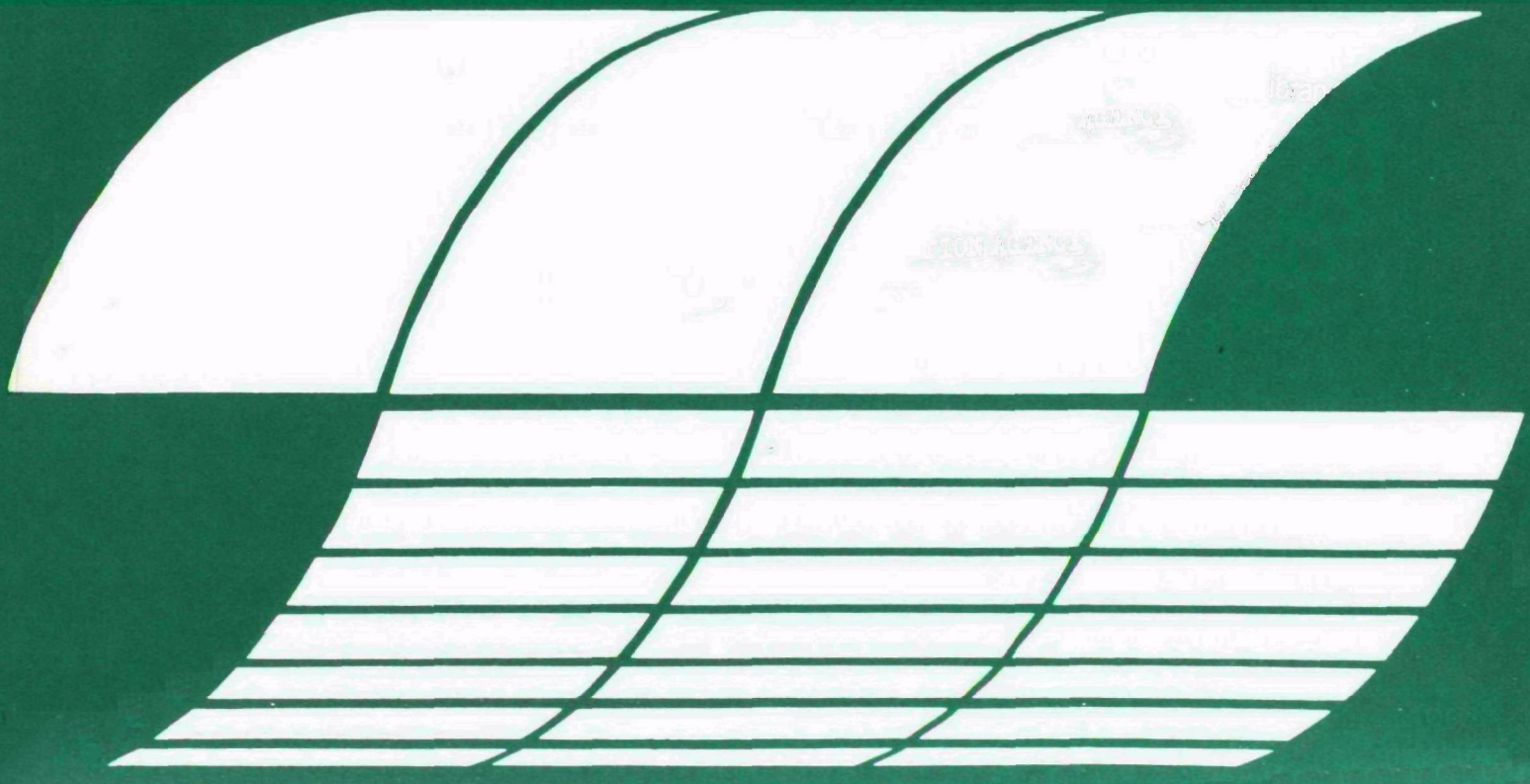

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RUNNING HEAD: Microcosm trophic structure

Trophic structure modifications by planktivorous fish in aquatic microcosms.[1]

A. Jassby, J. Rees, M. Dudzik, D. Levy, E. Lapan and J. Harte

Energy and Environment Division
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

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ABSTRACT

Two of 4 replicate 700-liter aquatic microcosms each were stocked with 2 mosquito fish (*Gambusia affinis*). The dominant zooplankter shifted from the large cladoceran *Simocephalus vetulus* to the smaller *Alona guttata*. The subsequent release of grazing pressure resulted in a rise in both phytoplankton and bacteria levels, which in turn were responsible for an increased rotifer biomass. Particulate organic carbon was higher and dissolved inorganic nitrogen was lower in the presence of *Gambusia*, reflecting a net shift of nutrients from inorganic to organic form, presumably because of smaller zooplankton respiratory losses. Ratios of particulate to dissolved organic carbon and of phytoplankton carbon to chlorophyll a were unnaturally high in the microcosms containing fish. An increase in total nitrogen was deduced for all 4 systems during the experiment; the increase could be explained by the presence of heterocystous *Anabaena* sp. and was independent of the presence of fish.

INTRODUCTION

Aquatic microcosms potentially are valuable tools for dissecting the important mechanisms operating in natural ecosystesm, as a number of studies have suggested (reviewed by Cooke, 1971). Microcosms also may have a central role to play in predicting the effects of human-derived disturbances on these mechanisms (Draggan, 1976). The applicability of microcosms for assessing human impacts on aquatic ecosystems ultimately depends on our ability to demonstrate basic analogies between microcosms and natural water bodies. Certain size-related differences between microcosms and natural systems, such as the surface-to-volume ratio of the containing structure, never will be bridged, but the usefulness of microcosms for a given research problem usually does not require the creation of a perfect analog to some naturally-occurring system. If microcosms are to fulfill their potential, detailed investigation of both the similarities and the insurmountable discrepancies between laboratory and natural water bodies is required. Only then can we have a basis for accepting or rejecting with confidence a specific prediction derived from research with microcosms.

Two kinds of experiment can be distinguished in the investigation of relationships between synthetic and natural ecosystems. In the first type of experiment the microcosms are allowed to develop without disturbance, the primary purpose being to determine whether or not the undisturbed system is capable of simulating production cycles or other basic features characteristic of any naturally-occurring counterpart. The second type of experiment, consists

of an experimental manipulation of the physical, chemical, or biological characteristics of a microcosm; the response of the microcosm then is compared with the response of natural ecosystems to the same disturbance. It is this latter kind of experiment that is a true test of the predictive value of microcosms.

The research reported here summarizes an experiment of the second kind, in particular, the addition of planktivorous mosquitofish (*Gambusia affinis*) to 2 of 4 replicate 700-liter freshwater microcosms. A number of studies have been published that document the effects of increasing or decreasing a planktivorous fish stock in natural water bodies (e.g., Hrbáček et al., 1961; Brooks and Dodson, 1965; Poštolková, 1967; Straškraba, 1967), so that adequate information exists to compare with the results of a similar trophic manipulation in aquatic microcosms. Detailed biological and chemical monitoring of the microcosms in this study permits such a comparison to be made, as well as providing new information on the results of manipulating a planktivorous trophic level.

METHODS

Each of the 4 700-liter systems (designated A,B,C,D) consisted of a fiberglass cylinder, 60.9 cm in radius, filled to a depth of 60.1 cm with demineralized water. The water was enriched with a modification of a common freshwater algal growth medium (Woods Hole MBL; Nichols, 1973) and inoculated with a 3.5 liter water sample collected from Lake Anza, a small eutrophic lake in the Tilden Park area of Berkeley, Ca. The enrichment levels of C, N, and P were 0.21 mmol liter^{-1} $NaHCO_3$, 1.7 μmol liter^{-1} $NaNO_3$, and 3.4 μmol liter^{-1} $Na_2HPO_4 \cdot 7H_2O$, respectively, a situation conducive to eventual N limitation of algal growth. The systems were maintained in a temperature controlled room at 19 ± 1 ^{o}C, illuminated by a bank of 8 4-ft high-output fluorescent lights on a 12:12 light:dark cycle, and aerated at a rate of 1.2 liter min^{-1}. On day 35 (enrichment was on Day 0), 2 mosquitofish (*Gambusia affinis*) of length 2.5 cm were added to each of A and C.

As demonstrated in other experiments (Jassby et al., 1977), the initiation conditions described above give rise to a phytoplankton bloom that is terminated by zooplankton grazing within 2 months, after which zooplankton grazing and phytoplankton growth remain loosely balanced for some time. Regular monitoring of each parameter for this experiment began sometime between Day 48 and Day 65, depending on the parameter, after the initial bloom had terminated and the *Gambusia* were given the opportunity to adjust to their new surroundings. The following parameters were measured twice per week: temperature, O_2, pH, inorganic carbon (IC), organic carbon (OC), NH_4, NO_3 + NO_2, chlorophyll a (Chl a), phytoplankton species and numbers, zooplankton species and numbers, and bacteria

numbers. Except for phytoplankton and zooplankton, all measurements were duplicated. Bacteria numbers were obtained with the Standard Plate Count method (APHA, 1971), and OC was fractionated into dissolved (DOC) and particulate organic carbon (POC) by measurement before and after filtration through a precombusted Whatman GF/C filter. Remaining analyses and the nutrient composition of the medium were as described elsewhere (Jassby et al., 1977). Monitoring was terminated on Day 100, except for bacterial numbers, which were followed until Day 117.

RESULTS

A detailed presentation of the temporal patterns that can be observed in these microcosms appears elsewhere in conjunction with a different set of experiments; the results of this trophic manipulation experiment thus are reported in summary form only.

The temperature of the microcosms remained at 18 ± 1 °C or approximately 1 °C below room temperature, presumably because of evaporative cooling. O_2 concentrations fluctuated between 0.28 and 0.31 mmol liter^{-1}. Aeration apparently was sufficient to prevent any significant biological modification of O_2 levels and most of the fluctuations can be ascribed to temperature variations in the microcosms. The pH values persisted at approximately pH 7.

The presence of *Gambusia* led to a significant decrease in the average occurence of the large cladoceran *Simocephalus vetulus* (Table 1), the adult female form of which reaches lengths of 2 to 3 mm. The copepod *Cyclops vernalis* (adult females approximately 1.5 mm in length) also decreased in concentration, but this decrease was much less significant. The mean levels of the small cladoceran *Alona guttata* (adult females approximately 0.5 mm in length) were similar both in the presence and absence of *Gambusia*. Among the 5 rotifers dwelling in the microcosms, only *Lecane* sp. responded significantly to the introduction of *Gambusia*, in the form of an order-of-magnitude increase in individual numbers. Changes in *Anuraeopsis* sp., *Keratella cochlearis*, *Keratella quadrata*, and *Trichotria* sp. were not significant.

Although Chl a concentrations were unaffected by *Gambusia*, total phytoplankton volume exhibited a large increase in the systems containing *Gambusia* and ratios of phytoplankton carbon to Chl a also were higher (Table 1). A

total of 31 phytoplankton species were recorded but no obvious relationship of community composition to the presence of *Gambusia* could be detected. The phytoplankton communities were dominated by 2 forms of *Synedra radians*, which was the dominant or second most dominant alga (by volume) in 79% of the 84 samples. *Synedra ulna* and a heterocyst-containing *Anabaena* sp. were next in importance, being most or second most dominant in 29% and 20% of the samples, respectively. In the 84 samples, the dominant and second most dominant algae accounted for an average of 86% of the community volume. A light periphytic growth on the sides of each container appeared by the end of the experiment and consisted primarily of *Mougeotia* sp.

Bacteria plate counts increased markedly in the systems containing *Gambusia* (Table 1). *Tanytarsus* larvae were observed first on the container sides of system C and apparently were present in the initial inoculum for C. The *Tanytarsus* spread via the adult flying stage to the remaining 3 systems (first to B and D, then to A) where larval populations also become established. The *Gambusia* in A and C were observed to feed on the larvae when they detached from the container sides and swam to the surface in preparation for their emergence as adults. The presence of *Gambusia*, however, did not alter significantly the occurrence of the midges within the water column (Table 1).

Mean levels of IC and DOC (Table 2) were unaffected by the presence of fish. On the other hand, stocking of systems A and C with *Gambusia* led to significant increases in POC and the ratio of POC to DOC, and to significant decreases in NH_4 and $NO_3 + NO_2$. It should be noted that POC values do not include the carbon content of *Gambusia*. Accordingly, the results of Table 1

indicate a shift of nitrogen from dissolved inorganic to particulate organic form upon the addition of a higher planktivorous trophic level. An increase in total N over the initial level of 1.7 μmol liter^{-1} NO_3 also can be deduced from the results for each treatment.

The *Gambusia* individuals in A and C, although starved for 48 h before addition, remained active and with normal coloration throughout the experiment. No quantitative observations on their size change were collected, but qualitative observation definitely suggested a size increase.

DISCUSSION

Biological effects. The average levels of the chemical parameters demonstrated good replicability within treatments (Table 2); this was not the case for the biological parameters, especially the zooplankton (Table 1). Part of the variability within pairs may be ascribable to the variable *Tanytarsus* colonization. However, other experiments, in which variability remained even when *Tanytarsus* larvae were stocked simultaneously in all systems, suggested that poor replication may result from random deviations of protozoa numbers in the initial inoculum (Jassby et al., 1977). Whatever the case, the poor replication entails that many of the potential treatment effects on zooplankton levels may not be extractable from the data.

Nonetheless, certain biological responses to the presence of a planktivore are evident (Table 1), and are consistent with the following explanation: the *Gambusia* fed on *Simocephalus* and reduced the average concentration almost 10-fold; *Cyclops vernalis*, being of smaller size, was fed on to a lesser extent, while the smallest crustacean, *Alona guttata*, appeared to have been ignored by the mosquitofish. The dominant zooplankters thus shifted from large cladocerans and cyclopoid copepods to smaller cladocerans. Similar transitions have been observed with heavy stocking of cyprinids in natural ponds (e.g., Hrbáček et al., 1961) and the introduction of the alewife *Alosa aestivalis* into Crystal Lake, Connecticut (Brooks and Dodson, 1965); and a transition in the opposite direction was observed when fish were excluded from the littoral areas of small natural lakes (e.g., Poštolková, 1967).

Despite the fact that they are larger than *Simocephalus* individuals, the midge largae did not show a significant response to the presence of

Gambusia. Most of the midge larvae were on the container sides where they remained free from from *Gambusia* predation. The larval population in the water column constantly was losing individuals that emerged from the water surface and gaining individuals that detached from the container sides. The resulting high turnover rate for larvae in the water column probably was sufficient to disguise the effects of *Gambusia* predation.

Because *Simocephalus* is capable of such high filtering rates (reported up to 90 ml ind^{-1} d^{-1}; Sushtchenia, 1958), the decrease of approximately 6 ind $liter^{-1}$ in mean *Simocephalus* concentrations without a corresponding increase in other crustaceans implies that much of the grazing pressure on phytoplankton and bacteria was eliminated with the introduction of *Gambusia*. Indeed, the bacteria plate counts increased by a factor of 3, and the phytoplankton community volume by a factor of 5. According to one trophic state classification based upon phytoplankton volume (Vollenweider, 1968), it may be said that the presence of *Gambusia* resulted in a shift from an oligotrophic to a mesotrophic system. A change in mean phytoplankton cell size was not observed in this study, although smaller sizes in the presence of planktivores have been reported in some of the studies referred to earlier.

In view of the large increase in mean phytoplankton levels where *Gambusia* were present, the lack of any difference in Chl a values may appear somewhat puzzling. However, these values were not corrected for phaeopignment interference and thus represent the fluorescence of grazed phytoplankton and other forms of detritus (Currie, 1962), as well as of living algae. Accordingly, the proportion of the Chl a attributable to living phytoplankton is least in B and D, where more grazing activity presumably is taking place. For similar reasons, the ratio of phytoplankton carbon to Chl a appears to be lower in the absence of *Gambusia*.

The increased levels of bacteria and phytoplankton imply a larger food source for rotifers, competition with crustaceans being ameliorated somewhat by the smaller *Simocephalus* populations. Only the numbers of *Lecane* sp. (Table 1) definitely reflect the increased food supply, but the mean value of the rotifer community volume increased from 0.013 mm^3 $liter^{-1}$ in B,C to 0.062 mm^3 $liter^{-1}$ in A,C. These results concur with the general observation from the above studies with natural systems that rotifers increase with increasing fish stock.

Chemical effects. Because of the aeration, biological activity in this experiment was not sufficient to alter the IC levels after the initial bloom, either in the presence or absence of the mosquitofish (Table 2). DOC also remained unchanged, suggesting that the dissolved compounds produced were of a labile nature that were processed quickly by bacteria before differences could arise between treatments. The increase in POC in A,C demonstrates that the loss of crustaceans in the presence of *Gambusia* was more than compensated for by increases in the rotifer, phytoplankton, and bacterial biomasses. This is to be expected, because the lowered grazing rates imply that a smaller amount of of primary production will be lost to zooplankton respiration and excretion.

The POC:DOC ratio of 0.1 in B,D is more characteristic of natural systems that the value of 0.3 observed in A,C (Wetzel and Rich, 1973). This probably is a reflection of the fact that the concentration of *Gambusia* in A and C was approximately 5 mm^3 $liter^{-1}$ wet weight, higher than that for phytoplankton and a level that would arise only rarely in a natural mesotrophic system. Similarly, the C:Chl a ratio of 21 observed in B, D is much more likely to be encountered in natural systems (Paerl et al., 1976) than the value for A,C of over 100. The abnormal POC:DOC and C:Chl a ratios in A,C underline the

fact that the effects attributable to the presence of mosquitofish can be accepted only in a qualitative manner; it is not possible to stock laboratory microcosms with realistic concentrations of planktovorous fish and obtain effects that are acceptable quantitatively.

Lower levels of NH_4 and $NO_3 + NO_2$ in A, C simply reflect the fact that POC (and, presumably, particulate organic nitrogen) increased when *Gambusia* was stocked in the microcosms. However, a marked increased over the initial N levels of 1.7 μmol liter^{-1} took place in all 4 systems. If we assume a C:N molar ratio of 12 for the POC (typical of autochthonous detritus; Wetzel, 1975), then the increase in mean total N (= $NH_4 + NO_3 + NO_2 + [POC/12] - 1.7$) was about 7 μmol liter^{-1} in both treatments. As mentioned previously a heterocystous *Anabaena* was the third most dominant alga in the microcosms and the possibility exists that the additional N was provided by algal fixation. An average fixation rate of only 0.004 μmol liter^{-1} h^{-1} is required to provide an additional 10 μmol liter^{-1} N by Day 100. Such rates are well within the range observed in nature (Tabel 11-3; Wetzel, 1975). The fact that the N increase was identical in both systems is consistent with this explanation; *Anabaena* filaments, by virtue of their size and mucilaginous sheaths, are not very susceptible to zooplankton grazing and so their population levels probably are unaffected by the presence of a planktivorous fish. Indeed, no significant differences were found in the *Anabaena* concentrations between treatments.

Conclusion. In sum, we conclude that the response of the microcosms to stocking of a planktivorous trophic level closely resembled the response of natural systems to the same manipulation. As has been observed in naturally-occurring

water bodies, introduction of planktivorous fish led to (i) a shift in the dominant zooplankters from large to small cladocerans; (ii) an increase in phytoplankton resulting from lowered grazing pressure; and (iii) a higher rotifer biomass in response to the increased phytoplankton. Additional responses observed in this study included (iv) an increase in bacteria, presumably for the same reason as the phytoplankton increase; (v) higher POC levels reflecting smaller losses to zooplankton respiration and excretion; (vi) lower inorganic N levels as the fish induce a net transition from inorganic to organic form; and (vii) increased ratios of POC:DOC and phyto C:Chl *a*. The agreement with observations from natural systems and the ability to suggest additional features of the response to trophic structure manipulation clearly establishes the usefulness of these microcosms for research on trophic structure.

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TABLE HEADINGS

Table 1. Biological parameters monitored in the microcosms. N is the number of times measurements of the parameters were taken in each system. Chl a and bacteria counts were duplicated. The entry for each pair of systems (A,C and B,D) is the mean ± standard error of the average value of the parameter for each member of the pair. The value of t is the t-statistic for the null hypothesis that the 2 pairs of systems (A,C with *Gambusia* and B,D without *Gambusia*) have equal means.

Table 2. Chemical parameters monitored in the microcosms. N is the number of times duplicate measurements of the parameter were taken in each system. The entry for each pair of systems (A,C and B,D) is the mean ± standard error of the average value of the parameter for each member of the pair. The value of t is the t-statistic for the null hypothesis that the 2 pairs of systems (A,C with *Gambusia* and B,D without *Gambusia*) have equal means.

Parameter (units)	N	Mean ± Standard Error A,C	B,D	t
bacteria (10^4 ml^{-1})	17	16 ± 4	5.0 ± 1.4	3.90*
Phytoplankton (mm^3 $liter^{-1}$)	14	3.4 ± 0.1	0.69 ± 0.37	10.05**
Chl a (μg $liter^{-1}$)	11	5.4 ± 2.0	5.1 ± 0.7	0.20
phyto C:Chl a[1]	11	113 ± 27	21 ± 7	4.68*
Rotifera (ind $liter^{-1}$)				
Anuraeopsis sp.	14	0.12 ± 0.00	0.59 ± 0.83	1.00
Keratella cochlearis	14	4.5 ± 4.4	66 ± 93	0.94
Keratella quadrata	14	130 ± 120	6.2 ± 6.7	1.48
Lecane sp.	14	42 ± 3	6.4 ± 6.4	7.15**
Trichotria sp.	14	23 ± 31	0.60 ± 0.85	1.00
Cladocera (ind $liter^{-1}$)				
Alona guttata	14	20 ± 4	15 ± 7	0.82
Simocephalus vetulus	14	0.80 ± 0.71	6.8 ± 1.0	6.96**
Copepoda (ind $liter^{-1}$)				
Cyclops vernalis	14	1.7 ± 0.1	7.2 ± 3.3	2.39
Diptera (ind $liter^{-1}$)				
Tanytarsus sp.	14	0.59 ± 0.83	1.8 ± 0.8	1.41

* Null hypothesis rejected at the 80% level of significance

** Null hypothesis rejected at the 90% level of significance

[1] Assuming that phyto C is 10% of wet weight

Table 1

Parameter (units)	N	Mean ± Standard error A,C	B,D	t
IC ($mmol\ liter^{-1}$)	12	0.21 ± 0.04	0.23 ± 0.04	0.57
DOC ($mmol\ liter^{-1}$)	11	0.32 ± 0.01	0.31 ± 0.01	1.34
POC ($mmol\ liter^{-1}$)	11	0.76 ± 0.011	0.022 ± 0.001	6.70**
POC:DOC	11	0.29 ± 0.01	0.090 ± 0.003	19.61***
NH_4 ($\mu mol\ liter^{-1}$)	14	2.2 ± 0.2	5.6 ± 1.5	3.21*
$NO_3 + NO_2$ ($\mu mol\ liter^{-1}$)	12	0.50 ± 0.05	0.90 ± 0.14	3.82*

* Null hypothesis rejected at the 80% level of significance

** Null hypothesis rejected at the 90% level of significance

*** Null hypothesis rejected at the 95% level of significance

Table 2