This is to certify that the

thesis entitled

The Effects of Food Availability and Temperature

on the Specific Growth Rate of Daphnia pulex

presented by

Abdel M. Abdalla

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Fisheries and Wildlife

Major professor

Date 2/22/85

THE EFFECTS OF FOOD AVAILABILITY AND TEMPERATURE ON THE SPECIFIC GROWTH RATE OF *D. PULEX*

By

Abdel Moez A. F. Abdalla

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1985

ABSTRACT

THE EFFECTS OF FOOD AVAILABILITY AND
TEMPERATURE ON THE SPECIFIC GROWTH RATE OF D. PULEX

by

Abdel Moez A. F. Abdalla

The population size and growth rate of D. pulex was observed at three temperatures (12, 20, 26°C) and five difference concentrations of torula yeast (cells/ml/day) at each temperature. The population size and specific growth rate of Daphnia increased with increasing food concentration and temperature. Yeast concentrations had a greater effect on Daphnia population densities than temperature. The relationship between the specific growth rate of D. pulex and the amount of torula yeast available per individual Daphnia per day followd the Michaelis-Menton equation with a correction for the threshold concentration. The predicted specific growth rate of D. pulex increased with increasing food concentration (yeast cells/individual Daphnia/day). The lowest and highest threshold yeast concentration (when growth rate is equal to zero) occurred at 20°C and 12°C respectively. The lowest and highest concentrations of yeast (cells/individual/day) at maximum efficiency occurred at 26°C and 12°C, respectively.

ACKNOWLEDGMENTS

I sincerely wish to thank Dr. Donald L. Garling, Jr. my major advisor for his help in constructing the equipment used and also for reviewing this manuscript and for solving all the problems which I faced during this study.

Warmest thanks are expressed to Dr. Darrell L. King, my committee member for his guidance and assistance, without which this research would never have been possible.

Heartfelt thanks are expressed to Dr. Alevin L. Rogers, another committte member, for his techinical assistance and understanding. Sincere thanks go to Dr. James D. Hall for all the advice and time which he gave me. I would also like to thank Dr. Niles R. Kevern for facilitating the use of his limnology lab.

Thanks are extended to my fellow graduate students in the Aquaculture Laboratory, Anthony Ostrowski, Douglas Sweet and Ibrahim El Shishtawi, who made my days with them more enjoyable. Sincere thanks are expressed to my friend El-Eraki Mohamed for his statistical assistance.

Special thanks are expresed to D. Hicks for the support and encouragement.

Finally, much appreciation is expressed to the American Agency for International Development (AID) for supporting me financially during my stay in the United States.

Dedication

This thesis is dedicated to the soul of my kind and lovely father (1923-1983).

TABLE OF CONTENTS

LIST OF TABLES

Appendix Tables

LIST OF FIGURES

INTRODUCTION

One of the major problems to expanding pond fish culture in less developed countries (LDC's) is the lack of an adequate supply of fingerlings for stocking. Increasing the availability of fingerlings is a single factor that could result in a rapid increase in pond fish production in many LDC's. Increasing the fingerlings supply will depend on a better understanding of the reproductive biology, better techniques for the rearing of larvae and fry and better production hatcheries (Colt 1983, Torans 1983).

Production of certain larval fishes in LDC's has been constrained by a limited understanding of the physical, chemical, and biological processes in pond, and the availability of appropriate sized and quality feed items in culture ponds throughout the rearing period (Yamada 1983, King and Garling 1983). However, in most LDC's, only supplemental feeding with locally available products will be economically feasible (Colt 1983, Lim 1983) since fish diets rely heavily on high quality protein meal which is not readily available in these countries (Mertiz 1972). LDC's should depend on natural productivity to solve their problem of the deficiency in high quality protein diet since the loss of edible protein with either fish or chickens fed

protein rich food stuffs can lead to reductions in the protein level of the human diets in these countries in that the increased market cost of the higher grade protein is often beyond the means of a significant portion of the population (King and Garling 1983).

Since larve need food items that are visible, suspended in the water, small enough to be ingested, high in protein and essential amino acid and are easily digested, it is no surprise that larvae of most fish feed initially on zooplankton (Sadykov 1975, Arnemo et al. 1980, Lasker 1975). Fry normally consume 40-80% of their body weight daily (Gorbunova and Lipskaya 1975, Stephen 1976). However, as fish grow, they will usually select progressively larger prey items (Detwyler and Houde 1970, Stephen 1976, Tamas and Horvarth 1976).

Daphnia (cladocera: Daphnidae) is a genus which is among the dominant consumers of primary producers in fresh water (Herbert 1978, Lampert 1977 III). It is found in oligotrophic and eutrophic lakes, in ponds and reservoirs where it forms a source of food for both invertebrate and vertebrate predators (Brooks 1959). However, the organisms within an ecosystem may be grouped into a series of more or less discrete trophic levels as primary producers, primary consumers, secondary consumers, etc., each successively dependent upon the preceding level as a source of energy,

with the primary producers dependent upon the rate of incident solar radiation (Lindeman 1942).

Generally, the importance of fertilizers (inorganic or organic) for enhancing pond production in modern fish culture is indisputable (Yamada 1983). Tang (1970) outlined three pathways through which the organic material entering the pond food web: Firstly, the materials enter as a source of nutritive substances (*e.g. carbon, phosphorous) for photosynthesis in chlorophyll bearing plants, secondly as an organic substrate for micro organisms which, in turn, support a zooplankton population, and finally it may be consumed directly by fish, crustaceans or insects. However, overfertilization of ponds can result in low dissolved oxygen, high ammonia and high hydrogen sulfide at the same time (Boyd et al. 1979, Hollerman and Boyd 1980).

Walleye fry (7-8 mm) reared in the U.S. can serve as a model for developing extensive larval culture techniques in LDC's. They accept live feeds more readily than milled diets (Kraise and Meade 1982), therefore even the walleye fingerlings used in intensive culture still have to be raised extensively from the fry stage in rearing ponds with natural food. However, high rates of growth and survival in walleye fries were achieved by stimulating Daphnia production by weekly application of sheep or horse manure at

32 ppm plus torula and brewers yeast at 5.3 ppm (Beyerle 1979). Also, Nandy et al. (1976) obtained high yield of D. lumholtzi when feeding 0.1% uniform.

Our research goal is to develop improved methods for extensive culture of walleye through natural feed production of D. pulex which can serve as a model for larval rearing techniques in LDC's.

The objective of this study was to determine the effects of food availability (torula yeast) at three different temperatures (12, 20, 26°C) on the specific growth rate of Daphnia pulex.

LITERATURE REVIEW

Daphnia Biology

Daphnia species are well suited to laboratory studies. The life cycle is in the order of days. There are no free eggs or larval stages in the life cycle which would complicate the census of the population. Although males are produced, they are in such a small minority that sex ratio can be ignored in the analysis of the results. Except for possible mutations or position effects due to crossing over, the offspring of a single female are genetically identical (Slobodkin 1954).

In favorable conditions, Daphnia species reproduce by parthenogensis. The eggs which are laid into the brood pouch develop into free swimming young in a few days. The young are liberated a few hours before the mother moults. Soon after moulting, another clutch of eggs is laid into the brood pouch. The young produced in this way are all females which mature and reproduce in the same manner. In unfavorable conditions, males appear among the offspring and some of the females produce eggs which require fertilization. In each instar, only one of these eggs is produced by each ovary. They pass into a modified brood

pouch which darkens and eventually becomes black. This modified brood pouch is called an ephippium and the eggs in it can withstand being dried and frozen. The ephippium with its eggs is cast separately from the rest of the carapace when the female moults. Females which produce ephippia may return to parthenogenetic reproduction when conditions improve. Some arctic populations produce ephippia without any males appearing so that the ephippial eggs appear to be unfertilized. The ephippial eggs are often termed resting eggs since they may not develop for several months or even years (Green 1956, Brooks 1959).

However, the egg size in Daphnia species may vary with the nutritional state of the mother, and the larger species have larger eggs and species with larger eggs have larger young (Green 1956).

The average caloric value of newborn (0.7 mm), last pre-adult instar (1.3 mm) and actively reproducing D. pulex is 4059, 4124 and 5075 cal/gm respectively. The increased amount of energy in the adults is due to increasing stored fat in the adults (Richman 1958).

Daphnia species store energy for reproduction and survival in the form of triglycerides droplets. The amount of energy stored in adults is lowest just after egg production and increases during the intermolt period. Thus, based on the lipid index (the number and size of droplets carried within cells in the cavity), each animal can be

rank-scored from zero to three. Minimum lipid values occur during the peak and initial decline of population number. Growth, survival and reproductive success are paralleled with the magnitude of the lipid index in D. pulex (Holm and Shaprio 1984). In D. catawba, the amount of lipid stored in the neonates was decreasing as the population was increasing, that is because when food was limited, the adults put less lipid into each egg or the neonates were using up that lipid more quickly under such conditions. However, lipid is stored in adult cladocerans whenever an animal achieves a positive net energy balance which means that the metabolic energy costs are less than the energy assimilated (Tessier and Gaulden 1982).

Dissolved oxygen and pH are very important environmental factors which affect the Daphnia population. Lack of oxygen inhibits the growth and reproductive capability in D. obtusa and D. magna (Fox 1951, Green 1956). Oxygen was found to have a significant effect on the filtering rate of D. pulex in that below a concentration of 3 mg/l, the rate decreased sharply (Kring and O'brien 1976). But, at the same time, hemoglobin can be produced at very low concentration of oxygen (0.6 mg/l) in Daphnia, thus enabling Daphnia to survive (Fox 1948, Fox et al. 1951, Kring and O'brien 1976, Landon and Stasiak 1983). The optimal pH for D. pulex and D. magna is between 7-8 (O'brien 1976, Leonard and Lawrence, unpublished data). Changes in

illumination did not appear to affect Daphnia (MacLaren 1963, McMahon 1965).

Population Growth and Feeding Behavior

The greatest growth increment (measured as carpace length) in seven species of Daphnia didn't always occur at the end of the adolescent instar, but it might occur at the end of the pre-adolescent instar or more rarely even earlier (Green 1956). As the initial size increased, the animals tended to become mature in earlier instars. A direct correlation was observed between the size of the females and the number of eggs carried in their brood pouches. A mature female of D. magna can assimilate enough material during each instar to produce eggs with a dry weight at least equal to that of her body after the eggs have been laid However, growth measured by total length, carpace length and height in D. pulex was sigmoid. The point of inflection in all curves came during the fourth instar. The increments increased up to the fourth instar, then decreased gradually to the eleventh instar, after which they remained low and relatively constant. However, the number of young released during the adult instars increases to a maximum at the tenth instar followed by a gradual decrease (Anderson et al. 1937, Richman 1958).

D. longispina which were starved throughout life, live about 40% longer than those well fed throughout life, but those starved for 11, 14 or 17 instars and then well fed

until their death live significantly longer. However, Daphnia starved from birth to various periods after reproductive maturity and well fed for the remainder of life grew slowly during the period of starvation but markedly increased in body length after being given an abundance of food, even though the increased supply of food was provided as late as the eighteenth instar by which time all the well fed sisters have died. Also, when Daphnia were fed abundantly even after being starved, they promptly produced many more young in each brood and the frequency of moults increased and also the frequency of heart beats increased (Ingle 1937). Dunham (1938), Banta et al. (1939) also found that poorly fed animals in corresponding instars attained sizes below those of well fed individuals.

Small D. pulex have a higher production rate than large ones production is the accumulation of organic matter in body material and this relation is clear especially at high food concentration. The higher production rates of small animals are exclusively concerned with body growth, but after the animal reaches maturity 20-30 Ug C), most of the produced substance is incorporated in the offspring (Lampert 1977 b). This can be explained also in terms of energy consumed as growth. The percent energy consumed as growth in preadult Daphnia was higher at any food concentration used than in the adult Daphnia. However, the adult Daphnia consumed more food than the preadult and most of the

consumed energy went into the production of offspring (Richman 1958).

D. magna population at 12°C persisted only for few weeks of faltering growth due to decreased metabolic activity which was not high enough to insure the reproductive and survival rates required for population growth and maintenance (Pratt 1943, MacArthur and Baillie 1929). Pratt (1943) stated that the population at 18° C showed a large initial peak followed by a relatively equiliberated phase which might be due to a series of overlapping generations. At 25°C the population continued to oscillate with no apparent approach to equilibrium and without any apparent environmental change. The source of oscillation is a lack of synchronization of a physiological state with the forces that provoke it. Also, he noticed that the life expectancy of the animals at 25°C was so short that each population peak represented a separate generation.

The effect of temperature on longevity of Daphnia was practically the opposite to the effect of temperature on the metabolic process (MacArthur and Baillie 1929, Pratt 1943, Mclaren 1963). In D. magna, an increase in temperature up to 28°C increased the initial growth rate by shortening the duration of the instars (Brown 1927). Below 7°C the developmental time was too long to allow good population growth in D. pulex and above 27°C the cultures died out after a few weeks (Lampert 1977b). At low temperature

females of dD. magna increased in size more slowly, but reached a large final size than females kept at higher temperature (MacArthur and Baillie 1929, Smith 1963). However, Hall (1964) observed an increase in the size of D. galeata mendota with increasing temperature up to 25°C.

The effects of temperature on egg production have been studied by many authors. Berg (1931) found that if the temperature remained below 3°C to 5°C for a long period, egg production by D. magna stopped, but if the temperature rose to 6°C to 10°C, it started again. In D. pulex temperature of 15° to 25°C were favorable for egg production, but above and below these .pa temperatures, there was a considerable reduction in the number of eggs produced (Tamson 1930).

Daphnia species can graze on a broad range of high quality food particles and translate them into high rates of production and growth (Allen 1976). Rotifers and large cladocerans (Daphnia) and calanoids are all able to collect particles of the 1-15 U range. The competitive success of the larger plankton herbivores is probably due to the greater effectiveness of food collection and a relatively reduced metabolic demand per unit mass which permit the assimilation to go to egg production (Brooks and Dodson 1965). However, D. pulex and D. magna are able to select food of a certain size and the maximum particle size ingested by cladocerans, increases linearly with increasing carapace length (Berman and Richman 1974, Burn 1968, Gliwicz

1980). Log phase Chlorella vulgaris didn't inhibit feeding, but senescent cells caused D. magna to decrease the filtering rate and its maximum feeding rate (McMahon and Rigler 1965). However, Taub and Dollar (1968) concluded that D. pulex failed to reproduce normally when fed either Chlorella pyrenoidos or Chlamydomonas reinhardtii because these algae appeared to be deficient in meeting the nutritional requirements of D. pulex especially with respct to reproduction. Also, Metz (1973) obtained a low assimilation rate when feeding yeast to D. pulex. However, there is some evidence that the animals used in his study were in insufficient condition because there was not any eggs at the beginning of the experiment, and also because of low carbon content and up to 75% mortality during the experiment (Lampert 1977 IIb). Although, it was believed that D. pulex never utilize the blue green algae, Holm et al. (1983) observed that D. pulex can utilize the blue green algae Aphanizomenon Flos Aquae even if it was put in a mixture of green and blue green algae.

Filtering rate of D. Rosea (measured in natural lake water and in pure culture of Rhodotorula glutinis yeast) increased with increasing body length and increasing temperature up to 20°C, also above a concentration of 0.25 x 10^5 yeast cells/ml, filtering rate decreased with increasing cell concentration and below this concentration, filtering rate was maximal (Burns and Rigler 1967). Similar results

were obtained for D. pulex (Burn 1969, Lynch 1977). These results coincide with Haney (1973) who stated that the grazing rate in the summer time for natural zooplankton communities exceeded 100% day^{-1} and became less than 10% day^{-1} during the winter.

Peterson et al. (1978) calculated filtering rate for four different Daphnia species grazing on natural bacteria by counting bacteria before and after incubation (for 0.5-2 hours). He used the following formula to calculate the filtering rate: filtering rate (ml $animal^{-1}$ hr^{-1}) = 1/t. ln (Co/C1) ml/animal rate is independent of the concentration (McMahon and Rigler 1965). However, as the size of D. magna was increasing, both where:

t = incubation time in hours

Co = concentration of bacteria or yeast cells at the beginning of incubation

C1 = concentration of bacteria or yeast cells at the end of incubation.

ml $animal^{-1}$ = volume of water per experimental animal.

Filtering rates for D. pulex, D. longermis and D. middendorffiana ranged from 0.2 - 1.5 ml $animal^{-1}$ hr^{-1} depending on the size of the animal and temperature.

Feeding rate (cells, $animal^{-1}$ hr^{-1}) is obtained by multiplying filtering rate (ml $animal^{-1}$ hr^{-1}) by the concentration of cells in the food suspension (cells/ml) (MacMahon 1965, MacMahon and Rigler 1967).

Feeding rate of D. magna measured on four different kinds of feed, showed that below a certain concentration of each feed, the feeding rate is proportional to the concentration of feed and above this concentration, feeding rate is independent of the concentration (McMahon and Rigler 1965). However, as the size of D. magna was increasing, both maximum feeding rate and maximum filtering rate increased. The "incipient limiting level" (the external level above which there is no limiting effect of food supply) also increased as the size of Daphnia increased. Temperature also affected the feeding rate of D. magna. The maximum feeding rate of D. magna occurred at 24°C (McMahon 1965).

Feeding rate was higher in females of D. schoedleri bearing an average of 10 eggs or embryos than in those bearing only two or no eggs apparently as a compensation for a greater metabolic demand of egg bearing females (Hayward and Gallup 1976). The can be explained by Slobodkin's (1954) observations on D. obtusa. He noticed that the organism with a high filtering rate would show a higher reproductive rate than an organism with low filtering rate.

The size dependence of the assimilation rate in D. pulex can be described by the power function of the relation $A = a.L^b$ where L is the length of Daphnia in mm. The numerical value of the exponent "b" is mainly influenced by the ability of small and large animals to ingest a certain

diet. However, the curves relating assimilation rate to food concentration are very similar to those reported for feeding rates. At low food concentration, the assimilation rate is proportional to concentration, reaching a plateau above the "incipient limiting level." Daphnids smaller than 3 mm showed a maximum assimilation rate at 20°C and in the animals 3 mm long, the maximum assimilation rate at 25°C (Lampert 1977a).

Generally, the ingestion rate of 200 plankton increased significantly with increasing animal size, food concentration and temperature. The filtering rate also increases with the increase in animal size and temperature, but declines as the food concentration increases (Peters and Downing 1984, Frost 1972).

Slobodkin (1954) in his study on the population dynamics of D. obtusa demonstrated the absence of a direct density effect in D. obtusa because the population size was linearly related to food supply. The population didn't follow a sigmoid population growth curve, thus the population oscillated due to the difference in ecology between different age-size categories of animals. However, this was in agreement with the results of Pratt (1943) and Frank (1952) who found the same oscillations under similar condition for D. magna and D. pulicaria. Generally, time lags were of two types an individual lag which was the time required for an individual animal to adjust its

physiological state to an environmental change, and a population lag which was the time required for the age and size distribution in a population to adjust to some environmental change affecting the entire population. However, at equilibrium, the populations maintained a constant size frequency distribution and reproductive rate while an oscillatory population showed a changing size frequency distribution (Slobodkin 1954).

The rate of increase of the population in the field may be estimated if the birth, death, emmigration and immigration rates are known. Although no animals reproduce continuously, many do reproduce frequently enough so that, the instantaneous (observed) growth rate can be calculated from the equation: $Nt = N_o e^{rt}$, where No, Nt denote the initial and time (t) population size respectively, and r is the instantaneous rate of increase. However, if the effects of death, emigration and immigration are ignored, the growth equation becomes: $Nt = Noe^{bt}$, where "b" is the instantaneous birth rate (Hall 1964). Generally, under stable age condition, the instantaneous rate of increase remains constant (Lotka 1925), whereas under a continually changing age distribution, the instantaneous rate of increase will change accordingly (Hall 1964, Slobodkin 1954).

The rate of increase "rs" measured by life table and fecundity table for D. galeata mendota ranged from 0.07 to 0.51 day^{-1} depending on the temperature level used

(11°C, 20°C, 25°) and the food concentration used measured in Klett units (16K, 1K, 1/4K) (Hall 1964). However, temperature influences were greater than food level under the conditions examined. Frequency of molting, duration of egg development and physiological life span were influenced principally by temperature (reproduction occurred every 2 days at 25°C, every 2.6 days at 20°C and every 8 days at 11° C). The growth per instar, maximum carpace length and brood size were influenced principally by food.

Generally, there are three variants of rate of increase: the first one is the observed rate of increase "r" which is measured by regressing Log_e of population size on time. The slope of this line estimates "r" which will be positive, zero, or negative depending on whether the population is increasing, stable or declining over the period it is observed (Cruchley and Birch 1977). The second one is the rate implied by the life table combined with the fecundity table "rs" (Brich 1948, Leslie and Park 1949, Evans and Smith 1952). Finally, the third one "rm" which is the intrinsic rate of natural increase. It is the rate of increase per head under specified physical conditions in an unlimited environment where the effects of increasing density don't need to be considered. It is the maximum rate at which a population with a stable age distribution can increase in a specified environment in the absence of predators. However, it can be calculated as a special case

of "rs which is obtained when all the individuals have access to more food, shelter, water and other requirements than they need, or it can be calculated from the logistic curve as follows:

$$\text{Log}_e \; K\text{-}N/K = a - rmt$$

where

K = maximum population size

N = population size at any time of the census

a = constant

rm = intrinsic rate of increase

t = time

(Birch 1948, Cruchly and Birch 1971).

Buening (1978) proposed a model predicting population growth of laboratory cultures of D. magna as a function of food availability. Combining food availability, initial density and time, the model estimated the finite birth rate, death rate, age of first reproduction, life expectancy, net reproductive rate and intrinsic rate of natural increase as a function of food supply per Daphnia per day. The intrinsic rate of increase appeared as a convex function of food availability. Wright (1965) proposed a model based on chlorophyll concentration, predator density and a constant death rate of D. schodleri.

A negative relation between density and the growth rate of Daphnia has been observed. Smith (1963) found a concave

relation between density and growth rate whether numbers or dry weights were used to measure density in D. magna. He proposed a new model for growth of D. magna derived from the logistic curve. The Verhaulst-Pearl logistic $dn/dt = rn(1-N/k)$ applies to ecology the principles that are used in the construction of rate models for chemical reaction and its appropriate for a single species population which have a single limiting factor in a constant environment. In order to compare the logistic model with any real system, either lags have to be introduced in the model or time free data have to be extracted from the system. However, Frank (1957) found that increasing density in D. pulex was accompanied by increased survival (over a wide range, decreased birth rate and lowered growth rate. The relation between numerical density and the intrinsic rate of natural increase was linear, thus, at maximum density the growth rate equalled zero.

However, neither the exponential form of the growth rate nor the logistic form of the growth rate relate the amount of food the organism used with the specific growth rate of the organism. Therefore, Monod (1949) related the specific activities rates of organisms to the substrate concentration as follows:

$$U = U_{max} \quad S/S+K_s$$

where

U = specific rate of activity or growth at existing conditions of limiting substrate or factors.

Umax = maximum specific rate of activity or growth

K_s = the condition or concentration of s at which $u = 0.5 U_{max}$.

This equation is best studied linearly in the form:

$$S/U = K_s/Umax + 1/Umax \quad (S)$$

(King, unpublished data, Williams 1973, Wright and Hobbie 1966).

There must be a minumum quantity of nutrients required to initiate the specific growth rate of the organism which is the threshold concentration. Young and King (1979) studied the interacting limits to algal growth: light, phosphorus and carbon dioxide availability to show the difference in the threshold concentration of carbon dioxide. They revealed that algae incubated at high light with ample phosphorus (915Lx, 580 Mg P l^{-1}) grew faster, over a wide range of carbon dioxide concentrations, and to lower concentration of carbon dioxide than algae grow under other conditions. In contrast, algae incubated at low light with limited phosphorus (280 Lx, 53 Ug P l^{-1}) grew at a lower rate, over a narrower range of concentrations of carbon dioxide, and ceased growth at a higher concentration of carbon dioxide compared to other conditions. However, algae yield (mg/day) decreased at high cell concentrations under

continuous sunlight irradiance since almost all the light is absorbed and further increase in cell concentration can add only to the overhead metabolism or respiration (Myers and Graham 1959).

Generally, the threshold food concentration is necessary for an individual D. pulex to keep its body mass constant or for a population to equalize all the biomass losses due to natural mortality and predation (Lampert 1977). She stated that from the balance equation (Production = Assimilation - Metabolic losses), it is clear that a high production, which would mean more fitness can be obtained by an increase in ingestion efficiency (higher assimilation rate and by a lower rate of metabolic expenditures. Moreover, if the food concentration is so low that the assimilation rate can not equalize the metabolic losses, a lower metabolic rate would mean better survival. The threshold was measured graphically by the point of intersection between assimilation rate and metabolic losses (when production is zero. There was a slight difference in the threshold concentration between 10° and 20° C, but the threshold increased at temperatures above 20°C. This increase is more profound for larger animals, so that the highest values occurred for animals 3 mm in length at 25°C. Thus, the threshold concentration were lower for small daphnids and it is also depended on the diet species.

However at very low concentrations of food, the collecting effort is reduced in copepods because more energy is lost than gained in the feeding process, thus, the maximum filtration effort may be predicted to occur at intermediate food concentration as copepod began to collect more food than they can physically or physiologically utilize efficiently (Lam and Frost 1976, Lehman 1976). This is in agreement with Beklenishev (1962) who believed that with increasing food, the ingestion rate increases and animals consumed food " in quantities far greater than could be utilized."

Field experiments on natural particles in sea water by Adams and Steel (1966) and Parsons et al. (1967) suggested that there is a threshold food concentration below which there is no feeding. Corner et al. (1972), Frost (1975) observed reduced grazing rates when copepods fed on very low concentrations of food cells. The copepod Acostia tomsa dana had a maximum grazing rate at about 10 mg chlorophyll a/l) decreasing to zero below 1 mg chl a/l (Reeve and Walter 1977).

If zooplankton cease feeding at a low food concentration in nature, then there is a "refuge" for phytoplankton in which they are free from mortality. The existence of such a "refuge" appearing as a positive x intercept of the curve relating ingestion to concentration

of food has been claimed by those using the modified Ivelv equation $I = Im\ [(1-e^{o(P-P^{-})})]$, or those using the modified Michalis-Menton equation $[I = Im(P-P'^{-})/(K-P^{-}) + (P-P'^{-})]$ (Mullin and Fuglister 1975)

where:

I = the rate of ingestion

Im= the maximum ingestion rate

P = the concentration of phytoplankton

o = constant

P^{-}= the concentration at which ingestion ceases

K = the concentration at which I = 0.5 Im.

However, Porter et al. (1982) stated that D. magna had no feeding threshold or reduced filtering activity at low concentrations as predicted by optimal foraging models.

Generally, the advantage of fitting the hyperbolic ingestion curves to the Michaelis-Menton model in Anuran larvae (Genera: Hyla, Bufo and Rana) feeding on the blue green algae is that it allows calculating important aspects of the curve to be summarized simply with three quantitative parameters the threshold concentration, the half saturation constant and the maximum ingestion rate (Seale and Beckvar 1980).

King and Garling (1983) used the same modified Michalis-Menton equation to relate the specific net carbon fixation rate of algae and submersed aquatic plants to the existing carbon dioxide concentration from the equation:

$$U = U_{max} \; (C - Cq) / (Kc - Cq) + (C - Cq)$$

where:

u = specific net carbon fixation rate (time^{-1})

c = existing carbon dioxide concentration (U moles CO_2/l)

Kc = carbon dioxide concentration at which U = 0.5 Umax (U moles CO_2/l)

Cq = threshold carbon dioxide concentration required to initiate net carbon fixation (U moles CO_2/l)

And thus from this equation, one can relate the specific growth rate of any organism to the amount of food required plus the calculations of the maximum growth rate of the organism and also the calculation of the threshold concentrations which is the amount of food below which there is no growth.

MATERIALS AND METHODS

Stock Culture of *D. pulex*

D. pulex were collected from ponds at Michigan State University, Aquaculture Laboratory with a simple conical tow net. *D. pulex* were identified under the microscope by the method of Brooks (1959). The Daphnia were then cultured in 9 and 30 l aquaria filled with well water. Temperature was controlled around 16-17° C. Daphnia were fed ground Purina Trout Chow every other day. Overcrowding of Daphnia in the aquaria was avoided by reducing the number of Daphnia whenever very high population densities occurred.

Transferring the neonates to the experimental units:

Individual adults were transferred from stock culture tanks to a depression slide under the microscope. Adults with eggs or embryos in the brood chamber were isolated by placing individual adult Daphnia in a separate 250 ml beaker filled with well water. Then on the second day, new born neonates were transferred to the experimental units.

Experimental Units:

All experiments were run in 1000 ml Erlenmeyer flasks using ten randomly chosen new born Daphnia. Three temperature levels (12 $\pm$ 0.5^{o}C, 20$\pm$ 1 ^{o}C, 26 $\pm^{o}$ C) were run separately with five different concentrations of torula (yeast supplied by the MDNR). Each yeast concentration was run in duplicate. Well water was used with all type experiments (Table 1). The light was continuously supplied by a fluorescent lamp.

During each experimental run, additional five Erlenmeyer flasks stocked with 10 newborn Daphnia were maintained with the same yeast concentrations used in each experiment. The Daphnia in these flasks were used to replace any Daphnia which died during the first 48 hours of the experiment.

Changing the medium and counting Daphnia:

During the experimental runs, Daphnia populations were transferred daily. Any dead Daphnia were removed and counted. An appropriate volume of the new medium was placed in clean flasks. The medium was drawn down to few cc's by suction through silk bolting cloth.

In experiment 1, the suction apparatus was made of fine mesh bolting silk which retained newborn Daphnia (134 U mesh nitex net), fastened over the large end of a small plastic funnel with an opening one inch in diameter. Hose from the

Table 1. Total dissolved metal concentration in well water.

Sensitivity ppm	Element		Concentration ppm
0.05	Aluminum	AL	NDA
0.05	Arsenic	AS	NDA
	Calcium	Ca	85
0.01	Cadmium	Cd	NDA
0.01	Chromium	Cr	NDA
0.005	Copper	Cu	NDA
0.1	Iron	Fe	NDA
0.05	Mercury	Hg	NDA
	Potassium	K	1.8
	Magnesium	Mg	28
0.01	Manganese	Mn	NDA
0.01	Molybdenum	Mo	NDA
	Sodium	Na	7.1
0.1	Phosphorus	P	NDA
0.05	Lead	Pb	NDA
0.05	Selenium	Se	NDA
0.05	Thallium	Tl	NDA
0.005	Zinc	Zn	NDA

NDA = no detectable amount

small end of the funnel lead to an aspirator which was connected from its other side by another hose attached to a tap. By operating the aspirator (opening the tap), the Daphnia were concentrated in a few cc's. Then the funnel was flushed with little water to get the attached Daphnia. No injuries or deaths for the Daphnia in the experiment were due to the suction system. Daphnia were transferred after this to white porcelain plates (12 cavities) by large mouth pipette with a rubber bulb and counted and transferred to the new medium (Figure 1).

In experiments 2 and 3, the technique used to change the old medium (Figure 2) was modified. The suction apparatus used in experiments 2 and 3 was similar to that used in experiment 1. It was made of fine mesh bolting silk which retained newborn Daphnia (130 U mesh nitex net), fastened over the large end of a small plastic funnel with an opening one inch in diameter. Hose from the small end of the funnel was attached to a rubber bulb, and the hose was controlled by a valve to control the flow of water when it was being drawn down.

Experiment 1:

Experiment I was run from 3/3/84 to 4/3/84 (32 days). The experiment was terminated after the population reached its maxima, then stabilized or declined. The average well water temperature was 12$\pm$ 0.5^{o}C. The ten Erelemyer flasks

Figure 1. The technique used to transfer Daphnia in the first experiment.

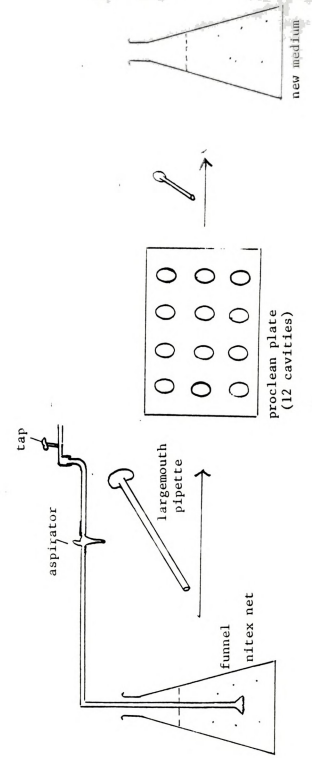
tap
aspirator
largemouth pipette
funnel
nitex net
proclean plate (12 cavities)
new medium

Figure 1.

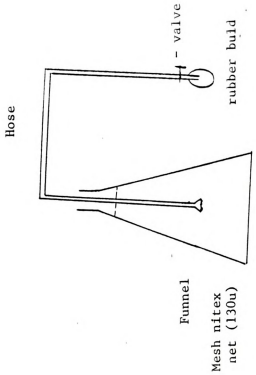

Figure 2. The suction apparatus used to transfer D. pulex in the second and third experiment.

were placed on a tray which was fixed in a large aquarium. Very little air was supplied to each flask. Ten newborn Daphnia (1-5 days old) were placed in each flask and fed on one of the five concentrations of torula yeast. Suspensions of yeast (1000 ml) were made with well water and counted on a Levi-Hauser Hemocytometer to establish concentrations of $1x10^4$, 2×10^4, 0.5×10^5, 1×10^5 and 2.5×10^5 number of yeast cells per ml per day.

Experiment 2:

Experiment 2 was run from 6-21-84 to 7-30-84 (40 days). The experiment was terminated after the population reached its maxima and then began to decline. The average temperature was $20 \pm 1^{o}C$. The water which was used in this experiment was a well water which was heated in large aquarium (30 l) with a submersed heater (200 W) to $19^{o}C$ and aerated with an air stone. The temperature increased $2^{o}C$ every 24 hours and became 21^{o} C at the day of changing the old medium. The ten Erlenmeyer flasks were placed on a tray which was fixed in a large aquarium. No air supply was used in this experiment because oxygen levels were never less than 7 mg O_2/l measured by Winkler method (Kring and O'Brien (1976) observed 3 mg O_2/l as the limiting concentration for D. pulex). Ten newborn Daphnia (1-3 days old) were placed in each flask and fed one of the five concentrations of torula yeast.

To prepare the suspensions of yeast, a standard was performed relating the number of yeast cells in a known weight of the torula yeast. We found that 5 mg yeast contained 286 x 10^5 cells, thus 40 mg yeast contained 2.288 x 10^8 cells. Our original suspension was composed of 40 mg yeast mixed very well with 800 ml well water. This solution had a concentration of 2.86 x 10^5 cells/ml. From this solution, the five concentrations of the yeast (0.5 x 10^4, 1 x 10^4, 1.5 x 10^4, 2 x 10^4 and 2.5 x 10^4 cells/ml/day) were prepared.

Experiment 3:

Experiment 3 was run from 8-9-84 to 9-10-84 (34 days). The experiment was terminated after the population reached its maxima and began to decline. The average temperature was 26$\pm$ 1^{o}C. All the procedures used were the same as the procedures used in the second experiment except temperature regulation concern over a potential decrease of temperature during the course of the experiment. A water bath was set up by filling the aquarium with water and controlled the temperature by a submersed heater (200 W) at 25^{o}C. Water was circulated by an aquarium pump to insure even heat distribution throughout all the aquarium (Figure 3).

Statistical analysis:

A two-way analysis of variance (ANOVA) and multiple classification analysis were performed with the SPSS program

Figure 3. The system used to raise D. pulex in the third experiment.

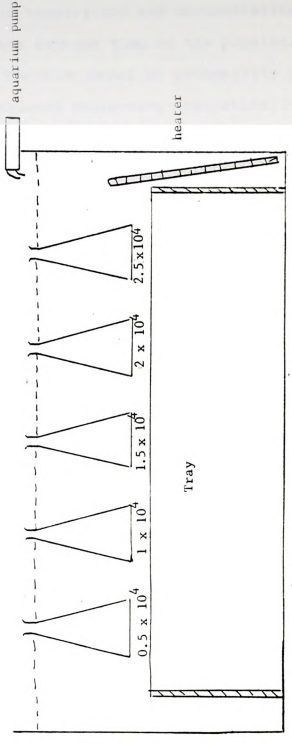
aquarium pump
heater
Tray
0.5 x 10^4
1 x 10^4
1.5 x 10^4
2 x 10^4
2.5 x 10^4

Figure 3.

by the Computer Laboratory at Michigan State University to test for the effects of temperature and concentrations of food and the interaction between them on the population density of D. pulex. The 0.05 level of probability provides the basis for all statements concerning statistically significant differences.

Outline of general procedures: -

a. Count the average number of D. pulex daily within each concentration of torula yeast.

b. Calculate the specific growth rate of D. pulex by the formula:

$$U = \frac{\ln N_2 - \ln N_1}{\Delta t}$$

where

U = specific growth rate of Daphnia (day^{-1}).

N_{+1} = number of organisms at time 1

N_{+2} = number of organisms at time 2

Δt = difference in time between t_2, t_1.

c. Calculate the number of yeast cells per individual Daphnia per day by the formula:

$$\text{cells/individual/day} = \frac{\text{concentration (cells/ml/day)} \times 1000(\text{flask volume})}{\text{Number of Daphnia}}$$

d. Combine the information relating the number of cells per individual per day (S/N) with the observed specific growth rate of D. pulex from each concentrations used at the specified temperatures.

e. Calculate the threshold concentration (sq) of D. pulex at this temperature by averaging all sq's used with different concentrations. However, the threshold concentration (sq) is the value of S/N when the growth rate is equal to zero, and it is determined by dividing the amount of yeast cells available per day by the maximum number of Daphnia attained (Sq = S/K).

f. Manipulate the main equation which predicts the threshold concentration as follows:

$$U = Umax \frac{S-Sq}{(S-Sq) + (Ks-Sq)} \qquad 1$$

or

$$U = Umax \frac{S/N - S/Nq}{(S/N-S/Nq) + (K_{s/n}-S/Nq)} \qquad 2$$

where

U = specific growth rate of D. pulex per day

Umax = Maximum growth rate of D. pulex per day

S/N = number of yeast cells per individual Daphnia per day

$K_{s/n}$ = number of yeast cells per individual Daphnia per day when U is equal to 0.5 Umax.

S/Nq = Threshold concentration or the number of yeast cells per individual Daphnia per day required to initiate the growth rate of D. pulex.

or

$$\frac{U}{S/N - S/Nq} = \frac{Umax}{(S/N-S/Nq) + (K_{s/n} - S/Nq)} \qquad 3$$

or

$$\frac{S/N - S/Nq}{U} = \frac{K_{s/n} - S/Nq}{Umax} + \frac{1}{Umax}\ (S/N - S/Nq) \qquad 4$$

Equation (4) takes the form of $Y = a + bx$ and can be regressed between $\frac{S/N - S/Nq}{U}$ and $(S/N - S/Nq)$, with a slope equal to $(\frac{1}{Umax})$. The reciprocal of the slope is equal to Umax). The Y intercept $(\frac{K_{s/n} - S/Nq}{Umax})$ multiplied by the reciprocal of the slope (Umax) yields $K_{s/n}$ - S/Nq). Thus, from this step, the parameters of the equation Umax, $K_{s/n}$ and S/Nq can be calculated.

RESULTS

Three experiments were run at three different temperatures (12, 20, 26°C). Five different concentrations of torula yeast (yeast cells/ml/day) were used at each temperature level. Each concentration of yeast was fed in duplicate 1000 ml Erlenmyer flasks containing ten D. pulex. Each experiment was ended when population size stabilized or began to decrease.

Experiment one:

Five concentrations of torula yeast (1×10^4, 2.5×10^4, 0.5×10^5, 1×10^5 and 2×10^5 yeast cells/ml/day) were maintained at 12 ± 0.5°C in the first experiment (Appendix Tables 1,2 and Figure 4). The population densities of D. pulex increased at yeast concentration of 1×10^4, 2.5×10^4 and 0.5×10^5 yeast cells/ml/day. At higher yeast concentrations (1×10^5, 2×10^5 yeast cells/ml/day, the population density of D. pulex was less than that at lower concentrations.

The threshold concentrations (S/Nq = the amount of torula yeast available per individual D. pulex per day when the growth rate is equal to zero) at 12°C were 2.63×10^5

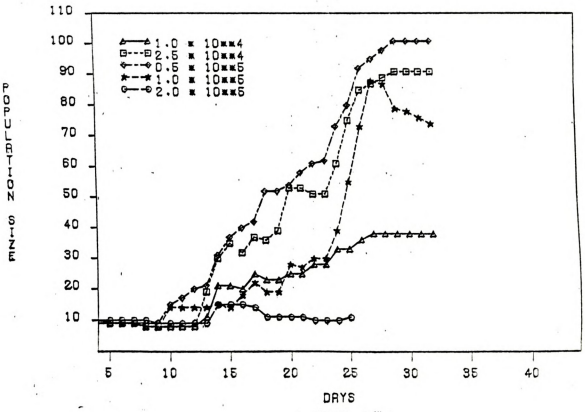

Figure 4. Population size of D. pulex at 12 degrees celsius with five different concentrations of torula yeast (cells/ml/day).

Table 2. The relationship between the observed growth rate of D. pulex and the number of torula yeast cells per individual D. pulex per day at 12°C.

a. for 1 x 10^4 cells/ml/day

Day	Number of Daphnia	U	(S/N) cells/ individual/day
12	8	0.229	12.5 x 10^5
16	20	0.056	5 x 10^5
20	25	0.069	4 x 10^5
24	33	0.035	3.03 x 10^5
28	38	0	2.63 x 10^5
30	38	0	2.63 x 10^5
32	38	0	2.63 x 10^5

b. for 2.5 x 10^4 cells/ml/day

Day	Number of Daphnia	U	(S/N) cells/ individual/day
12	8	0.347	31.25 x 10^5
16	32	0.126	7.81 x 10^5
20	53	0.035	4.72 x 10^5
24	61	0.094	4.1 x 10^5
28	89	0.011	2.81 x 10^5
30	91	0	2.75 x 10^5
32	91	0	2.75 x 10^5

Table 2. (cont'd.)

c. for 0.5×10^5 cells/ml/day

Day	Number of Daphnia	U	S/N/cells/ individual/day
8	9	0.187	55.6×10^5
12	19	0.186	26.3×10^5
16	40	0.075	12.5×10^5
20	54	0.075	9.26×10^5
24	73	0.073	6.85×10^5
28	98	0.015	5.10×10^5
30	101	0	4.95×10^5
32	101	0	4.95×10^5

yeast cells/individual/day at the 1 x 10^4 yeast cells/ml/day concentration and 2.75 x 10^5 yeast cells/individual at the 2.5 x 10^4 cells/ml/day concentration (Table 2). The average 2.7 x 10^5 yeast cells/individual/day was considered as the threshold concentration at 12°C.

The maximum growth rate of D. pulex (Umax) increased with increasing food concentrations (yeast cells/ml/day) (Table 8). Umax calculations were based on the Michaelis-Menton equation corrected for the threshold concentration at each concentration of food (yeast cells/ml/day). Consequently, the results of the three highest yeast concentrations (0.5 x 10^5, 1 x 10^5, 2 x 10^5 yeast cells/ml/day) were omitted from the sequence analysis of data regarding the predicted values from the Michaelis-Menton equation at 12°C.

The combined results from both yeast concentrations (1 x 10^4, 2.5 x 10^4 yeast cells/ml/day) and the observed specific growth rate (U) are shown in Table 3. The observed growth rate (U) increased in most cases with increasing food concentration.

The three constant parameters (Umax, Ks/n, S/Nq) of the Michaelis-Menton equation at 12°C corrected for the threshold concentration were Umax = 0.46 day^{-1}, Ks/n = 13.28 x 10^5 yeast cells/individual/day, S/Nq = 2.7 x 10^5 yeast cells/individual/day. The equation relating the predicted specific growth rate of D. pulex at 12°C to the

Table 3. The relationship between the number of torula yeast cells per individual D. pulex per day (S/N), the observed growth rates of D. pulex and the efficiency in the combined results of two concentrations of yeast (1×10^4, 2.5×10^4 cells/ml/day) at 12°.

Order	S/N cells/individual/day	Calculated μ	Efficiency μ/S/N	Observed growth rate
1	31.25×10^5	0.336	1.0752×10^{-7}	0.347
2	12.5×10^5	0.224	1.792×10^{-7}	0.229
3	7.81×10^5	0.15	1.92×10^{-7}	0.126
4	5×10^5	0.082	1.64×10^{-7}	0.056
5	4.72×10^5	0.074	1.57×10^{-7}	0.035
6	4.1×10^5	0.054	1.32×10^{-7}	0.094
7	4×10^5	0.05	1.25×10^{-7}	0.069
8	3.03×10^5	0.014	0.462×10^{-7}	0.035
9	2.81×10^5	0.005	0.178×10^{-7}	0.011
10.	2.7×10^5	0	0	0

amount of yeast available per individual Daphnia per day is:

$$U = 0.46 \quad \frac{S/N - 270000}{S/N + 788000}$$

From this equation, Table 3, and Figure 5, it is evident that the predicted specific growth rate of D. pulex increased with increasing food concentrations S/N (yeast cells/individual/day). However, the effect of changing a unit of food concentration (S/N) on the growth rate of D. pulex at high food concentrations was less effective than the changes at low food concentration. The predicted growth rate at a food concentration of 24 x 10^5 yeast cells/individual/day was 0.308 day^{-1} and at 25 x 10^5 yeast cells/individual/day was 0.312 day^{-1}, thus an increase of one unit of (S/N) caused a 0.004 day^{-1} increase in the growth rate. Also, at a food concentration of 3 x 10^5 yeast cells/individual/day, the growth rate was 0.013 day^{-1} and at 4 x 10^5 yeast cells/individual/day. The growth rate was 0.050, thus an increase in one unit of yeast concentration (S/N) caused a 0.037 day^{-1} increase in the growth rate.

The maximum efficiency was predicted to occur at a concentration of 7.81 x 10^5 yeast cells/individual/day (Figure 6, Table 3). At high concentrations of torula yeast, the efficiency was low and increased with decreasing food concentration until the efficiency approached its apex at an intermediate food concentration, after which the

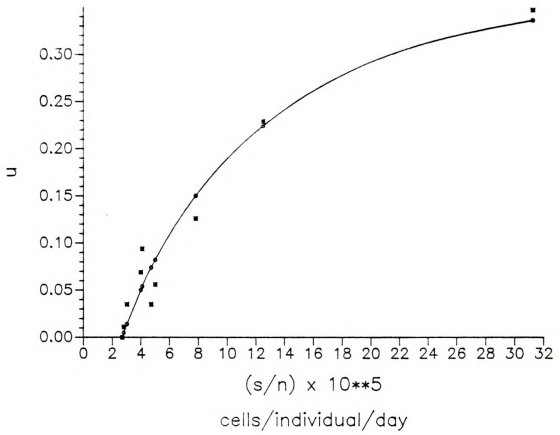

Figure 5. The relationship between the observed growth rates (*), the predicted specific growth rate and the amount of torula yeast available per individual D. pulex per day at 12°C.

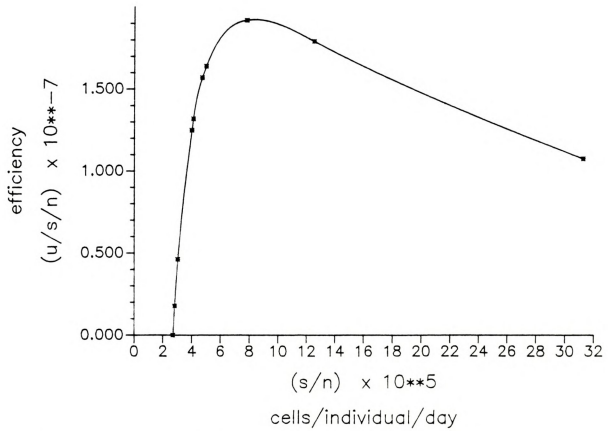

Figure 6. The relationship between the efficiency and the amount of torula yeast available per individual D. pulex per day at 12°C.

efficiency started to decrease again with decreasing food concentration (S/N).

Experiment 2:

Five concentrations of torula yeast (0.5×10^4 x 1 x 10^4, 1.5×10^4, 2×10^4 and 2.5×10^4) were maintained at $20 \pm 1^{o}C$ in the second experiment (Appendix Tables 3, 4, Figure 7). The size of D. pulex population increased at any yeast concentration with increasing the culture age, then stabilized (0.5×10^4 x 1 x 10^4 yeast cells/ml/day). The population size of D. pulex increased with increasing food concentration (yeast cells/ml/day). The population size increased slowly during the first 27-30 days of the experiment, after which the population increased very fast and reached its apex at day 36. After day 36, the population either stabilized or declined. However, the population size of D. pulex growing at a concentration of 0.5×10^4 yeast cells/ml/day reached its apex at day 23 then stabilized.

The threshold concentrations (S/Nq) at 20^{o} C were 1.42×10^5, 0.72×10^5, 0.62×10^5 and 0.66×10^5 yeast cells/individual/day for the concentrations of 0.5×10^4, 1 x 10^4, 1.5×10^4, 2×10^4 and 2.5×10^4 yeast cells/ml/day (Table 4). The average 0.82×10^5 yeast cells/individual/day was considered as the threshold concentration at $20^{o}C$.

The maximum growth rate (Umax) of D. pulex increased with increasing food concentration (yeast cells/ml/day)

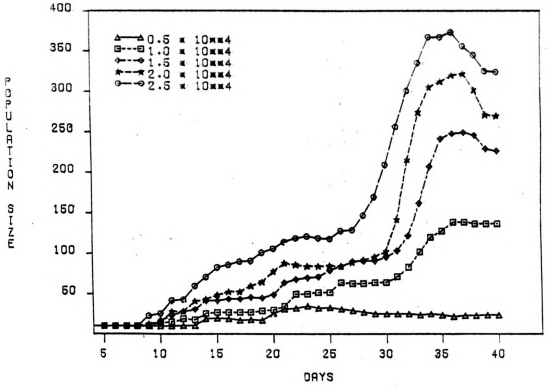

Figure 7. Population size of D. pulex at 20 degrees celsius with five different concentrations of torula yeast (cells/ml/day).

Table 4. The relationship between the observed growth rate of D. pulex and the number of torula yeast per individual per day at 20°C.

a. for 0.5×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
8	10	0.107	5×10^5
14	19	0.05	2.63×10^5
15	20	0.02	2.5×10^5
20	26	0.176	1.92×10^5
21	31	0.03	1.61×10^5
22	32	0.09	1.56×10^5
23	35	0	1.42×10^5

b. for 1×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
8	10	0.168	10×10^5
10	14	0.153	7.14×10^5
12	19	0.156	5.26×10^5
14	26	0.02	3.85×10^5
19	29	0.09	3.45×10^5
21	35	0.099	2.85×10^5
25	52	0.052	1.92×10^5
31	71	0.18	1.4×10^5
33	102	0.113	0.98×10^5
35	128	0.03	0.78×10^5
37	137	0	0.72×10^5

Table 4. (cont'd.)

c. for 1.5×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
8	10	0.257	15×10^5
12	28	0.113	5.35×10^5
16	44	0.027	3.4×10^5
20	49	0.093	3.06×10^5
24	71	0.06	2.11×10^5
28	91	0.073	1.64×10^5
32	122	0.171	1.23×10^5
36	249	0.004	0.6024×10^5
37	250	0	0.6×10^5

d. for 2×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
8	10	0.257	20×10^5
12	28	0.16	7.14×10^5
16	53	0.1	3.77×10^5
20	78	0.019	2.56×10^5
24	84	0.019	2.38×10^5
28	92	0.023	2.17×10^5
32	216	0.08	0.9×10^5
37	323	0	0.62×10^5

Table 4. (cont'd.)

e. for 2.5×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
8	10	0.365	25×10^5
12	43	0.173	5.81×10^5
16	86	0.05	2.9×10^5
20	106	0.03	2.35×10^5
24	119	0.05	2.1×10^5
28	147	0.179	1.7×10^5
32	302	0.05	0.8×10^5
36	374	0	0.66×10^5

(Table 8). Umax's calculations were based on the linear transformation of the Michaelis-Menton equation corrected for the threshold at each concentration of yeast. The Umax's of D. pulex were 0.11, 0.176, 0.318, 0.53 and 0.55 day^{-1} for the population growing at concentrations of 0.5 x 10^4 x 1 x 10^4, 1.5 x 10^4, 2 x 10^4 and 2.5 x 10^4 yeast cells/ml/day respectively.

The combined results from the five concentrations of yeast (0.5 x 10^4 x 1 x 10^4, 1.5 x 10^4, 2 x 10^4 and 2.5 x 10^4 yeast cells/ml/day) and the observed specific growth rate are shown in Table 5. In general, the observed growth rate of D. pulex increased with increasing food concentration (S/N). However, the observed growth rate increased in some cases with decreasing food concentration (s/n).

The three constant parameters (Umax, Ks/n, S/Nq) of the Michaelis-Menton equation at 20°C corrected for the threshold concentration were: Umax = 0.5 day^{-1}, Ks/n = 16.32 x 10^5 yeast cells/individual/day, S/Nq = 0.82 x 10^5 yeast cells/individual/day. The equation relating the predicted specific growth rate of D. pulex at 20°C to the amount of yeast available per individual Daphnia per day is:

$$U = 0.5 \frac{S/N - 82000}{S/N + 1468000}$$

From this equation, Table 5 and Figure 8, it is evident that the predicted specific growth rate of D. pulex increased

Table 5. The relationship between the number of torula yeast cells per individual D. pulex per day (S/N), The observed growth rates of D. pulex and the efficiency in the combined results of five concentration of yeast (0.5×10^4, 1×10^4, 1.5×10^4 2×10^4 and 2.5×10^4 cells/ml/day) at 20°C.

Order	S/N x 10^5	Observed growth rate (U)	Calculated growth rate	Calculated efficiency
1	25	0.365	0.305	1.22×10^{-7}
2	20	0.257	0.277	1.39×10^{-7}
3	15	0.257	0.239	1.59×10^{-7}
4	10	0.16	0.186	1.86×10^{-7}
5	7.14	0.16	0.145	2.03×10^{-7}
6	5.81	0.173	0.122	2.1×10^{-7}
7	5.35	0.113	0.113	2.11×10^{-7}
8	5.26	0.09	0.111	2.11×10^{-7}
9	5	0.107	0.106	2.12×10^{-7}
10	3.77	0.1	0.08	2.12×10^{-7}
11	3.7	0.03	0.078	2.11×10^{-7}
12	3.4	0.027	0.071	2.08×10^{-7}
13	3.3	0.14	0.069	2.09×10^{-7}
14	3.06	0.093	0.063	2.06×10^{-7}
15	2.9	0.05	0.059	2.03×10^{-7}
16	2.63	0.05	0.052	1.98×10^{-7}
17	2.56	0.019	0.05	1.95×10^{-7}
18	2.5	0.02	0.049	1.96×10^{-7}
19	2.38	0.019	0.046	1.93×10^{-7}
20	2.35	0.03	0.045	1.91×10^{-7}

Table 5. (cont'd.)

Order	$S/N \times 10^5$	Observed growth rate (U)	Calculated growth rate	Calculated efficiency
21	2.17	0.023	0.04	1.84 x 10
22	2.11	0.06	0.038	1.8 x 10
23	2.1	0.05	0.038	1.81 x 10
24	1.92	0.176	0.033	1.72 x 10
25	1.9	0.05	0.033	1.74 x 10
26	1.7	0.179	0.027	1.59 x 10
27	1.64	0.073	0.025	1.52 x 10
28	1.61	0.03	0.024	1.49 x 10
29	1.6	0.07	0.024	1.5 x 10
30	1.56	0.09	0.023	1.47 x 10
31	1.23	0.171	0.013	1.06 x 10
32	1.2	0.11	0.012	1 x 10
33	0.9	0.213	0.003	0.33 x 10
34	0.82	0		0

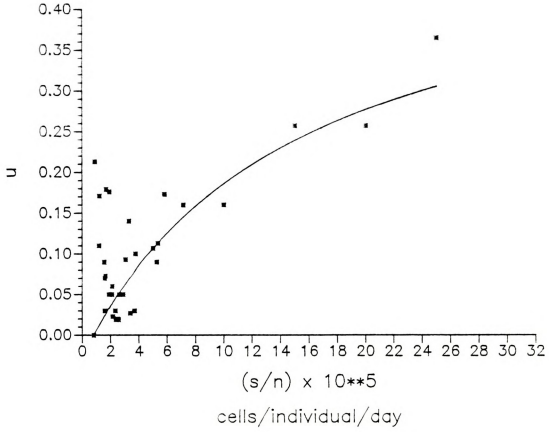

Figure 8. The relationship between the observed growth rate (*), the predicted specific growth rate and the amount of torula yeast available per individual D. pulex per day at 20°C.

with increasing food concentration (S/N). However, the effect of changing one unit of food concentration (S/N) at high food concentrations on the growth rate of D. pulex was less effective than that at low food concentrations. The predicted specific growth rate at a food concentration of 24 x 10^5 yeast cells/individual/day was 0.300 day^{-1}, and at a concentration of 25 x 10^5 yeast cells/individual/day was 0.305 day^{-1}, thus an increase of one unit of food (S/N) at high food concentration caused a 0.005 day^{-1} increase in the growth rate. At a food concentration of 3 x 10^5 yeast cells/individual/day, the growth was 0.062 day^{-1}, and at food concentration of 4 x 10^5 yeast cells/individual/day was 0.085 day^{-1}. Thus an increase of one unit of food (S/N) at low food concentration caused a 0.023 day^{-1} increase in the predicted specific growth rate.

The maximum efficiency was predicted to occur between concentrations of 5 x 10^5 and 3.77 x 10^5 yeast cells/individual/day, therefore a concentration of 4.385 x 10^5 yeast cells/individual/day was considered the concentration at which maximum efficiency occurred (Table 5, Figure 9). At higher concentrations of torula yeast, the efficiency was low and increased with decreasing food concentration until the efficiency approached its apex at a concentration of 4.39 x 10^5 yeast cells/individual/day, after which the efficiency started to decrease again with decreasing food concentration (S/N).

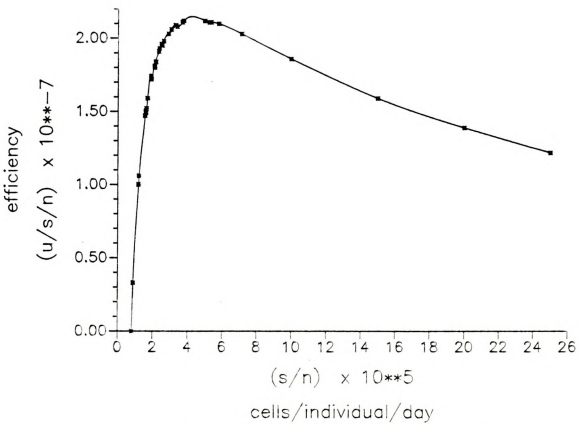

Figure 9. The relationship between the efficiency and the amount of torula yeast available per individual D. pulex per day at 20°C.

Experiment 3:

Five concentrations of torula yeast (0.5×10^4 x 1 x 10^4, 1.5×10^4, 2×10^4 and 2.5×10^4 yeast cells/ml/day) were maintained at 25±1°C in the third experiment (Appendix Tables 5, 6; Figure 10). The population size of Daphnia reached its maxima after 14 days in the three lowest concentrations of yeast (0.5×10^4 x 1 x 10^4, 1.5×10^4 yeast cells/ml/day). However, at the two highest concentrations (2.0×10^4, 2.5×10^4 yeast cells/ml/day, the population reached its maxima after 14 days, attained short equilibrium for 6-7 days. After day 22, the population started to increase again until day 28, after which the population started to decline again.

The threshold concentrations (S/Nq) were 1.04×10^5, 0.89×10^5, 0.76×10^5, 0.81×10^5 and 0.87×10^5 yeast cells/individual/day for the concentrations: 0.5×10^4 x 1 x 10^4, 1.5×10^4, 2×10^4 and 2.5×10^4 yeast cells/ml/day (Table 6). The average 0.87×10^5 yeast cells/individual/day was considered as the threshold concentration for the population of D. pulex growing at 26°C.

The maximum growth rate (Umax) of D. pulex increased with increasing food concentrations (yeast cells/ml/day) (Table 8). Umax's calculations were based on the Michaelis-Menton equation corrected for the threshold concentration at each concentration of food (yeast cells/ml/day). The Umax's

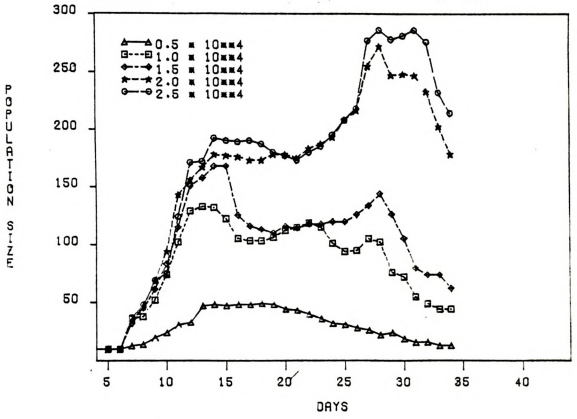

Figure 10. Population size of D. pulex at 26 degrees celsius with five different concentrations of torula yeast (cells/ml/day).

Table 6. The relationship between the observed growth rate of D. pulex and the number of torula yeast cells per individual D. pulex per day at 26°C.

a. for 0.5×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
6	10	0.168	5×10^5
8	14	0.269	3.57×10^5
10	24	0.159	2.08×10^5
12	33	0.187	1.52×10^5
14	48	0	1.04×10^5
16	48	0	1.04×10^5

b. for 1×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
6	10	0.668	10×10^5
8	38	0.333	2.63×10^5
10	74	0.278	1.35×10^5
12	129	0.011	0.78×10^5
14	132	0	0.76×10^5

c. for 1.5×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
6	10	0.752	15×10^5
8	45	0.306	3.33×10^5
10	83	0.299	1.8×10^5
12	151	0.053	0.99×10^5
14	168	0	0.89×10^5
15	168		0.89×10^5

Table 6. (cont'd.)

d. for 2×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
6	10	0.763	20×10^5
8	46	0.357	4.35×10^5
10	94	0.253	2.13×10^5
12	156	0.066	1.2820×10^5
14	178	0.008	1.12×10^5
24	193	0.056	1.03×10^5
26	216	0.045	0.93×10^5
29	247	0	0.81×10^5
31	247		

e. for 2.5×10^4 cells/ml/day

Day	Number of Daphnia	U	S/N cells/ individual/day
6	10	0.784	25×10^5
8	48	0.223	5.2×10^5
10	75	0.412	3.3×10^5
12	171	0.058	1.46×10^5
14	192	0.002	1.3×10^5
24	195	0.056	1.2821×10^5
26	218	0.136	1.14×10^5
28	286	0	0.87×10^5
31	286	0	0.87×10^5

of D. pulex at 26°C were 0.18, 0.772, 0.87, 0.92, and 0.98 day^{-1} for the population growing at concentrations of 0.5 x 10^4 x 1 x 10^4, 1.5 x 10^4, 2 x 10^4 and 2.5 x 10^4 yeast cells/ml/day respectively.

The combined results from the five concentrations of yeast (0.5 x 10^4, 1 x 10^4, 1.5 x 10^4, 2 x 10^4 and 2.5 x 10^4 yeast cells/ml/day) and the observed specific growth are shown in Table 7. Generally, the observed growth rate increased with increasing food concentration (yeast cells/individual/day).

The three constant parameters (Umax, Ks/n and S/Nq) of the Michaelis-Menton equation at 26°C corrected for the threshold concentration were: Umax = 0.91 day^{-1}, Ks/n = 5.6 x 10^5 yeast cells/individual/day, S/Nq = 0.87 x 10^5 yeast cells/individual/day. The equation relating the predicted specific growth rate of D. pulex at 26° to the amount of yeast available per individual Daphnia per day is:

$$U = 0.91 \frac{S/N - 87000}{S/N + 386000}$$

From this equation, Table 7 and Figure 11, it is evident that the predicted specific growth rate of D. pulex increased with increasing food concentration (S/N). However, the effect of changing a unit of food concentration (S/N) on the growth rate of D. pulex at high food concentrations was less effective than the changes at low food concentrations. The predicted growth rate at a food

Table 7. The relationship between the number of torula yeast cells per individual D. pulex per day (S/N), The observed growth rates of D. pulex and the efficiency in the combined results of five concentrations of yeast (0.5×10^4, 1×10^4, 1.5×10^4, 2×10^4 and 2.5×10^4 cells/ml/day) at 26°C.

Order	$S/N \times 10^5$	Observed growth rate (U)	Calculated growth rate	Calculated efficiency
1	25	0.784	.0761	3.044×10^{-7}
2	20	0.763	0.73	3.65×10^{-7}
3	15	0.52	0.682	4.55×10^{-7}
4	10	0.668	0.56	5.6×10^{-7}
5	5.2	0.223	0.435	8.36×10^{-7}
6	5	0.168	0.424	8.48×10^{-7}
7	4.35	0.357	0.386	8.87×10^{-7}
8	3.57	0.269	0.331	9.27×10^{-7}
9	3.33	0.306	0.311	9.34×10^{-7}
10	3.3	0.412	0.301	9.12×10^{-7}
11	2.63	0.333	0.247	9.39×10^{-7}
12	2.13	0.253	0.191	8.97×10^{-7}
13	2.08	0.159	0.185	8.9×10^{-7}
14	1.8	0.299	0.15	8.3×10^{-7}
15	1.52	0.187	0.11	7.24×10^{-7}
16	1.46	0.058	0.1	6.85×10^{-7}
17	1.35	0.278	0.084	6.22×10^{-7}
18	1.3	0.002	0.075	5.77×10^{-7}
19	1.2821	0.056	0.073	5.7×10^{-7}
20	1.2820	0.066	0.073	5.7×10^{-7}
21	1.14	0.136	0.049	4.3×10^{-7}
22	1.12	0.008	0.046	4.11×10^{-7}
23	1.03	0.056	0.03	2.9×10^{-7}
24	0.99	0.053	0.023	2.3×10^{-7}
25	0.93	0.045	0.04	1.18×10^{-7}
26	0.87	0	0	0

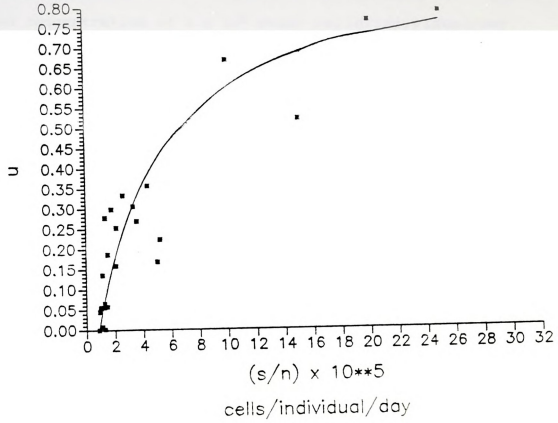

Figure 11. The relationship between the observed growth rate (*), the predicted specific growth rate and the amount of torula yeast available per individual D. pulex per day at 26°C.

concentration of 24 x 10^5 yeast cells/individual/day was 0.756 day^{-1}, and at 25 x 10^5 yeast cells/individual/day was 0.761 day^{-1}, thus an increase of one unit of food (S/N) at high food concentration caused a 0.005 day^{-1} increase in the growth rate. At a food concentration of 3 x 10^5 yeast cells/individual/day, the growth rate was 0.283 day^{-1} and at food concentration of 4 x 10^5 yeast cells/individual/day, the growth rate was 0.362 day^{-1}, thus an increase of one unit of food (S/N) at low food concentration caused a 0.079 day^{-1} increase in the growth rate.

The maximum efficiency was predicted to occur at a concentration of 2.63 x 10^5 yeast cells/individual/day (Figure 12, Table 7). At higher concentrations of torula yeast, the efficiency was low and increased with decreasing food concentration until the efficiency approached its apex at intermediate food concentration, after which the efficiency started to decrease again with decreasing food concentration (S/N).

Comparative results of the three experiments:

The size of D. pulex population increased with increasing the food concentration (yeast cells/ml/day) at each temperature used (12, 20, 26°C). In general, the population size of D. pulex increased with increasing the temperature. However, up to day 25, the higher the temperature, the higher the population size of D. pulex at any food concentration used. After day 27, the population

Table 8. The relationship between different concentrations of yeast (yeast cells/ml/day), temperature and the maximum growth rate[1] (Umax day^{-1}) for D. pulex.

Temperature	Concentration				
	0.5×10^4	1×10^4	1.5×10^4	2×10^4	2.5×10^4
12°C		0.377			0.47
20°C	0.11	0.176	0.318	0.53	0.55
26°C	0.18	0.772	0.87	0.92	0.98

[1]Umax was calculated at each yeast concentration (yeast cells/ml/day) by using the Michaelis-Menton equation corrected for the threshold.

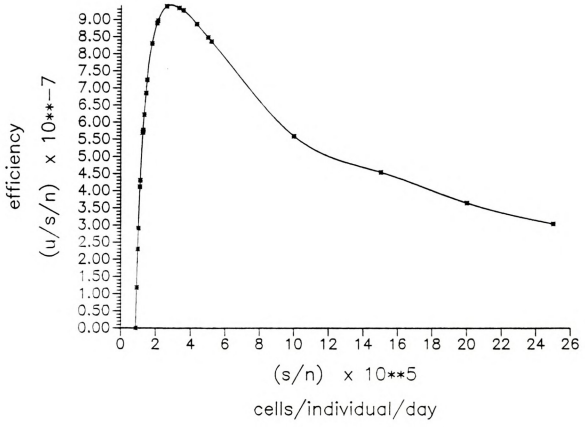

Figure 12. The relationship between the efficiency and the amount of torula yeast available per individual D. pulex per day at 26°C.

size at 20°C started to increase very fast and reached a larger final population size than that of populations growing at 12° and 26°C. The effect of food concentration (yeast cells/ml/day on the population size was proved to be more determined than the effect of temperature (Table 9). The food alone had a significant effect on the population size of D. pulex (P = 0.011). The temperature alone had a significant effect on the population size of D. pulex (P = 0.011). The interaction between food and temperature also had a significant effect on the population size of D. pulex (P = 0.011). It is worth noting that the effect of food concentration was more profound than the effect of temperature on population size of D. pulex since 21% of the variance of population size were due to the food concentration, while only 14% of the variance of population size were due to temperature.

The predicted specific growth rates of D. pulex at 26°C was much higher than that at 12° or 20°C (Figure 13) at any concentration, while at high food concentration (S/N), the growth rates for the population growing at both temperatures (12, 20°C) were almost similar. The threshold concentration (S/Nq) was higher at 12°C (2.7×10^5 yeast cells/individual/day) than at 26°C (0.87×10^5 yeast cells/individual/day) or at 20°C (0.82×10^5 yeast cells/individual/day).

Table 9. Summary of the analysis of variance and multiple classification analysis of the population size of D. pulex at the three levels of temperature (12, 20, 26°C) and the concentrations of food (yeast cells/ml/day).

Source of variation	Significance of F	Beta (R)	R^2
Food*	0.011	0.46	0.2116
Temperature	0.011	0.37	0.1369
Food and Temperature	0.011		

*Two concentrations of yeast used at 12°C (1×10^4, 2.5×10^4 yeast cells/ml/day) and five different concentrations of yeast used at 20°C and 26°C (0.5×10^4, 1×10^4, 1.5×10^4, 2×10^4, and 2.5×10^4 yeast cells/ml/day).

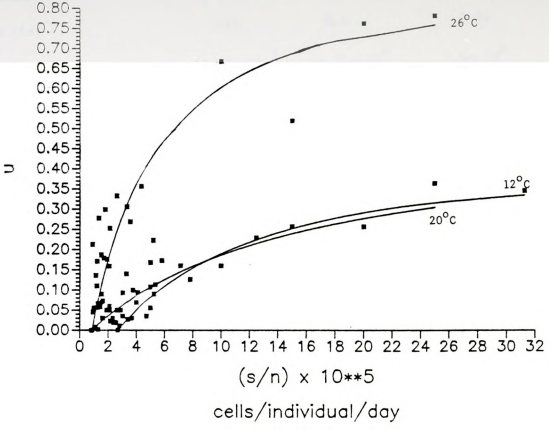

Figure 13. The relationship between the predicted growth rate of D. pulex with the amount of torula yeast available per individual D. pulex per day at the three different temperatures used (12°, 20°, 26°C).

Table 10. A comparison between all the predicted parameters of the three experiments (12°, 20°, 26°C).

Parameter	12°C	20°C	26°C
Umax (day^{-1})	.046	0.5	0.91
$K_{S/N}$ (cells/individual/day)	13.28×10^5	16.32×10^5	5.6×10^5
Maximum efficiency (number of Daphnia/ yeast concentration)	1.92×10^{-7}	2.12×10^{-7}	9.39×10^{-7}
S/N at maximum efficiency (cells/individual/day)	7.81×10^5	4.39×10^5	2.63×10^5
U at maximum efficiency (day^{-1})	0.15	0.093	0.247
Doubling time at maximum efficiency (days $\ln 2/U$ at maximum efficiency	4.62	7.45	2.8
Doublind time at Umax (days) ln2/Umax	1.51	1.39	0.76

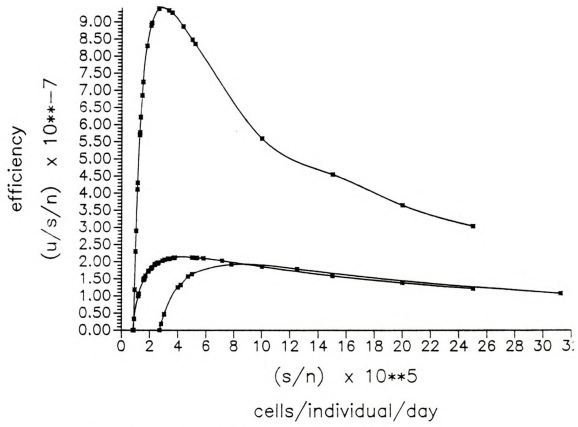

Figure 14. The relationship between the efficiency and the amount of torula yeast available per individual D. pulex per day at the three different temperatures used (12°, 20°, 26°C).

At each temperature, the efficiency (u/s/n) was low at high food concentration (S/N) and increased with decreasing food concentration until the efficiency reached its maxima. The efficiency started to decrease after that with decreasing food concentrations. The efficiency was higher at 26°C than at 12°C or 20°C at any food concentration used (S/N) Figure 14. The efficiency was higher at 20°C than at 12°C. However, at high food concentration the efficiency was almost the same at 12°C and 20°C. However, all the predicted parameters from the three experiments at 12, 20 and 26°C are summarized in Table 10. The amount of torula yeast required per individual Daphnia per day to achieve the maximum efficiency was higher at 12°C (7.81×10^5 yeast cells/individual/day than at 20°C (4.39×10^5 yeast cells/ individual/day) or at 26° C (2.63×10^5 yeast cells/ individual/day). The predicted specific growth rate at maximum efficiency was higher at 26°C (0.15853 day^{-1}) than at 12°C (0.15 day^{-1}) or at 20°C (0.093 day^{-1}). The doubling time (ln 2/U) at maximum efficiency was 4.62 days for the population growing at 12°C, 7.45 days for the population growing at 20°C and 2.8 days for the population growing at 26°C. However, the doubling time at the maximum specific growth rate (Umax) was 1.51 days for the population growing at 12°C, 1.39 days for the population growing at 20° and 0.76 days for the population growing at 26°C.

DISCUSSION

Larvae of most fish feed initially on zooplankton (Sadykov 1975, Arnemo et al. 1980, Lasker 1975). The role of zooplankton in fish culture is important. Zooplankton abundance and composition are clearly affected by fish standing stocks and they can influence the fish yield. A strong correlation has been noted between food conversion rate (Kg food supplied : Kg fish yield) and zooplankton standing stock at zooplankton densities of 0.1 to 1.1 mg dry weight/l. This correlation appeared to be weakest at the highest zooplankton densities (Schroeder 1973).

Fertilizers (organic or inorganic) are very important in enhancing food production for natural feeds by increasing phytoplankton and zooplankton which, in turn, support fish populations. Organic fertilizers are very important in fish culture especially in LDC's where the high quality protein required for fish feeds cost more than these countries can afford. However, the estimation of required nutrients for a pond fertilization program depends on the pond's morphology, hydrology, bottom material, water quality, type of fish cultured and type of fertilizer employed (Yamada 1983).

High rate of survival and growth of walleye fry were achieved by stimulating Daphnia production in ponds by applying sheep and horse manure plus torula or brewers yeast as fertilizers (Merna 1977, Beyerl 1979).

Our observed and predicted growth rates of D. pulex fed different concentrations of torula yeast at three temperatures (12°, 20°, 26°C) are discussed separately.

The batch cultures:

Three experiments were run at three levels of temperature (12°, 20°, 26°C) and five different concentrations of torula yeast at each temperature. Each experiment was terminated when the population stabilized for a short period of time, or when the population density (number of D. pulex per liter) began to decline. In other words, the experiment was terminated when the indirect density effect resulting from interaction between animals and their external environment began to appear (Birch 1948, Slobodkin 1954, Smith 1952).

The number of dead animals was recorded (Appendix Tables 1, 3, 5) in the three experiments. Mortalities were rare in all the experiments until the populations approached the maximum densities, after which the mortalities increased due to a shortage in food supply or build up of metabolities. Since mortalities were minimal, the population growth rate was considered to be equal to the birth rate (Hall 1964).

The mean size of the population densities over time can not be used in comparisons in place of the daily values because mean values are subject to random error of sampling, therefore observed daily variation in Daphnia population density was compared at different concentrations of yeast and levels of temperature (12^{o}, 20^{o}, $26^{o}C$) (Slobodkin 1954).

At each temperature level tested, Daphnia population densities increased with increasing food concentration (yeast cells/ml/day) (Appendix Tables 1, 2, 3, 4, 5, 6,; Figures 4, 7, 10). However, at $12^{o}C$ experiment, the population densities, unexpectedly, decreased at yeast concentrations above 0.5×10^{5} cells/ml/day and the population growth rate decreased above concentration 2.5×10^{4} cells/ml/day (Umax = 0.21 day^{-1} at 0.5×10^{5} yeast cells/ml/day, Umax = 0.377 day^{-1} at 1×10^{4} yeast cells/ml/day and Umax = 0.469 day^{-1} at 2.5×10^{4} yeast cells/ml/day. The reason for this decline might have been the result of decreased water quality with increasing food concentration. Generally, the higher the food concentration, the higher the reproductive rate (Ingle et al. 1937, Fox et al. 1949, Slobodkin 1954). Starvation decreases growth in two ways, it increases the duration of the instars and reduced the increment in each moult.

The effect of changing food concentrations on population densities appeared to be more profound than the effect of changing temperatures based on multiple

classification analysis (Table 9). Twenty one percent of the variance of population size were due to yeast concentration and 14% of the variance in population size were due to temperature. This observation is in agreement with those of Ingle (1937), Richman (1958), Allen (1976) and Lampert (1977b) who stated that the food concentration is regarded as the most important environmental factor influencing the production of D. pulex.

At 12°C (Figure 4), the Daphnia populations grew slowly and the final population density was lower than that at 20° or 26°C possibly due to decreased metabolic activities of Daphnia at 12°C (MacArthur and Baillie 1929, McLaren 1963).

At 20°C, the Daphnia population growth was unexpectedly slow during the first 25 days of the experiment. From day 25 to 36 the population increased very fast, followed by a decline (Figure 7). The slow growth at the beginning of this experiment decreased the final growth rate of the population at 20°. The reason for this decline might have been the age-size frequency distribution (Slobodkin 1954). The adults began to reproduce slowly at the beginning. By day 25, the population had a large number of reproducing Daphnia which caused the numerical maxima. Consequently, the food supply decreased which caused high mortalities. As a result, the growth rate at 20°C was almost the same as the growth rate at 12°C despite the large difference in final population sizes.

At 26°C (Figure 10), the Daphnia populations approached its maxima after 14 days in the first three yeast concentrations (0.5×10^4, 1×10^4 and 1.5×10^4 cells/ml/day), then began to decrease possibly due to shortage in food supply. In higher yeast concentrations (2×10^4, 2.5×10^4 cells/ml/day), the populations increased up to day 14, attained a short equilibrium period from day 14 to day 22, after which they started to increase again till a second maxima at day 28, then they started to decrease. The short equilibrium period might have resulted from the adult Daphnia exhausting their energy supply through massive reproduction during the first 14 days of the experiment (Richman 1958, Green 1956). The new generation produced prior to day 22 started to reproduce to form the second maxima of the population density, after which the populations started to decrease possibly due to the shortage in food supply. Pratt (1943), Slobodkin (1954) and Frank (1952) studied single species population of Daphnia under somewhat similar conditions and observed that a population after reaching an initial peak declined somewhat and then irregularly fluctuated without any apparent changes in environmental factors. Population lags (age-size frequency distribution) and individual lag play a very important role in the oscillation process in Daphnia population (Slobodkin 1954). However, the differences in the genetic composition

of experimental population may be responsible for the oscillation (Herbert 1978, Weider 1984).

Generally, population peaks coincided with the maximum proportion of small animals and the population troughs with the maximum proportion of large animals (Slobodkin 1954, Pratt 1943, Frank 1952). However in this study in the early stages of population growth, the few adult animals had a higher reproductive rate, resulting in a size frequency distribution that was skewed towards the small end (personal observation). This early population increase eventually gave rise to a population which was sufficient to reduce the food supply of all the animals so that reproduction almost stopped. At this point the population was at the apex of its initial numerical peak. It consisted largely of small animals.

The experiment at 12°C was started with 1-5 day old Daphnia and they began to reproduce after 10 days. The experiment at 20°C was started with 1-3 day old Daphnia and they began to reproduce after 9 days. At 26°C, the experiment was started with 1-2 day old Daphnia and they began to reproduce after 7 days. This suggested an inverse relationship between temperature and the development time of the organism as noted by (Pratt 1943, McLaren 1963, MacArthur and Baillie 1929, Allan 1976).

A population with an infinitesimally low rate of increase may reach a greater asymptote than that developed in a shorter

time with a much higher rate of increase (Pratt 1943). We found that the final population size at 20°C was greater than that at 26°C despite the large difference in the maximum rate of population growth at 20°C and at 26°C (Umax at 20°C = 0.5 day^{-1}, Umax at 26°C = 0.91 day^{-1}) (Table 10). However, the neonates from a slow growing population grew to larger size and lived for a longer period of time than the neonates from fast growing populations (Smith 1963).

The population increased more rapidly at 26°C and the population growing at the other temperatures (12°, 20°C). This may have been due to increased metabolic activities of Daphnia at 26°C. However, MacArthur and Baillie (1929) stated that at normal temperatures and in both sexes the product of length of life x heart rate was approximately a constant, that is nearly 15,400,000 heart beats will occur in a daphnid's life regardless of temperature or sex, therefore the duration of life varied inversely with the intensity of the metabolism in D. magna.

At each temperature level, D. pulex developed increased reddish coloration which meant an increase in the lipid index with increasing food concentrations (Tessier and Goulden 1982, Holm and Shapiro 1984). The population growing at 20°C had the greatest reddish coloration which meant an increase in the lipid index which might be because of optimal fitness conditions compared to populations at 12° or 26°C as noted by Lynch (1977). [Fitness is equal to feeding

rate (energy ingested/animal/time) divided by the basal metabolic rate (energy expended/animal/time.]

Generally, the optimal life history strategy of an organism is to allocate its resource to maintenance, growth and reproduction so as to maximize its contribution to the persistence of the population (Lynch 1977). For any age class, it becomes advantageous to devote a large share of resources to growth and survival when future reproductive output is significantly enhanced. Increasing reproductive effort at any age will augment fecundity at that age, but only at the expense of future growth ($26^{o}C$ experiment). Thus, while cladocerans continue to grow throughout their life, the rate of growth declines with the onset of reproduction (Anderson et al. 1937, Green 1956, Richman 1958).

Prediction for the continuous culture:

The continuous culture technique is designed to put the population at a selected specific growth rate (Novick and Szilord 1950) and thus at a constant food level (number of yeast cells/individual Daphnia/day). A modified Michaleis-Menton equation with a correction for the threshold concentration which predicts the relationship between food availability or resource availability and the specific growth rate of D. pulex is:

$$U = Umax \quad \frac{S/N - S/Nq}{S/N + Ks/n - 2S/Nq}$$

where

U = specific growth rate of *D. pulex* (day^{-1})

U_{max} = maximum growth rate of *D. pulex* (day^{-1})

S/N = number of yeast cells per individual Daphnia per day

$K_{s/n}$ = number of yeast cells per individual Daphnia per day when U is equal to 0.5 Umax.

S/Nq = number of yeast cells per individual Daphnia per day required to initiate the growth rate of *D. pulex* (the threshold concentration)

The prediction of population densities depend on the equation:

$$N_2 = N_1 e^{U\Delta t}$$

where

N_1 = numbers of the population at time 1

N_2 = numbers of the population at time 2

Δt = difference in time

U = specific growth rate of *D. pulex*

or

$$N_2 = N_1 e^{\left(U_{max}\frac{S/N - S/Nq}{S/N + K_{s/n} - 2S/Nq}\right) \cdot \Delta t}$$

Thus, we can calculate the constant requirements of food available per organism per day at any value of the growth rate of the organism.

In ecology, the Michaelis-Menton or Monod equation (Monod 1949)

$$R = R_k * C/C + C_1$$

where

R = specific growth rate of organism or the rate of uptake of a substrate

Rk = maximum growth rate of organism, or the maximum rate of uptake of a substrate

C = substrate concentration

C_1 = concentration of C when R = 0.5 RK

has been used to study complex phenomena as rate of uptake of organic and inorganic nutrients by natural population (Strickland 1962, Wright and Hobbie 1966, Dugdale 1967, MacIsaac and Dugdale 1969, Krembeck 1979). This sort of analysis is instructive if the equation is obeyed, but if the results don't appear to fit the equation, as has happened in some studies of heterotrophic processes (e.g. Vaccaro and Jannasch 1967, Hamilton and Presland 1970), there is a dilemma. Vaccaro and Jannasch (1967) suggested that the failure to fit the Michealis-Menton equation may have stemmed from the heterogenous nature of the indigenous marine population. At low substrate concentration, the observed rates were higher than the predicted ones. The discrepancy between observed and predicted values increased as the population became more diverse (Williams 1973). However, Fogg (1975) studied the relationship of relative growth rate of algae to nutrient concentration. He stated that this relation is more complicated than the simple hyperbolic expression of Monod equation (without correction for the threshold. The Michaelis-Menton kinetics may

accurately describe the uptake of nutrients but relative growth rate is dependent more directly on intracellular concentration rather than the rate at which the nutrients enter the cell.

The maximum specific growth rate (Umax) of D. pulex at 20°C (0.5 day^{-1}) was almost equal to the maximum specific growth rate at 12°C experiment (0.46 day^{-1}) (Figure 13, Table 10). The population at 20°C grew slowly at the beginning of the experiment which caused a decrease in the growth rate despite the fact that they grew very fast at the end of the experiment.

The threshold concentration from the Michaelis-Menton equation is the amount of food required to maintain the organism (e.g. movements, locomotion, standard metabolism). It is the amount of food required to initiate the growth rate of the organism (King and Garling 1983). The threshold value calculated by dividing the concentration of the food (number of yeast cells/day) by the maximum number of D. pulex (Sq = S/K) when the growth rate equaled zero based on the assumption that the growth rate equals zero after the population reaches the maximum population number. Beyond this point the growth rates became negative after the D. pulex reached the maximum population number in most of my experiments resulting from a decline in the population size.

The threshold concentration was higher at 12°C than at 20°C or 26°C (Figure 13, Table 10). This may have resulted from two reasons.

Firstly, the population density of Daphnia was lower at 12°C than in the other temperatures tested (20, 26°C) which increased the S/K value. Secondly, at 12°C there was a higher percentage of large animals presumably due to decreased reproductive ability at that temperature. Lampert (1977c) observed that larger Daphnia required a higher threshold concentration.

The threshold concentration at 26°C was higher than at 20°C (Table 10, Figure 13). This might have been due to an increase in the metabolic activities and, consequently, energetic needs at 26°C compared to 20°C (Richman 1958). However, Lampert (1977c) found that the threshold concentration for D. pulex increased with increasing the temperature up to 25°C. The threshold concentration for D. pulex increased with increasing the temperature up to 25°C. The threshold concentration at 20°C was lower than at the other temperatures tested. The low threshold value was expected at 20°C since the largest maximum population size (K) occurred at 20°C or perhaps as a result of maximum feeding efficiency, which is defined as feeding rate/basal metabolic rate which was high at 20°C as stated by (Burns 1968, 1969; Lynch 1977). However, the relationships of assimilation efficiency to food quantity and quality,

temperature and body size in D. pulex are not clear due to conflicting observations between experiments (Lynch 1977, Leonard and Lawrence 1981).

The efficiency of a machine is the ratio of the output to the input (Slobodkin 1960). The term efficiency here (U/s/n) represented the food conversion rate as number of D. pulex divided by the amount of food available per time as compared to

the definition given by Reeve (1963) where food conversion rate was equated with the weight of animals produced/weight of the food conversion rate was equated with the weight of animals produced/weight of the food consumed. There was a definite peak efficiency at each temperature (Figure 14). The peak always occurred at low food concentrations. Reeve (1962) observed that the food remained in the gut of Artemia progressively longer at low food concentrations, presumably from less pressure in the absence of larger amounts of the incoming food to force it through the gut. The longer stay in the gut should result in greater digestive efficiency. At very low concentrations of food, the efficiency was low since there was greater effort involved in feeding process which was not compensated adequately by the food ingested. Lower efficiency at high feed concentrations may have been caused by an increase in the food rejection rate and respiration rate (Porter et al. 1982), or by an increase in food ingestion rate which exceeded the capacity of the gut

to digest food (Slobodkin 1960). However, growth efficiency is related to the physical and biological conditions of any particular laboratory experiment, and can not be directly related to observations from fluctuating natural habitats (Reeve 1963). Strict comparison should not even be made between growth efficiency estimates from different laboratory observations where units measured have included dry weight (Ivlev 1939, Ricker 1946), calorific value (Richman 1958, Slobodkin 1960) and radioactive carbon (Lasker 1960). The increase in size of a given animal associated with increasing age would be expected to increase the energy cost of its maintenance, and to reduce correspondingly the total efficiency of growth unless the increase in maintenance is compensated for by an increase in growth rate (Brody 1945). Efficiency increased with increasing temperatures from 12°C to 20°C to 26°C (Figure 14). This might have been due to an increased growth rate of with Daphnia at increasing temperatures at any level of food concentration used (Figure 13).

The doubling time is the length of time necessary to double population size at any value of the specific growth rate (Hall 1964). It is calculated by dividing ln 2 by the growth rate. Thus, the higher the growth rate, the lower the doubling time of the population. The doubling time at maximum growth rate was longer at 12°C (1.51 days) than at 20°C (1.39 days) or at 26°C (0.76 days). This occurred as a

result of the increase in the maximum growth rate from 12°C to 200C to 26°C (Table 10).

Also, the doubling time at maximum efficiency was longer at 20°C (7.45 days) than at 12°C (4.62 days) or at 26°C (2.8 days). The increase of doubling time at maximum efficiency at 20°C was due to decreased growth rate at 20°C at maximum efficiency.

Based on our experimental results, it appears that:

1. If torula yeast is at a reasonable cost, readily available in large quantities, high concentrations of yeast should be used to insure high growth rate. However, very high concentration of food may lead to depletion of oxygen, an increase in ammonia concentration and other disadvantages in the system.
2. If the yeast is not available in large quantities or expensive, it should be used at maximum efficiency ($.7.81 \times 10^5$, 4.39×10^5 and 2.63×10^5 yeast cells/individual D. pulex per day for temperatures 12, 20 and 26°C respectively). However, the point of maximum dollar efficiency (maximum yield in terms of dollars) must be considered as the best concentration which can be used despite the fact that it might be higher than the concentration of food required at maximum efficiency.

Generally, the prolonged exposure of D. pulex to any constant yeast concentration may result in unpredictable population changes and growth characteristics (Jannasch 1974). Therefore, more research is needed to clearly establish the relationship between prolonged exposure to different concentrations of yeast and the population dynamics of D. pulex.

Finally, more research is needed to determine the long term relationship between zooplankton densities and fish fry requirements before generally recommendations can be made with confidence.

SUMMARY

1. As the age of the culture increased, the population size increased then stabilized or declined.

2. As the food concentration increased, the population size and the growth rate increased.

3. As the temperature increased, the growth rate of D. pulex increased and the population size increased for a short period of time then declined.

4. The effect of food concentration and temperature and the interaction between them on population size of D. pulex was significant.

5. The effect of yeast concentration (cells/ml/day) on Daphnia population size was more profound than the effect of temperature.

6. The relationship between the specific growth rate of D. pulex and the amount of torula yeast available per individual D. pulex per day followed the Michaelis-Menton equation with a correction for the threshold concentration.

7. The lower threshold yeast concentration of *D. pulex* occurred at 20°C while the larger one occurred at 12°C.

LITERATURE CITED

LITERATURE CITED

Adams, J. A., and J. H. Steele. 1966. Shipboard experiments on the feeding of Calanus finmarchicus (Gunnerus), p. 19-35 In. H. Barnes (ed.), some contemporary studies in marine science. Allen and Unwin.

Allen, J. D. 1976. Life history pattern in zooplankton. Am. Nat. 110: 165-180.

Anderson, B. G., H. Lumer, and L. J. Zupancic, Jr. 1937. Growth and variability in Daphnia pulex. Biol. Bull. 73: 444-463.

Arnemo, R., C. Puke, N. G. Steffner. 1980. Feeding during the first weeks of young salmon in a pond. Arch. Hydrobiol. 89 (42): 265-273.

Banta, A. M. 1939. Studies on the physiology, genetics and evolution of some Cladocera. Pap. Dep. Genet. Garneg. Instn. No. 39.

Beklemishev, C. W. 1962. Superfluous feeding of marine herbivorous zooplankton. Rappt. Proces-Verbaux Reunions, Conseil Perm. Internat. Exploration Mer. 153: 108-113.

Berg, K. 1931. Studies on the genus Daphnia O. F. Muller with especial reference to the mode of reproduction. Vidensk. Medd. dansk naturh. Foren. Kbh. 92: 1-222.

Berman, M. S., and S. Richman. 1974. The feeding behavior of Daphnia pulex from Lake Winnebago, Wisconsin. Limnol. Oceanogr. 19: 105-109.

Beyerle, G. B. 1979. Extensive culture of walleye fry in ponds at the Wolf Lake Hatchery. Mich. Dept. Nat. Resour., Fish. Res. Rep. 1874. 28 pp.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Animal Ecol. 17(1): 15-26.

Boyd, C. E. and F. Lichtokopplier. 1979. Water quality management in pond fish culture. Auburn University Experiment Station, Alabama. 30 pp.

Brody, S. 1945. Bioenergetic and growth. Reinhold Publishing Co., New York.

Brooks, J. L. 1957. The systematic of month American Daphnia. Mem. Comm. Acad. Arts and Sci. 13. pp. 58-66.

_____. 1959. Cladocera, p. 587-656. In: W. T. Edmonson, (ed). Freshwater biology, 2nd Ed. Wiley, New York, NY.

Brooks, J. L. and S. I. Dodson. 1965. Predation, body size and composition of plankton. Science (Washington, D.C.) 150: 28-35.

Brown, L. A. 1927. Temperature characteristics for duration of an instar in Cladocerans. J. Gen. Physiol. 10: 111-119.

Buening, I. 1978. A model predicting population growth of laboratory cultures of Daphnia magna as a function of food availability (Abstract). Ohio J. Sci. 78 (suppl.) 89.

Burns, C. W. 1968. The relationship between body size of filter feeding Cladocera and the maximum size of particles ingested. Limnol. Oceanogr. 13: 675-678.

_____. 1969. Relation between filtering rate, temperature, and body size in four species of Daphnia. Limnol. Oceanogr. 14: 693-700.

Burns, C. W., and F. H. Rigler. 1967. Comparison of filtering rates of Daphnia rosea in lake water and in suspensions of yeast. Limnol. Oceanogr. 12: 492-502.

Caughley, G., and L. C. Birch. 1971. Rate of increase. J. Wildl. Management 35(4): 658-663.

Colt, J. 1983. Pond culture practices. pp: 187-197. In: Lannon, J. E., Smitherman, R. O.; Tchobanoglous, G. (eds). Principles and practices of Pond Aquaculture: A state of the art review. USAID Title XII CRSP in Pond Dynamics/Aquaculture Report (Grant No. AID/DSNA-G-0264). Oregon State University, Marine Science Center, Newport, OR.

Corner, E. D., R. N. Head, and C. C. Kilvington. 1972. On the nutrition and metabolism of zooplankton. The grazing of Biddulphia cells by Calanus helgolandicus. J. Mar. Biol. Assoc. U. K. 52: 847-861.

Detwyler, R. and E. D. Houde. 1970. Food selection by laboratory-reared larvae of the scaled sardine Harengula pensacolae (Pisces, Clupeidae) and the bay anchovy Anchoa mitchilli (Pisces, Engraulidae). Mar. Biol. 7: 214-222.

Dugdale, R. C. 1967. Nutrient limitation in the sea: dynamics, identification and significance. Limnol. Oceanogr. 12: 685-695.

Dunham, H. H. 1938. Abundant feeding followed by restricted feeding and longevity in Daphnia. Physiol. Zool. 11: 399-407.

Evans, F. C., and F. E. Smith. 1952. The intrinsic rate of natural increase for the human louse, Pediculus humanus L. Amer. Nat. 86: 299-310.

Fogg, G. E. 1975. The characteristics of algal growth in cultures of limited volume. In Algal culture and phytoplankton ecology. 2nd ed. Madison: University of Wisconsin Press, 1975: 21-36.

Fox, H. M. 1948. The haemoglobin of Daphnia. Proc. R. Soc. Lond. B. Biol. Sci. 135: 195-212.

_____. 1949. Haemoglobin in crustacea. Nature 164: 59.

Fox, H. M., B. M. Gilchrist and E. A. Phears. 1951. Functions of haemoglobin in Daphnia. Proc. R. Soc. Lond. B. Biol. Sci. 138: 514-528.

Frank, P. W. 1952. A laboratory study of intraspecies and interspecies in Daphnia pulicaria (Forbes) and Simocephalus vetulus O. F. Muller. Physiol. Zool. 25: 178-204.

Frank, P. W., C. D. Bell, and R. W. Kelly. 1953. Vital statistics of laboratory cultures of Daphnia pulex De-Greer as related to density. Physiol. Zool. 30: 287-305.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod Calanus pacificus. Limnol. Oceanogr. 17: 805-815.

Gorbunova, N. N. and N. Y. Lipskaya. 1975. Feeding of larvae of blue marlin, Makaina nugricans (Pisces, Istiophoridae). J. Ichthyol. 15: 95-101.

Green, J. 1956. Growth, size and reproduction in Daphnia (Crustacea: Cladocera). Proc. Zool. Soc. London. 126: 173-204.

Guilliermond, A. 1920. The yeasts. New York: John Willey and Sons, Inc. pp. 12-122.

Hall, D. J. 1964. An experimental approach to the dynamics of a natural population of Daphnia Galeata Mendotae. Ecology 45: 94-112.

Hamilton, R. D., K. M. Morgan, and J. D. H. Strickland. 1966. The glucose uptake kinetics of some marine bacteria. Can. J. Microbiol. 12: 995-1003.

Haney, J. F. 1973. An in situ examination of the grazing activities of natural zooplankton communities. Archiv. Hydrobiol. 72: 87-132.

Hayward, R. S., and D. N. Gallup. 1976. Feeding and filtering and assimilation in Daphnia schoedleri affected by environmental conditions. Arch. Hydrobiol. 77: 139-163.

Herbert, P. D. N. 1978. The population biology of Daphnia (Crustacea, Daphnidae). Biol. Rev. 53: 387-426.

Hollerman, W. D. and C. E. Boyd. 1980. Nightly aeration to increase product of channel catfish. Trans. Am. Fish. Soc., 109(4): 446-452.

Holm, N. P., G. G. Ganf, and J. Shapiro. 1983. Feeding and assimilation rates of Daphnia pulex fed Aphanizomenon flos-aquae. Limnol. Oceanogr. 28(4): 677-687.

Holm, N. P., and Shapiro, J. 1984. An examination of lipid reserves and the nutritional status of Daphnia pulex fed Aphanizomenon flos-aquae. Limnol. Oceanogr. 29(2): 1137-1140.

Ingle, L., T. R. Wood, and A. M. Banta. ____. A study of longevity, growth, reproduction and heart rate in Daphnia longispina as influenced by limitations in quantity of food. J. Exper. Zool. 76: 325-352.

Ivlev, V. S. 1939. Transformation of energy by aquatic animals. Int. Rev. Hydrobiol., 38: 449-458.

Jannasch, H. W. 1974. Steady state and the chemostat in ecology. Limnol. Oceanogr. 19: 716-720.

King, D. L., and Garling, D. L. 1983. A state of the art overview of aquatic fertility with special reference to control exerted by chemical and physical factors. pp.: 67-76 In: Lannon, J. E.; Smitherman, R. O.; Tchobanoglous, C. (eds). Principles and Practices of Pond Aquaculture: A State of the Art Review. USAID Title XII CRSP in Pond Dynamics/Aquaculture Report (Grant No. AID/DSAN-G-0264). Oregon State University, Marine Science Center, Newport, OR.

Kring, R. L. 1976. Accommodation of Daphnia pulex to altered pH conditions as measured by feeding rate. Limnol. Oceanogr. 21: 313-315.

Kring, R. L. and W. J. O'brien. 1976. Effect of varying oxygen concentrations on the filtering rate of Daphnia pulex. Ecology 57: 808-814.

Krombeck, C. 1979. Applicability and limitations of the Michaelis-Menton equation in microbial ecology. Arch. Hydrobiol. 12: 64-76.

Lam, R. K., and B. W. Frost. 1976. Model of copepod filtering response to changes in size and concentration of food. Limnol. Oceanogr. 21: 490-500.

Lampert, W. 1977a. Studies on the carbon balance of Daphnia pulex de Geer as related to environmental conditions. II. The dependence of carbon assimilation on animal size, temperature, food concentration and diet species. Arch. Hydrobiol. Suppl. 48: 310-335.

_____. 1977b. Studies on the carbon balance of Daphnia pulex de Geer as related to environmental conditions. III. Production and production efficiency. Arch. Hydrobiol. Suppl. 48: 336-360.

_____. 1977c. Studies on the carbon balance of Daphnia pulex de Geer as related to the environmental conditions. IV. Determination of the threshold concentration as a factor controlling the abundance of zooplankton species. Arch. Hydrobiol. Suppl. 48: 361-368.

Landon, M. S. and R. H. Stansiak. 1983. Daphnia haemoglobin concentration as a function of depth and oxygen availability in Arco Lake, Minnesota. Limnol. Oceanogr. 28(4): 731-737.

Laska, R. 1960. Utilization of organic carbon by a marine crustacean. Science 131: 1098.

_____. 1975. Field criteria for survival of anchovy larvae: The relation between inshore chlorophyll maximum layers and successful first feeding. Fishery Bulletin 73(3): 453-4628

Lehman, J. T. 1976. The filter feeders as an optimal forager, and the predicted shapes of feeding curves. Limnol. Oceanogr. 21: 501-516.

Leonard, S. L., and S. G. Lawrence. (Unpublished data).

Lin, C. K. 1983. Biological principles of pond culture: Phytoplankton and macrophytes. pp. 39-43. In: Lannan, J. E.; Smitherman, R. O.; Tchobanoglous, G. (eds). Principles and Practices of Pond Aquaculture: A State of the Art Review. USAID Title XII CRSP in Pond Dynamics/Aquaculture Report (Grant No. AID/DSAN-G-0264). Oregon State University, Marine Science Center, Newport, OR.

Lindeman, R. L. 1942. The trophic dynamic aspect of ecology. Ecology 23: 399-418.

Lotka, A. J. 1925. Elements of physical biology. Baltimore, Williams and Wilkins. 460 pp.

Lynch. M. 1977. Fitness and optimal body size in zooplankton populations. Ecology 58: 763-774.

MacArthur, J. W., and W. H. T. Baillie. 1929a. Metabolic activity and duration of life. I. Influence of temperature on longevity in Daphnia magna. J. Exp. Zool. 53: 221-242.

MacIsaac, J. J., and R. C. Dugdale. 1969. The kinetics of ammonia uptake by natural populations of marine plankton. Deep Sea Res. 16: 45-57.

McMahon, J. W. 1965. Some physical factors influencing the feeding behavior of Daphnia magna straus. Can. J. Zool. 43: 603-611.

McMahon, J. W. and F. H. Rigler. 1965. Feeding rate of Daphnia magna straus in different foods labelled with radioactive phosphorus. Limnol. Oceanogr. 10: 105-113.

McLaren, I. A. 1963. Effects of temperature on growth of zooplankton and the adaptive value of vertical migration. J. Fish. Res. Board Can. 20: 685-727.

Merna, J. W. 1977. Food selection by walleye fry. Mich. Dept. Nat. Resour., Fish Res. Rep. 1847. 12 pp.

Mertiz, E. T. 1972. The protein and amino acid needs. In J. E. Haver (ed.), Fish Nutrition, pp. 106-143. Academic Press, Inc., New York.

Metz, H. 1973. Kohlenstoffinholt, Aufnahme und Abgabe des Kohlenstoffs bei Daphnia pulex. Arch. Hydrobiol., Suppl. 42(2): 232-252.

Monod, J. 1949. The growth of bacterial cultures. Ann. Rev. Microbiol. 3: 371-393.

Mullin, M. M., E. Fuglister-Stewart and F. J. Fuglister. 1975. Ingestion by planktonic grazers as a function of concentration of food. Limnol. Oceanogr. 20(2): 259-262.

Myers, J., and L. B. Clark. 1944. Culture conditions and the development of the photosynthetic mechanism. II. An apparatus for the continuous culture of chlorella. J. Gen. Physiol. 28: 103-112.

Myers, J., and J. Graham. 1959. On the mass culture of algae. II. Yield as a function of cell concentration under continuous sunlight irradiance. PL. Physiol. 34: 345-352.

Nandy, A.C., P. R. Das and S. K. Majumder. ____ Technique to obtain sustained culture of a cladoceran: Daphnia Holtzi sars: Proceeding of the Symposium on Warmwater Zooplankton, 1976 Oct. 14-19. National Institute of Oceanography, Dona Paula, GOA. India (Abstract).

Novick, A., and L. Szilard. 1950. Description of the chemostat. Science. 112 (715-716).

Parsons, T. R., and J. D. H. Strickland. 1962. On the production of particulate organic carbon by heterotrophic processes in sea water. Deep Sea Res. 8: 211-222.

Parsons, T. R., and J. D. Fulton. 1967. Some observations on the dependence of zooplankton grazing on the cell size and concentration of phytoplankton blooms. J. Oceanogr. Soc. Jap. 23: 10-17.

Peters, R. H., and J. A. Downing. 1984. Empirical analysis of zooplankton filtering and feeding rate. Limnol. Oceanogr. 29(4): 763-784.

Peterson, B. J., J. E. Hobbie and J. F. Haney. 1978. Daphnia grazing on natural bacteria. Limnol. Oceanogr. 23: 1039-1044.

Porter, K. G., J. Gerritsen and J. D. Orcutt, Jr. 1982. The effect of food concentration on swimming patterns, feeding behavior, ingestion, assimilation and respiration by Daphnia. Limnol. Oceanogr. 27(5): 935-949.

Pratt, D. M. 1943. Analysis of population development in Daphnia at different temperatures. Biological Bulletin 85: 116-140.

Richman, S. 1958. The transformation of energy by Daphnia pulex. Ecol. Monogr. 28: 273-291.

Ricker, W. E. 1946. Production and utilization in fish populations. Ecol. Monogr. 16: 375-391.

Reeve, M. R. 1963. The feeding of artemia. I. In pure culture. J. Exp. Biol. 40: 195-205.

_____. 1963. Growth efficiency in artima under laboratory conditions. Biol. Bulletin 125: 133-145.

Reeve, M. R., and M. A. Walter. 1977. Observations on the existence of lower threshold and upper critical food concentrations for the copepod Acartia Tonsa Dana. J. Exp. Mar. Biol. Ecol. 29: 211-221.

Sadykhov, D. A. (deceased), I. B. Bogatova, V. I. Filatov. 1975. The amino acids in certain representatives of the fresh water zooplankton. Hydrobiol. J. 11(6): 39-42.

Schroeder, G. L. 1973. Factors affecting feed conversion ratio in fish poinds. Bamidgeh 25(3): 104-113.

Seale, D. B., and N. Beckvar. 1980. The comparative ability of Anuran larvae (Genera: Hyla, Bufo, and Rana) to ingest suspended blue-green algae. Copeia 3: 495-503.

Slobodkin, L. B. 1954. Population dynamics in Daphnia obtusa kurza. Ecol. Monog. 24: 69-88.

Slobodkin, L. B. 1960. Ecological energy relationships at the population level. The American Naturalist. XCIV: 213-236.

Smith, F. E. 1952. Experimental methods in population dynamics: A critique. Ecology. 33: 441-450.

Smith, F. E. 1963. Population dynamics in Daphnia magna and a new model for population growth. Ecology: 44: 651-663.

Stephen, W. P., Jr. 1976. Feeding of laboratory-reared larval of the sea bream Archosargus rhombiodalis (Sparidae). Mar. Biol. 38: 1-16.

Tamas, G. and L. Horvath. 1976. Growth of cyprinids under optimal zooplankton conditions. Bamidgeh 28: 50-56.

Tang, Y. A. 1970. Evaluation of balance between fishes and available fish foods in multispecies fish culture ponds in Taiwan. Trans. Am. Fish. Soc. 99: 708-718.

Taub, F. B. and A. M. Dollar. 1968. The nutritional inadequacy of Chlorella and Chlamydomnas as food for Daphnia pulex. Limnol. Oceanogr. 13: 607-617.

Tamson, A. 1930. Die wirkung der au sseren Bedingungen auf die Veranderung des Geschlechts und auf die Entwicklung von Daphnia pulex de Geer. Roux Arch. Entw. Mech. Organ. 123: 80-131.

Tessier, A. J., and C. E. Goulden. 1982. Estimating food limitation in Cladoceran population. Limnol. Oceanogr. 27 (4): 707-717.

Torrans, E. L. 1983. Fish plankton interaction. p. 77-88 In Lannon, J. E; Smitherman, R. O.; Tchobanoglous, G. (eds.). Principles and Practices of Pond Aquaculture: A State of the Art Review. USAID Title XII CRSP in Pond Dynamics/Aquaculture Report (Grant No. AID/DSAN-G-0264). Oregon State University, Marine Science Center, Newport, OR.

Vaccaro, R. F., and H. W. Jannasch. 1967. Variations in uptake kinetics for glucose by natural populations in sea water. Limnol. Oceanogr. 12: 540-542.

Weider, L. J. Spatial heterogeneity of Daphnia genotypes. Vertical migration and habitat partitioning. Limnol. Oceanogr. 9(2): 225-235.

Williams, P. J. 1973. The validity of the application of simple kinetic analysis to heterogenous microbial populations. Limnol. Oceanogr. 18: 159-165.

Wright, J. C. 1965. The population dynamics and production of Daphnia in Canyon Ferry Reservoir, Montana. Limnol. Oceanogr. 10: 583-590.

Wright, R.T., and J. E. Hobbie. 1965. The uptake of organic solutes in lake water. Limnol. Oceanogr. 10: 22-28.

_____, and _____. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystem. Ecology 47(3): 447-464.

Yamad, R. 1983. Pond production system: Fertilization practices in warm water fish pond. pp. 103-115. In: Lannon, J. E.; Smitherman, R. O.; Tchobanoglous, G. (eds.). Principles and Practices of Pond Aquaculture: A State of the Art Review. USAID Title XII CRSP in Pond Dynamics/Aquaculture Report (Grant No. AID/DSAN-G-0264). Oregon State University, Marine Science Center, Newport, OR.

Young, T. C., and D. L. King. 1980. Interacting limits to algal growth: light, phosphorus and carbon dioxide availability. Water Research, Vol. 14: 409-412.

APPENDICES

Appendix Table 1. Daily numbers of D. pulex living (L), dead (D) and replaced (R) concentrations of torula yeast cultured in duplicate (1, 2) at 12°C.

		Concentration of torula yeast (ml/day)														
Day		1×10^4			2.5×10^4			0.5×10^5			1×10^5			2×10^5		
		L	D	R	L	D	R	L	D	R	L	D	R	L	D	R
1	1	10			10			10			10			10		
	2	10			10			10			10			10		
2	1	9	1	1	9	1	1	10	0	0	10	0	0	8	2	2
	2	9	1	1	8	2	2	10	0	0	10	0	0	10	0	0
3	1	8	2	2	8	1	1	10	0	0	10	0	0	10	0	0
	2	9	1	1	7	3	3	9	1	1	9	1	1	10	0	0
4	1	8	2		10	0		10	0		10	0		10	0	
	2	9	1		10	0		9	1		9	1		10	0	
5	1	8	0		9	1		10	0		10	0		10	0	
	2	9	0		9	1		8	1		8	1		10	0	
6	1	8	0		9	0		9	1		10	0		10	0	
	2	9	0		9	0		8	0		8	0		10	0	
7	1	8	0		8	1		9	0		10	0		10	0	
	2	9	0		8	1		8	0		8	0		10	0	
8	1	8	0		8	0		9	0		9	1		10	0	
	2	8	1		8	0		8	0		8	0		10	0	
9	1	8	0		8	0		9	0		9	0		10	0	
	2	8	0		8	0		8	0		8	0		9	1	
10	1	8	0		8	0		20	0		16	0		10	0	
	2	8	0		8	0		9	0		12	0		9	0	
11	1	8	0		8	0		18	2		16	0		10	0	
	2	8	0		8	0		16	0		9	3		9	0	
12	1	8	0		8	0		24	0		16	0		10	0	
	2	8	0		8	0		16	0		9	3		9	0	

Appendix Table 1. (cont'd.)

Day		Concentration of torula yeast (ml/day)														
		1×10^4			2.5×10^4			0.5×10^5			1×10^5			2×10^5		
		L	D	R	L	D	R	L	D	R	L	D	R	L	D	R
13	1	8	0		16	0		25	0		17	0		9	1	
	2	13	0		21	0		17	0		9	0		9	0	
14	1	15	0		22	0		39	0		17	0		9	0	
	2	27	0		38	0		23	0		10	0		20	0	
15	1	17	0		29	0		46	0		17	0		9	0	
	2	25	2		41	0		28	0		10	0		20	0	
16	1	17	0		25	4		49	0		21	0		9	0	
	2	23	2		37	4		31	0		12	0		16	4	
17	1	31	0		41	0		53	0		30	0		11	0	
	2	29	0		33	4		31	0		13	0		15	1	
18	1	21	0		39	2		56	0		27	3		9	2	
	2	25	4		33	0		48	0		12	1		12	3	
19	1	20	1		40	0		56	0		27	0		9	0	
	2	26	0		38	0		48	0		12	0		12	0	
20	1	23	0		49	0		62	0		28	0		9	0	
	2	27	0		57	0		46	0		28	0		12	0	
21	1	23	0		49	0		70	0		28	0		9	0	
	2	27	0		52	0		44	2		26	2		12	0	
22	1	24	0		49	0		70	0		36	0		8	1	
	2	32	0		53	4		52	0		23	3		11	1	
23	1	23	1		50	0		71	0		36	0		8	0	
	2	33	0		53	0		53	0		23	0		11	0	
24	1	36	0		69	0		82	0		47	0		10	0	
	2	30	3		53	0		64	0		30	0		12	0	

Appendix Table 1. (cont'd.)

Day		Concentration of torula yeast (ml/day)														
		1×10^4			2.5×10^4			0.5×10^5			1×10^5			2×10^5		
		L	D	R	L	D	R	L	D	R	L	D	R	L	D	R
25	1	36	0		87	0		92	0		60	0		10	0	
	2	30	0		63	0		68	0		50	0		12	0	
26	1	42	0		91	0		101	0		66	0		10	0	
	2	30	0		79	0		83	0		80	0		18	0	
27	1	42	0		95	0		101	0		85	0		10	0	
	2	34	0		79	0		89	0		92	0		18	0	
28	1	40	2		92	3		103	0		81	4		10	0	
	2	35	0		86	0		94	0		93	0		17	0	
29	1	40	0		96	0		109	0		77	4		9	1	
	2	25	0		86	0		93	0		81	12		15	2	
30	1	40	0		96	0		102	7		76	1		9	0	
	2	36	0		86	0		99	0		80	1		15	0	
31	1	42	0		92	4		100	2		70	0		9	0	
	2	36	0		88	0		103	0		81	0		15	0	
32	1	41	1		92	0		100	0		71	0		9	0	
	2	34	2		88	0		103	0		77	4		15	0	

Appendix Table 2. The average daily number of living Daphnia at 12°C cultured in five different duplicate concentrations of torula yeast.

Day	Concentration of torula yeast cells/ml/day				
	1×10^4	2.5×10^4	0.5×10^5	1×10^5	2×10^5
1	10	10	10	10	10
2	10	10	10	10	10
3	10	10	10	9	10
4	9	10	10	9	10
5	9	9	9	9	10
6	9	9	9	9	10
7	9	9	9	9	10
8	8	8	9	8	10
9	8	8	9	8	9
10	8	8	15	14	9
11	8	8	17	14	9
12	8	8	20	14	9
13	11	19	21	14	9
14	21	30	31	15	15
15	21	35	37	14	9
16	20	32	40	18	15
17	25	37	42	22	13
18	23	36	52	19	11
19	23	39	52	19	11
20	25	53	54	28	11
21	25	53	58	27	11
22	28	51	61	30	10
23	28	51	62	30	10
24	33	61	73	39	10
25	33	75	80	55	11
26	36	85	92	73	14
27	38	87	95	88	14
28	38	89	98	87	14
29	38	91	101	79	12
30	38	91	61	78	12
31	38	91	101	76	12
32	38	91	101	74	12

Appendix Table 3. Daily numbers of D. pulex living (L), dead (D), and replaced (R) cultured in duplicate (1,2) concentractions of torula yeast at 20°C.

		Concentration of yeast cells/ml/day														
Day		0.5×10^4			1×10^4			1.5×10^4			2×10^4			2.5×10^4		
		L	D	R	L	D	R	L	D	R	L	D	R	L	R	D
1	1	10			10			10			10			10		
	2	10			10			10			10			10		
2	1	7	3	3	7	3	3	9	1	1	10			10	0	0
	2	10	0	0	8	2	2	9	1	1	9	1	1	10	0	0
3	1	7	3	3	10	0	0	9	1	1	10	0	0	10	0	0
	2	9	1	1	9	1	1	10	0	0	10	0	0	10	0	0
4	1	10	0		10	0		10	0		10	0		10	0	
	2	10	0		10	0		10	0		10	0		10	0	
5	1	10	0		10	0		10	0		10	0		10	0	
	2	10	0		10	0		10	0		10	0		10	0	
6	1	10	0		10	0		10	0		10	0		10	0	
	2	10	0		10	0		10	0		10	0		10	0	
7	1	10	0		10	0		10	0		10	0		10	0	
	2	10	0		10	0		10	0		10	0		10	0	
8	1	10	0		10	0		10	0		10	0		10	0	
	2	10	0		10	0		10	0		10	0		10	0	
9	1	10	0		10	0		10	0		10	0		27	0	
	2	10	0		10	0		10	0		15	0		18	0	
10	1	10	0		14	0		15	0		12	0		26	1	
	2	10	0		14	0		18	0		18	0		25	0	
11	1	20	0		15	0		23	0		22	0		39	0	
	2	10	0		15	0		22	0		34	0		44	0	
12	1	10	0		20	0		27	0		22	0		39	0	
	2	11	0		18	0		28	0		34	0		46	0	

Appendix Table 3. (cont'd.)

Day		Concentration of yeast cells/ml/day														
		0.5×10^4			1×10^4			1.5×10^4			2×10^4			2.5×10^4		
		L	D	R	L	D	R	L	D	R	L	D	R	L	D	R
13	1	11	0		18	2		31	0		35	0		48	0	
	2	11	0		18	0		31	0		46	0		72	0	
14	1	21	0		24	0		49	0		40	0		53	0	
	2	17	0		28	0		34	0		47	0		88	0	
15	1	21	0		24	0		49	0		44	0		62	0	
	2	18	0		30	0		34	0		53	0		104	0	
16	1	20	1		24	0		53	0		44	0		71	0	
	2	17	1		30	0		35	0		62	0		101	3	
17	1	18	2		24	0		53	0		44	0		79	0	
	2	16	1		30	0		35	0		62	0		101	0	
18	1	18	0		23	1		57	0		44	0		79	0	
	2	17	0		30	0		34	1		75	0		102	0	
19	1	18	0		27	0		54	3		50	0		93	0	
	2	15	2		30	0		35	1		80	2		108	0	
20	1	33	0		30	0		55	0		60	0		94	0	
	2	19	0		30	0		43	0		96	0		118	0	
21	1	36	0		33	0		75	0		76	0		110	0	
	2	26	0		36	0		52	0		99	0		118	0	
22	1	37	0		49	0		80	0		75	1		108	2	
	2	26	0		51	0		56	0		96	3		130	0	
23	1	38	0		50	0		85	0		74	1		108	0	
	2	31	0		50	1		54	1		94	2		133	0	
24	1	36	2		51	0		89	0		76	0		108	0	
	2	27	4		53	0		53	1		92	2		130	3	

Appendix Table 3. (cont'd.)

Day		Concentration of yeast cells/ml/day														
		0.5×10^4			1×10^4			1.5×10^4			2×10^4			2.5×10^4		
		L	D	R	L	D	R	L	D	R	L	D	R	L	D	R
25	1	38	0		51	0		90	0		79	0		108	0	
	2	27	0		53	0		67	0		90	2		128	2	
26	1	36	2		60	0		95	0		79	0		121	0	
	2	26	1		67	0		73	0		89	1		134	0	
27	1	34	2		60	0		95	0		86	0		121	0	
	2	23	3		66	1		85	0		92	0		136	0	
28	1	33	1		60	0		98	0		86	0		133	0	
	2	20	3		65	1		84	1		97	0		161	0	
29	1	30	3		60	0		98	0		94	0		157	0	
	2	19	1		67	0		84	0		97	0		182	0	
30	1	30	0		60	0		98	0		94	0		181	0	
	2	19	0		67	0		92	0		110	0		238	0	
31	1	29	1		73	0		110	0		133	0		233	0	
	2	20	0		69	0		96	0		151	0		281	0	
32	1	27	2		82	0		131	0		186	0		267	0	
	2	23			83	0		112	0		246	0		336	0	
33	1	25	2		104	0		172	0		215	0		296	0	
	2	23	0		99	0		151	0		334	0		375	0	
34	1	25	0		113	0		223	0		264	0		339	0	
	2	24	0		126	0		193	0		348	0		396	0	
35	1	23	2		123	0		253	0		282	0		339	0	
	2	24	0		132	0		231	0		343	0		396	0	
36	1	20	3		135	0		265	0		291	0		352	0	
	2	24	0		143	0		233	0		351	0		396	0	

Appendix Table 3. (cont'd.)

Day		Concentration of yeast cells/ml/day														
		0.5×10^4			1×10^4			1.5×10^4			2×10^4			2.5×10^4		
		L	D	R	L	D	R	L	D	R	L	D	R	L	D	R
37	1	21	0		134	1		267	0		295	0		350	2	
	2	25	0		143	0		233	0		351	0		363	33	
38	1	21	0		130	4		260	7		262	33		352	0	
	2	25	0		143	0		233	0		343	9		340	23	
39	1	22	0		134	0		221	39		252	10		341	11	
	2	26	0		140	3		239	0		291	52		311	29	
40	1	23	0		131	3		221	0		250	2		335	6	
	2	26	0		142	0		233	6		291	0		315	0	

Appendix Table 4. The average daily numbers of living Daphnia at 20°C cultured in five difference duplicate concentrations of torula yeast.

Day	Concentration of yeast cells/ml/day				
	0.5×10^4	1×10^4	1.5×10^4	2×10^4	2.5×10^4
1	10	10	10	10	10
2	10	10	10	10	10
3	10	10	10	10	10
4	10	10	10	10	10
5	10	10	10	10	10
6	10	10	10	10	10
7	10	10	10	10	10
8	10	10	10	10	10
9	10	10	10	13	23
10	10	14	17	15	26
11	10	15	23	28	42
12	11	19	28	28	43
13	11	18	31	41	60
14	19	26	42	44	71
15	20	27	42	49	83
16	19	27	44	53	86
17	17	27	44	53	90
18	18	27	46	60	91
19	17	29	45	65	101
20	26	30	49	78	106
21	31	35	64	88	114
22	32	50	68	86	119
23	35	50	70	84	121
24	32	52	71	84	119
25	33	52	79	85	118
26	31	64	84	84	128
27	29	63	90	89	129
28	27	63	91	92	147
29	25	64	91	96	170
30	25	64	95	102	210
31	25	71	103	142	257
32	25	83	122	216	302
33	24	102	162	275	336
34	25	120	208	306	368
35	24	128	242	313	368
36	22	139	249	321	374
37	23	139	250	323	357
38	23	137	247	303	346
39	24	137	230	272	326
40	24	137	227	271	325

Appendix Table 5. Daily numbers of D. pulex living (L), dead (D) and replaced (R) concentrations of torula yeast cultured in duplicate (1, 2) at 12°C.

		Concentration of torula cells/ml/day														
Day		0.5×10^4			1×10^4			1.5×10^4			2×10^4			2.5×10^4		
		L	D	R	L	D	R	L	D	R	L	D	R	L	D	R
1	1	10			10			10			10			10		
	2	10			10			10			10			10		
2	1	9	1	1	9	1	1	10	0	0	10	0	0	10	0	0
	2	9	1	1	10	0	0	10	0	0	9	1	1	8	2	2
3	1	8	2	2	10	0	0	10	0	0	10	0	0	10	0	0
	2	10	0	0	10	0	0	10	0	0	10	0	0	10	0	0
4	1	10	0	0	10	0		10	0		10	0		10	0	
	2	10	0	0	10	0		10	0		10	0		10	0	
5	1	10	0		10	0		10	0		10	0		10	0	
	2	10	0		10	0		10	0		10	0		10	0	
6	1	10	0		10	0		10	0		10	0		10	0	
	2	10	0		10	0		10	0		10	0		10	0	
7	1	13	0		39	0		27	0		31	0		36	0	
	2	12	0		32	0		37	0		37	0		38	0	
8	1	13	0		39	0		35	0		40	0		42	0	
	2	14	0		36	0		54	0		52	0		54	0	
9	1	29	0		53	0		56	0		66	0		65	0	
	2	21	0		51	0		65	0		70	0		72	0	
10	1	20	0		66	0		72	0		83	0		70	0	
	2	27	0		82	0		93	0		105	0		80	0	
11	1	26	0		109	0		101	0		147	0		122	0	
	2	36	0		94	0		129	0		139	0		126	0	
12	1	28	0		150	0		152	0		157	0		168	0	
	2	37	0		107	0		150	0		154	0		173	0	

Appendix Table 5. (cont'd.)

Day		Concentration of torula yeast cells/ml/day														
		0.5×10^4			1×10^4			1.5×10^4			2×10^4			2.5×10^4		
		L	D	R	L	R	D	L	D	R	L	D	R	L	D	R
13	1	42	0		150	0		160	0		164	0		168	0	
	2	51	0		115	0		155	0		169	0		175	0	
14	1	42	0		148	2		165	0		165	0		199	0	
	2	54	0		115	0		171	0		191	0		184	0	
15	1	43	0		136	0		163	2		163	2		196	3	
	2	51	3		108	7		172	0		190	1		184	0	
16	1	44	0		110	26		140	23		163	0		195	0	
	2	51	0		99	9		110	62		189	1		182	2	
17	1	46	0		110	0		134	6		161	2		192	3	
	2	50	0		95	4		97	13		185	4		188	0	
18	1	41	5		110	0		130	4		160	1		193	0	
	2	56	0		95	0		96	1		186	0		180	8	
19	1	41	0		110	0		119	11		160	0		187	6	
	2	54	2		102	0		101	0		195	0		173	7	
20	1	38	3		116	0		115	4		160	0		179	0	
	2	50	4		108	0		116	0		195	0		174	0	
21	1	38	0		116	0		114	1		158	2		174	5	
	2	48	2		114	0		114	2		191	4		171	3	
22	1	37	1		122	0		119	0		167	0		175	0	
	2	43	5		115	0		116	0		199	0		184	0	
23	1	29	8		115	7		121	0		173	0		183	0	
	2	42	1		115	0		114	2		200	0		186	0	
24	1	25	4		103	12		122	0		181	0		181	0	
	2	39	3		98	17		117	0		205	0		198	0	

Appendix Table 5. (cont'd.)

Day		Concentration of torula yeast cells/ml/day														
		0.5×10^4			1×10^4			1.5×10^4			2×10^4			2.5×10^4		
		L	D	R	L	D	R	L	D	R	L	D	R	L	D	R
25	1	22	3		97	6		122	0		199	0		195	0	
	2	39	0		90	8		117	0		216	0		220	0	
26	1	21	1		96	1		136	0		211	0		201	0	
	2	35	4		93	0		116	1		220	0		235	0	
27	1	20	1		103	0		150	0		239	0		271	0	
	2	31	4		106	0		117	0		270	0		283	0	
28	1	16	4		104	0		155	0		259	0		281	0	
	2	27	4		99	7		132	0		284	0		290	0	
29	1	16	0		70	34		121	34		241	18		270	11	
	2	32	0		82	17		130	2		253	31		285	5	
30	1	11	5		64	6		111	10		245	0		267	3	
	2	27	5		79	3		98	32		250	3		295	0	
31	1	9	2		58	6		78	33		280	0		261	6	
	2	22	3		32	27		32	16		213	37		310	0	
32	1	9	0		49	9		77	1		261	19		254	7	
	2	23	0		49	3		71	11		204	7		298	12	

Appendix Table 6. The average daily numbers of living Daphnia at 26°C cultured in five difference duplicate concentrations of torula yeast.

Day	Concentration of yeast cells/ml/day				
	0.5×10^4	1×10^4	1.5×10^4	2×10^4	2.5×10^4
1	10	10	10	10	10
2	10	10	10	10	10
3	10	10	10	10	10
4	10	10	10	10	10
5	10	10	10	10	10
6	10	10	10	10	10
7	13	36	32	34	37
8	14	38	45	46	48
9	20	52	61	68	69
10	24	74	83	94	75
11	31	102	115	143	124
12	33	129	151	156	171
13	47	133	158	167	172
14	48	132	168	178	192
15	47	122	168	177	190
16	48	105	125	176	189
17	48	103	116	173	190
18	49	103	113	173	187
19	48	106	110	178	180
20	44	112	116	178	177
21	43	115	114	175	173
22	40	119	118	183	180
23	36	115	118	187	185
24	32	101	120	193	195
25	31	94	120	208	208
26	28	95	126	216	218
27	26	105	134	255	277
28	22	102	144	272	286
29	24	76	126	247	278
30	19	72	105	248	281
31	16	55	80	247	286
32	16	49	74	233	276
33	13	44	74	202	232
34	13	44	62	178	214