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SEASONAL PHENOTYPIC CHANGE AND

ITS EFFECT ON SOME LIFE-HISTORY CHARACTERISTICS

IN _Daphnia_ _retrocurva_ (Cladocera)

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

by

Julie A. Bebak

1983

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

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ABSTRACT

Life history characteristics were compared between round-headed and helmeted _Daphnia_ _retrocurva_ at a high and a low food level. Carapace length, the intrinsic rate of natural increase (r), clutch size per instar, and day of first reproduction were measured.

For a given total length, helmeted animals possessed a smaller carapace than round-headed animals. The growth rate of the carapace was significantly greater in the round-headed than in the helmeted animals. Food level did not affect carapace length. There was a significant positive correlation between carapace length and brood size for all treatments except low-food helmeted, which was negative and non-significant.

Helmeted animals at low food exhibited the smallest intrinsic rate of natural increase. High-food helmeted, high-food round, and low-food round had greater r's, and were all similar to each other.

At high food, there was no difference in average brood size per instar between helmeted and round-headed animals. At low food, helmeted animals exhibited a significantly smaller average brood size per instar.

Day of first birth was significantly later in helmeted animals at low food. Because of this delay in day at first birth. low-food helmeted animals did not begin reproduction until the fifth instar. High-food helmeted and high- and low-food round-headed animals began reproduction in the fourth instar.

At low food, the energy available is not sufficient to allow a helmeted animal to maintain the same reproductive output as a round-headed animal.

Animals producing helmets decrease their vulnerability to predation, but also decrease their reproductive potential. This "trade-off" does not occur under all conditions. Cost-benefit models and theories must allow for variability in an animal's response to its environment.

SEASONAL PHENOTYPIC CHANGE AND ITS

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IN Daphnia retrocurva (Cladocera)

INTRODUCTION

Many animal species, both vertebrate and invertebrate, exhibit seasonal changes in morphology that involve alterations in color and/or structure. In vertebrates, seasonal alteration in color is a common, well-known, phenomenon. Color change in the Himalayan rabbit is one example. This change, which occurs on different areas of the body, is influenced by environmental temperature (Schmalhausen 1949).

In invertebrates, species of the Aphididae (Homoptera) exhibit complex life cycles in which changes in phenotype and sex are influenced by photoperiod, crowding, food quality, and possibly by temperature and humidity. Water striders exhibit seasonal variability in wing length, which is thought to be related to temporal habitat stability. Seasonal morphological change in invertebrates also occurs in butterflies, locusts, leafhoppers, crickets, and moths, to name just a few. (Shapiro 1976)

Seasonal phenotypic change in algae, rotifers and crustaceans is commonly called cyclomorphosis (Wesenberg-Lund 1908). Hutchinson (1967) summarizes

cyclomorphic patterns in aquatic (planktonic)
organisms, and discusses some of the stimuli which
induce these changes in structure. In the Cladocera
(Crustacea), the structural changes involve alterations
in head shape, tail spine length, and, as a result of
these changes, carapace size. Cyclomorphosis in some
species is induced by increased water temperature and
turbulence, and may also be affected by food and light
(Brooks 1946, 1947; Jacobs 1961; Hrbacek 1959).
Chemical factors may also be important in induction of
cladoceran cyclomorphosis (Grant and Bayly 1981;
Krueger and Dodson 1981).

Daphnia retrocurva exhibits the typical seasonal
pattern of cyclomorphosis. In the early spring,
individuals possess the round head and shorter tail
spine similar to many non-cyclomorphic species
of Daphnia. In late spring and early summer, head
shape and tail spine length change. With each
successive generation. individuals develop a more
recurved helmet and a longer tail spine until peak size
of these structures is reached around mid-summer.
Temperatures greater than 18 C, turbulence, and
photoperiod are factors related to helmet growth in
D. retrocurva (Brooks 1947). No chemical induction
resulting from predator presence has yet been found
(J. Havel, personal communication).

The ultimate cause of cyclomorphosis remains
controversial. Wesenberg-Lund (1908) (cited in Jacobs

1961) suggested that the larger head and longer tail spine may increase buoyancy, thus enabling an animal to expend less energy while in the upper waters of the lake. This theory has been rejected because experimental and theoretical work has shown that sinking rate is dependent on density and size of an animal rather than shape (Brooks and Hutchinson 1950).

Brooks (1947), Jacobs (1961) and Hutchinson (1967) review the buoyancy hypothesis as well as the other earlier theories relating to the adaptive advantage of cyclomorphosis. Since these reviews, theoretical considerations and experimental evidence have suggested that cyclomorphosis may function as a means by which cladocerans decrease their susceptibility to vertebrate and/or invertebrate predation, which tends to be lowest in intensity in winter and early spring and highest in intensity in late spring and summer. Increased lake temperature and turbulence are also occurring at these times, so it is possible that these factors have been selected as the cues to indicate increased predation.

In 1965, Brooks proposed that the formation of a helmet decreases body size and, therefore, visibility to vertebrate predators that tend to select prey greater than 1 mm in length. Brooks's theory was modified by Dodson (1974), who suggested that cyclomorphosis may be an adaptive response to invertebrate as well as to vertebrate predation. Dodson points out that invertebrate predators are very

specific in their selection of prey size (usually less
than 1 mm long) and in their handling of prey after
capture. Dodson's view is that the cyclomorphic
structure functions as an "antilock and key mechanism
to foil invertebrate predators". Dodson also briefly
discusses vertebrate predation, agreeing with Brooks'
(1965) contention that the cyclomorphic structure may
reduce visible body size. According to Dodson,
cyclomorphosis is a double strategy dealing with two
selective pressures: a large size will foil
invertebrates, while a smaller visible body size will
provide protection from vertebrates.

More recent studies have suggested that
cyclomorphic animals are less susceptible to capture
and/or ingestion by fish, and especially,
invertebrates. Zaret (1972a) showed that in the field
the piscine predator Melanirus ate proportionately more
unhorned than horned forms of Ceriodaphnia cornuta.
Laboratory experiments (Zaret 1972b) suggested that the
larger eye of the unhorned form was attracting the
predator, resulting in the differential predation.

Kerfoot (1975a, 1975b) demonstrated that
invertebrate and vertebrate predators interact to
regulate the morph composition of two populations of
the same Bosmina species living in the same body of
water. Fish differentially prey on the "long-
featured", more conspicuous morph, and also eat the
copepods which prey on the "short-featured" morph, thus

allowing both morphs to coexist.

Kerfoot (1977a) showed that the invertebrate _Epischura nevadensis_ (Copepoda) has greater difficulty handling the "long-featured" morph of _Bosmina longirostris_ during predation attempts.

Using _Chaoborus_ larvae (Diptera) as predators, Krueger and Dodson (1981) demonstrated that the predation rate coefficient (k) is lower for Nackenzahne (neck-toothed) _Daphnia pulex_ than it is for the non-Nackenzahne morph. The predation rate coefficient is an instantaneous feeding rate constant in units of liters·predator^{-1}·day^{-1} (Dodson 1975).

Grant and Bayly (1981) and O'Brien and Vinyard (1978) have shown that crested _D. carinata_ are better at evading notonectid predators than are the uncrested forms.

The above studies are just a few that have demonstrated that cyclomorphic structures decrease predator effectiveness. Others are readily available (Jacobs 1965; O'Brien and Kettle 1979; O'Brien et al. 1980).

Using _Daphnia retrocurva_ as a research animal this study is an attempt to answer two major questions related to the structural changes involved in cyclomorphosis:

1.) Does the presence or absence of the helmet affect life history characteristics of the animal such as survivorship and reproduction? How is growth, using

carapace length as an index, affected?

2.) If the presence of a helmet does incur a cost, does this cost occur in all situations? That is, do survivorship, reproduction, and growth change with a change in food concentration?

Brooks (1946), in addressing the question of energy requirements of the helmet, suggested that *Daphnia retrocurva* may compensate for the cost of possessing a helmet by reproducing one instar earlier than it would when round-headed. O'Brien et al. (1980), Zaret (1972b), O'Brien and Vinyard (1978), and Kerfoot (1977b) have attempted to address the above questions using *Daphnia longiremus*, *Ceriodaphnia cornuta*, *Daphnia carinata*, and *Bosmina longirostris*. The results and conclusions of their studies are not consistent, probably because of differences in experimental conditions as well as in the species used.

METHODS AND MATERIALS

Daphnia retrocurva were obtained from Fine Lake -
a eutrophic, hardwater lake in Barry County, Michigan.
The surface area of the lake is 129 hectares. maximum
water depth is 15 meters, and mean water depth is
approximately six meters.

During experimentation, all animals were fed the
green alga Ankistrodesmus falcatus. Depending on the
laboratory facilities available when a particular
experiment was being run, A. falcatus was cultured in
either a batch or semi-continuous system. In the batch
culture method, the algae were raised in 100 mls
nutrient media plus vitamins at 20 C in constant light
until it reached a density of approximately three
million cells/ml. In preparation for feeding the
daphnids, the contents of the flask were centrifuged,
the supernatant removed and the algae resuspended in
filtered lake water. In the semi-continuous system,
the algae were cultured in a large chemostat with a
four litre capacity. As in the batch system, the
culture was raised in continuous light. supplied with
air, and constantly stirred with a magnetic stirring
rod. When the algae reached the same density as above.
they were partially drained off, nutrient media was
gravity fed into the chemostat from a stock solution,
and vitamins were injected. The harvested algae were
then treated similarly to the batch culture method.

Vitamins and nutrients are added to the algae to supply the necessary algal and daphnid food requirements (Goulden et al. 1982). Appendix A describes the composition of the vitamin and nutrient solutions used.

As shown in Table 1, experiments were conducted under laboratory conditions, with as many variables controlled as possible. Temperature was kept a constant 20 C. Photoperiod was also constant, at 16 hours light:8 hours dark. Two food levels were used in the experiment. High food and low food were 25,000 cells/ml (1.25 ug dry weight) and 4,000 cells/ml (0.20 ug dry weight), respectively. Algal density was determined with the use of a hemacytometer. Fresh algae, usually no more than two days old, was used to feed experimental and acclimating animals.

Experimental and acclimating D. retrocurva were cultured in lake water filtered with a 0.3 um Gelman A/E glass fibre filter. Lake water was never kept in the lab for more than six weeks and usually for only two to four weeks.

To obtain the two different morphs (helmeted and round-headed), the following procedure was used:
1.) Daphnia from helmeted mothers - Using a large plankton net with a Mason jar attached to catch the zooplankton. I towed vertically and horizontally in Fine Lake. Animals were brought back to the laboratory, gravid females isolated, and the young born

TABLE 1

SUMMARY OF EXPERIMENTAL TREATMENTS

1		2	3	4	5	6	7
Experiment		Start : End Date Date	n(start n)	Date Daphnia Brought in	Water Source	Genetic Composition	Acclimation
High-food Helmeted	I	6/3:7/8 1982	20(36)	6/2/82	Fine Lake	Mixed	Fine Lake
	II	7/22:9/17 '82	24(35)	7/21/82	Gull Lake/ Tredyferin	Mixed	Fine Lake
Low-food Helmeted	I	7/22:8/30 '82	26(34)	7/21/82	Gull/Tredy.	Mixed	Fine Lake
High-food Round	I	12/8:2/22 '83	37(51)	6/28/82	Lake Galena	1 summer clone (#2)	High food
	II	1/24:3/20 '83	21(54)	12/9/82	Lake Galena	1 fall clone (#8)	High food
Low-food Round	I	12/13:3/10 '83	23(36)	6/28/82	Lake Galena	#2	High food
	II	12/28:3/17 '83	27(34)	6/28/82	Lake Galena	#2	Low food
	III	2/3:3/20 '83	16(34)	12/9/82	Lake Galena	#8	Low food

Photoperiod - 16:8   High food - 25,000 cells/ml (1.25 ug dry weight) A. falcatus
Temperature - 20 C   Low food - 4,000 cells/ml (0.20 ug dry weight) A. falcatus

during the night were used to begin an experiment.

2.) Daphnia from round-headed mothers - When turbulent conditions and possible chemical stimuli are absent, round-headed adults will result (Banta 1939; Brooks 1946. 1947; Jacobs 1961). Therefore, the round-headed morph was obtained from laboratory cultures which had been kept for several generations. The day before beginning a life table study, I isolated gravid females, keeping them at the experimental food level, and used the young they produced in the next 12 to 16 hours to begin a cohort.

Figure 1 provides an example of helmeted and round-headed mothers and their respective juveniles.

Experimental vessels consisted of 40 ml shell vials containing 30 mls water and algae, with one animal per vial. Algae were added to the vial every day to maintain food levels and the water was changed every two days.

Table 1, column 7, gives the acclimation food levels for the mothers of neonates that were used for the life table/birth schedule experiments. Mothers of the helmeted young were "acclimated" in the lake. Except for low-food round (I), the mothers of which were mistakenly acclimated at high food, mothers of the round-headed young were acclimated at the food level which was to be used for a particular experiment. Acclimating animals were kept in individual vials at the experimental food levels from their day of birth.

Figure 1. Camera lucida drawing of helmeted (a) and round-headed (b) adults and juveniles. CL = carapace length measurements.

(a) Helmeted

CL

Adult

CL

Juvenile

|1mm|

(b) Round-headed

Adult

Juvenile
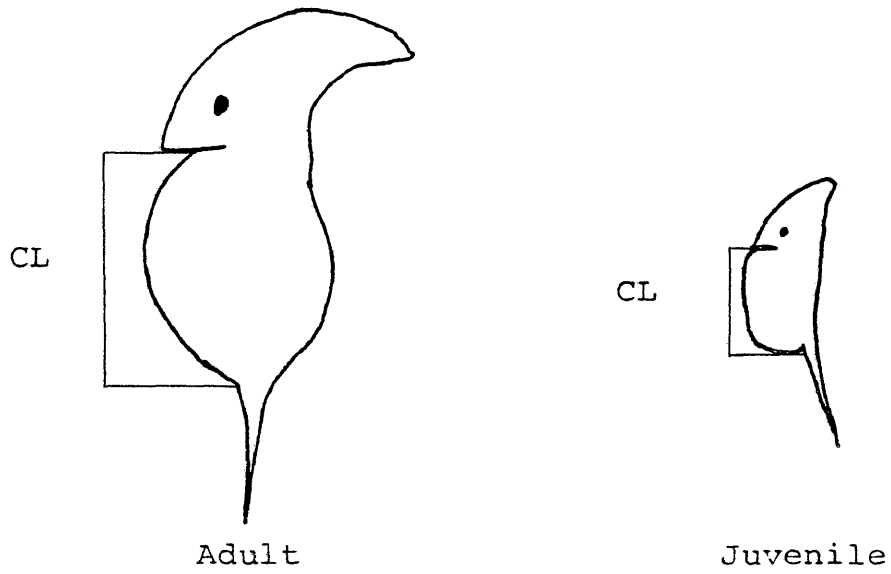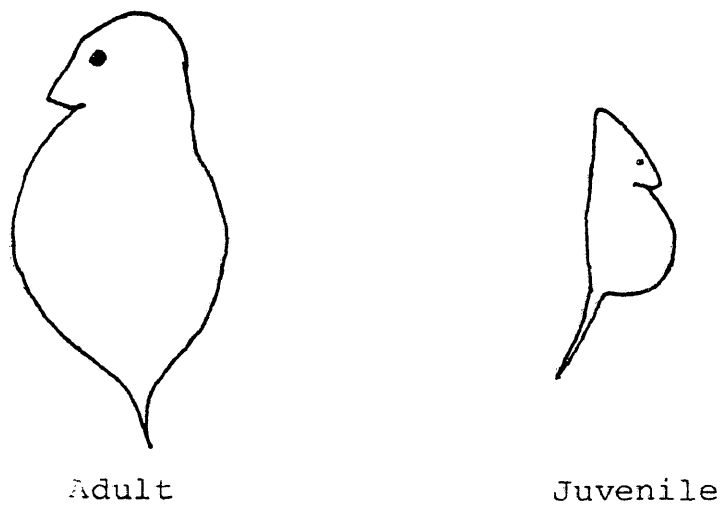
As can be seen from Table 1, column 5, four different water sources were used for the experiments. This variability in water source was unavoidable because the summer experiments were conducted in Michigan, whereas the winter experiments were conducted in Pennsylvania. During one period, water from Tredyferin Well, near Philadelphia. PA. was used. I experienced much difficulty in culturing individual animals in this water. Juveniles died as they molted into the second instar, and both juveniles and adults possessed very soft carapaces.

Table 1 also shows the dates animals were brought into the laboratory and the dates each experiment began and ended. It also gives information on the number of clones present in a particular experiment (column 6).

In column 3 of Table 1 are the total numbers of animals used for each experiment, with the starting number of animals in parentheses. Both morphs tended to become stuck in the surface film of the water, which caused carapace deformities and may have had other adverse effects. Because of this problem, I developed the following criteria:

If an animal was stuck in the surface film for greater than two days or had a deformed carapace valve, it was discarded and the data not used. Fortunately, deformities and surface film problems usually disappeared after the animals started reproducing. Also, one morph did not seem to be more adversely

affected than the other.

Each day, in addition to changing water and/or adding food, I recorded deaths and live births. Using a Wild dissecting microscope and an ocular micrometer, I also measured the lengths of the molted carapaces (Fig. 1).

Data analysis

Data on mortality, reproduction and carapace length was used to run the following analyses:

1. Growth.

To analyze growth, a regression of carapace length on age was run and a Biomedical Data Processing (BMDP) (Dixon and Rrown 1979) Analysis of Covariance Program (P1V) was used to analyze differences between regression lines. (For each regression line, sample sizes were: high-food helmeted, n=45; high-food round, n=63; low-food helmeted, n=69; low-food round, n=63.) Because I made five comparisons, I utilized Bonferroni's Inequality (Bickel and Doksum 1977). I used a probability level of $p=0.01$, which, after the five comparisons are made, increases p to 0.05.

2. Intrinsic rate of increase.

Using Rirch's (1948) method, I calculated the intrinsic rate of population increase (r) for all treatments. The equation used was:

$$\sum l_x m_x e^{(-rx)} = 1.00$$

where x is the mid-point of each age group. l_x is the probability at birth of being alive at age x. and m_x is the mean number of female offspring produced in a unit of time by a female aged x. To make these calculations, I used a computer program available at the Academy of Natural Sciences in Philadelphia- PA (E. Perry, personal communication).

Using Tukey's Jackknife Procedure (Mosteller and Tukey 1977; Sokal and Rohlf 1981) I calculated 95% confidence intervals for each r.

3. Survivorship curves.

Age-specific survivorship was calculated with the use of Deevey's (1947) method. Using a cohort of 1000 individuals, survivorship (l_x) is obtained by successive subtraction of deaths in the age interval from survivors at the beginning of the age interval. (Treatment sample sizes for each analysis are equal to treatment sample sizes in Table 1.)

4. Day of first birth.

For the analysis of variance at day of first birth, alive or dead young, I used BMDP-P2V program for the Analysis of Variance. A log transformation was necessary to meet the assumptions of the ANOVA. Transformed data were normally distributed. Homogeneity of variances was $p=0.10$, tested with the

use of the F_{max} test for homogeneity of variances
(Sokal and Rohlf 1981). (See Appendix B for sample
sizes, means and variances for each treatment used.)

5. Association of carapace length with brood size.

To test correlation of brood size with carapace
length, I used BMDP-P3D for the calculation of
correlation. This program calculates the Pearson
product-moment correlation coefficient (r). Sokal and
Rohlf's (1981) tabular method was used to test the
significance of r.

6. Reproduction at low food.

A BMDP one-way analysis of variance program (P7D)
was used to test differences between helmeted and round-
headed animals in reproduction for instars 5-10 at low
food. Instars 5-10 were chosen because the helmeted
animals did not reproduce in the fourth instar. After
instar 10, sample sizes for the helmeted animals were
so low that I could not get enough brood sizes to make
a valid comparison to the round-headed morph at those
instars. (For low-food round (I, II, and III
combined), n=175, $\bar{x}\pm s.e._{\bar{x}}$ was 3.43±1.03. For low-food
helmeted (I), n=49, $\bar{x}\pm s.e._{\bar{x}}$ was 2.05±1.07.)

RESULTS

1. Growth.

Figure 2 (a and b) represents mean length (mm) of the molted carapace vs. age in days for life table animals of both morphs and food levels (each point represents measurement of 5-10 animals). It is apparent that for the first 10 to 12 days, round-headed animals possessed a larger carapace.

If a regression of carapace length on age is run for the linear portion of growth (the first nine days in Figure 3), high- and low-food helmeted appear to be very similar and possess a smaller slope than high- and low-food round animals. The latter also appear similar to each other.

The analysis of the equality of slopes of the four regression lines is presented in Table 2. Between morphs, the slopes are significantly different ($p < 0.001$). Within a morph, between the two food levels, there is no difference in slope ($p = 0.6290$ for helmeted, $p = 0.5529$ for round). Therefore, not only do the two morphs exhibit different carapace growth rates, but food level does not change the growth rate of the carapace in either morph.

Figure 2. Average carapace length (mm) with 95%
confidence intervals vs. age (in days) for
D. *retrocurva* (a) High-food helmeted and high-food
round (b) Low-food helmeted and low-food round.
Roman numerals indicate treatment number.

Figure 3. Regression of carapace length (mm) on
age (in days) for D. retrocurva. Roman numerals
indicate treatment number. (m = slope of the line)
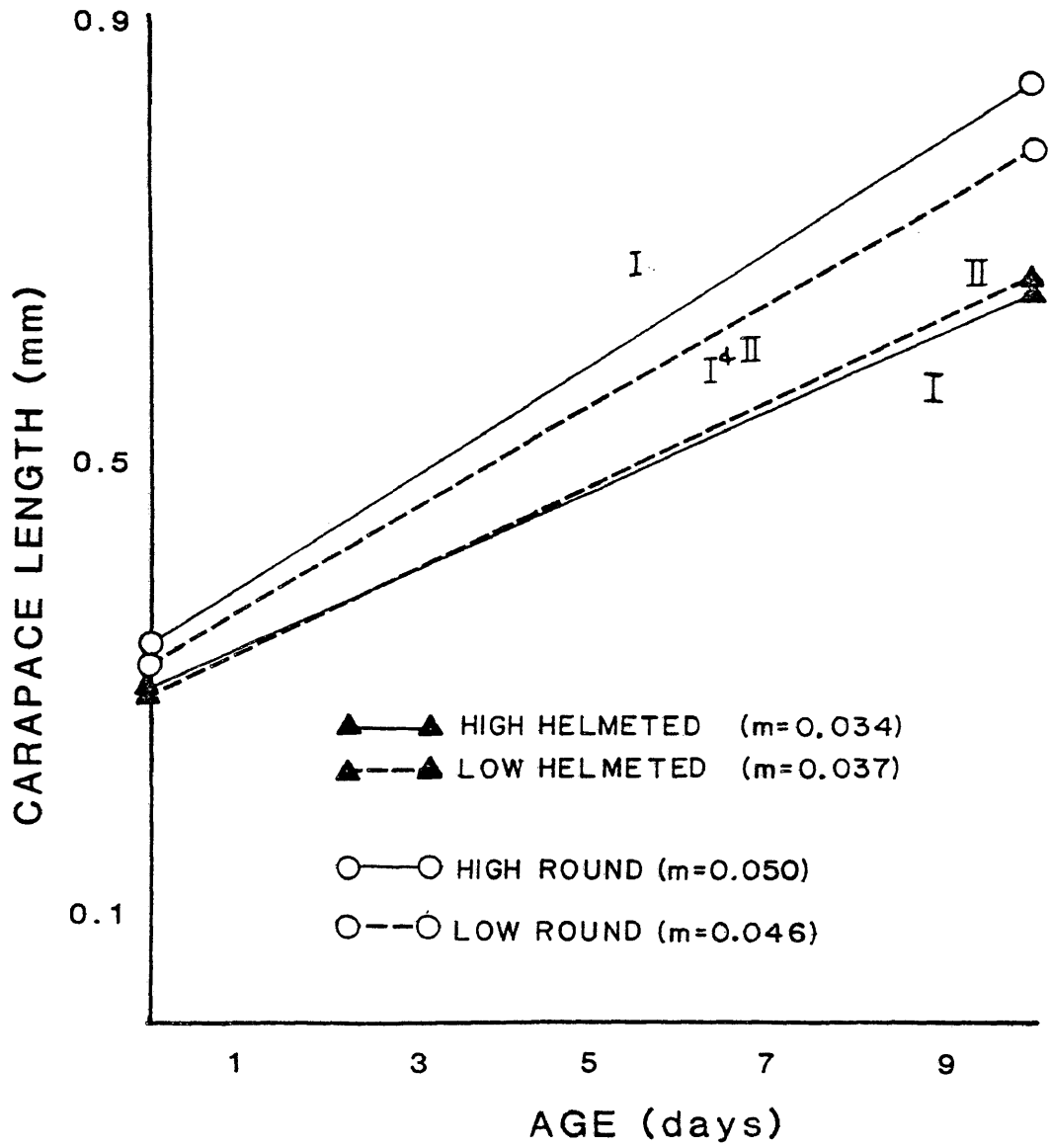
TABLE 2

Analysis of the Equality of Slopes for Growth (mm)
of the Carapace vs. Age

Comparison	Degrees of freedom	Sum of Squares	F- Value	Prob Value
1. All 4 lines	3	0.1101	22.8	<0.001
2. High helmeted vs. low helmeted	1	0.0002	0.23	0.63
3. High round vs. low round	1	0.0008	0.35	0.55
4. High helmeted vs. high round		0.0592	32.75	<0.001
5. Low helmeted vs. low round	1	0.0496	34.22	<0.001

2. Intrinsic rate of increase.

Figure 4 presents the values for the intrinsic rate of population increase (r) and 95% confidence intervals for all treatments. Within the round-headed treatments, for both high and low food, the 95% confidence intervals overlap. Confidence intervals are non-overlapping for high-food helmeted. High-food helmeted (II) appears to be lower than one would predict.

The trend in Figure 4 is that all treatments are similar except low-food helmeted (I). Its r of 0.102 ± 0.033 is much lower than r for the other treatments [except for low-food round (I)]. This difference in r suggests that under low food conditions, helmeted animals exhibit a potentially lower population growth rate than round-headed and helmeted animals under high food conditions. (For table of r values, see Appendix C.)

3. Survivorship.

Figure 5 (a and b) represents survivorship for each treatment. From day 1 to 7, juvenile survivorship is equal for all treatments. From day 8 to day 35, adult survivorship is equal for all groups except low-food round (I), which have a higher mortality than low-food round (II and III) animals (Komolgorov-Smirnov, $p < 0.05$). After day 35, round-headed animals exhibit

Figure 4.   The intrinsic rate of population increase
(r) for high-food helmeted, low-food helmeted,
high-food round and low-food round D. retrocurva
with 95% confidence intervals. Roman numerals indicate
treatment number.

Figure 5. Survivorship (per 1000) vs. age (in days
for D. retrocurva (a) High-food helmeted and
high-food round (b) Low-food helmeted and low-food
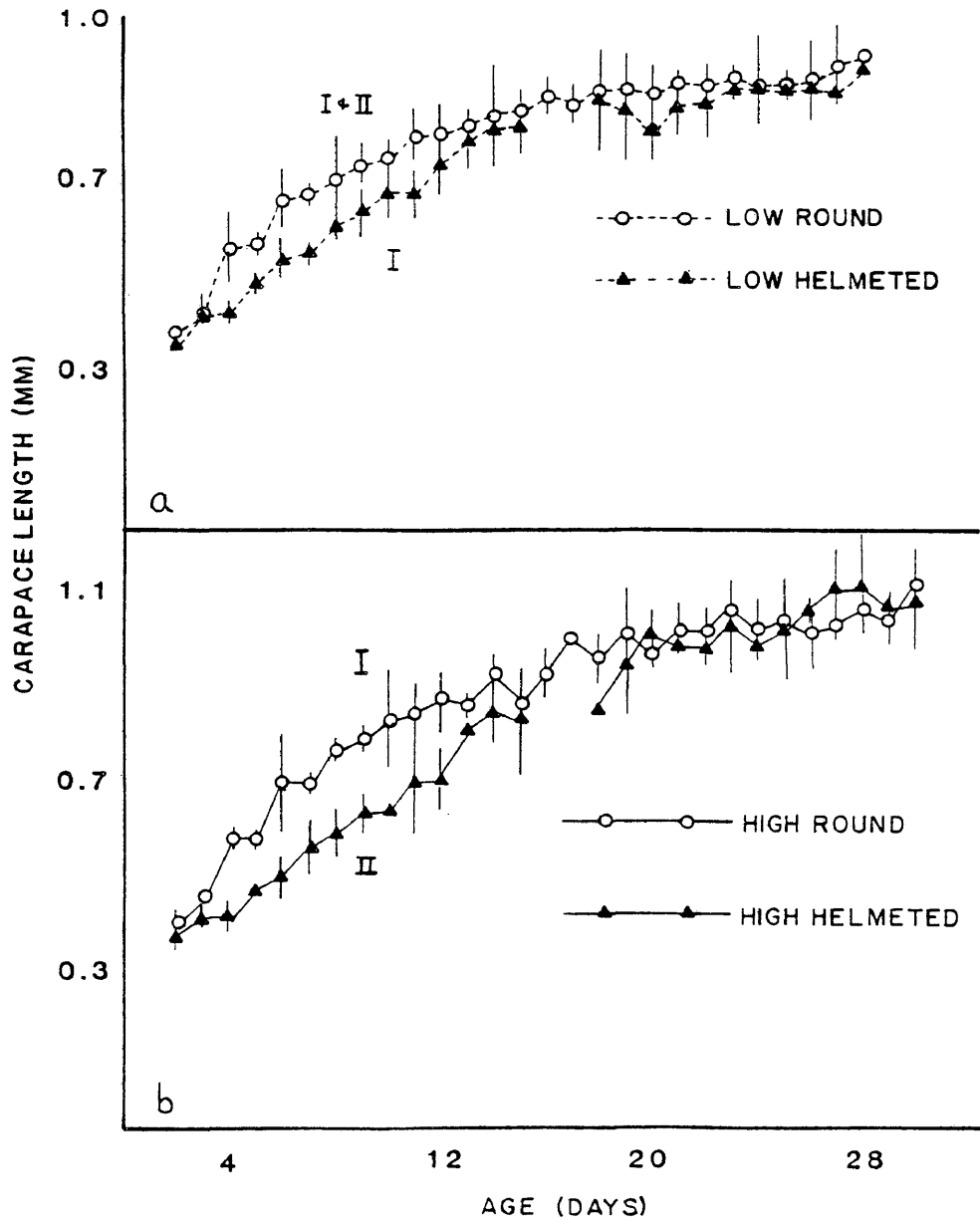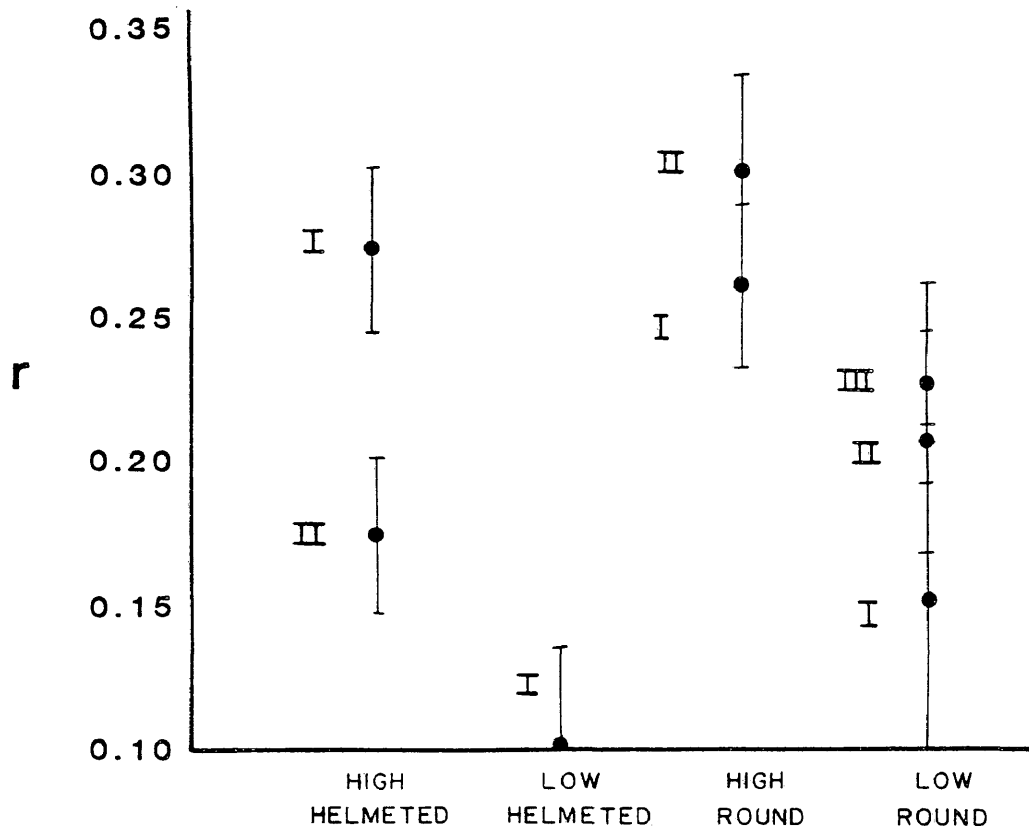round. Roman numerals indicate treatment number.

(a)

1000

I_x

500

100

30

▲——▲ HIGH HELMETED
○——○ HIGH ROUND

I

II

I

II

(b)

1000

I_x

500

100

10

III

II

I

I

▲---▲ LOW HELMETED
○---○ LOW ROUND

10   30   50   70
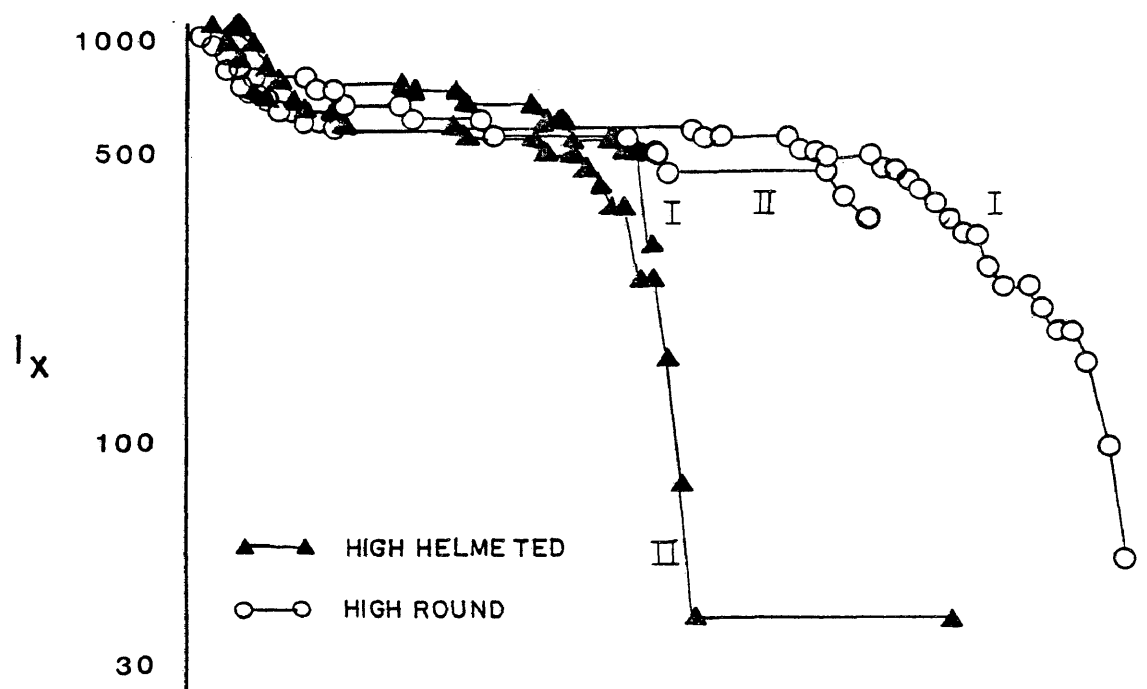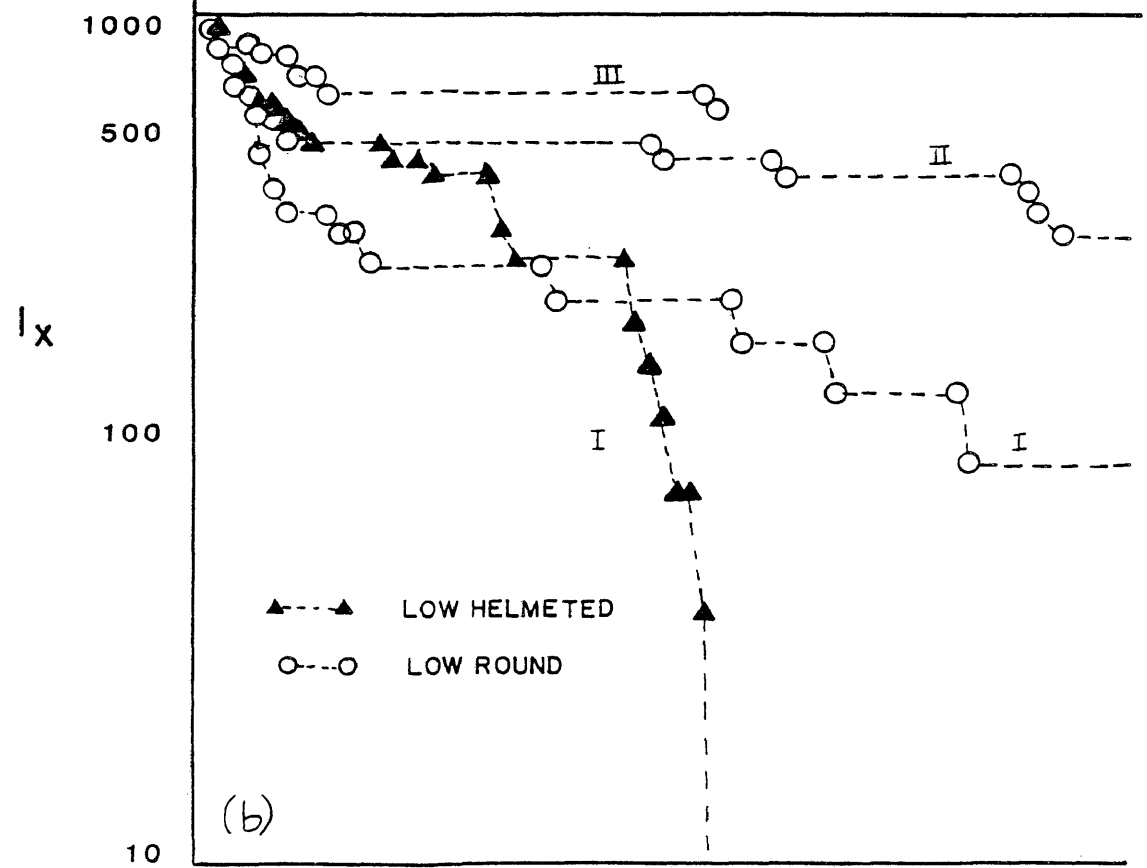
AGE (days)

better adult survivorship than helmeted animals.
Helmeted animals for both food levels died around days
35-40. The Komolgorov-Smirnov two-sample test is
significant at p<0.05 when testing helmeted against
round at both food levels. (See discussion.)

4. Day at first birth.

The results for the two-way ANOVA (Appendix B) for
differences between helmeted and round animals in the
day at first birth are presented in Table 3. Day at
first birth is significantly different between morph
and between food levels, but morph contributes more to
the variation.

5. Association of carapace length with brood size.

To determine whether brood size may be related to
carapace length, I calculated a correlation between the
two (Table 4). For all treatments except low-food
helmeted, r is positive and significant at p<0.01. For
low-food helmeted, r is negative and non-significant,
with p>0.10.

6. Brood size at each instar.

Figures 6 and 7 represent average brood size vs.
instar with 95% confidence intervals for high-food
helmeted and high-food round replicates. (Average
sample sizes for each point were: high-food
helmeted(I) and (II), n=10-15 (Figure 6); high-food

TABLE 3

Analysis of Variance for Age at First Birth

Source	Degrees of freedom	Sum of Squares	F-Value	Prob. Value
Morph	1	0.4523	112.19	<0.001
Food	1	0.0899	22.31	<0.001
Morph x Food	1	0.0118	2.93	0.090

TABLE 4

Correlation of Brood Size with Carapace Length

Treatment	Sample Size	r	p-level
High helmeted(II)	63	0.549	<0.01
Low helmeted(I)	38	-0.253	>0.10
High Round(I,II)	265	0.648	<0.01
Low Round (I,II)	138	0.762	<0.01

Figure 6. Average brood size vs. instar for helmeted
D. retrocurva at high food with 95% confidence
intervals. Roman numerals indicate treatment number.

High-food Helmeted
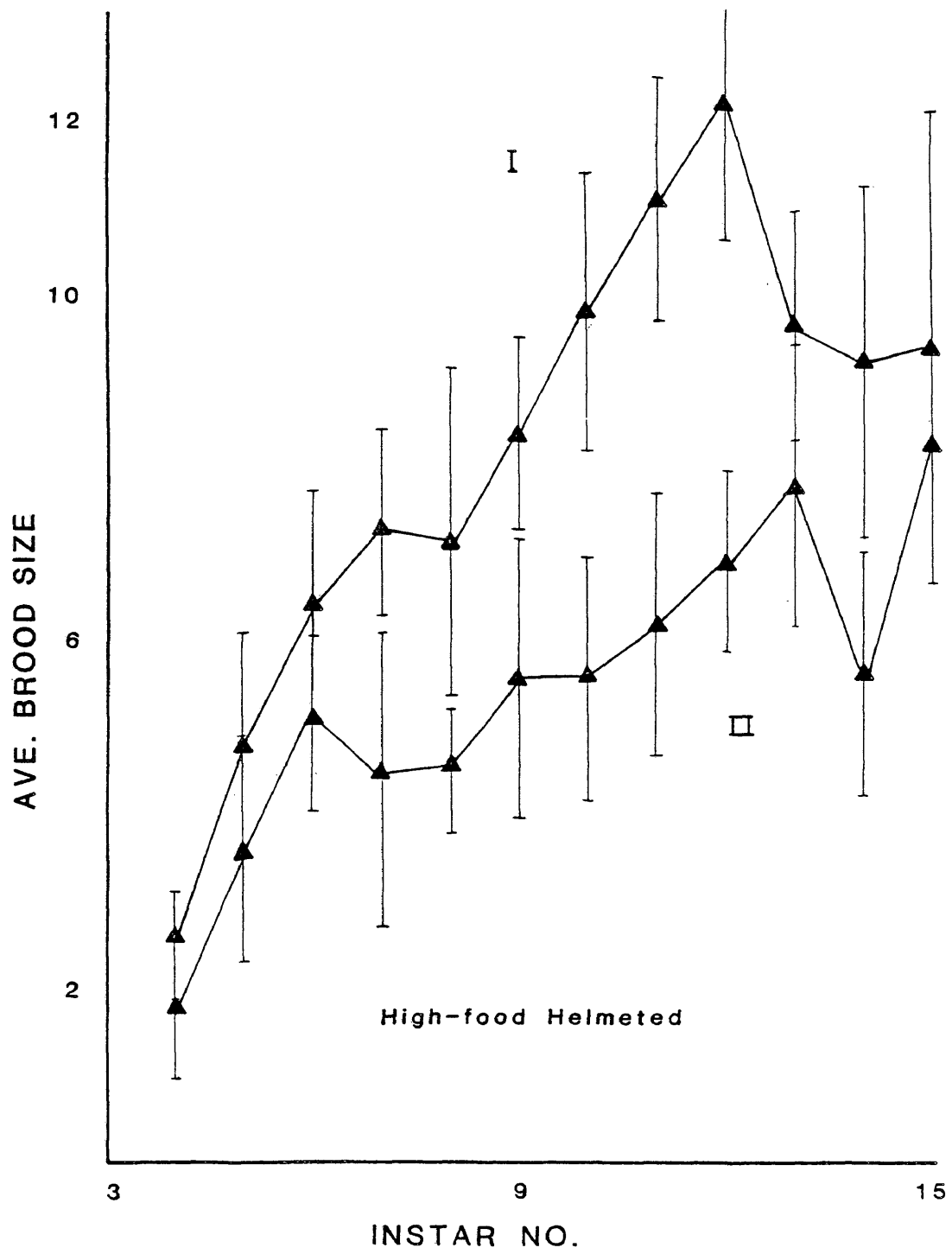
Figure 7. Average brood size vs. instar for round-
headed <u>D</u>. <u>retrocurva</u> at high food with 95%
confidence intervals. Roman numerals indicate
treatment number.

Figure showing AVE. BROOD SIZE versus INSTAR NO., with labels "III", "I and II", and "High-food Round".
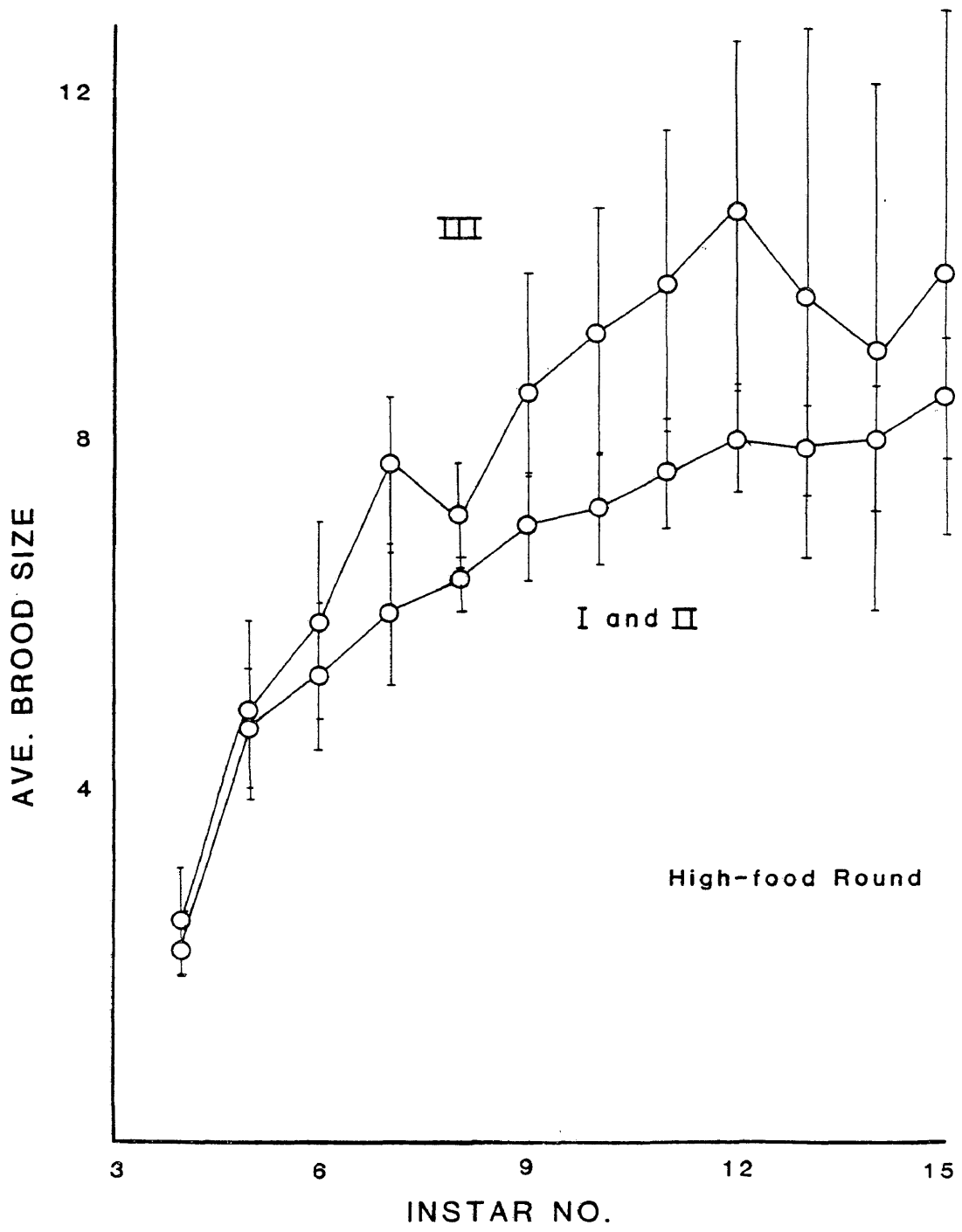
round(I and II). n=25-30; high-food round(III), n=10-15
(Figure 7).) At high food there is no difference
between morphs. So, when helmeted _Daphnia_
retrocurva are present in a high food environment,
their reproductive potential will be the same as it
would be if they possessed a round head.

At low food (Fig. 8). round-headed animals possess
a consistently larger average brood size than helmeted
animals. (Average sample sizes for each point were:
low-food helmeted(I), n=5-10; low-food round, n=10; low-
food round(I and II), n=19.) When the two round
replicates are combined and tested against helmeted
with an analysis of variance for instars 5-10 (Table
5), the helmeted morph is significantly lower than the
round in average brood size for that period.

7. First instar of reproduction.

At high food, helmeted and round-headed animals
reproduce in the fourth instar (Figures 6 and 7). At
low food (Figure 8), reproduction for the helmeted
animals begins one instar later than it does for the
round-headed _D_. _retrocurva_, which, like the high food
animals, begin reproduction in the fourth instar.

TABLE 5

One-Way Analysis of Variance for Brood Size for Low-food Round vs. Low-food Helmeted (Instars 5-10)

Source	Degrees of Freedom	Sum of Squares	F-Value	Prob. Value
Between groups	1	1.882	54.64	<0.001
Within groups	222	7.649		
Total	223	9.531		
Levene's Test for Equal Variances	1,222		0.02	0.90

Figure 8. Average brood size vs. instar for helmeted and round-headed D. retrocurva at low food. Roman numerals indicate treatment number. (--O--O-- Low Round; --▲--▲-- Low helmeted)
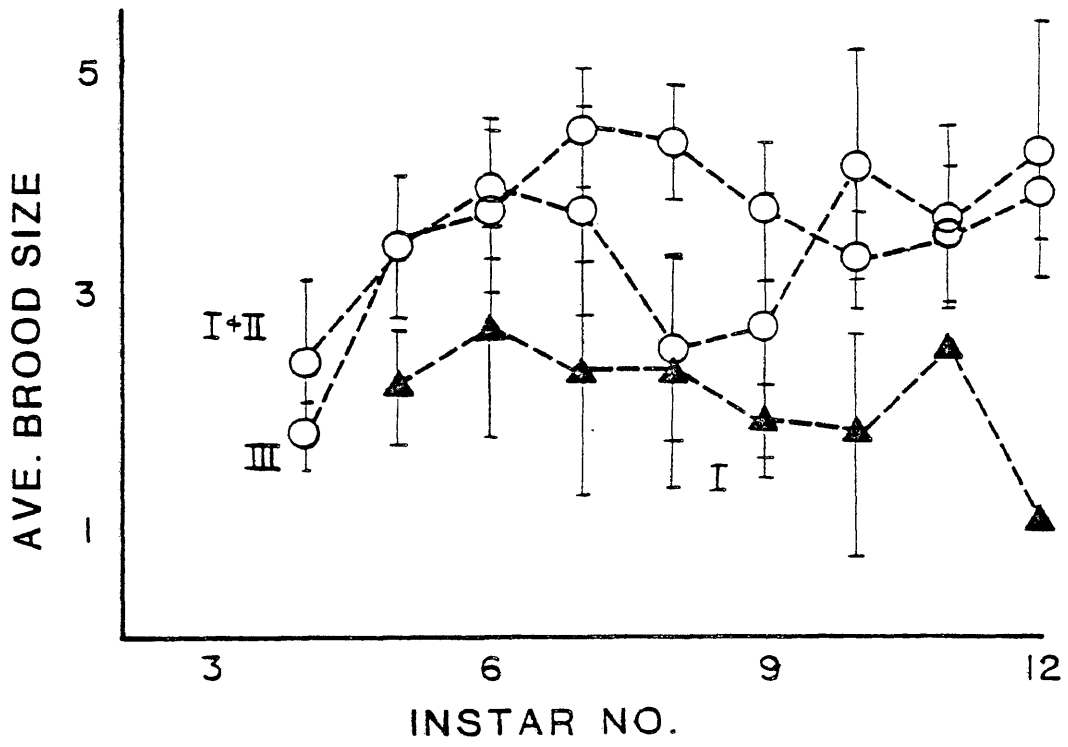
DISCUSSION

I could not measure helmet size of the life
table/birth schedule animals because they would have
been adversely affected. As a result, no helmet size
documentation exists (I did occasionally inspect stock
cultures and experimental animals of all ages, and
could see differences in helmet length between
"helmeted" and "round-headed" animals.). However,
since growth of the helmet with respect to the carapace
is an allometric relationship, helmeted animals should
possess a smaller carapace than round-headed animals
(Brooks 1947).

Figures 2 (a and b) and 3 show that helmeted
animals did possess a smaller carapace than the round-
headed animals for the first 10-12 days. Because
mechanical and chemical stimuli from turbulence and
predators were absent in the experimental vials, the
helmeted animals became more round-headed after day 12,
and attained a larger carapace size.

In Daphnia retrocurva a helmet is present on both
helmeted and round-headed neonates (Figure 1). Jacobs
(1961), Brooks (1947) and Coker and Addlestone (1938)
found that temperature is the major determinant of
helmet growth in embryos. Once an animal is born,
turbulence must be present for helmet growth to be
maintained.

The values for the intrinsic rate of increase

suggest that low-food helmeted animals have a smaller r than all the other treatments (Figure 4). There are some apparent exceptions to this observation. High-food helmeted (II) appears to be lower than one would predict. I think this discrepancy occurred because 6 of 14 adults produced dead young for their first brood. Note. also. from Figure 6, that high-food helmeted (I) animals had a consistently higher brood size per instar after instar 6 than did high-food helmeted (II). The mothers of high-food helmeted (I) animals were collected from Fine Lake on 2 June 82, while mothers of high-food helmeted (II) animals were collected 7 July 82 (Table 1, column 4). It is possible that peak helmet size had not been reached by the high-food helmeted (I) mothers, and so their offspring were able to attain larger clutch sizes.

The intrinsic rate of increase also seems low for low-food round (I) animals. These animals came from mothers which, inappropriately, were acclimated at high food levels, and they exhibited significantly higher mortality around day 10, which affected r (Figure 5b).

Figure 5 (a and b) suggests that round-headed adults exhibit better adult survivorship than helmeted adults do. But, day 35-40 is a period during which I transferred the high-food helmeted (II) and low-food helmeted (I) animals into a different water source, the Tredyferin Well water, from Upper Merion, PA. This water may have affected the survivorship of the high-

and low-food helmeted animals. Therefore. I am not convinced that the difference in survivorship of the helmeted animals is due to the presence of the cyclomorphic structure.

Figure 5b shows a significantly greater juvenile mortality for the low-food round (I) treatment. The high-food acclimation of the mothers of this cohort may have somehow affected its ability to survive in a low food environment.

In _Daphnia_ _retrocurva._ at high food levels, helmeted and round animals possess the same reproductive ability. The intrinsic rate of natural increase (r), average brood size/instar and day of first birth remain the same, whether the animal possesses a helmet or not.

In low food conditions, however, reproduction decreases when D_ _retrocurva_ possesses a helmet. The intrinsic rate of population increase is less, average brood size is significantly smaller, and helmeted animals reproduce at a significantly later day than round-headed animals. Contrary to Brooks (1946) suggestion that helmeted animals will reproduce one instar earlier than round-headed animals, this later day of first birth also affects first instar of reproduction, making it one instar later in the helmeted morph.

Why do helmeted D_ _retrocurva_ decrease reproduction under low food conditions? To answer this

question, the allocation of the energy consumed by helmeted and round-headed D. retrocurva under high and low food conditions must be considered.

Energy gained from the food consumed by an organism may be digested and absorbed. or egested. Of that portion digested and absorbed, a fraction is used by the organism for growth and reproduction and the rest is lost as heat (Calow 1977). There are five major areas in which the two morphs may differ in their intake and use of energy gained from food:

1.) Energy intake
2.) Egestion
3.) Heat loss
4.) Reproduction
5.) Growth

Energy intake

It is possible that the two morphs differ in their rate of food intake. In his study of energy transformation by Daphnia pulex, Richman (1958) found that there is an increase in filtering rate with an increase in body length and absolute body weight. On a unit weight basis, filtration rate decreases with an increase in size.

Burns (1969) studied filtration rates in four species of Daphnia. and like Richman. found that filtering rate increases with body weight and body length. She found, however, variability in filtering rates on a unit body weight basis - for a given species, the smaller animal did not always have a

higher filtration rate.

Neither Richman or Burns measured filtration rates below the equivalent of the high food level used in this experiment. But, for the algal species he used, Richman found no differences in filtration rates between food levels.

Based on the information above, it is possible that, because they have a shorter carapace length, helmeted animals may have a lower filtering rate. However, carapace length for helmeted animals increases after day 12-14, and, at that age, equals carapace length of the round-headed animals. Nevertheless, at low food, average brood size per instar remained significantly lower. These results suggest that filtering rate was already equal in both morphs. Therefore, they may be ingesting the same amount of food but are utilizing it differently.

Egestion

Assimilation efficiency will determine the amount of food egested by an organism. It is possible that both morphs ingest the same amount of food, but that a lower assimilation efficiency decreases the amount of energy available for growth and reproduction in the helmeted morph. As Hall et al. (1976) point out, the effect of animal size on assmilation efficiency has not been well studied in cladocerans.

Heat Loss

Energy lost as heat is a vague term, the components of which vary depending on whether the organism is a homeotherm or poikilotherm. Respiration and O consumption are two components of heat loss in Daphnia that can be compared between the two morphs (Richman 1958) to try and get an idea of whether the helmeted morph may be expending more energy than the round-headed morph.

O'Brien and Vinyard (1978) have pointed out that the antennal muscles in cyclomorphic cladocera look larger than the antennal muscles in non-cyclomorphic cladocera. This greater musculature may facilitate swimming and increase the probability of escape from predators. However, it may incur a cost by increasing active metabolism, the needs of which cannot be met by cyclomorphic cladocera in low food conditions.

Reproduction

It is clear that under low food conditions there are differences in allocation of energy to reproduction between the two morphs, which results in low-food helmeted animals exhibiting significantly smaller brood sizes, later day of first reproduction, and smaller rates of population increase than low-food round-headed animals. It is probable that this difference in allocation is occurring because some other aspect of energy transformation is being affected by low food

levels and is using energy normally available for reproduction.

Growth

Given the results of my experiment, it appears that the energy normally available for reproduction is being used for growth and probably maintenance of the helmet in D. retrocurva.

Biomass is a better parameter than body length when making growth comparisons between the two morphs. The allometric changes in growth between the two morphs make the situation more complex. and makes it much more difficult to compare D. retrocurva with non-cyclomorphic species. Even though absolute growth with respect to total length does not change, absolute growth in biomass may change. Before making valid comparisons with the literature on energy transformation in Daphnia, biomass measurements should be done.

It appears that. in Daphnia retrocurva, a trade-off is occurring between protection from predation and reproductive capacity. This trade-off, however, does not occur under all conditions. This point is an important one, for it stresses the need for the realization that cost and benefit situations are not strict and rigid, with only one possible outcome. Cost-benefit models and theories must allow for variability in an animal's response to its environment.

What are the ecological implications for the results of this experiment? First, and most obvious: by developing structures to protect itself against predation, D. retrocurva decreases its ability to prosper in a low food environment, especially in the face of intense competition from other species. Hutchinson (1967) points out the variability in the helmet size and abundance, from lake to lake, of cyclomorphic animals, even in lakes located close to each other. Information on the productivity of lakes with and without D. retrocurva would provide insight into this suggestion. Therefore, low productive lakes should have small, or even non-existent, populations of D. retrocurva. Helmet size should also vary, with animals in low-productive lakes possessing smaller helmets (as long as a smaller helmet still affords sufficient predator protection).

Second, D. retrocurva's decreased reproduction in a low food environment may give us a clue as to the reason for the yearly cycle cyclomorphic zooplankton undergo. The non-cyclomorphic morph is present in lakes during periods of low predation (ie. early spring and winter). It is, however, also present during periods of lower productivity, indicating that conflicting pressures may be regulating the seasonal morphological change.

APPENDIX A

Vitamin mixture added to algae cultures.[a][b]

Nutrient	Concentration of Stock Solution, mg/l
Biotin	5
Thiamine	100
Pyridoxine	100
Pyridoxamine	3
Calcium pantothenate	250
B_{12} (as mannitol)	100
Nicotinic acid	50
Nicotinamide	50
Folic acid	20
Riboflavin	30
Inositol	90

[a] One millilitre of stock solution is added to each litre of culture medium.
[b] Modified from Shiraishi and Provasoli (1959).

Nutrient Solution Used for Culture of A. falcatus.[c]

Nutrient	Stock Solution (g/l in H_2O)	Culture Solution (ml/l)
$NaNO_3$	85.010	2.0
K_2HPO_4	8.710	2.0
$MgSO_4$ H_2O	24.074	2.0
$CaCl_2$ $2H_2O$	36.760	0.8
Na EDTA	4.360	1.7
$FeCl_3$ $6H_2O$	1.080	1.0
H_3BO_3	2.474	1.0
$CuSO_4/ZnSO_4$	0.0002/0.92	1.0
$MnCl_2/CoCl_2/NaMoO_4$	1.385/0.019/0.01	1.0
$MgCl_2$ $6H_2O$	40.660	1.0

[c] Carmichael and Gorham (1974).

APPENDIX B

Matrix for Two-Way ANOVA for Day at First Birth

MORPH

Helmeted   Round-headed

High
$\bar{x} = 8.59$ $\bar{x} = 6.61$
$s^2 = 0.0071$ $s^2 = 0.0021$
$n = 29$ $n = 24$

Food

Low
$\bar{x} = 10.38$ $\bar{x} = 7.22$
$s^2 = 0.0058$ $s^2 = 0.0031$
$n = 13$ $n = 38$

APPENDIX C

"r" Values for all Treatments

```
High-food Helmeted        I = 0.272±0.027 (n=20)
                         II = 0.174±0.026 (n=24)

Low-food Helmeted         I = 0.102±0.033 (n=26)

High-food Round           I = 0.259±0.028 (n=37)
                         II = 0.297±0.034 (n=21)

Low-food Round            I = 0.151±0.057 (n=23)
                             (High food accli.)
                         II = 0.205±0.038 (n=27)
                        III = 0.225±0.034 (n=16)
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LITERATURE CITED

Banta, A.M. 1939. Studies on the physiology, genetics and evolution of some Cladocera. Paper No. 39. Dept. of Genetics, Carnegie Institution of Washington, Publication No. 513.

Bickel, P.J. and K.A. Doksum. 1977. Mathematical Statistics. Holden-Day, San Francisco, California. USA.

Birch. L.C. 1948. The intrinsic rate of natural increase of an insect population. Journal of Animal Ecology 17:15-26.

Brooks, J.L. 1946. Cyclomorphosis in Daphnia I. An analysis of D. retrocurva and D. galeata. Ecological Monographs 16(4):409-447.

_____. 1947. Turbulence as an environmental determinant of relative growth in Daphnia. Proceedings of the National Academy of Sciences 33(5):141-148.

_____. 1965. Predation and relative helmet size in cyclomorphic Daphnia. Proceedings of the National Academy of Sciences 52(1):119-126.

Brooks. J.L., and G.E. Hutchinson. 1950. On the rate of passive sinking of Daphnia. Proceedings of the National Academy of Sciences 36:272-277.

Burns, C.W. 1969. Relation between filtering rate, temperature, and body size in four species of Daphnia. Limnology and Oceanography 14(5):693-700.

Calow, P. 1977. Ecology, evolution and energetics: a study in metabolic adaptation. Advances in Ecological Research 10:1-62.

Carmichael, W.W., and P.R. Gorham. 1974. An improved method for obtaining axenic clones of planktonic blue-green algae. Journal of Phycology 10:238-240.

Coker, R.E., and H.H. Addlestone. 1938. Influence of temperature on cyclomorphosis of Daphnia longispina. Journal of the Elisha Mitchell Scientific Society 54:45-75.

Deevey, E.S., Jr. 1947. Life tables for natural populations of animals. Quarterly Review of Biology 22:283-314.

Dixon, W.J., and M.B. Brown, editors. 1979. BMDP Biomedical Computer Programs, P-Series. University

of California Press, Berkeley, California, USA.

Dodson, S.I. 1974. Adaptive change in plankton
     morphology in response to size-selective
     predation: a new hypothesis of cyclomorphosis.
     Limnology and Oceanography 19(5):721-729.

_____. 1975. Predation rates of zooplankton in
     arctic ponds. Limnology and Oceanography
     20:426-433.

Goulden, C.E., R.M. Comotto, J.A. Hendrickson, Jr.,
     L.L. Hornig, and K.L. Johnson. 1982. Procedures
     and recommendations for the culture and use of
     Daphnia in bioassay studies. Pages 139-160 in
     J.G. Pearson, R.B. Foster, and W.E. Bishop,
     editors. Aquatic Toxicology and Hazard Assessment:
     Fifth Conference, ASTM STP 766, American Society
     for Testing and Materials, Philadelphia,
     Pennsylvania, USA.

Grant. J.W.G., and I.A.E. Bayly. 1981. Predator
     induction of crests in morphs of the Daphnia
     carinata King complex. Limnology and Oceanography
     26(2):201-218.

Hall, D.J., S.T. Threlkeld, C.W. Burns, and P.H.
     Crowley. 1976. The size-efficiency hypothesis and
     the size structure of zooplankton communities.
     Annual Review of Ecology and Systematics
     7:177-208.

Hrbacek, J. 1959. Circulation of water as a main factor
     influencing the development of helmets in Daphnia
     cucullata Sars. Hydrobiologia 13:170-185.

Hutchinson, G.E. 1967. A Treatise on Limnology, Vol.
     II. Introduction to Lake Biology and the
     Limnoplankton. John Wiley and Sons, New York, New
     York USA.

Jacobs, J. 1961. Cyclomorphosis in Daphnia galeata
     mendotae Birge, a case of environmentally
     controlled allometry. Archiv fur Hydrobiologia
     58(1):7-71.

_____. 1965. Significance of morphology and
     physiology of Daphnia for its survival in
     predator-prey experiments. Die Naturwissenschaften
     52(6):141-142.

Kerfoot, W.C. 1975a. Seasonal changes
     of Bosmina (Crustacea, Cladocera) in Frains Lake,
     Michigan: laboratory observations of phenotypic
     changes induced by inorganic factors. The Journal

of Freshwater Biology 5:227-243.

_____. 1975b. The divergence of adjacent
populations. Ecology 56:1298-1313.

_____. 1977a. Implications of copepod predation.
Limnology and Oceanography 22:316-325.

_____. 1977b. Competition in Cladoceran
communities: the cost of evolving defenses against
copepod predation. Ecology 58:303-313.

Krueger, D.A., and S.I. Dodson. 1981. Embryological
induction and predation ecology in Daphnia pulex.
Limnology and Oceanography 26(2):219-223.

Mosteller, F., and J.W. Tukey. 1977. Data Analysis and
Regression. A Second Course in Statistics.
Addison-Wesley, Boston, Massachusetts, USA.

O'Brien, W.J., and D. Kettle. 1979. Helmets and
invisible armor: structures reducing predation
from tactile and visual planktivores. Ecology
60(2):287-294.

O'Brien. W.J., D. Kettle, H. Riessen, D. Schmidt. and
D. Wright. 1980. Dimorphic Daphnia longiremus:
predation and competitive interactions between the
two morphs. Pages 497-506 in W.C. Kerfoot, editor.
Evolution and Ecology of Zooplankton Communities.
Special Symposium Volume 3, American Society of
Limnology and Oceanography. University Press of
New England, Hanover- New Hampshire, USA.

O'Brien, W.J., and G.L. Vinyard. 1978. Polymorphism and
predation: the effect of invertebrate predation on
the distribution of two varieties of Daphnia
carinata in South Tndia ponds. Limnology and
Oceanography 23(3):452-460.

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

Schmalhausen, I.I. 1949. Factors of Evolution. The
Theory of Stabilizing Selection. Blakiston.
Philadelphia, Pennsylvania, USA.

Shapiro. A.M. 1976. Seasonal polyphenism. Pages 259-333
in M.K. Hecht, W.C. Steere, and B. Wallace,
editors. Evolutionary Biology. Vol. 9. Plenum, New
York, New York, USA.

Shiraishi, K., and Provasoli, L. 1959. Tohoku Journal
of Agriculture Research 10:89-96.

Sokal, R.R., and F.J. Rohlf. 1981. Biometry. W.H.
    Freeman, San Francisco, California, USA.

Wesenberg-Lund. 1908. Plankton investigations of Danish
    lakes. General Part. -Danish freshwater biology
    laboratory, No. 5; Nordisk Forlag, Copenhagen.

Zaret, T.M. 1972a. Predator-prey interaction in a
    tropical lacustrine ecosystem. Ecology
    53(2):248-257.

_____. 1972b. Predators, invisible prey, and the
    nature of polymorphism in the Cladocera (Class
    Crustacea). Limnology and Oceanography
    17(2):171-184.

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