Laboratory Culture, Growth Rate, and Feeding Behavior of a Planktonic Marine Copepod

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LABORATORY CULTURE, GROWTH RATE, AND FEEDING BEHAVIOR OF A PLANKTONIC MARINE COPEPOD[1]

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ABSTRACT

Rhincalanus nasutus was cultured through seven consecutive generations in 19-liter carboys when provided with a mixture of diatoms and *Artemia salina* nauplii as food. The mean generation length was 8.7 weeks, similar to that of the local field population of this species during some seasons. Fecundity of laboratory-reared animals was lower than that of the field population. Instantaneous coefficients of individual exponential growth (k in the expression, $W_t = W_0 e^{kt}$, where W is body weight of organic carbon and t is days) were 0.24 to 0.12/day, depending on the age of the individual. About 10 μg of detrital carbon were produced as exuviae during the growth of an individual. Even young nauplii fed preferentially on large food particles. The suggestion that the copepods' first antennae are used in the feeding process was not supported by an experimental test. *R. nasutus* nauplii are apparently active enough to avoid being eaten by their parents.

INTRODUCTION

Marine benthic harpacticoid copepods such as *Tigriopus fulvus* (Fraser 1936; Provasoli, Shiraishi, and Lance 1959) and *Tisbe furcata* (Johnson and Olson 1948) appear to be relatively amenable to laboratory culture; culture of the pelagic harpacticoid *Euterpina acutifrons* has also been successful (Bernard 1963; Neunes and Pongolini 1965). The coastal cyclopoid *Oithona nana* (Murphy 1923) and the estuarine calanoid *Pseudodiaptomus coronatus* (Jacobs 1961), the latter not being truly planktonic, have been cultured, and recently Zillioux and Wilson (1966) cultured the coastal calanoid *Acartia tonsa* through twelve generations. Conover (personal communication) has successfully cultured *Centropages hamatus*, another coastal calanoid, for several generations. Species of the neritic-oceanic genus *Calanus* have been raised from egg to adult (Crawshay 1913, *see also* Lebour 1916; Conover 1965), but subsequent generations were not produced in the laboratory, apparently because the copepods did not copulate. Mortality during molting has also been a major problem. Rae's (1958) plea for continuous culture of typical oceanic copepods is therefore still unanswered. Ideally, such culture would include not only the rearing under controlled conditions of consecutive generations of individuals morphologically similar to those found in the field, but also the duplication of natural parameters such as individual growth rate and fecundity.

Ability to culture the copepods also provides a supply of individuals of different developmental stages for such studies as the measurement of rates of growth and production of exuviae in terms of organic carbon and for experiments on selective feeding.

MATERIALS AND METHODS

The planktonic copepods, *Calanus helgolandicus* (*pacificus*) and *Rhincalanus nasutus*, are found in the California Current and are readily obtained a few kilometers offshore from Scripps Institution of Oceanography. They are relatively large, which facilitates identification and handling, and are abundant in several major areas of the world ocean. Marshall and Orr (1955) reported extensively on the biology of *Calanus* in the North Atlantic.

The copepods were kept at 12C in semidarkness in glass carboys containing 19 liters of membrane-filtered seawater to
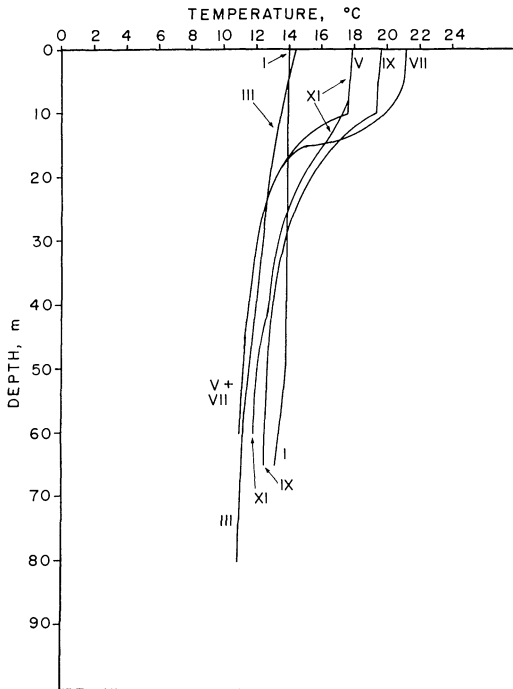
FIG. 1. Vertical distribution of temperature at the sampling station in various months, indicated by roman numerals.

which a mixture of food equivalent to 1 to 5 mg of particulate organic carbon per liter had been added. The food consisted of mixtures of the diatoms *Cyclotella nana*, *Thalassiosira fluviatilis*, *Ditylum brightwellii*, occasionally *Coscinodiscus wailesii*, all grown in pure culture, and the naupliar brine shrimp, *Artemia salina*, the last used only when older copepodite stages were being raised. The temperature of 12C was chosen as a seasonal average for the subsurface water at the sampling station from which animals were obtained (Fig. 1). Initially, 50 mg/liter of streptomycin sulfate or penicillin "G" (1,625 units/mg) were added, but this did not appear to improve survival and was discontinued. The water in the carboys was stirred continuously by a large Lucite propeller suspended on a Lucite shaft near the bottom of the carboy. The shaft was driven at approximately 2 rpm by a motor outside the carboy. Air was introduced through a glass tube as a trickle of bubbles.

Each week most of the water was very slowly siphoned out of each carboy through a filter screen which retained the copepods, plus any eggs that had been produced, in the last liter or so of water. This water was then transferred to a beaker, and the animals and eggs were removed with a pipette. The animals were examined under a dissecting microscope to determine their stages of development and were then pipetted into cooled, filtered seawater containing a fresh mixture of food in the rinsed carboy.

Up to 100 adults, or several hundred nauplii, were maintained successfully under these conditions, representing a concentration of *R. nasutus* about 100 times that found in local waters. Gravid females captured in the field with plankton nets survived well and produced eggs for several weeks. These eggs were used to start the first laboratory generation.

To compare laboratory growth rates with the growth rates of natural populations, samples were taken at approximately 2-week intervals about 5 km from Scripps Institution of Oceanography in water 100–300 m deep. Two unmetered nets (0.5-m mouth diameter), one with 363-μ mesh and one with 103-μ mesh, were lowered side by side to 90 m. The cod ends were attached to the wire so that the nets sank backwards. The nets were then hauled vertically to the surface, and the catches were preserved in formalin. Temperature was measured with a thermistor or bathythermograph.

It is doubtful that all the copepods (or even a predictable fraction) in the water column through which the nets were hauled were caught. Because of the low speed at which the nets were hauled to the surface, avoidance of the nets by the copepods and possible loss of catch by mechanical backwashing as the skiff responded to the swell were both possible. The samples are presumably somewhat biased in that older copepodite stages may be more capable of escaping capture than younger stages, and in that species differ in their ability to avoid towed nets (Fleminger and Clutter 1965). Since the entire water column was not sampled, the collections may

be further biased because the older developmental stages often live in deeper water than the younger stages (Russell 1927). Finally, there is no assurance that the same population of animals was sampled in successive weeks. Nevertheless, the samples should indicate the relative abundance of the various developmental stages of the copepods with sufficient accuracy for our purposes.

All of the copepodite stages of *C. helgolandicus* and *R. nasutus* were counted in the sample from the 363-μ net; if there were less than 100 individuals of either species, the copepodites of that species in the sample from the 103-μ net were also counted. The stage I copepodites of *C. helgolandicus* and nauplii of both species were counted in the sample from the 103-μ net; the sample was quantitatively subsampled with a Stempel pipette until at least 100 nauplii of each species were counted. When nauplii were rare, the whole sample was examined. The six naupliar stages of *R. nasutus* were identified according to Gurney (1934), Steuer (1935), and Johnson (1937). Lebour (1916) shows the naupliar stages of *C. helgolandicus*.

GROWTH OF *C. HELGOLANDICUS* AND *R. NASUTUS* IN THE FIELD AND IN CULTURE

The local field population of *R. nasutus* apparently completed four successive generations in the 29 weeks between 23 March and 12 October 1965, averaging 7.25 weeks per generation (Fig. 2). The results for 1966 were less clear, especially during spring. One generation was apparently completed in about 7.5 weeks between 26 April and 17 June, but there was a long delay in the early naupliar stages of the next generation so that a third generation was not produced until October. Hence, only two generations seem to have been produced in 1966 in the same period in which four generations had apparently been produced in 1965.

Fig. 2 indicates that the local *C. helgolandicus* breed more or less continuously so that the population is dominated by the naupliar stages during most of the year.

TABLE 1. *Approximate cumulative time (in days) required for development at 12C in the laboratory, based on three stocks of* Calanus helgolandicus *(Ch) and 13 of* Rhincalanus nasutus *(Rn)*

Stage	Ch	Rn
Egg	0	0
Nauplius IV–VI	<7	<7
Copepodite I–II	7–14	7–14
Copepodite III	15	14–21
Copepodite IV	22	21–28
Copepodite V	30	21–35
Adult	36	28–49

Only occasionally (as between 12 April and 10 May or between 24 August and 20 September 1966) was there an indication of a *discrete* generation maturing and producing offspring. These indications suggest a generation time of about four weeks, but this interpretation is questionable because the simultaneous occurrence of juveniles with older copepodites that are neither their parents nor their siblings seems likely.

Table 1 shows that the time required to attain various developmental stages in the laboratory was similar in the two species. In general, the males molted from the immature condition to the adult stage somewhat before the females and also died off in the cultures sooner than the females.

In laboratory cultures of *R. nasutus* in which eggs were produced, the eggs were laid two to three weeks after the appearance of adult females. This and the rate of development shown in Table 1 give a minimum egg-to-egg generation time of seven to eight weeks, similar to that of the field population during summer 1965 (Fig. 2). Egg production in cultures continued for an average of seven weeks, so that the time from an egg to the completion of egg production by the resulting female was about 15 weeks, although the life span of these females might be almost twice this.

A stock of *R. nasutus* started as eggs in the 19-liter carboys on 2 August 1965 was cultured successfully through seven generations in the laboratory without further introduction of "wild" animals. The adults of the seventh generation were present on 28 September 1966 after 61 weeks of
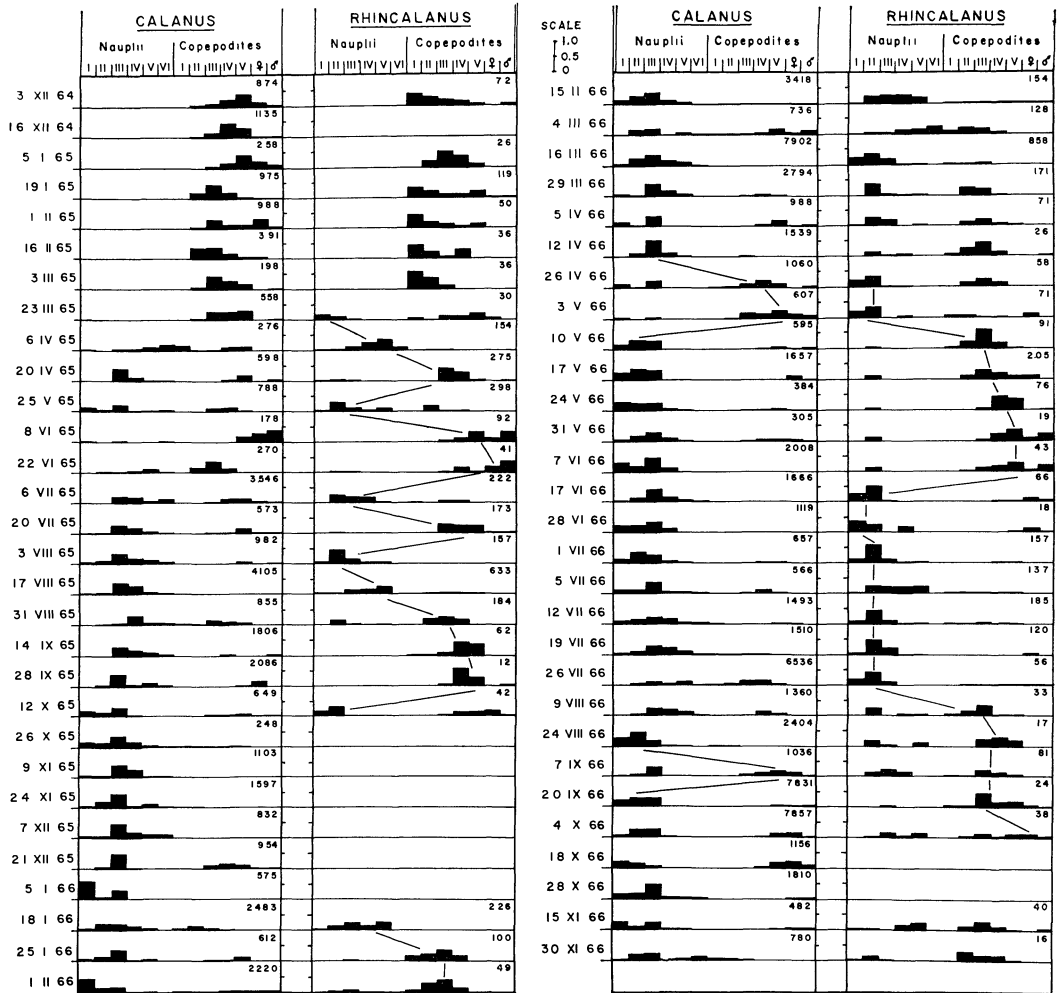
FIG. 2. Relative abundance of the developmental stages of *Calanus helgolandicus* and *Rhincalanus nasutus* at various times over a 2-yr period. The number associated with each histogram shows the total number of individuals of all stages of that species caught in a 90-m vertical tow with a single 0.5-m diam net on that date. The height of the shaded bar associated with each developmental stage shows the relative contribution of that stage to this total. If less than 10 individuals were found in the samples from any date, no histogram was drawn. Before 23 March 1965, no nauplii were sampled, so frequencies are based on copepodite stages only. Diagonal lines connect supposed generations referred to in the text.

continuous culture—equivalent to a mean generation time of 8.7 weeks. The null hypothesis that there was no difference in the number of adult males and females produced in culture (that is, a sex ratio of 1:1) is acceptable ($p > 0.2$ by Wilcoxon signed rank test). In no case did more than 30% of the animals survive from eggs to seven-week-old adults, and the maximum

fecundity was 150 eggs/surviving female. Because of the high mortality and an overall mean fecundity of only 66 eggs/female, the laboratory population remained virtually constant in number until June 1966, during the fifth generation. Mortality then increased sharply, as did the occurrence of inviable eggs, and the seventh generation produced no offspring. The increase in

mortality did not appear to be associated with parasitism. Whether this marked decline in the culture during summer arose from a genetic weakness caused by inbreeding, a nutritional deficiency accumulated over several generations (Provasoli, Shiraishi, and Lance 1959), or a change in quality of the seawater used in the cultures, cannot be determined. A mixture of foods was used and the cultures were not bacteria-free, so a nutritional deficiency seems unlikely.

Fecundity in the laboratory

The total number of eggs produced by wild *C. helgolandicus* and *R. nasutus* under ideal conditions was estimated by counting all the eggs laid by females believed to have copulated only a short time before capture; many were still carrying spermatophores. Four groups of *R. nasutus*, involving 57 females, gave average values of 103, 194, 214, and 355 viable eggs/female for 10 weeks of egg production, while two groups of 15 female *C. helgolandicus* each gave means of 613 and 691 eggs/female over nine weeks. This is somewhat higher than the egg production reported for *C. helgolandicus* by Marshall and Orr (1955). These estimates must be considered minimal because some of the females used may have laid eggs before capture and because some eggs may have been eaten by the females or overlooked in routine counting.

Attempts to duplicate this level of fecundity in laboratory populations in unstirred containers of less than 2-liter volume kept in the dark with the standard mixture of food were unsuccessful, in part because of high mortality during molting. Even when molting was successful and both sexes were produced, there was no evidence of breeding by either *C. helgolandicus* or *R. nasutus*. Stirring these small containers slightly or providing sufficient continuous light for the phytoplankters to photosynthesize improved survival during molting and sometimes even increased the rate of development, but breeding still did not occur. No eggs were produced by animals raised in a 4-liter container.

Animals of three *C. helgolandicus* stocks survived only moderately well during development in the 19-liter carboys and did not produce viable eggs, possibly because of a paucity of males. Several additional stocks were started with animals captured as copepodite stages IV and V, but although these animals molted successfully to adults of both sexes, no eggs were produced. We have thus far not found conditions permitting (or inducing) *C. helgolandicus* to breed in the laboratory, even though rearing from egg to adult has proved feasible if gravid females are obtained from nature.

Initial stocks started with wild *R. nasutus* copepodite stages IV and V had a maximum fecundity of 43 eggs/female in the 19-liter carboys. The fecundity was higher in a stock maintained under standard culture conditions than in a stock exposed to greater light intensities during the day. To test the effect of antibiotics, two stocks were started from eggs and maintained under the usual conditions, except that antibiotics were omitted from one of the cultures. Survival was good in both cases, and females carrying spermatophores were found in both stocks, showing that copulation had occurred. Mean fecundity was similar (about 45 eggs/female) in both cultures, although one female maintained in antibiotics laid a total of 363 eggs. Females known to have been impregnated had a mean fecundity of 63 eggs/female.

Other stocks were started with wild copepodite stages III and IV in three carboys, all provided with the same food. One carboy was stirred and aerated in the standard way, while a second was not stirred and only weakly aerated to test the effects of reduced turbulence. A third carboy was treated in the standard way, but the seawater was enriched with a mixture of dissolved trace metals and organic growth factors used by Lewis (1967) in rearing the naupliar stages of the predatory copepod, *Euchaeta japonica*. Only three of the resulting 25 females produced more than 200 eggs, and there was no significant increase in average fecundity either in the

carboy enriched with growth factors or in the carboy which was not stirred.

It is reasonable to assume from our observations and by analogy to other crustaceans that copulation occurs shortly after the female molts to adult condition. The accumulated observations indicate that not all females in the laboratory stocks copulate successfully. Even when copulation occurs, "normal" fecundity is not always achieved, although some individuals may produce as many eggs as the wild animals. In view of the failure of the animals to copulate in 4-liter containers (or smaller), it may be that volumes of water greater than 19 liters would permit more successful copulation and thereby increase fecundity. It is also possible that a special dietary factor is necessary at the time of gonad maturation and copulation. On the standard diet, females impregnated in the field laid abundant eggs, but even copepods that had lived most of their juvenile lives in the field had a lowered fecundity in the laboratory.

The most efficient program of culture, since small containers are easier to handle than large, would probably involve changing containers during the life cycle. The naupliar and early copepodite stages can be readily raised in 1- or 2-liter containers; transferring the animals when they reach copepodite stages IV–V to 19-liter containers will permit copulation. Impregnated females may then be returned to small containers from which eggs are easily collected.

GROWTH RATE AND EXUVIAE OF
R. NASUTUS

The body length, dry weight, and organic carbon content of the six copepodite stages of R. nasutus were determined using 45 animals collected in the field. Some laboratory-hatched nauplii were also analyzed. The animals were washed quickly with distilled water over a vacuum filter, dried at 60C (Lovegrove 1962), and weighed on an electrobalance. Carbon was determined with a carbon-hydrogen-nitrogen analyzer (F and M, model 180, kindly

provided by Dr. Reuben Lasker). All the carbon was assumed to be organic. The linear regression equations for dry weight vs. body length and carbon content vs. body length, all transformed to logarithms, are as follows:

$$\log_{10} (\mu g \text{ dry weight}) = 3.6 \log_{10} (\text{mm length}) + 0.14, \quad (1)$$

standard error of estimate = 0.11, 95% confidence limits on slope = ±0.2.

$$\log_{10} (\mu g \text{ carbon}) = 4.3 \log_{10} (\text{mm length}) - 0.47, \quad (2)$$

standard error of estimate = 0.14, 95% confidence limits on slope = ±0.3. Because both slopes are significantly greater than 3.0, growth in weight and carbon content may be termed positive allometry (see Gould 1966). It is evident from the relative slopes of the two equations that the carbon per unit dry weight increased with increasing body size. This is possibly because of the increased storage of lipid in the older copepodites. The regression equations may be expected to vary with different degrees of fatness in different populations.

Although growth of copepods is discontinuous in a sense because of discrete molting events, growth in weight is probably more nearly continuous because organic weight increases during a long intermolt period rather than during molting. Individual growth may therefore be approximated by the equation, $W_t = W_0 e^{kt}$, where W is the weight of organic carbon in the animal and t is the time in days of some period of life. Using the life history data in Table 1, the juvenile life of R. nasutus in our cultures can be divided into three periods arbitrarily chosen to involve equal intervals of time. During the initial 13 days of growth from the first nauplius through copepodite stage II, the animal increases from about 0.17 to 3.8 μg in body carbon content, equivalent to a coefficient of exponential growth, k, of 0.24/day. The middle 13 days of growth from copepodite stage II to a stage IV of 35 μg of body carbon implies a coefficient of growth of 0.17/day, while the final 13 days of growth

TABLE 2. *Selective feeding by* Rhincalanus nasutus *offered a mixture of food organisms*

Food organism	Artemia salina nauplius	Coscinodiscus wailesii	Ditylum brightwellii	Thalassiosira fluviatilis	Cyclotella nana
Approx initial concn	0.13/ml	0.95/ml	120/ml	1,080/ml	7,090/ml
Approx vol, μ^3/organism	2.9×10^7	1.6×10^7	5.0×10^4	4.3×10^3	313
Approx carbon, $\mu\mu$g C/organism	5×10^5	1.5×10^5	1.9×10^3	3.0×10^2	41
Contrib. to total C available in mixture	6%	14%	22%	30%	28%

R. nasutus stage	Total μg C ingested/ 18 hr	μg C ingested/ 18 hr-μg C of copepod	Contribution of each food organism to total C ingested by each stage (%)				
Nauplius II and III	0.2	0.6	—	0	100	0	0
Nauplius IV	1.9	1.7	—	0	46	54	0
Nauplius V and VI	1.3	0.7	—	62	37	0	0
Copepodite I	2.2	1.2	0	14	49	37	0
Copepodite II	4.4	1.2	0	13	51	32	4
Copepodite III	5.7	0.4	0	0	28	40	31
Copepodite IV	8.2	0.2	0	95	3	2	0
Copepodite V	21.8	0.4	4	54	23	19	0
Adult male	4.7	—	25	75	0	0	0
Adult female	16.5	0.1	37	45	18	0	0

to an adult female of 160 μg of body carbon is equivalent to a coefficient of 0.12/day. This calculation does not consider the amount of carbon lost as an exuvia when each developmental stage molts.

By weighing and measuring exuviae (cast exoskeletons) and using equation (1) to estimate the weight of the copepodites producing them, it was possible to determine the percentage of weight a copepodite loses through molting. The dry exuviae averaged 0.125 ($n = 25$, SD = 0.056) times the dry weight of the copepodites. The dry exuviae contained about 35% carbon. Thus, a copepod weighing 100 μg (dry) and containing 45 μg of carbon loses about 10% of its carbon during one molting. An individual *R. nasutus* therefore produces a total of about 10 μg of detrital organic carbon (30 μg of dry detrital seston) as exuviae in growing from egg to adult. Lasker (1964) first recognized the importance of euphausiid exuviae as a source of oceanic detritus.

SELECTIVE FEEDING BY NAUPLII AND COPEPODITES OF R. NASUTUS

The ability to raise *R. nasutus* from egg to adult in the laboratory has facilitated investigation of selective feeding by the nauplii and early copepodites of this particle-grazing species; adults from the Indian Ocean had been examined previously (Mullin 1966). The nature of the food of late copepodite stages and adults of several groups of planktonic copepods is known, but only in a few cases, such as *Calanus*, have juveniles been studied (Gauld 1959; Marshall and Orr 1956).

The method of setting up selective feeding experiments was that used previously (Mullin 1966). Experiments were run for about 18 hr in the dark at 12C. Table 2 shows the food organisms used in making up test mixtures and their initial concentration, volume, and carbon contribution. Carbon content of the phytoplankters was computed from cell volume by the equation of Mullin, Sloan, and Eppley (1966);

the carbon content of A. salina nauplii was determined by oxidation with chromic acid followed by back-titration of the excess dichromate with acidic ferrous ammonium sulfate. The total food initially available to the copepods in the experimental mixture was equivalent to approximately 1 mg C/ liter. A. salina were omitted from the mixture offered the naupliar R. nasutus.

Table 2 presents pooled data from seven experiments involving several hundred nauplii of each stage, 50 to 100 individuals of each of the early copepodite stages, and 20 to 50 individuals each of copepodite stage V and the adults. The stage I nauplius was never observed to feed. Stage II and III nauplii have both been observed to contain food in laboratory cultures; these and the older naupliar stages, which first possess a molariform process on the mandible, ate larger phytoplankters in the mixture in preference to the smallest (Table 2). The copepodites, with the exception of stage III, showed during development an increasing degree of preferential feeding on large food particles, as was noted in the genus Calanus (Mullin 1963). The rate of ingestion of food also increased during development (see Gauld 1951; Marshall and Orr 1956), but the rate of ingestion per unit body size tended to decrease.

The male R. nasutus have reduced mouthparts and feed at low rates, but their preferential feeding was similar to that of the females. Males lived for shorter periods as adults than females, even in the presence of excess food.

THE ROLE OF THE FIRST ANTENNAE IN SELECTIVE FEEDING

Cushing (1959) suggested, from indirect evidence, that swimming Calanus may detect food particles with the outstretched first antennae. This use of tactile encounter was thought to enable the copepod to scan more water for food than it could mechanically filter with the mouthparts. Such a method of feeding would presumably be most efficient in capturing large, widely scattered food particles. Although this encounter hypothesis has been attacked on morphological grounds, it has not been

tested experimentally. R. nasutus feeds selectively on large particles as does C. helgolandicus and might therefore be expected to use its even longer first antennae to detect food. If so, loss of the first antennae should reduce the rate of feeding on large particles, although mechanical filtration of small particles might not be directly affected.

Female R. nasutus were picked out of a plankton catch and anaesthetized in 1 g/ liter of m-aminobenzoic acid, ethyl ester, methane-sulfonate salt (MS-222) in cold seawater. Both first antennae of half of these animals were amputated, leaving about ⅓ of the original length. All animals were revived in cold seawater containing 50 mg/liter each of penicillin and streptomycin and were kept at 12C for five days to ensure complete recovery. Although the animals with antennae removed showed abnormal postural orientation and often had positive buoyancy, mortality was slight and both groups produced copious fecal pellets when fed T. fluviatilis.

A mixture of A. salina nauplii and T. fluviatilis cells was offered as food. Moderately low concentrations of food and short durations were used to minimize the possibility that normal animals would feed at a high rate until satiated and then stop feeding while the altered animals fed at a lower rate but continued to feed for a longer time, giving the same final feeding rate.

The grazing rates of both groups of animals increased with decreasing concentration of food and duration of experiment, as expected from previous results (Mullin 1963). The data (Table 3), while indicating a slight depression in the grazing rate of the altered animals, show that the first antennae are not necessary for selective feeding to occur, at least in the present experimental conditions. The encounter hypothesis is not supported by this result.

CANNIBALISM BY FEMALE R. NASUTUS ON THEIR NAUPLII

Adult R. nasutus eat newly hatched A. salina nauplii voraciously, and one wonders to what extent they also devour their own

TABLE 3. *Selective feeding by 10 normal and 10 altered* Rhincalanus nasutus *in three experiments. Each value in the table is a mean of two determinations per experiment*

Duration of expt (hr)	Food organism	Initial concn/ ml	Grazing rates, ml day^{-1} copepod^{-1}	
			Normal	Altered
11	A. salina	0.62	98	122
	T. fluviatilis	570	0	0
6	A. salina	0.32	350	317
	T. fluviatilis	1,300	0	0
3	A. salina	0.13	669	584
	T. fluviatilis	1,300	0	0

nauplii. If *R. nasutus* can "recognize" and spare nauplii of its own species, or if these nauplii can escape the predatory activities of their parents, cannibalism will not seriously affect population dynamics.

One hundred *A. salina* nauplii and 34 *R. nasutus* nauplii of similar size were placed in cooled seawater in each of two 900-ml containers; in one, all the nauplii had been killed by mild heating before addition to the container. Five female *R. nasutus* were placed in each of the two containers, which were then slowly stirred in the dark at 12C for 24 hr. At the end of the experiment, 97 *A. salina* and four *R. nasutus* nauplii had been eaten in the container with the living nauplii; 98 *A. salina* and 24 *R. nasutus* nauplii had been eaten in the container in which all of the nauplii had been killed. This experiment was repeated, using 100 *A. salina* and 75 *R. nasutus* nauplii in each of four containers, with essentially the same result.

The prevention of cannibalism probably depends largely on the ability of living nauplii to avoid the feeding activity of adult *R. nasutus*; *A. salina* nauplii are apparently unable to do this. Tactile stimuli elicit a darting, escape response by *R. nasutus* nauplii, while *A. salina* nauplii are only capable of rather sluggish, steady swimming. There may also be a partial rejection of their own offspring by feeding *R. nasutus* even when these offspring are not alive.

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