

THE RELATIONSHIP BETWEEN DIET AND LARVAL DEVELOPMENT OF PALAEMONETES^{1, 2}

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Larval development of crustaceans consists, in its simplest form, of growth and the addition of somites and limbs. The primitive pattern has been obscured among higher crustaceans by the degree of development achieved by the embryo before hatching and by the magnitude of developmental change which may become evident after each larval molt. Gurney (1942) and most authors think of the mode of development for each species as fixed and regard the number and sequence of definitive larval stages as constant.

Variation in the number and form of larval intermolts of *Palaemonetes pugio* Holthuis and *Palaemonetes vulgaris* (Say) reared in the laboratory has been described by Broad (1957). The present paper is a consideration of the relationships between diet and survival, molting frequency, rate of larval development and the number and form of intermolts during development of these species.

The author is indebted to Professor C. G. Bookhout, under whose guidance this work was done. He also wishes to express his thanks to Dr. T. R. Rice, who kindly furnished stocks of all species of unicellular marine algae used.

METHODS

Larvae dealt with were hatched in the laboratory from eggs carried by adult females and were reared through metamorphosis. Culture methods have already been discussed (Broad, 1957) and need not be repeated in detail.

Each larva was fed the same diet throughout its life, but several different foods were offered to different individuals. The diets differed from one another generally and specifically. There were five general diet categories: no food; unicellular marine algae; algae and non-living animal matter; non-living animal tissue alone; and living *Artemia* nauplii. Specific differences in diet were between the several combinations of the available foods in each general category.

Larvae which were not fed nevertheless had available whatever food might be obtained from the raw sea water in which they were reared. The form of the mouthparts of these larvae makes it extremely unlikely that particles not visible to the unaided eye could be utilized as food.

Some individuals were fed species or combinations of species of unicellular marine algae. These species were maintained in unialgal culture in the laboratory.

¹ Supported in part by a contract with the Office of Naval Research, Nonr-1232(00), and a National Science Foundation grant, NSF-G-1214.

² Part of a thesis submitted to the graduate faculty of Duke University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Clumps of cells which settle in older cultures were used as food. The algae available were from T. R. Rice's stocks of *Nitzschia closterium* and *Nitzschia* sp., (Bacillareae), *Chlamydomonas* sp.; *Thorocomonas* sp.; *Nannochloris* sp. (Chlorophyta); *Porphyridium* sp. (Rhodophyta) and *Pyramimonas* sp. (Chrysophyta).

Other larvae were fed non-living animal tissues alone or in combination with one or more of the algal species. Animal matter used was obtained largely from the plankton. Zooplankton, collected by net, was killed by immersion in distilled water to prevent the fortuitous inclusion of living larvae which might later be confused with individuals being reared. The general zooplankton (and possibly a few contaminating phytoplankton cells) was fed to some larvae. Others were fed on chaetognaths removed from the killed plankton. A few individuals were fed macerated gonad of the mud snail, *Nassarius obsoletus*.

The larvae usually hatched at night. The day on which free-swimming individuals were found was called the first day of larval life. Larvae and the bowls in which they were held were inspected daily. Only the presence of an exuvium was accepted as evidence of molting. Molts were considered to have occurred the day on which the cast exoskeleton was found. Uneaten food was removed and fresh food added at the time of daily inspection.

RESULTS

Palaemonetes larvae were found to ingest almost any particulate matter with which they came in contact. There was no evidence of chemoreception or of selection of any type of food. Cannibalism was infrequently observed. In feeding, the zoea larvae grasp and hold objects with the maxillipeds while the maxillae and mandibles function as jaws. There was no indication of ability to obtain food by filtering.

Larvae fed diets which differed in general composition showed different rates of survival and development and molted at different frequencies, but those fed diets which differed only in specific composition did not. All the larvae are grouped for treatment of data into five general categories according to the diet received.

Diet and survival of larvae

Figures 1 and 2 show the per cent of mortality observed at each molt among *P. pugio* and *P. vulgaris* larvae fed various diets. Most of the deaths occurred at the time of molting, but some individuals were lost during the intermolt phase. These are included among the larvae which died at the time of the next molt. The per cent of larval mortality at molt N is that fraction of the total number of larvae which completed molt N-1 but did not survive molt N, and includes larvae lost during intermolt N. Loss of some intermolt larvae was due to removal of specimens for preservation or study, and the apparent mortality accordingly is biased upward when the total number of larvae involved is small or in the later molts.

Larvae which were not fed did not survive nor did those which were fed only phytoplankton cells. Sixty *P. pugio* larvae were starved. Forty-two of these survived one molt and two individuals molted twice, but none lived longer than 10 days. Among 80 *P. vulgaris* larvae which were not fed, none molted and all died within 5 days. Feeding any of a variety of unicellular marine algae did not seem

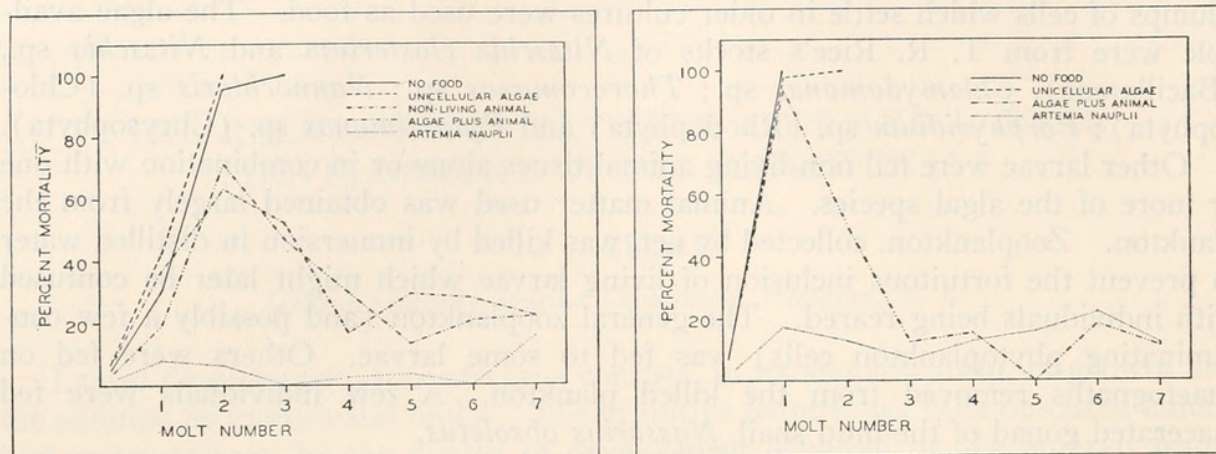


FIGURE 1. Mortality at each of the first seven molts among *Palaemonetes pugio* larvae fed various diets. Per cent mortality at each molt is based on the number of larvae which survived the last molt.

FIGURE 2. Mortality at each of the first seven molts among *Palaemonetes vulgaris* larvae fed various diets. Per cents computed as for *P. pugio* (Fig. 1).

to improve survival or molting in either 280 *P. pugio* or 100 *P. vulgaris* larvae. The survival of algae-fed larvae shown in Figures 1 and 2 closely approximates that of starved larvae.

Larvae that were fed foods of animal origin, either living or non-living, alone or in combination with algae, were able to survive through metamorphosis in the laboratory. The mortality of 608 *P. pugio* larvae that were fed diets of non-living animal matter, mostly zooplankton, combined with phytoplankton cells is shown by the dash-and-two-dots line in Figure 1. Sixteen of these individuals metamorphosed. The dash-and-dot line in Figure 1 shows the mortality of 732 *P. pugio* larvae that were fed non-living animal matter alone. In general the two curves are alike. Both reflect, in peaks at the second molt, the ability of this species to survive a single molt without food. Only 6 individuals metamorphosed on a diet of freshly killed zooplankton or other non-living animal matter. By far the best survival was shown by larvae fed living *Artemia* nauplii. The mortality of 100 of these is shown by the dotted line in Figure 1. Sixty-five individuals survived metamorphosis. Another 40 *P. pugio* larvae, for which it was not possible to determine daily the state of development of each individual, were fed living *Artemia* nauplii. Mortality at each molt of these individuals is not shown, but 33 metamorphosed.

The mortality of 667 *P. vulgaris* larvae that received a diet of non-living animal matter combined with algal cells is shown by the dash-and-two-dots line in Figure 2. The initially high mortality shown by starved larvae is also evident for these larvae, and contrasts with the early independence of available food shown by *P. pugio*. Six postlarvae survived metamorphosis. The dotted line in Figure 2 shows the mortality observed among 390 *P. vulgaris* larvae fed living *Artemia* nauplii. One hundred twenty-two of these metamorphosed in the laboratory.

Diet and the frequency of molting

Molting of *P. pugio* larvae is shown in Figure 3. The differences in molting frequencies between larvae which survived and most of those which did not on

each diet are insignificant, although those individuals which did not maintain a regular molting schedule did not survive. In general, the range of days during which specific molts occurred among larvae which lived is more restricted for the earlier than for the later molts. This most likely results from some variation in the molting frequencies of larvae fed similarly.

Since, except for the first two molts, there is little or no overlap in the means and standard deviations, or sometimes even the ranges, of corresponding molts by larvae fed different diets, the frequencies suggested by the diagram seem to be statistically separable. Average molting frequencies may be computed, although these become more or less meaningless since the interval between hatching and the first molt and that between the last larval molt and the molt of metamorphosis must be included. The interval between hatching and the first molt is usually somewhat longer than that between subsequent molts. The molt of metamorphosis, although not exactly comparable to other molts, is also included in the computations of average molting frequencies. Among larvae which survived metamorphosis on a diet of mixed plant and animal matter, the frequency of molting varied from one molt every 3.15 to 4.0 days with an average frequency of one molt every 3.70 days. Those larvae which survived on a diet of non-living animal matter alone molted once every 2.36 to 2.67 days with an average frequency of one molt every 2.51 days. Molting among larvae which survived metamorphosis on a diet of living *Artemia*

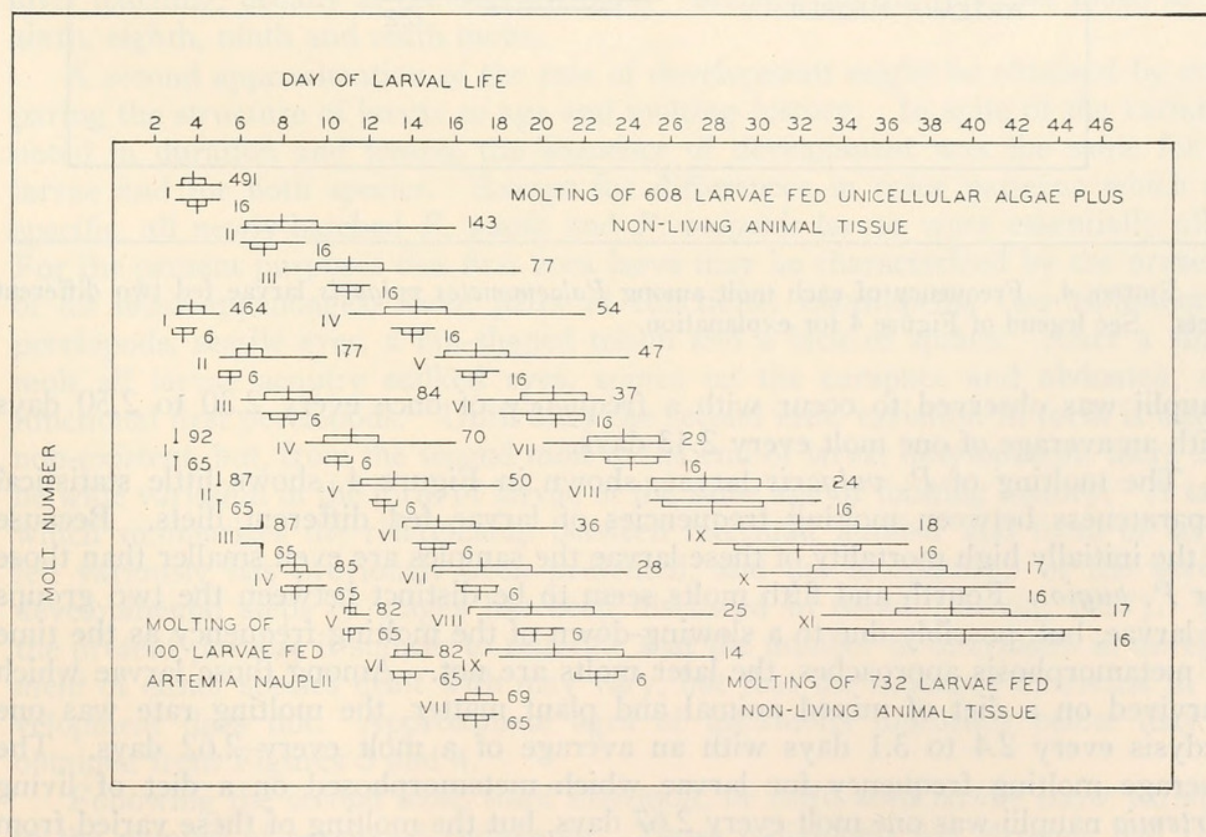


FIGURE 3. Frequency of the several molts among *Palaemonetes pugio* larvae fed three different diets. Number of each molt is given by Roman numbers. Upper line of each pair shows days during which molt occurred. Lower line gives days on which those larvae which completed metamorphosis molted. Means shown by vertical lines. Box on each side of mean is one standard deviation. Arabic numbers show sample size.

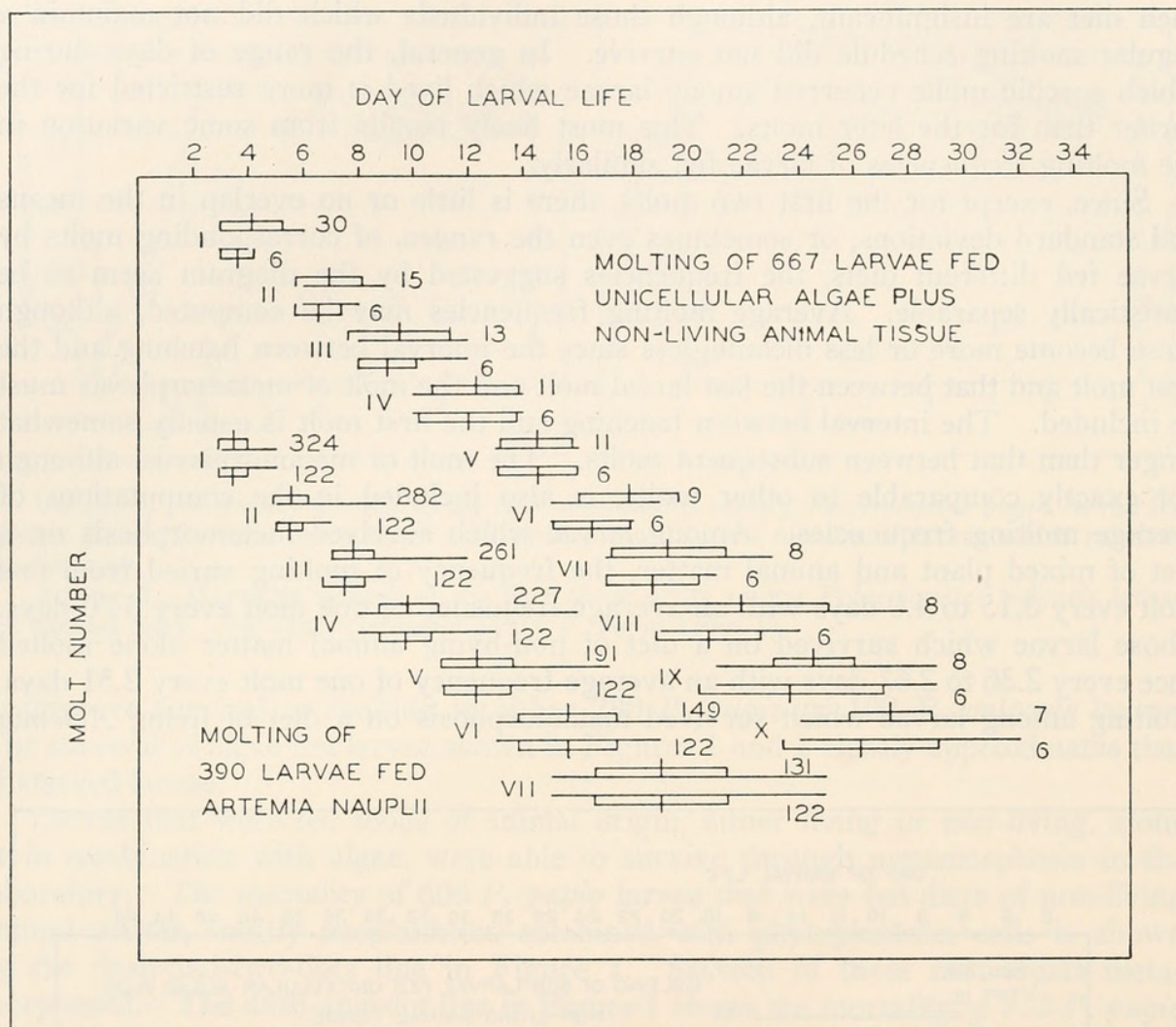


FIGURE 4. Frequency of each molt among *Palaemonetes vulgaris* larvae fed two different diets. See legend of Figure 4 for explanation.

nauplii was observed to occur with a frequency of once every 2.30 to 2.50 days with an average of one molt every 2.43 days.

The molting of *P. vulgaris* larvae, shown in Figure 4, shows little statistical separateness between molting frequencies of larvae fed different diets. Because of the initially high mortality of these larvae the samples are even smaller than those for *P. pugio*. Fourth and fifth molts seem to be distinct between the two groups of larvae, but, possibly due to a slowing-down of the molting frequency as the time of metamorphosis approaches, the later molts are not. Among those larvae which survived on a diet of mixed animal and plant matter, the molting rate was one ecdysis every 2.4 to 3.1 days with an average of a molt every 2.62 days. The average molting frequency for larvae which metamorphosed on a diet of living *Artemia* nauplii was one molt every 2.67 days, but the molting of these varied from one molt every 2.1 to 3.6 days.

Diet and the rate of larval development

An approximation of the rate of larval development may be obtained from the number of days between hatching and metamorphosis. This, however, is not

completely satisfactory since metamorphosis need not always occur at what appears from morphological criteria to be the end of larval development. Thus, Faxon (1879) found "last stage" *P. vulgaris* larvae which molted in the laboratory gave rise to other "last stage" larvae morphologically indistinguishable from their previous form. The larvae of both *P. pugio* and *P. vulgaris* reared in the present study sometimes passed through two or more identical intermolts at the end of development and before metamorphosis. At this time, depending upon other conditions not presently understood, the larva may metamorphose, as most of them do, or possibly may remain larval for some time.

P. pugio larvae fed a diet of mixed animal and plant matter metamorphosed from 29 to 49 days after hatching. Most of the larvae metamorphosed at the eleventh or twelfth molt, but a few metamorphosed as early as the ninth or tenth molt, or as late as the thirteenth molt. Larvae of this species fed a diet of non-living animal matter alone metamorphosed from the nineteenth to the twenty-eighth day after hatching and at the eighth, ninth, tenth or eleventh molt. Those individuals fed a diet of living *Artemia* nauplii metamorphosed from 17 to 21 days after hatching, usually at the seventh, but sometimes at the eighth molt.

P. vulgaris larvae fed a diet of mixed animal and plant matter metamorphosed from 24 to 34 days after hatching. The molt of metamorphosis was most often the tenth, but two individuals metamorphosed at the eleventh and thirteenth molts. Those larvae fed a diet of living *Artemia* nauplii metamorphosed from 14 to 30 days after hatching, usually at the seventh molt. Metamorphosis was also noted at the sixth, eighth, ninth and tenth molts.

A second approximation of the rate of development might be obtained by comparing the structure of larvae to age and molting history. In spite of the variation noted in duration and tempo, the sequence of development was the same for all larvae and for both species. Except for differences in color patterns which are specific, all newly-hatched *P. pugio* and *P. vulgaris* larvae were essentially alike. For the present purposes this first zoea larva may be characterized by the presence of all head appendages, three pairs of functional maxillipeds, two rudimentary pereopods, sessile eyes, a fan-shaped telson and a lack of spines. After a single molt all larvae acquire stalked eyes, spines on the carapace and abdomen, and functional first pereopods. Until after the second molt variation in form is almost non-existent, but, from the second molt to the end of larval development, there may be wide variation in the form of larvae of the same age or molting history. A table which summarizes the relationship between intermolt number and form of larvae fed variously has previously been presented, and the morphology of the several developmental steps or stages has been discussed in detail (Broad, 1957). For the present purpose it suffices to reiterate that the number of intermolts in development of either species dealt with may vary, but that the sequence of events in development does not. Approximate ages of intermolts discussed below may be obtained from Figures 3 and 4.

Following the second molt, third intermolt, or third zoea larvae show variation in the number of pereopod rudiments. Those individuals fed *Artemia* had rudiments of pereopods 3, 4 and 5 while those fed other diets lacked pereopods 4 and 5. Great variation was evident among fourth intermolt larvae. Those individuals fed *Artemia* bore rudiments of the pleopods but none of the others did. Among larvae fed non-living animal foods alone, pleopod buds first appeared in the fifth or

sixth intermolt and not until the seventh intermolt in larvae fed diets which combined algae with animal matter. The intervening intermolts, 4, 5 and 6, differed from one another in the development of pereopods.

Fifth intermolt larvae which had been fed *Artemia* had pleopod rudiments and chelae. The pleopod buds of fifth or sixth larval intermolts fed non-living animal tissue alone became rudiments after a single molt, but chelae did not appear until after two molts. Some of the eighth larvae fed algae and animal matter bore chelae, but others had none.

The pleopods of all sixth intermolt larvae which had been fed *Artemia* bore setose exopods. These were first evident in seventh or eighth intermolts which had received non-living animal food and in ninth zoea larvae which had been fed algae plus non-living animal foods.

The final larval form has been characterized by both setose endopods and *appendices internae* on pleopods. Most larvae fed *Artemia* nauplii achieved this final form in the seventh intermolt, although some individuals required two steps after the sixth zoea for *appendices internae* to appear. Metamorphosis occurred at the seventh or eighth molt. *Appendices internae* made their appearance in ninth intermolt larvae fed non-living animal matter, and metamorphosis occurred at the tenth molt. Among larvae fed algae and animal matter, the final larval form was reached in the tenth, eleventh, or even sometimes the twelfth intermolt. Metamorphosis usually occurred at the following molt.

DISCUSSION

Since survival of larvae fed algal diets alone did not differ from that of zoeae which were not fed, it would seem that the algal species available had no value as food for either *P. pugio* or *P. vulgaris* larvae. In order to survive, the larvae must find some particulate food, probably animal in nature.

A physiological distinction between *P. pugio* and *P. vulgaris* is possible in the ability of the former species to molt once and survive up to ten days without food while the latter neither molts nor lives longer than five days without food. Speculation regarding the survival value which might be associated with this relatively greater independence of trophic conditions, though interesting, is futile in view of the presently limited knowledge of the geographic and ecological distribution of the species of *Palaemonetes*. The ability of either species to adjust development to external conditions and the rather indefinite time of metamorphosis are both of positive survival value.

It is possible that the varying rates of survival, molting and development noted among larvae reared in the laboratory may be due to differences in the total quantity rather than in the quality of food available. Two factors limited the amount of non-living animal tissue available to larvae fed this diet. In order to retard fouling of the water in the bowls, the total quantity of food added daily had to be kept within limits. Since food was always left, the quantity was at first thought to be sufficient. Non-motile food, however, sank to the bottom of the bowls. The larvae swam near the surface. It has already been stated that contact between zoea and food seemed to be the result of chance encounter rather than active search on the part of the larva. The low probability of encounter between larvae swimming near the surface and food lying on the bottom might account for the uneaten food left each day.

If clumps of algal cells were added to the diet, a third limiting factor is also added. Larvae fed algae actually ingested the material offered. The red, green or brown color of the cells could be seen in the gut and feces of the larvae. Since the algal species used have been shown to be of no nutritive value, it seems possible that, where algae plus another food is offered, the ingestion of the nutritively inert material may restrict the intake of other foods which can be digested and utilized.

No restrictions were placed on the total number of *Artemia* nauplii fed. The nauplii swam near the surface with the zoea larvae. The probability of encounter between larva and food was greatest when the diet consisted of living animals.

It has been suggested that intraspecific variation in crustacean larvae may arise from extrinsic causes (Broad, 1957). Sandoz and Rogers (1944) found that poorly nourished *Callinectes* zoeae molted only after a relatively long period and were smaller than other larvae which had received more food. Templeman (1936a, 1936b) found reducing the amount of food given *Homarus* larvae lengthened the intermolt period and sometimes resulted in the production of an "extra" larval intermolt. The present data suggest that the amount of food available to *Palaemonetes* during its larval life may affect both the rate of development and the frequency of ecdysis. The independence of these variables is suggested by the differences in the magnitude of response to sub-optimal feeding. Although the longest regular interval between molts was only 1.9 times the least, the duration of larval life was extended 3.5 times over the most rapid successful development.

Heegaard (1953) has suggested that different developmental rates among decapod larvae may be caused by "external as well as internal" factors. The amount of available food may be an external factor which affects both the rate of development and, independently, the frequency of molting. Variation in the form of larvae under these conditions might be restricted only by the morphological limitations of species. If norms of developmental stages exist among decapods, as Fraser (1936) has found among Euphausiids, constancy of environment might be considered of prime importance in their establishment. Extra or abnormal larval stages found in nature or in the laboratory as well as stages skipped in development may reflect an adaptability to environmental variation during development.

SUMMARY

1. Larvae of *Palaemonetes pugio* Holthuis and *Palaemonetes vulgaris* (Say) reared in the laboratory showed differences in survival, frequency of molting and rate of development which may be associated with the amount of food available.

2. Larvae were unable to survive if fed diets of either single species or combinations of species of several unicellular marine algae or if not fed. Starved *P. pugio* larvae were able to survive one molt without food but starved *P. vulgaris* larvae died without molting.

3. Larvae of both species lived through metamorphosis if fed a diet which included animal tissue. The best survival was obtained by feeding living *Artemia* nauplii.

4. The frequency of molting, the duration of larval life and the number of larval intermolts in the development of *P. pugio* and *P. vulgaris* vary according to the quantity of food available. The frequency of molting and the rate of development are suppressed by a reduction in intake of food.

5. Variation in molting frequency independent of the rate of development makes possible variation in the form and number of larval intermolts.

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<https://doi.org/10.2307/1539194>.

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