Larval Development Of Pagurus Longicarpus Say Reared In The Laboratory. V. Effect Of Diet On Survival And Molting

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LARVAL DEVELOPMENT OF *PAGURUS LONGICARPUS* SAY REARED IN THE LABORATORY. V. EFFECT OF DIET ON SURVIVAL AND MOLTING [1]

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During the last several decades, papers too numerous to list here have been published describing larval stages of decapod crustaceans reared in the laboratory. Through this work techniques for rearing these larvae have become standardized, making a variety of physiological and ecological experiments possible (for example, Costlow, 1961, 1964, 1966, 1967; Costlow and Bookhout, 1968; Roberts, 1971a, 1971b).

Yet relatively little is known about the nutritional requirements of decapod larvae. Lebour (1922) performed a limited number of stomach content analyses on planktonic zoeae and reported that decapod larvae are principally phytophagous. Subsequent laboratory study led her to conclude that, contrary to her first report, many decapod larvae are actually carnivorous and that diatoms and other phytoplankters observed in stomach content analyses were ingested adventitiously (Lebour, 1927; Gurney, 1942).

Hart (1935, 1937) and Sandoz and Rogers (1944) in their early efforts to culture decapod larvae simply concentrated plankton and isolated desirable (or plentiful) components to feed to larvae. Through these and other studies, it has been verified that many decapod larvae require animal material in their diet, although it was suggested that *Callinectes sapidus* may utilize a dinoflagellate (Sandoz and Rogers, 1944).

A standard diet of *Artemia* nauplii has been used by most recent workers for culturing decapod larvae (for example, Costlow and Bookhout, 1960 and numerous more recent studies; Forss and Coffin, 1960; Chamberlain, 1962; Broad, 1957; Regnault, 1969a, 1969b; Provenzano, 1967a, 1967b). This diet has numorous practical advantages for culture works: (1) it is available in large quantities regardless of season, (2) it is a suitable size for many decapod larvae, and (3) it permits complete development to the juvenile or beyond with reasonably consistent mortality rate, intermolt duration, morphogenetic sequence, *etc.*

Nevertheless, *Artemia* is not a "natural diet" and there is no certainty that larvae reared on this diet follow the same morphogenetic sequence as larvae in the plankton. Furthermore, for commercial production operations, *Artemia* represents a high production cost. When planktonic and laboratory-reared specimens have been compared, minor discrepancies in morphological detail have been noted (LeRoux, 1966; Roberts, 1968; McDonald, Pike and Williamson, 1957; Bookhout, 1964; Chamberlain, 1962). This is quite different from the variability in number of instars observed under presumably constant culture conditions among the carid

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shrimp (Broad, 1957; Hubschman, 1969; LeRoux, 1963; Regnault, 1969a) and *Upogebia* (Webb, 1921; Roberts, unpublished).

A few authors have investigated the food value of specific unicellular algae and zooplankters for decapod larvae selected on the basis of availability. Algae prolong survival but do not permit complete development (Broad, 1957a; Chamberlain, 1962; Regnault, 1969b), except for certain species of *Pinnotheres* (Atkins, 1955), *Thor floridanus* (Broad, 1957b) and penaeid shrimp (Cook and Murphy, 1969). Sandoz and Rogers (1944) indicated that a dinoflagellate was a major prey species in their food mixture used for rearing *Callinectes sapidus*. No studies of the possible role of algae in the nutrition of anomurans have been conducted.

Copepods and other zooplankters promote complete development. Studies with these organisms have been hampered by technical difficulties in providing various diets in sufficient quantity when needed. Nauplii of *Artemia* have been selected as the standard laboratory diet for decapod larvae to overcome these difficulties, even though it is not a "natural" diet.

This paper describes some preliminary studies of suitable diets for the larvae of the anomuran, *Pagurus longicarpus*, conducted at the Virginia Institute of Marine Science, Gloucester Point, Virginia. Responses of larvae presented with several potential food sources were observed directly. Selected diets which could be provided in quantity over a suitably long period were then tested in culture experiments to determine whether larvae could survive and develop to or beyond the megalopa.

MATERIALS AND METHODS

Direct observations of feeding by *P. longicarpus* larvae were made using a dissecting microscope. Several zoeae grown in mass culture to various stages of development were placed in a Syracuse watch glass with *ca.* 10 ml filtered sea water containing a number of food organisms and observed periodically for at least one hour. Care was taken not to heat the container with the microscope illuminator. The food organisms supplied were copepod nauplii, copepodites, *Balanus* nauplii, *Polydora* 3–5 setiger larvae, and pelagic stages of several polychaete worms (unidentified). These organisms were all collected from plankton in the seawater system.

In a series of three culture experiments, selected algae and invertebrate larvae were used to feed larvae of *P. longicarpus*. Mortality rate and intermolt duration for larvae fed experimental diets were compared to those for unfed and *Artemia* nauplii fed control groups. The control *Artemia* diet is known to permit complete development of *P. longicarpus* larvae (Roberts, 1970, 1971a, 1971b).

The experimental algal diets were (1) a mixture of microflagellates (*Cyclotella, Dunaliella,* and *Monochrysis*) and (2) the dinoflagellate, *Amphidinium klebsii.* Microflagellates, cultured by the Microbiology Department, were used in Experiment 2. A volume of algal culture was added to filtered sea water to give a final algal concentration of *ca.* 10^4 cells/ml. The dinoflagellate, *Amphidinium,* used in Experiment 3, was grown in mass culture on an enriched sea water medium. During the course of the experiment, the culture was found to be contaminated with *Dunaliella* (20–50%). No estimate of the concentration of dinoflagellate provided each zoea was made, although some cells always remained at the end of each

24 hour period. In neither case were the algal cells washed free of the original culture medium before use.

The animal diets tested were shelled larvae of *Crassostrea virginica* and the post-trochophore stage of *Arenicola marina*. The *Crassostrea* larvae, obtained from oysters collected at Horsehead, Virginia were cultured by the Malacology Department. The larvae used in Experiment 1 ranged from early umbo stage (80–150 μ) to presettlement stage (250–320 μ). Approximately 20–40 larvae were given to each zoea daily. The *Arenicola* post-trochophores used in Experiment 3 were obtained as follows: several gelatinous egg strings were collected daily at low tide from the beach in front of the laboratory, washed in filtered sea water, and placed in sea water in large finger bowls. The post-trochophores emerged from the egg strings and accumulated on the lighted side of the culture dish. These larvae were sticky, adhering to the glass dish and to each other. In addition they carried with them some diatoms (*Nitschia sigmoides*) which were numerous in the egg strings. To remove the gelatin and diatoms, the larvae were washed by vigorous pipetting in filtered sea water. This procedure was not completely successful since many *Arenicola* post-trochophores still adhered to the culture vessels used in the feeding experiments. Each *Pagurus* zoea was given 200–400 *Arenicola* post-trophores daily.

The experiments were carried out in compartmented plastic boxes. The general procedures for obtaining and handling *Pagurus* larvae, both control and experimental groups, were described previously (Roberts, 1970, 1971a). The range of salinity was 18–20‰ (mean: 19‰) in Experiment 1, 20 to 21‰ (mean: 20.5‰) in Experiment 2, and 22 to 23‰ (mean: 22.3‰) in Experiment 3. The cultures were maintained at ambient sea water temperatures on a sea table. The range of temperature was 18 to 22° C (mean: 21° C) in Experiment 1, and 24 to 28° C (mean: 26° C) in Experiments 2 and 3. These conditions were within the acceptable range for this species (Roberts 1971a). The larvae were examined daily and the presence or absence of material in the digestive tract was noted. In all experiments, food was in excess. No efforts were made to quantify the amount ingested.

RESULTS

Pagurus zoeae were observed to feed successfully on copepod nauplii, copepodites, *Balanus* nauplii, *Polydora* 3–5 setiger larvae and pelagic stages of several unidentified polychaete worms. The zoeae handled each of these much the same way as *Artemia* nauplii and *Arenicola* post-trochophores (see below). Further testing of these potential foods was not feasible because of the time required to procure adequate amounts of the experimental foods.

Larvae in the unfed control group always had empty guts. These larvae never molted to Zoea II in any experiment. The survivorship curves for the unfed control group in the three experiments show no major differences. In all experiments there was a period of high survival lasting 3–4 days (slightly less in Experiment 1) followed by high mortality. Only 50‰ of the population was alive after 3.5–4.2 days. All of this group were dead by day 5–7.

Larvae in the *Artemia*-fed control group always had some material in the gut. Direct observations of feeding revealed that each *Artemia* nauplius is captured by the maxillipeds by chance encounters during normal swimming activity and pushed
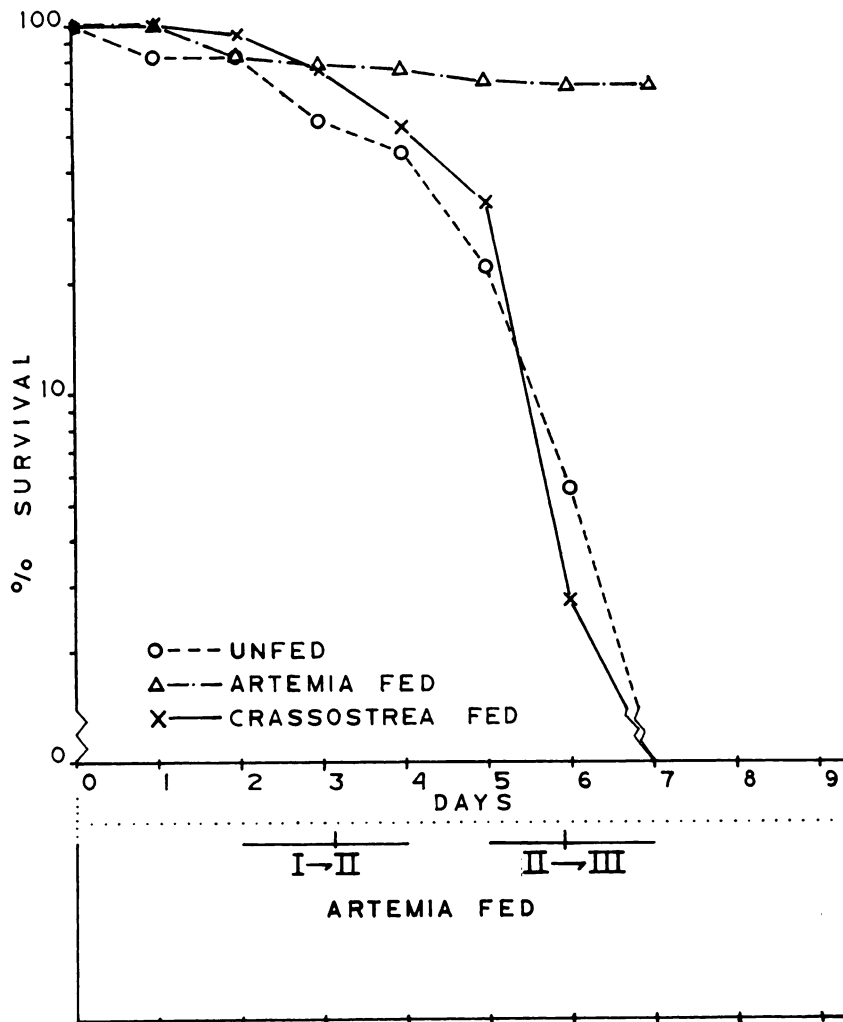
FIGURE 1. Survivorship curves for Experiment 1 comparing *Pagurus* larvae fed oyster larvae, a control group fed *Artemia* nauplii, and an unfed control group. Below the survivorship curve is a bar graph indicating the time during which molting occurred and the mean time to molt.

against the maxillae, maxillules and mandibles. The mandibles "chew" the nauplius, but generally only one or more appendages are removed before the nauplius escapes. Late stage zoeae are more efficient, but still rarely ingest an entire nauplius. Fecal production was undetected during the early stages, pronounced in late Zoea III and Zoea IV. The *Artemia*-fed control group always exhibited a high rate of survival. Experiment 1 was terminated after day 7 and when 53% of the remaining larvae had reached the third instar. In Experiment 2, 31% of the larvae reached the megalopa after a mean of 10.5 days, in Experiment 3, 58.9% after 9.9 days.
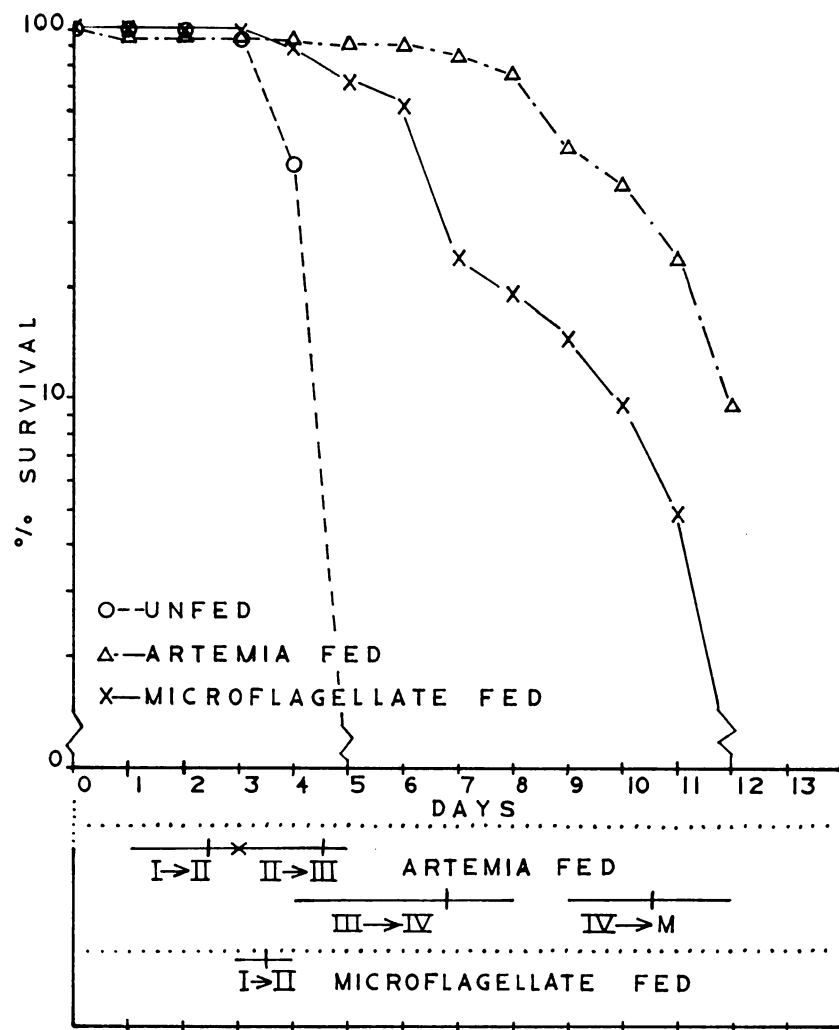
FIGURE 2. Survivorship curves for Experiment 2 comparing *Pagurus* larvae fed microflagellates and control groups. Below the survivorship curves is a bar graph indicating the molt record.

The algal diets were only marginally suitable for larval culture. Both were ingested by zoeae of *P. longicarpus* as indicated by the green color of the gut but details of ingestion could not be observed. The bulk of the ingested material seemed to be defecated. Both algal diets permitted a small number of zoeae to molt to the second instar (9.5% and 11.1%). The *Amphidinium*-fed and unfed control groups had nearly identical survivorship curves (Figure 3) until day 5 when the curve for the *Amphidinium*-fed larvae leveled off following the molt to Zoea II. Too few larvae survived to provide information on the value of *Amphidinium* as a food for Zoea II and older *Pagurus* larvae.

Shelled *Crassostrea* larvae proved unsuitable as food for *Pagurus* larvae. Zoeae which captured an oyster larva with their maxillipeds were unable to break the shell with the maxillae, maxillules and mandibles. Oyster larvae would frequently close their valves on the maxillae thus impeding further feeding activity and to some extent swimming of the larva. The inability to deal with oyster larvae was not an artifact of the method of observation since in culture, oyster-fed zoeae had a survivorship curve nearly identical with that of the unfed control group. The unfed control group and oyster-fed group exhibited 50% mortality on day 3.5 and 4.1 respectively, with 100% mortality on day 7 for both groups, while 68% of the *Artemia*-fed control group survived and molted to Zoea III (Figure 1).

Arenicola post-trochophores were readily handled by *Pagurus* zoeae and, in contrast to *Artemia* nauplii, were usually totally ingested once captured. This

TABLE I

Mean post-hatch time and intermolt duration for Artemia-*fed* (control) *and*
Arenicola-*fed* (experimental) *larvae* (from Experiment 4)

Instar	Artemia-fed		Arenicola-fed	
	Mean posthatch time	Intermolt duration	Mean posthatch time	Intermolt duration
I		1.57		3.34
	1.57		3.34	
II		2.16		3.10
	3.73		6.44	
III		2.47		3.06
	6.20		9.50	
IV		3.70		3.63
	9.90		13.13	
M				

diet was the only one, aside from *Artemia* which permitted complete development to the megalopa. The mean post-hatch time and intermolt duration for *Artemia*-fed and *Arenicola*-fed groups are given in Table I. The intermolt durations for the *Artemia*-fed control group increased from 1.57 days for Zoea I to 3.70 days for Zoea IV, while the intermolt duration for *Arenicola*-fed zoeae was uniformly 3.1–3.6 days for each instar. This trend of increasing intermolt duration from Zoea I to IV for *Artemia*-fed larvae, although not as uniformly progressive, was noted previously (Roberts, 1971a). The consistently long intermolt duration for *Arenicola* post-trochophores may reflect inadequate amounts of food in the early zoeal stages. With regard to mortality, the survivorship curve for these two groups is not significantly different until day 10, which corresponds to the molt of the *Artemia*-fed group from Zoea IV to megalopa, at which time they showed a marked decrease in survival rate. This break in the survivorship curve for
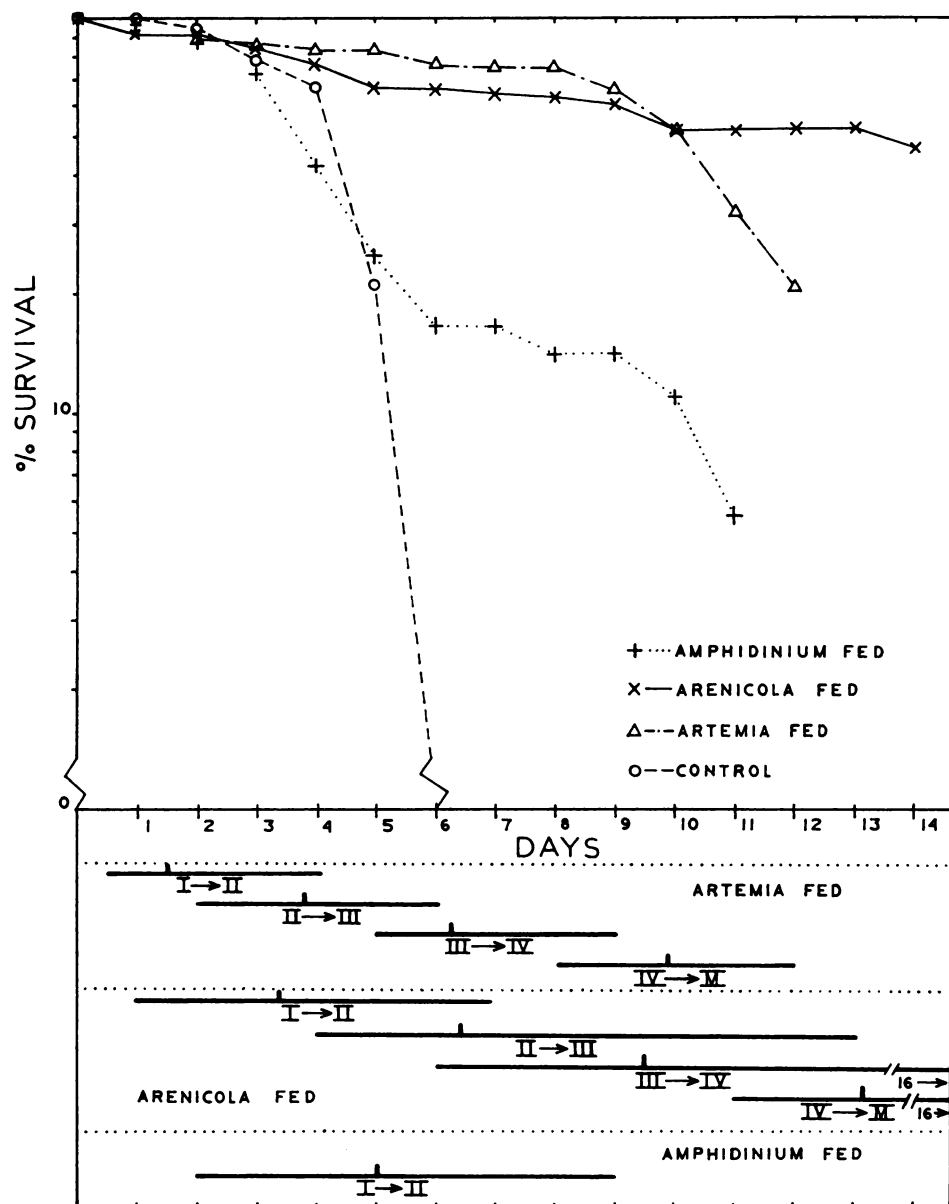
FIGURE 3. Survivorship curves for Experiment 3 comparing *Pagurus* larvae fed *Amphidinium* or *Arenicola* larvae to control groups. Below the survivorship curves is a bar graph indicating the molt record.

Artemia-fed larvae (Figures 2 and 3) was also noted previously (Roberts, 1971a). No similar decline in survival of *Arenicola*-fed larvae was associated with this molt (Figure 3).

DISCUSSION

Unfed larvae of this and other decapod species survive a period slightly greater than the normal intermolt duration at the first instar. Only carid shrimp are reported to exhibit some molting to the second instar when unfed (Regnault, 1969b; Broad, 1957a; Roberts, unpublished). Regnault (1969a) demonstrated an inverse correlation between starvation resistance of *Hippolyte inermis* and temperature. Rice and Provenzano (1966) obtained a similar result for *Dromidia antillensis*. However, Provenzano (1967b) obtained the opposite result for *Trizopagurus magnificus*. Regnault (1969a) also presented evidence that eggs produced early in the year had greater yolk reserves than those produced later. In the study reported here there was no conclusive evidence of a relationship between starvation resistance and temperature or season.

The *Artemia* diet promoted complete development to the megalopa. The survival rate was very high until the Zoea IV-Megalopa molt at which time mortality increased rapidly. The intermolt duration of Zoeae IV was always prolonged, and in Experiment 3, the intermolt was progressively longer for each instar. This phenomenon has been reported previously (Roberts, 1971a). Bookhout and Costlow (1970) have reported that larvae of four brachyuran species fed *Artemia* nauplii hatched from eggs collected in Utah had poorer survival than larvae fed *Artemia* hatched from eggs collected in California. Further, two species (*Rhithropanopeus harrisii* and *Callinectes sapidus*) showed some abnormalities in the megalopal or first crab stages. They also reported the concentration of DDT in *Artemia* nauplii hatched from cysts collected in Utah to be three times higher than in those from cysts collected in California. These observations may explain the discontinuity in the survivorship curve observed in the present study in which the *Artemia* cysts originated from Utah.

The microflagellate and dinoflagellate species tested were not suitable diets for *Pagurus longicarpus* in that they did not promote complete development. There were some benefits of an algal diet in prolonging survival and permitting a few animals to molt once. One might suspect that this relates to the quantity of material provided by an algal diet rather than quality. The fact that the ingested algae are largely defecated whereas animal material is nearly completely assimilated suggests that the deficiency of algal diets is a matter of nutritive value.

Crassostrea larvae (or other molluscan larvae) can be provided in the laboratory in adequate quantities by use of techniques developed by Loosanoff and Davis (1963) and their co-workers. These would therefore seem quite suitable as food for decapod larvae in culture. The inability of *Pagurus* larvae to ingest oyster larvae was unexpected since Lebour (1927) and Hart (1937) had reported using *Ostrea* larvae as food for decapod larvae. These workers may have used only *Ostrea* trochophores rather than shelled larvae. Decapod larvae are probably incapable of ingesting any shelled larvae of molluscs and hence molluscan larvae used as food for crustacean cultures would have to be produced daily.

Arenicola post-trochophores are smaller than *Artemia* nauplii and hence may be handled more readily. They proved equally nutritious for pagurid larvae and did not cause increased mortality after Zoea IV which was observed among *Artemia*-fed larvae. However, *Arenicola* is not especially valuable for culture studies since they are only seasonally available, and are not as readily caught because they tend

to adhere to the culture dish. Larvae of polychaete annelids may be very significant in the diet of naturally occurring decapod larvae as they are very abundant during the breeding season of most estuarine decapods.

Pagurus larvae have been observed to ingest several other organisms in the laboratory. Nauplii of *Balanus* were readily ingested as were small copepods and copepod nauplii. No culture experiments were attempted because these organisms were not available in sufficient quantity. Chamberlain (1962) tested the value of copepod nauplii as food for *Rhithropanopeus* zoeae and found them equal in value to *Artemia* nauplii. Copepod nauplii were obtained from the plankton, which is a very tedious and uncertain process.

Techniques are available which permit continuous culture of selected calanoid copepod species in the laboratory though not yet in quantities sufficient to feed crustacean, fish, or other carnivorous larvae (Zillioux, 1969; Zillioux and Lackie, 1970). A copepod diet may be desirable when culturing decapod species which cannot handle food the size of *Artemia* nauplii or species which are especially sensitive to pesticide residues found in *Artemia* or when the experimental design requires a "natural" diet. Naturally occurring decapod larvae must ingest large quantities of copepods, perhaps the single most abundant zooplankter.

Many fish and invertebrates consume detritus when their normal diets are unavailable. In some cases detritus has been found to be a major source of nourishment. Much of this material is decomposing fecal material (Darnell, 1968).

Broad (1957a) used non-living animal material as a source of food in his study of dietary requirements of *Palaemonetes pugio*. He collected zooplankton (presumably including much fecal material), and killed it by immersion in distilled water. This dead animal material was then fed to zoeae. He also tried macerated gonad of *Nassarius*. This diet permitted complete development but was not as good as *Artemia* nauplii though much better than algae or no food. Broad made no attempt to control decomposition of the food. If bacterial growth had been controlled, perhaps his results with non-living material would have been better. Nevertheless, non-living material may be significant in the diet of naturally occurring larvae.

If decapod larvae can develop on a diet of non-living material, then artificial diets may be suitable. Hubschman and Schmitt (1969) reported an attempt to develop a defined diet for larval *Palaemonetes kadiakensis*. This medium permitted development to Form III larvae, whereas unfed larvae only reach Form II in this species. If an artificial diet can be developed, the dietary requirements of decapod larvae can then be studied in detail. However, the necessity of maintaining sterile cultures when using non-living diets precludes the general use of such foods except in large volume cultures where anaerobic conditions are not likely to develop.

SUMMARY

The value of several flagellate algal species, shelled oyster larvae (*Crassostrea virginica*) and annelid larvae (*Arenicola marina* post-trochophores) as food for larvae of *Pagurus longicarpus* was determined. The flagellate algae were ingested by *Pagurus* larvae but did not allow development to the second instar. Shelled oyster larvae were an inadequate diet because *Pagurus* larvae could not break the shell. *Arenicola* post-trochophores allowed complete larval development with survival to the fourth zoeal stage comparable to *Artemia*-fed controls. Survival of *Arenicola*-fed larvae through the megalopal stage was superior to *Artemia*-fed controls. *Pagurus* larvae were shown to be capable of ingesting several micro-crustaceans and polychaete larvae, but culture tests of suitability for complete development were not performed.

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