EFFECT OF SEVERAL DIETS ON SURVIVAL, DEVELOPMENT TIME, AND GROWTH OF LABORATORY-REARED SPIDER CRAB, *LIBINIA EMARGINATA*, LARVAE

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ABSTRACT

Survival, development time, and growth were determined for larvae of the spider crab, *Libinia emarginata*, reared with nine diet combinations of algae, rotifers, copepods, ciliates, and *Artemia*. Percent survival was greater and development times shorter for diets of *A. salina* nauplii, either alone or in combination with other food sources. Zoeal survival was higher in diets of *Artemia* at 6 nauplii/ml than at 3 nauplii/ml. Megalopal survival was more variable, being highest in cultures with *Artemia* and the rotifer *Brachionus plicatilis* as food. No significant differences were noted in carapace measurements of larvae reared on the six diets which supported development beyond stage I zoea.

The literature includes many descriptions of decapod crustacean larval culture in the laboratory. Much of this work has been directed at deriving culture techniques and optimum levels of factors such as temperature and salinity. The "standard" diet has been newly hatched *Artemia* nauplii, a highly successful, convenient, but increasingly expensive food source. Research trends have been to seek substitute or supplemental diets for the brine shrimp. Foods investigated have included barnacle nauplii (Ławiński and Pautsch 1969; Reed 1969), the rotifer *Brachionus plicatilis* (Brick 1974; Sulkin 1975; Sulkin and Epifanio 1975), various ciliates (Sulkin 1975), polychaete larvae (Roberts 1974; Sulkin 1975), and oyster larvae (Roberts 1974).

This study was designed to evaluate possible diets, in addition to *Artemia* nauplii, which will support larval development of the spider crab, *Libinia emarginata* Leach. Normal larval development of this species consists of two zoeal stages and one megalopa (Johns and Lang 1977). Parameters used to estimate diet success were survival of larvae to each stage, time to each molt, and carapace size.

Development of a satisfactory diet, in combination with the short larval development time, could establish *Libinia* as a very suitable bioassay organism. The culture methodology described is relatively simple, further increasing the potential for continued laboratory study.

MATERIALS AND METHODS

Ovigerous female *L. emarginata* were collected by otter trawl in Narragansett Bay, during July and August 1976. Females were placed in containers of aerated seawater and immediately transported to the laboratory; storage in the laboratory was in a 1.2-m diameter (195-l volume) Fiberglas[2] tank provided with flow-through ambient temperature (approximately 20°C) seawater. As the eggs ripened, the females were transferred into tubs containing 8 l of filtered seawater at 20° and 29-31‰. After hatching occurred the female was removed and the water changed.

Within several hours of hatching, the larvae were placed 5/dish in 8.75-cm diameter culture dishes containing 75 ml of filtered seawater. Temperature and salinity were maintained at 20°C and 29-31‰. This type of static system has been used commonly to rear other species of crabs (Brick 1974; Sulkin and Norman 1976; Sulkin et al. 1976). The density of 1 larva/15 ml was chosen to allow sufficient room for developing megalopae.

Food organisms used included newly hatched San Francisco Bay Brand *Artemia salina* nauplii, the ciliate *Euplotes vannus*, the copepod *Eurytemora affinis*, the green flagellate algae *Dunaliella viridis*, and the rotifer *Brachionus plicatilis* (Table 1). These organisms are available at the Environmental Research Laboratory (Narragansett, R.I.)

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[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA or USEPA.

Manuscript accepted May 1977.
FISHERY BULLETIN: VOL. 76, NO. 1, 1978.

TABLE 1.—Laboratory diets used in rearing *Libinia emarginata* larvae.

Diet symbol	Diet	Size (μm)	Concentration (no./ml)	Replicates and no. of larvae used
A_1	*Artemia salina*	350-400	3	2/80
A_2	*A. salina*	350-400	6	1/40
D	*Dunaliella viridis*	15-20	10^2-10^3	1/40
BD	*Brachionus plicatilis*	55-200	25	
	D. viridis	15-20	10^1-10^2	2/80
BD/ABD	Stage I			
	B. plicatilis	55-200	25	2/80
	D. viridis	15-20	10^1-10^2	
	Stage II - M			
	A. salina	350-400	3	
	B. plicatilis	55-200	15	
	D. viridis	15-20	10^1-10^2	
EED	*Eurytemora affinis*	140-243	5	
	Euplotes vannus	80-100	5	1/40
	D. viridis	15-20	10^1-10^2	
ABD	*A. salina*	350-400	3	
	B. plicatilis	55-200	15	2/80
	D. viridis	15-20	10^1-10^2	
ABDE	*A. salina*	350-400	3	
	B. plicatilis	55-200	15	
	D. viridis	15-20	10^1-10^2	1/40
	Eurytemora affinis	140-243	5	
S	Starved		0	2/80

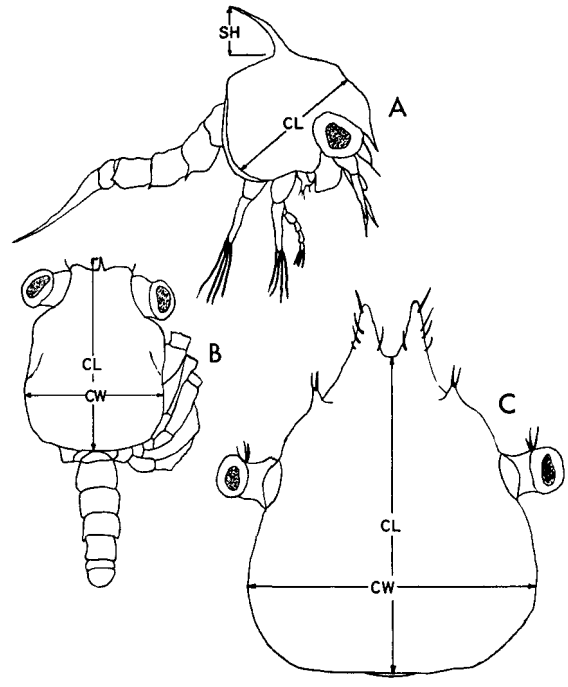

FIGURE 1. Body proportions of *Libinia emarginata* measured and the lines of measurement used. (A) zoea, (B) megalopa, (C) juvenile, (SH) spine height, (CW) carapace width, (CL) carapace length.

in mass cultures. Each species is an active swimmer, thereby satisfying the raptorial feeding requirements of *Libinia*. As noted in Table 1, several of the diets were replicated with 40 larvae (8 dishes of 5) in each of two trials; the remaining diets were investigated only once. Different trials utilized zoeae from different hatches; all 40 larvae in each diet replicate were from the same hatch. Concentrations of food organisms listed in Table 1 remained constant and were not adjusted as mortality occurred. One exception was diet BD/ABD, where the food organism composition was altered after the molt into stage II to include *Artemia* nauplii. Food and culture water were changed daily. Culture dishes were scrubbed clean in freshwater twice weekly. Larvae were transferred by wide-bore pipette to minimize body damage. Molts were recorded when exuviae appeared in the dishes and were verified under a compound microscope. The criteria for death was complete absence of a heartbeat.

Larvae and juvenile crabs were preserved in 10% buffered Formalin for carapace measurements. These measurements were determined with an ocular micrometer, with the carapace lengths and widths taken at maximum dimensions (Figure 1). Comparisons of development times and measurements were made by one-way analysis of variance, with significant differences ($P<0.05$) between diets tested by a Scheffe posterior comparison test (Nie et al. 1970).

RESULTS

Survival

Figure 2 shows the survival of spider crab larvae reared on each of the nine diets. Experiments continued for 25 days, at which time all larvae had either metamorphosed into the first crab stage or died. Survival data after each stage are shown in Table 2. Only six of the nine diets permitted development to proceed beyond stage I; in three diets (EED, D, and S) all zoeae died in the first stage.

Starved control larvae survived a maximum of 7 days, by which time mortality was 100% (Figure 2). After day 3, all larvae were moribund.

Addition of *Dunaliella viridis* (D) did not enhance either survival or molting. All stage I zoeae were motionless by day 4, but a heartbeat was observed up to day 10. No molts occurred. The dark red or orange chromatophores typically observed

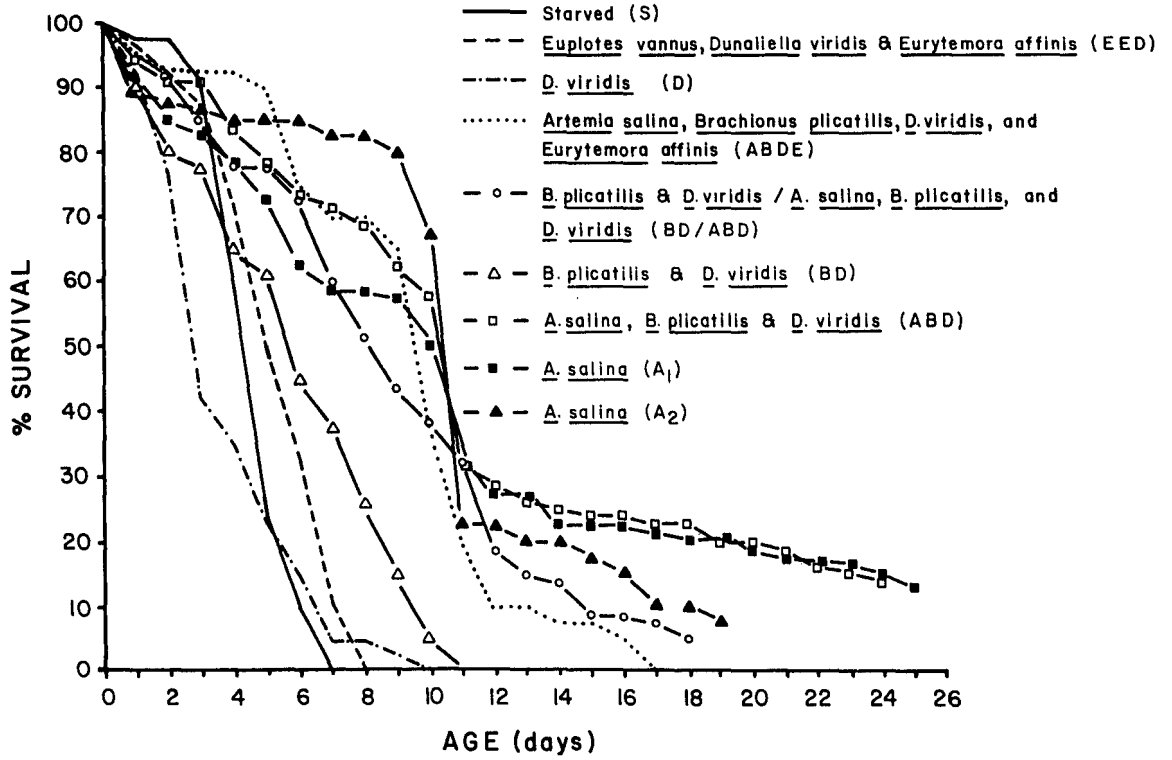

FIGURE 2. Percent survival at each day for *Libinia emarginata* larvae reared on nine laboratory diets. Refer to Table 1 for concentrations and sizes of food organisms in each diet.

TABLE 2.—Survival data and percentages to each stage of *Libinia emarginata* on the diets permitting larval development past stage I. N_I, N_{II}, and N_M represent number of larvae surviving to each stage; N_0 equals initial number.

	Molt					
	I→II		I→M		I→J	
Diet	N_I/N_0	%	N_{II}/N_0	%	N_M/N_0	%
A_1	59/80	74	34/80	43	8/80	10
A_2	33/40	83	30/40	75	3/40	8
ABD	58/80	73	40/80	50	10/80	13
BD/ABD	36/80	45	3/80	4	3/80	4
ABDE	32/40	80	18/40	47		
BD	29/80	36				

on the carapace were absent in nearly all larvae reared on diet D.

Survival on diet EED (ciliate, copepod, and algae) was only slightly higher than the starved controls (Figure 2). No molts were observed. Mortality was 100% by day 8.

A diet of *Brachionus* and *Dunaliella* (BD) allowed development into stage II. With this diet 36% (29/80) of the stage I zoeae molted into stage II, but all died by day 11.

Food organisms offered during stage I in diet BD/ABD were identical to diet BD. *Artemia* nauplii were added for all ensuing stages. Survival was 45% to stage II and 4% to both the megalopae and juvenile stages.

Diet ABD, identical to diet BD/ABD after stage I, allowed 73% survival to stage II, 50% to the megalopae, and 13% to the first crab stage.

Higher survival to stage II was achieved by diet ABDE, which included copepod subadults. On this diet, 80% of the zoeae molted successfully into stage II; 47% molted into megalopae. No larvae metamorphosed into the crab stage although several died during ecdysis.

Two diets of newly hatched *Artemia* nauplii were tested. Diet A_1, with 3 nauplii/ml, yielded 74% survival to stage II, 43% to megalopae, and 10% to the first stage crab. A second diet, A_2 (6 nauplii/ml), yielded higher survivals to stage II and megalopae, 83% and 75%, respectively, than any other diet. Survival to the juvenile stage was 8%.

Development Times

Diets supplying *Artemia* nauplii in stage I resulted in highest survival to stage II and the shortest development times (Table 3). Of the four diets grouped in the first subset (Table 4) for the molt into stage II, diet ABDE was the best. Diets BD and BD/ABD, although identical in content during stage I, were significantly different.

For the molt from zoeal stage II into megalopae diet ABDE again resulted in the shortest development time. Grouped with ABDE in homogeneous subset I was A_2, with the latter diet sufficiently similar in molt time to diet A_1 to also be included in subset II. As in the first molt, diet BD/ABD had the longest time to molt.

TABLE 3.—Development times of *Libinia emarginata* larvae from hatching to each molt for each diet. Diets EED, D, and S did not allow development past stage I.

Diet		Molt I→II	Molt I→M	Molt I→J
A_1	$\bar{x}$	4.66	10.29	18.86
	SD	0.60	1.14	2.48
	Range	4-7	9-14	16-22
A_2	$\bar{x}$	4.42	9.87	18.67
	SD	0.50	0.51	3.79
	Range	4-5	9-11	16-23
ABD	$\bar{x}$	4.62	10.30	19.00
	SD	0.64	0.85	2.21
	Range	4-6	9-12	16-24
BD/ABD	$\bar{x}$	6.56	13.00	21.67
	SD	1.36	1.73	3.06
	Range	5-9	11-14	19-25
ABDE	$\bar{x}$	4.25	9.39	
	SD	0.44	0.50	
	Range	4-5	9-10	
BD	$\bar{x}$	5.72		
	SD	1.28		
	Range	4-8		

In the last molt, from megalopae to the first crab stage, all four diets tested were grouped as one subset. Of the four, A_1 was ranked as the best in terms of development times and BD/ABD was the worst.

Carapace Measurements

Spine height, carapace length, and carapace width measurements were analyzed by a Scheffe posterior comparison test (Table 5). Zoeal stage II and juvenile crab measurements were not significantly ($P = 0.05$ level) different and were grouped into one homogeneous subset; carapace lengths of megalopae were similar in all diets. Only the carapace widths of megalopae proved statistically different, with two subsets describing the measurements of the larvae reared on different diets.

Ranking within each subset provides an indication of possible trends in size with respect to the diets tested. This trend is most evident in zoeal stage II; in both spine height and carapace length the ordering of diets was identical, with A_2 superior and ABD second. In megalopae and

TABLE 4.—Homogeneous subsets of diets tested on *Libinia emarginata* larvae as determined by analysis of variance and Scheffe posterior comparisons ($P < 0.05$) of development times. Shortest times are listed in subset I and longest in subset III.

Subset	Stage I→II	II→M	M→J
I	ABDE, A_2, ABD, A_1	ABDE, A_2	A_1, A_2, ABD, BD/ABD
II	BD	A_2, A_1, ABD	
III	BD/ABD	BD/ABD	

TABLE 5.—Carapace measurements for stage II, megalopa, and juvenile *Libinia emarginata* reared on various diets. Mean values (in millimeters) of spine height (SH), carapace width (CW), and carapace length (CL) are given in ranked order within each homogeneous subset of similar values. Roman numerals following the diet symbol denote replicate number, if applicable. Diets not represented, e.g., A_1 in stage II, could not be analyzed because of insufficient data.

Larval stage	Parameter measured	Subset	Ranked order of means					
Zoea II	CL	I	BD/ABD-II	ABDE	ABD-II	A_2		
			0.859	0.865	0.936	0.970		
	SH	I	BD/ABD-II	ABDE	ABD-II	A_2		
			0.311	0.316	0.338	0.360		
Megalopa	CW	I	A_1-II	A_2	A_1-I	ABD-I		
			0.938	1.037	1.044	1.100		
		II	A_2	A_1-I	ABD-I	ABDE	ABD-II	
			1.037	1.044	1.100	1.136	1.153	
	CL	I	ABDE	A_1-II	A_1-I	ABD-II	A_2	ABD-I
			1.232	1.258	1.260	1.265	1.289	1.380
Juvenile	CW	I	BD/ABD-I	A_1-II	A_1-I	ABD-II	ABD-II	A_2
			1.233	1.284	1.340	1.347	1.393	1.420
	CL	I	A_2	ABD-II	BD/ABD-I	A_1-II	ABD-I	A_1-I
			1.560	1.567	1.575	1.644	1.690	1.705

juveniles, diet ABD (replicates I and II) often resulted in the largest measurements.

DISCUSSION

Survival

Based on survival, laboratory diets that included *Artemia salina* nauplii were better than diets consisting solely of rotifers, algae, ciliates, or copepod nauplii. However, when offered in combination with brine shrimp nauplii, rotifers and copepods may provide some nutritional value to the larvae. Survival percentages to zoeal stage II were very high with diet ABDE: diet ABD produced the best survival to the first stage juvenile. Johns and Lang (1977; unpubl. data), using an excess diet of *A. salina* and a compartmented box culture system, got 20% survival to first stage crab.

The success of *Artemia* nauplii as a laboratory diet is well documented (e.g., Brick 1974; Sulkin et al. 1976). Studies by Brick (1974) also showed that survival of *Scylla serrata* to megalopae increased as the concentration of *Artemia* nauplii was increased. Results showed a 25% survival to megalopae at concentrations of 5 nauplii/ml and 44% at 16 nauplii/ml.

Differences in survival on various diets is commonly observed in laboratory studies. Diets that permit partial development, e.g., diet BD in this study, normally yield correspondingly lower survival. This trend has also been observed in diet studies on larvae of the sand shrimp, *Crangon septemspinosa* (Bigford[3]).

Development Times

The diets resulting in the shortest development times closely parallel those yielding the highest survival percentages. These diets all include *Artemia* nauplii (Tables 3, 4).

For the molt from zoeal stage I to stage II the shortest development times were recorded for diets ABDE and A_2, which also are the diets yielding maximum survival to stage II. These same diets continue to rate high in terms of survival and molt time for the second molt also.

Division of molt times into three subsets during zoeal development infers that *L. emarginata* may prefer certain food types or sizes at different stages. Diets including *Artemia* also consisted of the largest size food particles, with copepods, rotifers, ciliates, and algae being smaller. This possible discriminate particle selection was not observed in megalopae; all diets were consumed equally and development times were similar. All larvae surviving to first stage crabs were reared on *Artemia,* alone or in combination, during stage II and megalopae.

The lack of development observed in diets D, EED, and S, plus the partial development in BD, is supported by the literature. Studies by Sulkin (1975) have shown that algae and ciliates do not satisfy the nutritional requirements of brachyuran zoeae. Broad (1957) concluded that various algal diets were similar to starved controls, with the addition of animal matter required for metamorphosis in grass shrimp, *Palaemonetes,* larvae. Particle size and biochemical composition, among other factors, may limit development and survival. Conversely, rotifers have been found to enhance survival and development of several other decapod larvae, most notably the blue crab, *Callinectes sapidus* (Sulkin and Epifanio 1975). Food size appears to be the controlling factor in selection of the rotifer as food for early stage zoeae of the blue crab.

Although ABDE was a successful diet in the zoeal stages, it did not sustain metamorphosis to the crab stage in this study. Perhaps at differing concentrations of *Artemia* and *Eurytemora* the diet would prove more successful for megalopae.

Carapace Measurements

There does not appear to be a significant difference in carapace size between the diets studied. Instead, the effects of diets were manifested in terms of development rate. Larvae apparently molt upon reaching a certain biomass, with the postmolt sizes similar in most cases.

Carapace length measurements for second stage zoeae and megalopae (Table 5) for diets A_1 and A_2 compare favorably with the values reported by Johns and Lang (1977) in their description of the larvae reared on excess concentrations of *Artemia.* Their mean measurements of 0.94 mm and 1.21 mm, respectively, were only slightly below the values reported here. Differences in measuring

[3]Bigford, T. E. 1975. The effects of diet on larval development of the early stages of the sand shrimp *Crangon septemspinosa* Say. Unpubl. manuscr. U.S. Environmental Research Lab., Narragansett, R.I.

techniques could account for the larger megalopa carapace lengths reported in this paper.

CONCLUSIONS

The results of this experiment suggest that a combined diet including at least 5 *Artemia* nauplii/ml would produce highest survival in the zoeae. Additional food organisms may be required by megalopae. Faster development times associated with diet A_2, compared with A_1, emphasize the importance of food concentration in addition to food type.

Limited success of diet ABDE in the zoeal stages implies that *Eurytemora affinis* subadults may provide some nutritional substance to spider crab larvae. Replication of the copepod diet alone would be required to verify the potential of *Eurytemora*.

Each of the diets permitting development to proceed through metamorphosis resulted in a low percent survival. This could be partially explained by the static dish system used to culture the larvae. Flow-through designs would control water quality and perhaps microbial infestations. With an improved culture design, a satisfactory diet, and the short development time, *L. emarginata* could prove to be a very satisfactory bioassay organism.

The biochemical content of *Artemia* nauplii may account for their value in the diet of spider crab larvae. As determined by Sulkin (1975), *A. salina* contain 30 total lipid/unit dry weight, a value far superior to that of *Brachionus plicatilis* (9%). A diet of fertilized polychaete *(Hydroides dianthus)* eggs, containing 20% total lipid, also sustained complete development of *Callinectes sapidus* in Sulkin's experiments. The lipid content of *Eurytemora* was not determined.

Each of the diets tested in this experiment resulted in a normal progression of larval development for *L. emarginata* (Johns and Lang 1977). No supernumerary stages or characters appeared

ACKNOWLEDGMENTS

I thank Allan D. Beck, Richard Brooks, Neal Goldberg, D. Michael Johns, William H. Lang, and Leslie Mills, all of the Environmental Research Laboratory at Narragansett, for assistance during the course of the experiment. The graph was drawn by Annette Doherty; photographs were completed by James Brennan. The manuscript was typed by Josephine DeVoll.

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