Synthetic sea water as a medium for raising crab larvae

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KURZFASSUNG: Synthetisches Meerwasser als ein Medium für die Aufzucht von Krebs-larven. Der brachyure Krebs *Rhithropanopeus harrisii* (Gould) wurde bis zum Megalopa-Stadium in natürlichem Meerwasser und in zwei synthetischen Medien („Instant Ocean" und „Utility Seven Seas Marine Mix") bei Salinitäten von 3 ‰ und 11 ‰ aufgezogen. Die Über-lebensrate der Zoea-Larven wurde weder durch die Art des Mediums noch durch den Salzgehalt beeinflußt, die der Megalopa-Larven war jedoch bei 3 ‰ in allen drei Medien signifikant ge-ringer als bei 11 ‰. Die Megalopa-Stadien zeigten bei 11 ‰ in allen Versuchsreihen keine we-sentlichen Unterschiede in bezug auf Entwicklungsgeschwindigkeit und Sterblichkeit. Die beiden getesteten, in den USA kommerziell vertriebenen synthetischen Kulturmedien erwiesen sich so-mit zur Aufzucht von *R. harrisii* als brauchbar. Es wird empfohlen, die Eignung derartiger Medien – sofern sie für Massenkulturen bestimmter Arten eingesetzt werden sollen – anhand möglichst verschiedener Kriterien (Einfluß auf die Entwicklung, spezifische ionale Zusammen-setzung, Kaufpreis etc.) zu bewerten.

INTRODUCTION

The availability of commercially prepared synthetic sea salts as culture media for marine invertebrate larvae promises to expand the potential research effort to labora-tories and investigators with no ready access to natural sea water. Species that have been successfully raised in synthetic sea salts include the oyster *Ostrea edulis* (Millar & Scott 1968), the sea urchin *Lytechinus variegatus* (Mazur & Miller 1971), and the calanoid copepod *Acartia tonsa* (Heinle 1969). Reproductive success was poor in another calanoid copepod *Eurytemora affinis*, reared in synthetic sea water (Heinle 1969).

Synthetic sea water could provide a useful tool for examining the influence of specific media constituents on development, since components may be controlled. There is evidence to suggest that water quality does influence larval development. Wilson (1951, 1958) reported that polychaete and echinoderm larvae fared better in Celtic Sea water than in water collected in the English Channel. Ewald (1965) found that in simultaneous laboratory experiments, late larval stages of the decapod *Penaeus duorarum* exhibited a higher survival in open ocean Gulf Stream water than in Bis-cayne Bay water of the same temperature, salinity, and pH. It is not clear whether such differential rearing success is due to the presence of beneficial components in one

medium, or to harmful components in the other. Such components, where they exist, may be either organic or inorganic in nature.

In this study, larvae of the xanthid crab *Rhithropanopeus harrisii* were raised in natural sea water and in two commercially available synthetic sea salts, to determine whether synthetic sea water is a suitable culture medium for experimental studies on crab development.

MATERIALS AND METHODS

Rhithropanopeus harrisii (GOULD) was chosen for experimentation because gravid specimens were readily available, and the complete larval development has been described (CONNOLLY 1925, CHAMBERLAIN 1962). Rates of development and survival in various temperature-salinity combinations have been reported (CHAMBERLAIN 1962, COSTLOW et al. 1966). The development is characterized by four zoea stages and one megalopa.

Culture media used during the course of the experiments included natural sea water, "Instant Ocean", (Aquarium Systems, Inc., 33208 Lakeland Blvd., Eastlake, Ohio 44094, USA), and "Utility Seven Seas Marine Mix", (Utility Chemical Company, 145 Peel Street, Paterson, N.J., USA).[*] Larvae were raised in each of the above-mentioned media diluted to 3 ⁰/₀₀ and 11 ⁰/₀₀. These two salinities were chosen because the former is near the lower limit for good survival, and the latter is within optimal rearing conditions (COSTLOW et al. 1966).

Fresh sea water was collected in the surf near Indian Inlet, Delaware, USA. It was transported to the laboratory at Solomons, Maryland, USA, in 5-gal linear polyethylene jerricans. The water was passed through a 5 μ mesh filter bag into a 55-gal linear polyethylene storage tank. The salinity was 32 ⁰/₀₀. Each of the two synthetic blends was prepared in 55-gal linear polyethylene storage tanks. Salinity was measured at 34 ⁰/₀₀ for each mixture. All three stock solutions were aerated periodically.

Each stock solution was diluted to 3 ⁰/₀₀ and 11 ⁰/₀₀ S, and kept in 5-gal polyethylene jerricans. Sufficient quantities of each dilution were prepared for the entire experiment. The six test solutions were stored in a culture cabinet set at 25° C.

Ovigerous females of *Rhithropanopeus harrisii* were collected from oyster trays in the Patuxent River, Maryland. Ambient salinity and temperature at time of collection was 9.7 ⁰/₀₀ S and 25.9° C. Ovigerous crabs were placed in large glass bowls filled with filtered Patuxent River water and kept at room temperature (25° C) and under natural light conditions for two days. Egg masses were then inspected for stage of development. In order that acclimation time be approximately equal for all groups, further experimentation was conducted only on those egg masses in which the zoea eyes were well developed. Egg masses chosen for further study were teased apart with dissecting needles. Each egg mass was divided evenly among six 125 ml Erlenmeyer flasks, each containing 50 ml of one of the test solutions. The flasks were then placed upon a reciprocating variable speed shaker set at 110 rpm (COSTLOW & BOOKHOUT

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[*] Hereafter, the following abbreviations will be used to designate the three media: nsw = natural sea water; IO = "Instant Ocean"; 7-S = "Utility Seven Seas Marine Mix".

1960). Eggs were examined daily, but the water was not changed. Successful hatching occurred in all six tests solutions.

When hatching occurred, larvae in each test solution were separated into a number of small glass bowls, 10 zoeae per bowl. The bowls were examined daily for the number and stage of living and dead larvae. Living zoeae were transferred to clean bowls containing the appropriate test solution, and *Artemia salina* nauplii were added as food. When the megalopa stage was reached, each individual was kept in a separate compartment of a sub-divided plastic box. All stages were kept in culture cabinets set at 25° C and 12 hrs of light for the duration of each experiment.

Survival and rates of development are the criteria used in comparing the various solutions. Methods of data analysis are described with the results. Values obtained are based on six independent tests with larvae from three different crabs.

RESULTS

Hatching

Although no quantitative data were collected, it was apparent that hatching occurred successfully in all six test solutions. Larvae produced were vigorous and viable.

Survival

A high percentage of the deaths occurred at the time of molt. Dead larvae were characterized by a greatly swollen carapace or, in some cases, by being halfway out of the old carapace. These larvae were recorded as reaching the stage after the molt.

Figure 1 shows survivorship plotted as a function of stage of development for larvae raised in all six test solutions. Each point is an average of values obtained in six different rearing experiments. The relationship between survival and stage of development is evident. Survival is high through the four zoea stages in all test solutions but drops sharply during the megalopa stage.

In order to compare the per cent mortality that occurred during each larval stage among the various experiments, calculations were made using the following equation (derived from ROBERTS 1971):

$$M_i = \frac{\sum\limits_{1}^{y} \left[\dfrac{N_{i-1} - N_i}{N_{i-1}} \right]}{y} \times 100 \tag{1}$$

where M_i = per cent mortality during stage i, i = a particular stage of development, N_i = number of larvae surviving stage i, N_0 = number of larvae at beginning of experiment (i = o), Y = number of experimental repetitions.

The per cent mortalities that occurred during each stage of development are shown in Table 1. The zoea stages show low and similar mortalities among the six test solutions, as indicated by the survivorship curves (Fig. 1). Mortality ranged from 0 to 15 %, with the highest values obtained during the first zoea stage. A two-way
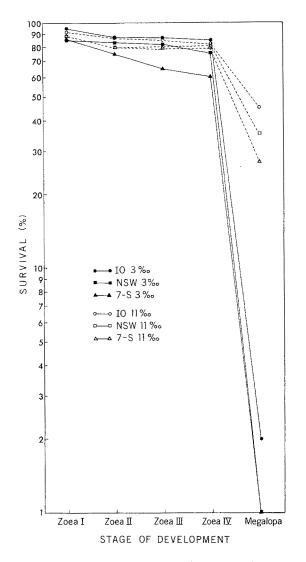
Fig. 1: Survivorship curves for larvae raised in six different test solutions. Survivorship plotted as a function of stage of development

analysis of variance on cumulative zoea mortality indicates no significant differences due either to salinity or to medium, and no interaction between these factors (Table 2). A two-way analysis of variance indicates highly significant differences between megalopa mortality at 3 $\%_0$ and at 11 $\%_0$ S. Although differences in mortality among the three media are apparent, this analysis of variance shows only a marginally significant difference. No interaction between medium and salinity is evident (Table 2). The difference in survivorship between 3 $\%_0$ and 11 $\%_0$ S is due almost entirely to the extremely high megalopa mortality at the lower salinity.

Table 1

Per cent mortality for each larval stage, for cumulative zoea development,
and for cumulative development to the first crab stage

Salinity	Medium	Zoea I	Zoea II	Zoea III	Zoea IV	Cumulative zoea mortality	Mega-lopa	Cumulative larval mortality
	nsw	15	2	1	9	25	100	100
3 ‰	IO	5	7	1	2	15	98	98
	7-S	13	14	13	8	40	100	100
	nsw	13	8	0	0	20	56	65
11 ‰	IO	8	5	2	2	17	46	55
	7-S	13	8	0	0	20	66	73

Table 2

Probabilities associated with the two-way analysis of variance on the effects of salinity
and culture medium on mortality

	Per cent mortality*	
	Cumulative zoea development	Megalopa development
Salinity	$0.10 < p < 0.25$	$p < 0.001$
Medium	$0.25 < p < 0.50$	$0.05 < p < 0.10$
Interaction	$0.50 < p < 0.75$	$0.25 < p < 0.50$

* Per cent mortality values subjected to angular transformation before statistical tests

Rate of development

Rate of development was calculated as mean intermolt time for each stage, and on a cumulative basis for zoea and megalopa development. Intermolt time for each stage of development was calculated on the basis of six independent determinations as follows:

$$R_j = \frac{\sum\limits_{1}^{y} (T_j - T_{j-1})}{Y} \qquad (2)$$

where R_j = intermolt duration for stage j, Y = no. of experimental repetitions, T_j = cumulative time of development through stage j, calculated as follows:

$$T_j = \frac{\sum\limits_{1}^{dj} (n_k k)}{N_j} \qquad (3)$$

where k = a specific day of development (e.g., $k = 1$: first day of development), n_k = number of larvae molting on day k, N_j = total number of molts for stage j, d_j = number of days of development (k) on which molts to stage j occurred.

Table 3 shows the intermolt time for each developmental stage, for cumulative zoea development, and for cumulative development to first crab. Rates of cumulative

Table 3

Intermolt time (days) for each larval stage, for cumulative zoea development
and for cumulative development to the first crab stage

Salinity	Medium	Zoea I	Zoea II	Zoea III	Zoea IV	Cumulative zoea development	Megalopa	Cumulative larval development
3 ‰	nsw	4.5	2.2	2.6	3.8	13.1	—	—
	IO	4.8	2.2	3.0	4.0	14.0	7.0	21.0
	7-S	4.6	2.9	3.0	3.9	14.4	—	—
11 ‰	nsw	4.4	2.2	2.5	3.8	12.9	5.8	18.7
	IO	4.5	2.2	2.4	3.9	13.0	5.5	18.5
	7-S	4.6	2.5	2.5	3.9	13.5	6.5	20.0

Table 4

Probabilities associated with the two-way analysis of variance on the effects of salinity
and culture medium on rate of development

	Rate of development	
	Cumulative zoea development	Megalopa* development
Salinity	$p < 0.005$	
Medium	$0.005 < p < 0.01$	$0.05 < p < 0.10$
Interaction	$0.10 < p < 0.25$	

* One way analysis of variance

zoea development for all six test solutions were subjected to a two-way analysis of variance. Significant differences were found, due both to salinity and to medium. No interaction was evident between these factors (Table 4). The data was subjected to the STUDENT-NEUMANN-KEUL test to determine where the statistical differences fell. The results showed that both IO and 7-S had a longer zoea development than did nsw at 3 ‰. The two synthetic media at 3 ‰ were also significantly different from either nsw or IO at 11 ‰. The position of 7-S at 11 ‰ S is equivocal (IO, 3 ‰ = 7-S, 3 ‰ ≧ 7-S, 11 ‰ ≧ nsw, 3 ‰ = nsw, 11 ‰ = IO, 11 ‰).

The results for the megalopa at 3 ‰ are difficult to analyze. The value shown in Table 3 is based on only one individual that survived to the first crab stage. Although the groups raised in 7-S medium at 11 ‰ S appeared to take slightly longer to molt than did those raised in the other two media, a one-way analysis of variance shows only marginal statistical difference (Table 4).

DISCUSSION

Using fitted response curves, COSTLOW et al. (1966) have estimated per cent mortality and rate of development for all larval stages of *Rhithropanopeus harrisii* in

natural sea water of various salinity-temperature combinations. Our results showed slightly lower zoea mortality and higher megalopa mortality at 3 $^0/_{00}$ than they reported. Mortality rates at 11 $^0/_{00}$ S were comparable to published results, as were rates of development at both salinities.

The fact that larvae required a longer time to reach the megalopa stage in synthetic sea water at 3 $^0/_{00}$ S than they did in either synthetic sea water at 11 $^0/_{00}$ S, or in natural sea water at 3 $^0/_{00}$ S may indicate that there is a threshold level of some constituent, or constituents. The concentration of such constituent(s) apparently was sufficient at 11 $^0/_{00}$ S, but may have been present in suboptimal amounts at 3 $^0/_{00}$. That megalopa development was characterized by very high mortality in all three media at 3 $^0/_{00}$ S is somewhat surprising, since zoeae survived well at 3 $^0/_{00}$ S and adults are found at these salinities (WURTZ & ROBACK 1955, RYAN 1956, PINSCHMIDT 1963).

These results indicate that commercially available synthetic sea salts are suitable for raising the larvae of at least one species of xanthid crab. Several factors must be weighed when choosing the proper medium. Its influence on the rate of development and survival is, of course, important. Cost should also be considered. If mass-rearing techniques will produce numbers in excess of experimental needs, a less expensive medium may be suitable even if it results in lower survival. Specific ionic composition, purity, and ease of preparation are also of importance.

If differences in developmental patterns exist due to the culture medium, a useful research tool is provided. In this study, two potential differences in development were noted that could be attributed to the culture medium. Synthetic sea water at low salinity delayed zoea development, and a marginally significant difference in mortality rate was noted among megalopa raised in the three media at 11 $^0/_{00}$. Such information can be used in at least two ways in further research. The influence of specific ion compostion and concentration on survival and development rate can be studied directly. In addition, specific media which produce different characteristic developmental rates can be used to study the influence of other factors, such as diet and temperature, on growth.

SUMMARY

1. Larvae of the xanthid crab *Rhithropanopeus harrisii* (GOULD), were raised from hatching through the megalopa in natural sea water, and in two commercially available synthetic sea salts. Groups of larvae from the same hatch were raised at two salinities for each medium.
2. Hatching occurred successfully in all six media-salinity combinations tested.
3. Survival was high through the four zoea stages in all six test solutions. Statistical tests on cumulative zoea mortality showed no significant differences due either to salinity or to medium, and no interaction between them.
4. Survival dropped during the megalopa in all test solutions. Megalopa mortality was significantly higher in 3 $^0/_{00}$ than in 11 $^0/_{00}$. Although differences in mortality among the three media were apparent, statistical tests showed only marginal significance. No interaction between salinity and medium was evident.

5. Rate of development through the zoea stages was affected by both salinity and medium although no interaction between these factors was evident. The following relationships among the six test solutions were demonstrated (from longest to shortest development): IO, 3 $^o/oo$ = 7-S, 3 $^o/oo$ ≥ 7-S, 11 $^o/oo$ ≥ nsw, 3 $^o/oo$ = nsw 11 $^o/oo$ = IO, 11 $^o/oo$.

6. Differences among the three media in rate of megalopa development at 11 $^o/oo$ were only marginally significant (only one individual survived at 3 $^o/oo$).

7. It is concluded that either of the two synthetic media used is suitable for raising larvae of *Rhithropanopeus harrisii*.

8. The following criteria should be considered in choice of medium for raising larvae: influence on rate of development and survival, cost, specific ion composition, purity, and ease of preparation.

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