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Nutritional effects of *Artemia* from different locations on larval development of crabs

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EXTRAIT: **Effets nutrionnels des *Artemia* de diverses localités sur le développement larvaire des crabes.** Quatre espèces de crabes ont été élevées avec des *Artemia salina* (L.) des étangs salés de Californie et du Great Salt Lake, Utah, pour déterminer s'il existe des différences dans le développement et la survie se rapportant aux deux sources de nourriture. Les *Rhithropanopeus harrisii* (GOULD) ont très bien survecu jusqu'au stade mégalope avec les nauplii d'*Artemia* des deux localités. Ceux qui ont été nourris avec les nauplii d'*Artemia* de Californie se sont normalement développés et 94 à 98 % du nombre total de zoés sont allés jusqu'au premier stade crabe. Ceux qui ont été nourris avec les nauplii d'*Artemia* d'Utah ont un stade mégalope anormal et aucun d'entre eux n'atteint le premier stade crabe. Les nauplii des *Artemia* d'Utah ont un effect nuisible à la fois sur les stades zoé et mégalope d'*Hexapanopeus angustifrons* (BENEDICT & RATHBUN) et aucun n'atteint le premier stade crabe. Soixante neuf pour cent des larves des crabes qui ont été nourries avec les nauplii de Californie ont survécu jusqu'au premier stade crabe. La survie jusqu'au stade mégalope fut très élevée chez les zoés de *Libinia emarginata* LEACH nourries à la fois avec les *Artemia* de Californie et d'Utah, mais seulement 5 %, nourries avec les *Artemia* d'Utah, ont survécu en comparaison des 63 % des larves qui ont été nourries avec les *Artemia* de Californie. Les larves de *Callinectes sapidus* RATHBUN ont montré des différences dans le taux de survie en faveur des nauplii d'*Artemia* de Californie mais elles ne sont pas importantes. Des explications possibles relatives aux anormalités du développement et aux différences de survie sont discutées en rapport avec ces différences dans les proportions, du DDT a été trouvé dans les *Artemia* de Californie et d'Utah.

INTRODUCTION

KUENEN (1939) wrote a comprehensive article on the systematics and variability of *Artemia*, the brine shrimp, from various locations in the world. He concluded that the results of scientists working on brine shrimp from different localities often varied because of differences in the source of material, more than because of different circumstances under which the experiments were conducted. He found that the California *Artemia* from Monterey differed from the brine shrimp from the salt works near Caglairi, Sardinia, in reaction to external stimuli, cellular and nuclear size, and in reproductive organs. He could not cross the California and Italian animals. Tentatively, he concluded that *Artemia salina*, as previously understood, is not one species. He suggests that two species might be distinguished:

(1) *Artemia salina* (L) – *Cancer salinus* LINNÉ, 1758, which has two lateral hairs on each abdominal segment, and

(2) *Artemia gracilis* Verrill, 1869, with no such hairs on the abdomen.

Verrill's original description of *Artemia gracilis* was based on material from Utah. It agreed with the characteristics of brine shrimp Kuenen (1939) studied from California and from Molla Kary near the Caspian Sea. Kuenen advised that the second species not be adopted until the question is solved with more certainty.

Rollefsen (1939) discovered that *Artemia* nauplii were satisfactory food for post-larval plaice, flounder and cod. In subsequent years they have been used widely by numerous investigators throughout the world for rearing fish, shrimp, hermit crabs, and crabs. They are also used extensively for various types of physiological and biochemical studies.

Despite the warning by Kuenen (1939) that *Artemia* from different geographical locations may give different experimental results, most investigators fail to mention the source of *Artemia* used in experiments. Hence, comparison and verification of results of different authors is difficult.

Until 1966 we used *Artemia* eggs from a supplier in San Francisco who obtained his eggs from salt pools in California, and we found the newly hatched nauplii to be ideal food for crab zoeae. When the San Francisco dealer went out of business, *Artemia* eggs had to be procured from another supplier who obtained *Artemia* eggs from Great Salt Lake, Utah. The survival rates of various species of crabs took a marked drop with the use of *Artemia* eggs from Utah. The larvae would usually survive through zoeal stages to megalopa, but died in the latter stage. Little (1969) also reported that *Palaemon macrodactylus* could be reared on San Francisco Bay *Artemia* nauplii, but not on *Artemia* nauplii from Great Salt Lake, Utah.

Early in 1967 we were again able to obtain *Artemia* eggs from California. Hence, in the summer of 1967, *Rhithropanopeus harrisii* (Gould), *Hexapanopeus angustifrons* (Benedict & Rathbun), *Libinia emarginata* Leach and *Callinectes sapidus* Rathbun were reared on *Artemia salina* (L) nauplii from California salt pools and Great Salt Lake, Utah, to determine if there are any differences in development and survival related to the two sources of food. If there are differences, are there any clues as to why *Artemia* nauplii from different locations give different results?

METHODS

Ovigerous *Rhithropanopeus harrisii* and *Hexapanopeus angustifrons*, small mud crabs belonging to the family Xanthidae, were collected in the Newport River estuary in the vicinity of Beaufort, North Carolina. Ovigerous *Libinia emarginata* and *Callinectes sapidus* were obtained outside the Beaufort Inlet.

The small mud crabs, *R. harrisii* and *H. angustifrons*, were placed in finger bowls containing seawater of 25 ‰ and 30 ‰ respectively, the salinities to be used during rearing of larvae. They were maintained in a culture cabinet at 25° C until the larvae hatched. These salinities and temperatures were used because they had previously been found to be close to optimum for rearing *R. harrisii* (Costlow et al. 1966) and *H. angustifrons* (Costlow & Bookhout 1966).

The method for developing the eggs of *Libinia emarginata* and *Callinectes sapidus* was the same as COSTLOW & BOOKHOUT described in 1960. Pleopods bearing eggs were removed, placed in finger bowls of seawater at 35 ‰ for *L. emarginata* and 30 ‰ for *C. sapidus,* the salinities to be used for rearing the larvae. Strands of eggs were cut with fine scissors from pleopods. The eggs were further dissociated, with glass needles, into groups of 100–1000 and transferred to freshly filtered seawater. The eggs were washed in a third bowl and then placed in compartments of plastic boxes containing approximately 20 ml of filtered seawater. The compartmented boxes were placed on an EBERBACH variable-speed shaker and the assembly was kept in a culture cabinet at 25° C.

When hatching occurred, the first stage zoeae of the four species of crabs were segregated to Carolina finger bowls, 88 mm in diameter, 10 zoeae per bowl. As shown in Tables 1, 2, 3 and 4, duplicate series of larvae were maintained from a single female of each species; one series was fed freshly hatched *Artemia* nauplii from Utah and the other given *Artemia* nauplii from California. The amount of food supplied each bowl per day was one concentrated drop of *Artemia* nauplii. (In the remainder of the text when *Artemia* is mentioned it will mean freshly hatched *Artemia* nauplii.)

Each day the bowls were examined for the number of crab larvae alive, the number dead, and the number of molts. Immediately afterwards the larvae were transferred to clean bowls of filtered sea water, were fed, and returned to culture cabinets. When a zoea molted into a megalopa, it was placed into one of the compartments of a plastic box. Megalopa were transferred daily to clean, compartmented boxes.

Brine shrimp nauplii, hatched from eggs from Great Salt Lake, Utah, and from eggs from salt pools near San Francisco Bay, California, were analyzed for pesticides by the Southern Testing and Research Laboratories, Wilson, North Carolina, U.S.A. The following methods were used. Each sample was extracted with water and acetonitrile using a high speed blender. Pesticides were removed from the extraction mixture by shaking with petroleum ether. The petroleum ether containing the pesticides was placed on a chromatographic column of florisil. The pesticides were eluted from the column by: (1) 6 % ethyl ether in petroleum ether and (2) 15 % ethyl ether in petroleum ether. These separate elutions were then evaporated to a suitable volume for injection into a gas chromatograph.

RESULTS

In the small mud crab, *Rhithropanopeus harrisii,* survival to megalopa was equally good, 95 % to 100 % on California and Utah *Artemia.* Normality of the megalopa, however, and further development differed markedly. Those fed on California *Artemia* developed into normal megalopa (Fig. 1) and 94 % to 98 % of them went to the first crab stage (Tab. 1). In the Utah-fed series, the megalopa were abnormal in that the legs projected backwards in an extended condition (Fig. 2) and none of them reached the first crab stage.

When the narrow mud crab, *Hexapanopeus angustifrons*, was reared on California *Artemia*, 74 % reached the megalopal stage and 69 % molted to the first crab stage (Tab. 2). When they were reared on Utah *Artemia* only 10 % reached the

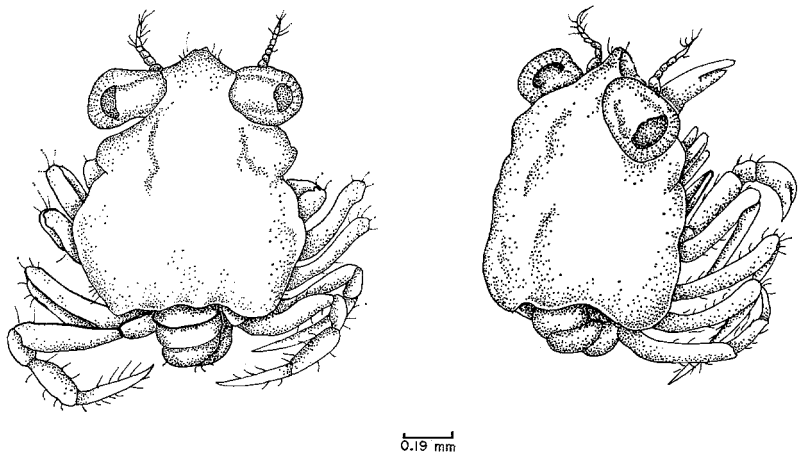

Fig. 1: Normal megalopa of *Rhithropanopeus harrisii* reared on California *Artemia* nauplii

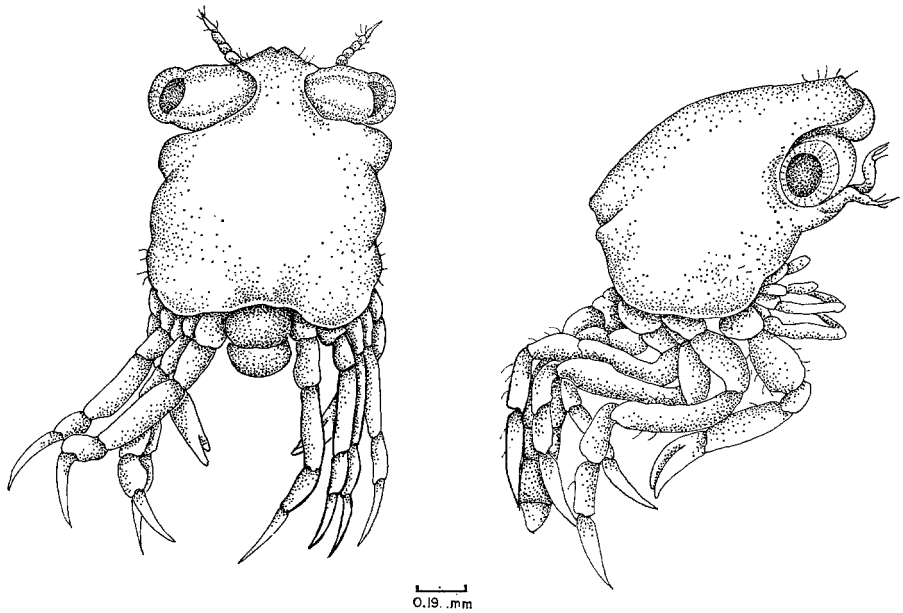

Fig. 2: Abnormal megalopa of *Rhithropanopeus harrisii* reared on Utah *Artemia* nauplii

megalopal stage and 2 % of these were abnormal. Apparently, *Artemia* from Utah had a detrimental effect on both zoeal and megalopal stages. No megalopa reached the first crab stage.

The large spider crab, *Libinia emarginata*, passes through two zoeal stages and a megalopal stage before molting to the first crab stage. Survival to the megalopal stage was high in the zoeae fed on both the California and Utah *Artemia*, but

Table 1

Comparison of survival and normality of *Rhithropanopeus harrisii* larvae fed on *Artemia* nauplii from California and from Utah (*Rhithropanopeus harrisii:* salinity 25 ‰, temperature 25° C)

Source of *Artemia*	Crab	Original number of zoeae	Number of megalopa		Number of crabs		Days to megalopa	Days to 1st crab
			norm.	deform.	norm.	deform.		
California	1	100	100	0	98	–	10.0	4.75
California	2	100	97	0	94	–	10.1	5.4
Utah	1	100	0	98	0	–	9.91	–
Utah	1	100	0	99	0	–	10.1	–
Utah	2	100	0	98	0	–	10.62	–
Utah	2	100	1	95	0	–	10.4	–

Table 2

Comparison of survival and normality of *Hexapanopeus angustifrons* larvae fed on *Artemia* nauplii from California and from Utah (*Hexapanopeus angustifrons:* salinity 30 ‰, temperature 25° C)

Source of *Artemia*	Crab	Original number of zoeae	Number of megalopa		Number of crabs		Days to megalopa	Days to 1st crab
			norm.	deform.	norm.	deform.		
California	1	100	74	0	69	–	14.21	7.27
Utah	1	100	8	2	0	–	15.7	–

Table 3

Comparison of survival of *Libinia emarginata* larvae fed on *Artemia* nauplii from California and from Utah (*Libinia emarginata*: salinity 35 ‰, temperature 25° C)

Source of *Artemia*	Crab	Original number of zoeae	Number of megalopa		Number of crabs		Days to megalopa	Days to 1st crab
			norm.	deform.	norm.	deform.		
California	1	100	95	0	63	–	7.61	4.95
Utah	1	100	82	0	5	–	7.47	6.11

survival to the first crab stage was only 5 % in those which were fed on Utah *Artemia* as compared to 63 % in those which were fed on California *Artemia* (Tab. 3).

The larvae of the commercial blue crab, *Callinectes sapidus*, seemed to be less affected by Utah *Artemia* than other species. Fifty percent of the zoeal larvae fed on California *Artemia* and 31 % of those fed on Utah *Artemia* molted into normal

megalopa (Tab. 4). Survival to the first crab stage was 34 % in those maintained on California *Artemia* and 24 % in those fed Utah *Artemia*. All first crab stages were normal except one of the latter.

Table 4

Comparison of survival of *Callinectes sapidus* larvae fed on *Artemia* nauplii from California and from Utah
(*Callinectes sapidus:* salinity 30 ‰, temperature 25° C)

Source of *Artemia*	Crab	Original number of zoeae	Number of megalopa		Number of crabs		Days to megalopa	Days to 1st crab
			norm.	deform.	norm.	deform.		
California	1	100	50	0	34	0	37.35	9.18
Utah	1	100	31	0	23	1	34.6	11.4

The length of time for zoeae to reach the megalopal stage was approximately the same for each of the four species, whether they were fed on Utah or California *Artemia larvae* (See Tables 1–4).

Brine shrimp nauplii from California, when analyzed for pesticides, were found to have 2,300 ppb DDT, whereas those from Utah had 7,050 ppb DDT, or approximately three times as much DDT as the nauplii from California.

DISCUSSION

There is evidence that the difference in normality and survival of developmental stages of four species of crabs may be due to different quantities of DDT in the *Artemia* nauplii from California and Utah. If complete inorganic and organic analyses were made, other differences in the *Artemia* nauplii from the two sources might be found.

Slobodkin (1968) reported that *Artemia* eggs from Utah produced nauplii which were toxic to plaice larvae reared in Great Britain. Although he stated the toxicity of the Utah nauplii had been traced to residual insecticides from surrounding agricultural regions of the Great Salt Lake basin, he did not give any information concerning the type or amount of pesticides in the eggs or nauplii.

In this investigation the amount of DDT in *Artemia* from the two sources is known, but the amount of pesticide absorbed and accumulated throughout larval development of the four species of crabs is not known. Woodwell et al. (1967) have shown that there is usually an increase in concentration of DDT residues from smaller to larger organisms and from lower to higher trophic levels.

It is suggested that by the time *Rhithropanopeus harrisii* larvae fed on *Artemia* nauplii from Utah have developed to the megalopal stage they have accumulated enough DDT to cause abnormalities and to bring about paralysis and lack of coordination. There are numerous references in the literature which reveal that DDT affects the nervous system of various adult animals. Wurster & Wingate (1968) reported that DDT interferes with normal calcification of the arthropod nerve axon

and produces similar effects to those resulting from calcium deficiency. ODUM et al. (1969) found that fiddler crabs *(Uca pugnax)* fed on detritus containing 10.0 ppm DDT residues developed poor co-ordination on day 4 and were unco-ordinated on day 5. LOWE (1965) found that some juvenile blue crabs, *Callinectes sapidus,* died in 0.5 ppb solution. These exhibited extreme irritability, increased sensitivity to external stimuli and finally paralysis before death. Although there seems to be no information on the effect of DDT on zoeae and megalopa of crabs, the behavior of *R. harrisii* megalopa fed on Utah *Artemia* is somewhat similar to the behavior of adult Crustacea poisoned by pesticides.

If our assumption is correct that the abnormalities, lack of coordination, and eventual death of *R. harrisii* megalopa are due to DDT poisoning, it follows that the amounts present in zoeal stages were sublethal because survival to the megalopa stage was equally high in those fed California and Utah *Artemia.* In *Hexapanopeus angustifrons,* the DDT in *Artemia* nauplii from Utah was lethal to both zoeal and megalopal stages for only 10 % reached the megalopal stage and 2 % were abnormal. None molted to the first crab stage, whereas 74 % of the original number of zoeae fed on California *Artemia* reached the megalopal stage and 69 % the first crab stage.

It is difficult to understand why the large spider crab, *Libinia emarginata,* which has only two zoeal stages and one megalopal stage, should be more drastically affected by *Artemia* from Utah. It passes through the larval stages and reaches the first crab stage in a relatively short time (12.56 to 13.58 days), as compared to the blue crab, *Callinectes sapidus,* which passes through seven zoeal stages and one megalopal stage (COSTLOW & BOOKHOUT 1959) in 46.0 to 46.53 days, and presumably consumes a greater quantity of Utah *Artemia* than *Libinia* larvae. To answer this question and others, there are plans to analyze eggs and each larval stage until the first crab stage is reached to determine the amount of DDT which is absorbed and stored as development progresses.

SUMMARY

1. Four crabs, *Rhithropanopeus harrisii, Hexapanopeus angustifrons, Libinia emarginata* and *Callinectes sapidus,* were reared from the egg to the first crab stage on freshly hatched *Artemia salina* nauplii from two sources: salt pools in California, and the Great Salt Lake in Utah. All larvae showed better survival on California nauplii than on Utah nauplii. The crab larvae fed on California nauplii showed normal development, whereas all *R. harrisii* megalopa and some *H. angustifrons* showed a particular set of abnormalities.
2. There is evidence that the difference in normality and survival of developmental stages of the four species of crabs may be due to a difference in the quantity of DDT in *Artemia* nauplii from California and from Utah. There was approximately three times as much DDT in *Artemia* nauplii from Utah as in the nauplii from California. The nature of the abnormalities which appeared in larvae fed on Utah nauplii resembled DDT poisoning which has been reported in juvenile and adult crabs.

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