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# A comparison of two diets in the laboratory culture of the zoeal stages of the brachyuran crabs *Rhithropanopeus harrisii* and *Neopanope* sp.*

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KURZFASSUNG: **Ein Vergleich zweier Ernährungsformen bei der Laboraufzucht von Zoëa-Stadien der brachyuren Krebse *Rhithropanopeus harrisii* und *Neopanope* sp.** Wenn Nauplien des Salinenkrebses *Artemia salina* Zoëa-Stadien von *Rhithropanopeus harrisii* und *Neopanope* als Nahrung geboten werden, vollzieht sich die Metamorphose zum Megalopa-Stadium bei einem wesentlich größeren Prozentsatz der Larven als bei Verfütterung des Rotators *Brachionus plicatilis*. Die Häutungsfrequenz der Larven beider Krebsarten ist verlangsamt, wenn die Zoëen ausschließlich mit *Brachionus plicatilis* ernährt werden. Die unterschiedlichen Ansprüche an die qualitative Beschaffenheit des Futters, die bei den Larven verschiedener Decapoden zu verzeichnen sind, werden erörtert. Die an *Rhithropanopeus harrisii, Neopanope* sp. sowie an *Callinectes sapidus* gewonnenen Befunde weisen darauf hin, daß *Brachionus plicatilis* als alleiniger Futterorganismus für die Aufzucht von Decapodenlarven nicht geeignet ist.

## INTRODUCTION

SULKIN & EPIFANIO (1975) demonstrated that the early larval stages of the portunid crab *Callinectes sapidus* RATHBUN showed good survival when fed the rotifer *Brachionus plicatilis* MULLER. However, SULKIN (1975) subsequently reported that the rotifer alone would not sustain development to the megalopa. Completed development was accomplished when larvae were fed either trochophores of the polychaete *Hydroides dianthus* (VERRILL) or a combination of rotifers for the first 14 days, followed by nauplii of the brine shrimp *Artemia salina* L. SULKIN concluded that a dietary requirement exists which is satisfied by either polychaete trochophores or brine shrimp nauplii, but is lacking in rotifers.

*Callinectes sapidus* development is characterized by a comparatively longer larval development (35–47 days) and a greater number of zoeal instars (7) than species of

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other brachyuran families (COSTLOW & BOOKHOUT 1959; SULKIN, 1975). In addition, the number of instars appears more variable with intermediate and supernumerary zoeal stages not uncommon (COSTLOW, 1965; SULKIN et al., 1976; SULKIN, unpublished data). These features may represent a primitive developmental condition characteristic of portunid crabs (LE BOUR, 1928; COSTLOW, 1965).

By contrast, species belonging to the family Xanthidae are characterized by comparatively short zoeal duration (14–18 days), smaller numbers of instars (4–5), and little reported variability in instar number. The hatching stage is generally larger than is the case with portunid crabs. In general, xanthid crabs adapt well to laboratory culture. *Rhithropanopeus harrisii* (GOULD), for example, has been widely used for behavioral and physiological studies, because of its hardiness (COSTLOW, 1966; SULKIN & MINASIAN, 1973; SULKIN & PICKETT, 1973; WELCH & SULKIN, 1974; FORWARD, 1974; FORWARD & COSTLOW, 1974).

Successful laboratory culture of *R. harrisii* has been achieved using nauplii of a variety of species, including brine shrimp and copepods (CHAMBERLAIN, 1962) and barnacles (LAWINSKI & PAUTSCH, 1969).

The purpose of this study was to determine whether the inadequacy of rotifers as a diet is characteristic of brachyurans or whether it demonstrates a dietary requirement particular to the primitive Portunidae. To examine this question, we have raised in the laboratory larvae of the xanthid crabs *Rhithropanopeus harrisii* and *Neopanope* sp. on either *A. salina* nauplii or the rotifer *B. plicatilis*.

## MATERIALS AND METHODS

Ovigerous *Rhithropanopeus harrisii* were obtained from submerged oyster trays at the mouth of the Patuxent River, Maryland. Specimens were maintained in the laboratory in small dishes until the larvae hatched. Salinity (S) was 15 ppt, photoperiod was 14:10 light/dark, and temperature was approximately 25° C. Ovigerous *Neopanope* sp. were collected from pier pilings near the mouth of the Delaware Bay. Specimens were maintained in the laboratory in small dishes until hatching. Salinity was 30 ppt; photoperiod and temperature were as described for *R. harrisii*.

When the larvae hatched, they were placed in 80 mm diameter dishes, 10 individuals per dish. Medium was 15 ppt S for *R. harrisii* larvae and 30 ppt S for *Neopanope* larvae. The cultures were maintained at 25° C. Daily examinations were made for evidence of dead larvae and exuviae. The living larvae were then transferred to dishes of fresh medium and fed. Two diet treatments were used for each species. Freshly hatched nauplii of *A. salina* were obtained daily. The technique for obtaining the second diet treatment, the rotifer *B. plicatilis*, has been described in detail in a previous report (SULKIN & EPIFANIO, 1975). Both diets were applied daily in excess. Available larvae from each hatch (siblings) were divided equally between the two diet treatments.

Values obtained for each diet include daily survivorship, stage survivorship, and mean day of each molt. The *A. salina* nauplii diet was considered the "control".

## RESULTS AND DISCUSSION

### *Rhithropanopeus harrisii*

The graph in Figure 1 shows daily survival for groups of sibling larvae raised on each diet. Data for 150 larvae from five different crabs are included. It is obvious that the rotifer diet causes prolonged zoeal development and lower survival to the megalopa. In Figure 2 mean percent survival calculated for the fifteen groups of larvae at each stage is presented. A significant difference in survival is seen only during the

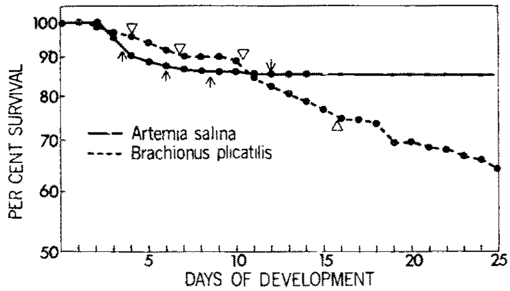


Fig. 1: *Rhithropanopeus harrisii.* Daily survivorship of zoeae raised on either nauplii of *Artemia salina* or the rotifer *Brachionus plicatilis.* Mean day of succeeding molts are indicated by open triangles for the rotifer-fed larvae and arrows for *Artemia*-fed larvae. Zoeal development had reached completion by day 14 on the *Artemia* diet

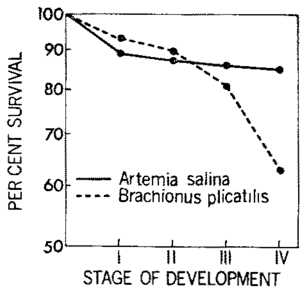


Fig. 2: *Rhithropanopeus harrisii.* Stage survivorship of zoeae raised on nauplii of *Artemia salina* or the rotifer *Brachionus plicatilis*

fourth (last) zoeal stage (T-test, $p < 0.005$). Although increased mortality occurs on the rotifer diet, considerable survival to the megalopa is noted (74 % of control survival).

Figure 3 shows the days on which successive zoeal molts occur for each diet. A delay in development is obvious for rotifer-fed larvae. Table 1 presents a comparison of intermolt duration for each stage on both diets. Significant differences occur at each stage, with prolonged development apparent for rotifer-fed larvae.

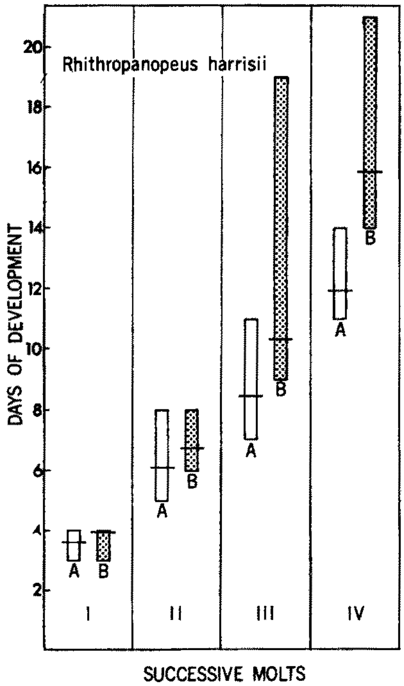


Fig. 3: *Rhithropanopeus harrisii.* Days on which successive zoeal molts occurred for larvae fed either nauplii of *Artemia salina* (A) or the rotifer, *Brachionus plicatilis* (B). The bars represent the range of days on which molts occurred. Horizontal lines represent mean day of molt

Table 1

*Rhithropanopeus harrisii.* Intermolt duration for each zoeal stage for larvae fed either the brine shrimp *Artemia salina* or the rotifer *Brachionus plicatilis*

| Zoea stage | Mean intermolt duration (days) *Artemia salina* | *Brachionus plicatilis* | T-test probability |
|---|---|---|---|
| I | 3.6 | 4.0 | $p < 0.005$ (sig.) |
| II | 2.5 | 2.8 | $p < 0.05$ (sig.) |
| III | 2.3 | 3.6 | $p < 0.005$ (sig.) |
| IV | 3.6 | 5.3 | $p < 0.005$ (sig.) |

## *Neopanope* sp.

Similar experiments were conducted on a smaller sample of *Neopanope.* Data for 50 larvae from two crabs are included. Figure 4 compares daily survial on the two diet treatments. Again it is apparent that the rotifer diet causes prolonged development and lower survival to the megalopa. Figure 5 shows the data calculated on a stage basis. As with *R. harrisii,* most of the increased mortality occurs during the

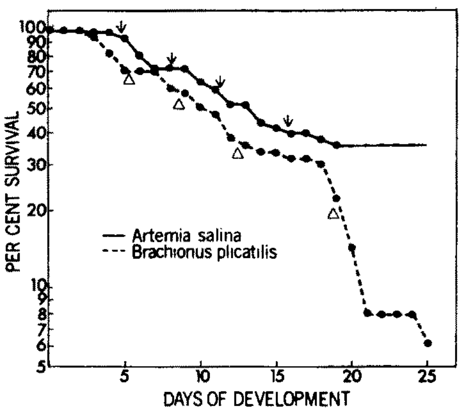


Fig. 4: *Neopanope* sp. Daily survivorship for larvae raised on the diets indicated in the legend. Open triangles indicate mean day of successive molts for larvae fed *B. plicatilis;* arrows indicate mean day of molt for larvae fed *A. salina* nauplii

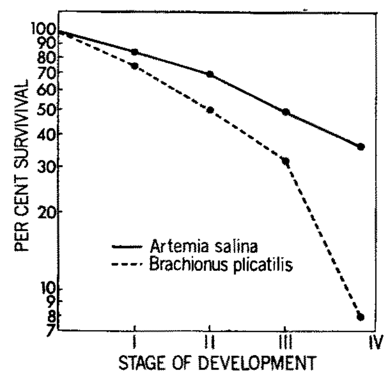


Fig. 5: *Neopanope* sp. Stage survivorship of zoeae raised on indicated diets

fourth and final stage. Survivorship to the megalopa is significantly lower on the rotifer diet as compared to the *A. salina* diet (t-test with arc sine transformation: $p < 0.01$). In contrast to *R. harrisii*, however, only 22 % of control survival is noted for rotifer-fed *Neopanope*.

Figure 6 shows the days on which successive zoeal molts occur for each diet. A delay in development is again obvious for rotifer-fed larvae. Table 2 presents a comparison of intermolt duration for each stage on both diets. A significant delay is seen in the first two stages, but not in the last two.

Sulkin (1975) reported that the greatest mortality for rotifer-fed *Callinectes sapidus* larvae occurred during what is normally the last zoeal stage. Of *C. sapidus* larvae entering the seventh stage, half died and half molted to a supernumerary zoeal stage (all of which died). The results reported here for two species of xanthid crabs are similar in that the greatest increase in mortality for rotifer-fed larvae occurred during the terminal zoeal stage. In contrast to *C. sapidus*, however, no supernumerary

Table 2

*Neopanope* sp. Intermolt duration for each zoeal stage for larvae fed either the brine shrimp *Artemia salina* or the rotifer *Brachionus plicatilis*

| Zoea stage | Mean intermolt duration (days) *Artemia salina* | *Brachionus plicatilis* | T-test probability |
|---|---|---|---|
| I | 4.8 | 5.1 | $p < 0.05$ |
| II | 3.1 | 3.7 | $p < 0.025$ |
| III | 3.2 | 3.8 | $p > 0.05$ |
| IV | 4.4 | 5.7 | $p > 0.05$ |

zoeal stages occurred. Furthermore some development to the megalopa was possible with the two species studied here.

In the present study a delay in molting occurred immediately in rotifer-fed larvae, even before significant increase in mortality was observed. In *R. harrisii* delays occurred for all four stages while in *Neopanope*, only the first two exhibited statistically significant delay. While the latter circumstance may reflect a comparatively small sample size and a broad range in values (Fig. 6), the results are similar to those reported for *C. sapidus* (SULKIN, 1975).

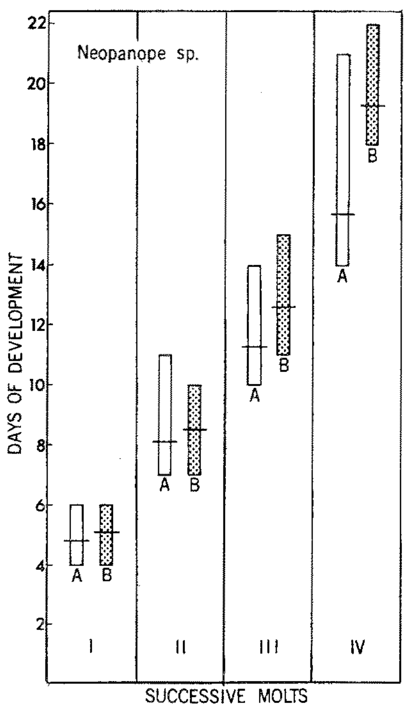


Fig. 6: *Neopanope* sp. Days on which successive zoeal molts occurred for larvae fed either nauplii of *Artemia salina* (A) or the rotifer, *Brachionus plicatilis* (B). The bars represent the range of days on which molts occurred. Horizontal lines represent mean day of molt

It is apparent that the response to what may be called "dietary stress" varies considerably among the species tested. Although the species used in this study fared better than the portunid *C. sapidus*, it is clear that they do not do as well on a rotifer diet as on the *A. salina* diet. In addition to increased mortality induced directly by inadequate diet, a prolonged period of development could lead to additional mortality in nature due to increased predation.

Hard evidence on the reason for the inadequacy of the rotifer diet does not yet exist. Based on the studies of *C. sapidus*, SULKIN (1975) suggested that a dietary requirement exists for some form of lipid. He reported that *A. salina* nauplii contain three times as much lipid per unit dry weight as do rotifers. In subsequent research, SULKIN (unpublished) has found that late zoeal stages of *C. sapidus* can survive and complete normal development when fed only rotifers, if they have previously been fed a diet high in lipid such as polychaete trochophores or *A. salina* nauplii. These results suggest that the significant considerations are not energetic, but more likely reflect a requirement for certain structural constituents or perhaps, fat-soluble vitamins.

The need either to synthesize required nutrients or receive them via the diet may depend upon the amount provided in the egg and utilized by the embryo prior to hatching. As mentioned previously, the hatching stage of *C. sapidus* is very small as compared to *R. harrisii* and *Neopanope*. If these species "pass through" the early developmental period in the egg, it is not unreasonable to speculate that they may be provided the nutrients that appear to be required in the diet by early stages of *C. sapidus*.

Additional adaptive significance thus may be applied to a developmental strategy which delays the hatching stage to a more advanced condition. Such strategy may have been accompanied by biochemical adaptations which reduce the newly hatched larva's dependence on the presence of favorable prey.

## SUMMARY


1. The zoeal stages of *Rhithropanopeus harrisii* and *Neopanope* sp. (Family Xanthidae) were raised in the laboratory on a diet of proven value, *Artemia salina* nauplii, and an experimental diet, the rotifer *Brachionus plicatilis*.
2. Survival to the megalopa was achieved on both diets for both species; however, survival was significantly lower for rotifer-fed larvae. Rotifer-fed *Neopanope* sp. larvae showed lower percent survival of control (*A. salina* nauplii) than did rotifer-fed *R. harrisii* larvae.
3. Delay in molting was immediately apparent in both species when fed rotifers. Intermolt duration was significantly longer for rotifer-fed larvae for all zoeal instars in *R. harrisii* and in the first two zoeal stages of *Neopanope* sp.
4. The results are compared with similar studies previously reported for the portunid crab *Callinectes sapidus*.


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