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Influence of starvation on the larval development of *Hyas araneus* (Decapoda, Majidae)*

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ABSTRACT: The influence of starvation on larval development of the spider crab *Hyas araneus* (L.) was studied in laboratory experiments. No larval stage suffering from continual lack of food had sufficient energy reserves to reach the next instar. Maximal survival times were observed at four different constant temperatures (2°, 6°, 12° and 18 °C). In general, starvation resistance decreased as temperatures increased: from 72 to 12days in the zoea-1, from 48 to 18 days in the zoea-2, and from 48 to 15 days in the megalopa stage. The length of maximal survival is of the same order of magnitude as the duration of each instar at a given temperature. "Sublethal limits" of early starvation periods were investigated at 12 °C: Zoea larvae must feed right from the beginning of their stage (at high food concentration) and for more than one fifth, approximately, of that stage to have at least some chance of surviving to the next instar, independent of further prey availability. The minimum time in which enough reserves are accumulated for successfully completing the instar without food is called "point-of-reserve-saturation" (PRS). If only this minimum period of essential initial feeding precedes starvation, development in both zoeal stages is delayed and mortality is greater, when compared to the fed control. Starvation periods beginning right after hatching of the first zoea cause a prolongation of this instar and, surprisingly, a slight shortening of the second stage. The delay in the zoea-1 increases proportionally to the length of the initial fasting period. If more than approximately 70 % of the maximum possible survival time has elapsed without food supply, the larvae become unable to recover and to moult to the second stage even when re-fed ("point-of-no-return", PNR). The conclusion, based on own observations and on literature data, is that initial feeding is of paramount importance in the early development of planktotrophic decapod larvae. Taking into account hormonal and other developmental processes during the first moult cycle, a general hypothesis is proposed to explain the key role of first food uptake as well as the response pattern of the zoea-1 stage to differential starvation periods.

INTRODUCTION

Meroplanktonic larvae have particular significance as a link between pelagic and benthic communities (Costlow & Bookhout, 1970). Their survival, mainly controlled by food limitations, temperature, and predation, is the principal key for understanding variations in recruitment and establishment of benthic marine communities (Thorson, 1946, 1966).

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There is some evidence that the amount of suitable planktonic food organisms, available during this critical period, can limit the size of year classes in fish and decapod populations (for recent discussion see Paul et al., 1979). This is not unlikely, since the authors observed a lower threshold for successful daily feeding response in larvae of three different decapod species. This threshold was much higher than average zooplankton concentrations in the sea (cf. also Omori, 1979). A number of other authors also reported unnaturally high food densities as optimal for laboratory rearing of several decapod species (e.g. Templeman, 1936; Sandoz & Rogers, 1944; Reeve, 1969; Brick, 1974; Knowlton, 1974; Mootz & Epifanio, 1974; Roberts, 1974; Welch & Sulkin, 1974; Provenzano et al., 1976; Bigford, 1978; Johns & Pechenik, 1980). It may be argued that the most commonly used diet, *Artemia salina*, which is not a natural prey organism, might be qualitatively inferior to natural prey items. However, it is well suited to support planktotrophic development in many decapod species in the laboratory, whereas natural zooplankton is generally at least two orders of magnitude less concentrated in the sea, and much of its biomass is not available to crustacean larvae, because potential prey organisms may have an unsuitable size, quality, swimming speed, defense mechanism or other characteristics preventing predation (cf. Herrnkind, 1968; Roberts, 1974; Sulkin & Heukelem, 1980).

Although Thorson (1950) "expected that most pelagic larvae living under natural conditions would starve", the ecological factor "starvation" has not received much attention as opposed to abiotic variables such as temperature, salinity, oxygen etc. The fact that recruitment in benthic communities does take place despite a presumable chronic lack of food, can be explained by the combination of two phenomena: the existence of considerable patchiness in plankton (for review see e. g. Parsons et al., 1977) and adaptation mechanisms for survival under highly fluctuating resources. Ikeda (1974, 1977 and earlier papers), Mayzaud (1973, 1976), and Holland (1978) reported on physiological and biochemical changes during starvation conditions. However, not much information exists about ultimate limits of starvation resistance in carnivorous zooplankton, and even less about adaption to temporary lack of suitable prey.

Starvation was recognized very early as a factor severely influencing development of fish larvae (for review see May, 1974; Ehrlich et al., 1976), but hardly anything is known about its significance for other meroplankton organisms.

In several cultivation experiments with decapod larvae, starvation was used to test the sufficiency of yolk reserves for larval development. These experiments revealed a considerable specific variation in the degree of dependence on prey, especially among Natantia larvae. Besides a number of species which positively need food during their whole larval development, there are others which are able to complete at least parts of their pelagic phase independent of food (Broad, 1957; Dobkin, 1971 and a number of earlier papers; Regnault, 1969; Fiedler, 1970; Foxton & Herring, 1970; Choudhury, 1971; Omori, 1971, 1979; Greenwood et al., 1976). Similar instances have been reported in Anomura, especially in late larval stages (Coffin, 1958; Bookhout, 1964, 1972; Rice & Provenzano, 1965; Provenzano, 1968a; Schatzlein & Costlow, 1978; Dawirs, 1980).

In Brachyura larvae, reserves are usually not sufficient to allow development under starvation conditions. One exception was reported by Provenzano & Brownell (1977) and by Brownell et al. (1977) for the tropical spider crab *Mithrax spinosissimus*, but not enough details on the methods were given to evaluate its significance. Wear (1967)

described and reviewed cases of aberrant, more or less direct development in some Brachyura from New Zealand and Australia. Those larvae mostly subsist on yolk reserves.

The potential to resist starvation not only depends on the species and on the particular larval stage considered, but also on environmental variables. The most important factor controlling metabolism and thus, the rate of reserve utilization, is temperature. However, its effect is by no means clear. In the few experiments which have been carried out, results are equivocal: In some cases, survival time under starvation increases with decreasing temperature (Rice & Provenzano, 1966; Gore, 1968; Regnault, 1969), in other instances there is the opposite trend (Rice & Provenzano, 1965; Provenzano, 1967; Gore, 1970) or survival time is independent of temperature over a wide range (Gore, 1972) or shows a maximum at an intermediate temperature (Provenzano, 1968b). In the literature reviewed above, only survival under long-term starvation was considered. Those investigations as well as rearing experiments under more or less optimal food conditions provide the extreme ends of the scale, in which natural development of decapod larvae must be expected. The next step toward understanding meroplankton survival and development duration in a variable environment is the search for "sublethal limits" and "sublethal effects" of starvation. The existence of internal developmental processes during each stage (see e.g. Costlow & Sastry, 1966; Freeman and Costlow, 1980) and the high probability of only short-term absence of suitable prey, together suggest that such sublethal effects must occur in nature and that their kind and extent depend on the time w h e n starvation takes place and h o w l o n g it lasts.

There are a few references to such effects: Kurata (1959), Yatsuzuka (1962), Modin & Cox (1967), and Kon (1979) observed a particularly critical period in the very beginning of larval life in decapods: if first feeding was delayed, growth and survival of the larvae were lowered. Paul & Paul (1980) observed in king crab zoeae a significantly decreasing ability to catch prey after early starvation. Sublethal effects of fasting periods on behaviour patterns were also reported (Burton, 1979; Cronin & Forward, 1980).

The present work is the first attempt to analyze systematically not only the ultimate limits of starvation resistance, but also the sublethal effects of early lack of food on later viability and development duration in a marine invertebrate: in larvae of the spider crab *Hyas araneus*. These larvae are common in the plankton of the German Bight from midwinter until early summer. Their development in this area presumably lasts ca. 12 to 16 weeks (Anger & Nair, 1979); the wide geographic distribution of *H. araneus* (cf. Christiansen, 1971) is probably related to this long pelagic phase (Thorson, 1961: "longdistance larvae"). Since *H. araneus* belongs to the most common species in the waters around the Island of Helgoland (North Sea), it is one of the subjects investigated within a joint research project (Anger & Nair, 1979).

The questions on which this study concentrated were: (1) Does any larval stage of *H. araneus* under starvation possess sufficient energy reserves to survive and to moult successfully to the next stage at any ecologically relevant temperature? If not: (2) What is the order of magnitude in the maximal survival time, and how strong is the influence of environmental temperature on starvation resistance? (3) How long, at least, must an early larva feed well, until it has accumulated enough reserves to moult successfully to the next stage (in which it might get the chance to switch to some new kind of prey)? How is survival and development time affected in this case?

(4) Is there a "point-of-no-return" (sensu Blaxter & Hempel, 1963) after which starved larvae cannot recover, when re-fed? If so: How close is this point to maximal survival time under starvation? (5) Which effects do early starvation periods exert on later development duration and on viability, when applied at different times within the first zoeal stage? Are there particularly sensitive periods within this stage?

The terminology used in this paper follows that of Williamson (1969). The misleading term "postlarva", which mostly refers to the megalopa (clearly a larval stage), but sometimes to juvenile stage, is not used; it should be generally avoided (see also Wear, 1967). The term "stage" always refers to an instar (zoea 1, zoea 2, megalopa, first crab), not to a unit of the moult cycle.

MATERIAL AND METHODS

Obtaining the larvae

In January 1979 and 1980, ovigerous females were dredged from a depth of ca. 30 to 50 m off the island of Helgoland (North Sea) and thereafter maintained in a laboratory recirculating system. The seawater had a temperature of 2 °C and a salinity of ca. 33 ‰. When the first hatching prezoeae were observed, the female releasing larvae was placed in a flow-through aquarium (ca. 5 l, water temperature ca. 6 °C). The larvae were collected in a sieve standing in a second aquarium and receiving the water from the overflow of the first one. In this way it was assured that all zoeae used in an experiment originated from the same mother animal and hatched the same day (within maximally 6 h).

Experiments on maximal starvation resistance

For all experiments, freshly hatched zoea-1 stage larvae (Z-1) were pipetted individually into numbered vials containing ca. 15 to 20 ml of filtered seawater (Millipore membrane filter, 0.4 μm pore size) with a salinity of ca. 33 ‰. Individual confinement was necessary, since Anger & Nair (1979) have shown that otherwise cannibalism or necrophagy severely falsify survival times under starvation. Sets of 50 or 100 larvae each (100 only at 12 °C) were placed into temperature-controlled rooms, where they acclimatized within a few hours to the experiment temperatures: 2°, 6°, 12° and 18 °C. The experiments were checked daily at the same time; water was changed regularly every other day.

Several hundred zoeae were reared at 12 °C to later stages using the same technique of individual maintenance as described above, but feeding a mixture of *Brachionus plicatilis* and freshly hatched *Artemia salina* nauplii ad libitum (ratio ca. 10:1, ca. 50 to 100 food organisms per ml). This rearing method was found to be superior to the mass rearing techniques previously used by Anger & Nair (1979) because both mortality and development duration could be substantially reduced.

The brine shrimp eggs came from Kew, Melbourne, Australia; the rotifers were cultivated and prepared in the same way as described by Anger & Nair (1979). No antibiotics or algae were added to the culture medium. Water and food were changed every second day. The vials were checked every 24 h for exuviae or dead larvae. An artifical day-night rhythm L:D 12:12 was provided.

As soon as possible after the larvae had moulted to the stage desired, they were transferred to filtered seawater and treated as described above for the Z-1 stage. In this way, the individual survival time of 50 to 75 (75 only at 12 °C) zoea-2 larvae (Z-2) and of 25 megalopae was also observed at each experimental temperature, i.e. a total of 250 Z-1, 225 Z-2, and 100 megalopae. Larvae were considered dead when opaque or when no movement of any appendage or internal structure could be seen under moderate magnification.

Experiments on the effects of early starvation on later development and survival

Basically the same experiment techniques as described above were used to evaluate "sublethal effects" of early starvation. The experimental design is shown in Figure 1.

Each experiment consisted of two to seven sets (subexperiments) with 25 (in one case 50) individually maintained larvae. Thus, one experiment comprised 50 to 175 larvae, each in its own numbered vial. This technique later allowed a detailed statistical analysis. Special care was taken to follow exactly the respective time schedule for controls and manipulations in a 24 h rhythm. Larvae which were to be switched from feeding to starving conditions were transferred to clean vials, only after being washed in baths of filtered seawater, in order to avoid accidental transfer of food organisms.

All experiments referred to in Figure 1 were carried out with Z-1 larvae. As soon as they moulted to the second instar, they fed regularly, and their later development was recorded daily (same culture methods as described above), until they died or

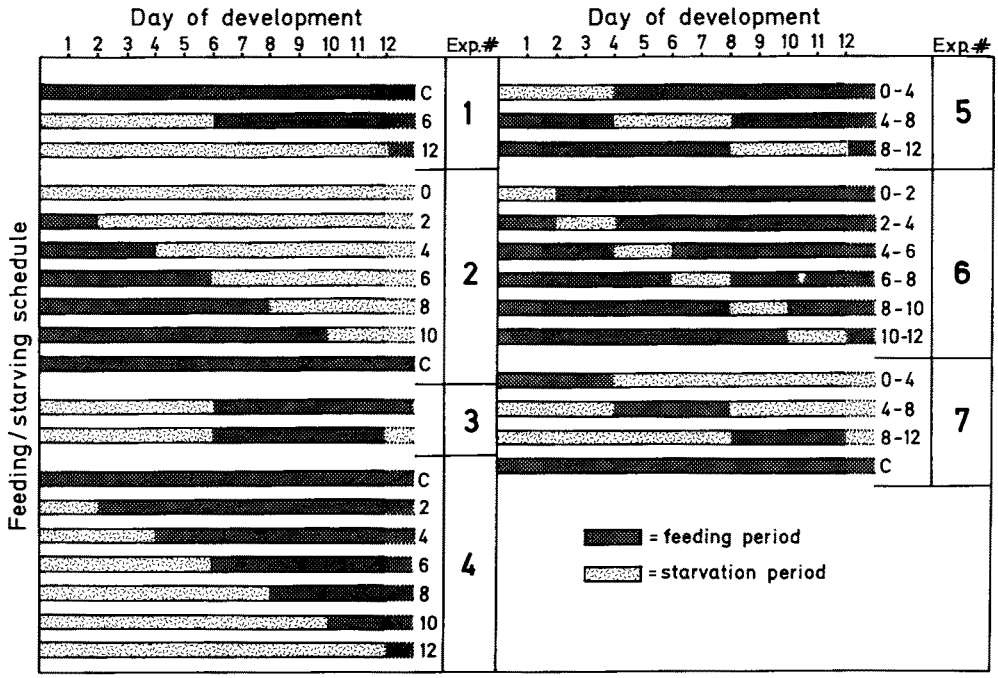

Fig. 1. Experimental design. Numbers and C (=control) beside bars correspond to Figures 3 to 9 and refer to feeding or starvation periods in experiments (exp.)

metamorphosed to the first crab stage. All larvae used in one experiment originated from the same hatch. This is also true for all sets in experiments Nos. 4, 5, and 8, which were started simultaneously. Experiments Nos. 1 to 3 were carried out in spring 1979, the others one year later.

In 1980, the first experiment of type No. 2 (Fig. 1) was carried out with Z-2 larvae: Zoeae from one hatch had been reared as described above, until they moulted to the second stage. Moulting, however, was not completely sychronized, and it could not be excluded that viability of faster developing larvae was different from those reaching the Z-2 stage later. In order to avoid a possible systematic error, on each of the three days in which moulting took place, freshly moulted Z-2 larvae were equally distributed among all subexperiments, until the whole experimental series was complete (25 larvae per set). This difficulty will occur in all further experiments with later stages unless evidence is provided that there is no relationship between individual stage duration and viability.

Statistical procedures

In Figures 3 to 9 and in the text, average values for development durations are given as arithmetic mean (indicated in graphs as small horizontal line or as height of a bar $\pm$ 95 % confidence limits (vertical bar) (calculated according to Sachs, 1974; pp. 195–197); in the graphs the range is also given (vertical line).

Time-dependent trends were described, if possible, by means of least-square regressions, either using a linear model ($y = b + m \cdot x$) or in some instances logarithmic transformation ($\ln y = b' + m \cdot \ln x$). Correlation (r) and regression coefficient (m) were tested for significant differences from zero with the statistic t (Sachs, 1974; pp. 329 and 339). In one case (experiment 4), an empirically found slope m was compared with a theoretical $m' = 1.0$, employing a test described by Sachs (p. 339). Comparison of mean values with equal or unequal variances (after an F-test) was carried out using different t-statistics (Sachs, pp. 209–210 or p. 212). Again, another t was employed as test statistic to compare mortalities in percentages (relative frequencies; Weber, 1972; pp. 194–197).

Calculated regressions were drawn as solid lines or curves. Curves and lines fitted by eye, and those indicating an average of values considered statistically equal (horizontal lines) were dotted in graphs. Statistically significant differences (see above) are referred to in the text giving the level of significance (P) or they can be recognized in graphs as nonoverlapping confidence intervals. Single observations (as opposed to mean values) are displayed as points (in Figs. 4 and 5).

RESULTS

Maximal survival time under starvation at different temperatures

At no stage and at no temperature to which larvae of *Hyas araneus* were exposed, did they have sufficient reserves to reach the next instar without food.

For statistical analyses the following difficulty arose: In some experiments, the mortality pattern did not reveal a sudden increase and thus a clear limit of starvation resistance. In some cases, at 18° and 2 °C, there was a bimodal mortality-frequency

distribution, apparently related to strong temperature changes at the beginning of the experiments. Although, in most experiments (especially at 12 °C), there was a clear response indicating a rather narrow range of time during which most larvae died due to depletion of energy reserves, a uniform statistical treatment was prevented by the above exceptions. Therefore, only the maximum values observed for survival under starvation are considered here as a measure of energy reserves and utilization.

These values were not falsified by single individuals surviving considerably longer than most larvae in the experimental population, and thus they can be used as an index for the potential of a given developmental stage to withstand starvation at a given environmental temperature (Fig. 2).

The longest possible survival time under starvation (72 days) was observed in the Z-1 stage at 2 °C, the second longest (48 days) by the Z-2 at 2° and 6 °C, and by the megalopa at 2 °C. With the exception of the second zoeal stage at these two temperatures, there was a conspicuous decrease in survival time with increasing temperature in all three larval stages. It was most striking in the first zoea (Fig. 2).

The Z-1 displayed the far longest survival among the larval stages at 2 °C; at all higher temperatures its resistance potential was weaker than that of the later instars. The Z-2 survived the longest at 6° and 18 °C, the megalopa at 12 °C. At the two highest temperatures, however, the survival maxima did not differ much.

The effect of early starvation on later development and chance of survival

The above experiments showed that *Hyas araneus* larvae possess sufficient energy reserves to survive starvation for almost two weeks at the highest and up to ten weeks at the lowest temperature occurring in their environment, but they do not have enough reserves to reach the next stage. These findings raise the question whether starved larvae are able to recover when re-fed later.

Experiment 1 was a preliminary step in the investigation of "sublethal effects"

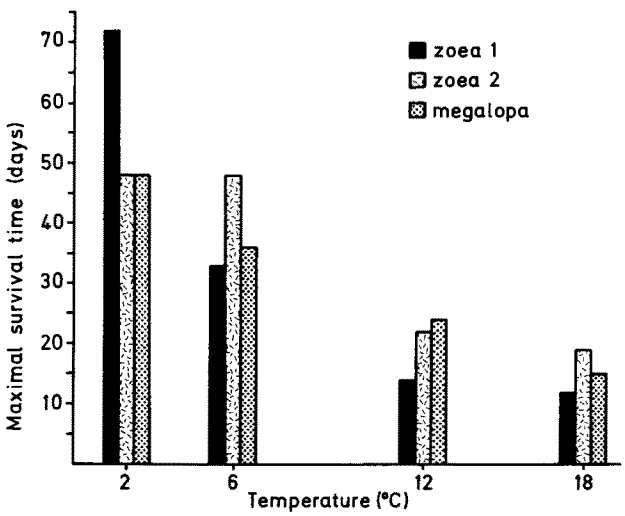

Fig. 2. *Hyas araneus:* Maximal survival time (days) of starved larvae in relation to temperature

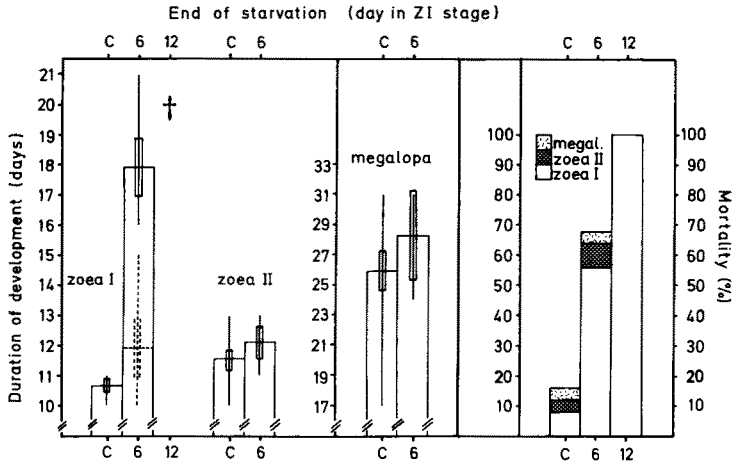

Fig. 3. *Hyas araneus:* Experiment 1. Larval development (mean values ± 95% confidence limits and range of single observations) and mortality in relation to differential starvation. Cross: no survival to following stage. Further explanations see Figure 1 and text

of starvation (Figs 1 and 3): One set of Z-1 larvae (in this exceptional case, 50 instead of 25 individuals) was starved for 12 days. This is only slightly below the lethal threshold at 12 °C, but 26 zoeae survived to the first day of feeding. Another set fasted for only six days before being fed; all 25 larvae survived to this day, as in the control (C in Fig. 3; no starvation).

None of the larvae which had been starved for 12 days recovered and reached the second stage. Some of them lived for up to 29 days before they died. This indicates that they had not completely lost their ability to take up and to convert food, but they did lose their ability to moult.

Six days of initial starvation also caused significantly higher mortality, after commencement of feeding, than in the fed control group ($P < 10^{-3}$).

The survivors needed seven days longer than the control larvae to reach the Z-2 stage. If it is assumed that during starvation no developmental processes take place and therefore, six days are subtracted from the intermoult duration (dotted range, mean, and confidence limits in Fig. 3), then there still remains a significant difference in development rate, when compared with the control ($P = 0.02$).

Slight differences in development rate and mortality, observed in later stages, were not statistically significant.

Experiment 2 was an attempt to estimate the time necessary to accumulate sufficient energy reserves for moulting successfully to the second zoeal stage without further food supply. As shown in Figures 1 and 4, feeding ceased after differential periods in five subexperiments, which can be compared with a starved (0) and a fed control group (C).

The starved zoeae again died after 12 to 14 days without reaching the second stage (Fig. 4). In the group fed for only two days after hatching, two individuals successfully moulted to the Z-2 after 14 days (i.e. after starving for 12 days). Both larvae, however, died later without reaching the megalopa stage.

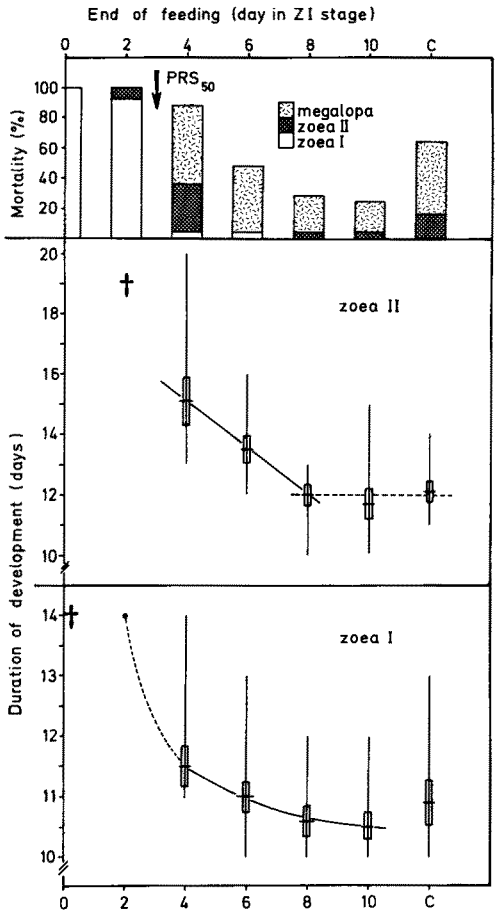

Fig. 4. *Hyas araneus:* Experiment 2. Explanations see Figures 1 and 3

After four days or more of feeding, starvation did not influence survival to the second stage: There was practically no mortality in the Z-1. Unfortunately, the fed control group had unusually poor survival in the Z-2 and in the megalopa stages and thus, interpretation of the mortality figures is difficult in this experiment.

Although there was an unusually high variation in the duration of stage 1 in the fed control too, the values for development rate in Figure 4 show clear trends: The earlier the first zoeal stage was starved, the longer its development lasted. This effect became even more evident in the Z-2, in which no lack of food occured during this experiment. These trends are reflected in statistically significant regressions (solid lines in Fig. 4): Z-1: $\ln D = 2.58 - 0.10 \cdot \ln t$ ($r = -0.9998$; $P = 0.01$); Z-2: $D = 18.18 - 0.78\,t$ ($r = -0.9998$; $P = 0.01$) where D = development duration, t = time (day) during Z-1 stage at which starvation began.

The mean values of stage duration in single subexperiments were not significantly different from the control group unless feeding ceased rather soon (4 days after hatching

for the Z-1, 6 days or sooner for the Z-2 duration). Astonishingly, the control group developed even slower in both zoeal stages than the groups starved during the last 2 to 4 days before moulting to the Z-2.

The duration of the megalopa stage was virtually unaffected by early starvation. It fluctuated between 23.5 ± 1.5 and 24.7 ± 0.9 days in the remaining five groups. Since the above effects in the zoeal stages add to each other and the megalopa had a rather constant duration, total development (D) to the first crab stage was also significantly delayed due to early starvation (t) (values rising from 45.5 ± 1.6 to 49.0 ± 1.4 days): $D = 51.55 - 0.58\ t$ ($r = -0.9770$; $P = 0.02$).

For comparative ecological considerations the minimum time necessary to accumulate enough reserves for reaching the next instar independent of further food supply might be of interest. We call this value here "point of reserve saturation" (PRS). Since there is no condition guaranteeing a 100 % survival to any later stage, the 50 % point in the mortality curve will be considered a measure for the degree of dependence on external energy supply in a given stage under defined conditions. This PRS_{50} value is, according to the above experiment, ca. 3 days or ca. 30 % of development time for the Z-1 stage at 12 °C and feeding conditions as described above.

Experiment 3: The last experiment revealed that six days of feeding in the beginning of the Z-1 stage allow almost a normal development to the Z-2 and later instars. Experiment 3 was designed as a test of whether this period is sufficient in any case or only if applied soon after hatching (Fig. 1).

As in experiment 1, one set of 25 zoeae was starved for six days and then fed until the larvae reached the second stage or died. The results were similar, but mortality was higher (88 % to the first crab stage, in contrast to 68 % in exp. 1), and the duration of the Z-1 stage was somewhat more delayed (19.6 vs. 17.9 days). The differences can be explained by the fact that experiment 3 was carried out at the end of the artificially prolonged hatching season (May, 1979), and the last hatching larvae were apparently less viable than earlier ones.

A parallel set of larvae was treated in the same way until day 12: After fasting six days and then feeding six days, they were starved again. None of these larvae reached the Z-2 stage; the last zoea died after 32 days without moulting. This lack of flexibility indicated the existence of particularly sensitive periods and thus of interference with an internal programme running during the first zoeal instar. Therefore, one year later, new experiments were started to elucidate the significance of this developmental programme for the resistance against starvation periods.

Experiment 4 (Figs 1 and 5) was an extension of experiment 1 and the reversal of experiment 2. The question to be answered was: How long can an early larva resist starvation without losing its ability to recover and to moult successfully to the next stage?

From Figure 5 it becomes evident that any lack of food in the beginning of larval life causes a delay in development: The duration of the Z-1 stage (D) showed a strong correlation with the time of starvation (t) in its beginning. $D = 10.31 + 1.44\ t$ ($r = 0.9985$; $P < 10^{-4}$)

The slope of this regression (solid line in Fig. 5, lower part) is not only significantly different from zero ($P < 10^{-4}$), but also ($P = 0.002$) from the theoretical relationship (dotted line in Fig. 5, lower part), which can be expressed as: $D = D_c + t$, where D_c = duration of Z-1 development in the control (C)

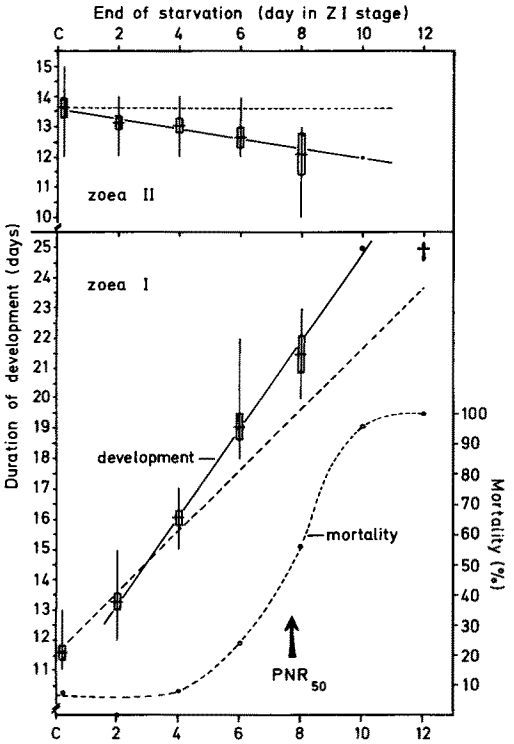

Fig. 5. *Hyas araneus:* Experiment 4. Explanations see Figures 1 and 3

This means that a significantly increasing time (t') in addition to D_c + t is necessary to compensate for the period of starvation (t).

Mortality did not follow the same linear pattern, but rather a sigmoidal relationship (Fig. 5). Neighbouring values were not significantly different from each other except in the range 6 to 10 days of starvation (P = 0.02 to 0.002). Survival was generally higher than in the comparable 1979 experiments 1 and 3, but it showed a similar upper limit of resistance: After 10 days of starvation, only one survivor recovered and reached the next moult (and even metamorphosis). After 12 days fasting, no more larva recovered and moulted to the Z-2 stage; the last one died after 34 days.

Such limit for early starvation resistance was also found in fish larvae; in accordance with Blaxter & Hempel (1963) we apply the term "point-of-no-return" (PNR) to this limit. For 50 % of the population considered, this value (PNR_{50}) was found after somewhat less than 8 days or slightly more than 50 % of the maximum possible survival time under starvation.

Once the first zoeal moult is successfully passed, later survival is obviously not influenced by early starvation periods: In all subexperiments practically no more mortality occurred in the later stages (three individuals out of 103 which had reached the Z-2).

A surprising phenomenon, however, was noted in the Z-2 stage: There is a statisti-

cally significant decline in the duration of this instar (D) with increasing starvation time at the beginning of the Z-1 stage (t): $D = 13.58 - 0.16\ t$ ($r = 0.9822$; $P < 10^{-3}$)

This trend (solid line in Fig. 5, upper part) is significantly different from the control (dotted horizontal line in Fig. 5, upper part), but it is much weaker than the increase in Z-1 duration and thus, can compensate for only little part of this delay.

In the megalopa stage there is a slight (statistically insignificant) increase from 26.3 ± 2.0 to 28.6 ± 1.9 days. Total development (D) to the first crab stage clearly increases with early starvation time (t) from 51.7 ± 2.0 to 64 days. The regression equation describing this trend is: $D = 50.79 + 1.33\ t$ ($r = 0.9894$; $P < 10^{-3}$)

In experiment 4, as in all later 1980 experiments, the development rate in the two zoeal stages was significantly slower than one year before, whereas the megalopa stage had not changed. This observation will be discussed below.

Experiment 5 (Figs 1 and 6): It became evident that starvation periods in the beginning and at the end of the first zoeal stage each had different effects on later development and survival (cf. exp. 2 and 4). Therefore. a more detailed analysis of the

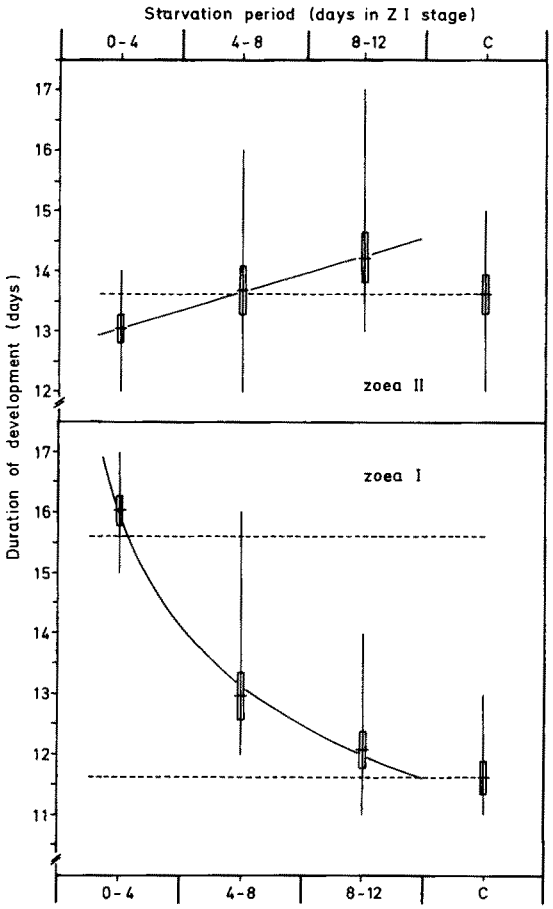

Fig. 6. *Hyas araneus:* Experiment 5. Explanations see Figures 1 and 3

interaction between the internal development programme and starvation effects was needed.

In experiment 5 a constant 4-day fasting period was shifted from the beginning to the middle and to the end of the Z-1 stage. The later this period of starvation occurred, the less it delayed the duration of the Z-1, but the more it influenced the Z-2. The regressions of the mean days of the 4-day periods (t) on the durations of the zoeal stages (D) are: Z-1: $\ln D = 2.90 - 0.18 \cdot \ln t$ ($r = -0.9972$; $P = 0.048$) Z-2: $D = 12.76 + 0.15\ t$ ($r = 0.9988$; $P = 0.03$)

As in experiment 4, there was a weak trend in megalopa development opposed to that of the Z-2 stage. It was again statistically insignificant. Only starvation in the very beginning of the Z-1 stage had a significant; delaying influence on total development time to the fist crab: 55.3 ± 1.5 versus 51.3 ± 1.6 to 51.7 ± 2.0 days.

Mortality was not influenced by these short starvation times: During total larval development it amounted to 10 % in the whole experiment.

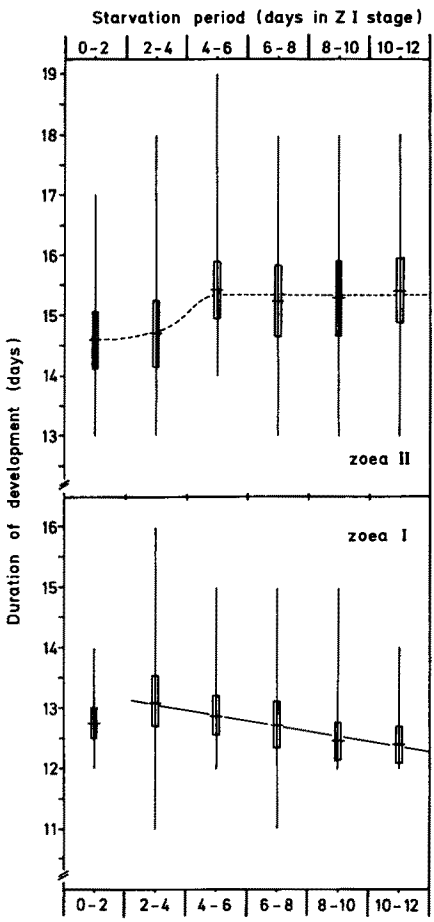

Fig. 7. *Hyas araneus:* Experiment 6. Explanations see Figures 1 an 3

Experiment 6 was carried out in the same way as the last one, only the starvation periods were shortened from 4 to 2 days (Figs 1 and 7). As might be expected, no statistically significant differences between single mean values could be found, since only two days of starvation hardly influenced development and survival. However, there was an unexpected finding: Short starvation immediately after hatching had apparently less delaying influence on the Z-1 stage than the same short period applied a little later (Fig. 7). Although the difference between these two mean values was not statistically significant, it might have a meaning which will be discussed later. This first value was the only one not fitting the trend shown by all the other figures. They revealed a similar decline (only weaker) to that observed in experiment 5 for Z-1 duration (D) in relation to starvation time (mean of the period, t): $D = 13.34 - 0.09\ t$ ($r = -0.9884$; $P = 10^{-3}$)

Short starvation occurring beyond the first four days of Z-1 development caused a slight delay in the Z-2. In contrast to experiment 5 there was no further difference in the extent of this delay.

Mortality was higher than in the other experiments (12 to 28 % died before metamorphosis), but did not show any trend.

Experiment 7 was an extension of exp. 3 and the reversal of 5 and 6 (Fig. 1): Instead of a starvation period appearing at different times in early development, a rather short feeding period (4 days) shifted within a long starvation period.

The results complement and confirm all above observations (Fig. 8): Four days of feeding immediately after hatching were sufficient for most of the larvae to reach the next stage without any further food supply (cf. PRS_{50} in experiment 2).

When this 4-day feeding period followed an equally long starvation period, mortality increased significantly ($P = 0.04$), and the intermoult duration of Z-1 stage was

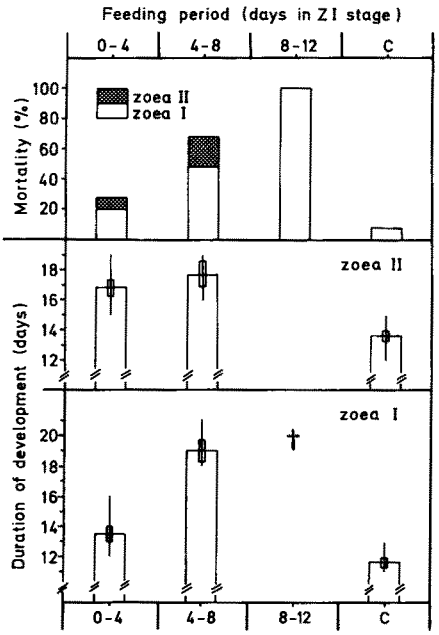

Fig. 8. *Hyas araneus:* Experiment 7. Explanations see Figures 1 and 3

considerably prolonged; still 48 % of the larvae reached the Z-2 and 32 % the first crab stage. In both subexperiments also, the Z-2 development was strongly delayed.

After eight days of starvation, a 4-day feeding period was insufficient to support development to the second stage. The latest larva died after 40 days (28 days of starvation following only 4 days of feeding!).

Survival in the second zoeal stage was significantly reduced in the group fed from day 4 to 8 as opposed to the control ($P = 0.02$). In the megalopa stage there was no mortality in any group.

Experiment 8 followed exactly the same design as experiment 2 (Fig. 1), except there were no control groups (corresponding to 0 and C in Fig. 1), and the second zoea was considered instead of the first stage (Fig. 9). This experiment was planned as an attempt to provide a preliminary idea about the general behaviour of the Z-2 to be expected in future investigations.

The PRS_{50} value was found to be ca. 5 days. This was (absolutely) later than in the Z-1, but about the same in relation to its duration (ca. one third). Development duration in the Z–2 was only prolonged when feeding ceased already after four days; in this instance

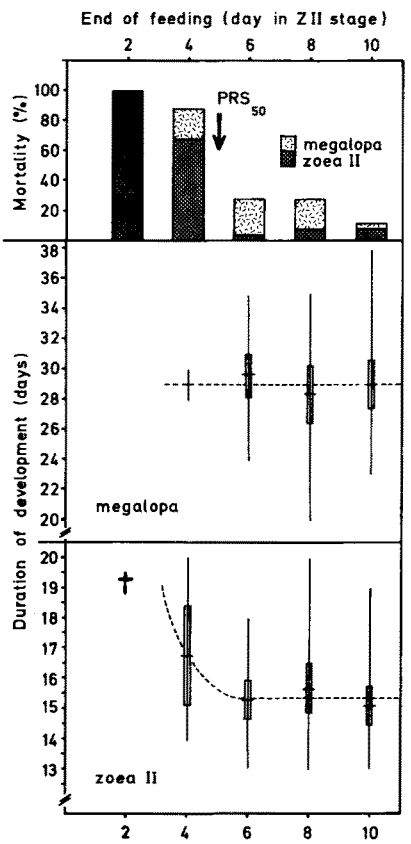

Fig. 9. *Hyas araneus:* Experiment 8. Explanations see Figure 3

mortality was significantly higher ($P < 10^{-4}$) than in the group fed two days longer. If starvation commenced even sooner, no larva reached the megalopa stage.

The duration of the megalopa stage was surprisingly unaffected by starvation during the Z-2 instar. Also In the total development time to the first crab only insignificant differences were observed (52.5 ± 1.4 to 53.7 ± 1.6 days).

DISCUSSION

The first question in the section "Introduction" can be answered as follows: All larval stages of *Hyas araneus* depend on the availability of food organisms for further development, regardless of temperature. However, they are apparently well adapted to temporary lack of food, as maximal survival times under starvation conditions are considerably longer than shown by other figures hitherto reported for crustacean larvae (only exception: Kon, 1979).

The limits for starvation resistance vary in relation to temperature and stage. In all stages, there is a more or less clearly developed inverse relationship between survival time and temperature. This finding might be explained simply by the well-known temperature dependence of metabolic processes; however, this explanation would be too simple. As shown by Rice & Provenzano (1965), Provenzano (1967, 1968b), and Gore (1970), not necessarily the lowest, but often the o p t i m a l temperature allows longest survival in starved decapod larvae. Some tropical species, for example, are killed by temperatures of 10° to 15° C, long before their reserves have been used up (Provenzano, pers. comm.). There are obviously two mechanisms superimposed on each other: (1). generally decreasing metabolism with decreasing temperature, and (2). a temperature range in which enzymatic and other biochemical processes function optimally due to genetic or non-genetic adaptation (cf. Kinne, 1964, Rosenberg & Costlow, 1979).

Since *H. araneus* is a cold-water species, these two phenomena act principally together and cannot be clearly distinguished. On the other hand, modifications of this general trend should be expected concerning the different larval stages. Hatching takes place during the season with coldest water temperature, larval development during rising temperatures in spring. This means that the Z-1 stage should be adapted to the lowest (ca. 3°–7° C), the Z-2 to intermediate (ca. 5°–10° C), and the megalopa to the highest temperatures (ca. 8°–15° C) (see the graphical model proposed by Anger & Nair, 1979). The survival pattern in Figure 2 corresponds rather well to these assumptions. Differential starvation resistance in the larval stages appears to be related to the temperature optima estimated above.

If the values from Figure 2 are plotted against the mean stage durations summarized by Anger & Nair (1979), plus some recently obtained figures (Anger & Dawirs, unpublished) for 18° C (8 days for Z-1, 10 days for Z-2), then the following regression is obtained: $S = 5.2 + 0.9\ D$ ($r = 0.9387$; $P < 10^{-3}$)

This gives an answer to the second question in the "Introduction". Maximal survival time (S) under starvation is in the same order of magnitude as the "normal" duration (D) of each stage at a given temperature, mostly S is usually slightly higher than D. Similar relationships ($S \sim D$) were also observed by Knowlton (1974), Roberts (1974), Yaqoob (1977), and Provenzano (1978). Deviations from this rule were observed at temperatures below the optimal range (Gore, 1968, 1970; Provenzano, 1967, 1968b). In these cases,

survival was considerably shorter than development (if this was, at all, possible); in the optimal range, S and D were again similar. If the general validity of this relationship can be confirmed, it should be possible to estimate the approximate maximal survival times of brachyuran larvae from their stage durations.

Experiments carried out in 1979 and 1980 indicated some systematic differences in development duration which cannot yet be explained definitely. The latter experiments started about one month earlier in the year than the former series. Since the berried females were always kept in cold water (2 °C), an irreversible non-genetic adaptation (Rosenberg & Costlow, 1979) might have caused a decrease in intermoult duration with increasing time of adaptation. Another possible explanation might concern the amount of yolk reserves in larvae, hatched at different times, as carbon measurements and starvation resistence were higher in larvae observed in February 1980 than in May 1979.

It is still an open question, whether these differences resulted from different females, periods within the spawning season or from different years. If there was no temperature adaptation, a decrease in yolk reserves during one season might be the most probable explanation! This would be supported by similar observations by Pandian & Schumann (1967) and Regnault (1969). The significance of those phenomena will be investigated before further experiments on starvation resistance and "sublethal effects" of starvation are started.

Question number 3 in the "Introduction" is answered in Figures 4 and 9: Both zoeal stages need at least one third approximately, of their development duration to accumulate enough reserves for about 50 % of the larvae to moult successfully to the next stage, independent of food availability (PRS_{50}). If starvation lasts from the PRS_{50} to the moult, survival is reduced and development is delayed. The time at which n o larva has yet accumulated sufficient reserves to survive starvation to the next moult (PRS_0) is only little earlier. In both zoeal stages it appears to be about 20 % of stage duration. In other words: Zoea larvae must feed right after hatching (or moulting) for more than one fifth of their normal duration in order to have at least some chance of surviving later starvation; if more than one third of the stage duration has elapsed under good feeding conditions, every second larva probably survives starvation to the next moult.

In experiment 2 (Fig. 4) starvation during the last 2 to 4, days of the first zoeal stage resulted in somewhat faster development as compared to the control (C). This might suggest that food organisms in some way disturb rather than support moulting zoeae. The differences were statistically not significant, and they were not found again in experiment 5 (Fig. 6); therefore this possible effect must remain in question.

The fourth question in the "Introduction" deserves a clear "yes": The point-of-no-return for 50 % of the Z-1 larvae (PNR_{50}) is reached after passing more than half of the maximally possible survival time under starvation (Fig. 5). If more than 70 % of this maximum time has elapsed without food supply, apparently no larva has a chance to recover when re-fed (PNR_{100}). These figures indicate an unusually good adaptation to fluctuating prey availability as compared to fish larvae (see Ehrlich et al., 1976). They correspond closely to the only comparable data for zoeae of a majid crab provided by Kon (1979).

The regression line for Z-1 duration (D) against starvation (t) in Figure 5 suggests a basic internal mechanism in early development: The programme which has to be completed to permit moulting to the second stage, does not start as long as the larva has

not fed for the first time. Normal development duration (D_C) is prolonged by the starvation time (t) plus an additional amount of time (t') which is probably necessary to compensate for energy losses during starvation. Since t' is proportional to t, another linear regression can be calculated to express the relationship between D and t (solid line in Fig. 5, lower part). In this new regression the slope is significantly steeper than in the theoretical $D = D_C + 1\ t$ (dotted in Fig. 5, lower part). Kon (1979) found such additional prolongation (t') only after more than one week of initial starvation. The general response of Majid zoeae to early starvation as described in that paper is basically the same as found in our experiments: Development starts only after first feeding. A similar observation was also made in king-crab zoeae (Kurata, 1959).

The new linear relationship indicates a constant energy loss per unit of starvation time for a certain range. There is, however, a restriction. The mechanism assumed above is not fully valid for very short starvation periods directly after the prezoeal moult. For starvation periods shorter than about three days, t' appears to equal zero or it becomes even negative! The same effect was also observed in experiment 6 (Fig. 7). This means that a small part (about one day) of the developmental programme mentioned above must run, independent of external energy supply, in the very beginning of the stage. Since freshly hatched larvae possess enough reserves to run a greater part of their developmental programme without food (Anger & Nair, 1979), the above mechanism cannot be explained exclusively with energetics. A possible explanation for the key role of first feeding in early larval development of decapods will be given below.

The existence of a critical period at the beginning of larval development was

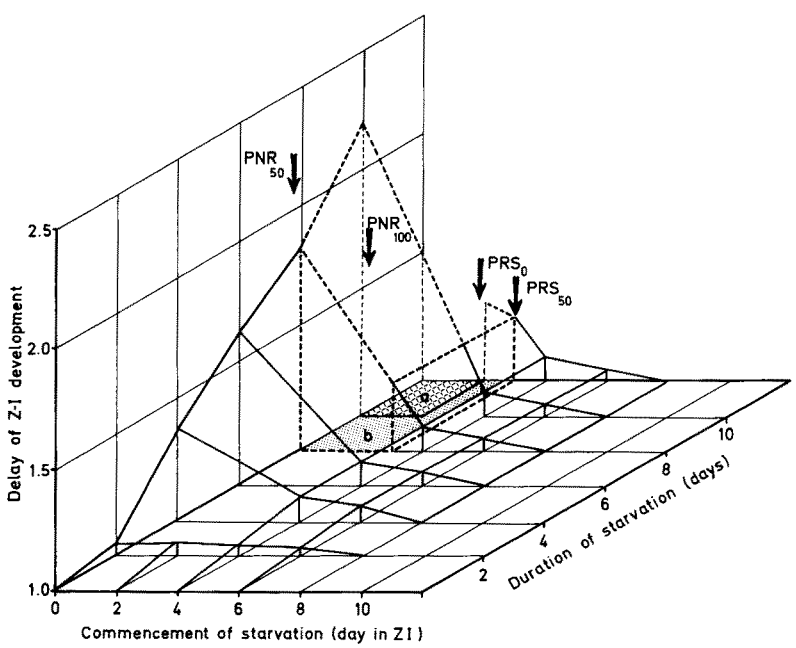

Fig. 10. *Hyas araneus:* Response pattern in the zoea-1 stage(ZI) to differential starvation. Delay expressed as multiple of development duration in fed controls. Further explanations see text

already suggested by observations reported by Yatsuzuka (1962), Modin & Cox (1967), and Kon (1979). These authors described lowered survival in decapod larvae, when feeding commenced too late following hatching. An analogy exists in larval fishes (Ehrlich et al., 1976). Also Chamberlain's (1957) observations most probably based on the same mechanism: Xanthid larvae fed exclusively on diatoms during the first days (later they received animal food) showed considerably delayed development as opposed to sibling larvae fed with zooplankton from the beginning. It is now generally accepted that brachyuran larvae are strictly carnivorous and cannot subsist on algal food. This means that Chamberlain starved the zoeae involuntarily in a similar way as in our experiment 1 (Fig. 3), and he obtained similar results.

Figure 10 summarizes the response patterns of the first zoeal stage of *H. araneus* to starvation: The delay in Z-1 development, expressed as prolongation factor using the control as unit (C = 1), shows differential effects of starvation.

The delay increases with duration of the starvation period, but even more drastically with its advancing commencement within the stage. This pattern confirms the critical-point concept (see above) and it answers the last question in the section "Introduction". If starvation starts before the PRS_{50} is reached or if it ends later than the PNR_{50} (shaded area, b in Fig. 10), then less than half of all larvae have a chance to reach the second stage. The PNR_{100} and the PRS_0 define the ultimate limit for any further development (hatched area, a).

A similar response pattern as shown in Figure 10 is obtained, if mortality instead of developmental delay is taken as a measure for starvation effects. However, zoeal mortality is a less accurate, sensitive, and reliable index (cf. Costlow & Bookhout, 1970) and therefore was only taken as additional information in this study. Also the observation reported by Wickins (1972), who provided food of different quality during differential periods in larval development of penaeid shrimps, correspond very well to our findings.

With the exception of experiment 8, there was never any starvation applied to the second zoea, but effects of early starvation on later stages did occur. Lack of food in the final phase of the Z-1 stage did not significantly affect the duration of this stage (Figs 4, 6, 7, 10), but that of the next instar. The effects (delayed development and increasing mortality) depend again on the duration of the fasting period.

A surprising phenomenon is observed when lack of food occurs in the beginning of the Z-1: the duration of the second stage is shortened (Figs 5 and 6). We cannot find sensible energetic explanations for this partial compensation in development rate.

From our experimental observations and from literature data the following hypothesis is derived and proposed as a possible explanation:

The first day of the moult cycle in the Z-1 stage is presumably characterized by the postmoult periods A and B. According to the only comparable figures for crab larvae (Freeman & Costlow, 1980) we assume them to last, taken together, ca. 10 % of the stage duration. During this time, water and minerals are taken up, and the endocuticle is secreted. The latter step involves chitin synthesis, which is independent of food uptake (Anger & Nair, 1979). Starvation, therefore, presumably does not affect further development, if suffered only during postmoult. Moult inhibiting hormone (MIH) is probably secreted into the haemolymph during this phase, independently of nutrition.

As the larva approaches intermoult (C), food uptake is necessary to initiate further

development. All reconstruction processes cease, if this initial cue is missing (see above), and protein reserves are utlized as main energy source during this time (Anger & Nair, 1979). The length of starvation time (t, measured from the beginning of phase C_o) corresponds to the minimum prolongation of intermoult duration (experiment 4, cf. Kurata, 1959; Kon, 1979). The longer initial starvation lasts during phase C, the more additional time (t') is added for compensation of protein loss. There is an upper limit (ca. 70 % of maximally possible survival time) which cannot be exceeded without losing the ability to recover and to moult. The actual cause of this point-of-no-return can only be speculated: The ability to catch prey is certainly weakened (Paul & Paul, 1980), but insufficient food uptake alone can hardly explain the PNR. Survival after initial starvation and recommencement of feeding is mostly much longer than maximum survival under complete lack of food (exp. 1, 3, 4, 7; see also Kon, 1979). Therefore some irreversible biochemical or histological damage must be suffered which prevents recovery and moulting.

When prey is available during intermoult, tissue growth and accumulation of organic reserves take place (Yamaoka & Scheer, 1970). This period is assumed to last ca. one third of Z-1 duration (estimation based on figures given by Freeman & Costlow, 1980), i. e. somewhat less than four days. If starvation (in the widest sense) precedes or interrupts phase C, it will last longer (of. Figs 5–8), because protein losses have to be compensated for.

There are several substances essential for growth in Crustacea (Provasoli, 1976). The fact that the intermoult phase (C) needs a starting mechanism suggests that there might be a single key substance. We assume that sterols taken from the first diet may play a crucial role. They cannot be synthesized by the larvae (Whitney, 1969; Gilbert & O'Connor, 1970; Provasoli, 1976), but they are needed as precursors of steroid hormones. It is possible that β-ecdysone (= β-ecdysterone, crustecdysone) synthesis is the actual starting point of further development. If starvation sets in after the start of phase C, development does not immediately cease; if at this time the pool of necessary reserves is already "saturated" ("point-of-reserve-saturation", PRS, see above), i. e. possibly a sterol pool sufficient for Z-1 development has been accumulated, then the β-ecdysone will suffice to initiate premoult and ecdysis, independent of further prey availability. If starvation begins too soon during phase C to allow successful completion of the moult cycle, then some development still takes place automatically. It will cease later at some point until feeding recommences. In this case the delay in development is shorter than the actual starvation period (Figs 4 and 6).

Intermoult (C) is completed when the β-ecdysone has exceeded that level of activity which is necessary to eliminate the influence of MIH and to start the first premoult phase (D_O). Beyond this point, further development is programmed by the moulting hormone; its automatic course is not significantly affected any more by prey absence or presence. If postmoult and intermoult, combined, amount to ca. 40 % of the stage duration (cf. Freeman & Costlow, 1980), then D_O should commence after about five days. This assumption corresponds to the observations in experiments 2 and 5. It would mean that the Z-1 of *H. araneus* undergoes a diecdysic type of moult (Knowles & Carlisle, 1956).

Experiments 2 and 5 also suggest that during premoult some substances taken from the diet are accumulated which are important for the second zoeal stage. If starvation lasts for the whole premoult period, the duration of the Z-2 stage is significantly

prolonged. The key substance might be again β-ecdysone or some precursory sterol stored during this period. Another possibility might be lipid, which is needed as a pool of precursors for chitin synthesis in the Z-2 (Holland, 1978). It should be recalled that this effect was not observed during the transition from the Z-2 to the megalopa (Fig. 9), suggesting that the long-lasting megalopa stage is much less dependent on such reserves.

Since the MIH is most probably secreted during the postmoult (Freemann & Costlow, 1980), the strange shortening effect in the Z-2 development (experiment 4; Fig. 5, upper part) might be explained in the following way: The MIH is, according to Rangaro (1965), a peptide. It is also known that under starvation, amino acids can become an important source of energy, i. e. they are catabolized (Munday & Poat, 1971). Thus, it is possible that part of neurosecretory MIH is used up during early starvation. This would, later change the ratio between the MIH and the β-ecdysone synthesized during intermoult (after recommencement of feeding). This disturbance in the hormone system should increase with the duration of the starvation period. Since the effects of delayed intermoult initiation and that of compensation for energy loss in the Z-1 (see above) are far stronger than that of a reduced MIH pool, the latter may become visible only in the Z-2 stage.

So far, our experiments have shown that early larvae of *H. araneus* have a considerable capacity to survive in an environment with highly variable zooplankton concentration. The technique used to evaluate limits and changes of this potential (Fig. 1) is also a suitable complementary means for analyzing developmental processes in crustacean larvae. Future investigations will have to scrutinize this hypothetical course of events using biochemical as well as histological methods. Such analyses may also permit an explanation for the point-of-no return: Why does a starved larva become unable to moult? Which irreversible damage is responsible for this effect? Furthermore, similar studies on different species should help to distinguish between species-specific effects and general rules. Finally, later larval stages must also be investigated, since they may reveal response patterns different from those in the first zoea (cf. Figs 4 and 9), and they lead to the most important transition in decapod development: to metamorphosis.

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