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Methodical aspects of rearing decapod larvae, *Pagurus bernhardus* (Paguridae) and *Carcinus maenas* (Portunidae)

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ABSTRACT: Improved methods for experimental rearing of *Pagurus bernhardus* and *Carcinus maenas* larvae are presented. Isolated maintenance was found essential for reliable statistical evaluation of results obtained from stages older than zoea-1. Only by isolated rearing is it possible to calculate mean values ± 95 % confidence intervals of stage duration. Mean values (without confidence intervals) can only be given for group-reared larvae if mortality is zero. Compared to group rearing, isolated rearing led to better survival, shorter periods of development and stimulated growth. Due to different swimming behavior *P. bernhardus* zoeae needed larger water volumes than *Carcinus maenas* larvae. *P. bernhardus* zoeae were reared with best results when isolated in Petri dishes (ca. 50 ml). They fed on newly hatched brine shrimp nauplii (*Artemia* spp.). *P. bernhardus* megalopa did not require any gastropod shell or substratum; it developed best in glass vials without any food. *C. maenas* larvae could be reared most sucessfully in glass vials (ca 20 ml) under a simulated day-night regime (LD 16 : 8); constant darkness had a detrimental effect on development, leading to prolonged stage-duration times. *C. maenas* larvae were fed a mixture of newly hatched brine shrimp naupli and rotifers (*Brachionus plicatilis*).

INTRODUCTION

Costlow & Bookhout (1959) developed simple, effective and reproducible rearing methods for decapod larvae. Though numerous studies have been published on decapod larval ecology since then, we are still far from understanding completely decapod crustacean larval dynamics in the sea. Coordinated laboratory and field data are required for analyzing larval dynamics. There is great need for further development of rearing methods.

This paper presents improved rearing methods for *Pagurus bernhardus* and *Carcinus maenas*. Both species are common in the German Bight (North Sea). (1) For both species two methods were tested: rearing of larvae in isolation and in groups. (2) Different types of "enclosures" (cf. Kinne, 1976) were examined as to their usefulness for laboratory larval maintenance. (3) It was further investigated whether presence or absence of light per se could improve rearing conditions. (4) The question of whether the development of *P. bernhardus* megalopa is affected by the surface of the glass vials is discussed.

Culture techniques for decapod crustacean larvae were reviewed by Provenzano (1967), Rice & Williamson (1970), Roberts (1975), and Kinne (1977).

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MATERIAL AND METHODS

Obtaining and handling of larvae

Egg-carrying females of *Pagurus bernhardus* were dredged from "Helgoländer Tiefe Rinne" a deep (ca 30 to 50 m) channel southwest of Helgoland. Ovigerous *Carcinus maenas* females were collected from the rocky intertidal region in the northern part of the island. In the laboratory, females of both species were maintained in flow through systems, where newly hatched larvae were automatically separated from adults by an overflow. These systems were checked twice a day. Since larvae mostly hatched at night, those found in the morning checks were called newly hatched on that day. Only these were used for the experiments.

The rearing medium for both species was filtered (ca 1 µm), natural sea water from Helgoland (31–33 ‰ S) without any further treatment. *P. bernhardus* larvae were fed newly hatched brine shrimp nauplii, *Artemia* spp. (ca 10 nauplii ml^{-1}, source: Kew, Melbourne, Australia, World Ocean Ltd., 87 Packington St.). *C. maenas* larvae were fed a mixture of newly hatched brine shrimp nauplii and the rotifer, *Brachionus plicatilis* (ratio: ca 10 : 1, ca 100 food organisms ml^{-1}). The rotifers were taken from mass rearing systems, where they fed on yeast. Before they were used as food they fed on *Dunaliella* spp. (grown in F/2 medium; see Guillard & Ryther, 1962). At least about 30 min were assumed to be long enough to supply vegetable vitamins.

Only healthy looking, actively swimming, newly hatched larvae, which were selected from the surface of the collecting chamber in the flow through systems, were used for all rearing experiments. Checks of all experimental sets were conducted at about the same time of the day as they were started. Larvae were checked once a day in any setup.

Rearing enclosures

Four different enclosures were used and partly compared as to their utility in rearing *Carcinus maenas* and *Pagurus bernhardus* larvae: glass vials, glass beakers, net bottom tubes, and petri dishes. The latter were employed only for rearing trials with *P. bernhardus*.

25 glass vials (2.2 cm × 6 cm) with about 20 ml sea water were put in hard plastic basins (20.5 × 10.5 × 3.0 cm), thus forming small sets, easy to handle. Each vial contained one single larva.

500 ml glass beakers containing 40 larvae each, were used for comparison of single and group rearing. Equal distribution of the food organisms was achieved by gentle aeration.

Transparent plexiglass tubules (1.6 × 7 cm) with 300 µm net bottoms (net bottom tubules) were put, interchangeably, into 50 holes of a plastic plate that covered a tub filled with about 19 l natural, filtered sea water. The lower part of the tubules reached into the water providing a separated volume for isolated larvae of about 10 ml.

Petri dishes were only used in the isolated rearing of *P. bernhardus* larvae. They were about 10 cm in diameter and filled with about 50 ml sea water.

Water exchange and checks

If not otherwise stated, water in the glass vials, beakers and petri dishes was renewed every other day. Larvae were considered dead when they turned opaque and no movement of appendages could be observed or provoked. In the vials, checks for molts were conducted by looking for the exuviae. In the beakers, where larvae were kept in groups, single larvae had to be checked under a dissecting microscope in order to determine stage numbers.

Due to the huge water reservoir in the tubs communicating with the plexiglass tubules' volumes, "water exchange" was accomplished by moving the entire set (plate with the 50 tubules) up and down once a day. Thus water was circulated and debris in the tubules rinsed through the bottom net. The whole water body was exchanged once a week. Checks were carried out by taking the tubules out of the plate individually and holding them into a small sea water-filled beaker (ca 50 ml), and thus looking for the exuviae.

Because of the relatively large size of *P. bernhardus* larvae, checks in the petri dishes were simply done by looking into the dishes from above. In every case, food organisms were provided dropwise by pipette after water renewal.

Experiments

In order to measure and compare working effort of both methods (individual and group maintenance of *Pagurus bernhardus* and *Carcinus maenas* larvae), the total time expenditure for each operation, commencement of experiments, subsequent water exchanges and checks were taken by means of a stop watch. Values were rounded off to full and half minutes. Thus the mean working effort of producing one newly molted larva of respectively successive stages could be calculated. 200 larvae tested in groups of 40 in 5 beakers were compared to 200 isolated larvae maintained in glass vials. Mortality and development time were also recorded in this setup.

50 *P. bernhardus* larvae reared in net bottom tubules were compared to 25 larvae kept in glass tubules, and 50 *C. maeanas* larvae reared in net bottom tubules were compared to two different hatches kept in sets of 200 (glass tubule 1) and 50 (glass tubule 2). The latter was the only case in the present study, where larvae from different hatches were compared in one experiment.

25 *P. bernhardus* larvae reared in petri dishes were compared to 25 larvae reared in glass tubules in constant darkness. The latter was achieved by simply using a plastic basin, like the one in which the single glass vials were put, as a lid upside down on the lower basin.

The same technique was used to darken *P. bernhardus* and *C. maenas* larvae in the direct tests on constant darkness and cyclic light conditions. In the latter, a day-night regime of LD 16 : 8 was simulated using a 40 Watt neon tube with 5000 lux dayligth. If not otherwise stated, all the rearing was run under these light conditions. Larvae kept in complete darkness were exposed to light only for the very short period of water exchange and checking. 25 larvae of both species were maintained in each condition.

All *P. bernhardus* megalopa used for experiments in the present study derived from one single hatch (March 20th 1980; cf. Dawirs, 1981). Two types of sediments were

tested: fine sand (177–250 µm) and medium sand (354–500 µm). Both were obtained by straining. The sands were treated with hydrochloric acid, dried and stored. Before usage, the sediments were thoroughly washed with ion-exchanged water, and finally with sea water. The bottoms of the glass vials were covered with sediment after they had been filled with sea water. 25 *P. bernhardus* megalopae were tested on either sediment; the water was never exchanged. For further comparison, 50 megalopae were maintained on the net bottom (300 µm) of the plexiglass tubules in one of the described tubs and 155 megalopae were kept in glass tubules (cf. Dawirs, 1981).

In order to determine dry weight, and content of carbon, nitrogen and hydrogen the same methods were applied as described in detail in Anger & Nair (1979) and Dawirs (1980). The "Elemental Analyzer Model 1106" (Carlo Erba Science) was used instead of Model 1104. 200 newly hatched larvae of *Carcinus maenas* were analysed in groups of 20. Larvae from the same hatch were maintained in groups (non-aerated glass dishes) and in isolation (glass vials). After 3 days at 18 °C, 200 Zoea-1 from each set were analysed in groups of 20 larvae. Energy equivalents were calculated using the formula given by Salonen et al. (1976) and expressed in Joules (1 J = 0,239 cal).

All rearing was done in a constant temperature of 18 °C. Differences in mortality were discussed using a test for different frequencies after Weber (1972). Other t-Tests (for equal and unequal variances), preceding F-tests, and curve fitting were done according to Sachs (1968). In the present study all mean values are given including ± 95 % confidence intervals.

RESULTS

Single and group rearing

To have a closer look at the utility of either single or group rearing, developmental time and mortality as well as the experimental working effort of both methods were examined (Figs 1, 2). The latter was defined as working time per newly molted individual of a particular larval stage. In both figures, Z2 to C1 denote cumulative development up to a respective stage, e.g. C1 stands for development up to metamorphosis. Larval growth of *Carcinus maenas* Zoea-1 was measured and compared in both methods.

Throughout the larval development of *C. maenas*, mortality was significantly lower in individual maintenance ($P < 10^{-10}$). The zoea-1 was mainly responsible for that difference. Only 31 % of the communally reared zoea-1 successfully molted to zoea-2 (Fig. 1). Mortality rate was lower in the following stages, hence cumulative mortality ascended steadily but less steeply than in the beginning. 59 % of the larvae completed development in single rearing, whereas only 5.5 % succeeded in molting to the first crab stage when maintained in groups.

Development was delayed in communally kept zoeal stages of *C. maenas*. The differences between both methods were highly significant. 2.6 days up to the zoea-2 ($P < 10^{-10}$), 3.7 days to zoea-3 ($P < 10^{-10}$), and 3.4 days to zoea-4 ($P < 10^{-10}$). Though still significant ($P < 0.04$), differences in cumulative development to the megalopa was less distinct: 1.4 days. No difference could be found between both methods in total development time. Metamorphosis took place after an average time of 34.1 ± 1.1 days in communally reared larvae and after 34.3 ± 0.7 days in individually reared larvae.

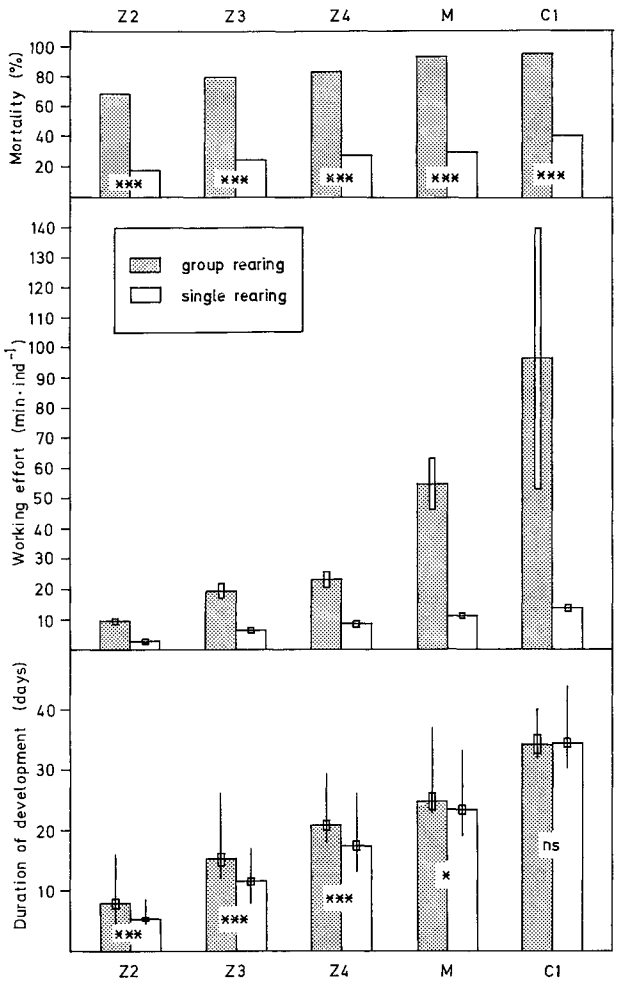

Fig. 1. *Carcinus maenas:* Rearing in groups (group rearing) and in solitary confinement (single rearing). Cumulative mortality (%), working effort (min per individual newly molted stage), and developmental time (days). Cumulative development up to respective stages: zoea-2 (Z2) to zoea-4 (Z4), megalopa (M), and first crab stage (C1). Mean values (columns) ± 95 % confidence intervals (bars) and ranges (vertical lines). Statistical differences are ns: not significant; *: $P \leq 0.05$; ***: $P < 0.0001$

Obviously, the earlier delay in development time was compensated in later development. Table 1 gives the stage durations where only single rearing delivered exact mean values and 95 % confidence intervals. The same is not possible for stages other than zoea-1 in group rearing, unless no mortality occurs. Thus respective data in Table 1 were estimated by differences in successive cumulative times. Obviously, communally reared zoea-4 and megalopae need less time for development than their individually kept siblings (Fig. 1).

Table 1. *Carcinus maenas*: Mean values of stage duration ± 95 % confidence intervals of larvae maintained by single rearing (SR). * Data for group rearing (GR) were estimated by differences of successive cumulative times (cf. Fig. 1)

	Stage duration (days)				
	Z1	Z2	Z3	Z4	M
SR	5.4 ± 0.2	6.4 ± 0.4	5.8 ± 0.3	6.2 ± 0.3	11--.2 ± 0.4
GR*	7.9	7.4	5.5	4.0	9.3

Table 2. *Carcinus maenas*: Growth of zoea-1 after 3 days in single rearing (SR) and group rearing (GR). Individual dry weight (DW), carbon (C), nitrogen (N) and hydrogen (H) (µg). Individual energy content ($J \cdot ind^{-1}$) and weight specific energy ($J(mg \cdot DW)^{-1}$). Mean values ± 95 % confidence intervals

	Day 0	Day 3		t-Test
		SR	GR	SR/GR P <
DW	11.0 ±0.2	17.3 ±0.6	14.6 ±0.4	10^{-7}
C	4.3 ±0.1	6.4 ±0.3	5.4 ±0.1	10^{-5}
N	0.9 ±0.02	1.4 ±0.1	1.2 ±0.1	10^{-4}
H	0.5 ±0.01	0.9 ±0.1	0.6 ±0.1	10^{-4}
$J \cdot ind^{-1}$	0.16 ± 0.002	0.23 ± 0.01	0.19 ± 0.01	10^{-5}
$J(mg \cdot DW)^{-1}$	14.05 ± 0.24	13.67 ± 0.89	13.01 ± 0.30	0.05

Larval growth was significantly stimulated in single rearing. Table 2 shows individual dry weight, carbon, nitrogen, hydrogen, and energy content of *Carcinus maenas* Zoea-1 immediately after hatching and after 3 days in single and group rearing. Percentage gain in all components was more than 20 % higher in isolated larvae. Even weight specific energy was significantly lower in group reared larvae.

Measurement of the working effort needed for either method of rearing larvae of *C. maenas* delivers unequivocal results. Thus statistical tests were regarded as quite superfluous. Throughout larval development much more time was spent on rearing larvae in groups than on rearing them individually (Fig. 1).

In the present experiment it was not possible to rear isolated *P. bernhardus* larvae beyond zoea-4 using the described glass vials (Fig. 2). Cumulative mortalities were 26 % up to zoea-2, 54 % up to zoea-3, and 96 % up to zoea-4. All remaining larvae died during this stage. Though not significant, mortality of zoea-1 was about 10 % lower than in communally reared larvae. Cumulative mortality up to the beginning of zoea-4 development was significantly lower in group rearing ($P < 10^{-6}$). 39 out of 200 communally reared larvae survived beyond metamorphosis, i.e. 19.5 %.

The duration of zoea-1 development did not differ significantly in both methods: 6.2 ± 0.2 days in communally and 6.3 ± 0.3 days in individually maintained larvae. In group rearing, stage duration of subsequent stages cannot be calculated directly as in single rearing, but was estimated indirectly comparing cumulative developmental times (Table 3). Thus isolated zoea-2 took almost twice the time their communally

Table 3. *Pagurus bernhardus*: Mean values of stage duration ± 95 % confidence intervals of zoea larvae maintained by single rearing (SR). * Data for group rearing (GR) were estimated by differences of successive cumulative times (cf. Fig. 2)

	Stage duration (days)				
	Z1	Z2	Z3	Z4	M
SR	6.3 ± 0.3	9.5 ± 0.6	14.3 ± 6.1	–	–
GR*	6.2	5.6	5.4	8.5	7.4

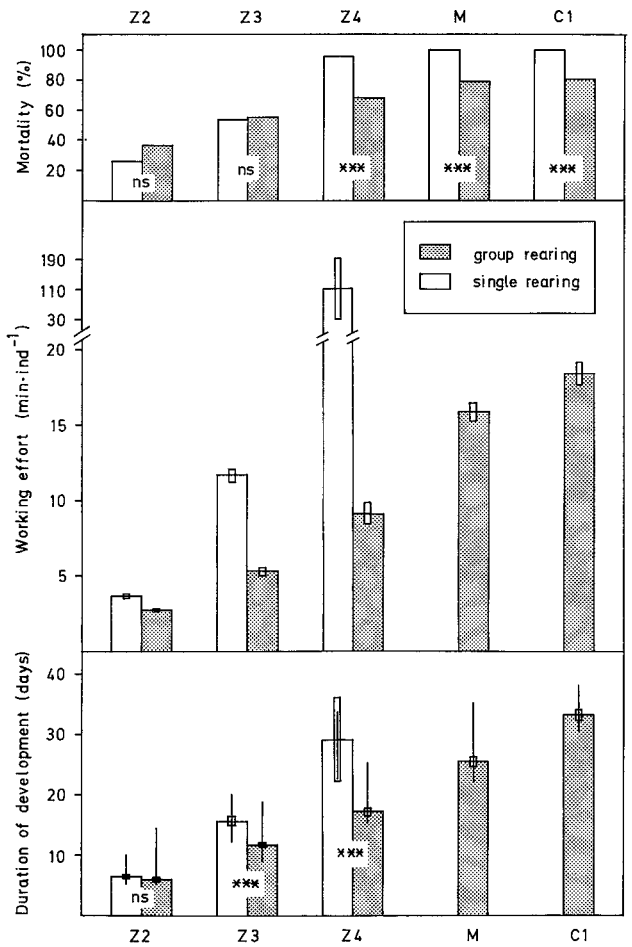

Fig. 2. *Pagurus bernhardus:* Rearing in groups (group rearing) and in solitary confinement (single rearing). Cumulative mortality (%), working effort (min per individual newly molted stage), and developmental time (days). Cumulative development up to respective stages: zoea-2 (Z2) to zoea-4 (Z4), megalopa (M), and first crab stage (C1). Mean values (columns) ± 95 % confidence intervals (bars) and ranges (vertical lines). Statistical differences are ns: not significant; ***: $P < 0.0001$

reared siblings needed to reach the zoea-3 ($P < 10^{-12}$). Stage duration of individually maintained zoea-3 was almost three times longer than that of larvae kept in groups ($P < 10^{-11}$).

Net bottom tubules and glass vials

All attempts to employ the above described net bottom tubules to raise *Pagurus bernhardus* larvae failed. This method was compared to maintenance in glass vials. Though neither method delivered satisfactory results, there were some differences (Fig. 3).

Cumulative and stage mortalities were always significantly higher in the net bottom tubules ($0.002 \leq P \leq 0.02$). Thus 2 % and 52 % of initial numbers molted to megalopae respectively. With the exception of the one megalopa that succeeded in metamorphosing on the gauze, all others died during or immediately after molt, so only dead megalopae were found in the checks.

This mortality pattern was accompanied by differences in stage duration times. The zoea development always took longer in the net bottom tubules. Due to relatively few remaining zoea-4 larvae in the net bottom tubules, differences in development of the zoea-3 larvae were not significant (Fig. 3).

The overall delay in *P. bernhardus* stage duration, and higher mortalities indicate even worse rearing conditions in the net bottom tubules. Besides this, both methods are far from optimal. In both systems extra stage "zoea-5" were observed. Three zoea-5

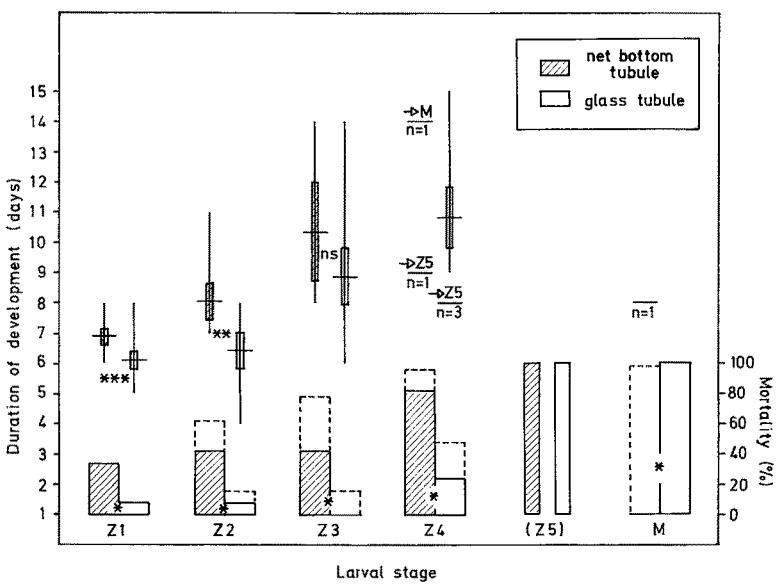

Fig. 3. *Pagurus bernhardus:* Rearing in net bottom tubules and glass tubules. Stage mortalities (solid columns) and cumulative mortalities (dotted columns). Mean values of stage duration (horizontal lines) ± 95 % confidence intervals (bars), and ranges (vertical lines). Zoea-1 (Z1) to zoea-4 (Z4), extra stage zoea-5 (Z5), megalopa (M). Zoea-4 developed to megalopa (→ M) and extra stage zoea-5 (→ Z5). Statistical differences are ns: not significant; *: $P \leq 0.05$; **: $P < 0.001$; ***: $P < 0.0001$

larvae that developed in the glass vials died immediately after molting, whereas the one in the net bottom tubule lived for another 8 days. Neither here, nor elsewhere, has a zoea-5 of *P. bernhardus* ever been observed molting to a megalopa (Fig. 3).

Net bottom tubules and glass vials, both isolating single larvae, were also tested as rearing enclosures for *Carcinus meanas* larvae (Fig. 4). Larvae which were kept in glass tubules originated from two different hatches, denoted glass tubule 1 (gt-1) and glass tubule 2 (gt-2). Hatching dates lay close together, between the end of June and the beginning of August. Developmental data of larvae reared in the glass tubules are more closely related to each other than to larvae in the net bottom tubules. Differences in mortality at the zoea-1 stage were not too conspicuous, but were found crucial in the following stages. Thus in gt-1 59 %, and in gt-2 78 % of initial larval numbers succeeded to metamorphose, whereas just one single larva (2 %) molted to the first crab stage on the gauze.

With similar mortalities in the glass tubules and the net bottom tubules, development of zoea-1 was significantly delayed in the tubules (Fig. 4). It took the zoea-1 about 2.3 days longer to molt in the net bottom tubules than in gt-1 ($P < 10^{-11}$), and about 2.5

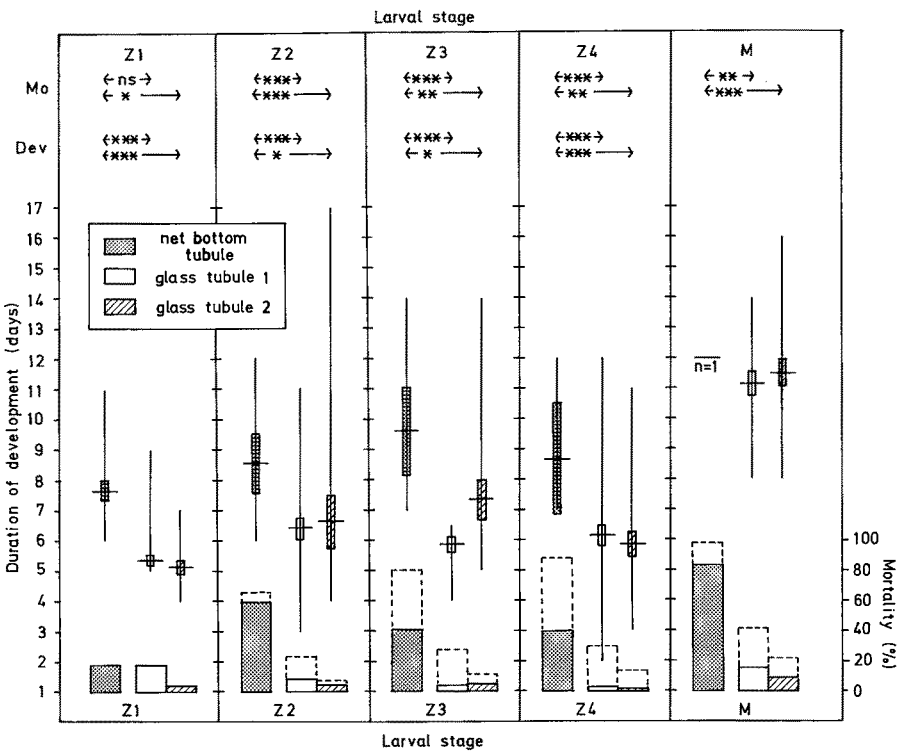

Fig. 4. *Carcinus maenas:* Rearing in net bottom tubules and two sets of glass tubules. Stage mortalities (solid columns) and cumulative mortalities (dotted columns). Mean values of stage duration (horizontal lines) ± 95 % confidence intervals (bars) and ranges (vertical lines). Zoea-1 (Z1) to zoea-4 (Z4), megalopa (M). Statistical differences (arrows) are ns: not significant; •: $P \leq 0.05$; ••: $P < 0.001$; •••: $P < 0.0001$. Mortality (Mo) and development time (Dev)

days longer than in gt-2 ($P < 10^{-12}$). That clearly indicates a sublethal detrimental effect of the net bottom tubules on this stage. The development of successive stages was also significantly delayed (Fig. 4). Thus megalopae appeared on an average after 24.8 ± 1.1 days (gt-1) and 25.0 ± 1.4 days (gt-2), i.e. about 8 days earlier than in the net bottom tubules (33.3 ± 3.2 days). Total larval duration was 34.1 ± 1.1 days (gt-1) and 36.2 ± 1.4 days (gt-2) in the glass vials, whereas the only surviving crab in the net bottom tubule had a total larval life span of 42 days.

Illumination

Larvae of *Pagurus bernhardus* and *Carcinus meanas* were reared in total darkness (DD) and in a simulated day-night regime of LD 18 : 6. Throughout *C. meanas* zoeal development no significant differences in stage mortality could be found (Fig. 5). Mortality was high in the zoea-1 and low in the following stages. Only the megalopa revealed a significantly higher mortality under dark than under cyclic light conditions ($P < 0.05$).

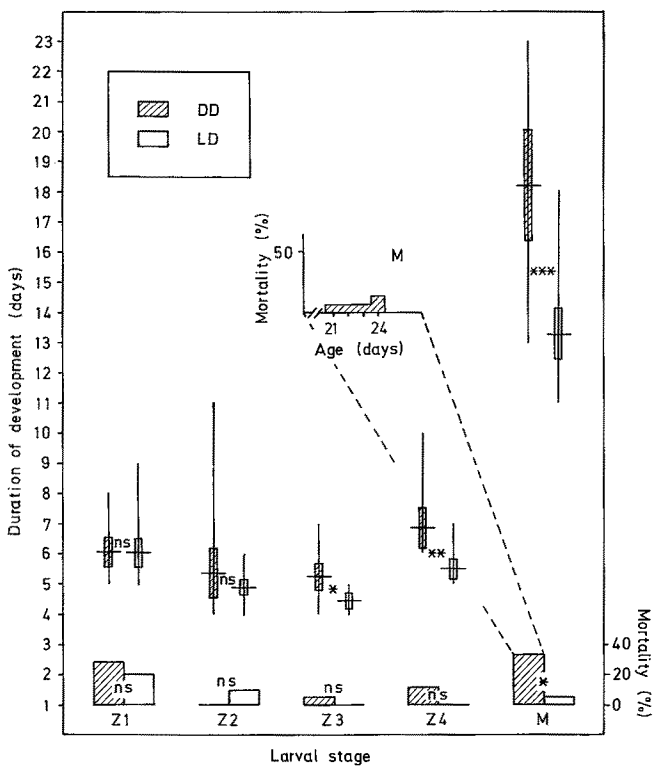

Fig. 5. *Carcinus maenas:* Rearing in continuous darkness (DD) and under cyclic light conditions (LD 16 : 8). Stage mortalities (columns). Mean values of stage duration (horizontal lines) ± 95 % confidence intervals (bars) and ranges (vertical lines). Zoea-1 (Z1) and zoea-4 (Z4), megalopa (M). Mortality of megalopae in DD is related to their age (follow dotted lines). Statistical differences are ns: not significant; *: $P \leq 0.05$; **: $P < 0.001$; ***: $P < 0.0001$

The duration of *C. maenas* zoea-1 development was found to be identical, 6.1 ± 0.5 days in both sets. In further development there was a clearly increasing trend to a prolongation of stage duration under constant darkness (Fig. 5). Thus constant darkness seems to bear some distinct sublethal detrimental influence on rearing *C. maenas* larvae under the described conditions.

The megalopae died when they were 22.8 ± 1.6 days old on an average, i.e. about 4.6 days after the surviving larvae had metamorphosed to crabs (18.2 ± 1.8 days). Though only few larvae died, this difference is statistically significant ($P < 10^{-2}$). Thus, besides a sublethal effect, a slightly lethal influence of continuous total darkness led to partial failure in metamorphosis of this stage (Fig. 5).

In Figure 6 cumulative data for larval duration and mortality are given for each stage. It must be stressed that these data can not be derived from the stage durations and mortalities given in Figure 5 and vice versa. Data of particular stages and cumulative values were both independently derived from original data. They reveal different

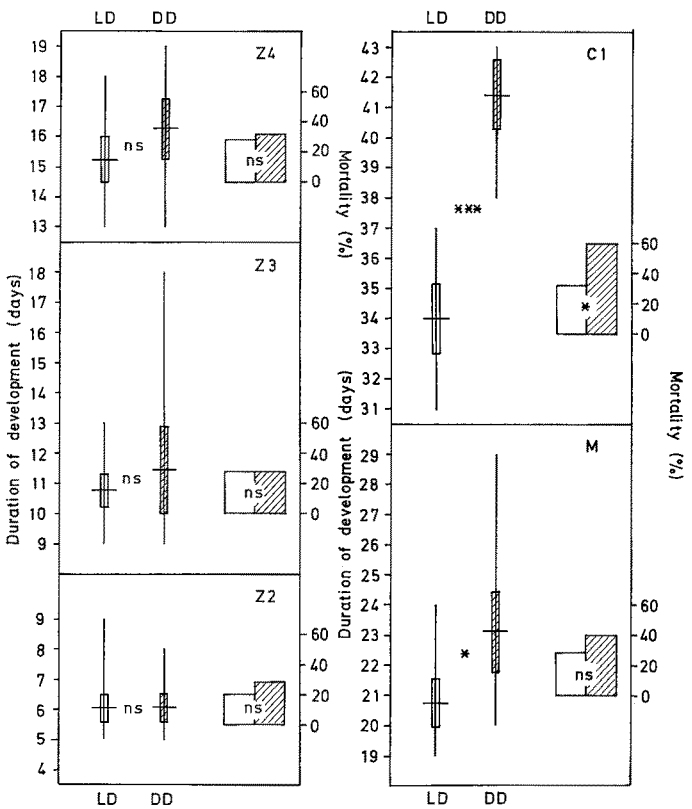

Fig. 6. *Carcinus maenas:* Rearing in continuous darkness (DD) and under cyclic light conditions (LD 16 : 8). Cumulative stage mortalities (columns). Mean values of cumulative developmental time (horizontal lines) ± 95 % confidence intervals (bars) and ranges (vertical lines). Development up to respective stages, zoea-2 (Z2) to zoea-4 (Z4), megalopa (M), and first crab stage (C1). Statistical differences are ns: not significant; *: $P \leq 0.05$; ***: $P < 0.0001$

information, which can only be gained by maintaining larvae in isolation. Z2 in Figure 6 denotes development up to zoea-2, i.e. data are identical with those given in Figure 5 under Z1, whereas Z4, for example, stands for larval development including zoea-1 to zoea-3 (Fig. 6).

Thus we see that the difference in megalopa mortality was strong enough to reveal a significant difference in total larval mortality. 68 % of the initial numbers metamorphosed under cyclic light conditions, while only 40 % completed larval life under constant darkness ($P < 0.05$). Nevertheless this difference is rather weak, compared to the time taken for larval development.

In darkness, the megalopae of *C. maenas* metamorphosed after a mean time of 41.4 ± 1.1 days; whereas their siblings, which were exposed to light for 8 h per day, molted to the first crab stage about 7.4 days earlier, after 34.0 ± 1.1 days of larval life ($P < 10^{-8}$). This trend is obvious throughout larval development.

In the larval development of *P. bernhardus*, the above observed trend is reversed. Figure 7 gives the duration and mortality of all larval stages of *P. bernhardus*, maintained in two sets, in darkness and under cyclic light conditions.

Mortality up to zoea-4 was rather low compared to earlier findings using glass vials as rearing containers, and did not reveal any significant difference between methods. Beyond this, there was a 100 % mortality, with megalopa dying immediately after molt, so that only dead megalopa were found in the checks of both sets (Fig. 7).

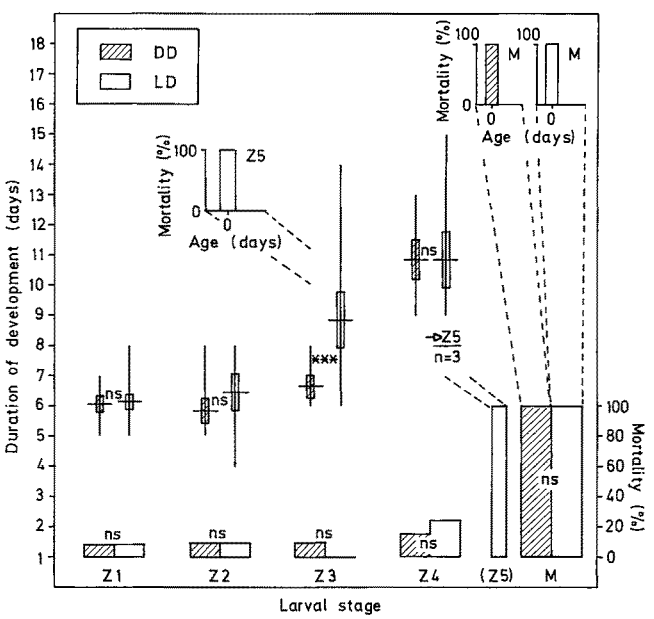

Fig. 7. *Pagurus bernhardus:* Rearing in continuous darkness (DD) and under cyclic light conditions (LD 16 : 8). Stage mortalities (columns). Mean values of stage duration (horizontal lines) ± 95 % confidence intervals (bars) and ranges (vertical lines). Zoea-1 (Z1) to zoea-4 (Z4), extra stage zoea-5 (Z5), megalopa (M). Zoea-4 developed to megalopa and extra stage zoea-5 (→ Z5). Mortality of zoea-5 and megalopa is related to their age (follow dotted lines). Statistical differences are ns: not significant; ***: $P < 0.0001$

Zoea-1 stage duration in darkness (DD) and in light-dark cycles (LD) were very similar: 6.0 ± 0.3 days (DD) and 6.1 ± 0.3 days (LD). The zoea-2 had a slightly, but not significantly, shorter development in the former. This trend is increased in the zoea-3, where larvae reared in continuous darkness needed about 2.2 days less than their siblings kept in a simulated day-night regime. This trend did not continue in the zoea-4, where stage duration was similar in both sets. These values only take into account zoea-4 larvae, which developed to megalopae. Besides these, three extra stage "zoea-5" occurred in the latter set, each after 8 days, all of which died immediately after molt.

Cumulative duration of development, first including zoea-3, was 2.9 days shorter in larvae, which were maintained without light, compared to larvae kept in light cycles (Fig. 8). Total zoeal development was about 1.8 days faster in the first set. Since zoea survival rates corresponded very well (DD: 64 %, LD: 52 %), a sublethal effect of light, at least regarding zoea-2 and zoea-3, can be assumed.

Petri dish and glass vial

One possible explanation of the rather unsatisfactory results in maintaining *Pagurus bernhardus* zoea in glass tubules could be that they require more space. This might be

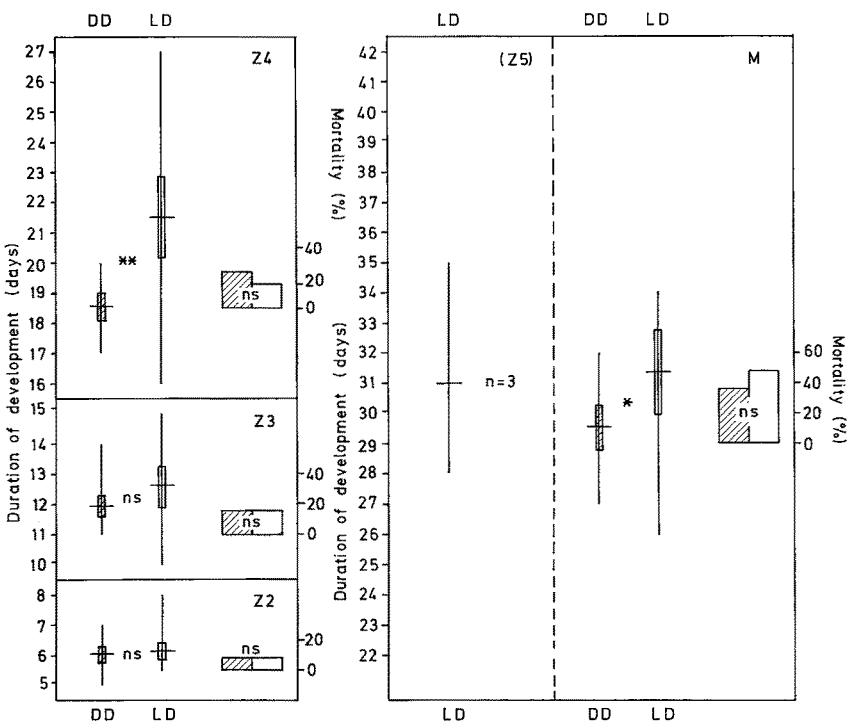

Fig. 8. *Pagurus bernhardus:* Rearing in continuous darkness (DD) and under cyclic light conditions (LD 16 : 8). Cumulative stage mortalities (columns). Mean values of cumulative developmental time (horizontal lines) ± 95 % confidence intervals (bars) and ranges (vertical lines). Development up to respective stages, zoea-2 (Z2) to zoea-4 (Z4), extra stage zoea-5 (Z5) and megalopa (M). Statistical differences are ns: not significant: *: $P \leq 0.05$; **: $P < 0.001$

assumed from their swimming behavior. In order to have a closer look at this problem, larvae isolated in glass tubules (gt) were compared to those reared individually in petri dishes (pd) (Fig. 9). In order to determine even very small differences between methods, the former were maintained under constant darkness, due to the better results compared to those of the light cycles (see above).

Mortality was very low throughout zoeal development in both enclosures without any significant difference. Only the megalopa revealed a notable mortality, which was two times higher in those that molted in the glass vials. 80 % of the inital numbers survived to the megalopa in both sets (Fig. 10). 32 % of the larvae reached metamorphosis in the vials, and in the petri dishes 56 % survived to crabs. Megalopae mainly died immediately after molt; only one died after 8 days, obviously with difficulties in metamorphosis (Fig. 9).

The zoea-1 of *P. bernhardus* did not show any difference in stage duration: 5.6 ± 0.2 days (gt) and 5.6 ± 0.3 days (pd) (Fig. 9). In further zoeal development there was an increasing trend to a shorter stage duration in petri dishes. The megalopa then showed the same but weaker trend, though megalopae which molted in the petri dishes were all transfered into the vials. Megalopae which had developed in the petri dishes before needed about 0.6 days less to metamorphose, compared to those kept in the vials throughout larval life ($P < 0.03$).

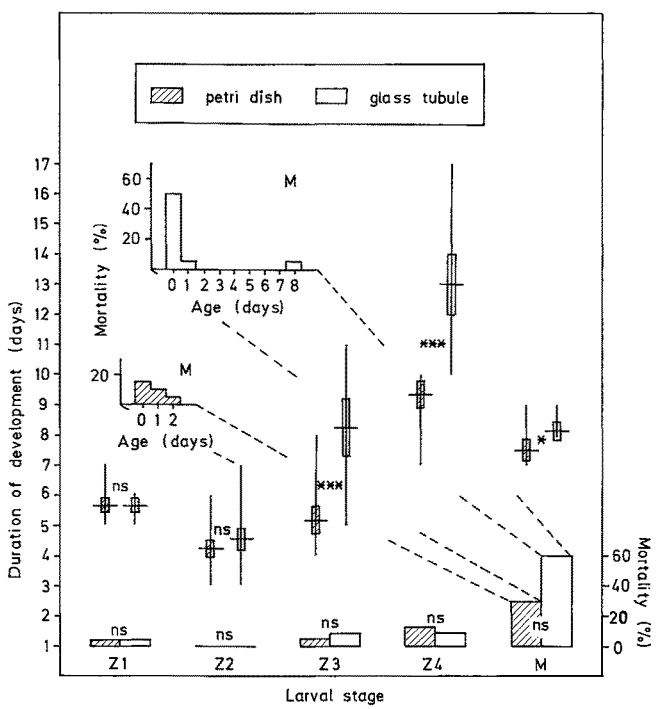

Fig. 9. *Pagurus bernhardus:* Rearing in petri dishes and glass tubules. Stage mortalities (columns). Mean values of stage duration (horizontal lines) ± 95 % confidence intervals (bars) and ranges (vertical lines). Zoea-1 (Z1) to zoea-4 (Z4), megalopa (M). Mortality of megalopa is related to their age (follow dotted lines). Statistical differences are ns: not significant; *: $P \leq 0.05$; ***: $P < 0.0001$

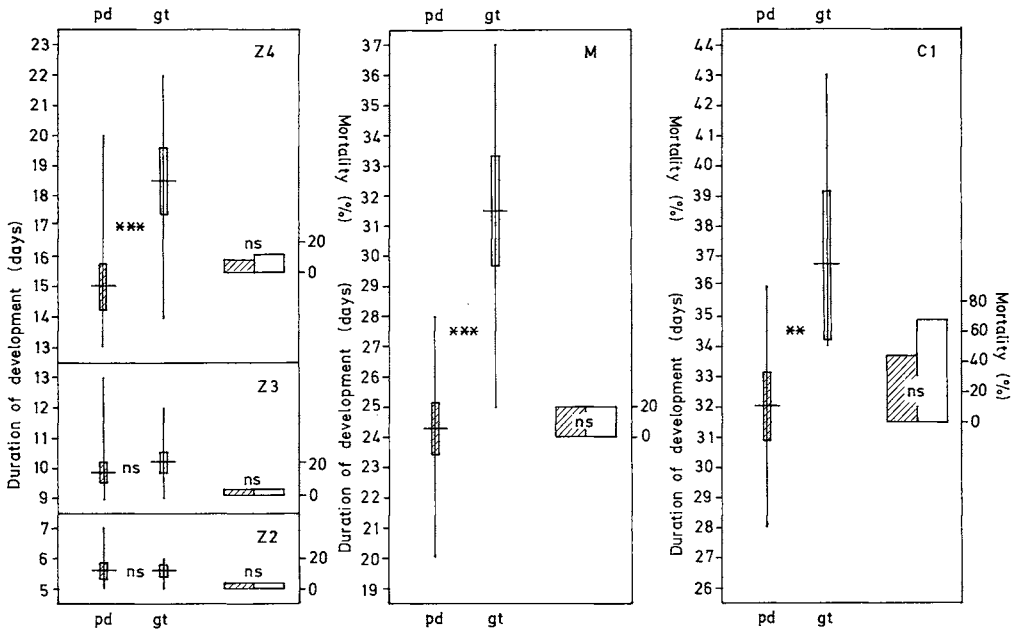

Fig. 10. *Pagurus bernhardus:* Rearing in petri dishes (pd) and glass tubules (gt). Cumulative stage mortalities (columns). Mean values of cumulative developmental time (horizontal lines) ± 95 % confidence intervals (bars) and ranges (vertical lines). Development up to respective stages, zoea-2 (Z2) to zoea-4 (Z4), megalopa (M), and first crab stage (C1). Statistical differences are ns: not significant; **: $P < 0.001$; ***: $P < 0.0001$

The essential differences in cumulative developmental times are given in Figure 10. Development up to zoea-4 was highly significantly shorter in the petri dishes than in the vials ($P < 10^{-5}$). Total zoeal life was 7.5 days shorter in the petri dishes compared to duration times in the glass tubules ($P < 10^{-8}$). Thus metamorphosis was achieved about 4.7 days earlier ($P < 10^{-3}$) by the former method than by using the described vials.

Pagurus bernhardus – megalopa

Megalopae of *Pagurus bernhardus* were reared with good results by keeping them in small vials, without food and without offering a snail shell (Dawirs, 1980, 1981). 137 megalopae were reared to metamorphosis by this method. Stage duration was found to be 7.3 ± 1.0 days, with a range of 6 to 9 days. This stage is likely to have a rather fixed developmental program and hardly shows greater variances. Nevertheless, even here, a delay in development might be a response pattern, although less distinct than in prior stages (see above). Since the megalopa of *P. bernhardus* does not need a food supply at all, it was assumed that the substratum might have some adverse effect on development of this stage.

Thus megalopae of the same hatch as the above 137 larvae were maintained on three types of substratum: fine sand (177–250 µm), medium sand (354–500 µm), and a 300 µm gauze (bottom net of the plexiglass tubules in the tubs). Stage duration on fine

sand was 7.8 ± 0.2 and on medium sand 8.0 ± 0.2 days. In both cases the range was narrow, 7–9 days. Only one individual out of 50 died. As no significant difference could be calculated, data of both sets were pooled. Thus megalopa stage duration was found to be 7.9 ± 0.1 days on sediments, i.e. 0.6 days longer than in glass vials without sediment. This difference is statistically highly significant ($P < 10^{-9}$).

In the tubs, where the megalopae had to live on the screen, stage duration was found to be 7.5 ± 0.2 days, i.e. about 0.4 days shorter than on the sediments. This difference is also statistically significant ($P < 10^{-3}$). A very narrow range of 7 to 9 days was found here too. Development in the net bottom tubules took therefore about 0.2 days longer than in the glass vials. The significance of this difference is $P = 0.05$.

Mortality did not play a major role in *P. bernhardus* megalopa. 16 out of 155 individuals died in the glass vials, one out of 50 died on the sediments, and no megalopa out of 50 died in the net bottom tubules. Though revealing only very small delays, 5 h, 10 h, and 14 h respectively, this points to possible detrimental effects of different substrata.

DISCUSSION

Single and group rearing

Since Costlow & Bookhout (1959) established reliable rearing methods for decapod crustacean larvae, a great number of papers an larval development of decapod larvae has been published. In most of these studies larvae were kept in groups, either in mass rearing systems of up to several hundred larvae per beaker, or in smaller batches, mostly about 10 per beaker or dish. Several authors kept decapod larvae in isolation (see below). The overwhelming majority of workers who maintained larvae in isolation did so in order to describe the morphology of successive larval stages. This would not be necessary for most brachyurans and anomurans, because of their largely known and rather stable developmental pattern (Knowlton, 1974). Only individual maintenance allows us to follow the morphogenesis of every single larva. Shrimps and prawns reveal a high variability in larval stage number, depending on various internal and external conditions (e.g. Broad, 1957a, b; Boyd & Johnson, 1963; Costlow, 1965; Reeve, 1969a; Knowlton, 1974; Fincham, 1979). Therefore, a meaningful description of larval stages and experimental examination of ecological parameters depend to a great extent on isolation of single larvae, in order to record individual molt history (Reeve, 1969a; Knowlton, 1974; Sandifer, 1974; Provenzano, 1978).

Only by isolated maintenance can the duration of successive larval stages be recorded and statistically verified. Some of the authors using isolated maintenance give information about intermolt duration of different species: Gore (1973, 1977) reported only ranges of larval stage duration times. Provenzano & Rice (1964), Knight (1970), Lang & Young (1977), and Provenzano (1978) gave mean values and ranges. Reeve (1969) and Knowlton (1974) only gave mean values of intermolt durations. Hubschman & Broad (1974) calculated single stage duration as mean values ± standard deviation, whereas Balestra et al. (1977–1978) published larval stage duration as mean values ± 95 % confidence intervals.

Beyond these more descriptive reports, there was a slight tendency to measure and

compare intermolt duration in relation to experimental conditions such as temperature, salinity or feeding conditions, by using isolated larvae (Templeman, 1936; Rice & Provenzano, 1965; Robertson, 1968; Gore, 1968; Sandifer, 1973; Young & Hazlett, 1978). They partly gave stage mortality and durations, but unfortunately without statistical evaluation. The only standard deviations Sandifer (1973) gave in this repect were related to overall mean instar durations. Only Boyd & Johnson (1963) compared stage durations ± 95 % confidence intervals of larvae reared in isolation at various temperatures.

Thus very few authors used the method of single maintenance to improve data from laboratory rearing (Boyd & Johnson, 1963; Hubschman & Broad, 1974; Balestra et. al., 1977–1978). Young (1979) kept larvae of *Clibanarius vittatus* in isolation to ensure that larvae of successive stages, which he needed for experiments on osmoregulation, were exactly of the same age. Anger & Nair (1979) kept larvae of *Hyas araneus* isolated in order to get reliable data for biochemical analyses.

Only little attention was focused upon a comparison of both methods, group and single maintenance. Poole (1966) tried to keep larvae of *Cancer magister* isolated in plastic containers (175 ml), after they had shown high mortality when reared in groups of 5 to 6. Nevertheless mortality could only be reduced by using antibiotics. Knight (1970) found molting frequency unaffected by larval density and volumes of sea water used for rearing. Dalley (1980b) reported a slight delay in larval life of *Crangon crangon* reared in groups, compared to larvae kept isolated. Anderson & Ford (1976) found high survival rates of *Cancer anthonyi* larvae in individual containers when compared to the survival rates of larvae maintained in groups, though he could not find any larval interaction in the latter. Reeve (1969a, b) among others, directly compared individually and communally maintained *Palaemon serratus* larvae. He found higher survival rates in single rearing, stressing the exclusion of cannibalism. Isolated larvae even showed better growth and shorter developmental times. Boyd & Johnson (1963) reported a clear delay in development of isolated larvae of *Pleuroncodes planipes*. They assumed a possible positive group effect and pointed out that in spite of identical relations of larvae and water volume in either method, total water volume was much smaller in individual containers (GR: 10 larvae per 500 ml; SR: 1 larva per 50 ml). Sandoz & Rogers (1944) checked effects of larval concentration on molting. They found that isolated blue crab zoeae failed to molt and discussed a probable positive group effect by the metabolites of the siblings. Sastry (1970) compared the overall efficiency of single and mass rearing methods, with regard to numbers of metamorphoses achieved in *Cancer irroratus* and *Panopeus herbstii*. A recirculating sea water system was used for mass rearing, while larvae were isolated in the famous "tackle boxes", which were first introduced by Costlow & Bookhout (1959).

Cannibalism can be an important factor in laboratory rearing of decapod larvae. Its role may be of varying significance, and may be accepted in those species where no statistical evalution of intermolt duration is sought. At any rate, as soon as megalopa was reached, larvae were isolated in most studies to prevent cannibalism (e.g. Costlow et al., 1960; Bookhout, 1964; Costlow & Bookhout, 1967; Williams, 1968; Bookhout & Costlow, 1974; Christiansen & Yang, 1976; Christiansen et al., 1977).

In the present study the advantage of rearing *Carcinus maenas* larvae in isolation was shown by higher survival, faster developmental rate, stimulated growth and less working time connected with the experiments.

Mortality in the beakers mainly occurred during times of molting to the zoea-2. Rearing conditions in the beakers also revealed a clear sublethal effect. This is only true in the first two stages (Table 1), while zoea-4 and megalopa stage duration is about 2 days shorter in the beakers. Thus overall developmental time is similar in both methods. Due to high early mortality in the groups, larval density was rather low during zoea-4 and megalopa development, i.e. detrimental interaction was lowered. Few larvae shared a relatively large volume of 500 ml. At the same time when space was restricted to less than 20 ml in the vials, this seemed to be detrimental because of an increase in larval mobility. Nevertheless mortality seemed to be unaffected, but development of these larvae was delayed compared to that of larvae in the beakers (Table 1). Thus the method employing glass vials was most efficient and time saving when raising *C. maenas* larvae, and can be used for ecological examinations at least of the first three stages. Due to probably detrimental effects in the zoea-4 and the megalopa, slightly larger vials should be used when these stages are maintained for experimental purposes.

Larval growth in terms of dry weight and elemental composition (CHN) also proved to be a very sensitive quality criterion for the comparison of different rearing conditions. In the present case, individual maintenance led to an increase of more than 20 % in dry weight, total carbon and nitrogen when compared to group rearing, only after 3 days of zoea-1 development. This indicates a very significant negative group effect. Similar findings were made for the zoea-1 of *Hyas araneus* (Dawirs et al., unpublished).

With regards to *Pagurus bernhardus* zoea-1, the described glass vials showed good results. In the following stages detrimental effects were always greater than in the groups kept in beakers. All zoea-4 died in the vials. Although metamorphosis could be reached in later tests in these glass tubules (see below), it became very obvious that conditions in these vials were far from being optimal for zoea-2 and higher stages. Intermolt durations were longer than in groups kept in beakers. On the other hand, group rearing delivered reproducible results. Unfortunately, survival was relatively low in the beakers (20 %) due to cannibalism. It is most likely space limitation in the tubules that disturbs *P. bernhardus* larvae, which are very active swimmers. Through this high mobility, larvae came into contact with the wall of the vials at least several times per minute. Thus, results in single rearing could be very much improved by offering more space, e.g. using petri dishes as rearing containers (see below).

Comparing the two tests with *P. bernhardus* und *C. maenas*, it becomes evident that optima of different factors controlling laboratory maintenance have to be examined for each species separately. An ideal standard rearing method will hardly be found. So far the group rearing method must be recommended for *P. bernhardus* larvae but not for *C. maenas*. If we do not want to dispense with information like stage duration and individual molt history, more time consuming methods demanding larger volumes per isolated larvae have to be used (see below). If we just want to produce sufficient numbers of newly molted larvae of successive stages, group rearing could be employed for *P. bernhardus*, whereas *C. maenas* would be most efficiently mass reared in isolation. Checks for molts are most easily done by looking for the exuviae (see above). Oxygen is not a limiting factor in the vials. Water surface is relatively large in relation to total volume and swimming activity of single larvae provides enough turbidity for gas exchange. Food organisms like *Brachionus plicatilis* and *Artemia* spp. were always found equally distributed in the glass tubules. Once an experimental or mass rearing set

up is started, further time expenditure is minimized. Even the water exchange procedure costs only seconds.

When handling larvae in groups, checks for molts are much more complicated and time consuming, because each larva has to be examined under a dissecting microscope. In most cases at least a gentle stream of air bubbles is necessary to prevent food organisms from settling or concentrating according to light conditions. The only exact statements as to the age of communally reared larvae can be made in the first stage. As a rule, molts do not occur on one day. Therefore the whole batch has to be checked, individual by individual, to separate the molted ones.

In my judgement there is no real alternative to individual rearing of decapod crustacean larvae, in order to record and evaluate reliable data of response patterns to various parameters in subsequent stages. By no other means can individual molt history be recorded. Thus even if the working effort is very much higher than when maintaining larvae in groups, it is worthwhile establishing methods for the isolated rearing of various species.

A positive group effect of larval stages was never found in my studies and would be quite surprising, considering the low natural larval density of *C. maenas* and *P. bernhardus* in the German Bight (cf. e.g. Rees, 1955).

Enclosures for isolated rearing

Most of the isolated rearing of decapod larvae from various families was done in compartmented plastic boxes. These "tackle boxes" were first introduced by Costlow & Bookhout (1959). Single compartments are cubic and were employed in different sizes. Different authors used volumes between 15 and 175 ml per larva.

Containers differing from this were used, for example, by Templeman (1936): "glass beakers", 250 ml; Costlow (1965): syracuse watch glasses; Nyblade (1970): "small beakers"; Sandifer (1971): plastic vials, 15 ml; Hubschman & Broad (1974): stacking caroliner culture dishes; Lang & Young (1977): 6 cm dishes, 15 ml; Anger & Nair (1979): glass vials, 30–50 ml.

The present study employs the above described glass vials with a capacity of about 20 ml as a standard tool for the rearing of *Carcinus maenas* larvae in isolation. These vials only lead to poor results when applied to *Pagurus bernhardus* larvae (see above). Regarding the latter, it was noted that almost all food organisms (brine shrimp nauplii) were lying on the bottom of the vials within one day or less, heavily damaged or dead. *P. bernhardus* larvae older than zoea-1 obviously injured and killed most of the nauplii by their swimming activities. In contrast, *C. maenas* larvae only killed nauplii they actually fed on and considerable amounts of dead bodies were rarely found on the bottom of the vials. In order to avoid detrimental effects of decomposing food organisms on larvae of *P. bernhardus* the above described net bottom tubules were employed (Fig. 3).

Though the plexiglass tubules only had a capacity of about 10 ml, this relatively small water column communicated with a large reservoir of about 19 liters. Thus water quality was no longer expected to influence larval development. Nevertheless, this method turned out to be even worse than the latter (Fig. 3), showing higher mortalities and delay in intermolt durations. In larger water bodies, zoeae of *P. bernhardus* could be

observed swimming on helical lines with a radius larger than the diameter of the vials and tubules. *P. bernhardus* larvae could be observed swimming telson first with velocities of 1 to 2 cm s^{-1} (cf. Foxon, 1934; Warner, 1977). The high frequency of collisions with the wall and especially the bottom net is assumed to be mostly responsible for the bad results. Larvae were frequently found with their rostrum broken.

In both systems, an extra stage, "Zoea-5", occurred (Fig. 3). This extra stage was never observed when *P. bernhardus* larvae were reared in groups and in larger volumes. It can be assumed that extreme stress conditions lead to that supernumerary stage (for discussion cf.: Costlow, 1965; Knowlton, 1974). This zoea-5 revealed a setation of its pleopods, while the zoea-4 stage has no setae attached to the pleopods.

The results for *P. bernhardus* larvae indicated that water quality in rearing containers could not be the major problem. As continuous darkness improved rearing conditions (for discussion see below), development of larvae kept in the vials under total darkness was compared to larvae kept in petri dishes under cyclic light conditions (Figs 9, 10). This was done to see whether best calibrated rearing conditions in the vials could be improved by employing the petri dishes. Though mortality was low in both sets, the detrimental effect of the glass tubules on larval development was crucial. In both sets, 80 % of initial zoea-1 succeeded in molting to the megalopa but were less viable in the vials, where about 60 % of the megalopae died immediately after molting. One sees, it was possible to have *P. bernhardus* metamorphosed in the glass tubules, but results showed very low reproducibility (cf. Figs 2, 3, 7). Newly molted zoea-2 must be immediately transferred to the dishes, if the vials were employed in earlier development, as only the larger space in the dishes allowed further successful development. Mainly the zoea-3 and zoea-4 were strongly hampered by restricted space, leading to a 7.5 days longer total zoeal life in the tubules. The advantage of a larger volume in isolated rearing of *P. bernhardus* zoea is most likely due to the larger diameter of the dishes, which suits the space consuming swimming behavior of *P. bernhardus* zoeae. Total larval development as well as single intermolt duration time in the dishes correspond very well with conditions in the group rearing beakers (Fig. 2). Whichever method was used, whether group maintenance of *P. bernhardus* larvae in beakers, or isolation in petri dishes sublethal effects were minimized to a comparable level. But due to cannibalism mortality was higher in the former. Mortality could be reduced to about 40 % in the dishes. This rate tallies with the rate of mortality found in isolated *C. maenas* individuals (ca. 20 to 40 %).

As a sufficient diameter of the container seems to be essential in the isolated rearing of *P. bernhardus* zoea, more convenient dishes with regards to handling procedure are desirable. "Carolina finger bowls" or "stacking carolina culture dishes" or similar containers might also be useful.

C. maenas larvae could be reared in small water bodies (20 ml) with very satisfactory results. The net bottom tubules were employed in order to further reduce working effort. As described above, it was no longer necessary to control the water exchange in single tubules, but the entire water body of the tub was changed. Larvae of *C. maenas* were often entangled in the bottom net of the tubules by the dorsal spine and were not able to free themselves. Under these circumstances molting became detrimentally affected (Fig. 4). Bearing in mind that in the natural environment zoeal molts happen in the water column, this is not really surprising. In the glass vials molting took place when

larvae were attached to the surface film or were lying on the bottom. In both cases, almost no frictional resistance hindered molting procedure.

Similar net-bottom systems have been employed in very few other decapod larvae rearing experiments. Reed (1969) and Modin & Cox (1967) both used compartmented styrine containers with synthetic screening bottom (202 µm). Modin & Cox (1967) succesfully raised larvae of *Pandalus jordani* individually in order to describe larval stages. Nevertheless, survival rate was rather low, 0.2 % metamorphosed to the last instar. The same method failed in rearing larvae of *Cancer magister* (Reed, 1969). It is reported in that study that *C. magister* zoeae were caught in the bottom screen, making other methods necessary. Anderson (1978) and Anderson & Ford (1976) used the same method for rearing larvae of *Cancer anthonyi*. They employed plexiglass tubing with bottom nets. In Anderson (1978), this method was obviously sufficient to have enough material for the description of all larval stages. As in Reed (1969) and in the present paper, Anderson & Ford (1976) reported that zoea larvae of *Cancer anthonyi* were often entrapped by the bottom screen, but were then obviously able to free themselves by rapidly flexing their abdomens. Under these conditions, individually kept larvae revealed survival rates up to metamorphosis of about 36 % (22 °C) and 21 % (18 °C).

Although the net bottom tubules were no great help in laboratory maintenance of *C. maenas* and *P. bernhardus* larvae, they may be succesfully employed in other species, and actually were used in rearing the juveniles with good results.

Illumination

Some authors examined the influence of light conditions on the larval development of decapod crustaceans. Templeman (1936) found that larvae of *Homarus americanus* maintained in almost total darkness had a shorter development, higher survival rates and "significantly larger zoea-4", when compared to larvae "reared in the light of a northwest window". Costlow & Bookhout (1962) tested various photoperiods in the larval development of *Sesarma reticulatum*. No influences on mortality, duration times, and number of stages were evident. Robertson (1968) did not find any difference in mortality of *Scyllarus americanus* larvae, kept in light-dark regimes and continuous darkness. In contrast, *Palaemon serratus* revealed higher mortalities in constant darkness (Reeve, 1969b). A comparison of two light regimes in *Palaemonetes vulgaris* (LD 18 : 6, LD 9 : 15) gave evidence of the tendency to develop through an extra stage under short day simulation, leaving survival and growth unaffected (Knowlton, 1974). Fitch & Lindgren (1979) could not prove any influence of various LD regimes on mortality and development times of the larval life of *Pagurus hirsutiusculus*. Dalley (1980a) reported significantly less growth and higher mortality of *Palaemon elegans* larvae in non-circadian (LD 8 : 8) as compared to circadian light rhythms (LD 12 : 12). The same comparison for *Crangon crangon* revealed no differences in growth and developmental times, but mortality was higher in LD 8 : 8 (Dalley, 1980b). Thus no unique response to various light regimes can be given for larval decapod crustacean development.

In the present study, opposing trends were found in the larval life of *Carcinus maenas* and *Pagurus bernhardus*. The question was asked whether constant darkness could give better rearing conditions than a simulated day-night regime of LD 16 : 8.

Using the above described techniques in the isolated maintenance of *C. maenas*

larvae, the zoea-1 was unaffected by constant total darkness. In the subsequent four stages some light period became necessary to avoid sublethal stress (Figs 5, 6). It is well known that several external rhythmic conditions can serve as a "Zeitgeber", regulating internal physiological processes (e.g. Sulkin et al., 1979). Periodical light exposure does not seem to be essentially necessary for larval development of *C. maenas*. But the experiments showed that larvae were nevertheless heavily stressed by lack of any light regime, leading to the observed 7.4 days delay in larval life (Fig. 6).

The glass vials used were assumed to be space limiting for larvae of *P. bernhardus*. As light is one possible stimulus for swimming, exclusion of this stimulus was expected to lower space requirements of the larvae. While the *C. maenas* zoea-1 was unaffected by either treatment (Fig. 7), it was obvious for zoea-2 and highly significant for zoea-3 that darkness indeed improved rearing conditions of *P. bernhardus* in the vials. It can be assumed that lack of light lowers swimming activities, hence zoea-2 and zoea-3 collide less often with the walls of the tubules, and so this main detrimental influence of limited space was reduced. Remembering that no differences in zoea-1 mortality and stage duration was found in the vials either when compared to the experiments in optimal dishes (Fig. 9), the glass vials seem to offer enough space for the first stage, and light conditions can be assumed to be without any direct effect. As larvae grow, reduced mobility in darkness resulted in better rearing conditions. This trend was terminated when the zoea-4 was developed. The volume obviously became too small and conditions could not be improved even by reduced swimming activity. After rather low mortalities in zoeal life, no megalopa was found alive in both sets; all died during or immediately after molting of the zoea-4.

Results of the present experiments led to the employment of larger volumes (Figs 9, 10) and finally underlined the need of sufficient space for the very active swimming of *P. bernhardus* zoeae.

Pagurus bernhardus-megalopa

The different bottom structures were examined in order to find optimal rearing conditions for *Pagurus bernhardus* megalopa. It could be found that metamorphosis does not depend on inhabiting a gastropod shell (cf. Dawirs, 1981). Bottom structures did not affect metamorphosis either, since no influence on mortality was seen. Nevertheless, there were some differences regarding developmental times. All tested types of bottom structures, except bare glass bottoms, significantly delayed metamorphosis. This prolongation of stage duration was caused more by natural sediments than by the artificial plastic screen. Though the observed delays were only in the magnitude of a couple of hours they were nevertheless significant. Thus the bare glass bottom showed best results with the shortest developmental time. So optimal maintenance of *P. bernhardus* megalopa turned out to be rather simple, remembering that they are independent of an energy supply by food uptake (Dawirs, 1981). Even as part of a mass rearing system the megalopae can easily be kept in hundreds of isolated vials, and water need not necessarily be changed at all.

With regard to delay of metamorphosis on any structural surface, it can be argued that metamorphosis normally happens inside a snail shell in nature, with the nacreous inside surface of the shell corresponding very well to the glass surface in the way of low friction. Though megalopae are part of benthic communities during and, for a certain

period of time, before metamorphosis, *P. bernhardus* megalopae survive best without sediments and when maintained without a gastropod shell in the laboratory.

CONCLUDING REMARKS

When larvae of decapod crustaceans are used in experimental sets to test the importance of ecological factors or pollution impacts, and to record physiological response patterns of successive larval stages, it is most useful to maintain single larvae in isolation. This is the only way to record individual life and molt history as well as to obtain a reliable statistical evaluation of the results. For various problems it is essential to know the exact age of single larvae of respective stages. This is impossible to determine if larvae are kept in groups, unless individuals can be labelled. In any case, the problem of possible intraspecific interaction would still be unsolved if larvae were kept in groups.

There are other purposes which do not require isolated rearing, such as mass production of higher stages or maintenance of a laboratory stock for all kinds of investigations, including biochemical analyses and behavioral observations, etc.

The present study shows that the components of a special rearing method have to be assessed for various crustacean species separately. In conclusion, highest survival rates and shortest stage duration indicate the best rearing method.

In this respect the use of antibiotics should be mentioned. Antibiotics have been used to promote survival in rearing decapod crustaceans (e.g. Boyd & Johnson, 1963; Rice et al., 1970; Christiansen, 1971; Brick, 1974; Rice & Ingle, 1975; Raymond Ally, 1975; Anderson & Ford, 1976; Fisher & Nelson, 1977, 1978; Motoh et al., 1977; Fincham, 1979). D'Agostino (1975) reviewed the application of antibiotics in cultures of invertebrates. Sometimes the toxic effect of different antibiotics on the larvae was greater than on the microorganisms. Negative effects were described, for example, by Brick (1974), D'Agostino (1975), Fisher & Nelson (1977), and Fincham (1979). Thus antibiotics may be used to keep mortality low in order to obtain specimes of higher larval stages for descriptive purposes. In all other cases no antibiotics should be used, unless it can be proved that they do not show any sublethal effect.

In decapod crustaceans (especially in brachyurans and anomurans), larval stage duration is a sensitive indicator of sublethal effects. Therefore, experimental rearing must be highly reproducible and show low variability. Both could be realized by the above tested methods.

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