INCUBATION IN BRITISH DECAPOD CRUSTACEA, AND THE EFFECTS OF TEMPERATURE ON THE RATE AND SUCCESS OF EMBRYONIC DEVELOPMENT

By ROBERT G. WEAR

Zoology Department, Victoria University of Wellington, Wellington, New Zealand*

(Text-figs. 1–7)

Twenty-one species of female decapod Crustacea with gravid ovaries were collected from the field and spawned naturally in the laboratory. Newly spawned eggs and incubating females were kept at temperatures between 3 °C and 24 °C constant at 3 °C increments until the eggs hatched after completing their embryonic development.

Observations on developing eggs confirmed that in decapods which hatch at a zoea stage, the pre-zoeal cuticle is associated with the metanauplius stage relegated to embryonic life, rather than to the preceding nauplius. During embryonic development, the rate of egg volume increase (elsewhere attributed to osmotic uptake of water) is considered slower in the eggs of species with a long development period than in those which develop rapidly. In all species here studied the rate of increase of egg volume accelerated during development, especially in the latter stages. The rate of yolk metabolism varies from species to species according to the time taken for egg development, but in all species it was slower during early development than during the last few days before hatching. In very early development (around gastrulation), the eggs of four unrelated species were found to possess a form of diapause which could not be shortened significantly by raising the water temperature. It is suggested that diapause has evolved in certain species as a response to the availability of food in the plankton and to enhance larval survival.

The development times from spawning to hatching of eggs (D), are described as functions of temperature (T) by Bělehrádek's temperature equation $D = a (T - \alpha)^b$, using the calculated value of $b = -2.3$ applying to 12 species with continuous development, and for which adequate experimental data were obtained. The small range in the computed values of α ($\alpha = 0.0$ to $\alpha = -4.7$) is consistent with all species having been collected from the same temperature regime, but a good fit could still have been obtained for all curves had the value of α been set equal to zero. From limited data, it is possible to suggest that increasing egg size slows down the rate of development and increases the value of a between closely related species only. Bělehrádek's function applied to species with an inherent diapause only after this stage had been passed.

Experiments with *Macropipus depurator* (L.) showed that a threefold decrease in egg development time can occur naturally in successive batches of eggs incubated during the early spring to mid-summer breeding season of a single species in the one locality. However, a rapid rise in water temperature of as little as 3 °C can disrupt the natural sequence in the spawning and incubation of successive egg batches, and also reduce fecundity by more than 90 %. Small eggs were able to survive in higher temperatures than larger, more yolky eggs, and at temperatures of 3 °C and below, the tolerance limits were probably determined by excessive slowing down of development rather than by any directly harmful effect. Larval viability is reduced when the entire period of egg development has taken place in temperatures outside the normal spring and summer range (8 °C to 16 °C), especially among species with large eggs incubated at higher than normal temperatures.

* Present address (to 1976) – Marine Science Laboratory, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

INTRODUCTION

The embryology of various species of decapod Crustacea has been well documented in early morphological works (Brooks & Herrick, 1891; Herrick, 1896), and more recently by Kajishima (1950), Ling (1967), Shiino (1950) and Wear (1967). A full account of decapod embryology known up to 1937 appears in Dr H. G. Bronns 'Klassen und Ordnungen des Tierreichs' (Korscheldt, 1944). Up to the present time, little attention has been given to the physiological requirements of the decapod embryo during its period of incubation which in most cool to temperate water species may occupy from 3 weeks to almost 9 months depending on the species. On the other hand, the often much shorter period of larval life has been the subject of a great number of experimental studies dealing with survival and growth rates under varying conditions of temperature and salinity (e.g. Coffin, 1958, on *Pagurus*; Costlow, 1965, and Sandoz & Rogers, 1944, on *Callinectes*; Costlow & Bookhout, 1962*a*, and Costlow, Bookhout & Monroe, 1960, on *Sesarma*; Costlow & Bookhout, 1962*b*, on *Hepatus*; Costlow & Fagetti, 1965, on *Cyclograpsus*; Provenzano, 1968, on *Panulirus*; Templeman, 1936, on *Homarus*).

Incubation in other crustacean groups such as the Cirripedia (Patel & Crisp, 1960*a*, *b*) and the Copepoda (Corkett & McLaren, 1970; McLaren, Corkett & Zillioux, 1969; Marshall & Orr, 1955) has been studied in relation to temperature. Excepting a paper by Reeve (1969) on the laboratory culture of *Palaemon*, similar work has not yet been extended to the decapods, probably because females ready to spawn are difficult to obtain alive in sufficient numbers, and because incubation frequently occupies several weeks during which the ovigerous females usually require substantial aquarium space and constant feeding.

The present study was stimulated by the opportunity to obtain adequate numbers of female decapods with gravid ovaries and representing a wide range of natant and reptant species. Laboratory facilities at Plymouth were excellent for this work which was carried out over a 4-month period (April to July, 1970) and later continued at Port Erin (Isle of Man) during August 1970.

MATERIALS AND METHODS

Female decapods with gravid ovaries were obtained alive by dredging, trawling and shore collecting from the Plymouth and Port Erin marine laboratories, and the eggs were spawned naturally in the laboratory sea-water circulation system. It was found that crabs with a flat abdomen (e.g. Portunidae, Cancridae and Goneplacidae) required a soft substrate to ensure successful attachment of eggs to the pleopod hairs during spawning. Ovigerous females were kept in temperatures between 3 and 24 °C constant at about 3 °C increments during the entire incubation period. The lower limit of this range was 4 °C below the minimum March (1970) sea surface temperatures and 8 °C above the maximum sea surface temperatures recorded in June and July by the City of Plymouth Health and Welfare Department Meteorological Station. Similar recordings for Port Erin were not available.

Animals were kept in tanks equipped with thermostat controlled heaters. Tanks at 12 °C and below were kept in cold-rooms. Temperature measurements were made not less than twice daily. Salinity remained between 34 and 35 ‰ and pH varied between pH 7·52 and pH 7·90 during the experimental period, with the mean pH at 7·70.

Egg volumes were calculated as $\frac{4}{3}\pi abc$ where a, b and c are the half diameters measured on three perpendicular axes. The volume of yolk remaining in the eggs at various stages in develop-

ment was obtained as the sum of the volumes of a number of imaginary, regularly shaped solid bodies which when fitted together would give good approximation to the irregular yolk mass. This was expressed as a percentage of the original egg volume. After this time-consuming procedure had been completed throughout the embryonic development of one specimen from each of the species studied, it was possible, by comparison, to estimate visually the percentage of yolk remaining. Spot calculations were occasionally made to ensure that these estimations were within an accuracy of $\pm 2\%$ of the calculated volume. A minimum of ten eggs were removed daily from each experimental animal to obtain mean volume measurements and for morphological observation. Because the time taken in incubation varied appreciably with the temperature, the morphological sequence in embryonic development was related to the volume of yolk remaining, rather than to time.

The overall viability of the egg mass was assessed using at least 100 eggs removed daily from those species carrying a large number of small eggs. For those species (mainly Galatheidae and most crabs of the family Majidae) which typically carried fewer and larger eggs less were taken. At room temperature and above, moribund eggs in an early stage of development were immediately obvious by their loss of yolk colour and the activity of adherent or associated microorganisms. Eggs in early development held at lower temperatures, in which the onset of visible deterioration was naturally delayed after death, were observed after about 12 hours in water at room temperature. The viability of eggs in more advanced stages of development was checked by observing the embryonic heartbeat.

The terms 'hatch viability' and 'moult viability' refer respectively to the percentage which hatches out of total remaining progeny, and to the percentage still living after shedding the pre-zoeal cuticle in the moult to the first stage zoea larva. In those species with many eggs, the percentages of surviving larvae were determined from a 50 ml. subsample of the total number of larvae distributed relatively evenly in 1000 ml. of seawater.

All terminology used in reference to embryonic development follows that employed in a previous paper by this author (Wear, 1967).

Decapod Crustacea studied in this work are as follows:

> Macrura Natantia
> > *Crangon crangon* (Linnaeus)
> > *Palaemon serratus* (Pennant)
>
> Macrura Reptantia
> Anomura
> > *Galathea dispersa* Bate
> > *Galathea squamifera* (Leach)
> > *Pisidia longicornis* (Linnaeus)
> > *Porcellana platycheles* (Pennant)
> > *Pagurus prideauxi* Leach
>
> Brachyura
> > *Corystes cassivelaunus* Pennant
> > *Carcinus maenas* Linnaeus
> > *Macropipus depurator* (Linnaeus)
> > *Macropipus holsatus* (Fabricius)
> > *Macropipus pusillus* (Leach)
> > *Cancer pagurus* Linnaeus
> > *Goneplax rhomboides* (Linnaeus)
> > *Eurynome aspersa* (Pennant)
> > *Hyas coarcticus* Leach
> > *Inachus dorsettensis* (Pennant)
> > *Macropodia longirostris* (Fabricius)
> > *Macropodia rostrata* (Linnaeus)
> > *Maia squinado* (Herbst)
> > *Ebalia tuberosa* (Pennant)

RESULTS

Morphological observations

The morphological sequence in decapod embryology is well known (Kajishima, 1950; Korscheldt, 1944; Shiino, 1950; Wear, 1967) and will not be described here in detail. The embryonic development of all species considered in this work is similar, with yolk cleavages followed by invagination, gastrulation, formation of a tissue cap, and subsequent stages recognized as nauplius and metanauplius both relegated to embryonic life as in all Decapoda hatching at a zoeal stage in development (Gurney, 1942).
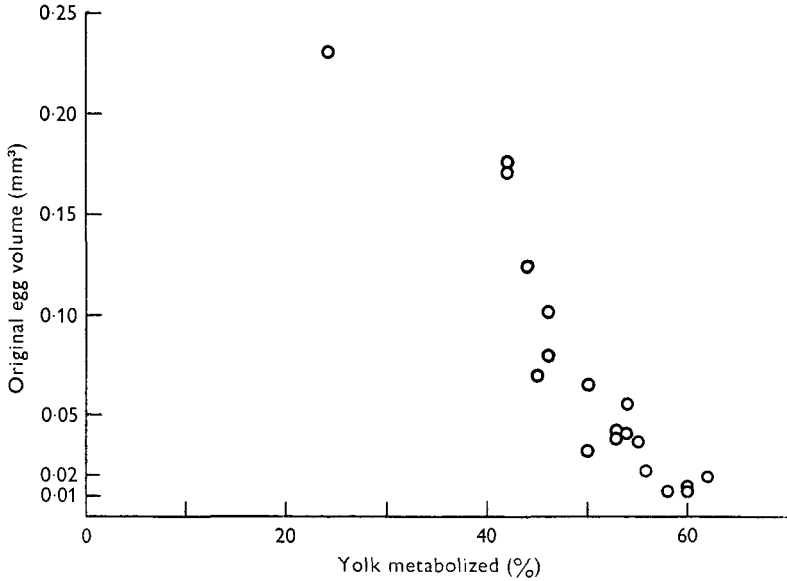

Fig. 1. Relationship between original egg volume and the percentage of yolk metabolized when heartbeat, chromatophores and visual pigment first appear in the development of some decapod Crustacea. Individual species concerned can be determined from egg volumes listed in Table 1.

In a previous paper (Wear, 1967) various theories regarding the ontogenetic position of the embryonic cuticle which surrounds the unhatched zoea larva and all its appendages were discussed. This thin membrane which is shed either during the process of hatching or very shortly afterwards, was named the 'pre-zoeal' cuticle by Lebour (1928), and this convenient term has been used almost universally by subsequent authors since it implies nothing more than its actual position in ontogeny. On limited evidence it was suggested that the pre-zoeal cuticle represents the metanauplius stage confined within the egg (Wear, 1967). In all species listed above, the appearance of this cuticle is in fact post-naupliar, as it first forms with the metanauplius with which it can now be more confidently associated.

The order of appearance of embryonic larval appendages and other morphological features did not vary from species to species, although there was some variation between species in the percentage of the original yolk volume metabolized relative to the degree of development of the embryo. Characters such as chromatophores, black eye pigment and

the first indications of heartbeat appear almost simultaneously in the development of all species. However, in *Maia squinado* which has very large eggs (0·23 mm³), these characters appeared with about 24 % of the original yolk volume metabolized compared with about 60 % in the very small eggs (0·013 mm³) of *Macropipus spp* (Fig. 1). An approximately linear relationship exists between these two extremes.
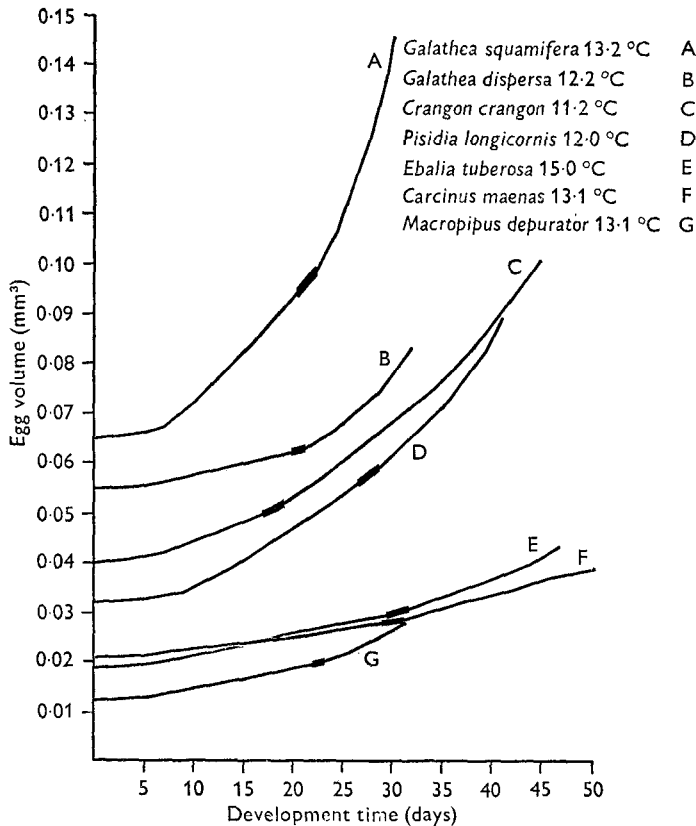

Fig. 2. Increase of egg volume in 7 species of decapod Crustacea. Heavy bars indicate the time over which heartbeat, chromatophores and visual pigment first appear in the embryo.

The eggs of aquatic invertebrates increase their volume significantly during embryonic development by a slow but steady osmotic uptake of water (Davis, 1968). An overall increase in decapod egg dimensions from the time they are first laid to the time of hatching has been recorded by many authors working on decapod larval development. The rate at which egg volume increased in the species studied here was also relatively steady (except between the time of spawning and the first appearance of a tissue cap when only a very slight increase was recorded) but there is some evidence of increase in the rate of water uptake from about the time the embryonic heartbeat is first observable, since the volume of the eggs increases more rapidly from this time (Fig. 2). From Fig. 1 it can be seen that in most species, this is the point at which about 50 % of the original yolk has been metabolized.
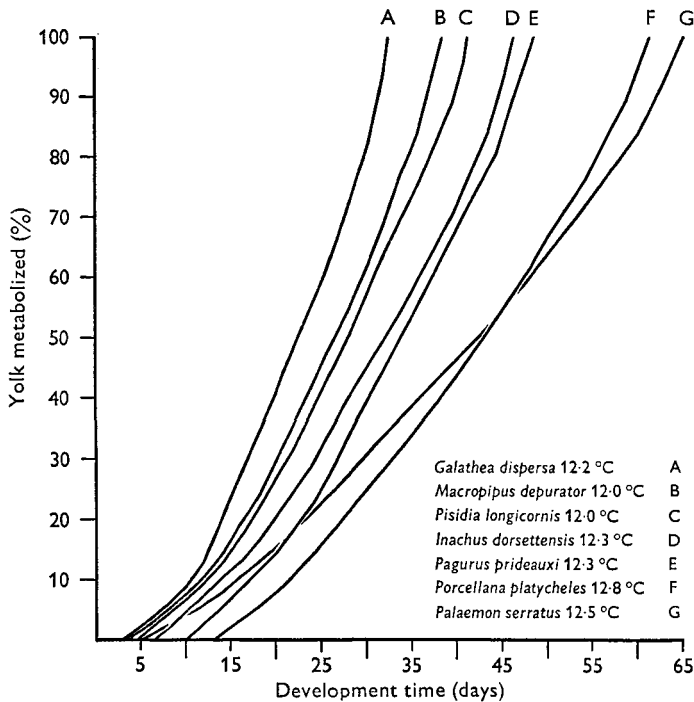
Fig. 3. Yolk metabolism during the embryonic development of 7 species
of decapod Crustacea.

TABLE 1. PERCENTAGE VOLUME INCREASE OF DECAPOD CRUSTACEAN EGGS
DURING DEVELOPMENT RELATED TO ORIGINAL EGG VOLUME AND DEVELOP-
MENT TIME AT 12·5 °C

Species	No. of specimens	Value of a (see Table 2)	Development time (days) at 12·5 °C	Original egg volume (mm³)	Egg volume at hatching (mm³)	% volume increase
Macropipus holsatus	4	21086	32·9	0·0125	0·029	135
M. pusillus	3	24144	39·4	0·0125	0·029	130
M. depurator	8	19783	33·4	0·013	0·029	120
Ebalia tuberosa	3	—	≃68	0·019	0·042	120
Carcinus maenas	5	29309	51·6	0·021	0·038	80
Pisidia longicornis	2	19689	35·5	0·032	0·088	175
Goneplax rhomboides	2	—	≃60	0·036	0·072	100
Eurynome aspersa	1	—	≃57	0·038	0·074	95
Crangon crangon	3	20437	34·0	0·040	0·098	145
Cancer pagurus	3	—	?80	0·042	0·063	50
Galathea dispersa	6	15803	29·3	0·055	0·094	70
G. squamifera	2	—	≃41	0·065	0·169	160
Macropodia rostrata	2	—	48·0	0·070	0·154	120
Porcellana platycheles	3	23744	60·8	0·080	0·200	150
Inachus dorsettensis	4	25386	43·1	0·110	0·204	85
Macropodia longirostris	2	—	≃48	0·123	0·289	135
Palaemon serratus	4	27129	66·4	0·170	0·383	125
Pagurus prideauxi	3	14297	43·1	0·175	0·429	145
Maia squinado	3	—	?100	0·230	0·380	65

At constant temperature, the rate of yolk metabolism during the greater part of embryonic development varies only slightly (Fig. 3). The overall rate differs from species to species according to the time taken for incubation of the eggs. However, in each species, yolk is metabolized more slowly during early embryonic development (egg nauplius stages) and a little more rapidly during the last few days before hatching. The percentage increase in egg volume is evidently not related to either original volume or total incubation period (Table 1). The rate of water uptake is slower in species with a long incubation period than in those with a short incubation period.

The presence or absence of a resting period or form of diapause was the main cause of major variation between species in the length of the incubation period. Diapause was found to occur in *Hyas coarcticus* for a period of 16 weeks, *Corystes cassivelaunus* (14 weeks), *Cancer pagurus* (8 weeks) and *Maia squinado* (6 weeks), with seawater temperatures between 11 and 15 °C. The eggs of these species ceased to develop beyond the gastrula stage which was achieved within 3 or 4 days after spawning, and this resting period could not be shortened significantly by raising the water temperature. There is no relationship between the presence of diapause and egg size. In all other species studied for this work there was no resting period in egg development, or if it occurred it was less than 7 days' duration and embryonic development was considered to be continuous. Continuous embryonic development proved to be slower in some species than in others held at the same temperature. Changes in the mean incubation temperature appeared to be the main cause of variation in the time taken for embryonic development in any single species.

Effects of temperature on incubation

Incubation rate

In the analysis of data obtained during this study, use is made of Bělehrádek's (1935, 1957) equation with Bělehrádek's original rate of metabolic function (V) here replaced by development time in days (D) to give:

$$D = a\,(T-\alpha)^{b},$$

with D being the observed development time (= incubation period) in days, T the recorded mean temperature throughout incubation in degrees Celsius, and with a, b and α being fitted constants. For the resulting curves, a is a scaling constant reflecting the change in incubation period due to changes in temperature for a given species thereby defining shifts along the development (D) axis, b is a negative coefficient expressing the degree of change in the shape of the curves or the degree of curvilinearity in response to temperature change within the limits of egg viability, and α defines the positions of the curves on the temperature scale. The constant α, which has been termed the 'biological zero' by Bělehrádek (1935, 1957) may be further defined as the temperature at which the development time (D) becomes infinite (i.e. the rate is zero). This value may be well below the natural temperature range, thereby occupying an essentially arbitrary position on the Celsius scale. McLaren (1963), McLaren *et al.* (1969) and Corkett & McLaren (1970) considered that this equation revealed differences between 'rate' – temperature

curves in the simplest and most direct way, although other three-constant equations may be equally accurate.

Statistical analysis was carried out using the experimental data from all species for which four or more observations of both D and T were available, giving a total of 72

TABLE 2. CONSTANTS a and α WITH CORRELATION COEFFICIENTS FOR THE CURVE $D = a(T-\alpha)^{-2\cdot3}$ FOR TWELVE SPECIES OF DECAPOD CRUSTACEA.

	Species	a	α	Correlation coefficient (r)
A	Galathea dispersa	15803	−2·9	0·999
B	Macropipus depurator	19783	−3·5	0·998
C	Macropipus pusillus	24144	−3·8	0·997
D	Carcinus maenas	29309	−3·3	0·998
E	Palaemon serratus	27129	−1·2	0·993
★	Macropipus holsatus	21086	−4·1	0·998
★	Porcellana platycheles	23744	−0·9	0·998
★	Pisidia longicornis	19689	−3·1	0·997
★	Pilumnus hirtellus	32446	−4·7	0·999
★	Pagurus prideauxi	14297	0·0	0·992
★	Inachus dorsettensis	25386	−3·5	0·999
★	Crangon crangon	20437	−3·6	0·996

Notes: 1. Curves for species A to E plotted in Figure 4.
2. Asterisk denotes species not plotted in Fig. 4.

observations covering 12 species. The data were treated in three basic steps as follows:

(1) A value for a and b was estimated, first assuming that α was equal to zero. To do this, we write for the kth species:

$$D = a_k (T-\alpha_k)^b,$$

but in order to use a linear regression model, logarithms were taken giving:

$$\log D = \log a_k + b \log (T-\alpha_k).$$

In this equation the error term was not considered.

(2) After step 1 had been completed for each species, visual scanning of the data suggested that it was possible to derive a single satisfactory estimate for b applying to all 12 species without introducing an excessive error component. A constant b would imply that the magnitude of response shown by D to temperature change is the same in all species. By pooling all observed values for D and T, together with those for a obtained in step 1, an estimate of $b = -2\cdot3$ was obtained.

(3) Using individual values of D, T and a, and with b held constant at $-2\cdot3$, α_k was estimated by successively adding values for α between $-5\cdot0$ and $+5\cdot0$ in increments of $0\cdot1$ to the observed temperature (T). The sums of squares of the deviations of observed from calculated development times were recorded, and the value for α which resulted in the minimum sums of squares deviation was selected for each species.

In this way, the individual estimates of a and α given in Table 2 were produced and a constant value for b of $-2\cdot3$ was obtained. The calculated curves for five species selected to cover the experimental range, together with their respective experimental data points are plotted to a linear scale in Fig. 4. The seven remaining species have not been graphed

for reasons of clarity. Correlation coefficients of the logarithmic regressions for all 12 species are included in Table 2. These all exceed 0·99, indicating that the computed curves fit the real data well. However, little store should be set by the precisely calculated values for α, since in most cases a good fit could still have been obtained had α remained set equal to zero.
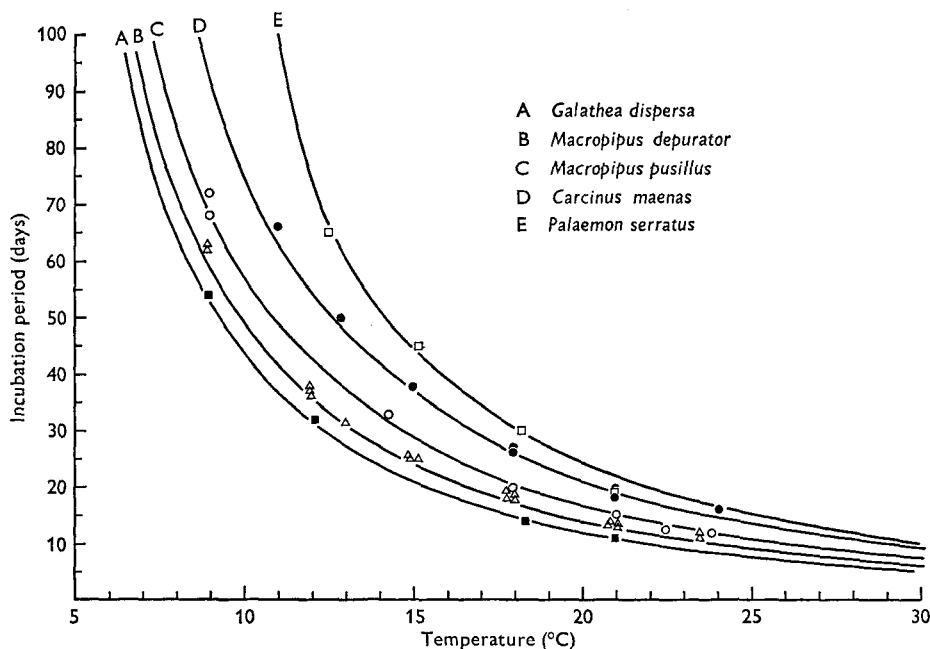

Fig. 4. Incubation period ($=$ development time) in days (D) between spawning and hatching of eggs in 5 species of decapod Crustacea at different temperatures. Experimental data points indicated. Curves fitted as described in text. Note*: Curves for a further 7 species fall within this range but have not been graphed for reasons of clarity. Computed data for plotting all curves and all experimental data are in Table 3. Derived constants and correlation coefficients are given for all twelve species in Table 2.

Data for plotting the curve for $D = a(T-\alpha)^{-2\cdot3}$ in all 12 species are given in Table 3. Table 4 summarizes experimental data obtained for these 12 and all other species studied. Also, in the 12 species for which sufficient experimental data are available, D has been plotted against $(T-\alpha)$ on a logarithmic scale thereby avoiding the complication caused by shifts of α along the horizontal axis (Fig. 5). This enables us to make a clearer visual comparison between the species concerned, with differences in the value of a now determining differences between the respective points of intersect with the D axis, as illustrated here by the lateral displacement between each of the parallel lines.

The relatively small range in the values of α listed in Table 2 ($\alpha = 0\cdot0$ to $\alpha = -4\cdot7$) is largely a reflexion of the fact that all experimental animals are from the same temperature regime. It is probable that decapods from the tropics have higher values for α, and those from colder waters have low values for α as has been shown for copepods by McLaren et al. (1969).

Laboratory work carried out with *Cancer pagurus*, *Corystes cassivelaunus*, *Hyas*

coarcticus and *Maia squinado* showed that it was not possible to shorten the inherent diapause period significantly, simply by raising the water temperature. Their response to increasing temperature followed that of other species only after the diapause period had been passed. Hence, in species possessing a diapause period, the equation $D = a(T-\alpha)^{-2\cdot3}$ does not apply over the whole of their embryonic development.

TABLE 3. COMPUTED POINTS FOR PLOTTING CURVES TO SHOW THE RELATION-SHIPS BETWEEN DEVELOPMENT TIME (D) AND TEMPERATURE (T) ACCORDING TO THE EQUATION $D = a(T-\alpha)^{-2\cdot3}$ IN 12 SPECIES OF DECAPOD CRUSTACEA

Species	Temperature °C (T) ...	Incubation period (days) (D)										
		5·0	7·5	10·0	12·5	15·0	17·5	20·0	22·5	25·0	27·5	30·0
Crangon crangon		144	80·0	50·2	34·0	24·5	18·3	14·2	11·2	9·11	7·52	6·29
Palaemon serratus		416	190	106	66·4	45·1	32·4	24·3	18·8	14·9	12·1	10·0
Pagurus prideauxi		360	140	72·2	43·1	28·3	19·9	14·6	11·1	8·73	7·01	5·73
Galathea dispersa		136	72·1	44·0	29·3	20·7	15·3	11·8	9·3	7·46	6·13	5·11
Pisidia longicornis		161	86·5	53·1	35·5	25·2	18·7	14·4	11·4	9·17	7·54	6·29
Porcellana platycheles		402	178	97·8	60·8	41·0	29·3	21·9	16·9	13·3	10·8	8·89
Carcinus maenas		228	124	76·7	51·6	36·8	27·4	21·1	16·7	13·5	11·1	9·25
Inachus dorsettensis		184	102	63·6	43·1	30·9	23·1	17·8	14·1	11·4	9·41	7·88
Macropipus depurator		142	78·9	49·3	33·4	23·9	17·9	13·8	11·0	8·88	7·32	6·12
M. holsatus		131	75·1	47·9	32·9	23·8	18·0	14·0	11·1	9·05	7·49	6·29
M. pusillus		163	91·7	57·8	39·4	28·4	21·3	16·5	13·1	10·6	8·78	7·36
Pilumnus hirtellus		188	108	68·9	48·2	34·4	26·4	20·2	16·4	13·1	11·0	9·15

TABLE 4. EXPERIMENTAL DATA FOR THE INCUBATION TIME FOR EGGS OF 19 SPECIES OF DECAPOD CRUSTACEA AT DIFFERENT TEMPERATURES

Species	Temperature (°C) (incubation period in parentheses)
Crangon crangon	11·2 (45·0), 13·2 (32·0), 18·0 (19·0), 20·8 (13·5), 23·8 (12·0)
Palaemon serratus	12·5 (65·0), 15·3 (45·0), 18·2 (30·0), 21·0 (19·0)
Pagurus prideauxi	11·5 (54·0), 12·3 (48·0), 12·4 (44·0), 16·4 (24·0)
Galathea dispersa	9·0 (54·0), 12·2 (32·0), 18·2 (14·0), 21·0 (11·0)
Pisidia longicornis	12·0 (41·0), 16·0 (23·0), 18·3 (18·0), 21·0 (15·0)
Porcellana platycheles	12·8 (61·0), 15·0 (43·0), 21·0 (20·0), 24·0 (16·5)
Carcinus maenas	11·0 (66·0), 13·1 (50·0), 15·0 (38·0), 18·0 (27·0, 26·5), 21·0 (19·0, 18·5), 24·0 (16·0)
Inachus dorsettensis	9·0 (78·0), 12·3 (46·0), 18·0 (23·0), 21·0 (16·0)
Macropipus depurator	9·0 (63·0, 62·0), 12·0 (38·0, 38·0, 37·25), 12·1 (36·0), 13·1 (31·5), 15·0 (25·5), 15·1 (25·25), 15·2 (25·0), 17·8 (19·5), 17·9 (18·5, 18·25), 18·0 (17·75), 20·8 (13·5, 13·25), 20·9 (13·25), 21·0 (13·0), 23·5 (12·0, 11·75)
M. holsatus	9·5 (56·0), 12·5 (34·5), 18·0 (18·0), 23·8 (12·0)
M. pusillus	9·0 (72·0, 68·0), 14·4 (33·0), 18·0 (20·0), 21·0 (15·0), 22·5 (12·5), 23·8 (12·0)
Pilumnus hirtellus	9·0 (80·0), 12·0 (52·0), 15·0 (35·0), 21·0 (18·c)
Galathea squamifera	13·2 (30)
Macropipus puber	12·5 (44)
Macropodia rostrata	12·5 (48)
M. longirostris	12·0 (52)
Ebalia tuberosa	15·0 (47)
Eurynome aspersa	14·0 (46)
Goneplax rhomboides	17·4 (29)

Incubation success

Evidence obtained from nine species from shallow water having an annual environmental temperature range between 7 and 16 °C points to a relationship between egg volume and the ability of these eggs to survive higher than normal temperatures (Fig. 6).
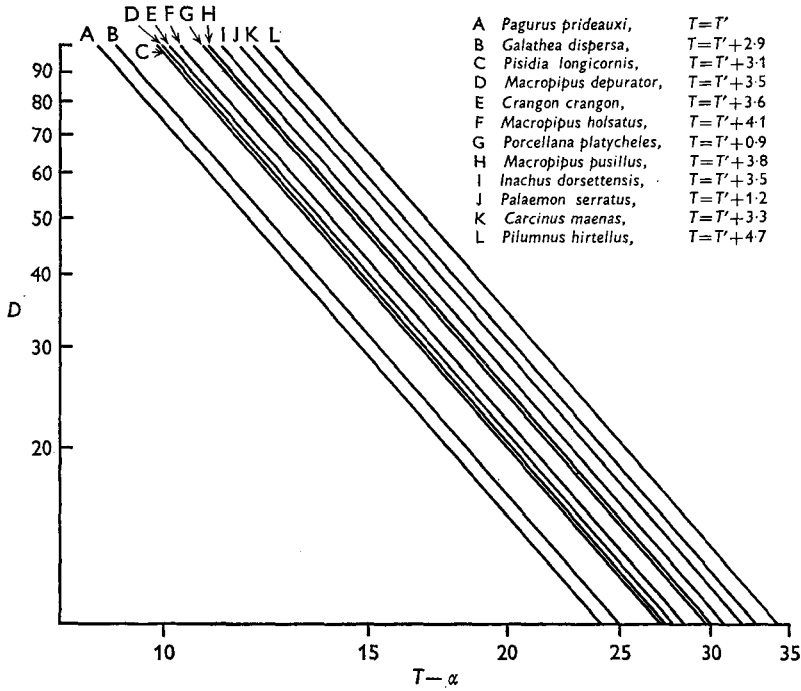
	A	Pagurus prideauxi,	$T=T'$
	B	Galathea dispersa,	$T=T'+2\cdot9$
	C	Pisidia longicornis,	$T=T'+3\cdot1$
	D	Macropipus depurator,	$T=T'+3\cdot5$
	E	Crangon crangon,	$T=T'+3\cdot6$
	F	Macropipus holsatus,	$T=T'+4\cdot1$
	G	Porcellana platycheles,	$T=T'+0\cdot9$
	H	Macropipus pusillus,	$T=T'+3\cdot8$
	I	Inachus dorsettensis,	$T=T'+3\cdot5$
	J	Palaemon serratus,	$T=T'+1\cdot2$
	K	Carcinus maenas,	$T=T'+3\cdot3$
	L	Pilumnus hirtellus,	$T=T'+4\cdot7$

Fig. 5. Regression lines for 12 species of decapod Crustacea derived as log plots of incubation period or development time (D) against $T\,°C-\alpha$.
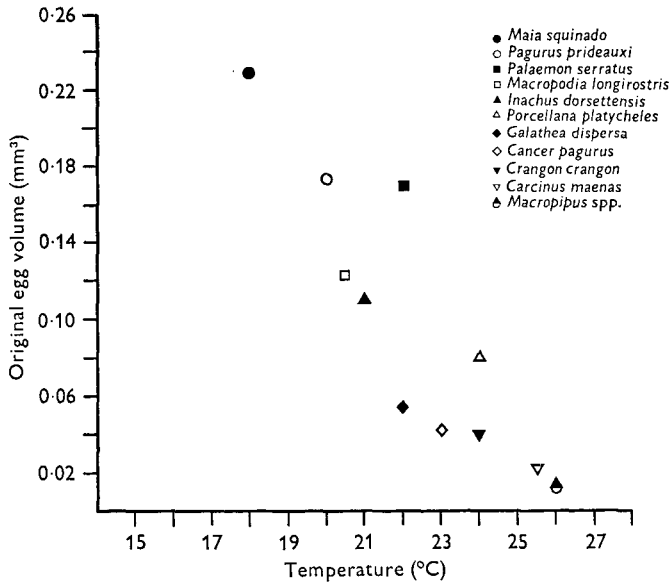
Fig. 6. Relationship between egg volume and high temperature tolerance during egg development in some decapod Crustacea. Points plotted indicate 10 % cytolysis among eggs sampled.

Daily viability assessments of eggs indicated when and at what temperature death occurred, but percentage viability figures thus obtained were misleading, as when the increasing numbers of moribund eggs exceeded the capability of the female to remove them, the entire egg mass cytolysed very rapidly due to the activity of bacteria and micro-organisms. Hence, the graphed points are temperatures at which approximately 10 % of the eggs removed for assessment were moribund after having increased the water temperature through 1 °C daily from a starting point of 9 °C (Fig. 6).

Smaller, less yolky eggs are able to survive higher temperatures than large eggs (Fig. 6). In the case of *Macropipus* spp. the females died at 24·8 °C, yet isolated eggs were able to survive in temperatures up to 26 °C. At abnormally high temperatures the most vulnerable time in the development of the eggs appeared to be during gastrulation or shortly afterwards.

A similar series of experiments were conducted with the same species of *Macropipus* by lowering the ambient temperature from 9 to 3 °C through 1 °C every 2 days. The results obtained were rather less conclusive than when the temperature was raised. Little or no mortality occurred among either the eggs or adults above 4 °C. The adults of all species other than *Macropipus depurator* died between 3 and 4 °C but mortality among isolated eggs was not significant as low as 3 °C in any of the species studied. In the eggs therefore, lower temperatures merely slow down the rate of development, and the present experiments did not reach the lowest limits of egg tolerance.

Temperature-induced spawning irregularities

Macropipus depurator is one of several species from the Plymouth region found to incubate three or more batches of eggs over the spring and summer breeding season without an intervening moult. Females of this species carrying newly spawned eggs were obtained from the field early in the breeding season (March). By placing these immediately in water 3 °C or more higher than the recorded sea temperature, it was possible to disrupt the normal serial sequence of spawning, incubation and hatching successive batches of eggs.

One specimen collected from water at 9 °C, and introduced to the laboratory at 12 °C with an anticipated incubation time of 38 days at that temperature spawned a second, third and fourth batch of normal and viable fertilized eggs after 15, 20 and 24 days respectively. A second specimen from the same collection introduced to the laboratory water at 18 °C (18 days normal incubation time) spawned a second and third batch of eggs after 10 and 13 days respectively. Under these conditions eggs of the second and subsequent spawnings were numerically less than 10 % of the original (first) egg mass. Hatching of all these eggs occurred in series according to the normal incubation period in relation to temperature.

Hatch and moult viability

The temperature range over which eggs would survive and continue to develop (Fig. 6) was greater than that for successful hatching and subsequent development.

From Fig. 7 it can be seen that few species could hatch 50 % of their eggs successfully below 8 °C, and none of those studied achieved 50 % moult viability below this tempera-

ture. At high incubation temperatures, 50 % hatch and moult viabilities extended over a greater range (15–25 °C) from species to species. However, in most cases, 50 % hatch viability was not achieved at the maximum temperature at which eggs were able to complete their development (cf. Figs. 6 and 7), and the maximum temperature for 50 % moult viability was about 3 °C below the upper limit for 50 % hatch viability. A further trend is that species with small eggs give rise to viable larvae after incubation over a greater range of temperatures than those with large eggs (Fig. 7). The reasons for this are not known.
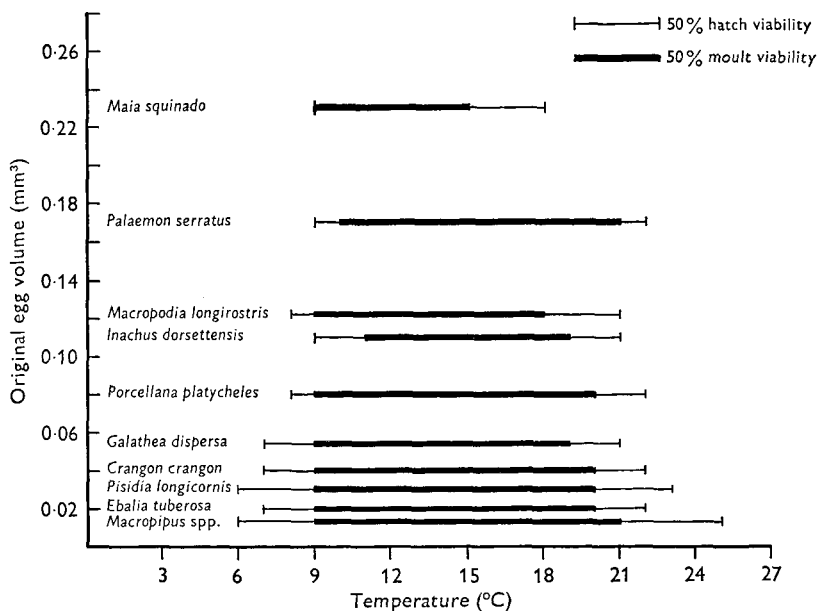
Fig. 7. Viability of newly hatched and newly moulted decapod larvae after development at different temperatures.

DISCUSSION

In a series of studies on the hatching process in aquatic invertebrates, Davis (1964 a, b, 1965 a, b, 1966) found that in decapod crustaceans the egg size increase during incubation was due to either a slow but steady osmotic swelling of the inner egg membrane or to swelling of the embryo itself. In both cases Davis considers that, the size increase is brought about by uptake of water which increases the internal pressure of the egg up to the time of hatching. The more rapid rate of egg volume increase recorded here from the time at which the embryonic heartbeat is first observed suggests, therefore, that later embryonic development is accompanied by osmotic changes leading to a more rapid uptake of water.

Patel & Crisp (1960b), in a study on the effects of temperature on the embryonic development of several species of barnacle, observed that at a given temperature all species develop at approximately the same rate. This agrees with some similar studies on other groups of animals. Fox (1939) found only slight differences in development rate

among three species of the genus *Psammechinus* (Echinodermata) and between three species of the genus *Aplysia* (Mollusca), while Moore (1942) has shown that five species of the genus *Rana* (Amphibia) differ only in the low temperature range where eggs of colder water species develop more rapidly. However, when egg size is considered, Berrill (1935) has shown that large tunicate eggs develop more slowly than small ones, and McLaren (1965) concludes that for different races of *Rana pipiens* from the United States, a single estimate of *b* for all races reveals a simple proportionality between egg diameter and *a* of Bĕlehrádek's equation. There is evidence for a similar relationship between egg size and development rate among copepods for different populations of the same species or among closely related species (McLaren, 1966; McLaren *et al.* 1969).

In the species considered for this study, where there is little close relationship between species beyond their common grouping as decapod Crustacea, no overall correlation between original egg volume (which is almost entirely yolk at this stage) and the various values of *a* is apparent. However, in the family Portunidae, there is an increase in *a* values between the three species of *Macropipus* with very small eggs on one hand, and *Carcinus maenas* with somewhat larger eggs on the other. Similarly, *Porcellana platycheles* (Porcellanidae) has larger eggs and a higher value of *a* than *Pisidia longicornis* (Fig. 4, Table 2). From the limited data available it is therefore possible to suggest that among decapod Crustacea which are closely related, increasing egg size (and consequently yolkiness) slows down the rate of development and increases the value of *a*.

Patel & Crisp (1960*b*) concluded that in barnacle species, the rate of embryonic development at the lower part of the temperature range at which breeding could occur increased three to four times for every 10 °C rise in temperature. Toward the upper part of the viable temperature range the rate of development increased more slowly, and at the highest temperatures at which development could take place the development time became constant. Hence, the equation $D = a(T-\alpha)^b$ cannot be applied to development of barnacles, since factors other than temperature appear to increase significantly the value of D towards the upper part of the temperature range over which development can occur.

In a general study on the laboratory culture of *Palaemon serratus*, Reeve (1969) included some observations on the period of incubation at three different temperatures. Reeve found that for 10 newly-berried females held at each of 10, 15 and 20 °C, the mean incubation time was 95+, 58 and 28 days respectively. The experiment at 10 °C was accidentally curtailed at 95 days, but the eggs were in an advanced stage of development at that time. These figures compare reasonably well with results obtained from this present study. From application of the equation $D = a(T-\alpha)^{-2\cdot3}$ to *P. serratus*, egg development time for 10, 15 and 20 °C would be 106, 45·1 and 24·3 days respectively.

It is clear from the relationship between temperature and incubation period that species such as *Macropipus depurator* which have each breeding season more than one batch of eggs will carry the first batch spawned in March (mean daily surface water temperature 7·9 °C in 1970) for a significantly longer period of time than the final batch laid in June (15·0 °C). Allowing for a mean temperature of 8·9 °C in April (1970), eggs spawned at the beginning of March would not hatch until early May (about 9 weeks

development time), whereas a second batch spawned in early May would hatch after 5 to 6 weeks. A third batch spawned after the first week in June, when in 1970 the average surface water temperature at Plymouth for the remainder of this month was 15·3 °C, would hatch in only 3 weeks. Hence, a threefold decrease in incubation time can occur naturally during the breeding season of a single species in the one locality.

As shown in laboratory experiments with *Macropipus depurator*, a rapid rise in water temperature can disrupt the normal interval between the spawning of successive egg batches and cause significant reduction in egg numbers. Since environmental factors such as temperature and sometimes photoperiod generally affect neurosecretory activity in arthropods (Highnam & Hill, 1969), a sharp rise in temperature would undoubtedly influence the eyestalk neurosecretory system controlling ovarian development. It is suggested here, that by way of the neurosecretory system, a rapid rise in temperature has a more drastic affect on oogenesis and vitellogenesis than on incubation itself, thereby reducing the normal 4 or 5-day interval between successive egg batches to a degree where extensive telescoping occurs.

During ovarian development in crustaceans, substantial quantities of protein and more especially of lipid are required. These organic substrates are synthesized from food intake, but during the peak of breeding activity or when successive broods are produced, there are indications that stored lipid may be transferred from the hepatopancreas to the ovaries, and protein mobilised from the muscles (Pillay & Nair, 1973). Hence, the smaller number of eggs occurring in successive batches produced by *Macropipus depurator* under rapidly increased temperature conditions may reflect the female's inability to mobilize sufficient protein and lipid reserves to keep pace with the artificially induced acceleration of her breeding cycle.

It is apparently not always accurate to assume that the relationship between development rate and temperature can be simply expressed as a linear regression of '$T_0 + 10$' ($= \alpha + 10$) for the eggs and embryos of all animals. It has been shown by McLaren *et al.* (1969) that the development rate of copepod eggs is approximately a square function ($b = 2$). This present study indicates that in a wide range of cool temperate decapods with continuous embryonic development, the response to temperature is a function in the order of 2·3 rather than being a simple linear response.

The significance of the diapause period occurring in species such as *Cancer pagurus*, *Corystes cassivelaunus*, *Hyas coarcticus* and *Maia squinado* is uncertain. *Hyas coarcticus* and *Corystes cassivelaunus* both have an incubation period of around 10 months which is among the longest known in the Brachyura (Hartnoll, 1963, 1972). In these two species, eggs laid in May to July would lie in diapause until October or November, then develop at a very slow rate during the winter months so that larvae are hatched in the early spring (March and April) when ample food is available. It is therefore tempting to suggest that diapause has evolved in certain species to enhance the chances of larval survival, and perhaps in a wider sense to ensure the continuity of planktonic food chains.

The immediate value of this paper is in the application of the equation $D = a(T - \alpha)^{-2·3}$ to the laboratory rearing of decapod larvae, where in the species so far considered it should be possible to achieve hatching of larvae at predetermined intervals from a single collection of newly berried females, simply by adjusting the mean temperature of

incubation of eggs. The results also hint at the likely effects of localized thermal discharge on the breeding biology of benthic decapod species, as it is clear that only a slight change in temperature is sufficient to alter the time taken for egg development significantly and consequently the natural timing of larval liberation into the plankton. Breeding abnormalities which reduce fecundity could also occur, as shown here in *Macropipus depurator*.

REFERENCES

BĚLEHRÁDEK, J., 1935. Temperature and living matter. *Protoplasma Monographien*, **8**, 277 pp. Berlin: Borntraeger.

BĚLEHRÁDEK, J., 1957. Physiological aspects of heat and cold. *Annual Review of Physiology*, **19**, 59–82.

BERRILL, N. J., 1935. Studies in tunicate development. Part III. Differential retardation and acceleration. *Philosophical Transactions of the Royal Society, London. Series B*, **225**, 255–326.

BROOKS, W. K. & HERRICK, F. H., 1891. The embryology and metamorphosis of the Macroura. *Memoirs of the National Academy of Sciences*, **5**, 319–576.

COFFIN, H.G., 1958. The laboratory culture of *Pagurus samuelis* (Stimpson) (Crustacea, Decapoda). *Walla Walla College Publications of the Department of Biological Sciences and the Biological Station*, **22**, 1–5.

CORKETT, C. J. & MCLAREN, I. A., 1970. Relationships between development rate of eggs and older stages of copepods. *Journal of the Marine Biological Association of the United Kingdom*, **50**, 161–8.

COSTLOW, J. D., 1965. Variability in larval stages of the blue crab, *Callinectes sapidus*. *Biological Bulletin. Marine Biological Laboratory, Woods Hole, Mass.*, **128**, 58–66.

COSTLOW, J. D. & BOOKHOUT, C. G., 1962a. The larval development of *Sesarma reticulatum* Say reared in the laboratory. *Crustaceana*, **4**, 281–94.

COSTLOW, J. D. & BOOKHOUT, C. G., 1962b. The larval development of *Hepatus epheliticus* (L.) under laboratory conditions. *Journal of the Elisha Mitchell Scientific Society*, **78**, 113–25.

COSTLOW, J. D., BOOKHOUT, C. G. & MONROE, R., 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. *Biological Bulletin. Marine Biological Laboratory, Woods Hole, Mass.*, **118**, 183–202.

COSTLOW, J. D. & FAGETTI, E., 1967. The larval development of the crab, *Cyclograpsus cinereus* Dana, under laboratory conditions. *Pacific Science*, **21**, 166–77.

DAVIS, C. C., 1964a. A study of the hatching process in aquatic invertebrates. X. Hatching in the fresh-water shrimp, *Potimirim glabra* (Kingsley) (Macrura, Atyidae). *Pacific Science*, **18**, 378–84.

DAVIS, C. C., 1964b. A study of the hatching process in aquatic invertebrates. XIII. Events of eclosion in the American lobster, *Homarus americanus* Milne Edwards (Astacura, Homaridae). *American Midland Naturalist*, **72**, 203–10.

DAVIS, C. C., 1965a. A study of the hatching process in aquatic invertebrates. XIV. An examination of hatching in *Palaemonetes vulgaris* (Say). *Crustaceana*, **8**, 233–8.

DAVIS, C. C., 1965b. A study of the hatching process in aquatic invertebrates. XX. The blue crab, *Callinectes sapidus* Rathbun. *Chesapeake Science*, **6**, 201–5.

DAVIS, C. C., 1966. A study of the hatching process in aquatic invertebrates. XXIII. Eclosion in *Petrolisthes armatus* (Gibbes) (Anomura, Porcellanidae). *Internationale Revue der gesamten Hydrobiologie u Hydrographie*, **51**, 791–6.

DAVIS, C. C., 1968. Mechanisms of hatching in aquatic invertebrate eggs. *Oceanography and Marine Biology Annual Review*, **6**, 325–76.

FOX, H. M., 1939. The activity and metabolism of poikilothermal animals in different latitudes. III. *Proceedings of the Zoological Society of London*, **108**, 501–5.

GURNEY, R., 1942. In: *Larvae of Decapod Crustacea*, viii, 306 pp. Ray Society, London: Wheldon and Wesley.

HARTNOLL, R. G., 1963. The biology of Manx spider crabs. *Proceedings of the Zoological Society of London*, **141**, 423–96.

HARTNOLL, R. G., 1972. The biology of the burrowing crab, *Corystes cassivelaunus. Bijdragen tot de dierkunde*, **42**, 139–55.

HERRICK, F. H., 1896. The American lobster: a study of its habits and development. *Bulletin of the United States Fish Commission*, **15**, 1–252.

HIGHNAM, K. C. & HILL, L., 1969. *The Comparative Endocrinology of the Invertebrates*, 270 pp. London: Edward Arnold.

KAJISHIMA, T., 1950. Studies on the embryonic development of *Leander pacificus* Stimpson. 2. Early development. 3. Later embryonic development. *Zoological Magazine, Tokyo*, **59**, 82–6, 108–11.

KORSCHELDT, E., 1944. *Ontogenie der Decapoden. Dr H. G. Bronns, Klassen und Ordnungen des Tierreichs*, **5** (1), 671–861.

LEBOUR, M. V., 1928. The larval stages of the Plymouth Brachyura. *Proceedings of the Zoological Society of London*, **2**, 473–560.

LING, S. W., 1967. The general biology and development of *Macrobrachium rosenbergi* (de Man). *Proceedings of the World Scientific Conference on the Biology of Shrimps and Prawns. F.A.O. Fisheries Report*, **57**, 589–606.

McLAREN, I. A., 1963. Effects of temperature on growth of zooplankton, and the adaptive value of vertical migration. *Journal of the Fisheries Research Board of Canada*, **20**, 685–727.

McLAREN, I. A., 1965. Some relationships between temperature and egg size, body size, development rate, and fecundity, of the copepod *Pseudocalanus. Limnology and Oceanography*, **10**, 528–38.

McLAREN, I. A., 1966. Predicting development rate of copepod eggs. *Biological Bulletin. Marine Biological Laboratory, Woods Hole, Mass.*, **131**, 457–69.

McLAREN, I. A., CORKETT, C. J. & ZILLIOUX, E. J., 1969. Temperature adaptations of copepod eggs from the arctic to the tropics. *Biological Bulletin. Marine Biological Laboratory, Woods Hole, Mass.*, **137**, 486–93.

MARSHALL, S. M. & ORR, A. P., 1955. *The Biology of the Marine Copepod Calanus finmarchicus Gunnerus*. 188 pp. Edinburgh: Oliver and Boyd.

MOORE, J. A., 1942. The role of temperature in speciation of frogs. *Biological Symposia*, **6**, 189–213.

PATEL, B. & CRISP, D. J., 1960a. Rates of development of the embryos of several species of barnacles. *Physiological Zoology*, **33**, 104–19.

PATEL, B. & CRISP, D. J., 1960b. The influence of temperature on the breeding and the moulting activities of some warm-water species of operculate barnacles. *Journal of the Marine Biological Association of the United Kingdom*, **39**, 667–80.

PILLAY, K. K. & NAIR, N. B., 1973. Observations on the biochemical changes in gonads and other organs of *Uca annulipes, Portunus pelagicus* and *Metapenaeus affinis* (Decapoda; Crustacea) during the reproductive cycle. *Marine Biology*, **18**, 167–98.

PROVENZANO, A. J., 1968. Recent experiments on the laboratory rearing of tropical lobster larvae. *Proceedings of the Gulf and Caribbean Fisheries Institute*, **21**, 152–7.

REEVE, M. R., 1969. The laboratory culture of the prawn *Palaemon serratus. Fishery Investigations, Ministry of Agriculture, Fisheries and Food*, Ser. 2, **26**, 1–38.

SANDOZ, M. & ROGERS, R., 1944. The effect of environmental factors on hatching, moulting and survival of zoea larvae of the blue crab, *Callinectes sapidus* Rathbun. *Ecology*, **25**, 216–28.

SHIINO, S. M., 1950. Studies on the embryonic development of *Panulirus japonicus* (von Siebold). *Journal of the Faculty of Fisheries, Prefectural University of Mie-Tsu*, **1**, 1–168.

TEMPLEMAN, W., 1936. The influence of temperature, salinity, light and food conditions on the survival and growth of the larvae of the lobster (*Homarus americanus*). *Journal of the Biological Board of Canada*, **2**, 485–97.

WEAR, R. G., 1967. Life-history studies on New Zealand Brachyura. 1. Embryonic and post-embryonic development of *Pilumnus novaezelandiae* Filhol, 1886, and of *P. lumpinus* Bennett, 1964 (Xanthidae, Pilumninae). *New Zealand Journal of Marine and Freshwater Research*, **1**, 482–535.