HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 46, 9–28 (1992)

Growth patterns, chemical composition and oxygen consumption in early juvenile *Hyas araneus* (Decapoda: Majidae) reared in the laboratory

K. Anger[1], J. Harms[1], M. E. Christiansen[2], U. Süsens[1] & B. Wilmes[1]

[1] *Biologische Anstalt Helgoland, Meeresstation; D-W-2192 Helgoland, Federal Republic of Germany*

[2] *Universitetet i Oslo, Zoologisk Museum; N-0562 Oslo, Norway*

ABSTRACT: Early (instar I and II) juveniles of the spider crab *Hyas araneus* were reared under constant conditions (12 °C, 32 ‰S) in the laboratory, and their growth, biochemical composition, and respiration were studied. Every second day, dry weight (W), ash-free dry weight (AFW), and contents of ash, organic and inorganic carbon (C), nitrogen (N), hydrogen (H), protein, chitin, lipid, and carbohydrates were measured, as well as oxygen consumption. Changes in the absolute amounts of W, AFW, and C, N, and H during the moulting cycle are described with various regression equations as functions of age within a given instar. These patterns of growth differ in part from those that have been observed during previous studies in larval stages of the same and some other decapod species, possibly indicating different growth strategies in larvae and juveniles. There were clear periodic changes in ash (% of W) and inorganic C (as % of total C), with initially very low and then steeply increasing values in postmoult, a maximum in intermoult, and decreasing figures during the premoult phase of each moulting cycle. Similar patterns were observed in the chitin fraction, reaching a maximum of 16 % of W (31 % of AFW). Ash, inorganic C, and chitin represent the major components of the exoskeleton and hence, changes in their amounts are associated with the formation and loss of cuticle material. Consequently, a high percentage of mineral matter was lost with the exuvia (76 % of the late premoult [LPM] ash content, 74 % of inorganic C), but relatively small fractions of LPM organic matter (15 % of AFW, 11 % of organic C, 5–6 % of N and H). These cyclic changes in the cuticle caused an inverse pattern of variation in the percentage values (% of W) of AFW, organic C, N, H, and biochemical constituents other than chitin. When these measures of living biomass were related to, exclusively, the organic body fraction (AFW), much less variation was found during individual moulting cycles, with values of about 43–52 % in organic C, 9–10 % in N, 6–9 % H, 31–49 % of AFW in protein, 3–10 % in lipid, and < 1 % in carbohydrates. All these constituents showed, on the average, a decreasing tendency during the first two crab instars, whereas N remained fairly constant. It cannot be explained at present, what other elements and biochemical compounds, respectively, might replace these decreasing components of AFW. Decreasing tendencies during juvenile growth were observed also in the organic C/N and in the lipid/protein weight ratios, both indicating that the proportion of lipid decreased at a higher rate than that of protein. Changes were observed also in the composition of inorganic matter, with significantly lower inorganic C in early postmoult (2–4 % of ash) than in later stages of the moult cycle (about 9 %). This reflected probably an increase in the degree of calcification, i.e. in the calcium carbonate content of the exoskeleton. As a fraction of total C, inorganic C reached maximum values of 17 and 20 % in the crab I and II instars, respectively. The energy content of juvenile spider crabs was estimated independently from organic C and biochemical constituents, with a significant correlation between these values. However, the former estimates of energy were, on the average, significantly lower than the latter (slope of the regression $\neq 1$). Since organic C should be a reliable

integrator of organic substances, but the sum of protein, lipid, chitin, and carbohydrates amounted to only 60–91 % of AFW, it is concluded that the observed discrepancy between these two estimates of energy was caused by energy from biochemical constituents that had not been determined in our analyses. Thus, energy values obtained from these biochemical fractions alone may underestimate the actual amount of organic matter and energy. Respiration per individual in juvenile spider crabs was higher than that in larval stages of the same species (previous studies), but their W-specific values of oxygen consumption (QO_2) were lower than in conspecific larvae (0.6–2 $\mu g\ O_2 \cdot [mg\ W]^{-1}$). QO_2 showed a consistent periodic pattern in relation to the moult cycle: maximum values in early postmoult, followed by a rapid decrease, and constant values in the intermoult and premoult phases. This variation is interpreted as an effect mainly of cyclic changes in the amounts of cuticle materials which are metabolically inactive. From growth and respiration values (both expressed in units of organic C), net growth efficiency, K_2, values may be calculated. In contrast to previous findings in larval stages, K_2 showed an increasing trend during growth of the first two juvenile instars of *H. araneus*.

INTRODUCTION

Growth patterns, changes in the elemental (CHN) or proximate biochemical composition, and oxygen consumption have been studied only seldom during the course of individual moulting cycles in juvenile brachyuran crabs. The most comprehensive accounts of biochemical changes during a moulting cycle of a crab were given by Renaud (1949) for *Cancer pagurus,* and by Heath & Barnes (1970) and Spindler-Barth (1976) for *Carcinus maenas.* Oxygen consumption has been measured in many crustacean species (McMahon & Wilkens, 1983), but seldom in relation to the moulting cycle (Bulnheim, 1974).

The spider crab *Hyas araneus* (L.) has been investigated quite intensively as to its larval physiology and biochemistry (Anger et al., 1989), but only a few chemical aspects such as CHN, inorganic matter, protein, and nucleic acids were studied during its early juvenile growth (Anger, 1984; Anger & Hirche, 1990). In the present study, growth (in terms of changes in the elemental and proximate biochemical composition) and respiration were measured in the first two juvenile instars of this species, and compared with patterns that had been found in other decapods, as well as with those in the larval stages of the same species, *H. araneus.*

MATERIAL AND METHODS

Hyas araneus larvae were obtained from ovigerous females and mass-reared in the laboratory at 32‰ salinity and a constant temperature of 12 °C, applying standard techniques (Anger et al., 1983). Freshly hatched *Artemia* sp. (San Francisco Bay Brand™) nauplii were given as food; seawater and food were changed every second day. After metamorphosis to the first juvenile stage, crabs were reared individually in net-bottom tubules (for details see Kunisch & Anger, 1984), where they received somewhat larger (ca 2-day old) *Artemia* sp. nauplii. Temperature and salinity were the same as during larval development.

Oxygen consumption, dry weight (W), ash-free dry weight (AFW), organic and inorganic carbon (C), nitrogen (N), and hydrogen (H), as well as total protein, lipid, carbohydrates, and chitin were measured in regular intervals during early juvenile development. W, AFW, and CHN were determined also in exuviae of the first crab stage. The first moults to the crab II instar occurred 20 days after metamorphosis, and first

ecdyses to the next stage 18 days later. After a total of > 500 juveniles had been sacrificed in these experiments, not enough material for their continuation was left after day 16 of the second instar; measurements of lipid and chitin were suspended after day 14, and those of respiration already after day 8 of the crab II moult cycle (in the latter case a technical failure prevented the use of later data).

Oxygen consumption was measured with a Winkler technique in 8 replicate experiments (with 3 individuals each, and 4 replicate blanks without animals; see Anger et al., 1989).

W and CHN measurements followed standard techniques (Anger et al., 1983), with 5 replicate analyses (1 individual each). Ash, AFW, and inorganic C were measured after freeze-drying, subsequent determination of W, and ashing at 500 °C for 4 h (Hirota & Szyper, 1975; Anger, 1984).

Biochemical measurements were made in three replicate samples: protein after Lowry et al. (1951) with bovine serum albumin (Serva 11 930) as a standard; lipids photometrically with a Merckotest® (Merck™, Darmstadt) reagent kit, utilizing the sulfophosphovanillin reaction (Zöllner & Kirsch, 1962); carbohydrates after Holland & Gabbot (1971) using glucose as a standard; chitin gravimetrically after Raymont et al. (1964).

Energy content was calculated independently from organic C (Salonen et al., 1976) and biochemical constituents (Winberg, 1971). Metabolic losses of organic C were calculated using a conversion factor of 0.3375 µg C/µg O_2, assuming an average RQ value of 0.9 (Anger, 1990).

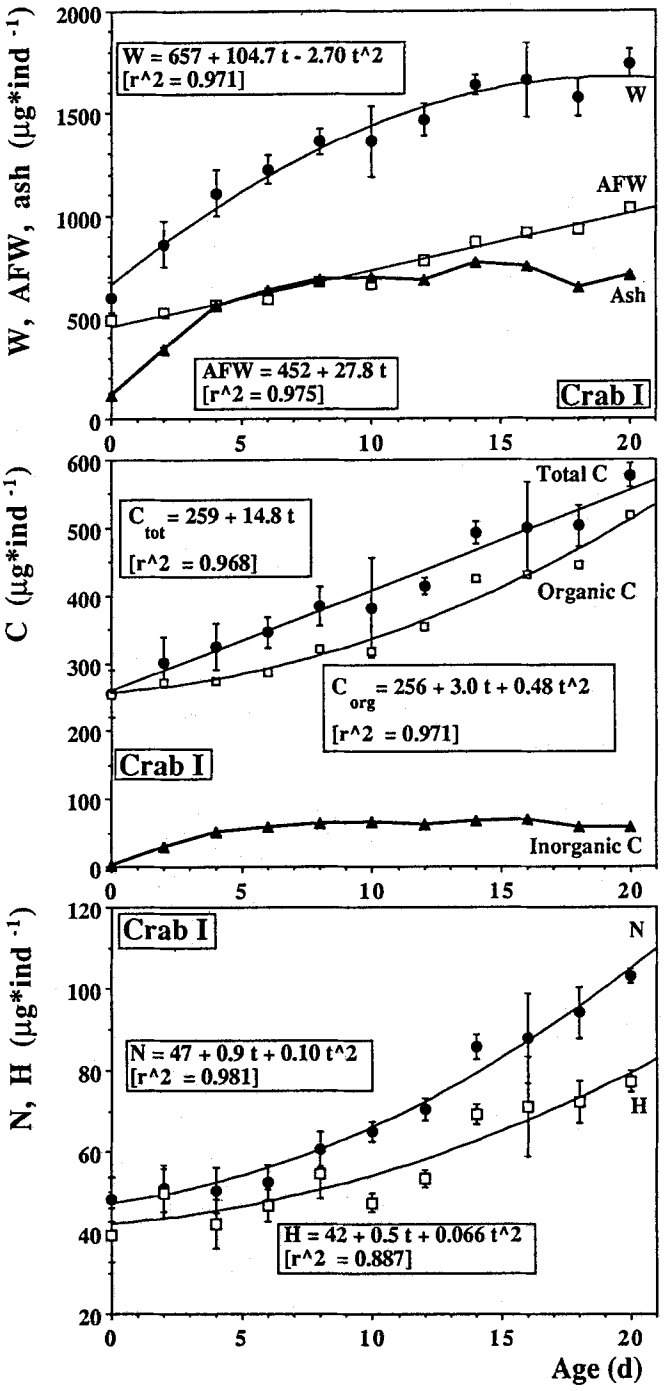
Statistical tests were carried out according to Sachs (1984): comparisons of mean values (H_0: $\bar{x}_1 = \bar{x}_2$; Student's t-test, after F-test; pp. 212–214); significance of correlation and regression coefficients (H_0: r = 0, m = 0; pp. 329, 339); differences of regression coefficients and intercept values (a) from a theoretical value (H_0: m = 1 or m = 6.25, a = 0; pp. 339–340); linearity of regressions (pp. 335–338). H_0 was rejected when $P < 0.05$. Error bars ($\bar{x} \pm$ SD) are given in Figures only when directly measured values are displayed, not in those calculated by difference or as a percentage from two independently measured data sets (for instance, µg inorganic C, from mean values of ash [% of W] and of C [% of ash]).

RESULTS

Dry weight (W), elemental composition (CHN), and ash content

Growth patterns, i.e. the increase in absolute biomass values (per individual) during time (t, in days) of the first two juvenile moulting cycles are shown in Figures 1 and 2. Dry weight (W) increased in both instars at a higher rate during the beginning than in later parts of the moult cycle, following a parabola-shaped curve. This recurrent pattern may be described by a quadratic equation as a function of time, t (regression equations given in Figs 1, 2; upper graphs). It appears highly influenced by changes in the ash fraction, which showed in both instars a steep increase during the first third of the moulting cycle (postmoult, early intermoult), followed by rather constant values later on. When ash was subtracted from total W, the organic fraction (AFW) could be seen to increase as a linear function of age (Figs 1, 2; upper graphs).

The increase in total carbon (C) may also be described as a linear function of time


W, AFW, ash (µg*ind -1)
W = 657 + 104.7 t - 2.70 t^2
[r^2 = 0.971]
W
AFW
Ash
AFW = 452 + 27.8 t
[r^2 = 0.975]
Crab I
C (µg*ind -1)
Total C
C tot = 259 + 14.8 t
[r^2 = 0.968]
Organic C
C org = 256 + 3.0 t + 0.48 t^2
[r^2 = 0.971]
Crab I
Inorganic C
N, H (µg*ind -1)
Crab I
N
N = 47 + 0.9 t + 0.10 t^2
[r^2 = 0.981]
H
H = 42 + 0.5 t + 0.066 t^2
[r^2 = 0.887]
Age (d)

within a given moulting cycle (Figs 1, 2; middle graphs). In contrast, the fraction of inorganic C showed a similar pattern to that of total ash, i.e. a steep increase during the beginning of the moult cycle followed by constant values. When only organic C was considered, it revealed a slightly parabolic, or an almost linear increase with time in the first two crab stages (regression equations in Figs 1, 2; middle graphs).

The lower graphs in Figures 1 and 2 show the patterns of growth in total nitrogen (N) and hydrogen (H). No anorganic N or H were detected in our analyses. The increase in these fractions could be described with best fit as a non-linear (parabolic) function of age; however, the curvature in H was very weak and not significantly different from linearity.

Figures 3–6 show changes in the relative (percentage of W) CHN and ash contents of juvenile crabs. The ash content increased in both instars dramatically during the early postmoult period: from ca 20 % after ecdysis to a maximum, a few days later, of 50–52 % (crab I instar) or 56 % (crab II) (Fig. 3). During the second half of the moult cycle a significant decrease occurred, leading to final values of 40 % (crab I) or 47 % (crab II). These patterns of change in the ash content were very similar in the first two juvenile instars, but the average level was somewhat higher in the second than in the first stage (Fig. 3).

The carbon content of ash (inorganic C) was low in early postmoult juveniles (2 and 4 % in the first and second stage, respectively), but then it increased rapidly and remained constant throughout the rest of the moulting cycle, amounting to about 8–9 % of ash (Fig. 3). As a fraction of total C, inorganic C increased during the postmoult phase from initial values of 1–2 %, to a maximum of ca 17 % (crab I) or 20 % (crab II), before it decreased to final values of ca 10 or 14 %, respectively (Fig. 3).

As a consequence of a rapid postmoult increase in the percentage of ash (Fig. 3), the fractions (% of W) of both total and organic C decreased concurrently during this early phase of the moulting cycle (Fig. 4). Since the absolute amounts of minerals (ash) remained constant after reaching a maximum, and those of organic constituents (AFW, C) continued to increase (Figs 1, 2), the fractions (% W) of both total and organic C increased during the second half of the moulting cycle (Fig. 4).

The C content of the organic fraction changed relatively little, ranging between 43 and 52 % of AFW, and without showing a clear pattern within the crab I or II moulting cycle (Fig. 4). However, a decreasing trend may be seen in these values during growth in these two instars (Fig. 4), indicating a gradual shift in their average biochemical composition.

Changes in the nitrogen and hydrogen contents of juvenile crabs (as a percentage of W; Fig. 5) were also highly influenced by changes in the ash content und thus, were similar to those in C (cf. Fig. 4). As a fraction of AFW, however, N remained rather constant (9–10 %), whereas the H content appeared to show, like organic C (Fig. 4), a slightly decreasing trend (Fig. 5).

The C/N weight ratio may be a useful index of the relative elemental composition of

---

Fig. 1. *Hyas araneus*, crab I instar. Growth patterns in the absolute values (in $\mu g \cdot individual^{-1}$) of dry weight (W), ash-free dry weight (AFW), ash, carbon (total, organic, and inorganic C), nitrogen (N), and hydrogen (H) in relation to age (days, d) within the moulting cycle. Error bars: $\bar{x} \pm SD$. Regression equations: quantities of single constituents (in $\mu g \cdot individual^{-1}$) as functions of time (t, in days); r^2: coefficient of determination

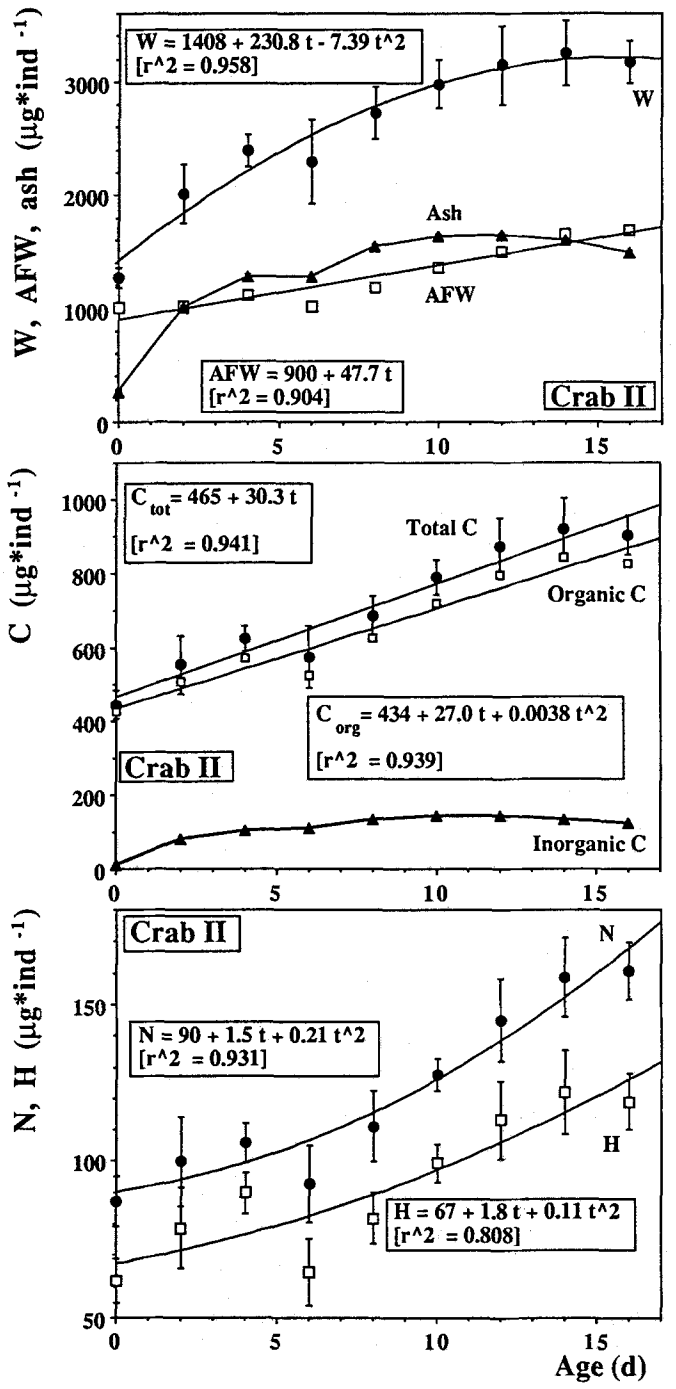


Fig. 2. *Hyas araneus*, crab II instar. Growth patterns in the absolute values (µg·individual^{-1}) of biomass. For explanation see Fig. 1

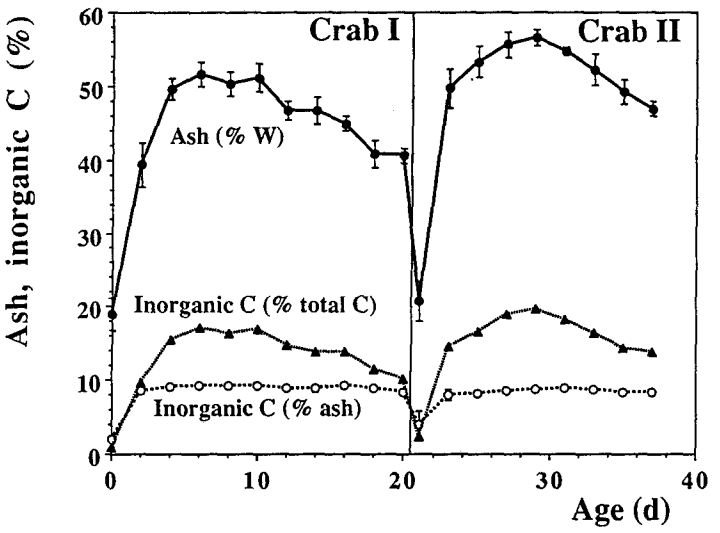


Fig. 3. *Hyas araneus*, crab I and II instars. Ash (% of W) and inorganic C (in % of total C, and of ash) in relation to age (days, d). Error bars: $\bar{x} \pm SD$

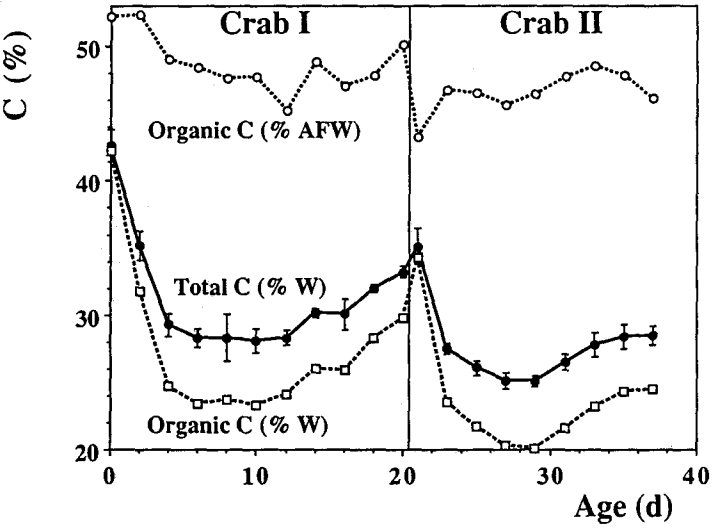


Fig. 4. *Hyas araneus*, crab I and II instars. Total C (in % of W) and organic C (% of W, and of AFW) in relation to age (days, d). Error bars: $\bar{x} \pm SD$

an organism. When total C was considered, it showed during the first two crab instars significant changes in relation to N, with a steep increase during the postmoult phase and a decrease during premoult (Fig. 6). However, when exclusively organic C was related to N, the organic C/N ratio was, on the average, much lower (except in early postmoult, where ash and inorganic C were very low), and changes during individual moulting

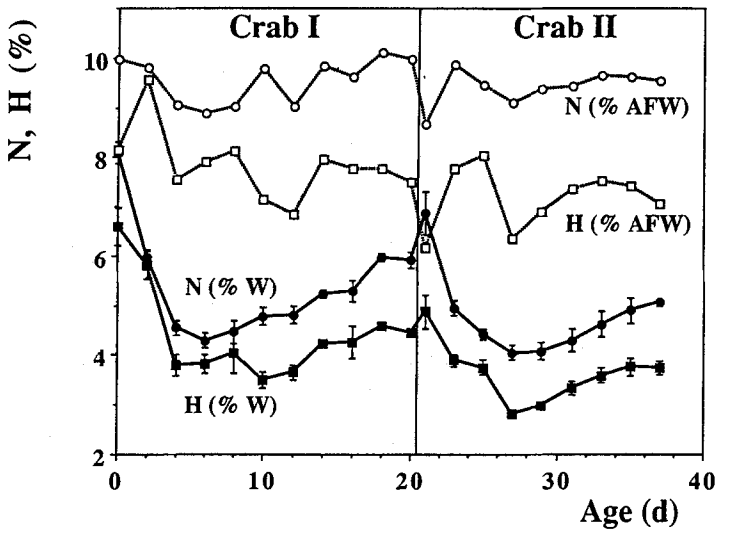


Fig. 5. *Hyas araneus*, crab I and II instars. Nitrogen (N) and hydrogen (H) (in % of W, and of AFW) in relation to age (days, d). Error bars: $\bar{x} \pm SD$

cycles were weaker than those found in total C/N (Fig. 6). As a consequence of decreasing organic C, accompanied by a constant percentage of N (as % AFW), the organic C/N ratio showed a decreasing tendency during the first two juvenile instars (Fig. 6).

Since N remained constant and both organic C and H revealed a decreasing trend during early juvenile growth, there was a decrease also in the sum of these elements

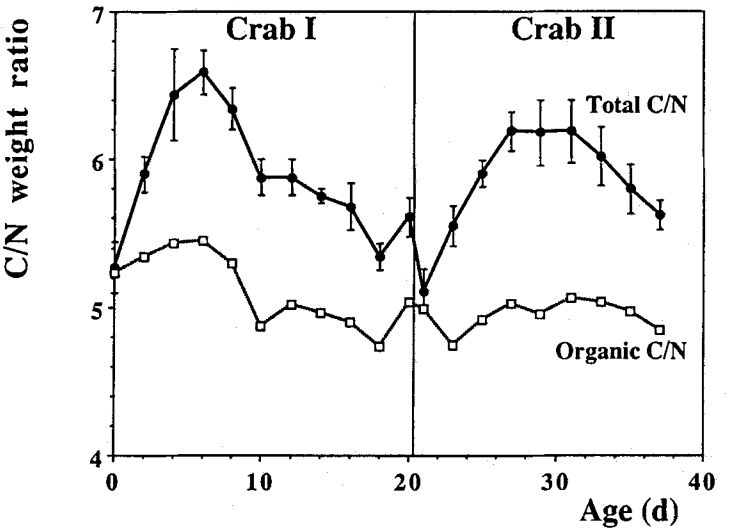


Fig. 6. *Hyas araneus*, crab I and II instars. Total and organic C/N weight ratios in relation to age (days, d). Error bars: $\bar{x} \pm SD$

within AFW. This tendency suggests an increase in the percentage of organic substances that contained proportionally lower amounts of C, N, and H, and higher quantities of other elements (such as phosphorous, oxygen).

Proximate biochemical composition

Changes in the absolute amounts (per individual) of the major biochemical constituents protein, lipid, chitin and carbohydrates are shown in Figure 7. Since no clear recurrent growth patterns were found therein, no attempts were made to describe these changes with regression equations as functions of juvenile age.

Protein constituted consistently the greatest body fraction, showing an almost constant increase during growth. Lipid was present in much smaller quantities, increasing

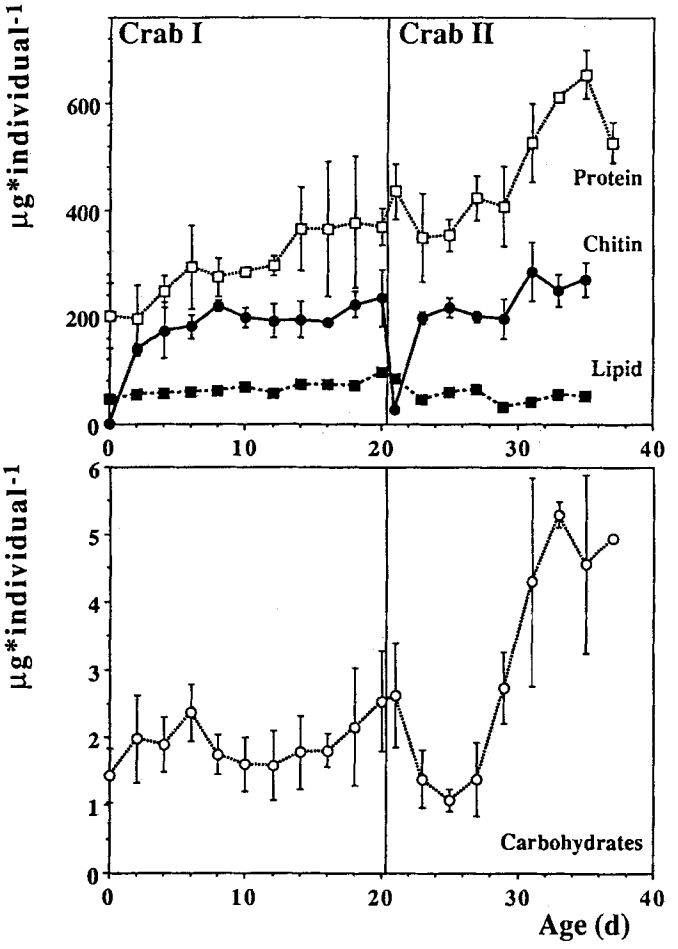


Fig. 7. *Hyas araneus*, crab I and II instars. Growth in the absolute values (in µg·individual^{-1}) of protein, chitin, lipid, and carbohydrates in relation to age (days, d). Error bars: $\bar{x} \pm SD$

during the first, but less in the second instar. Carbohydrates constituted by far the smallest of the biochemical fractions that we measured (consistently below 1 % of AFW). The absolute quantity of carbohydrates increased mainly in the second crab stage, after passing a transitory minimum, 4 days after ecdysis (Fig. 7, lower graph). Chitin showed a clear cyclic pattern, with very low values immediately after ecdysis, a steep increase during the postmoult phase, and constantly high values throughout the rest of the moulting cycle. On the average, chitin was the second largest fraction after protein.

Changes in the relative (% of AFW) biochemical composition of juvenile crabs are shown in Figure 8. Both the fractions of protein and lipid showed a statistically significant decrease with age, with values ranging from 31–50 of AFW in protein, and 3–10 % in lipid. Since this decrease was stronger in the latter class of constituents, also the lipid/

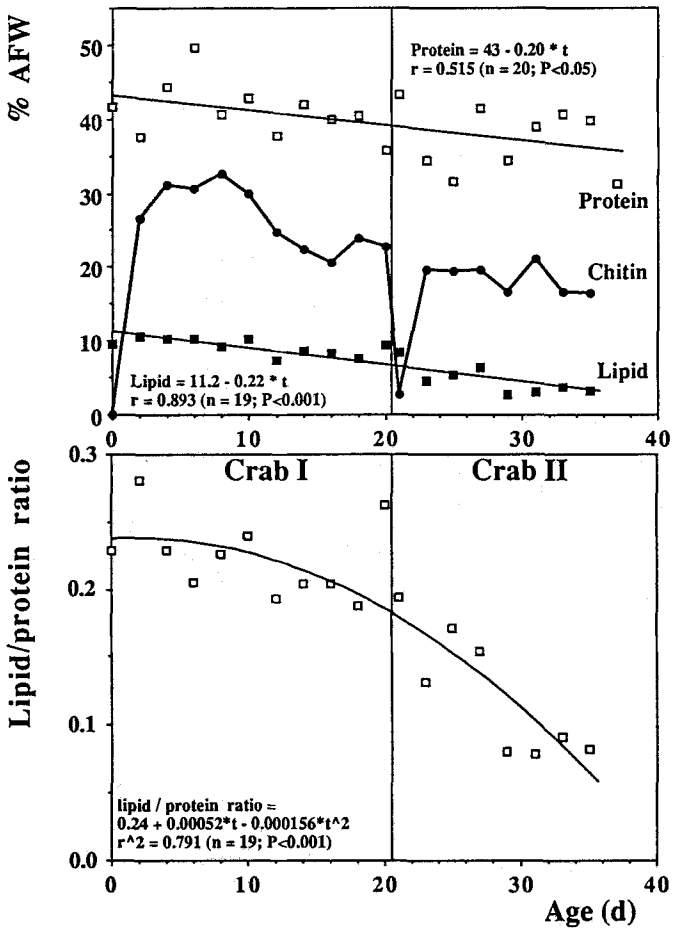


Fig. 8. *Hyas araneus*, crab I and II instars. Protein, chitin, lipid (in % of AFW), and the lipid/protein weight ratio in relation to age (days, d). Regression equations: percentage of protein, lipid, or the lipid/protein ratio as functions of time (t, in days); r: correlation coefficient (in linear regressions); r^2: coefficient of determination; n, P: number of observations, level of significance for $r \neq 0$

protein weight ratio decreased significantly, in particular during the second crab stage (Fig. 8, lower graph). This indicates again (cf. C/N; Fig. 6) a shift in the biochemical composition during early juvenile growth, with a relative increase in the proportion of protein and a decrease in that of lipid.

The average chitin content decreased as well, reaching maximum values of about 30 % and 20 % of AFW, respectively, in the first two instars. Since all major biochemical fractions (protein, lipid, chitin; expressed as % of AFW) decreased during early juvenile growth, and the quantity of carbohydrates did not play a significant role, compounds other than these must have concurrently increased within the organic matter of juvenile crabs.

Protein has in the literature often been estimated from N, using a conversion factor of 6.25. When total N was plotted against protein, a linear relationship was found, however, with a slope that was significantly lower than 6.25 (Fig. 9, upper graph). The slope was slightly steeper (3.95 vs. 3.36), and the coefficient of determination somewhat higher (r^2 = 0.893 vs. 0.875), when non-chitin N was used as a predictor of protein, instead of total N (chitin-N estimated as 6 % of chitin weight; Fig. 9, upper graph). Again, the slope of this regression line was significantly different from 6.25, and the intercept with the y-axis was significantly different from zero. This indicates that there must have been significant amounts of nitrogenous compounds other than protein or chitin in *Hyas araneus* juveniles, or some protein-like substances were not detected with the Lowry method.

Energy content

The energy content of juvenile spider crabs was estimated independently in two ways: (1) from organic C and (2) from biochemical constituents. Figure 10 (upper graph) shows that energy values calculated from C were mostly higher than those obtained from biochemical constituents. There was a significant linear relationship between these values, but the slope was significantly different from a theoretical value of 1 (Fig. 10, lower graph). This means that either C-derived values significantly over-estimated the actual energy content, or biochemical constituents other than those measured in the present study contributed significantly to the total energy content and thus, the sum of the energy contents calculated for the fractions of protein, lipid, chitin, and carbohydrates was lower than the actual energy content.

When the contribution of the major biochemical fractions to the total energy content (calculated from these components) was considered, protein was found to be the greatest contributor (50–73 % of total energy), and its relative importance showed an increasing tendency during early juvenile growth (Fig. 11). Chitin was normally the second most important fraction (up to 30 %), except for early postmoult periods, where its amounts were very low and consequently, there was proportionally more energy in the lipid fraction (up to 28 %). The latter showed a clearly decreasing trend (down to 9 %) during the period of observation (Fig. 11). The pool of carbohydrates contributed consistently < 1 % of total energy.

Exuvial losses

Exuviae were analysed only in the crab I instar. Since their W, AFW, and CHN values (including inorganic C) were very similar both in absolute terms and in their relative

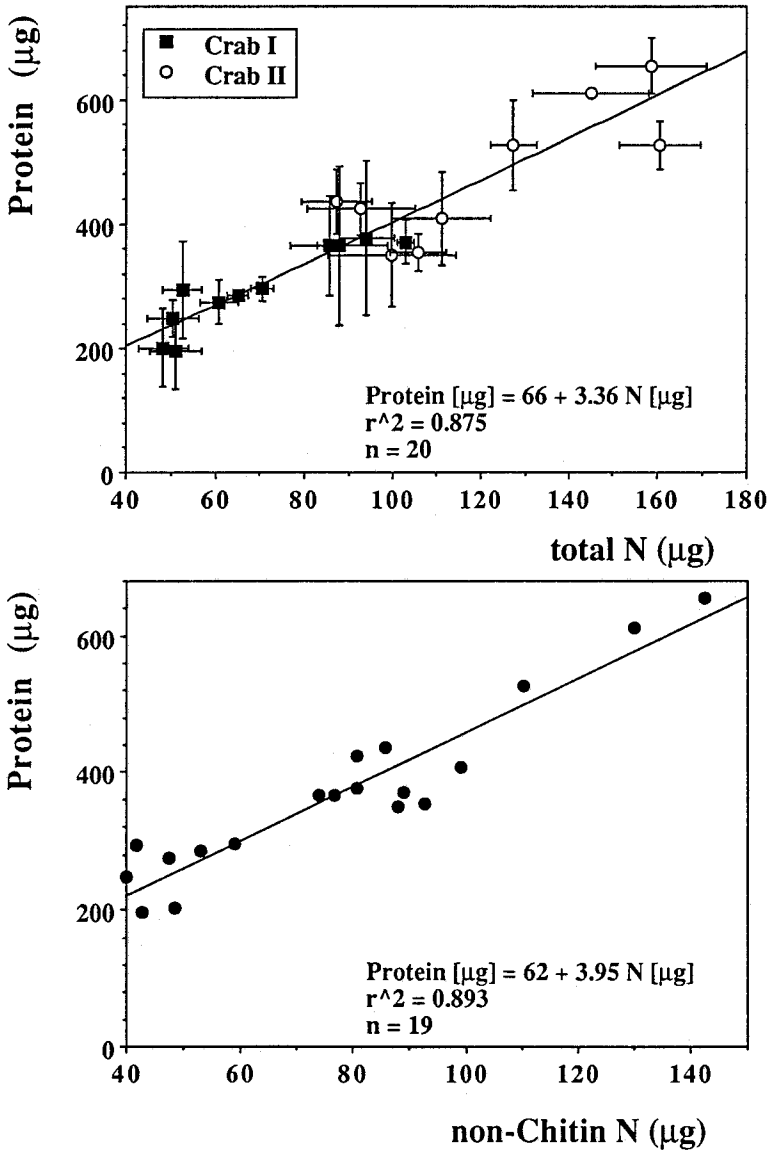


Fig. 9. *Hyas araneus*, crab I and II instars. Protein in relation to total and non-chitin N (all in µg), linear regression equations. r^2: coefficient of determination; n: number of observations ($P < 0.001$)

composition with those obtained in a previous study (Anger, 1984), the new data are not shown in a table or a graph. The organic fraction (AFW in % of W) of exuviae was somewhat higher in our present material as compared to that in the previous study (24 vs. 21–22 %). The ash content of crab I exuviae amounted to 76 % of W, with 9 % of the ash

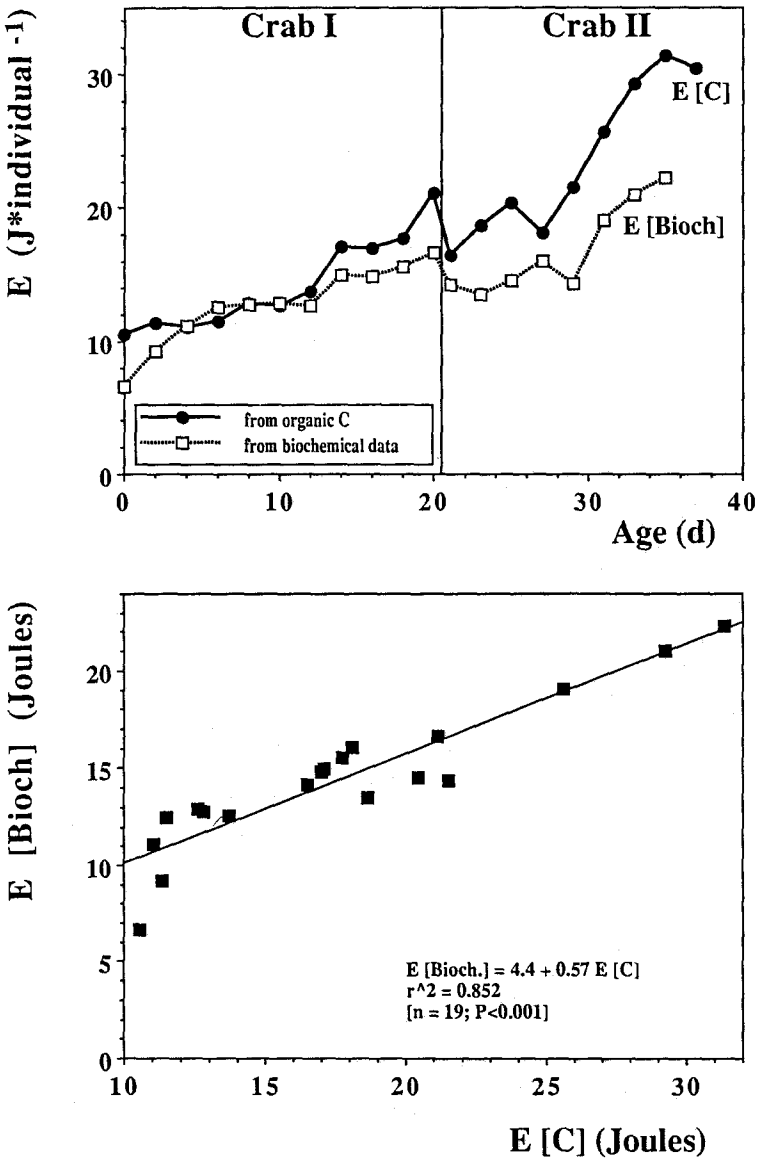


Fig. 10. *Hyas araneus*, crab I and II instars. Upper graph: Energy content (E, in Joules·individual^{-1}) in relation to age (days, d); E [C] calculated from organic C (Salonen et al., 1976); E [Bioch] from biochemical constituents (Winberg, 1971). Lower graph: relationship between E [C] and E [Bioch], linear regression equation; r^2: coefficient of determination; n, P: number of observations, level of significance for $r \neq 0$

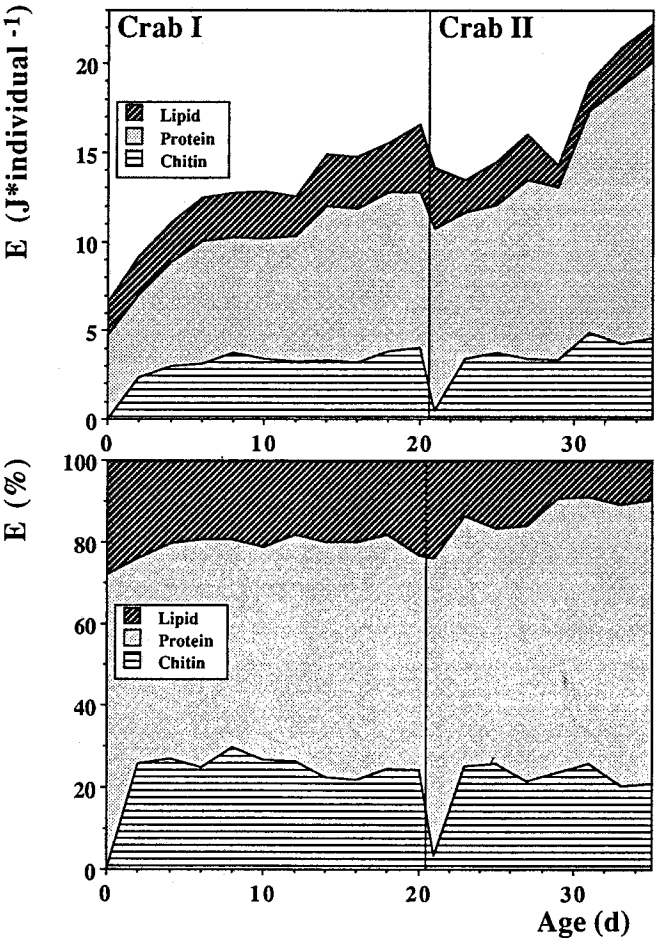


Fig. 11. *Hyas araneus*, crab I and II instars. Energy content (E) of major biochemical constituents in relation to age (days, d). Upper graph: E in Joules-individual^{-1}; lower graph: E given in % of total energy calculated from biochemical constituents

being inorganic C. As a consequence, as much as 43 % of total C in the exuviae was inorganic.

From these data on exuvial matter and composition, percent ecdysial loss may be calculated, related either to LPM matter or to previous growth. Exuvial loss amounted to 37 % of LPM values of body W. Most of this loss comprised inorganic matter, since 76 % of total LPM ash was cast with the exuvia, whereas most organic matter (AFW) was retained by the crabs (15 % loss). The same pattern could be observed in C: as much as 74 % of LPM inorganic C, but only 11 % of organic C were lost with the shed exoskeleton. Exuvial losses in N and H amounted to 5–6 % of LPM values.

When exuvial loss was expressed as a percentage of the growth that had been

achieved during the crab I moulting cycle, again high losses were found in total W (57 %), ash (83 %), and inorganic C (77 %), but low figures in precedingly accumulated organic matter (29 % AFW; 21 % organic C; 18 % N; 24 % H).

Respiration and net growth efficiency

Oxygen consumption per individual (R) did not show a consistent pattern of change during an individual moulting cycle. R values were significantly higher in early postmoult crab II than at any time during the preceeding instar (Fig. 12). When R was expressed as a fraction of body C respired per day (carbon-specific respiration rate), it was found to vary in the range of ca 2–4 % $C \cdot d^{-1}$.

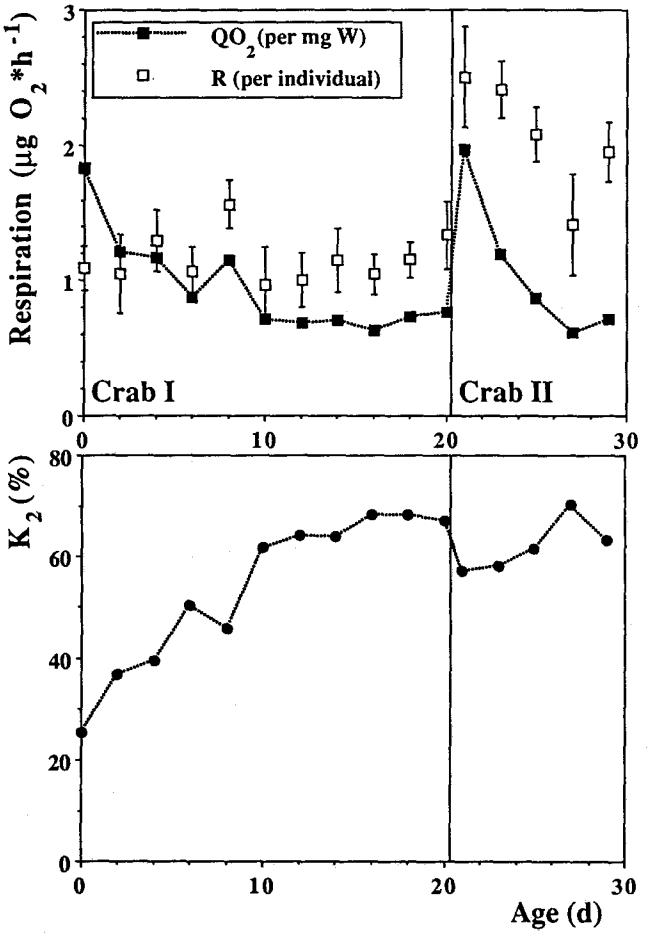


Fig. 12. *Hyas araneus*, crab I and II instars. Upper graph: Respiration (µg $O_2 \cdot h^{-1}$) per individual (R) and per mg W (QO_2) in relation to age (days, d). Error bars: $\bar{x} \pm$ SD. Lower graph: Net growth efficiency (K_2: growth in % of carbon-assimilation)

Weight-specific respiration rate (QO_2), which is a measure of the metabolic intensity of tissues, was consistently high in early postmoult, decreasing subsequently, and constantly low in later stages of the moult cycle (however, the latter part of this pattern is not certain in the second instar, because too few data were available here; Fig. 12).

Instantaneous growth rates (expressed as µg organic C per individual per day) may be calculated by first derivation of the regression equations describing organic C as a function of age in each juvenile instar (Figs 1, 2). If excretion is assumed to comprise exclusively ammonia production (or urea is neglected), the sum of respiration and growth should represent assimilation, and C-based net growth efficiency (K_2) may be estimated. Since growth rates showed an increasing trend (Figs 1, 2), while respiration was rather constant (Fig. 12, upper graph), K_2 showed an increasing tendency during juvenile growth in the first two juvenile instars of *Hyas araneus* (Fig. 12, lower graph).

DISCUSSION

Growth of early juvenile *Hyas araneus* was measured primarily as an increase in absolute biomass (W, AFW, CHN, biochemical constituents). It was in the present material higher than that observed by Anger et al. (1983) and Anger (1984), but lower than the values reported by Anger & Hirche (1990), although in all these studies juvenile crabs had been reared under identical conditions.

In addition to this intraspecific variation in the average absolute amounts of biomass, variation was found also in the relative proportions of single constituents, and in the patterns of absolute change. For instance, Anger & Hirche (1990) found parabolic growth curves (with decreasing instantaneous growth rates) in W and total C of the crab I instar, and a linear increase (i.e. a constant growth rate) in the N fraction. These patterns are very common also in larval growth of decapod crustaceans, including *H. araneus* (Anger, 1991). The material used in the present study, in contrast, showed in the first two crab stages a linear growth pattern in total C, and parabolic curves (with increasing instantaneous growth) in N (Fig. 1). Since no other data with a high temporal resolution of sampling during individual moulting cycles of early juvenile crabs is available, it must remain uncertain which of these patterns is typical, and by what internal or external factors growth patterns are influenced.

Variability occurs mainly in the amounts of organic constituents per individual, whereas the absolute quantities of ash and inorganic C were found to be very similar in different studies. This was particularly obvious in the composition of exuvial dry matter, two thirds of which are presumably calcium carbonate (estimated from inorganic C; Anger, 1984). The major components of exuviae, i.e. ash and inorganic C, accumulate very rapidly (faster than organic matter) during the early postmoult phase of the moult cycle. Thereafter they remain constant, while organic constituents continue to increase, as long as growth conditions (e.g. food availability) remain favourable. These patterns lead to an initial increase in the percentage of inorganic matter, subsequently followed by a decrease (Fig. 3).

Besides direct effects of food or other external factors on juvenile growth of crabs, their elemental and biochemical body composition may be influenced by previous conditions of life, such as temperature during embryonic development (Kunisch & Anger, 1984), or nutrition during larval development (Harms et al., 1991). Growth of decapod

crustaceans is also subject to significant genetic variation (e.g. van Olst et al., 1980), which may be reduced to some degree by differential mortality in under-sized, possibly also in over-sized individuals of a population (Kunisch & Anger, 1984).

Besides changes in the relation between organic and inorganic constituents, there are obvious shifts in the elemental and biochemical composition of both the whole body and the exuvial matter of juvenile crabs. Changes in the percentage of inorganic C within the ash fraction (Fig. 3) indicate that the mineral composition of ash changes during the moulting cycle, with very low calcium carbonate values after ecdysis, a rapid increase during the postmoult period, and a constantly high level thereafter. Later, shed exuviae showed the same mineral composition (inorganic C amounting to about 9 % of ash) as complete intermoult and premoult crabs. This indicates that almost all inorganic C had been accumulated in and then lost with the exoskeleton. Data presented by Anger (1984) suggest that these patterns remain consistent also in later juvenile instars. Such periodic changes in the mineral content of juvenile crabs exert a strong influence on the percentage of W values of all elements and compound classes that are associated with organic matter (Figs 4, 5). Similar moult-cycle related variation in elemental and biochemical composition has been found also in the larval stages of *H. araneus* (Anger et al., 1989) and many other decapod species (Anger, 1991), suggesting similar periodic patterns of growth throughout their life cycle.

Changes within the organic fraction can be made visible in the organic C/N ratio (Fig. 6), or when biochemical constituents are expressed as a percentage of AFW (Fig. 8). These data (% AFW) showed in the two first juvenile stages of *H. araneus* a steady decrease in the major organic constituents, protein, lipid, and chitin; the same applied to organic C and H (Figs 4, 5). The sum of all four biochemical constituents that were measured in this study decreased from maximum values of about 90 % of AFW in the early crab I instar, to minimum values of ca 60 % at the end of the second crab stage. These values compare favourably with a total of 85 % measured by Speck et al. (1972) in a crayfish, *Orconectes limosus*. Since lipid decreased in *H. araneus* at a higher rate than protein, and C faster than N, decreasing tendencies were detected also in the lipid/protein and C/N ratios (Figs 6, 8). Carbohydrates (present paper) and nucleic acids (Anger & Hirche, 1990) occur only in minor quantities and, thus, cannot explain this decline. Hence, no explanation can be given at present of what other organic constituents may replace the compounds and elements that decrease within the AFW fraction.

Besides inorganic constituents, only chitin showed a clear periodic pattern related to the moulting cycle (Figs 7, 8). Similar changes were found by Spindler-Barth (1976) in *Carcinus maenas*. All these fractions are associated with the formation of the exoskeleton.

A cyclic pattern occurred also in carbohydrates; however, this was clearly visible only in the second crab instar (Fig. 7). Similar patterns were observed by Renaud (1949) in *Cancer pagurus* and Spindler-Barth (1976) in *C. maenas*. Decreasing values during the postmoult period were probably caused by a high turnover rate, due to polymerisation of free sugars into chitin macromolecules of the cuticle. This decrease was followed by increasing carbohydrate values in the intermoult period, probably indicating a decreasing turnover and, hence, an increasing pool of glucose and glucosamin which are needed in the premoult phase for chitin synthesis, when a new cuticle is secreted beneath the old one (Speck & Urich, 1972; Gwinn & Stevenson, 1973). In late premoult, a part of the old

cuticle is resorbed and utilized both as an energy source and as a pool of precursors for the synthesis of new cuticle material (Speck & Urich, 1971, 1972).

The average biochemical composition of juvenile *H. araneus* is on the average similar to that in other juvenile or adult decapods, as well as to that found in the larval stages of the same species (Anger et al., 1989): protein was consistently the dominant fraction of AFW, followed by chitin (except in the early postmoult periods of the moulting cycle), and lipid. Carbohydrates other than chitin play only a minor role in the biochemical composition of crabs. Protein amounted to 31–50 % of AFW (or 15–34 % of W), which is similar to values found in previous studies on larval and juvenile *H. araneus* (Anger et al., 1983; Anger & Hirche, 1990), and comparable with an average value of 42 % of AFW (31 % of W) found in a crayfish (Speck et al., 1972).

The maximum (intermoult) chitin content of whole spider crab AFW was 33 % in the first, and 21 % in the second crab instar (corresponding to 16 and 10 % of W, respectively). Speck et al. (1972) found ca 22 % of AFW (16 % of W) in *O. limosus,* and Hornung & Stevenson (1971) 13 % of W in another crayfish, *O. obscurus.* In the edible crab, *Cancer pagurus,* and the shore crab, *Carcinus maenas,* only ca 6–7 % of W were determined as chitin (Drach & Lafon, 1942; Spindler-Barth, 1976).

The lipid fraction within AFW amounted in juvenile spider crabs to only 3–10 % (i.e. 1.2–7.7 % of W). This is similar to a value of 3.3 % of AFW (2.4 % of W) measured in a crayfish (Speck et al., 1972), whereas Renaud (1949) found in *C. pagurus* a higher lipid content (8–16 % of W). Higher values, ranging between 18 and 30 % of AFW (8–24 % of W) were found also in the larval stages of *H. araneus* (Anger et al., 1983, 1989). Like the data on juvenile growth (cf. Fig. 8, lower graph), the latter data suggest that there is during larval development a decreasing tendency in the proportion of lipids in relation to other biochemical constituents, gradually leading to a protein-oriented metabolism. This is corroborated by a decreasing average atomic O/N ratio (oxygen respired in relation to nitrogen excreted; Anger et al., 1989).

Quantitative relationships between elemental and biochemical constituents of biomass may be used to convert such data from the one to the other. The most commonly used conversion is that of nitrogen to protein, applying a simple theoretical factor of 6.25 (e.g. Raymont et al., 1964). In decapod larvae, including those of *H. araneus,* much lower factors were found (Anger & Harms, 1990), suggesting significant amounts of non-protein N. The same was observed also in juvenile crabs, even after chitin-N was substracted from total N (Fig. 9).

While organic C may be considered a useful integrator of biomass, the sum of protein, lipid, chitin, and carbohydrates covered only 60–91 % of AFW. The main pool of energy was found in the protein fraction (Fig. 11), although lipids have almost double the energy content per unit of weight (Winberg, 1971). Significant amounts (up to about 30 %) of biochemical energy are bound in chitin of the exoskeleton (Fig. 11) and, thus, lost with the exuvia. The gap between energy values estimated from C and those from biochemical constituents (Fig. 10, upper graph), as well as the slope < 1 in the regression line that links these values with each other (Fig. 10, lower graph), may be explained with a significant contribution to total energy by biochemical compounds other than those measured here.

Individual oxygen consumption (R) of juvenile *H. araneus* was, on the average, higher than that of the preceding megalopa and other larval stages of the same species,

while carbon-specific and weight-specific respiration (QO_2) values were lower (cf. Anger, 1990; Anger et al., 1989). As in all larval stages of this, and of other decapod species (Anger, 1991), QO_2 was maximum in early postmoult, then it decreased, and eventually remained fairly constant in the intermoult and later phases of the moulting cycle (Fig. 12). This correspondence suggests a consistent pattern of metabolic activity in tissues of decapods, possibly throughout their life cycle. It is mainly caused by the formation of chitin and inorganic materials in the cuticle which do not take part in metabolic activity of the animal.

When instantaneous growth rates (expressed as units of organic carbon per individual and per unit of time) are calculated from regression curves (Figs 1, 2) and related to metabolic combustion of organic C, C-based net growth efficiency, K_2, may be calculated (Fig. 12, lower graph). The same can be done in energy units instead of C (Gnaiger, 1983). The result is in both cases an increasing trend in K_2 during growth of the first two juvenile instars of *H. araneus*. This is at variance with previous findings in larvae of this and other decapod species (Anger, 1991) and might indicate a major difference between food conversion and growth strategies of larval and juvenile stages. However, the body of bioenergetic data on early juvenile crabs is at present very poor, and further comparative studies are needed for safe conclusions.

LITERATURE CITED

Anger, K., 1984. Gain and loss of particulate organic and inorganic matter in larval and juvenile spider crabs (*Hyas araneus*) during growth and exuviation. – Helgoländer Meeresunters. *38*, 107–122.

Anger, K., 1990. Modelling developmental changes in the carbon and nitrogen budgets of larval brachyuran crabs. – Helgoländer Meeresunters. *44*, 53–80.

Anger, K., 1991. Developmental changes in the bioenergetics of decapod larvae. – Mem. Qd Mus. *31*, 289–308

Anger, K. & Harms, J., 1990. Elemental (CHN) and proximate biochemical composition of decapod crustacean larvae. – Comp. Biochem. Physiol. *97B*, 69–80.

Anger, K. & Hirche, H. J., 1990. Nucleic acids and growth of larval and early juvenile spider crab *Hyas araneus*. – Mar. Biol. *105*, 403–411.

Anger, K., Harms, J., Püschel, C. & Seeger, B., 1989. Physiological and biochemical changes during the larval development of a brachyuran crab reared under constant conditions in the laboratory. – Helgoländer Meeresunters. *43*, 225–244.

Anger, K., Laasch, N., Püschel, C. & Schorn, F., 1983. Changes in biomass and chemical composition of spider crab (*Hyas araneus*) larvae reared in the laboratory. – Mar. Ecol. Prog. Ser. *12*, 91–101.

Bulnheim, H.-P., 1974. Respiratory metabolism of *Idotea balthica* (Crustacea, Isopoda) in relation to environmental variables, acclimation processes and moulting. – Helgoländer wiss. Meeresunters. *26*, 464–480.

Drach, P. & Lafon, M., 1942. Études biochimiques sur le squelette tégumentaire des décapodes brachyoures (variation au cours du cycle d'intermue). – Archs Zool. exp. gén. *82*, 100–118.

Gnaiger, E., 1983. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Polarographic oxygen sensors. Ed. by E. Gnaiger & H. Forstner. Springer, Berlin, 337–345

Gwinn, J. F. & Stevenson, J. R., 1973. Role of acetylglucosamine in chitin synthesis in crayfish. I. Correlation of ^{14}C-acetylglucosamine incorporation with stages of the molting cycle. – Comp. Biochem. Physiol. *45B*, 769–776

Harms, J., Anger, K., Klaus, S. & Seeger, B., 1991. Nutritional effects on ingestion rate, digestive enzyme activity, growth, and biochemical composition of *Hyas araneus* L. (Decapoda: Majidae) larvae. – J. exp. mar. Biol. Ecol. *145*, 233–265.

Heath, J. R. & Barnes, H., 1970. Some changes in biochemical composition with season and during the moulting cycle of the common shore crab, *Carcinus maenas* (L.). – J. exp. mar. Biol. Ecol. *5*, 199–233.

Hirota, J. & Szyper, J. P., 1975. Separation of total particulate carbon into inorganic and organic components. – Limnol. Oceanogr. *20*, 896–900.

Holland, D. L. & Gabbott, P. A., 1971. A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. – J. mar. biol. Ass. U.K. *51*, 659–668.

Hornung, D. E. & Stevenson, J. R., 1971. Changes in the rate of chitin synthesis during the crayfish molting cycle. – Comp. Biochem. Physiol. *40B*, 341–346.

Kunisch, M. & Anger, K., 1984. Variation in development and growth rates of larval and juvenile spider crabs *Hyas araneus* reared in the laboratory. – Mar. Ecol. Prog. Ser. *15*, 293–301.

Lowry, D. H., Rosenberg, N. J., Farr, A. L. & Randall, R. J., 1951. Protein measurement with the folin phenol reagent. – J. biol. Chem. *193*, 265–275.

McMahon, B. R. & Wilkens, J. L., 1983. Ventilation, perfusion, and oxygen uptake. In: The biology of Crustacea. Ed. by L. H. Mantel. Acad. Press, New York, *5*, 289–372.

Olst, J. C. van, Carlberg, J. M. & Hughes, J. T., 1980. Aquaculture. In: The biology and management of lobsters. Ed. by J. S. Cobb & B. F. Phillips. Acad. Press, New York, *2*, 333–384.

Raymont, J. E. G., Austin, J. & Linford, E., 1964. Biochemical studies on marine zooplankton. I. The biochemical composition of *Neomysis integer*. – J. Cons. perm. int. Explor. Mer *28*, 354–363.

Renaud, L., 1949. Le cycle des réserves organiques chez les crustacés Décapodes. – Annls Inst. océanogr. Monaco *24*, 259–357.

Sachs, L., 1984. Angewandte Statistik. Springer, Berlin, 552 pp.

Salonen, K., Sarvala, J., Hakala, I. & Viljanen, M.-L., 1976. The relation of energy and organic carbon in aquatic invertebrates. – Limnol. Oceanogr. *21*, 724–730.

Speck, U. & Urich, K., 1971. Quantitative Bedeutung der Reservestoffe für Chitinsynthese, Energiestoffwechsel und osmotische Vorgänge während der Häutung des Flußkrebses *Orconectes limosus*. – Z. vergl. Physiol. *71*, 286–294.

Speck, U. & Urich, K., 1972. Resorption des alten Panzers vor der Häutung bei dem Flußkrebs *Orconectes limosus*. Schicksal des freigesetzten N-Acetylglucosamins. – J. comp. Physiol. *78*, 210–220.

Speck, U., Urich, K. & Hahmann, R., 1972. Der Stoffbestand des Flußkrebses *Orconectes limosus*. Jahreszyklus und Organverteilung. – J. comp. Physiol. *77*, 287–305.

Spindler-Barth, M., 1976. Changes in the chemical composition of the common shore crab, *Carcinus maenas*, during the molting cycle. – J. comp. Physiol. *105*, 197–205.

Winberg, G. G., 1971. Methods for the estimation of production of aquatic animals. Acad. Press, London, 175 pp.

Zöllner, N. & Kirsch, K., 1962. Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion. – Z. ges. exp. Med. *135*, 545–561