

Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action

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ABSTRACT: Rates of clearance, ingestion, ammonia excretion, respiration and egg production were measured in food-acclimated (0 to 1700 $\mu\text{g C l}^{-1}$) planktonic copepods *Acartia tonsa* in relation to food concentration. Carbon and nitrogen budgets were constructed. Clearance peaked at a food concentration of 150 $\mu\text{g C l}^{-1}$, and decreased at both higher and lower concentrations. Ingestion and egg production rates increased sigmoidally with food concentration approaching plateaus equivalent to 180 and 64 % body C d^{-1} , respectively. Rates of ammonia excretion and respiration increased with algal concentration in a decelerating manner. Respiration and excretion rates of copepods fed at saturation food concentration were more than 4 times higher than those for starved individuals. The causality of the increased respiration rate in association with feeding (specific dynamic action, SDA) is discussed by considering the physiology and biochemistry of the processes that potentially contribute to SDA. The theoretical biochemical minimum costs of biosynthesis accounted for between 50 and 116 % of observed SDA, while assimilation costs equalled 18 to 28 %. Costs of feeding, digestion and excretion (~1 % of SDA), and the mechanical work required to transport food down the gut, contributed insignificantly to SDA. It is concluded that the increment in metabolic rate of feeding *A. tonsa* largely relates to biosynthesis ('growth') and transport, and that the efficiency of egg production in this species is near its theoretical maximum.

INTRODUCTION

An increment in metabolic rate or heat production in association with feeding has been termed 'specific dynamic action' (SDA) or 'heat increment of feeding'. It has been recorded in a variety of animals including man (e.g. Ganong 1973), ruminants (e.g. Webster 1981), reptiles (Coulson & Hernandez 1979), fish (e.g. Jobling 1981) and also among a number of invertebrates, such as molluscs (e.g. Bayne & Scullard 1977), echinoderms (e.g. Vahl 1984) and crustaceans (e.g. Wallace 1973, Abou Debs 1984). The phenomenon has attracted considerable interest among ecologists and nutritionists ever since it was first demonstrated about 200 yr ago by Seguin and Lavoisier (see e.g. Krebs 1964).

In spite of this interest, the causal relations of SDA are still incompletely understood. The SDA potentially

includes the cost of feeding (e.g. Bayne & Scullard 1977), intestinal work (e.g. Tandler & Beamish 1979), digestion, absorption and transport, amino acid oxidation and urea synthesis (Krebs 1964), and biomass formation/growth (e.g. Ashworth 1969, Jobling 1983). Formerly, SDA was considered to be the 'cost of feeding' by ecologists but it is now clear that it is mainly a post-prandial phenomenon (e.g. Coulson & Hernandez 1979, Jobling 1981, 1983). Ashworth (1969) suggested that SDA primarily represents the 'cost of growth' and that the observed increment in respiration is mainly due to biosynthetic work. However, the evidence is not unequivocal (e.g. Jobling 1981). To further elucidate the problem, Jobling (1981) called for studies relating metabolic rate to growth rate. However, since feeding and growth rate (and indeed all the processes listed above) are obviously interrelated, correlation analysis can not be used *per se* to establish definite causalities.

The biochemistry and physiology of the orchestration of processes that potentially comprise SDA must be considered as well.

The approach taken in this study was to measure as many of the relevant rate parameters as possible and, subsequently, to calculate the energetic cost of each of these (from biochemical and physiological considerations) in order to further elucidate the energetic composition of SDA. We selected adult females of the planktonic copepod *Acartia tonsa* for this purpose. Growth in adult females is mainly in the form of egg production; somatic growth is limited (no moltings) and zero in steady state. Thus, 'growth' was measured as rate of egg production in females in energetic equilibrium. Since *A. tonsa* may spawn up to 50 eggs d^{-1} , this permitted short-term experiments. More important, there is no turnover of deposited eggs, thus considerably simplifying the calculation of cost of biosynthesis. The parameters measured included clearance, ingestion, ammonia excretion (\sim protein catabolism), growth and respiration (\sim metabolism).

MATERIAL AND METHODS

Copepods were obtained from a culture of *Acartia tonsa*. Adult copepods were held in 200 l tanks at 16 to 18°C and 27 ‰ salinity and were fed the phytoplankton algae *Isochrysis galbana* and *Rhodomonas baltica*. Eggs spawned within 24 h were sucked from the bottom of the cultivation tank and transferred to a 100 l tank where hatching and subsequent growth and maturation took place. Egg-production in *A. tonsa* is high and stable during 3 to 4 wk after maturation, whereupon it declines (e.g. Parrish & Wilson 1978). Therefore, only fertilized females (i.e. females carrying a spermatophore) that had matured within the previous 1 to 2 wk were used for experiments.

In all experiments, *Rhodomonas baltica* were used as food. The algae were cultivated in a continuous culture at a constant dilution rate (approximately $1/3 d^{-1}$) and illumination ($120 \mu E m^{-2} s^{-1}$). Cell length and width were approximately 13 and 8 μm , respectively. All experiments were carried out at 18°C and 27 ‰ S.

Dry weight and cephalothoracic length of female copepods were determined in representative specimens and a length (L, mm cephalothorax length)–weight (W, μg dry wt) relation of $13.4 L^3$ was used to convert length measurements to weight estimates. Average cephalothorax length of females used was 0.82 mm. Dry weight, C and N content were measured in batches of 400–600 copepod eggs that were individually pipetted into glass beakers, and in samples of algae that were concentrated by centrifugation (Carlo-Erba CHN-analyses). Dry weight, C and N in eggs and algae are shown in Table 1.

Ingestion and egg production. Rate of ingestion was estimated by incubating adult females in glass jars. Ten to 15 individuals were added to each of four 600 ml screw-cap bottles containing a suspension of *Rhodomonas baltica* at the desired concentration. Two bottles without copepods served as controls. Concentrated algal growth medium was added to each bottle, sufficient to prevent nutrient limitation of algal growth during incubations. The bottles were fixed on a slowly rotating (2 rpm) wheel in a temperature controlled room with a light:dark cycle of 12 h:12 h. The copepods were incubated for 48 to 72 h. Food concentrations at onset and termination of grazing experiments were recorded by TAI Coulter Counter fitted with a 100 μm aperture tube. At the lowest food concentrations, changes in algal concentration during incubations were also measured by visual counts of cell numbers under the inverted microscope in order to correct for potential production of particles by grazing copepods. The concentration of algae decreased by 25 to 50 % during incubations. Upon termination of an experiment, the contents of the bottle were gently poured through a submerged 180 μm mesh sieve and number and activity of copepods controlled. Dead copepods were removed (mortality < 5 %). Thereupon copepods were transferred to another bottle containing the same initial concentration of phytoplankton cells. During the 4 subsequent days, egg production was measured. Copepods were transferred daily to new bottles containing fresh suspensions of algae. Numbers of copepods and eggs spawned during the previous 24 h were counted (empty shells included). Egg canni-

Table 1. Dry weight, C- and N-content of algae and copepod eggs. Average \pm SD; n = number of determinations

| Species | C | N | C/N | dry wt | n |
|---------------------------|---|--|-----------------|---------------------------------------|----|
| <i>Rhodomonas baltica</i> | pg cell ⁻¹ 36.7 \pm 1.9 | pg cell ⁻¹ 7.5 \pm 0.7 | 4.93 \pm 0.32 | pg cell ⁻¹ 81* | 12 |
| <i>Acartia tonsa</i> eggs | ng egg ⁻¹ 45.7 \pm 3.0 | ng egg ⁻¹ 9.1 \pm 3.0 | 5.05 \pm 0.08 | ng egg ⁻¹ 104 \pm 7.8 | 2 |

* Assuming a carbon content equal to 45% of dry weight

balism occurred in the bottles. However, empty shells are left by the copepods; they constituted less than 10 % of total egg number (average: 3.0 %). Upon termination of the experiment, cephalothoracic lengths of ca 10 females were measured. In some experiments, only ingestion or egg production was measured. Altogether, 15 grazing and 13 egg-production experiments (each with 4 replicates) were conducted at algal concentrations between 1000 and 45000 cells ml⁻¹.

Ingestion rate, clearance, and average concentration of algae during incubations in feeding experiments were calculated according to the equations of Frost (1972). In the case of egg-production experiments, average food concentration and ingestion rate during incubations were calculated from the previously measured clearances, again employing the formulae of Frost. Egg cannibalism was not included in ingestion estimates, but was always less than 5 % and averaged 1.1 % of algal grazing. The dependence of ingestion rate or egg production on food concentration was described by the sigmoid model

$$A = A_{\max}e^{-b/C} \quad (1)$$

where A_{\max} = maximum ingestion or egg-production rate; C = food concentration; b = a constant (Kiørboe et al. 1982). The model was fitted to experimental data by linear regression after logarithmic transformation. The clearance-food concentration relation is derived from the model by dividing by C on both sides of the equation.

Respiration rate. Females used for the respiration-rate measurements were acclimated to the food concentration tested for 2 d prior to measurements. We adopted the open-system technique developed by Møhlenberg & Kiørboe (1981) for measurement of respiration in feeding bivalves, here scaled down to copepod size.

The respiration chamber consisted of a glass tube (inner diameter: 0.7 cm, length: 1.5 to 2.0 cm) closed with a silicone stopper in each end. A needle of stainless steel inserted through each stopper allowed a constant flow of water (and food) through the chamber during measurement. Ten to 20 fertilized females were placed in the respiration chamber that was connected serially to a thermostated oxygen electrode (Radiometer) and a peristaltic pump. The tube between chamber and electrode was as short as possible (5 cm) and fitted with a copper thread to prevent bacterial growth (and respiration). The chamber and the inflow needle were submerged in a suspension of algae that was bubbled with air to ensure atmospheric air saturation. The concentration of algae in this suspension was adjusted so that the concentration in the respiration chamber would approximate the acclimation food level, taking into consideration flow rate and copepod clearance

rate. Numbers of copepods in the chamber and flow rate of water were selected so that oxygen partial pressure in outflow water was reduced by about 5 %.

The copepods were allowed to reduce the oxygen content in the chamber prior to start of the peristaltic pump for V/F hours (F = flow rate, ml h⁻¹; V = chamber volume, ml). After this length of time the subsequent equilibrium partial pressure is reached. With the flow rates (1 to 2 ml h⁻¹) and chamber volumes (0.6 to 0.8 ml) in question, this equaled on average about half an hour. After starting the pump, oxygen content was monitored for about 1 h to ensure that the system was in equilibrium. Blank measurements were made without copepods in the chamber and were run between every second measurement. Chamber respiration was normally low but values corresponding to up to 20 % of the respiration rate were accepted. Respiration rate was calculated by multiplying the percentage reduction in oxygen partial pressure in outflow water (blank adjusted), flow rate, and saturation oxygen concentration (considering pressure), and dividing by numbers of copepods in the chamber. Cephalothoracic lengths were measured in representative specimens. In all, 89 respiration rate measurements were made at 4 food concentrations, viz. 0, 2500, 10000 and 25000 cells ml⁻¹.

Additional measurements of respiration of individual females were obtained with the alternative gradient ampulla diver technique (Nexø et al. 1972, Zeuthen & Hamburger 1977, Riisgård et al. 1981). Again, copepods were acclimated for 2 d prior to experiments. The divers had the following average dimensions: total volume = 100 µl, volume of gas phase = 30 µl and volume of sea water with copepod = 15 µl. The positions of the divers were recorded photographically at intervals of 15 min for up to 20 h. Usually, the rate of migration of the diver was constant within the initial 5 h indicating a constant respiration rate. Straight lines were fitted by eye to the observations. From the slope of this line, the respiration was calculated. At the end of each experiment, the copepod was removed from the diver and the cephalothorax length was measured; 41 measurements were made on copepods acclimated to 2500 and 25000 cells ml⁻¹.

A series of measurements were made to study the effect of duration of starvation on respiration rate. Copepods fed at saturation food levels for 3 d were transferred to filtered seawater, and respiration of subsamples of copepods were measured daily (electrode) during the 4 subsequent days.

Ammonia excretion rate was measured in order to quantify the rate of protein catabolism, as ammonia constitutes more than 80 to 90 % of excreted nitrogen in copepods (Corner & Newell 1967, Mayzaud 1973,

Benson & Aldrich 1981). Copepods acclimated for 2 d to 0, 2500, 10000 or 25000 cells ml⁻¹ were gently washed in GF/C filtered water and then transferred to 150 ml beakers containing 100 ml filtered sea water. A sample of 25 ml was immediately taken to determine the initial NH₄⁺ concentration. An additional sample was taken after 1 to 2 h (pilot experiments showed that the NH₄⁺ excretion rate of food-acclimated copepods was constant during 2 to 3 h after which a decrease became evident). A blank without copepods was run in parallel. At the termination of an experiment, the number of copepods (50 to 100) was determined and cephalothorax length measured in 8 to 10 individuals. Ammonia was determined in the 1 µm filtered samples using the phenolhypochlorite method (Solarzano 1969). A total of 27 experiments was run.

RESULTS

Ingestion and egg production

Ingestion and egg production rates as well as food concentrations are expressed here in carbon units. Since eggs and algae had similar C:N ratios of about 5, conversion to nitrogen units can be obtained by dividing the axes by 5.

Ingestion rate increased sigmoidally with food concentration and approached a plateau of 0.71 µg C µg dw⁻¹ d⁻¹, equivalent to 180 % bodily carbon d⁻¹ (if a carbon content of 40 % of body dry weight is assumed; Parsons et al. 1977) (Fig. 1). Clearance peaked at a food concentration of about 150 µg C l⁻¹ and decreased at both higher and lower food levels (Fig. 2), thus providing the argument for applying a sigmoid model to describe ingestion rate. Ingestion rates and clearances

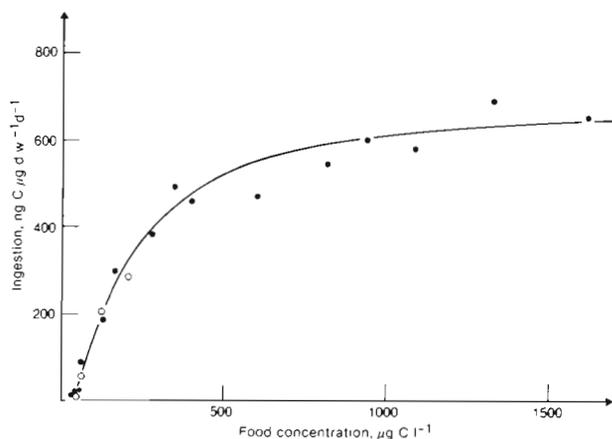


Fig. 1. *Acartia tonsa*. Ingestion rate (I) as function of food concentration (C). Each point represents the average of 4 replicates. Closed symbols: electronic cell counts; open symbols: microscopic cell counts. Fitted curve is $I = 713 e^{-157/C}$ ($r^2 = 0.97$)

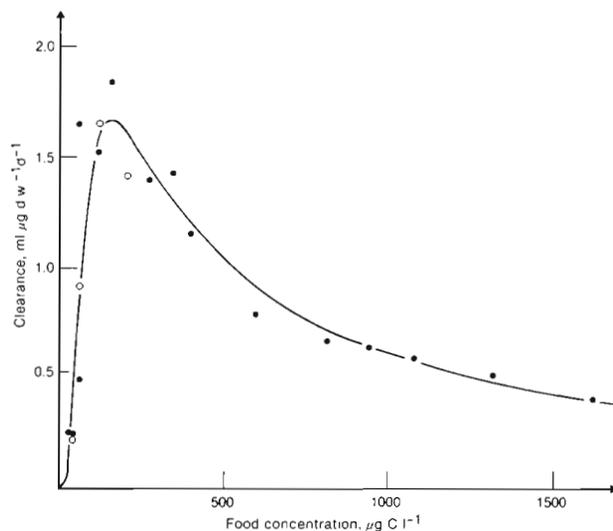


Fig. 2. *Acartia tonsa*. Clearance (F) as function of food concentration (C). Same data and symbols as in Fig. 1. Fitted curve is $F = 710 C^{-1} e^{-157/C}$

obtained from visual cell counts did not deviate significantly from the rates derived from electronic counting. Thus, production of *Rhodomonas baltica*-sized particles by grazing *Acartia tonsa* was apparently insignificant.

To estimate the duration of time necessary for egg production to come into equilibrium with ingestion rate, egg production was followed during the 4 d subsequent to shifts in food concentration (Fig. 3). Females fed at a saturation concentration (33000 cells ml⁻¹) for 3 d showed a rapidly declining rate of egg production

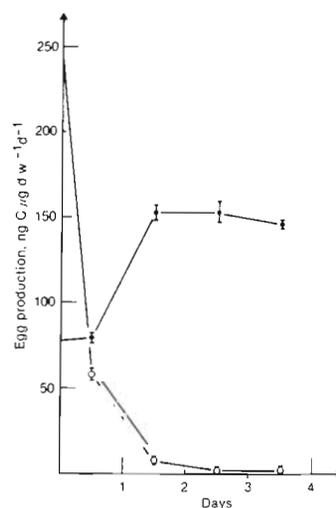


Fig. 3. *Acartia tonsa*. Rate of egg production relative to time after shift in food concentration. Average \pm SE of 4 measurements. Open symbols: copepods acclimated to 33000 cells ml⁻¹ for 3 d and transferred to 1 µm filtered seawater on Day 0. Closed symbols: copepods acclimated to 3000 cells ml⁻¹ for 2 d and transferred to 7500 cells ml⁻¹ on Day 0

when transferred to 1 μm filtered sea water. By Day 3, egg production was virtually zero. Similarly, copepods transferred from a low (3000) to a higher (7500 cells ml^{-1}) food concentration increased their rate of egg production. The second day following transfer, it had stabilized. Thus, 1 d seems sufficient for *Acartia tonsa* to reach energetic equilibrium. In the subsequent calculations of egg production rate, only data from Day 2 onwards (i.e. after 3 to 4 d of acclimatisation) have been utilized, and these females were assumed to be in energetic steady state (no somatic growth).

Rate of egg production of acclimated copepods showed a similar relation to food concentration as did

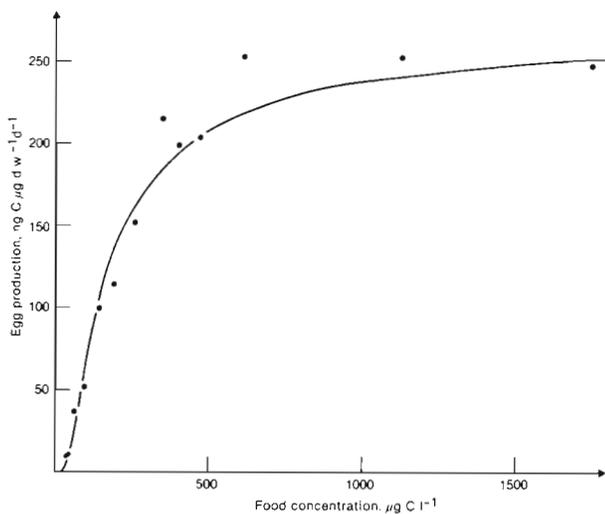


Fig. 4. *Acartia tonsa*. Rate of egg production (G) as function of food concentration (C). Each point represents the average egg production during 3 d for 4 replicates. Fitted line is $G = 272 e^{-134/C}$ ($r^2 = 0.98$)

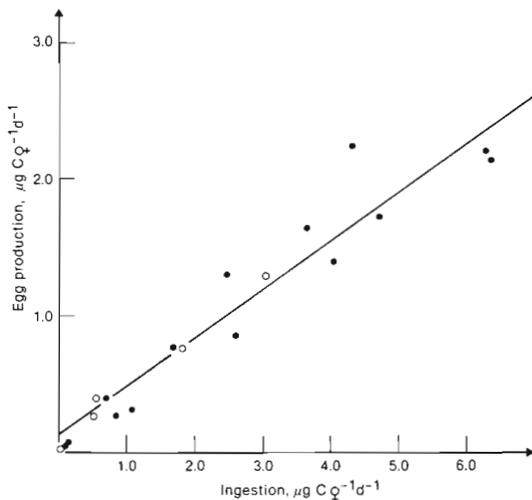


Fig. 5. *Acartia tonsa*. Egg production (G) as function of ingestion rate (I). Data from Fig. 1 and 4; symbols as in Fig. 1. Regression line is $G = 0.36 I + 0.10$ ($r^2 = 0.92$)

ingestion rate (Fig. 4). Maximum egg production rate was estimated as $0.27 \mu\text{g C } \mu\text{g dw}^{-1} \text{ d}^{-1}$, equivalent to 65 % body C d^{-1} .

Egg production rate varied nearly linearly with ingestion rate (Fig. 5) and ingested carbon (or nitrogen) was converted into egg-carbon (or nitrogen) by an efficiency of 36 % (slope of the linear regression). The alternative plot of egg production efficiency versus ingestion rate (not shown) yielded a slight, but insignificant negative slope ($r = -0.33$, $20\% > p > 10\%$), and the average efficiency so obtained equalled 41 %.

Respiration and ammonia excretion rates

Respiration rate of starved copepods was constant for the initial 8 h, whereupon it decreased drastically and reached a constant level from Day 2 onwards (Fig. 6).

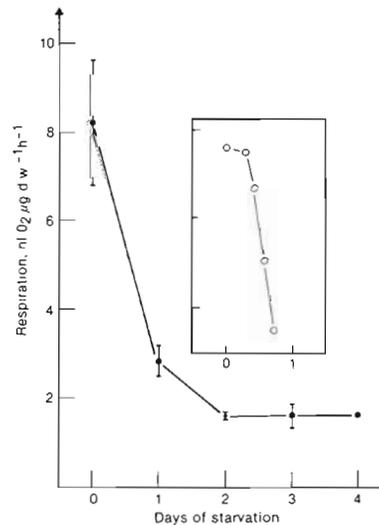


Fig. 6. *Acartia tonsa*. Respiration rate as function of duration of starvation. Average \pm SE of 1 to 4 measurements. Insert: respiration rate during the first 20 h of starvation derived from diver measurements

Thus, a starvation respiration rate can be defined, even though the copepods are not in steady state. Rate of respiration of acclimated copepods increased with algal concentration in a decelerating manner (Fig. 7). The respiration rate of copepods fed at saturating food concentrations was more than 4 times higher than for starved individuals. Rates obtained by the diver technique were slightly higher than electrode readings in both cases but the differences were statistically insignificant ($p > 5\%$); Mann-Whitney U-test).

Ammonia excretion rate followed a similar course with food concentration as did respiration rate (Fig. 8). Hence, the atomic O:N ratio was nearly constant and independent of food concentration (Table 2).

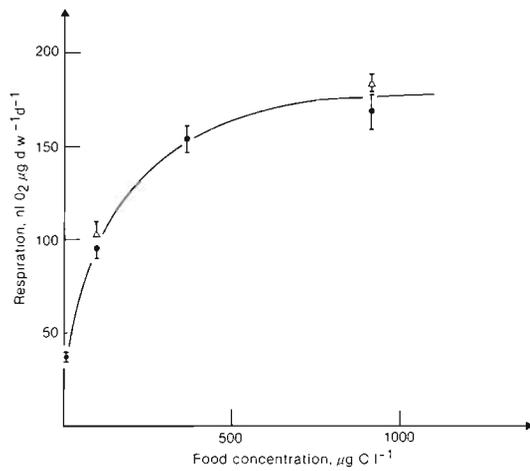


Fig. 7. *Acartia tonsa*. Respiration rate as function of food concentration. Average \pm SE. Closed symbols: electrode technique; open triangles: diver technique. Line fitted by eye

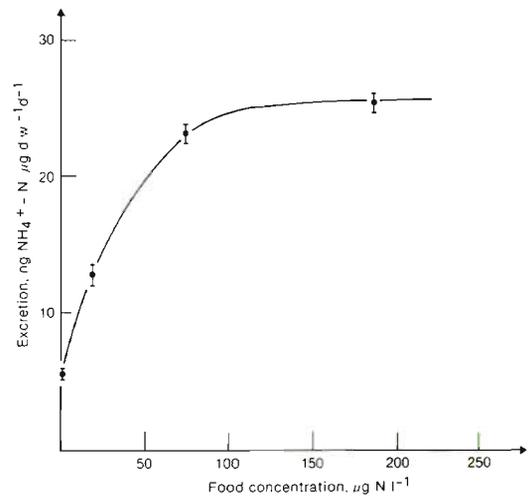


Fig. 8. *Acartia tonsa*. Ammonia excretion rate as function of food concentration. Average \pm SE. Line fitted by eye

Carbon and nitrogen budgets

The above observations can be summarized in carbon and nitrogen budgets (Table 3). Ingestion and egg production rates have been calculated from the fitted

Table 2. *Acartia tonsa*. Atomic ratio of oxygen consumed to ammonia-nitrogen excreted relative to acclimation food concentration

| Food concentration $\mu\text{g C l}^{-1}$ | O:N (atomic) |
|--|--------------|
| 0 | 8.17 |
| 92 | 8.89 |
| 367 | 7.80 |
| 916 | 7.88 |

models and the respiration and ammonia excretion rates are the ones actually measured. Oxygen was converted to carbon by assuming that lipids, carbohydrates and protein are oxidized in proportion to their occurrence in the diet. *Rhodomonas baltica* contains about 60 % protein (from CN analyses). The weight ratio of carbohydrates to lipids in phytoplankton algae averages about 3 (from data in Parsons et al. 1977). Thus, the respiratory quotient is assumed to be 0.86 (weighted average) and the carbon equivalent of 1 $\mu\text{l O}_2$ (18°C, 760 mm Hg) 0.43 μg .

Assimilation efficiencies (G + R/I) and net growth efficiencies (G/G + R) have been calculated on the (unrealistic?) assumption that no dissolved organics are excreted or otherwise lost by the copepods. The carbon assimilation efficiency was high at the lowest food level (81 %) but surprisingly low at the high food

Table 3. *Acartia tonsa*. Carbon and nitrogen budget. AE: assimilation efficiency; NGE: net growth efficiency; GGE: gross growth efficiency. Data in parenthesis from diver measurements

| Food concentration | I Ingestion | G Egg production | R Respiration or excretion | AE = G+R/I | NGE = G/G+R | GGE = G/I |
|------------------------|----------------|---|----------------------------------|---------------|----------------|--------------|
| $\mu\text{g C l}^{-1}$ | | ng C $\mu\text{g dw}^{-1}\text{d}^{-1}$ | | | | |
| 0 | 0 | — | *16 | — | — | — |
| 92 | 128 | 63 | 41 (44) | 0.81 (0.84) | 0.60 (0.59) | 0.49 |
| 367 | 464 | 188 | 66 | 0.55 | 0.74 | 0.41 |
| 916 | 600 | 234 | 73 (79) | 0.51 (0.52) | 0.76 (0.75) | 0.39 |
| $\mu\text{g N l}^{-1}$ | | ng N $\mu\text{g dw}^{-1}\text{d}^{-1}$ | | | | |
| 0 | 0 | — | 5 | — | — | — |
| 19 | 26 | 13 | 13 | 0.95 | 0.50 | 0.49 |
| 75 | 94 | 37 | 23 | 0.64 | 0.62 | 0.40 |
| 187 | 121 | 48 | 25 | 0.60 | 0.65 | 0.40 |

* 1 nl O₂ = 0.43 ng C

levels (51 to 55 %). Gross growth efficiency varied accordingly, as also noted above. Net growth efficiency increased with food concentration and ingestion rate from 60 % at the lowest food level to 76 % at the highest. The conversion efficiencies expressed in terms of nitrogen showed similar trends, except that nitrogen assimilation was more (60 to 95 %) and net growth less (50 to 65 %) efficient than the corresponding carbon conversions.

Specific dynamic action

Specific dynamic action, the increase in respiration rate associated with (but not necessarily caused by) feeding, is often expressed relative to ingestion or assimilation rate as a coefficient. In Fig. 9 respiration rate has been plotted against ingestion or assimilation (as carbon). With the exception of the starvation respiration rate, the observations fit linear relations, and the

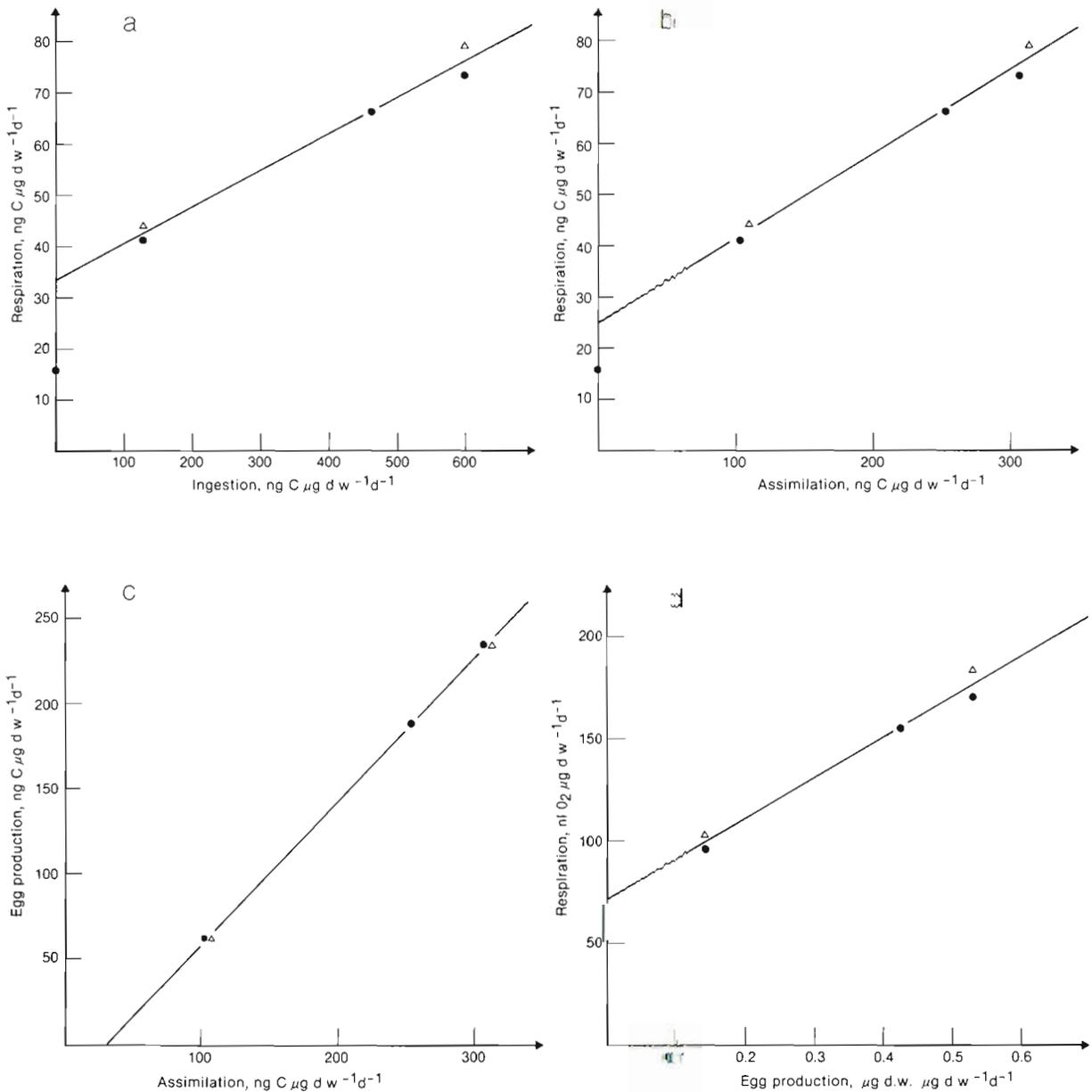


Fig. 9. *Acartia tonsa*. (a) Respiration (R) versus ingestion (I). Fitted regression (starvation respiration excluded) is $R = 0.07 I + 33.39$ ($r^2 = 0.98$). (b) Respiration versus assimilation (A). Fitted regression (starvation excluded) is $R = 0.16 A + 24.98$ ($r^2 = 0.99$). (c) Egg production (G) versus assimilation. $G = 0.84 A - 25.73$ ($r^2 = 1.00$). (d) Respiration versus egg production. $R = 197 G + 71$ ($r^2 = 0.98$). Closed symbols: respiration obtained by electrode technique; triangles: respiration by diver technique

slopes of the regressions correspond to the SDA-coefficients normally reported in the literature. Thus, SDA equals 7 % of ingested carbon or 17 % of assimilated carbon in *Acartia tonsa*.

Also shown in Fig. 9 are the plots of egg production versus assimilation and respiration versus egg production. The slope of the first of these regressions (dG/dA) is the efficiency of growth, where maintenance respiration (= ordinate intercept of Fig. 9d) has been taken into account. The slope of the latter regression (dR/dG) represents the apparent (direct or indirect) energetic cost of egg production and equals 197 nl O_2 consumed per μg dw of egg produced.

DISCUSSION

SDA is generally high in endothermic animals and, in particular, among ruminants where it represents 30 to 60 % of utilizable energy of the food (Webster 1981). In reptiles SDA-coefficients equal between 19 and 35 % of assimilated energy (Coulson & Hernandez 1979) and in fish, between 10 and 25 % (Jobling 1981, Soofiani & Hawkins 1982). For invertebrates, Wallace (1973) observed an SDA-coefficient of ca 6 % of ingested energy for the crab *Carcinus maenas*, whereas Bayne & Scullard (1977) found that the SDA in *Mytilus edulis* represented 28 and 56 % of ingested and assimilated energy, respectively. When considering copepods, a somewhat contradictory picture arises. Numerous authors have found that respiration decreases during starvation (e.g. Ikeda 1971, 1977, Mayzaud 1973). However, when relating respiration rate to feeding rate, Vidal (1980) found no significant relation in *Calanus pacificus*, whereas Abou Debs (1984) found a nearly 30-fold increase in respiration rate of *Temora stylifera* fed at saturating food concent-

ration in comparison to starved individuals. On the basis of Abou Debs' data an SDA-coefficient for saturation-feeding adult females amounting to 11.2 and 15.6 % of ingested and assimilated carbon, respectively, can be calculated. These data must, however, be considered with care since they, together with data on egestion and egg production, imply a specific daily growth rate of 170 %. Our data suggest an SDA-coefficient of 7 and 17 %, respectively, of ingested and assimilated carbon in saturation fed *Acartia tonsa*.

It is obvious from the above, that while increased metabolism in association with feeding is a general phenomenon among animals, the magnitude of increase varies considerably between species. Some of this variation may be caused by methodological differences but it does suggest that the components of SDA may differ between species.

Partitioning of SDA in *Acartia tonsa*

For the following considerations it is convenient to express oxygen consumption as equivalents of ATP synthesized (Table 4). For calculations in Table 4 total coupling between oxidation and phosphorylation was assumed, i.e. that 3 mol ATP are formed per mol $NADH_2$ oxidized (P/O-ratio = 3). If the P/O-ratio is only 2 (the lower likely limit), the rate of ATP-synthesis should be reduced by 1/3.

Formation of biomass

To calculate ATP-equivalents required for synthesis of egg biomass, egg composition must be known. From the CN- and dry-weight analyses we know that 55 % of egg biomass is protein ($N \times 6.25$). This agrees with the

Table 4. Conversion of oxygen consumed to ATP-equivalents synthesized

| Substrate | Cost of ATP-synthesis ^(a) Kcal mol ATP ⁻¹ | Oxycaloric coefficient ^(b) Kcal l O_2 ⁻¹ | ATP/Oxygen mmol ATP l O_2 ⁻¹ |
|--|---|---|--|
| Protein | 21 | 4.57 | 218 |
| Carbohydrate | 17.7 | 4.75 | 268 |
| Lipid | 18.5 | 4.41 | 238 |
| Food concentration $\mu g C l^{-1}$ | ATP-equivalents synthesized ^(c) nmol ATP $\mu g dw^{-1} d^{-1}$ | | |
| | Electrode | Diver | |
| 0 | 9 | — | |
| 92 | 23 | 24 | |
| 367 | 36 | — | |
| 916 | 40 | 43 | |

^(a) From Blaxter (1971) and Grisolia & Kennedy (1966)

^(b) At 18°C, 760 mm Hg; from Gordon (1972)

^(c) Assuming that protein, carbohydrate and lipid are catabolized in proportion 60:30:10 (cf. text), i.e. 235 mmol ATP l O_2 ⁻¹

composition of cirriped eggs (Aychituv & Barnes 1978). If the remaining part of the copepod egg has the same composition as a cirriped egg, then ca 5 % is carbohydrate, 35 % is lipid and 5 % is salt. This composition predicts a calorific content of *Acartia tonsa* eggs of 5.67 cal mg⁻¹, consistent with the calorific value of *Diap-tomus gracilis* eggs of 5.7 cal mg⁻¹ (Kibby 1971).

In the following we assume that the monomers required for synthesis are available in the food. The formation of a peptide bond requires 3 ATP-equivalents (e.g. Grisolia & Kennedy 1966). In addition, ATP is required for the regeneration of m-RNA, the amount of which depends on the turnover rate of RNA. A total requirement of 4 to 5 mol ATP per mol peptide bound is generally assumed in the literature (e.g. Grisolia & Kennedy 1966, Stouthamer 1973, Müller & Kirchgessner 1979). In Table 5, 4 mol ATP per mol peptide bond has been assumed. The ATP requirements for carbohydrate polymerisation and lipid formation from monomers assuming cheapest biochemical pathways have been outlined in Table 5 (information from Lehninger 1975). According to the calculations in Table 5, 22.9 nmol ATP is required for the formation of 1 µg dw of egg. This constitutes 50 to 74 % of SDA (P/O = 2 to 3).

If the monomers required for biosynthesis are not available in sufficient amounts in the food, the cost of biomass formation may increase. Thus, lipids are supposed to constitute only 10 % of the algae, but 35 % of the egg, whereas carbohydrates constitute 40 % of the algae and only 5 % of the egg. If all lipids in the eggs are formed from monosaccharids, and if the transportation costs of acetyl-CoA from mitochondria to cytoplasm is 1 mol ATP per mol acetyl-CoA (Lehninger 1975), then the cost of biomass formation increases to 31.6 nmol ATP µg dw⁻¹ (Table 5). If the cost of a peptide bond is 5 rather than 4 ATP, the total cost increases to 36.5. This represents 79 to 118 % of SDA (P/O = 2 to 3).

Assimilation

Protein, carbohydrate and lipid broken down to monomers in the gut lumen should be absorbed along the gut epithelium, transported by the body fluids and subsequently taken up by the metabolizing cells. From Fig. 9b, SDA in terms of µg carbon respired per µg carbon assimilated is 0.164. If we, for simplicity, assume that protein, carbohydrate and lipid are assimilated in proportion to their occurrence in the food (even though proteins are apparently assimilated more efficiently), then 0.17 µl O₂ is consumed or 26 to 40 nmol ATP synthesized (P/O-ratio 2 to 3) for each µg dw assimilated and transported.

For the calculation of energetic cost of assimilation and transport, we consulted recent reviews on intestinal absorption (Kimmich 1981, 1983, Munck 1981, Thomson & Dietschy 1981). Although the mechanisms for aminoacid and monosaccharid intestinal transport are different, the energetics of transport are similar. The intestinal absorption is primarily driven by the difference in electro-chemical potential for Na⁺. The stoichiometry of transport is 2 Na⁺ being exchanged for each aminoacid or sugar molecule, and that 1 mol of ATP is required for the back transport of 3 mol Na⁺. That is, 2/3 mol ATP is required for the assimilation of each mol aminoacid or monosaccharid. This is a minimum estimate since there is evidence (Kimmich 1981) that the sodium gradient does not provide sufficient energy, and that additional but unspecified amounts of energy are required. Other, more energy consuming, mechanisms for uptake have been suggested. Thus, the γ-glutamyl cycle of Meister (1973) is apparently responsible for aminoacid transport in the mammalian kidney but may function in the gut also. This cycle requires 3 mol ATP per mol aminoacid transported. Also, if monosaccharides are immediately phosphorylated in the gut epithelium cell (to prevent back diffusion) an additional mol of ATP is required for

Table 5. *Acartia tonsa*. Estimated ATP requirements for net biomass formation. Lipid composition of prawn (*Pandalopsis japonica*) eggs (Zama et al. 1976) were taken as representative of *A. tonsa* eggs. Values in parenthesis: estimated cost of lipid synthesis provided lipids are formed from monosaccharids (see text)

| Egg composition | ng µg ⁻¹ | Monomer mol wt | nmol µg ⁻¹ | mol ATP/monomer mol | nmol ATP µg ⁻¹ egg |
|--|---------------------|----------------|-----------------------|---------------------|-------------------------------|
| Protein | 550 | 112 | 4.91 | 4 | 19.64 |
| Carbohydrates | 50 | 162 | 0.31 | 2 | 0.62 |
| Lipid | | | | | |
| Triglycerides | 187 | 800 | 0.23 | 1 (22) | 0.23 (5.06) |
| Phospholipids | 139 | 700 | 0.20 | 8 (22) | 1.60 (4.40) |
| Sterols | 14 | 387 | 0.04 | 20 (40) | 0.80 (1.60) |
| Fatty acids | 9 | 256 | 0.04 | 0 (7) | 0 (0.28) |
| ATP requirements for biomass formation | | | | | 22.9 (31.6) |

each mol of monosaccharid transported. Once absorbed in the gut, the monomers should be transported in the body fluids and subsequently taken up by the metabolizing cells (e.g. the egg).

We do not know the energetics of this process, i.e. whether it is free or requires similar amounts of energy as the intestinal absorption. Therefore, 1 mol ATP for the uptake and transport of each mol aminoacid or monosaccharid probably provides a minimum estimate for transport energetics but is consistent with *in vitro* measurements of aminoacid uptake in the alligator (Coulsen & Hernandez 1979). The uptake of lipids is generally thought to be an energy-independent process. In Table 6, the energetic requirements for transport have been calculated on these assumptions and an estimate of 7.2 nmol ATP per $\mu\text{g dw}$ assimilated and transported is arrived at. This constitutes between 18 and 28 % of SDA (for a P/O-ratio between 2 and 3).

Cost of feeding, digestion and urea synthesis

From the above considerations, it is obvious that there is little energy left for the partitioning between other feeding and growth related activities.

From a comparison of clearance (Fig. 2) and respiration (Fig. 7) it is evident that the cost of filtration is largely independent of food concentration. Also, the similar respiration rates obtained by the electrode (feeding animals) and diver technique (non-feeding animals; gut evacuated before measurements were initiated, cf. Kjørboe et al. 1982) suggest that the costs of filtering, feeding and the mechanical work required to transport food down the gut are insignificant.

The cost of digestion is primarily due to the cost of synthesis of digestive enzymes. Nothing is known about the rate of enzyme synthesis in copepods. However, it is certainly small in comparison to egg protein syntheses and, therefore, of lesser quantitative importance.

The inefficiency of ATP synthesis from aminoacid oxidation (cf. Krebs 1964) has been taken into account

in Table 4 and, therefore, does not contribute to SDA when expressed in terms of ATP.

Urea constitutes less than 10 % of total N-excretion in copepods (e.g. Conover, 1978). If the synthesis of 1 mol urea requires 4 mol ATP (e.g. Müller & Kirchgessner 1979), and if urea-N excretion = $0.1 \times \text{NH}_4^+\text{-N}$ excretion, then 0.2 nmol ATP is required for urea synthesis for each ng-at $\text{NH}_4^+\text{-N}$ excreted. From the regression of oxygen consumption (R) on ammonia excretion (E), $R (\mu\text{l O}_2 \mu\text{g dw}^{-1} \text{d}^{-1}) = 0.09 E (\text{ng-at NH}_4\text{-N}) + 0.009$; $n = 6$, $r^2 = 0.98$, SDA in terms of $\mu\text{l O}_2$ consumed or nmol ATP synthesized per ng-at $\text{NH}_4^+\text{-N}$ excreted is 0.09 or 14 to 21 (P/O = 2 to 3), respectively. Thus, ATP requirements for urea synthesis constitute 0.9 to 1.4 % of SDA.

Efficiency of growth

The greater the cost of growth, the lower the efficiency. By a somewhat different approach than the above, Penning de Vries et al. (1974) attempted to account for all the potential costs in their considerations of heterotrophic plant biosynthesis, and McDermitt & Loomis (1981) calculated the theoretical maximum efficiency of biomass formation (from monomers) on basis of their findings. They showed that the maximum energetic efficiency of growth (excl. cost of maintenance) ranged from 0.79 to 0.86 depending on the composition of the biomass formed. With a composition similar to that of the copepod egg, an energetic efficiency of 0.82 is predicted, comparable to the present estimate of dG/dA (0.84) (Fig. 9c). This suggests that the production of eggs in *Acartia tonsa* runs near its theoretically maximum efficiency and, consistent with the above, that feeding related processes (cost of filtration, gut activity etc.) are of minor quantitative importance.

In the bioenergetic literature, growth efficiencies are most often expressed as net or gross growth efficiencies. dG/dA expresses the efficiency by which assimilated carbon in excess of maintenance requirements is

Table 6. *Acartia tonsa*. Estimated ATP requirements for uptake and transport of monomers

| Substrate | ng μg^{-1} of food | Monomer mol wt | nmol monomer μg^{-1} food | ATP mol $^{-1}$ | nmol ATP μg^{-1} food |
|---|----------------------------------|-------------------|---|--------------------|-------------------------------------|
| Protein | 600 | 112 | 5.36 | 1.0 | 5.36 |
| Carbohydrate | 300 | 162 | 1.85 | 1.0 | 1.85 |
| Lipid | 100 | — | — | 0 | 0 |
| ATP requirements for uptake and transport | | | | | 7.21 |

converted into egg carbon and is by definition larger than the net growth efficiency. However, the latter will approach the former asymptotically as food concentration rises and the relative importance of maintenance respiration decreases (cf. Table 3).

Carbon and nitrogen budgets

Carbon and nitrogen budgets were constructed on the assumption that no dissolved organics are lost from the copepods. Thus, the assimilation efficiency would be underestimated in case of a significant loss of dissolved organics. The present C-assimilation efficiencies at high food concentrations are, indeed, in the lower range of those reported for copepods in the literature (40 to 85 %; see review by Conover 1978). There are 2 potential errors: (1) loss of organics from the algae when grazed upon ('sloppy feeding'); (2) excretion of organics from the copepods. Both types of leakages have been demonstrated for zooplankton (e.g. Lampert 1978, Copping & Lorenzen 1980). If the lost organics do not participate in the energy metabolism, the basic conclusion regarding the components of SDA arrived at above will not be influenced (except that transportation costs may be slightly higher than assumed). If, however, potential organic exudates are anaerobic metabolites, respiration underestimates metabolism. If a 'true' assimilation efficiency at high food concentrations of 0.70 is assumed (cf. Landry et al. 1984) and, if organic exudates are solely anaerobic metabolites, then the energetic gain from metabolism will be increased by about 2 % and SDA, in terms of ATP synthesized, increased by 5 %. Part of this increase would, however, be consumed by increased transportation costs. A potential anaerobic contribution will, therefore, only slightly influence the above considerations.

When constructing the carbon budget, we also assumed that protein, lipid and carbohydrate are catabolized in proportion to their occurrence in the diet. However, the average O:N-ratio of 8.2 (cf. Table 2) indicates that protein may be catabolized to a higher degree than other components in the diet, since the O:N-ratio of pure protein catabolism is 6.8 (Ikeda 1977). In the case of pure protein metabolism, this will increase the oxygen to carbon and oxygen to ATP conversion factors by 5 and 8 %, respectively, and therefore, decrease the SDA-estimate (in ATP) by up to 8 %.

We also assumed an RQ independent of feeding rate. Lampert & Bohrer (1984) found that RQ increased from about 0.7 for starved daphnids to 1.1 for well-fed animals, indicating a concurrent shift from lipid to carbohydrate catabolism. If their finding was representa-

tive for *Acartia tonsa*, it would change the carbon budget and increase the SDA-estimate (in terms of ATP) by some 35 %. However, the lack of oil droplets in *A. tonsa*, the low lipid content of the food, and the high and constant O:N-ratio suggest that lipids are of minor importance in *A. tonsa* catabolism, that the composition of the catabolized substrate is independent of food concentration, and that the application of a constant RQ-ratio is warranted.

CONCLUSIONS

There are a number of uncertainties in the calculation of SDA in terms of ATP. Although some of these may outbalance the others, the overall trend may be that SDA is slightly underestimated. The calculations of the energetic requirements for biosynthesis and transport also involve some uncertainties but, again, the expenses are under- rather than overestimated. The estimates given in this paper suggest that SDA in *Acartia tonsa* is largely related to biosynthesis and transport and that feeding, gut activity, aminoacid oxidation and urea excretion are of minor importance. SDA, therefore, represents the 'cost of growth' rather than the 'cost of feeding'.

Acartia tonsa seems to be very efficient in transforming ingested material into eggs. Since the maximum weight specific rate of egg production is similar to the maximum weight specific growth rate of nauplii and copepodites (Sekiguchi et al. 1980), this may hold true during the entire life cycle, even though growth in juveniles is mostly in proteins and little in lipids. *A. tonsa* rapidly adapts energetically to changing food conditions (Fig. 3 & 6), its maintenance and starvation requirements are small and it is able to propagate even at very low ingestion rates. This is in partial agreement with Dagg (1977) who found that, among 3 species of neritic copepods, *A. tonsa* was intermediately adapted to a fluctuating (patchy) food environment: it survived starvation longer (6 to 10 d) than did *Centropages typicus* (3 to 6 d), but shorter than *Pseudocalanus minutus* (> 16 d). On the other hand, the 'incipient limiting food concentration' of *A. tonsa* when feeding on the small-sized *Rhodomonas baltica* is high (5 to 700 $\mu\text{g C l}^{-1}$) compared to natural food concentrations. Thus, this neritic species seems well adapted to the fluctuating but occasionally very high food concentrations characterizing coastal waters.

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LITERATURE CITED

- Abou Debs, C. (1984). Carbon and nitrogen budget of the calanoid copepod *Temora stylifera*: effect of concentration and composition of food. *Mar. Ecol. Prog. Ser.* 15: 213-223
- Achituv, Y., Barnes, H. (1978). Studies in the biochemistry of cirripede eggs. VI. Changes in the general biochemical composition during development of *Tetraclita squamosa* Pilsbry, *Balanus perforatus* Brug., and *Pollicipes cornucopia* Darwin. *J. exp. mar. Biol. Ecol.* 32: 171-176
- Ashworth, A. (1969). Metabolic rates during recovery from protein-calorie malnutrition: the need for a new concept of specific dynamic action. *Nature, Lond.* 223: 407-409
- Bayne, B. L., Scullard, C. (1977). An apparent specific dynamic action of *Mytilus edulis*. *J. mar. biol. Ass. U. K.* 57: 371-378
- Benson, F. W., Aldrich, J. C. (1981). A study of nitrogen excretion in the marine copepod *Temora longicornis*. *Kieler Meeresforsch. (Sonderh.)* 5: 186-190
- Blaxter, K. L. (1971). Methods of measuring the energy metabolism of animals and interpretation of results obtained. *Fed. Proc.* 30: 1436-1443
- Conover, R. J. (1978). Transformation of organic matter. In: Kinne, O. (ed.) *Marine ecology, Vol. IV, Dynamics*. Wiley, Chichester, p. 221-499
- Copping, A. E., Lorenzen, C. J. (1980). Carbon budget of a marine phytoplankton-herbivore system with carbon-14 as a tracer. *Limnol. Oceanogr.* 25: 873-882
- Corner, E. D. S., Newell, B. S. (1967). On the nutrition and metabolism of zooplankton. IV. The forms of nitrogen excreted by *Calanus*. *J. mar. biol. Ass. U. K.* 47: 113-121
- Coulson, R. A., Hernandez, T. (1979). Increase in metabolic rate of the alligator fed proteins and aminoacids. *J. Nutr.* 109: 538-550
- Dagg, M. (1977). Some effects of patchy food environments on copepods. *Limnol. Oceanogr.* 22: 99-107
- Frost, B. W. (1972). Effect of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* 17: 805-815
- Ganong, W. F. (1973). *Review of medical physiology*. 6th ed. Lange Medical Publications, Los Altos, p. 196-231
- Gordon, M. S. (1972). *Animal physiology: principles and adaptations*. Macmillan Publishing Co., Inc., New York, p. 44-71
- Grisolia, S., Kennedy, J. (1966). On specific dynamic action, turnover, and protein synthesis. *Perspect. Biol. Med.* 9: 578-585
- Ikeda, T. (1971). Changes in respiration rate and in composition of organic matter in *Calanus cristatus* (Crustacea Copepoda) under starvation. *Bull. Fac. Fish. Hokkaido Univ.* 21: 280-298
- Ikeda, T. (1977). The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton. IV. Changes in respiration and excretion rates of boreal zooplankton species maintained under fed and starved conditions. *Mar. Biol.* 41: 241-252
- Jobling, M. (1981). The influence of feeding on the metabolic rate of fishes: a short review. *J. Fish Biol.* 18: 385-400
- Jobling, M. (1983). Towards an explanation of specific dynamic action. *J. Fish Biol.* 23: 549-555
- Kibby, H. V. (1971). Energetics and population dynamics of *Diaptomus gracilis*. *Ecol. Monogr.* 41: 311-327
- Kimmich, G. A. (1981). Intestinal absorption of sugar. In: Johnson, L. R. (ed.) *Physiology of the gastrointestinal tract*. Raven press, New York, p. 1035-1061
- Kimmich, G. (1983). Coupling stoichiometry and the energetic adequacy question. In: Gilles-Baillien, M., Gilles, R. (ed.) *Intestinal transport*. Springer-Verlag, Berlin, p. 87-102
- Kjørboe, T., Møhlenberg, F., Nicolajsen, H. (1982). Ingestion rate and gut clearance in the planktonic copepod *Centropages hamatus* (Lilljeborg) in relation to food concentration and temperature. *Ophelia* 21: 181-194
- Krebs, H. A. (1964). The metabolic fate of amino acids. In: Munro, H. N., Allison, J. B. (ed.) *Mammalian protein metabolism*. Academic Press, London, p. 125-176
- Lampert, W. (1978). Release of dissolved organic carbon by grazing zooplankton. *Limnol. Oceanogr.* 23: 831-834
- Lampert, W., Bohrer, R. (1984). Effect of food availability on the respiratory quotient of *Daphnia magna*. *Comp. Biochem. Physiol.* 78A: 221-223
- Landry, M., Hassett, R. P., Fagerness, V., Downs, J., Lorenzen, C. J. (1984). Effect of food acclimation on assimilation efficiency of *Calanus pacificus*. *Limnol. Oceanogr.* 29: 361-364
- Lehninger, A. L. (1975). *Biochemistry. The molecular basis of cell structure and function*. Worth Publishers, Inc., New York
- McDermitt, D. K., Loomis, R. S. (1981). Elemental composition of biomass and its relation to energy content, growth efficiency, and growth yield. *Ann. Bot.* 48: 275-290
- Mayzaud, P. (1973). Respiration and nitrogen excretion of zooplankton. II. Studies of the metabolic characteristics of starved animals. *Mar. Biol.* 21: 19-28
- Meister, A. (1973). On the enzymology of amino acid transport. *Science* 180: 33-39
- Møhlenberg, F., Kjørboe, T. (1981). Growth and energetics in *Spisula subtruncata* (Da Costa) and the effect of suspended bottom material. *Ophelia* 20: 79-90
- Müller, H. L. von, Kirchgessner, M. (1979). Zur Energetik der Proteinsynthese beim Wachstum. *Z. Tierphysiol., Tierernährg. u. Futtermittelkde.* 42: 161-172
- Munck, B. G. (1981). Intestinal absorption of amino acids. In: Johnson, C. R. (ed.) *Physiology of the gastrointestinal tract*. Raven Press, New York, p. 1097-1122
- Nexø, B. A., Hamburger, K., Zeuthen, E. (1972). Simplified microgasometry with gradient divers. *C. r. Trav. Lab. Carlsberg* 39: 33-63
- Parrish, K. K., Wilson, D. F. (1978). Fecundity studies on *Acartia tonsa* (Copepoda: Calanoida) in standardized culture. *Mar. Biol.* 46: 65-81
- Parsons, T. R., Takahashi, M., Hargrave, B. (1977). *Biological oceanographic processes*. 2nd ed. Pergamon Press, Oxford, p. 40-64
- Penning de Vries, F. W. T., Brunsting, A. H. M., Laar, H. H. van (1974). Products, requirements and efficiency of biosynthesis: a quantitative approach. *J. theor. Biol.* 45: 339-377
- Riisgård, H. U., Randløv, A., Hamburger, K. (1981). Oxygen consumption and clearance as a function of size in *Mytilus edulis* L. veliger larvae. *Ophelia* 20: 179-183
- Sekiguchi, H., McLaren, I. A., Corkett, C. J. (1980). Relationship between growth rate and egg production in the copepod *Acartia clausi hudsonica*. *Mar. Biol.* 58: 133-138
- Solarzano, L. (1969). Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnol. Oceanogr.* 14: 799-801
- Soofiani, N. M., Hawkins, A. D. (1982). Energetic costs at different levels of feeding in juvenile cod, *Gadus morhua* L. *J. Fish Biol.* 21: 577-592
- Stouthamer, A. H. (1973). A theoretical study on the amount of ATP required for synthesis of microbial cell material. *Antonie van Leeuwenhoek.* 39: 545-565

- Tandler, A., Beamish, F. W. H. (1979). Mechanical and biochemical components of apparent specific dynamic action in large mouth bass, *Micropterus salmoides* lacépède. J. Fish Biol. 14: 343-350
- Thomson, B. R., Dietschy, J. M. (1981). Intestinal lipid absorption: major extracellular and intracellular events. In: Johnson, L. R. (ed.) Physiology of the gastrointestinal tract. Raven Press, New York, p. 1147-1220
- Vahl, O. (1984). The relation between specific dynamic action (SDA) and growth in the common starfish, *Asterias rubens* L. Oecologia (Berl.) 61: 122-125
- Vidal, J. (1980). Physioecology of zooplankton. III. Effects of phytoplankton concentration, temperature, and body size on the metabolic rate of *Calanus pacificus*. Mar. Biol. 56: 195-202
- Wallace, J. C. (1973). Feeding, starvation and metabolic rate in the shore crab, *Carcinus maenas*. Mar. Biol. 20: 277-281
- Webster, A. J. F. (1981). The energetic efficiency of metabolism. Proc. Nutr. Soc. 40: 121-128
- Zama, K., Maruyama, T., Takahashi, K. (1976). Lipids of the crustacea. 1. Lipids of the muscle and the egg of the prawn (*Pandalopsis japonica*). Bull. Fac. Fish. Hokkaido Univ. 27: 181-190
- Zeuthen, E., Hamburger, K. (1977). Microgasometry with single cells using ampulla divers operated in density gradients. In: Glick, D., Rosenbaum, R. R. (ed.) Techniques of biochemical and biophysical morphology, Vol. 3. Wiley Interscience, New York, p. 59-80

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