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Influence of two different green algal diets on specific dynamic action and incorporation of carbon into biochemical fractions in the copepod *Acartia tonsa*

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Previous studies have shown that the two green algae Tetraselmis sp. (Prasinophyceae) and Dunaliella tertiolecta (Chlorophyceae) induce high and low egg production rates in Acartia tonsa. The primary goal of the present study was to investigate if this is attributable to differences in the specific dynamic action (SDA) of the two diets. Secondly, we wanted to investigate if any qualitative differences in the incorporation of nutritional constituents from the two diets are influencing SDA. The functional response of ingestion was very different with the two diets. Ingestion of T. impellucida was relatively high even at low food concentrations with a maximum of 19 μg C ind^{-1} day^{-1}. The functional response was more clearly sigmoidal on D. tertiolecta with a maximum of 7.3 μg C ind^{-1} day^{-1}. The higher ingestion rate of T. impellucida also induced higher respiration rates. Maximum respiration rates were 3.0 nl O_2 ind^{-1} min^{-1} on T. impellucida and 1.5 nl O_2 ind^{-1} min^{-1} on D. tertiolecta. This created significantly different SDA coefficients: 0.19 on T. impellucida and 0.06 on D. tertiolecta, which implies that the magnitude of SDA is strongly influenced by the composition of the diet. The incorporation of carbon into lipids was significantly higher on D. tertiolecta. However, because of lack of longer chain fatty acids in D. tertiolecta the copepods did not benefit from this. Thus, the proportion of carbon allocated to egg lipids was much lower than when feeding on T. impellucida. Acartia tonsa incorporated relatively more carbon into proteins when feeding on T. impellucida than on D. tertiolecta. Since protein synthesis is energetically very demanding this is probably the reason for the higher SDA coefficient in those feeding on T. impellucida.

INTRODUCTION

The metabolism of marine copepods has been studied intensively during the last century. Carbon has been the primary denominator when dealing with intrinsic energy budgets and trophic interactions, and most of the attention has focused on the relationship between the amount of dietary carbon available and different energetic factors, e.g. ingestion, respiration, and growth (Vidal, 1980a,b,c; Abou Debs, 1984; Kiørboe *et al.*, 1985; Berggreen *et al.*, 1988). However, to the copepod the amount of food is not the only element of concern. It is also vital that the nutritional composition of the food meets the specific physiological needs (Tang and Dam, 1999). The fluxes of material and energy are therefore, to rephrase Kleppel, governed by the relationship between the nutritional composition of the food and the nutritional needs of the copepods (Kleppel, 1993). This applies on the individual level as well as on the ecosystem level.

When the copepod feeds, food items of nutritional value are absorbed through the gut epithelium. The major part of this is then assimilated in the cells and either built

into structural tissue and eggs or respired to generate energy. The energy generated by respiration is in turn used to fuel the physiological processes taking place during feeding (Jobling, 1983). When the rate of these processes increases during feeding, so does the respiration rate. Such an increase in respiration rate associated with feeding is termed the specific dynamic action [SDA; (Grisolia and Kennedy, 1966)]. From correlations and theoretical considerations, the main cause of SDA is thought to be the energetic expenses of biomass formation (Vahl, 1984; Kiørboe *et al.*, 1987; Carefoot, 1990). Thus, by calculating the theoretical energetic costs of absorption, assimilation and growth in *A. tonsa*, Kiørboe and colleagues (Kiørboe *et al.*, 1985) found that 50–74% of the measured SDA was caused by the energetic costs of biomass formation. The costs of absorption and assimilation amounted to 18–28% of the total SDA. The energetic costs of filtering and feeding, and of the mechanical work required to transport food down the gut were insignificant.

The total energy requirement of biomass formation depends primarily on two factors: the biochemical composition of the end product (somatic tissue and eggs) and the biochemical composition of the diet. Here we consider the latter. During feeding, the increased energy expenditure is the sum of the energy demand of the particular biochemical pathways that transform the ingested matter into somatic tissue or eggs (Lehninger, 1973). If the required nutritional constituents are readily available from the diet, then the energetic costs are low. But if some kind of transformation has to take place before the ingested matter can be used, the energetic costs increase. Moreover, the energetic expense of biosynthesis varies with the composition of the actual macromolecules being synthesized (Lehninger, 1973; Stryer, 1981). The SDA may therefore vary depending on the nutritional and biochemical composition of the diet.

According to previous studies the two green algae *Tetraselmis* sp. (Prasinophyceae) and *Dunaliella tertiolecta* (Chlorophyceae) have induced high and low egg production rates in *Acartia tonsa* (Cervetto *et al.*, 1999). The goal of the present study was to investigate intrinsic physiological factors contributing to this difference. First of all we wanted to investigate the influence on SDA of the two diets. Secondly we wanted to investigate if any qualitative differences in the incorporation of nutritional constituents from the two diets were influencing SDA.

METHOD

Acartia tonsa were collected by horizontal tows with a 200 μm mesh size WP2 net in Long Island Sound, USA (41°20′ N, 72°5′ W) during October and November 1998. Immediately after collection the copepods were acclimated to 20°C, 34‰ and a light : dark cycle of 12 h : 12 h. These were the experimental conditions throughout the experiments. Before the experiments adult females and males were acclimated for 24 h to the experimental food sources and food concentrations. *Tetraselmis impellucida* [mean equivalent spherical diameter (ESD) 7.0 μm] and *Dunaliella tertiolecta* (mean ESD 5.9 μm) were grown exponentially in Instant Ocean artificial sea water (*asw*) on f/2 growth medium at a light : dark cycle of 12 h : 12 h (100 μE cm^{-2} s^{-1}) at the experimental temperature and salinity. The ESD of the algal cells were measured in all experiments using an Elzone 280PC particle counter equipped with a 120 μm tube.

Ingestion rate

For both diets, 10 adult females were placed in triplicate 500 ml bottles at algal concentrations of 0, 25, 50, 75, 100, 300 and 500 μg C l^{-1}. The exact concentrations (cells ml^{-1}) were measured with the particle counter. For the calculation of specific metabolic rates the algal concentrations were converted to carbon equivalents using the regression $\log_{10}C = 0.76 \log_{10}V - 0.29$ [(Mullin *et al.*, 1966); see also (Verity and Robertson, 1992)], where C is carbon content in pg $cell^{-1}$ and V is volume in μm^3 obtained from the particle counter. The copepods were then incubated for 24 h on a rotating plankton wheel (2 r.p.m.) in the environmental chamber. After the incubation period the algal concentrations were again measured and the ingestion rates were calculated according to Frost (Frost, 1972).

Specific dynamic action

Specific dynamic action can be derived in two different ways. It can be measured directly as the increase in respiration rate during and after a feeding event [(Thor, 2000); Thor, in preparation], or it can be calculated from simultaneous measurements of respiration and ingestion or assimilation at varying food concentrations (Kiørboe *et al.*, 1985). The advantage of the former method is its direct approach and the possibility of measuring SDA on single individuals or groups of individuals receiving the same experimental treatment. The disadvantage is the dependency of the algal concentration on the feeding activity of the copepods. The latter method is indirect and dependent on groups receiving different treatments. However, because the respiration rate reacts slowly to changes in food concentration, it is possible to measure the respiration rate reflecting the feeding scenario in a previous acclimation. Here a far better control of the algal concentration can be obtained. In the present study, we chose the latter method.

The respiration rate was measured using two different techniques. Five to fifteen females from the acclimation prior to the ingestion experiments were incubated in *asw*

in Winkler's titration bottles (65 ml) for 24 h on a rotating plankton wheel (2 r.p.m.). Three replicates were used for each algal concentration. Eight replicates receiving the same treatment but without copepods were used as controls. After the incubation period, the oxygen concentration in each bottle was measured using the Winkler's titration. Alternatively, the respiration rates were measured using the flow through technique (Møhlenberg and Kiørboe, 1981) adapted to small animals. Five females from the acclimation were placed in small glass chambers (400 µl) fitted with silicone stoppers on both ends. A peristaltic pump maintained a steady flow of food medium (10 µl min^{-1}) through the chambers via stainless steel needles in the stoppers. A 200 µm mesh in the outflow end prevented the copepods from entering the outflow. Polarographic oxygen electrodes connected to the outflows, with a length of Tygon tubing never exceeding 5 mm, measured the oxygen concentration in the outflowing water. A total of seven experimental chambers (with copepods) and one reference chamber (without copepods) were monitored simultaneously. To minimize disturbance all chambers were kept dark during the experiments. Measurements lasted until the oxygen concentration of the outflows became steady. Following the measurements the copepods were recovered from each chamber and their physical appearance was examined under a microscope. Respiration rates were calculated according to Thor (Thor, 2000).

The amounts of oxygen consumed were transformed to carbon equivalents using a respiratory quotient (RQ) of 0.90 (Omori and Ikeda, 1984). We assumed that the excretory end product was ammonium which gives an RQ of 0.97 for protein (Gnaiger, 1983), and that the composition of the algae was approximately 60% protein, 30% carbohydrates and 10% lipids (Parsons *et al.*, 1961).

Carbon incorporation

Tetraselmis impellucida and *Dunaliella tertiolecta* growing exponentially were diluted to one-half with f/2 growth medium, inoculated with 600 µCi $NaH^{14}CO_3$ l^{-1} in 500 ml round bottles and grown for 3 days to ensure maximum labelling. After centrifugation at 1000 ***g*** for 5 min, the supernatant was replaced with filtered *asw* to remove extracellular isotopic activity. This rinsing was performed twice.

During the experiments, 15 to 20 adult females were placed in 500 ml bottles containing labelled algae (1000 µg C l^{-1}). Three replicates for each diet were then incubated for 2, 6, 12 and 24 h on a rotating plankton wheel (2 r.p.m.) in the environmental chamber. After the feeding period eggs were collected on a 30 µm mesh and all samples of copepods and eggs were frozen in 120 µl filtered *asw*.

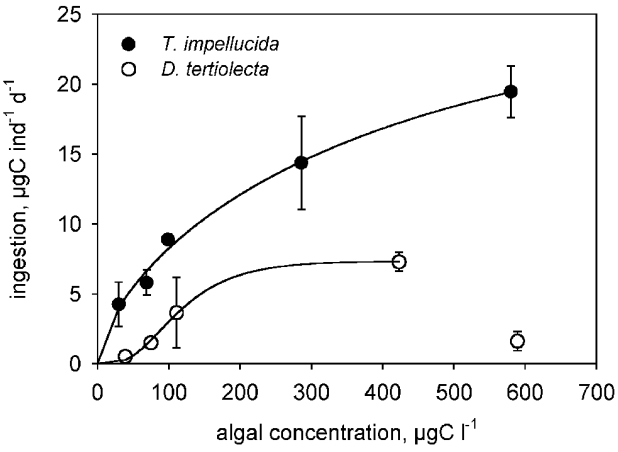

Fig. 1. Ingestion rates of *Acartia tonsa* fed *T. impellucida* and *D. tertiolecta*. Regression lines are based on all data: *T. impellucida*: $I = 24.0(1-e^{-C/465})^{0.63}$, $r^2 = 0.93$ and *D. tertiolecta*: $I = 7.12(1-e^{-C/34.5})^{15.6}$, $r^2 = 0.70$. Regression model was Chapman, three-parameter (from Sigma Plot 4.0, SPSS Inc.).

After the experiments, the samples were thawed and chemically fractionated into protein, lipid, polysaccharides and low molecular weight (LMW) substances. The LMW fraction contains various metabolites and some hydrolysed polysaccharides (Roman, 1991). The samples were homogenized and 300 µl methanol and 150 µl chloroform were added. The water content of the samples was higher than advisable, but the relative proportions of the extracting agents were 1 : 2 : 0.8 (chloroform : methanol : water), allowing a monophasic system for optimal extraction (Bligh and Dyer, 1959). After an extraction period of 10 min at 4°C, another 150 µl chloroform and 150 µl distilled water were added and the samples were centrifuged at 1000 ***g*** for 5 min. This created a biphasic system and the lower chloroform layer containing the lipids was removed for liquid scintillation counting (*LSC*). After centrifugation at 12 500 ***g*** for 15 min. the upper methanol/water layer containing the LMW was likewise removed for *LSC*. The non-extracted material was then heated to 90°C for 30 min in 0.3 M trichloroacetic acid followed by ultracentrifugation (12 500 ***g***, 15 min) to precipitate the protein and solubilize the polysaccharides (Zamer *et al.*, 1989). The supernatant containing the polysaccharides was removed for *LSC* and the pellet containing the proteins was dissolved in 500 µl 1 M NaOH and likewise removed for *LSC*.

RESULTS

The functional response of ingestion was very different with the two diets [two-way analysis of variance (ANOVA) $F_{1,5}$=201***; Figure 1]. The ingestion rate of *T. impellucida* was relatively high even at low food concentrations. It

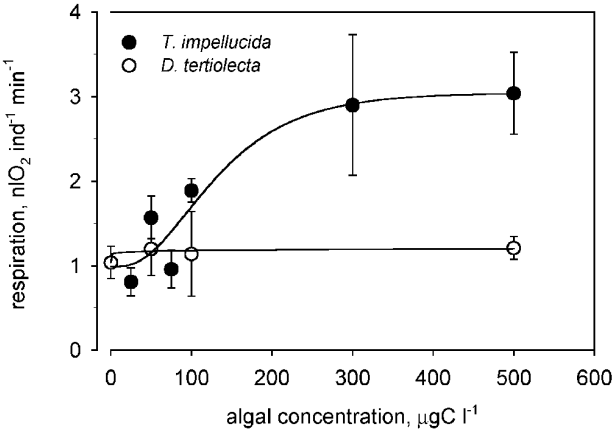

Fig. 2. Respiration rates of *Acartia tonsa* fed *T. impellucida* and *D. tertiolecta* measured with polarographic oxygen electrodes. Regression lines are based on all data: *T. impellucida*: $R = 0.99 + 2.05(1-e^{-C/76.9})^{3.52}$, $r^2 = 0.91$ and *D. tertiolecta*: $R = 1.04 + 0.21(1-e^{-C/11447})^{0.086}$, $r^2 = 0.86$. Regression model was Chapman, four-parameter (from Sigma Plot 4.0, SPSS Inc.).

continued to increase in the whole range of algal concentrations towards a maximum of 19 µg C ind^{-1} day^{-1}. This is equivalent to 380% body C day^{-1} using a carbon weight of 4.6 µg ind^{-1} of *A. tonsa* from Long Island Sound (Tang *et al.*, 1999). The functional response was more clearly sigmoidal on *D. tertiolecta* and there seemed to be a threshold concentration of approximately 40 µg C l^{-1} below which the copepods did not feed. This was not seen with *T. impellucida*. The maximum ingestion rate of *D. tertiolecta* was 7.3 µg C ind^{-1} day^{-1}, equivalent to 150% body C day^{-1}, and curiously it was very much lowered at the high concentration being only 22% of the maximum ingestion rate.

As with ingestion the functional response of respiration was significantly different in individuals fed the two algal diets (two-way ANOVA $F_{1,5}$=8.28**). Figure 2 shows the respiration rates measured with polarographic oxygen electrodes. There was a clear functional response of respiration on the *T. impellucida* diet with a maximum rate of 3.04 nl O_2 ind^{-1} min^{-1}. Those fed *D. tertiolecta* did not show any functional response and the respiration rate was relatively constant, around 1.15 nl O_2 ind^{-1} min^{-1}.

Respiration and ingestion rates were compared to calculate the SDA coefficient. Because the respiration measurements with the polarographic electrodes were conducted at different algal concentrations than the measurements of ingestion rate and respiration rate using Winkler titrations, no direct comparison could be made. The rates were therefore single logarithm transformed to give linear plots against algal concentration and the slopes of these plots were compared (Figure 3). For both diets the regressions of respiration vs. ln C (where C = algal concentration) were significant (*T. impellucida*: F_{44} = 66.4***; *D. tertiolecta*: F_{43} = 6.89*) which was also true for the regressions of ingestion vs. ln C (*T. impellucida*: F_{13} = 27.8***; *D. tertiolecta*: F_{10} = 9.9*).

The generalized equations from the regressions in Figure 3 were used for the calculations of SDA coefficients:

$$I = a_I \ln C + b_I$$

$$R = a_R \ln C + b_R,$$

where I is ingestion, C is algal concentration, R is respiration, a_I and a_R are the slopes of the regressions, and b_I, and b_R the intercepts with the y-axis. Isolating ln$C$ and combining the two equations yields:

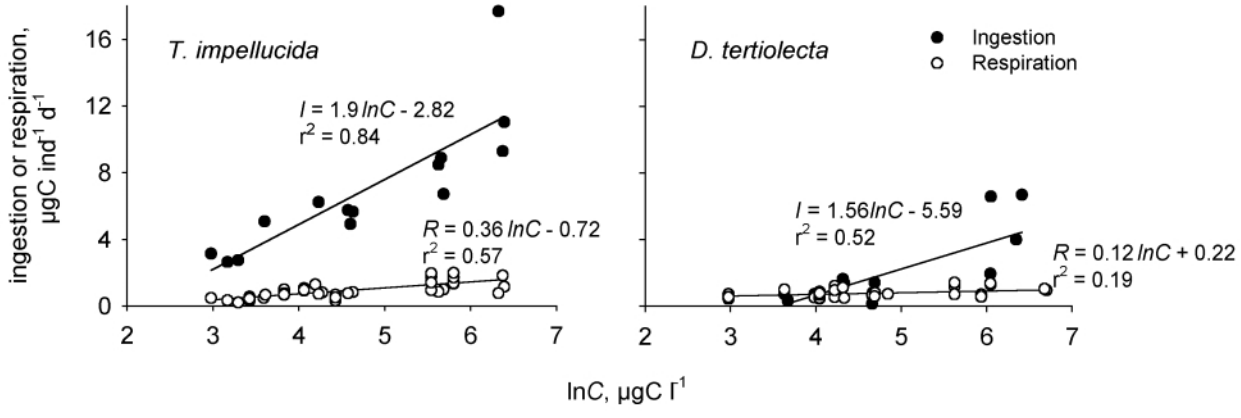

Fig. 3. Plot of rates of ingestion and respiration of *Acartia tonsa* fed *T. impellucida* and *D. tertiolecta* against the natural logarithm of algal concentration. The SDA coefficient is the ratio between the slope of the line depicting respiration rate and the slope of the line depicting assimilation rate. For the respiration, all data from Winkler titration and polarographic oxygen electrodes are plotted.

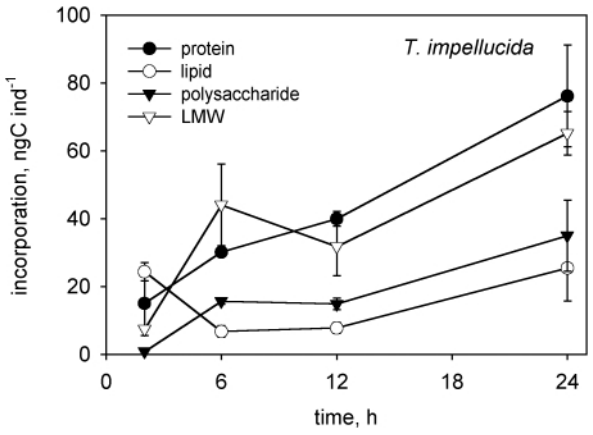

Fig 4. Incorporation of labelled carbon into the protein-, lipid-, polysaccharide- (PS), and 'low molecular weight'- (LMW) fractions of *Acartia tonsa* fed *T. impellucida*.

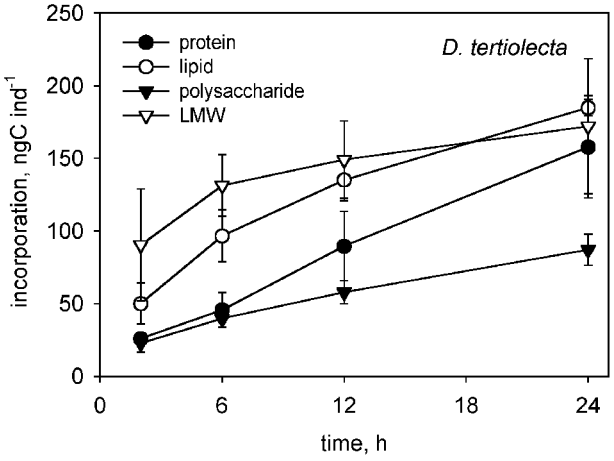

Fig 5. Incorporation of labelled carbon into the protein-, lipid-, polysaccharide- (PS), and 'low molecular weight'- (LMW) fractions of *Acartia tonsa* fed *D. tertiolecta*.

$$R = dI + \beta,$$

where the SDA coefficient is

$$\alpha = \frac{a_R}{a_I}$$

Thus, the SDA coefficient is the ratio between the slopes of the two regressions, α. The SDA coefficients were 0.19 ± 0.031 (α ± S.E.) on *T. impellucida*:

$$\alpha_{Tet} = \frac{0.36}{1.90} = 0.19,$$

and 0.06 ± 0.029 on *D. tertiolecta*:

$$\alpha_{Dun} = \frac{0.12}{1.56} = 0.06$$

These were significantly different (t_{21} = 2.84***). Standard errors of the SDA coefficients were propagated from standard errors of the regressions of R and I vs. ln$C$ (Meyer, 1975).

The labelled carbon was distributed equally into the biochemical fractions in the two algal species: 39% into protein, 39% into lipid, and 15% into the LMW fraction (Student's *t*-test: protein, t_4 = 0.49^{ns}; lipid, t_4 = 2.36^{ns}; LMW, t_4 = 2.00^{ns}). The polysaccharide fraction constituted 8% in *T. impellucida* and 6% in *D. tertiolecta* of the total label (t_4 = 6.99**; Figure 6).

Figures 4 and 5 shows the course of incorporation into the copepods during the 24 h incubation period. The main differences in the pattern of incorporation between the two diets were in the protein and lipid fractions (Figure 6). The percentage of carbon incorporation into protein resembled that of the algae in the copepods feeding on *T. impellucida*: 38% (Student's *t*-test, t_4 = 0.37^{ns}) but was significantly lowered to 26% on the *D. tertiolecta* diet (t_4 = 3.65*). Moreover, the incorporation into lipid on the *T. impellucida* diet was only 13% of the total carbon incorporated. This was significantly lower than on the *D. tertiolecta* diet (two-way ANOVA: F_{14} = 105.8***). Here it was 31%, resembling the composition of the algae to a greater extent although still significantly different from it (Student's *t*-test: t_4 = 13.04***). Looking at the percentage of labelled carbon that was incorporated into eggs a different picture emerges. Of the relatively low amount of label incorporated into the lipid fraction of copepods fed *T. impellucida*, ca. 80% was allocated to eggs. In the copepods fed *D. tertiolecta* it was under 20% (Figure 7).

Although the difference was not significant (t_{88} = 0.88^{ns}), the Winkler's titration method gave higher respiration rates than the electrode measurements at all algal concentrations (Figure 8). This is rather surprising since the copepods were held in filtered *asw* during the 24 h incubation for the Winkler's titration. This would lower the respiration rate to a starvation rate within the first 8–10 h (Thor, 2000) creating lower average rates. A plausible explanation is a higher level of stress during the electrode measurements. Stress due to crowding and confinement to small volumes has previously been observed and the reaction tends to be lowered rates of respiration and excretion (Le Borgne, 1986)

DISCUSSION

The two green algae *Tetraselmis impellucida* and *Dunaliella tertiolecta* created very different maximal ingestion rates as well as functional response curves in *Acartia tonsa*. The allocation of ingested carbon was also different between

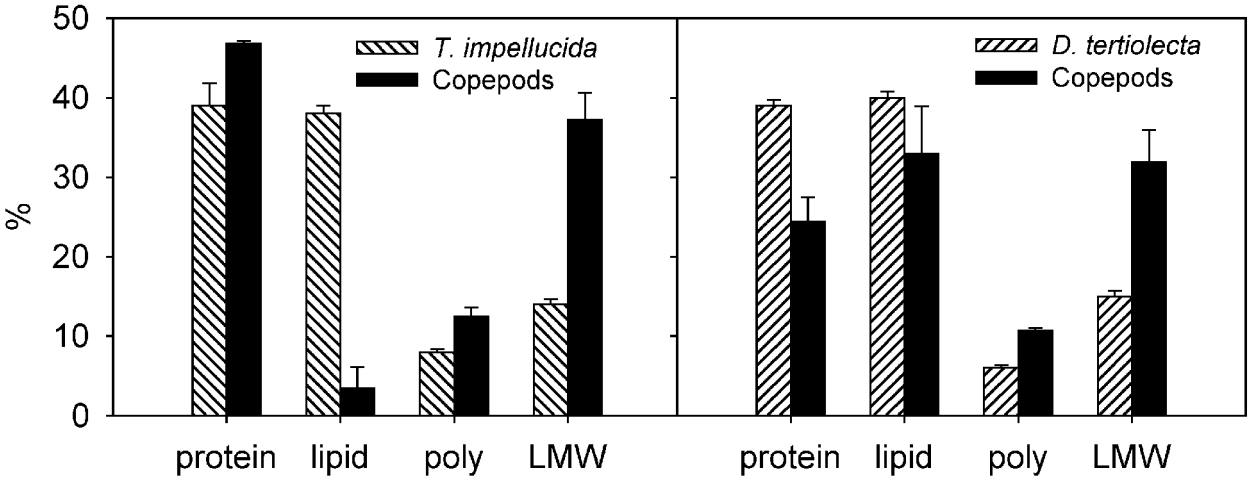

Fig. 6. Incorporation of labelled carbon into the protein-, lipid-, polysaccharide- (PS), and 'low molecular weight'- (LMW) fractions of algae and *Acartia tonsa* based on data from Figures 4 and 5. The total amount of label incorporated (protein + lipid + polysaccharides + LMW) after 24 h is set as 100%.

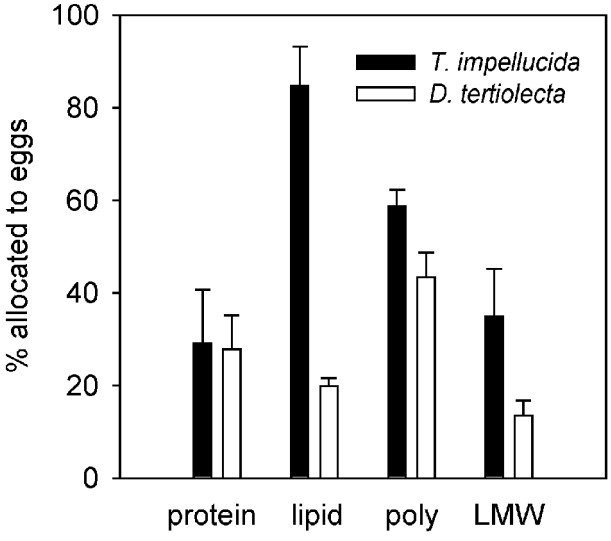

Fig. 7. Proportion of labelled carbon incorporated into eggs of *Acartia tonsa* fed either *T. impellucida* or *D. tertiolecta*. For each fraction, the total amount of label in eggs plus tissue at any given time is set as 100%.

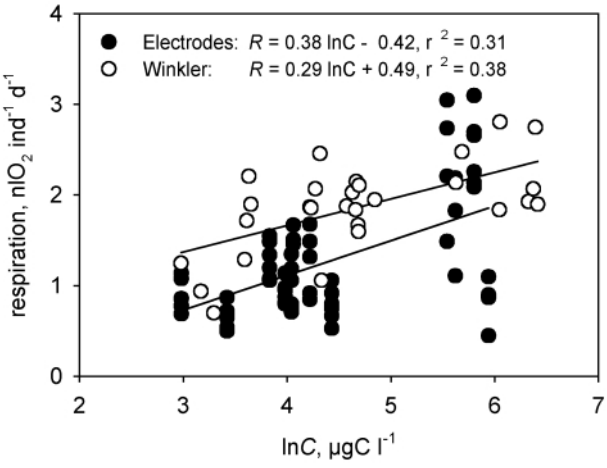

Fig. 8. Comparison of methods of respiration measurement on *Acartia tonsa*.

individuals feeding on the two algae indicated by significant differences in the incorporation into proteins, lipids and polysaccharides. Apparently this induced significant differences in the metabolism of the copepods since the SDA coefficients were much higher on the *T. impellucida* diet than on the *D. tertiolecta* diet.

The rates of ingestion of *D. tertiolecta* were much lower than of *T. impellucida* at all algal concentrations. Previously *D. tertiolecta* has generated low egg production rates in *A. tonsa* and might, therefore, be considered a low-quality diet (Cervetto *et al.*, 1999). For the discussion in the present paper, the quality of a given food source is called high if it induces high rates of ingestion or growth, for example. The functional response on the *D. tertiolecta* diet was identical to that previously found for *A. tonsa* feeding on this alga (Støttrup and Jensen, 1990). It reached a maximum of 150% body C day^{-1} at 450 µg C l^{-1} and, curiously, it decreased at the higher concentration. The functional response on the *T. impellucida* diet very much resembled that of *A. tonsa* feeding on *Isocrysis galbana*, another flagellate (Støttrup and Jensen, 1990) and the seemingly very high maximum ingestion rate of 380% body C day^{-1} is comparable with what has been found in *A. clausi* feeding on the same algae (Pagano and Saint-Jean, 1994). Feeding *A. tonsa* different algal species, Berggreen *et al.* was able to show that the retention efficiency during filtration depended greatly on algal cell size (Berggreen *et al.*, 1988). However, since the cell diameters of *T. impellucida* and

D. tertiolecta are almost identical any differences in retention efficiency are negligible. Sensory perception of the quality of a given alga has been shown in many studies [see review (Kleppel, 1993)]. Copepods are thought to be able to sense the chemical quality of the algal cells thereby selecting those of good quality and optimizing feeding. The main dietary component determining the dietary quality of an alga is thought to be protein or amino acids (Libourel Houde and Roman, 1987). Nevertheless, the protein content of two algal species in the same two genera, *T. maculata* and *D. salina*, is virtually the same—52% and 57%, respectively (Parsons *et al.*, 1961). So, if this also applies for *T. impellucida* and *D. tertiolecta* then the differences in ingestion cannot be caused by perception of the total amount of protein in the algae.

The SDA coefficients were based on ingestion rate rather than assimilation rate used previously (Kiørboe *et al.*, 1985, 1987). When the coefficients are based on ingestion rate they become sensitive to variations in assimilation efficiency. They are therefore not directly comparable with SDA coefficients based on assimilation but fortunately a recalculation is possible. The assimilation efficiency is likely to decrease with increasing algal concentration (Kiørboe *et al.*, 1985; Landry *et al.*, 1984) and if the assimilation efficiencies from Kiørboe *et al.* are used to calculate assimilation rates in our study the assimilatory SDA coefficients become 0.38 ± 0.11 and 0.12 ± 0.06 (Kiørboe *et al.*, 1985). These are still significantly different ($_{21}$ = 2.15*). The assimilatory SDA coefficients of aquatic crustaceans vary, being 0.06 in the Shore crab *Carcinus meanas* (Wallace, 1973), over 0.16 in the copepod *Temora stylifera* (Abou Debs, 1984) and 0.17 in *A. tonsa* fed *Rhodomonas baltica* (Kiørboe *et al.*, 1985), and up to 0.17–0.20 in the daphnid *Daphnia magna* (Lampert, 1986). Thus, the calculated assimilatory SDA coefficients of *A. tonsa* fed *T. impellucida* is unusually high, which may be a result of the somewhat pragmatic recalculations. Nevertheless, the results show that *A. tonsa* exhibit SDA coefficients varying from high to low when feeding on *T. impellucida*, the 'high-quality' diet, or *D. tertiolecta*, the 'low-quality' diet.

The significantly lower SDA coefficients of copepods fed *D. tertiolecta* indicate that the increase in metabolic rate during and after feeding was not responsible for the lower egg production rates previously found in *A. tonsa* on this diet. On the contrary it seems that the magnitude of the SDA coefficients was primarily governed by the amount of food ingested. However, nutritional differences between the two diets may also have been important. The magnitude of SDA of both *A. tonsa* and another calanoid copepod *Calanus finmarchicus* was tightly coupled to the rate of protein deposition in a previous study (Thor, 2000). Moreover, the magnitude of SDA has been shown to vary with developmental stage in *Calanus finmarchicus* (Thor, in preparation). Here the magnitude of SDA was higher in females allocating more carbon into proteins than in copedite V instars which allocated less carbon into proteins. In our study relatively higher amounts of carbon were allocated to proteins in copepods fed *T. impellucida* then in those fed *D. tertiolecta*. From this it seems that SDA is an attribute of growth primarily influenced by the physiological processes of egg production and growth rather than a factor acting independently competing for the ingested energy. Apparently protein synthesis was the physiological process of greatest importance.

The incorporation of carbon into lipids was significantly higher in copepods fed *D. tertiolecta*. The reason could be that *Dunaliella* species contain more lipid in total (Parsons *et al.*, 1961) and that the copepods therefore were acclimated to high rates of lipid assimilation during the acclimation period. Interestingly, the copepods did not benefit from this. The proportion of carbon allocated to egg lipids was much lower than in those fed *T. impellucida*. Thus, the lower egg production in *A. tonsa* fed *D. tertiolecta*, found previously (Støttrup and Jensen, 1990; Cervetto *et al.*, 1999), was not caused by higher SDA but probably by a low nutritional value of the lipids in this algal species. Støttrup and Jensen concluded that the cause was lack of essential longer chain fatty acids making *D. tertiolecta* less suitable for egg production (Støttrup and Jensen, 1990).

The ingestion of *D. tertiolecta* was depressed at the high algal concentration. It seems that there existed some kind of upper threshold beyond which *A. tonsa* reacted negatively to the alga. *Dunaliella tertiolecta* has been shown to be absorbed poorly by snail larvae (*Nassarius obsuletus*) as compared to larvae fed either the diatom *Thalassiosira pseudonana* or the flagellate *I. galbana* (Pechenik and Fisher, 1979). They argued that the low assimilation efficiency (or 'retention efficiency') might have been due to either the lack of some essential micronutrients or the production of toxins by the algae. We do not know of any records of toxins in *D. tertiolecta*. Moreover, feeding on a mixture of five algal species, including *D. tertiolecta*, *A. tonsa* had maximal egg production rates. If any inhibitory substances were present this should not have been the case.

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