JOURNAL OF PLANKTON RESEARCH | VOLUME 27 | NUMBER 6 | PAGES 557–568 | 2005

Relationship between functional response and gut transit time in the calanoid copepod *Acartia clausi*: role of food quantity and quality

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Received December 22, 2004; accepted in principle March 24, 2005; accepted for publication May 19, 2005; published online June 3, 2005

Communicating editor: K.J. Flynn

The relationship between ingestion rate and gut transit time of the calanoid copepod Acartia clausi was examined in laboratory experiments with five different diets: (i) living cells of the diatom Thalassiosira weissflogii, (ii) detrital cells of the same diatom, (iii) 50:50 mix of the two previous diets on a protein basis, (iv) dinoflagellate cells of Prorocentrum micans and (v) Prorocentrum minimum. Ingestion followed a Holling type 2 response for diets 1 and 4 and a linear one for diets 2, 3 and 5. Gut transit time varied with food abundance only when the copepods were fed with the living diatom. The gut evacuation rate increased with the concentration of T. weissflogii with values of 0.010, 0.020, 0.032, 0.042 min^{-1}, corresponding to gut transit time of 97, 50, 31 and 24 min, measured at 50, 110, 130 and 275 μg protein L^{-1}, respectively. Copepods fed with dinoflagellates, mixed and pure detrital diets exhibited longer and similar gut transit times ranging from 85 to 166 min, depending on diet. The coupling between ingestion rate and gut transit time measurements is discussed in the context of copepod feeding strategies.

INTRODUCTION

During the last two decades, interest in the influence of food quality on copepod metabolism has increased greatly and is now considered an essential parameter in studies of their feeding activity and fecundity (e.g. Checkley, 1980; Paffenhöfer and Van Sant, 1985; Libourel Houde and Roman, 1987; Donaghay, 1988; Vanderploeg *et al.*, 1990; Kleppel, 1993; Jónasdóttir, 1994; Jónasdóttir *et al.*, 1998; Mayzaud *et al.*, 1998; Miralto *et al.*, 1999; Irigoien *et al.*, 2002; Ianora *et al.*, 2004; Kuijper *et al.*, 2004; Jones and Flynn, 2005). Food type and concentration also affect copepod faecal pellet characteristics (Dagg and Walser, 1986; Feinberg and Dam, 1998; Griffin, 2000; Besiktepe and Dam, 2002) with important implications on estimation of organic matter export flux from the euphotic zone to deep waters. Nevertheless, the definition of food quality remains complex, and its role is yet not well understood (Mitra and Flynn, 2005).

Penry and Jumars (Penry and Jumars, 1986, 1987), modelling copepods gut as a chemical reactor, indicated gut transit time as one of the 'key processes' to study in order to understand strategies of adaptation to trophic conditions. Jumars *et al.* (Jumars *et al.*, 1989) suggested that, for a given cell type, when ingestion rate increases, the gut residence time decreases. As a result, they made the hypothesis that when food abundance is not limiting, it is in the animal's best interests to have lower conversion efficiency and faster gut transit time than when food is scarce Recently, Mitra and Flynn (Mitra and Flynn, 2005) pointed out that when food is of poor quality, predator may adopt two contrasting solutions: it may decrease throughput of ingested material allowing more time for food digestion or increase throughput increasing

doi:10.1093/plankt/fbi031, available online at www.plankt.oxfordjournals.org

the ingestion of the limiting nutrients. This second option had been already applied to mesozooplankton by Paffenhöfer and Van Sant (Paffenhöfer and Van Sant, 1985), who suggested that if a copepod cannot obtain or extract much from ingested matter, then the residence time in the gut should be short. On the other hand, if a grazer obtains much energy/nitrogen from ingested material, then food residence time in the gut should be long.

Even if it seems reasonable that food quality should be an important factor in the regulation of digestion time, evidence of its effect on copepod gut transit times is scarce and discordant. Tsuda and Nemoto (Tsuda and Nemoto, 1987) observed that *Pseudocalanus minutus* did not change gut transit time when fed two species of dinoflagellates of similar cell volume, *Prorocentrum triestinum* and *Scripsiella trochoidea*, even if the former was ingested at a higher rate than the latter. Copepods of the species *Pseudocalanus* sp., fed with the same concentrations of different algae (melted ice algae and pelagic under-ice algae), had gut transit times significantly different (Head, 1988). In the review about gut clearance rate constant, temperature and initial gut contents, Irigoien (Irigoien, 1998) showed that copepods fed with phytoplanktonic cultures had shorter gut transits time than copepods used in experiments immediately after the catch, which probably had a more variable diet (phytoplankton but also detritus and/or microzooplankton) than laboratory acclimated animals. Besiktepe and Dam (Besiktepe and Dam, 2002) found that *Acartia tonsa* had shorter gut transit time when fed with diatoms than with dinoflagellates, flagellates or scuticociliates.

Conflicting evidences have been reported also about the influence of food concentration on gut transit time. Some authors have reported gut transit times inversely related to food abundance (Murthaug, 1984; Wang and Conover, 1986; Dagg and Walser, 1987; Tsuda and Nemoto, 1987; Pasternack, 1994; Mayzaud *et al.*, 1998; Besiktepe and Dam, 2002), whereas others have not found any relationships (Head *et al.*, 1988; Ellis and Small, 1989; Tseitlin *et al.*, 1991). The same disagreement is reported in the literature concerning the link between gut transit time and gut content. In some studies copepods have been reported to slow down the gut transit time when the amount of food in their guts increases (Baars and Oosterhuis, 1984; Head, 1986; Irigoien, 1994; Tseitlin, 1994; Perissinotto and Pakhomov, 1996; Tirelli and Mayzaud, 1999). However, in other experiments this behaviour has not been observed (Wang and Conover, 1986; Head *et al.*, 1988; Morales *et al.*, 1990).

Experimental studies carried out considering the coupling between ingestion and gut transit time of copepods as function of food quantity and quality are still scarce (Dagg and Walser, 1987; Mayzaud *et al.*, 1998; Besiktepe and Dam, 2002). This article is an extension of a previous work (Mayzaud *et al.*, 1998) dedicated to the influence of food quality on the nutrition of the copepod *Acartia clausi*. Mayzaud *et al.* (Mayzaud *et al.*, 1998) observed that gut transit time displayed different adaptive changes with food regime depending on protein concentration and suggested that *Acartia*-type copepods optimize nitrogen or protein uptake. Their experiments were carried out with four diets (the diatom *Thalassiosira weissflogii*, detritus obtained from the same diatom culture, 50:50 mix of the two previous diets on protein basis and the dinoflagellate *Prorocentrum micans*) and gut transit time was measured at two food concentrations (limiting and saturating concentration). The range of diets used reflected food quality variation due to taxonomic differences (diatom–dinoflagellate) and due to differences in cell composition associated to cell physiological state (diatom-detritus). In this study, we present more comprehensive results of experiments on ingestion rate and gut transit time in *A. clausi*, fed with all the previous tested diets and the dinoflagellate *Prorocentrum minimum*. The coupling of these two processes is used to obtain information on feeding strategies in this species.

METHOD

Zooplankton were collected in the Bay of Villefranche sur Mer (France: 43°42′N, 7°18′E) with a 10-min haul from 10 m to the surface, using a 333 μm mesh size net. The copepods were diluted into a 5-L plastic cooler immediately after capture and brought back to the laboratory within 30 min. Adults and few stage-V copepodites of *A. clausi* were then sorted under a dissecting microscope. All the experiments were carried out in darkness at the *in situ* collection temperature, which ranged between 14 and 15°C. Before the start of each experiment, sorted animals were acclimated to laboratory conditions for 6 h in 1-L jars filled with natural sea water.

Food quality

Five different diets were used: (i) the diatom *T. weissflogii*, (ii) detritus obtained from the same diatom culture, (iii) 50:50 mix of the two previous diets on protein basis, (iv) the dinoflagellates *P. micans* and (v) *P. minimum*. Cultures were obtained using the F/2 medium (Guillard and Ryther, 1962), with silicate for the diatom and without silicate for the dinoflagellates. Only cultures in exponential growth phase were used in the experiments. In this study, we utilized heat-killed diatoms as an example of detritus. The procedure to obtain detritus was adapted from that used by Paffenhöfer and Van Sant (Paffenhöfer and Van Sant, 1985), as described in Mayzaud *et al.* (Mayzaud *et al.*, 1998). Dead cells retained the shape and dimensions of living cells but had much

lower protein content (50–75%) (Table I). Experimental food media were prepared by adding known concentrations of phytoplankton to seawater passed through 0.45 μm Millipore membrane filter. Phytoplankton abundance was assessed either from microscopic counts using a counting cell or by fluorescence analysis. For chlorophyll measurements, known volumes of living phytoplankton and detritus were filtered onto Whatman GF/C filters, extracted in 90% acetone and analysed with a Turner-design 10 fluorometer (Holm-Hansen *et al.*, 1965).

Ingestion rate

As many previous studies have indicated that nitrogen rather than carbon represents the limiting element for copepods (Roman, 1984; Paffenhöfer and Van Sant, 1985; Libourel-Houde and Roman, 1987), in this study we chose total protein as the unit for food concentration. Food concentration offered varied from 50 to 500 μg protein per litre. Proteins were measured following the procedure described by Lowry *et al.* (Lowry *et al.*, 1951), using albumine as the standard, after filtration of known volumes of each diet on precombusted (450°C, 12 h) GF/C filters. For each ingestion experiment, nine 1-L jars were filled with experimental food medium at a specific protein concentration. Three jars were used to estimate the initial concentration of chlorophyll, three were inoculated with 40–50 *A. clausi*, and three were incubated as controls (without copepods). Feeding activity with increasing food concentrations was studied during the same experiment. Incubations took place on a rotating wheel for a period of 5–15 h (Table II). Duration of incubations was organized to obtain a detectable decrease of fluorescence, due to feeding activity, and to keep them to a minimum compatible with a 20% decrease in phytoplankton standing stock in the experimental containers compared to the control.

For *P. minimum* the feeding activity of copepods acclimated at low food concentration (40 μg protein L^{-1} of *P. minimum*) for 24 h before the experiment was also measured. In this instance, the incubation period was of 26 h.

Ingestion rates were first measured as changes in chlorophyll concentrations and calculated according to the equations of Frost (Frost, 1972). Rates were then converted to protein using the protein/chlorophyll ratio measured for each diet during each experiment.

Table I: Concentration in nanograms per cell of protein and volume of the algae used as food for Acartia clausi

Diet	Protein content (ng protein/cell)	Cell volume (μm^3)
Prorocentrum micans	48×10^{-2}	82.4×10^3
Prorocentrum minimum	12×10^{-2}	9.16×10^3
Thalassiosira weissflogii	$6–7 \times 10^{-2}$	1.3×10^3
Thalassiosira weissflogii—detritus	$\sim 3 \times 10^{-2}$	1.3×10^3

The volume was calculated considering the shape of *Thalassiosira weissflogii* and dinoflagellates similar to a cylinder and an ellipsoid respectively.

Gut transit time

Gut transit time was measured for each diet, at several food concentrations (Table III). For each experiment, the gut pigment content of 700–900 copepods was measured. Initially, 12–20 groups of 60 copepods were placed in 1-L jars and fed for 3 h. The animals were confined in the jar within a chamber consisting of a cylinder with a 200 μm mesh net at the bottom. This system permitted the transfer with minimum stress of the animals from the food medium to a jar filled with food-free filtered seawater. At fixed time intervals, copepods from one jar were removed, rinsed and filtered onto Millipore 5 μm membrane filter. These animals were immediately counted under cold-dim light (optic fibre), homogenized and extracted in 6 mL of 90% acetone for at least 1 hour at 4°C. Samples were then centrifuged at 3250 *g* for 20 min. The fluorescence of the supernatant was measured on a Turner-design 10 fluorometer, before and after acidification, using Sigma Chl standard. No corrections for background fluorescence and pigment destruction were applied. The gut pigment contents were calculated as the sum of chlorophyll *a* and phaeopigments. The decline in gut fluorescence over time was fitted with a negative exponential equation using the Systat statistical package (Wilkinson *et al.*, 1992) and gut transit time (K, min^{-1}) was calculated as:

$$G = G_0 e^{-Kt}$$

where G is gut pigment after time t (in minutes) and G_0 is initial gut pigment.

RESULTS

Ingestion rate

Over the range of food concentration considered, the ingestion of food types with different qualities was best described by different functional curves (Fig. 1). A Holling type 2 response (Holling, 1959) was observed for both the living diatom *T. weissflogii* and the dinoflagellate *P. micans* (Fig. 1A and B). The I_{max} (maximum ingestion rate) obtained by fitting an Ivlev (Ivlev, 1951) equation to the experimental

Table II: Parameters of the Ivlev (I, $I_{max}{}^{*}1 - e^{-\alpha conc.}$) and linear ($I$, a·concentration) equations used to describe the relationship between food concentration and ingestion rate at different food quality

Diet	Acclimation period	Incubation period	Model	I_{max} (μg prot. cop. $^{-1}h^{-1}$)	< 95% >	α	< 95% >	r^2
Thalassiosira weissflogii	6 h natural SW	5 h	Ivlev	0.38 ± 0.032	$0.32 < I_{max} > 0.45$	3.5×10^{-3}	$2.3 \times 10^{-3} < \alpha > 4.8 \times 10^{-3}$	0.99
Thalassiosira weissflogii – *detritus*	6 h natural SW	5 h	Linear			26×10^{-5}	$24 \times 10^{-5} < \alpha > 28 \times 10^{-5}$	0.99
Detritus	6 h natural SW	18 h	Linear			5×10^{-5}	$5 \times 10^{-5} < \alpha > 6 \times 10^{-5}$	0.98
Prorocentrum minimum	6 h natural SW	12 h	Linear			26×10^{-5}	$22 \times 10^{-5} < \alpha > 29 \times 10^{-5}$	0.90
	24 h at 40 μgL^{-1} *P.* minimum	26 h	Linear			8×10^{-5}	$7 \times 10^{-5} < \alpha > 8 \times 10^{-5}$	0.98
Prorocentrum micans	6 h natural SW	15 h	Ivlev	0.095 ± 0.007	$0.077 < I_{max} > 0.112$	9.8×10^{-3}	$5.4 \times 10^{-3} < \alpha > 14 \times 10^{-3}$	0.97

I_{max}, maximum ingestion rate (μg prot. cop.$^{-1}h^{-1}$); α for Ivlev model, the rate at which saturation is achieved with increasing food levels (μg prot. L^{-1}); α for linear model, clearance rate (L cop.$^{-1}h^{-1}$); concentration, food concentration (μg prot. L^{-1}). r^2, coefficient of determination.

Table III: Initial gut pigment content (G_0), gut evacuation rate (K) and gut transit time (1/k) obtained adjusting the experimental data with a negative exponential equation $G = G_0 e^{-Kt}$

Diet	Food concentration (μg prot. L^{-1})	G_0 (ng pig. Cop.$^{-1}$)	Gut evacuation rate K(min^{-1})	Gut transit time 1/K (min)	N	r^2
Prorocentrum minimum	50	0.208 ± 0.016	0.0091 ± 0.003	109	14	0.97
	170	0.269 ± 0.022	0.0095 ± 0.003	105	11	0.98
	370	1.000 ± 0.079	0.0094 ± 0.003	106	16	0.96
Prorocentrum micans	50*	0.092 ± 0.003	0.007 ± 0.001	135	18	0.99
	110	0.300 ± 0.004	0.007 ± 0.004	135	17	0.91
	275*	0.798 ± 0.056	0.007 ± 0.002	142	15	0.96
Thalassiosira weissflogii	50*	0.445 ± 0.024	0.010 ± 0.004	97	18	0.98
	110	0.748 ± 0.051	0.020 ± 0.007	50	14	0.97
	130	0.782 ± 0.056	0.032 ± 0.009	31	15	0.97
	275*	2.197 ± 0.158	0.042 ± 0.010	24	20	0.95
Thalassiosira weissflogii—detritus	50*	0.535 ± 0.028	0.011 ± 0.002	85	17	0.98
	275*	1.984 ± 0.092	0.012 ± 0.002	88	17	0.98
Detritus	50	Nd	Nd	Nd	14	—
	100*	0.179 ± 0.011	0.0058 ± 0.002	172	12	0.98
	220*	0.601 ± 0.033	0.0062 ± 0.002	166	15	0.98
	300	0.156 ± 0.013	0.0060 ± 0.003	161	13	0.97

G is gut pigment after time t, in minutes.
Nd, no defecation; N, number of groups of copepods examined; r^2, adjusted coefficient of determination.
*Data already published by Mayzaud *et al.* (Mayzaud *et al.*, 1998).

data ranged from 0.095 ± 0.007 μg protein cop.$^{-1}$ h^{-1} for *P. micans* to 0.38 ± 0.032 μg protein cop.$^{-1}$ h^{-1} for the diatom cells. The α coefficient (Table II) indicates that saturation was reached faster with *P. micans* than any other diet and food saturation concentration (food concentration corresponding to an ingestion rate equivalent to 95% of I_{max}) was higher for the diatom (856 μg protein L^{-1}) than the dinoflagellate (306 μg protein L^{-1}).

Acartia clausi fed on detritus and mixed diets showed a progressive increase in ingestion rates with food abundance, never reaching saturating plateau within the range of experimental food concentrations (Fig. 1C and D). The mixed diets were consumed at a rate on average five times higher than pure detritus.

The functional response observed for ingestion of *P. minimum* was linear, notwithstanding the type of copepod acclimation (Fig. 1E and F), but ingestion rates were higher for animals acclimated for only 6 h in natural sea water. During this experiment, a sharp decrease in ingestion activity at dinoflagellate concentrations higher than 400 μg protein L^{-1} (Fig. 1E) was observed.

Gut transit time

The rate of decrease in gut pigments of copepods pre-fed with different food qualities and quantities is presented in Figs. 2–6. During the incubations in filtered sea water, the quantity of gut pigments decreased to a minimum of 60% of its initial value. Gut transit time varied with food abundance only when copepods were fed with living diatom cells (Fig. 2, Table III). The gut evacuation rate increased with the concentration of *T. weissflogii* with values of 0.010, 0.020, 0.032, 0.042 min^{-1}, equating to gut transit time of 97, 50, 31.25 and 24 min, measured at 50, 110, 130 and 275 μg protein L^{-1}, respectively (Table III). Copepods fed with mixed (Fig. 3), pure detrital (Fig. 4) and dinoflagellates (Figs.5 and 6) diets exhibited longer and invariant gut transit times: mean gut transit times were 86.5 ± 2.1, 166.3 ± 5.5, 106.7 ± 2.1, 137.3 ± 4.0 min for mixed diet, detritus, *P. minimum* and *P. micans* respectively (Table III). No defecation was observed when copepods were pre-fed at 50 μg protein L^{-1} of detritus (Fig. 4A).

The initial gut contents obtained with the exponential equation (G_0) were always in the range of the real values of gut pigment contents measured at the beginning of the experiment and increased according to the food concentration offered to the copepods (Table III). The only exception to this trend was the G_0 of the experiment carried out at the highest concentration of detritus (Fig. 4D). Indeed copepods pre-fed at 300 μg protein L^{-1} showed the lowest gut pigment content (0.156 ng

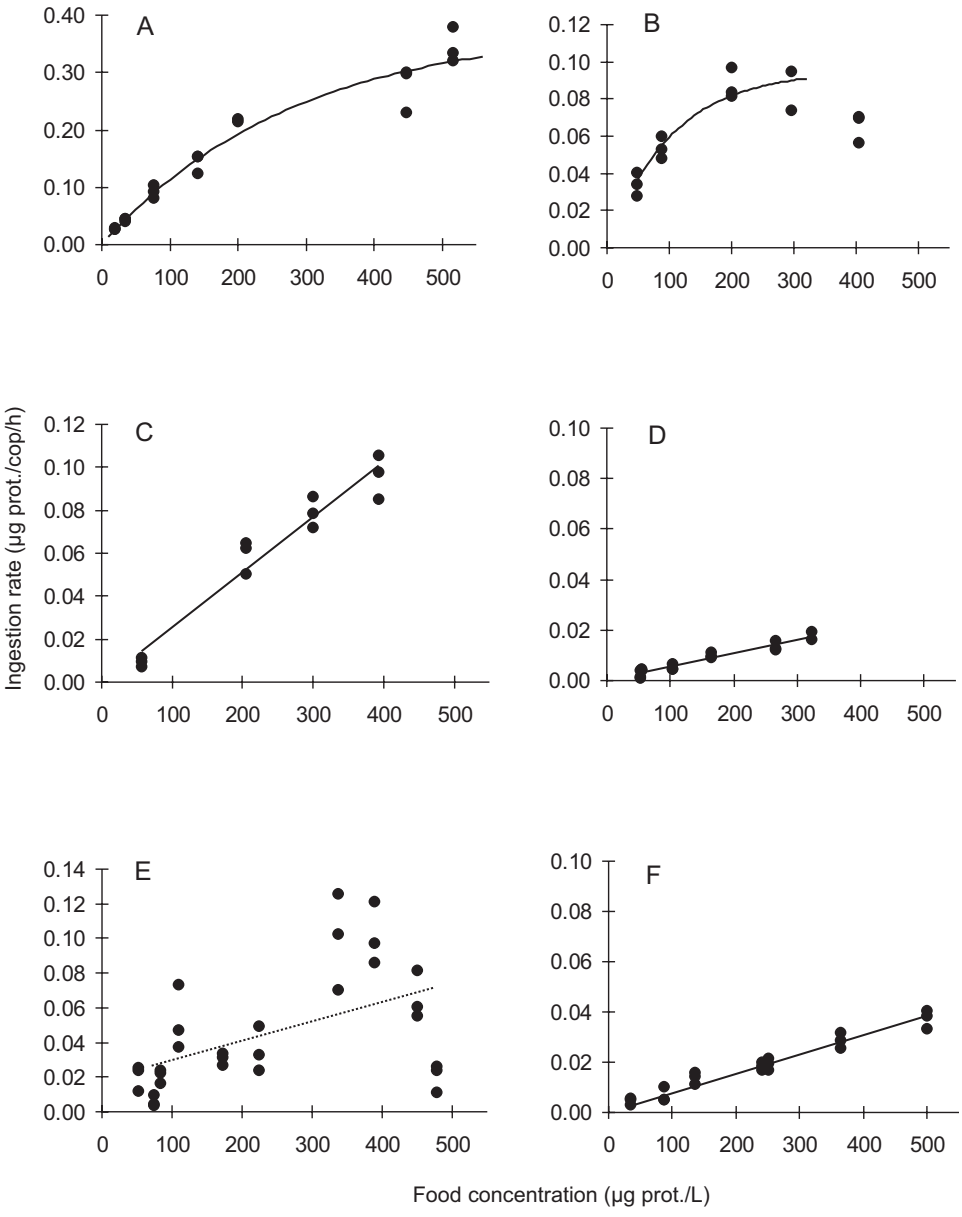

Fig. 1. Ingestion rates *Acartia clausi* fed at several concentrations of different-quality food after an acclimation period of 6 h in natural seawater: (**A**) the diatom *Thalassiosira weissflogii* at exponential growth phase (**B**) the dinoflagellate *Prorocentrum micans*, (**C**) 50:50 mix of live diatom and detrital cells obtained from *Thalassiosira weissflogii*, on a protein basis, (**D**) pure detrital cells obtained from *T. weissflogii* and (**E**) the dinoflagellate *Prorocentrum minimum*. (**F**) Ingestion rates *A. clausi* fed with *P. minimum* after an acclimation of 24 h at low concentration of the same species of dinoflagellate.

pigments cop.$^{-1}$) obtained with this diet, probably due to the low chlorophyll content of the detritus used in this experiment.

DISCUSSION

Food quality influenced both the ingestion process and the gut transit time of *A. clausi* during these experiments. The functional response of ingestion was fitted well by the Ivlev equation only when the copepods were fed with the diatom *T. weissflogii* and the dinoflagellate *P. micans*. In agreement with Frost (Frost, 1972) and Libourel-Houde and Roman (Libourel-Houde and Roman, 1987) we observed that the maximum ingestion rate (I_{max}, µg prot./cop./h) occurred at lower concentrations (µg protein L^{-1}) for larger size cells. In fact *A. clausi* reached saturation faster when fed with *P. micans,* which has a volume 68 times higher than *T. weissflogii.* On the other hand, I_{max} was higher for *T. weissflogii* than for *P. micans,* supporting the hypothesis proposed by Paffenhö-

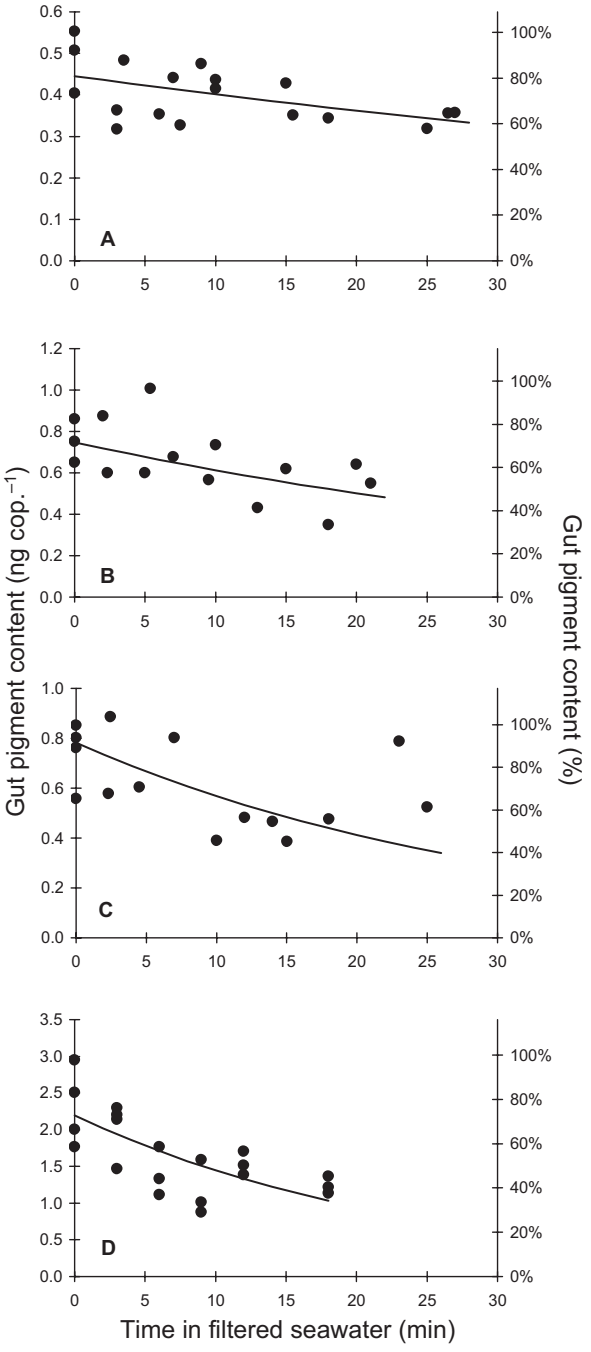

Fig. 2. *Acartia clausi*. Decline in gut pigments (Chl *a* + Phaeopigments) following transfer to filtered seawater, after feeding at increasing concentrations of the diatom *Thalassiosira weissflogii*: (**A**) 50 μg prot. L^{-1}, (**B**) 110 μg prot. L^{-1}, (**C**) 130 μg prot. L^{-1} and (**D**) 275 μg prot. L^{-1}.

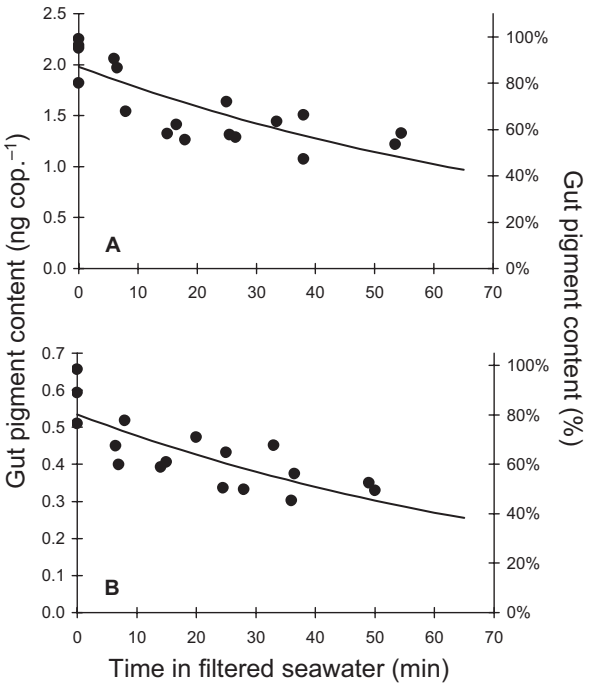

Fig. 3. *Acartia clausi*. Decline in gut pigments (Chl *a* + Phaeopigments) following transfer to filtered seawater, after feeding at low (A:50 μg prot. L^{-1}) and high (B:275 μg prot. L^{-1}) concentrations of the 50:50 mix, on a protein basis, of diatom *Thalassiosira weissflogii* and detritus.

fer and Vant Sant (Paffenhöfer and Vant Sant, 1985) and Libourel Houde and Roman (Libourel Houde and Roman, 1987), that copepods optimize the uptake of protein ingesting at higher maximal rates cells with a lower protein content. *A. calusi* has a mean N content of 0.68 ± 0.06 μg N cop.$^{-1}$ (Cataletto and Fonda Umani, 1994) and, adopting a protein:N of 6.25, a mean protein content of 4.23 ± 0.36 μg prot.cop.$^{-1}$. Considering these values, we can calculate that the I_{max} measured when *A. clausi* was fed on *T. weissflogii* corresponds to the maximal daily specific ingestion rate of 2.16 μg prot./μg prot. $_{copepod}$ /d and, for a gross growth efficiency of 0.33, to a growth rate of 0.7 day^{-1} (value in the range of growth rate present in literature for *Acatia*-type copepods).

Over the range of concentrations considered, copepods fed pure detritus and mixed diets increased progressively their ingestion rate with increasing food abundance, without reaching any saturation (Fig. 1C and D). A linear functional response in the presence of detrital particles has been already observed by Roman (Roman, 1984), Paffenhöfer and Van Sant (Paffenhöfer and Vant Sant, 1985), Ayukai (Ayukai, 1987) and Mayzaud *et al.* (Mayzaud *et al.*, 1998) under both experimental and natural food conditions. Ingestion rates on the mixed diet were on average 5 times higher than on detritus (Table II), suggesting that *A. clausi* might have selected preferentially living cells or increased the feeding activity in order to enhance its chance of ingesting cells rich in protein. The shape of the functional response observed for *T. weissflogii*, *P. micans* and detritus supports the results obtained by Mayzaud *et al.* (Mayzaud *et al.*, 1998). The

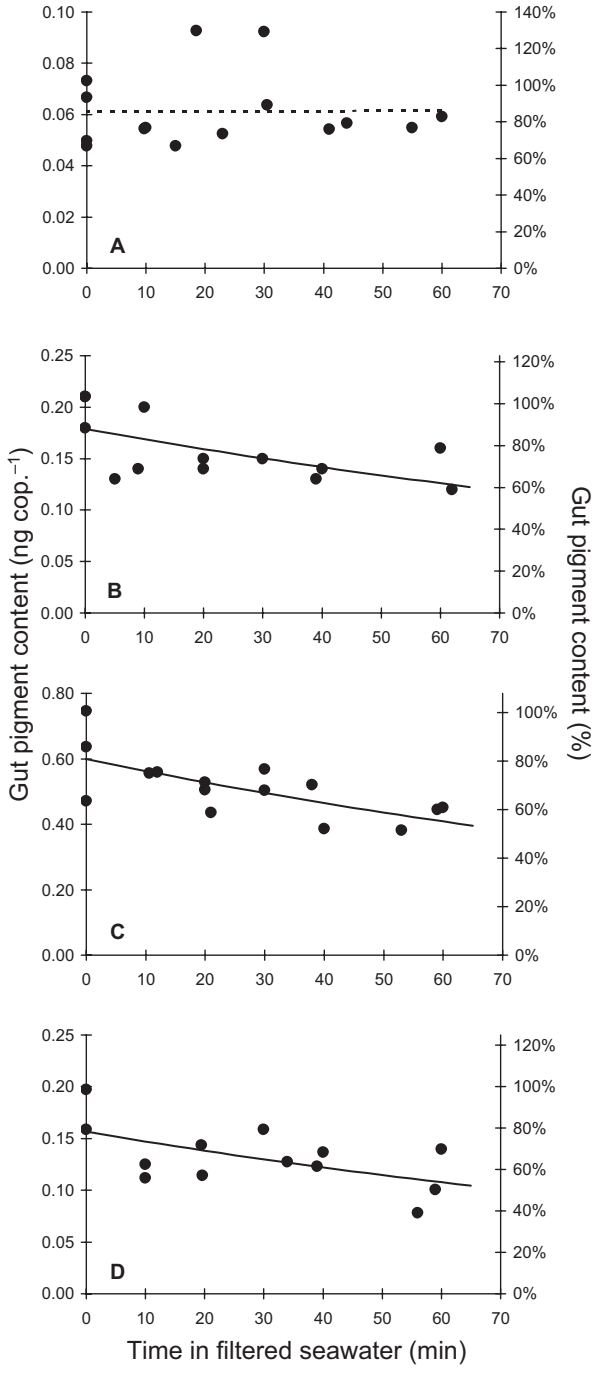

Fig. 4. *Acartia clausi*. Decline in gut pigments (Chl *a* + Phaeopigments) following transfer to filtered seawater, after feeding at increasing concentrations of the detritus: (**A**) 50 µg prot. L^{-1}, (**B**) 100 µg prot. L^{-1}, (**C**) 220 µg prot. L^{-1} and (**D**) 300 µg prot. L^{-1}.

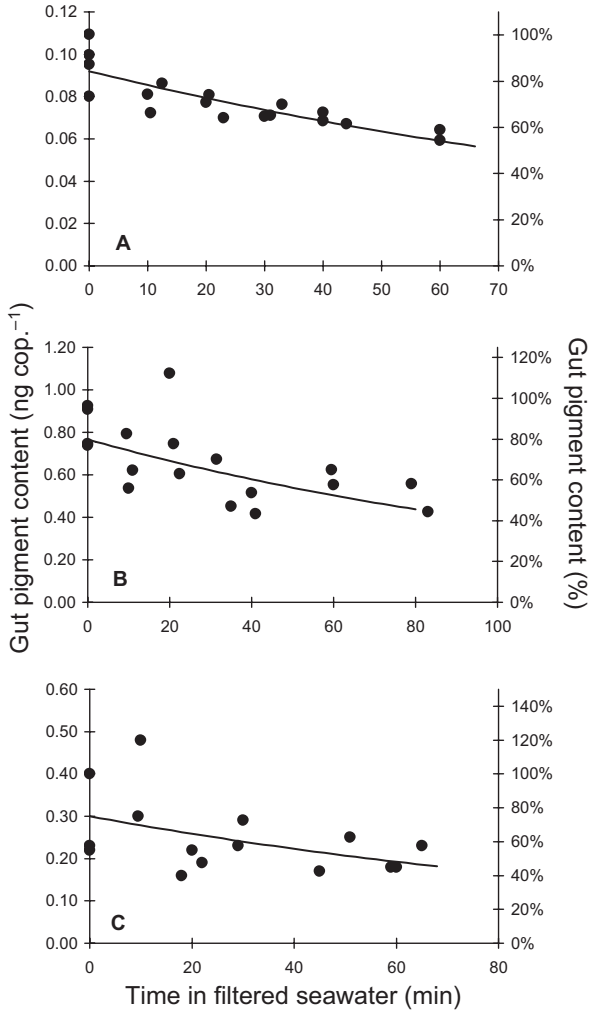

Fig. 5. *Acartia clausi*. Decline in gut pigments (Chl *a* + Phaeopigments) following transfer to filtered seawater, after feeding at increasing concentrations of the dinoflagellate *Prorocentrum micans*: (**A**) 50 µg prot. L^{-1}, (**B**) 110 µg prot. L^{-1}, (**C**) 275 µg prot. L^{-1}.

functional response of *A. clausi* fed a mixed diet obtained by Mayzaud *et al.* (Mayzaud *et al.*, 1998), was considered best described by a Holling type 2 response with very low a coefficient (K coefficient in Table II of Mayzaud *et al.*, 1998) and very high I_{max} (on average 4 times higher than that obtained with *T. weissflogii* and *P. micans*). However, if a linear equation is applied to the same experimental data set we obtain a functional response similar to that observed in this study (α coefficient of $24x10^{-5}$; r^2 = 0.95; $P < .05$), suggesting that, in the range of food concentrations considered, the linear response is probably the simplest representation of the behaviour of *A. clausi* in response to this food quality.

Compared to the other live diets used in this study, *P. minimum* had an intermediate cell size (volume ~7 times that of *T. weissflogii*, but only 1/9 of *P. micans*) and cellular protein content (2 times that of the diatom and 1/4 of *P. micans*). This species of dinoflagellate is often considered a 'good' diet for copepods, as several studies have shown that calanoid copepods fed *P. minimum* produced a large quantity of eggs (Ianora and Poulet, 1994; Jónasdóttir,

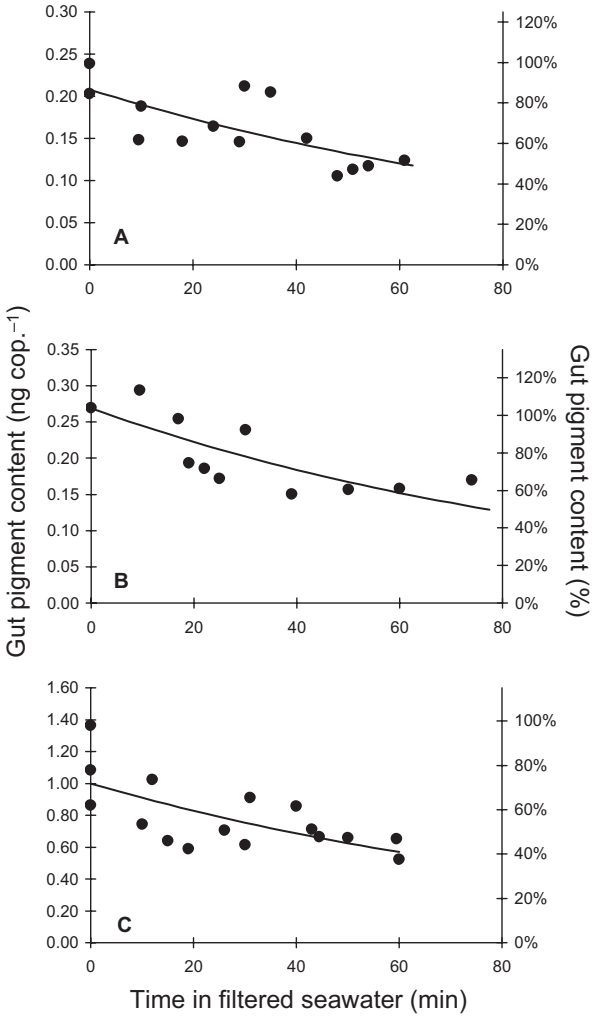

Fig. 6. *Acartia clausi*. Decline in gut pigments (Chl *a* + Phaeopigments) following transfer to filtered seawater, after feeding at increasing concentrations of the dinoflagellate *Prorocentrum minimum*: (**A**) 50 μg prot. L^{-1}, (**B**) 170 μg prot. L^{-1}, (**C**) 370 μg prot. L^{-1}.

1994; Poulet *et al.*, 1994; Ianora *et al.*, 1995, 1996). Nevertheless, in our experiments the ingestion rate (μg prot./cop./h) on *P. minimum* cells by *A. clausi* was lower than the ingestion rate shown with other live diets. Saturation was never reached (Fig. 1E and F). When copepods were acclimated for 24 h with this food type, they decreased ingestion to the point that the time of incubation had to be prolonged in order to obtain a measurable decrease of fluorescence. When copepods were acclimated for only 6 h in natural seawater, ingestion rates dropped drastically at food concentrations higher than 400 μg protein L^{-1}. Probably, as we will discuss below, cell size and protein content are not sufficient to explain the feeding behaviour exhibited by *A. clausi* with this food type.

This study supports the preliminary results of Mayzaud *et al.* (Mayzaud *et al.*, 1998) and points out that *A. clausi* may change the duration of its digestive process by decreasing the gut transit time with an increasing concentration of *T. weissflogii* and, consequently increases its gut content (G_0). This relationship appears to be characteristic of copepods fed live diatoms because when *A. clausi* was fed other diets, it adopted a constant gut passage time, notwithstanding the changes in food quantity occurring in its gut (Fig. 7).

If we consider cellular protein content as an index of food quality, our results contradict the hypothesis of Paffenhöfer and Van Sant (Paffenhöfer and Van Sant, 1985). In fact these authors suggested that copepods should adopt a short gut transit time when fed poor food quality, in order to save on the energetic costs of digestion, but *A. clausi* fed cells of identical size but of different protein/cell content (live and detrital cells of the diatom *T. weissflogii*), increased gut transit time when fed on detritus, the 'poorer diet'. The longest gut transit time was measured in copepods fed pure detritus and the shortest in animals fed saturating concentrations of live diatom. Copepods fed a mixed diet had intermediate K values (Table III) and at low food concentration they exhibited a gut transit time similar to that obtained when fed the solution of pure live cells (Fig. 7). This suggests that at low food concentration (~50 μg prot.L^{-1}) copepods may be selecting against detrital cells and ingest mainly live cells.

The gut transit time measured in copepods fed the dinoflagellates were longer than those obtained with live diatom diet (Table III). A study carried out on eleven species of centric diatoms and eight species of dinoflagellates showed that, for similar cell volumes, diatoms had less chlorophyll, proteins, carbohydrates and lipids than dinoflagellates (Hitchcock, 1982). Thus it may be suggested that *A .clausi* gains more proteins by ingesting *P. micans* and *P. minimum* rather than *T. weissflogii*, but it may need more time to digest the dinoflagellate cells. The latter hypothesis is supported also by the results of Besiktepe and Dam (Besiktepe and Dam, 2002) who observed, at food concentration lower than 50 μgCL^{-1} (corresponding to less than 52 μg protein L^{-1} at C:N of ca. 6), longer gut passage time for *A. tonsa* feeding on *P. minimum* than on *T. weissflogii*. Moreover, in studies carried out to estimate copepod fecundity, *Temora stylifera* (Ianora *et al.*, 1995) and *Calanus pacificus* (Uye, 1996) had a lower egestion rate (measured as number of faecal pellets produced during a day) when fed the dinoflagellate *P. minimum* than when fed diatoms (*Thalassiosira rotula* and *T. weissflogii*).

Our experiments with dinoflagellate-fed copepods indicate also that *A. clausi* may digest the smallest cells faster. In fact, considering that the *Prorocentrum* species used may have similar biochemical composition, we observed that *P. micans*, which has a volume 9 times

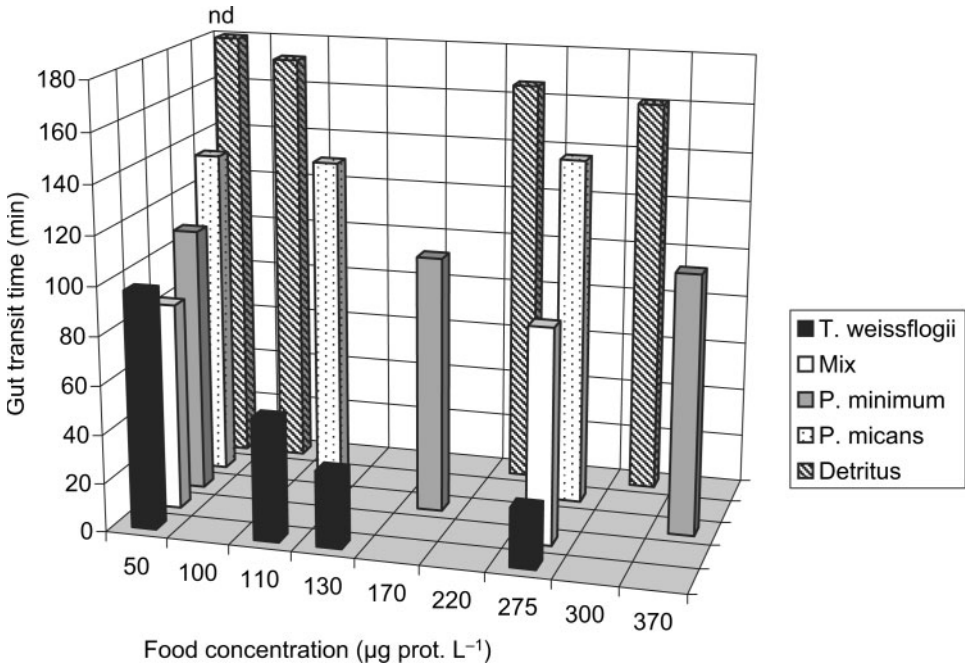

Fig. 7. Gut passage time (min) for each diet versus total available protein concentration (µg prot. L^{-1})

that of *P. minimum* (Table I), is processed more slowly than the other (gut transit time: 137 ± 4 min for *P. micans* and 107 ± 2 min for *P. minimum*).

With the exception of the studies of Penry and Jumars (Penry and Jumars, 1986, 1987), Jumars *et al.* (Jumars *et al.*, 1989), Mayzaud *et al.* (Mayzaud *et al.*, 1998) and Besiktepe and Dam (Besiktepe and Dam, 2002), investigations about zooplankton nutrition have often considered ingestion and digestion process separately. Nevertheless, a global approach is required in order to interpret the large variation in gut transit time and functional response. As pointed out by Mayzaud *et al.* (Mayzaud *et al.*, 1998), ingestion may be regulated by two groups of processes: (i) mechano- and chemo-reception to detect the trophic environment and food suitability, and (ii) internal control by digestion and assimilation to match energy/nutrient requirements with food intake (feed-back). On the other hand, digestion and assimilation are not only a function of food ingestion but also of food nutrient content, gut fullness, digestibility and availability of organic compounds, digestive enzyme activity and assimilation capability of the copepods. According to data available in the literature, a diet rich in proteins allows the animal to satisfy its energetic needs and thus the functional response assumes an asymptotic shape. In contrast, a diet poor in proteins does not satisfy the energetic demand of the animal, which has to increase progressively its ingestion rate with an increase in food supply.

In this context, a functional response achieving a saturation level with increasing food concentration, coupled with a short gut transit time, as observed with the *T. weissflogii* diet, may be interpreted as the situation when copepods have an easy digestible and rich food available. This can rapidly provide all the essential elements for the metabolism of the animal. Otherwise, an asymptotic functional response associated with a long gut transit time, as that observed with the dinoflagellate *P. micans*, is consistent with the hypothesis that food, even if rich in proteins, may not be digestible. Essential and structural elements would not be fully extracted, digested and assimilated unless residence time in the gut is sufficiently long to cope with energetic demands. For *P. micans* this is likely related to the presence of a cellulose-rich theca, which implies the action of additional tool enzymes, i.e. cellulose (Mayzaud, 1986) to ensure full hydrolysis of the cellular content.

Conversely, the linearization of ingestion and the high K value measured when copepods were fed detritus may be explained as the need to prolong the gut transit time to better exploit a food that is digestible, as live diatoms, but has a low protein content. A similar strategy (linearization of ingestion + long gut transit time) was observed also when *A. clausi* was fed *P. minimum*, even if this diet consists of cells reach in protein. To explain this result we suggest that *P. minimum* cells may be indigestible because of the presence of a theca (similar to the cells of *P. micans*) but they may also be poor in some essential elements that are limiting for the copepod and inhibit the saturation of its ingestion rate.

ACKNOWLEDGEMENTS

Special acknowledgement is due to R. Perissinotto for his fundamental support and valuable suggestions. We thank A. Atkinson, K. Flynn and four anonymous referees for improving the article with their comments. We also thank D. Betti for his help in collecting zooplankton. This research was supported by CNRS (grant EP 017), the MAST 2 European program (contract MAS2-CT94-5015) and the Ministère des Affaires Etrangers (France).

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