# Comparative utilization of phytoplankton and vascular plant detritus by the cockle *Cerastoderma edule*: digestive responses during diet acclimation

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ABSTRACT: Physiological components of food absorption (rates of particle clearance and ingestion and absorption efficiencies) were recorded in the cockles *Cerastoderma edule* fed variable concentration diets of phytoplankton (*Isochrysis galbana*) and detritus from salt-marsh vascular plants (*Spartina maritina*), after 2 and 12 d of acclimation to these diets. Corresponding digestive processes were also analysed, including determinations of gut passage time of food, gut fullness (GF) and digestive investments, the latter measured in terms of both metabolic faecal losses (MFL) and enzyme activities of the digestive glands. Digestive responses to increasing availability of phytoplankton included faster processing of food particles coupled to improved hydrolytic capacity of digestive glands that resulted in increased gut performance. Compared to phytoplankton, detritus utilization was constrained in the short-term by its refractory behaviour to digestion and the magnitude of metabolic faecal losses experienced by cockles fed these food materials. However, increased GF achieved during acclimation to detrital diets compensated for reduced digestibility, allowing for more food to be processed and thus cancelling out the effects of reduced passage time (and the consequent decline in absorption efficiency). Induction of the appropriate set of carbohydrases during the acclimation process resulted in increased amounts of reducing sugars released from natural substrates, with the additional benefit of diminishing the losses of endogenous materials accompanying each cycle of intracellular digestion.

KEY WORDS:  Food absorption · Phytoplankton · Detrius · Digestive enzymes · Gut passage time · Bivalve molluscs · *Cerastoderma edule*

## INTRODUCTION

Seston accessible to marine filter feeders includes a diversity of organic particles varying in nutritional value, from living phytoplankton to detritus of different origins. Contribution of detritus to growth and production of benthic populations, particularly marine bivalves, has been a subject of special attention, since this material constitutes a significant fraction of suspended particulate organic matter (POM), particularly in estuarine systems and during the seasons not dominated by phytoplankton (Wienke & Cloern 1987). For instance, phytoplankton biomass estimated from chlorophyll concentration explains <40% of POM, on aver-

age, even in the highly productive Galician rías (Cabanas et al. 1979, Navarro et al. 1996, Figueiras et al. 2002), while this proportion would appear considerably reduced in coastal areas affected by resuspension of bottom sediments: viz. 3 to 12% in Marennes-Oleron Bay (Prou et al. 1994, Urrutia et al. 1996, Kang et al. 1999); 6 to 45% in the Oosterschelde estuary (Smaal et al. 1986, Smaal & Haas 1997). In the area where the present study was conducted, this figure has been found to fluctuate, on a seasonal/tidal basis, between 3 and 28% (authors' unpubl. data).

There has been considerable discussion in the literature concerning both the origin of detritus present in the different coastal systems and its nutritional value

for suspension feeders (Kirby-Smith 1976, Seiderer et al. 1982, Stuart et al. 1982, Mann 1988, Fielding & Davis 1989, Langdon & Newell 1990, Alber & Valiella 1994,1995, Bustamante & Branch 1996, Levinton et al. 2002, Huang et al. 2003, Newell et al. 2005). Information on the subject has benefited extraordinarily from the generalized use of stable isotopes as 'signatures' to trace the origins of nutrients that contribute to tissue growth in marine species. Most of these studies have revealed that macrophyte-derived detritus might explain a significant amount of nutrients assimilated by bivalve populations growing in areas where these plants are relatively abundant (55 to 85% for kelp detritus: Bustamante & Branch 1996; 40 to 50% for salt-marsh vascular plants: Langdon & Newell 1990). Peterson et al. (1985, 1986) estimated 30 and 80% contribution of *Spartina alterniflora* to the nutrition of *Mytilus edulis* and *Geukensia demissa*, respectively, while Riera & Richard (1996, 1997) and Kang et al. (1999) reported spatial and seasonal variations in stable isotope composition of oyster *Crassostrea gigas* and cockle *Cerastoderma edule* tissues that reflected the incorporation of detritus of terrestrial origin into the water column.

Comparison of the above figures with direct physiological measurements of detritus utilization by marine bivalves reveals a complex situation whereby processing rates and assimilation efficiency vary according to both bivalve species and detritus origin. Tenore et al. (1982) suggested that vascular plants contain a high percentage of unavailable energy that can only be utilized after microbial decomposition, whereas detritus from seaweeds would be less refractory to digestion by bivalves and so could be more readily utilized. Accordingly, various authors have reported absorption efficiencies as high as 40 to 60% in the mussels *Aulacomya ater* and *Choromytilus meridionalis* (Stuart 1982, Stuart et al. 1982, Fielding & Davis 1989) and up to 87% in the scallops *Placopecten magellanicus* (Cranford & Grant 1990) fed kelp detritus (but see Charles 1993, Charles et al. 1996, concerning the deposit feeder *Abra ovata*). Meanwhile, measurements of carbon gross absorption efficiencies (GAEs) in other bivalves fed lignocellulosic detritus obtained from *Spartina alterniflora* ranged between 3% in the oyster *Crassostrea virginica* (Crosby et al. 1989) and 4 to 25% in the case of the mussel *Geukensia demissa* (Kreeger et al. 1990, Charles & Newell 1997, Kreeger & Newell 2001).

Regarding the ecological significance of salt-marsh plant detritus, the integration of physiological data with data about the environmental availability of cellulosic detritus in Canary Creek marsh (Langdon & Newell 1990) has revealed that the contribution to carbon and energy requirements of bivalves ranged from 0.7 to 2.1% in the oysters *Crassostrea virginica* and

from 5.2 to 8.6% in the mussels *Geukensia demissa*. Although these figures might increase up to 20% in other systems, on account of the much higher detritus concentration in the seston (Crosby et al. 1989), they differ greatly from comparable estimations based on stable isotope enrichment. Consequently, a reconsideration of physiological data seems opportune, particularly data that concern assimilation efficiency values.

The aim of the present paper was to compare the utilization of phytoplankton and organic detritus from vascular plants by the infaunal bivalve *Cerastoderma edule*, a species living in food environments characterized by broad seasonal and spatial fluctuations in the relative importance of these 2 food sources (Navarro et al. 1997, Kang et al. 1999). Food assimilation was analyzed under a set of experimental conditions that resulted in a range of feeding rates and variable degrees of diet acclimation in an attempt to explore the effect of these factors on digestive performance for both food types. This approach is thought to provide physiological information useful in modeling growth and production of bivalve species inhabiting coastal systems submitted to a complex food regime.

## MATERIALS AND METHODS

**Collection and maintenance of animals.** Cockles *Cerastoderma edule* measuring around 28 mm in shell height were collected in an intertidal mudflat in the Plentzia Estuary (Biscay, northern Spain) in February (Expt 1) and May 1999 (Expt 2). On each occasion, some 50 specimens were collected, immediately transported to the laboratory and placed in individual containers immersed in feeding tanks filled with seawater provided from a re-circulating system set at environmental temperature (12 and 16°C, for Expts 1 and 2, respectively) and 33‰ salinity. Cockles were divided into different groups each receiving a diet which differed in terms of either composition (phytoplankton or particulate organic detritus) or particle concentration; they were fed these diets for up to 12 d.

**Characteristics of experimental diets.** Diets were designed to have a common organic content, OC (~80%), but to be based on 2 different types of organic particles, either phytoplankton cells of the species *Isochrysis galbana* (Diet P) or particles of organic detritus (Diet D). The latter were obtained from freshly collected specimens of *Spartina maritima* that were freeze-dried and ground and the resulting powder was sieved to select the particle fraction <45 μm. OCs of the particulate suspensions of both phytoplankton and detritus were adjusted to the chosen values by adding ashed silt particles <45 μm obtained from natural sediments from the estuary.

Concentrated stocks of these diets were dosed into the feeding tanks at rates set to provide for stable particle concentrations that were checked by frequent monitoring of particulate volumes with a Coulter Multisizer. In Expt 1, Diets P and D were provided at a single particle concentration of 1.5 mm$^3$ l$^{-1}$. In Expt 2, each composition was dosed at 2 concentrations of 1 (low concentration) and 2 mm$^3$ l$^{-1}$ (high concentration). No pseudofaeces production was observed with any of these diets.

On 6 occasions, duplicated water samples taken from the feeding tanks were filtered onto ashed pre-weighted GF/C glass-fiber filters, which were subsequently processed to determine concentrations (mg l$^{-1}$) of total particulate matter (TPM), particulate inorganic matter (PIM) and POM (mg l$^{-1}$) in the feeding suspensions. Salts retained in the filters were rinsed out with a solution of ammonium formate seawater isotonic, and then filters were dried at 110°C, weighed, ashed at 450°C and weighed again. TPM and PIM were estimated, respectively, as the dry and ash weight increments of the filters, and POM was estimated as the weight loss of this filtered material on ashing. OC was finally estimated as POM/TPM.

**Physiological measurements.** Physiological determinations were carried out after 2 d (short term) and 12 d (long term) of acclimation to the above dietary conditions. Clearance rate (CR, l h$^{-1}$), ingestion and absorption rates (IR and AR, mg h$^{-1}$) and net absorption efficiency (NAE) were determined during Expt 1 according to the biodeposition method (Iglesias et al. 1998). To this effect, faeces produced by individual cockles were collected over defined time intervals, filtered onto GF/C filters and processed for inorganic and organic weight determinations of faecal material. The biodeposition method assumes conservative processing of inorganic particles within the gut of marine bivalves and, hence, the inorganic egestion rate (IER) would equal the inorganic ingestion rate (IIR, mg h$^{-1}$), whence CR could be estimated as IIR/PIM. Total IR and organic ingestion rate (OIR) were then calculated as the product of CR and TPM and CR and POM, respectively. The net absorption rate of organics (NAR, mg h$^{-1}$) was computed as the difference between OIR and the rates of organic egestion (OER), and NAE, as the ratio AR/OIR. Technically this measurement of absorption efficiency corresponds to the Conover method (Conover 1966) as it is based on the same principles (see Iglesias et al. 1998). Determinations of gut passage time of food (GPT, h) and GAE, performed during Expts 1 and 2, and IRs in the case of Expt 2, were based on the radioactive labeling of food particles using $^{14}$C-formaldehyde (López & Crenshaw 1982, Charles et al. 1995). Pure cultures of *Isochrysis galbana* and concentrated suspensions of detrital particles ob-

tained from *Spartina maritima* in seawater were incubated for 48 h with $^{14}$C-formaldehyde (1.1 µm Ci mg$^{-1}$ organics), then concentrated by centrifugation; the pellet was then re-suspended in 0.45 µm filtered seawater, in order to remove unreacted formaldehyde. This label was checked to remain stable for at least 48 h. OC of these diets was finally adjusted to the intended 80% by adding the appropriate amount of silt particles.

Radiolabeled diets were supplied to cockles in 1 h pulses at the particle concentrations specified above. Cockles were then transferred to unlabeled diets, and the faeces produced individually were collected at frequent intervals for up to 30 h. For radioactivity determination, samples of food and faeces were filtered on GF/C glass-fiber filters, processed in a Sample Oxidizer (Packard, Model 307) and then counted by liquid scintillation. A curve of cumulative isotope egestion (dpm) was fitted to each individual set of data, and GPTs were estimated as the time required for 95% $^{14}$C egestion (Hawkins et al. 1990). $^{14}$C ingestion (= $^{14}$C added – $^{14}$C remaining) was occasionally (Expt 2) used to compute IR using the specific radioactivity of diets. The efficiency of isotope absorption [($^{14}$C ingested – $^{14}$C egested) / $^{14}$C ingested] was taken to represent the absorption efficiency of dietary organics (GAE), and the gross absorption rate (GAR, mg h$^{-1}$) was computed as the product OIR × GAE.

The difference between gross and net rates of absorption (GAR – NAR) has been termed metabolic faecal loss (MFL) (Hawkins & Bayne 1985, Bayne & Hawkins 1990), representing the endogenous materials contributed to digestion that are not reabsorbed.

Gut fullness (GF) was computed as the product of organic ingestion and GPT, to represent the amount of food (mg) held by the gut each moment. Digestive performance (DP) was defined as the ratio GAR/GF, or absorbed energy per unit weight of food held in the gut. By construction, it also represents the absorption efficiency (GAE) per unit GPT.

**Digestive enzyme activities.** Once physiological determinations were concluded, digestive glands of 5 to 7 ind. per condition were dissected, freeze-dried and stored at –40°C for further analysis. Individual digestive glands were homogenized in cold 0.01 M phosphate-citrate buffer (pH 6.9), centrifuged at 5100 × *g* for 30 min, and the clear supernatant was used for *in vitro* enzyme assays.

Two kinds of assays were performed with this extract: (1) standard carbohydrase and protease activity determinations based on commercial substrates (Expts 1 and 2) and (2) determinations of reducing sugar release from 'natural substrates' (Expt 2).

During standard determinations, crude extracts were tested against commercial starch, carboximethylcellu-

lose, laminarin and casein (for details see Ibarrola et al. 1996) in order to ascertain amylase, cellulase, laminarinase and total protease activities.

In enzyme activity determinations on 'natural substrates' crude extracts were assayed for total carbohydrase activity on lipid-free extracts of *Isochrysis galbana* cells and detrital particles of *Spartina maritima* that were obtained as follows: 60 l of phytoplankton culture were concentrated by continuous centrifugation up to a final volume of 200 ml; on the other hand, 2 g of the particulate *S. maritima* material used in the confection of detrital diets was suspended in 100 ml of filtered (0.45 µm) seawater. These concentrated stocks were lipid extracted with a mixture of chloroform/ methanol/water (2:2:1), according to Bligh & Dyer (1959). The chloroform phase was discarded, and the procedure was repeated until total absence of coloration in the lipidic phase was observed. After centrifugation (20 min at 3300 × *g*), the supernatant was discarded and the pellet was rinsed 3 times with 0.2 M phosphate-citrate buffer (pH 6.9), and finally re-suspended in 100 ml of the buffer. Before the enzymatic assays, these re-suspensions were boiled for 30 min in order to obtain a homogeneous solution. Carbohydrase activity was measured following the Nelson-Somogyi method (Nelson 1944, Somogyi 1952).

**Size standardization.** At the end of experiments, the soft body of cockles was dissected out of the shell, dried at 80°C for 48 h and weighed. For cockles used in enzyme analysis, freeze-dried digestive glands and dried remaining tissues were weighed separately and total dry weight was obtained by addition. Physiological measurements, as well as the size of digestive glands, were standardized to an equivalent 200 mg dry-weight cockle according to the expression:

$$Y_s = (200/W_e)^b Y_e \qquad (1)$$

where $Y_s$ represent the standardized measurement, $W_e$ is the actual dry weight, $Y_e$ is the uncorrected measurement and b is the specific weight power that scales each measurement to body weight: b = 0.56 for rates of food processing by cockles (Newell & Bayne 1980), b = 0.41 for GPT (Hawkins et al. 1990) and b = 0.82 for dry weight of digestive gland (Ibarrola et al. 2008).

Digestive enzyme activities were expressed as specific activity (micrograms of released product per milligram of dry weight of digestive tissue) and total activity (micrograms of released product per standardsized digestive gland).

**Statistical procedures.** Physiological measurements and digestive enzyme activities were compared for differences between treatments by means of multiple factor ANOVA, after homogeneity of variances was confirmed by means of a Bartlett test (Zar 1984). Nonlinear regression analyses were performed using SYS-

TAT 5.2 statistical package (SYSTAT 1992), and significant differences between coefficients were tested using a *t*-test (Zar 1984).

# RESULTS

## Physiological measurements

### Expt 1

Characteristics of diets recorded during Expt 1 are reported in Table 1. Both total concentration (TPM and POM) and OC of suspended particles were kept constant irrespective of dietary composition (P vs. D). Differences in length of acclimation (short- vs. long-term), however, were significant for TPM and POM, but not for OC (Table 2).

Mean values of physiological variables measured in cockles *Cerastoderma edule* fed P and D diets for 2 and 12 d are given in Table 3. A 2-factor ANOVA applied to test the significance of mean differences for primary measurements (CR, NAE, GAE and GPT; Table 4) reveals the following. (1) Diet composition and time of acclimation per se had no significant effect on CR,

Table 1. Expt 1: characteristics of experimental diets recorded in short-term (2 d) and long-term (12 d) acclimation to diets of phytoplankton (P: *Isochrysis galbana* cells) and detritus (D: detrital particles from *Spartina maritima*). TPM: total particulate matter (mg l$^{-1}$); POM: particulate organic matter (mg l$^{-1}$); OC: organic content (fraction). Values are means of 6 to 13 determinations (±95 % CI)

| Diet | Acclimation | TPM | POM | OC |
|------|-------------|-----|-----|-----|
| P | Short-term | 1.54 ± 0.20 | 1.28 ± 0.15 | 0.83 ± 0.04 |
| P | Long-term | 1.78 ± 0.14 | 1.52 ± 0.09 | 0.86 ± 0.06 |
| D | Short-term | 1.57 ± 0.25 | 1.29 ± 0.21 | 0.81 ± 0.04 |
| D | Long-term | 2.18 ± 0.13 | 1.79 ± 0.14 | 0.82 ± 0.02 |

Table 2. Expt 1: Analysis of variance (ANOVA). Post hoc test (Fisher's protected least significant difference, PLSD) for mean significant differences in TPM, POM and OC. Abbreviations in Table 1. *p < 0.05

| Effect | Mean difference | Critical difference | p |
|--------|-----------------|---------------------|---|
| **TPM** | | | |
| Diet | −0.162 | 0.217* | 0.1324 |
| Acclimation time | −0.396 | 0.222 | 0.0009* |
| **POM** | | | |
| Diet | −0.104 | 0.177 | 0.2427 |
| Acclimation time | −0.356 | 0.181 | 0.0003* |
| **OC** | | | |
| Diet | 0.021 | 0.043 | 0.3365 |
| Acclimation time | −0.017 | 0.044 | 0.4517 |

Table 3. *Cerastoderma edule*. Expt 1: physiological variables measured following short-term (2 d) and long-term (12 d) acclimation to diets of phytoplankton (P: *Isochrysis galbana* cells) and detritus (D: detrital particles from *Spartina maritima*). CR: clearance rate (l h$^{-1}$); OIR: organic ingestion rate (mg POM h$^{-1}$); GAE: gross absorption efficiency (fraction); NAE: net absorption efficiency (fraction); GPT: gut passage time (h); MFL: metabolic faecal loss (mg h$^{-1}$). Values are means of 5 determinations (10 to 15 for CR) (±95% CI)

| Diet | Acclimation | CR | OIR | GAE | NAE | GPT | MFL/OIR |
|---|---|---|---|---|---|---|---|
| P | Short-term | 0.50 ± 0.12 | 0.54 ± 0.13 | 0.88 ± 0.03 | 0.76 ± 0.06 | 12.68 ± 2.31 | 0.12 ± 0.05 |
| P | Long-term | 0.37 ± 0.07 | 0.54 ± 0.10 | 0.86 ± 0.02 | 0.79 ± 0.02 | 9.13 ± 2.30 | 0.07 ± 0.02 |
| D | Short-term | 0.30 ± 0.06 | 0.33 ± 0.06 | 0.77 ± 0.09 | 0.51 ± 0.10 | 15.25 ± 2.67 | 0.26 ± 0.11 |
| D | Long-term | 0.48 ± 0.05 | 0.85 ± 0.09 | 0.68 ± 0.05 | 0.56 ± 0.04 | 12.34 ± 3.31 | 0.12 ± 0.05 |

whereas the interaction of both did, indicating that CR tended to decrease (but not significantly) with acclimation in cockles fed P diets, while it increased significantly with acclimation to D diets. In other words, reduced CR values recorded in cockles fed *Spartina maritima* detritus are shown to increase following acclimation, to reach values characteristic of phytoplankton diets. (2) Composition of the diet had a very strong effect on GAE and NAE, detrital diets being absorbed with a reduced efficiency. (3) Both composition and ac-

Table 4. *Cerastoderma edule*. Expt 1: summary of ANOVAs to test significance of both diet (POM in the case of OIR) and acclimation time on the physiological measurements reported in Table 3. *p < 0.05

| Source of variation | df | SS | MS | F | p |
|---|---|---|---|---|---|
| **CR** | | | | | |
| Diet | 1 | 0.026 | 0.026 | 1.071 | 0.3062 |
| Acclimation time | 1 | 0.006 | 0.006 | 0.241 | 0.6256 |
| Interaction | 1 | 0.283 | 0.283 | 11.672 | 0.0014* |
| Residual | 45 | 1.091 | 0.024 | | |
| **GAE** | | | | | |
| Diet | 1 | 0.101 | 0.101 | 29.170 | 0.0001* |
| Acclimation time | 1 | 0.016 | 0.016 | 4.492 | 0.0501 |
| Interaction | 1 | 0.006 | 0.006 | 1.949 | 0.1928 |
| Residual | 16 | 0.055 | 0.003 | | |
| **NAE** | | | | | |
| Diet | 1 | 0.286 | 0.286 | 60.496 | 0.0001* |
| Acclimation time | 1 | 0.008 | 0.008 | 1.685 | 0.2127 |
| Interaction | 1 | $5 \times 10^{-4}$ | $5 \times 10^{-4}$ | 0.104 | 0.7516 |
| Residual | 16 | 0.076 | 0.005 | | |
| **GPT** | | | | | |
| Diet | 1 | 37.148 | 37.148 | 4.852 | 0.0449* |
| Acclimation time | 1 | 46.443 | 46.443 | 6.067 | 0.0273* |
| Interaction | 1 | 0.451 | 0.451 | 0.059 | 0.8117 |
| Residual | 14 | 107.175 | 7.655 | | |
| **OIR** | | | | | |
| POM | 1 | 0.005 | 0.005 | 0.202 | 0.6591 |
| Acclimation time | 1 | 0.128 | 0.128 | 5.339 | 0.0345* |
| Interaction | 1 | 0.167 | 0.167 | 6.939 | 0.0180* |
| Residual | 16 | 0.384 | 0.024 | | |
| **MFL/OIR** | | | | | |
| Diet | 1 | 0.047 | 0.047 | 8.229 | 0.0111* |
| Acclimation time | 1 | 0.045 | 0.045 | 8.027 | 0.0120* |
| Interaction | 1 | 0.014 | 0.014 | 1.830 | 0.1949 |
| Residual | 16 | 0.091 | 0.006 | | |

climation had significant effects on GPT: phytoplankton diets are processed faster than detrital diets in the guts of cockles, and GPT tends to decrease with acclimation.

Since IR is directly affected by food concentration, possible effects of increased rations recorded along the acclimation period were analyzed by testing the effects of POM and acclimation time on OIR. Results of ANOVA (Table 4) led us to conclude that there were no effects of food concentration per se and significant effects of both acclimation and the interaction term. The latter results reflected opposite trends shown by CR during acclimation in the 2 diets (see above): a time-dependent reduction in phytoplankton-fed cockles appears compensated for by increased POM, while the strong increment of CR during acclimation to detrital diets enhances the effect of increased POM. In conclusion, feeding-response adjustments would be more important than differences in food ration concerning the overall increment of IR achieved during acclimation.

Computed values of MFLs per unit of organic ingestion (MFL/OIR), representing digestive costs per unit of food intake are also shown in Table 3. Being a relative magnitude, values taken by this index would not be affected by differences in rates of food processing associated with POM differences between diets, making comparisons particularly meaningful. These comparisons (Table 4) show that both food composition and acclimation exert significant effects. Thus, digestive costs per unit of ingestion are much higher for Diet D and tend to decrease with time of acclimation in both types of diets.

### Expt 2

During this experiment cockles were fed 2 rations of either phytoplankton or detrital particles for 2 or 12 d before the labeled diets were used to determine physiological parameters. Characteristics of the diets are presented in Table 5. As intended, high rations had particle concentrations approximately twice those of low rations, except in the case of Diet D with long-term

Table 5. Expt 2: characteristics of experimental diets recorded in short-term (2 d) and long-term (12 d) acclimation to diets of phytoplankton (P: *Isochrysis galbana* cells) and detritus (D: detrital particles from *Spartina maritima*), dosed at 2 rations. TPM: total particulate matter (mg l$^{-1}$); POM: particulate organic matter (mg l$^{-1}$); OC: organic content (fraction). Values are means of 7 to 12 determinations (±95% CI)

| Diet | Acclimation | Ration | TPM | POM | OC |
|------|-------------|--------|-----|-----|-----|
| P | Short-term | High | 1.15 ± 0.23 | 0.88 ± 0.21 | 0.76 ± 0.05 |
| | | Low | 0.62 ± 0.11 | 0.45 ± 0.06 | 0.75 ± 0.05 |
| P | Long-term | High | 1.33 ± 0.19 | 0.99 ± 0.16 | 0.77 ± 0.03 |
| | | Low | 0.74 ± 0.09 | 0.54 ± 0.06 | 0.73 ± 0.05 |
| D | Short-term | High | 1.13 ± 0.16 | 0.88 ± 0.14 | 0.78 ± 0.03 |
| | | Low | 0.62 ± 0.15 | 0.49 ± 0.13 | 0.77 ± 0.05 |
| D | Long-term | High | 1.70 ± 0.17 | 1.29 ± 0.14 | 0.76 ± 0.03 |
| | | Low | 0.61 ± 0.10 | 0.44 ± 0.09 | 0.72 ± 0.03 |

exposure, where larger differences were obtained. Mean comparisons (Table 6) revealed that food concentration (both TPM and POM) increased significantly during acclimation, while OC decreased.

Values of physiological parameters (IR, GAE and GPT) estimated by using $^{14}$C-labeled diets are given in Table 7. Variance analysis applied to these data (Table 8) reveals a complex picture arising from the interplay of the 3 different factors (e.g. dietary composition, ration and exposure time) acting upon the physiological variables.

(1) Food ration exerted significant effects on variables related to food processing (IR and GPT), either per se or in combination with other factors (type of diet and acclimation time). Parallel behaviour of both variables suggests a functional relationship based on the expectation that, in continuous feeders, gut residence time of food particles would decline with increased rates of feeding.

Table 6. Expt 2: ANOVA. Post hoc test (Fisher's PLSD) for mean significant differences in TPM, POM and OC. Abbreviations in Table 5. *p < 0.05

| Effect | Mean difference | Critical difference | p |
|--------|-----------------|---------------------|---|
| **TPM** | | | |
| Ration | 0.678 | 0.107 | <0.0001* |
| Diet | 0.001 | 0.106 | 0.9908 |
| Acclimation time | −0.164 | 0.106 | 0.0030* |
| **POM** | | | |
| Ration | 0.537 | 0.088 | <0.0001* |
| Diet | −0.008 | 0.088 | 0.8497 |
| Acclimation time | −0.094 | 0.088 | 0.0369* |
| **OC** | | | |
| Ration | 0.022 | 0.029 | 0.1472 |
| Diet | −0.006 | 0.029 | 0.6893 |
| Acclimation time | 0.039 | 0.029 | 0.0090* |

Table 7. *Cerastoderma edule*. Expt 2: physiological variables measured following short-term (2 d) and long-term (12 d) acclimation to diets of phytoplankton (P: *Isochrysis galbana* cells) and detritus (D: detrital particles from *Spartina maritima*), dosed at high and low food rations. IR: ingestion rate (mg TPM h$^{-1}$); GAE: gross absorption efficiency (fraction); GPT: gut passage time (h). Values are means of 5 to 7 determinations (±95% CI)

| Diet | Acclimation | Ration | IR | GAE | GPT |
|------|-------------|--------|-----|-----|-----|
| P | Short-term | High | 0.78 ± 0.14 | 0.83 ± 0.05 | 14.82 ± 2.12 |
| | | Low | 0.45 ± 0.07 | 0.78 ± 0.03 | 16.66 ± 1.52 |
| P | Long-term | High | 0.87 ± 0.25 | 0.78 ± 0.05 | 13.27 ± 2.20 |
| | | Low | 0.21 ± 0.05 | 0.79 ± 0.15 | 15.63 ± 4.76 |
| D | Short-term | High | 0.48 ± 0.19 | 0.64 ± 0.07 | 11.46 ± 2.20 |
| | | Low | 0.60 ± 0.07 | 0.53 ± 0.04 | 13.32 ± 3.00 |
| D | Long-term | High | 0.50 ± 0.22 | 0.72 ± 0.15 | 19.41 ± 3.08 |
| | | Low | 0.38 ± 0.12 | 0.59 ± 0.02 | 12.27 ± 2.52 |

To illustrate such dependence, individual values of GPT recorded in both experiments were plotted against IR and points were fitted with exponential functions of the form:

$$Y = Y_{max} \times e^{-B1X} \tag{2}$$

where $Y_{max}$ represents the maximum GPT, $X$ is IR, and $B1$ is the rate at which GPT declines with rising IR.

Table 8. *Cerastoderma edule*. Expt 2: summary of ANOVAs to test significance of diet, food ration and acclimation time on the physiological measurements reported in Table 7. *p < 0.05

| Source of variation | df | SS | MS | *F* | p |
|---------------------|-----|-----|-----|-----|---|
| **IR** | | | | | |
| Diet (A) | 1 | 0.090 | 0.090 | 2.161 | 0.286 |
| Ration (B) | 1 | 0.801 | 0.801 | 19.142 | <0.0001* |
| Acclimation time (C) | 1 | 0.099 | 0.099 | 2.358 | 0.1312 |
| Interaction AB | 1 | 0.889 | 0.889 | 21.230 | <0.0001* |
| Interaction AC | 1 | 0.001 | 0.001 | 0.017 | 0.8961 |
| Interaction BC | 1 | 0.255 | 0.255 | 6.085 | 0.0173* |
| Interaction ABC | 1 | 0.017 | 0.017 | 0.404 | 0.5281 |
| Residual | 48 | 2.010 | 0.042 | | |
| **GPT** | | | | | |
| Diet (A) | 1 | 12.999 | 12.999 | 1.151 | 0.2896 |
| Ration (B) | 1 | 1.928 | 1.928 | 0.171 | 0.6816 |
| Acclimation time (C) | 1 | 32.052 | 32.052 | 2.838 | 0.0997 |
| Interaction AB | 1 | 52.758 | 52.758 | 4.672 | 0.0366* |
| Interaction AC | 1 | 61.867 | 61.867 | 5.478 | 0.0242* |
| Interaction BC | 1 | 60.244 | 60.244 | 5.334 | 0.0260* |
| Interaction ABC | 1 | 52.903 | 52.903 | 4.684 | 0.0363* |
| Residual | 41 | 463.029 | 11.293 | | |
| **GAE** | | | | | |
| Diet (A) | 1 | 0.368 | 0.368 | 41.687 | <0.0001* |
| Ration (B) | 1 | 0.060 | 0.060 | 6.855 | 0.0122* |
| Acclimation time (C) | 1 | 0.007 | 0.007 | 0.771 | 0.3850 |
| Interaction AB | 1 | 0.034 | 0.034 | 3.875 | 0.0556 |
| Interaction AC | 1 | 0.019 | 0.019 | 2.167 | 0.1474 |
| Interaction BC | 1 | 0.002 | 0.002 | 0.198 | 0.6588 |
| Interaction ABC | 1 | 0.003 | 0.003 | 0.378 | 0.5422 |
| Residual | 42 | 0.370 | 0.009 | | |

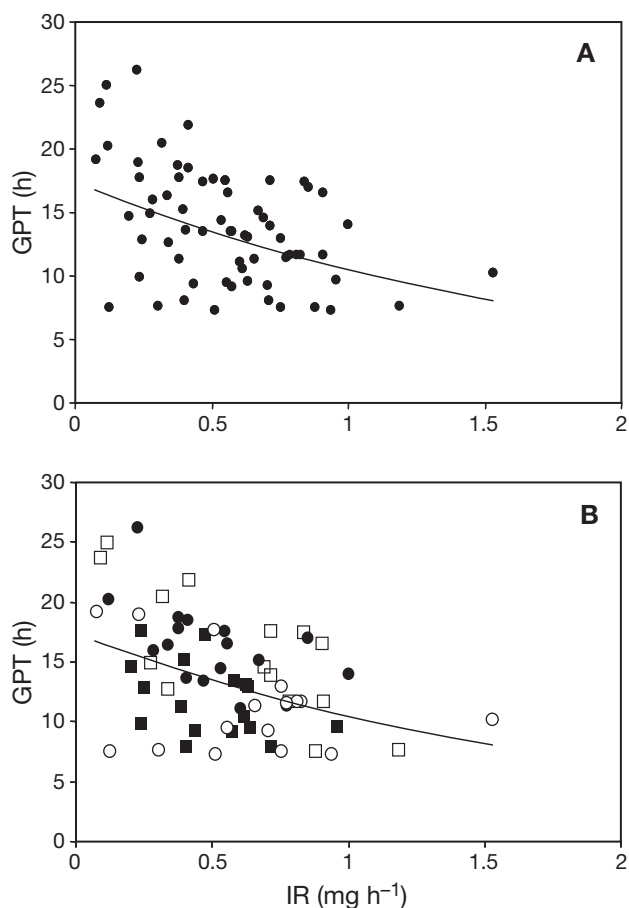Fig. 1. *Cerastoderma edule*. Gut passage time (GPT, h) as a function of ingestion rate (IR, mg h$^{-1}$). (A) Pooled data for all combinations of diet and acclimation times in Expts 1 and 2. (B) Same data as in Panel A, with different symbols identifying the different experimental groups—circles: phytoplankton diets; squares: detritus diets; solid symbols: 2 d of acclimation; open symbols: 12 d of acclimation. Data were fitted with the exponential function (parameter value ± asymptotic standard error): GPT = $17.28 \pm 1.46 \times e^{-0.49 \pm 0.16 \times IR}$, $R^2 = 0.182$, $p < 0.001$

Data for each combination of diet and acclimation time were independently fitted, and computed values were compared according to their confidence limits, to conclude lack of significant differences in either $Y_{max}$ ($t = 0.875$) or $b$ parameters ($t = 0.562$) ($t_{0.05} = 2.03$). Consequently, a single relationship could be fitted to all data pooled (Fig. 1).

From Tables 5 & 6, it is clear that actual values of food concentration (TPM and POM) were consistently lower after short-term acclimation compared with long-term acclimation, which could account for significance interactive effects of ration × time on IR and GPT. When POM is introduced as a covariate (Table 9), comparison of means reveals no significant differences for any comparison criteria (diet or acclimation time).

Table 9. Expt 2: ANOVA. Summary of post hoc test (Fisher's PLSD) of IR, GPT and GAE, with POM as a covariate. Abbreviations in Table 7. *p < 0.05

| Effect | Mean difference | Critical difference | p |
|---|---|---|---|
| **IR** | | | |
| Diet: | | | |
| P vs. D | 0.080 | 0.110 | 0.1483 |
| Acclimation time: | | | |
| 2 vs. 12 d | 0.084 | 0.110 | 0.1315 |
| **GPT** | | | |
| Diet: | | | |
| P vs. D | 0.930 | 1.943 | 0.3392 |
| Acclimation time: | | | |
| 2 vs. 12 d | −1.737 | 1.949 | 0.0793 |
| **GAE** | | | |
| Diet: | | | |
| P vs. D | 0.167 | 0.056 | <0.0001* |
| Acclimation time: | | | |
| 2 vs. 12 d | −0.034 | 0.056 | 0.2228 |

(2) Type of diet and food ration both exert significant effects on GAE (Table 8). Since GPT represents the available time for food digestion and absorption, ration effects on GAE very likely reflect the modification of rates of food processing within the gut. In modeling this relationship GAE was assumed to increase with rising GPT according to an asymptotic function that, according to Willows (1992), is written as:

$$Y = Y_{max} [1 - (1 + bX) \times e^{-B2X}] \qquad (3)$$

where $Y_{max}$ represents the maximum GAE, $X$ is GPT and $B2$ is the rate at which this maximum is reached with rising GPT. Data for each combination of diet and acclimation time were independently fitted, and computed values were compared according to their confidence limits. The conclusion was that maximum GAE did not significantly differ between diet groups ($t = 0.158$) and the $b$ parameter differed between diet groups (P vs. D, $t = 2.535$), but not within diet groups (i.e. 2 vs. 12 d of acclimation, $t = 0.147$) ($t_{0.05} = 2.04$). This confirms the results shown in Table 9 as to the significance of differences between GAE means for diet and lack of significance for acclimation time. Consequently, 2 different expressions were fitted for P and D diets (Fig. 2).

## Digestive enzyme activities

### Expt 1

Dry weight of the digestive gland and protein content of the digestive extract, together with data on enzyme activities recorded in this organ, are presented in Table 10. Three carbohydrase (amilase, cellulase
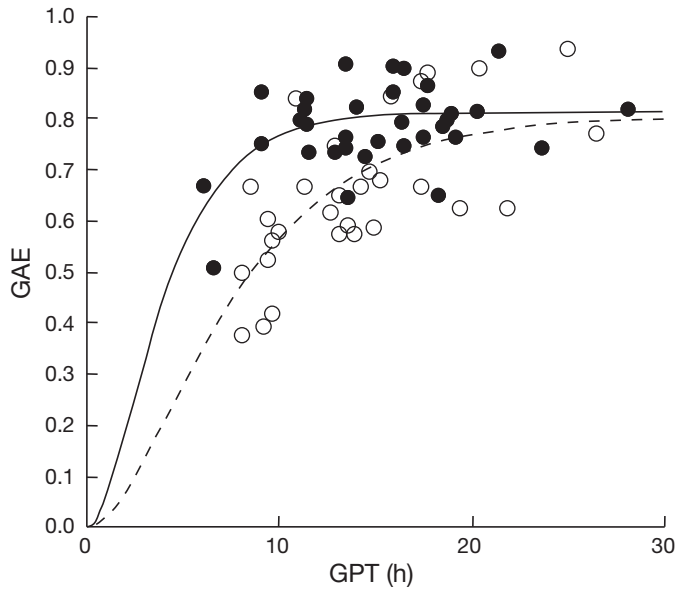
Fig. 2. *Cerastoderma edule*. Gross absorption efficiency (GAE, fraction) as a function of gut passage time (GPT, h). Solid symbols and continuous line: phytoplankton diets; open symbols and dashed line: detritus diets. Fitted parameters (± asymptotic standard error) were: phytoplankton diets: $Y_{max}$ = 0.8125 ± 0.0182, $B2$ = 0.4166 ± 0.0600, $R^2$ = 0.183, p < 0.001; detritus diets: $Y_{max}$ = 0.8040 ± 0.0507, $B2$ = 0.2450 ± 0.0314, $R^2$ = 0.460, p < 0.001

Table 11. Expt 1: summary of ANOVAs to test significance of both diet and acclimation time on digestive gland (DG) size and their protein content and total digestive enzyme activities. No significant differences were found in specific activities (omitted). *p < 0.05

| Source of variation | df | SS | MS | $F$ | p |
|---|---|---|---|---|---|
| **Weight of DG** | | | | | |
| Diet | 1 | 0.274 | 0.274 | 0.030 | 0.8641 |
| Acclimation time | 1 | 17.504 | 17.504 | 1.941 | 0.1853 |
| Interaction | 1 | 0.2464 | 0.2464 | 0.027 | 0.8711 |
| Residual | 14 | 126.230 | 9.017 | | |
| **Protein content** | | | | | |
| Diet | 1 | $8 \times 10^{-4}$ | $8 \times 10^{-4}$ | 1.429 | 0.2517 |
| Acclimation time | 1 | $1 \times 10^{-4}$ | $1 \times 10^{-4}$ | 0.120 | 0.7337 |
| Interaction | 1 | $1 \times 10^{-7}$ | $1 \times 10^{-7}$ | $2 \times 10^{-4}$ | 0.9884 |
| Residual | 14 | $7.4 \times 10^{-3}$ | $5 \times 10^{-4}$ | | |
| **Total amylase** | | | | | |
| Diet | 1 | 19.278 | 19.278 | 3.735 | 0.0738 |
| Acclimation time | 1 | 0.331 | 0.331 | 0.064 | 0.8038 |
| Interaction | 1 | 8.019 | 8.019 | 1.553 | 0.2331 |
| Residual | 14 | 72.261 | 5.161 | | |
| **Total cellulase** | | | | | |
| Diet | 1 | 0.800 | 0.800 | 0.861 | 0.3692 |
| Acclimation time | 1 | 3.518 | 3.518 | 3.784 | 0.0721 |
| Interaction | 1 | 3.177 | 3.177 | 3.417 | 0.0857 |
| Residual | 14 | 13.015 | 0.939 | | |
| **Total laminarinase** | | | | | |
| Diet | 1 | 0.089 | 0.089 | 0.356 | 0.5601 |
| Acclimation time | 1 | 0.469 | 0.469 | 1.863 | 0.1938 |
| Interaction | 1 | 1.260 | 1.260 | 5.004 | 0.0421* |
| Residual | 14 | 3.525 | 0.252 | | |
| **Total protease** | | | | | |
| Diet | 1 | 0.189 | 0.189 | 1.240 | 0.2842 |
| Acclimation time | 1 | 0.117 | 0.117 | 0.771 | 0.3949 |
| Interaction | 1 | 0.139 | 0.139 | 0.914 | 0.3552 |
| Residual | 14 | 2.137 | 0.153 | | |

and laminarinase) and total protease activities are presented as specific (milligrams of released maltose or tyrosine per milligram protein per hour) and total standard activities (milligrams of released maltose per hour per digestive gland). A 2-factor ANOVA (Table 11) showed that neither the type of diet nor acclimation had any significant effect on the size of the digestive gland, its protein content, or specific enzyme activities. Concerning total activities, the only significant effect refers to the interaction of diet and acclimation on laminarinase activity, accounting for a reduced laminari-

nase value in the long-term acclimation to diets of phytoplankton (Table 10). Importantly, whilst not statistically significant, cellulase showed consistent increments during acclimation to detrital diets, both in terms of specific and total activities.

Table 10. *Cerastoderma edule*. Expt 1: dry weight ($W_{DG}$, mg), protein content (PC, fraction) and enzyme activities of digestive glands (DG) of standard-sized cockles fed phytoplankton and detrital diets for 2 d (short-term acclimation) and 12 d (long-term acclimation). Ae, Ce, Le, Pe: specific amylase, cellulase, laminarinase (mg released maltose $mg^{-1}$ protein $h^{-1}$) and protease (mg released tyrosine $mg^{-1}$ protein $h^{-1}$) activities, respectively; $A_{DG}$, $C_{DG}$, $L_{DG}$, $P_{DG}$: total amylase, cellulase, laminarinase (mg released maltose digestive gland$^{-1}$ $h^{-1}$) and protease (mg released tyrosine digestive gland$^{-1}$ $h^{-1}$) activities, respectively. Values are means of 5 measurements (±95% CI)

| Diet | Acclimation | $W_{DG}$ | PC | Ae | Ce | Le | Pe | $A_{DG}$ | $C_{DG}$ | $L_{DG}$ | $P_{DG}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| P | Short-term | 19.036 ± 1.791 | 0.198 ±0.078 | 1.336 ±0.525 | 0.443 ±0.101 | 0.387 ±0.120 | 0.189 ±0.078 | 5.738 ±2.104 | 1.926 ±0.452 | 1.703 ±0.610 | 0.854 ±0.468 |
| P | Long-term | 20.785 ±1.917 | 0.242 ±0.053 | 0.857 ±0.345 | 0.389 ±0.175 | 0.179 ±0.073 | 0.242 ±0.053 | 4.123 ±1.724 | 1.970 ±1.164 | 0.846 ±0.388 | 1.194 ±0.398 |
| D | Short-term | 18.552 ±4.201 | 0.118 ±0.042 | 1.435 ±0.436 | 0.328 ±0.178 | 0.232 ±0.063 | 0.187 ±0.042 | 6.478 ±2.653 | 1.505 ±0.941 | 1.028 ±0.356 | 0.825 ±0.224 |
| D | Long-term | 20.772 ±2.702 | 0.163 ±0.035 | 1.478 ±0.151 | 0.651 ±0.157 | 0.259 ±0.120 | 0.163 ±0.035 | 7.548 ±1.1613 | 3.240 ±0.740 | 1.236 ±0.338 | 0.810 ±0.151 |

Expt 2

In this experiment, in addition to analyzing the effect of food concentration on enzyme activities, we tested the possibility that an assay system based on substrates present in natural food particles might be a more adequate procedure to reveal some digestive responses (of the type suggested by enhanced cellulase activities with prolonged exposure to detrital diets). To this effect, crude extracts of the digestive gland of specimens fed the different diets were simultaneously assayed for standard protease and cellulase activities and total carbohydrase activity using 'natural substrates' obtained as lipid-free extracts of *Isochrysis galbana* cells and detrital particles of *Spartina maritima*, as described in 'Materials and methods'. Results obtained in these assays (Table 12) can be summarized as follows. (1) Both food concentration and type of diet have a significant effect on the size of digestive glands (Table 13), these being consistently bigger in cockles fed phytoplankton at high ration. This helps to explain higher total protease activities with diets of phytoplankton and higher cellulase activity with the diet of phytoplankton at high concentration (significant interactive effect of diet and ration: AB in Table 13), since differences in specific activities are not significant. (2) Protein content of digestive glands significantly increases with detrital diets compared with phytoplankton diets. (3) The amount of maltose released in assays with 'natural substrates' is less than in assays with standard substrates (Tables 11 & 12), which could be attributed to a substrate concentration effect. This same effect could account for the fact that activities measured with detritus extracts are consistently higher than with phytoplankton extracts. (4) In both kinds of assays with 'natural substrates' a combined effect of diet and acclimation time is evident (Table 13), which is accounted for by the fact that digestive enzyme

Table 13. *Cerastoderma edule*. Expt 2: summary of ANOVAs to test significance of diet, particle concentration and acclimation time on digestive gland size and their protein content and total digestive enzyme activities reported in Table 12. Only significant interactions are included. $^*$p < 0.05

| Source of variation | df | SS | MS | $F$ | p |
|---|---|---|---|---|---|
| **Weight of DG** | | | | | |
| Diet (A) | 1 | 183.144 | 183.144 | 8.786 | 0.0047$^*$ |
| Ration (B) | 1 | 169.798 | 169.798 | 8.146 | 0.0064$^*$ |
| Acclimation time (C) | 1 | 77.744 | 77.744 | 3.729 | 0.0594 |
| Residual | 46 | 1000.517 | 20.844 | | |
| **Protein content** | | | | | |
| Diet (A) | 1 | 0.0076 | 0.0076 | 9.009 | 0.0043$^*$ |
| Ration (B) | 1 | 0.0004 | 0.0004 | 0.472 | 0.4955 |
| Acclimation time (C) | 1 | 0.0003 | 0.0003 | 0.296 | 0.5891 |
| Residual | 46 | 0.389 | 0.0008 | | |
| **Total cellulase** | | | | | |
| Diet (A) | 1 | 1.629 | 1.629 | 3.828 | 0.0565 |
| Ration (B) | 1 | 0.095 | 0.095 | 0.223 | 0.6393 |
| Acclimation time (C) | 1 | 0.008 | 0.008 | 0.021 | 0.8857 |
| Interaction AB | 1 | 1.764 | 1.764 | 4.145 | 0.0475$^*$ |
| Residual | 46 | 19.578 | 0.425 | | |
| **Total protease** | | | | | |
| Diet (A) | 1 | 2.086 | 2.086 | 4.235 | 0.0455$^*$ |
| Ration (B) | 1 | 0.248 | 0.248 | 0.503 | 0.4817 |
| Acclimation time (C) | 1 | 0.245 | 0.245 | 0.498 | 0.4839 |
| Residual | 46 | 21.279 | 0.493 | | |
| **Total carbohydrase (phytoplankton extracts)** | | | | | |
| Diet (A) | 1 | 0.041 | 0.041 | 0.621 | 0.4348 |
| Ration (B) | 1 | 0.003 | 0.003 | 0.042 | 0.8388 |
| Acclimation time (C) | 1 | 0.546 | 0.546 | 8.181 | 0.0063$^*$ |
| Interaction AC | 1 | 0.518 | 0.518 | 7.764 | 0.0070$^*$ |
| Residual | 46 | 3.071 | 0.067 | | |
| **Total carbohydrase (detritus extracts)** | | | | | |
| Diet (A) | 1 | 0.012 | 0.012 | 0.041 | 0.8405 |
| Ration (B) | 1 | 0.003 | 0.003 | 0.009 | 0.9215 |
| Acclimation time (C) | 1 | 1.403 | 1.403 | 0.223 | 0.6392 |
| Interaction AC | 1 | 0.184 | 0.184 | 4.820 | 0.0332$^*$ |
| Residual | 46 | 32.569 | 0.528 | | |

Table 12. *Cerastoderma edule*. Expt 2: dry weight ($W_{DG}$, mg), protein content (PC, fraction) and enzyme activities of digestive glands (DG) of standard-sized cockles fed phytoplankton and detrital diets for 2 d (short-term acclimation) and 12 d (long-term acclimation), dosed at high and low particle concentrations. $C_{DG}$ and $P_{DG}$: total cellulase (mg released maltose digestive gland$^{-1}$ h$^{-1}$) and protease (mg released tyrosine digestive gland$^{-1}$ h$^{-1}$) activities, respectively; CHase: total carbohydrase activities (mg released maltose digestive gland$^{-1}$ h$^{-1}$) recorded with lipid-free extracts of phytoplankton (*Isochrysis galbana* cells) and detrital particles of *Spartina maritima* acting as substrates (P and D, respectively). Values are means of 7 measurements (±95% CI)

| Diet | Acclimation | Ration | $W_{DG}$ | PC | $C_{DG}$ | $P_{DG}$ | CHaseP | CHaseD |
|---|---|---|---|---|---|---|---|---|
| P | Short-term | High | 30.673 ± 5.738 | 0.224 ± 0.024 | 1.679 ± 0.631 | 2.004 ± 0.394 | 0.368 ± 0.216 | 0.790 ± 0.437 |
| | | Low | 25.544 ± 2.180 | 0.244 ± 0.031 | 1.508 ± 0.273 | 1.814 ± 0.707 | 0.225 ± 0.183 | 0.871 ± 0.261 |
| P | Long-term | High | 26.224 ± 4.152 | 0.217 ± 0.017 | 1.611 ± 1.033 | 1.596 ± 0.525 | 0.321 ± 0.204 | 0.678 ± 0.287 |
| | | Low | 22.554 ± 3.608 | 0.221 ± 0.023 | 0.888 ± 0.378 | 1.262 ± 0.441 | 0.282 ± 0.218 | 0.476 ± 0.312 |
| D | Short-term | High | 23.616 ± 2.278 | 0.255 ± 0.016 | 0.797 ± 0.422 | 1.076 ± 0.195 | 0.079 ± 0.096 | 0.475 ± 0.355 |
| | | Low | 22.641 ± 1.613 | 0.239 ± 0.014 | 1.056 ± 0.479 | 1.242 ± 0.659 | 0.009 ± 0.018 | 0.599 ± 0.508 |
| D | Long-term | High | 24.213 ± 4.185 | 0.246 ± 0.015 | 1.071 ± 0.389 | 1.4171 ± 0.503 | 0.288 ± 0.163 | 0.961 ± 0.568 |
| | | Low | 20.027 ± 1.309 | 0.261 ± 0.029 | 1.369 ± 0.356 | 1.264 ± 0.283 | 0.597 ± 0.335 | 0.899 ± 0.488 |

activity increases with acclimation in cockles fed detrital diets. As a result, released maltose that was initially lower in cockles fed *Spartina maritima* particles reached the amount released by phytoplankton-fed cockles after long-term acclimation to the detrital diet.

## DISCUSSION

The present results have revealed that detritus derived from salt-marsh vascular plants can be assimilated with high efficiency (>50%), comparable to the efficiencies exhibited by bivalves feeding on natural seston (Bayne & Newell 1983). When values of $^{14}C$ are considered, representing the carbon GAE, these efficiencies reach 65%, whereas 82% is achieved for phytoplankton.

Discrepancies with the above-quoted results by Langdon, Newell and co-workers (Newell & Langdon 1986, Crosby et al. 1989, Kreeger et al. 1990, Langdon & Newell 1990), concerning the low AE values reported by these authors for mussels and oysters fed salt-marsh detritus (see 'Introduction'), may be interpreted in several ways. First, most of their results were obtained using lignocellulosic preparations (crude fiber extracts) obtained from vascular plant tissues. This rather refractory particulate material was chosen, since it appeared to more closely represent naturally occurring detritus after the labile components had been eliminated by fragmentation, eroding and colonization of organic particles by microorganisms (Newell & Langdon 1986). Available data seem insufficient to resolve the question as to whether composition differences between fresh and extracted detritus might have caused such large differences in absorption efficiency. Secondly, variable physiological conditions of bivalves induced by environmental factors might also play an important role. For example, Charles & Newell (1997) reported absorption efficiencies of ribbed mussels *Geukensia demissa* that were 72% higher than previous values in specimens of the same species collected from the same geographical location and tidal height (Kreeger et al. 1990). They attributed this difference to the induction of cellulase activity associated with the induction of absorption due to the presence of cellulose in the diet as well as to seasonal variability in cellulase activity. On the other hand, intertidal mussels absorbed lignocellulosic material with a higher efficiency than did subtidal mussels (Kreeger et al. 1990, Charles & Newell 1997), which was explained by the fact that, because of emersion, longer retention times of food within the gut occur in intertidal mussels, providing increased action time for digestive enzymes and favoring absorption efficiency (Bayne et al. 1988). Consequently, both diet acclimation and rates of food pro-

cessing are acknowledged to influence the use of detrital energy by bivalves, most especially by affecting absorption efficiency.

As to the precise nature of plant detritus that is actually available to bivalves in the field, it should be pointed out that the objectives of the present work were primarily focused on exploring the suite of feeding–digestive adjustments that enable bivalves to exploit different vegetable sources rather than on the immediate ecological relevance of the results.

### Digestive adjustments: gross absorption

Digestive adjustments are more clearly represented by the behaviour of GAR, as this measurement constitutes the overall component of energy gain from digestive processes. When GAR is plotted against food concentration (Fig. 3, Table 14) a clear difference between phytoplankton and detritus emerges. The capacity for obtaining energy from increasing amounts of suspended food appears to be greatly constrained in the case of *Spartina maritima* detritus in comparison with phytoplankton; however, the situation is clearly improved for detrital diets after 12 d of acclimation, when rates of absorption showed a rising tendency with increasing food concentration irrespective of food source (see ANOVA results in Table 14). Such beneficial effects of acclimation have been broadly documented in the cockles *Cerastoderma edule* (Ibarrola et al. 2000a,b) (for mussels *Mytilus edulis* see also Bayne 1993, Bayne et al. 1993). The digestive mechanisms underlying such acclimation responses can also be considered to differ between food types. GF, representing the total amount of food held by the gut at each moment, was independent of food concentration and remained invariable throughout the course of acclimation to phytoplankton diets, while it increased in cockles acclimated to moderate and high concentrations of detritus (Fig. 3, Table 14).

In continuous feeders, gut capacity and gut residence time of food together determine how much food can be processed per unit time (Sibly & Calow 1986, Willows 1992). Implicit to this statement is the existence of a functional relationship between IR and GPTs of food, which has been modeled as an exponential decaying function in bivalve mollusks (Bayne et al. 1984, 1989, Navarro & Iglesias 1993, Navarro et al. 1994). Present data for this relationship were fitted to a single function including pooled values for both food types after 2 and 12 d of acclimation (Fig. 1A), on account of the lack of differences between groups. A re-examination of data (Fig. 1B), however, suggests that, for a given rate of ingestion, GPTs tend to increase with acclimation time in detritus-fed cockles,
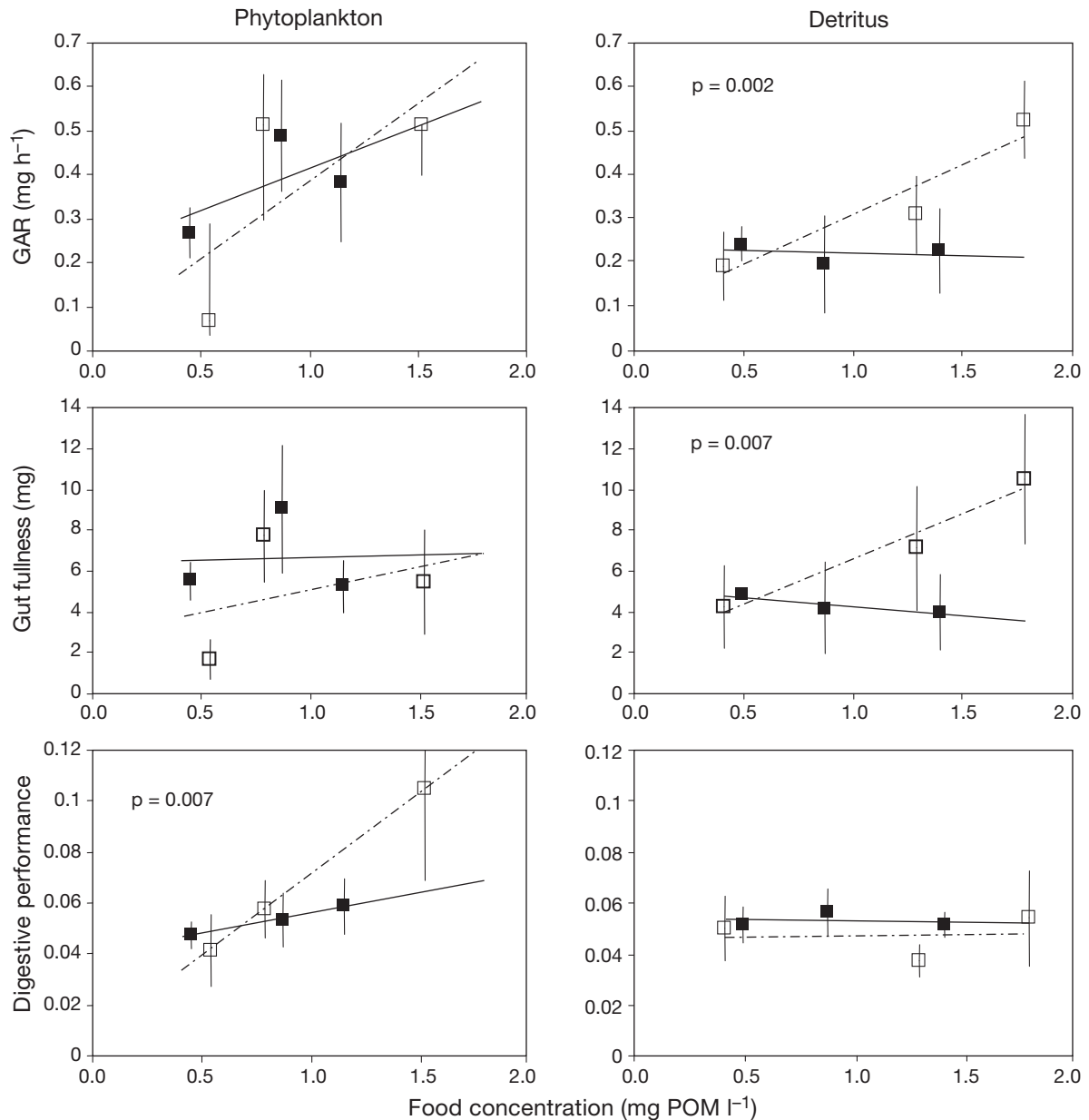
Fig. 3. *Cerastoderma edule.* Gross absorption rate (GAR), gut fullness (GF) and digestive performance (DP = GAE/GPT), as a function of food concentration in phytoplankton and detritus diets (see 'Discussion' for details). Points are mean values for the different experimental groups (±2 SE). Solid symbols: 2 d of acclimation; open symbols: 12 d of acclimation. To account for seasonal differences in feeding rates between Expts 1 (February) and 2 (May), thermal effects were assumed and data were standardized to a common temperature (16°C) applying a thermal coefficient ($Q_{10} = 2.5$) computed using data on seasonal variation of clearance rates of cockles (Newell & Bayne 1980, Ibarrola et al. 2008). Significant interactions between food concentration and acclimation time are indicated. ANOVA testing the effect of acclimation time with particulate organic matter (POM) as covariate given in Table 14

while they decrease in the case of phytoplankton diets, as revealed by significant interaction between diet and acclimation time (p = 0.02; see Tables 8 & 9). It is very likely that these opposite trends resulted in the cancellation of significant differences between food groups. In conclusion, enlarged gut capacity after complete acclimation to detrital diets (see ANOVA results in Table 14) would have enabled cockles to retain food longer in order to increase absorption efficiency.

The question now is why phytoplankton-fed cockles do not behave according to this 'strategy'. A possible explanation would be the different susceptibility of phytoplankton and detrital particles to digestive pro-

Table 14. ANOVA for testing the effect of acclimation time on GAR, GF and DP, with POM as covariate (see Fig. 3 for abbreviations). *p < 0.05

| Factor | Phytoplankton | | | | | | Detritus | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GAR | | GF | | DP | | GAR | | GF | | DP | |
| | $F$ | p | $F$ | p | $F$ | p | $F$ | p | $F$ | p | $F$ | p |
| Acclimation time | 1.57 | 0.218 | 1.24 | 0.274 | 4.68 | 0.039* | 5.09 | 0.031* | 2.41 | 0.132 | 0.60 | 0.444 |
| POM | 10.57 | 0.002* | 1.23 | 0.275 | 23.95 | <0.0001* | 8.47 | 0.007* | 3.41 | 0.076 | 0.059 | 0.809 |
| Interaction | 0.75 | 0.392 | 0.18 | 0.675 | 8.46 | 0.007* | 11.44 | 0.002* | 8.58 | 0.007* | 0.059 | 0.811 |

cesses, resulting in significantly different functions relating GAE and GPT for cockles fed microalgal and detrital diets (Fig. 2). Absorption efficiency has been proposed to increase asymptotically with increases in the residence time of food within the gut (Sibly 1981, Sibly & Calow 1986, Willows 1992), representing a basic feature of continuous-flow digestive systems that has been empirically proven in suspension-feeding bivalves (Bayne et al. 1987, 1988, Navarro & Iglesias 1993, Navarro et al. 1994). According to the present data (Fig. 2), cockles attained the same maximum GAE (81%) irrespective of food type, but the rate at which this value was approached with increasing GPT was almost halved in the case of *Spartina maritima* detritus (0.24) compared to *Isochrysis galbana* cells (0.42). Such reduced absorption efficiency per unit GPT could simply reflect the reduced digestibility of vascular plant detritus compared with phytoplankton. López & Levinton (1987) postulated similar digestive behaviour in deposit feeders with regards to the processing of different classes of food particles (benthic diatoms and seaweed and lignocellulosic detritus) present in marine sediments. Furthermore, the curves in Fig. 2 also served to illustrate that changes in GPT in the range of from 8 to 25 h (the empirical range observed in the present work) would produce a minimal effect in the GAE of phytoplankton (asymptotic zone), while dramatically affecting the efficiency with which detrital food is absorbed. In other words, cockles would greatly benefit from any increment in the gut residence time of detrital particles during the course of acclimation, while the improvement would be much less important in the case of phytoplankton diets. Indeed, different approaches based on optimality principles have predicted that extended gut residence times of food are associated with poor diets, accounting for the widespread finding that animals sustaining themselves on less digestible food items tend to develop larger guts (Sibly 1981, Sibly & Calow 1986).

Conversely, the virtual independence of GAE from GPT may help explain why acclimation to microalgal diets could, in spite of producing a faster passage of food, enhance digestive performance (see ANOVA results in Table 14), very likely enabling the exploitation of a further increase in food concentration.

## Digestive enzyme activities

Compensatory adjustments considered up to this point, namely the increased digestive performance with reduced GPT in the highly concentrated phytoplankton diets, and the maintenance of digestive performance with increasing amounts of food processed by the gut in the course of acclimation to detritus diets, would imply an increment in the hydrolytic capacity of digestive structures. With respect to the digestive gland, such expectations are largely met by the present results on enzyme activity. For instance, cockles exposed to high rations of *Isochrysis galbana* showed increased protease and cellulase total activities that were partly based on the size increment of the digestive gland of these individuals. These results are coincident with those reported by Ibarrola et al. (1996, 2000b,c), who showed that the increment in various digestive enzyme activities (non-specific protease, cellulase and laminarinase) compensated for the decreased GPT of food produced by increasing the ration of *Tetraselmis suecica*, making absorption efficiency virtually independent of IRs (Ibarrola et al. 2000a,b). Also Bayne (1993) and Bayne et al. (1993) have reported that increments in carbohydrase activity correlated with the increment in ARs of both carbohydrates and total organics achieved during the acclimation of mussels *Mytilus edulis*, and Hawkins & Bayne (1992) reported changes in α-amilase and laminarinase activities in mussels held at high and low levels of ration. In the case of cockles, these changes have been found to involve the modification of several morphometric parameters of the digestive gland, as well as the volume fractions of digestive cell lysosomes and basophilic cells associated, respectively, with protease and carbohydrase activities (Ibarrola et al. 2000c).

On the other hand, maltose released from 'natural substrates' (lipid-free extracts of microalgal and detrital particles) in the assays performed with digestive gland extracts of cockles-fed detritus increased significantly in the course of acclimation. The induction of carbohydrases during the process of acclimation is strongly supported by the higher protein content of these glands. Fernández-Reiriz et al. (2004) reported that total carbohydrase activity in both the digestive

gland and crystalline style of *Argopecten purpuratus* significantly increased between 24 and 72 h of acclimation to detritus-rich diets, in contrast with the behaviour of scallops acclimated to diets in which phytoplankton was abundant. These changes mainly involved amilase and cellulase activities. According to results obtained in the present assays with standard substrates, carbohydrase induction would mainly be based on the increased cellulase activity that was shown to consistently occur in the course of acclimation to detrital diets (Tables 10 & 12).

Quantification of the activity of a few selected carbohydrases might not represent all digestive adjustments in bivalves. Since the composition of carbohydrates in available food is naturally variable, multiple types of glucanases are required for the breakdown of food particles. Consequently, other activities in addition to those of amylase, cellulase and laminarinase have been reported in the digestive system of bivalves (for review see Vonk & Western 1984). Although a significant increase in a given glucanase type is indicative of induction of this particular enzyme, no direct correspondence needs to exist between the increased rate of synthesis of a given enzyme and the rate of sugar release from food particles. In addition, total release of sugars depends on the sequential action of glucanases and glycosidases, so that slight increments in the activity of a given glucanase are likely to exponentially enhance total sugar release due to its synergic effects on glycosidases. This is especially true for those glucanases acting on structural carbohydrates, such as cellulase, because cell wall breakdown (Brock 1989) might improve substrate availability for other enzymes.

In this respect, the use of natural substrates represents a holistic approach, allowing the measurement of the overall activity of the whole pool of glucanases and glycosidases acting on a natural mixture of carbohydrates. The significant increment in the activity towards these natural substrates in the cockles acclimated to detrital diets (Table 12) might be a mere consequence of slight increases in cellulase activity that have been recorded, or, alternatively, it might come from changes in other (not determined) activities. In any case, reported results undoubtedly indicate the existence of digestive adjustments that allow cockles to improve detritus absorption.

## Digestive adjustments: net absorption

Net rates of absorption are constrained by the magnitude of MFLs, that component of endogenous products contributing to digestion which is not reabsorbed (Hawkins & Bayne 1985, Bayne & Hawkins 1990). In

animals, such as bivalves exhibiting predominantly intracellular digestion, the main component of endogenous matter defecated would derive from the fragmentation and subsequent release of digestive cell apices in the form of membranous 'fragmentation spherules' (Purchon 1977, Morton 1983), which are reputed to have a high lipid content (Owen 1972). This is consistent with the finding that assimilatory balance of lipids in cockles was reduced under feeding conditions characterized by high MFL (Ibarrola et al. 1996, 1998).

The magnitude of MFL increases with increasing rates of food processing; hence, for comparative purposes, the use of a relative index, such as MFL per unit of ingested organics or fractional costs of digestion, would be preferable. In the short-term exposure a clear difference between diets was evident, since these costs represented 12 % in phytoplankton-fed cockles versus 26 % in those fed detritus, but acclimation exerted an important reduction of the latter, to the point of a virtual cancellation of these differences (7 vs. 12 %). It is important to note that this reduction of digestive cost induced by acclimation in detritus-fed cockles occurred concomitantly with the improvement of the digestive potential of digestive gland extracts measured in terms of the amount of reducing sugars released from 'natural' substrates. This coincidence suggests that increased efficacy in the intracellular processing of such refractory materials, mediated by the induction of the appropriate set of enzymes, would produce the additional benefit of reducing the losses of cell materials that accompanies each cycle of intracellular digestion. Whatever the precise mechanisms, both factors appear to function synergistically in order to greatly improve digestive capabilities during acclimation of cockles to detrital diets.

## Concluding remarks

Concerning the practical question of the role played by different food types in sustaining growth of bivalves, it should be noted that the present study focused only on the carbon-energy balance, while algae and detritus might have different nutritional values due to differences in C:N composition. In particular, the relatively low nitrogen content of *Spartina maritima* might constitute a limiting factor, and the improved absorption of detrital organic matter shown in the present paper does not in itself enable cockles to exploit detritus effectively as a nutrient source. In this respect, results reported by Grant & Cranford (1991) indicated that, in the scope of growth measurements of the scallop *Placopecten magellanicus*, the actual growth rates recorded under nitrogen-limited diets of kelp detritus were greatly overestimated. Attempts to culture the scallop *Argo-*

*pecten irradians* (Kirby-Smith 1976) and the mussel *Mytilus edulis* (Williams 1981) on marsh-grass detritus alone were unsuccessful, although it is unclear from such studies whether poor growth was due to the nutritional limitations of detritus or due to its refractory behaviour to digestion. The present results account for some adjustments enabling cockles to compensate for the poor digestibility of *Spartina maritima* detritus. On the other hand, compensatory responses to reduced nitrogen availability have also been reported in mussels on a seasonal basis (Kreeger 1993).

Trophic relationships of suspension feeders in coastal environments revealed by recent studies based on stable isotope enrichment (Decottignies et al. 2007a,b, Dubois et al. 2007) depicted a complex structure: variability recorded in the relative contribution of different food sources (phytoplankton, microphytobenthos and plant detritus) to tissue growth included seasonal and site differences, as well as differences in selective behaviour between competitor species. Thus, for these relationships to be fully understood broad information on particulate food availability in the environment should be framed together with the knowledge of those aspects of feeding behaviour and nutrition concerning the differential utilization of living microalgae and plant detritus, including the assessment of species-specific variability.

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