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Finding new diatoms for intensive rearing of the pacific oyster (*Crassostrea gigas*): energy budget as a selective tool

Laurent Barillé [a,*], Joël Haure [b], Emmanuelle Pales-Espinosa [a], Michèle Morançais [a]

[a] *Laboratoire de Biologie Marine, Faculté des Sciences, ISOMER, 2 rue de la Houssinière, B.P. 92208, 44322 Nantes Cedex 3, France*
[b] *IFREMER, URAPL, Station de Bouin, Polder des Champs, 85230 Bouin, France*

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Abstract

Intensive shellfish rearing in the polders of Bourgneuf Bay on the French Atlantic coast (46–47°N, 1–2°W) relies on the diatom *Skeletonema costatum* (Grev.) Cleve produced in nutrient-rich saline ground water. An episodic reduction in biomass production caused by an unidentified protozoon stressed the need to find new microalgal species as substitutes for *S. costatum* and diversify the microalgae used by the bivalve industry. Three species met the requirements for year-round availability in Bourgneuf Bay coastal waters and growth potential in saline ground water: *Nitzschia acicularis* (Kützing), *Nitzschia closterium* (Ehrenberg) Wm Smith and *Nitzschia gandersheimiensis* Krasske = *Nitzschia tubicola* Grunow. These microalgae were tested as food sources for adult Pacific oysters (*Crassostrea gigas*) by comparing short-term bivalve physiological responses with those obtained with *S. costatum* as reference. Suspended particulate matter concentrations in experimental diets ranged from 9.3 to 18.6 mg l^{-1} and particulate organic matter concentrations from 3.3 to 5.7 mg l^{-1}. Significant differences were observed, with clearance rates ranging from 4.0 l h^{-1} g^{-1} for *N. acicularis* to 7.3 l h^{-1} g^{-1} for *N. gandersheimiensis*. The filtration rate for organic matter was significantly higher for *N. gandersheimiensis* than the other species, but this algae was also significantly more rejected in pseudofaeces. No differences were found among the four mean faeces production rates. Net energy balance differed significantly among the four microalgae tested, ranging from 282 J h^{-1} g^{-1} for *S. costatum* to 27 J h^{-1} g^{-1} for *N. closterium*. However, no differences were found between *S. costatum*, *N. acicularis* and *N. gandersheimiensis*. These results suggest that

* Corresponding author. Tel.: +33-2-51-12-56-55; fax: +33-2-51-12-56-68.
E-mail address: barille@isomer.univ-nantes.fr (L. Barillé).

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N. acicularis and *N. gandersheimiensis* could be tested on a larger scale involving the production of microalgae in 50-m^3 outdoor tanks in association with Pacific oyster growth experiments.

Keywords: *Crassostrea gigas*; Energy budget; *Nitzschia acicularis*; *Nitzschia gandersheimiensis*; *Skeletonema costatum*

1. Introduction

Bourgneuf Bay on the French Atlantic coast (46–47°N, 1–2°W) is an important shellfish production area characterised by traditional aquaculture of the Pacific oyster *Crassostrea gigas* (Thunberg) (Robert, 1990). This area is the third leading production site in France for the Pacific oyster, with an annual output of around 12,000 tons (Barillé-Boyer et al., 1997). In addition to extensive oyster farming on large intertidal flats, former salt ponds in neighbouring marshes are traditionally used for oyster fattening from October to December to improve product value just before the annual sales peak during the Christmas period (Korringa, 1976).

The discovery around 1980 of nutrient-rich saline ground water in marshes (Moreau, 1996) allowed the development of outdoor batch cultures of a large biomass of *Skeletonema costatum* (Grev.) Cleve, a diatom widely found in ponds and coastal waters of Bourgneuf Bay (Rincé, 1978; Robert et al., 1987). Low-cost production was conducive to new cultural practices, such as intensive rearing of juvenile and adult clams and oysters (Baud and Bacher, 1990; Bacher and Baud, 1992; Sauriau et al., 1997). In addition, a project for controlled oyster fattening in ponds was recently undertaken (Baud et al., 1995; Méléder et al., 2001) to provide a daily supply of *S. costatum* and ensure that the gain in soft tissues and glycogen was not solely dependent on the occurrence of natural microalgal blooms.

Thus, several research projects and related economic activities rely on the monospecific production of this diatom. However, a recent episodic reduction in biomass production was apparently caused by an unidentified protozoon. Although this pathologic event appears to be limited, producers and scientists have recognised the need to find microalgal species that could be substituted for *S. costatum* to diversify the food sources used by the bivalve industry. These new species should meet three main requirements: year-round availability in Bourgneuf Bay and adjacent ponds, growth potential with saline ground water, and suitability as a food source for adult Pacific oysters. The first two requirements, briefly considered in this work and reported in detail by Pales-Espinosa (1999), limited the possibilities to three diatoms of the genus *Nitzschia*. The purpose of the present study was to test these microalgae as possible food sources for adult *C. gigas* by comparing short-term bivalve physiological responses with those obtained with *S. costatum* as reference. The integration of these responses was used to calculate an energy budget on an hourly time scale, i.e. the widely used ‘‘scope for growth’’ (Bayne, 1998). Comparison of the different growth predictions, in conjunction with analysis of the physiological processes involved in the energy budget, constituted a first step in identifying new microalgae of aquacultural interest preliminary to detailed analysis of nutritional quality, such as the polyunsaturated fatty acid composition of the diet (Thompson and Harrisson, 1992; Thompson et al., 1993).

2. Materials and methods

2.1. Isolation and production of algal species

Natural populations of microalgae were collected monthly during 1996 in the shellfish ecosystem of Bourgneuf Bay south of the Loire River estuary (France). Each species was isolated in the laboratory using an inverstoscope and the Pasteur pipette method (Hoshaw and Rosowski, 1973). Non-axenic strains were grown and produced in Conway enriched seawater medium (Helm et al., 1979) at 15 °C under a 14-h light/10-h dark cycle, with 50 μE m^{-2} s^{-1} light intensity. The ability of these populations to grow in nutrient-rich underground saline water, diluted with one-third natural seawater, was investigated using algal growth tests (Pales-Espinosa and Moreau, 1997). Five diatoms met the requirements of year-round availability and growth with underground waters: three from the *Nitzschia* genus [*Nitzschia acicularis* (Kützing), *Nitzschia closterium* (Ehrenberg) Wm Smith and *Nitzschia gandersheimiensis* Krasske = *Nitzschia subcapitellata* Hustedt = *Nitzschia tubicola* Grunow], *Navicula* sp. and *Amphora* sp. These five species were maintained in the culture collection of the Marine Biology Laboratory of the College of Sciences of the University of Nantes. Algal cultures were then produced in 500-l batches with stirring in order to obtain a large amount of biomass. At this stage, *Navicula* sp. and *Amphora* sp. could not be produced in high volume. *N. closterium* grew well, but rapidly formed clusters of several cells that were subsequently broken up by the pumps connected to the flow-through system. Consequently, the ecophysiological responses of Pacific oysters fed the three *Nitzschia* species were compared with those obtained with a diet of *S. costatum*, the species currently produced by oyster breeders in Bourgneuf Bay. All details concerning algal growth experiments have been reported by Pales-Espinosa (1999).

2.2. Measurements of individual oyster physiological responses

One hundred adult Pacific oysters (*C. gigas*) grown in Bourgneuf Bay were bought from an oyster farmer at the Polder des Champs (Bouin, Département of Vendée, France), cleaned of epibionts, and acclimated for 15 days during October 1996 using natural seawater maintained at 20 °C. This temperature is close to the optimal level for the clearance rate of Pacific oysters cultivated on the French Atlantic coast (Bougrier et al., 1995). Our objective was to obtain high feeding responses to ensure better expression of the physiological responses recorded with each microalga. For each of the four microalgae, 10 oysters were randomly chosen from the experimental population and tested (mean dry weight = 1.1 g, S.D. = 0.2 g, $n = 40$). The experimental flow-through system, described by Barillé et al. (1994), consisted of five individual trays each containing a single oyster. A sixth tray was occupied by an empty shell to take the sedimentation effect into account. Each tray, equipped with a flow meter, received a mean flow rate of 10 l h^{-1}. Peristaltic pumps were used to adjust food concentration to comparable values of particulate organic matter (POM). Clay (kaolinite, BS1, AGS, Clérac, 17270 Montguyon, France) was mixed with each algal culture in filtered seawater (0.45 μm) to obtain suspended particulate matter (SPM) and organic fractions comparable to those encountered in oyster ponds

(Sornin et al., 1987) and the fattening production system (Baud et al., 1995). The clay size chosen mimicked the natural distribution in coastal seawater (Barillé et al., 1993). Cell concentration was checked with a Multisizer particle counter fitted with a 100-μm aperture.

Samples of suspended matter from the outflow of the six trays, as well as pseudofaeces and faeces collected separately after 1 h of production, were filtered on pre-weighed Whatman GF/C filters, dried at 60 °C for 24 h, weighed, and then ashed at 450 °C for 1 h to determine total SPM and POM. POM concentrations were corrected for loss of kaolinite structural water during calcination (Dankers and Laane, 1983). The concentrations obtained with the tray containing the empty shell were used as inflow values. The chlorophyll *a* concentration at inflow was determined by fluorometric analysis.

The components of the energy budget were calculated separately, as follows: the clearance rate, which represents the volume of seawater cleared of particles per unit of time, was calculated by measuring particle concentrations with a particle counter at the inflow and outflow of the trays. At least three measurements were performed for each oyster. Particular attention was paid to the size range for which the particle number was measured. To avoid underestimation of the clearance rate because of small particles cleared from suspension by the bivalve but not retained on the gills and released into the tray (Barillé et al., 1993), concentrations were counted only for particles above 10 μm in equivalent spherical diameter (ESD). The threshold was set at 6 μm for *N. closterium* because of its size distribution. The formula used by Bayne (1971) to calculate clearance rate was retained after checking that there was no significant difference with the formula proposed by Hildreth and Crisp (1976). Considering the mean flow and individual tray volume, the clearance rate (l h^{-1}) was calculated as:

$$[(\text{inflow} - \text{outflow})/\text{inflow}] \times \text{flow rate}$$

Inactive individuals were systematically replaced. Retention efficiencies were computed for each size in the 2–30 μm ESD range, as described in Barillé et al. (1993). The filtration rate (mg h^{-1}), which represents the mass of particles filtered per unit of time, was calculated as the product of the clearance rate multiplied by POM concentration at inflow. This calculation was possible because organic particles of the diets are located above the thresholds set for the calculation of clearance rates. The ingestion rate (mg h^{-1}) was the difference between the filtration rate and pseudofaeces production (mg h^{-1}), and the absorption rate (mg h^{-1}) was the difference between the ingestion rate and faeces production (mg h^{-1}). All these physiological responses were based on POM. An energy conversion factor of 23.5 J mg^{-1} of absorbed organic matter was used (Widdows et al., 1979). Scope for growth (Bayne, 1998), which might better be referred to as net energy balance (NEB, J h^{-1}), was calculated as the difference between the absorption rate expressed in J h^{-1} and a respiration rate (R) estimated from the model of Bougrier et al. (1995) based on the dry weight of the animal (W) and temperature (T): R (mg O_2 h^{-1})= $[(0.613 \times 1.042^{T}) - 0.432]$. $W^{0.8}$ can be expressed in J h^{-1} by multiplying the value in mg O_2 h^{-1} with a conversion factor of 0.7×20.08 (Gnaiger, 1983). Excretion was not taken into account in the calculations.

Table 1
Characteristics of the experimental diets fed to adult Pacific oysters

	Chlorophyll *a* (μg l^{-1})	SPM (mg l^{-1})	POM (mg l^{-1})	Organic content (%)
N. acicularis	40.1 (3.5)	18.6 (1.5)	5.0 (1.3)	27
N. closterium	29.5 (1.6)	9.6 (2.8)	3.3 (0.5)	34
N. gandersheimiensis	84.4 (5.0)	15.6 (1.4)	5.7 (0.8)	36
S. costatum	49.8 (10.8)	9.3 (0.9)	3.3 (0.5)	36

SPM: suspended particulate matter; POM: particulate organic matter. Values between brackets are standard deviation. Number of measurements = 3.

2.3. Standardisation of physiological rates and statistical analysis

All physiological responses were standardised to those of an equivalent oyster of 1 g soft tissue weight, as follows: $Y_s=(W_s/W_e)^b Y_e$, where Y_s is the standardised variable, W_s is the standard weight (1 g), W_e is the weight of the experimental animal, Y_e is the uncorrected variable, and b is the corresponding weight exponent of 0.67 for feeding processes (Shpigel et al., 1992) and of 0.8 for oxygen consumption (Bougrier et al., 1995).

Feeding processes were compared using one-way analysis of variance (ANOVA). After the normality and homogeneity of SNK variances were checked, a posteriori tests were run with ANOVA data.

3. Results

SPM concentrations were adjusted to around 15 mg l^{-1} for the four algae tested (Table 1), but with some significant variations (9.3 to 18.6 mg l^{-1}; ANOVA, $P<0.05$). POM

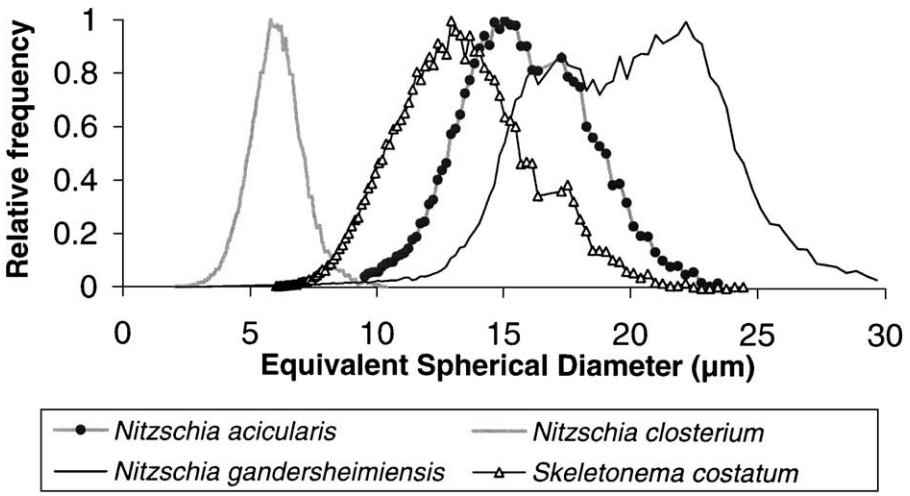

Fig. 1. Particle counter size distributions for the experimental diets.

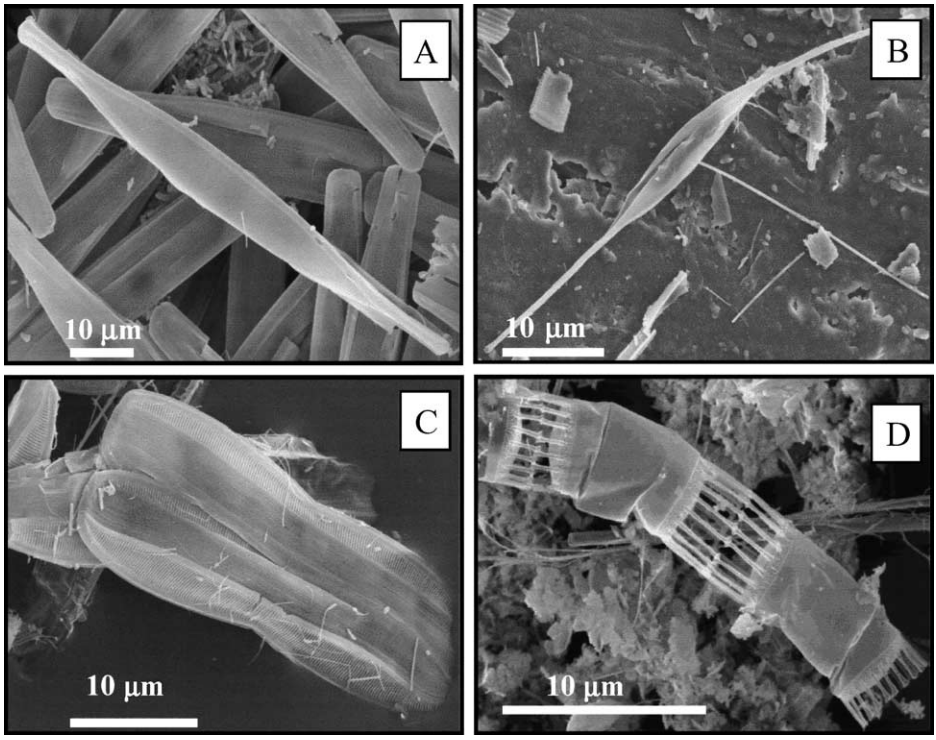

Fig. 2. Scanning electron microscopy of the four diatom species used in the study. (A) *N. acicularis* (Kützing). (B) *N. closterium* (Ehrenberg) Wm Smith. (C) *N. gandersheimiensis* Krasske = *N. tubicola* Grunow. (D) *S. costatum* (Grev.) Cleve.

concentrations did not differ statistically (3.3 to 5.7 mg l^{-1}; ANOVA, $P>0.05$). Organic contents of 27–36% were obtained with the four diets (Table 1). Statistical differences were observed for chlorophyll *a* concentrations (up to 84.4 µg l^{-1} for *N. gandersheimiensis*; ANOVA, $P<0.05$). Microscopy studies indicated that *N. gandersheimiensis* had the largest chloroplasts relative to its overall size.

Size spectra obtained with the particle counter and expressed in ESD showed that *N. gandersheimiensis* was the largest of the four algae tested (Fig. 1). This form of

Table 2
Characteristics of microalgal size distributions

	Mode obtained with a particle counter (equivalent spherical diameter, µm)	Mode of the apical length of the frustule (µm)
N. acicularis	15	65
N. closterium	6	40
N. gandersheimiensis	15 and 25[a]	40
S. costatum	12[b]	50

[a] Bimodality is due to cell division.
[b] Chain length of five cells for *S. costatum*.

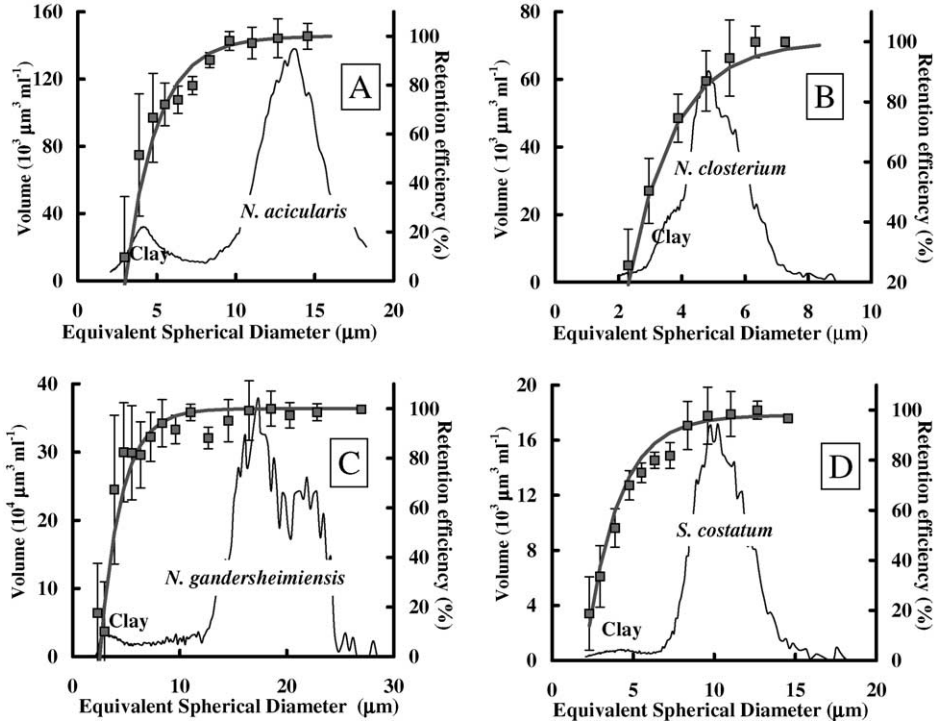

Fig. 3. Retention efficiency of *C. gigas* on size classes of suspended particles for the four diets tested (mean ± 95% confidence intervals). The lines have been fitted by eye to data. Size distributions are represented by particle volume. (A) *N. acicularis*. (B) *N. closterium*. (C) *N. gandersheimiensis*. (D) *S. costatum*.

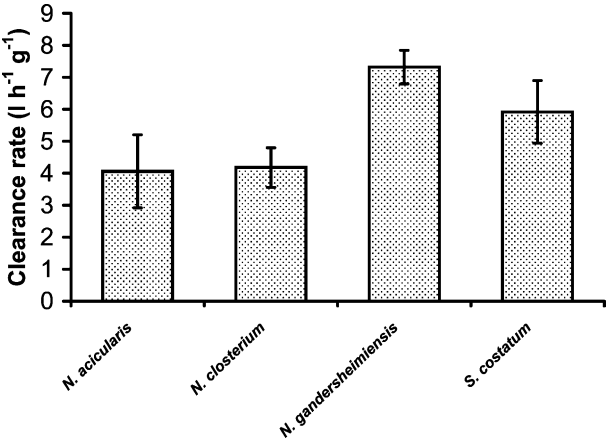

Fig. 4. Mean clearance rates of Pacific oysters for the four diets tested (mean ± 95% confidence intervals).

representation gives a better image of the volume occupied by an alga and provides data complementary to linear measurements of frustule apical length (pervalvar length for *S. costatum*) obtained with a light microscope. Interestingly, *N. closterium* and *N. gandersheimiensis*, which have approximately the same valve length of around 40 μm (Fig. 2), can easily be differentiated by measurements of their respective volumes (Fig. 1). Table 2 indicates the equivalences for size distribution obtained with the particle counter and the light microscope.

The retention efficiency curves show that *N. acicularis*, *N. gandersheimiensis* and *S. costatum* were cleared with 100% efficiency (Fig. 3). However, for *N. closterium*, oysters were only maximally efficient for sizes larger than 6–7 μm ESD, in which case, a small fraction of the algal size distribution was not retained on the gills. The clay added to the four diets, which showed a distribution characterised by a mode of around 3–4 μm ESD, was

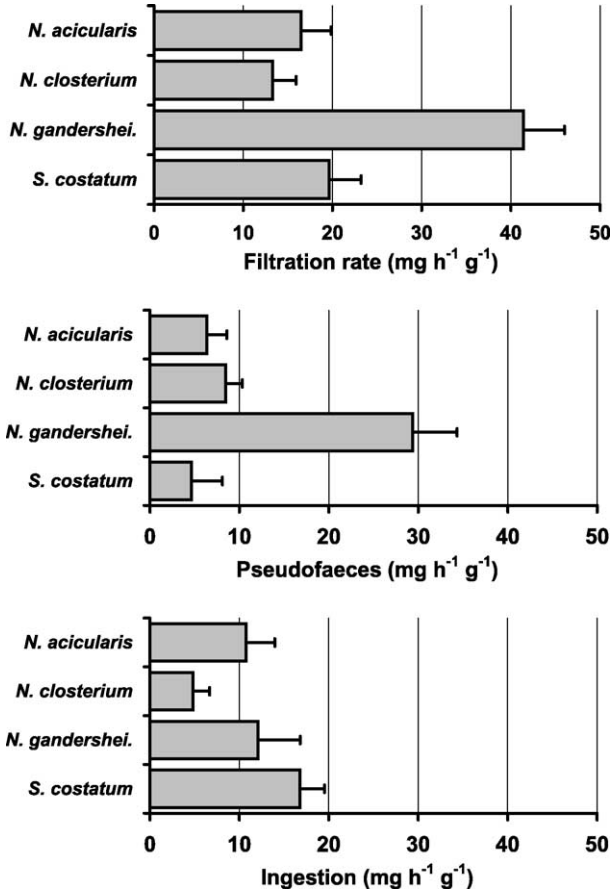

Fig. 5. Mean filtration rates, pseudofaeces production and ingestion rates of Pacific oysters for the four diets tested (mean ± 95% confidence intervals).

poorly retained by the oysters (Fig. 3). As clay contributed to SPM concentrations, its low retention minimised the significance of SPM variations in the diets.

A comparison of the mean clearance rate (CR) calculated with the four microalgae showed significant statistical differences (Fig. 4; ANOVA, $P < 0.05$), ranging from 4.0 l h^{-1} g^{-1} for *N. acicularis* to 7.3 l h^{-1} g^{-1} for *N. gandersheimiensis*. Mean CRs obtained with *N. acicularis* and *N. closterium* (4.1 l h^{-1} g^{-1}) were significantly lower than those for *S. costatum* (5.9 l h^{-1} g^{-1}) and *N. gandersheimiensis* (SNK, $P < 0.05$). The latter CR was also significantly higher than that measured for *S. costatum*. Because of its high CR, *N. gandersheimiensis* had a significantly higher filtration rate for organic matter than the other species (Fig. 5; ANOVA $P < 0.05$). However, rejection in pseudofaeces was significantly greater for this diatom than the others (Fig. 5; ANOVA $P < 0.05$). Although this reduced differences among the four algae at the ingestion level (Fig. 5), mean values remained significantly different (ANOVA, $P < 0.05$). In fact, higher amounts of *N. closterium* were

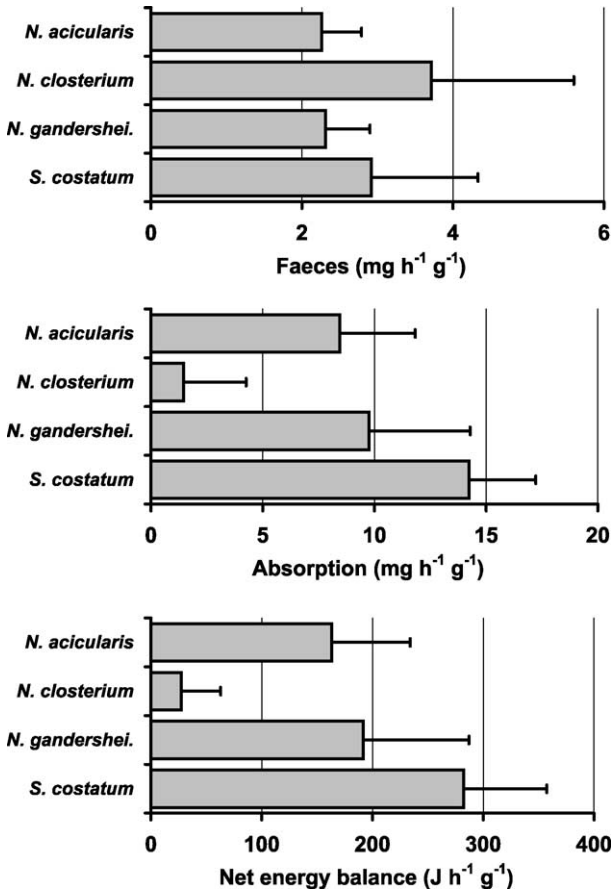

Fig. 6. Mean faeces production, absorption rates and net energy balance of Pacific oysters for the four diets tested (mean ± 95% confidence intervals).

rejected in pseudofaeces relative to filtered quantities, resulting in the lowest ingestion rate (Fig. 5). Mean organic ingestion was higher for *S. costatum* than *N. gandersheimiensis*, although the difference was not statistically significant (SNK, $P>0.05$). No differences were found for the mean faeces production of the four algae (Fig. 6; ANOVA, $P>0.05$). The higher value for *N. closterium* suggests that this alga was digested less.

A comparison of the absorbed ration which integrates the different feeding processes revealed a hierarchy among the four algae tested (Fig. 6). The highest absorption was recorded for *S. costatum* (14.2 mg h^{-1} g^{-1}) and the lowest for *N. closterium* (1.5 mg h^{-1} g^{-1}). However, the mean absorptions of *N. acicularis*, *N. gandersheimiensis* and *S. costatum* did not differ significantly (SNK, $P>0.05$). Metabolic expenditures were estimated using a respiration model (Bougrier et al., 1995) based on the temperature and dry weight of the animals. As all diets were subject to a temperature of 20 °C, the respiration rate of a standard oyster (1 g dry tissue weight) was 13.5 J h^{-1}. Therefore, the trend observed with standardised absorption (Fig. 6) was conserved when the net energy balance (NEB) was calculated (Fig. 6). NEB differed significantly among the four microalgae (ANOVA, $P<0.05$), ranging from 282 J h^{-1} g^{-1} for *S. costatum* to 27 J h^{-1} g^{-1} for *N. closterium*. NEB for *N. acicularis*, *N. gandersheimiensis* and *S. costatum* did not differ significantly (SNK, $P>0.05$).

4. Discussion

The availability of nutrient-rich saline ground water in the polders of Bourgneuf Bay has allowed low-cost production of microalgae and diversification of shellfish cultures (Robert, 1990). For more than a decade, the diatom *S. costatum* has been successfully produced year-round in outdoor ponds and used in intensive rearing systems (Sauriau and Baud, 1994; Sauriau et al., 1997). This microalga supports good bivalve growth, especially for oysters (Walne, 1970; Rodhouse et al., 1983; Enright et al., 1986). However, in the context of a new project to achieve adult oyster fattening within 2 months (Baud et al., 1995; Méléder et al., 2001), diversification of the food source is being considered.

Our study shows that two of the diatoms tested, *N. acicularis* and *N. gandersheimiensis*, have suitable characteristics for commercial exploitation. These two species are found year-round in neighbouring coastal waters and grow very well in 500-l cylinders filled with ground water diluted 1:3 with seawater (Pales-Espinosa, 1999). Both developed dense cultures throughout the water column when bubbling was applied. Moreover, based on the short-term physiological responses of Pacific oysters, these two diatoms showed an estimated net energy balance (scope for growth) comparable to that obtained with *S. costatum* as reference. To our knowledge, this report is the first to suggest that these microalgae are suitable for bivalve aquaculture. Pelagic, euryhaline *N. acicularis* (Van der Werff and Huls, 1976) has been found in gut contents of flat oysters feeding on seston in an estuarine area (Paulmier, 1972). However, no information is available for *N. gandersheimiensis*. Lange-Bertalot and Simonsen (1978) noted the high degree of morphologic plasticity of this euryhaline diatom (for which they found 16 different designations). It would appear that the same species, described by Nicotri (1977) as *Nitzschia frustulum* var. *perminuta*, was less efficiently digested than other benthic diatoms by intertidal gastropods.

A notable result in our study was the variability of feeding responses of adult Pacific oysters relative to different species of algae delivered by a flow-through system under similar conditions. This phenomenon was observed by Tenore and Dunstan (1973) with *Crassostrea virginica* fed four species of phytoplankton, including *S. costatum* and *N. closterium*. In our study, a first difference was observed for clearance rates (CRs), as the value of 7.3 l h^{-1} g^{-1} for *N. gandersheimiensis* was almost twice that obtained with *N. acicularis* and *N. closterium*. This variability cannot be explained by size differences because all species except *N. closterium* were fully retained by oyster gills. Even for *N. closterium*, CR calculations were based on counts of cells larger than 6 μm (ESD), so that all CR data were comparable in terms of pumping rates. Moreover, microalgae can excrete organic substances, which may depress bivalve CR (Ward and Targett, 1989). However, CRs were not particularly low in our study with respect to the reference value of 4.8 l h^{-1} g^{-1} provided by the model of Bougrier et al. (1995) for a temperature of 20 °C. The reasons for this variability remain unclear, as in the study of Newell et al. (1989) concerning *Mytilus edulis*, which showed variable CRs for fluorescent versus nonfluorescent particles of the same size. Differences among microalgae were also observed in our study for pseudofaeces production relative to the filtration rate. This was clearly apparent for *S. costatum* and *N. acicularis*, which were rejected less in pseudofaeces than *N. closterium* in proportion to the amounts filtered. Similarly, Tenore and Dunstan (1973) found that *N. closterium* gave the highest rate of biodeposition and the second lowest feeding rate among four microalgae tested. In our study, this species was digested with the least efficiency, which suggests that the strain isolated in coastal waters of Bourgneuf Bay is unsuitable for adult oyster aquaculture. However, this diatom has been frequently described in the gut content of bivalves (Leroux, 1956; Paulmier, 1972; Newell et al., 1989), and the early work of Loosanoff and Engle (1947) does not indicate that it is particularly unfavourable for oysters. The rapid formation of clusters of several cells in our cultures (see Materials and methods) may account for our poor results with this species, even though these clusters were subsequently separated in the flow-through experimental trays.

This study determined energy budgets on the basis of short-term laboratory experiments as a tool for testing microalgae that could eventually be substituted for *S. costatum*. Net energy balance or scope for growth can be used to predict long-term growth performance in bivalves (Beiras et al., 1993, 1994). The range of mean NEB variations for the three species, *N. acicularis*, *N. gandersheimiensis* and *S. costatum*, ranging from 163 to 282 J h^{-1} g^{-1}, is higher than the range found by Soletchnik et al. (1997), i.e. 110–170 J h^{-1}, for post-spawned Pacific oysters with a mean dry weight of around 2.5 g. However, our NEB values are comparable to those reported by Beiras et al. (1994) for the post-metamorphic oyster *Ostrea edulis*, reared at 20 °C with a ration of 4 mg POM l^{-1}. Nonetheless, our objective was not to use NEB values to extrapolate long-term growth. The temperature of 20 °C chosen to allow detection of differences among physiological responses would certainly be lower in industrial applications (Bacher and Baud, 1992). In fact, our approach focused on two microalgae suitable for further experiments. The growth performance of *N. acicularis* and *N. gandersheimiensis* will now be tested in 50-m^3 outdoor tanks filled with ground water, which oyster producers currently use for *S. costatum*. The potential of microalgae for oyster growth will be studied during 1- or 2-month experiments under economically optimised conditions. These short-term phyto- and zootechnical investigations, required to

validate the choice of a substitute microalga, will be carried out prior to detailed studies of algal nutritional value, as reported for *S. costatum* (Piveteau et al., 1999, 2000).

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