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Phytoplankton composition and selective feeding of the pearl oyster *Pinctada margaritifera* in the Takapoto lagoon (Tuamotu Archipelago, French Polynesia): *in situ* study using optical microscopy and HPLC pigment analysis

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ABSTRACT: The *in situ* diet of the pearl oyster *Pinctada margaritifera* was determined in the lagoon of Takapoto Atoll by comparing the phytoplankton composition of water and bivalve gut contents using 2 different methods, optical microscopy and HPLC pigment analysis. In order to evaluate the available food resources for pearl oysters in the water column, a new method for estimating the pigment/chlorophyll *a* (chl *a*) ratio (based on an inverse analysis) was developed which allowed us to determine the contribution of the main phytoplanktonic groups in terms of chl *a*. In the water, picocyanobacteria and nanoflagellates predominated, the latter being mainly chlorophytes and prymnesiophytes. Comparisons between the results obtained by the 2 methods of investigation indicated that most of the dinoflagellates are unpigmented and, therefore, heterotrophic. An examination of the gut contents showed that picocyanobacteria were only weakly ingested by the oyster and, thus, nanoflagellates constituted the main food resource. Cryptophytes, although poorly represented in the water, were preferentially ingested. Chlorophytes were inefficiently digested since they were found alive and motile in the faeces of the oyster. The ecological implications of this feeding behaviour are discussed.

KEY WORDS: Pearl oyster *Pinctada margaritifera* · Selective feeding · Natural diet · Phytoplankton pigments · Coral reefs · French Polynesia

INTRODUCTION

The question of how much food must be available to sustain a given population is frequently raised in the mariculture of filter-feeders. Food resources are generally considered as a limiting factor required for achieving high density rearings (Grant 1996, Rheault & Rice 1996). Many studies have been carried out on bivalve shellfish in temperate areas and used to establish predictive models of trophic capacity for rearing zones (e.g. Bacher 1989, Raillard & Menesguen 1994, Campbell & Newell 1998, Grant & Bacher 1998). Moreover, the impact of mariculture on modifications of the ecosystems has already been investigated and concerned either the nutrient cycling (Kautsky & Evans 1987, Dame 1993, Herman 1993, Smaal & Prins 1993) or the grazing pressure (Gerritsen et al. 1994, Baker et al. 1998, Makarewicz et al. 1999). In contrast, few studies have been undertaken in tropical environments (Hawkins et al. 1998).

Even though the production of black pearls constitutes the main economic resource of French Polynesia, the mariculture of the mother-of-pearl *Pinctada margaritifera* (Linné, 1758) has been, for a long time, based on an empirical knowledge of this bivalve and its natural environment. The mortality, which occurred in 1985 and affected pearl oysters rearings in the Taka-

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poto lagoon, demonstrated the lack of precise knowledge on this bivalve. It induced the development of a multidisciplinary research program on pearl oyster (Programme Général de Recherche sur la Nacre [PGRN]). Assessing the carrying capacity of the lagoon for pearl oyster farming implies determining the food resource available to the oysters. It is generally assumed that filter-feeding species rely on phytoplankton as their main source of energy, but only a few studies have been concerned specifically with the feeding behaviour of the pearl oyster (Chellam 1983, Nasr 1984, Hawkins et al. 1998, Yukihira et al. 1998a,b, 1999, Pouvreau et al. 1999, 2000a). Because these benthic bivalves are reared in a suspended position they interact with the planktonic food web. In the oligotrophic environment of the atoll lagoons, the structure of the planktonic food web is characterized by a predominance of pico- and nanophytoplankton (Charpy 1996, Niquil et al. 1998); the former being weakly retained by the pearl oyster (Pouvreau et al. 1999, Yukihira et al. 1999). This apparently unfavourable trophic environment currently supports high densities of vigorously growing farm-reared oysters (Pouvreau et al. 2000b). Several mechanisms may explain this paradox, e.g. a high clearance rate. However, it implies an efficient exploitation of the trophic resource.

The objectives of the present study were: (1) to qualitatively and quantitatively characterize the planktonic community in the water column, and (2) to determine the *in situ* feeding behaviour of *Pinctada margaritifera* by comparing the composition of suspended particles in the lagoonal water and that of the gut contents of reared animals. Two different methods were used; optical microscopy and HPLC pigment analysis.

MATERIAL AND METHODS

Study sites. Takapoto Atoll (14° 30' S, 145° 20' W) is located in the Tuamotu Archipelago, in the north of French Polynesia (Fig. 1). Since 1977, this lagoon has been the most frequently used site for studies concerning the pearl oyster, firstly during the UNESCO Man And Biosphere program (Salvat 1976) and later as a part of PGRN. The lagoon has a surface area of 81 km^2 and an average depth of 25 m (Zanini 1997). The main feature of this atoll is the absence of a pass, which restricts water flow between the lagoon and the ocean. During high swells and/or strong winds, oceanic waters can enter the lagoon through reef-flat spillways which are mostly situated on the eastern part of the atoll. The residence time of water in the lagoon was estimated to be 4.2 yr (Sournia & Ricard 1976). Prevailing winds come from the east and are considered to be the main forcing factor within the lagoon since tides are negligible (Rougerie 1979).

Three sampling sites representing different environmental conditions were chosen (Fig. 1). The location of the sites depended on logistic constraints (e.g. sampling at nighttime a short distance from the laboratory). Stn 1 was located in the south part of the lagoon. It was investigated during the first phase of PGRN (Charpy et al. 1992) and was the reference site for the experiments on the ecophysiology of the pearl oyster (Pouvreau et al. 2000a). Because Stn 1 was located in a somewhat confined part of the lagoon—due to the existence of an east-to-west submerged coral structure—Stn 2 was located further north, in the eastern part of the lagoon. Stn 2 had also been sampled during the first part of PGRN (Charpy 1996) and was simultaneously sampled in a companion study on the planktonic food-web (Sakka et al. 1999). Stn 3 was situated approximately on the central axis of the lagoon, near pearl oysters rearings.

Sampling procedure. Sampling was performed from 17 to 24 April 1997. Water was sampled every 3 h during 24 h from 06:00 h. The gut contents were collected every 6 h. Water samples were collected at 7 m depth (i.e. same depth as oysters in our experiment) using an electric pump. For HPLC analysis, triplicates of 2 l water samples were filtered firstly on a 200 µm nylon net to discard large zooplankton, then through glass-fibre filters Whatman GF/F (nominal porosity 0.7 µm, 25 mm Ø). The filters were kept frozen in liquid nitrogen pending HPLC analysis. For microscopic analysis, triplicates of 250 ml water samples were preserved by

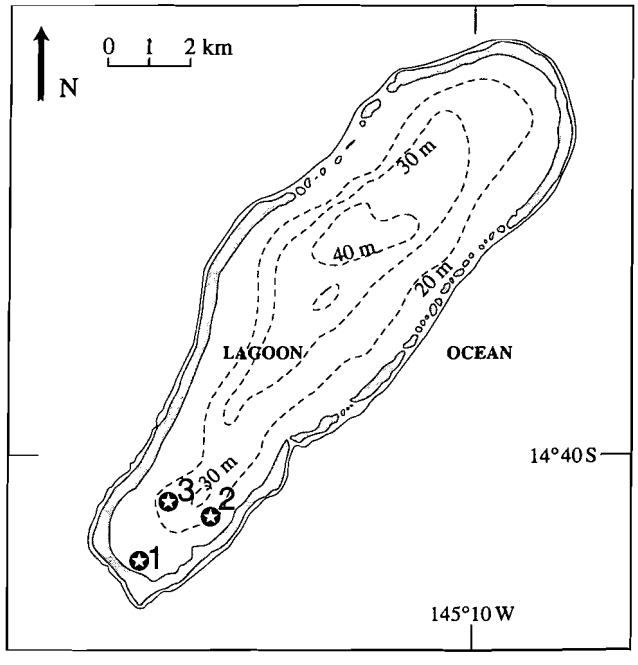

Fig. 1. Map of the Takapoto Atoll with the 3 sampling sites (Stns 1, 2, 3)

the addition of formaldehyde (final concentration 3.7%) and kept in the dark pending enumeration under the optical microscope.

Pearl oysters originated from rearings of the Service des Ressources Marines (SRM). They were suspended on ropes at 7 m depth (i.e. the usual depth for reared pearl oysters) and thus kept in their natural water environment. In order to analyse digested food separately from recently ingested food, the digestive tracts of the bivalve were dissected into 2 parts. The proximal part of the gut was considered as the portion of the digestive tract between oesophagus and stomach, whereas the distal part corresponded to the intestine between the digestive gland and the anus. The material collected in 1 gut of bivalve did not contain enough pigment material to be detected and analysed with the HPLC technique. Consequently, the distal and proximal parts of the gut contents of 4 oysters were pooled by suspension in known volumes of GF/F filtered seawater and kept frozen in liquid nitrogen.

Microscope analysis. Water and gut samples were left to settle for 24 h in a 100 ml and a 5 ml sedimentation chamber (Hydro-Bios combined plate chambers), respectively. Enumeration was achieved according to Utermöhl (1958), using an inverted microscope Olympus IMT at magnifications of 100× and 400×. As epifluorescence was not available, picoplankton were not enumerated. The phytoplankton cells numbers were determined for the major taxonomic groups. Additionally, the cell size (µm) was determined on 100 randomly selected cells in both water and gut content samples. For each taxonomic group, cell counts were expressed as percent of total enumerated cells.

Pigment analysis by HPLC. The pigments were extracted in 100% methanol for 1 h at 4°C in the dark, sonicated for 30 s (Wright et al. 1991), then centrifugated at 1000 × *g* for 10 min. The marker pigments of phytoplankton for the different algal groups were analysed by HPLC using a method described by Kraay et al. (1992) which allows effective separation of lutein and zeaxanthin. The system consisted of 3 Kontron HPLC Pumps (series 400) monitored by a Kontron computer and 2 detectors in series. Absorption measurements were performed using a photodiode array detector (Kontron, model 440) at 440 nm. The fluorescence of chlorophylls and phaeopigments were measured using a spectrofluorimetric detector (Kontron Analytical SFM 25) with an excitation wavelength of 430 nm and a 663 nm cut-off secondary filter for emission. The pigments were analysed by reverse-phase HPLC using a BIO-RAD Bio-Sil C18 HL 90-5 S (150 mm long, 4.6 mm Ø and 5 µm particle size) column and HPLC-grade solvents.

Some standards were obtained commercially: chlorophylls (chl) *a* and *b*, β,β-carotene (Sigma Chemical Company) and 19'-hexanoyloxyfucoxanthin from VKI (Water Quality Institute, Denmark). Lutein and zeaxanthin were kindly provided by Hoffman LaRoche Co. (Basel, Switzerland). Other pigments were obtained from monospecific algal cultures and then purified according to Pastoureaud et al. (1996). These standards were transferred to the adequate solvent and measured on a Shimadzu UV-160 spectrophotometer, using specific absorption coefficients as indicated by Bidigare (1991).

HPLC data analysis. ***Water column:*** There is already abundant literature about the contributions of the major phytoplanktonic groups in terms of chl *a* determined using the marker concentrations. Our study aimed to accurately determine the value of the pigment ratio in order to link the concentration of 1 pigment to the corresponding chl *a* concentration. An earlier method by Gieskes et al. (1988) used a multiple regression analysis to infer the values of the pigment ratios from the measured dataset. A new program for estimating class abundances from chemical markers, so-called CHEMTAX, was developed by Mackey et al. (1996) and applied by Wright et al. (1996) and Meyer-Harms et al. (1999). But this method was not applicable to our dataset because it requires a previous knowledge of the pigment ratios of the local populations. In contrast, the technique proposed by Goericke & Montoya (1998) was based on the use of ratios derived from the literature. However, this approach lacked an objective statistical analysis. Letelier et al. (1993) considered that not all species had a unique specific marker pigment, but these authors insufficently explicited their use of the inverse method to solve this problem. Using the Letelier et al. (1993) statements, we developed a new method to determine the pigment ratios.

If a given marker pigment M*i* could be assigned to a specific algal group (Table 1), the relationship between chl *a* and the *n* concentrations of the detected marker pigments M*i* would be written as follows:

$$\text{chl } a = x_1[\text{M1}] + x_2[\text{M2}] + x_3[\text{M3}] + \ldots x_n[\text{M}n] \quad (1)$$

where x_i represent the chl *a*:M*i* ratio for a given algal group *i*.

Table 1. Main pigments, used as taxonomic markers, and size of the corresponding algal groups. Zeaxanthin (zea), Fucoxanthin (fuco), chlorophyll *b* (chl *b*), Peridinin (per), Alloxanthin (allo), 19'-hexanoyloxyfucoxanthin (19'hf)

Phytoplankton group	Marker pigment	Size (µm)
Cyanophytes	Zea	<1
Diatoms	Fuco	>20
Chlorophytes	Chl *b*	1–40
Dinoflagellates	Per	>10
Cryptophytes	Allo	5–10
Prymnesiophytes	19'hf	5–20

This equation did not take into account the fact that 2 markers are each contained in 2 different groups of algae. Diatoms and prymnesiophytes contain fucoxanthin and cyanobacteria and chlorophytes contain zeaxanthin. Thus, for the 6 marker pigments detected in Takapoto lagoon, Eq. (1) became

$$\text{chl } a = x_1[\text{allo}] + x_2[\text{perid}] + x_3[19'\text{hf}] + x_4([\text{zea}] - x_5[\text{chl } b]) + x_6[\text{chl } b] + x_7([\text{fuco}] - x_8[19'\text{hf}]) \quad (2)$$

where the contribution of chlorophytes to the zeaxanthin concentration (x_5 = zea/chl *b*) are subtracted, as well as the contribution of prymnesiophytes to the fucoxanthin concentration (x_8 = fuco/19'hf).

The model described by Eq. (2) is non-linear and consequently it was impossible to find a solution using a multiple-linear regression. Besides, as some explanatory variables are repeated with different parameters, (e.g. [chl *b*] and [19'hf]), Eq. (2) provided an infinity of solutions. Additional criteria allowed the interval of acceptable solutions to be restrained. Firstly, all the contributions, including the terms [zea] – x_5[chl *b*] and [fuco] – x_8[19'hf], should be positive. Secondly, the previous knowledge on the range of pigment ratios (Table 2) was used as inequality constraints. We explored the space of the solutions through a simple, empirical and iterative computation. Considering that x_8 is accurately known due to the narrow range reported in the literature (0.03 to 0.05, Table 2), the redundancy of the parameters could be overcome by considering *a priori* values of x_5. Since the only information on x_5 came from the known range of values (Table 2), we drew 100 values of x_5 at random. For each value of x_5, Eq. (2) became linear and could be solved with the use of a least-square algorithm in order to minimise differences between chl *a* values observed and calculated (linear regression with constraints). This method was applied to the 72 data of chl *a* obtained at the 3 sampling sites. It therefore provided 100 sets of parameters x_i, which were acceptable solutions to Eq. (2) from which we computed the mean and standard deviations of each pigment ratio. The pigment ratios were used to calculate the contributions of algal groups in terms of chl *a* in the water column.

Table 2. Literature ranges of chl *a* to marker pigment ratios used to determine the different amounts of the algal classes contributing to total chl *a*. See Table 1 legend for marker pigment abbreviations

Equation parameter	Pigment ratio	Literature range	Source
x_1	Chl *a*/allo	1.8–4.2	a, b, c
x_2	Chl *a*/per	1.4–2.8	b, d, e, f
x_3	Chl *a*/19'hf	0.7–3.2	g, h, i, j
x_4	Chl *a*/zea	0.5–2.1	a, k, l
x_6	Chl *a*/chl *b*	1.5–2.7	m, n
x_7	Chl *a*/fuco	1.3–2.3	h, o, p
x_8	Fuco/19'hf	0.03–0.05	i, j, l

(a) Hager & Stransky (1970), (b) Burkill et al. (1987), (c) Claustre et al. (1994), (d) Vesk & Jeffrey (1977), (e) Bjornland & Tangen (1979), (f) Jeffrey et al. (1975), (g) Wright & Jeffrey (1987), (h) Bidigare (1989), (i) Buma et al. (1991), (j) Vaulot et al. (1994), (k) Kana et al. (1988), (l) Letelier et al. (1993), (m) Hooks et al. (1988), (n) Simon et al. (1994), (o) Bustillos-Guzmàn et al. (1995) and (p) Tester et al. (1995)

Gut contents: At the 3 sites, the proportions of each algal group in the water and the gut contents were determined every 6 h throughout the 24 h survey. During each sampling, we compared the proportions in the water and in the gut contents (chi-square test); assuming that each marker pigment was specific to 1 algal group (Table 1). Because chl *b* co-eluted with a chl *c*-like, the lutein was used instead of chl *b* to evaluate the contribution of chlorophytes. The method based on the chl *a* pigment ratios developed for the water column could not be applied to the gut contents because of the scarcity of informations on the chl *a*: lutein ratio. Consequently, the proportions of the main phytoplanktonic groups were calculated as percentages of the total marker carotenoids. The same calculation was applied to water samples for comparison with the gut contents.

RESULTS

Lagoonal water composition

The HPLC analysis provided the elution pattern of chlorophylls and carotenoid pigments as illustrated by the absorbance chromatogram presented in Fig. 2. The major pigments were chls *a* and *b*, chl c_{1+2}, 19'-hexanoyloxyfucoxanthin (19'hf), zeaxanthin and β,β-carotene. In contrast, peridinin, alloxanthin and fucoxanthin showed minor peaks. It is important to emphasise that we considered that the 19'hf was the exclusive marker of the prymnesiophytes in the Takapoto lagoon. In fact, several types of pigment composition were identified in prymnesiophytes (Jeffrey & Wright 1994). In our samples, the presence of 19'hf and chl c_3, the absence of 19'bf and the low amount of fucoxanthin clearly indicated that the prymnesiophytes of Takapoto lagoon belonged mainly to the 19'hf-chl c_3 group.

The contributions of the main phytoplanktonic groups (Fig. 3) were calculated from the estimations of pigment ratios evaluated by the data analysis method (Table 3). Stn 1 was dominated by cyanobacteria, which represented more than 55% of total chl *a*. At this station, the second major phytoplanktonic class was chlorophytes which contributed 15% of the total chl *a*.

Table 3. Mean and standard deviation (SD) of the pigment ratio values estimated by our model and used to determine the contributions of the main phytoplanktonic groups in the water column. See Table 1 legend for marker pigment abbreviations

Equation parameter	Pigment ratio	Mean	SD
x_1	Chl *a*/allo	4.20	0.15
x_2	Chl *a*/per	1.40	0.09
x_3	Chl *a*/19'hf	1.28	0.05
x_4	Chl *a*/zea	1.38	0.03
x_5	Zea/chl *b*	0.20	0.09
x_6	Chl *a*/chl *b*	1.53	0.06
x_7	Chl *a*/fuco	1.30	0.12
x_8	Fuco/19'hf	0.03	0.001

However, Stns 2 and 3 were characterized by 3 main algal classes (prymnesiophytes, cyanobacteria and chlorophytes), whose contributions were respectively estimated to 40, 25 and 20% of total chl *a*.

Microscopic analyses (Fig. 4) showed that chlorophytes and dinoflagellates were predominant in lagoon water. Among chlorophytes, *Chlamydomonas* sp., ca 10 to 12 µm Ø, was the most frequently identified genus—whereas dinoflagellates were represented by *Gymnodinium* sp. and *Prorocentrum* sp., whose size equalled 18 and 25 µm, respectively. Small pennate diatoms and coccolithophorids were less frequently observed. Chlorophytes made the bulk of nanophytoplankton at Stn 1, whereas dinoflagellates were predominant at Stns 2 and 3. No nycthemeral variation could be outlined using either microscopy or HPLC methods.

Composition of the gut contents

The absorbance chromatogram (Fig. 2) showed the diversity of pigments detected using HPLC. All the marker pigments of the algal groups encountered in the water were present in the guts. In addition, 2 pigments which were absent in the water column were detected: the diatoxanthin, a carotenoid involved with diadinoxanthin in photoprotection processes, and a pigment, identified as a chl *c*-like, which coeluted with chl *b*. Its absorption spectrum (444-582-630.5) showed maxima very near from those of chl c_2. This pigment was present mainly in the distal part of the gut where its concentration, calculated using the chl c_2 coefficient, corresponded to 1–5% of the total pigments. The degradation forms of either chlorophylls (both phaeophorbides and phaeophytins) or carotenoids represented a very small part of the detected pigments. This was also true in the distal part of the guts where significant amounts of native chl *a* were encountered.

The proximal part of the guts was characterized by high amounts of alloxanthin, 19'hf and chls *a* and *b*. Three major pigments—19'hf, alloxanthin and chl *b*—were detected in the proximal part of the gut at Stns 2 and 3 (Fig. 5), whereas the alloxanthin was the major carotenoid detected at Stn 1 (20 to 90% of the total biomarkers). The number of marker pigments detected in the distal part of the guts was higher than in the proximal one (Fig. 5), although the food had already been subjected to the digestive processes.

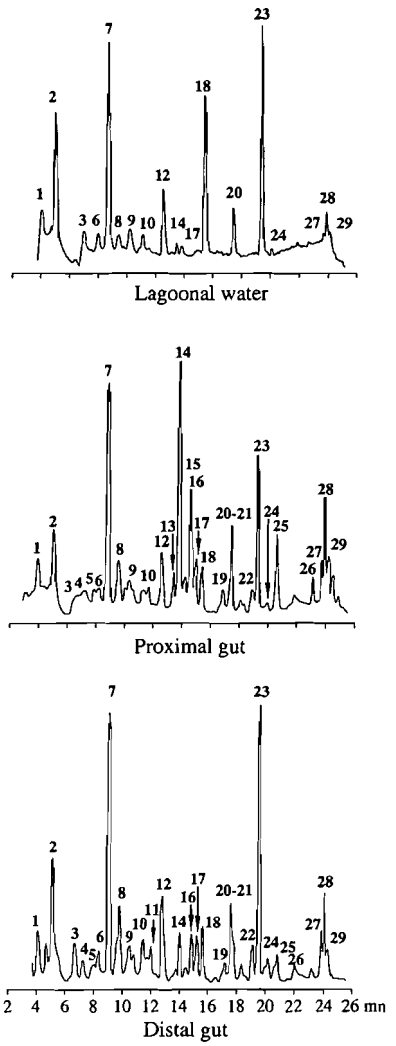

Fig. 2. Absorbance chromatograms of phytoplankton pigments at Stn 3 in (a) water, (b) in the proximal and (c) distal parts of the gut of *Pinctada margaritifera*. Pigment identifications: (1) chl c_3; (2) chl c_2; (3) peridinin; (4) siphonaxanthin; (5) chl *c*; (6) fucoxanthin; (7) 19'hexanoyloxyfucoxanthin; (8) 9'-cis-neoxanthin; (9) prasinoxanthin; (10) violaxanthin; (11) phaeophorbid *a*-like; (12) diadinoxanthin; (13) antheraxanthin; (14) alloxanthin; (15) monadoxanthin; (16) diatoxanthin; (17) lutein; (18) zeaxanthin; (19) chl *b*-like; (20) chl *b*; (21) chl *c*-like; (22) chl *a* allomer; (23) chl *a*; (24) and (25) chl *a* epimers; (26) phaeophytin *a*; (27) β,α-carotene; (28) β,β-carotene; (29) β,ψ-carotene

The predominant food items identified during microscopic examination of the gut contents are summarized in Table 4. Six algal groups ranging in size from 6 to 50 µm were encountered in the gut of oysters; consisting mainly of chlorophytes at Stn 1 and dinoflagellates at Stns 2 and 3 (Fig. 4). *Pinctada margaritifera* also ingested zooplankton, e.g. bivalve larvae, invertebrate eggs, small copepods and protozoans.

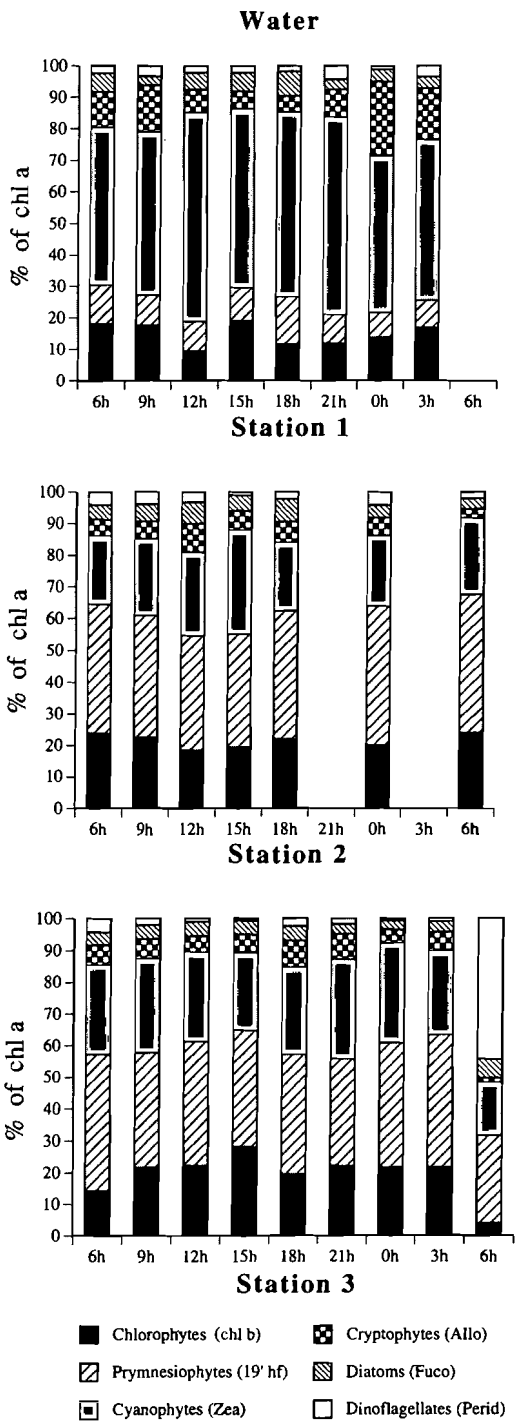

Fig. 3. Contribution of the main phytoplanktonic groups in the lagoonal water at each sampling station estimated by HPLC pigment analysis and expressed in percent of total chl *a*

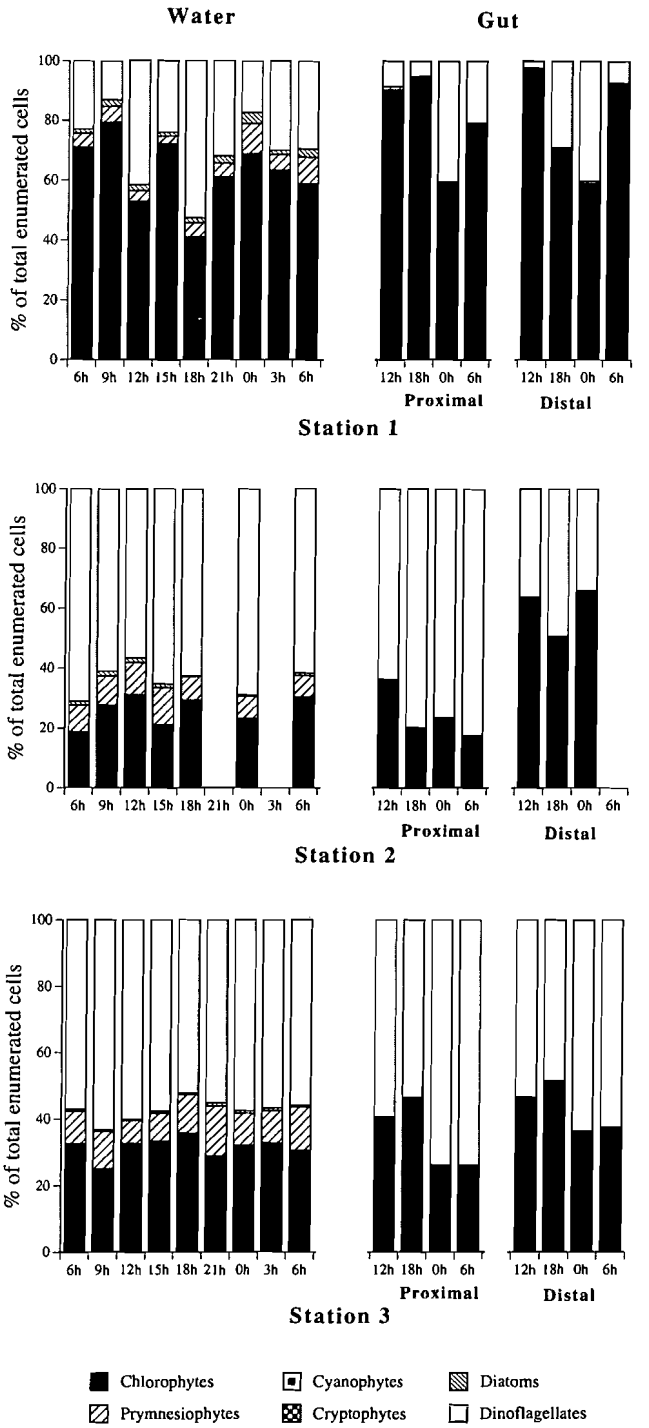

Fig. 4. Contribution of the main phytoplanktonic groups in the lagoonal water and in the proximal and distal parts of the gut at each sampling station using optical microscope and expressed in percent of the total enumerated cells. Picoplankton and small nanoflagellates were not taken into account

Table 4. Main phytoplanktonic species (with their size) identified in the gut contents of the pearl oysters *Pinctada margaritifera* in the Takapoto lagoon

Algal group	Dominant phytoplankton species	Cell diameter (µm)
Chlorophytes	*Chlamydomonas* sp.	10–12
Prasinophytes	*Nephroselmis* sp.	6–8
Prymnesiophytes	*Cricosphaera* sp.	18
Cryptophytes	*Leucocryptos* sp.	10
Diatoms	*Navicula* sp.	30
	Nitzschia sp.	25
Dinoflagellates	*Gymnodinium* sp.	18
	Oxytoxum sp.	14
	Prorocentrum scalpellum	45–50
	Prorocentrum sp.	35

Comparisons between the gut contents and the water

The comparison of the proportions of the marker pigments between the water and the proximal part of the gut contents (Fig. 5) showed significant differences ($p < 0.05$). For each marker pigment, the ratio between the proportions estimated in the water and in the guts was calculated (Table 5). The values of the ratio ranged from 0 to 79.7. The highest value was obtained for alloxanthin. It showed evidence of a high cryptophyte accumulation in the proximal part of the gut contents, regardless which station and which sampling hour was considered. On the contrary, the ratios of the other marker pigments were low, except for peridinin at Stn 3.

The comparison between the proportions of the algal groups determined by the microscope did not show significant differences between water and gut contents. The composition of the gut contents appeared quite similar to that of the water column (i.e. dominance of chlorophytes or dinoflagellates). Diatoms and prymnesiophytes which were observed as minor groups in the water were not identified in the gut contents.

DISCUSSION

Two methodological approaches were used to assess the phytoplankton composition and abundance in the water and in the gut contents of the pearl oyster. Although the advantages and limits of microscope and HPLC methods have already been widely discussed (e.g. Wilhelm et al. 1991, Schlüter & Havskum 1997), these methods were sucessfully used to investigate the ingestion rate of the copepod *Acartia bifilosa* (Meyer-Harms & von Bodungen 1997). The present study reports the first *in situ* study on the qualitative composition of the diet of a filter-feeding bivalve using the combined approach of HPLC pigment analysis and microscopy. As expected, microscopic examination and HPLC analysis did not provide identical results.

The HPLC method identified and quantified the nano- and picophytoplanktonic organisms which dominated the autotrophic community in the Takapoto lagoon. As most of the cyanobacteria were ca 1 µm ∅ (Charpy & Blanchot 1998), they were not detected under the optical microscope without fluorescence.

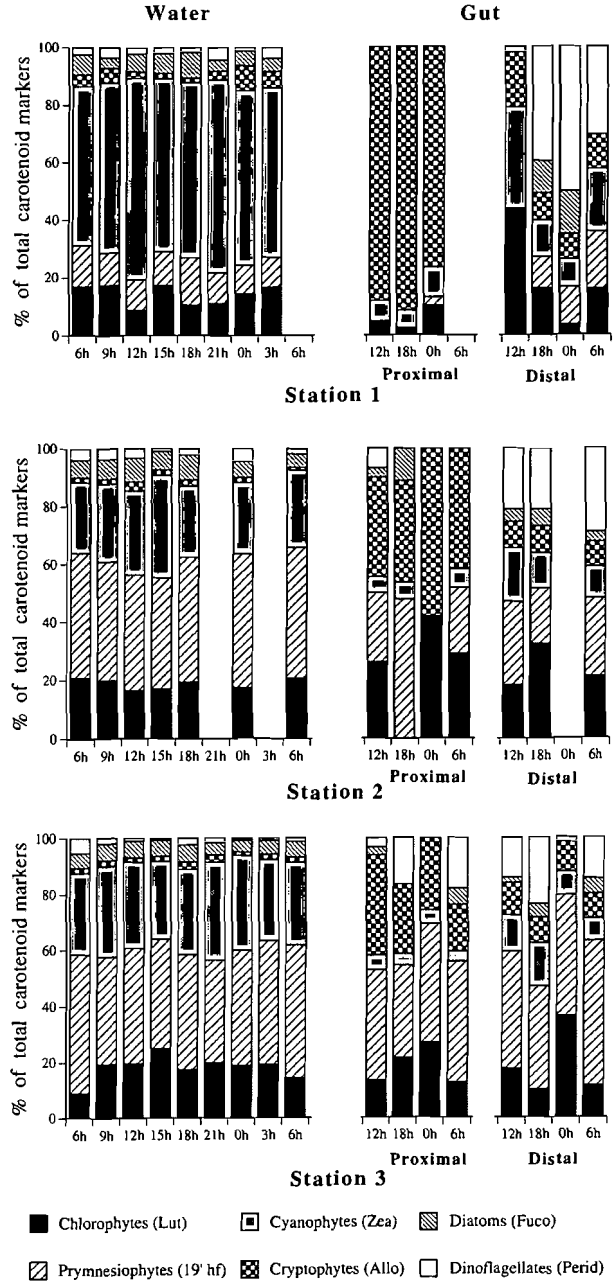

Fig. 5. Contribution of the main phytoplanktonic groups in the lagoonal water and in the proximal and distal parts of the gut at each sampling station estimated by HPLC pigment analysis and expressed in percent of total carotenoid markers

Table 5. Ratios between the proximal part of the gut and the water column for the main marker pigments at the 3 sampled stations at different sampling times. Lutein (lut), see Table 1 for other marker pigment abbreviations

	Stn 1				Stn 2				Stn 3			
	12 h	18 h	0 h	6 h	12 h	18 h	0 h	6 h	12 h	18 h	0 h	6 h
Per	0.0	0.0	4.6	–	1.8	0.0	–	0.0	1.6	21.5	0.0	22.0
Fuco	0.0	0.0	0.0	–	0.5	1.7	–	0.0	0.4	0.0	0.0	1.1
19'hf	0.0	0.0	13.8	–	0.6	1.2	–	0.5	1.0	0.7	1.1	1.0
Allo	16.2	45.6	79.7	–	19.8	17.2	–	40.7	17.9	11.6	9.0	8.6
Lut	0.3	0.1	2.6	–	1.3	0.0	–	1.3	0.7	0.7	1.2	0.7
Zea	0.1	0.1	0.8	–	0.2	0.2	–	0.2	0.2	0.1	0.1	0.1

Similarly, the nanoflagellates, which constituted a major phytoplanktonic community in the pearl oysters diet, were mostly detected by the pigment analysis. Formaldehyde preservation often damages these small and fragile algae (Gieskes & Kraay 1983). In particular, the algae lacking a theca, or a thick cell wall (e.g. cryptophytes or chromulins) can be deformed and thus escape microscopic enumeration. The microscope detection and identification of ingested algae are hindered in the gut of the oysters as they are mixed with detritus, and, are partly degraded. Considering the digestive process, even if the ingested material is partially digested, we assume that the pigment degradation is minimal in the proximal part of the guts.

The HPLC-pigment analysis seems to be a suitable method to obtain a realistic quantitative composition of either lagoonal phytoplankton or gut contents. However, it implies that the amount of a detected pigment precisely reflects the contribution of the corresponding algal class to the total biomass. As already mentioned ('Material and methods'), various techniques have been developed in order to link pigments concentrations to the relative abundance of an algal class. However, none of them have proved to be completely satisfactory. We do not claim that the model that we developed for the water samples is superior to previously formulated models, especially because our model likewise assumed that the pigment ratios were little varying at all the sampling sites during the nycthemeral cycle. A confirmation of this assumption would require further studies involving cultures of algal strains isolated from different places in the lagoon. Our model, however, differed in the way that the estimated pigment ratios were based on a statistical analysis of the pigment dataset.

As mentioned, the HPLC method presents several advantages. Nevertheless, the present study demonstrated the interest of the microscopic method in such a lagoonal environment. For example, in water samples, the dinoflagellates were only detected using a microscope — whereas their specific marker, the peridinin — is one of the minor peaks on the HPLC chromatograms. This discrepancy indicates that, in the Takapoto lagoon, most of the dinoflagellates were heterotrophic. This observation was confirmed by Sakka et al. (1999) in a companion study. The absence of pigmentation and the lack of peridinin are known to characterize a great number of dinoflagellate species (Hallegraeff & Jeffrey 1984), and this heterotrophy has been underlined in reef environments (Sournia & Ricard 1976). Abundant literature is available on the trophic status of the dinoflagellates (Gaines & Taylor 1984, Lessard & Swift 1985, Gaines & Elbrächter 1987, Stoecker et al. 1997). They are particularly concerned with the dinoflagellates *Gymnodinium* and *Prorocentrum*, which were the predominant genus identified in the water column and are known to contain auto-, mixo- and heterotrophic species. From the above observations, it can be concluded that both methods are necessary in order to completely describe the planktonic community in the Takapoto lagoon: The HPLC method showed the predominance of small forms (picocyanobacteria and nanophytoflagellates) among the autotrophic communities and the microscopy confirmed the importance of heterotrophic dinoflagellates presence.

Analysis of the water samples indicated that the mother-of-pearls *Pinctada margaritifera* were open to a choice in different food items, according to their location in the lagoon. The picocyanobacteria predominated at Stn 1. Those algae <1 µm were still present (ca 20%) but a complex of nanoflagellates (chlorophytes and prymnesiophytes) and microplankton (dinoflagellates) were predominant at Stns 2 and 3. The quantitative importance of picocyanobacteria in Takapoto lagoon (and particularly *Synechococcus* sp.) has been evidenced from fractionated carbon, chlorophyll and primary production measurements (Charpy et al. 1992, Charpy & Blanchot 1996, 1998). At the time of our study, this dominance applied only in the south part of the lagoon. A small chlorophyte *Chlamydomonas* sp. predominated in the >3 µm fraction at Stn 1 and was also reported from the Takapoto lagoon in a companion study (Sakka et al. 1999). However, chlorophytes were still present at Stns 2 and 3 but in lower proportions compared with prymnesiophytes. Small phytoflagellates had not been recorded in the waters of the Takapoto lagoon since Sournia & Ricard's (1976) pioneer studies, which mainly focused on diatoms and dinoflagellates. Due to the limited examination of phytoplankton during more than 20 yr, it cannot be stated if those drastic changes in the phytoplankton composition reflect a temporal evolution, or if they could be attributed to more sophisticated investigation methods. The high abundance of prymnesiophytes has not been pre-

viously reported. Toxic species of this class with a similar pigment signature are frequently involved in algal blooms (Jeffrey & Wright 1994).

A spatial homogeneity in the composition of the phytoplanktonic communities was observed at Stns 2 and 3 (Fig. 3) and is in agreement with the lack of spatial variability mentioned by Buestel & Pouvreau (2000) at the lagoon scale. The differences observed in the composition of these communities at Stn 1 could be explained by the influence of anthropic outputs from the village, located in the south part of the atoll (Charpy et al. 1997). No significant differences (ANOVA, $\alpha = 5\%$) in the phytoplanktonic composition of the water column and of the gut contents were observed during the nycthemeral cycles. As a consequence, the temporal variability of the phytoplankton composition in the lagoon was low. It does not corroborate to the observations carried out in the lagoon of atolls which showed an increase in the autotrophic biomass and production at the end of the day (Charpy 1996, Buestel & Pouvreau 2000).

Pearl oysters poorly ingest picocyanobacteria as the content of zeaxanthin in the proximal part of the gut (Fig. 5) is low and may, partly, be attributed to chlorophytes. The low abundance of cyanobacteria in the gut of the bivalve could be related to the low retention of particles <2 µm demonstrated by the previous studies of Pouvreau et al. (1999) and Yukihira et al. (1999). This low retention is attributed to the absence of eulatero-frontal cirri on the gill filaments. However the indirect ingestion of the picoplanktonic biomass can be mediated by heterotrophic protists which then represent a trophic link between cyanobacteria and suspension-feeders (Le Gall et al. 1997, Dupuy et al. 1999).

In the oyster gut, the HPLC analysis and the microscope examination showed that the phytoplankton >3 µm are composed mainly of 3 classes, size ranges given in Table 1; cryptophytes, chlorophytes and prymnesiophytes. The microscopic analysis evidenced a high proportion of heterotrophic dinoflagellates (not detected by the HPLC method) in the proximal part of the guts. Among the phytoplanktonic groups present in the water column, pearl oysters preferentially ingested cryptophytes (Fig. 5): alloxanthin was accumulated in the proximal part of the gut contents. These algae were poorly represented in the water samples (<15% of total chl *a*), whereas they were always found in the proximal part of the gut and even dominated at Stn 1. The selective feeding is evidenced by highly significant differences in the alloxanthin concentration — the specific marker pigment of cryptophytes — between the water column and the gut. Until now, few studies have focused on defining the available food and the feeding behaviour of the pearl oyster *Pinctada margaritifera* in its natural environment (Hawkins et al. 1998, Pouvreau et al. 1999, 2000a, Yukihira et al. 1999). A positive selection (i.e. preferential ingestion) of cryptophytes by any species of bivalves studied under *in situ* conditions has not, according to our knowledge, been mentioned before. At the time of our study, this phytoplanktonic group played a key role in the diet of *Pinctada margaritifera*.

The selection of cryptophytes does not seem to be based on the size of the particles. Although being of a similar size range, the prymnesiophytes and the chlorophytes, especially the 10 µm *Chlamydomonas* sp., are not preferentially ingested because they are encountered in relatively same proportions in the water as in the gut. Two processes can be involved in the selective feeding on cryptophytes: (1) a preferential retention on the ctenidia and/or (2) a sorting on the ctenidia or labial palps (Ward et al. 1998). At present, it is impossible to evaluate the relative importance of these 2 processes. On the one hand, the preferential retention has been reported for several bivalves using flow-cytometry (Shumway et al. 1985, Newell et al. 1989, Bougrier et al. 1997). However, the mechanisms involved in this distinction have yet to be determined. On the other hand, the pre-ingestive selection of organic matter has been proved to be low for *Pinctada margaritifera* when compared to other bivalves (Hawkins et al. 1998, Yukihira et al. 1999, Pouvreau et al. 2000a). Although further evidence is required for making precise conclusions about the processes involved in the selection, our results demonstrate, for the first time, the capability of this bivalve to select the ingested food on a qualitative basis.

Cryptophytes are known to be high nutritive algae (Stewart & Wetzel 1986). Yukuhira et al. (1998b) showed the importance of energy-rich and digestible particles for the energy gain of pearl oysters. Cryptophytes are considered as easily digestible organisms (Porter 1973) probably because they contain small amounts of structure material (Stewart & Wetzel 1986). Shumway et al. (1985) and Newell et al. (1989) reported that the cryptophyte *Chroomonas salina* was easily digested by several bivalves.

The relative abundance in the proximal to the distal parts of the gut of the different algal classes was not identical. This was particularly characteristic in the case of peridinin, which was absent in the proximal part of the gut and abundant in the distal part. On the contrary, no significant differences could be detected for the 19'hf. The relative abundance of a given marker pigment in the distal part of the gut must, however, be considered with great care as it results from 2 processes occurring during the transit in the gut. Firstly, the whole algae can be more or less efficiently digested by the oyster, e.g. because of its structure. Secondly, the marker pigment itself can more or less resist

the digestive attack. Because peridinin is considered to be a fragile pigment due to its chemical structure (Repeta & Gagosian 1987), and as dinoflagellates present a resistant cell wall, the accumulation of peridinin in the distal part of the gut indicates that the autotrophic dinoflagellates were probably not efficiently digested by the oyster. Similarly, chlorophytes are weakly digested, as attested by the presence of intact cells in the distal part of the gut, and living and motile cells in the faeces. The absence of chlorophytes digested could be related to their glycoprotein envelope (van den Hoek 1995). For chlorophytes, the 2 methods — HPLC analysis and microscope examination — collaborate to formulate evidence of their low digestibility performed by the oyster. No similar conclusion could be drawn for the other algal groups, which were only detected using HPLC analysis and whose contribution did not significantly differ between the 2 parts of the gut.

The abundance of intact dinoflagellates and chlorophytes justifies the low amount of degradation products of chlorophylls. The pigment degradation during digestive processes has been largely investigated in the grazing experiments of copepods (Kleppel & Pieper 1984, Nelson 1989, Swalding & Marcus 1994, Buffan-Dubau et al. 1996). In contrast, few data are available on the pigment destruction in the gut of bivalves (Numagushi 1985, Hawkins et al. 1986, Bayne et al. 1987, Pastoureaud et al. 1996). From our results, the digestive efficiency of the pearl oyster *Pinctada margaritifera* seems to be low. However, further studies are required to gain a better insight into such selective digestibility.

A chl *c*-like was identified in the gut content whereas it was undetected in the water samples. The chromatographic and absorption properties of this non-polar chl *c*-like pigment suggest that it could derive from phytol-substituted chl *c* observed in pure cultures of some prymnesiophytes (Nelson & Wakeham 1989, Bidigare et al. 1990, Kraay et al. 1992, Zapata & Garrido 1997, Garrido & Zapata 1998). Kraay et al.'s (1992) analytic methods obtained the phytol-substituted chl *c* eluting just after β-carotene. Our chl *c*-like differed from this one as it eluted earlier and with chl *b*. Thus we did not identify the same pigment as these authors, but probably rather a degraded form. This hypothesis is reinforced by the fact that this pigment was mainly detected in the distal part of the gut.

To conclude, the 2 complementary approaches used in this study aided the determination of the feeding behaviour of the pearl oyster *Pinctada margaritifera* under natural conditions. Selection processes were demonstrated, either pre-ingestive or post-ingestive. As a consequence, all algal groups potentially available to the bivalve were consumed different. The ecological implications of this selective feeding behaviour might be considered, regarding the carrying capacity of the Takapoto Atoll lagoon for pearl oyster farming and the long-term evolution of the phytoplankton composition.

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