

Pearl oysters *Pinctada margaritifera* grazing on natural plankton in Ahe atoll lagoon (Tuamotu archipelago, French Polynesia)

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Abstract:

In atoll lagoons of French Polynesia, growth and reproduction of pearl oysters are mainly driven by plankton concentration. However, the actual diet of black-lip pearl oysters *Pinctada margaritifera* in these lagoons is poorly known. To fill this gap, we used the flow through chamber method to measure clearance rates of *P. margaritifera* in Ahe atoll lagoon (Tuamotu Archipelago, French Polynesia). We found: (i) that pearl oysters cleared plankton at a rate that was positively related to plankton biovolume, (ii) that nanoflagellates were the main source of carbon for the pearl oysters, and (iii) that the quantity and origin of carbon filtrated by pearl oysters was highly dependent on the concentration and composition of plankton. These results provide essential elements for the comprehension of growth and reproduction variability of pearl oysters in atoll lagoons of French Polynesia.

Highlights

► Atoll lagoons of French Polynesia. ► Clearance rates of natural pico- to micro-plankton communities by *Pinctada margaritifera*. ► High day to day fluctuations of plankton composition. ► Nanoplankton was the main source of carbon for the pearl-oyster. ► Plankton fluctuations lead to fluctuation in origin and quantity of carbon filtrated.

Keywords: *Pinctada margaritifera* ; Clearance rates ; French Polynesia ; Phytoplankton ; Protists

1. Introduction

For the last 40 years, farming of the black-lip pearl oyster *Pinctada margaritifera* has been the main aquaculture activity in French Polynesia atoll lagoons. In 2010, production and annual exportation of black pearls reached up to 12 metric tons, worth approximately 50 million Euros, making this industry the 2nd source of income for French Polynesia after tourism (Service de la Perliculture, pers. com.). However, this industry entirely relies on spat collection successes, which strongly depends on natural reproduction rates and on environmental conditions (Pouvreau et al., 2000a; Thomas et al., This issue).

1 French Polynesian atoll lagoons have been characterized in the past by stable and homogeneous
2 temperature and salinity (*e. g.* Buestel and Pouvreau, 2000). The planktonic biological processes are
3 controlled by the hydrodynamic regime and specifically by the water residence time (Charpy et al.
4 1997; Delesalle and Sournia, 1992; Torrétion et al., 2002), which is closely linked to atoll
5 geomorphology and water exchanges through the reef rims (Andréfouët et al., 2001; Charpy and
6 Blanchot, 1998; Sournia and Ricard, 1976; Dumas et al. this issue).

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11 The same lagoons were also characterized by concentrations of chlorophyll *a* and particulate
12 organic carbon that rarely exceed $0.6 \mu\text{g l}^{-1}$ and 0.4mg l^{-1} , respectively (Buestel and Pouvreau,
13 2000; Charpy et al., 1997); and by the dominance of planktonic particles inferior to $5 \mu\text{m}$ size which
14 represented more than 70% of the total planktonic biomass (Buestel and Pouvreau, 2000; Charpy
15 and Charpy-Roubaud, 1990; Niquil et al., 1998).

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22 In the 1990s the feeding strategy of *P. margaritifera* was investigated with various methods
23 including laboratory and *in situ* experiments : (1) batch and flow-through chamber methods were
24 used by Pouvreau et al. (1999) and Yukihiro et al. (1998b) to measure clearance rates of *P.*
25 *margaritifera* on various species of cultured algae, (2) batch method was used by Loret et al.
26 (2000a) to study clearance rates of pearl oysters on natural assemblage of ciliates and
27 dinoflagellates, (3) the biodeposit method was used by Pouvreau et al. (2000b) to measure *in situ*
28 clearance rates of pearl oysters in Takapoto lagoon and, finally, (4) direct sampling of *P.*
29 *margaritifera* gut content and HPLC analysis was used to determine which phytoplankton taxa
30 were contributing to the pearl oysters' diet (Loret et al. 2000b).

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39 These experiments demonstrated that (i) planktonic particles $< 2\mu\text{m}$ were not efficiently retained,
40 (ii) the diet of *P. margaritifera* included both autotrophic and heterotrophic plankton and (iii) *P.*
41 *margaritifera* compensated the low concentration of efficiently retained planktonic particles ($>$
42 $2\mu\text{m}$) by relatively high pumping rates to meet its energy requirements. However, this knowledge
43 remained too limited to fully characterize, quantitatively, the pearl oysters' diet.

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50 In this context, this study aims to measure the clearance rates of pearl oysters for six types of
51 autotrophic and/or heterotrophic plankton (picoplankton, nanoflagellates, dinoflagellates, ciliates,
52 phytoplankton $< 2\mu\text{m}$ and phytoplankton $> 2\mu\text{m}$), and to assess their relative contribution to the
53 pearl oysters' diet in Ahe lagoon.

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57 We selected the flow-through chamber method to measure clearance rates for two reasons: (i) it
58 allows keeping the pearl oysters under the influence of natural fluctuations of environmental
59 parameters and (ii) it facilitates repetitive sampling.

Complementary techniques such as flow cytometry, microscope counts and chlorophyll *a* extraction were used to measure the plankton concentration in the flow-through chambers.

MATERIAL AND METHODS

Study site

This study was conducted in Ahe atoll lagoon, located 500 km north of Tahiti Island in the north of the Tuamotu Archipelago (Figure 1). Ahe lagoon measures 142 km² with a mean depth close to 42 m. Ahe is defined as a semi-enclosed atoll. One active pass is located in the west part of the lagoon and several reef-flat spillways (less than 50 cm depth) are distributed along the reef rim, mainly in the south and west parts of the lagoon. The average water renewal time (ratio of lagoon volume to average water input rate) was estimated at 80 days (Dumas et al., This issue). With nearly 1350 spat collection stations and almost 11% of the lagoon dedicated to black-lip pearl oyster rearing, Ahe lagoon is a remarkable site for pearl culture and spat collection in French Polynesia.

Our study site and experimental set up were located in the northeast of the lagoon, 30 m off the coast, in a small pile building (Figure 1). Lagoon depth was approximately 2.5 m. Experimental devices were protected from direct sunlight and rain. Pearl oysters were subjected to natural light regimes and experiments were conducted after an acclimation period of four days in the flow-through grazing chambers.

The experiments took place in May 2008 (from 15th- 23rd), October 2008 (from 10th-23rd) and April/May 2009 (from 28th-10th). The rate at which pearl-oysters cleared phytoplankton from lagoon water (chlorophyll *a* used as a proxy) was measured during each of these three experimental periods. The rate at which pearl-oysters cleared picoplankton, nanoflagellates, dinoflagellates and ciliates from lagoon water were only measured during October 2008 experiments.

Environmental parameters

Hourly wind direction and velocity were obtained from Takaroa atoll meteorological station (Météo France data) located about 120 km east of Ahe (145°3'4''W, 14°28'57''S). Lack of any orographic effects around atolls allows using this distant measurement, which was in good agreement with local value and numerical models output at Ahe atoll (Dumas et al., This issue).

Water temperature (°C) and salinity (PSU) were obtained from a Sea Bird probe (SBE V19 plus) immersed at a 10 meter depth, next to an experimental breeding station located approximately 3 km away from our study site (Figure 1).

Phytoplankton concentration

1 Water samples (200ml) were filtered firstly on Millipore filters (2 μm of pore size) and then on
2 GF/F Whatman filters (ca. 0.7 μm pore size). Chlorophyll *a* (Chl *a*) retained on these filters was
3 extracted from phytoplankton cells during 4h in the dark at 4°C in 6 ml of methanol 100%. Chl *a*
4 concentration in these extracts was determined using a Turner design TD 700 fluorimeter calibrated
5 with Chl *a* standard (Sigma) and equipped with the set of optical filters recommended by
6 Welshmeyer (1994) for direct measurement of Chl *a*.
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12 We measured concentration of phytoplankton $< 2 \mu\text{m}$ (Chl *a* $< 2 \mu\text{m}$, in $\mu\text{g l}^{-1}$) and $> 2 \mu\text{m}$ (Chl *a* $>$
13 $2 \mu\text{m}$, in $\mu\text{g l}^{-1}$) respectively from the Chl *a* concentration measured in GF/F filters extracts and
14 from the Chl *a* concentration in Millipore filters extracts. To convert Chl *a* $> 2 \mu\text{m}$ and Chl *a* < 2
15 μm concentrations into carbon biomass, we used ratios equal to 50 $\mu\text{gC } \mu\text{gChl}a^{-1}$ and to 82 μgC
16 $\mu\text{gChl}a^{-1}$, respectively (Charpy and Charpy-Roubaud, 1990; Charpy, 1996).
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Picoplankton concentration

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25 In this study, picoplankton abundance (Pico. in cell l^{-1}) is defined as the sum of bacteria,
26 cyanobacteria (*Synechococcus sp.* and *Prochlorococcus sp.*) and picoeukaryotes abundances.
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31 Bacteria and picoautotrophic cells were fixed with 0.2 μm filtered formaldehyde (final
32 concentration 2%) and frozen in liquid nitrogen (N_2). Bacterial cells were counted by flow
33 cytometry using the method described by Marie et al. (1997). One mL formaldehyde-fixed
34 subsamples were incubated with DAPI at a final concentration of 1/10,000 for 15 min at room
35 temperature in the dark. Each subsample was counted using a MoFlo cytometer (Dako Colorado
36 Inc., Fort Collins, CO, USA). Stained bacterial cells, excited at 488 nm, were enumerated according
37 to their right-angle light scatter (RALS) and green fluorescence (FL1) measured using a 530/30 nm
38 filter. These cell parameters were recorded on a 4 decade logarithmic scale mapped onto 1024
39 channels. Fluorescent beads (0.94 μm , Polysciences Inc., Warrington, PA, USA) were
40 systematically added to each sample. Standardized RALS and FL1 values (cell RALS and FL1
41 divided by 0.94 μm beads RALS and FL1, respectively) were used as an estimation of the relative
42 size and nucleic acid content of bacterial cells, respectively (Troussellier et al., 1995). Listmode
43 files were analyzed using SUMMIT software (Dako Colorado Inc., Fort Collins, CO, USA).
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57 Picophytoplankton (*Prochlorococcus sp.* and *Synechococcus sp.* cells) and autotrophic
58 picoeukaryotes counts were performed with the same flow cytometer set up as described above.
59 Cells excited at 488 nm were detected and directly enumerated according to their FALS and RALS
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1 properties and their orange fluorescence (585/42 nm) and red fluorescence (>650 nm) due to
2 phycoerythrin and chlorophyll pigments, respectively. Fluorescent beads (0.94 μm) were also
3 systematically added to each sample. Listmode files were analyzed using SUMMIT software (Dako
4 Colorado Inc., Fort Collins, CO, USA).
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7 To calculate an average carbon conversion factor for picoplankton, we used conversion factors of
8 14 fgC cell⁻¹ (Gundersen et al., 2002), 60 fgC cell⁻¹, 178 fgC cell⁻¹ (Charpy and Blanchot, 1998) and
9 836 fgC cell⁻¹ (Verity et al., 1992) for bacteria, *Prochlorococcus sp.*, *Synechococcus sp.* and for
10 picoeukaryotes respectively. These values were averages and weighted by the mean abundance of
11 heterotrophic bacteria, *Prochlorococcus sp.*, *Synechococcus sp.* and picoeukaryotes measured
12 during this study. This community-scale conversion factor was then used to convert the total
13 picoplankton concentration (cell l⁻¹) into carbon biomass ($\mu\text{gC l}^{-1}$).
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22 Similarly, to calculate an average biovolume (BV, μm^3) per picoplankton cell we used biovolumes
23 of 0.035 μm^3 , 0.11 μm^3 , 0.38 μm^3 and 1.2 μm^3 per heterotrophic bacteria (Sakka et al., 2000),
24 *Prochlorococcus sp.* cell, *Synechococcus sp.* cell (Charpy and Blanchot, 1998), and picoeukaryote
25 cell, respectively. These values were weighted by the mean abundance measured for each plankton
26 type.
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32 **Nanoplankton and microplankton concentration**

34 The taxonomic determination of protists was carried out in accordance with systematics literature
35 (Kahl, 1931; Lee, 1985; Nezan, 1996; Paulmier, 1997; Ricard, 1987; Sournia, 1986).
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40 For microplankton counts (dinoflagellates and ciliates), water samples (1 liter) were fixed with
41 alcalin lugol iodine (2% final concentration). A first period of sedimentation was conducted during
42 24h after which the top 900 mL of sample was slowly siphoned off with small-bore tubing. The
43 remaining 100 mL was then stored at 4 °C in the dark before enumeration. A second sedimentation
44 of 24h was carried out in Utermöhl settling chamber (Hydro-Bios combined plate chamber) and cell
45 enumeration was made at 400 magnification using a Leica DMI 3000B inverted microscope with
46 interference contrast. Cells were counted in every microscope field (at least 60 fields per samples)
47 for five transversal bands covering the settling chamber width and disposed at equal distance of
48 each other.
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58 For nanoplankton counts, water samples (25 ml) were fixed and preserved with paraformaldehyde
59 (1% final concentration). Samples were concentrated to 10 ml with a filtration tower mounted with
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0.8 μm pore size black polycarbonate filters (Nuclepore) and stained with DAPI ($2.5 \times 10^{-4} \text{ g l}^{-1}$ final concentration). Enumeration of stained nanoplanktonic cells was made under UV light excitation on at least 15 randomly selected fields, at the magnification of x1000.

Nanoplankton and microplankton abundances (in cell l^{-1}) were computed using the following equation :

$$A = (N_C / (N_{MF} \times S_{MF})) \times S_{SC} \times 1000 / V_S$$

where A = abundance of nanoplankton or microplankton (cell l^{-1}), N_C = total number of cells (in cell), N_{MF} = number of counted microscopic fields, S_{MF} = area of one microscopic field (mm^2), S_{SC} = area of settling chamber or filter (mm^2), V_S = sample volume (l).

An average biovolume for dinoflagellates (200 cells) and ciliates (about 50 cells) was calculated using the mean length and width of cells, which were determined with a calibrated ocular micrometer.

Using these mean biovolumes and the biovolume to carbon content relationship from Menden-Deuer and Lessard (2000), we calculated the carbon conversion factors for both dinoflagellates and ciliates. These conversion factors were then used to convert dinoflagellates and ciliates concentration (cell l^{-1}) into carbon biomass ($\mu\text{gC l}^{-1}$).

For nanoplankton cells, we assumed an average biovolume of $509 \mu\text{m}^3$ which was calculated from the cell diameters of chlorophytes, prasinophytes and cryptophytes measured by Loret et al. (2000b) in Takapoto lagoon and we assumed an average conversion factor of $4.7 \times 10^{-6} \mu\text{gC}$ per cell of nanoplankton as in Ferrier-Pagès and Furla (2001).

Flow through chambers

After a critical analysis of the methodological shortcomings and possible misinterpretations related to the different methods of studying bivalve feeding processes (Bayne, 2004; Filgueira et al., 2006; Pascoe et al., 2009; Petersen, 2004 and Riisgård, 2004 for the most recent reviews) we selected the flow through chamber method for the measurement of *in situ* clearance rates of *Pinctada margaritifera*.

Water was pumped from the lagoon at 1 meter deep to a 80 liter reservoir tank at a flow rate of approximately 300 l h^{-1} . We used a peristaltic pump to avoid the destruction of fragile planktonic organisms. From this tank, lagoon water was distributed by gravity into flow through grazing chambers. Flow rates were adjusted (between 5 l h^{-1} and 68 l h^{-1}) in each flow through chamber to

1 prevent the pearl oyster from removing more than 30% of chlorophyll *a* (Hawkins et al., 1999). A
2 control flow through chamber without pearl oyster was maintained in the same configuration as our
3 grazing chambers.
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5 To avoid “recirculation issues” and to ensure “sufficient mixing of the exhalant flow with the flow
6 bypassing the bivalve” (Riisgård, 2001), our grazing chambers were divided into three
7 compartments (Figure 2): the inflow compartment where the water was entering, the grazing
8 compartment where the pearl oyster was filtering and the outflow compartment where the water was
9 siphoned off. Inflow and grazing compartments were separated out by 2 homogenization grids. In
10 the grazing compartment, one pearl oyster was maintained on a PVC support at mid height of the
11 water column and the exhalant flow was directed to the outflow compartment through a PVC
12 reduction of 5cm of diameter. In the outflow compartment, water was siphoned off 5 cm under the
13 surface.
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24 The siphoned water was sampled with 500 ml graduated test tube simultaneously from the control
25 chamber and from the grazing chambers.
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29 Experiments with the smallest pearl oysters (25 to 30 mm in height) were conducted in 25 liters
30 grazing chambers (20 cm in diameter and 50 cm in length) whereas experiments with pearl oysters
31 measuring 41mm to 115 mm in height were conducted in 50 liters grazing chambers (20 cm in
32 diameter and 100 cm in length).
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37 All flow trough chambers were emptied and cleaned every single day to remove faeces and
38 pseudofaeces produced by the pearl oysters.
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43 **Pearl Oysters**

44 A total of 16 pearl oysters were used during these experiments. In May 2008, experiments were
45 conducted with pearl oysters measuring 42 ± 1 mm in height (mean \pm sd) (n= 4) and with 113 ± 4 mm
46 height pearl oysters (n=2). In October 2008, experiments were conducted with 28 ± 2 mm height
47 pearl oysters (n= 4), and in May 2009, with 75 ± 6 mm height pearl oysters (n=6). For each size class
48 of pearl oysters, sampling strategy is indicated in Table 1.
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55 The smallest pearl oysters (25 to 43 mm in height) were bred at the Ifremer center's hatchery of
56 Vairao (Tahiti Island) and were stored in Ahe lagoon at one meter deep at least one week before
57 starting the experiments. Pearl oysters from 74 mm to 115 mm came from the “Motu Tahiri” pearl
58 farm in Ahe. All epibionts were cleaned off and pearl oysters were allowed to recover from any
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potential stress during three days before starting the starting the experiments. Sampling was then conducted at least once a day during the experimental periods.

At the end of the experiments, each pearl oyster height was measured and the flesh was freeze dried and weighted. Freeze dried fresh weight (DW in g) was used to normalize clearance rates per g of dry flesh.

Clearance rates

Clearance rate is defined as the volume of water entirely cleared of plankton by one pearl oyster per unit of time. It was calculated for each plankton type with the following equation, modified from Hildreth and Crisp (1976) :

$$CR_i = Fr \times (C_c - C_g) / C_g$$

with CR_i = clearance rates of pearl-oyster (in $l\ h^{-1}$, per individual), C_c and C_g = concentration of plankton at the exit of the control (C_c) and grazing (C_g) flow trough chambers (in $\mu g\ Chl\ a\ l^{-1}$ or in $cell\ l^{-1}$), Fr = flow rate in the grazing flow through chamber ($l\ h^{-1}$).

Clearance rates is known to follow an allometric relationship of the type $CR = aDW^b$ with DW = freeze dried fresh weight (in g), a and b = linear regression coefficients of $\log(CR_i)$ vs $\log(DW)$ (e.g. Pouvreau et al., 1999). Following this relationship, all CR_i values were divided by DW^b and standardized clearance rates (CR) were expressed in $l\ h^{-1}\ g^{-1}$.

Carbon retention rates

Clearance rates can be defined as the capacity of pearl oysters to filter and retain particles from their environment. However, the amount of carbon retained by *P. margaritifera* also depends on the plankton biomass. To assess the contribution of each plankton type to the diet of *P. margaritifera*, we estimated carbon retention rates by the following equation :

$$RR = CB \times CR,$$

where RR = Retention Rates of carbon in $\mu gC\ g^{-1}\ h^{-1}$, CB = Carbon Biomass in $\mu gC\ l^{-1}$, CR = Clearance Rates in ($l\ h^{-1}$).

Statistics

All analysis were conducted with the R freeware (<http://www.r-project.org/>). All data sets were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test). In most cases, data had to be log-transformed (natural log of X).

1 As wind velocity, salinity and water temperature data were not normal , highly heteroscedastic and
2 were also highly asymmetric between surveys, we only used their mean and associated 95%
3 confidence interval (Efron and Tibshirani, 1986) for each survey.
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6 Two-way analysis of variance (ANOVA) were used (i) to compare concentration of Chl *a* among
7 surveys and within size class ($> 2\mu\text{m}$ and $< 2\mu\text{m}$) and (ii) to compare CR of pearl-oysters among
8 surveys and within size-class of Chl *a* ($> 2\mu\text{m}$ and $< 2\mu\text{m}$). *A posteriori* multiple comparisons were
9 carried out using Tukey HSD tests.
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14 We used Pearson's correlation to examine relationships between Chl *a* concentration ($> 2\mu\text{m}$ and $<$
15 $2\mu\text{m}$), flow rates in the flow trough grazing chambers and clearance rates of pearl oysters.
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20 For each survey, exact binomial tests were used to compare the percentage of carbon retained by
21 pearl oysters from Chl. *a* $< 2\mu\text{m}$ and Chl. *a* $> 2\mu\text{m}$.
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26 Non parametric Kruskal-Wallis tests were used to compare (1) abundance of ciliates,
27 dinoflagellates, nanoplankton and picoplankton measured in October 2008, (2) clearance rates of
28 ciliates, dinoflagellates, nanoplankton and picoplankton measured in October 2008. *A posteriori*
29 multiple comparisons were carried out using the non parametric Steel-Dwass test (Critchlow and
30 Fligner, 1991; Spurrier, 2006).
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37 In all tests, significance was determined with an alpha level of 0.05.
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42 **RESULTS**

43 **Temperature, salinity, wind direction and speed**

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46 Mean water temperature ranged from 26.82 ± 0.01 °C (in October 2008) to 29.1 ± 0.05 °C (in May
47 2009). Mean salinity ranged from of 36.16 ± 0.03 (in October 2008) to 36.87 ± 0.09 in (May 2008)
48 (Table 2).
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52 East and southeast winds blew continuously in October 2008 with the highest velocity of the three
53 surveys (8.63 ± 0.41 m s⁻¹). In May 2008 and in May 2009, winds were predominantly blowing from
54 the northwest and northeast (more than 75% of the time) with lower velocity (3.0 ± 1.7 m s⁻¹ and
55 2.7 ± 1.4 m s⁻¹ respectively) (Table 2).
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61 **Chlorophyll *a* : concentration and clearance rates and carbon retention**

1 Variations of Chl $a < 2\mu\text{m}$ and Chl $a > 2\mu\text{m}$ concentrations and of clearance rates are presented in
2 Figure 3. Both Chl $a > 2\mu\text{m}$ and Chl $a < 2\mu\text{m}$ concentrations showed significant variations between
3 surveys (Tables 3 and Table 4). Chl $a > 2\mu\text{m}$ was lower than Chl $a < 2\mu\text{m}$ in May 2008 and in May
4 2009, while concentrations were not significantly different in October 2008, a period when we
5 observed the highest Chl $a > 2\mu\text{m}$ concentration ($1.31\ \mu\text{g l}^{-1}$).
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9 Conversion factors presented in Table 5 were used to convert mean Chl $a > 2\mu\text{m}$ and Chl $a < 2\mu\text{m}$
10 concentration into carbon biomass. Phytoplankton biomass ranged from $23\ \mu\text{gC l}^{-1}$ (May 2009) to
11 $42\ \mu\text{gC l}^{-1}$ (October 2008).
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14 The biomass temporal trends were similar to concentration trends. In May 2008 and May 2009,
15 biomass of Chl $a < 2\mu\text{m}$ was higher than biomass Chl $a > 2\mu\text{m}$ while there was no significant
16 difference between biomass Chl $a < 2\mu\text{m}$ and Chl $a > 2\mu\text{m}$ in October 2008.
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22 The allometric relationship between Chl $a > 2\mu\text{m}$ clearance rates and freeze dried dry flesh weight
23 is presented in Figure 4. Linear regression of $\log(\text{CRi})$ on $\log(\text{DW})$ was significant ($r^2 = 0.87$ and
24 $p < 0.001$, $n = 16$) and we established that $\text{CRi} = 13.3\ \text{DW}^{0.62}$. This relationship was further used to
25 standardize clearance rates for a 1g DW pearl oyster ($\text{CR} = \text{CRi} / \text{DW}^{0.62}$).
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31 In all surveys, pearl oysters cleared Chl $a > 2\mu\text{m}$ at a higher rate than Chl $a < 2\mu\text{m}$. Mean CR did
32 not show any significant variations between surveys (Table 4). CR of pearl oysters was not
33 influenced by variations of Chl $a < 2\mu\text{m}$ and Chl $a > 2\mu\text{m}$ concentration, neither by flow rates
34 (Table 6).
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40 In May 2008, pearl oysters retained significantly higher quantities of carbon from Chl $a < 2\mu\text{m}$
41 than from Chl $a > 2\mu\text{m}$. In October 2008, it was the opposite : pearl oysters retained significantly
42 higher quantities of carbon from Chl $a > 2\mu\text{m}$ than from Chl $a < 2\mu\text{m}$. In May 2009, pearl oysters
43 retained similar quantities of carbon from Chl $a > 2\mu\text{m}$ and from Chl $a < 2\mu\text{m}$ (Table 3).
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50 **Planktonic microorganisms : concentration, clearance rates and carbon retention**

51 This section presents results of the October 2008 survey, when the contribution of all plankton types
52 to the pearl oysters diet was assessed. Variations of plankton concentrations and clearance rates are
53 presented in Figure 3. Mean plankton concentration, mean clearance rates and mean carbon
54 retention rates are presented in Table 3.
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58 In October 2008, picoplankton and nanoflagellates were the two most abundant plankton types
59 (Table 3). Picoplankton concentration ranged from $1.92 \times 10^8\ \text{cell l}^{-1}$ to $3.09 \times 10^8\ \text{cell l}^{-1}$ with a
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1 mean of $2.64 \pm 0.71 \times 10^8$ cell l^{-1} . We calculated an average carbon content per cell of picoplankton
2 of 1.1×10^{-7} $\mu\text{gC Cell}^{-1}$ (Table 5). Nanoflagellates concentration ranged from 3.77×10^7 cell l^{-1} to
3 6.04×10^7 cell l^{-1} with a mean concentration of $5.25 \pm 0.80 \times 10^7$ cell l^{-1} . Dinoflagellates
4 concentration ranged from 0.86×10^4 cell l^{-1} to 12.3×10^4 cell l^{-1} with a mean concentration of
5 $5.09 \pm 4.32 \times 10^4$ cell l^{-1} . From their mean length (14.1 ± 5.0 μm) and width (10.9 ± 3.7 μm), we
6 calculated an average biovolume of $1,600$ μm^3 and an average carbon content per cell of 2.2×10^{-4}
7 $\mu\text{gC Cell}^{-1}$ (Table 5). Ciliates concentration ranged from 282 cell l^{-1} to 1093 cell l^{-1} with a mean of
8 740 ± 354 cell l^{-1} (Table 3, Figure 3). From their mean length (30.3 ± 13.4 μm) and width (23.4 ± 9.4
9 μm) we calculated an average biovolume of $18,000$ μm^3 and an average carbon content per cell of
10 2.1×10^{-3} $\mu\text{gC Cell}^{-1}$ (Table 5).
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19 The mean concentration of picoplankton, nanoflagellates, dinoflagellates and ciliates were
20 converted into carbon biomass using the conversion coefficients presented in Table 5. The total
21 carbon biomass was 288 $\mu\text{gC } l^{-1}$ and nanoflagellates constituted the bulk of total plankton biomass
22 (85%).
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25 Mean clearance rates of pearl oysters increased with the size of plankton (from 0.5 $l \text{ h}^{-1} \text{ g}^{-1}$ for
26 picoplankton to 18.7 $l \text{ h}^{-1} \text{ g}^{-1}$ for ciliates) and there was a significant relationship ($r^2 = 0.71$, $p =$
27 0.000 , $n=16$) between mean clearance rates of pearl oysters and biovolumes of plankton cells : CR
28 $= 0.42 \ln (BV) + 0.35$ (Figure 5).
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33 Nanoflagellates were the dominant source of carbon retained by pearl oysters in October 2008
34 (93%). The second source of carbon for pearl oysters were dinoflagellates (6%).
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39 DISCUSSION

40 41 42 43 Plankton concentration

44 Phytoplankton concentration measured during this study was in the upper range of phytoplankton
45 concentration measured in Ahe lagoon and in several other Tuamotu atoll lagoons (Table 7).
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47 In October 2008 phytoplankton concentration reached values > 1 $\mu\text{gChl } a \text{ } l^{-1}$ with a mean
48 concentration above 0.65 $\mu\text{gChl } a \text{ } l^{-1}$. In October 2008, we also observed (i) concentration of
49 dinoflagellates being in the upper range of values measured in other lagoons (Takapoto and
50 Tikehau) (Table 7), (ii) concentration of nanoflagellates that were approximately 10 times greater
51 than those measured in Rangiroa, Tikehau and in Ahe lagoon at other sites/periods (Table 7), (iii)
52 concentrations of picoplankton in the lower range of values reported in other atolls (Table 7).
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54 Thus, during the October 2008 experiments, the biomass of > 2 μm planktonic particles
55 (nanoplankton + dinoflagellates + ciliates) represented more than 90% of the total planktonic
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1 biomass. These observations are unusual in Tuamotu atoll lagoons where the biomass of $> 2\mu\text{m}$
2 planktonic particles is approximately 36% of the total planktonic biomass, as reviewed by Pouvreau
3 et al. (2000a).

4
5 Previous studies in French Polynesian atolls have shown that plankton concentration variations can
6 be significant at small spatial and/or temporal scale, despite the average low concentration of
7 plankton and despite weak seasonal trends (Buestel and Pouvreau, 2000; Charpy et al., This issue;
8 Fournier et al, This issue; González et al., 1998; Pagano et al., This issue; Sournia and Ricard, 1976;
9 Thomas et al., 2010). However, the exact mechanisms responsible for these changes remain unclear.
10 Changes in hydrodynamic regimes are likely causal factors and warrant further investigations. The
11 availability of 3D circulation numerical models will allow in a near future a better understanding of
12 these processes (Dumas et al. This issue).
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24 Clearance rates

25 Mean CR of pearl oysters ranged between $11.8 \text{ l h}^{-1} \text{ g}^{-1}$ and $18.7 \text{ l h}^{-1} \text{ g}^{-1}$ for plankton $> 2\mu\text{m}$ (Chl a
26 $> 2\mu\text{m}$, nanoflagellates, dinoflagellates and ciliates). These values are in the range of CR measured
27 by Yukihiro et al. (1998b) ($12.3 \text{ l h}^{-1} \text{ g}^{-1}$) and Pouvreau et al. (1999) ($25.9 \text{ l h}^{-1} \text{ g}^{-1}$) during laboratory
28 experiments with a monospecific solution of *Isochrysis galbana* retained at 98% by *P.*
29 *margaritifera*.
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33 Clearance rates of *P. margaritifera* are also close to clearance rates of the oyster *Crassostrea gigas*
34 measured under low seston load conditions in Thau lagoon in France ($16 \text{ l h}^{-1} \text{ g}^{-1}$ for $> 5 \mu\text{m}$
35 flagellates) (Dupuy et al., 2000).
36

37 During our experiments, we did not measure any influence of plankton concentration variations on
38 clearance rates (Table 6). However, bivalves filtration performances are known to decrease when
39 seston load increases (e.g., Pouvreau et al., 2000b for *P. margaritifera*). Species inhabiting high
40 seston load environments display lower clearance rates than species in low seston load
41 environments (Jørgensen, 1996; Yukihiro et al., 1998a, Trottet et al., 2008). The low load of atoll
42 lagoons compared to many temperate coastal environments explains the typically high, and stable,
43 CR of *P. margaritifera* (and *C. gigas* when in a low seston load environment).
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53 Clearance of picoplankton by pearl oysters was extremely low compared to clearance of
54 nanoplankton and microplankton. Moreover, there was a clear positive relationship between
55 clearance rates of *P. margaritifera* and biovolume of plankton cells (Figure 5). This relationship,
56 obtained *in situ*, is in agreement with the relationship between retention efficiency and particle size
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obtained in laboratory by Pouvreau et al. (1999). Finally, numerous studies have shown that this relationship was explained by the gill structure, and especially by the disposition of cirri on gill filaments (e.g Pouvreau et al., 1999; Silverman et al., 1996; Wright et al., 1982).

For *P. margaritifera*, *in situ* clearance rates data are scarce in literature. However, comparisons between clearance rates values measured during our experiments and clearance rates values measured by Loret et al. (2000a) in Takapoto lagoon again highlight this obvious relationship between clearance rates and particle size / biovolume.

Indeed, mean CR of small (length : 14.1 μm ; width : 10.9 μm) dinoflagellates ($16 \text{ l h}^{-1} \text{ g}^{-1}$) measured during this study was half lower than CR ($33 \text{ l h}^{-1} \text{ g}^{-1}$) of large (length : 83 μm ; width : 35 μm) dinoflagellates measured by Loret et al (2000a).

Conversely, mean CR of small (length : 30.3 μm ; width : 23.4 μm) ciliates ($19 \text{ l h}^{-1} \text{ g}^{-1}$) measured during this study was in the range of CR of $10 \text{ l h}^{-1} \text{ g}^{-1}$ for *Amphileptus sp* (length : 55 μm ; width : 21 μm) and of $20 \text{ l h}^{-1} \text{ g}^{-1}$ for *Strombidium sp.* (length : 50 μm ; width : 30 μm) measured by Loret et al. (2000a) in Takapoto lagoon.

Carbon retention rates

Obviously, plankton concentration measured in October 2008 was exceptionally high and did not represent the average plankton concentration in Ahe lagoon. Thus, to assess the average amount of carbon retained by pearl oysters in Ahe lagoon, we calculated the average concentration of Chl. *a* < 2 μm , Chl. *a* > 2 μm , picoplankton, nanoflagellates, dinoflagellates and ciliates from literature data (Table 8). Then, we converted these average plankton concentrations into their respective carbon biomass using the conversion factors in Table 5. Finally, we calculated the average carbon retention rates of pearl oysters for each plankton fraction using clearance rates measured in October 2008.

The average biomass of phytoplankton in Ahe was $26 \mu\text{gC l}^{-1}$ and Chl *a* > 2 μm represented 27% of this biomass. However, pearl oysters retained similar amounts of carbon from Chl *a* < 2 μm and from Chl *a* > 2 μm (ca. $100 \mu\text{gC h}^{-1} \text{ g}^{-1}$) (Table 8).

The average total planktonic carbon biomass was $103 \mu\text{gC l}^{-1}$ (Table 8). Picoplankton represented 69% of this total carbon biomass and nanoflagellates represented 24%. Finally, dinoflagellates and ciliates represented only 7%. In contrast, carbon retained by pearls oysters originated mainly from nanoflagellates (64%), then from dinoflagellates and ciliates (27%), and finally from picoplankton (8%).

In October 2008, pearl oysters retained almost 8 times more planktonic carbon than average (ca. $3000 \mu\text{gC h}^{-1} \text{ g}^{-1}$ and $400 \mu\text{gC h}^{-1} \text{ g}^{-1}$, respectively).

1 In Takapoto lagoon, pearl oysters retained similar quantities of carbon from dinoflagellates ($64 \mu\text{gC h}^{-1} \text{g}^{-1}$) compared to the average in Ahe ($70 \mu\text{gC h}^{-1} \text{g}^{-1}$). Dinoflagellates were larger in Takapoto
2 lagoon but their concentration was lower than in Ahe lagoon (Loret et al., 2000a).

3
4 Pearl oysters retained higher quantities of carbon in Takapoto from ciliates ($86 \mu\text{gC h}^{-1} \text{g}^{-1}$)
5 compared to the average in Ahe ($55 \mu\text{gC h}^{-1} \text{g}^{-1}$), where they were smaller and less abundant than in
6 Takapoto (Loret et al., 2000a).

7
8 To our knowledge, there is no comparable *in situ* study that has measured the relative contribution
9 of pico- nano- and micro- plankton to the diet of a tropical bivalve. In temperate environments,
10 Trottet et al. (2008) and Dupuy et al. (2000) investigated the relative contribution of pico- nano- and
11 micro- plankton in the blue mussel diet (*Mytilus edulis*) and in the cupped oyster diet (*Crassostrea*
12 *gigas*), respectively. In Thau lagoon (France), *C. gigas* retained a total of $1634 \mu\text{gC h}^{-1} \text{g}^{-1}$, and in
13 Grand Entrée lagoon (Canada), total carbon retention of *M. edulis* ranged from $160 \mu\text{gC h}^{-1} \text{g}^{-1}$ to
14 $1467 \mu\text{gC h}^{-1} \text{g}^{-1}$.

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16 In Thau lagoon, diatoms represented 87% of the total planktonic biomass and 80% of the carbon
17 retained by *C. gigas* while in Grande Entrée lagoon, ciliates represented at least 50% of the total
18 planktonic biomass and at least 70% of the carbon retained by *M. edulis*.

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20 Similarly to these two species, we report for *P. margaritifera* that (i) natural variations in the
21 composition and abundance of plankton lead to important feeding variations (ii) particles of size $>$
22 $2\mu\text{m}$ are the main source of carbon.

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The grazing experiments conducted in Ahe lagoon with the flow trough chamber method confirmed
the *in situ* high clearance rates of *P. margaritifera* and highlighted the strong relationship between
clearance rates and plankton size/biovolume. Our results also clearly demonstrated that, even if atoll
lagoons of Tuamotu Archipelago are characterized by a low average biomass of plankton, the
variations of this biomass and the variations in the structure of planktonic communities have a
major influence on the feeding of pearl oysters. This will help on the long run to understand the
inter-lagoon differences of pearl oysters' ecophysiology (growth, reproduction, see Fournier et al.,
This issue) and therefore the inter-lagoon differences in aquaculture an pearl farming potential.

However, food sources of *P. margaritifera* are highly diversified (Loret et al., 2000a; Nasr, 1984)
and it is obvious that several plankton taxa/types were not considered in the present study due to
their low concentration such as diatoms, small metazooplankton, coccolithophorids.

Despite their average low abundance, transitory peaks of diatoms, bivalve larvae and other
metazoan larvae concentration have been observed in atoll lagoons (Fournier et al., this issue;
Pagano et al, this issue; Sournia and Ricard, 1976). These plankton fractions may therefore

represent significant food sources for pearl oysters.

For these reasons, further studies on pearl oysters nutrition should focus on the measurement of clearance rates and carbon retention rates of small metazooplankton, coccolithophorids and diatoms.

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Table 1 : Mean height \pm standard deviation (in mm) and number of oysters (between parentheses) used during our experiments. Sampling strategy (n) for the measurement of clearance rates of pearl oysters (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. $a < 2 \mu\text{m}$ and Chl. $a > 2 \mu\text{m}$ = phytoplankton $< 2 \mu\text{m}$ and $> 2 \mu\text{m}$)

Survey	Oysters Height	Chl. a (n)	Pico. (n)	Nano. (n)	Dino. (n)	Cili. (n)
May 2008	42 \pm 1 (4)	8				
	113 \pm 4 (2)	9	-	-	-	-
October 2008	28 \pm 2 (4)	30	22	15	10	10
May 2009	75 \pm 6 (6)	50	-	-	-	-

Table 2 : Mean \pm 95% confidence interval of wind velocity (m s^{-1}), water temperature ($^{\circ}\text{C}$) and salinity (PSU) measured in May 2008, October 2008 and April/May 2009.

Survey	Wind Velocity	Water Temp.	Salinity
May 2008	2.83 \pm 0.86	28.22 \pm 0.03	36.87 \pm 0.09
October 2008	8.63 \pm 0.41	26.82 \pm 0.01	36.16 \pm 0.3
May 2009	2.65 \pm 0.62	29.10 \pm 0.05	36.23 \pm 0.01

Table 3 : Abundance (in Cell l^{-1} or in $\mu\text{gChl } a \text{ l}^{-1}$), Carbon biomass (CB in $\mu\text{gC l}^{-1}$ and B in %), Clearance Rates of pearl oysters (in $\text{l h}^{-1} \text{g}^{-1}$) and carbon retention rates (Carbon Retained in $\mu\text{gC h}^{-1} \text{g}^{-1}$ and Carb. in %) measured in May 2008, October 2008 and April/May 2009 at our study site (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. $a < 2 \mu\text{m}$ and Chl. $a > 2 \mu\text{m}$ = phytoplankton $< 2 \mu\text{m}$ and $> 2 \mu\text{m}$).

Survey	Plankton Type	Abundance	CB	B (%)	Clearance Rates	Carbon Retained	Carb (%)
May 2008	Chl $a > 2 \mu\text{m}$	0.10 \pm 0.03	5	13	14.4 \pm 6.0	72	32
	Chl $a < 2 \mu\text{m}$	0.41 \pm 0.08	34	87	4.5 \pm 6.2	152	68
October 2008	Chl $a > 2 \mu\text{m}$	0.34 \pm 0.32	17	40	13.7 \pm 7.8	233	72
	Chl $a < 2 \mu\text{m}$	0.30 \pm 0.06	25	60	3.7 \pm 2.5	92	28
	Pico.	2.64 \pm 0.71 $\times 10^8$	28	10	100% 0.5 \pm 5.4	15	0
	Nano.	5.25 \pm 0.80 $\times 10^7$	247	85		2918	93
	Dino.	5.09 \pm 4.32 $\times 10^4$	11	4		179	6
	Cili.	740 \pm 354	2	1		29	1
May 2009	Chl $a > 2 \mu\text{m}$	0.14 \pm 0.06	7	30	14.6 \pm 5.0	102	53
	Chl $a < 2 \mu\text{m}$	0.20 \pm 0.06	16	70	5.6 \pm 2.5	92	47

Table 4 : Analysis of variance table for statistical comparisons of concentration Chl. a and standardized clearance rates of pearl oysters within size class of Chl. a ($> 2 \mu\text{m}$ and $< 2 \mu\text{m}$) and between survey.

Analysis	Source	df	F	p
Chl. a concentration among survey and size class of Chl a	Size class	1	25.3	0.000
	Survey	2	17.9	0.000
	Interaction	2	21.0	0.000

CR of pearl oysters among survey and size class of Chl <i>a</i>	Size class	1	154.6	0.000
	Survey	2	2.4	0.089
	Interaction	2	0.3	0.766

Table 5 : Average biovolumes (BV in μm^3) and carbon content (C.C. in $\mu\text{gC Cell}^{-1}$ or in $\mu\text{gC } \mu\text{gChl}a^{-1}$) computed from our data and from literature data (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. *a* < 2 μm and Chl. *a* > 2 μm = phytoplankton < 2 μm and > 2 μm).

Plankton type	B.V.	C.C.
Pico.	0.25	1.1×10^{-7}
Nano.	509	4.7×10^{-6}
Dino.	1606	2.2×10^{-4}
Cili.	18091	2.1×10^{-3}
Chl. <i>a</i> > 2 μm	-	50
Chl. <i>a</i> < 2 μm	-	82

Table 6 : Relationship between clearance rates of pearl oyster ($\text{CR}_{\text{Chl } a < 2\mu\text{m}}$ and $\text{CR}_{\text{Chl } a > 2\mu\text{m}}$), concentration of phytoplankton < 2 μm (Chl *a* < 2 μm), of phytoplankton > 2 μm (Chl *a* > 2 μm), and flow rates in the grazing chambers. Pearson's product moment correlation (*r*) and p-values (*p*) are indicated for each analysis.

	$\text{CR}_{\text{Chl } a < 2\mu\text{m}}$	$\text{CR}_{\text{Chl } a > 2\mu\text{m}}$
Chl <i>a</i> > 2 μm	<i>r</i> = -0.08 <i>p</i> = 0.434	<i>r</i> = 0.09 <i>p</i> = 0.348
Chl <i>a</i> < 2 μm	<i>r</i> = 0.01 <i>p</i> = 0.970	<i>r</i> = -0.04 <i>p</i> = 0.717
Flow rates	<i>r</i> = 0.20 <i>p</i> = 0.050	<i>r</i> = 0.08 <i>p</i> = 0.415

Table 7 : Range of plankton concentration (in $\mu\text{gChl } a \text{ l}^{-1}$ or in Cell l^{-1}) measured during our experiments (This study), at other sites/periods in Ahe atoll lagoon (Ahe) and in other French Polynesian atoll lagoons (Other atolls).

	This study	Ahe	Other atolls
Chlorophyll <i>a</i>	0.25-1.76	0.08-0.85 ^a	0.02-1.24 ^c
Picoplankton. ($\times 10^8$)	1.9-3.1	1.0-5.1 ^b	2.2-23.2 ^e
Bacteria. ($\times 10^8$)	0.6-1.9	2.6-7.8 ^b	2.2-20.7 ^e
<i>Synechococcus</i> . ($\times 10^8$)	0.6-1.4	0.8-1.2 ^b	<0.1-2.8 ^e
<i>Prochlorococcus</i> . ($\times 10^8$)	0.1-0.8	0.6-1.4 ^b	<0.1-1.7 ^e
Picoeukaryotes. ($\times 10^6$)	1.4-5.3	2.8-4.6 ^b	<0.1-4.9 ^e
Nanoflagellates. ($\times 10^6$)	37.0-67.0	5.5-8.5 ^c	0.7-2.0 ^f
Dinoflagellates. ($\times 10^5$)	0.09-1.2	<0.01-0.03 ^d	<0.01-1.90 ^g
Ciliates. ($\times 10^3$)	0.3-1.1	<0.01-0.9 ^d	<0.01-4.0 ^g

^(a) Thomas et al. (2010), Fournier et al. (this issue), Charpy et al. (this issue); ^(b) Thomas et al. (2010); ^(c) Dupuy, (Pers. Com.); ^(d) Fournier et al. (this issue); ^(e) Charpy & Blanchot (1998), Torreton et al. (2002); ^(f) González et al. (1998); ^(g) González et al. (1998), Loret et al. (2000a).

Table 8 : Average abundance (in Cell l⁻¹ or in µgChl *a* l⁻¹), Carbon biomass (CB in µgC l⁻¹ and B in %), and carbon retention rates of pearl oysters (Carbon Retained in µgC h⁻¹ g⁻¹ and Carb. in %) in Ahe lagoon. (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. *a* < 2 µm and Chl. *a* > 2 µm = phytoplankton < 2µm and > 2µm).

Plankton Type	Abundance	CB	B (%)	Carbon Retained	Carb (%)
Chl <i>a</i> >2µm	0.14 ^(a)	7	27	102	49
Chl <i>a</i> <2µm	0.23 ^(a)	19	73	105	51
Pico.	6.5 x 10 ⁸ ^(b)	71	69	38	8
Nano.	5.3 x 10 ⁶ ^(c)	25	24	293	64
Dino.	2.0 x 10 ⁴ ^(a)	4	4	70	15
Cil.	1.4 x 10 ⁴ ^(a)	3	3	55	12

^(a) Fournier et al. (this issue); ^(b) Thomas et al. (2010); ^(c) Dupuy C., (Unp. data.)

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Figure 1 : Location of Ahe atoll. Location of the sites where filtration experiments were carried out (site F) and where we measured water temperature and salinity (site T) in Ahe lagoon (map by courtesy of Yoann Thomas).

Figure 2 : Flow through grazing chambers were divided into 3 compartments : inflow compartment, grazing compartment and outflow compartment.

Figure 3 : Abundance of plankton (graphs a, c and e) and clearance of pearl oysters (graphs b, d and f) measured in May 2008, October 2008 and April/May 2009 in Ahe lagoon. (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. $a < 2 \mu\text{m}$ and Chl. $a > 2 \mu\text{m}$ = phytoplankton $< 2 \mu\text{m}$ and $> 2 \mu\text{m}$).

Figure 4 : Allometric relationship between clearance rates (CRi in l h^{-1}) and freeze dried flesh weight (DW in g) of pearl oysters. Each point represents the mean individual clearance rates of pearl-oysters (Chl $a > 2 \mu\text{m}$) with bars corresponding to standard deviation. The curve corresponds to the equation $\text{CRi} = 13.3 \times \text{DW}^{0.62}$.

Figure 5 : Relationship between standardized clearance rates of pearl oysters (CR in $\text{l h}^{-1} \text{g}^{-1}$) and plankton biovolume (in μm^3). Each point represents the mean CR of pearl-oysters measured in October 2008 in Ahe lagoon. (picoplankton = full circle, nanoplankton = empty triangle, dinoflagellates = empty square and ciliates = empty circle). Bars represent standard deviation. Curve represents the equation $\text{CR} = 0.42 \ln(\text{BV}) + 0.35$.

Figure 1

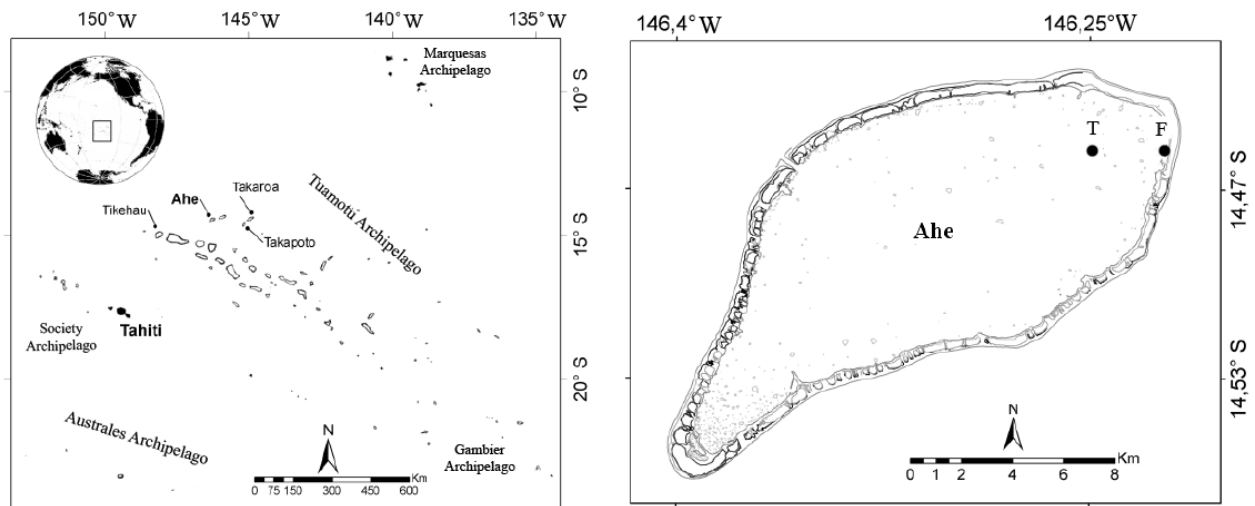


Figure 2

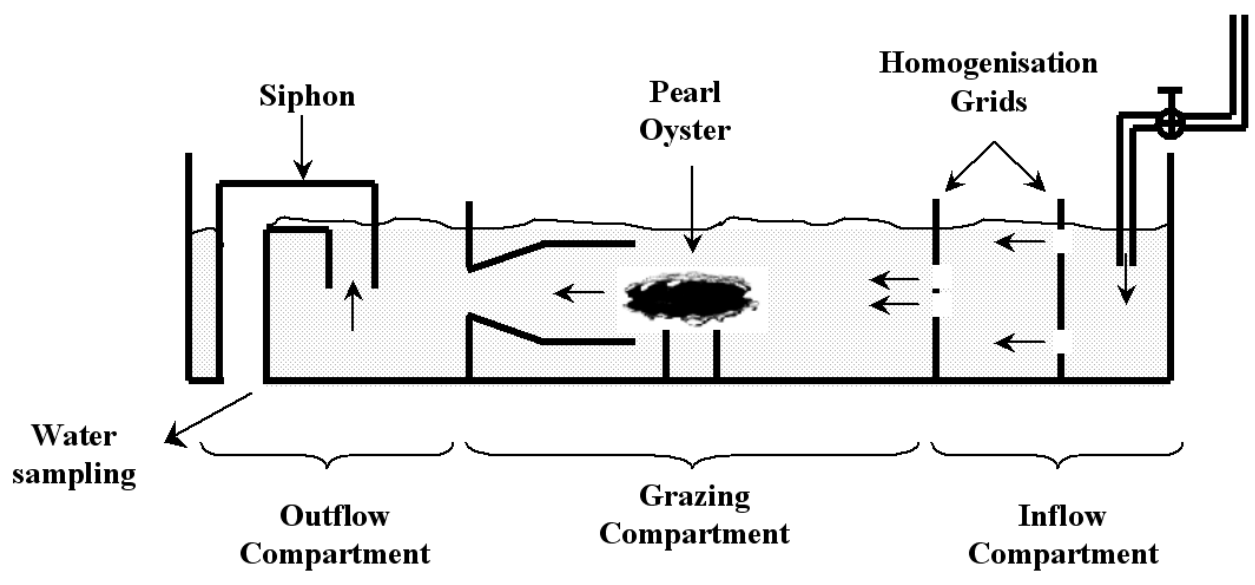


Figure 3

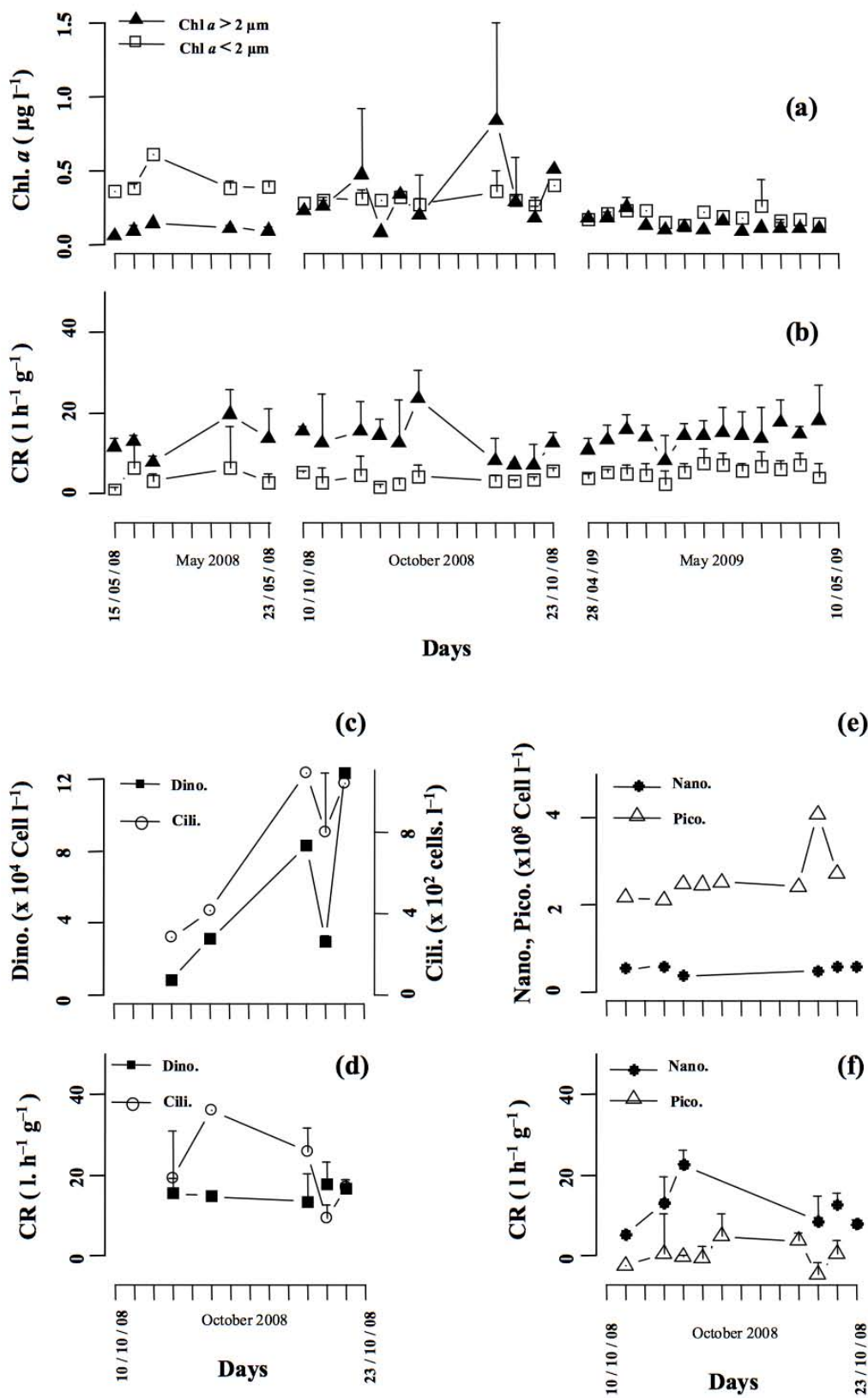


Figure 4

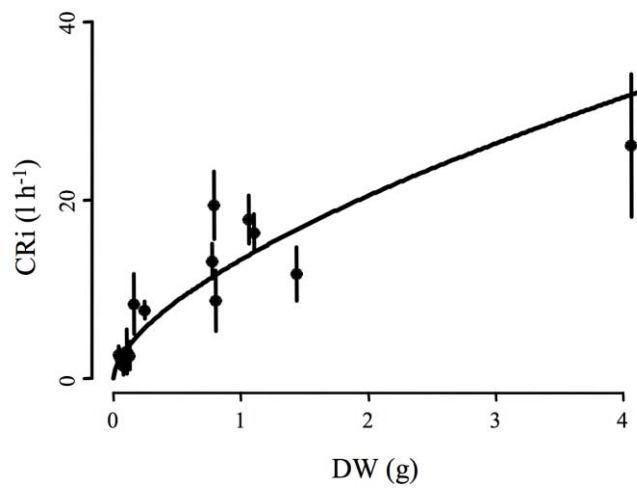


Figure 5

