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Different food particle sources in the pearl oyster *Pinctada margaritifera* and its epibionts

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ABSTRACT

Suspended bivalve culture (e.g. longlines) transfers benthic biomass - including bivalves and various epibionts - toward the water column, creating strong trophic interactions with the planktonic compartment. Trophic interactions are of central interest for ecologists, yet not well understood in coastal lagoon food webs, especially in tropical areas. Using stable isotope (SI) analyses, this study explored the trophic relationships between marine particulate organic matter (POM), pearl oysters and epibionts, in two contrasted production areas in French Polynesia. Different size classes of POM (0.7–2 μm, 2–20 μm, 20–80 μm, 80–250 μm and >250 μm) were well discriminated both by their $\delta^{13}C$ and $\delta^{15}N$ signature and results showed a low dietary overlap between pearl oysters and epibionts, likely due to assimilation of large particles in greater proportion by pearl oysters. Pearl oyster diet may vary in time in relation with variations of the basal trophic resource and selective feeding, such that their isotopic signature is more variable compared with that of epibionts. Pearl oysters and epibionts might alter nutrient cycling in a different way as reflected by their different tissue C:N.

1. Introduction

Suspension-feeders dominate in most benthic ecosystems where they process large amounts of organic material (hereafter OM) through strong filtering capacities. Many of those organisms are also of high economic interest and are cultivated worldwide using different farming technics. Suspended longline systems, often used for bivalve culture, induce the transfer of benthic biomass toward the water column. As a consequence, cultivated bivalves strongly interact with the planktonic food web by exerting a negative control (top-down control) on the OM pool. The impact of cultivated bivalves on the planktonic compartment is often measured quantitatively, with the final objective of defining the production carrying capacity of systems, such as the maximum commercial production that could be sustained without exceeding the renewal rate of food resources (Filgueira et al., 2015). Due to its ease of measurement, chlorophyll-*a* is the most widely proxy used to describe phytoplankton biomass in this context (e.g. Ogilvie et al., 2000; Petersen et al., 2019; Pinkerton et al., 2018). However, this may not be the most useful indicator since heterotrophic organisms may also represent a large proportion of the food resource for bivalves (Dupuy et al., 2009; Fournier et al., 2012a; Trottet et al., 2006), and strongly contribute to the carbon flow in the ecosystem. Moreover, bivalves are capable of particle size selection and consume mainly particles > 5 μm (Rosa et al., 2018) whereas measured chlorophyll-*a* often represents organisms > 0.7 μm.

In addition to commercial species, many associated organisms that colonize artificial structures and bivalve shells contribute to the removal of particulate OM (hereafter POM) in farming areas (Lacoste and Gaertner-Mazouni, 2015). These epibiont organisms have been shown to contribute up to 18% to the total clearance rate in a mussel farm (Woods et al., 2012). Among epibionts, ascidians are able to extract resources more efficiently than mussels due to their lower metabolic cost and higher filtration capacity (Filgueira et al., 2019). These co-occurring species may thus compete for food with reared bivalves at small spatial scale (Daigle and Herbinger, 2009; Petersen, 2007; Sievers et al., 2013), and modify the impact on the planktonic compartment due to differential filtration abilities (Lacoste et al., 2016). In Canada, Comeau et al. (2015) showed that the solitary tunicates *Styela clava* or *Ciona intestinalis* increased the clearance rate per unit lease area by 30–47%. Trophic interactions between commercial bivalves and epibionts have therefore been widely investigated in temperate ecosystems (Decottignies et al., 2007; Dubois et al., 2007; Kang et al., 2009) whereas tropical

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systems have received less attention.

In French Polynesia, numerous lagoons are exploited for pearl oyster farming that represents the second most important economic resource for the country. Pearl oysters are reared in the water column on longline systems. Due to the submersion of pearl oysters on longline system in deep areas (>15 m most of the time) and the absence of riverine input in atolls, the main food source available to pearl oysters is pelagic marine POM, principally derived from plankton. In oligotrophic lagoons of French Polynesia, POM is mostly composed of pico-sized particles (0.2–2.0 μm) (Charpy, 1996) that are only marginally consumed by pearl oysters, while nanoplankton (2–20 μm) is thought to represent their main carbon source (Dupuy et al., 2009; Fournier et al., 2012a). Pearl oyster retention has been shown to vary from 15% for 1 μm particles to 98% for 5 μm particles in the laboratory (Pouvreau et al., 1999). Pearl oysters might also be able of pre-ingestive selection dependent upon the characteristics of available food (e.g. nutritional value) (Loret et al., 2000). Although variability in resources can impact the ecological performance of pearl oysters in the field, sources of organic matter and their relative contribution to the diet of pearl oysters under natural conditions have received little attention (Fournier et al., 2012a; Lacoste et al., 2016; Loret et al., 2000). Overall, the organic carbon input into the food web has been overlooked in tropical areas.

Due to the constant immersion of longlines, a wide range of epibionts can be found associated with pearl oysters, colonizing rearing structures and shells (Lacoste et al., 2014b). For a long time, those – mainly suspension feeder – organisms have been considered to be trophic competitors of pearl oysters. However, some studies showed no negative impact of biofouling on the mortality, growth or reproduction of pearl oysters (Hulot et al., 2019; Lacoste et al., 2014b). Differential sorting abilities between pearl oysters and their main epibionts, with non-limiting food, are proposed to explain the neutral role of biofouling (Lacoste et al., 2016; Niquil et al., 2001). Trophic functioning and potential interactions among suspension feeders in French Polynesian lagoons are however still poorly documented.

Thus, along with another work (Lacoste et al., 2016), the present study appears to be the first step in the assessment of trophic interactions between POM, pearl oysters and associated suspension-feeders in contrasted lagoons of French Polynesia, exploited for pearl farming. The stable isotope (SI) ratio analyse was used since it is a useful approach to determine carbon flows in aquatic ecosystems and has been widely used to explore the diet of bivalves in coastal areas (Briant et al., 2018; Fukumori et al., 2008; Nerot et al., 2012). Analyses of $\delta^{13}C$ and $\delta^{15}N$ SIs provide knowledge of organisms food sources and trophic position respectively. Carbon and nitrogen SI also allow comparison of food sources used by bivalves and potential filter-feeder competitors (Decottignies et al., 2007; Dubois et al., 2007; Riera et al., 2002). Thus, after quantifying the relative contributions of different plankton groups to the POM pool, we have tested the hypothesis that pearl oysters and epibionts have a different isotopic niche, as a proxy of trophic niche, based on their different sorting abilities. Due to their higher particle selection capacities, it was also expected that individual variability in isotope signature would be higher for pearl oysters (Dubois and Colombo, 2014).

2. Materials and methods

2.1. Study sites

Sampling was carried out in 2 lagoons in French Polynesia (Fig. 1). These 2 lagoons were chosen for their contrasted ecological conditions, due to their natural configuration (atoll, high island) and latitudinal position (Mangareva: 23°06'S, Arutua: 15°14'S), and for their well-developed pearl farming industry. On these 2 islands, more than 2000 ha are exploited for pearl oyster culture (ISPF, 2018). Mangareva, the main high island of the Gambier Archipelago, is located 1700 km southeast of Tahiti (Fig. 1). Because of its latitudinal position, high thermal amplitude is observed in this area, and seawater temperature can fall by up to 22 °C (Lacoste et al., 2014b). The coral reef is highly dispersed and the ocean opening very wide. In 2016, 84 pearl farms, covering a surface area of 1600 ha, were counted in this archipelago (ISPF, 2018). Arutua is an atoll located 380 km northeast of Tahiti (Fig. 1) with an area of ca. 516 km^2 and only one active channel that allows exchanges with the ocean. Little information is available concerning environmental conditions in this area, however mean temperature may be assumed to be around 28 °C as observed in other Tuamotu atolls. In 2018, 72 farms were in activity in this atoll lagoon, covering an area of ca. 1227 ha (data from *Direction des Ressources Marines*, 2018). In the 2 islands, experiments were done in collaboration with pearl farmers, benefiting from their facilities and pearl oysters livestock.

2.2. Trial design and field collection

At Mangareva and Arutua, 200 one-year-old pearl oysters were placed on experimental long lines in May 2016 and September 2016

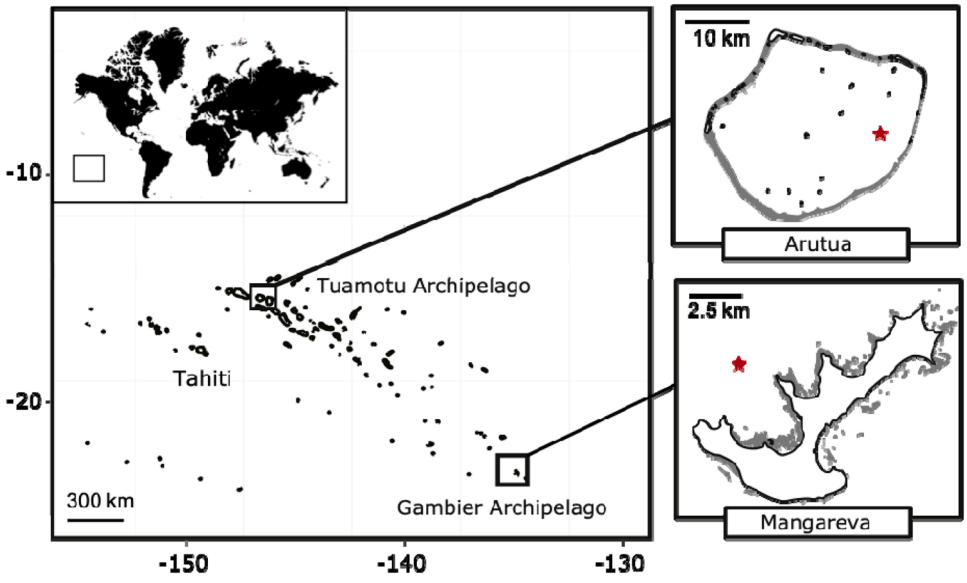

Fig. 1. Location of the 2 study sites in French Polynesia. Stars indicate the experimental sites in the lagoons of Arutua and Mangareva.

respectively. Pearl oyster spat at Arutua originated from Ahe, another Tuamotu atoll. The pearl oyster cultivation procedure was similar at both sites: 10 pairs of pearl oysters were fixed on specific ropes and placed inside a rigid net so as to be protected against predators. These experimental ropes were stocked on long lines at 6 m depth, 0.5–1 m apart from each other for the duration of the experience, without any cleaning. Depth under longlines was approximately 25 m at both sites. Three campaigns were done at each site, covering the dry and wet seasons over a year (Table 1). During each campaign, 16 pairs of pearl oysters were sampled directly on experimental longlines for isotopic analysis. On nine randomly selected pairs (i.e. 18 pearl oysters), all epibionts were carefully removed before being sorted at the laboratory. To determine particulate organic matter (POM), water was sampled nearby, at the same depth where pearl oysters are reared.

Due to the depth of the lagoon where pearl oysters are reared in suspension (>15 m), and the absence of riverine input at Mangareva and Arutua, plankton (phytoplankton and heterotrophic organisms) is assumed to constitute their main food resource. Isotopic signature of POM, as a proxy of plankton, was therefore used to identify food sources available to filter feeders (Michener and Faufman, 2007). To assess the proportion of different size classes of POM available to filter feeders (pearl oysters and associated epibionts), samples were separated into 5 size classes: POM > 250 μm and 250 μm > POM > 80 μm (mesoplankton), 80 μm > POM > 20 μm (microplankton), 20 μm > POM > 2 μm (nanoplankton) and 2 μm > POM > 0.7 μm (picoplankton). Large POM (> 20 μm) was collected using a plankton net and several mesh size sieves (20, 80 and 250 μm). The material collected on each sieve was filtered on pre-combusted GF/F (4 h 500 °C). POM from 3 nets was pooled for each sample and three samples were collected per site. For POM < 20 μm, 18 L of seawater were sampled using a Niskin bottle and pre-filtered with a 20 μm sieve. Water was then filtered on 2 μm Nucleopore™ filters and the remaining water was finally passed through 0.7 μm GF/F filters to obtain the fraction > 0.7 μm and < 2 μm. POM collected on filters was freeze-dried before being analyzed.

The taxonomic composition of the living part of the POM was given by Hulot (2019) from a single sample at each site. Briefly, picoplankton was identified with flow cytometry (CytoFLEX) on 1.6 mL samples fixed with buffered formalin (2%) stored in liquid nitrogen. Bacteria cells were stained with SYBRGreen I (Molecular Probes, Eugene, OR, USA) while picophytoplankton was enumerated depending on their SSC properties and red and orange fluorescence. Samples for nanoplankton were filtered through black polycarbonate membrane filters (0,8 μm pore size, 25 mm diameter, Nuclepore), stained with DAPI and counted by epifluorescence microscopy. Autotrophic and heterotrophic nanoflagellates were enumerated in 2 size classes 2–10 μm and 10–20 μm. Microphytoplanktonic (20–80 μm) organisms were identified and counted using sedimentation chambers and an inverted microscope (Olympus IX70), following the Utermöhl method (Utermöhl, 1958) on samples fixed with 4% formaldehyde. Microzooplankton (ciliates) were counted using the same method on samples fixed with lugol (2%). Finally, samples for mesoplankton (> 80 μm) were collected on a 63 μm mesh net, fixed in 4% formaldehyde and identified and counted under a binocular microscope. Conversion of plankton abundance to carbon biomass was applied using appropriate references (Hulot, 2019, Table S1).

2.3. Stable isotope analysis

Muscle tissues of pearl oysters and whole organisms of epibionts (without shell for small bivalves) were analyzed for SI ratios. Tissues were thoroughly rinsed with filtered seawater (0.2 μm) to prevent any contamination by shell carbonates before being freeze-dried.

C and N stable isotope analyses were conducted on 1–5 mg of each sample. Samples were placed in tin capsules and acidified with 100 μl sulfuric acid (0.25 N) to remove any potential residual inorganic carbon, and dried at 60 °C, following the method of Raimbault et al. (2008). The isotopic composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) was then measured with a SI ratio mass spectrometer (INTEGRA CN, Sercon). Carbon and nitrogen SI ratios were expressed in conventional δ unit notation in relation to international standards (Vienna-PeeDee Belemnite for carbon; atmospheric N_2 for nitrogen), with the formula:

$$\delta X(‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where $X = {}^{13}C$ or ${}^{15}N$, and R is the corresponding ${}^{13}C$:${}^{12}C$ or ${}^{15}N$:${}^{14}N$ ratio. Analytical precision based on the standard deviation of replicates of internal and certified (IAEA) standards was 0.3‰ for nitrogen and 0.2‰ for carbon.

2.4. Data analysis

Variations of SI values were explored using a multivariate approach on normalized data. PERMANOVAs based on Euclidean distance matrices were run (9999 permutations) to test the effect of date, size class (for POM) or taxon (for epibionts) on SI values for each site separately. Multivariate dispersion of data was verified using the R version (package vegan) of the PERMDISP routine (Anderson et al., 2008).

It has been proposed that the variability of isotopic composition of a population or a species (i.e. its isotopic niche) can be used as a proxy to

Table 1
Sampling performed each date at the 2 sites (gray cells). Each size class (μm) of particulate organic matter (POM) was sampled in triplicate except for periods with no data ("na"). Number of epibionts and pearl oysters sampled is given for each taxa (n). Mean pearl oysters mass (wet flesh in g) is given below sample size. "abs" for absence of epibionts.

		Mangareva			Arutua		
		2016-09	2017-04	2017-11	2016-05	2016-11	2017-08
Season		Dry	Dry	Wet	Dry	Wet	Dry
POM (μm)	Pico (0.7 – 2)						
	Nano (2 – 20)						
	Micro (20 – 80)				na		
	Meso (80 – 250)						
	Meso (> 250)	na					
Epibionts (n)		abs	3 bivalves 3 sponges 3 ascidians	3 bivalves 3 sponges 3 ascidians	abs	abs	3 bivalves 3 sponges
Pearl oysters (n / g)		18 / 10.7±2.3	34 / 19.3±5.8	43 / 24.1±7.0	14 / 4.1±1.0	31 / 7.8±2.2	33 / 12.8±4.3

assess the trophic niche of this population or species, and/or the degree of individual specialization in the population (Jackson et al., 2011). Thus, trophic niche extent (or diet diversity) of pearl oysters and epibionts was compared using Bayesian standard ellipse area (SEA_B) as implemented in the R SIBER package (Stable Isotope Bayesian Ellipses in R; Jackson et al., 2011). Comparison of $\delta^{15}N$ and $\delta^{13}C$ of ascidians and small bivalves indicated low interspecific variations for both $\delta^{15}N$ and $\delta^{13}C$. Given the low number of samples for epibionts, we thus considered the pooled epibionts isotope data (ascidians and small bivalves) and only analysed Mangareva results. Since there is no test value in the Bayesian framework, ellipses were compared using probabilities tests (Jackson et al., 2011).

3. Results

3.1. Spatio-temporal characterization of the planktonic resource

While picophytoplankton represented 99% of the total plankton abundance in all samples, nanoplankton was largely dominant in terms of carbon biomass during the second campaign at Mangareva (ca. 80%) and during the 3 campaigns at Arutua (Fig. 2a,b). During campaigns 1 and 3 at Mangareva, picophytoplankton (mainly *Synechococcus* sp., Table S2) represented a high proportion of the relative biomass (> 40%, Fig. 2b) and mesoplankton contributed more than 25% to the relative carbon biomass during the first campaign (September, Fig. 2b), mainly due to the presence of copepods during this period. Total biomass increased continuously between campaigns 1 and 3 at Mangareva with values from 45 µgC l^{-1} to 135 µgC l^{-1} and was more constant at Arutua with ca. 98 µgC l^{-1} during campaign 2 and 146 µgC l^{-1} during campaigns 1 and 3 (Fig. 2c).

3.2. Variations of stable isotope signatures

POM $\delta^{13}C$ and $\delta^{15}N$ varied between -27.5 ± 0.4‰ and -19 ± 1.1‰ and 3.8 ± 0.3‰ and 10.6 ± 1.1‰ respectively, depending on the site and size class considered (Table 2). The interaction date × size had a significant effect at Mangareva ($df = 7$, $F = 11.990$, $p < 0.001$) and at Arutua ($df = 7$, $F = 4.211$, $p < 0.005$). Significant differences indicated an overall depletion in ^{13}C and ^{15}N for small POM compared with larger POM. At Mangareva, C3 showed lower $\delta^{15}N$ values compared with C1 & C2 for the largest particles (> 20 µm) (Table 2, Fig. S1). C/N ratios varied between 15.1 ± 2.0 and 4.3 ± 0.1, and showed the lowest values for mesoplankton (Table S3).

Pearl oysters SI values varied between -19.3 ± 0.2‰ and -17.4 ± 0.4‰ for $\delta^{13}C$ and between 3.4 ± 0.7‰ and 7.4 ± 0.7‰ for $\delta^{15}N$. No significant difference was observed between dates at Arutua ($df = 2$, $F = 2.177$, $p = 0.087$) whereas the 3 dates were significantly different at Mangareva ($df = 2$, $F = 85.535$, $p < 0.001$), with a progressive depletion in ^{13}C from C1 to C3 (Table 2, Fig. S1). We should note that the dispersion test was significant at Mangareva (ANOVA, $F = 19.308$, $p < 0.001$). Given the decreasing carbon values from C1 to C3 (Table 2, Fig. S1) it is however not unreasonable to state that there are both date and dispersion effects. The highest variability in values concerned $\delta^{15}N$ during C2 (Table 2, Fig. S1).

Difference of SI ratios was tested for epibionts at Mangareva at C2 and C3. Main taxonomic groups observed were *Herdmania* sp. for ascidians and *Pinctada maculata* for small bivalves. No significant effect of interaction (group × date: $df = 2$, $F = 0.242$, $p = 0.903$) or date ($df = 1$, $F = 0.845$, $p = 0.425$) was observed, but sponges were significantly different from other filter-feeders (group effect, $df = 2$, $F = 4.605$, $p = 0.01$). Sponges were both ^{13}C and ^{15}N enriched compared with ascidians and small bivalves (Table 2, Fig. S1). Sponges also showed the highest C/N ratio among epibionts (sponges > ascidians > small bivalves, Table S3). Pearl oysters presented the lowest values of C/N ratios (Table S3).

3.3. Diet diversity among filter-feeders

Bivariate standard ellipses (Fig. 3), representing core isotopic niches of consumers, show ellipses of pearl oysters and epibionts occupying different parts of the isotopic space with small overlapping (< 2%). Overall, pearl oysters are more enriched in ^{13}C and could also show high ^{15}N enrichment compared with epibionts (Fig. 3). Pearl oyster ellipses varied both along the carbon and nitrogen axes between the 3 campaigns whereas the position of epibiont ellipses was almost the same between the 2 campaigns.

Ellipse size of *pearl oysters* in the isotopic space changed drastically according to sampling dates, with the maximum width observed during C2. During this campaign, pearl oysters showed in particular high variations in their nitrogen signature. For pearl oysters, SEA_B calculations suggested that, in over 99% of model runs, the ellipse area of C1 and C3 was smaller than that of C2. For epibionts, only 55% of estimations suggest that SEA_B from C2 and C3 was different. Comparison of ellipses between pearl oysters and epibionts showed opposite results for the 2 campaigns, with a slightly larger ellipse of pearl oysters during C2 (63% of estimations) but larger SEA_B of epibionts compared with pearl oysters during C3 (93% of estimations).

4. Discussion

4.1. Isotopic characterization of the POM pool

To be efficient, the isotopic method requires food sources displaying contrasted SI composition. In this study, the carbon and nitrogen

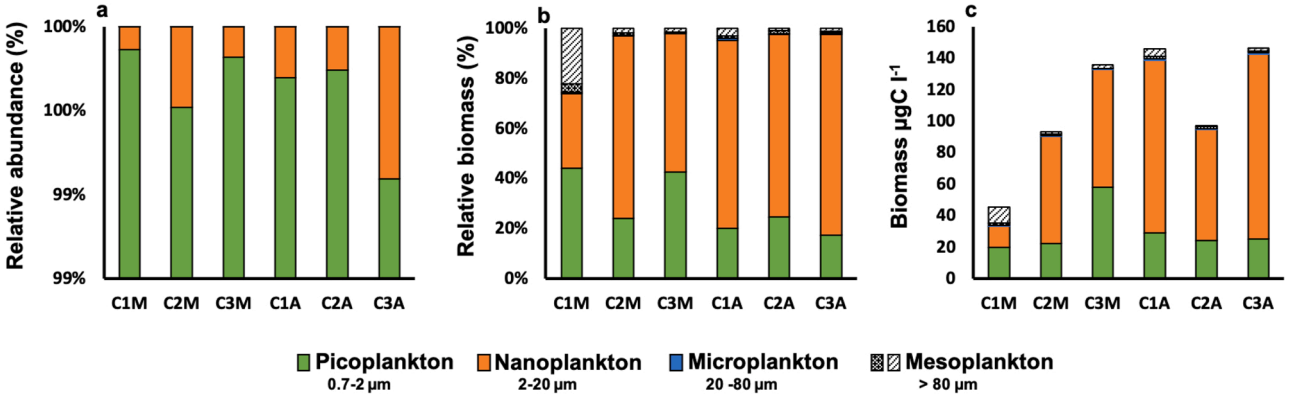

Fig. 2. (a) Relative abundance, (b) relative carbon biomass, and (c) total carbon biomass of the 5 size classes of living POM sampled during the 3 campaigns at Mangareva (C1M, C2M, C3M) and Arutua (C1A, C2A, C3A). Data from Hulot (2019). Conversion of plankton abundance to carbon biomass was applied using appropriate references (Hulot, 2019, Table S1).

Table 2
Mean ± standard deviation of isotopic values ($\delta^{13}C$ and $\delta^{15}N$, ‰) for pearl oysters, several epibionts and particulate organic matter (POM) as a proxy of plankton, collected in the lagoons of Mangareva and Arutua during the 3 campaigns (C1, C2, C3).

			C1		C2		C3	
			$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Mangareva	Pearl oysters		-17.4 ± 0.4	3.4 ± 0.7	-18.3 ± 0.4	6.2 ± 2.0	-19.3 ± 0.2	4.4 ± 0.4
	Epibionts	Ascidian	abs		-20 ± 1.6	3.4 ± 0.6	-20.7 ± 1.9	3.6 ± 0.4
		Sponge			-19.2 ± 0.8	4.3 ± 0.5	-19.4 ± 1.7	4.5 ± 0.2
		Bivalvia			-20.1 ± 0.2	3.6 ± 0.2	-21.2 ± 0.0	3.5 ± 0.2
	POM (µm)	Pico (0.7–2)	-25.0 ± 0.1	7.1 ± 0.5	-23.9 ± 1.1	4.7 ± 0.5	-27.5 ± 0.4	4.8 ± 0.7
		Nano (2–20)	-24.7 ± 0.5	4.7 ± 1.3	-25.2 ± 0.9	5.8 ± 0.3	-24.7 ± 0.3	5.3 ± 0.4
		Micro (20–80)	-19.0 ± 1.1	5.8 ± 0.6	-20.5 ± 0.3	5.7 ± 0.1	-21.4 ± 1.1	4.5 ± 0.8
		Meso (80–250)	-20.9 ± 0.6	10.2 ± 0.7	-21.6 ± 1.4	7.8 ± 0.3	-20.3 ± 1.1	3.8 ± 0.3
		Meso (>250)	na		-22.2 ± 1.2	8.7 ± 0.4	-21.0 ± 0.5	5.4 ± 0.3
Arutua	Pearl oysters		-17.7 ± 0.4	7.1 ± 1.4	-18 ± 0.5	7.1 ± 1.5	-17.9 ± 0.2	7.4 ± 0.7
	Epibionts	Sponge	abs				-18.2 ± 1.1	8.7 ± 1.2
		Bivalvia					-20.0 ± 0.2	5.9 ± 0.1
	POM (µm)	Pico (0.7–2)	na		na		-26.6 ± 0.6	6.2 ± 0.3
		Nano (2–20)	-26.4 ± 0.1	5.7 ± 1.1	-25.8 ± 2.8	6.7 ± 0.3	-23.8 ± 0.3	5.7 ± 0.5
		Micro (20–80)	na		-22.8 ± 1.9	6.7 ± 0.4	-20.5 ± 0.3	8.4 ± 1.5
		Meso (80–250)	-20.9 ± 0.4	10.6 ± 1.1	-20.2 ± 0.7	7.7 ± 0.7	-21.0 ± 0.3	8.6 ± 1.0
		Meso (>250)	-22.1 ± 0.3	8.8 ± 0.2	-20.4 ± 0.9	8.4 ± 0.3	-20.9 ± 0.8	10.6 ± 0.7

Note: C1, C2 and C3 are independent for the 2 sites. abs: absence of epibionts, na: contaminated samples not determined.

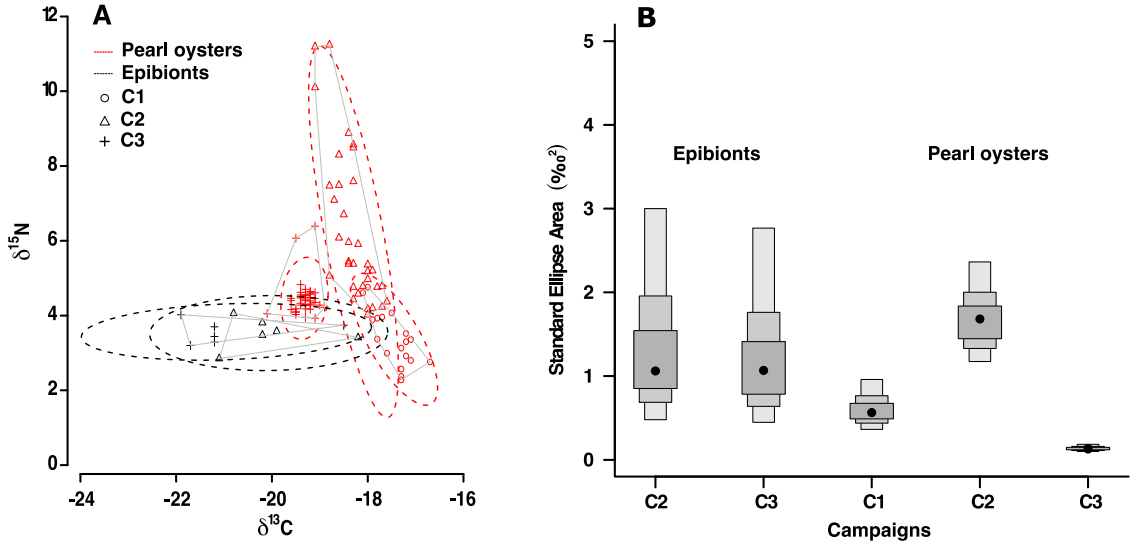

Fig. 3. A: Biplots of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic values of pearl oysters (red) and epibionts (black) during the 3 campaigns (C1, C2, C3) at Mangareva, representing the isotopic niche. Gray lines and ellipses represent respectively group hulls and the 95% confidence interval around the bivariate mean. B: Bayesian Standard Ellipse Area (SEA_B, ‰^{2}) for pearl oysters and epibionts. SEA_B are obtained from biplots of $\delta^{15}N$ and $\delta^{13}C$ values.

isotopic composition of POM fall within the range of values previously reported in tropical areas (Briand et al., 2015; Letourneur et al., 2013), with high variations in ^{13}C and ^{15}N enrichment, in relation with particle size. It is common that small particles are depleted in ^{13}C compared to large particles, due to different carbon fixation along the trophic levels and the balance auto-heterotrophy (Bănaru et al., 2014; Rau et al., 1990; Rolff, 2000). In this study, $\delta^{13}C$ especially discriminate particles < 20 µm and > 20 µm and most of the time larger POM fractions were also ^{15}N enriched. Enrichment in ^{15}N is usually explained as reflecting size-related consumption patterns in plankton food web (Rolff, 2000). This seems in line with the taxonomic composition of POM observed in this study (Table S2) since mesoplankton (> 80 µm) mainly comprised copepods and chaetognaths predators. Nano-sized particles comprised a mix of hetero- and autotroph nanoflagellates whereas pico-sized particles are mainly photosynthetic organisms. It should however be noted that the non-living part of POM (fecal pellets, degraded plankton) may also influence variations in isotopic signature (Bănaru et al., 2014) and C/N stoichiometry such that POM fractions may not represent exactly discrete trophic levels.

Mean isotopic signature of POM fractions was in the same range between the 2 sites. While isotopic values were constant at Arutua during the 3 campaigns,temporal variation was observed at Mangareva with lower $\delta^{15}N$ values during the third campaign. POM isotopic variations may reflect changes in the planktonic community composition (Briand et al., 2015) and nutrient forms and concentrations assimilated by phytoplankton, which may vary during the course of a phytoplankton bloom (Descolas-Gros and Fontugne, 1985; Savoye et al., 2003). The variability of POM isotopic signature in Mangareva may therefore come from more temporal variability in the plankton community composition compared with Arutua. The different contribution of the largest particles to the relative carbon biomass between the 3 campaigns at Mangareva support such changes in the composition of the plankton community. During a year of monitoring pearl oysters growth at Mangareva, Cochard et al. (2003) assumed a decrease of the trophic resource at the beginning of the winter period, that may arise from changes of the plankton concentration and/or community composition. Additionally to species-specific variations (), environmental conditions such as light, temperature or pH can also affect ^{15}N and ^{13}C fractionation pattern and

thus $\delta^{15}N$ and $\delta^{13}C$ signature in algae cell (Aberle and Malzahn, 2007; Montoya and Mccarthy, 1995; Thompson and Calvert, 1994). Thus, the geographic position of Mangareva, under oceanic influence and with a strongly marked seasonality (Cochard et al., 2003; Lacoste et al., 2014b; Pouvreau and Prasil, 2001) compared with the relative "stability" of environmental conditions in the Tuamotu lagoons, may also contribute to explain higher changes in the isotopic signature of POM. Data are however still scarce for these sites and a detailed explanation requires more information.

4.2. Resource use by pearl oysters and epibionts

Analysis of Mangareva data and the difference in the $\delta^{15}N$ and $\delta^{13}C$ signature between pearl oysters and epibionts give grounds for saying that they did not assimilate the organic constituent in the same proportion. This is in agreement with previous studies showing that filter-feeders clearly partition POM while receiving the same mixture from the water column (Dubois and Colombo, 2014; Richoux et al., 2014). Pearl oysters were ^{13}C and ^{15}N-enriched compared with epibionts, likely indicating an assimilation of large particles in greater proportion for pearl oysters. Isotopic differences between suspension-feeding invertebrates were already reported, due to interspecific differences in sorting abilities and preferential uptake of different POM components (Decottignies et al., 2007; Dubois et al., 2007; Lesser et al., 1992), firstly based on the size of food particles (Ward and Shumway, 2004). In this sense, our results seem to confirm an important contribution of nanoplankton to the diet of pearl oyster (Fournier et al., 2012a) given the high carbon biomass of this size class in the total plankton pool. Conversely, the depleted ^{13}C signature of epibionts suggests a high assimilation of smaller particles (picophytoplankton) which is the main constituent of POM in terms of particle abundance. The low overlap of isotopic niches between pearl oysters and epibionts also suggests a sufficient amount of POM for these communities such that in these trophic conditions, pearl oysters may select preferred particles in the POM pool whereas epibionts mainly capture small particles that are the most abundant in French Polynesian lagoons.

Epibionts are non-selective filter-feeders and mostly assimilate the most abundant fraction of POM that is available throughout the year (picophytoplankton), such that their trophic niche did not show high variability in time. This confirms previous observations (Lacoste et al., 2016) which showed POM consumption of two species of ascidians to be dependent on the POM pool in ambient water. Conversely, pearl oysters showed variations in their trophic niche both between dates (mainly $\delta^{13}C$) and a high variability between individuals at the same date at Mangareva (mainly $\delta^{15}N$). Dubois and Colombo (2014) hypothesize that species able to selectively sort particles based on their quality show smaller temporal variations of their trophic niche compared with non-selective species. However, changes in their own diet and/or changes of the basal trophic resource signature can modify the trophic niche of bivalves (Briant et al., 2018; Decottignies et al., 2007). Since we showed a relative variability in POM signature at Mangareva, it is suggested that temporal variability of pearl oysters isotopic niche is related to the variations of the basal resource isotopic signature and the selective feeding of pearl oysters in the pool of available food. More information on the time of assimilation in the pearl oyster tissues would help to better describe changes in their diet in relation with food sources variations in time.

A high capacity in particle selection is supposed to increase the size of the trophic niche, due to a broader spectrum of possible diet (Cresson et al., 2016; Dubois and Colombo, 2014). During this study, the trophic niche width of pearl oysters was alternatively bigger and smaller compared with epibionts, depending on the sampling period. A small trophic niche width could reflect a low diversity in the plankton pool and/or a strong affinity of all individuals for a specific component of the plankton pool, as observed during phytoplankton blooms in temperate areas (Decottignies et al., 2007; Dubois and Colombo, 2014). Although pearl oysters has already been shown to select particles based on their digestibility and nutritive potential (Loret et al., 2000; Yukihira et al., 1998), food selection by pearl oysters under natural conditions is not well described and requires further investigations. The larger trophic niche width observed during the second campaign could illustrate a higher diversity of food sources at this time and a variability in particle selection by pearl oysters. Oceanic influences and temperature variations could be responsible of variations in the composition of the plankton pool through the seasons, and thus modify the isotopic signature of pearl oysters. Further investigations are necessary to better describe seasonal variations of food sources in this area and the subsequent evolution of pearl oyster isotopic signature depending on their particle selection capacities. Finally, intra-specific variability in pearl oysters isotopic signature could also be linked with individual variations in the storage strategy. This could, for example, be a consequence of different reproductive status, as this species has been shown to be very asynchronous (Fournier et al., 2012b; Lacoste et al., 2014b; Le Moullac et al., 2012). The variability of pearl oysters C/N values during the three campaigns prevents however to be affirmative.

4.3. Perspectives

Whereas most studies rely on the characterization of sources of nutrition from bulk marine POM, we showed that SI ratios among discrete size classes of POM is of relevance to elucidate food sources and trophic relationships among suspension feeders in a tropical oligotrophic environment. While too much splitting may induce errors (and is time consuming), it seems not unreasonable to use 3 size classes: < 20 μm, 20–80 μm and > 80 μm, to elucidate the role of nanosized particles vs larger plankton in the diet of pearl oyster. Other food sources that have not yet been analyzed in French Polynesia could also be sampled such as microphytobenthos that could deposit on longline structures and pearl oysters shells.

Pearl oysters and epibionts have been shown to modify nutrient availability in the water column (Lacoste et al., 2014a) with potential consequences on the whole planktonic ecosystem. In bivalves, C/N ratio could be used as a proxy of lipid content and reflect the reproduction cycle (Briant et al., 2018; Post et al., 2007). Whereas reproduction of pearl oyster (and thus lipid storage) at Mangareva is supposed to be seasonal, the constancy of the C/N ratio between seasons might suggest adaptation in feeding and a regulation of N content through variations in the excretion of this product to maintain a constant ratio of C/N in the tissues (Bayne, 2009). On the other hand, epibionts may release more N than pearl oysters in the environment as reflected by their higher tissue C/N. Such differences between filter-feeders and the likely seasonal variation in nutrient excretion by pearl oysters could thus modify nutrient stoichiometry and add to the complexity of interactions between pearl farming and the environment. To further explore the relationships between pearl oysters, epibionts and the environment (water column and benthic compartment), we could recommend measurement of biodeposits and dissolved excretion products C/N ratios from pearl oysters and epibionts at different seasons and for different trophic conditions.

CRediT authorship contribution statement

Élise Lacoste: Methodology, Formal analysis, Writing - Original draft, **Nabila Gaertner-Mazouni**: Resources, Writing - review & editing, **Patrick Raimbault**: Supervision, Investigation, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2021.100887.

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