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Effects of oyster age on selective suspension-feeding and the chemical composition of biodeposits: insights from fatty acid analysis

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ABSTRACT: The study objective was to clarify how the growth stages of the Pacific oyster *Crassostrea gigas* affect selective suspension-feeding of particulate organic matter (POM) and the composition of biodeposits. A day-long (22 h), continuous-flow mesocosm experiment was conducted with 3, 15, and 27 mo old oysters. The suspended particulate matter (PM), settled PM (mostly biodeposits in the oyster mesocosms), and oyster soft tissues were analysed to determine the content of fatty acids, organic carbon, and nitrogen, as well as the carbon and nitrogen stable isotope ratios to trace compositional changes in POM through oyster biodeposition. Regardless of oyster age, the stable isotope ratios of biodeposits were similar to those of the body tissues but not to those of the suspended PM, indicating that oysters selectively fed on assimilable fractions of POM. Compared with the suspended PM, a higher concentration of long-chain polyunsaturated fatty acids was found in the body tissues and, consequently, in the biodeposits; in contrast, the concentrations of shorter-chain fatty acids were generally lower in the biodeposits. Furthermore, the biodeposits produced by the older oysters had higher carbon, nitrogen, and fatty acid contents compared with the biodeposits produced by the 3 mo old oysters. The oxygen consumption rate of biodeposits was positively related to organic carbon content, but less so to fatty acid composition. Our findings demonstrate that older oysters not only produce larger amounts of biodeposits, but that these biodeposits have higher organic and fatty acid contents, potentially exhibiting greater effects on biogeochemical and ecological processes in nearby benthic habitats.

KEY WORDS: *Crassostrea gigas* · Eicosapentaenoic acid · EPA · Docosahexaenoic acid · DHA · Trophic marker · Assimilation efficiency · Size fraction · Oxygen depletion · Suspended aquaculture

1. INTRODUCTION

Suspension-feeding macro-sized consumers collect particulate organic matter (POM) from the water columns and reject some POM as pseudofaeces or egest it as faeces, which eventually reaches the sea floor as biodeposits. Such processes substantially affect various biogeochemical and ecological processes in coastal marine systems (Vaughn & Hoellein 2018). For instance, suspension-feeding by macroconsumers has been reported to significantly reduce the POM, including phytoplankton, in coastal waters (e.g. Cloern 1982, Greene et al. 2011), and further affect material budgets in coastal systems, particularly when coastal waters have longer hydraulic residence times (e.g. Rowe et al. 2017). Biodeposits produced by suspension-feeding consumers further alter chemical properties of sediments and nutrient cy-

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cling at the sediment–water interface (e.g. Newell et al. 2002, Hoellein et al. 2015, Smyth et al. 2016). Pollet et al. (2015) experimentally demonstrated that mussel biodeposits altered the metabolic activities of benthic prokaryotic communities and their functional diversity in terms of organic matter metabolism.

Aquacultured bivalves also produce substantial biodeposits and notably alter the dynamics of POM and nutrients in both the water column and bottom sediment (e.g. Newell 2004, Forrest et al. 2009). Because bivalves are generally cultivated at much higher densities than in their natural habitats, they greatly change the local biogeochemical processes in the vicinity of farming facilities (e.g. McKindsey et al. 2006). Furthermore, bivalve aquaculture has been reported to reduce phytoplankton and weaken the energy transfers to higher trophic levels (e.g. Guyondet et al. 2013, Umehara et al. 2018). Particularly in locations where the currents and waves are weak, biodeposits from aquacultured bivalves excessively accumulate in sediments, increase oxygen consumption, increase hydrogen sulphide emission (e.g. Hatcher et al. 1994, Giles et al. 2006), and adversely affect benthic communities (e.g. Grant et al. 1995, Nugues et al. 1996). However, a low-level addition of biodeposits by suspension-feeders can enhance the production of benthic consumers due to the increase in available food resources (Lu & Grant 2008).

The chemical composition of POM greatly affects biogeochemical and ecological processes of coastal marine systems. For instance, the stoichiometric compositions of suspended POM (including phytoplankton) greatly affect the productivity of zooplankton in marine and freshwater systems (e.g. Sterner & Elser 2002). The chemical composition and origin of sedimentary organic matter also affect nutrient cycling at the sediment–water interface (e.g. Eyre et al. 2013, Kelaher et al. 2013) and dietary use by benthic consumers (e.g. Darnaude et al. 2004, Sakamaki et al. 2010). Thus, the roles of suspension-feeders in biogeochemical and ecological processes need to be evaluated with respect to their qualitative and quantitative alterations of POM. In addition, bulk POM in coastal marine systems consists of POM with various spatial (e.g. river or offshore) and biological (e.g. bacteria, algae, or higher plant) origins. The feeding and metabolism of POM by local consumers are expected to differ according to the properties of POM, and suspension-feeders presumably selectively utilise POM and modify the composition of the surrounding POM via their feeding and biodeposition processes.

Suspension-feeding and biodeposition processes are considered to change ontogenetically, because consumption and assimilation rates generally depend on consumer age or body size, or both (Maino & Kearney 2015). Bivalves with larger body sizes produce higher amounts of biodeposits, and also biodeposits with larger grain sizes and higher settling velocities (e.g. Callier et al. 2006, Ehrich & Harris 2015). It remains unclear how suspension-feeders alter the composition of POM through feeding and biodeposition or how the ontogenetic changes in these processes occur. The synthetic, empirical modelling study of Maino & Kearney (2015) demonstrated that both consumption and assimilation rates ontogenetically increased with consumer body mass, but the assimilation rate did not increase with ontogenetic mass to the same degree as the consumption rate. Furthermore, lipase, a digestive enzyme that acts in lipid hydrolysis, has been shown to be less active in older scallops than in younger individuals (Pichaud et al. 2009). Such a decrease in enzymatic activity with growth might increase the egestion rate of undigested organic materials (Wotton & Malmqvist 2001). These findings suggest that the contents of nutritional, organic components in biodeposits possibly increase with the growth of suspension-feeders. However, the feeding organs of consumers, such as gills and palps, develop ontogenetically, and the physical and chemical properties of ingestible materials also differ according to the different growth stages (e.g. Hentschel 1996, Cannuel & Beninger 2006, Rosa & Padilla 2020). Thus, pre-ingestive selection by suspension feeders and the composition of POM excluded as pseudofaeces may also change with ontogeny, potentially complicating the ontogenetic response of biodeposit composition.

Numerous studies have investigated the biogeochemical and ecological effects of suspension-feeders with a focus on the quantitative alterations of POM fluxes; many of these studies applied element-based analyses involving carbon and nitrogen. However, those elements do not have specificity for biological origins, and analysis based on a few elements is not considered to provide sufficient information to describe the wide variation in the chemical composition of POM. Meanwhile, the fatty acids, being a major and ubiquitous type of biomolecule, include various compounds with different numbers of carbon atoms and unsaturated bonds. Some fatty acids that are produced by only a limited group of producers have been used as tracers to estimate the relative contributions of POM of different biological origins to bulk POM (e.g. Mayzaud et al. 1989, Canuel 2001). In addition, essential fatty acids, although they are not completely conserved between trophic levels, flow

through food chains and can be used as trophic markers in food web analyses (e.g. Meziane & Tsuchiya 2000, Kelly & Scheibling 2012). As fatty acids can be simultaneously analysed using gas chromatography, they have the potential to conveniently provide multidimensional information of POM composition, as well as its compositional change through the feeding processes of consumers.

Evidence from previous studies has supported that the fatty acid composition of sediment organic matter affects the community structure of benthic micro- and macro-organisms (e.g. Quintana et al. 2015, Fujibayashi et al. 2019). In addition, the fatty acid composition of POM is associated with its lability; for instance, highly labile material derived from microalgae is generally high in polyunsaturated fatty acid content (e.g. Wakeham et al. 1997, Quintana et al. 2015). These results further suggest that biogeochemical processes of POM, such as oxygen consumption and nutrient regeneration, are also potentially linked with fatty acid dynamics. Thus, a better understanding of the compositional changes of POM associated with suspension-feeding and biodeposition by macroconsumers from the perspective of fatty acid dynamics has the potential to enhance our understanding of biogeochemical processes in coastal marine systems and ecological roles of suspension-feeders. The objectives of this study were to elucidate how the growth stages of oysters affect their selective suspension-feeding of POM and quality of their biodeposits particularly in terms of fatty acid composition. In particular, we hypothesised that the fatty acid content of biodeposits would increase with oyster age, based on previous studies showing an ontogenetic decrease in assimilation efficiency. To test this, we conducted a 22 h mesocosm field experiment involving 3 age classes of Pacific oysters *Crassostrea gigas* and conducted fatty acid analyses of the POM samples from the mesocosms to trace compositional changes in POM via suspension-feeding and biodeposition.

2. MATERIALS AND METHODS

2.1. Mesocosm experiment

Continuous-flow mesocosms were established in a fishing harbour of Shizugawa Bay (38° 56′ N, 27° 07′ E) on the northeast coast of Honshu, Japan, in early winter (25–26 November) of 2015. The mesocosms were made of a transparent plastic sheeting that were shaped into 130 l cylinders with plastic frames. A 2 l sediment trap was connected to the centre of the bottom of each mesocosm to collect the settled particulate matter (PM). A total of 12 mesocosms were deployed on the sides of a fishing boat moored in the harbour. Seawater was pumped up to a head tank placed on the boat and flowed continuously into each mesocosm at a flow rate of 120 l h^{-1}. Seawater overflowed from 2 holes of each mesocosm at several centimetres above the surrounding water level.

Pacific oysters of 3 age classes, 3 (Oys-3), 15 (Oys-15), and 27 (Oys-27) mo old, were collected from oyster farms in the bay. All oysters used in the experiment were acclimated for 1 wk in the harbour where the mesocosm experiment was conducted and were introduced into the mesocosms at the start of the experiment. Triplicate mesocosms were established for each oyster age class (Oys-3, Oys-15, and Oys-27) and for the control treatment, which did not contain any oysters. Oysters weighing 1.3 to 1.7 kg (wet mass of oysters including shells), which comprised 220 ± 20, 20 ± 0, and 10 ± 0 ind. (mean ± SD) for Oys-3, Oys-15, and Oys-27, respectively, were put into baskets made of 1 cm mesh plastic and introduced to the experimental mesocosms (Table S1 in Supplement 1 at www.int-res.com/articles/suppl/m644p075_supp.pdf). The oysters had a shell length of 3.4 ± 0.4, 8.5 ± 2.1, and 15.0 ± 1.7 cm for Oys-3, Oys-15, and Oys-27, respectively. The dry mass (DM) of the soft tissues of oysters was 0.09 ± 0.03, 1.41 ± 0.38, and 2.86 ± 0.69 g for Oys-3, Oys-15, and Oys-27, respectively.

Oyster biodeposits were collected for 22 h, beginning at 14:30 h on 25 November 2015. The water temperature during the mesocosm experiment ranged from 12.6 to 13.1°C. Seawater samples were collected from the head tank and mesocosms at the beginning and end of the mesocosm operations. The collected seawater was immediately filtered through 250 µm sieves on site and stored in portable tanks. Settled PM was collected in the sediment traps immediately after the operation. In addition, to compare the chemical composition of oyster soft body tissues between the 3 age classes, 3 oysters were randomly collected from each mesocosm and dissected to obtain their soft tissues.

Suspended PM in the seawater samples was filtered with precombusted glass fibre filters (Whatman GF/F, pore size ~0.7 µm) using low-vacuum pressure aspirators (<100 mm Hg) in a temporary laboratory near the harbour. For size-fractionated analyses of the suspended PM, seawater samples from the control and Oys-15 treatments were also filtered through 75 µm and 20 µm sieves as well as membrane filters with a pore size of 2 µm. The size-fractionated suspended PM was collected on precombusted GF/Fs. Using

these filtration treatments, suspended PM in 4 size fractions, 0.7–2.0, 0.7–20, 0.7–75, and 0.7–250 µm, was sampled. All filtered samples were immediately preserved in a –30°C freezer for chemical analyses.

Settled PM retrieved from each mesocosm was separated into 2 subsamples; one was collected using precombusted GF/Fs for chemical analyses, and the other was used to measure their oxygen consumption rate. The PM samples were immediately transported in an ice box to the laboratory.

2.2. Measurement of oxygen consumption rate of biodeposits

The oxygen consumption rates of settled PM collected from the mesocosms were measured in the laboratory less than 1 d after the samples were collected. Approximately 1 g wet weight of the settled PM from each mesocosm was enclosed in a 100 ml Erlenmeyer flask filled with oxygen-saturated seawater and fitted with an oxygen sensor spot (SP-PSt3-YAU-D5, PreSens). The flasks were shaken with a shaker at 100 rpm and incubated at 20°C in the dark for 6 h. The dissolved oxygen concentration of seawater in the flasks was non-destructively measured using an oxygen sensor (Fibox3, PreSens) every hour throughout the incubation period. As the decrease in dissolved oxygen concentration in the vials was found to be a zero-order reaction, the oxygen consumption rate was estimated from the slope obtained in univariate regressions for oxygen concentration vs. time.

2.3. Chemical analyses

The samples of oyster soft tissues as well as the samples of suspended and settled PM for analyses of fatty acids were freeze-dried in the laboratory. The one-step method proposed by Abdulkadir & Tsuchiya (2008) was applied to the freeze-dried samples to prepare the fatty acid methyl esters (FAMEs). Tricosanoic acid was used as an internal standard to quantify each fatty acid. The FAME composition was analysed using a gas chromatograph (GC2014, Shimadzu) equipped with a capillary column (Select-FAME, 0.25 mm, 100 m; Agilent) under the analytical conditions reported by Fujibayashi et al. (2018). To identify the fatty acid peaks in chromatography, retention times of FAMEs in commercial authentic standard mixtures were compared with those of the peaks from the samples (Fujibayashi et al. 2018).

In advance of the analyses of the carbon and nitrogen content and their stable isotope ratios, the samples of suspended PM and settled PM on the GF/Fs were acidified by dropping 10% HCl thoroughly over the filters to remove the carbonate, rinsed by filtering with deionized water, and oven-dried at 60°C. Those filter samples and oyster tissue samples were analysed using a stable isotope ratio mass spectrometer equipped with an elemental analyser (Delta-V + Flash2000, Thermo Scientific). The carbon and nitrogen stable isotope ratios were noted using $\delta^{13}C$ and $\delta^{15}N$ (‰).

2.4. Data analysis

When analysing the fatty acid data, we focussed on 20 specific fatty acids found in the suspended PM, settled PM, and oyster tissues, including 5 nutritionally important essential fatty acids (e.g. Kainz et al. 2004, Glencross 2009) and other fatty acids commonly used as markers to assess the relative contributions of organic matter with different biological origins (e.g. Parrish 2013 and references therein, Kelly & Scheibling 2012) (Table 1; Supplement 2).

Differences in the chemical properties of the suspended PM, settled PM, and oyster tissues between the 3 oyster ages were tested using a 1-way ANOVA. The settled PM in the oyster mesocosms was considered to consist of not only oyster biodeposits but also PM via the gravitational deposition. Thus, the chemical properties (i.e. the content of C, N, and fatty acids, the stable isotopic ratios [$\delta^{13}C$ and $\delta^{15}N$], and the oxygen consumption rate) of the oyster biodeposit fraction in the settled PM of the oyster mesocosms were estimated based on the assumption that the settled PM in the control mesocosms was similar to the PM gravitationally settled in the oyster mesocosms. Likewise, the content of chemical constituents and the stable isotopic signatures of the fraction of POM ingested by the oysters were estimated based on the assumption that the difference in the concentration of suspended PM between the control and oyster mesocosms was all due to the suspension-feeding by the oysters. To assess the quantitative effects of oysters on POM, the rate of suspended PM removal from the water column by individual oysters and the rate of biodeposit production by individual oysters were estimated from the differences in the suspended/settled PM between the control and oyster mesocosms. For details of the estimation methods, including equations, see Supplements 3 & 4.

Table 1. Fatty acids (FAs) selected for statistical analysis. See Supplement 2 for more details and references

FA	Biomarker for:	Essential FA
Sum of branched-chain saturated FAs, *i*15:0, *ai*15:0, *i*17:0, and *ai*17:0	Bacteria	
16:1ω7	Diatoms, bacteria	
18:1ω7	Bacteria, cyanobacteria	
18:2ω6 (LOA)	Green algae, vascular plants, cyanobacteria, terrestrial inputs	Yes
18:3ω3 (LNA)	Green algae, vascular plants, cyanobacteria terrestrial inputs	Yes
18:4ω3	Cryptophytes, haptophytes, dinoflagellates, cryptomonads, seagrass, kelp	
20:1 and 22:1[a]	Zooplankton	
20:4ω6 (ARA)	Macroalgae, Rhodophyta, Phaeophyta	Yes
20:5ω3 (EPA)	Diatoms	Yes
22:6ω3 (DHA)	Dinoflagellates	Yes
Sum of long-chain FAs, 24:0, 25:0, 26:0, 27:0, 28:0, 30:0, and 31:0	Vascular plants, terrestrial plants	

[a]We used only 20:1ω9 because 22:1 was not identified in this study

To assess the change in carbon, nitrogen, and fatty acid contents in PM through suspension-feeding and biodeposition processes, we calculated the content ratio, defined as the ratio of content of each focal chemical constituent in biodeposits or oyster tissues to those in the suspended PM collected from the head tank. The content ratio was calculated in 2 cases; both the numerator (settled PM or oyster tissues) and denominator (suspended PM in the head tank) in the calculation of the ratio were the chemical contents per unit DM and per unit carbon.

To estimate the concentrations of particulate organic carbon and nitrogen and fatty acids in 4 size fractions (0.7–2.0, 2–20, 20–75, and 75–250 µm) in the water of the control and Oys-15 mesocosms, some of the fractions were estimated by subtracting one fraction from another; for example, the 20–75 µm fraction was determined as the difference between the concentrations of the 0.7–75 and 0.7–20 µm fraction samples. To compare the fatty acid composition between the size fractions, principal component analyses (PCAs) were conducted. Furthermore, PCAs were conducted to compare the fatty acid composition between the suspended PM, settled PM, and oyster tissues. Because the absolute fatty acid contents markedly differed between the size fractions as well as between the suspended PM, settled PM, and oyster tissues, the percentage of each type of fatty acid to the total studied fatty acids (n = 20), i.e. the relative abundances of the fatty acids, were used as the PCA variables to properly compare the fatty acid composition among the samples.

Regression analyses were used to examine the effects of the quantity and quality of organic matter contained in the settled PM on their oxygen consumption. Regression analyses were performed for the 2 cases: the oxygen consumption rate (dependent variable) and the content of each chemical component (explanatory variable) per unit DM of the settled PM or per unit carbon in the settled PM. Furthermore, we evaluated how the age-dependent quantitative and qualitative changes in the oyster biodeposits affected their short-term oxygen consumption. The hourly increment in the oxygen consumption rate of biodeposits produced by an individual oyster of each age class was estimated by multiplying the oxygen consumption rate of the biodeposits by the biodeposit production rate.

All statistical analyses were conducted using R (ver. 3.1.3).

3. RESULTS

The amount of settled PM at the end of the mesocosm experiment was 7.2 ± 1.3, 7.1 ± 1.9, and 3.8 ± 0.6 g DM (mean ± SE, n = 3) in Oys-3, Oys-15, and Oys-27, respectively, compared to 0.3 ± 0.1 g DM in the control. For the settled PM, the mean content of most of the fatty acids was lower in all oyster age mesocosms than in the control mesocosms. The estimated content of the fatty acids in oyster biodeposits was generally up to 30% lower than the fatty acids from the settled PM but by a maximum of 87% for 18:2ω6 (Fig. S1 in Supplement 3). However, the rela-

tive abundance of the fatty acids showed less difference between estimates for oyster biodeposits and the settled PM (Fig. S2). The estimates of the stable isotopic signatures and oxygen consumption rate of the oyster biodeposits did not differ from the settled PM (Tables S2 & S3).

The concentration of suspended PM in the water column at the end of the mesocosm experiment was 2.82 ± 0.17, 3.65 ± 0.50, and 4.45 ± 0.60 mg DM l^{-1} (mean ± SE) in Oys-3, Oys-15, and Oys-27, respectively, compared to 6.51 ± 0.24 mg DM l^{-1} in the control. The estimated contents of organic carbon, 18:2ω6, 18:3ω3, 20:1ω9, 20:4ω6, 20:5ω3, and 22:6ω3 in the PM selectively ingested by the oysters were estimated to be higher than those in the suspended PM collected from the oyster mesocosms (Fig. S1). Conversely, the estimated contents of branched saturated fatty acids (BSFAs), 16:1ω7, 18:1ω7, and long-chain fatty acids (LCFAs) in the suspended POM selectively ingested by oysters were lower than those in the suspended PM from the oyster mesocosms. The estimated stable isotope ratios of carbon and nitrogen in suspended PM selectively ingested by oysters were similar to the ratios in the suspended PM from the oyster mesocosms (Table S2).

The Oys-3 oysters showed much lower rates of both suspended PM removal from the water column and biodeposit production than the Oys-15 and Oys-27 oysters (Table S4 in Supplement 4). The content of carbon, nitrogen, and fatty acids in the suspended PM did not differ significantly among the 3 oyster age treatments (ANOVA: $F_{2,6}$ = 0.01–1.09, p = 0.39–0.99) (Fig. 1). However, the contents of BSFAs, 16:1ω7, and 18:1ω7 in the suspended PM were generally higher in the oyster mesocosms than in the control. In the settled PM, the contents of carbon, 18:2ω6, 18:3ω3, 18:4ω3, and LCFAs were significantly lower in the mesocosms with younger oysters than in those with older oysters (ANOVA: $F_{2,6}$ = 5.3–15.3, p = 0.004–0.047). In addition, the mean content of all chemical constituents of settled PM was lower in the Oys-3 mesocosms than in the Oys-15 and Oys-27 ones. Meanwhile, the concentration of fatty acids with 20 or more carbon bounds ($C_{\geq 20}$) in the tissues of younger oysters was significantly higher than in older oysters (ANOVA: $F_{2,24}$ = 4.6–18.7, p < 0.001–0.047).

Both DM and carbon-based contents of the C_{16} or C_{18} fatty acids in the settled PM was lower than that in the suspended PM entering the mesocosms (fatty acid content ratio of <1 for settled PM to suspended PM), whereas the content of the C_{20} or C_{22} fatty acids in the settled PM was higher than that in the suspended PM (ratio >1) (Fig. 2). The content of all fatty acids, except for 16:1ω7 and LCFAs, was higher in the oyster tissues than in the suspended PM (ratio of the fatty acid content of oyster tissues to suspended PM was >1). In particular, the content of the unsaturated fatty acids with C_{20} or C_{22} was substantially higher in the oyster tissues than in the suspended PM. In contrast, the contents of 16:1ω7 and LCFAs in the oyster tissues were slightly lower than those in the suspended PM.

The particle-size-fraction analysis of the suspended PM from the control and Oys-15 experiment revealed that the concentrations of organic carbon, nitrogen, and all fatty acids, except for 16:1ω7, 20:4ω6 and LCFAs, were significantly different between the size fractions (ANOVA: $F_{3,14}$ = 6.1–56.6, p < 0.0071) and were particularly higher in the 2–20 µm fraction than in the other size fractions (Fig. S3 in Supplement 5). The C/N ratio of the suspended PM increased with particle size (ANOVA: $F_{3,13}$ = 23.9, p < 0.001). The mean concentrations of organic carbon and all fatty acids in the 2–20 µm fraction were lower in the Oys-15 mesocosms than in the control. The fatty acid composition also differed between the size fractions of suspended PM (Fig. 3). The 2 smaller size fractions of suspended PM, 0.7–2.0 and 2–20 µm, had higher relative contents of 16:1ω7, 18:1ω7, and 18:4ω3 compared with other fatty acids. The 20–75 µm fraction had a higher relative content of LCFAs. The largest fraction, 75–250 µm, had higher relative contents of 18:2ω6, 20:1ω9, 20:4ω6, 20:5ω3, and 22:6ω3.

The fatty acid composition distinctly differed between the suspended PM, settled PM, and oyster tissues (Fig. 4). In particular, the oyster tissues had high relative contents of 20:5ω3 and 22:6ω3, whereas the suspended PM was higher in 16:1ω7, 18:1ω7, and 18:3ω3. These 5 fatty acids were found in relative contents in the order of suspended PM < settled PM < oyster tissues, or oyster tissues < settled PM < suspended PM. The settled PM was also relatively higher in other fatty acids such as 18:2ω6, 20:1ω9, and 20:4ω6. Meanwhile, in the oyster mesocosms, both the $\delta^{13}C$ and $\delta^{15}N$ of the settled PM overlapped with those of the oyster tissues (Fig. 5). Both the $\delta^{13}C$ and $\delta^{15}N$ were generally found in the following order, suspended PM < settled PM < oyster tissues.

The relationship between the oxygen consumption rate of the settled PM and the contents of the chemical constituents was more significant in the regression analyses based on chemical contents per unit DM, than in those based on chemical contents per

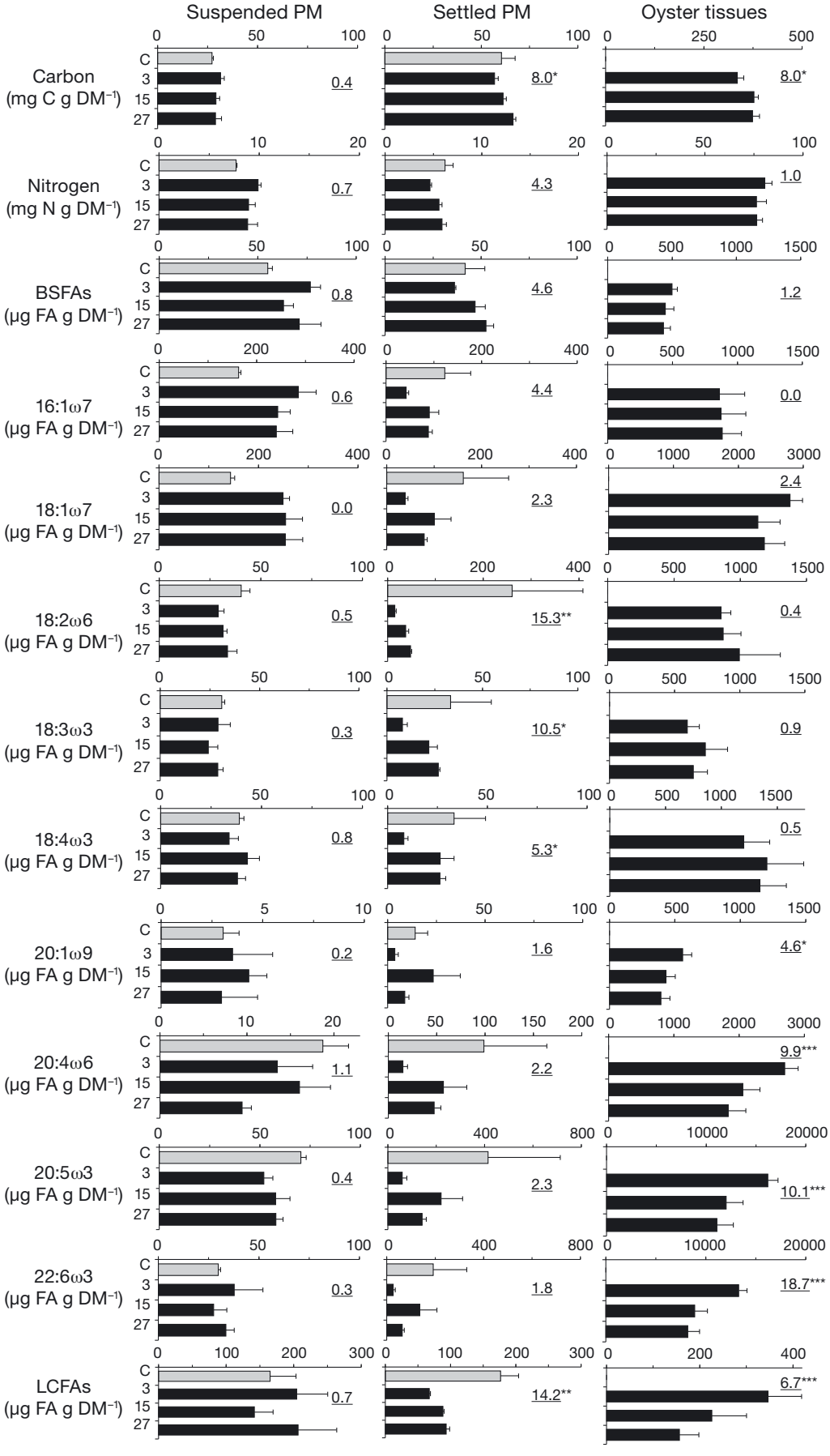

Fig. 1. Content per dry mass (DM) of carbon, nitrogen, and fatty acids (BSFAs: branched-chain saturated fatty acids, LCFAs: long-chain fatty acids) in suspended particulate matter (PM), settled PM, and oyster soft tissue at the end of mesocosm experiment. Data are mean ± SE (n = 3 mesocosms treatment^{-1}). Underlined values in the panels are *F*-values from 1-way ANOVA, which tested the effect of oyster age: 3, 15, or 27 mo, excluding the control (C). df in the *F*-value calculations were (2, 6), (2, 6), and (2, 24) for the suspended PM, settled PM, and oyster tissues, respectively. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

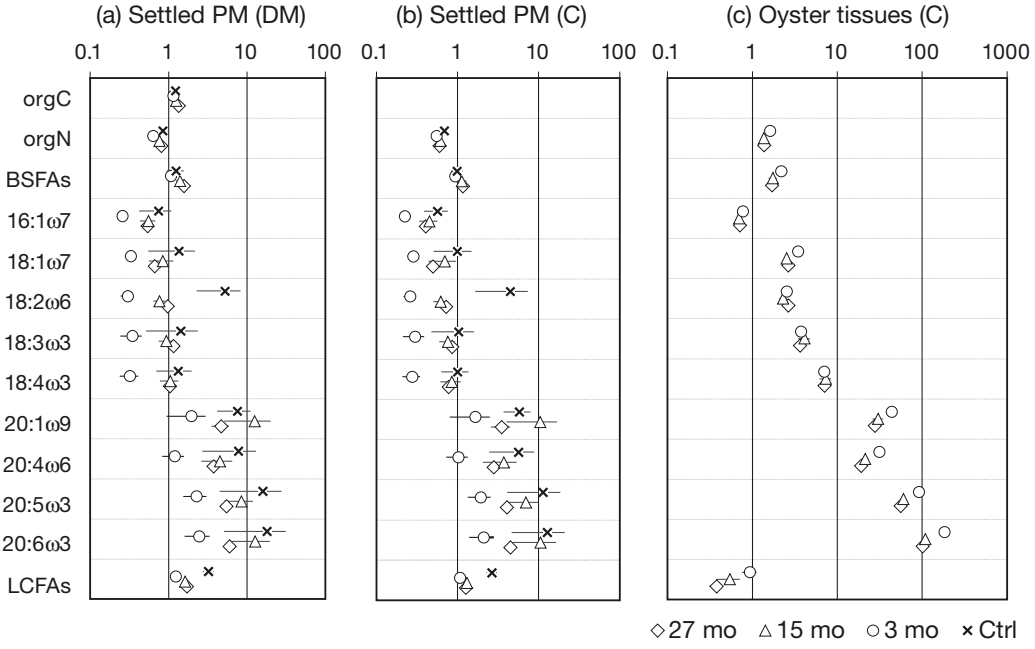

Fig. 2. Ratios of organic carbon (orgC), organic nitrogen (orgN), and fatty acid content (BSFAs: branched-chain saturated fatty acids, LCFAs: long-chain fatty acids) in biodeposits or oyster tissues vs. those in suspended particulate matter (PM) of the head tank. (a) Settled PM vs. suspended PM based on the contents per unit dry mass (DM); (b) settled PM vs. suspended PM based on the contents per unit carbon (C); and (c) oyster soft tissues vs. suspended PM based on the contents per unit carbon (C). Data are mean ± SE (n = 3 mesocosms treatment^{-1})

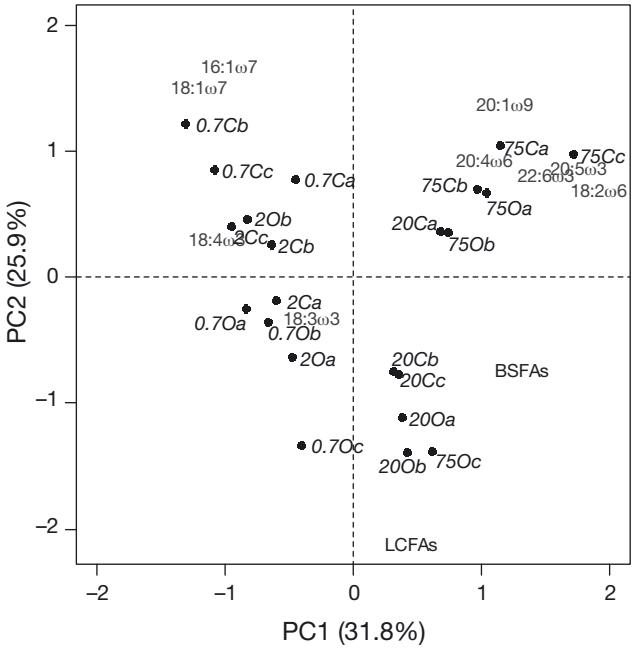

Fig. 3. Relative abundance of fatty acids in size-fractionated suspended particulate matter in the water columns of the mesocosms at the end of the 22 h experiment. 0.7: 0.7–2.0 µm, 2: 2–20 µm, 20: 20–75 µm, 75: 75–250 µm fraction, O: 15 mo old oysters introduced, C: control with no oysters, a–c: triplicate mesocosms for each treatment

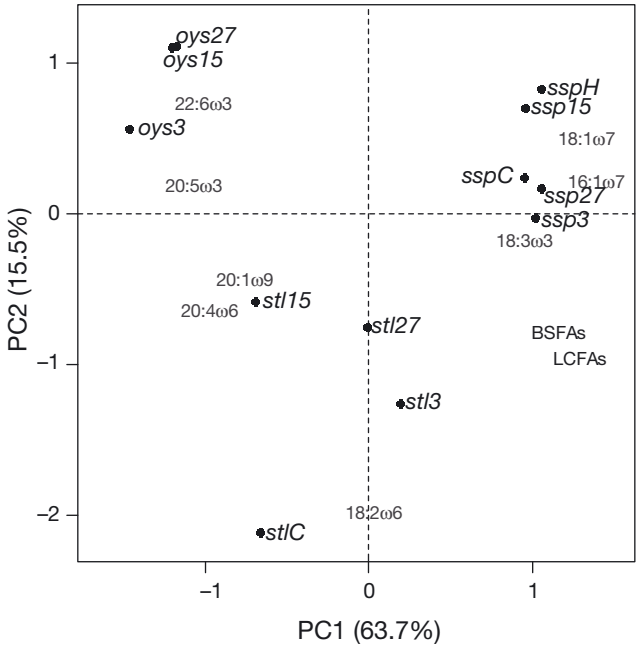

Fig. 4. Correlations between the fatty acids and the characteristics of fatty acid composition of suspended particulate matter (ssp), settled particulate matter (stl), and oyster soft tissues (oys) at the end of the mesocosm experiment, as revealed by PCA. In the sample labels, 3, 15, and 27: oyster age (mo); C: control with no oyster; H: inflow seawater from the head tank

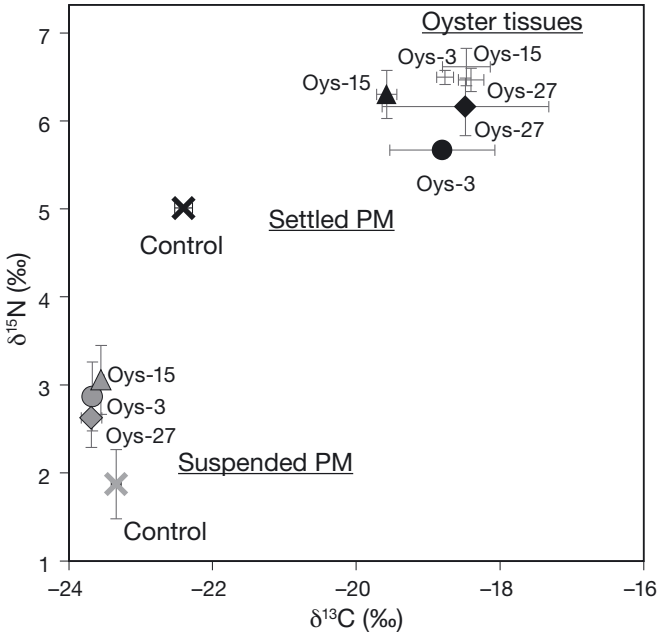

Fig. 5. Stable isotope ratios of carbon and nitrogen of suspended particulate matter (PM), settled PM, and oyster soft tissues at the end of the mesocosm experiment. Data are mean ± SE (n = 3 mesocosms treatment^{-1})

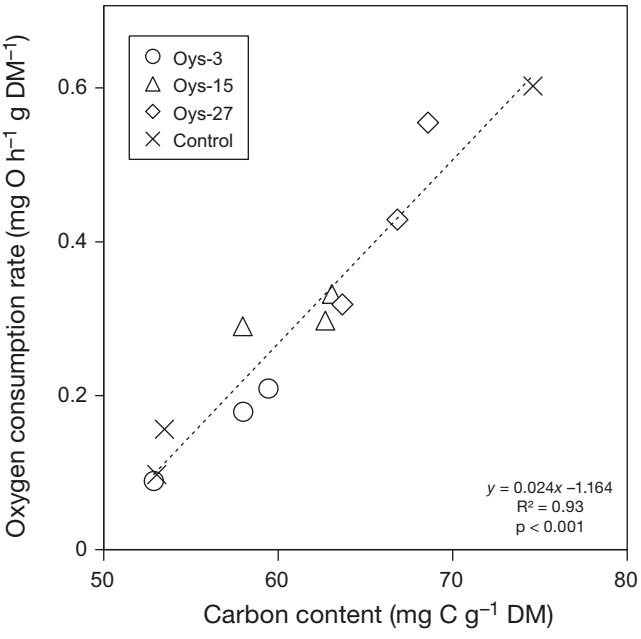

Fig. 6. Relationship between the organic carbon content of settled particulate matter (PM) and oxygen consumption rate for each oyster age class: 3, 15, and 27 mo old, and for the control treatment, which did not contain any oysters

Table 2. Results of univariate regression analyses for the oxygen consumption rate of settled particulate matter (dependent variable) vs. the organic carbon, organic nitrogen, and fatty acid content (explanatory variables). R^2 values are shown for 2 cases of regressions: both dependent and explanatory variables are per unit dry mass (mg DM^{-1}) and per unit carbon (mg C^{-1}). ***p < 0.001, **p < 0.01, *p < 0.05

Explanatory variables	Per unit DM	Per unit C
Organic carbon	0.93***	
Organic nitrogen	0.50**	0.00
Sum of *i*15:0, *ai*15:0, *i*17:0, and *ai*17:0	0.49*	0.17
16:1ω7	0.48*	0.32
18:1ω7	0.40*	0.29
18:2ω6	0.00	0.01
18:3ω3	0.62**	0.53**
18:4ω3	0.48*	0.35*
20:1ω9	0.07	0.05
20:4ω6	0.42*	0.33*
20:5ω3	0.39*	0.30
22:6ω3	0.33	0.25
Sum of 24:0, 25:0, 26:0, 27:0, 28:0, 30:0, and 31:0	0.08	0.00

unit carbon (Table 2). Particularly for the DM-based analysis, the oxygen consumption rate of the settled PM had the most significant, positive relationship with its organic carbon content among the tested chemical variables (R^2 = 0.93, p < 0.001, Fig. 6). Both the organic carbon content and oxygen consumption rate of the settled PM increased with oyster age. The oxygen consumption rate of the settled PM was also significantly related to the contents of BSFAs, 16:1ω7, 18:1ω7, 18:3ω3, 18:4ω3, 20:4ω6, and 20:5ω3 (R^2 = 0.39–0.62, p = 0.002–0.031). For the carbon-based regression analyses, the oxygen consumption rate of settled PM was significantly related only to the contents of 18:3ω3, 18:4ω3, and 20:4ω6 (R^2 = 0.33–0.53, p = 0.008–0.049).

The hourly increment in oxygen consumption rate of biodeposits produced by an individual oyster, considering increases in both the amount and carbon content of biodeposits with oyster age, was estimated to be 0.13 ± 0.04, 4.7 ± 1.3, and 6.7 ± 1.6 µg O_2 ind.$^{-1}$ h^{-1} (mean ± SE, n = 3) for Oys-3, Oys-15, and Oys-27, respectively. The specific ratio of the estimated increments was 1:37:53 (Oys-3:Oys-15:Oys-27). When considering only the increase in biodeposits but not the oyster age effect (i.e. assuming consistency of quality and oxygen consumption rate of unit biodeposit mass across oyster ages), the ratio then became equivalent to the ratio of the biodeposit production rate: 1:19:19 (Oys-3:Oys-15:Oys-27).

4. DISCUSSION

4.1. Alterations in the chemical composition of POM by oysters

The chemical composition (e.g. carbon content, stable isotopes, and fatty acids) of the settled PM differed from that of suspended PM in the control; therefore, the gravitational deposition selectively transported specific fractions of POM downward. In particular, organic carbon was slightly more concentrated in the settled PM than in the suspended PM in the control, while nitrogen content was lower in the settled PM than in the suspended PM. In general, larger particles tend to have higher settling velocities (e.g. Guidi et al. 2008). Thus, the larger-sized POM with higher C/N ratios may have deposited faster and led to the relatively higher C/N ratio observed in the settled PM in the control. Furthermore, the largest particle size fraction, 75–250 µm, showed relatively higher contents of fatty acids with relatively longer carbon chains and multiple unsaturated bounds, including eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3). Based on the results from our size-fraction measurement, these fatty acids are considered to have higher settling velocities compared to other fatty acids due to their larger particle size. This may partially explain their relatively high content in the settled PM compared to the suspended PM in the control. The biodeposits produced by the oysters were considered to be predominant in the settled PM collected from the oyster mesocosms, since the amount of the settled PM was ca. >90% higher in the oyster mesocosms than in the control ones. This result may explain the relatively small differences in the chemical properties between the estimations of the biodeposits produced by oysters and the analysis results of the settled PM samples. The chemical properties of the settled PM from the oyster mesocosms are considered to represent those of biodeposits from oysters, but the content of some fatty acids in the oyster biodeposits was underestimated by up to 30%.

The different chemical composition of PM between the control and oyster mesocosms demonstrated that oyster suspension-feeding affected not only the amount of suspended PM and settled PM but also the chemical composition of PM. Our results indicated that oysters fractionated fatty acids by suspension-feeding. Oysters were considered to have higher affinity for the fatty acids with $C_{\geq 20}$ and unsaturated bonds, including EPA and DHA, since the contents of those fatty acids were much higher in the biodeposits and oyster tissues than in the suspended PM. EPA and DHA act as cell membrane components and hormone precursors (Bell et al. 1986, Sargent et al. 1999). In general, essential fatty acids influence consumers, including oysters, and this influence has been reported to be more profound in the early growth stages (Knauer & Southgate 1997, Glencross 2009). Because diatoms and dinoflagellates contain an abundance of EPA and DHA, respectively, oysters probably selectively ingest and digest these types of phytoplankton to efficiently obtain these nutritionally important fatty acids.

The particle size and surface chemical properties may have interactively affected the efficiency of particle ingestion by oysters, and fractionated POMs from different biological origins (e.g. bacteria, diatoms, dinoflagellates, other algal groups) in the feeding processes (Tamburri & Zimmer-Faust 1996, Ward & Shumway 2004). The content of fatty acids associated with bacteria (e.g. BSFAs, 16:1ω7, 18:1ω7) in the suspended PM was relatively higher in the oyster mesocosms than in the control, which is consistent with the findings of a previous study on oyster mesocosms (Mostajir et al. 2015). The contents of fatty acids commonly used as microalgal markers, including EPA and DHA, of the suspended PM in the water column were not significantly different between the control and oyster mesocosms. These findings suggest that bacteria had a high passage rate during the pre-ingestive processes of suspension-feeding by oysters, and that the oysters probably retained bacteria with less efficiency compared to phytoplankton. In general, bacteria are smaller than a few micrometres (e.g. Nevejan et al. 2018) and can pass through bivalve suspension-feeding, resulting in lower retention rates in most bivalve species (e.g. Jørgensen 1996, Prasetiya et al. 2017). However, Sonier et al. (2017) demonstrated that eastern oysters *Crassostrea virginica* assimilated carbon from picophytoplankton (0.2–2.0 µm). This implies that selective ingestion by oysters cannot be explained solely by their low retention efficiency of smaller particles. The chemical properties of the surface of organic particles are also cues for the pre-ingestive selection by oysters (e.g. Ward & Shumway 2004, Rosa et al. 2013). Furthermore, the particle size and chemical properties of POM are not independent in natural aquatic systems; in this study, for instance, the C/N ratio and fatty acid contents differed among the size fractions of POM.

The selective absorption of nutrients in oyster digestive processes may also explain the compositional differences between suspended PM and biodeposits found in this study. The types of enzymes

and the gastrointestinal microbiome composition in the digestive organs of consumers control the digestion of POM and the composition of egested faeces (e.g. Navarro et al. 2009, Arambalza et al. 2010, Karasov et al. 2011). Fatty acids exhibit differences in digestibility. In particular, unsaturated fatty acids (e.g. EPA and DHA) are more digestible than saturated fatty acids (e.g. Glencross 2009). The high digestibility and/or high absorption may be responsible for the lower content of fatty acids with a higher number of carbon and unsaturated bonds in the oyster biodeposits than in the PM settled by gravitational deposition in the control mesocosms, as well as the higher content of these fatty acids in the oyster tissues. Overall, the selective assimilation of fatty acids and compositional changes to POM by biodeposition may be attributed to the selection of POM in both the ingestion and digestion processes of oysters.

Our results suggest that the oysters may have selectively ingested PM rich in EPA, and there are some potential mechanisms for oyster affinity to fatty acids. The high content of fatty acids with $C_{\geq 20}$ and unsaturated bonds in the biodeposits could be due to pre-ingestive exclusion of excess PM rich in those fatty acids and/or limited assimilation capacity following excessive ingestion of those fatty acids. In contrast to the $C_{\geq 20}$ unsaturated fatty acids, the contents of C_{16} and C_{18} fatty acids were lower in the biodeposits than in the suspended PM (<1 in the content ratio). This could be attributable to low ingestion efficiency of those fatty acids (low affinity) or high assimilation efficiency of those fatty acids after ingestion (high affinity). Selective ingestion by oysters and the mechanisms underlying it may differ even between the fatty acids with C_{16} and C_{18}. For instance, the estimated 18:2ω6 content of ingested POM was higher than that of the sampled, suspended POM (content ratio >1.6), so the oysters may have higher assimilation efficiency of 18:2ω6 and lower egestion of this fatty acid. However, the estimated contents of 16:1ω7 and 18:1ω7 of ingested POM were lower than those of the sampled suspended PM (content ratio <0.5), so oysters probably have a lower affinity for those fatty acids particularly at ingestion. In our study, the faeces and pseudofaeces were not distinguished due to technical difficulties in our experimental design. To answer the remaining questions on mechanisms for the different affinity of oysters between fatty acids, the relative importance of pre- and post-ingestive processes for the control of biodeposit composition needs to be clarified by further examinations with separate analyses between faeces and pseudofaeces.

While fatty acids showed diverse responses to oyster feeding, the stable isotopic signatures of the settled PM were like those of the oyster tissues but not the suspended PM. In particular, the trophic fractionation of carbon stable isotopes is lower between primary consumers and the diets (e.g. DeNiro & Epstein 1978), so the stable isotope result indicates that the oysters had a strong selectivity for particle intake. Nevertheless, the stable isotopic signatures of suspended PM did not distinctly differ between the control and oyster mesocosms, leading to almost no difference between the estimated stable isotopic signatures of the suspended PM ingested by oysters and the signatures of the sampled suspended PM. These results suggest that the oysters may have ingested only a small fraction of bulk POM, and this may have resulted in the similarity in the stable isotopic signatures between the control and oyster mesocosms. Furthermore, the fatty acids are also a minor fraction of suspended POM (e.g. generally several %) as our results and previous studies have shown (e.g. Galois et al. 1996, Canuel & Zimmerman 1999). Therefore, the selective acquisition of fatty acids by oysters is considered not to substantially alter the stable isotopic signatures of bulk suspended POM. The substantially different stable isotopic signatures between the suspended PM and biodeposits could have been attributable to oyster selective feeding of POM components other than lipids.

Our results revealed the compositional changes in POM through suspension-feeding and biodeposition by oysters. However, our study was conducted only on a single day during the fall season. The composition of POM in seawater as well as the feeding behaviour and metabolic functions of oysters vary temporally due to various factors (e.g. season and hydrological conditions). This point needs to be considered when interpreting our study results, and future studies should examine the consistency or variability of selective feeding and assimilation by suspension feeders as well as the quantitative and qualitative changes of their biodeposits associated with temporal factors.

4.2. Effects of oyster age on the chemical composition and oxygen consumption of biodeposits

Our results revealed that the Oys-27 oysters showed much higher rates per individual of both suspended PM clearance and biodeposit production compared with the younger oysters, which is consistent with the findings of many other studies (e.g. Cal-

lier et al. 2006, Cranford et al. 2011, Ehrich & Harris 2015). The higher suspension-feeding ability of older individuals is possibly due to their larger body size and relatively lower limitation in morphological constraints, such as a larger gut volume and weaker water friction associated with larger feeding organs (e.g. Sherrard & LaBarbera 2005, Cranford et al. 2011). Our results highlighted that biodeposit quality, in addition to quantity, significantly differed between oyster age classes. Specifically, the carbon, nitrogen, and fatty acid content of biodeposits from the Oys-3 oysters was lower than that from older oysters, thus supporting our hypothesis that the fatty acid content of biodeposits would increase with oyster age. Older oysters probably exhibited a lower assimilation efficiency and increased the content of those chemical constituents in the biodeposits. These results are consistent with an empirical modelling study on energy allocation of consumers demonstrating that the assimilation rate of consumers does not increase with their ontogenetic mass to the same degree as the consumption rate does (Maino & Kearney 2015), and also another study of digestive enzymes for a scallop species showing that lipase was more active in younger rather than older individuals (Pichaud et al. 2009). However, the feeding organs of consumers develop ontogenetically, and pre-ingestive selection processes are also considered to change with growth (e.g. Hentschel 1996, Rosa & Padilla 2020). Thus, the lower ability of POM clearance or POM rejection as pseudofaeces during the ingestion processes also might be responsible for the relatively lower contents of chemical constituents in biodeposits from younger oysters. The compositional change of pseudofaeces with growth stage and its effects on the bulk biodeposits needs to be further examined to determine the mechanisms of ontogenetic variation in biodeposit composition.

Since the organic content of biodeposits better explained their oxygen consumption rates compared with other chemical indicators (e.g. the contents of nitrogen and fatty acids), the oxygen consumption rate of biodeposits was considered to be primarily controlled by their organic content. As the oxygen consumption rate of biodeposits were also positively related to the contents of most of the studied fatty acids, the biological components in biodeposits linked to those fatty acids are considered to have contributed to the oxygen consumption of biodeposits. There are 2 interpretations for the observed significant relationships of oxygen consumption of biodeposits with their fatty acid content. First, the organic matter in the biodeposits originating from specific biological components, linked to specific fatty acids, may have had high degradability, and thus served as exogenous substrates for heterotrophic microbes (e.g. bacteria) which readily consumed oxygen. Second, some microbes are resistant to digestion in the guts of consumers (Wotton & Malmqvist 2001). Thus, living microbes (e.g. microalgae and heterotrophic bacteria) contained in the biodeposits, which are also reflected by the fatty acid contents, may have consumed oxygen via their endogenous respiration. Endogenous and exogenous respiration of microbes can occur concurrently at either the within-cell or community levels (e.g. Kratz & Myers 1955, Gronlund & Campbell 1966, Glazier 2009), and both may have contributed to the oxygen consumption by biodeposits.

Our results suggest that fatty acids have the potential to be a good indicator for which components of POM contribute to its oxygen consumption. In the analysis in which tested variables were chemical contents per unit carbon and represented the qualitative aspect of organic matter contained in the biodeposits, only the contents of 18:3ω3, 18:4ω3, and 20:4ω6, which are used as makers of macroalgae, showed significant, positive relationships with the oxygen consumption rate. These findings imply that the fine POM originating from fragmented or decomposed macroalgae may have had relatively higher contributions to the oxygen consumption among various origins of organic matter in our experiment. However, the contribution of POM originating from macroalgae to oxygen consumption could vary spatiotemporally, since, in general, POM from macroalgae is relatively less labile compared to that of microalgae (e.g. Enríquez et al. 1993). The relationships of fatty acids with oxygen consumption probably depend on some spatiotemporally variable factors characterising POM, such as the contents of fatty acids in POM and lability of organic compounds. Further examination of these factors is necessary to understand the general patterns or mechanisms for the relationship between oxygen consumption by POM and its fatty acid composition.

4.3. Implications

From the perspective of food web ecology, biodeposition can be considered to increase the resource supply for benthic communities and may enhance the production of benthic consumers (e.g. McKindsey et al. 2006, Lu & Grant 2008). We showed that the concentration of fatty acids in oyster biodeposits was

lower than that in the naturally settled PM, but that oysters increased the amount of fatty acids transported to the sea floor. In addition, the fatty acid composition also significantly differed between the naturally settled PM and biodeposits produced by oysters. The fatty acid composition of deposits potentially affects the fatty acid acquisition by benthic consumers, and thus impacts their production and community structure (e.g. Silina & Zhukova 2007, Glencross 2009, Fujibayashi et al. 2019). The fatty acid composition of deposits also affect ecosystem functions at bottom habitats, including oxygen consumption associated with metabolism by all heterotrophic components of the communities (e.g. bacteria, meiofauna and macrofauna) (e.g. Pollet et al. 2015). Our results highlighted that the quality of settled POM, particularly fatty acid composition, can be altered by suspension feeders and that the growth stages of suspension feeder populations affect these processes. Although our study was conducted in only 1 d, the quantity and composition of suspended POM as well as biodeposits are potentially variable at various time scales (e.g. oyster production cycle, seasonal, meteorological scales). Thus, biodeposit production by oysters is also considered to change over time. Further investigations at different time scales are needed to find out a general pattern of variation in fatty acid composition of biodeposits, and to properly evaluate the ecological functions of biodeposition.

Our findings demonstrated the importance of considering the increase in organic content and oxygen consumption of biodeposits with oyster age, and that if these factors are not considered, the effects of biodeposition on surrounding environments can be substantially misestimated. From the perspective of an aquaculture operation, Helm (2006) reported that the duration of oyster cultivation before harvest differs regionally and ranges between 18 and 30 mo. In the present study region, many oyster farmers tend to intentionally prolong cultivation duration to harvest larger oysters, which generally have a higher market price (according to personal communication with local fishermen). To avoid adverse impacts of bivalve aquaculture on surrounding environments, and considering that not only quantity but also the organic content of biodeposits increases with oyster growth, the duration of oyster cultivation should be properly controlled. Specifically, our study results support that accelerating the production cycle in oyster farms (i.e. shortening the duration of oyster culture and retaining younger oysters) helps to mitigate the organic pollution and oxygen consumption in bottom habitats.

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