

## Supplementary Information

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**Supplement 1. Oysters used in the mesocosm**

Table S1. The mass and dimensions of oysters introduced into the mesocosms (mean  $\pm$  SD). The numbers of oyster individuals sampled from each oyster age were 9 and 21 for the measurements of soft tissue mass and dimensions, respectively.

Oyster age treatment (month)	Oys-3	Oys-15	Oys-27
Total oysters introduced			
Individual numbers	220 $\pm$ 20	20 $\pm$ 0	10 $\pm$ 0
Total wet mass including shells (kg)	1.29 $\pm$ 0.12	1.46 $\pm$ 0.05	1.68 $\pm$ 0.01
Soft tissues (g ind <sup>-1</sup> )			
Wet mass	0.9 $\pm$ 0.5	10.1 $\pm$ 2.3	21.6 $\pm$ 4.7
Dry mass	0.09 $\pm$ 0.03	1.41 $\pm$ 0.38	2.86 $\pm$ 0.69
Shell dimensions (cm)			
Length	3.4 $\pm$ 0.4	8.5 $\pm$ 2.1	15.0 $\pm$ 1.7
Width	2.1 $\pm$ 0.3	4.8 $\pm$ 0.4	5.7 $\pm$ 0.5
Thickness	0.9 $\pm$ 0.4	2.8 $\pm$ 1.3	3.0 $\pm$ 0.3

## Supplement 2. Fatty acids analysed in the present study

For data analysis in the present study, we selected fatty acids that are recognised as nutritionally important for animals or that are commonly used as biomarkers (Table 1 in the main text). The 18:2 $\omega$ 6 and 18:3 $\omega$ 3 fatty acids are considered to be essential because animals cannot synthesise them (Glencross 2009). Although the nutritional roles of these two fatty acids in marine animals are not well understood, they are the precursors of nutritionally important fatty acids, such as 20:4 $\omega$ 6, 20:5 $\omega$ 3, and 22:6 $\omega$ 3 (Glencross 2009), which are known to be necessary for the growth, survival, and stress resistance of aquatic animals (Parrish et al. 2000, Parrish 2013 and references therein). These fatty acids can be synthesised by animals but only in the presence of certain enzymes. However, most animals, especially marine animals and predators, are limited in their ability to convert 18:2 $\omega$ 6 and 18:3 $\omega$ 3 to these nutritionally important fatty acids to meet their requirements. In turn, 20:4 $\omega$ 6, 20:5 $\omega$ 3, and 22:6 $\omega$ 3 are also considered to be essential fatty acids (Kainz et al. 2004).

Essential fatty acids are also used as biomarkers. For instance, 18:2 $\omega$ 6 and 18:3 $\omega$ 3 are abundant in green algae, vascular plants, and cyanobacteria (Cobelas & Lechado 1989, Napolitano 1999, Kelly & Scheibling 2012). As these fatty acids are mainly produced in terrestrial ecosystems, >2.5% detection from marine samples (e.g. suspended particulate matter, sediments) is assumed to indicate a significant input of terrestrial origin organic matter (Budge et al. 2001). The 18:4 $\omega$ 3 fatty acid has been used as a marker for dinoflagellates (Napolitano et al. 1997). The 20:5 $\omega$ 3, 22:6 $\omega$ 3, and 20:4 $\omega$ 6 fatty acids have been considered as and used as biomarkers for diatoms, dinoflagellates, and macroalgae, respectively (Napolitano 1999, Graeve et al. 2001, 2002).

The 16:1 $\omega$ 7 fatty acid is also one of the major fatty acids produced by diatoms (Cobelas & Lechado 1989, Kelly & Scheibling 2012). Although 16:1 $\omega$ 7 has also been detected from bacteria (Kelly & Scheibling 2012), the ratio of 16:1 $\omega$ 7 to 16:0 being greater than 1 is considered an indication of dominance of diatoms (Budge et al. 2001). Branched fatty acids and 18:1 $\omega$ 7 are contained in bacteria (Kaneda 1991, Napolitano 1999), in turn these fatty acids have been used as bacterial markers in ecological studies (e.g. Mfilinge et al. 2005). The 18:1 $\omega$ 7 fatty acid is also contained in cyanobacteria (Napolitano 1999). Long-chain fatty acids (LCFAs), which are  $\geq$ 24 carbons in length, are used as biomarkers of terrestrial plants (Colombo et al. 1996, Wang et al. 2013, Derrien et al. 2017). Furthermore, 20:1 and 22 $\omega$ 1 have been used as zooplankton biomarkers (Parrish et al. 2000, Kelly & Scheibling 2012).

**Supplement 3.** Estimation of chemical properties of POM processed by oysters**3.1. Estimation methods**

The chemical properties (i.e., the contents of C, N and fatty acids, the stable isotopic ratios [ $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ], and the oxygen consumption rate) of the oyster biodeposit fraction in the settled particulate matter (PM) of the oyster mesocosms were estimated excluding the effects of settled PM via gravitational deposition. The estimation was based on the assumption that the same amounts of settled PM obtained from the control mesocosms deposited in the oyster mesocosms. Each property of oyster biodeposits ( $X_{BD}$ ) was estimated using the following equations;

$$\begin{aligned}\bar{X}_{BD} &= (\bar{X}_{Oys} - \bar{f}_{Ctrl} \cdot \bar{X}_{Ctrl}) / \bar{f}_{BD} \\ \bar{f}_{BD} &= 1 - \bar{f}_{Ctrl} = 1 - \overline{DEP}_{Ctrl} / \overline{DEP}_{Oys}\end{aligned}$$

where  $\bar{X}_{Oys}$  is the mean of the property of settled PM obtained from the oyster mesocosms of each age treatment,  $\bar{X}_{Ctrl}$  is the property of settled PM from the control mesocosms,  $\bar{f}_{Ctrl}$  is the fraction of settled PM due to the gravitational deposition,  $\bar{f}_{BD}$  is the fraction of oysters' biodeposits in the settled PM of the oyster mesocosms,  $\overline{DEP}_{Ctrl}$  is the amount of the settled PM in the control mesocosms, and  $\overline{DEP}_{Oys}$  is the amount of the settled PM in the oyster mesocosms. The upper bars indicate the mean of triplicate mesocosms of each treatment.  $\overline{DEP}_{Ctrl}$  and  $\overline{DEP}_{Oys}$  were at the C or N mass for the estimations of the stable isotopic signatures, and at the dry mass for the other estimations (i.e., the contents of C, N, and fatty acids, and the oxygen consumption rate). The variance around  $\bar{X}_{BD}$  was estimated by the error propagation law.

Likewise, the contents of the chemical constituents and the stable isotopic signatures of the POM fraction fed by oysters in the water columns of the oyster mesocosms ( $Y_{Fed}$ ) were estimated. Assuming 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,  $Y_{Fed}$  was estimated using the following equations;

$$\begin{aligned}\bar{Y}_{Fed} &= (\bar{Y}_{Ctrl} - \bar{f}_{Oys} \cdot \bar{Y}_{Oys}) / \bar{f}_{Fed} \\ \bar{f}_{Fed} &= 1 - \bar{f}_{Oys} = 1 - \overline{SPM}_{Oys} / \overline{SPM}_{Ctrl}\end{aligned}$$

where  $\bar{Y}_{Ctrl}$  is the property of suspended POM obtained in the control mesocosms,  $\bar{Y}_{Oys}$  is the property of suspended POM obtained in the oyster mesocosms,  $\bar{f}_{Fed}$  is the fraction of suspended POM fed by the oysters in the oyster mesocosms,  $\bar{f}_{Oys}$  is the fraction of suspended POM remaining in the water column of the oyster mesocosms,  $\overline{SPM}_{Oys}$  is the concentration of POM in the oyster mesocosms, and  $\overline{SPM}_{Ctrl}$  is the concentration of POM in the control mesocosms. The upper bar indicates the mean of triplicate mesocosms of each treatment.  $\overline{SPM}_{Oys}$  and  $\overline{SPM}_{Ctrl}$  were at the carbon or nitrogen mass for the estimations of the stable isotopic signatures, and at the dry mass for the other estimations. The variance around  $\bar{f}_{Fed}$  was estimated by the error propagation law.

### 3.2. Results

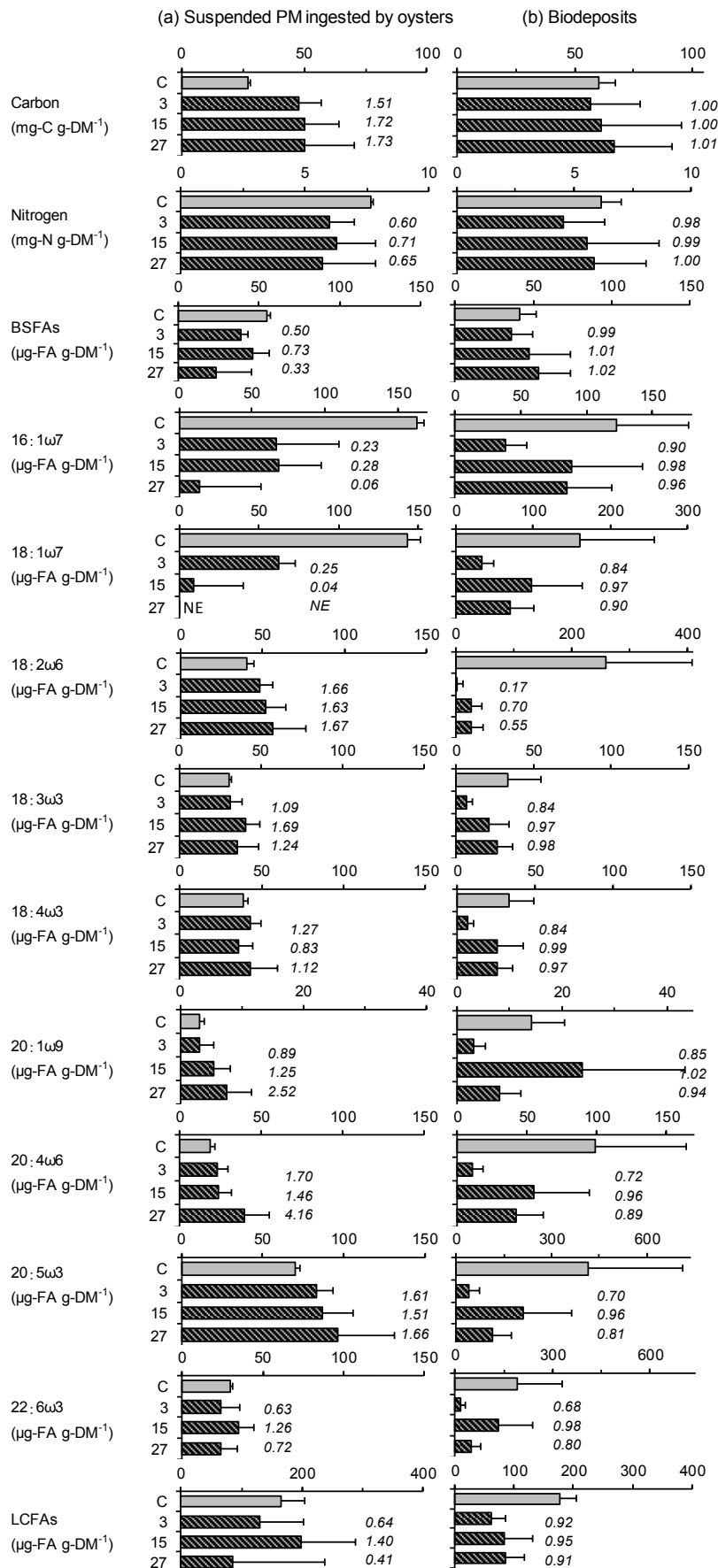


Fig. S1. The estimated contents (per dry mass) of carbon, nitrogen, and fatty acids in (a) suspended particulate matter (PM) ingested by oysters and (b) biodeposits produced by oysters. For comparison, the contents of those chemical constituents of suspended PM and biodeposits in the control with no oyster are also shown. The values in italic show the ratio of the estimated contents of the chemical constituents to the contents in (a) suspended PM and (b) settled PM sampled from the oyster mesocosms. Data are mean  $\pm$  SE ( $n = 3$  mesocosms treatment<sup>-1</sup>). BSFAs: branched-chain saturated fatty acids, LCFAs: long-chain fatty acids.

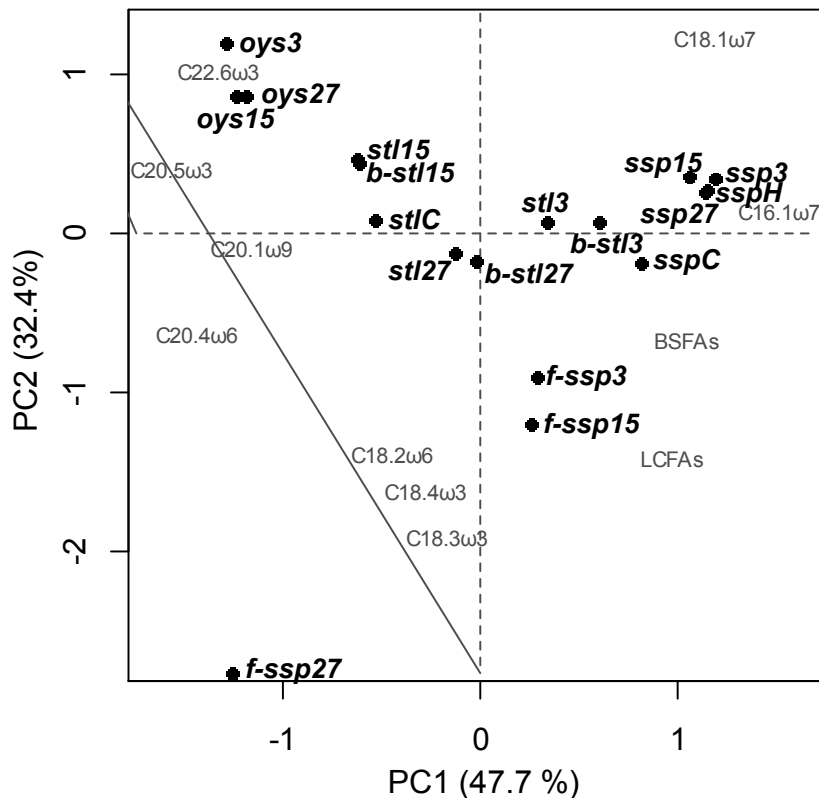


Fig. S2. The correlations between the contents of fatty acids and the characteristics of fatty acid composition of suspended particulate matter (ssp), settled particulate matter (stl), and oyster soft tissues (oys) at the end of the mesocosm operation, as revealed by the PCA. The analysis includes estimated fatty acid compositions for the suspended particulate matter ingested by oysters (f-) and biodeposits produced by oysters (b-), which are additional to Fig.4 in the main text. In the sample labels, “3”, “15”, “27”: oyster age (months), C: control with no oyster, H: inflow seawater from the head tank.

Table S2 Estimated  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of (a) suspended particulate matter (PM) ingested by oysters and (b) biodeposits produced by oysters (mean  $\pm$  SE,  $n = 3$ ). The results from sample analyses of suspended PM and settled PM are also shown for the purpose of comparison.

Run	Data type	(a) Suspended PM		(b) Biodeposits	
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Oys-3	Estimated	$-23.0 \pm 4.6$	$0.6 \pm 1.1$	$-18.6 \pm 5.0$	$5.7 \pm 1.5$
	Sample	$-23.7 \pm 0.1$	$2.9 \pm 0.4$	$-18.8 \pm 1.3$	$5.7 \pm 0.1$
Oys-15	Estimated	$-23.0 \pm 5.0$	$-0.2 \pm 1.4$	$-19.4 \pm 7.5$	$6.4 \pm 2.5$
	Sample	$-23.6 \pm 0.0$	$3.1 \pm 0.4$	$-19.6 \pm 0.2$	$6.3 \pm 0.5$
Oys-27	Estimated	$-22.5 \pm 7.0$	$-0.6 \pm 2.0$	$-18.1 \pm 4.7$	$6.3 \pm 1.8$
	Sample	$-23.7 \pm 0.1$	$2.6 \pm 0.3$	$-18.5 \pm 2.0$	$6.2 \pm 0.6$
Control	Sample	$-23.3 \pm 0.1$	$1.9 \pm 0.4$	$-22.4 \pm 0.2$	$5.0 \pm 0.0$

Table S3. The estimated oxygen consumption rate and organic carbon content of biodeposits produced by oysters (mean  $\pm$  SE, n = 3). The results from the sample analysis of settled particulate matter retrieved from the experimental mesocosms were also shown for the purpose of comparison.

Run	Data type	Oxygen consumption rate mg-O <sub>2</sub> g-DM <sup>-1</sup> h <sup>-1</sup>	Carbon content mg-C g-DM <sup>-1</sup>
Oys-3	Estimated	0.15 $\pm$ 0.06	56.6 $\pm$ 21.5
	Sample	0.16 $\pm$ 0.04	56.8 $\pm$ 2.0
Oys-15	Estimated	0.31 $\pm$ 0.12	61.3 $\pm$ 34.1
	Sample	0.31 $\pm$ 0.01	61.2 $\pm$ 1.6
Oys-27	Estimated	0.45 $\pm$ 0.13	67.0 $\pm$ 24.8
	Sample	0.43 $\pm$ 0.07	66.4 $\pm$ 1.4
Control	Sample	0.29 $\pm$ 0.16	60.4 $\pm$ 3.6

## Supplement 4. Rates of removal of particulate matter from the water column and biodeposit production by oysters during the mesocosm experiments

### 4.1. Calculation methods

The rate of suspended particulate matter removal from the water column (RW) by individual oysters in the mesocosms was estimated as  $\Delta C \cdot V / I \cdot T$ , where  $\Delta C$  is the difference in the concentration of each chemical constituent between the oyster-introduced and control mesocosms,  $V$  is the water volume of the mesocosms (130 L),  $I$  is the number of oyster individuals introduced in each mesocosm, and  $T$  is the duration time of the mesocosm experiment (22 h). The rate of biodeposit production (BP) by individual oysters was estimated as  $(D_O - D_C) / I \cdot T$ , where  $D$  is the settled amount of each chemical constituent in the oyster treatments (O) and the control with no oyster (C) at the end of the mesocosm experiment. The variance around the mean of the estimates were calculated following the error propagation law.

### 4.2. Results

Table S4. Estimates for the rates of removal of suspended particulate matter from the water column (RW) and biodeposit production (BP) by individual oysters of three age classes during the mesocosm experiment. Data are mean  $\pm$  SE ( $n = 3$  mesocosms treatment<sup>-1</sup>). For 18:1 $\omega$ 7 in the treatment with 27-month old oysters, the negative estimates of RW were due to a higher concentration of this specific fatty acid in the water column compared with the control; BP/RW was not estimated for this case (n.e.). Units: mg ind<sup>-1</sup> h<sup>-1</sup> for total solids, organic C, and organic N; and  $\mu$ g ind<sup>-1</sup> h<sup>-1</sup> for fatty acids. BSFAs: the sum of branched-chain saturated fatty acids, *i*15:0, *ai*15:0, *i*17:0, and *ai*17:0. LCFAs: the sum of long-chain fatty acids, 24:0, 25:0, 26:0, 27:0, 28:0, 30:0, and 31:0.

Constituents	Oyster age (months)	RW	BP	BP/RW
Total solids	3	1.13 $\pm$ 0.10	0.80 $\pm$ 0.15	0.70 $\pm$ 0.15
	15	17.18 $\pm$ 3.31	15.40 $\pm$ 4.28	0.90 $\pm$ 0.30
	27	24.80 $\pm$ 7.70	15.55 $\pm$ 2.88	0.63 $\pm$ 0.23
Organic C	3	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.83 $\pm$ 0.22
	15	0.87 $\pm$ 0.17	0.94 $\pm$ 0.26	1.09 $\pm$ 0.37
	27	1.24 $\pm$ 0.33	1.04 $\pm$ 0.19	0.84 $\pm$ 0.27
Organic N	3	0.01 $\pm$ 0.00	0.00 $\pm$ 0.00	0.53 $\pm$ 0.13
	15	0.11 $\pm$ 0.02	0.09 $\pm$ 0.02	0.79 $\pm$ 0.26
	27	0.14 $\pm$ 0.03	0.09 $\pm$ 0.02	0.64 $\pm$ 0.19
BSFAs	3	0.04 $\pm$ 0.00	0.03 $\pm$ 0.01	0.65 $\pm$ 0.13
	15	0.79 $\pm$ 0.08	0.73 $\pm$ 0.22	0.92 $\pm$ 0.29
	27	0.58 $\pm$ 0.52	0.83 $\pm$ 0.17	1.45 $\pm$ 1.33
16:1 $\omega$ 7	3	0.08 $\pm$ 0.05	0.03 $\pm$ 0.01	0.40 $\pm$ 0.28
	15	1.17 $\pm$ 0.45	1.36 $\pm$ 0.51	1.17 $\pm$ 0.63
	27	0.35 $\pm$ 1.04	1.33 $\pm$ 0.31	3.84 $\pm$ 11.54
18:1 $\omega$ 7	3	0.07 $\pm$ 0.01	0.03 $\pm$ 0.01	0.37 $\pm$ 0.13
	15	0.16 $\pm$ 0.53	1.50 $\pm$ 0.71	9.61 $\pm$ 33.17
	27	-1.87 $\pm$ 0.59	1.10 $\pm$ 0.30	n.e.

(table continued from the last page)



Constituents	Oyster age (months)	RW	BP	BP/RW
18:2 $\omega$ 6	3	0.05 $\pm$ 0.01	0.00 $\pm$ 0.01	0.04 $\pm$ 0.14
	15	0.88 $\pm$ 0.13	0.41 $\pm$ 0.23	0.46 $\pm$ 0.27
	27	1.40 $\pm$ 0.24	0.41 $\pm$ 0.30	0.30 $\pm$ 0.22
18:3 $\omega$ 3	3	0.04 $\pm$ 0.01	0.01 $\pm$ 0.00	0.15 $\pm$ 0.08
	15	0.71 $\pm$ 0.05	0.33 $\pm$ 0.12	0.47 $\pm$ 0.17
	27	0.89 $\pm$ 0.14	0.41 $\pm$ 0.09	0.46 $\pm$ 0.12
18:4 $\omega$ 3	3	0.05 $\pm$ 0.01	0.01 $\pm$ 0.00	0.11 $\pm$ 0.05
	15	0.62 $\pm$ 0.09	0.40 $\pm$ 0.16	0.65 $\pm$ 0.28
	27	1.06 $\pm$ 0.24	0.40 $\pm$ 0.09	0.37 $\pm$ 0.12
20:1 $\omega$ 9	3	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.69 $\pm$ 0.69
	15	0.09 $\pm$ 0.04	0.37 $\pm$ 0.25	3.97 $\pm$ 3.25
	27	0.18 $\pm$ 0.09	0.13 $\pm$ 0.05	0.69 $\pm$ 0.41
20:4 $\omega$ 6	3	0.03 $\pm$ 0.01	0.01 $\pm$ 0.01	0.34 $\pm$ 0.22
	15	0.40 $\pm$ 0.12	0.85 $\pm$ 0.46	2.11 $\pm$ 1.32
	27	0.97 $\pm$ 0.23	0.66 $\pm$ 0.21	0.68 $\pm$ 0.27
20:5 $\omega$ 3	3	0.09 $\pm$ 0.01	0.03 $\pm$ 0.02	0.35 $\pm$ 0.24
	15	1.50 $\pm$ 0.15	3.24 $\pm$ 1.73	2.17 $\pm$ 1.17
	27	2.38 $\pm$ 0.48	1.78 $\pm$ 0.71	0.75 $\pm$ 0.33
22:6 $\omega$ 3	3	0.03 $\pm$ 0.01	0.01 $\pm$ 0.01	0.52 $\pm$ 0.47
	15	0.59 $\pm$ 0.13	2.05 $\pm$ 1.30	3.45 $\pm$ 2.30
	27	0.60 $\pm$ 0.17	0.79 $\pm$ 0.33	1.33 $\pm$ 0.68
LCFAs	3	0.15 $\pm$ 0.08	0.05 $\pm$ 0.01	0.33 $\pm$ 0.19
	15	3.42 $\pm$ 1.38	1.29 $\pm$ 0.38	0.38 $\pm$ 0.19
	27	2.12 $\pm$ 3.70	1.32 $\pm$ 0.30	0.62 $\pm$ 1.10

**Supplement 5. Size-fractionated concentrations of the chemical constituents of suspended particulate organic matter**

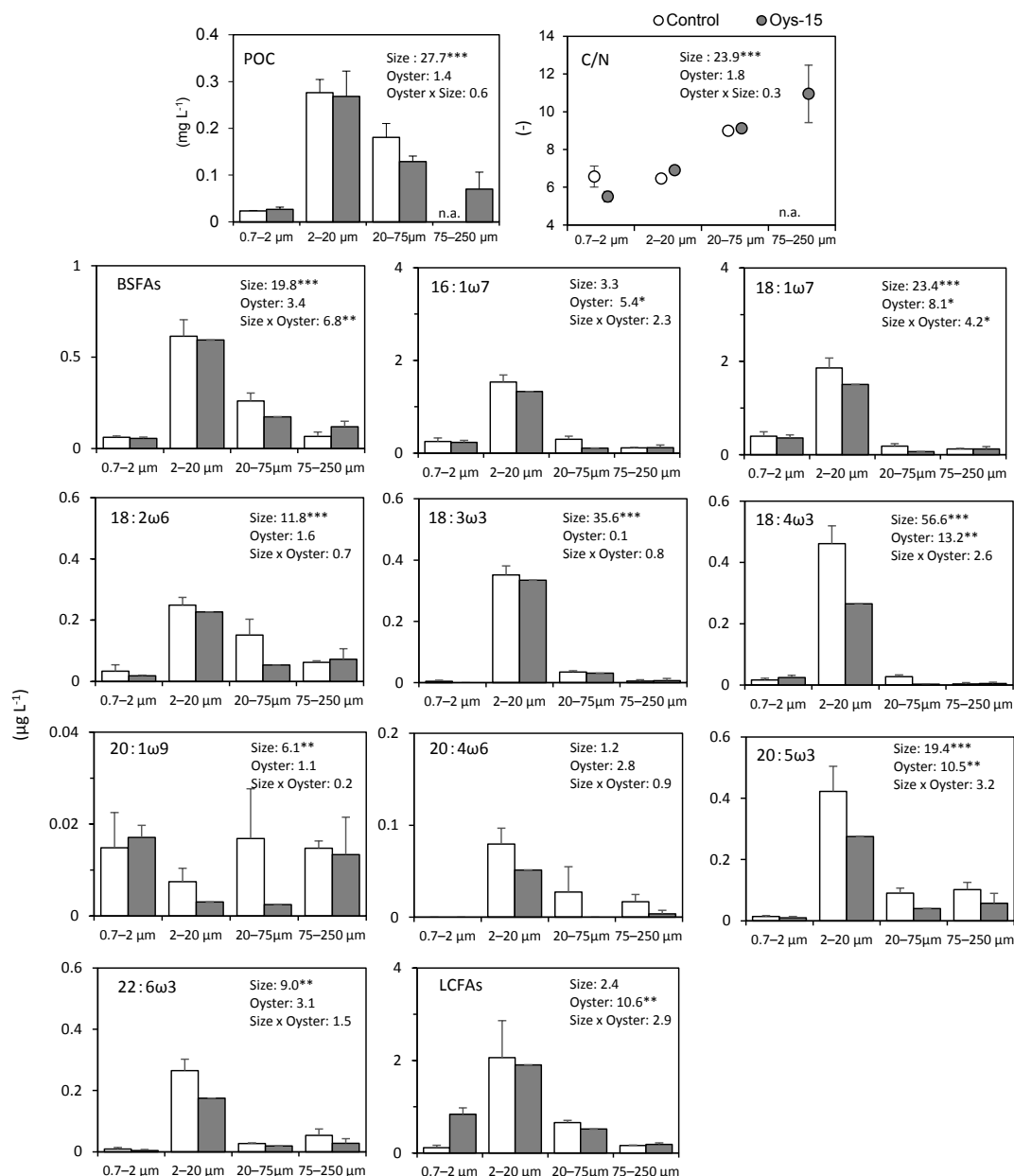


Fig. S3. Size-fractionated concentrations of particulate organic carbon (POC) and fatty acid markers as well as C/N ratio of suspended particulate organic matter in the control and Oys-15 (with 15-month-old oysters) mesocosms at the end of the 22 h experiment. Data are mean  $\pm$  SE ( $n = 3$  mesocosms treatment<sup>-1</sup>). Due to analytical failure, SE for fatty acid concentrations of the 2–20  $\mu\text{m}$  and 20–75  $\mu\text{m}$  fraction in Oys-15 was not calculated. Data are not available (n.a.) for the POC and C/N of the 75–250  $\mu\text{m}$  fraction due to analytical failure. The F-values for each factor, obtained from a two-way ANOVA testing effects of particle size and oyster presence, are shown in the panels. The DFs in the F-value calculations (numerator, denominator) were generally (1, 14), (3, 14), and (3, 14) for the experimental treatments (with or without oyster), size fraction of POM, and their interaction, respectively; but the DFs for POC and C/N were (1, 13), (3, 13), and (2, 13). The asterisks indicate significant differences associated with the factors, as follows, \*\*\*:  $p < 0.001$ , \*\*:  $p < 0.01$ , \*:  $p < 0.05$ . BSFAs: the sum of branched-chain saturated fatty acids, *i15:0*, *ai15:0*, *i17:0*, and *ai17:0*. LCFAs: the sum of long-chain fatty acids, 24:0, 25:0, 26:0, 27:0, 28:0, 30:0, and 31:0.

## LITERATURE CITED

- Budge SM, Parrish CC, McKenzie CH (2001) Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. *Mar Chem* 76:285–303.
- Cobelas MA, Lechado JZ (1989) Lipids in microalgae. A review I. *Biochemistry. Grasas Aceites* 40:118–145.
- Colombo JC, Silverberg N, Gearing JN (1996) Lipid biogeochemistry in the Laurentian Trough: I–fatty acids, sterols and aliphatic hydrocarbons in rapidly settling particles. *Org Geochem* 25:211–225.
- Derrien M, Yang L, Hur J (2017) Lipid biomarkers and spectroscopic indices for identifying organic matter sources in aquatic environments: A review. *Water Research* 112:58–71.
- Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. *Rev Aquacult* 1:71–124.
- Graeve M, Dauby P, Scailteur Y (2001) Combined lipid, fatty acid and digestive tract content analyses: A penetrating approach to estimate feeding modes of Antarctic amphipods. *Polar Biol* 24:853–862.
- Graeve M, Kattner G, Wiencke C, Karsten U (2002) Fatty acid composition of Arctic and Antarctic macroalgae: Indicator of phylogenetic and trophic relationships. *Mar Ecol Prog Ser* 231:67–74.
- Kainz M, Arts MT, Mazumder A (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. *Limnol Oceanogr* 49:1784–1793.
- Kaneda T (1991) Iso- and anteiso-fatty acids in bacteria: Biosynthesis, function, and taxonomic significance. *Microbiol Rev* 55:288–302.
- Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. *Mar Ecol Prog Ser* 446:1–22.
- Mfilinge PL, Meziane T, Bachok Z, Tsuchiya M (2005) Litter dynamics and particulate organic matter outwelling from a subtropical mangrove in Okinawa Island, South Japan. *Estuar Coast Shelf Sci* 63:301–313.
- Napolitano GE (1999) Fatty acids as trophic and chemical markers in freshwater ecosystems. In: Arts MT, Wainman BC (ed) *Lipids in Freshwater Ecosystems*. Springer, New York.
- Parrish CC (2013) Lipids in Marine Ecosystems. *ISRN Oceanography* Article ID604045.
- Parrish CC, Abrajano TA, Budge SM, Helleur RJ, Hudson ED, Pulchan K, Ramos C (2000) Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky P (ed) *The Handbook of Environmental Chemistry*. Springer.
- Wang L, Wu F, Xion Y, Fang J (2013) Origin and vertical variation of the bound fatty acids in core sediment of Lake Dianchi in Southwest China. *Environ Sci Pollut R* 20:2390–2397.