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# First report on *in situ* biodeposition rates of ascidians (*Ciona intestinalis* and *Styela clava*) during summer in Sanggou Bay, northern China

Zhanhui Qi<sup>1,2,3</sup>, Tingting Han<sup>1,2</sup>, Jihong Zhang<sup>3</sup>, Honghui Huang<sup>1,2</sup>, Yuze Mao<sup>3</sup>,  
Zengjie Jiang<sup>3</sup>, Jianguang Fang<sup>3,\*</sup>

<sup>1</sup>Key Laboratory of Fishery Ecology and Environment, Guangdong Province and

<sup>2</sup>Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture;  
South China Sea Fisheries Research Institute, CAFS, Guangzhou 510300, PR China

<sup>3</sup>Yellow Sea Fisheries Research Institute, CAFS, Qingdao 266071, PR China

**ABSTRACT:** Ascidians are globally important members of marine fouling communities. We measured *in situ* biodeposition rates of *Ciona intestinalis* and *Styela clava*, common biofoulers of aquaculture infrastructure, in Sanggou Bay, northern China, during September. Ascidian numbers were recorded within a scallop *Chlamys farreri* farming zone to assess biodeposit loading. Both ascidians were most abundant on lantern nets and scallop shells in August and September. The average densities of *C. intestinalis* and *S. clava* in the farming zone in September were approximately 329 and 22 ind. m<sup>-2</sup>, respectively, and their biodeposition rates were 32.1 and 121.2 mg dry material ind.<sup>-1</sup> d<sup>-1</sup>, respectively. Total daily biodeposit production by ascidians in September within the scallop farming zone may amount to 13.24 g m<sup>-2</sup>, with daily organic matter, C, N, and P biodeposition rates of 1.88, 0.94, 0.11, and 0.98 × 10<sup>-2</sup> g m<sup>-2</sup>, respectively. The predicted daily biodeposit production by *C. intestinalis* and *S. clava* within the scallop farming zone in the bay during September was 105.9 t dry material, 7.52 t C, 0.86 t N, and 0.078 t P. By comparison, drop-off to the sea floor was approximately 143.0 t of dry matter for an entire growing season, which would be a relatively small input if averaged on a daily basis. However, some of the drop-off is expected to occur as a short-duration pulse of material (e.g. during cleaning), which may be relatively important in terms of benthic effects. The results suggest that the biodeposition processes and drop-off of *C. intestinalis* and *S. clava* may play an important role in coupling material fluxes from the water column to the seabed.

**KEY WORDS:** Biofouling · Ascidians · *Ciona intestinalis* · *Styela clava* · Biodeposition · Coastal suspension aquaculture · Impact

## INTRODUCTION

Ascidian species are often dominant members of fouling communities worldwide, and some are well-documented invaders (usually via anthropogenic vectors) of ecosystems outside of their native ranges, particularly on artificial structures (Lambert & Lambert 1998, 2003, Castilla et al. 2005, Robinson et al. 2005, Qi et al. 2010). They can form dense and heavy

aggregations within suspended aquaculture operations, as culturing infrastructure (e.g. buoys, anchors, ropes, and lantern nets) and bivalve shells provide an abundance of hard substrata for them to colonize (Grant et al. 1998, Costa-Pierce & Bridger 2002, Howes et al. 2007, Rocha et al. 2009, Woods et al. 2012).

The filtering-biodeposition process of bivalves can be extremely important in regulating water column processes and pelagic–benthic coupling when they

\*Corresponding author: fangjg@ysfri.ac.cn

are abundant in coastal waters (Prins et al. 1997, Newell 2004, Zhou et al. 2006, Lacoste et al. 2014). Like bivalves, ascidians have a relatively high filtration capacity (Randløv & Riisgård 1979, Lesser et al. 1992, Petersen & Riisgård 1992). Large populations of ascidians may generate enormous quantities of deposits (e.g. feces and pseudofeces), and act as a conduit for nutrients to the benthos. Haven & Morales-Alamo (1966) reported that biodeposition by fouling invertebrates (e.g. barnacles, tunicates, and other lamelli-branches) may exceed that of the oyster *Crassostrea virginica*. Therefore, the biodeposition characteristics of ascidians should be investigated to understand the potential importance of their ecological effects.

The ascidians *Ciona intestinalis* and *Styela clava* are common biofoulers of aquaculture infrastructure and crops, and have a well documented history of invading new ecosystems outside of their native ranges via anthropogenic vectors. McKindsey et al. (2009) found that the presence of *C. intestinalis* on small constructed mussel socks increased biodeposition by a factor of ~2 relative to mussel socks without tunicates. *S. clava* increased sedimentation rates relative to that of abiotic control socks. Unfortunately, despite their abundance and possible influences on the benthic environment, direct data on the biodeposition rates of these species are relatively limited (McKindsey et al. 2009). Such knowledge is necessary for both a basic understanding of their physiological traits and for the assessment of the ecological impacts of suspended bivalve culture as a result of colonization by ascidians.

In the present study, we aim to provide useful information for the quantitative evaluation of the potential benthic impacts of *C. intestinalis* and *S. clava*. We assessed the potential drop-off of these organisms to the seabed, and performed a field experiment to measure their biodeposition rates. This paper is intended as a starting point that contributes to understanding the fouling-related ecological impacts of suspended bivalve culture.

## MATERIALS AND METHODS

### Study site

The study was conducted in Sanggou Bay, a 140 km<sup>2</sup> coastal embayment in northern China (37° 01' to 37° 09' N, 122° 24' to 122° 35' E), where large-scale longline culture of bivalves, including the scallop *Chlamys farreri* and pacific oyster *Crassostrea gigas*, and kelp *Saccharina japonica* has been carried out

since the 1980s. The average water depth of the bay is 7–8 m, and seasonal water temperatures range from 0 to 25°C. *C. farreri* is the main cultured species in Sanggou Bay, where it is mainly grown in lantern nets (each lantern net is generally divided into 10 cells separated by plastic perforated plates 30 cm in diameter) suspended underneath buoyed longlines. Approximately 2 million lantern nets are used for scallop culture within the bay.

### Densities of ascidians and potential drop-off to the seabed

As Sanggou Bay is located in a temperate zone, *Ciona intestinalis* and *Styela clava* are relatively short-lived in the bay. At our study site, their lifespan lasts from early summer to late autumn, with most of them dying with the onset of the cold winter. The numbers of *C. intestinalis* and *S. clava* on scallop lantern nets and shells were recorded monthly in 2009 from early summer (July) to late autumn (November). Three lantern nets were randomly collected monthly from a scallop farm in the bay, and were immediately transported to the seaside laboratory. One hundred scallops were randomly selected from each net and were carefully removed by hand to avoid disturbing the ascidians fouling the shells. The numbers of *C. intestinalis* and *S. clava* on the lantern nets and scallop shells were recorded. Fifty ascidians including those from both the lantern nets and the scallops were randomly selected, and their wet and dry (after drying to constant weight at 60°C for 48 h) weights were measured. Ash free dry weight (AFDW) was calculated by subtracting the weight following ashing in a furnace at 480°C for 4.5 h. Based on these data, the biomass of ascidian drop-off to the seabed was estimated. Drop-off was expected to primarily arise as a consequence of ascidians being cleaned from the cages during harvest and discarded in late autumn, and from natural die-back in early winter.

### Biodeposition measurement

*In situ* biodeposition rates of *C. intestinalis* and *S. clava* were measured during peak densities in September. Ascidians attached to the lantern net partitioning plates were collected while still attached to the plates to avoid disturbing the organisms. Ascidians and substrata were carefully cleaned of any visible fouling epibiota and other material using a soft brush. Before the experiment, the ascidians were

acclimated for 24 h in the ambient sea water conditions of the experimental site.

Biodeposition rates of ascidians were measured using PVC cylindrical biodeposit traps (diameter: 20 cm, height: 80 cm). The plastic plates (with ascidians attached) were cut into rounded plates with a diameter nearly 20 cm and then placed in collecting traps, with the ascidians facing downwards as they were within the lantern nets. The plates were hung inside the trap, ~3 cm under the top of cylinder. The ascidians were placed near the top of the traps so that a sufficient water flow and food supply was maintained, hence deposition rates were representative of actual *in situ* biodeposition.

The experiment involved 3 treatments with 4 replicates each. One treatment consisted of *C. intestinalis* (20–25 ind. replicate<sup>-1</sup>), the second of *S. clava* (10–14 ind. replicate<sup>-1</sup>), and the third was a control without ascidians. The densities of experimental ascidians were typical of natural densities in the study area. Control traps contained empty plastic plates of a size and shape similar to those in the former 2 treatments. Naturally sedimented material was collected in all of the traps, whereas biodeposits produced by ascidians were also collected in those containing *C. intestinalis* or *S. clava*. The biodeposition rate (mg ind.<sup>-1</sup> d<sup>-1</sup> or mg [g dry weight]<sup>-1</sup> d<sup>-1</sup>) was determined based on the amount of material collected in each trap containing ascidians compared to the control traps. All traps were suspended from a longline (located ~100 m from the nearest scallop farm) so that the experimental ascidians were at a depth of ~2.5 m, which corresponds to a routine culture depth for scallops in lantern nets. After the sediment traps had been deployed for ~3 d, they were carefully retrieved and the material in the traps was allowed to settle before the overlying water was siphoned off. After the experiment, ascidians in each treatment were collected and their wet and dry weights were measured.

The traps were taken to the laboratory, where the collected material was rinsed several times with distilled water to remove salts. All of the sedimented material was collected and then dried at 60°C for 4 d, weighed, and then crushed to a fine powder (100 mesh) with a mortar and pestle. Subsamples of the powder were taken to measure the chemical composition. The percentage of organic matter (OM) was determined as AFDW using the method described above. Subsamples were treated with 0.2 M HCl to remove carbonates for analysis of organic carbon (OC) and nitrogen with an Elementar Vario EL III CHN analyzer (Elementar Analysensysteme) stan-

dardized with acetanilide. Total phosphorus (TP) and organic phosphorus (OP) content were determined following the method of Zhou et al. (2003a, 2006).

Key physical and chemical parameters of the water column at the experimental site were measured 4 to 6 times every month from July to November. Water temperature, salinity, and pH were measured using a YSI 600 (Yellow Spring Instrument). Water samples were also collected daily during the 3 d experiment at 2.5 m depth. Total particulate matter (TPM), particulate organic matter (POM), and chlorophyll *a* were measured using Whatman GF/C filters as described by Mao et al. (2006).

### Data analysis

Variation in the chemical composition and element ratio of biodeposits and sediment (see data in Table 3) was evaluated using 1-way ANOVA. All data were graphically assessed for normality and homogeneity of residuals (Faraway 2002). When overall differences were significant at the 0.05 level, Duncan's multiple range test was used to compare the mean values of individual groups. Data are reported as means ± SE. All statistical analyses were performed using Statistica 6.0 (Statsoft).

## RESULTS

Water column characteristics at the study site are shown in Table 1. From July to November, water temperature ranged from 13.5 to 24.0°C and peaked in September. Salinity varied slightly, from 31.2 to 32.3, with the relatively lower values being observed during the summer months due to rainfall. Water pH values ranged from 8.10 to 8.24. The mean chlorophyll *a* (chl *a*) concentration was 1.43 µg l<sup>-1</sup>, with a minimum of 0.87 µg l<sup>-1</sup> in November and a maximum of 2.18 µg l<sup>-1</sup> in July. Seston concentrations varied markedly among months, with TPM ranging from 4.34 to 10.60 mg l<sup>-1</sup> and POM from 1.28 to 2.15 mg l<sup>-1</sup>.

### Densities of ascidians

*Ciona intestinalis* and *Styela clava* from lantern nets and scallop shells were most abundant in August and September, and markedly decreased thereafter (Fig. 1). Dense aggregations of ascidians were present on the underside of plates inside the lantern nets. The number of *C. intestinalis* and *S. clava* on

Table 1. Water column characteristics at the study site in Sanggou Bay, northern China. T: temperature; S: salinity; Chl *a*: chlorophyll *a*; POM: particulate organic matter; TPM: total particulate matter. Values are means  $\pm$  SE

Month	T (°C)	S	pH	Chl <i>a</i> ( $\mu\text{g l}^{-1}$ )	POM ( $\text{mg l}^{-1}$ )	TPM ( $\text{mg l}^{-1}$ )
July	21.6 $\pm$ 1.3	31.6 $\pm$ 0.1	8.10 $\pm$ 0.08	2.18 $\pm$ 0.47	2.15 $\pm$ 0.45	8.97 $\pm$ 2.10
August	23.8 $\pm$ 2.2	31.2 $\pm$ 0.6	8.23 $\pm$ 0.13	1.90 $\pm$ 0.32	1.62 $\pm$ 0.36	7.13 $\pm$ 2.36
September	24.0 $\pm$ 2.8	31.3 $\pm$ 0.3	8.15 $\pm$ 0.12	1.23 $\pm$ 0.35	1.28 $\pm$ 0.32	4.34 $\pm$ 0.98
October	20.2 $\pm$ 3.1	32.1 $\pm$ 0.2	8.24 $\pm$ 0.10	0.98 $\pm$ 0.26	1.76 $\pm$ 0.41	5.13 $\pm$ 1.21
November	13.5 $\pm$ 2.7	32.3 $\pm$ 0.4	8.20 $\pm$ 0.09	0.87 $\pm$ 0.30	1.81 $\pm$ 0.22	10.60 $\pm$ 3.24

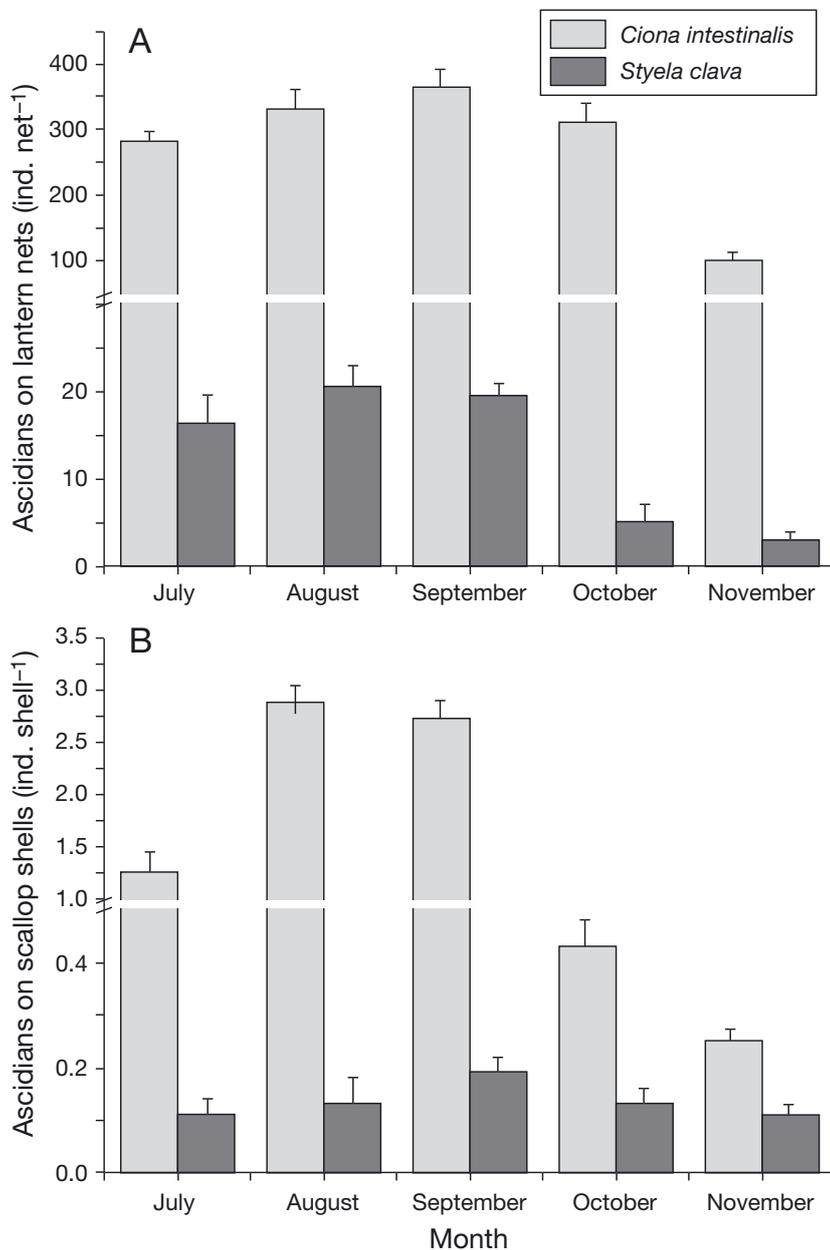


Fig. 1. Mean number ( $\pm$  SE) of ascidians *Ciona intestinalis* and *Styela clava* on (A) lantern nets and (B) shells of the scallop *Chlamys farreri* in Sanggou Bay, northern China

lantern nets ranged from 100.0 to 364.3 and from 3.0 to 20.5 ind. net<sup>-1</sup>, respectively (Fig. 1A). The number of *C. intestinalis* and *S. clava* on scallop shells ranged from 0.25 to 2.88 and from 0.11 to 0.19 ind. shell<sup>-1</sup>, respectively (Fig. 1B). In Sanggou Bay, the scallop *Chlamys farreri* is cultured at a density of  $\sim$ 350 ind. net<sup>-1</sup> (with each lantern net covering 4 m<sup>2</sup>) or  $\sim$ 88 ind. m<sup>-2</sup>. Based on these data, the mean densities of *C. intestinalis* and *S. clava* in the scallop farming zone during September were  $\sim$ 329 ind. m<sup>-2</sup> (364.3 ind. net<sup>-1</sup>  $\times$  1 net [4 m<sup>2</sup>]<sup>-2</sup> + 2.72 ind. shell<sup>-1</sup>  $\times$  350 scallops [4 m<sup>2</sup>]<sup>-2</sup>) and 22 ind. m<sup>-2</sup> (19.5 ind. net<sup>-1</sup>  $\times$  1 net [4 m<sup>2</sup>]<sup>-2</sup> + 0.19 ind. shell<sup>-1</sup>  $\times$  350 scallops [4 m<sup>2</sup>]<sup>-2</sup>), respectively. Therefore, the density of *C. intestinalis* during September was almost 4 times greater than that of cultured scallops.

The mean wet weights of *C. intestinalis* and *S. clava* in September were  $0.72 \pm 0.03$  and  $2.67 \pm 0.16$  g ind.<sup>-1</sup>, respectively. The mean dry weights (dw) of these ascidians were  $0.037 \pm 0.002$  and  $0.26 \pm 0.08$  g ind.<sup>-1</sup>, respectively, and the AFDWs were  $0.025 \pm 0.001$  and  $0.12 \pm 0.04$  g ind.<sup>-1</sup>, respectively. Based on these data, the ascidian drop-off to the seabed in September would amount to  $\sim$ 17.87 g dry matter m<sup>-2</sup> ( $329 \text{ ind. m}^{-2} \times 0.037 \text{ g ind.}^{-1} + 22 \text{ ind. m}^{-2} \times 0.259 \text{ g ind.}^{-1}$ ).

### Biodeposition

The biodeposition rates of dry matter, OM, organic carbon (OC), organic nitrogen (ON), total phosphorus (TP), and organic phosphorus (OP) by *C. intestinalis* and *S. clava* are shown in Table 2. The individual biodeposition rate ( $\text{mg ind.}^{-1} \text{ d}^{-1}$ )

Table 2. Biodeposition rates ( $\text{mg ind.}^{-1} \text{d}^{-1}$ ; means  $\pm$  SE) of dry material, organic matter (OM), organic carbon (OC), organic nitrogen (ON), total phosphorus (TP), and organic phosphorus (OP) by *Ciona intestinalis* and *Styela clava* in Sanggou Bay, northern China

Species	Dry material	OM	OC	ON	TP	OP
<i>Ciona intestinalis</i>	32.1 $\pm$ 0.7	4.61 $\pm$ 0.43	1.89 $\pm$ 0.19	0.087 $\pm$ 0.021	0.024 $\pm$ 0.005	0.009 $\pm$ 0.003
<i>Styela clava</i>	121.3 $\pm$ 19.8	16.90 $\pm$ 1.84	7.27 $\pm$ 0.68	0.557 $\pm$ 0.09	0.084 $\pm$ 0.016	0.033 $\pm$ 0.019

Table 3. Organic matter (OM), organic carbon (OC), organic nitrogen (ON), and organic phosphorus (OP) content, and C:N, C:OP, and N:OP ratios in sediments collected in the traps in Sanggou Bay, northern China, including controls (without ascidians) and traps with *Ciona intestinalis* or *Styela clava*. Values are means  $\pm$  SE. Values in the same column with the same superscript letter are not significantly different as determined by Duncan's test ( $p > 0.05$ )

Sediments	OM (%)	OC (%)	ON (%)	OP (%)	C:N	C:OP	N:OP
Control	14.36 $\pm$ 0.35	5.18 $\pm$ 0.18	0.29 $\pm$ 0.03 <sup>a</sup>	0.026 $\pm$ 0.004	8.88 $\pm$ 1.51	552.3 $\pm$ 46.2	31.1 $\pm$ 6.3
<i>Ciona intestinalis</i>	14.34 $\pm$ 0.15	5.17 $\pm$ 0.09	0.27 $\pm$ 0.05 <sup>a</sup>	0.028 $\pm$ 0.006	8.92 $\pm$ 2.13	512.1 $\pm$ 52.5	28.7 $\pm$ 5.9
<i>Styela clava</i>	13.77 $\pm$ 0.10	4.93 $\pm$ 0.06	0.46 $\pm$ 0.06 <sup>b</sup>	0.027 $\pm$ 0.002	8.06 $\pm$ 1.80	510.0 $\pm$ 53.6	31.6 $\pm$ 7.8

of dry matter, OM, OC, ON, TP, and OP by *C. intestinalis* were lower than that of *S. clava* (Table 2), whereas the biodeposition rate of *C. intestinalis*, based on per tissue dry weight ( $\text{mg} [\text{g dw}]^{-1} \text{d}^{-1}$ ), was higher than that of *S. clava* (see Table 4). The chemical composition (OM, OC, ON and OP) of the ascidian biodeposits is shown in Table 3. There were no statistically significant differences in the chemical composition of the ascidian biodeposits and the control sediment, except that the ON content of *S. clava* biodeposits was significantly higher ( $p < 0.05$ ) than that of *C. intestinalis* and the control sediment.

## DISCUSSION

Temperature is one of the key factors controlling the reproduction of ascidians in temperate environments. Recruitment of *Ciona intestinalis* and *Styela clava* usually peaks during the summer (Blum et al. 2007, Bourque et al. 2007, McCarthy et al. 2007). We have consistently found that the densities of both *C. intestinalis* and *S. clava* are highest in summer in Sanggou Bay. Some previous studies conducted in temperate areas have reported 2 recruitment peaks (e.g. Blum et al. 2007, Bourque et al. 2007), but these studies involved serial cleaning or replacement of the settlement substratum, in contrast to the present study where substrata (i.e. lantern nets and scallop shells) were not manipulated. The majority of the available settlement space in the lantern nets was occupied and retained by ascidians and other set-

tlers, leaving little available space on the substratum for recruitment of ascidians produced in subsequent reproductive cycles.

At present, direct data on biodeposition by *C. intestinalis* and *S. clava* are limited. McKindsey et al. (2009) used sediment traps to measure the biodeposition of *C. intestinalis* and *S. clava* from small groups of individuals on constructed mussel socks. Based on the dry mass of material collected and the mean abundance of tunicates on socks, the biodeposition rates of *C. intestinalis* and *S. clava* were approximately 7.12 and 20.53  $\text{mg ind.}^{-1} \text{d}^{-1}$ , respectively. The values in our study were approximately 5 times greater than those of McKindsey et al. (2009). However, it is difficult to perform direct comparisons of the biodeposition rates of filter feeders due to differences in body size and experimental conditions. Since water temperature is an important determinant of biodeposition rates, a possible explanation for the lower biodeposition rates recorded by McKindsey et al. (2009) is that the water temperature in their study area was lower than in the present study. Their measurements were carried out in October in Malpeque Bay, Canada (46°N), a location farther north than Sanggou Bay (37°N). Other factors such as water chl *a* and suspended particulate matter concentration are also important determinants of biodeposition rates. The biodeposition rates (based on dry tissue weight) of some filter feeders are summarized in Table 4, which shows the wide variation among species. The biodeposition rates of *C. intestinalis* and *S. clava* were higher than that of *Chlamys*

Table 4. Comparison of biodeposition rates (mg [g tissue dry wt]<sup>-1</sup> d<sup>-1</sup>) between bivalves and ascidians

Species	Dry wt	Biodeposition		Source
		N	P	
<i>Mytilus chilensis</i>	595	2.38	1.2	Jaramillo et al. (1992)
<i>Modiolus americanus</i>	2250	2.47	8.16×10 <sup>-2</sup>	Peterson & Heck (1999)
<i>Chlamys farreri</i>	420	0.52	0.18	Zhou et al. (2003b)
<i>Chlamys farreri</i>	122.3	0.80	0.17	Zhou et al. (2006)
<i>Ciona intestinalis</i>	659.0	1.78	0.49	Present study
<i>Styela clava</i>	467.8	2.15	0.32	Present study

*farreri* previously measured in Sanggou Bay (Zhou et al. 2003b) and Sishili Bay (Zhou et al. 2006) (Table 4). The biodeposition rates of *C. intestinalis* and *S. clava* are based on per unit of total dry weight. These values would be higher if based upon the unit of dry weight of body parts (without tunic) or AFDW, since a relatively high proportion of the tunic has no biological activity. Based upon the biodeposition rates and densities of ascidians, the total daily biodeposit production in September amounted to 13.24 g m<sup>-2</sup>, and daily C, N, and P deposition rates were 0.94, 0.11, and 0.0098 g m<sup>-2</sup>, respectively.

The scallop cultivation area covers about 8.0 × 10<sup>6</sup> m<sup>2</sup> in Sanggou Bay (data from the Fisheries Research Institute of Rongcheng City, Shandong Province; www.rchy.gov.cn). Therefore, the estimated daily biodeposit production by *C. intestinalis* and *S. clava* in September was 105.9 t dry weight, 7.52 t C, 0.86 t N, and 0.078 t P. Based on the biodeposition rate of the scallop *C. farreri* (Zhou et al. 2003b), the daily biodeposit production by 2 yr old scallops would be ~490 t dry weight, 24.5 t C, 3.43 t N, and 0.21 t P in September. The biodeposition of dry material, C, N, and P by *C. intestinalis* and *S. clava* amounts to 21.6, 30.7, 25.1, and 37.0% of that by cultured scallops. McKindsey et al. (2009) found that the presence of *C. intestinalis* significantly increased the benthic loading from suspended mussel culture. The data from the present study similarly indicate that when estimating the impact of suspended scallop aquaculture on the coastal ecosystem, the contribution of ascidians should be considered.

In addition to biodeposit production, ascidians may also impact the benthos by drop-off. Most ascidians are either eliminated by net cleaning activities by commercial producers in late autumn, or die during the winter, and subsequently fall to the seabed. According to the densities and individual weight of the 2 ascidians measured in the present study, drop-off to the sea floor would amount to ~143.0 t of dry matter ([329 ind. m<sup>-2</sup> × 0.037 g ind.<sup>-1</sup> + 22 ind. m<sup>-2</sup> ×

0.259 g ind.<sup>-1</sup>] × 8.0 × 10<sup>6</sup> m<sup>2</sup>). This figure indicates that the biomass of ascidians could be substantial. However, it is noteworthy that this is an estimation for an entire growing season. Therefore, it is a relatively small input to the benthos compared with biodeposition if averaged on a daily basis. On the other hand, the drop-off process would occur in short-duration pulses (e.g. during cleaning), and a short-term large

influx of OM may be relatively important in terms of benthic effects. The decomposition and nutrient release from ascidian drop-off may influence the chemical properties of the surface sediment, and further, potentially modify remineralization and biogeochemical processes.

In summary, the present study has demonstrated that high population densities of *C. intestinalis* and *S. clava* may have potential impacts on the culture environment through biodeposition and accumulation of dead and discarded ascidians. These 2 common aquaculture pest ascidians may play an important role in coupling material fluxes from the water column to the seabed, and their role in this process requires further investigation.

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