The Concentration of Chlorophyll-C in The Bottom Sediment of Sea Cucumber Rearing Cage

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Abstract. The total chlorophyll-c has been proved as indicator of the occurrence of diatom and green algal biomass in sediments. Those chlorophyll-c-containing algae acts as food of sea cucumber reared in the cage. The present study was aimed to examine the total chlorophyll-c in the bottom sediment of sea cucumber Holothuriaatra rearing cage with high stocking density (40 ind.4m-2). The sample of sediments were collected from 0-3 cm surface layer of bottom sediment during Mei-July. The chlorophyll-c were analysed spectrophotometrically. The result showed that the chlorophyll-c concentration in the bottom cage sediment fluctuated, decreased due to activity of sea cucumber feeding on microphytobenthos and increased due to their bioturbation activity.

1. Introduction
Microphytobenthos or benthic microalgae describes the group of photoautotrophic microorganisms inhabiting surficial sediments of shallow aquatic ecosystems such as diatoms, cyanobacteria and other chlorophytes[1]. Within shallow coastal waters microphytobenthos play an important role in system metabolism. They are significantly contribute to primary production[2]. Because much of the sediment surface resides within the euphotic zone, benthic autotrophs often are the dominant primary producers. They are able to photosynthesize at low light levels[3], taking advantage of the usually higher nutrient concentrations in the sediment[4] and therefore microphytobenthos fundamentally are able to alter sediment organic matter (SOM) quality and quantity[5]. Since biomass may accumulate at this layer, its contribution to the overall system productivity is often significantly higher than the integrated adjacent water-column[6][7]. It is not surprising that they are an important food source for benthic fauna such as sea cucumber[8] and many more estuarine consumers. Understanding the relationship between the food availability and the organism cultured is important as a key success of sea cucumber rearing.

The total chlorophylls c in sediments is a very sensitive indicator of the occurrence of chlorophyll c-containing algae in the over-lying water column[5]. Chlorophylls c were found in unicellular
chromophyte algae *i.e.* diatoms, dinoflagellates, prymnesiophytes and chrysophytes. Therefore the present works was aimed to measure the chlorophyll-c in the bottom sediment of sea cucumber cage.

2. Research Methods

The sediment samples were collected from 0-3 cm surface layer of bottom sediment of sea cucumber *H. atra* rearing cage during Mei-July. The chlorophyll-c were analysed spectrophotometrically. Pigments were extracted from 10 grams of wet sediment using 20 ml of 90% acetone in screw capped glass tub. Samples were kept in dark room at temperatures of 4°C for 24 hours, then extracts were centrifused at 2500 rpm for 5 min, filtrate then analyzed for Chlorophyll-c by spectrophotometer following procedures of [9] modified by [10] and [11]. A Perkin-Elmer Lambda 3BUV/VIS spectrophotometer with a 1 nm spectral bandwidth and optically matched 4 cm micro-cuvettes are used in the present work. The chlorophyll-c was calculated using following formula.

\[
\text{Chl-c (mg.g}^{-1}) = \frac{\left((55*A_{660}) - (4.64*A_{665}) - (16.3*A_{645})\right) \times 10000 \times 0.002}{L \times p}
\]

3. Results

Microphytobenthos inhabit the top few centimeters of the substrate layers (mud or sand) of marine sediment where has sufficient light for photosynthesis[7]. Benthic microalgae have an important role as a food source for higher trophic levels in shallow water as well as estuarine food webs. [12] also proved that a host of benthic consumers including omnivores, suspension feeders and deposit feeders (such as sea cucumber) mostly rely on benthic microalgae for food.

The contents of total chlorophylls c in bottom sediments of sea cucumber rearing cage are presented in Fig. 1. It showed that in the beginning, the chlorophyll-c in the sediment of the cage and control (without cage) were almost the same. In June, there was decreasing of chlorophyll-c concentration but during July there was significant increments of cell densities at the sediment. During this period because in the cage, the physical characteristic of the water may not give effect on the microphytobenthic community. Although actual current speeds were not measured, this event might have helped alleviate physical stress in the cage, and microphytobenthic could stay and grow well in the bottom layer of the cage and increase the chlorophyll-c on July. In the contrary, the chlorophyll-c were decreased in control site. There are many potential ecological consequences due to decreasing microphytobenthic production. One of them for example, biogeochemical processes such as nitrification and denitrification are affected by diel variations in oxygen content related to microphytobenthic metabolism as well as competition with microphytobenthos for dissolved N [13][14].
Chlorophylls c are very sensitive markers of chlorophyll c containing algae [5]. Moreover, the concentrations of these pigments in sediments can be treated as indicators of the diatoms living in sediments and the overlying waters regarding disturbances caused by local currents and the conditions of deposition. They are rather an indicator of biomass than of the number of cells or species. The ratio of total chlorophylls c and b to chlorophyll a could be a valuable indicator of diatom and green algae biomass. The diatom in a typical of shallow-water assemblages were represented by both attached (epipsammic) and motile (epipelic) species in which the former being strongly predominant. In their work [5] showed that the most abundant of them were Achnanthes delicatula, Opephora aspleni, Opephora aspleni, Fragilaria aposotensis, Navicula cryptocephala, N. germanoponita, N. paulschulzi. While in the deeper water (more than 5 meter), these sediments were predominantly inhabited by planktonic diatom taxa which settled onto the bottom from the water column. The sediments contained whole diatom cells, a certain amount of detritus and resting spores. The diatom flora was mainly composed of resting spores of Chaetoceros spp. or dominated by Thalassiosira decipiens and Cyclotella octactawhatcheeana.

As happen in nature [15] during periods of high grazing pressure (in this case during June), microphytobenthic community production was more than sufficient to supply food resources for meiofaunal consumers, i.e. sea cucumber. The similar result showed by [16] that in the intensive Holothurias c in the bottom sediment of H. atra rearing cage. The concentration of Chlorophyll-c in the bottom sediment of H. atra rearing cage

\[ \text{Chl-c} \text{ (mg/g)} \]

| Month | Cage | Control |
|-------|------|---------|
| May   | 1.0  | 0.8     |
| June  | 0.5  | 0.4     |
| July  | 1.5  | 1.3     |

Figure 1. The concentration of Chlorophyll-c in the bottom sediment of H. atra rearing cage.
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ments and convert organic detritus into animal tissue and nitrogenous wastes, which can be taken up by algae[30][31]. Higher grazing rates will also likely result in a redistribution of nutrients (as waste products from sea cucumber), enhanced rates of nutrient regeneration, and subsequent growth [32]. Microphytobenthos as measured by chlorophyll-c in the upper few millimeters of sediment seems to be limited primarily by the availability of resources (light, nutrients, etc.) [7]. [22] examined the interactive effects of consumers and resources on ecosystem structure and function, and showed that when consumers are present, peak diversity occurs at higher levels of nutrient supply.

Bioturbation and sediment reworking by meiofaunal activities, such as sea cucumber [33], may also increase porosity and solute transport rates, facilitating porewater exchange and nutrient supply to microphytobenthos[34]. Thus, diversity and primary productivity depend on the relative rates of nutrient supply and consumer pressure in many marine food webs. These results are inline with the general community structuring principles[22] and showed strong relationship between microalgae and meiofauna in the upper few millimeters of sediments. The trophic relationships are complex, with linked feedback mechanisms that operate over small spatio-temporal scales[35]. The coupling of measurements of rate and biomass responses for both producers and grazers has provided some useful insights into possible mechanisms underlying sea cucumber-microalgal trophodynamics in bottom cage. Aside from the light, the texture and relief of the sediment surface of bottom cage and its organic content also determine the vertical distribution of microphytobenthoscommunities[36][37][38]. As the top layers of the sediment represent a zone with such remarkably strong physicochemical gradients, most benthic microalgae show adaptive diurnal and tidal rhythms of vertical migration, moving in response to light, tide cycles, desiccation, predation and resuspension[39][40][41]. Microphytobenthos may be able to migrate vertically from 10 to 27 mm.h⁻¹[42]. Furthermore, in microscale horizontal gradients, nutrient, irradiance, water content and salinity may affect the vertical gradients, and their combination affect the growth of microphytobenthos communities[43]. A study on sea cucumber species of Australostichopus mollis revealed that nutrient release from holothuroids can increase benthic productivity[12]. Furthermore they said that losses of microalgae from consumption by sea cucumbers outweighed the increased productivity of microalgae from nutrients they excreted.

4. Conclusions
The concentration of chlorophyll-c as represented by microphytobenthic biomass in the sediment of bottom cage were more fluctuated during period of sea cucumber rearing that might be due to their feeding and their bioturbation activities compare to control samples.

References
[1] Sullivan MJ and Currin CA 2000 Community structure and functional dynamics of benthic microalgae in salt marshes. In: Weinstein MP, Kreeger DA (eds) Concepts and controversies in tidal marsh ecology. Kluwer Academic Publishers Dordrecht 81–106
[2] Hardiso AK, EA Canuel, IC Anderso, CR Tobias, B Veuger, MN Waters 2013 Microphytobenthos and benthic macroalgae determine sediment organic matter composition in shallow photic sediments Biogeosciences 10 5571–5588 www.biogeosciences.net/10/5571/2013/ doi:10.5194/bg-10-5571-2013.
[3] Blanchard GF and PA Montagna 1992 Photosynthetic response of natural assemblages of marine benthic microalgae to short- and long-term variations of incident irradiance in Baffin Bay Texas. J Phycol 287-14.
[4] Queiroz RL, FPBrandini, FMPellizzari 2004 Dynamics of microalgal communities in the water-column/sediment interface of the inner shelf off Parana State, Southern Brazil. Brazilian J of Oceanography 523(4)183-194.
[5] Kowalewska GA, B Witkowski, Toma, 1996 Chlorophylls c in bottom sediments as markers of diatom biomass in the southern Baltic Sea. OCEANOLOGIA 38(2) 227–249.
[6] Cahoon LB and J Cooke 1992 Benthic microalgal production in Onslow Bay, North Carolina, USA. Mar Ecol Prog Ser 84 185-196
[7] MacIntyre HL, RJ Geider, DC Miller 1996. Microphytobenthos: The ecological role of the "Secret Garden" of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. Estuaries 19 186-201

[8] Blanchard GF 1991 Measurements of meiofauna grazing rates on microphytobenthos: is primary production a limiting factor? J Exp Mar Biol Ecol 147 37-46

[9] Lorenzen G 1967 Determination of chlorophyll and phaeopigments: Spectrophotometric equations. Limnol Oceanogr 12 343-346

[10] Wellburn AR 1994 The Spectral determination of chlorophyll a and b as well as Total carotenoid, using various solvents with spectrophotometers of different resolutions. J Plant Physiol 144 307-313

[11] Dere S, G Toht, R Sivaci 1998 Spectrofotometric determination of chlorophyl-a, b and total carotenoid contents of some algae species using different solvents. Botany 22 1 13-17

[12] MacTavish T, J Stenton-Dozey, KVopel, C Savage 2012 Deposit-feeding sea cucumbers enhance mineralization and nutrient cycling in organically-enriched coastal sediments. PLoS ONE 7 e50031.

[13] An S and SB Joye 2001 Enhancement of coupled nitrificationdenitritification by benthic photosynthesis in shallow estuarine sediments. Limnol Oceanogr 46 62–74

[14] Rysgaard S, PB Christensen, LP Nielsen 1995 Seasonal-Variation in Nitrification and Denitritification in Estuarine Sediment Colonized by Benthic Microalgae and Bioturbating Infauna, Mar. Ecol.-Prog. Ser. 126 111–121

[15] Pinkney JL, KR Carman, SE Lumsden, SN Hymel 2003 Microalgal-microfaunal trophic relationships in muddy intertidal estuarine sediments. Aquat Microb Ecol 31 99–108

[16] Plotiau T, J Baele, R Vaucher, C Hasler, D Koudad, I Eeckhaut 2013 Analysis of the impact of Holothuriascabraintensive farming on sediment. Cah Biol Mar 54 703-711

[17] Slater MJ and AG Jeffs 2010 Do benthic sediment characteristics explain the distribution of juveniles of the deposit-feeding sea cucumber Austrolostichopusmolllis? J of Sea Res 64 241-249

[18] Yingst JY 1976 The utilization of organic matter in shallow marine sediments by an epibenthic deposit feeding holothurian. J Exp Mar Biol Ecol 23 55–69

[19] Moriarty DJW 1982 Feeding of Holoturia atra and Stichopus chloronotus on bacteria, organic carbon and organic nitrogen in sediments of the Great Barrier Reef. Australian J Mar Freshw Res 33 255–263

[20] Uthicke S 1999 Sediment bioturbation and impact of feeding activity of Holothuria (Halodeima) atra and Stichopus chloronotus, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef. Bull Mar Sci 64, 129–141.

[21] Carpenter SR, J Kitchell, JR Hodgson 1985 Cascading trophic interactions and lake productivity BioScience 35 634–639

[22] Worm B, HK Lotze, H Hillebrand, U Sommer 2002 Consumer versus resource control of species diversity and ecosystem functioning Nature 41 7848–851

[23] Connor MS, JM Teal, I Valiela 1982 The effect of feeding by mud snails, Ilyanass obsolete (Say), on the structure and metabolism of a laboratory benthic algal community. J Exp Mar Biol Ecol 65 29–45

[24] Montagna PA 1984 In situ measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. Mar Ecol Prog Ser 18 119–130

[25] Carman KR, JW Fleeger, SM Pomarico 1997 Response of a benthic food web to hydrocarbon contamination. Limnol Oceanogr 42 561–571

[26] Miller DC, RJ Geider, HL MacIntyre 1996 Microphytobenthos: the ecological role of the ‘secret garden’ of unvegetated, shallow-water marine habitats. II. Role in sediment stability and shallow-water food webs. Estuaries 19 202–212
[27] Barranguet C, J Kromkamp, J Peene 1998 Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos. Mar Ecol Prog Ser 173 117–126

[28] Jørgensen BB and DJ Des Marais 1986 A simple fiber-optic microprobe for high resolution light measurements: application in marine sediment. Limnol Oceanogr 31 1376–1383

[29] Kuhl M, C Lassen, BB Jørgensen 1994 Light penetration and light intensity in sandy marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes. Mar Ecol Prog Ser 105 139–148

[30] Uthicke S and DW Klumpp 1998 Microphytobenthos community production at a near-shore coral reef: seasonal variation and response to ammonium recycled by holothurian. Mar Ecol Prog Ser 169 1–11

[31] Uthicke S 2001 Interactions between sediment-feeders and microalgae on coral reefs: grazing losses versus production enhancement. Mar Ecol Prog Ser 210 125-138

[32] Mc Cormick PV 1994 Evaluating the multiple mechanisms underlying herbivore-algal interactions in streams. Hydrobiologia 291 47–59

[33] Purcell SW, C Conand, S Uthicke, M Byrne 2016 Ecological roles of exploited sea cucumbers. Oceanography and Marine Biology: An Annual Review 54 367-386

[34] Aller RC and JY Aller 1992 Meiofauna and solute transport in marine muds. Limnol Oceanogr 37 1018–1033

[35] Buffan-Dubau E and KR Carman 2000 Diel feeding behavior of meiofauna and their relationships with microalgal resources. Limnol Oceanogr 45 381–395

[36] Joergensen BB, Revsbech NP, Cohen Y 1983 Photosynthesis and structure of benthic microbial mats, Microelectrode and SEM studies of four cyanobacterial communities. Limnol Oceanogr 28 1075-1093

[37] Wiltshire KH 1992 The influence of microphytobenthos on oxygen and nutrient fluxes between eulittoral sediments and associated water phases in the Elbe Estuary. In: G Colombo, IF Ferrari, VU Ceccherelli, R Rossi (eds) Marine Eutrophication and Population Dynamics, Proc, 25th EMBS Olsen and Olsen Fredensborg 63-70

[38] Wiltshire KH 1993 The influence of photosynthetically produced oxygen by microphytobenthos on the oxygen and nutrient status of sediment water systems in the Elbe estuary. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 25 1141-1146

[39] Admiraal W 1984 The ecology of estuarine sediment inhabiting diatoms, Progr in Phycolog Res 3 269-270

[40] Pinckney J and RG Zingmark 1991 Effects of tidal stage and sun angles on intertidal benthic microalgal productivity. Mar Ecol Prog Ser 76 81-89

[41] Paterson DM, KH Wiltshire, A Miles, J Backburn, I Davidson, MG Yates, S McGorty, JA Eastwood 1998 Microbiological mediation of spectral reflectance from intertidal phytoplankton and microphytobenthos due to increased sediment fluxes in the Venice Lagoon, Italy. Estuar Coast Shelf Sci 54 773–792

[42] Chatterjee A 2014 Role of benthic microalgae in a coastal zone : biomass, productivity and biodiversity. Other. Université de Bretagne occidentale-Brest, 2014 English. <NNT : 2014BRES0005>,<tel-01668574>

[43] Wolff WJ 1979 Flora and vegetation of the Wadden Sea. Report 3 Final Report of the Section Marine Biology of the Wadden Sea. AA Balkema, Rotterdam, The Netherlands