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### 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 occurance 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<sup>-2</sup>). The sample of sediments were collected from 0-3 cm surface layer of bottom sediment during Mei-July. The chlorophyll-c were analysed using spectrophotometer. 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

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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  $4^{\circ}$  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.

Chl-c (mg.g<sup>-1</sup>) = 
$$\frac{((55*A_{630}) - (4,64*A_{665}) - (16,3*A_{645})) \times 10000 \times 0,002)}{L \times p}$$
(1)

#### 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 microphytopbenthic 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].

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Figure 1. The concentration of Chlorophyll-c in the bottom sediment of H. atra rearing cage

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 *Achnanthesdelicatula, Opephoraolsenii, Ophephorasp., Fragilariasopotensis, Naviculacryptocephala, N. germanopolonica N. paulschulzii*. 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 detritus and resting spores. the diatom flora was mainly composed of resting spores of *Chaetoceros* spp. or dominated by *Thalassiosira f. decipiens* and *Cyclotellachoctawhatcheeana*.

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 *Holothuriascabra* farming the concentration of photosynthetic microorganisms fell by up to 22% within sea farm pens and showed the grazing by sea cucumber. [17] also recorded the highest growth rate of *Australostichopusmollis* when microphytobenthic activity was the highest. Sea cucumber mostly digest bacteria, cyanobacteria, decaying plants (e.g., seagrass and algae) matter, some diatoms, foraminiferans, fungi, and other organic matter that constitute detritus [18][19][20][12].

It is therefore not surprising that the concentrations of bacteria and photosynthetic microorganisms (microphytobenthic) decreased in the pens. Microphytobenthos biomass, like most microbial communities, is regulated by both top-down and bottom-up controls[21][22]. Grazers can potentially limit their standing stock via high consumption rates[23][24][25] as well as high grazing pressure by deposit-feeding benthicfauna which may also reduce microphytobenthos abundance [26], while nutrients and light may regulate their biomass and productivity [27]. In addition, grazers may indirectly stimulate microphytobenthos production by enhancing nutrient availability while simultaneously 'thinning' the microalgaloverstory and allowing deeper penetration of light into the sediments[28][29]. *H. atra*, as many other aspidochirote sea cucumbers, feed on large quantities of

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sediments 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-microalgaltrophodynamics 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<sup>-1</sup>[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 Australostichopusmollis 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 controll samples.

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