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Bioremediation using *Gracilaria lemaneiformis* to manage the nitrogen and phosphorous balance in an integrated multi-trophic aquaculture system in Yantian Bay, China

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ABSTRACT

To reduce negative environmental impacts from human aquaculture activities, the red alga *Gracilaria lemanei-formis* was co-cultured with the fish *Pseudosciaena crocea* in an integrated multi-trophic aquaculture (IMTA) system for 35 d in Yantian Bay. The eutrophication index value decreased from 14.5 to 8.4 after seaweeds were co-cultured in cage farming areas, which indicated that the eutrophic water column in Yantian Bay could be mediated by IMTA. Total DIN and DIP of the tidal input and output were 9.23 kg, 0.19 kg and 11.08 kg, and 0.27 kg, respectively. Total 5.24 kg of dissolved N and 0.81 kg of dissolved P were released from IMTA system. These results indicate that *G. lemaneiformis* co-cultured in IMTA system could not completely remove all excess nutrients. In theory, at least 324.48 kg of seaweed seedlings would be required to balance excess nutrients generated from fish cages.

1. Introduction

As demand for marine products has steadily increased worldwide in recent decades, most countries have already reached their maximal capacity for aquaculture production (Chen and Qiu, 2014). The rapid expansion of intensive monoculture systems meets requirements for fish supplies (Wilfart et al., 2013; Ferreira et al., 2014), but the increase of human activities in coastal areas puts further pressure on already impoverished marine ecosystems (Maroni, 2000; Chopin et al., 2001). In areas where conventional marine cage farming occurs, excess nutrients from the cage system enter the environment as dissolved ammonia, feces, and uneaten feed (Chopin et al., 2001). Thus, long-term fishery activities may lead to eutrophication, which in turn can stimulate phytoplankton growth and harmful algal blooms. Such eutrophication has had catastrophic impacts on aquaculture and caused a series of health problems, such as anoxia and parasites, especially in enclosed and/or semi-enclosed bays (Troell et al., 1997; Troell et al., 2009; Abreu et al., 2011). Therefore, new mariculture systems are needed, and systems that use multiple species with disparate ecological niches can reduce wastes and costs while enhancing aquaculture benefits in terms of both productivity and environmental value.

The concept of integrated multi-trophic aquaculture (IMTA) systems that include macroalgae was introduced in the 1980s (Ryther et al., 1975) and became a predominant aquaculture system throughout the world (Neori et al., 2004). Macroalgae act as biological filters to remove nutrients from the water column and re-oxygenate to the culturing system, thereby providing a positive environment for cultured animals. In an IMTA system, seaweeds added to shrimp culture cages were found to assimilate excess inorganic pollutants, which improved their own growth rate and biomass production, which in turn ameliorated the animal culture environment (Dudley et al., 2000; Sara, 2007). This was not surprising, as the released forms of phosphorus and nitrogen are known to be suitable for seaweed uptake (Zhou et al., 2006; Nobre et al., 2010; Abreu et al., 2011). In recent years, much attention has been focused on integrated land-based culture, such as integrated bivalve-fish cultivation, integrated bivalve-shrimp cultivation, and algaefish/shrimp (Rawson et al., 2001, 2002; McVey et al., 2002). However, co-culture studies in marine areas are challenging, as hydrodynamic, chemical, physical, and biological conditions make it difficult to take measurements (Wu, 1995; Troell et al., 1997; Troell et al., 1999b;

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Cheshuk, 2001). Wu et al. (2015a,b) reported that the nutrient flux in the IMTA system using red algae *Gracilaria chouae* co-cultured with black snapper (*Sparus macrocephalus*) in Xiangshan Bay, China, and the optimal proportion of macroalgae to fish was obtained.

Sansha Bay in Fujian Province of China is a typical enclosed bay, and it is one of the most famous mariculture locations along the southeastern coast of the country (Wu et al., 2015a,b). Eutrophication in Sansha Bay due to excess phosphorus and nitrogen has become a serious problem. It has occurred due to the continuous increase in human culture activities and poor water exchange, and it is predicted to become a major threat to sustainable development of mariculture, especially fish cage farming (Hu et al., 2013). In 2015, 22,000 fish cages were present in Sansha Bay, most of which were used for large yellow croaker (*Pseudosciaena crocea*) culture. The end use of the largescale aquaculture of the red algae *Gracilaria lemaneiformis* in this area is primarily abalone feed and industrial raw materials (Fei, 2004; Neori et al., 2004). The duration of a whole culture-harvest cycle of *G. lemaneiformis* is almost 1 month in Sansha Bay during the autumn and early winter seasons.

The goals of the present study were to evaluate the bioremediation efficiency of *G. lemaneiformis* co-cultured with *P. crocea* in an IMTA system and to identify the ideal proportion of marine fish to seaweed. The results of this study may lead to a new technical method that uses macroalgae to turn nutrient-rich effluents into profitable resources, results in optimal numbers and sizes of mariculture species, and enhances commercial and ecological benefits for local citizens.

2. Materials and methods

2.1. Study area

This study was conducted at the cage aquaculture base in Yantian Bay($26.72^{\circ}-26.84^{\circ}$ N, $119.76^{\circ}-119.83^{\circ}$ E), one of enclosed bays inside the Sansha Bay, located along the northeast bank of Fujian Province (Fig. 1). Seawater in the experimental area normally has a salinity of 26–29, but at low tide it may decrease to 24–27. During the study period, surface seawater temperature and pH values ranged from 18.4 °C to 26.0 °C and 7.43 to 7.83, respectively. The tidal current in the

study area was a right track half-tide with an average tidal range of 5.35 $\pm~0.25$ m and an average tidal current rate of 0.90 $~\pm~0.02$ m/s.

Thirty-two cages located in the central part of the fish aquaculture area were selected for study. Twenty-four cages were used in the bioremediation experiment among which twelve cages contained fish and the others had seaweeds ropes tied (i.e., bioremediation area), and eight cages were chose with only fish cultured (i.e., controls). The dimensions of each fish cage were $3.3 \times 3.3 \times 4.0$ m, and mesh size was 2.0-3.5 cm. Each cage was stocked with 1200 *P. crocea* (average weight 72.8 \pm 0.5 g; average length 17.2 \pm 0.3 cm), and about 20 kg of fresh feed fish were provided to each cage every morning.

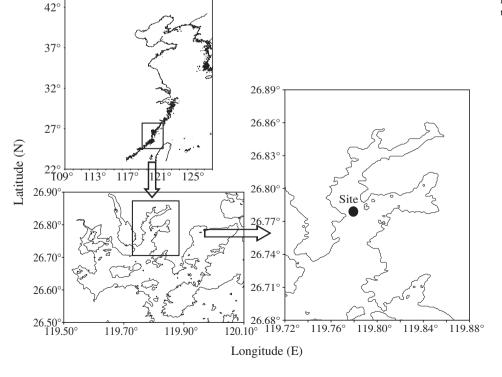
Polypropylene ropes with a diameter of 10 mm were soaked for 48 h in seawater before the experiment. Eight ropes were tied to each cage at intervals of 35–40 cm. Twenty-five clusters of 40–42 g of healthy *G. lemaneiformis* were attached to the ropes at 13–15 cm intervals. The seaweed ropes were suspended in the water column about 30–50 cm below the sea surface. The experiment began on 25 October 2014 and ended 35 d later.

2.2. Water samples collection and measurement of nutrient concentrations

Water samples were collected at the control area (C1–C3) and bioremediation area (C4–C6) after seaweed ropes were suspended on 30 October 2014 (Fig. 2). Samples at each site were collected every 5 d during 35 d experiment, resulting in 7 date sets. Surface temperature, salinity, pH, and dissolved oxygen (DO) concentration were measured in the field using a multi-parameter kit (MS5, HACH). Using Niskin bottles, three water samples were collected from three locations surrounding the central area of the bioremediation and control areas during slack tide time from a depth of 0.5–0.8 m below the seawater surface.

Chemical oxygen demand (COD) was measured directly by the method described by Parsons et al. (1984). Samples for dissolved inorganic nutrient measurements were filtered through GF/F glass-fiber filters (0.45 μ m) that were pre-soaked in 10% HCl for at least 24 h. Samples for Chlorophyll *a* (Chl *a*) determination were filtered onto GF/F glass-fiber filters (0.45 μ m). The filters were extracted with 90% acetone, and a Turner Designs fluorometer was used to measure the Chl

Fig. 1. Map showing the location of Yantian Bay and the experimental fish cage area in Yantian Bay.



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