The JSFS 85th Anniversary-Commemorative International Symposium
"Fisheries Science for Future Generations"
Symposium Proceedings, No. 04004

Original article

Protein hydrolysis activity in fish-farming area of Tanabe Bay, Wakayama

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Keywords: Hydrolysis; Fish farming impact.

Received: 18 July 2017 / Accepted: 11 September 2017

Introduction

In a fish-farming area, organic load is imposed on water bodies in the form of residual food and excreta of cultured fish. The burden of organic matter on the environment increases due to excessive overcrowding and overfeeding. When the environmental capacity of the water area is exceeded, water and sediment qualities deteriorate. In some cases, phenomena such as formation of anoxic water mass in the bottom layer and, reduction of benthic organisms occur [1,2]. Therefore, understanding the degradation process of the organic load in the fish-farming area becomes important to ensure sustainable aquaculture. Protein requirement of cultured fish is high. For instance, in the case of red sea bream, protein content in food was 46–48% [3]. This indicates that proteins is a major constituent of organic matter released to the environment by aquaculture activities. In this study, we focused on the hydrolysis of environmental proteins produced by aquaculture activities and released in the seawater and bottom sediments of the fish-farming area.

Materials and methods

From January 2015–May 2017, field surveys were carried out every odd month in the fish-farming area (Fig. 1 Station Kawakyuu, water depth=14 m) in Tanabe Bay, Wakayama. Seawater samples were collected from a depth of 1 m (surface) and 1 m above the seafloor (bottom layer). Bottom sediment samples were collected from 0–1 cm surface layer of the sea floor. Hydrolytic activities of the enzymes, trypsin and leucine aminopeptidase (LAPase), in the environmental samples were determined based on binding of fluorescent substrates (Fig. 2). The measurements of trypsin and LAPase activities were carried out from July 2016 and January 2015, respectively, to May 2017. Hydrolytic activity was measured by modifying the protocol of Obayashi and Suzuki [4].

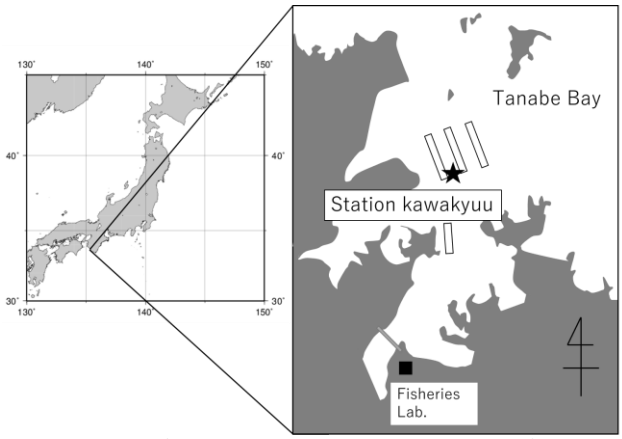

Fig. 1. Location of the sampling site in the Kogaura Inlet of Tanabe Bay.

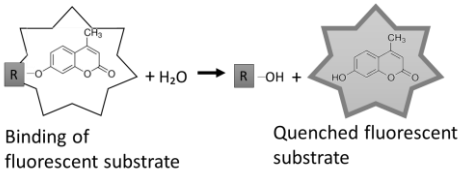

Fig. 2. Outline of enzyme activity measurement.

Results

Trypsin activity

During periods of lower water temperature (November–March), the average values of trypsin activity of surface water, bottom layer water and bottom sediments were 0.0948 ± 0.0519, 0.0723 ± 0.0519, and 51.0 ± 15.3 nmol cm^{-3} h^{-1}, respectively. During periods of higher water temperature (May – September), the average values of trypsin activity were 0.0596 ± 0.0426, 0.0477 ± 0.0494, and 53.2 ± 20.6 nmol cm^{-3} h^{-1}. Trypsin activity of surface water and bottom layer water tended to increase at lower water temperature.

LAPase activity

During the period with lower water temperature (November – March), the average values of LAPase

activity of surface water, bottom layer water and bottom sediments were 0.165 ± 0.130, 0.142 ± 0.111, 38.6 ± 27.1 nmol cm^{-3} h^{-1}, respectively. During the period with higher water temperature (May – September), the average values of LAPase were 0.300 ± 0.0835, 0.0741 ± 0.0411, 21.1±12.6 nmol cm^{-3} h^{-1}. The LAPase activity of the surface water tended to increase during the period with higher water temperature, and the LAPase activity of the bottom layer water and the sediment tended to increase during the period of lower water temperature.

Temporal variation in enzyme activity

Trypsin activity of surface water tended to increase during the period with lower water temperature, whereas that of LAPase tended to increase during the period with higher water temperature. However, the activities of both the enzymes of the bottom layer water tended to increase in the phase of lower water temperature. Trypsin activity of the sediment did not differ significantly between lower water temperature and higher water temperature periods, but LAPase activity tended to rise during the lower water temperature period.

Discussion

In the same fish farm, Yoshikawa et al. [5] showed a significantly higher LAPase activity in December than in October in the bottom layer water and a higher LAPase activity than chymotrypsin activity in the water column. Yoshikawa et al. [6] also investigated the mineralization activity and reported that the activity improved in the sediments in winter than in summer. For both reasons, they mentioned that the supply of dissolved oxygen (DO) was high in the bottom layer in December. Further, in this study, the activity of trypsin and LAPase activity of bottom layers, and LAPase activity of sediments, tended to increase during the phase of lower water temperature. Protein decomposition is likely to proceed in the bottom layer water during the winter when vertical mixing tends to occur and oxygen supply to the bottom layer is large. The LAPase activity of the bottom layer water showed a positive correlation with DO (r = 0.513, Fig. 3).

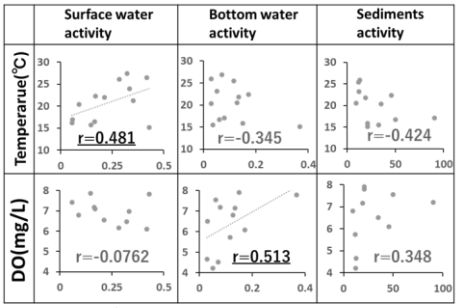

Fig. 3. Distribution of LAPase activity against water temperature and DO.

Many of the previous studies have measured LAPase activity as a representative of proteolytic enzyme activities. However, recent studies on oceans have reported trypsin activity, in addition to that of LAPase, is an important indicator of proteolysis [4]. Moreover, it has been pointed out that the environmental characteristics differ depending on the sea area, and the type of protease with high activity may differ [7]. LAPase activity tended to be higher than trypsin activity in the water column of the studied fish farming area of Tanabe Bay. In areas of the Tanabe Bay, where the influence of aquaculture is low, the LAPase activity reported earlier tended to be lower, whereas the trypsin activity tended to be higher. Higher LAPase activity may be peculiar to fish farms.

Acknowledgements

We are grateful to T. Katsuno and R. Miuchi of Kindai University for their cooperation in the analysis of enzymatic activities. Financial support was partially provided by the Strategic Project to Support the Formation of Research Bases at Private Universities: matching fund subsidy from MEXT, Japan, 2014-2016 (S1412003).

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