Rep. Suwa Hydrobiol., 9; 77~85 (1995)
Proceedings of the 7th International Symposium on River and Lake Environments, 1994, Matsumoto

The effects of the silver carp (*Hypophthalmichthys molitrix*) predation on the extracellular enzymatic activities

Seung-Ik CHOI, Myeong-Seop BYEON and Tae-Seok AHN
Dept. of Environmental Science, Kangwon National University, Chunchon, 200-701, Korea, Fax;+82-361-51-3991.

INTRODUCTION

The ecological character of the aquatic environments and the quality of water are determined by the amount of the production and the rate of organic materials decomposition. Particularly, in large reservoirs, water quality strongly depends on the structure and the function (energy transfer efficiency) of food webs. And these food webs are controlled by resource limitation ("bottom-up") and by predation ("top-down"). Solar energy, nutrient inputs and dynamics of an ecosystem set its overall level of production, so to that extent the control may be envisaged as bottom-up. However, among those limits, some of the "coarse-tuning" and much of the "fine-tuning" of structure and function result from the complexity of top-down effects [12]. Consequently, a deeper insight into that complexity of top-down processes is absolutely necessary if these processes should be used in biomanipulation as a new tool in water quality management [3]. By this time, most of the previous research on "top-down" processes were focused on direct effects of predation. However, impacts of the mediation on the nutrient regeneration and on the quality and quantity of food bases of different trophic levels have not been so widely studied [3].

The aim of this study was to define a small but important part of "top-down" process ; the effects of fish predation on phytoplankton, namely, the predation induced changes of the physicochemical and extracellular enzymes activities. We used a herbivorous fish (*Hypophthalmichthys molitrix*; silver carp) in the experiments.

MATERIAL AND METHODS

Condition of reserach ponds

Two ponds, L 3m x W 3m x H 1.5m, were used. The experimental

ponds were filled with water from Lake Paldang, and phosphate buffer solution (for BOD dilution) was added as final concentration to be 0.1 mg-P l^{-1} for good phytoplankton growth. Twenty *Hypophthalmichthys molitrix* (30 to 35 cm) were released to the fish pond on 2 June, 1993. No fish pond was used as a control.

Environmental factors

Water samples were collected weekly from 4 June to 29 June, 1993. Nutrient concentrations (total phosphorus, soluble reactive phosphate, total nitrogen, NH_4), water temperature, dissolved oxygen and chlorophyll *a* were measured by standard methods [1]. Bacterial cells were counted on 0.2 μm polycarbonate membrane filters after staining with acridine orange [13].

Enzyme activities

Phosphatase activity was determined by modified method of Hoppe [9], using the fluorogenic substrate, methylumbelliferyl (MUF)-phosphate (1mM). Cellobiohydrolase and ß-glucosidase activities were analyzed with MUF-cellobiopyranoside (5mM) and MUF-ß-glucoside (5mM), respectively [5, 11].

RESULT AND DISCUSSION

Environmental factors

Variations of temperature, dissolved oxygen and pH are shown in Table 1. Temperature did not change greatly, and DO and pH increased in fish pond and these might be caused by the increase of phytoplankton biomass. The nutrients except ammonia between two ponds had no difference (Table 2). This may suggest that silver carp did not affect the nutrient concentrations. But the concentration of ammonium ions showed a slight difference between two ponds.

Variations of total bacteria and chlorophyll *a* content

Variations of total bacterial numbers and chlorophyll *a* contents in the two ponds are shown in Fig. 1. Total numbers of the bacteria in the control pond and in the fish pond when experiment was started, were 3.0×10^6 cells ml^{-1} and 5.3×10^6

cells ml^{-1}, respectively. This values were similiar to the results which carried out in Lake Paldang by Ahn and Cho [2] in May 1989. During the experiment total number of the bacteria in the control pond ranged 1.5×10^6 - 3.9×10^6 cells ml^{-1} with little change. But the number was increased to 7.9×10^6 cells ml^{-1} at 22th day and peaked to 1.1 x 107 cells ml-1 at 29th day in fish pond. Chlorophyll *a* content was also not changed so as AODC in control pond, but it rapidly increased from 5.4 to 7.5 mg m^{-3} after 8 days and 12.6 mg m^{-3} after 27 days in fish pond. The increase of total bacterial number and chlorophyll *a* content suggest that rapid mineralization of fish feces might supply enough available nutrients.

Phosphatase activity(PA)

Phosphatase is the enzyme that hydrolyze the dissolved organic phosphorus and particulate organic phosphorus. Phosphatase activity is induced when inorganic phosphate in the water is exhausted and/or content of cellular phosphate decreased [6]. So, phosphatase actvity indicates the concentration of available phosphate in water column and the phosphate deficit of the phytoplankton cell [4]. The variation of PA of two ponds are shown in Fig. 2. At the first 8 days, no difference was found in the Vmax of PA in the two ponds, the values were constant of 120 nM l^{-1} hr^{-1}. However, in the fish pond, after 13 days Vmax increased to 251.2 nM l^{-1} hr^{-1}, two fold of the initial value. The highest value was shown after 20 days, as 453.9 nM l^{-1} hr^{-1}, and after then the values decreased gradually. In the other hand, in control pond, the Vmax peaked after 20 days and the variation range was relatively smaller than that of the fish pond.

Phosphatase are usually secreted from phytoplankton [10]. In our experiments, the variation of Vmax of phosphatase activity showed a same trend with the changes of the concentration of chlorophyll *a* (Fig. 1). Therefore, we could assume that the phosphatase was released from the phytoplakton. Since Kt+Sn, the sum of substrate transfer constant and concentration of available substrate rapidly decreased, so the turnover time of substrate dicreased.

Cellobiohydrolase activity

The passage of organic molecules across the microbial cytoplasmic membrane is an active process requiring specific transport enzymes (e.g. permease), and only small and simple molecules can be directly transferred from the environment into the cell [8]. In the lake ecosystem, the majority of organic substance is cellulose, cell wall component of phytoplankton and macrophyte. Cellobiohydrolase is one of the enzyme that catalyzes the hydrolysis of biopolymers ($[glucose]_n$, n = 2 - 5).

The Vmax of cellobiohydrolase showed a distinct pattern of activity. The Vmax of cellobiohydrolase activity ranged 4.4 - 78.0 nM l^{-1} hr^{-1} in both ponds. Vmax was low (*ca.* 20 nM l^{-1} hr^{-1}) during the first 8 days (Fig.3), and began to increase markedly till 13 days. After 20 days, the Vmax decreased in both ponds. In control pond, after 27 days, Vmax decreased as 4.4 nM l^{-1} hr^{-1}, one fifth of initial value (20 nM l^{-1} hr^{-1}). In fish pond, the Vmax showed higher value than that of the control pond. It might be due to the higher bacterial number observed in fish pond.

Because concentration and composition of the dissolved carbohydrates of natural environments are highly variable and changed rapidly [11], the substrates for cellobiohydrolase can be assumed as very small quantity. So the variation of Kt+Sn can be regarded as the variation of enzyme affinity to substrate, Km value [7]. The values of Kt+Sn ranged 9.6 - 231.4 μM l^{-1} in both ponds (Fig. 3). At the early stage, substrate affinity was extremely different between two ponds, highest in fish pond and lowest in control pond. In control pond, however, the affinities increased until the end of experiment. But, in fish pond, affinities were high during the early and late, and low during the middle of experiment. It can be explained as follows; Mainly the enzymatic affinity is affected by the temperature and pH condition. But during the experiment, the difference of water temperature and pH were less than 2 °C and *ca.* 0.3 only, respectively. So the difference of affinity was caused by the differences of carbohydrate composition, not by environmental factors. In control pond, the biomass of phytoplankton is the major source for organic compounds, and in fish pond, beside the phytoplankton biomass, fish feces is another important source.

So, the affinity at control pond was slightly increased throughout the bacterial adaptation to organic materials produced by phytoplankton within an enclosed space. While in fish pond, new substrates were released in the form of feces of silver carps, and zymogenous bacteria for these substrates were the dominants, so the affinity was extremely low. After 8 days, the bacteia were adapted to the organic materials from phytoplankton.

ß-glucosidase activity

ß-glucosidase is a broad-specificity enzyme that catalyzes the hydorlysis of ß-linked disaccharide of glucose, celluhexose, and carboxymethylcellulose [3] and it is very important and significant for glucose metabolism of microheterotrophs in aquatic ecosystem [5].

The Vmax of ß-glucosidase were ranged 16 - 252 nM l^{-1} hr^{-1} in both ponds (Fig. 4). Its activities were generally higher than that of cellobiohydrolase. During the first 8 days, the Vmax was not different between two ponds. However, later in fish pond, Vmax increased last until 27 days, while in control pond, Vmax decreased slightly after 13 days like cellobiohydrolase activity. Initial Km value in the fish pond was also lower than in the control pond like cellobiohydrolase activity. In both two ponds, the Km values slightly increased till the end of the experiment. Especially, in the fish pond, substrate affinity decreased slightly one hundred time at 15 days compared to initial value. According to Chróst [7], the value of affinity, was changed by various substrate, and the lowest affinity was found at glucose supplemented sample. Moreover, addition of cellobiose strongly induced ß-glucosidase synthesis, which means that bacteria produced the enzyme with high specific activity and affinity. So, the lower affinities of the final days, in two ponds were due to the diverse low molecule weight organic materials by bacterial degradation. And in the fish pond, the bacteria was more abundant, and the high Vmax could be sustained.

ACKNOWLEGMENT

This study was partly supported from Research Center for Molecular Microbiology, Seoul National University.

REFERENCES

1.APHA-AWWA-WPCF, 1989. Standard method for the examination of water and wastewater 17th ed., APHA. N.Y.

2.Ahn, T.S. and K.S. Cho, 1989. Bacterial distributions and activities in Lake Paldang. *J. Kor. WPRC.* 5:30-34.

3.Barman, T.E. 1969. Enzyme Handbook, vol. 2. Springer Verlag, Berlin. 928pp.

4.Choi, S.I., T.S. Ahn and B.C. Kim, 1992. Degradation rates of organic phosphate in Lake Soyang. *Kor. J. Microbiol.* 30:113-118.

5.Chróst, R.J., 1989. Characterization and significance of ß-glucosidase activity in lake water. *Limnol.Oceanogr.* 34:660-672.

6.Chróst, R.J. and J. Overbeck, 1987. Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in lake Plussee(north German eutrophic lake). *Microb. Ecol.*, 13:229-248.

7.Chróst, R.J., 1991. Environmental control of the synthesis and activity of aquatic ectoenzymes. *In* R.J. Chróst(ed.) *Microbial enzymes in aquatic environment.* pp. 29-59. Springer Verlag, NY.

8.Geller, A. 1985. Degradation and formation of refractory DOM by bacteria during simultaneous growth on labile substrates and persistent lake water contituents. *Swiss J. Hydrol.* 47:27-44.

9.Hoppe, H.G., 1983. Significance of exoenzymatic activities in the ecology of brackish water:measurements by means of methylumbelliferyl-substrate. *Mar. Ecol.*, 11:299-318.

10.Jansson, M., H. Olsson and K. Peterson, 1988. Phosphatase :Origin, characteristics and function in lakes. *Hydrobiologia*, 170:157-176.

11.Münster, U., 1991. Extracelluar enzyme activity in eutrophic and poly humic lakes. *In* R.J. Chróst(ed.) *Microbial enzymes in aquatic environment.* pp 96-122. Springer Verlag, NY.

12.Northcote, T.G., 1988. Fish in the structure and function of freshwater ecosystem: A "top-down" view. *Can. J. Fish. Aquat. Sci.* 45:361-379.

13.Parsons, T.R., 1984. A manual of chemical and biiological method for seawater analysis. Pergamon Press.

Table 1. The envronmental conditions of research ponds waters

Parameter	water Temp.(℃)		DO* (mg l^{-1})		pH	
Aquarium	C	F	C	F	C	F
June, 4	21	21	8.4	8.4	7.4	7.2
10	22	22	8.7	8.9	7.9	7.8
15	22	22	8.0	8.5	7.4	8.0
22	22	22	8.0	8.7	7.6	7.8
29	23	23	7.9	8.8	7.3	7.4

* C : Control pond without *Hypophthalmichthys molitrix*
F : Fish pond with *Hypophthalmichthys molitrix*
DO : dissolved oxygen

Table 2. The concentrations of nutrients of research pond waters

Nutrients	TP(mgP l^{-1})		SRP(mgP l^{-1})		TN(mgN l^{-1})		NH4 (mgN l^{-1})	
Aquarium	C	F	C	F	C	F	C	F
June, 4	0.101	0.101	0.06	0.06	1.79	1.79	0.394	0.394
10	0.101	0.105	0.06	0.06	1.79	1.80	0.290	0.294
15	0.100	0.103	0.06	0.06	1.80	1.90	0.350	0.280
22	0.103	0.113	0.06	0.06	1.88	1.90	0.300	0.286
29	0.094	0.101	0.06	0.06	1.79	1.90	0.300	0.350

* Same as Table 1.
TP:total phosphrus, SRP:soluble reactive phosphate
TN:total nitrogen, NH_4:ammonia

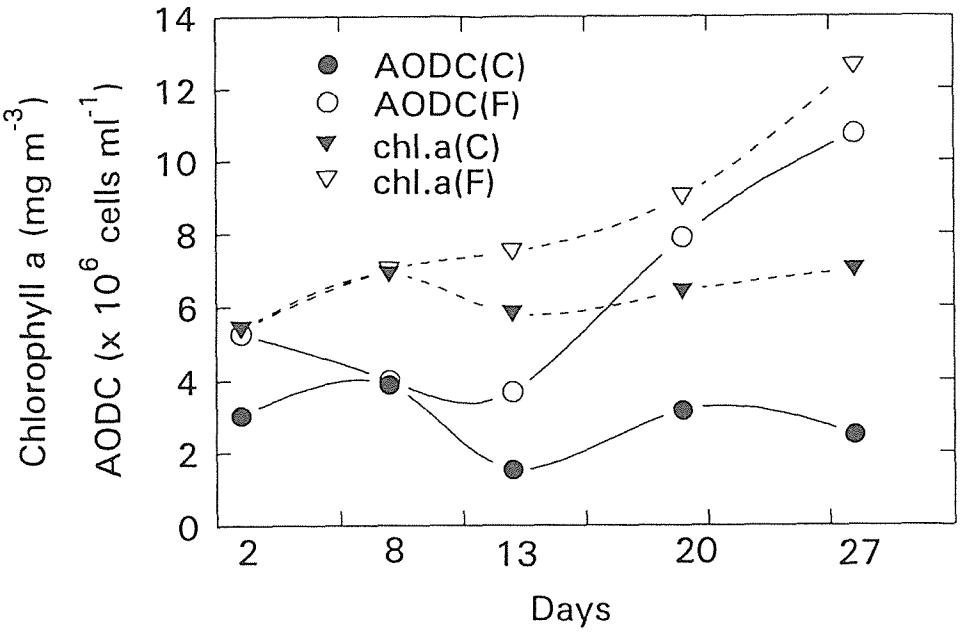

Fig. 1. The variations of bacterial number and chl. a concentration at research ponds.
(C:Control pond, F:Fish pond)

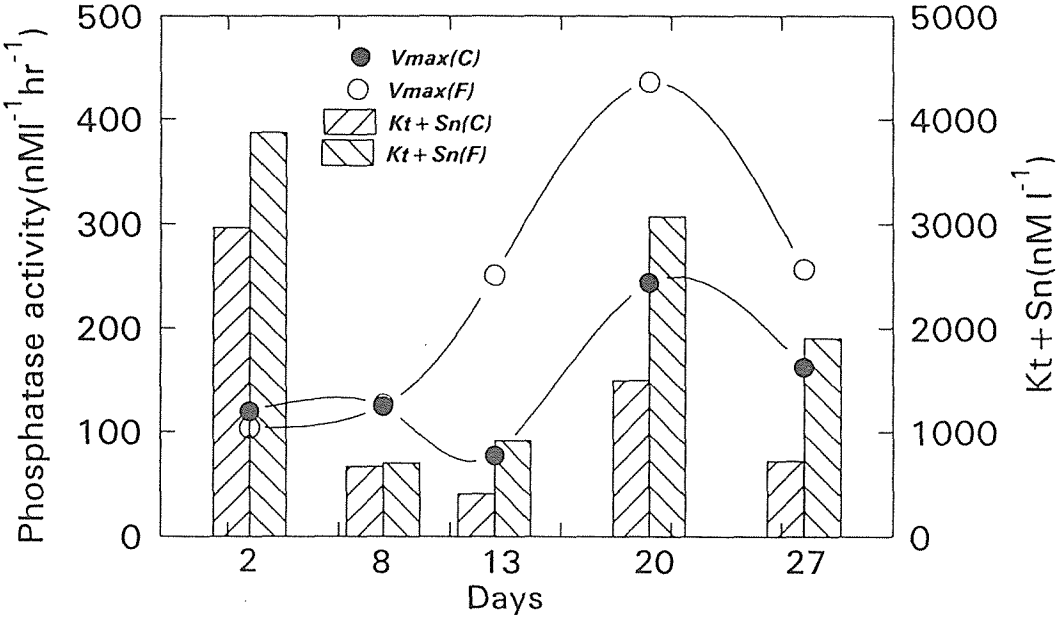

Fig. 2. Kinetics of phosphatase activity in research ponds.

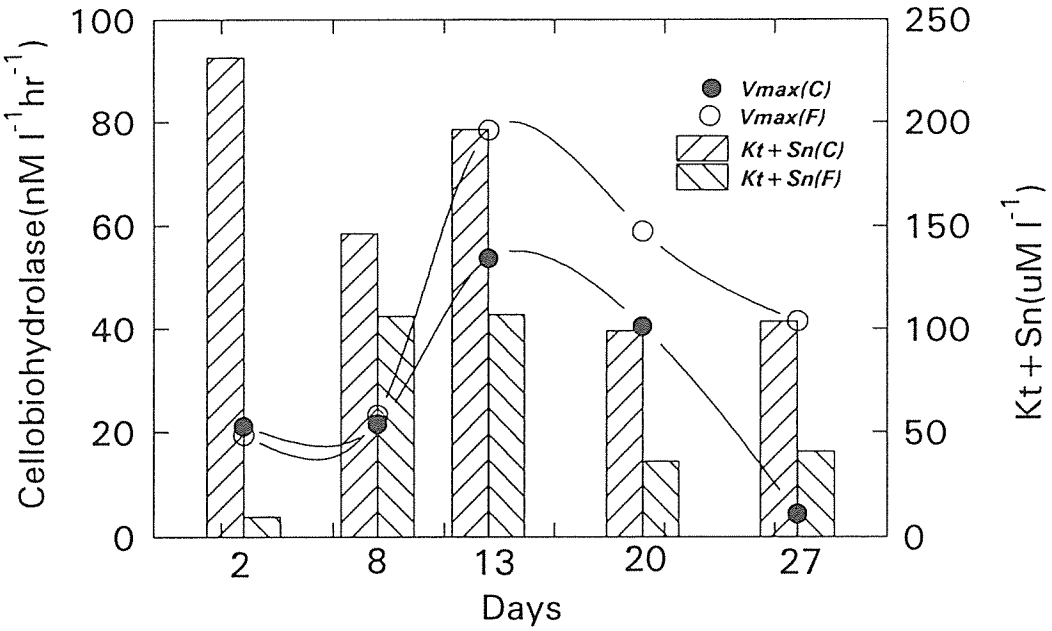

Fig. 3. Kinetics of cellobiohydrolase activity in research ponds.

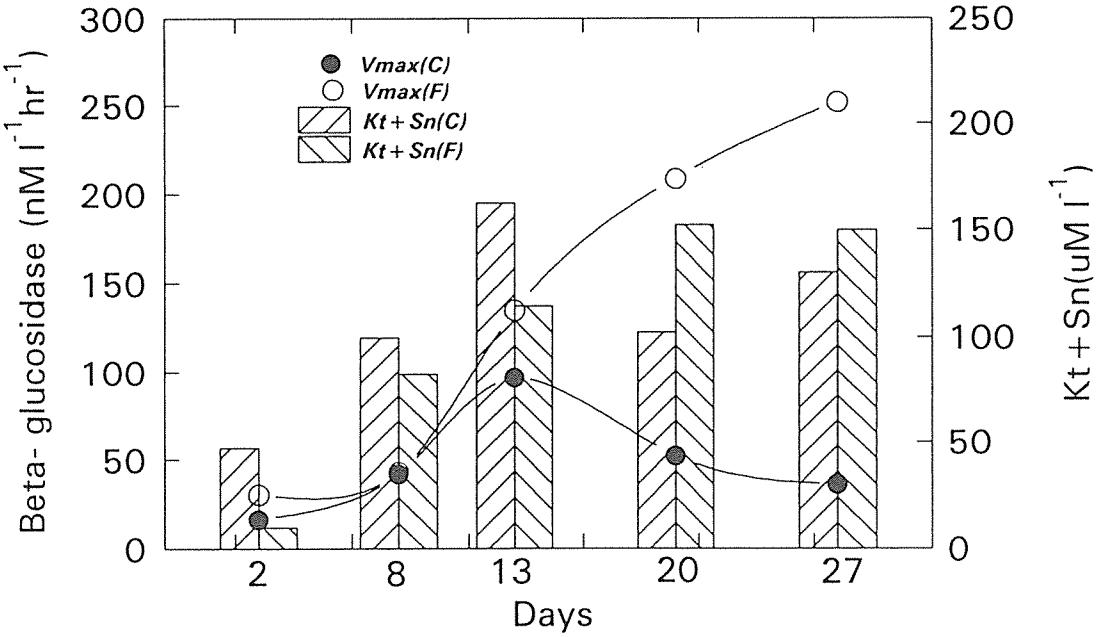

Fig. 4. Kinetics of beta-glucosidase activity in research ponds.