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The effect of environmental factors on filtration and the oxygen consumption rate of the rotifer *Brachionus plicatilis*: a primary exploration of red tide control

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Abstract

To evaluate the potential to control red tides using the mass-cultured heterotrophic grazer, rotifer *Brachionus plicatilis*, the effects of environmental factors (water temperature and salinity) on physical activities (filtration and oxygen consumption rate) of *B. plicatilis* were estimated. Experiments were conducted at different water temperatures (15, 20, 25, 30 and 35°C) and salinities (20, 25, 30, 35 and 40 PSU), in 25 different combinations (5 temperatures × 5 salinities).

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Results showed that water temperature and salinity had significant effects on the filtration rate of *B. plicatilis* (F=41.66, P<0.05). The results of multiple regression analysis yielded the following functional dependence of filtration rate (F) on water temperature (T) and salinity (S): F=-1.658+0.917T+0.63S (R^2=0.769, P<0.001). The highest filtration rate (4.23 ±0.74 µl rot^{-1} h^{-1}) was obtained at 30°C and salinity 35 PSU, and the lowest one (0.869 ±0.13 µl rot^{-1} h^{-1}) was observed at 15°C and salinity 20 PSU. Both water temperature and salinity had significant effects on the oxygen consumption rate of *B. plicatilis* (F=34.08, P<0.05). The results of multiple regression analysis yielded the following functional dependence of oxygen consumption rate (O) on water temperature (T) and salinity (S): O=-3.133+0.165T+0.81S (R^2=0.938, P<0.001). The highest oxygen consumption rate (5.38 ±0.66 ng rot^{-1} h^{-1}) was observed at 35°C and salinity 40 PSU, and the lowest one (1.01 ±0.15 ng rot^{-1} h^{-1}) was observed at 15°C and salinity 20 PSU. Results from this present study indicated that the filtration and oxygen consumption rate of the rotifer were significantly influenced by the water temperature and salinity. The utilization of rotifer for red tide control has to consider the influence of environmental factors.

INTRODUCTION

Coastal systems around the world have suffered a variety of environmental problems, including loss of seagrass habitats, coral reef degradation or destruction, loss of quality of coastal waters for recreational use, deaths of marine mammals, red tides, fish kills, and outbreaks of shellfish poisonings. The last five problems cited above can be attributable to what is called harmful algae blooms (HABs) (Masó and Garcés 2006). There is growing international recognition that HABs are affected by human activities.

HABs occur throughout the world as a result of high concentrations of marine algae. The significant adverse impacts of HABs on public health, economics and natural resources have led to intensive monitoring programs to detect the presence of HABs. Although such programs are essential for altering the public to potential dangers, the severity and growing threat of HABs and their impacts could justify bloom mitigation and direct control as approaches for protecting public health and the marine ecosystem.

Zooplankton, small animals that co-occur with algae and graze them as food, could conceivably be considered a biological control agent to control HABs (Wang et al. 2005; Xie et al. 2008, 2009). It grazes different phytoplankton cells at different rates and has different impacts on phytoplankton community development. The grazing activity will efficiently control HABs (Sun et al. 2004). In freshwater ecosystems the strategy of supporting large species of *Cladocera* by biomanipulation was successfully used to limit algal blooms and improve water quality in many cases (Lammens 2001).

Martin et al. (1973) suggested that marine ciliates could be cultured and used for control of *Gymnodinium breve* cells. Likewise, Shirota (1989) considered the use of zooplankton, such as *Acartia clause*, in controlling HABs.

A promising strategy for controlling HABs is the application of zooplankton to remove the algae from the water column through grazing. One of the factors contributing to the development of red tides is the inability of grazing pressure to suppress the growth of causative organisms. The introduction of mass-cultured rotifers into water parcels containing red tide organisms may increase grazing pressure and effectively reduce the natural population of these harmful species down to low concentrations (Jeong et al. 2008). The rotifer *Brachionus plicatilis* has been successfully artificially mass cultured.

In this study, to evaluate the potential to control red tides using mass-cultured heterotrophic protistan grazers, we estimated the effects of environmental factors (water temperature and salinity) on the physical activities (filtration and oxygen consumption rates) of rotifers.

MATERIALS AND METHODS

Adult rotifers (*B. plicatilis),* were maintained in the Division of Marine Technology of Chonnam National University, South Korea. Rotifer cultures were carried out using *Chlorella vulgaris* as the exclusive food (3×10^4 microalgal cells per rotifer) fed one time a day, at 25°C and salinity 28 in a 12:12 L/D photoperiod. Rotifers were taken for experiments when a density of 100 ind. ml^{-1} was achieved. Before experiments rotifers were kept in starvation for 24h.

Experiments were conducted at different water temperatures (15, 20, 25, 30 and 35°C) and salinities (20, 25, 30, 35 and 40) in 200 ml beakers in complete darkness over a two hour time period. The density of rotifer *B. plicatilis* was 3 ind. ml^{-1}. This low density allowed the avoidance of lethal effects during experiments. The number of rotifers was counted using a dissecting microscope at ×10 magnification. Three replicates were performed for each treatment in the experiments.

Filtration rate (*F*) was calculated using neutral red solution (earlier filtered with 0.45 μm filter membrane) (Cole and Hepper 1954) according to the equation:

$$F = \frac{V(\ln C_0 - \ln C_t)}{nt}$$

where C_0 is the initial concentration of neutral red, C_t is the neutral red concentration at time t, n is the number of rotifers and V is the culture volume.

Neutral red concentrations were determined at the beginning and end of experiments at 530 nm using a spectrophotometer (Ultrospec 3330 pro UV, Biochrom Ltd., Cambridge, UK).

Dissolved oxygen concentrations were determined at the beginning and end of the experiments. The decrease in dissolved oxygen in sealed bottles was measured using a digital oxymeter (YSI-550, YSI Ltd., Farnborough, UK). The respiration rate (*O*) was calculated by the following equation (Kang et al. 2004):

$$O = \frac{V(D_i - D_t)}{nt}$$

where D_i is the initial concentration of dissolved oxygen, D_t is the dissolved oxygen concentration at time *t*, *n* is the number of rotifers and *V* is the culture volume.

Data from the different treatments were subjected to one-way ANOVA. Tukey's HSD was used to determine which treatments were significantly different. Significance of differences was defined as $P<0.05$ in all cases. Statistics were performed using the statistical software SPSS for Windows (SPSS Inc).

RESULTS

Both water temperature and salinity showed significant effects on the filtration rate of *B. plicatilis* (one-way ANOVA, F=41.66, $P<0.05$, Fig. 1). The results of multiple regression analysis are shown in Table 1. Multiple regression analysis yielded the following functional dependence of filtration rate (*F*) on water temperature (*T*) and salinity (*S*): $F=-1.658+0.917T+0.63S$ ($R^2=0.769$, $P<0.001$). The highest filtration rate (4.23 ±0.74 µl rot^{-1} h^{-1}) was obtained at 30°C and salinity 35 PSU, and the lowest one (0.869 ±0.13 µl rot^{-1} h^{-1}) was observed at 15°C and salinity 20 PSU. For all tested temperatures the highest filtration rates were observed at salinity 35 (Fig. 1).

Both water temperature and salinity showed significant effects on the oxygen consumption rate of *B. plicatilis* (one-way ANOVA, F=34.08, $P<0.05$, Fig. 2). The results of multiple regression analysis (Table 1) showed the following functional dependence of oxygen consumption rate (*O*) on water temperature (*T*) and salinity (*S*): $O=-3.133+0.165T+0.81S$ ($R^2=0.938$, $P<0.001$). The highest oxygen consumption rate (5.38 ±0.66 ng rot^{-1} h^{-1}) was observed at 35°C and salinity 40 PSU, and the lowest one (1.01 ±0.15 ng rot^{-1} h^{-1}) was observed at 15°C and salinity 20 PSU. For all tested temperatures the highest oxygen consumption values were obtained at salinity 40 (Fig. 2).

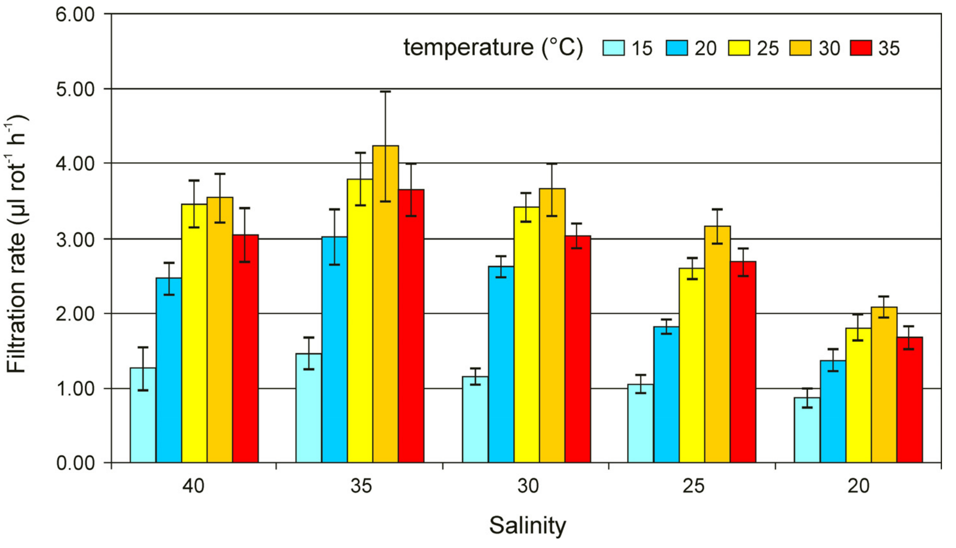

Fig. 1. Filtration rates of rotifer *Brachionus plicatilis* at different water temperature and salinity.

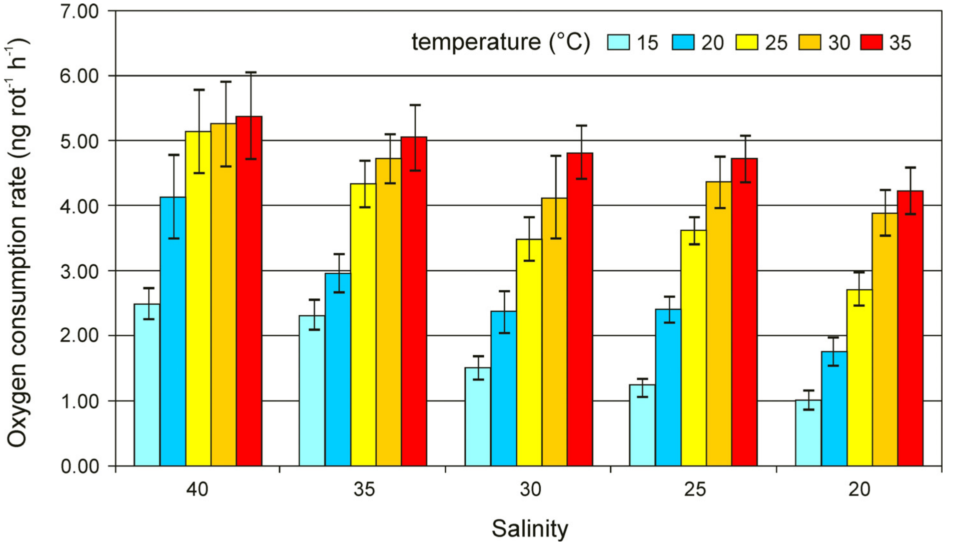

Fig. 2. Oxygen consumption rates of rotifer Brachionus plicatilis at different water temperature and salinity.

Table 1

Results for the multiple regression analysis among filtration rate (*F*) and oxygen consumption rate (*O*) of rotifer *Brachionus plicatilis* against water temperature (*T*) and salinity (*S*).

Model		R^2	F	P
Filtration rate (μl rot^{-1} h^{-1})	$F = -1.658+0.917T+0.63S$	0.769	22.187	<0.001
Oxygen consumption rate (ng rot^{-1} h^{-1})	$O = -3.133+0.165T+0.81S$	0.938	166.797	<0.001

Filtration rate and oxygen consumption rate are the dependent variable, and water temperature and salinity are the independent variables. All of the partial regression coefficients are significant ($P<0.05$).

DISCUSSION

Results showed that the filtration rate of *B. plicatilis* was significantly affected by water temperature and salinity, indicating that the number of rotifers which will be introduced into water parcels containing red tide organisms will change according to variations in water temperature and salinity.

In this study, we used neutral red to determine the filtration rate of *B. plicatilis* to eliminate the interference of food type. Vadstein et al. (1993) found lower filtration rates in *B. plicatilis* with latex beads than with algae or bacteria and Rothhaupt (1990) observed a 35% reduction in clearance rates of *B. rubens* with beads compared to algae. The filtration rate of *B. plicatilis* obtained in this study, then, might be lower than the actual filtration rate.

In the present study, the values obtained for filtration rates were in accordance with those obtained by Navarro (1999).

Although, some authors indicated that some zooplankton could be used for red tide control, actually, they arrived at an estimation that was completely unrealistic with respect to the coast, space, and facilities (Wang et al. 2005). For example, Shirota (1989) calculated that a 33,000 m^3 tank would be needed to hold sufficient zooplankton to treat a red tide 100 m long and only 1 m deep. Furthermore, this tank would have to be maintained constantly during the red tide season.

Results of the investigations of Xie et al. (2008, 2009) showed that three harmful algal bloom (HAB) species (*Prorocentrum donghaiense, Alexandrium tamarense* and *Heterosigma akashiwo*) could be used by *B. plicatilis* as a food source, but the first two algae species were more effectively ingested by *B. plicatilis.* It seems, then, that the effectiveness of the use of *B. plicatilis* will depend on which HAB species are present.

The rotifer *B. plicatilis* showed relatively higher filtration rates than other species previously studied, so it may be more suitable. Moreover, the adult rotifer has been successfully cryopreserved, so the cost will continuously decrease with the development of cryopreservation technology.

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