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Original Research Paper

Effects of Concentrations of *Prorocentrum donghaiense* and *Oxyrrhis marina* on the Feeding Behaviour of *Oithona brevicornis*

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ABSTRACT

In order to explore possible development process of red tides caused by *Prorocentrum donghaiense*, effects of concentrations of *P. donghaiense* and *Oxyrrhis marina* on the feeding behaviour of *Oithona brevicornis* were investigated. The results showed that within the concentration range of *P. donghaiense*, 1.0~5.0×10^4 cells·mL^{-1}, ingestion rates (IRs) and faecal pellet production rates (FPPRs) of *O. brevicornis* on *P. donghaiense* increased with increasing concentrations of *P. donghaiense*, the maximum IR and FPPR were 620 cells·ind^{-1}·h^{-1} and 31.67 pellet·$copepod^{-1}$·d^{-1}, respectively. When the concentration of *P. donghaiense* was 10.0×10^4 cells·mL^{-1}, the IR value decreased to 400 cells·ind^{-1}·h^{-1} and the FPPR value decreased to 13.33 pellet·$copepod^{-1}$·d^{-1}, respectively. Within the concentration range of *P. donghaiense*, 1.0~10.0×10^4 cells·mL^{-1}, filtration rates (FRs) of *O. brevicornis* decreased with increasing concentrations of *P. donghaiense*. The results also showed that *O. brevicornis* could ingest *O. marina* fed *P. donghaiense*, and within the concentration range of *O. marina*, IRs of *O. brevicornis* on *O. marina* increased with increasing concentrations of *O. marina*, while its FRs decreased, the maximum IR value and FR value were 300 cells·ind^{-1}·h^{-1} and 0.23 ml·ind·h^{-1}, respectively. Within the concentration range of *O. marina*, FPPRs of *O. brevicornis* increased with increasing concentrations of *O. marina*, the maximum FPPR was 21.67 pellet·$copepod^{-1}$·d^{-1}, and FPPRs had a good linear relationship with IRs. In this study, "Copepods-red tide algae" and "Copepods-protozoa-red tide algae" food chain models can provide references for the development process and regulating method of red tides caused by *P. donghaiense*.

INTRODUCTION

Oxyrrhis marina is an extensively studied morphospecies and a common protist model used to examine a range of ecological processes, exhibiting a wide geographic distribution (Watts et al. 2011). In China, *O. marina* is widely distributed in coastal environments of Qingdao, Qinhuangdao, Shenzhen and Shanghai (An et al. 2011, An et al. 2015). Owing to easy cultivation, *O. marina* has been used as a model organism to examine the feeding responses of heterotrophic protists to many marine microalgae, such as *Chattonella marina* (An et al. 2014, An et al. 2015), *Chlorella pyrenoidosa* (An et al. 2012), *Platymonas subcordiformis* (An et al. 2012, An et al. 2015), *Karenia mikimotoi* (An et al. 2012), bacteria (An et al. 2015), fungi (Jeong et al. 2010), and so on. Furthermore, a range of planktonic invertebrates can consume and grow on *O. marina*, including copepods and rotifers (Yang et al. 2011).

Prorocentrum donghaiense, a representative red tide organism in coastal waters of China, is one of the main species of red tide in the East China Sea, and caused red tides in the vicinity of Yangtze River Estuary and Zhejiang coastal waters almost in every spring in recent years (Zhu et al. 2009). We found that *O. marina* could grow well by feeding on *P. donghaiense*. *Oithona brevicornis*, a dominant species at some investigating sites, is widely distributed in coastal areas in China (Liu et al. 2013, Luo et al. 2013, Song et al. 2013, Zhang et al. 2000, Zhang et al. 2014, Zhou et al. 2013). Up to now, the feeding characteristics of *O. brevicornis* on *O. marina* and *P. donghaiense* have not been reported.

The goal of this study is to investigate the effects of concentrations of *P. donghaiense* and *O. marina* on the feeding behaviour of *O. brevicornis* for discussing the roles of "Copepods-red tide algae" and "Copepods-protozoa-red tide algae" food chain models in the developing processes of red tides caused by *P. donghaiense*.

MATERIALS AND METHODS

The Source of samples: Wild population of *O. marina* was collected from the coastal waters near Qinhuangdao in the Bohai Sea in 2010, China, and identified based on external morphology by light microscopy. Then the population was cultured in seawater on a diet of a natural bacterial assemblage grown in starch-enriched seawater in a 1000 mL conical flask, with culture temperature 20°C and light intensity 60 μE m^{-2} s^{-1} (An et al. 2015). Wild population of *P. donghaiense* was collected from the coastal waters near Ningbo in the East China Sea in 2005, China, and identified based on external morphology by light microscopy.

Then the population was cultured in f/2 medium, with culture temperature 20°C and light intensity 60 μE m^{-2} s^{-1}. Wild population of *O. brevicornis* was collected from the coastal waters near Qinhuangdao in the Bohai Sea in 2014, China, and identified based on external morphology using a dissecting microscope, cultured in the medium of *P. subcordiformis*.

Ingestion rates of *Oithona brevicornis* on *Prorocentrum donghaiense*: Triplicate 125 mL PC experiment bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up for each predator-prey combination. Dense cultures of *P. donghaiense* were added to all bottles, which were then filled to capacity with freshly sterilized seawater and capped. To determine actual prey densities at the beginning of the experiment, a 10 mL aliquot was removed from each bottle, fixed with 5% Lugol's solution and examined with a compound microscope to determine prey abundance by enumerating cells in three 1 mL Sedgwick-Rafter counting chambers (SRCs). The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on plankton wheels rotating at 1 rpm and incubated at 20°C under an illumination of 20 μE m^{-2} s^{-1} in a 12:12 h light:dark cycle. After counting, the initial concentrations of *P. donghaiense* were 1.0×10^4 cells mL^{-1}, 2.0×10^4 cells mL^{-1}, 3.0×10^4 cells mL^{-1}, 4.0×10^4 cells mL^{-1}, 5.0×10^4 cells mL^{-1} and 10.0×10^4 cells mL^{-1}, respectively, and initial amount of *O. brevicornis* was 10 in every bottle. After incubation for 24h, *O. brevicornis* and *P. donghaiense* were counted as above, and ingestion and clearance rates were calculated using the equations of Frost (1972).

Ingestion rates of *Oithona brevicornis* on *Oxyrrhis marina* fed *Prorocentrum donghaiense*: Triplicate 125 mL PC experiment bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up for each predator-prey combination. Dense cultures of *O. marina* growing on *P. donghaiense* were added to all bottles, which were then filled to capacity with freshly sterilized seawater and capped. To determine actual prey densities at the beginning of the experiment, a 10 mL aliquot was removed from each bottle, fixed with 5% Lugol's solution and examined with a compound microscope to determine prey abundance by enumerating cells in three 1 mL Sedgwick-Rafter counting chambers (SRCs). The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on plankton wheels rotating at 1 rpm and incubated at 20°C under an illumination of 20 μE m^{-2} s^{-1} in a 12:12 h light:dark cycle. After counting, the initial concentrations of *O. marina* were 1.0×10^2 cells mL^{-1}, 1.0×10^3 cells mL^{-1} and 1.0×10^4 cells mL^{-1}, respectively, and initial amount of *O. brevicornis* was 10 in each bottle. After incubation for 24h, *O. brevicornis* and *O. marina* were counted as above, and ingestion and clearance rates were calculated using the equations of Frost (1972).

Faecal pellet production rates of *Oithona brevicornis*: After incubation as above for 24h, faecal pellet amounts produced by each *O. brevicornis* were calculated (Yu et al. 2012).

RESULTS

Ingestion rates of *Oithona brevicornis* on *Prorocentrum donghaiense*: Ingestion and filtration rates of *O.brevicornis* on *P.donghaiense* concentrations are shown in Fig. 1. The ingestion rates of *O.brevicornis* increased with increasing *P.donghaiense* concentration between 1.0×10^4 and 5.0×10^4 cells·mL^{-1}, the maximum ingestion rate was 620 cells·ind^{-1}·h^{-1} at the *P.donghaiense* concentration of 5.0×10^4 cells·mL^{-1}; the ingestion rate decreased to 400 cells·ind^{-1}·h^{-1} at *P.donghaiense* concentration of 10.0×10^4 cells·mL^{-1} (Fig.1A). Within the concentrations designed in this experiment, filtration rates of *O.brevicornis* on *P.donghaiense* decreased with increasing *P.donghaiense* concentration and the maximum filtration rate was 0.31mL·ind^{-1}·h^{-1} at the *P.donghaiense* concentration of 1.0×10^4 cells·mL^{-1} (Fig.1B). The results showed that the ingestion rates of *O.brevicornis* on *P.donghaiense* could not increase unlimitedly with increasing *P.donghaiense* concentration, and *P.donghaiense* concentration could affect ingestion of *O.brevicornis* to a certain degree.

Ingestion rates of *Oithona brevicornis* on *Oxyrrhis marina* fed *Prorocentrum donghaiense*: Ingestion and filtration rates of *O.brevicornis* on *O.marina* concentrations are shown in Fig. 2. Within the concentrations designed in this experiment, ingestion rates of *O.brevicornis* on *O.marina* increased with increasing *O.marina* concentration and the maximum ingestion rate was 300 cells·ind^{-1}·h^{-1} at the *O.marina* concentration of 10×10^3 cells·mL^{-1} (Fig.2A). Filtration rates of *O.brevicornis* on *O.marina* decreased with increasing *O.marina* concentration and the maximum filtration rate was 0.23 ml·ind^{-1}·h^{-1} at the *O.marina* concentration of 1.0×10^2 cells·mL^{-1} (Fig.2B). The results showed that the *O.marina* concentration could affect ingestion of *O.brevicornis* to a certain degree.

Faecal pellet production rates of *Oithona brevicornis*: Faecal pellet production rates (FPPRs) of *O.brevicornis* are shown in Fig. 3. FPPRs of *O.brevicornis* increased with increasing *P.donghaiense* concentration between 1.0×10^4 and 5.0×10^4 cells·mL^{-1}, the maximum FPPR was 31.67 pellet·ind^{-1}·d^{-1} at the *P.donghaiense* concentration of 5.0×10^4 cells·mL^{-1} (Fig.3A). Within the concentrations designed in this experiment, FPPRs of *O.brevicornis* increased with increasing *O.marina* concentration and the maximum FPPR was 21.67

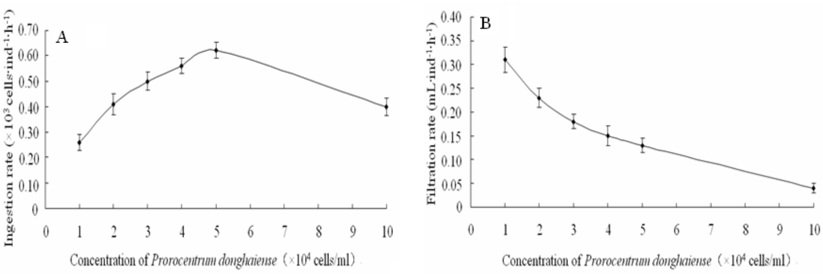

Fig.1: Effects of concentrations of *Prorocentrum donghaiense* on ingestion of *Oithona brevicornis.*

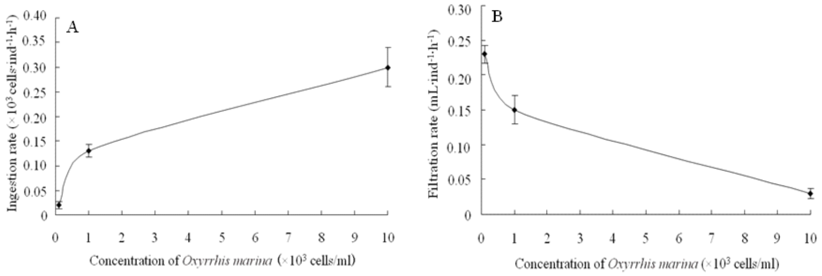

Fig. 2: Effects of concentrations of *Oxyrrhis marina* on ingestion of *Oithona brevicornis.*

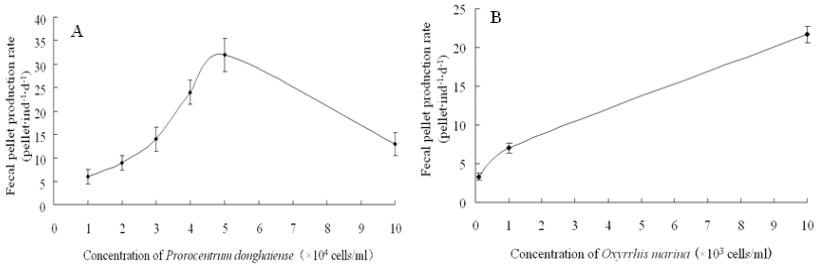

Fig. 3: Effects of concentrations of *Prorocentrum donghaiense* and *Oxyrrhis marina* on PPRs of *Oithona brevicornis.*

pellet·ind^{-1}·d^{-1} at the *O.marina* concentration of 10×10^3 cells·mL^{-1} (Fig.3B). The results showed that the FPPRs of *O.brevicornis* could not increase unlimitedly with increasing *P.donghaiense* concentration, and *P.donghaiense* concentrations could affect faecal pellet production of *O.brevicornis* to a certain degree.

DISCUSSION

Factors affecting ingestion rate of copepods include individual weight, development period, physiological state, activity, prey concentration and particle size, temperature, light, and so on (Calbet et al. 2007, Zhao et al. 2002). Zhao et al. (2002) revealed that ingestion and filtration rates of *O.similis* on *Dunaliella* sp. were 594 cells·ind^{-1}·h^{-1} and 0.05 ml·ind^{-1}·h^{-1} at 20°C, respectively; the ingestion rates of *O.similis* increased with increasing *Dunaliella* sp. concentration between 0 and 4.93×10^8 cells·L^{-1}, and then the ingestion rates decreased with further increasing *Dunaliella* sp. concentration. Calbet et al. (2007) revealed that ingestion rates of *Centropages typicus* showed a linear relationship with prey concentration, but the ingestion rates of *C.typicus* could not increase unlimitedly with increasing prey concentration. As revealed in this study, the ingestion rates of *O.brevicornis* on *P.donghaiense* increased with increasing *P.donghaiense* concentration between 1.0×10^4 and 5.0×10^4 cells·mL^{-1}, while the ingestion rate decreased at the *P.donghaiense* concentration of 10.0×10^4 cells·mL^{-1}; filtration rates of *O.brevicornis* on *P.donghaiense* decreased with increasing *P.donghaiense* concentration. It was also found that swimming movements of *O.brevicornis* in media of *P.donghaiense* concentration between 1.0×10^4 and 5.0×10^4 cells·mL^{-1} were normal, while their swimming movements became slow obviously at the *P.donghaiense* concentration of 10.0×10^4 cells·mL^{-1}. Wang et al. (2003) revealed that *P.donghaiense* concentration of 10.0×10^4 cells·mL^{-1} had a certain degree of adhesion and thus caused survival rate of *Brachionus plicatilis* decreased. Also, Han et al. (2006) revealed that high concentration *P.donghaiense* had a certain degree of adhesion and thus caused survival rate of *Calanus sinicus* decreased. Therefore, prey quality is another important factor influencing ingestion of copepods. Malzahn et al. (2010) indicated that the copepod was not affected when *Acartia tonsa* fed on *O.marina* that had been maintained on low-quality *Rh.salina*, but when they fed directly on *Rh.salina*, their respiration rates were higher and the development rate was lower. Jeong et al. (2001) revealed that *Acartia* spp. could not feed on a toxic strain of *Amphidinium carterae* directly, but it could feed on *O.marina* which was an effective grazer on the toxic strain of *A.carterae*. It proved that *O.marina* can transfer the materials of a toxic dinoflagellate to higher trophic levels. While in this experiment, *O.brevicornis* grew well on the *O.marina* fed *P.donghaiense*. Therefore, if the "*O.brevicornis-P.donghaiense*" food chain could not establish in the red tide areas with high density *P.donghaiense*, "*O.brevicornis-O.marina-P.donghaiense*" food chain could exist under suitable environmental conditions and play important role in the developing process of red tides caused by *P.donghaiense*.

FPPRs of copepods reflected their ingesting status and showed a linear relationship with ingestion rates (Nejstgaard et al. 2001). FPPRs of *C.sinicus* increased with increasing *Platymonas halgolankeca* var. *tsingtaoensis* and *Nitzschia closterium* concentration and had a good correlation with the preys concentration, reaching as high as 40-100 pellet·copepod^{-1}·d^{-1} (Zhang et al. 2000). Similarly, in this experiment, FPPRs of *O.brevicornis* increased with increasing *O.marina* concentration and had a good correlation with *O.marina* concentration, namely, y=1.7623x+4.1461 (R^2=0.9884), also, FPPRs of *O.brevicornis* had a good correlation with ingestion rates, namely, y=67.276x+ 0.5752 (R^2=0.9564).

CONCLUSIONS

1. "*O.brevicornis-O.marina-P.donghaiense*" food chain was established successfully in laboratory.
2. Within the concentrations designed in this experiment, *O.marina* and *P.donghaiense* concentrations affected ingestion, filtration and faecal pellet production of *O.brevicornis* to a certain degree.

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