Ecological Indicators 77 (2017) 261–266

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

A new method for evaluating defense of microalgae against protozoan grazing

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ARTICLE INFO

Article history:
Received 24 December 2016
Received in revised form 11 February 2017
Accepted 15 February 2017
Available online 27 February 2017

Keywords:
Bioassay
Body-size distinctness
Protozoan community
Nannochloropsis oceanica
Chlorella sp.

ABSTRACT

Body-size spectrum has proved to be a highly informative indicator to summarize the functional structure of a community at taxon-free resolution. In this study, an approach based on body-size spectrum of protozoan communities was used to detect the defense of microalgae against protozoan grazing. The biofilm-dwelling protozoan communities were used as a test predator system, and two algal species, *Chlorella* sp. and *Nannochloropsis oceanica*, were employed as test microalgae. A nine-day bioassay test was carried out by exposing biofilm-dwelling protozoan communities to a gradient of concentrations 10^0 (control), 10^4, 10^5, 10^6, and 10^7 cell ml^{-1} of both microalgae, respectively. Results showed that both algal species represented strong defense effects on the test predator system at different levels of concentration. The body-size distinctness of the protozoan assemblages showed a sharp decrease at high concentration level more than 10^6 cell ml^{-1} in both algal treatments. Based on the paired body-size distinctness indices of the protozoa, ellipse tests demonstrated that the body-size spectrum showed an increasing trend of departure from the expected pattern with increasing concentrations of both test algae. Thus, it is suggested that the body-size spectrum of protozoa may be used as a useful indicator to identify the defense of microalgae against protozoan grazing.

1. Introduction

Protozoa play an important role in the functioning of microbial food webs by feeding on bacteria and microalgae and transferring flux of energy to the high trophic-functional levels in aquatic ecosystems (Finlay and Esteban, 1998; Norf et al., 2009; Jiang et al., 2011; Xu et al., 2014). Because of short generation times and rapid responses to environmental changes, they have been widely used as a useful indicator for bioassay, especially at community level (Jiang et al., 2011; Xu et al., 2014).

Body-size spectrum, as an internal trait, has proved to be effective tool for summarizing the functional structure of a community (Sheldon et al., 1972; San Martin et al., 2006; Kamenir et al., 2010; Zhao et al., 2016). So far, several studies on bioassessment have been carried out using body-size spectrum of protozoan communities in marine ecosystems (Jiang et al., 2012; Xu et al., 2013, 2016; Xu and Xu, 2016). However, as to their use for evaluating defense of microalgae against protozoan grazing, little information was known.

In this study, a nine-day baseline bioassay was conducted using biofilm-dwelling protozoan communities as test predator assemblages by exposure to different concentrations of two microalgae, *Chlorella* sp. and *Nannochloropsis oceanica*. The objective of this study were to evaluate the feasibility for determining defense of microalgae against protozoan grazing using a potential indicator based on body-size spectrum of protozoa.

2. Materials and methods

2.1. Protozoan sample collection

Samples of protozoan communities were collected using glass slide method in coastal waters of the Yellow Sea, northern China (Fig. 1). The glass slide systems were designed, deployed, anchored, and sampled as described by Xu et al. (2011). A total of 20 microscopy glass slides were used for collect the protozoan communities at a depth of 1 m below the water surface. Two PVC frames were used to hold the 20 glass slides and all slides were collected at

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http://dx.doi.org/10.1016/j.ecolind.2017.02.026
1470-160X/

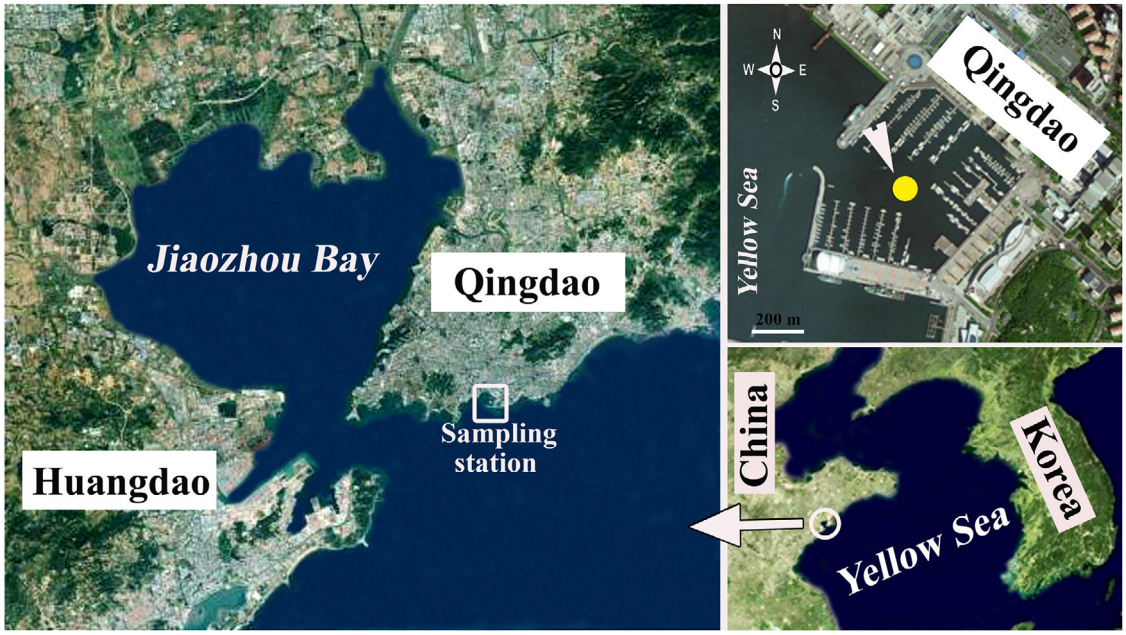

Fig 1. Sampling station, which was located in the harbor of the Olympic Sailing Center (OSC), a coastal area of the Yellow Sea, near Qingdao, northern China.

the exposure time of 14 days. The glass slides were transferred into Petri dishes containing *in situ* water and then stored in a cooling box before transporting to the laboratory within 2 h for test protozoan assemblages (Xu et al., 2014).

After 3-day domestication under laboratory conditions in an illumination culture cabinet (temperature 21.6 °C and illumination 3960 lx), a total of 18 glass slides with protozoan communities were used as test communities.

2.2. Experimental designation

Two algal species *Nannochloropsis oceanica* and *Chlorella* sp. were used as test microalgae, which were obtained from laboratory of applied microalgae biology, Ocean University of China.

All bioassay experiments were conducted in Petri dishes during a period of 9 days. For each of two test microalgae, five treatments with a same gradient of concentrations were designed as 10^0 (treatment 1 as a control), 10^4 (treatment 2), 10^5 (treatment 3), 10^6 (treatment 4), and 10^7 (treatment 5) cell ml^{-1}, respectively. For each of both control and a total of 8 treatments, one glass slide with protozoan communities was transferred into a Petri dish with 20 ml filtered seawater (FSW) without and with test microalgae, respectively. In each treatment, two replicates were used as parallel tests.

2.3. Identification and enumeration

Protozoa identification and enumeration were conducted following the methods outlined by Xu et al. (2011). Taxonomic classification of protozoa was based on the published references such as Song et al. (2009). The body-size rank system used was according to Xu et al. (2016).

The enumeration of protozoa *in vivo* was conducted at a 100-fold magnification under an inverted microscope (Xu et al., 2011). For recovering all species colonizing the glass slides, whole slide was examined to record both occurrences and individual abundances, using bright field illumination.

Equivalent spherical diameter (ESD) was used to evaluate the body-size spectrum of the protozoa. Biovolumes of the protozoan individuals were calculated by measurements of their linear dimensions according to the volume equations of appropriate geometric shapes (Winberg, 1971; Jiang et al., 2012; Xu et al., 2013).

2.4. Data analysis

Body-size diversity (Δ_z), body-size distinctness (Δ^*_z), average taxonomic distinctness (Δ^+_z) and variation in taxonomic distinctness (Λ^+_z) were used to summarize the body-size pattern of protozoan samples (Xu and Xu, 2016; Xu et al., 2016). All four indices were computed following the equations:

$$\Delta_z = [\Sigma\Sigma_{i<j}\omega_{ij}x_i x_j]/[N(N-1)/2]$$

$$\Delta^*_z = [\Sigma\Sigma_{i<j}\omega_{ij}x_i x_j]/[\Sigma\Sigma_{i<j}x_i x_j]$$

$$\Delta^+_z = [\Sigma\Sigma_{i<j}\omega_{ij}]/[S(S-1)/2]$$

$$\Lambda^+_z = [\Sigma\Sigma_{i<j}(\omega_{ij} - \Delta^+_z)]/[S(S-1)/2]$$

where x_i ($i = 1, 2, \ldots, S$) denotes the abundance of the i^{st} species; N is the total number of individuals in the sample; ω_{ij} is the "distinctness weight" given to the path length linking species i and j (with $i < j$, for sake of definiteness); S is the number of species (Warwick and Clarke 1995).

The distinctness weightings were according to Xu et al. (2016), in context of the Rk5 (logically "phylum-level"): $\omega = 1$ (species in the Rk1 level, logically "genus-level"), 2 (same Rk2, logically "family-level", but different Rk1), 3 (same Rk3, logically "order-level", but different Rk2), 4 (same Rk4, logically "class-level", but different Rk3) and 5 (same Rk5, logically "Phylum-level", but different Rk4). The distinctness of two species connected at the highest body-size rank level was set equal to 100 according to Xu et al. (2016). All four indices were calculated based on a trait hierarchy of body-size units using the routine 'DIVERDY' in the PRIMER v7.0.11 package (Xu et al., 2016).

Multivariate analyses of variations in the protozoan communities were analyzed using the PRIMER v7.0.11. Bray-Curtis similarity matrices were used for community analysis (Xu et al., 2014). The variations in protozoan body-size spectra at five concentration levels of both algal species were summarized using the routine dbRDA