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## Marine Pollution Bulletin

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# Insights into assessing water quality using taxonomic distinctness based on a small species pool of biofilm-dwelling ciliate fauna in coastal waters of the Yellow Sea, northern China



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#### ARTICLE INFO

Article history:
Available online 23 October 2014

Keywords: Biofilm-dwelling ciliate Environmental stress Maine bioassessment Microbial ecology Taxonomic diversity Taxonomic sufficiency

#### ABSTRACT

The aim of this study is to determine the feasibility of using a small species pool from a raw dataset of biofilm-dwelling ciliates for bioassessment based on taxonomic diversity. Samples were collected monthly at four stations within a gradient of environmental stress in coastal waters of the Yellow Sea, northern China from August 2011 to July 2012. A 33-species subset was identified from the raw 137-species dataset using a multivariate method. The spatial patterns of this subset were significantly correlated with the changes in the nutrients and chemical oxygen demand. The taxonomic diversity indices were significantly correlated with nutrients. The pair-wise indices of average taxonomic distinctness ( $\Delta^+$ ) and the taxonomic distinctness ( $\Delta^+$ ) showed a clear departure from the expected taxonomic pattern. These findings suggest that this small ciliate assemblage might be used as an adequate species pool for discriminating water quality status based on taxonomic distinctness in marine ecosystems.

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#### 1. Introduction

As a measure of biodiversity, taxonomic diversity has many desirable properties such as weak dependence on environment types and sampling-effort/sample-size, avoidance of species identifications, high sensitivity to environmental stress and anthropogenic impact, and the presence of a statistical framework for determining the significance of departure from expectation (Warwick and Clarke, 1995, 2001). Taxonomic distinctness indices have been widely used to assess environmental stress and anthropogenic impact (Leonard et al., 2006; Somerfield et al., 2008; Prato et al., 2009; Xu et al., 2011c; Jiang et al., 2014). Furthermore, the utility of these biodiversity indices based on presence/absence data for discriminating water quality status has been demonstrated for both macrofauna and microfauna (Mouillot et al., 2005; Xu et al., 2011c; Jiang et al., 2014).

As an important component of the benthic fauna, biofilm-dwelling ciliates play a crucial role to transfer energy from bacteria and microalgae to higher trophic levels in microbial food webs (Norf et al., 2009a,b; Xu et al., 2014a). They have a number of advantages,

such as short generation times, relative immobility, rapid responses to environmental stress and ease of sampling, and thus have widely been used as useful bioindicators of water quality in many aquatic ecosystems (e.g., Xu et al., 2014a). Xu et al. (2014a) reported that spatial variation in community patterns of biofilm-dwelling ciliates in coastal waters of the Yellow Sea is significantly correlated with environmental conditions. Recent investigations have demonstrated that early colonization patterns of biofilm-dwelling ciliate fauna can be used to discriminate the quality status of coastal waters (Xu et al., 2014b). However, determining a species pool that is sufficient for assessing water quality based on the taxonomic distinctness has received comparatively little attention.

In the present study, we investigated the utility of taxonomic distinctness of the biofilm-dwelling ciliate fauna for the discrimination of water quality status in coastal waters of the Yellow Sea. The main aim was to determine an adequate species pool that allows the reliable assessment of water quality based on the taxonomic distinctness of the biofilm-dwelling ciliate fauna.

#### 2. Materials and methods

#### 2.1. Data collection

Four sampling stations were located in coastal waters of the Yellow Sea, near Qingdao, northern China (Fig. 1A–D). The stations

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were selected according to their environmental status based on the marine water quality standards of China. Station A was located in the heavily stressed area in Jiaozhou Bay, the pollution being mainly organic compounds from domestic sewage and industrial discharge from several rivers; station B was within an area of Jiaozhou Bay that is moderately polluted by discharges from a small river; station C was in slightly polluted area near the mouth of Jiaozhou Bay, and relatively distant from the rivers entering the bay; station D was located in the least polluted area, outside the bay and more distant from the river discharges (Xu et al., 2014a) (Fig. 1).

The dataset was compiled based on 40 samples that were collected monthly at the four stations from August 2011 to July 2012. A total of 800 glass slides, each with an area of  $2.5 \times 7.5$  cm, were used as artificial substrates for collecting the biofilm-dwelling ciliates at a depth of 1 m below the water surface. For each station, two PVC frames holding a total of 20 glass slides were immersed in the water for 14 days to allow colonization by the ciliates. The glass slides were then retrieved, transferred into Petri dishes containing water from the station which were placed in a cooling box and transported to the laboratory for identification and enumeration (Xu et al., 2009).

Species identification and enumeration were conducted following the methods described by Xu et al. (2014). Taxonomic classification was based on published references such as Song et al. (2009). The taxonomic scheme used was according to Lynn (2008). Although the glass slides used in this study were also colonized by bacteria, algae and some micrometazoa, we have restricted our taxonomic analyses to ciliated protozoa.

The environmental variables nitrate nitrogen (NO<sub>3</sub>-N), nitrite nitrogen (NO<sub>2</sub>-N) and ammonium nitrogen (NH<sub>3</sub>-N), soluble reactive phosphate (SRP) and chemical oxygen demand (COD) were measured according to the "Standard Methods for the Examination of Water and Wastewater" (APHA, 1992). The measurements of water temperature (T), pH, salinity (Sal) and dissolved oxygen (DO) were recorded using WTW Multi 3500i sensor. Water transparency (Tra) was measured *in situ* using a transparent scale.

#### 2.2. Data analyses

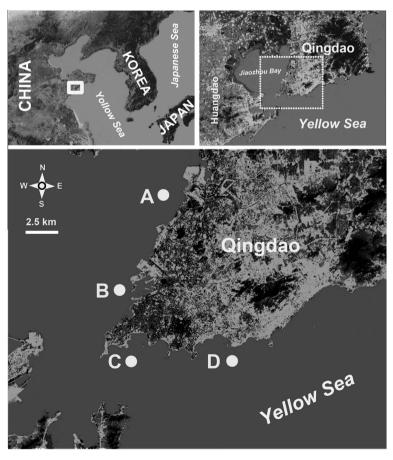
Taxonomic diversity ( $\Delta$ ), taxonomic distinctness ( $\Delta^*$ ), average taxonomic distinctness ( $\Delta^*$ ) and variation in taxonomic distinctness ( $\Delta^*$ ) of samples were computed using the following the equations:

$$\Delta = \left[\sum_{i < j} \sum_{\alpha_{ij}} x_i x_j\right] / [N(N-1)/2]$$

$$\Delta^* = \left[\sum_{i < j} \sum_{\alpha_{ij}} x_i x_j\right] / \left[\sum_{i < j} \sum_{\alpha_{ij}} x_i x_j\right]$$

$$\Delta^+ = \left[\sum_{i < j} \sum_{\alpha_{ij}} |f(S(S-1)/2)| \right]$$

$$\Delta^+ = \left[\sum_{i < j} \sum_{\alpha_{ij}} |f(S(S-1)/2)| \right] / [S(S-1)/2]$$



**Fig. 1.** Sampling stations in coastal waters of the Yellow Sea, near Qingdao, northern China. A: Station A, heavily stressed area in Jiaozhou Bay, the pollution being mainly in the form of organic pollutants and nutrients from domestic sewage and industrial discharge from several rivers; B: station B, moderately polluted area Jiaozhou Bay by minor discharges from a small river entering the bay; C: station C, slightly polluted area near the mouth of Jiaozhou Bay and relatively distant from the rivers entering the bay; D: station D, relatively clean area which was outside the bay and more distant from the river discharges.

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