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## Congruency analysis of biofilm-dwelling ciliates as a surrogate of eukaryotic microperiphyton for marine bioassessment



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#### article info abstract

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Biofilm-dwelling ciliates are primary components of the eukaryotic microperiphyton in both species composition and community structure. To evaluate the congruency of biofilm-dwelling ciliates as potential surrogates of the eukaryotic microperiphyton, a dataset was collected every month at four stations from the coastal waters of the Yellow Sea, northern China, and assessed. Sufficient species abundance data were obtained for ciliated protozoans at high taxonomic levels up to the family level, indicating a significant variation along the gradient of contamination. Correlation analyses revealed that the taxa richness of these matrices can explain  $>85\%$  of the variance in that of the full species dataset. The cost/benefit analysis showed that the protozoan subset at low resolutions up to the family level may be used as a potential surrogate of the original dataset. Thus, we suggest that the protozoan assemblages at genus- and/or family-level resolutions may be useful, cost-efficient surrogates of the original dataset for bioassessment in marine ecosystems.

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#### 1. Introduction

The eukaryotic microperiphyton is a primary contributor to the functioning of benthic microbial food loops in aquatic ecosystems [\(Norf et al., 2009; Zhang et al., 2012; Jiang et al., 2012; Liu et al., 2013;](#page--1-0) [Xu et al., 2015a](#page--1-0)). Photoautotrophic microalgae (mainly periphytic diatoms) act as primary producers and participate in primary production ([Duong et al., 2007; Debenest et al., 2009; Liu et al., 2014; Zhang](#page--1-0) [et al., 2014; Zhong et al., 2014; Xu et al., 2015a, b](#page--1-0)). As various trophicfunctional consumers, protozoans (mainly ciliates) play an important role in transferring the flux of elements and energy from plankton to benthos [\(Sherr and Sherr, 1987; Gosselin et al., 1995; Kchaou et al.,](#page--1-0) [2009; Xu et al., 2014a, b\)](#page--1-0). To date, they have been successfully used as effective bioindicators of water quality with several advantages [\(Coppellotti and Matarazzo, 2000; Ismael and Dorgham, 2003; Xu](#page--1-0) [et al., 2014a](#page--1-0)). However, traditionally, community-based bioassessment has been conducted on the full species abundance dataset ([Coppellotti](#page--1-0) [and Matarazzo 2000; Jiang et al., 2014; Xu et al., 2014c, d\)](#page--1-0). This regime limits their use in monitoring programs, as species identification is laborious [\(Bertasi et al., 2009; Xu et al., 2015c, d; Zhang et al., 2015\)](#page--1-0).

Taxonomic sufficiency (TS) is a feasible, cost-effective protocol to identify potential surrogates of the original species abundance data using coarse taxonomic resolutions for bioassessment, which has been increasingly studied at present (e.g., [Ellis, 1985; Vanderklift et al., 1998;](#page--1-0)

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[Dauvin et al., 2003; Mendes et al., 2007; Carneiro et al., 2010; Xu et al.,](#page--1-0) [2011a, b; Zhang et al., 2015\)](#page--1-0). Although the effectiveness of TS has been well established for many taxonomic taxa (e.g., metazoans, phytoplankton, and protozoans) ([Bertasi et al., 2009; Olsgard and Somer](#page--1-0)field, 2000; [Pagola-Carte et al., 2002; Carneiro et al., 2010\)](#page--1-0), further studies on the congruency of biofilm-dwelling ciliates (BDCs) as surrogates of full species dataset of the microperiphyton are warranted ([Xu et al., 2011a;](#page--1-0) [Zhang et al., 2015\)](#page--1-0).

In this study, a dataset of eukaryotic microperiphyton communities was collected from the coastal waters of the Yellow Sea near Qingdao, northern China, over a 1-year cycle. This was then investigated to determine the TS of the microperiphyton for bioassessment. The aims of this study were to determine potential surrogates of the original data using high taxonomic ranks, as well as heterotrophic or photoautotrophic assemblages at various taxonomic resolutions, for assessing the water-quality status of marine ecosystems.

#### 2. Materials and methods

#### 2.1. Dataset collection

The dataset of microperiphyton communities used in this study was collected at four stations from the coastal waters of the Yellow Sea, northern China, at a depth of 1 m using the glass slide method over 1 year (August 2011 to July 2012) [\(Fig. 1](#page-1-0), A–D). The dataset contains a total of 40 sample data points, each of which was collected using 20 microscope glass slides as two parallel samples. Thus, for the four stations, 40 polyvinyl chloride (PVC) frames were immerged to hold a total of

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Fig. 1. Sampling stations in coastal waters of the Yellow Sea near Qingdao, northern China. A-D: stations A-D.

800 glass slides. Samples were collected monthly after an exposure time of 14 days. All glass slides were transferred into Petri dishes with water from the station and immediately examined in the laboratory ([Xu et al.,](#page--1-0) [2015a](#page--1-0)).

Species identification and enumeration were performed according to the methods described by [Xu et al. \(2015a\)](#page--1-0). Taxonomic classification was carried out based on the already-published methods of [Song et al. \(2009\)](#page--1-0) and [Hasle and Syvertsen \(1997\).](#page--1-0) The taxonomic scheme was based on that used by [Lynn \(2008\)](#page--1-0) and [Round, Crawford and Mann \(1990\).](#page--1-0)

Water temperature (T), pH, salinity (Sal), and dissolved oxygen (DO) were measured with a WTW (Multi 3500i) sensor, and transparency (Tra) was recorded in situ using a transparent scale. The nutrients nitrate nitrogen ( $NO<sub>3</sub>–N$ ), nitrite nitrogen ( $NO<sub>2</sub>–N$ ), ammonium nitrogen (NH<sub>4</sub>–N), and soluble reactive phosphate (SRP), as well as  $SiO<sub>3</sub>$ –S, and chemical oxygen demand (COD), were measured following the "Standard Methods for the Examination of Water and Wastewater" [\(APHA, 1992](#page--1-0)).

#### 2.2. Data analysis

To evaluate the congruency of ciliated protozoans as potential surrogates of the microperiphyton, the sub-dataset BDCs were constructed at species, genus, family, order, and class resolutions based on both species abundance and presence/absence before analyses.

The spatial variations in community pattern and environmental conditions were summarized using the routine canonical analysis of principal coordinates (CAP) of PERMANOVA  $+$  on Bray–Curtis similarities from the transformed abundance data, and on Euclidean distance on log-transformed and normalized abiotic data, respectively ([Anderson](#page--1-0) [et al., 2005\)](#page--1-0). The differences between groups of samples were tested via a routine permutational analysis of variance (PERMANOVA) [\(Anderson et al., 2005\)](#page--1-0). The significance of biota–environment correlations was tested using the routine RELATE [\(Clarke and Gorley, 2015](#page--1-0)). The relationships between pairs of similarity matrices were analyzed using the Spearman correlation coefficients ( $\rho$  values), which were computed using the routine second STAGE and RELATE [\(Clarke and](#page--1-0) [Gorley, 2015](#page--1-0)). All multivariate analyses were conducted using the PRIMER package version 7.09 [\(Clarke and Gorley, 2015](#page--1-0)).

Linear regression analyses between numbers of species and those of higher taxonomic levels were conducted using SigmaPlot (v12.5).

A cost/benefit (C/B) ratio was used to select taxonomic levels with minimal loss of information and the least taxonomic resolutions, as calculated by the equation.

where  $CB_l$  is the cost/benefit ratio at the taxonomic level l,  $r_l$  is the correlation coefficient between taxonomic level l and species level,  $t<sub>l</sub>$  is the number of taxa at the taxonomic level *l*, and *S* is the number of species. The cost/benefit ratio ranges between 0 and 1: the value "0" indicates the highest correlation between the species level and the other taxonomic levels, as well as minimum loss of information [\(Karakassiss and Hatziyanni, 2000](#page--1-0)).

### 3. Results

### 3.1. Congruency analysis on community patterns of BDC and full species dataset

The taxa numbers of BDCs at the species, genus, family, order, and class levels are summarized in Table 1.

The CAP ordinations indicate that these matrices exhibit a similar community pattern to the original dataset; that is, the first canonical axis (CAP 1) separated the samples collected from the heavily polluted area (station A) from the data points in the clean area (station D), and the second canonical axis (CAP 2) may distinguish the data points in slightly/moderately polluted areas (stations B and C) from those in heavily polluted/clean areas (stations A and B) [\(Fig. 2\)](#page--1-0).

The PERMANOVA tests showed that only the matrices at the taxonomic resolutions up to the family level covered a significant variation on the spatial scales ( $P > 0.05$ ). Furthermore, the multivariate correlation analysis revealed that the correlation coefficients are  $>0.75$ between BDCs at five taxonomic levels and the full species matrices (see  $\rho$  values in [Fig. 2\)](#page--1-0). Thus, we proposed the use of these matrices at low resolutions up to the genus level as potential surrogates for the original dataset.

#### Table 1

Accumulative numbers of taxa across five taxonomic ranks (species, genus, family, order and class) of biofilm-dwelling eukaryotic microbiota in coastal waters of the Yellow Sea, near Qingdao, northern China during the study period.

Taxa	Full dataset	<b>BDC</b>
	183	144
lт	103	79
г	63	43
	33	18
	1.	

BDC: biofilm-dwelling ciliated protozoa; S: species; G: genus; F: family; O: order; C: class.

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