



Environmental status assessment using biological traits analyses and functional diversity indices of benthic ciliate communities



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ARTICLE INFO

Keywords:

Bio-indicator
Marine
Protozoans
Wetland
Yangtze estuary

ABSTRACT

In this study, we tested the hypothesis that the functional diversity of benthic ciliates has high potential to monitor marine ecological status. Therefore, we investigated the spatial and temporal variation of functional diversity of benthic ciliates in the Yangtze Estuary during one year using biological traits analyses and functional diversity indices. Traits and community compositions showed clear spatial and temporal variations. Among a variety of biological traits, feeding type and body size emerged as strongest predictable variables. Functional divergence (FDiv) had an advantage over two other functional diversity indices, as well as over classical diversity measures (i.e. richness, evenness, Shannon-Wiener) to infer environmental status. Significant correlations between biological traits, FDiv and environmental variables (i.e. nutrients, temperature, salinity) suggested that functional diversity of benthic ciliates might be used as a bio-indicator in environmental status assessments. Further mandatory researches need to implement functional diversity of ciliates in routine monitoring programs were discussed.

1. Introduction

As a component of biodiversity, functional diversity generally considers the range of biological traits (“things that organisms do”) in communities and ecosystems (Petchey and Gaston, 2006). Therefore, functional diversity is an important aspect of biodiversity to study and understand ecosystem processes (Díaz and Cabido, 2001). Several definitions exist for functional diversity, but there is a general agreement that functional diversity studies always consider organisms as “dynamic entities that interact with their environment” (Calow, 1987; Laureto et al., 2015). A wealth of trait information is available for different groups of organisms (Petchey and Gaston, 2006). The choice of traits to be used for the measurement of functional diversity depends on the specific aims of a particular study.

Traditional methods to measure functional diversity rely on functional group analysis such as feeding types and body size. These individual measures, however, ignore the functional difference among species within certain groups (Petchey and Gaston, 2002). Therefore, Bremner et al. (2003) introduced an alternative approach, first applied to marine benthic communities. This technique of biological traits analysis considered the variation patterns of a wide range of characteristics shared by different taxa based on multivariate ordinations.

Taking this strategy one step further, different authors introduced various functional diversity indices that estimate the extent, dispersion and relative abundance of species biological traits (Mouchet et al., 2010; Petchey and Gaston, 2006). Among these measures, FRic, FEve, and FDiv measure the three primary aspects of functional diversity (Mason et al., 2005): functional richness, functional evenness and functional divergence, respectively. These measures are complementary and independent to all the other indices (Mason et al., 2005; Mouchet et al., 2010; Villéger et al., 2008). Because species are not equal in their responses to environmental changes these measures have the potential to explore relationships between biodiversity and environmental conditions (Mouchet et al., 2010). Indeed, there is good experimental and analytical evidence that functional diversity can provide a link between organisms and ecosystem properties. For example, biological traits analyses and functional diversity indices were successfully applied to assess environmental changes across habitat gradients or anthropogenic impacts on macrobenthic organisms (Bady et al., 2005; Bremner et al., 2003; Dolbeth et al., 2013; Gusmao et al., 2016; Hussin et al., 2012; Tillin et al., 2006; Veríssimo et al., 2012; Wong and Dowd, 2015).

The vast majority of such studies in benthic ecosystems focused on macrofauna, even though microbial organisms such as unicellular eukaryotes (protists) may have better qualities as environmental

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indicators (Stoeck et al., 2018). This is because protists are highly sensitive to environmental changes, including for example organic enrichment (Stoeck et al., 2018), heavy metal contaminations (Madoni, 2000), acidification (Davidson et al., 2016; Meakin and Wyman, 2011), oxygen depletion (Orsi et al., 2012), hydrocarbon pollution (Echeveste et al., 2010), and UV-radiation (Sonntag et al., 2017). Also, they have very short generation times, resulting in much higher species turnover rates after environmental changes compared to macrofauna with longer generation times. Thus, protists can indicate environmental changes much faster than macrofauna. Furthermore, they have high population densities, which are characterized by an extremely high biodiversity, even under typically azoic environmental conditions (Stoeck et al., 2018). This provides a large choice of potential environmental indicators even in situations when most or all larger organisms have disappeared (Stoeck et al., 2018). Among protists, especially ciliates are used as environmental indicators (Berger and Foissner, 2003; Bick, 1972; Chen et al., 2008; Feng et al., 2015; Foissner, 1999, 2016; Foissner and Berger, 1996; Jiang et al., 2013; Xu et al., 2016). They play key roles in microbial food webs where they predate bacteria, algae, other protists and even some metazoans, while being prey by larger zooplankton (Hamels et al., 2005; Porter et al., 1979; Sherr and Sherr, 1988; Weisse, 2003, 2006; Weisse and Sonntag, 2016; Weisse et al., 1990). Because they are relatively large compared to other protists ciliates are well-studied for more than two decades and have a good taxonomic framework documented in many identification keys (Berger and Foissner, 2003; Bick, 1972; Carey, 1992; Foissner and Berger, 1996). In addition, a wealth of literature is available describing their biological traits (Lynn, 2008; Lynn and Small, 2002; Pratt and Cairns, 1985; Weisse, 2017), many of which are related to their roles in aquatic food webs (Caron and Goldman, 1988; Lischke et al., 2016; Sanders and Wickham, 1993). Accordingly, first studies showed the potential of functional groups of planktonic ciliates to assess water quality (Jiang et al., 2013; Xu et al., 2016, 2017).

Here, we investigate functional diversity of benthic ciliates in the Yangtze Estuary in relation to environmental conditions. We chose this proof-of-concept habitat because coastal and estuarine ecosystems belong to the most productive but also most exploited natural habitats on Earth. Increasing pressure on coastal environments come for example from land use, accelerated urbanization, eutrophication, global warming, aquaculture and tourism (Lotze et al., 2006). Our study was conducted at three contrasting sampling regions over a time course of 12 months to test the following hypothesis: The functional diversity of benthic ciliates has high potential to monitor marine ecological status. To test our hypothesis, we addressed the following specific questions: (1) how do the benthic ciliate community structure and its biological traits composition vary across environmental gradients?; and (2) how well do three standard functional diversity indices resolve the relationship between environmental status and ciliate communities?

2. Materials and methods

2.1. Study sites and sampling

Three typical stations with environmental gradients (e.g. salinity and sediment grain size) were selected in Dongtan wetland in Yangtze Estuary (Fig. 1): Station 1 (31°27'1.88"N, 121°53'40.03"E), which is close to the South Branch of the Yangtze Estuary and mostly influenced by the fresh water of Yangtze River, has sandy soil. Station 2 (31°30'35.00"N, 121°57'33.00"E) is about 10 km northwest of station 1 and facing the East China Sea. This station is characterized by mixed water masses from the Yangtze River and the China Sea. Station 3 (31°34'46.00"N, 121°54'28.16"E) is located ca. 15 km north of station 1, close to the North Branch and mainly influenced by marine waters from the China Sea (Dai et al., 2011). Station 2 and 3 have saline-alkaline clay soil.

Sampling of each station took place once during ebb tide in March

(spring), June (summer), September (autumn), and December (winter) of 2015. At each sampling, ten replicates were collected at each station along a short transect parallel to the water line in the mid-intertidal zone at low tide (120 samples in total). The upper 2 cm of the sediment (ca. 4 ml) were collected using a syringe and then mixed with glutaraldehyde (2% final concentration). For granulometric analyses, total organic carbon (TOC) and total nitrogen (TN) measurements, three additional samples were collected from the top 2 cm of the sediment surface. For analyses of nutrients, three sediment pore water samples were taken using Millipore syringe filters with a pore size of 0.45 µm. After addition of 1.5% v/v saturated HgCl₂ to the filtered pore water, samples were stored at 4 °C until further processing.

2.2. Ciliate identification

Ciliates were extracted and stained from all 120 samples according to Xu et al. (2010). Enumeration and identification were conducted using microscopy (Olympus BX53) at 200× to 1000× magnifications. Identifications followed mainly Carey (1992), Corliss (1979), and Lynn and Small (2002). For each taxon, cell lengths of at least five individuals (or all individuals encountered if fewer than five were found) were measured with an ocular micrometer. The species abundance dataset was reduced to retain only those taxa found in at least three samples and contributing at least 10% of total ciliate abundance in any sample, thus selecting taxa that were dominant in each sample and widely distributed over the regions.

2.3. Measurement of environmental parameters

Salinity and temperature of sediment pore water were measured in situ three times at each station using a multi-parameter kit (WTW Multi 3430). Sediment grain size was determined with a laser size analyzer (BECKMAN COULTER LS13 320). TOC and TN contents were determined using a Vario EL CHNOS Elemental Analyzer (as % dry weight of the sediment) according to Ryba and Burgess (2002). Nutrients (ammonia, nitrate, nitrite, phosphate and silicate) were determined using a segmented flow analyzer (Model: SKALAR San^{plus} System, Netherlands) according to Gao et al. (2009).

2.4. Biological trait analysis

Six biological traits sub-divided into 15 categories were chosen according to Xu et al. (2018) for our analysis (Suppl. Table 1). Information on biological traits were gathered both from our own observations and from the literature (Lynn, 2008; Lynn and Small, 2002; Pratt and Cairns, 1985). Fuzzy-coding procedure was used to account for multiple trait categories of an individual taxon, i.e. a scoring range of 0 to 3 was adopted, where 0 expresses no affinity for the given trait category, 1 or 2 express partial affinity and 3 expresses exclusive affinity (Bremner et al., 2003). For example, *Frontonia microstoma* feeds both on microalgae and bacteria but has a preference for the former, so it was coded 2 (algivores), 1 (bacterivores), and 0 (predators) for the “feeding type” trait.

After obtaining “taxa abundance by samples” and “taxa by traits” data matrices, we then calculated the “traits by samples” matrix using the *dbFD* function in the “*FD*” R package (Laliberté et al., 2014).

2.5. Diversity indices

Three classical diversity indices, i.e. Margalef's richness (*d*), Pielou's evenness (*J'*) and Shannon-Wiener (*H'*) indices, were calculated using the submodule DIVERSE in the PRIMER v7.0.11 package (Clarke et al., 2014). Three indices of functional diversity, i.e. FRic, FEve, and FDiv, were computed using the *dbFD* function in the “*FD*” R package (Laliberté et al., 2014). The Bray-Curtis (BC) index was used as a measure of similarity between the samples (beta-diversity).

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