Journal of Experimental Marine Biology and Ecology 469 (2015) 36–43

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Effects of plankton net characteristics on metagenetic community analysis of metazoan zooplankton in a coastal marine ecosystem

Junya Hirai [a,*], Motoshige Yasuike [a], Atushi Fujiwara [a], Yoji Nakamura [a], Soshi Hamaoka [b], Seiji Katakura [b], Yoshihito Takano [a], Satoshi Nagai [a]

[a] National Research Institute of Fisheries Science, Fisheries Research Agency, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa 236-8648, Japan
[b] City of Mombetsu, Kaiyo-koryukan, Kaiyo-koen, Mombetsu, Hokkaido 094-0031, Japan

ARTICLE INFO

Article history:
Received 12 February 2015
Received in revised form 8 April 2015
Accepted 13 April 2015

Keywords:
Coastal water
Community structure
Metagenetics
Plankton net
Zooplankton

ABSTRACT

Metagenetic analysis is a recently introduced, taxonomically comprehensive method for characterizing zooplankton communities; however, effects of plankton net characteristics (mesh and opening sizes) on metagenetic data, and biodiversity data in particular, have not been fully evaluated. To this end, we collected zooplankton samples from the subarctic coastal waters off Japan using two plankton nets: 1) Kitahara Quantitative Plankton Net (Kitahara net) with a 0.04-m^2 opening and 100-μm mesh and 2) North Pacific Standard Plankton Net (Norpac net) with 0.16-m^2 opening and 335-μm mesh. We then conducted 18S rDNA metagenetic and morphological analyses of the resulting catches. Molecular operational taxonomic units (MOTUs) at 97% similarity revealed higher diversity than did the morphological analysis, especially for morphologically unidentified taxa (e.g., Gastropoda and Polychaeta larvae), suggesting the effectiveness of the metagenetic method for characterizing zooplankton communities. Samples obtained with the Kitahara net produced more sequence reads of non-metazoan taxa, mainly derived from phytoplankton, leading to smaller numbers of available sequence reads for metazoan plankton. Numbers of morphological taxa were higher in the Norpac net samples. However, we expected metagenetic analysis to reveal higher diversity for the Kitahara net, due to larger MOTU numbers from smaller-sized taxa. Small-sized taxa also accounted for a larger proportion of sequence reads in the Kitahara net samples. In contrast, the diversity of large-sized taxa was better represented in the Norpac net sample. Although these differences were expected from the morphological analysis, effects of plankton net characteristics were more clearly reflected by metagenetic analysis than the morphological analysis.

1. Introduction

Marine plankton community samples collected by plankton nets are composed mainly of small metazoan plankton. Marine metazoan zooplankton are highly abundant and diverse, due to their key role in the food web structure and their rapid responses to environmental changes; monitoring their community structure is therefore important in understanding marine ecosystems (Bucklin et al., 2010; Hays et al., 2005). For example, long-term monitoring programs have shown that changes in zooplankton communities have been largely related to the recruitment of commercially important fishes (Beaugrand et al., 2003). Global monitoring programs are usually based on microscopic morphological classification, which is time-consuming and requires individual sorting and identification by taxonomic experts. Molecular techniques may facilitate species identification based on similarities to known sequences in public databases (Hebert et al., 2003). Molecular techniques also enable scientists to evaluate the hidden diversity of a wide range of metazoan plankton, including immature stages of development of a particular species, or cryptic species (Bucklin et al., 2011).

Among molecular approaches, metagenetic analysis is considered an especially effective method for monitoring zooplankton communities. Metagenetic analysis enables complete taxonomic information to be obtained from bulk environmental samples, based on specific genetic markers. Such markers include nuclear genes for the small and large subunits of ribosomal RNA (18S and 28S, respectively), and mitochondrial cytochrome c oxidase subunit I (COI). Metagenetic analysis techniques have been developed following the advent of high-throughput sequencing, which allows the production of large-scale sequence data from a single run (Sogin et al., 2006). In eukaryotes, metagenetic analysis has been applied to both unicellular organisms and metazoan communities (e.g., Creer et al., 2010; Hajibabaei et al., 2011; Yu et al., 2012). The use of metagenetic analysis for the study of zooplankton communities in the English Channel (Lindeque et al., 2013) and Red Sea reefs (Pearman et al., 2014) is an example of its effectiveness in this application.

In contrast to morphological data, which depends on the individual skills of taxonomists, data from metagenetic analysis can be easily

* Corresponding author. Tel.: +81 45 788 7819.
E-mail address: jhirai@affrc.go.jp (J. Hirai).

http://dx.doi.org/10.1016/j.jembe.2015.04.011
0022-0981/

standardized across different laboratories (Lindeque et al., 2013). Sequence data, deposited in public databases, can be directly compared among different studies if the same genetic region is targeted. For example, global metagenetic studies of the microbial community, using the V6 region of the 16S rRNA gene, have been conducted as part of the International Census of Marine Microbes (ICoMM; Amaral-Zettler et al., 2010; Zinger et al., 2011). However, sampling methods constitute a source of bias in metagenetic analysis of zooplankton because various sampling methods have been applied to study zooplankton communities over a long oceanographic history (Wiebe and Benfield, 2003).

Effects of sampling methods on characterizations of zooplankton communities have been well documented by morphological analysis. For example, smaller-sized zooplankton taxa are significantly underestimated using conventional mesh sizes (≥200 μm), while plankton nets with larger mouth openings and mesh sizes, allowing better water filtration, are appropriate for collecting larger-sized zooplankton capable of avoidance (Gallienne and Robins, 2001; McGowan and Fraundorf, 1966; Sameoto et al., 2000; Turner, 2004). Although metagenetic methods may be taxonomically comprehensive, data can be similarly affected by the sampling method employed. Metagenetic analyses of marine zooplankton, including studies using cloning techniques, have previously used various sampling plankton nets with different towing methods (Cheng et al., 2014; Hirai et al., 2015; Lindeque et al., 2013; Machida et al., 2009; Pearman et al., 2014). Currently, there is no standard method to sample zooplankton communities across the global range of the oceans. Because we expect that, in the future, metagenetic analyses will be applied to studies conducted on a wide range of temporal and spatial scales, it is important to evaluate how sampling methods affect outcomes of analysis.

In this study, we evaluated the effects of plankton net characteristics (opening and mesh sizes) on the biodiversity of marine zooplankton, as detected by metagenetic analysis. Zooplankton samples were collected in the subarctic coastal waters of the Okhotsk Sea in Japan. In this sea, both holoplankton and meroplankton are observed, and extensive zooplankton sampling has been performed for marine ecosystem monitoring (Hamaoka et al., 2010). In addition to comparing metagenetic and morphological methods, we compared species composition in samples obtained with two types of nets. Marked differences in species diversity were detected between samples collected using different nets; these are discussed here in terms of the effects of plankton net selection on outcomes of metagenetic analysis of marine zooplankton. Although metagenetic analysis is a promising method for monitoring zooplankton communities, no study has evaluated the effects of sampling methods on metagenetic data of zooplankton. The study presented here provides important insights into the selection of suitable sampling methods for metagenetic analysis of zooplankton.

2. Materials and methods

2.1. Sampling using two types of plankton nets (Kitahara and Norpac)

Zooplankton samples were collected at depths of 10 m around the Okhotsk Tower, located 1 km off the coast of Mombetsu in northeastern Hokkaido, Japan (44° 20.2′ N, 143° 22.9′ E). Sampling was conducted weekly between 10:00 a.m. and 12:00 noon (GMT + 9), from April 10, 2012, to June 25, 2012 (Table 1). Vertical water temperature profile was measured using the RINKO-Profiler ASTD102 (JFE Advantech Co., Ltd). We used two types of plankton nets to collect zooplankton: Kitahara Quantitative Plankton Net (Kitahara net; Nakai, 1962) and North Pacific Standard Plankton Net (Norpac net; Motoda et al., 1957). The Kitahara net is a Hensen-style plankton net with a 22.5-cm diameter mouth opening and a 45 cm diameter through the middle. The Norpac net is a simple conical plankton net with a 45 cm diameter at top and a mouth opening area (0.16 m^2) four times larger than that of the Kitahara net (0.04 m^2). In addition, the length of the Norpac net (180 cm) is greater than that of the Kitahara net (80 cm). The finer mesh size of 100 μm was selected for the Kitahara net, while 335 μm mesh was selected for the Norpac net. Due to structural differences, greater water filtration was expected for the Norpac net. In contrast, the Kitahara net was expected to be more suitable for collecting smaller organisms. For each plankton net, two vertical tows were performed, from the bottom to the surface; a total of four samples were thereby obtained on each collection date. For each net type, one sample was preserved in 5% borax-buffered formalin for morphological classification and the other was frozen at −20 °C for metagenetic analysis.

2.2. Morphological classification

Zooplankton samples preserved in formalin were morphologically identified to the species level, as per Chihara and Murano (1997). Developmental stage and sex were also recorded for each specimen, if possible. Any specimen that could not be fully identified to the species level was classified to the lowest-ranking taxon possible (e.g., *Saria* sp., Harpacticoida copepod, Gastropoda larva). The number of individuals was counted for each species or taxonomic group in each sample. In this study, we use the term "morphological group" to refer to both species and other taxonomic groups, in comparisons with metagenetic data.

2.3. DNA extraction, PCR amplification, and 454 pyrosequencing

Genomic DNA was extracted from bulk frozen samples using QuickGene-810 (Fujifilm). DNA concentration and quality were assessed using the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific).

Table 1
Information on collected samples. For each plankton sample collected, the net type, date of collection, water temperature, primer barcodes, and numbers of raw and filtered sequence reads are given. Temperature was measured at a depth of 2 m.

Sample no.		Date	Temperature (°C)	Barcode	Kitahara net		Norpac net	
Kitahara	Norpac				Raw	Filtered	Raw	Filtered
K1	N1	April 10, 2012	0.2	AGACTC	43,787	6978	42,535	24,234
K2	N2	April 16, 2012	1.7	AGATCA	41,623	6731	47,735	26,003
K3	N3	April 23, 2012	5.2	AGCACG	22,566	3251	22,933	12,241
K4	N4	April 30, 2012	7.0	AGCTGA	26,844	2240	34,287	6439
K5	N5	May 7, 2012	7.7	AGTAGC	31,262	4334	31,738	16,901
K6	N6	May 14, 2012	8.1	AGTGAG	31,988	4765	31,179	16,520
K7	N7	May 21, 2012	8.1	ATACAG	74,149	10,283	105,312	59,011
K8	N8	May 28, 2012	8.5	ATAGTC	52,872	5606	66,737	34,616
K9	N9	June 4, 2012	10.4	ATCATG	80,538	5723	80,447	40,203
K10	N10	June 11, 2012	11.7	ATCGAC	48,726	5798	46,955	21,665
K11	N11	June 18, 2012	12.6	CAGACA	6229	404	6709	3172
K12	N12	June 25, 2012	12.0	CAGCTG	5694	357	5973	2320