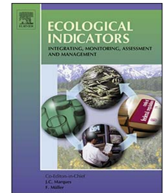
Ecological Indicators 85 (2018) 153–164

Contents lists available at ScienceDirect

Ecological Indicators

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



Original Article

Metabarcoding of benthic ciliate communities shows high potential for environmental monitoring in salmon aquaculture

Thorsten Stoeck[a,*], Rebecca Kochems[a], Dominik Forster[a], Franck Lejzerowicz[b], Jan Pawlowski[b]

[a] University of Kaiserslautern, Ecology Group, D-67663, Kaiserslautern, Germany
[b] University of Geneva, Department of Genetics and Evolution, 1211, Geneva, Switzerland

ARTICLE INFO

Keywords:
Benthic ciliates
Ecological indicators
Salmon farming
Aquaculture
Metabarcoding
SSU rDNA
Environmental impact assessment

ABSTRACT

The backbone of benthic marine monitoring programs is the biological component, traditionally the macrofauna inventory. Such macrofauna-based environmental impact assessments (EIA), however, are very time consuming and expensive. To overcome these shortcomings, we used environmental metabarcoding to test the potential of protists as bioindicators in EIAs. Therefore, we analyzed taxonomic metabarcodes (V9 region of the SSU rRNA), obtained from sediment samples collected along a 400-m transect extending from below salmon cages towards the open sea along the predominant current flow. The obtained genetic data of protistan communities were compared to benchmark data obtained from traditional macrofauna surveys of the same samples. Ciliates emerged as the most powerful indicators mirroring the macrofauna benchmark patterns with statistical significance. Ordination analyses showed that ciliate communities resolved impacted sampling sites below and in immediate vicinity of the salmon cages even better than macrofauna communities. It can be concluded that ciliates allow for a better fine-scale resolution of impact conditions than traditional monitoring methods. Other protistan taxon groups such as diatoms and chrysophytes were not as successful as marine benthic indicators compared to ciliates. We conclude that the implementation of ciliate metabarcoding can substantially improve EIAs. We discuss further mandatory research needs to make ciliate metabarcoding a routine tool in official regulations for EIAs in salmon farming.In contrast, o

1. Introduction

Coastal waters provide a variety of sociocultural and economic ecosystem services (Barbier et al., 2011). Keeping a healthy balance between ecosystem exploitation and ecosystem function(ing) requires frequent monitoring of coastal habitats subjected to human activities. One example is aquafarming. According to the most recent report of the Food and Agriculture Organization of the United Nations (FAO, 2016), global total capture fishery production in 2014 was 93.4 million tons, of which 81.5 million tons were from marine waters. Globally, the production of aquatic animals from aquaculture in 2014 amounted to 73.8 million tons, with marine aquaculture accounting for 26.7 million tons. The vast majority of the latter was finfish. However, along with the economic opportunity of marine finfish culture comes environmental risk.

Dominant seabed effects arise from the sedimentation of organic particles (feces and uneaten food) in the vicinity of farm cages (Buschmann et al., 2006; Carroll et al., 2003; Holmer et al., 2005; Keeley et al., 2013). Through continual organic enrichment, the receiving benthic environment may experience pronounced changes in sediment geochemistry and benthic communities (Bannister et al., 2014; Brown et al., 1987; Holmer et al., 2005; Keeley et al., 2013; Neofitou et al., 2010; Sweetman et al., 2014). More specifically, when waste deposition exceeds the natural rate of organic material breakdown, particulate organic material (POM) accumulates on the sediment surface. The seabed eventually becomes acidified and oxygen-depleted because of microbial degradation processes (Aranda et al., 2015). Toxic gases such as hydrogen sulfide and methane may be produced. Geochemical changes in the seabed structure are typically accompanied by changes in epifaunal and infaunal communities: less resistant and larger members of the benthic fauna are replaced by fewer, more tolerant, forms such as the smaller polychaete *Capitella capitata* (Holmer et al., 2005; Pearson and Rosenberg, 1978). This depositional footprint of a finfish farm is mostly localized within the first 25–100 m from the point of discharge below the cages in direction of the prevailing current (Keeley et al., 2012). With increasing distance from the cages, the environmental effects decrease gradually until they have reached conditions similar to a distant control site.

* Corresponding author.
E-mail address: stoeck@rhrk.uni-kl.de (T. Stoeck).

http://dx.doi.org/10.1016/j.ecolind.2017.10.041
Received 11 May 2017; Received in revised form 17 October 2017; Accepted 19 October 2017
1470-160X/ © 2017 Elsevier Ltd. All rights reserved.

Because there is a tradeoff between the acceptable environmental impact of aquaculture and socio-economic benefits, international regulatory systems for sustainable industrial development with minimal environmental impacts are in place worldwide (Borja et al., 2013). The backbone of environmental monitoring programs in marine finfish farming is the biological component (Borja et al., 2009). Several indicators of the effects of aquaculture on benthic communities have been proposed but not all are suitable for use across a wide range of different ecosystem types and farming conditions (Borja et al., 2009). Among the frequently used indices are for example the AMBI (AZTI's Marine Biotic Index, Borja et al., 2000; Muxika et al., 2007; Subida et al., 2012), the ITI (Infaunal Trophic Index, Maurer et al., 1999), and the NSI (Norwegian Sensitivity Index, Rygg and Norling, 2013). These indices rely on benthic macrofauna diversity and their sensitivity to environmental changes. Different indices correlate generally rather well, but if a dominant indicator species is classified differently by different methods, the results will diverge (Rygg and Norling, 2013). All forms of indices and indicators require each individual benthic macro-invertebrate species to be sampled, sorted and identified under a stereomicroscope. This process is excessively laborious and time-consuming (Goodwin et al., 2017). Moreover, this does not permit responding in a timely manner for effective adaptive management (Lejzerowicz et al., 2015). Furthermore, species identification requires a high level of taxonomic expertise that is increasingly rarely available (Jones, 2008; Menezes et al., 2010; Pawlowski et al., 2012, 2014; Goodwin et al., 2017). Errors in species identification often lead to incorrect classifications and misinterpretations of the data, eventually discrediting biotic indices (Martinez-Crego et al., 2010; Goodwin et al., 2017). Damaged specimens and immature life stages are further limitations hampering accurate morphological species surveys (Ranasinghe et al., 2012; Goodwin et al., 2017). Thus, based on the above-mentioned reasons, morphospecies based monitoring is very expensive. On the other hand, there is an increasing demand for aquaculture-produced seafood, which requires a higher number of EIAs to be conducted. Therefore, concerted efforts are ongoing to investigate new and cost-effective methods to monitor and assess marine waters (Aylagas et al., 2014; Lejzerowicz et al., 2015; Pawlowski et al., 2014; Pochon et al., 2015).

In this context, protists may be ideal candidates. These unicellular eukaryotes comprise some specific taxon groups that are well-known indicators. These include, for example, diatoms used as indicators for freshwater systems (Almeida et al., 2014; Desrosiers et al., 2013; Visco et al., 2015; Zimmermann et al., 2015) as well as ciliates (Carey, 1992; Chen et al., 2008; Foissner, 1999, 2016; Foissner and Berger, 1996; Jiang et al., 2013; Lara and Acosta-Mercado, 2012; Lee et al., 2004; Lynn and Gilron, 1992; Xu et al., 2014; Zhang et al., 2014). The latter are highly sensitive to local environmental conditions (including anoxia, organic enrichment, acidification and chemical pollution; see references in Lynn, 2008) and react much faster to environmental changes than metazoans (Lear et al., 2011; Madoni, 2005). However, the visual screening of environmental samples for exhaustive protist morphospecies identification is too challenging to be applied routinely in most ecological studies (Foissner, 2016; McManus and Katz, 2009). This is mainly because their identification relies on microscopy, which requires substantial experience in sample preparation and taxonomic training and thus is equally time consuming as the identification of macrofauna species.

To countervail this taxonomic impediment, and to make the properties of protists as bioindicators better available to ecologists, biological identification through DNA barcodes has been introduced (Pawlowski et al., 2012; Stoeck et al., 2014b; Zimmermann et al., 2015). Environmental DNA barcoding (metabarcoding) uses short, standardized gene regions obtained from environmental samples as internal species tags to allow for rapid identifications (Taberlet et al., 2012; Valentini et al., 2016). This technique is high throughput, technically straightforward and readily available. It has the potential to increase accuracy, resolution and speed while decreasing the cost of environmental monitoring (Aylagas et al., 2014; Bourlat et al., 2013; Ji et al., 2013; Lallias et al., 2015; Pawlowski et al., 2016b; Thomsen and Willerslev, 2015; Valentini et al., 2016).

In a previous study, DNA barcodes of foraminifera showed excellent potential for environmental impact assessments of aquaculture (Pawlowski et al., 2014, 2016a; Pochon et al., 2015). Therefore, the protistan community structures were compared against the traditional macrofauna communities and indices obtained from the same samples in order to explore the potential of other protistan groups to be used as bioindicators in the environmental monitoring of salmon aquaculture.

2. Materials and methods

Extensive details on sampling, RNA extraction, and macrofauna identification and benchmarking are available from Lejzerowicz et al. (2015). Brief information is provided below.

2.1. Study area and sampling

In May 2013, we sampled a salmon farm consisting of nine circular salmon cages located on the east side of the Isle of Lismore, on the west coast of Scotland. Sampling was conducted in the framework of a routine farm monitoring according to the "Benthic monitoring guidelines for aquaculture operations in Scotland" of the Scottish Environmental Protection Agency (SEPA, https://www.sepa.org.uk/media/114761/ffm_anx_f.pdf) and occurred at the peak of salmon production on the eve of harvest. Mean current velocity was maximal 10 cm s^{-1} and water depth ranged between 26 and 28 m. From the edge of the most southerly cage (cage center: −5.500, 56.502, decimal °, WGS84) we sampled five benthic stations (soft bottom) distributed along a transect (bearing 240°) in line with the cages and with the dominant current flow. These stages were at the cage edge (0 m), within the allowable zone of effect (AZE, 26 m), in the intermediate zone of impact (60 m) and at two reference sites (270 m and 400 m). At each station, sediment was collected using a Van Veen grab sampler (three replicate grabs per site). From two grabs, the redox potential was measured (redox probe CMPtr 106/300 mm; Russel pH Ltd, Auchtermuchty, UK) within the first 10 mm and three sediment replicates were subsampled for protistan DNA metabarcoding (6 g sediment preserved in MoBios LifeGuard solution). The remaining sediment from all three grabs was treated for macrofauna morphotaxonomic inventories. For the latter, the sediment was washed through a 1 mm sieve and the residue fixed in 4% borax-buffered formaldehyde prior to macrobenthic sorting and counting. The sieve-retained fauna was identified to species level under the National Marine Biological Quality Control Scheme (NMBAQCS) by Myriad Taxonomy (Campbeltown, Argyll) after pooling of replicates.

2.2. Molecular analyses

Total environmental RNA was extracted from sediment samples using MoBio's PowerSoil RNA kit. Residual genomic DNA was removed and RNA was transcribed into cDNA as described previously by Lejzerowicz et al. (2015). As a taxonomic marker gene for protists we amplified the hypervariable V9 region of the SSU rDNA according to the protocol of Stoeck et al. (2009, 2010). Primers were 1391F (5′-GTACACACCGCCCGTC-3′, *S. cerevisiae* NCBI GenBank nucleotide database accession number U53879 position 1629–1644) and EukB (5′-TGATCCTTCTGCAGGTTCACCTAC-3′, *S. cerevisiae* position 1774–1797). The PCR protocol for V9 amplification employed an initial activation step at 95 °C for 5 min, followed by 30 three-step cycles consisting of 94 °C for 30 s, 57 °C for 45 s, and 72 °C for 1 min; then a final 2-min extension at 72 °C. From the resulting PCR products, sequencing libraries were constructed using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA). The quality of the libraries was assessed with an

Download English Version:

https://daneshyari.com/en/article/8845658

Download Persian Version:

https://daneshyari.com/article/8845658

Daneshyari.com