Spatial patterns of meiofaunal assemblage at the Noho site in the Okinawa Trough, NW Pacific: traditional vs. semi-automated methods

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Submarine resources, such as seafloor massive sulfide (SMS) deposits, are expected to become developed in the near future. Mining activities would affect the surrounding seafloor environment and benthic ecosystem. It is necessary to evaluate such mining impacts in order to minimize the adverse effects on the ecosystems. Meiofauna is widely recognized as a useful indicator for assessing the effect of anthropogenic and natural disturbances on deep-sea ecosystems. However, traditional methods of investigating meiofauna, which includes counting and identifying small-sized meiofaunal specimens one by one under a microscope, is labor-intensive and time-consuming. Alternative methods, which can rapidly deal with an abundance of meiofaunal samples, are required when conducting long-term environmental impact assessments. The flow cytometer and microscope (FlowCAM, Fluid Imaging Technologies), originally developed to analyze microplankton semi-automatically, has the potential to resolve this need. We investigated the meiofaunal assemblages at the Noho site in the Sakai hydrothermal vent field, located in the middle of the Okinawa Trough, north-west Pacific Ocean. This site is a target area for the 2016 drilling expedition by the D/V *Chikyu*. The sediment samples were collected during the research cruise of the R/V *Kairei*, KR-15-17 in November 2015, using a push corer on the ROV *KAIKO Mk-IV*. The aim of this study was to collect pre-impact data on the meiofaunal assemblages at this site. In addition, we compared the assemblage data obtained with a semi-automated method with those obtained using traditional methods.

Fixing, storage time and DNA extraction applied to DT-RFLP for ecological and molecular studies of marine nematode assemblages

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The application of molecular methods offers an alternative faster than traditional methods based on morphology. Molecular approaches offer the potential to investigate questions that would be unmanageable using traditional approaches, in those cases where a large number of samples are being studied. It is almost impossible to process all the samples in a short period using traditional methods, especially given the rapid deterioration of marine sediment samples. The dT-RFLP (directed Terminal-Restriction Fragment Length Polymorphism) allows a rapid assessment of biodiversity changes in nematode assemblages. However, after the nematodes have

been extracted from benthic sediments, a high content of vegetable detritus remains. This could be a limitation of the DNA extraction process and furthermore, of the dT-RFLP analysis. Therefore, an experimental study was designed to investigate the best fixative, the level of DNA degradation over time and the best DNA extraction method for marine nematodes. Here, three different DNA extraction methods were tested (two commercial kits and one with phenol-chloroform) to investigate the best DNA extraction method for benthic nematodes. The fixation of benthic nematodes was tested using three different storage fixatives (70% ethanol, DESS and freeze drying) to investigate with which one benthic nematodes are better preserved for dT-RFLP analysis. The storage time was investigated using five different time points (0, 2, 4, 8 and 12 months) where in each one the DNA was extracted. This gave an opprtunity to study the level of DNA degradation over time. Benthic nematodes were collected from Mira Estuary (SW, Portugal) and preserved in different fixatives and during different time periods. DNA was extracted and PCR-amplification performed to test the suitability of the extracts for molecular applications. In order to test whether choice of DNA extraction method, storage fixative and storage time have an effect on nematode community composition, dT-RFLP was performed. Real-time PCR (qPCR) was also performed in order to investigate whether previous choices have an effect in benthic nematode quantification. Both the dT-RFLP and qPCR processes showed differences in nematode community following the different methods under investigation. This study demonstrates that dT-RFLP applied to marine nematodes communities facilitates a faster and more efficient approach for ecological and molecular studies.

P83 The use of marine nematode species in ecotoxicological bioassays

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Within ecotoxicology, different nematode species have been used to assess ecological impacts of contaminants. However, these studies have been conducted mainly with freshwater and terrestrial nematodes and the use of marine nematodes, despite their numerical and ecological importance, remain poorly explored. In this study, we tested the potential of two species of marine nematodes as useful indicators to be employed in ecotoxicological bioassays. *Litoditis marina* lives on macroalgae in the littoral zone of coasts and estuaries around the world and *Diplolaimella diavegatensis* in sediments. Both feed on bacteria and are easily cultivated in laboratory. We have investigated the effects of three sublethal concentrations of sodium dodecyl sulfate (SDS) in the growth, reproductive output and survival of these two nematode species. SDS is the most common chemical compound used in cleaning and personal hygiene products and therefore is frequently found in industrial and domestic sewage. The two species were synchronized and maintained in agar plates. Survival