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Spatial variability in recruitment of benthos near drilling sites in the Iheya North hydrothermal field in the Okinawa Trough

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ABSTRACT

Due to increasing anthropogenic impacts on deep-sea hydrothermal vent ecosystems, it is essential to understand population structure and maintenance through larval recruitment and recovery of vent faunas after disturbances. In this study, we quantified vent animal recruitment in the Okinawa Trough, in the western Pacific Ocean. This is the first study to investigate recruitment patterns at a man-made hydrothermal vent. Colonization plates were deployed at three sites. Site 1 manifested new hydrothermal shimmering with small chimneys, white bacterial mats, and some alvinocaridid shrimp that arrived after drilling. Site 2 showed no evidence of newly arrived foundation species after drilling, and Site 3 had pre-existing animal communities in the vicinity of the new vent. Twenty-two months after deployment, colonization plates were retrieved and recruited animals were inventoried. Species composition and abundance differed among sites, but relatively high similarity in species composition was observed at Sites 1 and 3, though not at Site 2. Newly established communities on the plates at Sites 1 and 2 (no pre-existing fauna) showed lower species richness and abundance than at Site 3. Differences in abundance and size-frequency distributions of major recruits on the plates (i.e. *Lepetodrilus nux*, *Bathymodiolus* spp.) suggest the importance of reproductive and early life-history characteristics in spatial variability of recruitment. *Lepetodrilus nux* populations established on the plates at Site 1 showed high genetic connectivity. These results illustrate the importance of localized recruitment, which may have a significant impact on sustainability of vent faunal populations, despite the existence of regional metapopulations.

1. Introduction

In deep-sea hydrothermal vent ecosystems, catastrophic eruptions, tectonic disturbances, and cessation of vent fluid discharges alter the geochemical environment and reset succession of benthic faunal communities (e.g. Shank et al., 1998; Mullineaux et al., 2012). Colonization by larvae is therefore critical to recovery of faunal communities and structure of those communities at the nascent stages. Larval dispersal, colonization, and recruitment in hydrothermal vent ecosystems have been studied mainly at eastern (e.g. Kelly et al., 2007; Thomson et al., 2003; Tunnicliffe et al., 1997) and northeastern Pacific vents (e.g. Van Dover et al., 1988; Mullineaux et al., 1998, 2003, 2005, 2010, 2012; Pradillon et al., 2005), and at the Mid-Atlantic Ridge (e.g. Comtet and Desbruyeres, 1998; Cuvelier et al., 2014; Teixeira et al., 2012). These studies found that larvae of certain species have the potential to disperse hundreds of kilometers (Epifanio et al., 1999; Marsh et al., 2001; Teixeira et al., 2012), but in other species, larvae are retained near their natal sites (Thomson et al., 2003), with localized dispersal on a scale of kilometers (Adams and Mullineaux, 2008). Moreover, colonizing species composition and abundance could be affected by larval availability, resident fauna, and local environmental conditions, e.g. vent fluid, temperature, sulfide concentration, pH (Cuvelier et al., 2014; Mullineaux et al., 2012). In spite of advances achieved by these studies in the above areas, western Pacific vents have been poorly studied relative to larval dispersal, colonization, and recruitment. Only one investigation has examined recruitment processes at western Pacific vents (Nakamura et al., 2014). In addition, a recent study demonstrated that distant vent communities in the western Pacific are potentially connected via larval dispersal, due to strong directional currents (Mitarai et al., 2016). With growing scientific and commercial interest in hydrothermal vents (Ramirez-Llodra et al., 2011; Van Dover, 2014), more studies at western Pacific vents are urgently required.

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The present study was performed after Integrated Ocean Drilling Program (IODP) Expedition 331 investigated the Iheya North hydrothermal field in the Okinawa Trough, Japan, to inventory diverse subseafloor microbial communities associated with hydrothermal activities (Takai et al., 2012). Expedition 331 drilled at five sites and induced the formation of new hydrothermal vents. One of the new vents was created at Site C0014, where hydrothermal fluid discharges had not been previously observed. Several *Calyptogena s.l.* clams were found on the fine-grained, brownish sediment, but most (> 90%) were dead (only shells) (Kawagucci et al., 2013). On one of the seven holes at Site C0014, a triangular, gimbaled guide base was mounted with stainless-steel pipe inserted into the hole to a depth of 136.7 m below the seafloor for sampling the fluid. There, high-temperature hydrothermal fluid discharge was recorded for 25 months after drilling: the highest recorded temperature was 311 °C. Eleven months after the drilling, the seafloor became whitish, probably due to a microbial population in the clay mineral substrate. On the white seafloor, several tiny chimneys and low density of galatheid crabs were observed around the site. Afterward, galatheid crabs became more numerous and alvinocaridid shrimps and some *Paralvinella* polychaetes were observed 16 months after drilling (Nakajima et al., 2015).

Post-drilling observations revealed changes in landscape, discharged fluid chemistry, and immigration of megabenthos (Kawagucci et al., 2013; Nakajima et al., 2015). However, no study has ever been made of recruitment at a new vent community. This drilling expedition presented an opportunity to investigate recruitment of vent fauna, community establishment, and succession in western Pacific vents. Therefore, we surveyed recruitment patterns around these artificial hydrothermal vents, comparing them with those occurring at natural hydrothermal vents, to address the question: How do recruitment patterns vary between sites with evidence of newly established venting and communities, with no evidence of either, and with pre-existing communities?

2. Materials and methods

This study was conducted at the Iheya North Field (27°45'-50'N, 126°53'-55'E) in the Okinawa Trough, where IODP Expedition 331 was implemented in September 2010 (Takai et al., 2012) (Fig. 1a). Recruitment surveys were conducted around Hole C0014G at Site C0014, one of drilling sites (Fig. 1b, c). This area was chosen because animal communities were almost nonexistent before drilling. Several *Calyptogena s.l.* clams were found on the fine-grained, brownish sediment, but most (> 90%) were dead (only shells). Discharges of hydrothermal fluids and vent endemic fauna were not observed before drilling (Kawagucci et al., 2013; Nakajima et al., 2015); thus, this site was useful for investigating establishment of community structure and succession in a hydrothermal vent community after discharge of hydrothermal fluids commenced, and for observing the effects of anthropogenic disturbances to the deep seafloor. In addition, Hole C0014G was continuously observed for changes in hydrothermal fluid chemistry, landscape, and megabethos species (Kawagucci et al., 2013; Nakajima et al., 2015).

Colonization plates were placed at three sites: a site where new hydrothermal shimmering was occurring and some foundation species arrived after drilling (Site 1, ~ 0.24 km west-southwest of Hole C0014G); a site with no new visible arrivals of foundation species on the soft substrate (Site 2, ~ 0.13 km east-northeast of Hole C0014G); and a site near Hole C0014G, where vent communities existed in the vicinity of natural hydrothermal fluid discharges before and after drilling (Site 3, ~ 0.47 km west of Hole C0014G) (Figs. 1 and 2, Table 1). Colonization plates were made of Lexan plastic (10 cm x 10 cm x 64 mm thick). These were structured as "sandwiches," like those used at the East Pacific Rise (EPR) (Mullineaux et al., 2010, 2012). Each set of colonization plates comprised three sandwiches made of six plastic plates with ~1 cm gaps between plates (Fig. 2). Three sets of colonization plates were deployed at each site during dives 537–538 of the ROV *Kaiko 7000 II* during the KR 12–02 cruise of R/V Kairei, conducted by JAMSTEC (Chief Scientist: Hiroyuki Yamamoto), 16 months after the drilling. Twenty-one months later, two sets of plates at each site (named P1 and P2) were retrieved during dives 1591–1596 of the ROV *Hyper-Dolphin* during the NT13–22 cruise of R/V Natsushima, conducted by JAMSTEC (Chief Scientist: Hiroyuki Yamamoto). Each set of plates was gently placed in an individual, sealed collection box to prevent loss of colonizing organisms prior to recovery and to prevent intermixing of samples.

All benthic animals on the plates were identified to the lowest taxonomic level possible, based on morphology, and were fixed in 99.5% ethanol. In addition, benthic animals found in the sealed collection boxes were included, since they fell off the plates. Abundances of all taxonomic groups were determined. A diversity index, the Shannon-Weaver index (H′), was calculated using the vegan package for R (ver. 3.2.1, R Development Core Team, 2014), and similarity of faunal composition among colonization sandwiches was calculated using the Bray-Curtis similarity index after overall transformation of abundance using the fourth root (PRIMER-E ver. 6, Clarke and Gorley, 2006). Results were plotted using non-metric multi-dimensional scaling, with an overlay of similarities estimated by group-average cluster analyses. In addition, abundances were also compared among sites, using univariate ANOVA, with the Tukey HSD post-test, for commonly observed animals at all sites,

The most abundant species on colonization plates at Sites 1 and 3, *Lepetodrilus nux,* was analyzed for size distribution and population genetic structure. Shell length of *L. nux*, the longest distance from the shell apex to the anterior edge, was measured according to Nakamura et al. (2014). Size distribution was compared among sets of plates (P1 and P2) and sites, using the Kolmogorov-Smirnov 2 sample test and modal decomposition analysis with the Mclust program, R environment (R Development Core Team, 2014). Population genetic structure was compared among cohorts of each plate resulting from modal decomposition analysis between two sites. DNA was extracted from foot tissue of *L. nux* using 10 µL of ice-cold PCR buffer II (500 mM KCl, 100 mM Tris-HCl, pH 8.3) and 1 µL of a 10 mg/mL proteinase K solution, with incubation for 3 h at 55 °C. Proteinase K was inactivated by incubating samples 5 min at 100 °C. Then 10 µL of Gene-Releaser were added to each tube as per the Gene-Releaser cycling protocol (Schizas et al., 1997). Each tube was centrifuged 1 min at 13,000 × *g*. Microsatellite markers developed by Nakajima et al. (2017) were used for population genetic analysis (Table 2). Extracted DNA was amplified using multiplex PCR, adding four primer sets to each PCR tube. Ampli Taq Gold 360 Master Mix (Thermo Fisher Scientific) was used with 20 µM fluorescent primers, DNA template, and nuclease-free water for a total reaction volume of 5 µL (Table 2). Amplification was conducted under the following conditions: 95 °C for 9 min followed by 35 cycles at 95 °C for 30 s, 54 °C for 30 s, 72 °C for 1 min, and a final extension of 5 min at 72 °C. Allelic variations of amplified products were analyzed with a DNA capillary sequencer (3130xl Genetic Analyzer, Thermo Fisher Scientific). The GenAlEx program (Ver. 6.5) (Peakall and Smouse, 2006) was used to calculate the number of alleles, allele frequencies, the number of private alleles, genetic differentiation by means of analysis of molecular variance (AMOVA, Excoffier et al., 1992) and pairwise F_{ST} values. Principal coordinate analysis (PCA) was also performed with GenAlEx.

3. Results

3.1. Diversity of benthic communities on colonization plates

Species composition and abundance in communities established on plates varied among sites (Fig. 3; Table 3), but relatively high similarity was found between Sites 1 and 3 (63.181–70.675), relative to Site 2, which was slightly more similar to Site 1 (46.203–53.357) than to Site 3 (31.825–40.885) (Fig. 4).