Research Note

Scientific Gear as a Vector for Non-Native Species at Deep-Sea Hydrothermal Vents

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Abstract: *The fauna of deep-sea hydrothermal vents are among the most isolated and inaccessible biological communities on Earth. Most vent sites can only be visited by subsea vehicles, which can and do move freely among these communities. Researchers assume individuals of the regionally homogeneous vent fauna are killed by the change in hydrostatic pressure the animals experience when the subsea vehicles, which collected them, rise to the surface. After an* Alvin *dive, we found 38 apparently healthy individuals of a vent limpet in a sample from a hydrothermally inactive area. Prompted by our identification of these specimens as* Lepetodrilus gordensis*, a species restricted to vents 635 km to the south of our dive site, we tested whether they were from a novel population or were contaminants from the dive made 36 h earlier. The 16S gene sequences, morphology, sex ratio, bacterial colonies, and stable isotopes uniformly indicated the specimens came from the previous dive. We cleaned the sampler, but assumed pressure changes would kill any organisms we did not remove and that the faunas of the 2 areas were nearly identical and disease-free. Our failure to completely clean the gear on the subsea vehicle meant we could have introduced the species and any diseases it carried to a novel location. Our findings suggest that the nearly inaccessible biological communities at deep-sea vents may be vulnerable to anthropogenic alteration, despite their extreme physical conditions.*

Keywords: Gorda Ridge, hydrothermal vent, *Lepetodrilus*, species introduction, stable isotope, subsea vehicle, Juan de Fuca Ridge

Equipo Científico como un Vector para Especies No Nativas en Conductos Hidrotermales de Aguas Profundas

Resumen: *La fauna de los conductos hidrotermales de aguas profundas esta entre las comunidades biológicas más aisladas e inaccesibles del mundo. La mayoría de los sitios en conductos solo pueden ser visitados por vehículos submarinos, que pueden moverse libremente entre estas comunidades. Los investigadores asumen que individuos de la fauna de conductos regionalmente homogénea mueren por el cambio de presión hidrostática que los animales experimentan cuando los vehículos submarinos que los recolectó suben a la superficie. Después de un viaje de* Alvin*, encontramos 38 individuos de lapa aparentemente sanos en una muestra de una zona inactiva hidrotermalmente. Motivados por nuestra identificación de estos especímenes como* Lepetodrilus gordensis*, una especie restringida a conductos 635 km al sur del sitio de muestreo, probamos si eran de una población nueva o eran contaminantes de nuestro muestreo realizado 36 horas antes. Las secuencias del gene 16S, la morfología, proporción de sexos, colonias de bacterias e isotopos estables indicaron uniformemente que los especímenes provenían del muestreo anterior. Limpiamos el muestreador, pero asumimos que los cambios de presión matarían a cualquier organismo que no removimos y que las faunas de las 2 áreas eran casi idénticas y no tenían enfermedades. Nuestro error de no limpiar el*

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equipo del vehículo submarino significó que pudimos haber introducido la especie y cualquier patógeno que portara a una localidad nueva. Nuestros hallazgos sugieren que las comunidades biológicas, casi inaccesibles, de los conductos en aguas profundas pueden ser vulnerables a la alteración antropogénica, no obstante sus condiciones físicas extremas.

Palabras Clave: Borde Juan de Fuca, Borde Gorda, conducto hidrotermal, introducción de especies, isotopo estable, *Lepetodrilus*, vehículo submarino

Introduction

Human transport of species beyond their current ranges presents one of the greatest challenges to the preservation of marine species and often results in ecological surprises as novel sets of species interact (Suchanek 1994; Bax et al. 2003; Lewis et al. 2003). Non-native species have been documented from the intertidal zone to the depths of continental shelves (Steneck & Carlton 2001; Carlton 2003). Despite implications that activities such as ocean drilling and submersible operations have introduced a substantial number of species to depths as great as that of the continental shelf (Steneck & Carlton 2001), introductions in the deep sea, and specifically in one of the most isolated and extreme environments on the planet, hydrothermal vents, are not currently considered a real threat to species. The study of hydrothermal vents has largely focused on their extreme physical features and temporal transience. The environmental changes vent animals are subjected to when they are transported from the seafloor to the surface have been assumed to be lethal. However, increasing evidence shows otherwise.

Two species of vent limpets occur in the northeastern Pacific, *Lepetodrilus fucensis* on the Juan de Fuca Ridge and *L. gordensis* on Gorda Ridge. The ridges and species are isolated by the 450-km-long Blanco Transform Fault (Fig. 1). These closely related congeners reach $10^5/m^2$ densities in areas of hydrothermal-fluid flow (Bates et al. 2005; Johnson et al. 2006). Despite the species' restriction to Gorda Ridge and its reliance on vent fluid flow for nutrition (Bates 2007), we collected 38 apparently healthy individuals of *L. gordensis* on a hydrothermally inactive sediment pond on Endeavour Segment, Juan de Fuca Ridge, 635 km north of its northernmost collection locality (Johnson et al. 2006). We assumed the specimens had 2 possible origins: a previously undiscovered population living in an area without hydrothermal activity or they were retained on the submersible after a dive at Gorda Ridge. To determine the origin of these limpets, we examined their 16S sequences, shell lengths, sex ratios, epibiont incidences, and tissue stable isotope signatures.

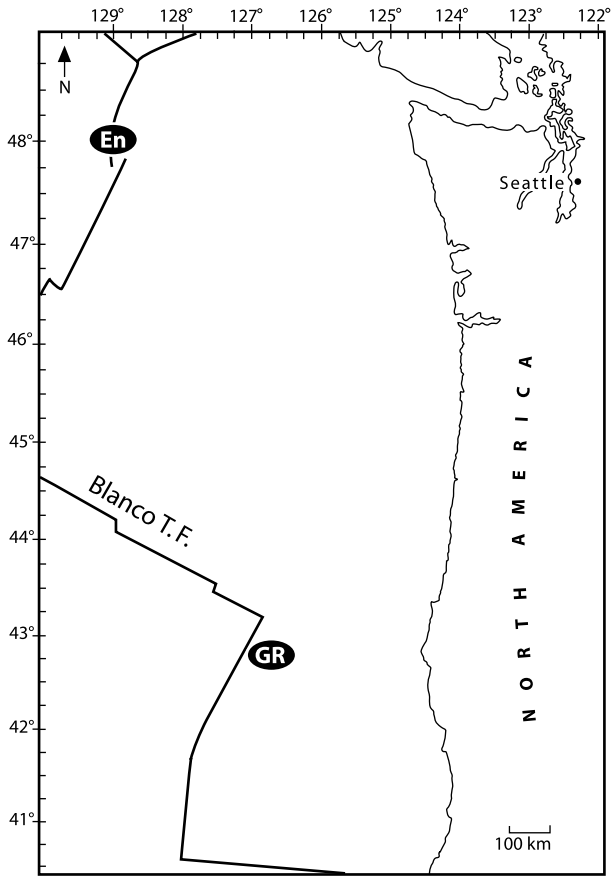

Figure 1. Location of GR-14 (GR), site of Alvin *Dive 4044, on Gorda Ridge (GR), which is separated by the Blanco Transform Fault (Blanco T. F.) from Juan de Fuca Ridge, which includes the Endeavour Main Field (En), site of* Alvin *Dive 4045.*

Methods

Among the sampling tools on the human-occupied submersible *Alvin* is a multichamber suction sampler. The sampler consists of a pump that creates suction, a corrugated hose, screens to minimize the chance of blockage, and 5 clear acrylic bins that retain samples. Near the end of *Alvin* Dive 4044 on 31 August 2004 at 2723 m depth on the GR-14 (Seacliff) hydrothermal vent field, Gorda Ridge (Fig. 1), we took a final sample with the suction sampler from a blue folliculinid mat that was sustained by hydrothermal fluid flow. The mother ship recovered *Alvin* and sailed 635 km in 30 h to Endeavour Segment, Juan de Fuca Ridge. We launched *Alvin* Dive 4045 on 2 September 2004 to recover 2, 24-month-old wood structures (hereafter deployments) deployed on an isolated

sediment pond near Endeavour Main Field at 2213 m depth (Voight 2007). Anoxic sediment surrounded the deployments. After securing both deployments, we used the suction sampler to collect animals dislodged from or living beneath the deployments. After each dive, researchers removed the contents of each suction-sampler bin and preserved each separately for onshore sorting and identification. We labeled all samples with collection method and deposited the specimens at The Field Museum of Natural History in Chicago (FMNH).

Among the specimens collected on *Alvin* Dive 4045 were 38 individuals of *Lepetodrilus* (catalogued as FMNH 308780). We initially attributed their presence to localized anoxia generated by the deployments. We photographed the limpets en masse under light microscopy and noted the unusual thinness of their shells. We preserved the limpets in 95% ethanol.

The limpets were present only in the first suction-sampler bin used during the dive. In the same bin were 12 individuals of the vent snail *Depressigyra globulus* and 2 small brown clumps of folliculinid protist. We later identified the limpets as *L. gordensis* on the basis of external morphology, including the species' thin shell (Johnson et al. 2006), and comparison with specimens collected from Gorda Ridge vents.

We sought to confirm our species identification by sequencing, with Folmer primers (Folmer et al. 1994), the COI gene of the *Lepetodrilus* collected at Endeavour. These attempts failed, likely because the primer amplified symbiont rather than limpet sequence (Vrijenhoek 2009). To sequence the 16S gene, we used a DNeasy kit (Qiagen, Valencia, California) to extract DNA from 10 specimens of *L. fucensis* from 2 vent fields on Endeavour Segment, Juan de Fuca Ridge (Raven, FMNH 293797 and Main Field, FMNH 297173), and from 8 specimens of *L. gordensis* from the 2 known vent fields on Gorda Ridge (Escanaba Trough, FMNH 308775 and GR-14 FMNH 308723) and 5 limpets collected from Endeavour. We used the primer pair 16Sar and 16Sbr (Palumbi 1996) to amplify the 16S gene and a thermal protocol that consisted of 92° for 2 min, 35 cycles of 94° for 30 s, 35 cycles of 55° for 30 s, 35 cycles of 72° for 45 s, and a final extension step of 72° for 7 min. We used the millipore vacuum procedure to purify PCR products and Big Dye Terminator (Applied Biosystems, Carlsbad, California) chemistry to sequence them. We edited raw sequences and assembled them in SEQUENCHER (version 4.2; Gene Codes, Ann Arbor, Michigan) and used the default parameters in MUSCLE (version 3.8) (Edgar 2004) to align them. We inspected the sequences by eye in MESQUITE (version 2.74) (Maddison & Maddison 2010) to identify and compare variable sites. We posted the sequences generated to GenBank (accession numbers JN936278-JN936295).

We tested the hypothesis that the specimens collected from Endeavour live in a hydrothermally inactive area and thus have morphological features that distinguish them from limpets that live on the folliculinid mat on GR-14. This hypothesis follows from observations that size and sex ratio of *L. fucensis* populations are a function of habitat quality (Bates 2008). We compared shell lengths of 38 specimens collected from Endeavour with those of 65 collected from the GR-14 folliculinid mat (FMNH 308694; 308723). We also compared the incidence of females and of "pustules or crusts of bacterial colonies" (Johnson et al. 2006) on the limpet shells between these groups. We used a Welch 2-sample t test to compare limpet shell length and the exact binomial test to compare the incidence of females and bacterial colonies. We used R (version 2.13.2) (R Development Core Team 2011) for statistical analyses.

The isotope signatures of specimens from Endeavour vents range from $-26.3‰$ to $-15.8‰$ for $\delta^{13}C$ and from $-1.2‰$ to $5.3‰$ for $\delta^{15}N$ (unpublished data). Expected values for animals that do not live at vents are roughly $\delta^{13}C = -22‰$ and $\delta^{15}N = +15‰$ (Van Dover & Fry 1994). Values for animals from inactive vent sediments are roughly $\delta^{13}C = -22‰$ and $\delta^{15}N = +7‰$ (Levin et al. 2009). If the specimens collected from Endeavour lived in the hydrothermally inactive area, we assumed their carbon and nitrogen isotope signatures would differ from those of limpets living in hydrothermal fluids. To test this assumption, we analyzed the carbon and nitrogen isotopes of 2 limpets collected from Endeavour and 20 limpets collected from GR-14 (FMNH 302163, 308694, 308723). We followed the standard methods, for example, Becker et al. (2010) to determine tissue-stable isotope values with an elemental analyzer (Costech, Valencia) that routes carbon dioxide and nitrogen gas from samples to a mass spectrometer (Isoprime, GV Instruments, Manchester, U.K.). Routine precision for replicate animal samples is $0.3‰$ for $\delta^{15}N$ and $0.1‰$ for $\delta^{13}C$.

Results

The external morphology of the Endeavour specimens was consistent with that of *L. gordensis* (Johnson et al. 2006) and with that of *L. gordensis* specimens collected previously from Gorda Ridge.

Of the 417 base pairs of the 16S gene successfully sequenced for 5 *L. fucensis*, 8 *L. gordensis*, and 5 Endeavour *Lepetodrilus* specimens, 6 sites (1.4% of base pairs sequenced) unambiguously supported our morphology-based species identification. Two distinguishing sites were single-base insertion or deletions; 4 sites were transitions. Sequences of the Endeavour specimens were the same as those of *L. gordensis*.

Shell length of Endeavour and GR-14 limpets did not differ (mean Endeavour limpets 9.0 mm; mean GR-14 folliculinid limpets 8.4 mm, $t = 1.62$, df $= 59.38$, $p = 0.11$). The proportion of females (Endeavour, 58.3%; GR-14, 52.3%) and proportion of limpet shells with bacterial

colonies (Endeavour, 95.8%; GR-14, 96.9%) did not differ (females: $p = 0.68$; bacterial colonies: $p = 0.53$). One limpet from Endeavour had a small patch of folliculinid on its shell.

The nitrogen isotope signatures of the 2 groups of *L. gordensis* were indistinguishable. Those of the 2 Endeavour specimens were 5.5‰ and 6.0‰; those of the 20 GR-14-collected specimens ranged from 4.9‰ to 7.7‰. The δ^{13}C isotope signature of the 20 GR-14 specimens ranged from −15.4‰ to −11.6‰. One Endeavour *L. gordensis* specimen was slightly more negative (−16.5‰); the others were within the signature range of GR-14 specimens (−14.4%).

Discussion

Although we recovered 38 specimens of *L. gordensis* from *Alvin*'s sampling gear after a dive on Juan de Fuca Ridge, 16S sequences, shell lengths, sex ratios, epibiont incidences, and stable isotope signatures indicated they were collected on the previous dive at Gorda Ridge and transported as contaminants in sampling gear. Our transport of these animals was inadvertent, although we were aware that it was the responsibility of the Science Party to clean the sampling gear between dives. At the time of collection, we assumed that the taxonomic treatments that listed *L. fucensis* as the only northeastern Pacific species in the genus were correct and that changes in pressure would be lethal to any vent animal brought to the surface. Both these assumptions were incorrect. Two years after *Alvin* Dive 4045, *L. gordensis* was recognized as a distinct species (Johnson et al. 2006). It is now known that depressurization alone is insufficient to kill many deep-sea animals, including *Lepetodrilus*. Individuals of at least 3 genera of vent gastropods tolerate transport to the surface and being maintained in aquaria, and when returned to depth, they behave normally and show high survival rates (Bates et al. 2005). Individuals of many taxa from hydrothermal vents survive if they are returned to the pressure of their original depths within days of collection. For example, Bates et al. (2010) list 14 species from 3 phyla with this capacity. Vent crabs of *Bythograea* from depths of 2500 to 2600 m on the East Pacific Rise have been reared from megalopa larvae at sea-level pressure (Epifanio et al. 1999). Some simpler organisms, such as microbial pathogens (e.g., barotolerant bacteria [Kato et al. 1995]), may also tolerate a broad range of hydrostatic pressures.

The consequences of human-mediated redistribution of animals across the globe have been marked. Of crucial concern in coastal environments (Cunningham 1996; Thompson et al. 2009) are pathogens and parasites introduced with non-native species that infect naïve hosts and cause mass mortalities. Although disease dynamics and epidemiological patterns are unknown for hydrothermal vent ecosystems, disease exists in these extreme habitats and is very likely an important but ignored aspect of vent ecology (Van Dover et al. 2007; Zielinski et al. 2009). *L. fucensis*, which has received far more study than has *L. gordensis*, hosts a parasitic copepod that strongly reduces an individual's reproductive fitness (Tunnicliffe et al. 2008). Bacteria, including a rickettsia and other possible infective agents, occur in the gills of *L. fucensis* and other limpets (Terlizzi et al. 2004).

Our small limpets and their associates accrued somewhere in the suction sampler, perhaps in the corrugated hose, where enough water pooled to keep them alive. Replacing the corrugated hose with a smooth hose may help prevent inadvertent transplants of biota, but any surface or crevice on the submersible or associated gear could provide refuge to macrofauna or bacteria. Spraying and flushing of gear by a member of the science party between dives is standard procedure, but in hindsight, may be insufficient.

We urge our colleagues to assume that physiologically tough stowaways are present on deep-sea research tools and to guard against transport of non-native species by clearing hoses and rinsing containers with freshwater, or even a peroxide solution, and drying tools before transporting them to different sites. Such measures may be especially important when working near marine reserves, such as Endeavour Hydrothermal Vents Marine Protected Area (Dando & Juniper 2001). Even when science operations are nonbiological, inadvertent collection of biota is likely, perhaps even unavoidable. The biological consequences of working with subsea vehicles are still being determined, and best practices are still being developed. Preventing introductions is of paramount importance in maintaining intact hydrothermal vent ecosystems.

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