A tale of two shrimps – Speciation and demography of two sympatric shrimp species from hydrothermal vents

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Abstract (200 words)

Hydrothermal vents can serve as natural laboratories to study speciation processes due to their fragmented distribution often with geographic barriers between habitats. Two sympatric species of *Rimicaris* shrimps occur at vents on the Izu-Bonin-Mariana volcanic arcs: *Rimicaris loihi* also found near Hawai'i and *R. cambonae* also present on the Tonga Arc. These two species biogeographically co-occur and are genetically highly similar, raising the question of the mechanisms of how they maintain distinct species, including the possibility of interbreeding. Here, we use barcoding and shotgun sequencing to test if hybridization is occurring. We also evaluate population demography over 10 years to assess population densities and sex ratios at vents. Our results support *R. cambonae* and *R. loihi* as two distinct species despite sympatry throughout part of their range and presence of hybrids. Different sex ratios between species populations suggest that life-history traits such as different mating systems might reinforce reproductive barriers. We observed fluctuations in shrimp densities alongside their genetic diversity, linked to variations in hydrothermal activity over years. We suggest that geographic isolation after a rare, historical long-distance dispersal across volcanic arcs, followed by

environmentally-driven genetic fluctuations may have fostered rapid divergence by allopatry, although sympatric speciation is not excluded.

Introduction

Speciation, the process by which two species emerge from an ancestral entity, is responsible for modern biodiversity. The formation of distinct species arise from their reproductive isolation that can result from geographical isolation via barriers (allopatric) or ecological barriers by a specialization for distinct niches (sympatric) [1,2]. Although some cases of sympatric speciation do occur, notably in cichlids fishes and spiny mice [3–5], allopatric speciation is generally considered as the predominant form of speciation, with geographic separation at least during part of the process [6,7]. Indeed, in the absence of geographic barriers, nascent species may still be capable of interbreeding and exchanging genes, promoting homogenization of their genetic pools [8]. However, efficient reproductive barriers can maintain recently diverged species, despite their capacity to interbreed and prevent recombination of genomes [2]. These barriers can be prezygotic and act before fertilization such as through distinct mate choice, mechanical incompatibility during copulation, or desynchronized reproductive cycles [9–11]; post-zygotic barriers, such as non-viability and sterility of hybrids or their counter-selection in the environment, can also reinforce speciation [12,13].

Within the deep ocean, hydrothermal vents constitute fragmented ecosystems with high endemism that form potentially-isolated "islands" along oceanic ridges, volcanic arcs and back arc basins [14,15]. Within a vent field, the steep thermal and chemical gradients generated by the mixing of vent fluids with seawater creates a variable mosaic of distinct habitats constraining the spatial distribution of faunal communities [16,17]. Chemical composition of vent fluids further differs due to the underlying geological context and water-rock interactions, with large variation among vent fields within the same region [18,19]. Moreover, many hydrothermal vents are also relatively unstable at ecological and evolutionary timescales, with tectonic rearrangements that can establish or break existing barriers to gene flow among populations favouring allopatric speciation followed by secondary contact where hybridization is possible [20–24]. Hence, these ecosystems provide excellent opportunities to study the role of geographic dynamics and local adaptation on speciation processes in natural populations.

Among the vent-endemic fauna, species of *Rimicaris* shrimps are commonly found living sympatrically in several regions, where two or three species often co-occur within the same vent fields [25–27]. In most cases, *Rimicaris* shrimps exhibit broad geographic distributions with high genetic connectivity along mid-ocean ridges [28–30] or back-arc basins [31–33], suggesting a high dispersal

potential. As with most decapods, *Rimicaris* shrimp are gonochoric brooders that maintain their eggs under their abdomen but also display a variety of reproductive cycles with seasonal or aperiodic brooding patterns [34–36]. In some *Rimicaris* species, brooding cycles were unrelated to variations in photosynthetic surface production, unlike other seasonal brooders that inhabit vents [35].

Two sympatric *Rimicaris* species co-occur at vents from the Izu-Bonin-Mariana (IBM) Volcanic Arc: *Rimicaris* (formerly *Opaepele*) *loihi* and the recently described *R. cambonae* [27]. *Rimicaris loihi* is a grazer foraging on bacterial mats covering the substratum and mussel shells near active vents [36,37]. The hydrothermally-active habitats of *R. loihi* and *R. cambonae* are highly diverse, characterized by areas of high degassing, pools of liquid CO_2 at NW Eifuku, liquid sulfur ponds at Nikko Seamount and other sites with an extensive record of historical eruptive events [18,38]. In particular, active eruptions occurred at NW Rota Volcano during each of the six visits between 2004 and 2010 prior to the last observations in 2014 [39–42]. *Rimicaris loihi*, was also reported from distant sites including the Loihi Seamount on the southeast coast off Hawai'i [43] and at West Mata Volcano north of Tonga [44]. West Mata and Loihi are also known for their high instability with seismic and eruptive activities, both of which were recorded at Loihi in 1996 and by *in situ* observations of explosive eruptions during dives at West Mata in 2008 and 2009 [44,45].

In addition to being sympatric, *R. cambonae* and *R. loihi* are also highly genetically similar, differing by just 2% in the barcoding region of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene fragment [27] and thus falling within the grey zone of speciation [8,46]. Given that these two species coexist within the same faunal assemblages, questions arise about the presence of gene flow and possible hybridization between them and, thus, the effectiveness of reproductive barriers despite the potential for reproduction. However, data on the reproductive biology of these species are limited to one single population of *R. loihi* from Suiyo collected in August 2019, that revealed a largely female-biased sex ratio, and presence of many juveniles and ovigerous females [36].

This study aims to answer the following questions: 1) Are nuclear genes of *R. loihi* and *R. cambonae* concordant with mitochondria and represent separate species-level lineages?; 2) Is there gene flow and hybridisation between *R. cambonae* and *R. loihi* or is there a complete reproductive isolation?; 3) To what extent does the eruptive and unstable geological context affect the population dynamics of these shrimps over the years at a given site?; 4) Could differences in the reproductive characteristics of these two species explain the maintenance of two closely related species in the face of possible hybridisation?; And 5) What is the most likely scenario for their speciation?

Materials and Methods

Animal sampling and processing

Alvinocaridid shrimps were collected during eight oceanographic expeditions between 2004 and 2023 using suction samplers or baited traps on either Remotely Operated Vehicles (ROVs) or Human Operated Vehicles (HOVs). In total, five hydrothermal vent fields from the IBM Volcanic Arc were visited once (Seamount X in 2006, Suiyo in 2019), twice (Nikko in 2006 and 2010, NW Eifuku in 2004 and 2023) or six times (NW Rota between 2004 and 2014) (Figure 1A). Additional shrimps were collected in 2004 at Loihi Seamount near Hawai'i, the type locality of *Rimicaris loihi* and in 2009 at the West Mata volcano (Figure 1A; see Table S1 for detailed sampling information).

Specimens were preserved either in 75% ethanol or frozen whole at -80°C on-board the ship. Additional specimens from NW Eifuku in 2023 were fixed with 4% paraformaldehyde (PFA) for 3h on board and stored in 50% phosphate-buffered saline (PBS)-ethanol solution for electron microscopy (see Supplementary Methods). We determined sex based on examination of 1st and 2nd pleopod shape and measured the size of each shrimp as carapace length (CL) and/or total length (TL) following [27]. We also distinguished *R. cambonae* and *R. loihi* morphologically based on [27] in conjunction with COI barcoding for specimens collected in 2023 at NW Eifuku. For the few ovigerous females encountered, we estimated realized fecundity from egg counts and sizes.

Shrimp Abundance

On NW Rota, where vents occurred only in the upper 80 m of the summit, fine-scale mapping with an SM2000 system, good ROV navigation, and physical markers (e.g., Schnur et al. 2017) supported comparative estimates of venting extent and shrimp distribution between years. Rugged terrain and radical changes in the volcano topography hampered regular transecting and overlays. Instead, we estimated shrimp abundance by assessing the total extent of the ROV tracks in which shrimps were visible in densities greater than ~20 individuals/m^2.

DNA extraction, COI barcode amplification, and Sanger sequencing

We performed DNA extractions on 597 alvinocaridid shrimps using the 4th/5th pleopods or pieces of abdominal tissues following manufacturer's instructions of the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). We amplified the 5' region of the *COI* gene with the Cari-COI-1F and Cari-COI-1R alvinocaridid primers [47] for shrimps collected from Nikko 2010, Suiyo 2019, and NW Eifuku 2023 (679 to 734 bp fragment) or with the COI-1f (5'-AGTAGGAACAGCCCTTAGTCTCCTA-3') and COI-1r (5'-TCCCGCTGGGTCAAAGAATGAGG-3') primers for the remaining shrimps (529 to 623 bp fragment).

We used Promega GoTaq polymerase in 25µl reactions for PCR amplifications with Cari-COI-1F and Cari-COI-1R and iProof High Fidelity DNA Polymerase (Bio-Rad) in 40µl reactions for PCR

amplifications with COI-1f and COI-1r using 1 µl of the DNA template for both. We conducted PCR with the following cycle conditions: initial denaturation at 95°C for 5 min, 35 cycles of 95°C (1 min), 50°C (1 min), 72°C (2 min), followed by a final extension stage of 72°C for 7 min with Cari-COI primers or initial denaturation at 95°C for 5 min, 35 cycles of 95°C (1 min), 50°C (1 min), 72°C (2 min), followed by a final extension stage of 72°C for 7 min with the COI-1f/ COI-1r primers.

Bi-directional Sanger sequencing was conducted by Eurofins (Köln, Germany) for PCR products amplified with Cari-COI-1F/ Cari-COI-1R primers and by the High Throughput Genomics Unit (Seattle, WA, USA) for PCR products amplified with COI-1f/COI-1r primers. We edited and aligned sequence chromatograms with Geneious Prime® 2023.1.2 (https://www.geneious.com).

Haplotype networks

To assign individual mitochondrial barcodes to putative species, we used the Assemble-Species-by-Automatic-Partitioning method (ASAP) on a 529-bp alignment, a method based on the detection of "barcode gaps" [48]. We estimated median joining haplotype networks with PopART v1.7 [49,50] with default settings. We used DnaSP v6 [51] to determine the number of variable sites (S), the number of haplotypes (h), haplotype diversities (H_d), nucleotide diversities (π), average number of nucleotide differences (k, [52], raw genetic distance (D_a), and pairwise F_{ST} values with 1000 permutations within and among putative species and populations.

Whole Genome Shotgun and Oxford Nanopore Sequencing

Following *COI* barcoding, we used whole genome shotgun (WGS) sequencing on a subset of animals along our sampling range to examine whether nuclear data were concordant with mitochondrial data and to explore connectivity amongst populations and lineages. We quantified genomic DNA with the DNA High-Sensitivity assay with the Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham MA, USA) and prepared 32 individuals for WGS sequencing with the Illumina DNA Prep kit (Illumina, Inc. San Diego, CA). Sequences were generated on the Element Bio AVITI instrument at UC Davis with a paired-end 2x150 protocol. Four older specimens from NW Rota were unsuccessful and were excluded from analyses. We also sequenced one individual (Ebi13 from cruise KM23-E05) for long reads on a M1kC device from Oxford Nanopore with the PCR Sequencing kit (SQK-PSK004) on a v9 flow-cell. Details on quality filtering of Whole Genome Shotgun and Oxford Nanopore sequencings are given in Supplementary Methods.

Molecular clock calibration for mitochondrial genomes

We used the programs Beauti v10.5.0 and BEASTX v10.5.0 [53–55] to calibrate a molecular clock and the estimated divergence times between *R. loihi* and *R. cambonae.* We included all available

mitochondrial genomes from alvinocaridid shrimps. We used the substitution rate for *COI* of 2.2-2.6/MY based on [46] with a GTR+I+Γ model and a relaxed uncorrelated yule prior. We ran BEASTX for 10 million generations and sampled every 1000 generations. Trees were visualized in TreeViewer v.2.2.0 [56].

Whole Genome Shotgun Sequencing: Statistics

We used the programs ANGSD, PCAngsd [57], and ngsTools v3.0 (https://github.com/mfumagalli/ngsTools), ðaði v. 2.3.7 [58] and dadi-pipeline v.3.1.7 [59] to estimate population and species differentiation, admixture, and demographic histories of populations. With ANGSD, we estimated genotype likelihoods (GL) and the minor allele frequency (MAF) to calculate the site frequency spectrum (SFS), population differentiation; (weighted and unweighted F_{ST}), and population admixture. Lacking an ancestral sequence to polarize data, we calculated Tajima's D, Waterson's Θ, and Nucleotide diversity (π) from the SFS with a 'folded' spectrum in ANGSD. We estimated population admixture of individual genotypes with NGSadmix v.32 [60] with a MAF of 0.5 and sites where at least 5 individuals had SNP data. We also used ANGSD and ngsDist [61] to calculate genetic distances among individuals for a multiple dimensional scaling analysis (MDS). We used PCAngsd to estimate principal component analyses (PCA) with a covariance matrix estimated from genotype likelihoods in ANGSD. We also used ANGSD and realSFS from each population SFS estimates to create an input file for ðaði and then formatted the input file with the perl script: realsfs2dadi.pl (https://github.com/z0on/2bRAD_denovo /blob/master/realsfs2dadi.pl). We used the dadi-pipeline two-population diversification model set script to estimate demographic parameters where we compared *R. loihi* and *R. cambonae* from Eifuku seamount where the spectra were folded [59,62]. We had large amounts of missing data so we down-sampled our projections to reflect our sample sizes of each population. Analyses were run from 10-40 replicates for four rounds each and then the lowest AIC value was compared with the Summarize_outputs.py script from dadi-pipeline to find the best fitting model. We plotted results with the Make_plots.py script. Results were visualized in the tidyverse [63] with RStudio and R [64].

Results

Distribution, densities, and habitat preference

The two identified species of *Rimicaris* had different distributions. We found *Rimicaris loihi* at five sites on the IBM arc across 1900 km and on Loihi Volcano more than 6000 km to the east (Figure 1A); This species occurrence is yet unknown at seven other IBM vent sites. It was the only species of shrimp on Suiyo, Nikko, and Loihi volcanoes, although the sample size at Loihi was small (7 individuals). *Rimicaris loihi* was the dominant species on NW Rota where, of the 332 specimens were confirmed via

COI identification, only three were *R. cambonae* (0.9%). In contrast in 2023, *R. cambonae* was the dominant species at NW Eifuku and constituted 90.3% of shrimps collected whereas, in the small 2004 sample from the same site, only two of the 25 specimens collected were *R. cambonae*. Finally, 12 *R. cambonae* were identified from the West Mata vent field in the Southwest Pacific.

R. loihi appeared most abundant on sulphur crusts at all collection sites but were also observed on surfaces of andesitic lavas at NW Rota and among mussels at both NW Eifuku and Suiyo. At NW Eifuku in 2023, *R. cambonae* co-occurred with *R. loihi* within mussel beds and on bare rocks.

Repeated exploration of NW Rota vent field between 2004 and 2010 revealed that, as eruption intensity and venting varied over the years, so did the abundance of shrimps (Table 2, Figure S1). For 2004, we estimated average shrimp densities around vents to be 386 m^{-2} (n=6; SD=165); maximum adult densities exceeded 900 m^{-2} in 2009 when shrimps were most abundant (Figure 2B). In the rugged terrain, it was not possible to map the distribution of shrimp species, however we observed that along the known sites, the abundance of shrimps mirrored the venting extent, peaking in 2009 at 45%. The lowest abundances of shrimps were in 2006 during intense eruptions when shrimps were tightly clustered around a few vent openings, and in 2014, when volcanic activity had ceased and hydrothermal venting contracted to the central eruption pit (Table 2).

COI haplotype network and genetic diversity

Our ASAP analysis to assess putative species revealed two partitions that had equal support (lowest ASAP score of 2.50; Figure S2): individuals fell into either two or three genetic groups. Our morphological identifications agreed with these two groups corresponding to *Rimicaris loihi* and *R. cambonae*. A third *COI* lineage identified in the three partitions result was formed by the divergence of two *R. cambonae* haplotypes from NW Rota from the rest of the *R. cambonae* haplotypes. Distribution of pairwise differences was bimodal with two distinct Gaussian distributions and showed no overlap (Figure S2). F_{ST} values also supported genetic differentiation for two groups, and possibly a third, with high values between *R. cambonae* and *R. loihi* and, to a smaller extent, between *R. cambonae* from NW Eifuku and from NW Rota (Table S2). The partitions were evident despite relatively low genetic distance (D_a = 0.023 between *R. cambonae* and *R. loihi*; D_a = 0.009 between *R. cambonae* from NW Eifuku and NW Rota).

Species delineation was also clear based on the *COI* haplotype network; it comprised 158 haplotypes from 597 barcoded individuals, of which 134 were assigned to *R. loihi* and 24 to *R. cambonae* (Figure 1D). *Rimicaris loihi* and *R. cambonae* showed distinct genetic characteristics with a much lower haplotype diversity for *R. cambonae* compared to *R. loihi* (H_d = 0.499 ± 0.052 and H_d = 0.877 ± 0.011, respectively) and almost three-fold lower nucleotide diversity for *R. cambonae* (π =

0.00190 and π = 0.00437; Table S3). Similarly, the average number of nucleotide differences was lower for *R. cambonae* (k= 1.048) than for *R. loihi* (k= 2.208) although these patterns could simply be a result of different sample sizes. The West Mata population of *R. cambonae* had higher levels of haplotype diversity (H_d), nucleotide diversity (π), and average nucleotide differences (k) compared to northern *R. cambonae* populations (Table S3) also with two intermediate haplotypes, although closer to *R. cambonae* than *R. loihi* (Figure 1D). Haplotype variability among populations of *R. loihi* was notably higher than the sympatric *R. cambonae*, where Suiyo was the most diverse and Nikko was the least diverse (Table S3).

To explore further the possibility of temporal metapopulation dynamics of *R. loihi*, we estimated a separate haplotype network and summary statistics for NW Rota data only (Figure 2A). Yearly data were similar except for 2006 and 2014 when there was slightly lower nucleotide diversity (π), as well as lower average nucleotide differences (k); (Table S4).

Mitochondrial Genome clock calibration

We aligned 26 mitochondrial genomes from *R. cambonae* and *R. loihi* recovered from WGS data. Mitochondrial genomic alignments showed the same pattern of divergence between the two species as the *COI* fragment and differed by 1.5% for the GTR substitution model. The mitochondrial genomes of both species had the same gene composition and arrangement as all other published mitochondrial genomes of the Alvinocarididae (Figure S3). Molecular clock calibrations indicated or suggested that *R. cambonae* and *R. loihi* diverged relatively recently ~0.04 Mya (0.002–0.092 HPD). However, these estimates were noted because estimates for divergence of other genera such as *Rimicaris* and *Alvinocaris* were much more recent than published accounts for a fragment of *COI.*

Whole Genome Shotgun Sequencing, species delineation, and gene flow

Our WGS sequencing of *Rimicaris* individuals resulted in 109 million reads that included 20 *R. loihi* from NW Eifuku, Suiyo, and Nikko seamounts and eight *R. cambonae* from NW Eifuku (Figure 3A). We used genomic data to assess whether nuclear loci were concordant with mitochondrial data supported *R. loihi* and *R. cambonae* as distinct species, and to infer demographic histories of the two species. Population genomic results for PCA and MDS both revealed clusters for *R. loihi* and *R. cambonae* with two putative hybrids from NW Eifuku. One 'hybrid' individual had the *R. loihi* mitotype and the other had the *R. cambonae* mitotype (Figures 3 and S4). Summary statistics including Tajima's D, Watterson's θ, and nucleotide diversity were most similar for *R. loihi* from Nikko and Suiyo, but differed between the two species from NW Eifuku. We found all populations had positive values for Tajima's D. We also found that *R. loihi* from NW Eifuku had a very narrow range for Watterson's θ and nucleotide diversity compared to the other populations (Figure S5).

We estimated admixture plots from two to five populations that revealed sharp subdivision between the species, with admixed genotypes for the same two 'hybrid' individuals. Admixture plots for three or more populations showed that *R. loihi* from NW Eifuku appeared somewhat divergent (Figure S4). However, weighted and unweighted F_{ST} estimates showed that while species were differentiated, populations of each were not (Table S5).

We used ∂a∂i to estimate the demographic histories of each species and population, however, we observed a large amount of missing data and the results must be interpreted with caution. We used the AIC to determine and plot the four most probable models from analyses. Despite the distinct lineages, we found three of the four most probable models involved 'symmetric migration' and particularly when directional migration was tested, it also was relatively symmetric (Table S6, Figure S6). Demographic estimates from ∂a∂i analyses also revealed that each species had relatively similar effective population sizes for most models (Table S6, Figure S7).

Symbiont colonization, population sex ratio and reproductive traits

Scanning electron microscopy revealed distinct patterns of symbiont colonization for the "head" cavity between the two species (Figure S8). The anterior third of *R. cambonae* "head" cavities were densely colonized by filamentous bacteria with little colonization on the rest of the cavities whereas only a few sparse filamentous and rod-shaped bacteria occupied the entire "head" cavities of *R. loihi*.

The sex ratio of *R. loihi* from NW Rota was significantly female skewed for each year (χ^2 = 25.64 – 259.69, $p < 0.001$; Table 1) as previously observed in the Suiyo population [36]. A similar trend occurs for the sex ratio of *R. loihi* from NW Eifuku, but was not statistically supported (χ^2 = 1.33, $p > 0.05$) probably due to a low sample size (Table 1). Conversely, the sex ratio of *R. cambonae* from NW Eifuku did not significantly deviate from 1:1 (χ^2 = 1.09, $p > 0.05$; Table 1). Despite large sampling sizes, ovigerous females of *R. loihi* were nearly absent from populations collected at NW Rota in March (2004 and 2009) and April (2006 and 2010) with only two ovigerous females out of 408 females in these years (Figure 4A). Similarly, no ovigerous females were collected among the 72 females of *R. cambonae* from NW Eifuku in March 2023 (Figure 4A). Conversely, four ovigerous females out of 380 females were collected at NW Rota in December 2014 (Figure 4A) and larger numbers were observed peripheral to venting (but not sampled; Figure 4B). With 110 to 387 eggs per brood (11.4 to 44.4 eggs mm^{-1}), relative fecundities of *R. loihi* ovigerous females from NW Rota were in the range reported at Suiyo with 61 to 356 eggs per brood (10.2 to 49.2 eggs mm^{-1}), (Figure 4C) [36].

Discussion

How do *Rimicaris loihi* and *R. cambonae* maintain genetic segregation?

Nuclear genomic data confirmed previous results based on the mitochondrial *COI* fragment and

morphology that *Rimicaris loihi* and *Rimicaris cambonae* constitute two separate species [27] that recently diverged ~0.04 Mya (0.002–0.092 HPD). Yet, we found symmetric gene flow between the two species, as evidenced by the identification of two putative hybrids with maternal inheritance from both *R. loihi* and *R. cambonae* at NW Eifuku where they live in sympatry. Hydrothermal vents are favourable environments for hybrid zones between closely-related species, as observed for *Bathymodiolus* mussels on the East Pacific Rise and the Mid-Atlantic Ridge [20,65] and for *Lepetodrilus* limpets at the Woodlark Basin in the Southwest Pacific [66]. However, unlike these species that co-occur and hybridize at the edges of their respective geographical ranges, *R. loihi* and *R. cambonae* represent an unusual case of closely related sister-species that coexist near the centre of their geographic ranges with ongoing hybridization.

Theoretical modelling of the "tension zone" predicts that maintenance of species coexistence despite introgression often results from a balance between recurrent influx of individuals from pure parental populations from both species and selection against hybrids [67]. Under this scenario, Mariana Arc vent fields would represent a large hybrid zone stabilized by larval immigrants from pure parental populations of *R. loihi* located further north, dispersing from Suiyo and Nikko on the Izu-Bonin Arc. However, the presence of a similarly regular gene flow from a pure parental population of *R. cambonae* remains unclear. Our genetic analyses show that previous reports of *R. loihi* at West Mata volcano [44], are, in fact, referable to *R. cambonae*. Thus, this vent field on the Tonga Arc, 5600 km southeast of the nearest vent on the Mariana Arc, constitutes the only known potential locality for a pure parental population for *R. cambonae*. Within the West Pacific, few hydrothermal vent species distributions cross the equator [14] with notable exceptions such as *Alvinocaris dissimilis* shrimps or *Bathymodiolus septemdierum* mussels [33,68]. Larval dispersal among distinct volcanic arc systems or back-arc basins in the region is inferred to be infrequent and directional, following oceanic currents [14,69]. However, sustained dispersal from south to north hemisphere by larvae is inhibited by the Equatorial Counter-current and deemed highly improbable, even from the two closest locations [69]. Therefore, it is unlikely that significant gene flow exists between the two regions unless unknown sites exist to form a dispersal corridor.

Speciation between these two *Rimicaris* shrimps could be maintained by strong reproductive barriers. Differences in reproductive mode – egg laying versus brooding – likely strengthen strong reproductive isolation between the syntopic sister species of intertidal snails *Littorina saxatilis* and *L. arcana* [70]. In coastal isopods of the *Jaera* species complex, sexual isolation between co-occurring species is reinforced by preferential mate choice despite some level of hybridization [11] while receiving no influx from pure parental populations [71]. As previously reported for Suiyo [36], the samples of *R. loihi* from the Mariana Arc were largely biased toward females whereas the proportion

of males and females was roughly equal for *R. cambonae*. Variation in adult sex ratios can strongly influence breeding systems and mate choice in many animal groups [72,73]. The discrepancy between the sex ratios of *R. loihi* and *R. cambonae* may reflect different mate choice systems that limit heterospecific mating pairs. Partially-desynchronized reproductive periods between *R. loihi* and *R. cambonae* could also act as an additional reproductive barrier, although evidence is limited. As with most carideans [74], alvinocaridid shrimps are gonochoric species that mate and brood their eggs under their abdomen until larval release [35,36]. However, a wide variety of reproductive cycles occur in this family, including aperiodic or periodic brooding patterns, with or without synchronicity to seasonal variations [34–36]. Taken together, current data on the brooding cycle of *R. loihi* do not seem to indicate any particular periodicity, with large numbers of ovigerous individuals in both August and December, despite a notable and repeated absence in March/April. However, there may be local variation between vent fields from different latitudes, for example, a brooding period in summer at Suiyo and in winter at NW Rota. Additional sampling at different periods is required to determine whether or not reproductive patterns of *R. cambonae* differ from its sister species.

Which speciation mode between *R. loihi* and *R. cambonae*?

Our observations over 10 years showed significant changes in the abundance of shrimps that reflected eruptive events and fluctuation of venting activities at NW Rota vent field. There were concomitant variations in genetic diversity that increased and contracted on a short timescale. This geological instability of some hydrothermal vent fields on the IBM volcanic arc [18,38] also characterizes vent fields on Loihi and West Mata [44,45]. The absence of both *R. loihi* and *R. cambonae* at several IBM vent sites [75] and on other volcanic arcs of the SW Pacific [14,75] suggests that these two species have specific habitat requirements and/or settlement cues related to volcanic emissions or sulphur substratum. The recent split of the two species (0.002–0.092 Mya) does not correspond to major tectonic events in the Northwest Pacific: current Mariana Arc volcanism initiated 3 – 4 Mya while volcanic activity on the Izu-Bonin Arc dates from ~25 Mya [76]. Similarly, along-arc splitting and rifting events in the Southwest Pacific, such as the opening of the Lau basin (~5 Mya) [77,78], all significantly pre-date our mean divergence estimate between the two shrimp species (~0.04 Mya). This estimate based on mitochondrial genomes, that were less divergent than *COI,* was very recent compared to previous phylogenies [46,79]. However, these previous phylogenies calibrated the origin of Alvinocarididae using the Jurassic fossil *Harthofia* which has since been removed from Alvinocarididae as 'family uncertain'; currently no fossils are available in this family for calibration [80]. Our method also has a caveat that we apply the estimated rate for *COI* to the whole mitogenome, but despite this the separation between *R. cambonae* and *R. loihi* should not be older than a few million years. A rare circumstance of long-distance dispersal between the two distant arcs could have established an

outpost population that gradually diverged from the ancestral genetic pool, driven by local fluctuations of venting activities affecting shrimp population sizes within each region as observed at NW Rota. Therefore, the current sympatric distribution of both *R. loihi* and *R. cambonae* in the Mariana Arc would result from a secondary contact by another rare larval dispersal event from south to north. This scenario of speciation by allopatry postulates that genetic exchanges via larval dispersal between the two regions was disrupted for a sufficiently long period to generate an isolation phase. However, our demographic analyses (supplementary material, figure S6-7) suggest that our data best fitted a speciation model with symmetric migration, even if caution should be exercised due to the amount of missing data.

A speciation mechanism between *R. loihi* and *R. cambonae* with migration would imply a larval supply maintained between regions throughout their separation. High genetic connectivity over long distances has been observed in many alvinocaridid shrimps [30–33], for example, *R. exoculata* lacks genetic structure over 7100 km, likely supported by Mid-Atlantic Ridge ocean circulation promoting connectivity [28]. Morphological features of hatched larvae from several alvinocaridid species including *R. loihi* supports a long pelagic larval duration (PLD) [36,81]. We can therefore assume that *R. loihi* and *R. cambonae*, like their common ancestor, have a high dispersal potential. That potential is demonstrated by the presence of a distal outpost of *R. loihi* on Loihi Volcano near Hawai'i, likely advected from the IBM Arc by the dominant eastward current of the North Pacific Drift. However, prevailing ocean currents and a 5600 km cross-equator distance remain a major barrier to connectivity for frequent larval exchange in the western Pacific. Conversely, a similar pattern of speciation with a maintained gene flow would also appear if divergence between species had arisen without geographic isolation in sympatry.

Although debated, sympatric speciation in some species, including cichlid fishes and mice [3–5], is supported by theory suggesting that speciation in sympatry is favoured by disruptive selection for distinct ecological niches, diets and selection on mating related traits [6]. Hydrothermal vents generate steep environmental gradients occupied by different species [16,17] that could support species divergence. However, the presence of *R. loihi* and *R. cambonae* in the same habitats (mussel beds and sulphur-coated rocks) suggests divergence in discreet ecological niches is unlikely. Thus, in addition to assortative mating (see discussion above), disruptive selection between *R. loihi* and *R. cambonae* could have been driven by trophic niche differentiation, as observed in many co-occurring alvinocaridids [33,47]. Microscopic observations of their "head" cavity showed different degrees of colonization by filamentous ectosymbionts that could provide an additional feeding source of a mixed diet for *R. cambonae* while *R. loihi* would rely on bacterial mat grazing (Figure S8; Methou et al. in prep). Acquisition of a larger pool of filamentous ectosymbionts for nutrition could have driven trophic

niche differentiation between vent shrimps and ultimately, participate in an ecologically driven speciation process. Further studies using larger genomic dataset across the entire range of the two species as well as investigations on their trophic and reproductive ecology will be required to clarify mechanisms leading to their speciation.

Conclusion

Rimicaris loihi and *R. cambonae* are two distinct, recently diverged species living in sympatry throughout a part of their geographic range. Analysing genetic data from shrimps collected from a single vent over a decade revealed high flexibility in genetic diversity alongside local eruption events, suggesting that population dynamics of these shrimps are impacted by their unstable habitats. The detection of two hybrid individuals via WGS points to the lack of complete separation of the two species. We suggest that the difference in sex ratios between the two species may reflect differences in mate choice as a reproductive barrier, among other possibilities. Although our demographic analyses with the data available remain inconclusive as to how speciation occurred among these two species, the geographic overlap and recent divergence of this species pair makes them an interesting case study for a future exploration into speciation mechanisms that may provide insights to how species arise in insular habitats overall.

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Data Availability.

All COI barcode sequences have been deposited in GenBank under accession numbers DQ328819 – DQ328837 and PQ643496 – PQ643653 (see Table S7 for details on haplotype numbers and localities with associated individual ID). Raw sequences of the WGS sequencing dataset are available in the NCBI SRA repository with the BioProject ID PRJNA1197504 (Accession numbers: SAMN45807939 – SAMN45807966). Details information on sampling localities, sampling date, sex, life stages and sizes of individuals analysed for population sex ratio and reproduction are compiled in a table sheet named “Population_dataset” in supplementary material.

Figure Captions

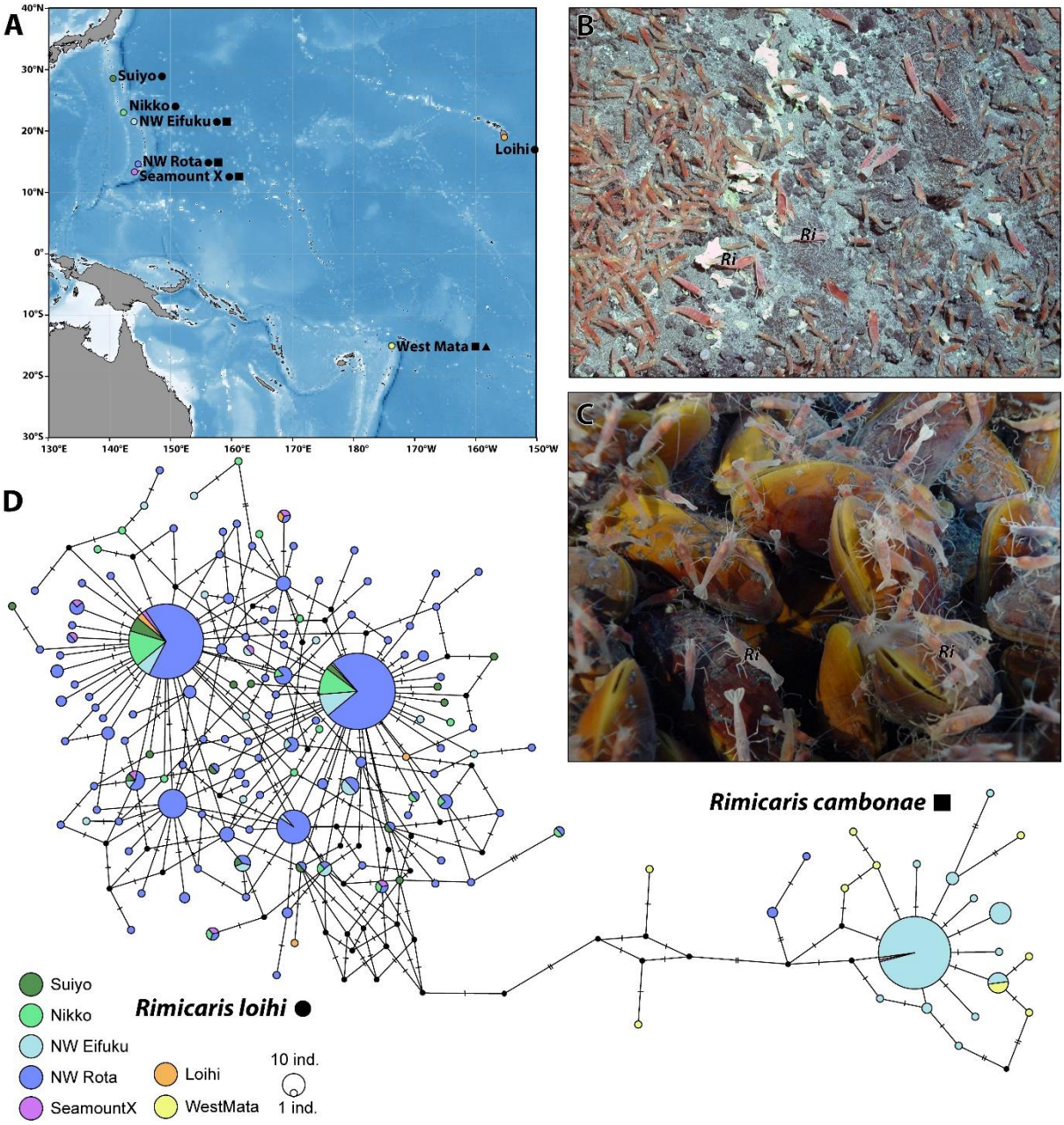

Figure 1. A. Geographic distribution of *Rimicaris cambonae* (square) and *Rimicaris loihi* (circle). Colours indicates sites and shape indicates species. **B.** and **C.** Typical habitats of *Rimicaris spp.* (*Ri*) on (**B**) sulfur crusts (NW Rota, 2009) and (**C**) mussel beds (NW Eifuku, March 2004). **D.** *COI* haplotype network (529 bp) of *Rimicaris* species from Loihi, West Mata and the Mariana Arc vent fields (n= 597) detailed per sampling site. Two clusters emerge with 24 haplotypes affiliated with *R. cambonae* and 134 haplotypes affiliated with *R. loihi*.

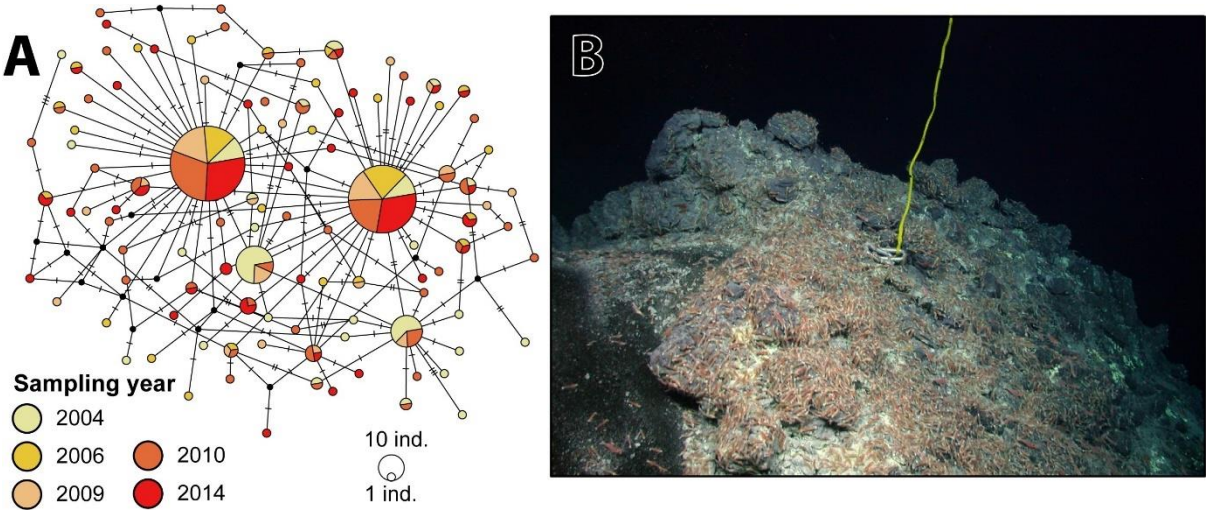

Figure 2. Temporal metapopulation dynamics of *Rimicaris loihi* at NW Rota between 2004 and 2014. **A.** *COI* haplotype network of *Rimicaris loihi* from NW Rota (n= 332) detailed per sampling year. **B.** Abundant shrimps near Marker 109 in April 2009.

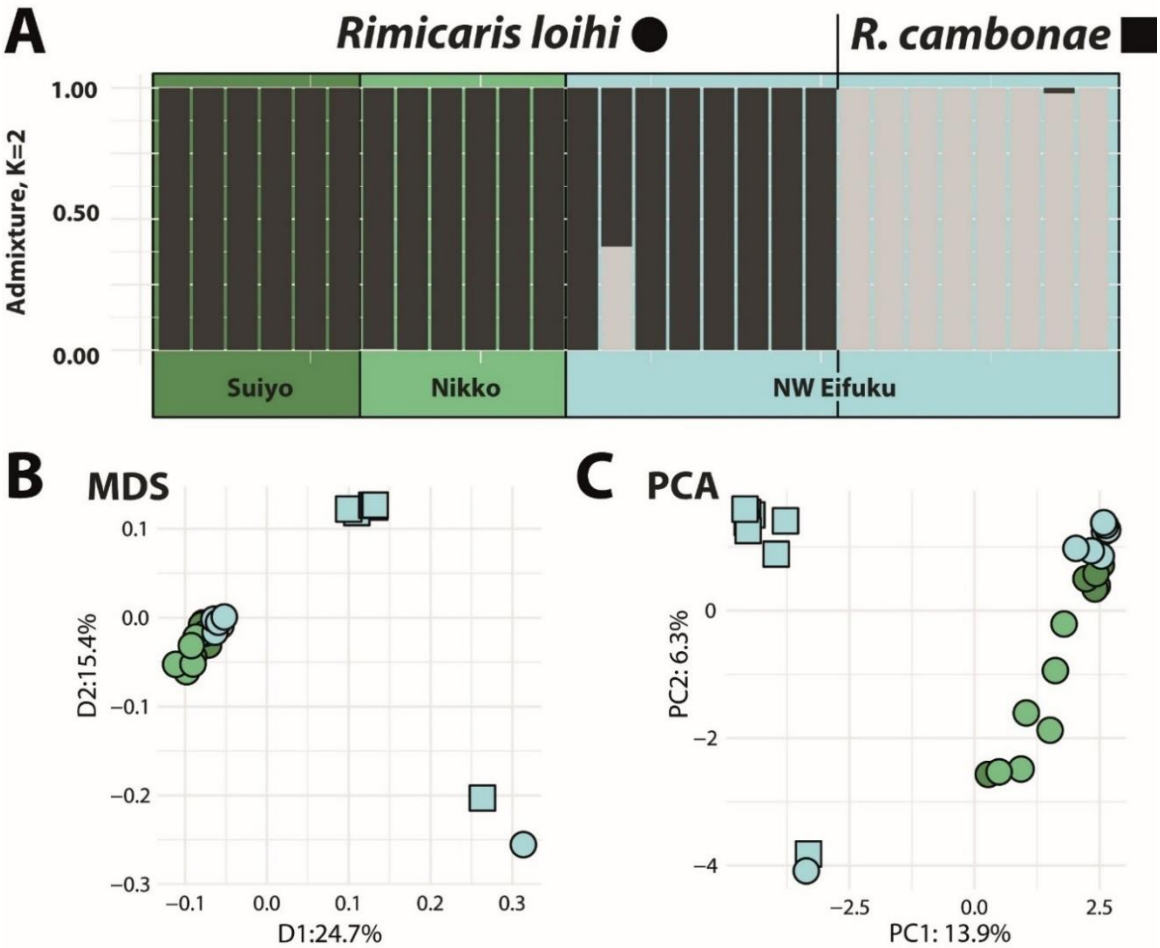

Figure 3. A. Admixture plot for two populations (K=2) including 28 individuals for 4028 loci; circles are *R. loihi* and squares are *R. cambonae,* **B.** plot of first two dimensions for multiple dimensional scaling (MDS) estimates from 4599 loci based on genetic distances, and **C.** plot of first two axes of principal component analyses (PCA) for 4178 loci. Two putative hybrids fall separately in latter two plots.

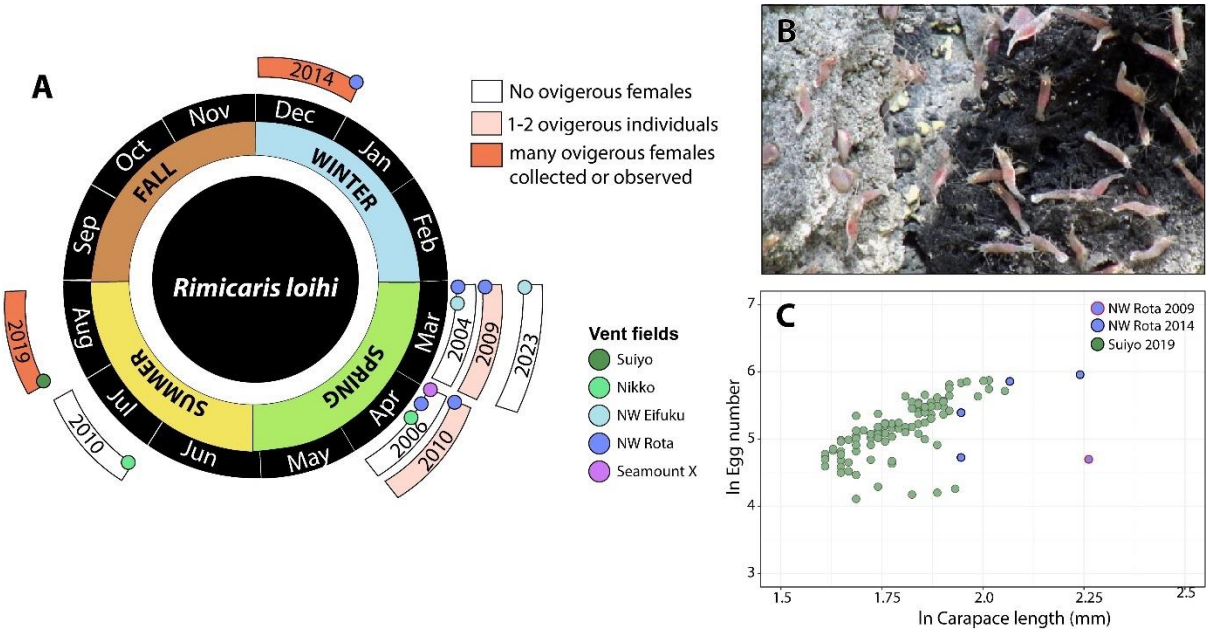

Figure 4. Reproductive characteristics of *Rimicaris loihi*. **A.** Diagrams summarizing the occurrence of *R. loihi* ovigerous females over sampling realized between 2004 and 2019. **B.** Numerous ovigerous females (reddish colour below the abdomen) observed, but not collected, at NW Rota in December 2014. **C.** Relative fecundity of *R. loihi* displayed by $\log_e$-transformed number of eggs with $\log_e$-transformed carapace length (CL). Data from Suiyo are from Methou et al. (2023). Data from NW Rota are from this study.

Vent field	Year	Species	M	F	M:F	χ^2	*p*
NW Rota	2009	*Rimicaris loihi*	119	211	0.56	25.65	***
NW Rota	2010	*Rimicaris loihi*	395	797	0.49	135.57	***
NW Rota	2014	*Rimicaris loihi*	47	380	0.12	259.69	***
Suiyo[(1)]	2019	*Rimicaris loihi*	145	235	0.62	22.94	***
NW Eifuku	2023	*Rimicaris loihi*	4	8	0.5	1.33	NS
NW Eifuku	2023	*Rimicaris cambonae*	60	72	0.83	1.09	NS

Table 1. Population characteristics with sex ratio of *Rimicaris cambonae* and *Rimicaris loihi* from NW Rota, Suiyo and NW Eifuku. [(1)] Data from Suiyo are from Methou et al. (2023). M: males, F: females; Significance levels: NS: > 0.05, *: < 0.05, **: < 0.01, ***: < 0.00.

Year	Volcanic state	Hydrothermal State	Shrimp population
2004	Volcanic vent in a deep pit with surging sulphurous plume and ejecta; mass wasting on volcano flank.[1]	13 discrete low temperature vents and seeping around rim of volcanic pit.	Extensive: dense at vents and scattered across summit; 37%
2006	Explosive eruptions from pit observed after sidewall collapse; ejecta volume to 100 m^3/hr.[2]	Discrete sites fewer; seeping through sediments near pit; eastern scarp more focussed.	Restricted: scattered near pit; concentrated at three sites; 12%.
2009	Up to 47 m of tephra accumulated since 2004[3]; ejecta evident.	Many discrete venting sites <50°C broadly distributed across summit; venting through sediments.	Very extensive: dense at vents and scattered across summit; 45%
2010	Recent eruption reshaped the summit[3]; now several volcanic orifices	Loss of several vents in landslide; exposed walls covered with sulphur;	Very extensive: dense at vents; scattered on summit and on new exposed walls; 40%.
2014	No eruptive activity[4]; likely quiescence >1 year.	No or negligible activity at all former sites; only notable temperature anomaly around pit.	Restricted mostly to one site; other locations limited or abandoned; 6%.

Table 2. History of observed volcanism and venting on NW Rota. The relative extent of the shrimp populations is assessed by estimating the % of ROV tracks in which shrimp were greater than 20 individuals/m^2. [1]Embley et al. 2006; [2]Chadwick et al. 2008; [3]Chadwick et al. 2012; [4]Schnur et al. 2014.

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