Reproductive biology of the original hydrothermal hairy snail, Alviniconcha hessleri, from the Mariana back-arc

Nova Hanson
nbhanson@gmail.com
Memorial University of Newfoundland https://orcid.org/0000-0001-9948-3425
Amanda Bates
University of Victoria
Suzanne Dufour
Memorial University of Newfoundland

Research Article

Keywords: Alviniconcha, gastropod, reproductive biology, population structure, gametogenesis, hydrothermal vent

Posted Date: September 27th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4883307/v1

Version of Record: A version of this preprint was published at Marine Biology on March 11th, 2025. See the published version at https://doi.org/10.1007/s00227-025-04619-2.

Abstract

Many life history traits of hydrothermal vent fauna are undescribed, yet this knowledge is crucial for the accurate assessment of the vulnerability of vent endemic species, which are endangered by anthropogenic impacts in the deep-sea. Here we advance the knowledge of life history traits in Hessler's hairy snail, *Alviniconcha hessleri*, a dominant gastropod harbouring chemosynthetic endosymbionts endemic to hydrothermal vents along the Mariana back-arc. Snails were collected from sites at four hydrothermal vent fields to quantify reproductive periodicity using histology, larval developmental mode using oocyte and protoconch-I size, and differences in oocyte size across diverse venting environments. Results indicate that *A. hessleri* shares some of the same traits as other vent gastropods: continuous oogenesis and consistent provisioning to oocyte size, regardless of local vent fluid chemistry. A maximum oocyte size of 93 μm indicates planktotrophy for this species but could not be further supported using protoconch size due to excessive shell degradation. Our results suggest that this species maximizes reproductive output and oocyte quality, expected as a response to unpredictable environmental conditions to ensure the continued maintenance of existing populations and the establishment of new populations. Although the life history traits of *A. hessleri* may convey high reproductive and dispersal potential, the realized range of this species is limited to seven vent fields. Thus, this species may be vulnerable to habitat disruption due to its reliance on a restricted habitat, even though it has life history traits adapted to high environmental variability.

INTRODUCTION

Hydrothermal vent systems provide an opportunity to understand the population dynamics and colonization potential of uniquely adapted invertebrate species among fragmented ecosystems that vary in energy availability spatially and temporally. While vents can be relatively stable, continuous sources of energy, they are fundamentally unreliable due to the dynamic geothermal processes regulating venting activity. Vent fluid chemistry can vary dramatically on macro- and micro-spatial scales (between geologic settings like spreading centers or back-arcs, vent fields, and individual vents) in terms of pH, dissolved gases, and metal concentrations, resulting in diverse environments for microbial primary production (Van Dover 2000; Trembath-Reichert et al. 2019). These ecosystems support dense invertebrate communities that may nevertheless thrive in the harsh and variable environment that vent fluids create, including outflow that is high in temperature, low in pH and high in heavy metal concentrations.

Hydrothermal vent invertebrates tend to share reproductive and life history traits that may be adaptive for the unique vent environment. Living in an unreliable, high-energy environment has likely selected for traits that facilitate exploitation of chemosynthetic resources paired with the ability to reach and settle at new vent outflows, possibly hundreds of kilometers away. Most vent invertebrate species appear to be dioecious (Tyler and Young 1999; Pradillon and Gaill 2007; Tyler et al. 2008; Castel et al. 2022), suggesting that reproductive investment to one gamete type may maximize reproductive output given the probable scenario of low larval survival and recruitment success. Species with "fast" (r-type) life history strategies (versus "slow", k-type) are distinguished by early maturity, high reproductive output and widespread larval dispersal. Indeed, most vent invertebrates fall on the fast side of the fast-slow continuum including gastropods (Tyler et al. 2008; Matabos and Thiebaut 2010; Marticorena et al. 2020; Poitrimol et al. 2024), bivalves(Berg 1985) and annelids (McHugh 1989; Faure et al. 2007).

Of the available literature on vent-endemic gastropods, most exhibit continuous or semi-continuous reproduction and lecithotrophic larval development (Kelly and Metaxas 2007; Tyler et al. 2008; Marticorena et al. 2020; Poitrimol et al. 2024). Continuous reproduction may be associated with relatively constant vent outflow, as the dominant factor controlling energy availability at most vents is likely venting activity rather than surface seasonal productivity (Scheltema 1994). Dispersal is believed to occur during the larval stage (Adams et al. 2012), and due to the fragmented nature of hydrothermal vents, larvae must be able to disperse to vent sites tens to hundreds of kilometers away, necessitating a long larval stage. Larvae of vent species have been suggested to remain at depth during development, utilizing slow bottom currents for dispersal (Gustafson and Lutz 1994a). Dispersal at depth may be especially important for lecithotrophic species with a shorter planktonic period, as cool, deep ocean water may slow metabolism and thus larval development, allowing dispersal over great distances (Tyler et al. 2008). However, planktotrophy is considered the ancestral condition for deep-sea gastropod species(Bouchet and Warén 1994) and could enable greater dispersal distances over a longer planktonic period, especially if those larvae utilize faster mid-ocean or surface currents. It is not known whether the traits of vent gastropods studied to date (largely limpet taxa) are shared by other less-studied gastropod families at vents, and if different venting environments interact with traits such as reproductive provisioning.

Variability in invertebrate reproductive strategies may undergo selection at the population (vent site) level as hydrothermal fluid chemistry between vents is highly variable and influences food availability. Fluid chemistry within and between vent fields can vary markedly due to differences in geothermal activity, affecting bacterial diversity and microbial chemosynthetic production. This dynamic may be of particular importance in species hosting nutritional bacterial symbionts that depend upon the reduced chemicals in vent fluids. Symbioses where bacteria are hosted in specialized tissues and are either digested directly or benefit the host by provisioning fixed carbon compounds are present in many vent gastropods and support diverse pathways for nutrient acquisition (Stein et al. 1988; Suzuki et al. 2005). Research on chemosymbiotic gastropods has largely focused on identifying the symbionts and their metabolic pathways, with little attention placed on the ecological relationship between symbionts and host. Because energy allocation to gamete production is related to nutritional state, examining reproductive traits in species with symbiotic bacteria may reveal differences in energy dynamics across variable environments.

One way to estimate symbiotic productivity is by examining energy allocation to reproduction in populations from different vents, as a positive correlation between maternal resources and offspring size is generally observed in marine invertebrates (Marshall and Keough 2007). Greater allocation of yolk reserves to oocytes presumably increases overall oocyte size, where a more successful holobiont has greater energy reserves for host reproduction. While some studies on symbiotic *Lepetodrilus spp*. have indicated that vent site does relate to oocyte size (Kelly and Metaxas 2007; Matabos and Thiebaut 2010), the link between fluid chemistry and reproductive quality (besides venting intensity) remains unknown for most vent species. Moreover, very few studies have measured fluid chemistry in detail (Poitrimol et al. 2024) to explore relationships between individual fluid components (e.g. reduced hydrogen sulfide or methane concentrations) and reproductive investment. Thus, it remains to be explored whether symbiotic gastropods occurring across vents that vary markedly in fluid chemistry exhibit differences in reproductive provisioning.

Most studies of vent gastropods focus on limpet lineages and are localized to vents from mid-ocean spreading ridges, such as the Juan de Fuca Ridge, East Pacific Rise and Mid-Atlantic Ridge (Kelly and Metaxas 2007; Tyler et al. 2008; Matabos and Thiebaut 2010; Marticorena et al. 2020). Here, we focus on the caenogastropod *Alviniconcha hessleri*, a dominant invertebrate from the relatively understudied Mariana back-arc. This back-arc system is particularly interesting, as it experiences differences in spreading rate from north to south (Baker et al. 2017), and features complex magmatic interactions due to the subducting oceanic plate at ocean depths of 2800–5000 m. Hydrothermal vents along the back-arc have diverse effluents that range widely in temperature, pH and metal concentrations, even at vents within short distances of each other, allowing simultaneous advances in characterizing the basic biology of *A. hessleri*, which is largely unknown, and responses across different vents with unique fluid chemistries.

Alviniconcha hessleri and its congeners have been of particular interest due to the chemosynthetic symbiotic bacteria in their gills. However, the host itself is largely understudied, with life history information for this species almost entirely lacking. Literature on *A. hessleri* prior to 2015 is somewhat misleading, as all collections of the moderately cryptic “hairy snails” from the Indo-Pacific Ocean were thought to represent a single species. However, genetic work revealed that the genus includes six species, and that *A. hessleri* is only found along the Mariana back-arc (Johnson et al. 2015). Consequently, most of the research effort on hairy snails was revealed to be on species other than *A. hessleri sensu stricto*. Further complications to the study of reproductive and life history characters in vent gastropods like *A. hessleri* include difficulty in locating egg masses and larvae, and an inability to maintain adults or culture larvae away from the natural environment. Thus, what remains feasible is a study of adult biological traits, which can nevertheless lead to a better understanding of the distribution and colonization potential of species such as *A. hessleri.*

The present study aims at new insights into the reproductive biology and recruitment patterns of a dominant vent gastropod. We investigate several adult traits in *A. hessleri* to estimate reproductive quality, larval developmental pattern, and allocation to reproduction. Reproductive periodicity is investigated using oocyte-size and population-size frequencies. Larval developmental mode is inferred using oocyte size and an estimate of protoconch-I size. Lastly, we explore whether vent fluid chemistry strongly relates to oocyte size. Similarly to other vent gastropods, we expect *A. hessleri* to exhibit continuous reproduction and lecithotrophic larval development. Further, we expect oocyte size to be greater at vents where temperatures and reduced chemical concentrations are higher, resulting in a more productive symbiosis and a higher energy budget for the host.

MATERIALS AND METHODS

Sample collection and preparation

Diffuse hydrothermal vent fluids and specimens of *Alviniconcha hessleri* were collected from four vent fields from the northern and central regions of the Mariana back-arc (~ 18.2 to 15.4° N) in the western Pacific Ocean during the December 2016 FK161129 Schmidt Ocean Institute cruise onboard the R/V Falkor using the remotely operated vehicle (ROV) SuBastian (Table 1, Fig. 1A). All vents are referred to by vent field, except in cases where multiple vents were sampled within a vent field. Fluid and faunal sampling was accomplished by hydrothermal fluid samplers, and scoop or suction, respectively. Fluid samples were taken directly from areas where clumps of snails were aggregated. Hydrothermal fluid was sampled as described by Trembath-Reichert et al. (2019) and chemically analyzed for hydrogen sulfide and dissolved methane gas concentrations, as described in Butterfield et al. (2004). Once on deck, specimen shell height (as an indicator of size) was measured using calipers to the nearest 0.1 mm before removing specimens from shells. Gonads were dissected and fixed in 10% buffered formalin.

Table 1

Sampling locations of *Alviniconcha hessleri* from the northern and central regions of the Mariana back-arc. Environmental samples were collected from within clusters of snails to capture a realistic environment. Environmental data was not available for Sequoia.

Vent Field	**Vent**	**Depth (m)**	**Latitude**	**Longitude**	**pH avg**	**T avg (°C)**	**H_2S avg (µM)**	**CH_4 avg (nM)**
Ilium	Snail Pile (Mkr 138)	3582	18.2136	144.7075	6.00	29.3	13.0	3.5
Burke	Snail Pit (Mkr 234)	3631	18.1826	144.7200	5.77	42.8	261.7	5.9
Hafa Adai	Sequoia	3274	16.9612	144.8670	-	-	-	-
Hafa Adai	Voodoo Crater (Mkr 171)	3278	16.9617	144.8692	6.10	22.9	160.3	1,580.9
Perseverance	Stump of Mystery	3909	15.4802	144.5077	6.08	15.3	159	10,295.0

CH_4 avg, average methane; H_2S avg, average hydrogen sulfide; Mkr, marker; pH avg, average pH; T avg, average temperature

In the laboratory, portions of gonads were serially dehydrated with a graded series of ethanol, ending with 100% ethanol for at least 6 hours, cleared in xylene substitute for 6 hours and embedded in paraffin. Histological sections were cut at a thickness of 7 µm with a microtome and stained with hematoxylin and eosin, which stains cell nuclei purple and the cytoplasm pink (Fischer et al. 2008). Sections were imaged with an AxioScan.Z1 digital slide scanner and only oocytes in which the boundaries of the oocyte were clear and the nucleus was visible were measured. The area of a minimum of 100 oocytes from each individual was measured from 1–2 sections separated by at least 50 µm using ImageJ and the feret diameter was calculated to avoid skewed measurements due to commonly seen distortions in oocyte shape due to tight packing of oocytes in the ovary, as is the standard among similar studies (Tyler et al. 2008; Matabos and Thiebaut 2010; Bayer et al. 2011; Marticorena et al. 2020).

For protoconch examination, a shell from the most intact juvenile of *A. hessleri* was dried in a 70°C oven for a minimum of 24 hours, sputter-coated with gold and visualized using a FEI MLA 650FEG SEM in apical and axial view. Due to high environmental degradation at the apex of the shell, only a hypothetical diameter of protoconch-I (the former embryonic shell) could be measured using ImageJ (Abramoff et al. 2012).

Statistical Analyses

Population size-frequency and oocyte size-frequency distributions were quantified from individual measurements at each vent site. Size-frequency distributions were assessed for normality using a Lilliefors test (Kolmogorov-Smirnov one-sample test) (Lilliefors 1967). Differences in these frequency distributions between specimens within and between vent sites were tested using nonparametric Kruskal-Wallis rank sum tests. Pairwise comparisons using the Tukey-Kramer-Nemenyi all-pairs test identified individuals contributing to observed significant differences. Gametogenic maturity was expressed as the percentage of vitellogenic oocytes out of the total number of oocytes measured within an individual. Differences in gametogenic maturity between vents were also investigated using a Kruskal-Wallis multisample test. We further used a linear mixed effects model to determine if shell height was a significant predictor of mean oocyte diameter and proportion of vitellogenic oocytes for each individual.

RESULTS

Diffuse fluid chemistry

Chemistry parameters of fluid taken from within snail aggregations are presented in Table 1. Among the vents, pH ranged from 5.77 to 6.10. The average temperature of diffuse flow was highest at the northernmost vents (29–42°C) and became progressively cooler moving southward (15.3°C at Perseverance). Reduced chemical concentrations deviated from expected trends: warmer vents did not consistently have a higher concentration of hydrogen sulfide. Diffuse fluid at Illium had the second highest temperature but the lowest hydrogen sulfide (13 µM compared to 159 µM at the coolest vent, Perseverance). Methane concentration increased dramatically from north to south: Illium and Burke had concentrations of 3–6 nM, whereas Perseverance had a concentration of 10,295 nM.

Population size-frequency distributions

The size (shell height) distributions of *Alviniconcha hessleri* varied across the five vent sites. Size-frequency distributions at all sites except Voodoo Crater differed significantly from a normal distribution (Lilliefors test, p values < 0.05, Table 2). Snails collected from Illium, Burke and Perseverance showed unimodal, left-skewed size distributions, whereas a strongly bimodal size distribution was observed in Sequoia (Fig. 2). Shell height was significantly different between sites (Kruskal-Wallis test, $H = 110.68$, df = 4, $p < 0.001$), due to individuals from both Illium and Voodoo Crater being significantly larger than those at all other sites (Fig. 2, Table 3), except each other ($p = 0.067$). Individuals collected from Sequoia were significantly smaller than snails from all other sites, except Perseverance (Table 3), and snails collected from Burke and Perseverance showed no significant difference in shell height ($p = 0.995$, Table 3).

Table 2

Number, shell height range, mean and median (mm) of individuals of *Alviniconcha hessleri* collected from the Mariana back-arc.

Vent	***N***	**Shell height min-max**	**Mean (± SD)**	**Median**	**Normality test *p* value**
Illium	36	7.5–50.7	39.4 ± 10.2	41.6	< 0.001
Burke	53	6.5–31.5	20.4 ± 6.1	21.7	0.017
Sequoia	85	1.3–28.3	14.3 ± 8.9	17.2	< 0.001
Voodoo Crater	40	12–40.6	26.6 ± 5.6	27.2	0.651
Perseverance	38	4.5–29.3	19.8 ± 5.8	21.9	< 0.001

N, number of individuals. Normality test: Lilliefors (Kolmogorov-Smirnov) test.

Table 3

Results of Tukey-Kramer-Nemenyi all-pairs test comparing population size distributions of *Alviniconcha hessleri* among vents from the Mariana back-arc.

Vent	**Illium**	**Burke**	**Sequoia**	**Voodoo Crater**
Burke	< 0.001	-	-	-
Sequoia	< 0.001	0.018	-	-
Voodoo Crater	0.067	0.003	< 0.001	-
Perseverance	< 0.001	0.995	0.134	0.002

Gametogenesis patterns

Histological sections identify most individuals as dioecious, with a small number of morphological hermaphrodites containing both male and female tissues at Illium (*n* = 2), Burke (*n* = 3) and Perseverance (*n* = 2). Female gametes exhibited various stages of oogenesis, with particularly obvious vitellogenic oocytes with ample lipid (yolk) droplets staining pink and nuclei staining dark purple (Fig. 3A). Male gametes predominantly stained indigo, with all stages of spermatogenesis evident (Fig. 3B). Hermaphroditic tissue appeared generally disorganized with some individuals showing a small degree of segregation of female and male gametes (Fig. 3C), while others showed no organization of tissue, including vitellogenic oocytes appearing sporadically throughout areas of male tissue (Fig. 3D). All stages of oogenesis and spermatogenesis were evident in hermaphrodites.

All three stages of oocyte development were evident in each female or hermaphroditic individual, resulting in a large range of oocyte sizes. Developing oogonia appeared throughout the ovary, arising from epithelial tissue (Fig. 3A). Oogonia differentiated into previtellogenic oocytes at ~ 9–14 µm and had a basophilic cytoplasm and a low nucleus-cytoplasm ratio. The onset of vitellogenesis ranged substantially, starting in oocytes as small as 17 µm and as large as 42 µm, and could be identified by the presence of lipid droplets in the cytoplasm and a large nucleus with a darkly stained nucleolus (Fig. 3). Early and mature vitellogenic oocytes showed the largest size range of 17–93 µm.

Oocyte size-frequency distributions

Individuals from all sites displayed a similar unimodal distribution pattern with a large left peak due a higher proportion of previtellogenic and small vitellogenic oocytes, and a right skew representing relatively larger vitellogenic oocytes (Fig. 4). Comparison of oocyte size-frequency distributions indicated a significant difference in oocyte size between sites (Kruskal-Wallis test, $H = 103.08$, df = 4, $p < 0.001$). Oocytes in individuals collected from Illium were significantly smaller than those from all other sites and there were no significant differences among the remaining sites (Table 4).

Table 4

Results of Tukey-Kramer-Nemenyi all-pairs test comparing oocyte size distributions of *Alviniconcha hessleri* among vents from the Mariana back-arc.

Vent	Illium	Burke	Sequoia	Voodoo Crater
Burke	< 0.001	-	-	-
Sequoia	< 0.001	0.510	-	-
Voodoo Crater	< 0.001	0.990	0.850	-
Perseverance	< 0.001	0.970	0.16	0.800

Oocyte sizes differed significantly across individuals within populations at Illium (Kruskal-Wallis test, $H = 70.09$, df = 4, $p < 0.001$), Sequoia ($H = 19.51$, df = 4, $p < 0.001$*)* and Voodoo Crater ($H = 22.10$, df = 2, $p < 0.001$) (Table 5). However, only one individual at Illium, two at Sequoia, and one at Voodoo Crater, contributed to the differences seen (Fig. 5, Table 5).

Table 5
Number of individuals examined and oocyte size-distribution comparisons for *Alviniconcha hessleri* from the Mariana back-arc.

Vent	**N**	**Oocyte diameter (μm)**	**KW**		**TKN**	**Prop Vo (%)**
			H	*p*		
Illium	5	10.6–85.7	70.09	< 0.001	1/5 different	29–70
Burke	3	9.2–91.9	3.43	0.180	-	47–61
Sequoia	5	11.6–93.2	19.51	< 0.001	2/5 different	54–81
Voodoo Crater	3	13.7–92.8	22.10	< 0.001	1/3 different	40–70
Perseverance	3	10.3–82.7	3.80	0.151	-	46–63

KW, Kruskal-Wallis rank sum test; N, number of individuals; Prop Vo, proportion of vitellogenic oocytes; TKN, Tukey-Kramer-Nemenyi all-pairs test.

Shell height and oocyte diameter were significantly larger at Illium, but this difference was not explained by fluid chemistry parameters and revealed no obvious relationship (Fig. 6). Even so, Illium differed the most in hydrogen sulfide (13 μM compared to > 150 μM for all other sites) (Table 1).

The percentage of vitellogenic oocytes (gametogenic maturity) in reproductively mature individuals ranged between 29–70% at Illium, 47–61% at Burke, 54–81% at Sequoia, 40–70% at Voodoo Crater and 46–63% at Perseverance (Table 5). However, there were no significant differences in gametogenic maturity across sites (Kruskal-Wallis test, $H = 2.922$, df = 4, $p = 0.571$). Shell height was a significant predictor of mean oocyte diameter (Linear mixed effects model, $p = 0.002$), but an insignificant predictor for the proportion of vitellogenic oocytes ($p = 0.053$) (Fig. 7).

Protoconch size estimates

The estimated diameter of protoconch-I was ~ 675 μm (dashed dimensions in Fig. 8C), whereas examination of the axial view showed ~ 1.5 intact larval whorls (Fig. 8D).

DISCUSSION

Alviniconcha spp. are iconic hydrothermal vent snails, yet almost no life history or population structure information exists for species within this genus. Here, we present biological trait data for the original hairy snail, *Alviniconcha hessleri*. We investigated reproductive periodicity, larval developmental mode, and allocation to reproduction in adults, including population size-frequency, oocyte size, and protoconch-I size. This species occurs in multiple vent fields from the Mariana back-arc, which differ in geothermal conditions, resulting in vent effluents with differing chemical compositions. *Alviniconcha hessleri* hosts diverse chemosynthetic bacteria (Stein et al. 1988; Breusing et al. 2020, 2022), and it is possible that the combination of symbiont identity and chemical and thermal conditions could impact the productivity of the symbiosis and affect reproductive partitioning in the form of oocyte size.

While not the focus of this study, the unexpected discovery of morphologically hermaphroditic individuals within this species is the second such occurrence within the genus. Castel et al. (2024) reported a high frequency of intersex individuals among *Alviniconcha kojimai* that were initially visually identified as female. Histological sections of *A. kojimai* individuals revealed mostly female gametic tissue, with the sporadic appearance of male tissues at various stages of development, which led Castel et al. (2024) to suggest that these individuals may represent an intermediate step in evolution between gonochorism and hermaphroditism. Interestingly, the histological sections of intersex individuals of *A. hessleri* in the present study more frequently revealed small patches of female tissue appearing sporadically throughout male tissue. Given the apparent lack of gametic organization and previous work revealing no significant relationship between snail size and sex within *A. hessleri* (seemingly ruling out sequential hermaphroditism; Warren 2019), it may be that the sexual systems of these species are indeed experiencing evolutionary change. The discovery of intersex individuals within *A. hessleri* and *A. kojimai* in particular is intriguing as these species are suggested to be the most closely related within the genus (Johnson et al., 2015) despite inhabiting geographically isolated back-arc systems.

Oocyte development indicates continuous reproduction and planktotrophy

Examination of histological sections revealed asynchronous oogenesis across individuals at all sites. Ovaries contained all three stages of oocyte development within a single female and all oocyte size-frequency distributions tended to be unimodal. Our results are consistent with continuous gametogenesis within females of *A. hessleri*, as in the majority of hydrothermal vent molluscs studied (Fretter 1989; Gage and Tyler 1991; Gustafson and Lutz 1994b; Tyler and Young 1999; Kelly and Metaxas 2007; Tyler et al. 2008; Matabos and Thiebaut 2010; Poitrimol et al. 2024). While the closest relative to *Alviniconcha, Ifremeria nautilei*, displays discontinuous gametogenesis and the novel use of a brooding pouch (Reynolds, 2009), other vent gastropod taxa, such as *Lepetodrilus* species, show continuous oogenesis with female gonads containing all oocyte developmental stages (Kelly and Metaxas 2007; Tyler et al. 2008; Poitrimol et al. 2024). Continuous or semi-continuous gametogenesis have also been noted in other dominant vent taxa, such as the bivalve *Calyptogena magnifica*(Berg 1985) and the alvinellids *Alvinella pompejana* (Faure et al. 2007), *Paralvinella pandorae* and *P. palmiformis* (McHugh 1989). In the hydrothermal environment where there is a mostly continuous energy supply, continuous reproduction is considered a strategy to take advantage of active venting. Maximizing reproductive output and larval dispersal would ensure the continual establishment of new populations at other vents, protecting against the unpredictability of geothermal activity, and thus food availability.

Maximum oocyte diameter was 93 µm in *A. hessleri*, which is on the lower end of reported oocyte sizes for vent gastropods. At least four *Lepetodrilus* species have maximum oocyte sizes between 80–100 µm (Tyler et al. 2008), while several other gastropod taxa have a maximum size of 100–150 µm (Kelly and

Metaxas 2007; Tyler et al. 2008; Matabos and Thiebaut 2010; Marticorena et al. 2020; Poitrimol et al. 2024). Oocyte size is often used as an indicator of life history, where larger eggs provisioned with more yolk imply direct development or lecithotrophy, and smaller oocytes indicate planktotrophy and the potential for wider dispersal (Gustafson and Lutz 1994b). However, given that these relationships between life history characters have mostly been observed in shallow species, it is unknown whether these patterns hold in the deep-sea.

While extensive studies linking oocyte size and mode of development do not exist for vent species, a comprehensive study of the larval biology of the caenogastropod genus *Conus* revealed a significant correlation between egg size and pelagic duration (Kohn and Perron 1994). Out of 64 *Conus* species, oocyte size for planktotrophic species ranged from 125–425 µm, one instance of lecithotrophy was found at an oocyte size of 390 µm, and the oocyte size of direct developers ranged from 470-1,000 µm (Kohn and Perron 1994). Of the hydrothermal vent gastropod taxa studied, most appear phylogenetically constrained to exhibit lecithotrophic development (Berg, 1985; Gustafson and Lutz, 1994; Lutz et al., 2008). Despite this, a feeding planktotrophic larva is believed to be the ancestral condition for deep-sea gastropods (Bouchet and Warén 1994) and oocyte size within this study suggests planktotrophy for *A. hessleri*. However, oocyte size should not be taken in isolation to indicate larval life history, but other parameters such as protoconch size, as well as the comparison of similar characters in congeners should be considered for a more definitive interpretation.

Recognized sources of error involved with histological processing may have influenced some our measuments, and should be considered in future studies which rely on our data for comparative purposes. For instance, we may have underestimated maximum oocyte size and relative abundance of vitellogenic oocytes, as larger oocytes were less likely to meet our requirements for inclusion. Due to their larger size, an obvious oocyte boundary was not always evident, and occasionally yolk droplets could be observed in isolation, suggesting that some oocytes may have lost their integrity during processing. Histological processing can also shrink tissues up to ~ 20% (Tyler et al. 2008). These sources of error combined highlight that histologically-based oocyte sizes may be an underestimate of actual size, further increasing the importance of using other characters to inform interpretations of larval life history among gastropod species.

Protoconch-I size estimate indicates excessive larval shell degradation

Few options exist for characterizing larval life history when larvae are not accessible and cannot be cultured, and most studies use protoconch size to estimate larval trophic strategy and planktonic duration (Bouchet and Warén 1994; Kohn and Perron 1994). Unfortunately, degradation of the larval shell in vent gastropods can occur during exposure to the acidic hydrothermal fluids, which can increase the difficulty of estimating protoconch size. This is particularly true for *Alviniconcha* species, where even the smallest specimen post-metamorphosis shows damage at the apex. As the juveniles grow, a calcareous plug is secreted to fill the apex and protect the snail. It is unknown whether the plug approximates the dimensions of the original protoconch, therefore we only used the smallest, most intact specimens available to measure protoconch size. In *A. hessleri*, the protoconch-teleoconch transition is not obviously demarcated by a sculptural change in the shell. Thus, the initial whorl of the shell can be used as an approximation of protoconch-I, as it would have needed to encompass the visceral mass of the developing embryo, including the majority of the endogenous yolk. Here, we presented a hypothetical measure of protoconch-I to investigate whether degradation of the larval shell occurs in a predictable way and to help inform on the life history of *A. hessleri.*

The hypothetical protoconch-I measurement for *A. hessleri* was ~ 675 µm, which is larger than in the original description by Okutani and Ohta (1988), who reported a protoconch of ~ 500 µm. In addition to this inconsistency, a study examining zooplankton community composition in the North Pacific Subtropical Gyre genetically identified the presence of *A. hessleri* larvae within 200 and 500 µm size classes from zooplankton tows located north of Hawaii (Sommer et al. 2017). Thus, hatching size (protoconch-I size) of *A. hessleri* larvae are most likely smaller than the sizes reported in this study and in the original description by Okutani and Ohta (1988). This indicates that more than one whorl degrades post-metamorphosis, and estimates of protoconch-I size in adults are not a reasonable substitute for intact larval shells. Beyond this, the capture of *A. hessleri* larvae roughly 6,000 km away from their only known habitat (Sommer et al. 2017) also supports the conclusion that this species has a long larval period, typical of planktotrophic larvae, and rasies important questions about the transport and vertical position of larvae within the water column.

Given that many vent species exhibit lecithotrophic development, vent larvae have been assumed to remain at depth during development (Gustafson and Lutz 1994a), which may facilitate retention within vent systems and explain the limited range of some species. Previously modelled dispersal patterns have indicated that *A. hessleri* larvae in the Mariana back-arc likely disperse using cold bottom currents, with larvae dispersing at a depth of 3000 m predicted to be 20% more likely to arrive at another vent destination within the back-arc than those dispersing at the surface (Breusing et al. 2021a). However, the record of *A. hessleri* larvae occurring thousands of kilometers away from their only known habitat (Sommer et al. 2017) suggests that the larvae of this species may utilize stronger mid-oceanic or upper-layer oceanic currents. Adams et al. (2012) speculated that vent species of the East Pacific Rise could potentially use upper ocean countercurrents to form a loop facilitating transport and return of larvae in the Pacific Ocean, however examples of this are lacking. Evidence of larvae dispersing in mid-water, and sometimes even in the photic zone, exists for a few comparatively shallow vent and cold seep species, such as alvinocaridid shrimp (Herring 2006), limpet snails (Yahagi et al. 2017) and bathymodiolus bivalves (Arellano et al. 2014). But, for a species occurring only at depths greater than 3000 m such as *A. hessleri*, this degree of vertical transport (and even greater horizontal transport) resulting in continued successful recruitment is unprecedented. While evidence from this study and published literature imply planktotrophy for this species, increased sampling effort is needed to clarify the larval ecology of this species.

Population size structure indicates irregular recruitment events

The population size structure distribution of *A. hessleri* varied across sites. All sites except Voodoo Crater differed from a normal distribution, with a strong bimodal population at Sequoia. Distributions other than unimodal may suggest periodic recruitment, which is difficult to reconcile with the continuous gametogenesis observed in all individuals in this study, but may be explained by periodic recruitment. For instance, the size structures observed herein are most likely due to environmental influences on larval recruitment, including directional currents and variability in venting activity. Breusing et al. (2021) used genetic data to assess larval dispersal patterns, which revealed that both our northern sites (Illium and Burke) contain source populations for the central and southern sites (Sequoia, Voodoo Crater, and Perseverance). The same work also indicates that Sequoia, Voodoo Crater and Perseverance receive larvae from southern vent fields outside this study, Forecast, Snail and Pika. This may explain why we see younger populations at Sequoia in particular, as it may be receiving larvae from both directions, leading to stronger recruitment pulses. Similarly, the inferred dispersal directionality could explain why there are fewer recruitment events at Illium, which also appeared to contain the oldest population, with snail sizes significantly larger than at most other sites.

Oocyte size may be conserved beyond a reduced chemical threshold

Given that sites along the Mariana back-arc differ substantially in parameters such as temperature, reduced hydrogen sulfide and methane concentrations, differences in fluid chemistry may influence the symbiosis between *A. hessleri* and its bacterial endosymbionts, thus impacting the available energy for host allocation to reproduction. However, the relationship between temperature and reduced chemical content was not as straightforward as expected, as the hotter sites of Burke and Illium did not consistently have higher reduced chemical concentrations. Illium had the lowest hydrogen sulfide and methane of all sites, suggesting that this vent may be older and is starting to senesce. Interestingly, the only site to show significant differences in *A. hessleri* characteristics was Illium, displaying significantly larger shell height and significantly smaller oocyte sizes, suggesting that the unique fluid chemistry at Illium may be influencing the biology of *A. hessleri*.

With a temperature of ~ 29°C, Illium should be optimal for the *A. hessleri* holobiont, as warmer fluid chemistries are typically associated with higher quantities of reduced chemicals (Van Dover 2000). However, Illium had a remarkably low hydrogen sulfide level of 13 µM, whereas all other sites exceeded 150 µM. Because the dominant symbiont type in *A. hessleri* is thiotrophic (Stein et al. 1988; Breusing et al. 2022), one interpretation of our results is that the symbiosis is indeed less productive at this site due to a lack of hydrogen sulphide, and that this may have downstream effects for provisioning to oocytes by the host. However, differential allocation of resources to reproduction could also take the form of variation in total fecundity, which should be accounted for in future studies for species within this genus. A confounding variable is that the Illium population was also significantly larger in body size based on shell height than all other sites (except Voodoo Crater), so it is possible that this population is older and may be decreasing provisioning to eggs due to age. However, gametogenic maturity did not differ among sites, and individuals at Illium still had a proportion of vitellogenic oocytes greater than 50%, except for one individual.

Research examining spatial variation in oocyte size is mixed for symbiotic vent gastropods.Kelly and Metaxas (2007) found that symbiotic *Lepetodrilus fucensis* from senescent vents on the Juan de Fuca Ridge showed a decrease in oocyte size, quantity, and proportion of vitellogenic oocytes compared to those from more actively venting sites. In contrast, three species of *Lepetodrilus* from the East Pacific Rise(Tyler et al. 2008) and two from the west Pacific(Poitrimol et al. 2024) showed no spatial variation in oocyte size. Thus, it might be that as long as a certain reduced chemical threshold is met, allocation to reproduction in vent gastropods is highly conserved. Two other factors could also convey resilience in reproductive allocations in *A. hessleri* across differing chemical environments: 1. its ability to graze as well as receive nutrition from symbionts, 2. its diverse endosymbiont population (the most diverse in the genus; Breusing et al., 2022). Given the relatively plastic nature of some life history traits despite claims of phylogenetic constraints in the Gastropoda, further reproductive studies on *Alviniconcha* and sister taxa are required to confirm whether different vent chemistries can impact host reproductive effort.

Conclusions

In the present work, we advanced the basic biology of *Alviniconcha hessleri*, a dominant, yet understudied gastropod from hydrothermal vents in the western Pacific. This study revealed that *A. hessleri* shares some of the life history traits found among vent gastropods such as continuous reproduction and a tendency to conserve reproductive quality across different venting environments. In contrast, the evidence currently available suggests that this species has planktotrophic larvae capable of widespread dispersal despite the realized range of adults being limited to the Mariana back-arc. These traits have likely been selected to maximize reproductive output in the face of low larval survival and recruitment success due to the highly specific habitat requirements of vent species. A continuous output of larvae would ensure the best chance at maintaining existing populations and most importantly, the establishment of new populations, protecting against the unpredictability of venting activity and senescence. The conservation of reproductive provisioning across venting environments suggests that high oocyte quality is a necessity for the survival of this species. Redundancy in modes of host energy acquisition through host feeding and chemosynthetic endosymbionts may convey the flexibility needed to ensure the continual production of high-quality oocytes. While the sum of these reproductive and life history traits may convey high resilience amongst venting sites, the number of suitable habitats for this species remains extremely low and patchy, meaning that all known sites to host *A. hessleri* along the Mariana back-arc require protection against anthropogenic disturbances.

Amassing knowledge on vent species is of the utmost importance due to an immense interest in the mining of these areas and rapid climate change in the deep ocean (Levin and Bris 2015). Currently, the exploitation of polymetallic sulfide deposits at active hydrothermal vents is being explored in the western

Pacific Ocean. Concerns regarding mining are related to the direct destruction of vents and those that would be adversely impacted due to far-reaching sediment plumes. Research indicates that sediment plumes from mining activity could disperse and smother adjacent vent communities as close as 60 to 70 km (Luick 2012; Miller et al. 2018), degrading already sparsely available habitat. While the results from this study indicate that *A. hessleri* may have high reproductive and dispersal potential due to the continual production of planktotrophic larvae, genetic data suggests that vent fields at the northern and southern boundaries of the range act as important sources of larvae for central populations (Breusing et al. 2021a). Given these dispersal patterns, mining-related destruction of vent habitat (direct or adjacent smothering of communities) could affect species that rely upon long-dispersing larvae for the maintenance and establishment of new populations disproportionately if source vents are destroyed.

While no exploratory mining licenses have yet been granted along the Mariana back-arc, this area resides within the Exclusive Economic Zone of the United States and does not currently benefit from any protective measures. Nearby, the Marianas Trench Marine National Monument provides some protection to the island chain, volcanic arc and trench, but excludes the back-arc, despite its unique features and endemic fauna. *Alviniconcha hessleri* is currently listed on the IUCN Red List of Threatened Species as 'vulnerable' (Thomas et al. 2019), based on its extremely restricted habitat and geographic area. Any decline in habitat quality and quantity may lead to extinction. Without the immediate implementation of more permanent protection, such as the formation of a Marine Protected Area, there is a future risk of mining-related extinction of this and other vent endemic species. Thus, it is fundamental that we continue investigating the traits and population dynamics of vent endemic species to adequately assess biodiversity patterns that can inform conservation in the face of anthropogenic threats.

Declarations

ACKNOWLEDGMENTS

The data collected in this study includes work supported by the NOAA Earth Ocean Interactions (EOI) Program and the Schmidt Ocean Institute during cruise FK161129 aboard R/V Falkor. We thank the captains and crews of the R/V Falkor and ROV SuBastian and the science team which participated in this cruise. We thank Dr. Verena Tunnicliffe, Dr. David Butterfield and Megan Warren for their contributions to the collection of data and initial work on this project.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by NBH and AEB. The first draft of the manuscript was written by NBH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

We gratefully acknowledge funding provided by the Natural Sciences and Engineering Research Council of Canada for a PGS-D awarded to NBH and a Discovery Grant awarded to SCD (grant number 2015-06548), and funding provided by University of Southampton to AEB.

Data Availability

The datasets generated and analysed from this study are available from the corresponding author on reasonable request. The biological data used in this study are available online (https://doi.org/10.17882/101552).

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

All applicable international, national, and/or institutional guidelines for sampling were followed and all necessary approvals were obtained.

Consent to publish

The authors consent to the publication of their work in all Springer publications. They guarantee that the work has not been published elsewhere in any form other than as a preprint, that it has not been submitted in parallel to any other publisher, and that it is not currently under review by any other publisher.

References

1. Abramoff MD, Magalhaes PJ, Ram SJ (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675
2. Adams DK, Arellano SM, Govenar B (2012) Larval dispersal: Vent life in the water column. Oceanography 25:256–268
3. Arellano SM, Van Gaest AL, Johnson SB, Vrijenhoek RC, Young CM (2014) Larvae from deep-sea methane seeps disperse in surface waters. Proceedings of the Royal Society B: Biological Sciences 281:1–8. 10.1098/rspb.2013.3276
4. Baker ET, Walker SL, Resing JA, Chadwick WW, Merle SG, Anderson MO, Butterfield DA, Buck NJ, Michael S (2017) The effect of arc proximity on hydrothermal activity along spreading centers: New evidence from the Mariana Back Arc (12.7°N–18.3°N). Geochemistry, Geophysics, Geosystems 18:4211–4228. 10.1002/2017GC007234
5. Bayer SR, Mullineaux LS, Waller RG, Solow AR (2011) Reproductive traits of pioneer gastropod species colonizing deep-sea hydrothermal vents after an eruption. Mar Biol 158:181–192. 10.1007/s00227-010-1550-1

6. Berg CJJ (1985) Reproductive strategies of mollusks from abyssal hydrothermal vent communities. Bull Biol Soc Wash 6:185–197
7. Bouchet P, Warén A (1994) Ontogenetic migration and dispersal of deep-sea gastropod larvae. In: Reproduction, larval biology, and recruitment of the deep-sea benthos. pp 98–118
8. Breusing C, Johnson SB, Tunnicliffe V, Clague DA, Vrijenhoek RC, Beinart RA (2020) Allopatric and sympatric drivers of speciation in Alviniconcha hydrothermal vent snails. Mol Biol Evol 37:3469–3484. 10.1093/molbev/msaa177
9. Breusing C, Johnson SB, Mitarai S, Beinart RA, Tunnicliffe V (2021a) Differential patterns of connectivity in Western Pacific hydrothermal vent metapopulations: A comparison of biophysical and genetic models. Evol Appl 1–14. 10.1111/eva.13326
10. Breusing C, Johnson SB, Mitarai S, Beinart RA, Tunnicliffe V (2021b) Differential patterns of connectivity in Western Pacific hydrothermal vent metapopulations: A comparison of biophysical and genetic models. Evol Appl 1–14. 10.1111/eva.13326
11. Breusing C, Castel J, Yang Y, Broquet T, Sun J, Jollivet D, Qian P, Beinart RA (2022) Global 16S rRNA diversity of provannid snail endosymbionts from Indo-Pacific deep-sea hydrothermal vents. Environ Microbiol Rep 14:299–307. 10.1111/1758-2229.13051
12. Butterfield DA, Roe KR, Lilley MD, Huber JA, Baross JA, Embley RW, Massoth GJ (2004) Mixing, reaction and microbial activity in the sub-seafloor revealed by temporal and spatial variation in diffuse flow vents at axial volcano. In: The subseafloor biosphere at mid-ocean ridges. pp 269–289
13. Castel J, Hourdez S, Pradillon F, Daguin-Thiébaut C, Ballenghien M, Ruault S, Corre E, Adrien Tran Lu Y, Mary J, Gagnaire PA, Bonhomme F, Breusing C, Broquet T, Jollivet D (2022) Inter-specific genetic exchange despite strong divergence in deep-sea hydrothermal vent gastropods of the genus Alviniconcha. Genes (Basel). 10.3390/genes13060985
14. Castel J, Pradillon F, Cueff V, Leger G, Daguin-Thiébaut C, Ruault S, Mary J, Hourdez S, Jollivet D, Broquet T (2024) Genetic sex determination in three closely related hydrothermal vent gastropods, including one species with intersex individuals. J Evol Biol. 10.1093/jeb/voae051
15. Faure B, Chevaldonné P, Pradillon F, Thiébaut E, Jollivet D (2007) Spatial and temporal dynamics of reproduction and settlement in the Pompeii worm Alvinella pompejana (Polychaeta: Alvinellidae). Mar Ecol Prog Ser 348:197–211. 10.3354/meps07021
16. Fischer AH, Jacobson KA, Rose J, Zeller R (2008) Hematoxylin and eosin staining of tissue and cell sections. Cold Spring Harb Protoc 3:4986–4988. 10.1101/pdb.prot4986
17. Fretter V (1989) The anatomy of some new archaeogastropod limpets (Superfamily Peltospiracea) from hydrothermal vents. J Zool 218:123–169. 10.1111/j.1469-7998.1989.tb02530.x
18. Gage JD, Tyler PA (1991) Reproduction, recruitment and growth of deep-sea organisms. In: Deep-sea biology: A natural history of organisms at the deep-sea floor. pp 301–335
19. Gustafson C, Lutz RA (1994a) Molluscan life history traits at deep-sea hydrothermal vents and cold methane/sulfide seeps. In: Reproduction, larval biology, and recruitment of the deep-sea benthos. pp 76–97

20. Gustafson C, Lutz RA (1994b) Molluscan life history traits at deep-sea hydrothermal vents and cold methane/sulfide seeps. In: Reproduction, larval biology, and recruitment of the deep-sea benthos. pp 76–97
21. Herring PJ (2006) Presence of postlarval alvinocaridid shrimps over south-west Indian Ocean hydrothermal vents, with comparisons of the pelagic biomass at different vent sites. J Mar Biol Association United Kingd 86:125–128. 10.1017/S0025315406012938
22. Johnson SB, Warén A, Tunnicliffe V, Van Dover C, Wheat CG, Schultz TF, Vrijenhoek RC (2015) Molecular taxonomy and naming of five cryptic species of Alviniconcha snails (Gastropoda: Abyssochrysoidea) from hydrothermal vents. Syst Biodivers 13:278–295. 10.1080/14772000.2014.970673
23. Kelly NE, Metaxas A (2007) Influence of habitat on the reproductive biology of the deep-sea hydrothermal vent limpet Lepetodrilus fucensis (Vetigastropoda: Mollusca) from the Northeast Pacific. Mar Biol 151:649–662. 10.1007/s00227-006-0505-z
24. Kohn AJ, Perron FR (1994) Life history and biogeography patterns in Conus. Oxford University Press, New York
25. Levin LA, Bris NL (2015) The deep ocean under climate change. Science (1979) 350:766–768. 10.1126/science.aad0126
26. Lilliefors HW (1967) On the Kolmogorov-Smirnov Test for Normality with Mean and Variance. Am Stat Association J 62:399–402
27. Luick JL (2012) Physical oceanographic assessment of the Nautilus EIS for the Solwara 1 Project
28. Lutz Ra, Jablonski D, Turner RD, Cash FE (2008) Larval Development and Dispersal at Deep-Sea Hydrothermal Vents Published by : American Association for the Advancement of Science Larval Development and Dispersal at Deep-Sea Hydrothermal Vents. 226:1451–1454
29. Marshall DJ, Keough MJ (2007) The Evolutionary Ecology of Offspring Size in Marine Invertebrates. Adv Mar Biol 53:1–60. 10.1016/S0065-2881(07)53001-4
30. Marticorena J, Matabos M, Sarrazin J, Ramirez-Llodra E (2020) Contrasting reproductive biology of two hydrothermal gastropods from the Mid-Atlantic Ridge: implications for resilience of vent communities. Mar Biol 167:1–19. 10.1007/s00227-020-03721-x
31. Matabos M, Thiebaut E (2010) Reproductive biology of three hydrothermal vent peltospirid gastropods (Nodopelta heminoda, N. subnoda and Peltospira operculata) associated with Pompeii worms on the East Pacific Rise. J Molluscan Stud 76:257–266. 10.1093/mollus/eyq008
32. McHugh D (1989) Population structure and reproductive biology of two sympatric hydrothermal vent polychaetes, Paralvinella pandorae and P. palmiformis. Mar Biol 103:95–106. 10.1007/BF00391068
33. Miller KA, Thompson KF, Johnston P, Santillo D (2018) An overview of seabed mining including the current state of development, environmental impacts, and knowledge gaps. Front Mar Sci 4:418. 10.3389/fmars.2017.00418
34. Poitrimol C, Matabos M, Veuillot A, Ramière A, Comtet T, Boulart C, Cathalot C, Thiébaut É (2024) Reproductive biology and population structure of three hydrothermal gastropods (Lepetodrilus

schrolli, L. fijiensis and Shinkailepas tollmanni) from the South West Pacific back-arc basins. Mar Biol 171:31. 10.1007/s00227-023-04348-4

35. Pradillon F, Gaill F (2007) Adaptation to deep-sea hydrothermal vents: Some molecular and developmental aspects. J Mar Sci Technol 15:37–53. 10.51400/2709-6998.2057
36. Scheltema RS (1994) Adaptations for reproduction among deep-sea benthic molluscs: An appraisal of the existing evidence. In: Reproduction, larval biology, and recruitment of the deep-sea benthos. pp 44–75
37. Sommer SA, Van Woudenberg L, Lenz PH, Cepeda G, Goetze E (2017) Vertical gradients in species richness and community composition across the twilight zone in the North Pacific Subtropical Gyre. Mol Ecol 26:6136–6156. 10.1111/mec.14286
38. Stein J, Cary SC, Hessler RR, Ohta S, Vetter RD, Childress JJ, Felbeck H (1988) Chemoautotrophic symbiosis in a hydrothermal vent gastropod. Biol Bull 174:373–378. 10.2307/1541963
39. Suzuki Y, Sasaki T, Suzuki M, Tsuchida S, Nealson KH, Horikoshi K (2005) Molecular phylogenetic and isotopic evidence of two lineages of chemoautotrophic endosymbionts distinct at the subdivision level harbored in one host-animal type: The genus Alviniconcha (Gastropoda: Provannidae). FEMS Microbiol Lett 249:105–112. 10.1016/j.femsle.2005.06.023
40. Thomas EA, Chen C, Sigwart J (2019) Alviniconcha hessleri. In: The IUCN Red List of Threatened Species
41. Trembath-Reichert E, Butterfield DA, Huber JA (2019) Active subseafloor microbial communities from Mariana back-arc venting fluids share metabolic strategies across different thermal niches and taxa. ISME J 13:2264–2279. 10.1038/s41396-019-0431-y
42. Tyler PA, Young CM (1999) Reproduction and dispersal at vents and cold seeps. J Mar Biol Association United Kingd 79:193–208. 10.1017/S0025315499000235
43. Tyler PA, Pendlebury S, Mills SW, Mullineaux L, Eckelbarger KJ, Baker M, Young CM (2008) Reproduction of gastropods from vents on the East Pacific rise and the Mid-Atlantic Ridge. J Shellfish Res 27:107–118. 10.2983/0730-8000(2008)27[107:ROGFVO]2.0.CO;2
44. Van Dover C (2000) The Ecology of the Deep-Sea Hydrothermal Vents. Princeton University Press, Princeton, New Jersey
45. Warren MD (2019) The Reproductive Biology of the Deep-sea Hairy Snail. Alviniconcha hessleri
46. Yahagi T, Kayama Watanabe H, Kojima S, Kano Y (2017) Do larvae from deep-sea hydrothermal vents disperse in surface waters? Ecology 98:1524–1534. 10.1002/ecy.1800

Figures

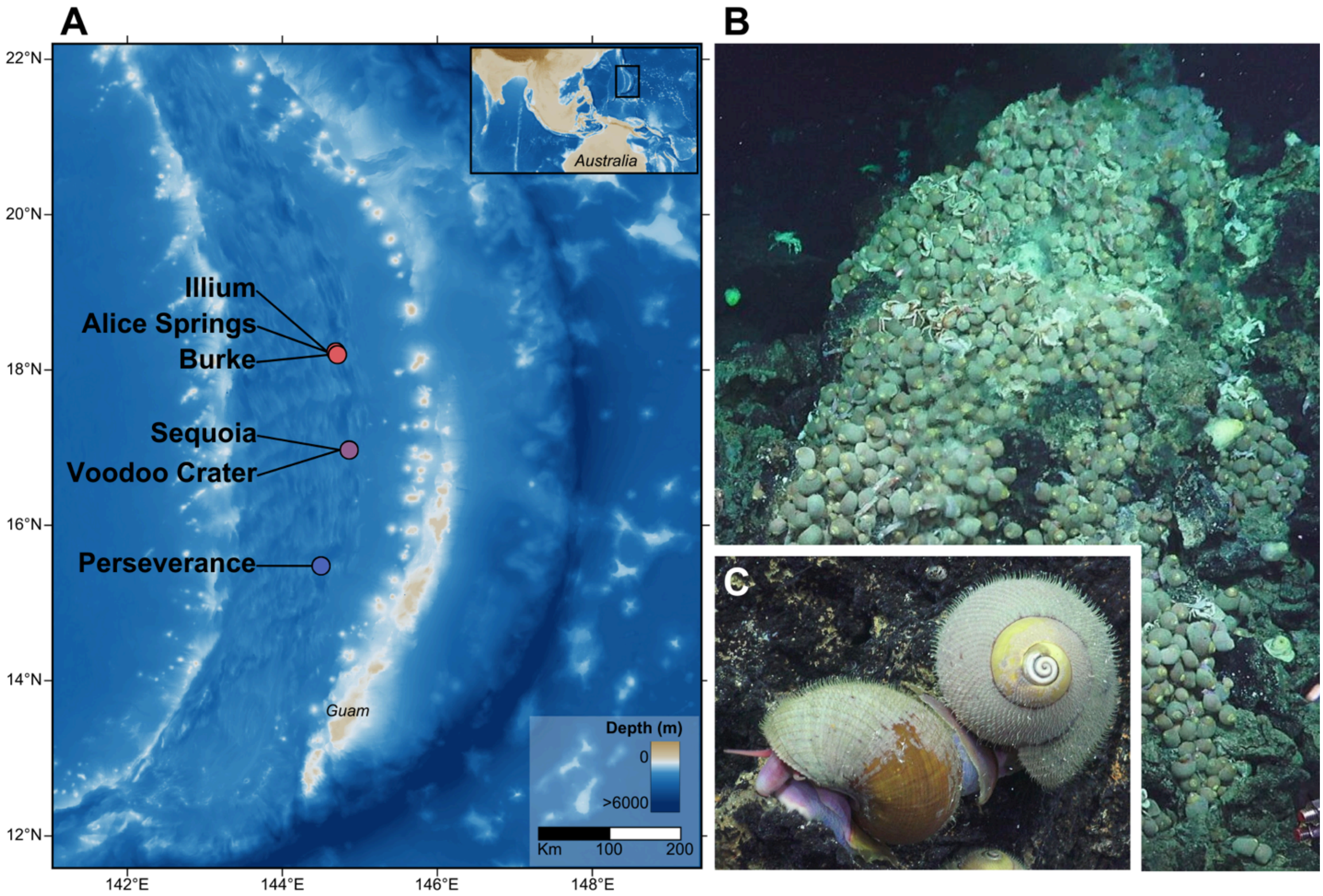

Figure 1

(A) Bathymetric map of hydrothermal vents sampled from the northern and central regions of the Mariana back-arc. (B) Illium hydrothermal vent at 3595 m depth. Pictured are masses of snails, shrimp, crabs and anemones piled on top of diffuse venting fluid. (C) Adult *Alviniconcha hessleri* at Illium hydrothermal vent

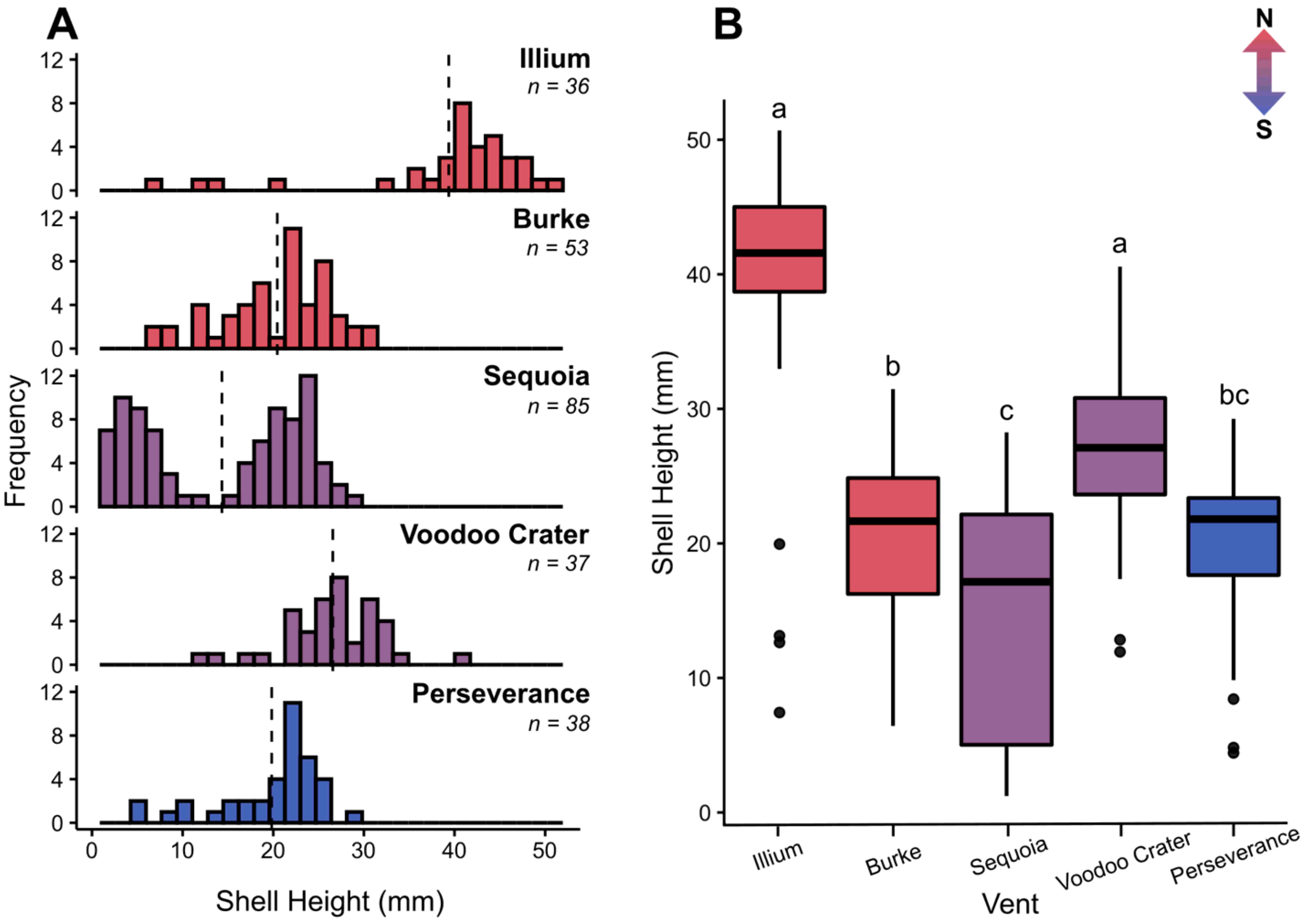

Figure 2

Histograms (A) and a box plot (B) comparing shell height in *Alviniconcha hessleri* across vents. Red to blue indicates vents occurring from north to south. Dashed lines represent mean, whiskers are 1.5x interquartile range and points represent outliers. Letters indicate significant differences detected with a Kruskal-Wallis multi-sample test and Tukey-Kramer-Nemenyi tests

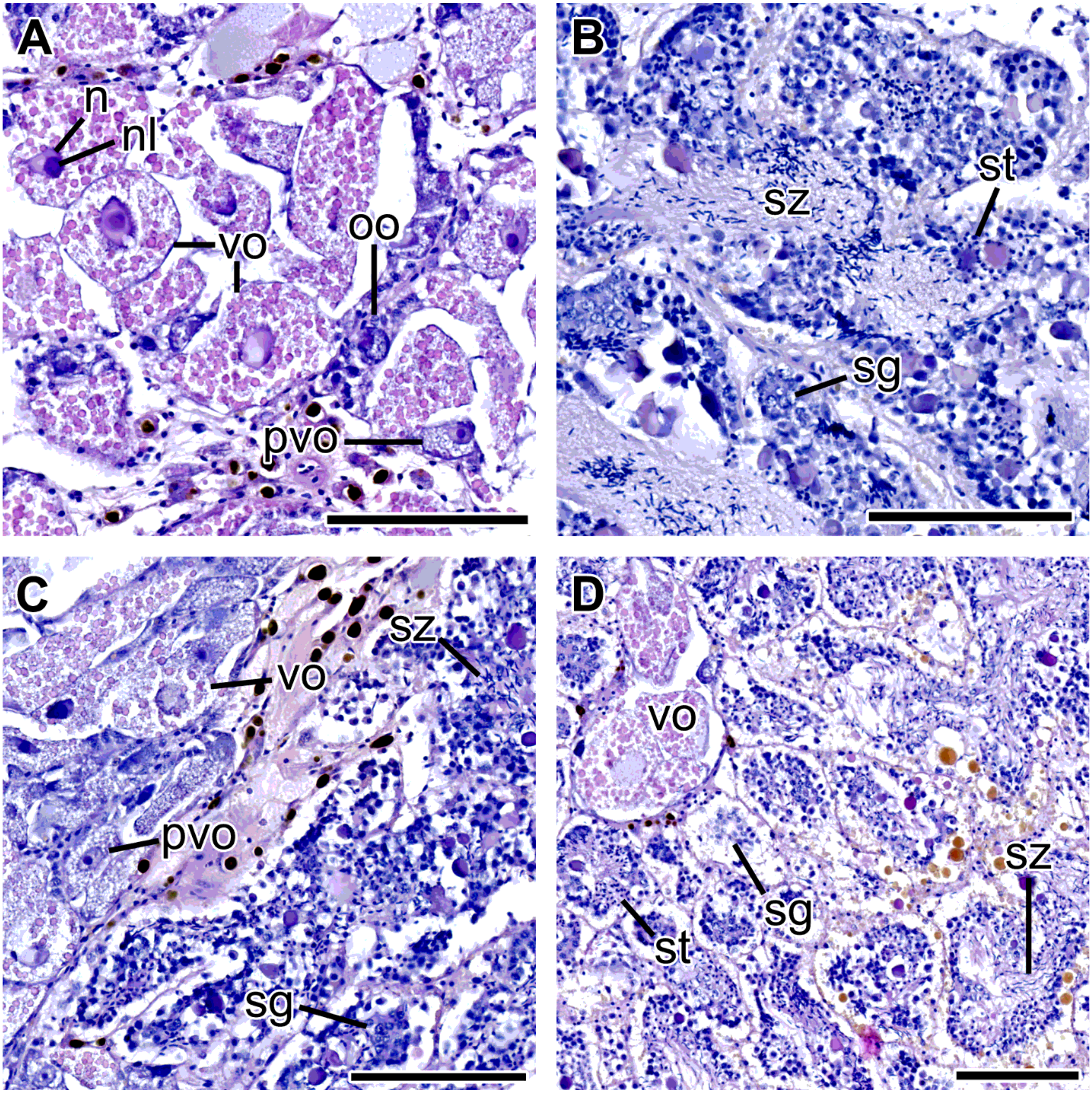

Figure 3

Gonads of *Alviniconcha hessleri* collected from Illium, Mariana back-arc, stained with eosin and hematoxylin. (A) Female, (B) male, (C-D) hermaphroditic. Abbreviations: n, nucleus; nl, nucleolus; oo, oogonia; pvo, previtellogenic oocyte; sg, spermatogonia; st, spermatid; sz, spermatozoa; vo, vitellogenic oocyte. Scale bars = 100 μm

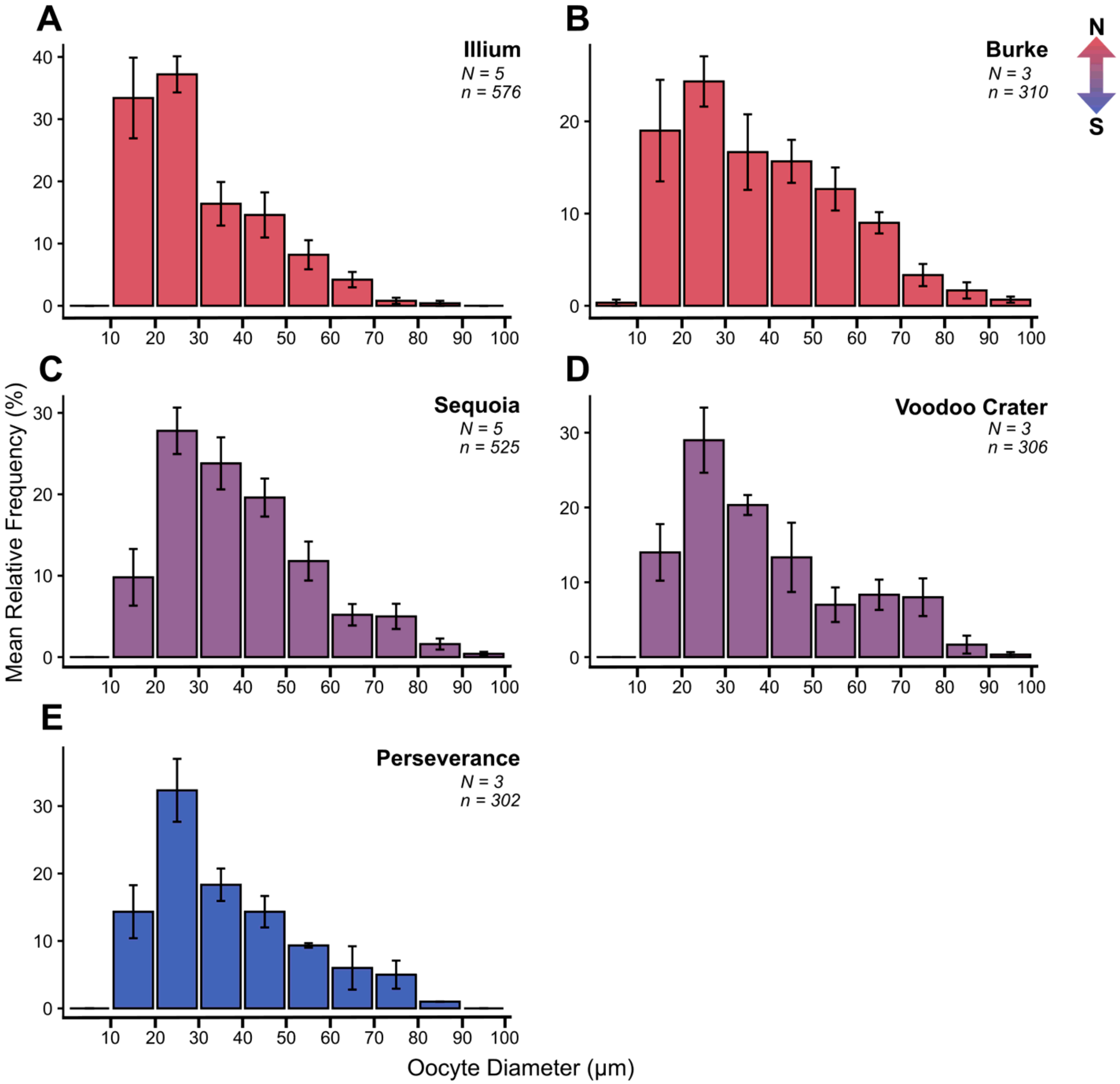

Figure 4

Mean oocyte size-frequency distributions (mean ± SE) of *Alviniconcha hessleri* for each vent site from the Mariana back-arc: (A) Illium, (B) Burke, (C) Sequoia, (D) Voodoo Crater, (E) Perseverance. Red to blue indicates vents occurring from north to south. Abbreviations: *N*, number of individuals; *n*, number of oocytes measured

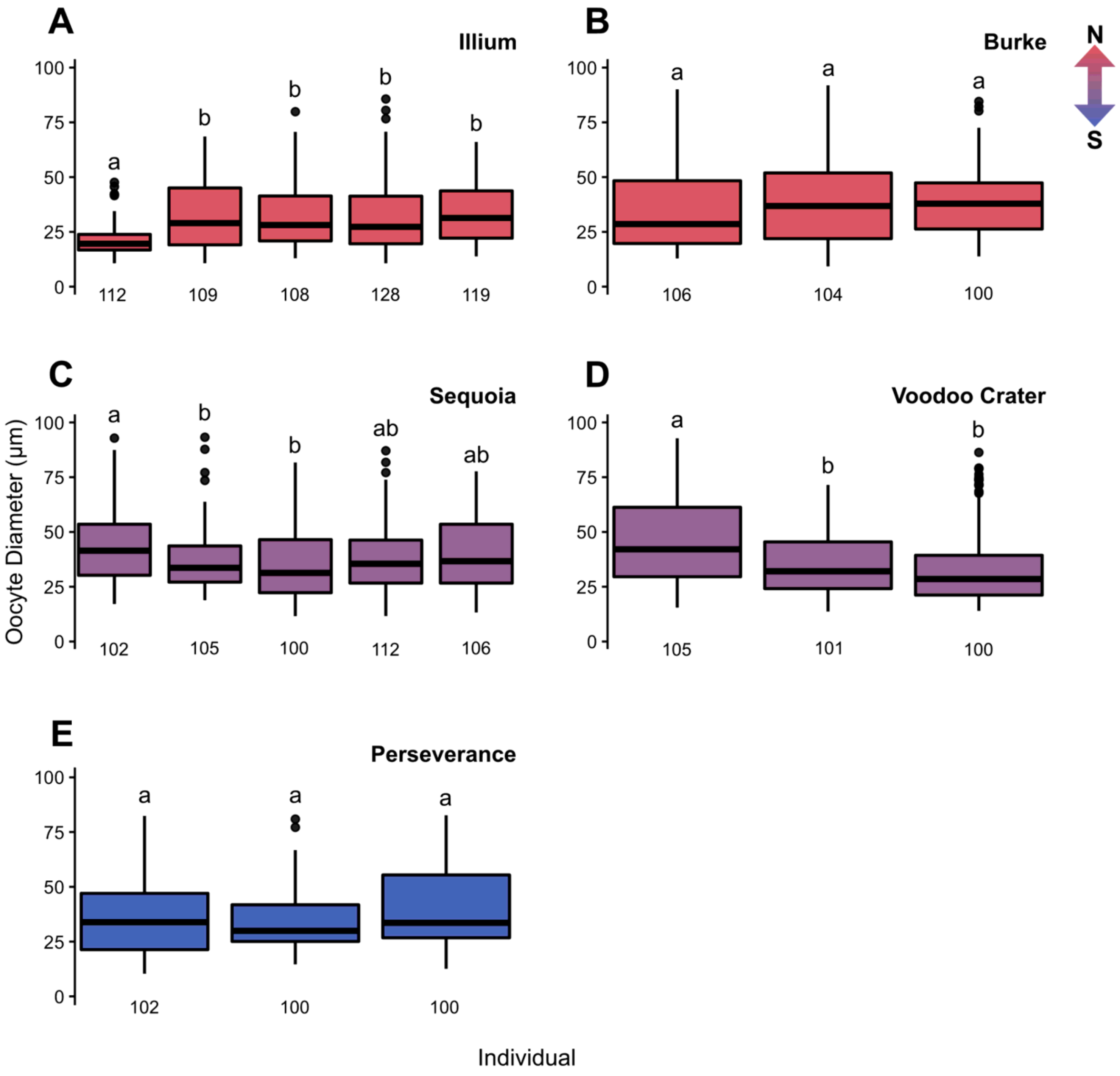

Figure 5

Box plots comparing oocyte diameter in individuals of *Alviniconcha hessleri* from each vent site along the Mariana back-arc: (A) Illium, (B) Burke, (C) Sequoia, (D) Voodoo Crater, (E) Perseverance. Red to blue indicates vents occurring from north to south. Each box represents oocytes measured within one individual, with the number of oocytes measured displayed below each box. Whiskers are 1.5x interquartile range and points represent outliers. Letters indicate significant differences detected with a Kruskal-Wallis multi-sample test and Tukey-Kramer-Nemenyi tests

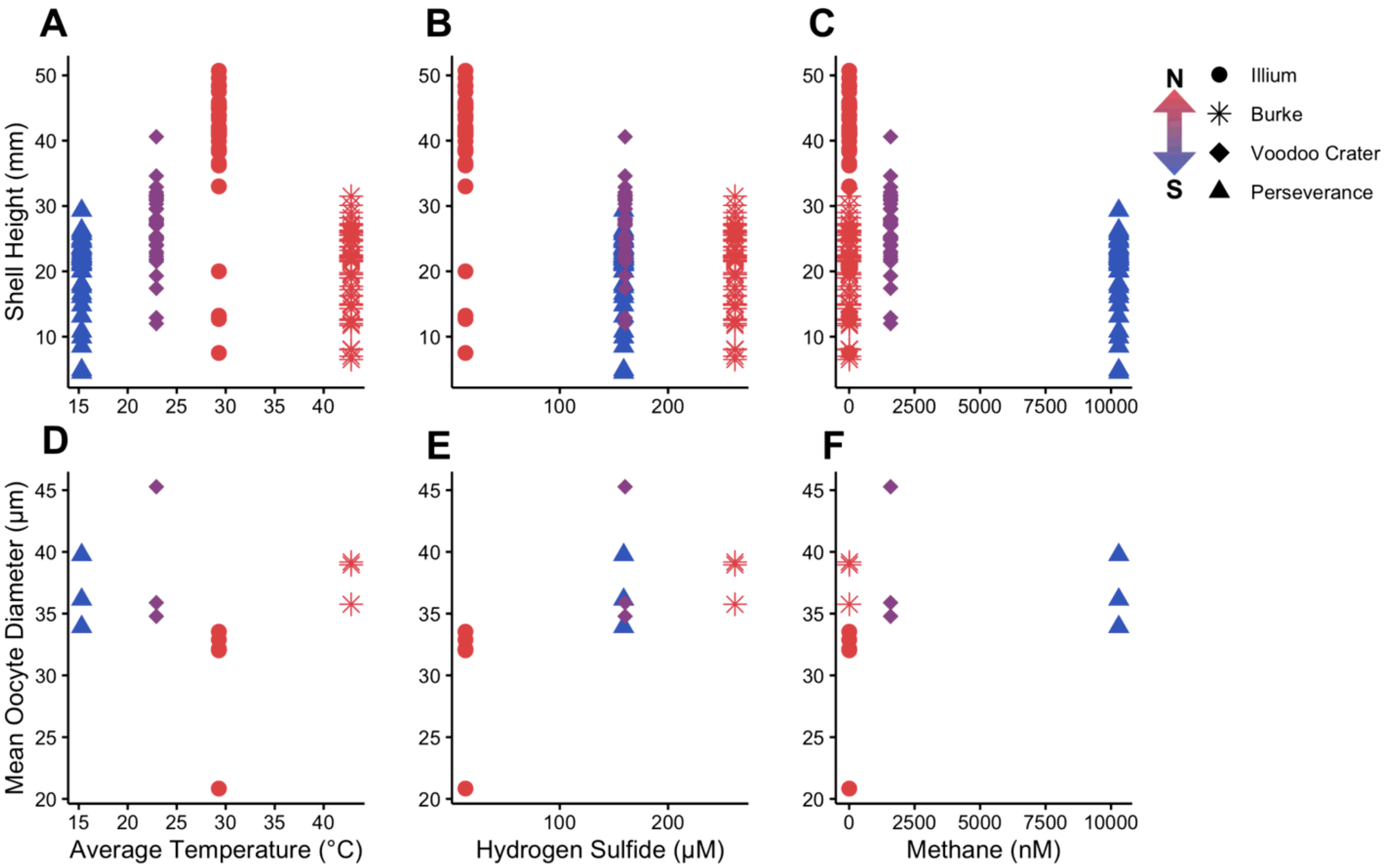

Figure 6

Shell height (A-C) and mean oocyte diameter (D-F) of *Alviniconcha hessleri* compared to different hydrothermal fluid parameters from vents along the Mariana back-arc. Red to blue indicates vents occurring from north to south. Environmental data was not available for Sequoia

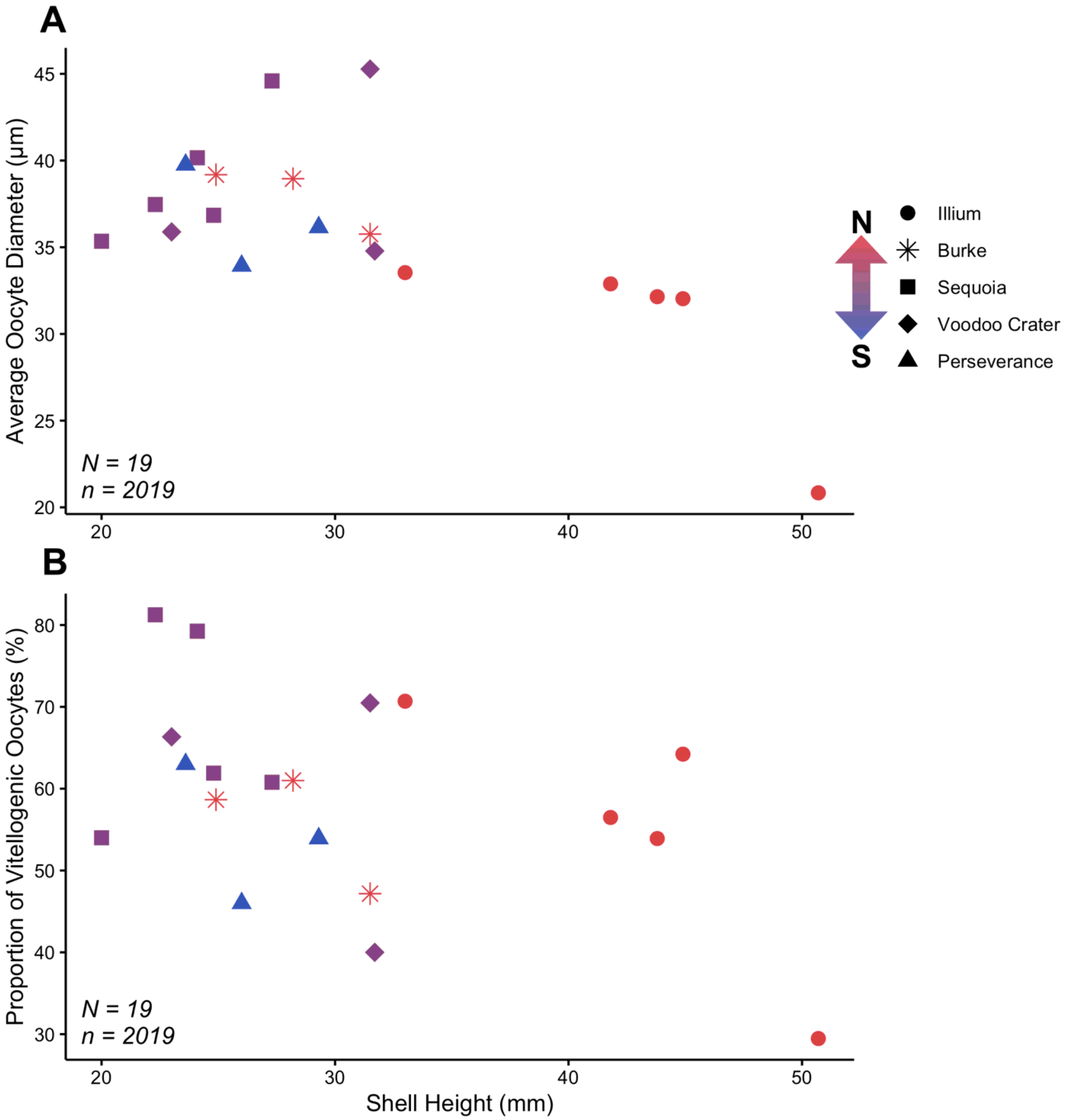

Figure 7

Shell height compared to average oocyte diameter (A) and percentage of vitellogenic oocytes (B) in *Alviniconcha hessleri* collected from the Mariana back-arc. The proportion of vitellogenic oocytes was determined as the percentage of vitellogenic oocytes from the total number of oocytes measured. Red to blue indicates vents occurring from north to south. Abbreviations: *N*, number of individuals; *n*, number of oocytes measured

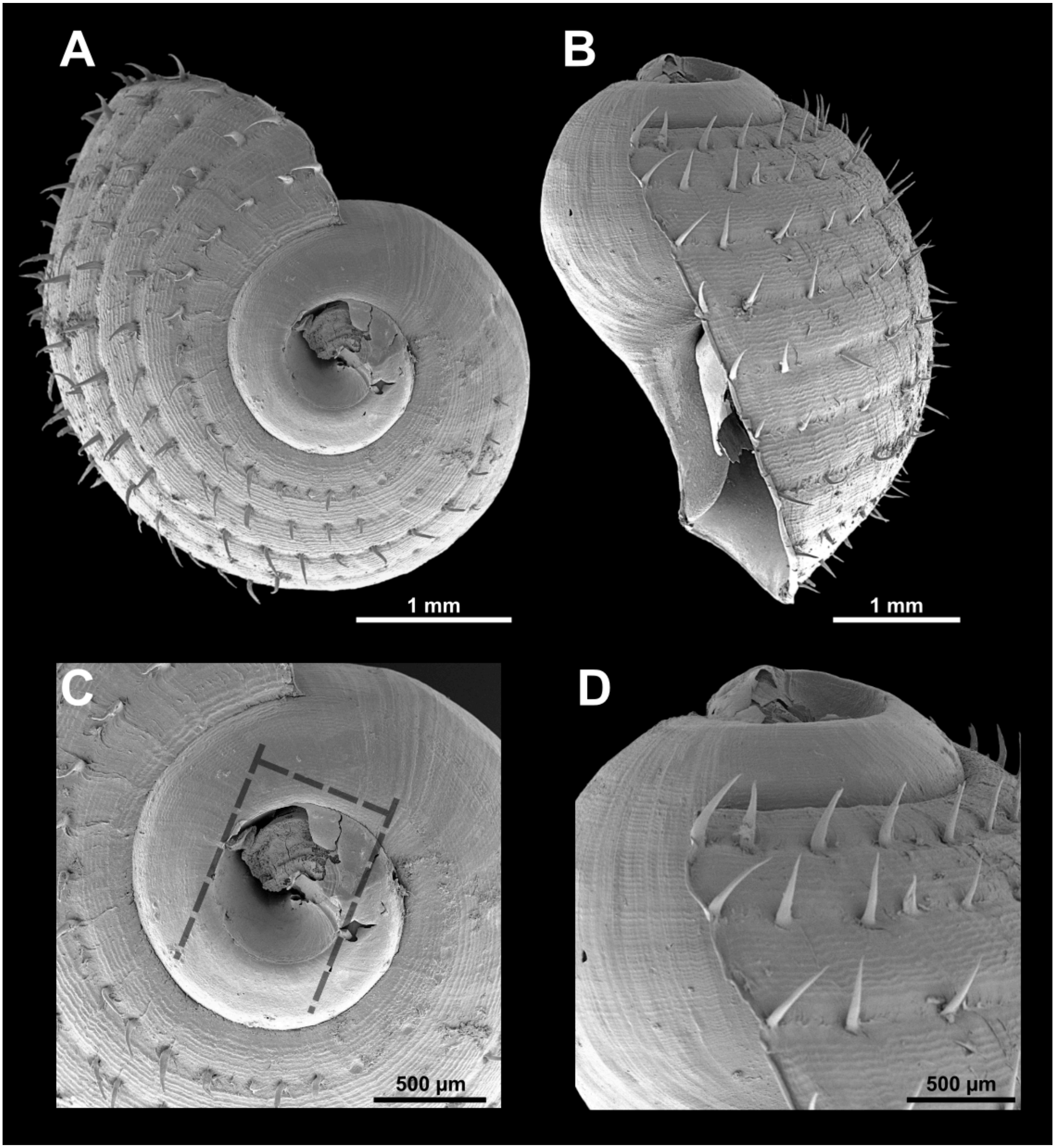

Figure 8

Apical (A) and axial (B) views of *Alviniconcha hessleri*. (C) Apical view of the protoconch with estimated dimensions (dashed) used for measuring protoconch-I. (D) Axial view of the protoconch showing the number of intact larval whorls present