UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Ocean and Earth Science

Volume 1 of 1

Life-history biology and biogeography of invertebrates in deep-sea chemosynthetic environments

by

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Thesis for the degree of Doctor of Philosophy

December 2013

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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LIFE-HISTORY BIOLOGY AND BIOGEOGRAPHY OF INVERTEBRATES IN DEEP-SEA CHEMOSYNTHETIC ENVIRONMENTS

Verity Nye

Globally-distributed, insular and ephemeral deep-sea hydrothermal vents with their endemic faunas provide 'natural laboratories' for studying the processes that shape global patterns of marine life. The continuing discovery of hydrothermal vents and their faunal assemblages has yielded hundreds of new species and revealed several biogeographic provinces, distinguished by differences in the taxonomic composition of their assemblages. However, the first-order question of how these provinces are separated remains unanswered. The recent discovery of the Beebe (~4600 m depth) and Von Damm (~2300 m depth) vent fields at the Mid-Cayman Spreading Centre and their faunas has provided a critical opportunity to further our understanding of the biodiversity, life-history biology and biogeography of vent species. Here I present *Rimicaris hybisae* sp. nov. (Caridea: Alvinocarididae), *Iheyaspira bathycodon* sp. nov. (Turbinidae: Skeneinae), and *Lebbeus virentova* sp. nov. (Caridea: Hippolytidae) from the Beebe and Von Damm vent fields. I elucidate the general reproductive features of the dominant species at Beebe and Von Damm (*R. hybisae*) and reveal a high degree of spatial variability in the population structure and reproductive features of this species at both these vent fields. I demonstrate inter-annual variation in the population structure and reproductive development of *R. hybisae*, superimposed upon the pattern of spatial variation, and hypothesise periodic or seasonal reproductive development in this species. Cluster analysis and the presence of *Rimicaris*-dominated faunal assemblages at the Beebe and Von Damm vent fields support higher-level taxonomic affinities with the fauna of Mid-Atlantic vents. These findings reveal previously unappreciated spatial variation in the reproductive development of a motile species at hydrothermal vents and expand our knowledge of the distribution of biodiversity. This work advances our understanding of biogeographic patterns of deep-sea chemosynthetic ecosystems, and provides a foundation to test the influence of various processes on the distribution of vent fauna at global scale.

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Declaration of authorship

I, Verity Nye declare that the thesis entitled *Life-history biology and biogeography of invertebrates in deep-sea chemosynthetic environments* and the work presented in the thesis is both my own, and has been generated by me as the result of my own original research. I confirm that this work was done wholly or mainly while in candidature for a research degree at this University. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated. Where I have consulted the published work of others, this is always clearly attributed. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work. I have acknowledged all main sources of help. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself. In these instances I confirm that I carried out the research reported in the publications and that I was predominantly responsible for writing the manuscripts. Co-authors assisted in the fieldwork and provided editorial assistance in the writing process. Sophie Plouviez provided molecular data for the description of *Lebbeus virentova* (Chapter 4). Parts of this work have been published as:

Nye V., Copley J. & Plouviez S. (2012) A new species of *Rimicaris* (Crustacea: Decapoda: Caridea: Alvinocarididae) from hydrothermal vent fields on the Mid–Cayman Spreading Centre, Caribbean. *Journal of the Marine Biological Association of the United Kingdom* 92, 1057-1072.

Nye V., Copley J., Linse K. & Plouviez S. (2013) *Iheyaspira bathycodon* new species (Vetigastropoda: Trochoidea: Turbinidae: Skeneinae) from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean. *Journal of the Marine Biological Association of the United Kingdom* 93, 1017-1024.

Nye V., Copley J., Plouviez S. & Van Dover C.L. (2013) A new species of *Lebbeus* (Crustacea: Decapoda: Caridea: Hippolytidae) from the Von Damm Vent Field, Caribbean Sea. *Journal of the Marine Biological Association of the United Kingdom* 93, 741-751.

Nye V., Copley J.T. & Tyler P.A. (2013) Spatial variation in the population structure and reproductive biology of *Rimicaris hybisae* (Caridea: Alvinocarididae) at hydrothermal vents on the Mid-Cayman Spreading Centre. *PLoS ONE* 8(3), e60319.

Nye V., Copley J.T. & Tyler P.A. (submitted) Fine-scale spatial variation and time-series of reproductive development in *Rimicaris hybisae* (Caridea: Alvinocarididae) at hydrothermal vent fields on the Mid-Cayman Spreading Centre. *PLoS ONE* (submitted).

Signed:

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Date: 11/12/2013

Acknowledgements

"When once [the deep ocean] has been seen, it will remain forever the most vivid memory in life, solely because of its cosmic chill and isolation, the eternal and absolute darkness and the indescribable beauty of its inhabitants." – WILLIAM BEEBE

I would like to acknowledge the Natural Environmental Research Council for funding this research (UK NERC award NE/F017774/1 to J. Copley, D. Connelly, P. Tyler, B. Murton and K. Stansfield).

I am more grateful than I can say to Jon Copley and Paul Tyler for granting me the opportunity to undertake this project and explore, discover and learn with you. It has been a privilege and joy to work and sail with you and to have such fine mentors and role models. Thank-you for your time, integrity and unwavering support, your ideas, discussions, enthusiasm and good humour, and the numerous contributions you've made to my work. Most of all, thanks for this experience! It has given me profound pleasure and moments of great joy and wonder that I shall cherish for the rest of my life.

I'm grateful to my panel chair Martin Sheader for your interest in my work and advice. Thanks to Cindy Van Dover for hosting me at Duke, and to Cindy, Chris German, and Ken Takai for their kind invitations to join research cruises, the provision of samples, and opportunity to dive in the *Shinkai6500*. Thanks to Katrin Linse, Sophie Plouviez and Anton Page for your guidance, encouragement and generosity of time. Special thanks to Pam, Ben, Elisa and Layla Nye for your patience, support and interest, and for keeping me grounded. Big cheers to Kirsty Morris, James Cooper, and Andrew Thaler for your friendship, loyalty, and good humour throughout. To my stars, Steve Nye, Joan Cartwright and Chris Andreani: I shine as brightly as I can in your honour.

1. Introduction

"Isn't the deep ocean supposed to be like a desert? Well, there's all these animals down here." – JACK CORLISS

1.1 Introduction

The process of chemosynthesis, whereby primary biological energy is produced from reduced inorganic chemicals rather than sunlight, has been recognised since the 19th century (Winogradski, 1887). Chemosynthesis was thought to make a very minor, insignificant contribution to the carbon budget of the marine environment (Kiel & Tyler, 2010). Furthermore, the deep ocean had long been perceived, in general, as being food-poor and devoid of any *in situ* primary productivity in the absence of sunlight, yielding only low levels of secondary production and biomass (Gage, 2003). This perception changed rapidly with the discovery of deep-sea hydrothermal vent systems and their lush faunal assemblages at the Galapagos Rift (Corliss & Ballard, 1977; Lonsdale, 1977; Corliss *et al.*, 1979), followed a few years later by the discovery of cold seeps on the Florida Escarpment, Gulf of Mexico (Brooks *et al.*, 1984; Paull *et al.*, 1984). It was soon recognised that chemosynthetic ecosystems could also be sustained in other chemically reducing environments, such as in association with large organic falls including wood (Distel, 2000) and whale falls (Smith *et al.*, 1989; Smith & Baco, 2003; Treude *et al.*, 2009), and in oxygen minimum zones (OMZs) (Gallardo, 1977; reviewed in Levin, 2003). Recent work has highlighted other sources of chemosynthetic primary production (e.g. nitrification) in non-benthic marine ecosystems (e.g. Glaubitz *et al.*, 2009; Molina *et al.*, 2012).

Although thermal springs were predicted to occur at seafloor spreading centres (Elder, 1965), the abundant invertebrate fauna associated with them, and the subsequent discovery of endosymbiotic relationships between invertebrate hosts and chemosynthetic bacteria capable of utilising the hydrogen sulfide emitted from hydrothermal vents (Cavanaugh *et al.*, 1981; Felbeck, 1981; Raugh, 1981), were profound revelations. These unanticipated discoveries altered one of the major paradigms in deep-sea biology, turning the view of the deep sea as a food-limited environment upside down, and commencing one of the most productive themes in deep-ocean science.

1.2 The physical properties of deep-sea chemosynthetic environments

To understand the ecology, evolution and biogeography of fauna inhabiting deep-sea chemosynthetic environments it is necessary to understand the physical properties of these ecosystems.

Deep-sea hydrothermal vents and cold seeps exhibit a patchy distribution through the world's oceans and occur in a variety of geological settings (Van Dover *et al.*, 2002). The global mid-ocean ridge (MOR) system is a nearly continuous, ~65, 000 km long volcanic chain that hosts a range of discontinuous hydrothermal settings (Van Dover *et al.*, 2002; Tyler *et al.*, 2003). Although much of the MOR remains unexplored for hydrothermal activity (German *et al.*, 2011), hydrothermal vents are now known to occur along all active mid-ocean ridges including the slowest spreading ridge crests (e.g. German *et al.*, 1998; Connelly & German, 2002; Edmonds *et al.*, 2003; Connelly *et al.*, 2012), at back-arc spreading centres and at some volcanic seamounts (Vrijenhoek, 2010).

Vent plume incidence along the MOR is positively correlated to spreading rate, though not always in a linear fashion (Baker *et al.*, 2008). For example, vent fields on the slow-spreading Mid-Atlantic Ridge (MAR) are spaced 100s of kilometres apart whereas those on the intermediate-spreading northern East Pacific Rise (EPR) may be separated by as little as 5 km (Van Dover, 1995). Other hydrothermal systems, such as those of Western Pacific back-arc basins and volcanic hotspots, are geographically and tectonically isolated from the MOR (Hessler & Lonsdale, 1991). Seeps occur worldwide on passive and active margins and are common deep-water habitats on the continental slope (reviewed by Sibuet & Olu, 1998, Tyler *et al.*, 2003; Levin *et al.*, 2005; Kühl, 2011). The patchy, discontinuous distribution of hydrothermal vents and cold seeps means that they are insular, isolated environments, analogous to 'island' habitats in the deep sea.

Hydrothermal vents are characterised by elevated temperatures relative to ambient deep-sea water, lower oxygen concentration and higher levels of toxic compounds (sulfide, metals) (Tunnicliffe, 1991). Most vent species, with few exceptions, live at temperatures similar to those of shallow-water habitats (Cuvelier *et al.*, 2011). Thus it is the chemistry and composition of the fluids, rather than elevated temperatures, that sustain life at these deep-ocean chemosynthetic environments (Jannasch, 1985). Temperatures at cold seeps are typically colder and less variable than

those found at vents and the interactions between biology and geology may be more complex at these lower temperatures (Sibuet & Olu, 1998). As a result of the chemistry and composition of fluids, rather than elevated temperatures, sustaining deep-sea chemosynthetic assemblages (Jannasch, 1985), variations in the fluid supply are an important factor in explaining the patchy and ephemeral occurrence of chemosynthetic fauna (Tunnicliffe, 1991; Sibuet & Olu, 1998).

Deep-sea chemosynthetic environments are remarkably heterogeneous, exhibiting spatial and temporal variability at a variety of scales (see below and Chapters 5-6). Hydrothermal vents exhibit steep thermal and chemical gradients and turbulent mixing between the hydrothermal fluids and cold surrounding seawater (e.g. Van Dover, 2000). Concentrations of oxygen and other oxidised compounds present in seawater (e.g. sulfate, nitrate) vary from ambient levels to undetectable (anoxic). Sulfide and reduced forms of other elements in diffuse flow vary from high concentrations (e.g. >80 mmol/kg) to undetectable (e.g. Von Damm, 2000; Fisher *et al.*, 2007). Gradients in pH range from acidic to alkaline seawater (e.g. Luther *et al.*, 2001; Le Bris *et al.*, 2006). Superimposed on these gradients are variations in fluid emission associated with tides and deep-sea currents (Johnson *et al.*, 1988a; Tivey *et al.*, 2002). The gradients result in high local variability on a scale of a few centimetres (e.g. Lutz & Kennish, 1993; Cuvelier *et al.*, 2011 references therein). This extreme environmental heterogeneity is often reflected in the microdistribution of vent assemblages (Copley *et al.*, 2003) and much of the vent fauna must be well adapted to these dynamic environmental conditions (Fisher *et al.*, 2007).

Cold seeps provide one of the greatest levels of local and regional habitat heterogeneity in any continental margin or deep-sea habitat (Cordes *et al.*, 2010). Although cold seeps lack the thermal gradients found at vents, sulfide and methane fluxes can be remarkably heterogeneous in space and time (Henry *et al.*, 1992; Sibuet & Olu, 1998), as exemplified by distinct patterns of faunal structure and distribution within seep habitats (e.g. MacDonald *et al.*, 1989; Barry *et al.*, 1997; Sahling *et al.*, 2002; Levin *et al.*, 2003; Van Dover *et al.*, 2003; Levin, 2005; Treude *et al.*, 2009).

Hydrothermal vents demonstrate temporal instability at many scales by a high frequency of disturbance (e.g. Van Dover, 2000). Temperature records from vent assemblages on a short time scale of days to seconds show rapid, unpredictable fluctuations, indicating that the fauna is subject to abrupt changes in vent flow and chemistry (Tunnicliffe *et al.*, 1985; Johnson *et al.*, 1988b; Gage & Tyler, 1991). More

extreme examples of disturbance are provided by descriptions of dead and senescent patches, and of nascent vent fields (Tunnicliffe *et al.*, 1997; Shank *et al.*, 1998), effectively illustrating the ephemeral nature of the vent environment.

Active hydrothermal vent activity may span years or decades (Lutz & Kennish, 1993), as revealed by studies of heat loss and rates of dissolution of giant white clam (*Calyptogena magnifica*) shells commonly found in massive death assemblages at previously active vent localities, by direct observations of assemblage changes through time, and by radiometric dating (Macdonald *et al.*, 1980; Turner & Lutz, 1984; Grassle, 1985; Lutz *et al.*, 1985, 1988; Fustec *et al.*, 1987; Tunnicliffe, 1991; Lalou *et al.*, 1993; 1995). The stability of the vent habitat (on an annual to decadal scale) is related to ridge spreading rate, with stability lowest at faster-spreading ridges (Juniper & Tunnicliffe, 1997). For example, Eastern Pacific vents are especially ephemeral, lasting for a few years to several decades before fluid conduits are blocked, magma supplies shift, or lava flows annihilate local faunal assemblages (Chevaldonné *et al.*, 1997). Vents at slow-spreading ridges are generally longer-lived, much bigger and more isolated than those at fast-spreading ridges (Fornari & Embley, 1995). New vents can be created by magmatic eruptions and earthquakes can open fluid conduits, re-activating old vents (Shank *et al.*, 1998).

Cold seeps are typically longer-lived and more stable over time than hydrothermal ecosystems on faster-spreading ridges and seepage is thought to provide a relatively stable source of reduced chemicals (Sibuet & Olu, 1998). For example, Gulf of Mexico seeps have been active since the last glaciation period and the area may have been seeping for 200,000 years (Aharon *et al.*, 1997). Craddock *et al.* (1995) suggested that this stability might create different selection pressures to those at hydrothermal vents, promoting local diversification and speciation, while Sibuet & Olu (1998) invoked long-term stability as a significant factor in the evolution of a diverse seep fauna.

The interaction of physical features of deep-sea chemosynthetic environments with other factors, such as life-history biology, play important roles in the distribution and adaptation of species inhabiting these environments (e.g. Shank *et al.*, 1998). The ephemeral and insular nature of vent and seep habitats raises a key question: how are populations of species established and maintained? Addressing this question has been a fundamental goal of hydrothermal vent and cold seep ecology (e.g. Tunnicliffe, 1991; Van Dover, 2000). Providing the answers to this question is essential for understanding

the biogeography, population connectivity and evolution of fauna in deep-ocean chemosynthetic environments (e.g. Chevaldonné *et al.*, 1997).

1.3 Invertebrate assemblages in deep-sea chemosynthetic environments

Hydrothermal vents and cold seeps are characterised by high chemosynthetic primary production (e.g. Lutz & Kennish, 1993). Both vent and seep assemblages require the presence of a chemically reduced compound (typically hydrogen sulfide, methane, or hydrogen) that can be oxidised by microbes to release energy for the formation of organic carbon from carbon dioxide, carbon monoxide, or methane (Van Dover *et al.*, 2002). These microbes may be present in the water column, at the seafloor as microbial mats, within sediments, fractures of crustal rocks or the sub-seabed, or in symbioses with larger multi-cellular organisms (Dubilier *et al.*, 2008). These microbiota mediate the transformation of chemical energy, thereby facilitating the development and maintenance of densely populated ecosystems in which both biomass and faunal abundances are very much greater than is typical at the deep seafloor (e.g. Lutz & Kennish, 1993; Smith *et al.*, 2008).

The dissolved oxygen required for aerobic microbial oxidation in deep chemosynthetic ecosystems originates from photosynthetic sources (Juniper & Tunnicliffe, 1997). Hydrogen is the principal electron donor under anaerobic conditions at vents, and nitrate, sulphate, and carbon dioxide act as electron accepters (Van Dover, 2000). The reduced compounds that fuel vent ecosystems are produced during convective circulation and geothermal heating of seawater through the upper ocean crust and are emitted in hydrothermal fluids (e.g. Van Dover *et al.*, 2000; Tolstoy, 2009; Coykendall *et al.*, 2011). Cold seeps are sustained by hydrocarbon reservoirs, methane hydrates, pore waters in sediments and sites of organic enrichment (e.g. see Tunnicliffe *et al.*, 2003 for review; Levin, 2005; Cordes *et al.*, 2010). In contrast to vents, the reduced fluids in seep ecosystems, generated from accumulations of terrestrial or marine organic matter, are derived from photosynthetic processes (Levin, 2005). The distribution of deep-sea chemosynthetic assemblages is therefore inherently tied to the processes that determine the nature and distribution of seepage and hydrothermalism.

Discoveries of deep-sea chemosynthetic habitats in the last four decades have significantly enhanced our understanding of the biodiversity and functioning of deep-

ocean ecosystems. In contrast to the majority of the deep sea, benthic vent assemblages are typically very high in biomass and relatively low in diversity (Hessler & Smithey, 1983; Lutz, 1984; Tunnicliffe, 1991), hence the analogy of vents as 'oases' in relatively barren submarine 'deserts' (Lutz, 1984). Like populations at vents the faunal assemblages hosted by cold seeps are high in biomass, but are generally more diverse than those inhabiting hydrothermal vents (Carney, 1994). Both vent and seep ecosystems exhibit high levels of endemicity: 70% in hydrothermal vents (Desbruyères *et al.*, 2006) and approximately 40% at seeps (Cordes *et al.*, 2006; Levin *et al.*, 2009). In contrast, endemicity in OMZs is considered to be relatively low (Levin *et al.*, 2009). Few (if any) of these endemics are globally cosmopolitan or even known throughout a particular ocean basin (Tunnicliffe *et al.*, 1996; Sibuet & Olu, 1998; Van Dover, 2011a and references therein.). Although some taxa are shared among chemosynthetic ecosystems (reviewed in Smith *et al.*, 2003), many genera and some higher taxa are known only from hydrothermal vents and not cold seeps and vice versa (Van Dover, 2000; see Wolff, 2005 for review). At the generic level, the bone-eating worms *Osedax* Rouse, Goffredi & Vrijenhoek, 2004 and wood-boring bivalves *Xylophaga* Turton, 1822 appear to be distributed broadly (Glover *et al.*, 2005; Voight, 2009).

As a result of the taxonomic novelty of deep-sea chemosynthetic assemblages, an impressive descriptive work has been conducted since the discovery of vents in 1977, producing more than 500 fully described new species (Wolff, 2005). A great number of additional species have been recorded from hydrothermal vents, including those also occurring in cold seeps, organic falls, and non-chemosynthetic environments (e.g. Wolff, 2005; Desbruyères *et al.*, 2006). Most of the species described to date belong to the mega- and macrofauna size classes, although meiofauna accounts for a considerable proportion of the total diversity in vents (Gollner *et al.*, 2010).

Less than 20% of Earth's entire ocean ridge system has been explored for hydrothermal activity, and although more than 200 vent, seep and organic-fall chemosynthetic sites have been visited and examined in some detail (Figure 1.1), there are an estimated order of magnitude more sites awaiting discovery (German *et al.*, 2011). Thus many species remain potentially to be discovered and described (Gollner *et al.*, 2010; Vanreusel *et al.*, 2010). Because chemosynthetic ecosystems are ultimately ephemeral and distributed sparsely across the deep seafloor, successful species must be capable of dispersal away from, and colonisation of these discrete 'islands' in the deep ocean (Bergquist *et al.*, 2003; Metaxas & Kelly, 2010; see below and Chapters 5-6).

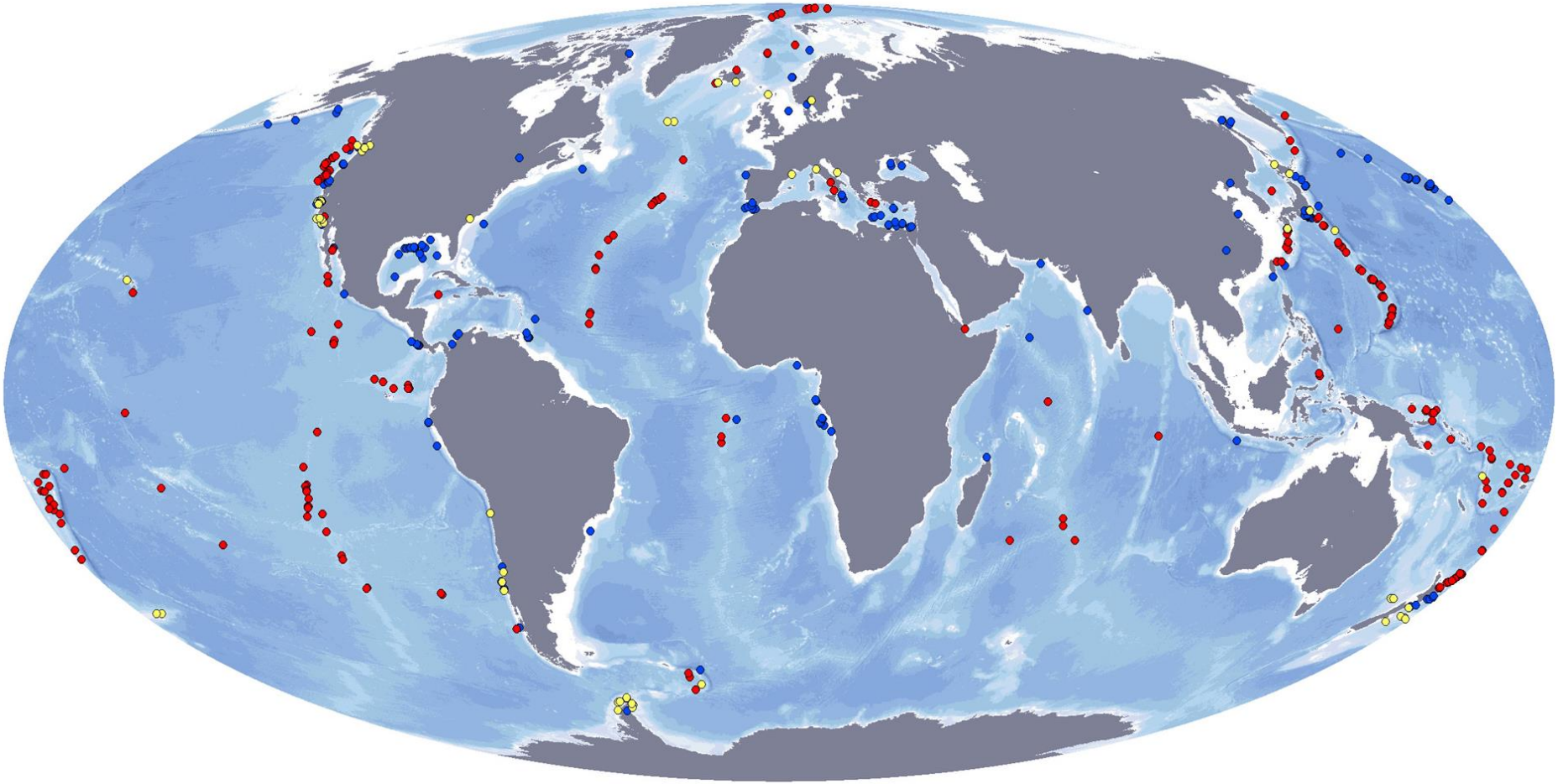


Figure 1.1. Global distribution of hydrothermal vent (red), cold seep (blue) and whale fall (yellow) sites that have been studied with respect to their fauna. Source: German *et al.* (2011) doi:10.1371/journal.pone.0023259.g002.

1.4 The biogeography of deep-sea hydrothermal vents

As a result of the lack of perceived environmental variability and geographical barriers, ranges of deep-ocean species were traditionally assumed to be exceedingly large. Consequently the general biogeographic paradigm discerned in the deep sea in the 19th Century was one of cosmopolitan faunas undifferentiated between ocean basins (e.g. Moseley, 1880; Van Dover, 2000). Exploration and discovery over the last 130 years has changed this perception radically, revealing a multitude of deep-ocean habitats (see Ramirez-Llodra *et al.*, 2010 for review), spectacularly high biodiversity (Sanders, 1968; Grassle, 1989) and extraordinary *in situ* evolutionary radiations (Little & Vrijenhoek, 2003).

Following extensive global sampling programmes in the 1950-1970s, many biogeographic classifications have been proposed (e.g. Vinogradova, 1979, 1997; Belyaev, 1989; Watling *et al.*, 2013; for reviews see e.g. Stuart *et al.*, 2003, McClain & Hardy, 2010; Ramirez-Llodra *et al.*, 2010), subdividing the deep-sea benthos into faunal regions or provinces, based on environmental parameters more than biological data (Ramirez-Llodra *et al.*, 2010). In contrast, vent biogeography has followed a biological-distribution approach (see below).

Chemosynthetic habitats with high endemicity challenged the concept of a single biogeographic realm for the deep ocean. The traits of hydrothermal vent assemblages - especially the high levels of endemism, their global distribution and constraint to discrete habitats separated at different spatial scales and by geological and environmental barriers, and their historical coupling to plate tectonics - and the ephemeral nature of hydrothermal circulation have probably had important implications for the composition, diversity and biogeography of their faunal assemblages and the dispersal and genetic structure of vent species (Van Dover, 2000; Vrijenhoek, 2010, 2013).

The initial discovery of vents at the Galapagos Rift (~2500 m depth) (Lonsdale, 1977; Corliss *et al.*, 1979) was rapidly followed by further discoveries of vents in the Pacific (Grassle, 1985; Tunnicliffe, 1988; Rona *et al.*, 1990). With the collection of hydrothermal sediments and shrimp from the Trans-Atlantic Geotraverse (TAG) region of the MAR in 1984 (Rona *et al.*, 1986), the first concept of vent biogeography arose, based on the observed distribution of vent taxa. The Pacific vents were dominated by tubeworms and alvinellid polychaetes whereas the alvinocaridid shrimp *Rimicaris*

exolculata Williams & Rona, 1986 was dominant at Atlantic vents, and vent mussels of the genus *Bathymodiolus* Kenk & Wilson, 1985 were found to be common to both biogeographic regions (Van Dover, 2000).

Although only a very small proportion of the vents and seeps that potentially exist in the worldwide deep sea have been explored, the continuing discovery of vent sites throughout the world's oceans has suggested the existence of several biogeographic provinces, described and hypothesised by several authors (Tunnicliffe *et al.*, 1996, 1998; Van Dover *et al.*, 2002; Tyler *et al.*, 2003; Shank, 2004; Ramirez-Llodra *et al.*, 2007; Bachraty *et al.*, 2009; UNESCO, 2009; Vrijenhoek, 2010; Moalic *et al.*, 2012; Rogers *et al.*, 2012; Desbruyères *et al.*, 2007 for back-arc basins).

Despite their global distribution, biogeographic relations among seep faunas are not well understood and seep biogeographic provinces have not been characterised definitively because seeps have been studied less extensively than hydrothermal vents (McArthur & Tunnicliffe, 1998; see Sibuet & Olu, 1998; Levin, 2005; Baco *et al.*, 2010). Depth-related patterns in assemblage composition have long been noted in the deep ocean (Gage & Tyler, 1991) and distinct differences among populations from different depths may be caused by environmental gradients that parallel depth (e.g. Jennings *et al.*, 2013 and references therein). A recent study on the biogeography of Atlantic Equatorial Belt seep faunas suggests that depth, rather than biogeographic region, may be the dominant factor structuring seep assemblages (Olu *et al.*, 2010). Depth has also been invoked as a factor determining the distribution of endemic taxa at hydrothermal vents (Tarasov *et al.*, 2005). However, Olu *et al.* (2010) highlight the need for the development of more molecular markers and population genetic studies to understand better the genetic connections among seep populations and regions. Furthermore, exploration and documentation of new areas would be required to test the hypothesis that depth is the dominant factor structuring seep assemblages.

Defining biogeographic provinces is challenging and typically requires *a priori* assumptions to be made (e.g. Moalic *et al.*, 2012). Vent provinces have generally been distinguished by differences in the taxonomic composition of their faunal assemblages (presence/absence). However, the number of vent fields analysed, the underlying hypotheses and the specific criteria used to define the provinces vary within the literature. Several authors (e.g. Van Dover *et al.*, 2002; Ramirez-Llodra *et al.*, 2007) do not specify the taxonomic level or specific criteria used to define the provinces, implying an interpretive approach based on correlations between the provinces and the

geographical locations of the ocean basins, degree of isolation along the MOR, bathymetry and vicariance events.

In contrast, Bachraty *et al.* (2009) proposed a statistical six-province model, based on multivariate analysis of species-level faunal composition from 63 hydrothermal vent fields. Concerns with the methodology used by these authors include the stability of the statistical model they employed and their choice of latitude and longitude as constraining variables for the cluster analysis (Rogers *et al.*, 2012). Studies of other deep-ocean ecosystems have demonstrated that analyses of presence/absence can miss significant differences in the structure of marine assemblages that can be resolved using species abundance or ranked abundance data (e.g. McClain *et al.*, 2009). Furthermore, present-day locations may not be good predictors for vent biogeography as a result of the fact that they do not reflect geographic proximity over evolutionary time scales nor consider other variables (e.g. topography, depth, currents and oceanic fronts which may act as filters to dispersal and gene flow: Won *et al.*, 2003; Hurtado *et al.*, 2004; Audzijonyte & Vrijenhoek, 2010; McClain & Hardy, 2010; Plouviez *et al.*, 2010; Vrijenhoek, 2010; Adams *et al.*, 2012) that are thought to influence deep-sea biogeography (Rogers *et al.*, 2012).

As a consequence of the different methodologies used, the delineations of provinces also vary within the literature. This is exemplified by the number of provinces hypothesised recently. For example, UNESCO (2009) and Vrijenhoek (2010), both modified from Van Dover *et al.* (2002), describe ten and six vent-provinces respectively. Debate concerns whether the shallow MAR vent fields, encompassing Menez Gwen (850 m), Lucky Strike (1700 m), and possibly Rainbow (2300 m), constitute a separate province (Van Dover *et al.*, 2002; Shank, 2004; Ramirez-Llodra *et al.*, 2007), or if they fall within the main MAR province (Ramirez-Llodra *et al.*, 2007). Also contentious is how many hydrothermal provinces exist in the Pacific (one, two or three) and whether Indian-Ocean vents constitute a novel biogeographic province or a subset of Pacific-vent fauna. A more recent analysis, based on multivariate analysis of a modified version of the Bachraty *et al.* (2009) dataset, suggests an 11-province model whereby vent ecosystems in the Southern Ocean represent a novel biogeographic province (Rogers *et al.*, 2012).

Moalic *et al.* (2012) applied network analysis to the Bachraty *et al.* (2009) dataset to infer that pathways between the five biogeographic provinces they proposed support the role of plate tectonics in the biogeographical history of hydrothermal vent

assemblages. These authors also suggested a possible ancestral position of the Western Pacific for the Annelida, Mollusca and Arthropoda, based on analyses at the genus and species levels for these phyla. However, whether Western-Pacific fauna occupy a basal position in the evolutionary history of these taxa remains to be tested by molecular analyses. The network topology they derived challenges the hypothesis of a major ancestral gateway between the MAR and EPR provinces through the Panama Isthmus (see below) and the connectivity proposed between the Southern Atlantic and Eastern Pacific via the Circumpolar Current (Van Dover *et al.*, 2002).

Despite the absence of a universal life-history adaptation to deep-sea chemosynthetic environments (see below and Chapter 5), some taxa are clearly more widespread than others in their distributions within and between these provinces. The similarities in hydrothermal vent assemblages are important because, in addition to helping define biogeographic provinces, they may also elucidate the links between different biogeographic provinces, which may further our understanding of their history and colonisation pathways, and the dispersal patterns of the fauna they contain (Moalic *et al.*, 2012). Despite thousands of publications on deep-sea chemosynthetic ecosystems in the literature in the last four decades, how their biogeographic regions are separated remains a first-order question.

As more of the global MOR is explored and additional vent assemblages are located and documented, and as under-sampled sites and cryptic species are given full account, the existing biogeographic classifications will evolve and become more refined. The strength of these classifications rests upon vigorous taxonomy, robust phylogenetic analyses and comprehensive sampling. Their value is as a tool for elucidating the mechanisms driving distribution patterns in these environments. Clear articulation of biogeographic classifications and processes is also essential to help balance exploitation and conservation in the deep ocean (e.g. Glover & Smith, 2003; Ramirez-Llodra *et al.*, 2011; Watling *et al.*, 2013). Hence the need to produce robust classifications of the biogeographic distribution patterns of vent fauna, incorporating sound morphological and molecular taxonomic data from the most recently discovered vent sites, with explicit statements of the methodology applied.

A wealth of potential factors shaping biogeographic patterns of extant vent taxa has been propounded in the literature (e.g. Tunnicliffe *et al.*, 1996, 1998; Van Dover *et al.*, 2002; Faure *et al.*, 2009). Although many genera and even some higher taxonomic groups are only known from cold seeps and not from hydrothermal vents and vice versa,

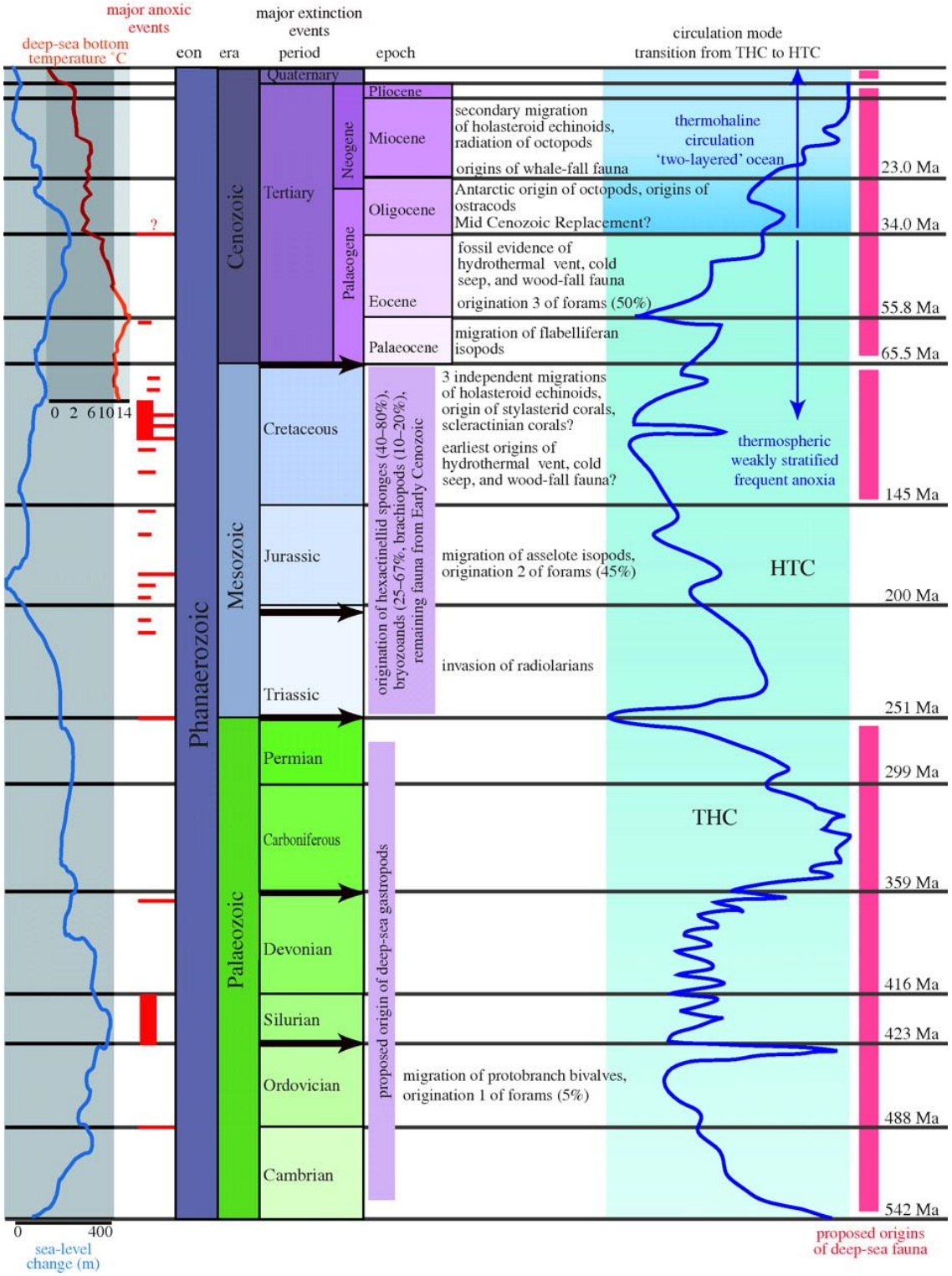
evidence for shared evolutionary histories, some shared species and phylogeographic relationships suggests that there are links between the invertebrate taxa of vents, seeps and organic falls on evolutionary and ecological timescales (Hecker, 1985; Sibuet & Olu, 1998; Van Dover, 2000; Little & Vrijenhoek, 2003; Smith *et al.*, 2003; Campbell, 2006).

The fossil record and molecular evidence suggest that many invertebrate taxa from contemporary chemosynthetic environments appear to have evolved recently (<100 Ma), with radiations of their contemporary crown taxa occurring mostly after the Palaeocene/Eocene thermal maximum, which led to a widespread anoxic/dysoxic event in deep-sea basins (Figure 1.2; Table 1.1; see Vrijenhoek, 2013 and Roterman, 2013 for reviews). Periods of anoxia/hypoxia have contributed to the distribution patterns and composition of fauna in the deep ocean (Rogers, 2000). The collective evidence challenges an early hypothesis that the diversity of taxa found in chemosynthetic environments is the result of a long and continuous evolutionary history (Newman, 1985). Evidence for the recent diversification of many taxa living in contemporary chemosynthetic environments supports the extinction/repopulation hypothesis and suggests that the faunal diversity may be the result of ecological opportunities created by periodic habitat destruction and renewal (Vrijenhoek *et al.*, 2013). Evidence of the evolutionary age of vent taxa has lead to the suggestion that Cenozoic tectonic history and oceanic circulation patterns have been significant in determining contemporary biogeographic patterns (Van Dover *et al.*, 2002).

The current distribution patterns of extant taxa are also the result of their respective dispersal potential, with or without the aid of evolutionary stepping stones such as whale falls and cold seeps (Van Dover *et al.*, 2002; Bachraty *et al.*, 2009; Vrijenhoek, 2010), which is determined in part by their life-history biology. Hence the need to determine the influence of life-history features on the biogeographic distributions of species in deep-sea chemosynthetic environments.

1.5 Reproduction in deep-sea chemosynthetic environments

The importance of population establishment and maintenance through reproduction, dispersal and recruitment in patchily-distributed and ephemeral deep-sea chemosynthetic environments was recognised immediately (Corliss *et al.*, 1979).



in degrees Celsius (orange line); major anoxic events (dark red blocks, width denotes regional vs. global); major extinction events (grey arrows within timescale), major migrations of fauna into the deep (text); circulation mode (dark blue line) and proposed origins of deep-sea fauna (light red blocks). Source: McClain & Hardy (2010) doi:10.1098/rspb.2010.1057.

Table 1.1. Estimated ages of groups of taxa living in contemporary chemosynthetic environments.

Group	Fossil record	Molecular evidence	References
Vestimentiferans (Polychaeta: Siboglinidae)	~400 Ma (based on fossilized tubes that may not be of Vestimentiferan origin)	~50-126 Ma	Black *et al.* (1997); Little *et al.* (1997); Halanych *et al.* (1998); Chevaldonné *et al.* (2002).
Osedax (Polychaeta: Siboglinidae)	~30 Ma	~42-45 Ma or Cretaceous (~65.5-145.5 Ma)	Rouse *et al.* (2004); Vrijenhoek *et al.* (2009); Kiel *et al.* (2010, 2011, 2013).
Amphisamytha (Polychaeta: Ampharetidae)	No record found	~44-55 Ma	Chevaldonné *et al.* (2002); Vrijenhoek *et al.* (2013).
Alvinellids (Polychaeta: Alvinellidae)	No record found	~41-51; split from *Paralvinella pandorae* ~98-121 Ma	Chevaldonné *et al.* (2002); Stiller *et al.* (2013); Vrijenhoek *et al.* (2013).
Vesicomyid clams (Vereroida: Vesicomyidae)	~45 Ma	~22-63 Ma; split between *Calyptogena*, *Abyssogena* and *gigas* spp. complex ~30 Ma	Peek *et al.* (1997); Amano & Kiel (2007); Vrijenhoek *et al.* (2013).
Bathymodiolin mussels (Mytiloida: Mytilidae: Bathymodiolinae)	~150 Ma (but may not be closely related to modern bathymodiolins) <40 Ma earliest confirmed	~20-94 Ma; split between 2 basal clades ~58 Ma; dominant clades of large vent & seep mussels split ~40 Ma	Campbell & Bottjer (1993); Little & Vrijenhoek (2003); Jones & Vrijenhoek (2006); Jones *et al.* (2006); Kiel (2010); Miyazaki *et al.* (2010); Kiel & Amano, 2013; Vrijenhoek *et al.* (2013); Lorien *et al.* (in press).
Neoamphaline limpets (Neoamphalina: Neoamphalidae + Peltospiridae)	~133 Ma	~59 Ma	Kiel & Campbell (2005); Campbell *et al.* (2008); Vrijenhoek *et al.* (2013).
Lepetodrilid limpets (Vetigastropoda: Lepetodrilidae)	No record found	~ 45-57 Ma	Vrijenhoek *et al.* (2013).
Abyssochysoids (Caenogastropoda: Abyssochysisae + Provannidae)	~50-180 Ma; ~85 Ma earliest verified	~110 Ma; *Alvinoconcha* & *Ifremeria* split ~40 Ma	Johnson *et al.* (2010); Kaim *et al*. (2008a, b); Kaim & Kelly (2009); Kiel *et al.* (2009); Vrijenhoek *et al.* (2013).

Stalked barnacles (Cirrepedia)	No record found	~20-70 Ma	Vrijenhoek *et al.* (2013).
Alvinocaridid shrimp (Decapoda: Caridea: Bresiliidae)	No record found	<20- ~70 Ma	Shank *et al.* (1999); Vrijenhoek *et al.* (2013); Yang *et al.* (2013).
Bythograeid crabs (Decapoda: Brachyura: Bythograeidae)	No record found	<20 ~56 Ma	Mateos *et al.* (2012); Vrijenhoek *et al.* (2013); Yang *et al.* (2013).
Kiwaidaeid crabs (Decapoda: Anomura: Chirostyloidea:Kiwaidae)	Cretaceous (~65.5-145.5 Ma)	~22.7-39.3 Ma; Pacific & non-Pacific lineage split ~13.4-25.9 Ma	Schweitzer & Feldman (2000); Roterman *et al.* (2013)

Understanding the reproductive biology of organisms inhabiting chemosynthesis-based assemblages is essential because it shapes our understanding of their dispersal among remote habitats (Parra *et al.*, 2009), and because the reproductive traits of an organism play a major role in the biogeography of the species (Van Dover, 2000; Ramirez-Llodra, 2002; Van Dover *et al.*, 2002; Tyler *et al.*, 2003). Evaluating dispersal capabilities and extent of gene flow may also aid in the identification of biogeographic barriers or filters to dispersal that act on evolutionary timescales (Van Dover *et al.*, 2002).

Life-history features (reproductive patterns, developmental mode) are very diverse in marine invertebrates (e.g. Ramirez-Llodra, 2002; Young, 2003). Based on the constant temperature regime of the deep ocean, Orton (1920) predicated that deep-sea species would not undergo the seasonal periodicities in their reproductive processes exhibited by their shallow-water counterparts subject to changes in water temperature. In shallow water most invertebrates display high fecundity and produce planktotrophic larvae which feed in the water and are dispersed by currents (Gage & Tyler, 1991). Studies have also highlighted the extreme heterogeneity in dispersal scale among shallow-water species and within functional groups (e.g. producers and herbivores) (e.g. Kinlan & Gaines, 2003). Thorson (1950) predicted that deep-sea species would be characterised by limited or no pelagic development. The expected lack of seasonal periodicities in reproductive processes in the deep sea has been revealed as invalid and studies demonstrating the existence of true planktotrophy among deep-sea species conflict with Thorson's theory (Gage & Tyler, 1991). The deep ocean and shallow marine realm are complex and varied environments that cannot be typified by a single

deep-sea or shallow-water life-history and dispersal pattern (e.g. Rex, 1979; Kinlan & Gaines, 2003).

There also arose an early prediction that there would be particular life-history adaptations to vent and seep environments because of their insularity and ephemerality. This prediction was soon refuted by observations of a variety of reproductive patterns (seasonal, periodic, continuous) and developmental modes (lecithotrophy, planktotrophy, direct development) in vent and seep species (reviewed by Tyler & Young, 1999; see Appendix 1). It was soon recognised that our traditional understanding of the relationships between developmental mode, dispersal potential, and geographic range may not always apply to deep-sea chemosynthetic systems (Lutz *et al.*, 1980, 1984), or even in the deep sea or shallow water in general (Kinlan & Gaines, 2003; Young, 2003). Consequently understanding dispersal in these environments remains a key area of research today (e.g. McGillicuddy *et al.*, 2010; Mullineaux *et al.*, 2010).

Despite the considerable amount of data that has been collected on the life histories of the main groups of deep-sea chemosynthetic fauna, the information on reproductive biology remains fragmented. More than 500 species have been described from these environments in the last four decades (Desbruyères *et al.*, 2006), yet just over 90 species have been the subject of reproductive studies and the entire life-history of a single species has been elucidated only recently (the bone-eating worm *Osedax japonicus* Fujikura, Fujiwara & Kawato, 2006 by Miyamoto *et al.*(2013). The reproductive biology and developmental modes of invertebrates in deep-sea chemosynthetic environments are reviewed in Appendix 1 (see also Chapters 5-6).

To address the first-order question of how the biogeographic provinces of deep-sea chemosynthetic ecosystems are separated, it is necessary to determine the influence of life-history features (reproductive patterns, modes of larval development and dispersal ability) on the biogeographic distributions of species in these environments.

1.6 Mid-Cayman Spreading Centre

Connections between the Atlantic and Indian Ocean ridge systems were severed approximately 90 Ma by closure of the Tethys Sea (Hessler & Lonsdale, 1991). The subsequent rise of the Panama Isthmus terminated deep-water exchange between the

Pacific and Atlantic Oceans (~10 million years ago (Ma)) (Burton *et al.*, 1997). This event resulted in the isolation of Pacific marine fauna from the Atlantic (Ramirez-Llodra *et al.*, 2007).

The only contemporary mid-ocean ridge connection between the biogeographic provinces of the eastern Pacific and the northern MAR is at high latitudes, via the Indian Ocean and Pacific-Antarctic ridge systems (Van Dover *et al.*, 2002). Prior to the complete closure of the Panama Isthmus by ~3.1 Ma, a low-latitude deep-water gateway connected the Pacific and Atlantic oceans via the Caribbean (Coates & Abando, 1996; Burton *et al.*, 1997). The close taxonomic similarities of seep fauna from the Gulf of Mexico and the margins of California and Oregon (Tunnicliffe *et al.*, 1996; Black *et al.*, 1998) suggest a dispersal pathway existed for seep species through the Panama Isthmus. Although this deepwater gateway lacked direct ridge connections, exchanges of vent fauna between the north EPR and MAR may have occurred through the seaway via other chemosynthetic environments, such as cold seeps, sunken wood and vertebrate bones (Bachraty *et al.*, 2009). This hypothesised exchange of vent fauna between the two oceans could have been facilitated further by ridge connections through the Caribbean (Van Dover, 1995). Alternatively, the Caribbean plate may not have facilitated faunal exchanges between the EPR and MAR because of its isolation from the Cocos and the North American plates (Bachraty *et al.*, 2009).

The presence of hydrothermal activity on ultraslow-spreading ridges (<2 cm yr^{-1}) was revealed at the end of the 1990s (German & Parson, 1998: Bach *et al.*, 2002; Edmonds *et al.*, 2003; Connelly *et al.*, 2007). Ultraslow-spreading ridges were previously considered incapable of hosting hydrothermal activity as a result of their reduced magmatic heat budget (Baker *et al.*, 2004). Slow and ultraslow ridges, however, represent half the global ridge system (Sinha & Evans, 2004). These ridges dominate the Atlantic, Arctic and southwest Indian Ocean (German *et al.*, 2010a) and represent a great diversity of venting styles (German & Lin, 2004). Observations made on these ridges have advanced our understanding of the tectonics, magmatic architecture, and hydrothermal evolution of oceanic crust, and are continuing to do so (Snow & Edmonds, 2007; Tao *et al.*, 2012). Ecological studies of vent fauna on ultraslow-spreading ridges are, however, in their infancy.

The Mid-Cayman Spreading Centre (MCSC) is a 110 km-long, ultraslow spreading (1.5 cm yr^{-1}) centre located along the Caribbean-North American plate boundary, in geographic isolation from the rest of the global ridge system (Figure 1.3)

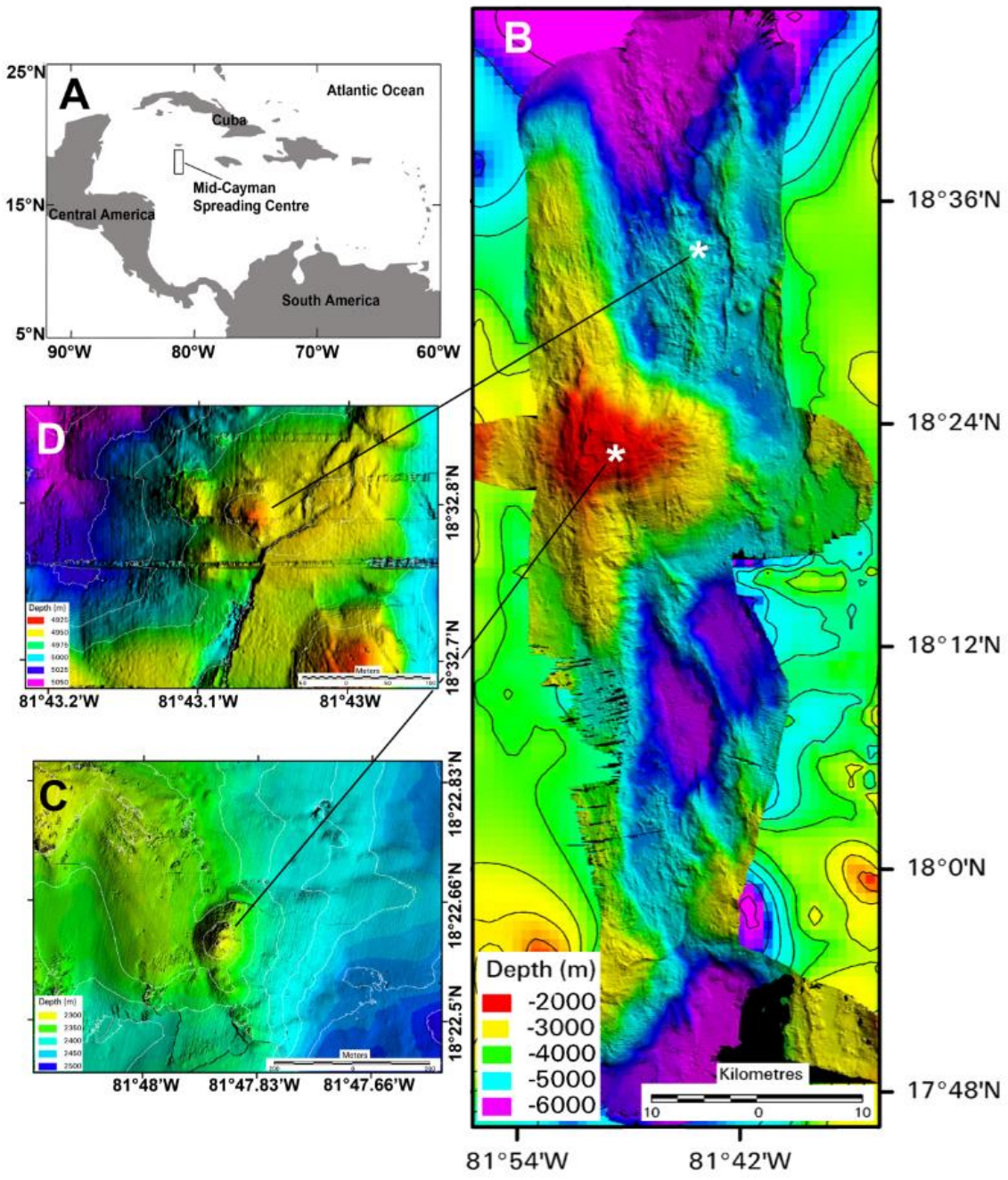


Figure 1.3. Location of the Mid-Cayman Spreading Centre (MCSC) and hydrothermal vent fields. A, Regional map of the MCSC; B, bathymetry of the MCSC; C, microbathymetry of the Von Damm Vent Field; D, microbathymetry of the Beebe Vent Field.
Source: Connelly *et al.* (2012) doi10.1038/ncomms1636.

(Ballard *et al.*, 1979; Hayman *et al.*, 2011). With an axis ranging in depth from 4200 m to >6000 m, it is the world's deepest seafloor spreading centre (ten Brink *et al.*, 2002). The MCSC lies along the pathway of the past deep-water connection that existed between the Atlantic and Pacific oceans prior to the closure of the Panama Isthmus, making it an essential study site to understand contemporary patterns of vent biogeography (German *et al.*, 2011). The MCSC was chosen as a key exploration and investigation area within the international Census of Marine Life ChEss programme. (2002-2010) to address the potential dispersal pathway of vent species between the MAR and the EPR-Galapagos Rift system.

In 2009, water column anomalies above the MCSC provided the first evidence for hydrothermal venting on the MCSC (German *et al.*, 2010b). In the following year, two active high-temperature vent fields in contrasting settings were discovered, visualised and sampled for the first time (Figure 1.3): the Von Damm Vent Field at 2,300 m depth on the uppers slopes of the Mount Dent Oceanic Core Complex; the Beebe Vent Field on the ridge axis at 4,960 m depth, which is the world's deepest known hydrothermal system (Connelly *et al.*, 2012). The discovery of these active hydrothermal vent fields and their chemosynthetic assemblages has provided a critical opportunity to further our understanding of the dispersal and evolution of vent species and the potential role of the closure of the Panama Isthmus as a vicariant event leading to the evolutionary divergence of Atlantic and Pacific faunas.

Characterisation of the fauna at MCSC vents will enable the following hypotheses to be tested: the fauna of MCSC vents is (1) similar to those of the northern EPR, from which they have been tectonically isolated since closure of the Isthmus of Panama (Tyler & Young, 2003); (2) similar to those of the MAR, from which they migrated over evolutionary history (or vice versa); (3) similar to those of Atlantic cold seeps from which they migrated over evolutionary history (or vice versa); (4) unique, having evolved in isolation from fauna of the northern EPR and Atlantic vents and seeps. These hypotheses are subordinate to the overall PhD thesis. Testing these hypotheses, however, has the potential to inform understanding of vent biogeography and elucidate factors influencing biogeographic patterns.

1.7 Aims and thesis outline

The main objective of this PhD was to investigate the composition, life-history biology and biogeographic affinity of the recently discovered faunal assemblages at the Beebe and Von Damm vent fields, MCSC, focusing on macrofaunal invertebrates. The thesis falls into three sections: the presentation of three species new to science, incorporating phylogenetic and biogeographic relationships with those of other taxa in other chemosynthetic sites (Chapters 2-4); the reproductive biology and ecology of the visually dominant species (Chapters 5-6); the biogeographic synthesis (Chapter 7).

The taxonomic characterisation of MCSC vent assemblages provides an initial indication of their affinities to existing biogeographic provinces. The phylogenetic analyses of MCSC vent species elucidate the evolutionary relationships of MCSC vent fauna with those of other regions. The determination of the gametogenic patterns in the visually dominant species advances knowledge of the reproductive biology of vent species and is used ultimately to examine whether taxa shared with other biogeographic provinces exhibit planktotrophic development, rather than other modes, and the influence of life-history biology on the biogeographic relationships among deep-sea hydrothermal vent faunas at the global scale.

Chapter 2 describes a new species of alvinocaridid shrimp (*Rimicaris hybisae* Nye, Copley & Plouviez, 2012) and emends the diagnosis of the genus *Rimicaris* Williams & Rona, 1986. This research incorporates new morphological, molecular and distributional data based on the examination of material collected recently from hydrothermal vent fields on the MCSC and type material. Data on the distribution of alvinocaridid shrimps in deep-sea chemosynthetic environments are reviewed and the biogeography of alvinocaridids is discussed.

Chapter 3 describes a new species of skeneid gastropod (*Iheyaspira bathycodon* Nye, Copley, Linse & Plouviez, 2013). This work includes new morphological, molecular and distributional data based on the examination of material sampled recently from the Von Damm Vent Field, MCSC. The distribution of turbinid gastropods described previously from deep-sea chemosynthetic environments is reviewed.

Chapter 4 describes a new species of hippolytid shrimp (*Lebbeus virentova* Nye, Copley, Plouviez & Van Dover, 2013). This research incorporates new morphological, molecular and distributional data based on the examination of material collected recently from the Von Damm Vent Field, MCSC. Data on the contemporary

geographical distribution and bathymetric range of the genus *Lebbeus* White, 1847 in hydrothermal vents are summarised.

Chapter 5 describes the general reproductive features of *Rimicaris hybisae* and examines the reproductive development and population structure of this species at the Beebe and Von Damm vent fields using spatially discrete samples collected in January 2012. The aims of this study were to: (1) examine variation in the population structure and reproductive features of *R. hybisae* between the Beebe and Von Damm vent fields; (2) assess spatial variation in the reproductive features of *R. hybisae* within the Beebe Vent Field; (3) discuss and compare the results with data available for other alvinocaridid species.

Chapter 6 investigates spatial variation in the population structure and reproductive development of *Rimicaris hybisae* at the Von Damm Vent Field for the first time, and extends studies of spatial variability of this species at the neighbouring Beebe Vent Field. In addition, it examines inter-annual variation in the population structure and reproductive development of *R. hybisae* between January 2012 and February 2013 at both vent fields. Using samples collected from the vent fields of the MCSC in February 2013, the aims of this study were: (1) to investigate spatial variation in the population structure and reproductive development of *R. hybisae* at the Von Damm Vent Field; and (2) to examine inter-annual variation in the population structure and reproductive development of *R. hybisae* between January 2012 and February 2013.

Chapter 7 provides a general synthesis of the previous chapters and examines the biogeographic relationships among deep-sea hydrothermal-vent faunas at global scale.

1.8 Wider implications

Globally distributed, ephemeral and insular deep-sea chemosynthetic environments with their endemic faunas provide ‘natural laboratories’ for studying the interactions between ecological and evolutionary processes that shape global patterns of marine life. Understanding interactions between ecological and evolutionary processes that determine global patterns of marine life are crucial to inform the sustainable use of marine resources and conservation strategies; this is particularly pertinent given the potential emergence of a vent mining industry in the foreseeable future (Hoagland *et al.*,

2010; Van Dover, 2011b). This work contributes to meeting several "challenges" in NERC's biodiversity and Earth system science themes, addresses NERC's science goal of "exploring ecosystems to discover novel biodiversity and increasing knowledge of the distribution of biodiversity", and advances our understanding of biogeographic patterns of chemosynthetic ecosystems, which was an objective of the international Census of Marine Life ChEss programme.

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2. A new species of *Rimicaris* (Crustacea: Decapoda: Caridea: Alvinocarididae) from hydrothermal-vent fields on the Mid-Cayman Spreading Centre, Caribbean

This work has been published as: Nye V., Copley J. & Plouviez S. (2012). A new species of *Rimicaris* (Crustacea: Decapoda: Caridea: Alvinocarididae) from hydrothermal vent fields on the Mid-Cayman Spreading Centre, Caribbean. *Journal of the Marine Biological Association of the United Kingdom* 92, 1057-1072. Doi: 1017/S0025315411002001. Published online 14 December 2011; code-compliant paper version published in August 2012.

Abstract

Rimicaris hybisae sp. nov. is described from hydrothermal-vent fields on the world's deepest seafloor spreading centre, the Mid-Cayman Spreading Centre (MCSC), Caribbean, at depths of 2300-4960 m. The new species is described and illustrated on the basis of 17 specimens. Brief notes on the distribution and habitat of the new species are provided. Molecular phylogenetic data from mitochondrial COI (460 bp), 16S ribosomal DNA (549 bp) and nuclear 18S ribosomal DNA (576 bp) regions are used to compliment the description. Morphological variation within *R. hybisae* sp. nov. and morphological affinities with previously described species are discussed. Based on morphological and molecular evidence, the new species is provisionally assigned to the genus *Rimicaris*, and differs from all known species in the genus by a distinctive pair of "pores" on the posterior lobes of its four-lobed dorsal organ. An emended diagnosis for *Rimicaris* is provided. *Rimicaris hybisae* sp. nov. is the first taxon to be described from MCSC vent fields. This record extends the known geographic range of *Rimicaris* into the Caribbean Sea and constitutes the deepest documented occurrence of alvinocaridid shrimp.

2.1 Introduction

All known species of the caridean family Alvinocarididae Christoffersen, 1986 occur exclusively in hydrothermal vents or cold (brine and hydrocarbon) seeps (see Table 2.1 and references therein). Alvinocaridids are known from a considerable number of vents and seeps in the Atlantic, Pacific and Indian Oceans (Table 2.1). Alvinocaridid shrimp are the dominant macrofaunal invertebrates at several vents along the Mid-Atlantic Ridge (MAR) (e.g. Van Dover *et al*., 1988; Segonzac, 1992; Gebruk *et al*., 1993, 1997; Van Dover, 2000; Martin & Shank, 2005) and at known vent fields in the Indian Ocean (Hashimoto *et al*., 2001; Van Dover *et al*., 2001; Watanabe & Hashimoto, 2002). The Alvinocarididae is presently comprised of 26 described species in 8 genera, all from chemosynthetic environments in the bathymetric range 252-4088 m (Table 2.1).

Alvinocaris Williams & Chace, 1982 is presently the only genus known to inhabit both hydrothermal vents and cold seeps (Table 2.1). *Alvinocaris*, *Rimicaris* Williams & Rona, 1986, *Chorocaris* Martin & Hessler, 1990 and *Opaepele* Williams & Dobbs, 1995 were originally assigned to the caridean family Bresiliidae Calman, 1896 (see Martin & Davis, 2001; Komai & Segonzac, 2003), whereas the family Mirocarididae Vereshchaka, 1997 was established to accommodate the genus *Mirocaris* Vereshchaka, 1997. Komai & Segonzac (2003) subsequently assigned all these genera to the Alvinocarididae, and synonymised the family Mirocarididae with the Alvinocarididae.

The family Bresiliidae is now only represented by two genera (De Grave *et al.*, 2009), *Bresilia* Calman, 1896 and the monotypic *Encantada* Wicksten, 1989, neither of which are known to occur in chemosynthetic habitats (Wicksten, 1989; Komai & Yamada, 2011). The family Alvinocarididae (Christoffersen, 1986, 1990; Segonzac *et al*., 1993; Komai & Segonzac, 2003) is now regarded as a valid monophyletic family (e.g. Martin & Haney, 2005; Komai & Segonzac, 2005, 2008; De Grave et al., 2009), morphologically distinct from opportunistic shrimp species recorded from vents and seeps (see Martin & Haney, 2005; Desbruyères *et al*., 2006 for recent reviews).

Recently, two high-temperature hydrothermal vent fields and chemosynthetic communities were discovered on the world's deepest seafloor spreading centre, the Mid-Cayman Spreading Centre (MCSC), Caribbean (Connelly *et al.*, 2012). The ~110 km long, ultraslow-spreading (15 mm yr^{-1}) MCSC has been active for approximately 49 My (Rosencrantz *et al*., 1988; German *et al*., 2010) and is located in a deep trough, geographically and tectonically isolated from the global mid-ocean ridge system

Table 2.1. Summary of known geographic distribution, bathymetric range and habitat of alvinocaridid shrimp species (confirmed locations and fully described species only).

Species	Site (s)	Depth (m)	Habitat	References
Alvinocaridinides formosa	NE Taiwan: Gueishandao	252-275	vent	Komai & Chan (2010)
Alvinocaris alexander	KR: Rumble V Seamount, Brothers Caldera	367-1346	vent	Ahyong (2009)
Alvinocaris brevitelsonis	OT: Minami-Ensei Knoll	705	vent	Kikuchi & Hashimoto (2000); Komai & Segonzac, (2005)
Alvinocaris chelys	NE Taiwan: Gueishandao	252-300	vent	Komai & Chan (2010)
Alvinocaris dissimilis	OT: Minami-Ensei Knoll	705	vent	Komai & Segonzac (2005)
Alvinocaris komai	ELSC: ABE, Kilo Moana, TowCam, Tu'i Malila	1880-2700	vent	Zelnio & Hourdez (2009)
Alvinocaris longirostris	OT: Iheya Ridge, Hatoma Knoll; SB: Off Hatsushima site	1053-1627	vent & seep	Kikuchi & Ohta (1995); Fujikura *et al*. (1995); Watabe & Miyake (2000); Ohta & Kim (2001)
Alvinocaris lusca	GR: Rose Garden; EPR: 9°N	2450-2520	vent	Williams & Chace (1982); Shank *et al*. (1999)
Alvinocaris markensis	MAR: Lucky Strike; Rainbow; Broken Spur; TAG; Snake Pit; Logatchev	1693-3650	vent	Williams (1988); Shank *et al*. (1999)
Alvinocaris methanophila	Blake Ridge Diapir	2155	seep	Komai *et al*. (2005)
Alvinocaris muricola	GoM: Florida Escarpment; Barbados Accretionary prism; West African equatorial margin, Congo Basin; Blake Ridge Diapir	1697-3277	seep	Williams (1988); Komai & Segonzac (2005); Komai *et al*. (2005)
Alvinocaris niwa	KR: Rumble V Seamount, Brothers Caldera	360-1538	vent	Webber (2004)

Alvinocaris stactophila	GoM: Louisiana Slope	534	seep	Williams (1988)
Alvinocaris williamsi	MAR: Menez Gwen	850-865	vent	Shank & Martin (2003)
Chorocaris chacei	MAR: Moytirra; Lucky Strike; TAG; Snake Pit; Logatchev	1600-3650	vent	Williams & Rona (1986); Komai & Segonzac (2008); Copley *et al*. (unpublished data)
Chorocaris paulexa	EPR: 17°-21°S	2573-2832	vent	Martin & Shank (2005)
Chorocaris vandoverae	MBAB: Alice Springs, Burke	3640-3660	vent	Martin & Hessler (1990)
Mirocaris fortunata	MAR: Moytirra; Menez Gwen; Lucky Strike; Rainbow; Broken Spur; TAG; Snake Pit, Logatchev; Ashadze; Turtle Pits	850-4080	vent	Martin & Christiansen (1995); Vereshchaka (1997); Shank *et al*. (1999); Komai & Segonzac (2003); Komai *et al*. (2007); Fabri *et al*. (2011); Copley *et al*., unpublished data
Mirocaris indica	CIR: Kairei, Edmond	2422-3300	vent	Komai *et al*. (2006)
Nautilicaris saintlaurente	NFB: White Lady, Mussel Valley; LB: Hine Hina, Vaï Lili; KR: KR: Brothers Caldera	1604-2000		Komai & Segonzac (2004); Ahyong (2009)
Opaepele loihi	Hawaii: Loihi Seamount	980	vent	Williams & Dobbs (1995)
Opaepele susannae	MAR: Sisters Peak; Lilliput; Semenov	1500-2986	vent	Komai *et al*. (2007); Beltenev *et al.* (2009)
Opaepele vavilovi	MAR: Broken Spur	~3090	vent	Lunina & Vereshchaka (2010)
Rimicaris exoculata	MAR: Moytirra; Rainbow; Lucky Strike; Broken Spur; Snake Pit; Logatchev; Ashadze; Mephisto	1700-4088	vent	Williams & Rona (1986); Komai *et al*. (2007); Komai & Segonzac (2008); Copley *et al*. (unpublished data)

Rimicaris kairei	CIR: Kairei; Edmond	2415-3320	vent	Watanabe & Hashimoto (2002)
Rimicaris hybisae sp. nov.	MCSC: Beebe; Von Damm	2300-4960	vent	Nye *et al*. (2012)
Shinkaicaris leurokolos	OT: Minami-Ensei Knoll	~700	vent	Kikuchi & Hashimoto (2000); Komai & Segonzac (2005)

CIR, Central Indian Ridge; ELSC, East Lau Spreading Centre; EPR, East Pacific Rise; GoM, Gulf of Mexico; GR, Galapagos Rift; KR, Kermadec Ridge, New Zealand; LB, Lau Basin; MAR, Mid-Atlantic Ridge; MBAB, Mariana Back-Arc Basin: MCSC, Mid-Cayman Spreading centre; OT, Okinawa Trough; SB, Sagami Bay.

(Ballard *et al*., 1979). The Beebe Vent Field consists of a sulfide mound (80 m diameter, 50 m height) surmounted with several actively venting sulfide chimneys and, at 4960m, is the worlds' deepest known vent field (Connelly *et al*., 2012). The Von Damm Vent Field occurs 30 km from the Beebe Vent Field on the upper slopes of an off-axis oceanic core complex, at 2300 m depth (Connelly *et al*., 2012). The Von Damm Vent Field is a conical mound (150 m diameter, 75 m height) venting predominantly clear, buoyant, high-temperature (>140ºC) fluids from orifices at its peak (Connelly *et al*., 2012). Study of the fauna inhabiting these unique vents has the potential to enhance current understanding of the dispersal, isolation, and evolution of vent taxa and patterns of vent biogeography.

The fauna at both vent fields is dominated by dense aggregations of *Rimicaris hybisae* (Figure 2.1), a new species of alvinocaridid shrimp, with a greatly reduced rostrum, and a four-lobed dorsal organ, similar to the photoreceptor of *Rimicaris* (Van Dover *et al*., 1989). *Rimicaris hybisae* sp. nov., described and illustrated herein, is the first taxon to be described from vent fields on the MCSC. In addition to enhancing existing knowledge of biodiversity, this record extends the known geographical range of *Rimicaris* (previously only recorded from Atlantic and Indian Ocean vents; Table 2.1) westwards into the Caribbean Sea, and extends the known bathymetric range of the Alvinocarididae by 872 m

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2.2 Materials and methods

The specimens were collected during the 44th cruise of the *RRS James Cook* in April 2010 from the Beebe (4960 m depth) and Von Damm (2300 m depth) vent fields

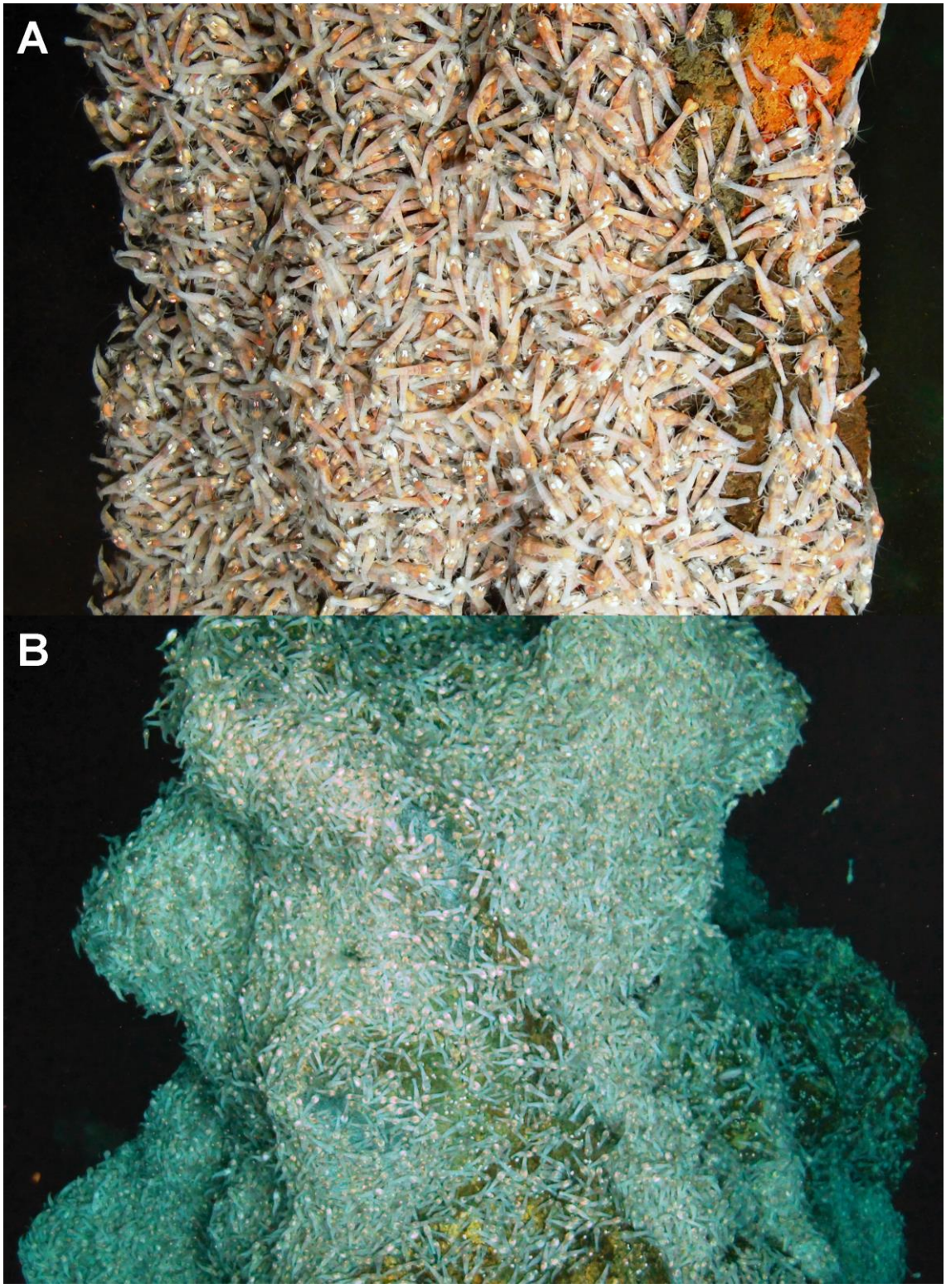

Figure 2.1. *Rimicaris hybisae* sp. nov., *in situ*. A, Beebe Vent Field; B, Von Damm Vent Field.

on the Mid-Cayman Spreading Centre (MCSC), Caribbean. Shrimp samples were taken using a grab (Von Damm) and suction sampler (Beebe) attached to the manoeuvrable TV grab *HyBIS* (Hydraulic Benthic Interactive Sampler), together with still photographs and video recordings of them *in situ* at both sites. Material for morphological study was immediately fixed in 10% neutralized formalin and subsequently transferred to 80% IMS (Industrial Methylated Spirits) on return to the laboratory. Material for molecular analysis was immediately placed in 95% ethanol.

The measurements taken for each specimen were measured to the nearest 0.1 mm using Vernier callipers. Post-orbital carapace length (CL) was measured from the midpoint of the posterodorsal margin to the level of the posterior margin of the orbit. Maximum total length (TL) was measured from the posterior margin of the telson to the anterior margin of the antennal scale. Maximum carapace width (CW) and maximum carapace depth (CD) were measured at the widest and deepest points of the carapace respectively. Specimen size is indicated herein by postorbital carapace length (CL).

Individuals were sexed under a dissecting microscope. Males were distinguished by an asymmetrical mesial extension on the endopod of pleopod 1 and the presence of the appendix masculina on the second pleopod (Willliams, 1988). The sex of specimens CL 6.7 mm and smaller could not be determined by this method; those specimens are referred to as juveniles.

Drawings were prepared with the aid of a camera lucida mounted onto a Leica MZ8 stereomicroscope, scanned and digitally inked using Adobe® Illustrator® and a WACOM™ digitiser, as described by Coleman (2003, 2009). The material examined is deposited in the Natural History Museum, UK (NHMUK). The descriptive terminology used follows that of Komai & Segonzac (2008).

One specimen from the Beebe Vent Field was used for scanning electron microscopy (SEM) and was not designated as a paratype. This specimen was dehydrated through a graded ethanol series, critical point dried and sputter coated with gold palladium prior to examination with a FEI Quanta 200 Scanning Electron Microscope at accelerating voltage of 10 kV.

Abdominal muscle for DNA extraction was cut from the shrimp of ethanol-preserved specimens (from both the Beebe and Von Damm vent fields) and the carapace removed. Genomic DNA was extracted using the CTAB (cetyltrimethyl ammonium bromide) procedure (Doyle & Dickson, 1987). Regions of the mitochondrial genes Cytochrome *c* Oxidase subunit I gene (COI) and 16S ribosomal DNA, and of the

nuclear 18S ribosomal DNA gene were amplified by performing polymerase chain reactions (PCR).

The COI region was amplified with universal primers LCO1490 and HCO2198 (Folmer *et al*., 1994). The 20 µl amplification mixture contained 1X buffer reagent (200 mM Tris pH 8.8, 500 mM KCL, 0.1% Triton X-100, 2 mg/ml bovine serum albumen), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 mM of each primer, 1 U Taq DNA polymerase (Bioline), 5 µl of template DNA and sterile H_2O to final volume. Thermal cycling conditions were: 94°C/2 min; followed by 5 cycles at (94°C/35 s; 45°C/35 s; 72°C/1:20 min) and 35 cycles at (94°C/35 s; 50°C/35 s; 72°C/1:20 min) with a final extension of 72°C/10 min.

For the 16S gene, PCR amplifications were performed using the universal primers 16Sar and 16Sbr (Palumbi, 1996) and a 20 µl amplification mixture: 1X reaction buffer (same as for COI), 2.5 mM $MgCl_2$, 0.13 mM of each dNTP, 0.38 mM of each primer, 1 U Taq DNA polymerase (Bioline), 2.5 µl of template DNA and sterile H_2O to final volume. Thermal cycling conditions were: 94°C/4 min; 30 cycles at (94°C/30 s; 52°C/1 min; 72°C/2 min) and 72°C/5 min.

PCR amplifications of the 18S gene were performed using universal primers 18SuniF and 18SuniR (Sogin, 1990) in an amplification mixture as described for COI. Thermal cycling conditions were: 95°C/5 min; 30 cycles at (94°C/1 min; 64°C/1 min; 72°C/2:30 min) and 72°C/10 min. Negative controls were included as standard and sterile procedures were consistently followed for all PCR experiments.

PCR products were purified with the ExoAP treatment by adding the following ExoAP mixture to 15 µl PCR product: 0.2 µl 10X ExoAP buffer (50 mM Bis-Tris, 1mM $MgCl_2$, 0.1 mM $ZnSO_4$), 0.05 µl 5000 U/ml Antarctic phosphatase (New England Biolabs: Ipswich, MA), 0.05 µl 20000 U/ml exonuclease I, and 3.7 µl sterile H_2O) and thermal-cycler incubation (37°C/60 min; 85°C/15 min). Sequencing reactions were performed using BigDye Terminator Reactions following the manufacturer's protocol (Applied Biosystems: Foster, CA) with the same primer sets used for amplifications. For COI, the thermal-cycler reaction was performed as: 94°C/30 s followed by 25 cycles at (94°C/15 s; 50°C/15 s; 60°C/3 min). For 16S and 18S the PCR conditions were identical to those described for COI, except for the use of a 52°C and 64 °C annealing temperature respectively. Sequencing reaction products were purified with the AMPure magnetic bead system (Agencourt: Morrisville, NC) following the

manufacturer's protocol and were subsequently run on an ABI 3730x1 DNA Analyzer (Applied Biosystems International).

The sequence strands for each gene were proof read and assembled with CodonCode Aligner, version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), to produce a continuous fragment. Sequences were compared with those in GenBank using the nucleotide BLAST program (NCBI Basic Alignment Search Tool) and manually aligned in BioEdit (Hall, 1999). Phylogenetic trees were constructed with *MEGA*5 (Tamura *et al*., 2011) using the neighbour-joining (NJ) (Saitou & Nei, 1987) and maximum-likelihood (ML) (Kimura, 1980) methods on 460- and 540-base pair (bp) alignments for COI and 18S respectively. Bootstrap values were calculated on 1000 re-sampling replicates.

GenBank accession numbers for partial sequences of the 16S, COI and 18S regions are JN850606, JN850607 and JN850608 respectively.

2.3 Systematics

Order DECAPODA Latreille, 1802

Infraorder CARIDEA Dana, 1852

Superfamily BRESILOIDEA Calman, 1896

Family ALVINOCARIDIDAE Christoffersen, 1986

Genus *Rimicaris* Williams & Rona, 1986

Rimicaris Williams & Rona, 1986: p.447 (in part); Martin & Hessler, 1990: p.8; Holthuis, 1993; Martin & Haney, 2005: p.467; *Iorania* Vereshchaka, 1996: p.952; Komai & Segonzac, 2008: p.22.

2.4 Type species

Rimicaris exoculata Williams & Rona, 1986

2.5 Diagnosis (emended)

Carapace greatly inflated laterally, distinctly broader than pleon, dorsal surface rounded, pitted with scattered, shallow punctuations. Pterygostomial expansion produced, exceeding antennal lobe, covering greater part of antennal bascicerite, rounded or blunt. Rostrum reduced to broadly rounded lobe. Eyes lacking pigment, eyestalks flattened, greatly reduced and medially fused. Antennal scale broadly oval, bearing distolateral transverse suture. Mandible with two-segmented palp, distinct separation between incisor and molar processes. Maxilla with scaphognathite greatly expanded anteriorly and conspicuously setose on dorsal and ventral surfaces. First maxilliped with greatly expanded exopod, similar to scaphognathite, conspicuously setose on dorsal and ventral surfaces. Third maxilliped with three long segments and coxa.

2.6 Composition

Rimicaris exoculata Williams & Rona, 1986 (Mid-Atlantic Ridge, 45°N-4°47S), *Rimicaris kairei* Watanabe & Hashimoto, 2002 (Central Indian Ridge, Kairei and Edmond vent fields) and *Rimicaris hybisae* sp. nov. (Beebe and Von Damm vent fields, Mid-Cayman Spreading Centre, Caribbean).

Rimicaris hybisae sp. nov.

(Figures 2.1-2.8)

2.7 Type material

Holotype: adult female, CL 15.3 mm. Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean Sea; co-ordinates: 18º 22.605' N 81º 47.875' W; water depth: 2300 m, [NHMUK 2011.8054]. Collected on the 44th voyage of *RRS James Cook*, on 18 April 2010.

Paratypes: adult male, CL 9.4 mm. Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean Sea; co-ordinates: 18º 22.605' N 81º 47.875' W; water depth: 2300 m, [NHMUK 2011.8055]. Collected during the 44th cruise of the *RRS*

James Cook, on 18 April 2010. Adult female, CL 10.8 mm; adult female with visible mature ovary, CL 7.0 mm; seven adult males, CL 7.0-11.8 mm; six juveniles, CL 3.6-6.7 mm. Beebe Vent Field, Mid-Cayman Spreading Centre, Caribbean Sea; co-ordinates: 18º 32.785' N 81º 43.080' W; water depth: 4960 m, [NHMUK 2011.8056-8070]. Collected on the 44th voyage of *RRS James Cook*, on 15 April, 2010.

2.8 Comparative material examined

Chorocaris chacei (Williams & Rona, 1986). Ten males, CL 9.7-13.0 mm [MNHN-Na17811]. Mid-Atlantic Ridge (Lucky Strike: Tour Eiffel; 37°17'N, 32°17'W). Collected by net, 1689 m depth.

Chorocaris vandoverae Martin & Hessler, 1990. Paratypes: Seven females, CL 8.0-12.6 mm [USNM 243947]. Mariana Back Arc Basin (Alice Spring Vent Field; 18°12.599'N, 144°42.231'E). Collected by net, 3640 m depth.

Rimicaris exoculata Williams & Rona, 1986. Paratypes: Ten females, CL 14.3-18.4 mm [USNM 228447]. Mid-Atlantic Ridge (TAG; 26°08.18'N, 44°49.36'W). Collected by dredge, 3620-2650 m depth. Paratypes: five juveniles, CL 8.4-9.2 mm [USNM 228454]. Labelled as *Rimicaris chacei*; classified as *R. exoculata* juveniles at stage B by Komai & Segonzac, 2008. Mid-Atlantic Ridge (TAG; 26°08.3'N, 44°49.6'W). Collected by dredge, 2650-3620 m depth. Twenty four additional specimens: Eleven males, CL 11.3-18.1; thirteen females, CL 8.8-18.4 mm from J. Copley's reference collection. Mid-Atlantic Ridge (TAG).

Rimicaris kairei Watanabe & Hashimoto, 2002. Paratype: One female, CL 19.3 mm [USNM 1005217]. Central Indian Ridge, Rodriguez Triple Junction (Kairei Vent Field; 25°19.16'S, 70°02.40'E). Collected by suction sampler, 2454 m.

Opaepele loihi Williams & Dobbs, 1995. Paratypes: Twenty females, CL 8.0-11.7 mm [USNM 1005217]. North Pacific Ocean (Loihi Seamount; 18°55 N, 155°16 W). Collected by baited traps, 990 m depth.

2.9 Diagnosis

Rostrum reduced to broadly rounded lobe, nearly reaching, reaching or slightly overreaching anterior margins of medially-fused eyes; ventral surface flat or slightly

convex. Carapace and pleon with scattered minute setae; antennal lobe broadly triangular or rounded; pterygostomial lobe rounded or blunt. Fourth pleonal pleuron posterolateral margin unarmed or armed with one tooth; produced into subacute posteroventral angle. Fifth pleonal pleuron posterolateral margin unarmed marginally or armed with 1-4 teeth; produced into acute posteroventral angle. Antennae not operculiform; distolateral tooth first antennal peduncle subacute or blunt; antennal scale distolateral tooth subacute. Scaphognathite of maxilla and caridean lobe of first maxilliped bearing numerous plumose setae-like structures on dorsal and ventral surfaces; exopodal flagellum of first maxilliped completely reduced. Appendix masculina tapering distally with 7-8 spiniform setae restricted to tip. Uropodal protopod posterolateral projection triangular with acute tip.

In life, with four-lobed dorsal organ; lobes fused anteriorly; posterior lobes with paired “pores” (Figure 2.2).

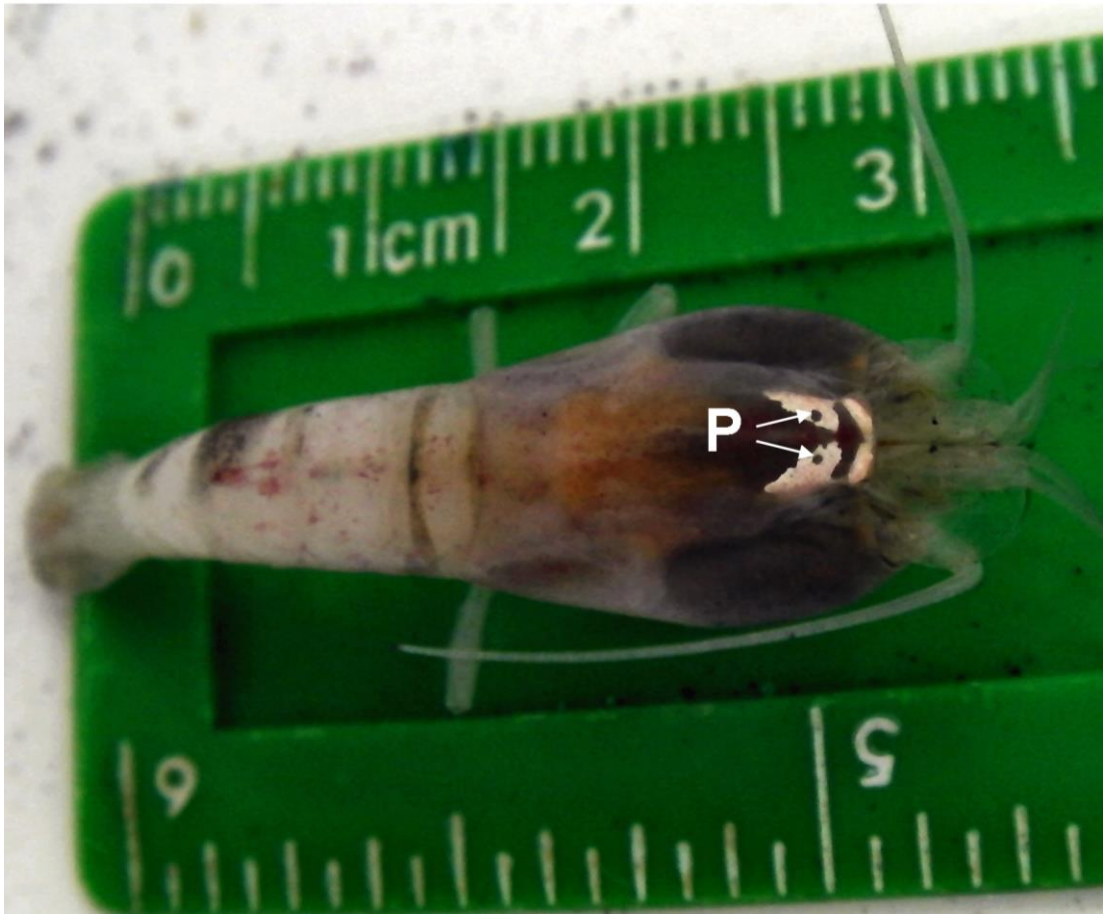

Figure 2.2. *Rimicaris hybisae* sp. nov., live specimen, from the Beebe Vent Field, Mid-Cayman Spreading Centre. P denotes twin pores in the dorsal organ.

2.10 Description

Body integument smooth, firm, pitted with scattered, very shallow punctuations bearing minute scattered setae (including rostrum). Carapace (Figure 2.3) ovate-oblong, generally broader than deep in greatest dimensions (Table 2.2), distinctly broader than pleon; branchial regions distinctly inflated but to a lesser extent than that of *Rimicaris exoculata* Williams & Rona, 1986. Rostrum reduced to broadly rounded lobe; nearly reaching, reaching or slightly overreaching anterior margins of fused eye-stalks; dorsal surface rounded, ventral surface flat or slightly convex. Carapace dorsal surface rounded; obsolescent epigastric ridge present, broader than breadth between antennal lobes, defined by conspicuous grooves extending from bases of antennal lobes; ventral margin reinforced by low submarginal ridge, most robust and farthest from margin posteriorly. Antennal lobe rounded or broadly triangular, tip blunt or subacute; anterolateral margin between antennal lobe and pterygostomial expansion slightly convex. Pterygostomial expansion produced, exceeding antennal lobe, covering greater part of antennal bascicerite in larger specimens, rounded or blunt. Posterior submarginal groove present, poorly defined.

Abdomen (Figure 2.4A) evenly rounded dorsally, without carination; anterior three pleonal pleura unarmed marginally, posteroventral angle rounded. Fourth pleonal pleuron posterolateral margin unarmed or armed with one tooth; produced into subacute posteroventral angle. Fifth pleonal pleuron posterolateral margin unarmed marginally or armed with 1-4 teeth; produced into acute posteroventral angle. Sixth pleonal pleura 1.5-1.8 times longer than fifth in dorsal midline, 1.5 times longer than high; broadly notched for insertion of uropods; posterolateral process terminating in acute triangular tooth overlapping base of telson, posteroventral corner subacute. Armature of pleonal sternites as described for *Chorocaris chacei* (Williams & Rona, 1986).

Telson (Figure 2.4B; C) 1.5-1.7 times length of sixth pleonal pleura in dorsal midline, slightly narrowed posteriorly, length 2.2-2.9 times greatest width; posterior margin broadly convex, bearing row of 8-35 plumose setae and 2 spines at both lateral ends; 4-8 (sometimes asymmetrical) dorsolateral spines arranged in sinuous row. Uropods (Figure 2.4B) with broad rami exceeding distal margin of telson; exopod with distinct transverse suture and two or three small spines at distolateral angle; endopod shorter and narrower than exopod; posterolateral projection of protopod triangular with acute tip.

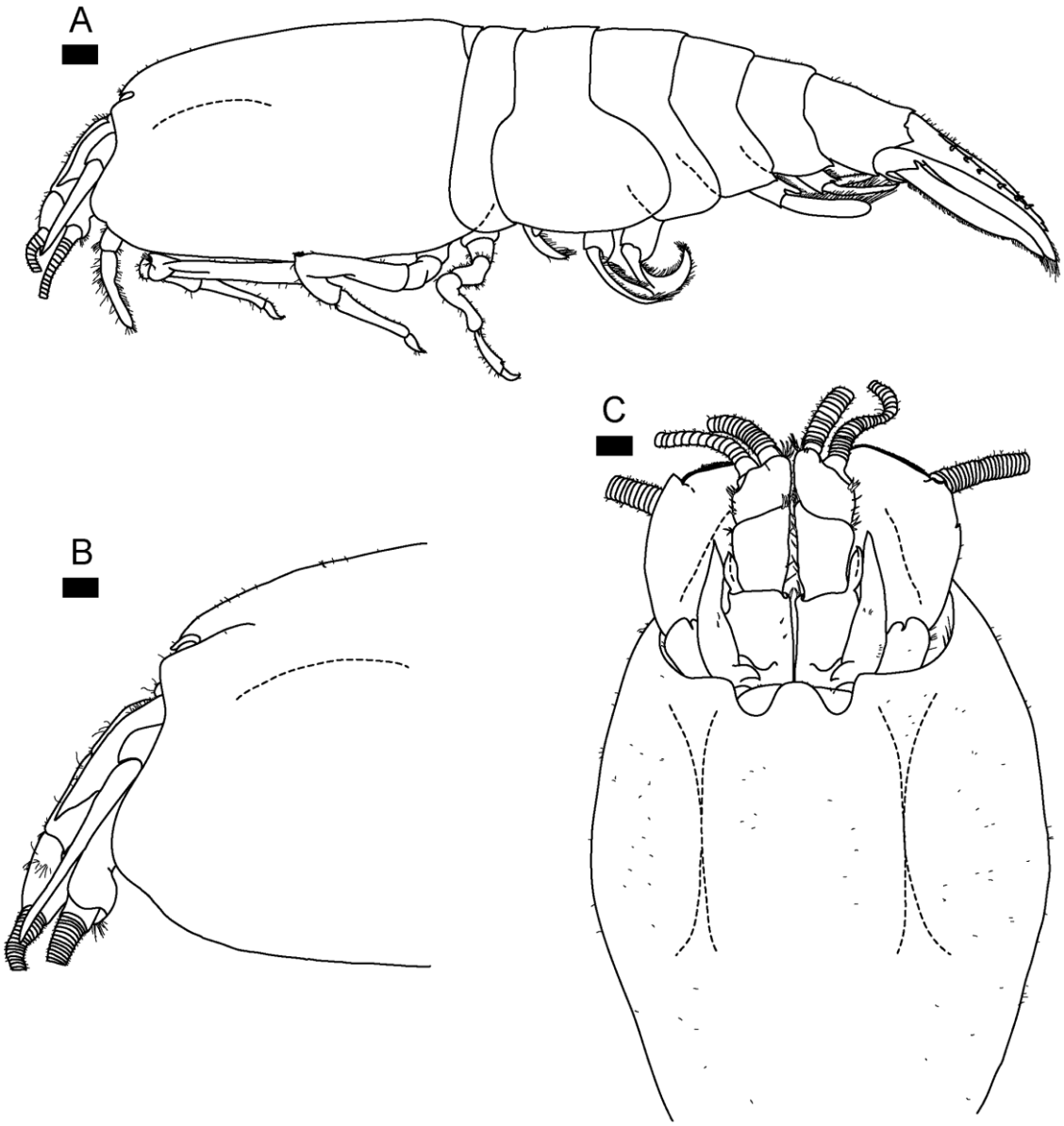

Figure. 2.3. *Rimicaris hybisae* sp. nov., holotype, female (CL 15.3 mm), [NHMUK 2011.8054], from the Von Damm Vent Field, Mid-Cayman Spreading Centre: A, entire animal, lateral view; B, carapace and cephalic appendages, lateral view; C, carapace and cephalic appendages, dorsal view. Scale bars = 2 mm.

Table 2.2. Morphological variation in *Rimicaris hybisae* sp. nov., Mid-Cayman Spreading Centre.

Site	Cat. No.	Sex	Type status	Measurements (mm)					Teeth			
				CL	CW	CD	CW/ CD	TL	AS4 left	AS4 right	AS5 left	AS5 right
VD	NHMUK 2011.805	♀	H	15.3	12.6	7.5	1.7	46.6	0	0	0	0
VD	NHMUK 2011.8055	♂	P	9.4	8.8	5.2	1.7	32.5	0	0	1	1
B	NHMUK 2011.8056	♀	P	10.8	9.3	5.4	1.7	39.7	0	0	0	0
B	NHMUK 2011.8057	♂	P	10.6	8.3	7.1	1.2	35.1	1	0	4	1
B	NHMUK 2011.8058	♂	P	10.0	7.4	6.4	1.2	36.6	0	0	0	0
B	NHMUK 2011.8059	♂	P	10.8	8.7	6.9	1.3	37.7	0	0	1	3
B	NHMUK 2011.8060	♂	P	10.0	8.1	5.8	1.4	33.7	0	0	1	1
B	NHMUK 2011.8061	♂	P	11.8	9.9	7.2	1.4	36.2	0	0	0	0
B	NHMUK 2011.8062	♀*	P	7.0	x	x	x	25.9	0	0	1	1
B	NHMUK 2011.8063	♂	P	10.9	x	x	x	35.2	0	0	1	2
B	NHMUK 2011.8064	♂	P	9.0	7.9	6.1	1.3	30.9	0	0	0	0
B	NHMUK 2011.8065	juv	P	6.7	4.9	4.1	1.2	22.6	0	0	2	3
B	NHMUK 2011.8066	juv	P	5.1	3.5	2.9	1.2	16.5	0	0	1	1
B	NHMUK 2011.8067	juv	P	3.9	2.7	2.2	1.2	13.1	0	0	2	2
B	NHMUK 2011.8068	juv	P	3.6	x	x	x	12.4	0	0	0	0
B	NHMUK 2011.8069	juv	P	4.6	3.4	3.3	1.0	15.1	0	0	3	1
B	NHMUK 2011.8070	juv	P	4.2	2.8	1.9	1.5	13.7	0	0	1	1

CL, post-orbital carapace length; CW, maximum carapace width; CD, maximum carapace depth, TL, total length; AS4, abdominal somite 4, posterolateral margin; AS5, abdominal somite 5, posterolateral margin; B, Beebe Vent Field; H, holotype; P, paratype; VD, Von Damm Vent Field; x: measurement could not be taken due to condition of carapace, * with visibly mature ovary.

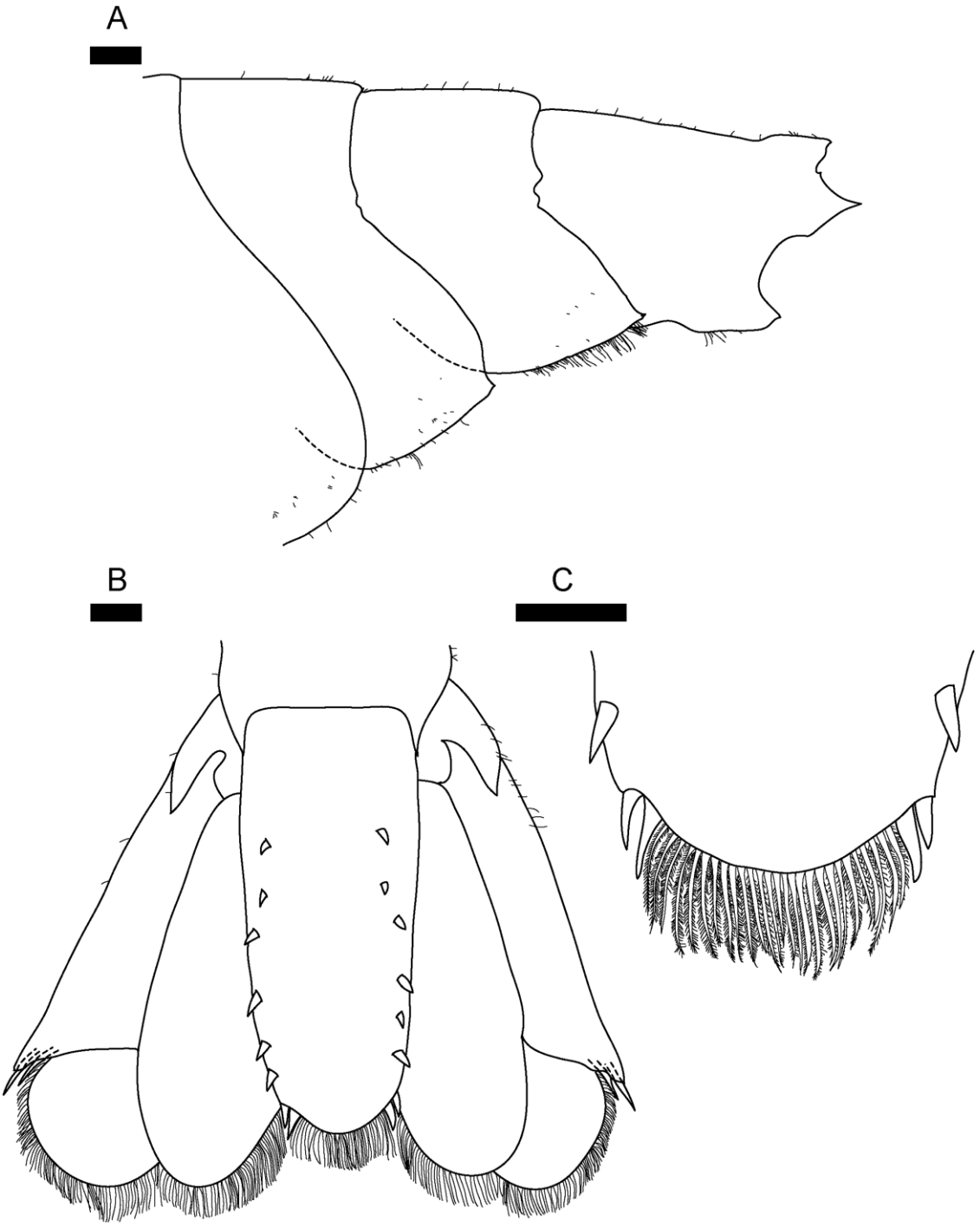

Figure 2.4. *Rimicaris hybisae* sp. nov., holotype, female (CL 15.3 mm), [NHMUK 2011.8054], from Von Damm Vent Field, Mid-Cayman Spreading Centre: A, third to sixth abdominal somites, lateral view; B, telson and uropods, dorsal view; C, posterior margin of telson, dorsal view. Scale bars = 1 mm.

Eye-stalks (Figure 2.3C) broadly fused mesially, lacking pigmentation, anterior surface without conspicuous tubercles.

Antennae (Figure 2.3) of normal structure. Antennular peduncles stout, dorsoventrally depressed, mesial surface rounded, not closely approximated; first and third segments nearly equal in length, second segment slightly longer; first segment with small distomesial tooth, strong distolateral tooth subacute or blunt, reaching or overreaching midlength of second segment, dorsal face convex; stylocerite moderately slender, lateral margin slightly convex, distinctly separated from first segment, extending beyond midlength of second segment; prominent proximolateral tubercle; second segment longer than broad or nearly as long as broad with small distomesial tooth. Antennular flagella robust, thick, similar in structure, tapering, sensory setae at joints between annuli, inserted next to each other on oblique terminal margin of third article, sweeping posterolaterally in gentle arc.

Antennal peduncles (Figure 2.3) shorter than antennular peduncles, both exceeded by antennal scale (scaphocerite); flagella larger (all dimensions) than those of antennule and of similar structure, sensory setae at joints of annuli, sweeping in gentle curve lateral to carapace, reaching as far as midlength of fourth pleonal pleuron in holotype female. Antennal scale (Figures 2.3; 2.5A) broadly oval; convex lateral margin ending in subacute tooth directed forward, short transverse suture extending mesially from base of tooth; plumose setae fringing broadly rounded distal margin and convex mesial margin. Antennal basicerite bearing acute ventrolateral and ventromesial tooth.

Mouthparts (Figure 2.5) typical of Alvinocarididae. Mandible (Figure 2.5B) bearing 7 unequal acute teeth on mesial margin of broad incisor process (4 large and 3 small in holotype female), distalmost tooth distinctly separated from remaining teeth; molar process slightly upcurved, slender, unarmed, not reaching incisor; palp biarticulate, proximal article weakly curved, bearing 2 long plumose setae on distolateral margin, distal article stout, distinctly shorter than basal article, bearing numerous plumose setae of variable lengths on all margins and ventral face.

Maxillule (first maxilla) (Figure 2.5C) with both endites strongly curved toward mouth; coxal endite semitriangular, bearing long, dense, stiff setae; basial endite more rounded dorsally and distally, armed with row of stiff setae (shorter than those of coxal endite) and 3 rows of spines along mesial margin, each successive row becoming more regularly spaced, bearing spines in greater number and size; lateral margin bearing row

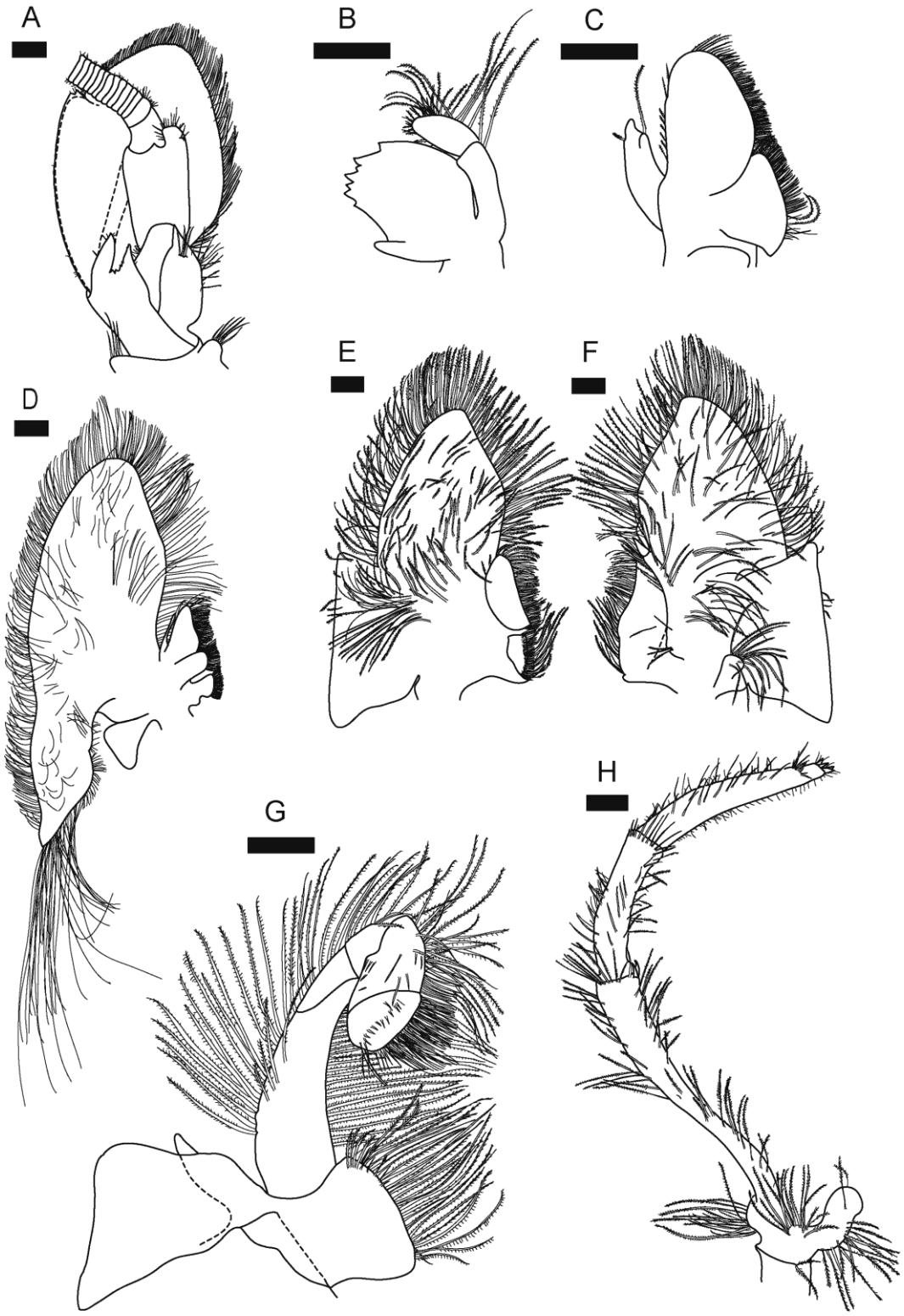

Figure 2.5. *Rimicaris hybisae* sp. nov., holotype, female (CL 15.3 mm), [NHMUK 2011.8054], from the Von Damm Vent Field, Mid-Cayman Spreading Centre: A, right antennal peduncle, scale and proximal part of flagellum, ventral view; B, right mandible, dorsal view; C, right maxillule (first maxilla), ventral view; D, right maxilla (second maxilla), ventral view; E, right first maxilliped, ventral view; F, right first maxilliped, dorsal view; G, right second maxilliped, ventral view; H, right third maxilliped, lateral view. Scale bars = 1 mm.

of long plumose setae; palp weakly curved, slightly bilobed distally with proximal lobe bearing long plumose seta, distal lobe bearing short plumose seta.

Maxilla (second maxilla) (Figure 2.5D) with densely setose mesial endites; proximal endite curved, straplike lobe; distal endite composed of two lobes, separated from each other by a deep notch and suture; flanked by dorsoventrally compressed tapering palp; scaphognathite enormously expanded, numerous plumose-setae like structures conspicuous on dorsal and ventral surfaces and lateral and mesial margins, supporting dense coverage of numerous filamentous bacteria-like structures (Figure 2.6); posterior lobe elongate, subtriangular, convex mesial margin distinctly notched, distomesial margin fringed with very long, wiry, tangled setae, preceded by much shorter plumose setae along proximomesial margin.

First maxilliped (Figure 2.5E, F) with irregularly fusiform, heavily setose mesial endite; palp concealed by caridean lobe, short and bilobed, proximal lobe straplike bearing plumose setae along margins, distal lobe triangular, without ornamentation; caridean lobe broad, similar in shape and ornamentation to scaphognathite, lacking flagellum; large epipod subrectangular, curving dorsally, scattered setae on distolateral margin only.

Second maxilliped (Figure 2.5G) composed of six segments as in other Alvinocarididae; coxa expanded mesially, bearing numerous plumose setae on mesial margin; merus and ischium-basis fused segments moderately stout with numerous plumose setae on curved lateral surfaces, row of dorsally curved setae on nearly straight mesial surface; articulation between merus and carpus oblique; carpus short, long plumose setae on outer face; propodus with moderately long plumose setae mesially; articulation between propodus and dactylus oblique; dactylus longer than propodus, tapering to blunt distal margin, bearing very dense patch of short setae on mesial to distal margins forming brush-like structure; triangular epipod with slender rudimentary protobranch overreaching distal margin of epipod.

Third maxilliped (Figure 2.5H) overreaching antennal peduncle by one third of ultimate segment, comprised of coxa and three long segments; coxa heavily setose, with large, bilobed epipod; epipod without strap-like process; antepenultimate segment longest (consisting of fused basis-ischium-merus, but fusion between basis and ischium incomplete with partial suture on dorsal surface and corresponding indentations on lateral and mesial margins), dorsoventrally flattened, sinuously curved in dorsal view,

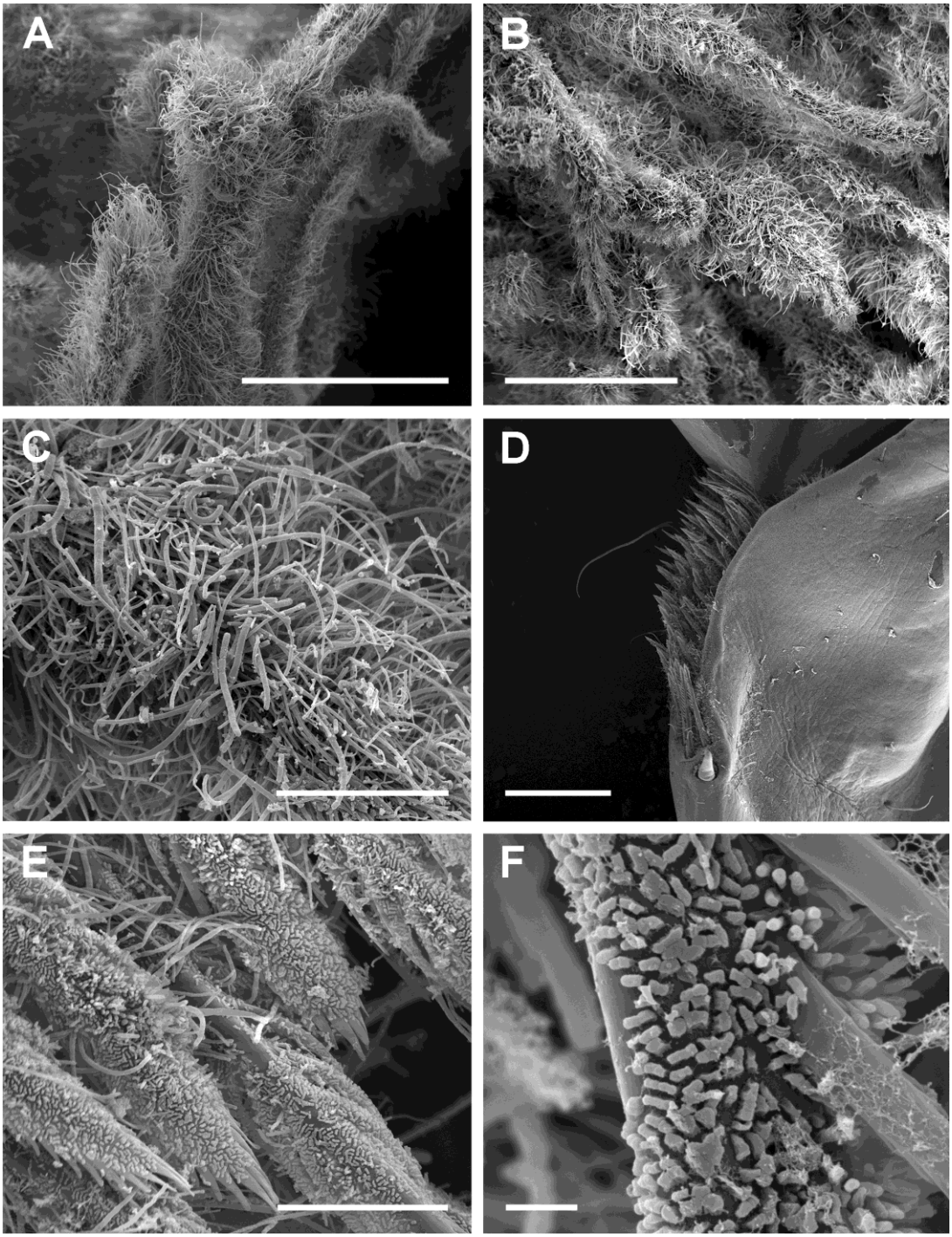

Figure 2.6. *Rimicaris hybisae* sp. nov., male, from the Beebe Vent Field, Mid-Cayman Spreading Centre. Scanning electron micrographs of: A, plumose-setae like structures, margin of maxilla; B, plumose-setae like structures, ventral surface of maxilla; C, close-up of filamentous bacteria-like structures on plumose-setae like structures, maxilla; D, carpal brush on distal segment of carpus, first pereopod; E, close-up of carpal brush setae and bacteria-like structures; F, close-up of dorsal surface of carpal-brush seta and setules with filamentous and non-filamentous bacteria-like structures. Scale bars: A = 300 µm; B, D = 200 µm; C =50 µm; E = 20 µm; F = 2 µm.

armed with 1-2 spines at distolateral ventral angle, bearing numerous plumose setae on margins, dense cluster of long setulose setae on low elevation at proximomesial section, elevation length one fifth of segment length, mesial margin notched where elevation terminates; penultimate segment with plumose setae on dorsolateral surfaces, rows of very dense, short, stiff setae on ventromesial face distal to one third length; ultimate segment strongly curved, stout, about 1.5 times longer than penultimate segment, tapering distally to truncate tip bearing several terminal spines (7 on holotype female), trigonal in cross-section, lateral surface longitudinally carinate, with row of stiff setae, ventromesial surface flat with rows of very dense, short, stiff setae.

Branchial formula identical to that described in *Rimicaris* and *Chorocaris* (Williams & Rona, 1986; Williams, 1988; Martin & Hessler, 1990; Komai & Segonzac, 2008).

Pereopods (Figure 2.7) without epipods. First pereopod typical of family, polymorphic, smaller than succeeding pereopods, typically folded upon itself at mero-carpal joint, reaching tip of antennal peduncle when extended; carpus distal margin obliquely truncate for accommodation of proximal part of palm, mesial face bearing grooming apparatus (carpal brush *sensu* Martin *et al*., 1998: Figs. 1-3) comprised of a triangular patch of serrate setae (some setules also serrate) arising from a recessed area, a row of distally-orientated serrate setae just proximal to the setal patch, and a stout spine proximal to the row of setae (Figure 2.6D); carpal brush setae bearing numerous filamentous and non-filamentous bacteria-like structures (Figure 2.6 E-F); dactyl slightly overreaching fixed finger.

Second pereopod more slender than first, slightly twisted, just overreaching tip of antennal peduncle if extended; merus and ischium unarmed; carpus and chela much weaker than those of first pereopod.

Third to fifth pereopods similar in structure, moderately stout, normally folded at mero-carpal articulation, meri and carpi somewhat twisted, meri and ischia unarmed, decreasing in length from third to fifth (e.g. 20.5-18.0-17.1 mm respectively in holotype female); carpi 1.1-1.2 length of propodi in third pereopod, 0.9-1.1 in second, 0.7-0.9 in third; propodi increasing in length from third to fifth, with irregular double (more or less) row of spinules on ventral surface and normally 4 spinules on ventrodistal margin; dactyli subconical, 0.2 length of propodi, each terminating in robust, curved, corneous unguis, flexor surface bearing marginal and plantar rows of distally curved, corneous spinules, increasing in strength distally.

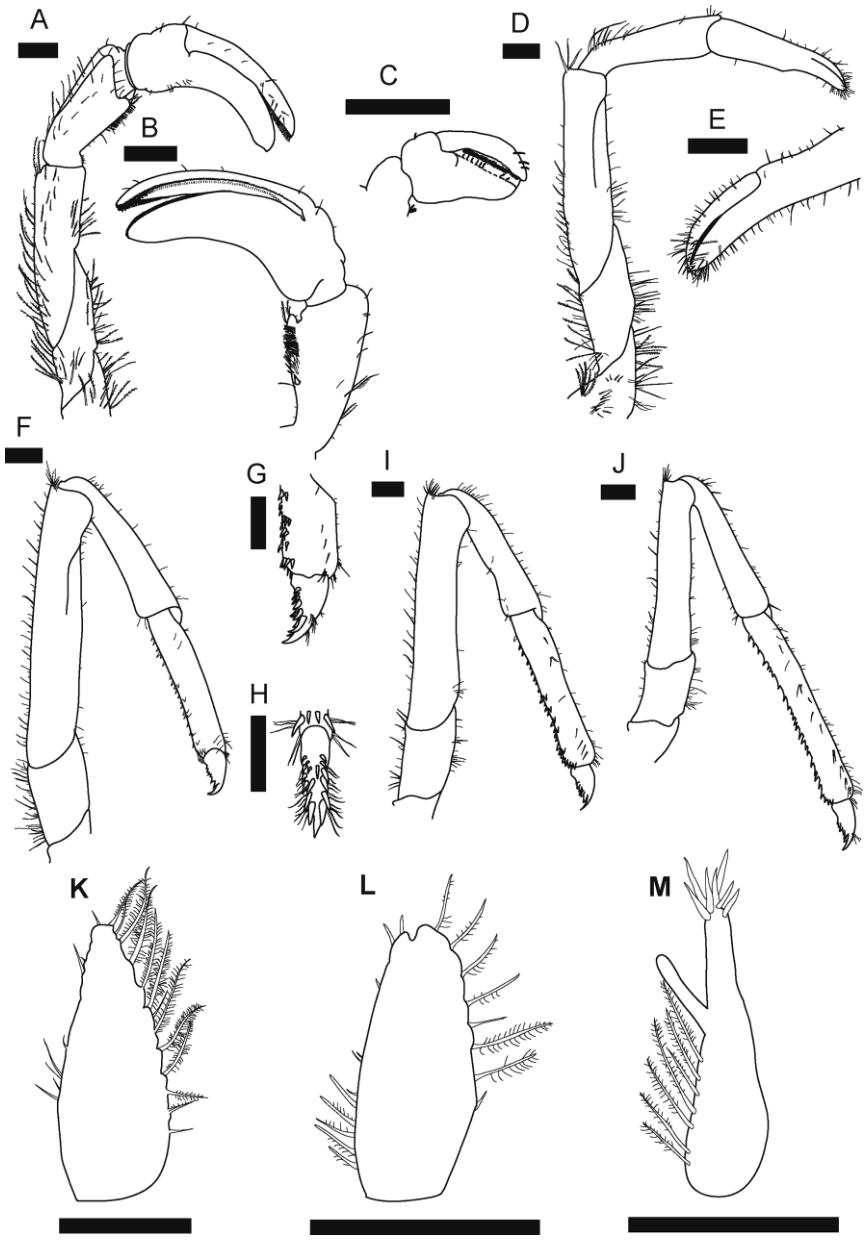

Figure 2.7. *Rimicaris hybisae* sp. nov., A-B, D-K, holotype, female (CL 15.3 mm), [NHM2011.8054], from the Beebe Vent Field, Mid-Cayman Spreading Centre. C, paratype male (CL 5.1 mm), [NHM 2011.808066], from the Von Damm Vent Field, Mid-Cayman Spreading Centre; L-M, paratype male (CL 9.4 mm), [NHMUK 2011.8055], from the Von Damm Vent Field. A, right first pereopod, lateral view; B, chela and carpus of right first pereopod, mesial view; C, chela of left first pereopod, inner view; D, right second pereopod, lateral view; E, chela of right second pereopod, mesial view; F, right third pereopod, lateral view; G, dactlyus and distal part of propodus of right third pereopod, lateral view; H, dactlyus of third right pereopod, posterior view; I, right fourth pereopod; J, right fifth pereopod; K, endopod of right first pleopod, female, ventral view; L, endopod of left first pleopod, male, ventral view; M, appendix masculina and appendix interna, lateral view. Scale bars = 1 mm.

First pleopod bearing sexually dimorphic endopod; in female, endopod (Figure 2.7K) simple, terminating in blunt apex; in male, endopod (Figure 2.7L) with asymmetrical distal notch separating much produced mesial lobe from smaller distolateral lobe. Second pleopod with slender appendix interna without cincinnuli; appendix masculina (Figure 2.7M) (males) tapering distally, bearing 7-8 spiniform setae distally. Third and fourth pleopods each with slender appendix interna, third without cincinnuli, fourth bearing a few tiny cincinnuli at tip. Fifth pleopod with stout, more robust appendix interna with many cincinnuli in subapical mesial cluster.

2.11 Colouration

In life, carapace mostly pale translucent white; internal tissues neutral to greyish; integument of juveniles more translucent, oily orange globules visible underneath, tissues more orange.

Specimens from the Beebe Vent Field with rust-coloured deposits, most notably under antero-lateral area of carapace, on ventral surfaces of thorax, abdomen, frontal region, all areas of maxilla and first maxilliped; blackening common under antero-dorsal area of carapace (Figure 2.2), on tips of dactyli, third maxilliped and carpal brush; black sparkly particles clustered between mouthparts. Specimens from the Von Damm Vent Field are all "clean" in appearance, lacking the rust-coloured deposits, blackened areas and black particulate matter observed on specimens collected from the Beebe Vent Field (Figure 2.1).

Eyes lacking colouration and pigment; juveniles brown pigmentation present in proximal area of fused eyes; orange tint in smallest juveniles (CL 3.6-4.2 mm).

Four-lobed dorsal organ (Figure 2.2) highly reflective with a slight pink tint; reflective property and colouration not preserved in death or after chemical fixation; white pigmentation visible in specimens preserved in 100% ethanol; brown pigmentation under carapace in juveniles (where dorsal organ is located in adults).

Gills typically bright white.

Third to fifth pereopods each ending in pale brown dactylus visible in preservation.

2.12 Variation

Specimens from both vent fields exhibit similar morphology. Variation of some features within and between specimens, most notably the posteroventral angle and armature of fourth and fifth pleonal pleura (Table 2.2), the number of dorsolateral spines on the telson and distolateral spines on the exopod of the uropod (noted above). Slight intra- and inter-specimen variation also observed in the degree of extension of the lateral lobe of the endopod of the first pleopod in males and the shape and reach of the distolateral tooth on the first antennal peduncle (noted above). Variability in these features does not appear to occur in any consistent combination, or in relation to sex or size.

The chela of the first pereopod appears to be polymorphic. In 16 specimens the chela of the first pereopod is slightly recurved, slender and somewhat delicate (Figure 2.7B). In contrast, one male (CL 5.1 mm), [NHMUK 2011.8066] from the Beebe Vent Field, has a stouter, more robust chela (Figure 2.7C).

No variation was observed between the female with a visible mature ovary (CL 7.0 mm) [NHMUK2011.8062] and other females.

One male specimen (CL 9.4 mm), [NHMUK 2011.8055] (Figure 2.8), from the Von Damm Vent Field has an acutely pointed rostrum, antennal lobe and pterygostomial expansion. All other specimens have a rounded or blunt antennal lobe and pterygostomial expansion with a rounded rostrum.

In juveniles (Table 2.2), carapace less inflated; rostrum distinctly pointed downwards, lateral and dorsal surfaces convex, anterior margin ornamented with small, distally-projecting setae; smallest juveniles (CL 3.6-4.2 mm) [NHMUK 2011.8067, NHMUK 2011.8068, NHMUK 2011.8070] medially-fused eyes with tiny tubercle on anterior surface medially.

Variation in colouration noted above.

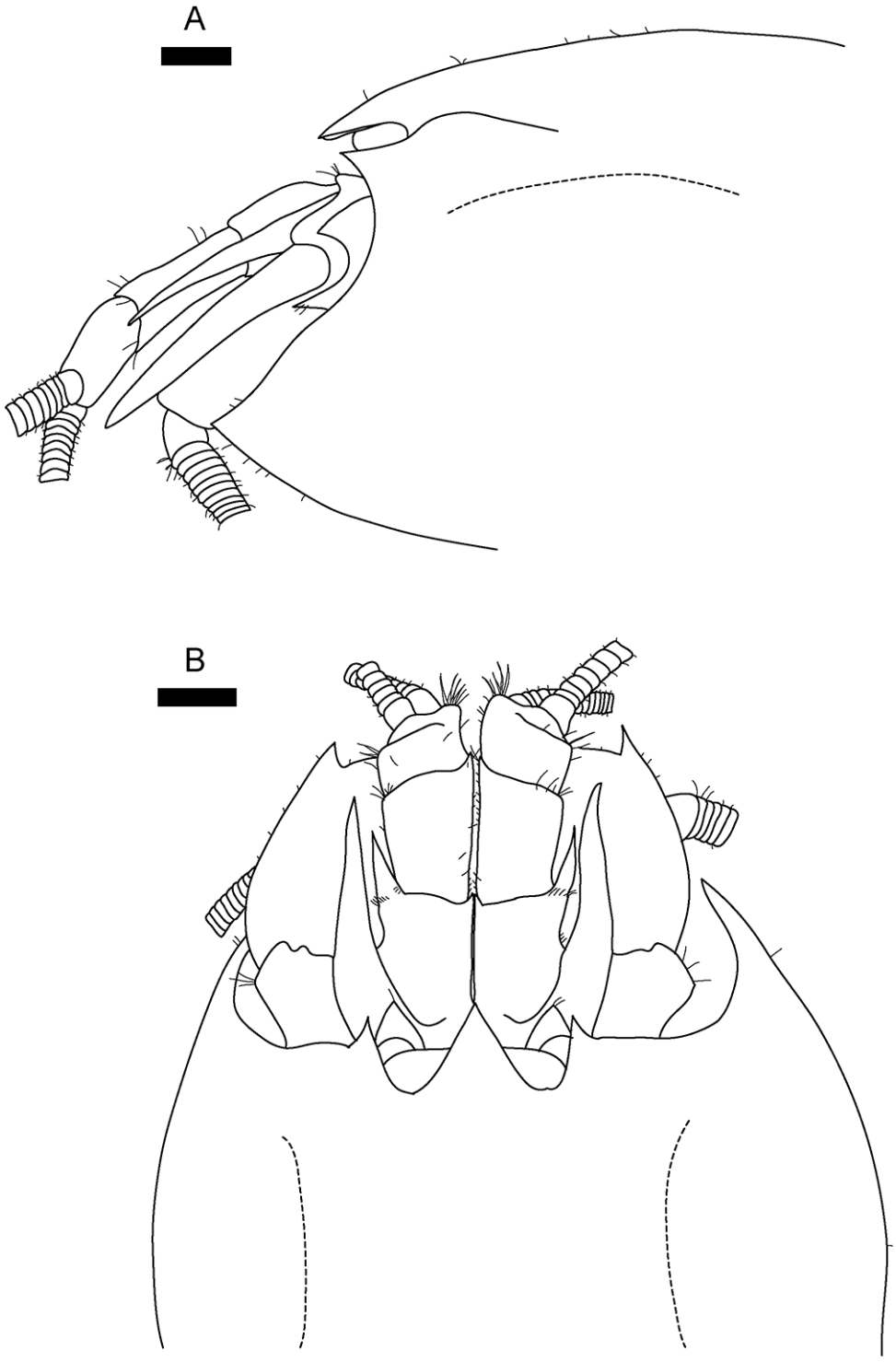

Figure 2.8. *Rimicaris hybisae* sp. nov., paratype male (CL 9.4 mm), [NHMUK 2011.8055], from the Von Damm Vent Field, Mid-Cayman Spreading Centre. A, carapace and cephalic appendages, lateral view; B, carapace and cephalic appendages, dorsal view. Scale bars = 1 mm.

2.13 Comparative remarks

The present new species has been mentioned previously in the literature as "a new morphospecies of alvinocaridid shrimp" and "the MCSC vent shrimp" (Connelly *et al*., 2012: Figures 4, 5A). It is morphologically most similar to *Rimicaris exoculata, R. kairei* and *Chorocaris chacei* because of the reduction of the rostrum to a broadly rounded lobe and nonacuminate antennal lobe of the carapace, the presence of plumose-seta like structures on the dorsal and ventral surfaces of the scaphognathite of the maxilla and caridean lobe of the first maxilliped, and an appendix masculina armed with distal setae only. The new species is easily differentiated from *C. chacei* by the more inflated anterolateral region of the carapace, possession of a four-lobed dorsal organ, and acute tip of the uropodal protopod. A four-lobed dorsal organ and inflated carapace are known only for *R. hybisae* sp. nov. and the two other known *Rimicaris* species. The new species, however, is the first example within the genus to possess paired "pores" on the posterior lobes of the dorsal organ. Furthermore, the carapaces of the new species and *R. exoculata* are ornamented with setae, whereas there are no setae on the carapace of *R. kairei*. The carapace of *R. hybisae* sp. nov. is slightly less inflated than that of *R. exoculata* and *R. kairei*. Of the three known *Rimicaris* species, the carapace is most strongly inflated in *R. kairei*. Other differentiating characters between *R. hybisae* sp. nov. and the two other *Rimicaris* species include the structure of the antennae and extent to which the eyes are fused and rostrum reduced. The characters differentiating between the new species and its closest relatives are discussed below.

2.14 Distribution and habitat

Known only from the type locality, the Von Damm (2300 m) and Beebe (4960 m) hydrothermal vent fields, Mid-Cayman Spreading Centre, Caribbean (Figure 2.1). For preliminary descriptions of both vent fields, see Connelly *et al*. (2012). Observed at the Beebe Vent Field in dense aggregations on the vent chimneys (> 2000 individuals m^{-2}) and with high abundances of anemones around crevices issuing visible diffuse flow in the central area of the sulfide mound (Connelly *et al*., 2012). At the Von Damm Vent Field, in dense aggregations around actively venting orifices of the edifice peak (> 2000

individuals m^{-2}, Connelly *et al*., 2012), along with another, numerically subordinate, morphotype of shrimp (see Chapter 4).

2.15 Etymology

From the name of the British manoeuvrable TV grab *HyBIS*, in celebration of her first, and highly successful, scientific mission. The gender is feminine.

2.16 Molecular phylogeny

Partial sequences of the mitochondrial COI (460 bp) and 16S (549 bp) regions and the nuclear 18S (576 bp) region of *Rimicaris hybisae* sp. nov. were consistent among specimens from both sites. Unique and fixed mutations were observed in the partial sequences of the COI and 18S regions. Based on NJ and ML phylogenetic analyses for COI sequences available in GenBank, *R. hybisae* sp. nov. exhibits the smallest evolutionary distance to *Alvinocaris* sp. [AY163260.1], with a 1.96% divergence from this species, compared with a 7.39% divergence between *R. hybisae* sp. nov. and both *R. exoculata* and *Chorocaris chacei*. In the 16S region, no substitution was found between *R. hybisae* sp. nov. and *C. chacei* (0% divergence) based on the 300-bp sequence in GenBank for the latter species, whereas *R. hybisae* sp. nov. exhibits 0.47% divergence (424 bp) with *R. exoculata*. Phylogenetic analyses on the 300-bp region common among the species shows high bootstrap values (100% and 95% for NJ and ML methods respectively) separating the *R. exoculata*-*R. hybisae* sp. nov.-*C. chacei* clade from the other alvinocaridid species. Within this clade *R. hybisae* sp. nov. and *C. chacei* are separated from *R. exoculata* (88% bootstrap support, NJ and ML methods).

In GenBank, partial sequences of the 18S region for alvinocaridids are limited to *Rimicaris exoculata*, *Chorocaris chacei*, *Alvinocaris muricola* Williams, 1988 and *Alvinocaris longirostris* Kikuchi & Ohta, 1995. Because there is only a single partial sequence in the database for both *R. exoculata* and *C. chacei*, it is not known if the mutations observed in the 18S partial sequences of these species are fixed within each of these species. Based on a 540-bp alignment, NJ and ML phylogenetic trees place *R. hybisae* sp. nov. in the same clade as *R. exoculata* and *C. chacei* (88% and 90% for NJ

and ML methods respectively) (Figure 2.9). For the available 18S partial sequences the new species is closest in evolutionary distance to *C. chacei* (0.56% and 0.88% divergence with *C. chacei* and *R. exoculata* respectively).

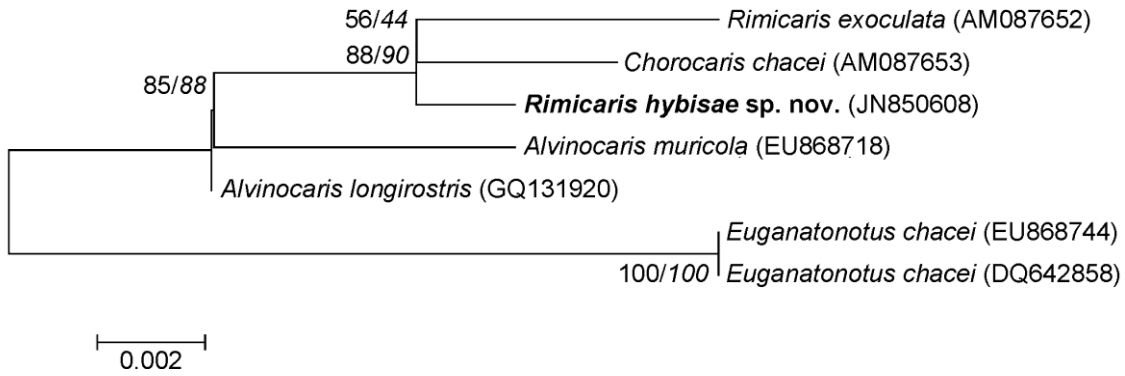

Figure 2.9. Neighbour-joining tree of the Alvinocarididae based on a 540-bp alignment of partial nucleotide sequences from the nuclear 18S ribosomal RNA region with *Euganatonotus chacei* Chan & Yu, 1991 (Nematocarcinidae) as an outgroup. Evolutionary distances computed using the Jukes-Cantor method (Jukes & Cantor, 1969) are represented by branch length; scale bar is proportional to inferred nucleotide divergence. Bootstrap support calculated on 1000 re-sampling replicates is shown by the numbers along the branches (NJ, plain text; ML, italic text). GenBank accession numbers are given after species names.

2.17 Discussion

The presence of unique and fixed mutations in the partial sequences of the COI and 18S region suggest that *Rimicaris hybisae* sp. nov. is genetically distinct from all other species in the GenBank database. This supports the morphological evidence that *R. hybisae* is a new species. Although morphological variation was present amongst the specimens studied, partial sequences of the COI, 16S and 18S regions were consistent between specimens, confirming that those analysed from both MCSC vent fields are monospecific.

Based on morphology, and supported by the results from the molecular analyses, *Rimicaris hybisae* sp. nov. belongs within the *Rimicaris-Chorocaris-Opaepele* clade. Common features within this complex include a greatly reduced rostrum, broadly fused

eyes, three or greater rows of accessory spines on the ventral surfaces of the dactyli of the third to fifth pereopods, the unarmed ischium of the third to fifth pereopods, and sinuous rows of dorsolateral spines on the telson (Komai & Segonzac, 2005, 2008). *Rimicaris hybisae* sp. nov. differs from *Opaepele* in possessing a more reduced rostrum, inflated carapace and non-acuminate antennal and pterygostomial lobes (Lunina & Vereshchaka, 2010). The assignment of the new species to a genus was a complex issue. Many morphological characteristics of *Chorocaris* are shared with *Rimicaris* (see Martin & Hessler, 1990; Komai & Segonzac, 2008), as exemplified by the original assignment of *C. chacei* to the genus *Rimicaris* by Williams & Rona (1986). However, a suite of morphological traits distinguish the two genera.

Two of the most striking morphological features of *Rimicaris hybisae* sp. nov. are its inflated carapace and four-lobed dorsal organ. These features are, to date, unique to *Rimicaris* and support placement of the new species within the genus. The carapace is marginally less inflated than that of *R. exoculata* (which is less inflated than that of *R. kairei* Watanabe & Hashimoto, 2002; personal observation). *Rimicaris hybisae* sp. nov. can be further distinguished from *R. exoculata* and *R. kairei* by the presence of paired "pores" on the posterior lobes of the dorsal organ. Variability has been documented in the shape of the dorsal organ in adult specimens of *R. exoculata* (O'Neill *et al*., 1995: Figure 5), but the presence of "pores" is, to our knowledge, a feature unique to *R. hybisae* sp. nov.

The "dorsal eye" of *R. exoculata* (and presumably *R. kairei*) is an extremely efficient photoreceptor, used for detecting light emitted from the vents (Pelli & Chamberlain, 1989; Van Dover *et al*., 1994, 1996; O'Neill *et al*., 1995). Shiny anterior spot-like organs have been described inside the carapaces of *Chorocaris chacei*, *Mirocaris fortunata* Martin & Christiansen, 1995, *Alvinocaridinides formosa* Komai & Chan, 2010, and species of *Opaepele* and *Nautilocaris* Komai & Segonzac, 2004 (Desbruyères *et al*., 2006; Tsuchida *et al*., 2008; Komai & Chan, 2010). These spot-like organs may be homologous to the "dorsal eye" found in species of *Rimicaris* (Lakin *et al*., 1997; Kuenzler *et al*., 1997; Komai & Chan, 2010), but are smaller and not comprised of four lobes. Histological examination of the dorsal organ in the new species and *R. kairei*, requiring the collection of further specimens, would be necessary to determine if this organ is homologous in all *Rimicaris* species or if this is an example of convergence.

Martin & Hessler (1990) hypothesized that *Rimicaris*, with its inflated carapace, opercular frontal region, dorsal organ, and dramatically reduced rostrum, is a derived genus that stemmed from *Chorocaris* or another morphologically similar deep-sea shrimp. The presence of an inflated carapace and four-lobed dorsal organ in *R. hybisae* sp. nov. suggest that this species may be more derived than species of *Chorocaris*. In contrast, the armature of the fourth and fifth pleonal pleura is a feature also found in more basal genera (*Opaepele*, *Alvinocaris*, and *Shinkaicaris* Komai & Segonzac, 2005; Komai *et al*., 2007). Within *Rimicaris* and *Chorocaris*, the fourth and fifth pleonal pleura are subacutely or acutely pointed only at their posteroventral angles and are not marginally armed (Komai & Segonzac, 2008).

Based on analyses of the COI gene (600 bp), Shank *et al*. (1999) proposed that *Chorocaris* is a paraphyletic assemblage with *C. chacei* being more closely related to *Rimicaris exoculata*. This is supported by the most recent and complete molecular phylogeny of the Alvinocarididae based on COI (600 bp), whereby *C. chacei* clusters with *R. exoculata* with strong statistical support (100% bootstrap values; Zelnio & Hourdez, 2009). In *R. hybisae* sp. nov., *Rimicaris* species and *C. chacei*, the scaphognathite of the maxilla and caridean lobe of the first maxilliped are ornamented with numerous plumose-seta like structures on both their dorsal and ventral surfaces, whereas in *C. vandoverae* Martin & Hessler, 1990 and *C. paulexa* Martin & Shank, 2005, their ventral surfaces are nearly naked (Martin & Hessler, 1990; Martin & Shank, 2005; Komai & Segonzac, 2008). The appendix masculina of *R. hybisae* sp. nov., *C. chacei* and the *Rimicaris* species bear distal setae only, whereas setae extend onto the dorsal surface of the appendix masculina in *C. vandoverae*. Komai & Segonzac (2008) suggest that this feature supports the close proximity between *C. chacei* and *Rimicaris* species and also consider *C. chacei* as a possible sister species of the *Rimicaris* clade. *R. hybisae* sp. nov., with shared morphological affinities with both *C. chacei* and *Rimicaris*, further supports the proximity between these taxa, although the more basal features of the new species are not shared with either genus.

Features common to *Chorocaris* and absent in *Rimicaris exoculata* and *R. karei* include: possession of a strong distolateral tooth, a small distomesial tooth, and a prominent proximolateral tubercle on the first segment of the antennal peduncle; a clear separation of the stylocerite from the antennal peduncle; a carpal brush on the first pereopod; the armed antepenultimate segment of the third maxilliped; blunt or subacute antennal teeth; an increase in length from the third to fifth pereopods and a well-

developed, functional appendix interna bearing cincinnuli on the fifth pleopod only (Martin & Hessler, 1990; Komai & Segonzac, 2008; personal observation). These traits are also exhibited by *R. hybisae* sp. nov., revealing some morphological affinities with the genus *Chorocaris*.

Notable autapomorphies of *Rimicaris* are: the complete fusion of the eyes into a transverse ocular plate; the reduction of the rostrum to a broadly rounded lobe, fitting closely to the posterior concavity of the ocular plate; the formation of the antennal components into an operculum-like structure and the presence of a mat of dense spinules on the flexor surface of the propodi of the third to fifth pereopods (Martin & Hessler, 1990; Komai & Segonzac, 2008). These features were not observed in *R. hybisae* sp. nov., despite the examination of specimens comparable in size to adult *R. exoculata* and *R. kairei*.

In consideration of the morphological and molecular evidence presented here, it appears that *Rimicaris hybisae* sp. nov. may be intermediate between *Chorocaris chacei* and *R. exoculata*. Given the uncertainty concerning the *Chorocaris* genus, the establishment of a new genus for *R. hybisae* sp. nov. is premature. In reverence to the fact that *C. chacei* was originally *Rimicaris chacei*, the assignment of *R. hybisae* sp. nov. to the genus *Rimicaris* is the most conservative approach available. As Komai & Segonzac (2008) have already suggested, a comprehensive and extensive molecular phylogenetic analysis of the whole *Rimicaris-Chorocaris-Opaepele* clade is required to clarify the relationships between these taxa. Such a study has the potential to markedly improve our current understanding of the evolution, radiation and biogeographic patterns of these shrimp among deep-sea chemosynthetic environments in the world's oceans.

Morphological variation is well documented in the Alvinocarididae and is acknowledged as common within alvinocaridid species (e.g. Kikuchi & Ohta, 1995; Martin & Shank, 2005; Komai & Segonzac, 2008). The variation described between specimens of *Rimicaris hybisae* sp. nov. does not appear to be related to size, gender, or collection site. Polymorphism of the chela of the first pereopod has also been described for species of *Chorocaris* (Martin & Shank, 2005; Komai & Segonzac, 2008). Specimens of *C. chacei* from Lucky Strike (one female in Komai & Segonzac, 2006: Plate 3, p. 241; one male in Komai & Segonzac, 2008: Figures 9 F- G) have been shown with an acutely pointed rostrum, antennal lobe and pterygostomial expansion; these specimens were considered by Komai & Segonzac (2008) to be an aberrant form of the

species, and although this identification may not be fully justified, it is reasonable given the sampling location. Consequently the specimen illustrated in Figure 2.8 [NHMUK 2011.8055] is interpreted here as an aberrant form of *R. hybisae* sp. nov. The "clean" appearance of specimens from the Von Damm Vent Field and presence of black and rust-coloured deposits on specimens from Beebe Vent Field may be due to the differences in the chemistry of their respective habitats (see Connelly *et al*., 2012).

Results from analysis of 460 bp of the COI region for *Rimicaris hybisae* sp. nov. suggest that for this gene, the new species is genetically closest to *Alvinocaris* sp. [AY163260.1] (*Alvinocaris methanophila* Komai, Shank & Van Dover, 2005 from Blake Ridge; see Van Dover *et al*., 2003). This result does not fit well with the evidence from morphology, 16S and 18S, thus highlighting the potential problem of relying on COI for molecular phylogenies. While it is possible that there is a problem with the COI sequence for *Alvinocaris* sp., a second possibility is that there is more than one gene in the COI region. Work is in progress to test the second hypothesis (Plouviez *et al*., in prep.). In the 16S region, *R. hybisae* sp. nov. exhibits fixed mutations that are identical to those observed in *Chorocaris chacei*. However, it was not possible to establish if these species share an identical nucleotide sequence for more than 300-bp of the 16S region. Results from analysis of the 18S region suggest that, for this slow-evolving gene, *R. hybisae* sp. nov. is closest in evolutionary distance to *C. chacei*, although this is based on a 576-bp alignment only.

Rimicaris hybisae sp. nov. is easily differentiated from *Chorocaris chacei* by the more inflated anterolateral region of the carapace, possession of a four-lobed dorsal organ, and acute tip of the uropodal protopod. Like juveniles of *C. chacei*, juveniles of *R. hybisae* sp. nov. bear plumose setae on the posterior margin of the telson, a conspicuous distolateral tooth on the first segment of the antennal peduncle and a carpal brush on the first pereopod. In a recent review, Komai & Segonzac (2008) noted differences in the morphology of ovigerous females in species of *Chorocaris* and *Rimicaris*. Samples of ovigerous females of *R. hybisae* sp. nov. are required to enable their morphology to be described and compared to other species.

In summary, morphological features and molecular analyses indicate that *Rimicaris hybisae* sp. nov. belongs within the *Rimicaris-Chorocaris-Opaepele* clade, closest to and intermediate between *C. chacei* and *R. exoculata*. The inflated carapace and four-lobed dorsal organ of *R. hybisae* sp. nov. are diagnostic and distinguishing morphological features, previously recorded only in *R. exoculata* and *R. kairei*, which

support placement of the new species within the genus *Rimicaris*. The molecular evidence reported here confirms that *R. hybisae* sp. nov. is a genetically distinct species, closest to *C. chacei*, which was originally placed in the genus *Rimicaris* as *R. chacei* (Williams & Rona, 1986). Phylogenies of the Alvinocarididae suggest that *Chorocaris* is polyphyletic, with *C. chacei* more closely related to *Rimicaris* than to other *Chorocaris* species (Shank *et al*., 1999; Zelnio & Hourdez, 2009). Moreover, on the basis of morphological traits shared between the two taxa, it has been proposed that *C. chacei* is a sister species of *Rimicaris* (Komai & Segonzac, 2008). The most conservative approach is therefore to expand the diagnosis of *Rimicaris* to incorporate *R. hybisae* sp. nov., rather than to erect a new genus in the absence of highly corroborated phylogeny. The presence of features previously considered diagnostic of *Rimicaris* spp. and *C. chacei* in *R. hybisae* sp. nov., and the low genetic divergence between these taxa suggest that reassimilation of *C. chacei* within *Rimicaris* could also be considered in the future.

2.18 Biogeography

Rimicaris hybisae sp. nov. represents the third named species in the genus, all of which are known only from hydrothermal vents in a particular area (Table 2.1). *Rimicaris karei* is so far restricted to its type locality in the Indian Ocean, whereas *R. exoculata* is present at vents along the MAR, where it occurs sympatrically with *Chorocaris chacei* (Table 2.1). *Rimicaris hybisae* sp. nov. extends the distribution of the genus approximately 4000 km westwards into the Caribbean and increases the bathymetric range of the Alvinocarididae by 872 m to 4960 m. This may also be the first record of the Alvinocarididae in the Caribbean. Escobar-Briones & Villalobos Hiriart (2003) reported an indeterminate species of *Alvinocaris* from non-chemosynthetic environments on the Banco Chinchorro, northern Caribbean, at depths of 176-203 m but provided no molecular evidence or catalogue details.

Before the complete closure of the Isthmus of Panama by 3.1 Ma (Burton *et al*., 1997), a deep-water connection existed between the eastern Pacific and Caribbean. Martin & Hessler (1990) proposed that the presence of *Chorocaris* species in the Atlantic and Pacific indicates a faunal connection between the eastern Pacific and Mid-Atlantic vents. However, no specimens of *Rimicaris* have been collected from the

Pacific Ocean, despite numerous active surveys at Pacific vents. In addition, *Chorocaris* may be paraphyletic, with the Atlantic species (*C. chacei*) belonging to *Rimicaris*. The recent discovery of hydrothermal vents and chemosynthetic communities on the MCSC provides an opportunity to test Martin & Hessler's (1990) hypothesis. The presence of shrimp-dominated faunal assemblages at the Beebe and Von Damm vent fields also indicate that the MCSC vent fauna shares similarities with MAR vent fauna.

2.19 Acknowledgements

Thanks to the Master and ship's company of the *RRS James Cook,* the UK National Marine Facilities staff, and fellow scientists on the 44th voyage of the *RRS James Cook*. Professors P.A. Tyler and C.L. Van Dover provided laboratory facilities at the University of Southampton and Duke University Marine Laboratory respectively. Dr A. Page provided SEM facilities at the Biomedical Imaging Unit (University of Southampton). Dr T. Horton (National Oceanography Centre Southampton) provided equipment and advice. Comparative material was obtained on loan from the Muséum National d'Histoire Naturelle (MNHN) collection, Paris and the National Museum of Natural History, Smithsonian (USNM) collection, Washington DC. This work benefitted from two anonymous reviewers who gave valuable comments for the improvement of this paper. This work was supported by a UK NERC award (NE/F017774/1) to J. Copley and NASA ASTEP Grant (NNX09AB75G) to C.L. Van Dover, which is gratefully acknowledged.

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3. *Iheyaspira bathycodon* new species (Vetigastropoda: Trochoidea: Turbinidae: Skeneinae) from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean

This work has been published as: Nye V., Copley J., Linse K. & Plouviez S. (2013) *Iheyaspira bathycodon* new species (Vetigastropoda: Trochoidea: Turbinidae: Skeneinae) from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean. *Journal of the Marine Biological Association of the United Kingdom* 93, 1017-1024. Doi:10.1017/S0025315412000823. Published online 13 August 2012; code-compliant paper version published in June 2013.

Abstract

Iheyaspira bathycodon sp. nov. is described from the Von Damm Vent Field on the world's deepest spreading centre, the Mid-Cayman Spreading Centre (MCSC), Caribbean, at 2300 m depth. The new species is defined and illustrated from 11 specimens, with brief notes on habitat and known distribution. Molecular phylogenetic data from partial COI mitochondrial DNA, 16S ribosomal DNA and nuclear 18S ribosomal DNA regions are used to analyse the species' phylogenetic position and its morphology is compared with previously described skeneid and vent taxa. The new species is distinguished from the most closely allied vent species, *Iheyaspira lequios* Okutani, Sasaki & Tsuchida, 2000 by morphological differences in radula diagnosis and appendage structure of the head-foot. *Iheyaspira bathycodon* sp. nov. is the tenth turbinid to be described from a hydrothermal-vent environment and the second species to be named from recently discovered hydrothermal vents on the MCSC. Determining the faunal composition of assemblages at the vent fields of the MCSC will help to elucidate the vent biogeography of the region.

3.1 Introduction

Gastropoda are one of the most species-rich macrofaunal taxa from hydrothermal vents (Warén *et al.*, 2006), and form a dominant component of assemblages at vent fields in western Pacific back-arc basins and on the Central and Southwest Indian Ridges (e.g. Desbruyères *et al.*, 1994; Galkin, 1997; Kojima *et al.*, 2001; Van Dover *et al.*, 2001; Tao *et al.*, 2012). A substantial research effort has sought to elucidate the systematics and higher phylogeny of vent/seep gastropods, and to characterise their diversity, biogeography and life-history biology (see Sasaki *et al.*, 2010 for recent review).

More than 200 species of gastropods from at least 100 genera and 35 families have been recorded from deep-sea chemosynthetic environments of the world's oceans (Sasaki *et al.*, 2010). Some of these species also inhabit non-chemosynthetic environments but have been recorded in much greater densities from chemosynthetic assemblages (see Sasaki *et al.*, 2010), whereas others are endemic to vents and seeps at the species and higher taxonomic levels (e.g. Warén & Bouchet, 2001).

Skeneinae Clark, 1851 was originally treated as a separate family ("Skeneidae") in Trochoidea Rafinesque, 1815 (e.g. Hickman & McLean, 1990). More recent anatomical and molecular studies showed, however, that "Skeneidae" was polyphyletic and that many genera should be reassigned (e.g. Bouchet *et al.*, 2005; Kano, 2008). Bouchet *et al.* (2005) ranked Skeneinae as a subfamily of the Turbinidae Rafinesque, 1815, an arrangement maintained by Williams *et al.* (2008) in the newly defined Turbinidae. The rank of the Skeneinae remains uncertain and under discussion (Williams, 2012).

To date, all turbinids endemic to deep-sea chemosynthetic environments belong to either the Skeneinae or the Margaritinae Thiele, 1924 (Sasaki *et al.*, 2010; see Table 3.1). Some colloniids, such as C*antrainea* Jeffreys, 1883, also live in seeps and vents (e.g. Warén & Bouchet, 1993; 2001; Sasaki *et al.*, 2010). The family Colloniidae Cossmann, 1917 was classified as a subfamily within the Turbinidae (e.g. Hickman & McLean, 1990; Bouchet *et al.*, 2005), but was moved recently to familial rank in the superfamily Phasianelloidea Swainson, 1840 (Williams *et al.*, 2008).

The Von Damm Vent Field is an active, high-temperature hydrothermal system, situated in a unique off-axis setting on the upper slopes of an oceanic core complex at 2300 m depth (Connelly *et al.*, 2012). The Von Damm Vent Field supports an abundant

Table 3.1. Turbinid gastropods described from extant hydrothermal vents/cold seeps up to the end of 2011 (confirmed locations and fully described species only).

Subfamily	Species	Site(s)	Depth (m)	Habitat	References
Margaritinae	*Gaza fisheri*	GoM: Louisiana Slope; Caribbean Sea: off St Lucia	600-1061	seep	Dall (1889); Warén & Bouchet (1993, 2001)
	Margarites huloti	Off Central Chile (36°S)	843-728	seep	Vilvens & Sellanes (2006)
	Margarites ryukyensis	OT: North knoll of Iheya Ridge	968-1053	vent	Okutani *et al.* (2000); Sasaki *et al*. (2005)
	Margarites shinkai	OT; SB	1110-1340	vent & seep	Okutani *et al.* (1992); Okutani *et al*. (1992, 1993); Sasaki *et al*. (2005)
Skeneinae	*Bruceiella athlia*	Aleutian Trench	Ca. 4800	seep	Warén & Bouchet (2001); Kiel (2004)
	Bruceiella globulus	LB; NFB	1750-2443	vent	Warén & Bouchet (1993, 2001); Warén *et al*. (2006)
	Bruceiella wareni	CIR: Kairei	2422-2443	vent	Okutani *et al*. (2004)
	Fucaria mystax	Edison Seamount	1483	vent	Warén & Bouchet (2001); Warén *et al*. (2006)
	Fucaria striata	JdFR: Middle Valley	2425	vent	Warén & Bouchet (1993); Warén & Bouchet (2001); Warén *et al*. (2006)
	Iheyaspira bathycodon sp. nov.	MCSC: Von Damm	2300	vent	Nye *et al*. (2013)
	Iheyaspira lequios	OT: North knoll of Iheya Ridge	968-1053	vent	Okutani *et al.*, (2000)
	Protolira thorvaldsonni	MAR: Menez Gwen to Snake Pit & Ashadze; off SW Iceland	850-4080	vent & whale bone	Warén (1996); Warén & Bouchet (2001); Warén *et al*. (2006); Fabri *et al.* (2011)
	Protolira valvatoides	MAR: Menez Gwen to Lucky Strike, Snake Pit	850-3478	vent	Warén & Bouchet (1993); Warén *et al*.(2006)

CIR, Central-Indian Ridge; GoM, Gulf of Mexico; JdFR, Juan de Fuca Ridge; LB, Lau Basin; MAR, Mid-Atlantic Ridge; MCSC, Mid-Cayman Spreading Centre; NFB, North Fiji Basin; OT, Okinawa Trough; SB, Sagami Bay.

faunal assemblage that is dominated by dense aggregations of the shrimp *Rimicaris hybisae* Nye, Copley & Plouviez, 2012 (see Chapter 2) and includes small skeneimorph gastropods. During a recent research cruise to the Mid-Cayman Spreading Centre a piece of chimney was sampled from the Von Damm Vent Field. On the surface of the sampled chimney were several small gastropods of one species, *Iheyaspira bathycodon* sp. nov., which is described herein. In addition to enhancing existing knowledge of biodiversity, characterising the composition of faunal assemblage at Mid-Cayman Spreading Centre vents has the potential to elucidate the factors determining vent biogeography of this region.

3.2 Materials and methods

Specimens were collected from the Von Damm Vent Field (2300 m) at the Mid-Cayman Spreading Centre, Caribbean, during the 44th voyage of *RRS James Cook* (April 2010). All specimens were picked from the surface of a sample of vent chimney, collected by the hydraulic grab of *HyBIS* (Hydraulic Benthic Interactive Sampler), a manoeuvrable TV grab sampler. Specimens for molecular analysis were immediately placed in 95% ethanol and the shell and operculum were subsequently removed. Specimens for morphological study were fixed in 10% neutralised formalin, subsequently transferred to 90% IMS (Industrial Methylated Spirits) and measured to the nearest 0.1 mm using Vernier callipers (see Table 3.2).

Specimen [NHMUK 20120076] was dissected for scanning electron microscopy (SEM) of shell, operculum and radula. The shell and operculum were placed in an ultrasonic cleaning bath for three minutes. The mantle tissue was dissolved in potassium hydroxide diluted in water to expose the radula. The shell, operculum, radula and ctenidium were mounted uncoated onto an aluminium stub and micrographs were taken with a Hitachi TM3000 tabletop microscope. For SEM of soft parts, specimen [NHMUK 20120070] was dehydrated through a graded ethanol series, critical point dried and sputter coated with gold palladium prior to examination with a FEI Quanta 200 Scanning Electron Microscope at accelerating voltage of 10 kV.

Genomic DNA was extracted from eight specimens using the CTAB (cetyltrimethyl ammonium bromide) extraction procedure (Doyle & Dickson, 1987). A

Table 3.2. Morphological variation in *Iheyaspira bathycodon* sp. nov.

Catalogue no.	Type status	Shell height (mm)	Shell diameter (mm)	No. whorls	Operculum diameter (mm)	Operculum no. rings
NHMUK 2012.0068	H	4.3	3.6	4.3	1.8	11
NHMUK 2012.0069	P	6.7	5.9	4.5	1.8	11
NHMUK 2012.0070**	P	5.2	4.3	4.5	2.1	13
NHMUK 2012.0071	P	5.4	5.1	4.2	1.8	14
NHMUK 2012.0072	P	5.9	5.5	4.3	2.2	17
NHMUK 2012.0073	P	4.8	4.2	4.3	2.3	12
NHMUK 2012.0074	P	3.7	3.6	3.6	1.4	9
NHMUK 2012.0075*	P	6.9	5.6	4.6	3.3	16
NHMUK 2012.0076**	P	6.7	6.5	4.6	2.6	13
NHMUK 2012.0077	P	6.4	5.0	4.6	2.5	14
NHMUK 2012.0078	P	7.2	6.1.	4.4	2.8	14

H, Holotype; P, Paratype; *soft parts dissected out of shell; ** soft parts dissected out of shell and used for scanning electron microscopy.

region of mitochondrial cytochrome oxidase subunit I gene (COI) was amplified by polymerase chain reaction (PCR) performed in 20 µl final volume using universal primers (Folmer *et al.*, 1994) and the following conditions: 1X buffer reagent (200 mM Tris pH 8.8, 500 mM KCl, 0.1% Triton X-100, 2 mg/ml bovine serum albumen), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 mM of each primer, 1 U Taq DNA polymerase (Bioline), 5 µl of template DNA and sterile H_2O to final volume. Thermal cycling conditions were: 94°C/2 min; followed by 5 cycles at (94°C/35 s; 45°C/35 s; 72°C/1:20 min) and 35 cycles at (94°C/35 s; 50°C/35 s; 72°C/1:20 min) with a final extension of 72°C/10 min.

For the 16S ribosomal DNA gene (16S), PCR amplifications were performed in 20 µl final volume using 16Sar and 16Sbr primers (Palumbi, 1996) and the following conditions: 1X buffer reagent (same as for COI), 2.5 mM $MgCl_2$, 0.13 mM of each dNTP, 0.38 mM of each primer, 1 U Taq DNA polymerase (Bioline), 2.5 µl of template DNA and sterile H_2O to final volume. Thermal cycling conditions were: 94°C/4 min; 30 cycles at (94°C/30 s; 52°C/1 min; 72°C/2 min) and 72°C/5 min.

PCR amplifications of the 18S ribosomal DNA gene (18S) were performed using the primer pair 5'-CACAGTGAAACTGCGAATGG-3'and 5'-CAAATGCTTTCGCT GTAGGG-3' (this study) in a 20 µl final volume amplification mixture as described for COI. Thermal cycling conditions were: 95°C/5 min followed by 30 cycles at (94°C/1 min; 60°C/1 min; 72°C/2 min; 72°C/2 min).

Purifications and sequencing were performed as described by Nye *et al.* (2012). Sequence strands were proof read and assembled with CodonCode Aligner, version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), to produce a continuous fragment. The 16S and 18S partial rDNA sequences were compared with those of other gastropods available in GenBank using the BLAST program (NCBI Basic Alignment Search Tool). The COI partial sequence of the new species was also compared with those of other trochoids of Suzanne William's published and unpublished dataset of deep-sea gastropods (Williams *et al.*, 2008; S.T. Williams, personal communication). Phylogenetic trees were constructed with *MEGA*5 (Tamura *et al.*, 2011) using both maximum-likelihood (ML) (Kimura, 1980) and neighbour-joining (NJ) (Saitou & Nei, 1987) methods on 425- and 803-base pair (bp) alignments for 16S and 18S respectively. Bootstrap values were calculated on 1000 re-sampling replicates.

The GenBank accession numbers for the partial sequences of COI, 16S and 18S regions from the new species are JQ306326, JQ306327 and JQ306328 respectively.

3.3 Systematics

Order VETIGASTROPODA Salvini-Plawen, 1980

Superfamily TROCHOIDEA Rafinesque, 1815

Family TURBINIDAE Rafinesque, 1815

Subfamily SKENEINAE Clark, 1851

Genus *Iheyaspira* Okutani, Sasaki & Tsuchida, 2000

Iheyaspira bathycodon sp. nov.

(Figures 3.1-3.6)

3.4 Type material

Holotype and paratypes deposited in the Natural History Museum, UK (NHMUK) [NHMUK 20120068-20120078]. All type material collected from the surface of a piece of vent chimney sampled from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean (18º 22.605' N 81º 47.875' W), water depth 2300 m.

3.5 Description

Shell (Figure 3.1). Rounded, skeneiform, sturdy, height greater than width (see Table 3.2); maximum dimensions 7.2 mm height, 6.1 mm width [NHMUK 20120078]. Surface smooth, lacking pigmentation, with thin, beige-white periostracum. Surface and apical region, including the protoconch and early teleoconch, are corroded in most specimens. Protoconch too corroded for any details to be seen (Figure 3.1 E-F). Teleoconch whorls more than 2.5 in number, body whorl large. No nacre or lustre visible on exterior or interior of the shell. Umbilicus open and deep, clearly visible in basal view. Peristome smooth. Aperture large and circular with smooth outer lip.

Operculum (Figure 3.2A) moderately thin, corneous, and yellowish-brown; multispiral with a central nucleus and short growing edge, with a good fit to the aperture. Opercula retraction is deep.

Soft parts (Figures 3.3-3.5). Animal pale white in colour. Head quite large, snout cylindrical, terminating in a broad tip with mouth positioned at the midline. One pair of cephalic tentacles of similar size to each other and equal in length to the snout; cephalic tentacles densely papillated with what appear to be sensory papillae (Figure 3.5D). Eyestalks subequal in length and width to cephalic tentacles, without visible papillae and eyes. Right eyestalk approximately one-half length of right cephalic tentacle (Figure 3.4 A, B, D), left eyestalk approximately one-third length of left cephalic tentacle (Figure 3.5 A-B). Cephalic lappets absent. Neck lobes arise from both basal sides of the head. Right neck lobe (Figure 3.4D) divided into elongate anterior (nl1) and posterior

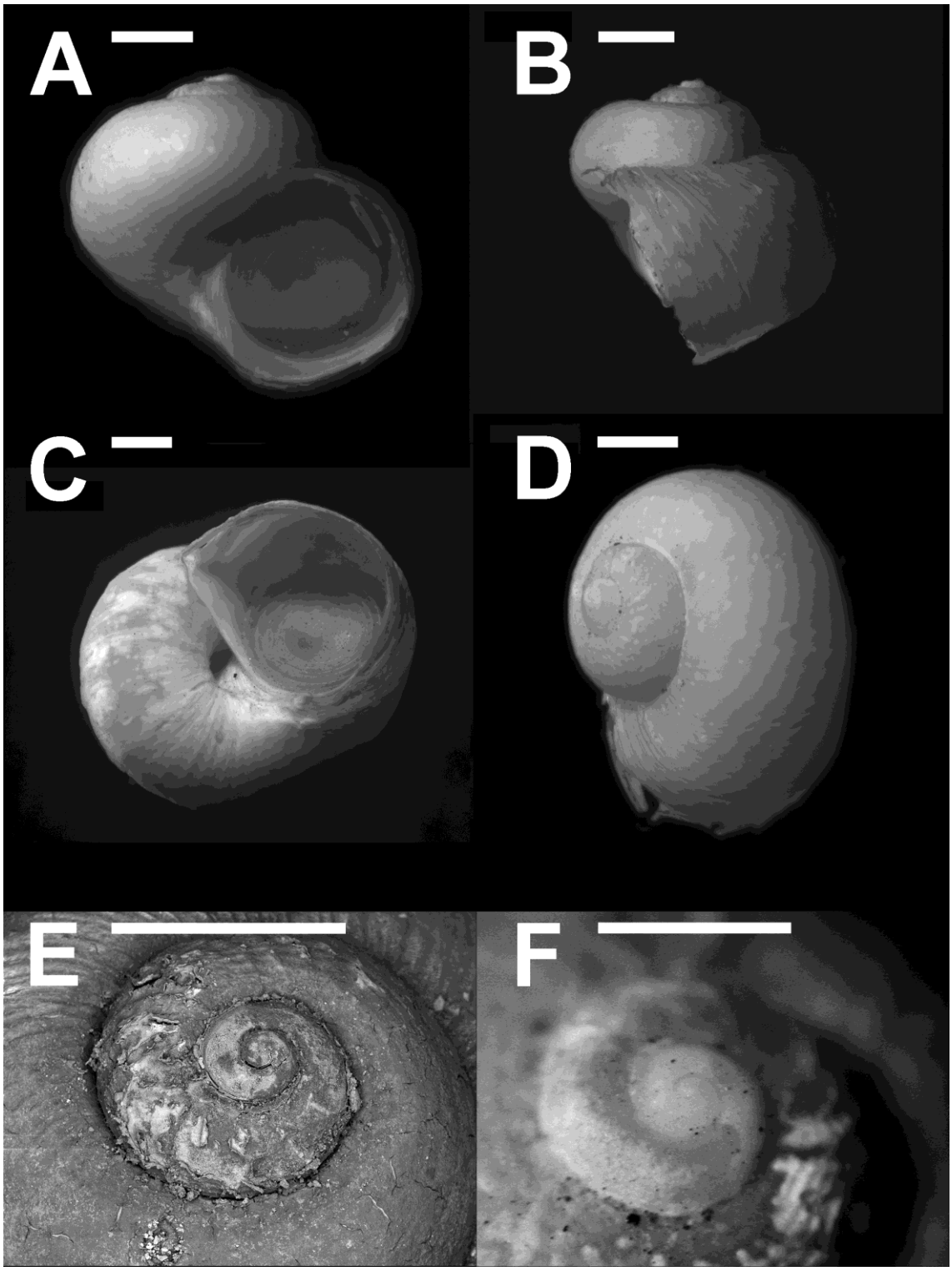

Figure 3.1. *Iheyaspira bathycodon* sp. nov., shell, from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. A, holotype [NHMUK 2012.0068]; B, holotype, lateral view [NHMUK 2012.0068]; C, holotype, basal view [NHMUK 2012.0068]; D, paratype [NHMUK 2012.0072]; E, holotype, apical view [NHMUK 2012.0068]; F, holotype, sub-apical view [NHMUK 2012.0068]. Scale bars = 1 mm.

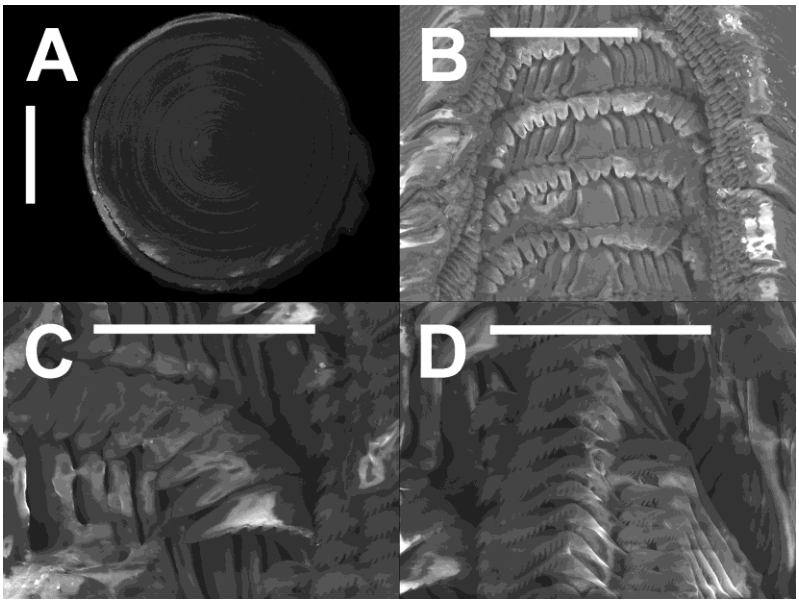

Figure 3.2. *Iheyaspira bathycodon* sp. nov., paratype [NHMUK 2012.0076] from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. A, operculum; B, radula; C, radula: lateral teeth; D, radula: marginal teeth. Scale bars: A = 1mm; B = 100 µm; C, D = 50 µm.

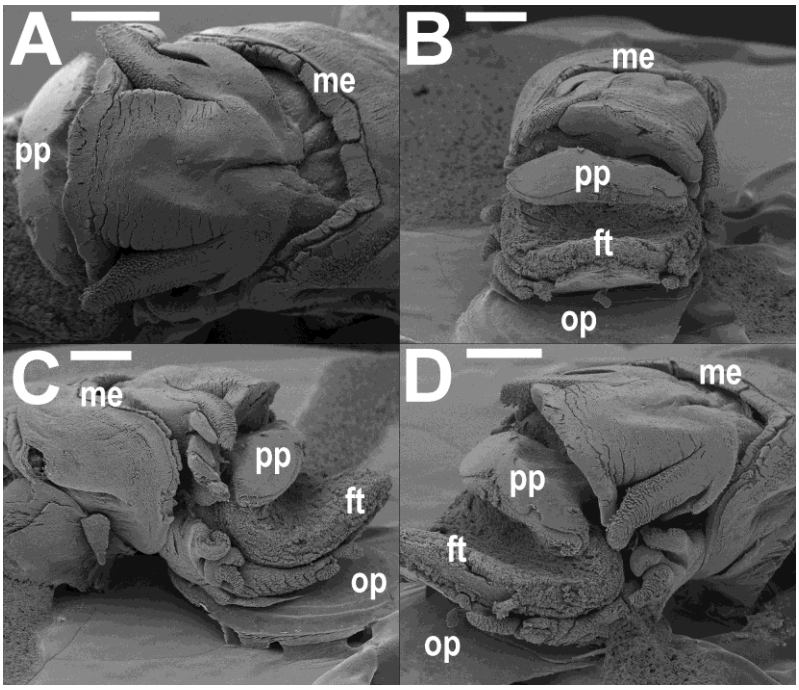

Figure 3.3. *Iheyaspira bathycodon* sp. nov., paratype [NHMUK 2012.0070] from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. SEM micrographs of head-foot. A, dorsal view; B, anterior view; C, right side, lateral view; D, left side, anterolateral view. Abbreviations used: ft, foot; me, mantle edge; op, operculum; pp, parapodium. Scale bars: A = 1mm; B-D = 500 µm.

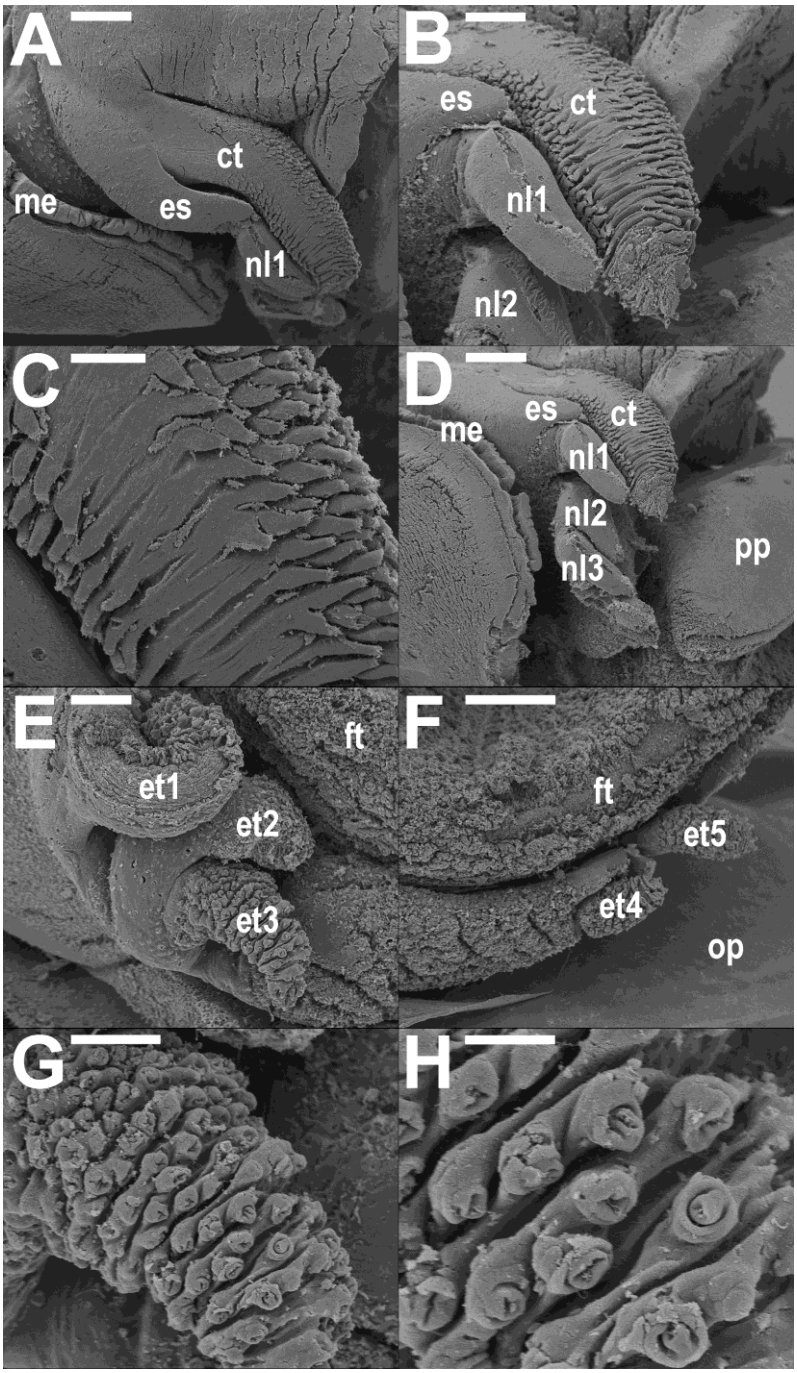

Figure 3.4. *Iheyaspira bathycodon* sp. nov., paratype [NHMUK 2012.0070] from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. SEM micrographs of head-foot, right side. A, head, dorsolateral view; B, head appendages, anterolateral view; C, close-up of cephalic tentacle, lateral view; D, head appendages and neck lobes, anterolateral view; E, epipodial tentacles 1-3, dorsolateral view; F, epipodial tentacles 4-5, lateral view; G, epipodial tentacle 3, anterolateral view; H, papillae on epipodial tentacle 3. Abbreviations used: ct, cephalic tentacle; es, eyestalk; et, epipodial tentacle; ft, foot; me, mantle edge; nl, neck lobe; op, operculum; pp, parapodium. Scale bars: A, D, F = 200 µm; B, E = 100 µm; C, G = 50 µm; H = 20 µm.

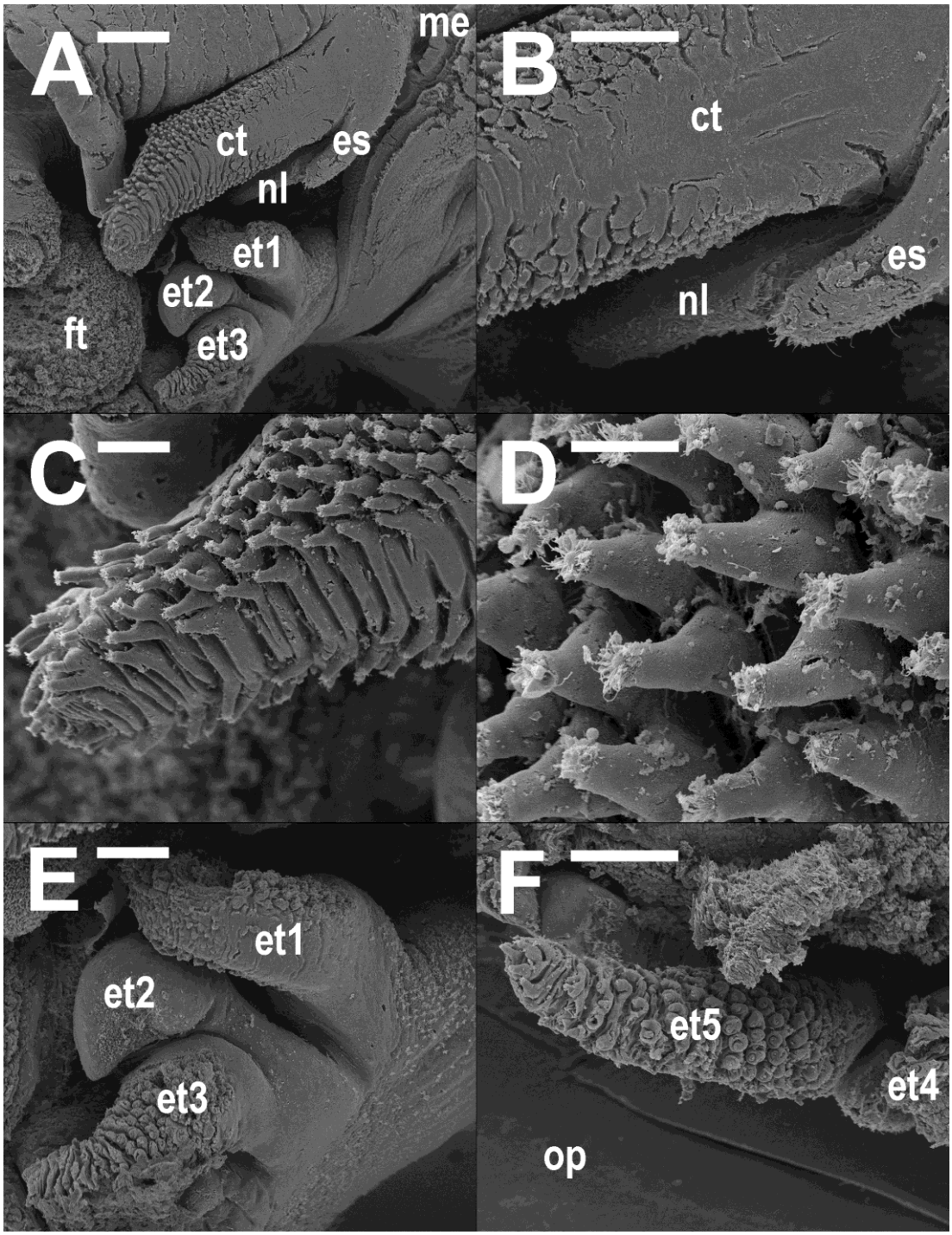

Figure 3.5. *Iheyaspira bathycodon* sp. nov., paratype [NHMUK 2012.0070] from the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, 2300 m. SEM micrographs of head-foot, left side. A, head, dorsolateral view; B, head appendages, lateral view; C, tip of cephalic tentacle, lateral view; D papillae on cephalic tentacle; E, epipodial tentacles 1-3, dorsolateral view; F, epipodial tentacles 4-5, dorsolateral view. Abbreviations used: ct, cephalic tentacle; es, eyestalk; et, epipodial tentacle; ft, foot; me, mantle edge; nl, neck lobe; op, operculum. Scale bars: A = 200 µm; B, E, F = 100 µm; C = 50 µm; D =20 µm.

(nl2, nl3) tentacles. Left neck lobe composed of at least one undivided tentacle beneath the left eyestalk (Figure 3.5 A-B). Foot equipped with five epipodial tentacles on both sides. Right side: first, second and third epipodial tentacles clustered together, similar in size, with dense papillae except on second (Figure 3.4E); fourth and fifth isolated from first three in middle part of epipodium between lobes of epipodial skirt, densely papillate (Figure 3.4F). Left side: first, second and third epipodial tentacles clustered together; first and third epipodial tentacles of similar size with dense papillae; second slightly smaller, without visible papillae (Figure 3.5E); fourth and fifth isolated from first three in middle part of epipodium, densely papillate (Figure 3.5F). Ctenidium monopectinate, attached along its whole length, with bursicles.

Radula (Figure 3.2 B-D). Rhipidoglossate, bilaterally symmetrical, with the formula ∞ - 9 - 1 - 9 - ∞ (>20). Length ~3.1 mm, width ~384 μm, with at least 60 transverse rows along total length in paratype [NHMUK 20120076]. Central tooth differentiated in form from lateral teeth; smooth-sided, bell-shaped, wider proximally than distally, with a single incurved central cusp (Figure 3.2B). Lateral teeth (Figure 3.2 B-C) of similar size to central tooth, increasing in size outwards; with a long, rounded single central cusp, and an outer apical margin with several flanking denticles (>7); dentition attenuates towards the cusp and is strongest on the outermost lateral. Marginal teeth (Figure 3.2B, D) exceed twenty in number on both sides; cutting plate concave, terminating in a single short cusp; apical margins oblique, each with about 10-14 denticles that are longer and finer than those on the lateral teeth. Outermost marginal teeth in a row are smaller, with weaker dentition and straighter shafts. Marginal rows overlap each other.

No jaws are present.

3.6 Comparative remarks

The shell of *Iheyaspira bathycodon* sp. nov. is superficially similar to those of several other skeneimorph taxa, but the radula pattern appears to be unique in number of teeth and shape.

The new species is closest in morphology to *Iheyaspira lequios* Okutani, Sasaki & Tsuchida, 2000 (Turbinidae: Skeneinae), the type species of a monotypic genus. Affinities with *I. lequios* include conchological similarity and shared radula characters,

most notably in the shape of the central tooth (see Okutuni *et al.*, 2000: Figure 2, p. 269). The new species does, however, exhibit several important dissimilarities to *I. lequios*: (1) Shell: small (max. 7.2 x 6.1 mm) rather than minute (max. 5.7 x 5.4 mm in *I. lequios*), umbilicate, teleoconch with more than two whorls; (2) Radula: central tooth bell-shaped, not rhombic/arrow-shaped; only nine (not twelve) pairs of lateral teeth; (3) Eyestalks: reduced, subequal in length and width to cephalic tentacles, as opposed to well-developed, thicker than cephalic tentacles; (4) Neck lobes: right neck lobe composed of three (not two) tentacles; (5) Epipodial tentacles: five (not four) on both sides; left ET1 and ET3 densely papillate (*I. lequios* ET1-3 lack papillae). In *Iheyaspira bathycodon* sp. nov., ET2 and ET3 are very close together, arising as a pair from the same base; this is similar to *I. lequios*, and may be an epipodial sense organ.

The new species is also comparable with the turbinid *Fucaria mystax* Warén & Bouchet, 2001 (Skeneinae), based on similarities in shell and radula characters, especially the shape of the central tooth (see Warén & Bouchet, 2001: Figure 11C, p. 135). *Iheyaspira bathycodon* sp. nov. is differentiated from *F. mystax* by: (1) Shell: umbilicus clearly visible in basal view, teleoconch with greater number of whorls (>2.5); (2) Radula: central tooth bell-shaped, lacking drawn out and narrow anterior support; only nine (not eleven) pairs of lateral teeth; (3) Eye stalks: do not encircle cephalic tentacles. Moreover, members of the genus *Fucaria* Warén & Bouchet, 1993 are equipped with a coat of sensory papillae on the snout, a feature not observed in the new species.

3.7 Distribution and habitat

Known only from the type locality, the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean in 2300m depth. See Connelly *et al*. (2012) for a description of the geological, geochemical and biological setting of the Von Damm Vent Field. Accompanying fauna observed in close proximity to the new species included the alvinocaridid shrimps *Rimicaris hybisae* Nye, Copley & Plouviez, 2012 (see Chapter 2) and *Alvinocaris* sp., the hippolytid shrimp *Lebbeus virentova* Nye, Copley, Plouviez & Van Dover 2013 (see Chapter 4), zoarcid fish and siboglinid polychaetes (Figure 3.6).

Figure 3.6. *Iheyaspira bathycodon* sp. nov. *in situ* at the Von Damm Vent Field, Mid-Cayman Spreading Centre. A, main mound with *Rimicaris hybisae*, *Alvinocaris* sp. and *Lebbeus virentova* (lasers = 10 cm apart); B, fumerole with *Rimicaris hybisae*; C, edge of Hole to Hell with *Rimicaris hybisae* and *Pachycara* sp.; D, tail area with *Alvinocaris* sp. and *Escarpia* sp.

3.8 Etymology

The species name *bathycodon* is derived from the Greek words for deep and bell, in reverence to the species' deep-sea habitat and bell-shaped rachidian tooth.

3.9 Molecular phylogeny

Partial sequences of the COI (549 bp), 16S (505 bp) and 18S (803 bp) regions of *Iheyaspira bathycodon* sp. nov. were consistent amongst specimens. Fixed and unique mutations were observed in the partial sequences of the COI, 16S and 18S regions in comparison with other trochoid taxa. When compared with partial sequences of COI in the gastropod dataset of Suzanne Williams (NHMUK) the partial COI sequence of the new species was near other trochoidean skeneimorph taxa and unique amongst any of the species available (Suzanne Williams, personal communication). Based on a 425-bp alignment of partial 16S sequences, NJ and ML phylogenetic trees have the same topologies and place the new species in the same clade (Turbinidae: Skeneinae) as *Dillwynella* cf. *vitrea* [AY163406.1], *Protolira valvatoides* [AY163405.1] and *Protolira* sp. [GQ160698.1], with 96% and 87% bootstrap support for NJ and ML methods respectively (Figure 3.7). Of the 16S partial sequences available in GenBank the new species is closest in evolutionary distance to *D.* cf. *vitrea* (14% divergence). Phylogenetic analyses on an 803-bp alignment of partial 18S sequences of turbinid species place the new species closest in evolutionary distance to *D. planorbis* [AB365310.1], with 39% and 42% bootstrap support for NJ and ML methods respectively. The new species exhibits 1.4% divergence from *D. planorbis* across an 803 bp of the 18S region.

3.10 Discussion

The molecular and morphological analyses of specimens of this trochoid gastropod reveal the presence of a new species. The new species is similar in morphology to *Iheyaspira lequios* and *Fucaria mystax* (see above), both of which are known only from hydrothermal vents in the Pacific at water depths less than 1500 m (see Table 3.1). It is, however, excluded from both species by differences in the radula and appendage structure of the head-foot (see above). It is closest in morphology to *I. lequios* and, therefore, the most conservative approach is to give the new species the generic name *Iheyaspira* to indicate the similarity between the two species. Consistency

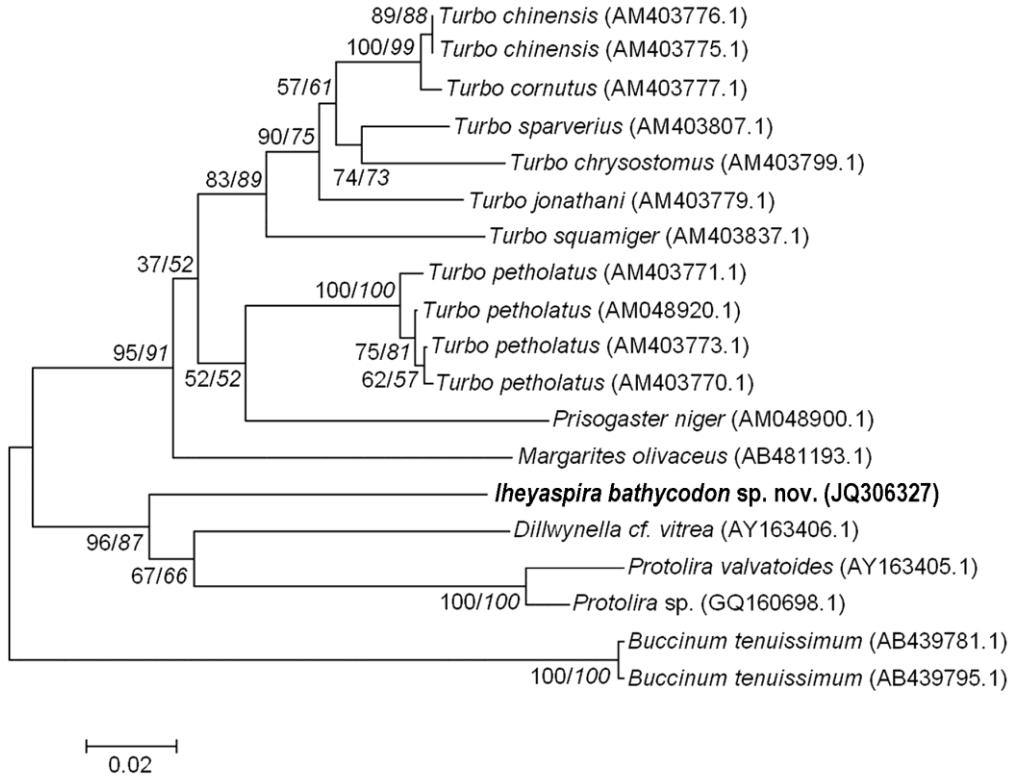

Figure 3.7. Neighbour-joining tree of turbinid gastropods based on a 425-bp alignment of partial nucleotide sequences from the mitochondrial 16S region with *Buccinum tenuissimum* (Caenogastropoda: Neogastropoda: Buccinoidea: Buccinidae) as outgroup. Evolutionary distances computed using the Jukes-Cantor method (Jukes & Cantor, 1969) are represented by branch length; scale bar is proportional to inferred nucleotide divergence. Bootstrap support calculated on 1000 re-sampling replicates is shown by the numbers along the branches (NJ, plain text; ML, italic text). GenBank accession numbers are given after species names.

between specimens of *I. bathycodon* sp. nov. in partial sequences of the COI, 16S and 18S regions confirm that they belong to a single species, but the presence of unique and fixed mutations in the sequences indicate that they are genetically distinct from all other genera and species in the GenBank database.

The systematic position of both *Iheyaspira* and *Fucaria* is uncertain because there are no sequences available in GenBank for either genus; however both genera are classified currently within the family Turbinidae and subfamily Skeneinae (e.g. Bouchet, 2010 a, b; Sasaki *et al.*, 2010). After redefining the Turbinidae, Williams *et al*. (2008) remarked that it is hard to determine morphological characters that are typical of

this family, and even suggested that the Skeneinae could be considered as a group distinct from (but most closely related to) Turbinidae (Williams *et al.*, 2008). The rank of the Skeneinae is still under discussion and further work will elucidate the systematic position of this taxon (Williams, 2012).

Morphological features of the Skeneinae shared with the new species include the monopectinate ctenidium and absence of any visible nacre. In addition, the shell of the new species bears superficial resemblance to that of other members of the Skeneinae, such as *Protolira*. In the GenBank database sequences for the Skeneinae are available presently for a few species only. Despite this impediment, comparative 16S results presented herein suggest the proximity of the new species to members of the Skeneinae, with strong bootstrap support for inclusion of the new species within this clade. This is supported further by comparative 18S results, whereby NJ and ML methods both place the new species closest in evolutionary distance to *Dillwynella planorbis* [AB365310.1], although this is with weak bootstrap support (39% and 42% for NJ and ML methods respectively).

The first right neck lobe tentacle (RNL1) in the new species may be modified (see Figure 3.4B, D). Warén & Bouchet (1989) described a modified neck lobe tentacle in *Bathymargarites symplector* Warén & Bouchet 1989 and interpreted this modified appendage as a penis. Collection of further specimens will enable the reproductive anatomy of the new species to be characterised.

The recent discovery of hydrothermal vents and chemosynthetic assemblages on the Mid-Cayman Spreading Centre has provided an opportunity to enhance existing knowledge of biodiversity in the deep sea. *Iheyaspira bathycodon* sp. nov. is the second new species to be described from the Von Damm Vent Field, and the tenth turbinid gastropod to be described from a hydrothermal vent environment to date (Table 3.1). Description of species from Mid-Cayman Spreading Centre vents, and further characterisation of their faunal assemblages by future collections has the potential to elucidate the factors determining vent biogeography of this region.

3.11 Acknowledgements

Thanks to those onboard the 44th voyage of the *RRS James Cook*, to P.A. Tyler and C.L. Van Dover for providing laboratory facilities at the NOCS and DUML, and to A. Glover (NHMUK) and A. Page (Biomedical Imaging Unit, University of Southampton) for use of their microscopy facilities. A. Warén and S. Williams are also thanked for commenting on aspects of this work, which is supported by a UK NERC award (NE/F017774/1) to J. Copley and NASA ASTEP Grant (NNX09AB75G) to C.L. Van Dover. This work benefitted from an anonymous reviewer who gave valuable comments for the improvement of this paper.

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4. A new species of *Lebbeus* (Crustacea: Decapoda: Caridea: Hippolytidae) from the Von Damm Vent Field, Caribbean Sea

This work has been published as: Nye V., Copley J., Plouviez S. & Van Dover C.L. (2013) A new species of *Lebbeus* (Crustacea: Decapoda: Caridea: Hippolytidae) from the Von Damm Vent Field, Caribbean Sea. *Journal of the Marine Biological Association of the United Kingdom* 93, 741-751. Doi:10.1017/S0025315412000884. Published online 13 August 2012; code-compliant paper version published in May 2013.

Abstract

A new species of the hippolytid shrimp genus *Lebbeus* White, 1847 is described from the Von Damm Vent Field on the Mid-Cayman Spreading Centre (MCSC), Caribbean, at 2294 m water depth. *Lebbeus virentova* sp. nov. is defined and illustrated from seven specimens, with brief notes on its distribution and habitat. Molecular phylogenetic data from the COI mitochondrial DNA region are used to analyse the species' phylogenetic position, and its morphology is compared with previously described species. This new species represents the second family of caridean shrimp to be reported from the Von Damm Vent Field. *Lebbeus virentova* sp. nov. is the eighth member of the genus to be described from hydrothermal vents and appears to be the first hippolytid shrimp at a vent field known from outside the Pacific Ocean.

4.1 Introduction

Following the discovery of aggregations of alvinocaridid shrimp at hydrothermal vents in the Pacific (Williams, 1980; Williams & Chace, 1982), and at cold seeps in the Gulf of Mexico (Williams, 1988), a substantial research effort has addressed the taxonomy, phylogeny, ecology, physiology and distribution of caridean shrimp from chemosynthetic ecosystems in the deep sea (e.g. Van Dover *et al.*, 1988, 1989; Gebruk *et al.*, 1993; Segonzac *et al.*, 1993; Shank *et al.*, 1998, 1999; Copley & Young, 2006; Komai *et al.*, 2010; Teixeira *et al.*, 2010).

At least fifty species from nine caridean families have been recorded from deep-sea vents and seeps (Martin & Haney, 2005; De Grave & Fransen, 2011). Members of the family Alvinocarididae Christoffersen, 1986 appear to be endemic to deep-sea chemosynthetic environments, and form a dominant component of the biomass at several vent fields in the Atlantic and Indian Oceans (see Nye *et al.*, 2012 for recent review). In contrast, the presence of other caridean families at vents and seeps is considered to be opportunistic (e.g. Martin & Haney, 2005; Desbruyères *et al.*, 2006).

The genus *Lebbeus* White, 1847, is composed of sixty species (Komai *et al.*, 2012), and represents the most diverse genus within the Hippolytidae Spence-Bate, 1888. Species of *Lebbeus* are found from shallow to deep waters (e.g. Chang *et al.*, 2010). The genus exhibits a cosmopolitan distribution from the tropics to high latitudes, but its species generally have narrow geographic ranges (Komai *et al.*, 2004).

The majority of *Lebbeus* species are described from the western North Pacific (e.g. Komai & Takeda, 2004; Komai *et al.*, 2004; De Grave & Fransen, 2011). *Lebbeus* is the only hippolytid recorded from deep-sea chemosynthetic environments, with several species documented from hydrothermal vents in the Pacific (see Table 4.1 and references therein).

Two high-temperature hydrothermal vent fields were discovered recently at the Mid-Cayman Spreading Centre (MCSC), Caribbean (Connelly *et al.*, 2012). The ultraslow-spreading MCSC is located in a deep trough, tectonically and geographically isolated from other mid-ocean ridges (Ballard *et al.*, 1979; German *et al.*, 2010). The Von Damm Vent Field is located away from the axis of the spreading centre on the upper slopes of an oceanic core complex at ~2300 m water depth (Connelly *et al.*, 2012). The Von Damm Vent Field is a conical mound approximately 150 m in diameter and 75 m high, venting clear, buoyant, high-temperature fluids at its peak, visually

dominated by swarming shrimp (Connelly *et al.*, 2012). Investigations of the fauna inhabiting vent fields on the MCSC have the potential to enhance current understanding of the dispersal and evolution of vent taxa, and vent biogeography of the region (Van Dover *et al.*, 2002).

In this study, a new species of *Lebbeus* from the Von Damm Vent Field is described and illustrated. *Lebbeus virentova* sp. nov. belongs to the second family (Hippoloytidae) of caridean shrimp to be reported from the Von Damm Vent Field, the other being the Alvinocarididae (see Chapter 2). In addition to enhancing existing knowledge of biodiversity in the deep sea, this appears to be the first record of a hippolytid shrimp from a vent field outside the Pacific Ocean.

Table 4.1. Summary of geographical distribution and bathymetric range of *Lebbeu*s species from hydrothermal vents.

Species	**Site(s)**	**Depth (m)**	**References**
Lebbeus bidentatus+	SE Pacific: southern East Pacific Rise	Not available	Martin & Haney (2005)
*Lebbeus laurente**	NE Pacific: East Pacific Rise 13°N	2618-2640	Wicksten (2010); Komai *et al.*(2012)
Lebbeus manus	SW Pacific: Manus Basin	1540-1577	Komai & Collins (2009)
Lebbeus pacmanus	SW Pacific: Manus Basin	1662	Komai *et al.*(2012)
*Lebbeus shinkaiae***	NW Pacific: Okinawa Trough	691-1491	Komai *et al.* (2012)
Lebbeus thermophilus	SW Pacific: Manus & Lau Basins	1512-1842	Komai *et al.* (2012)
Lebbeus virentova sp. nov.	Caribbean: Von Damm Vent Field, Mid-Cayman Spreading Centre	2294-2375	Nye *et al.* (2013)
Lebbeus wera	SW Pacific: Brothers Caldera, Kermadec Ridge	1208-1336	Ahyong (2009); Komai *et al.*(2012)

+ Record unverified. Known also from its type location, off Chile at 1680 m depth (Zarenkov, 1976); *a replacement name for *L. carinatus* De Saint Laurent, 1984, a junior homonym of *L. carinatus* Zarenkov (1976); ** Recorded as *L. washingtonianus* (Kikuchi & Ohta, 1995; Martin & Haney, 2005; Komai & Collins, 2009) before being referred to *L. shinkaiae* Komai, Tsuchida & Segonzac, 2012.

4.2 Materials and methods

Specimens were collected from the Von Damm Vent Field (2294-2375 m) at the Mid-Cayman Spreading Centre, Caribbean, during the 18th voyage of the *RV Atlantis* (16th leg, January 2012). All specimens were collected using a suction sampler attached to the remotely operated vehicle (ROV) *Jason II*, together with still photographs and video recordings of them *in situ*. Material for morphological study was fixed immediately in 10% neutralised formalin and subsequently transferred to 75% IMS (industrial methylated spirits). Material for molecular analyses was placed immediately in 95% ethanol.

Measurements of specimens were taken to the nearest 0.1 mm using Vernier callipers. Postorbital carapace length (CL) was measured from the posterior margin of the orbit to the posterior margin of the carapace and is used herein as an indication of specimen size. Individuals were sexed under a dissecting microscope.

Illustrations were prepared with the aid of a cameral lucida mounted onto a Leica MZ8 steromicroscope, scanned and inked digitally using a WACOM™ digitiser and Adobe® Illustrator® software, as described by Coleman (2003, 2009). Specimens are deposited in the invertebrate collection at the Smithsonian Institution, National Museum of Natural History (USNM), Washington DC. Morphological terminology generally follows Komai *et al.* (2012).

Abdominal muscle for DNA extraction was cut from ethanol-preserved specimens and the carapace removed. Total genomic DNA was extracted using the CTAB (cetyltrimethyl ammonium bromide) procedure (Doyle & Dickson, 1987). Regions of mitochondrial cytochrome c oxidase subunit I (COI) DNA and 16S ribosomal DNA were amplified by performing polymerase chain reactions (PCR).

The COI region was amplified with the universal primers LCO1490 and HCO2198 described by Folmer *et al*. (1994). The 20 µl amplification mixture contained 1X buffer reagent (200 mM Tris pH 8.8, 500 mM KCL, 0.1% Triton X-100, 2 mg/ml bovine serum albumen), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 mM of each primer, 1 U Taq DNA polymerase (Bioline), 5 µl of template DNA and sterile H_2O to final volume. The thermal cycling conditions were: 94°C/2 min; followed by 5 cycles at (94°C/35 s; 45°C/35 s; 72°C/1:20 min) and 35 cycles at (94°C/35 s; 50°C/35 s; 72°C/1:20 min) with a final extension of 72°C/10 min.

Amplifications of the 16S region were performed using the universal primers 16Sar and 16Sbr described by Palumbi (1996). The 20 µl amplification mixture contained: 1X reaction buffer (same as for COI), 2.5 mM $MgCl_2$, 0.13 mM of each dNTP, 0.38 mM of each primer, 1 U Taq DNA polymerase (Bioline), 2.5 µl of template DNA and sterile H_2O to final volume. The thermal cycling conditions were: 94°C/4 min; 30 cycles at (94°C/30 s; 52°C/1 min; 72°C/2 min) and 72°C/5 min.

The PCR products were purified with the ExoAP treatment by adding the following ExoAP mixture to 15 µl PCR product: 0.2 µl 10X ExoAP buffer (50 mM Bis-Tris, 1mM $MgCl_2$, 0.1 mM $ZnSO_4$), 0.05 µl 5000 U/ml Antarctic phosphatase (New England Biolabs: Ipswich, MA), 0.05 µl 20000 U/ml exonuclease I, and 3.7 µl sterile H_20) and thermal-cycler incubation (37°C/60 min; 85°C/15 min). Sequencing reactions were performed using BigDye Terminator Reactions following the manufacturer's protocol (Applied Biosystems: Foster, CA) with the primer sets used for amplifications. For COI, the thermal-cycler reaction was performed as: 94°C/30 s followed by 25 cycles at (94°C/15 s; 50°C/15 s; 60°C/3 min). The PCR conditions for 16S were identical to those described for COI, but with the use of 52°C and 64°C annealing temperatures. The sequencing reaction products were purified with the AMPure magnetic bead system following the manufacturer's protocol (Agencourt: Morrisville, NC) and were subsequently run on an ABI 3730x1 DNA Analyzer (Applied Biosystems International).

The sequence strands for each gene were proof read and assembled with CodonCode Aligner, version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), to produce a continuous fragment. Sequences were compared with those in GenBank using the nucleotide BLAST program (NCBI Basic Alignment Search Tool) and manually aligned in BioEdit (Hall, 1999). Phylogenetic trees were constructed with *MEGA*5 (Tamura *et al*., 2011) using the neighbour-joining (NJ) (Saitou & Nei, 1987) and maximum-likelihood (ML) (Kimura, 1980) methods on a 588-base pair (bp) alignment for COI. The bootstrap values were calculated on 1000 re-sampling replicates.

GenBank accession numbers for partial sequences of the COI and 16S regions are JQ837265 and JQ837266 respectively.

4.3 Systematics

Order DECAPODA Latreille, 1802

Infraorder CARIDEA Dana, 1852

Superfamily ALPHEOIDEA Rafinesque, 1815

Family HIPPOLYTIDAE Spence Bate, 1888

Genus *Lebbeus* White, 1847

Lebbeus virentova sp. nov.

(Figures 4.1-4.5)

4.4 Material examined

Holotype: female, CL 15.4 mm. Von Damm Vent Field, MCSC, Caribbean Sea; co-ordinates: 18°37.661'N 81°79.81W; water depth: 2294 m [USNM 1183692]. Collected on the 18th voyage (16th leg) of the *RV Atlantis*, on 19 January 2012.

Paratypes: six females, CL 11.12-15.6 mm [USNM 1183693-1183698]. Same data as holotype.

4.5 Description

Body moderately robust; integument glabrous.

Rostrum (Figure 4.1, 4.2 A-B) straight, directed forward, 0.30-0.48 CL; reaching beyond midlength but not to distal margin of first segment of antennular peduncle; laterally compressed, tapering to bifurcate apex; dorsal margin armed with 4-6 teeth (2-3 widely spaced teeth on rostrum proper; 2-3 larger, widely spaced postrostral teeth), posteriormost tooth arising at 0.16-0.26 CL; ventral margin armed with 2-4 teeth in distal 0.25, ventral lamina poorly developed. Carapace (Figure 4.1, 4.2 A-B) with low but distinct median portrostral carina extending to posterior two-thirds of carapace;

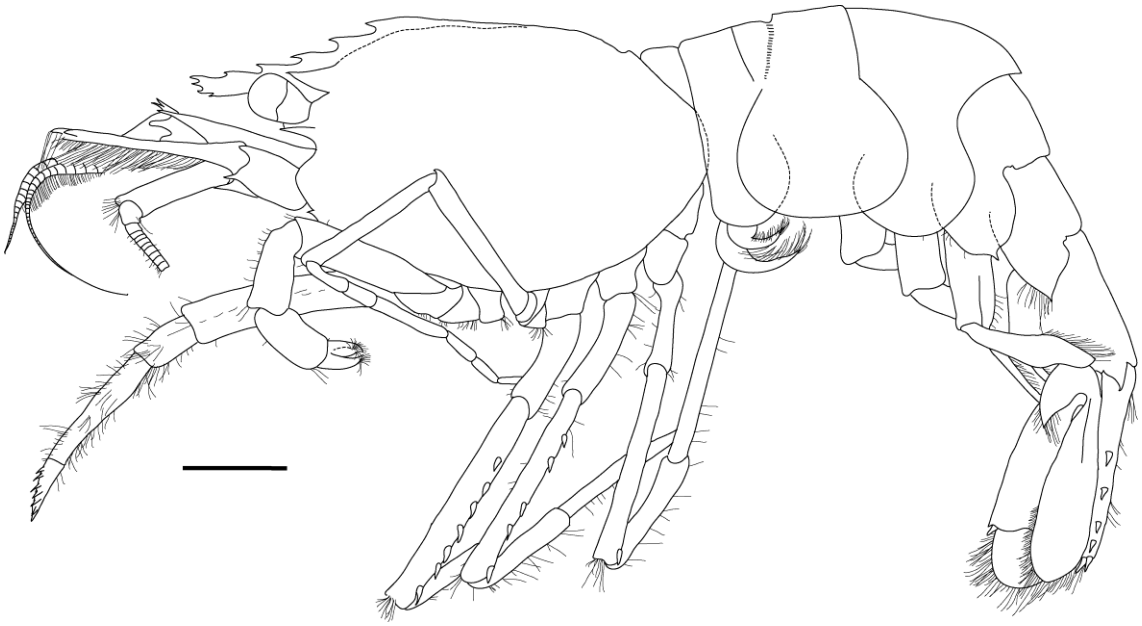

Figure 4.1. *Lebbeus virentova* sp. nov., holotype, female (CL 15.4 mm), [USNM 1183692], from the Von Damm Vent Field, Mid-Cayman Spreading Centre: entire animal, lateral view. Scale bar = 5 mm.

dorsal profile in lateral view gently convex. Supraorbital tooth strong, arising level with posterior margin of orbit, directed forward, reaching tip of suborbital lobe and antennal tooth; deep V-shaped notch inferior to base of supraorbital tooth. Orbital margin weakly concave; suborbital lobe bluntly triangular. Antennal tooth well-developed, acute, reaching tip of suborbital lobe. Pterygostomial tooth small, not reaching antennal tooth. Anterolateral margin between antennal tooth and pterygostomial tooth strongly sinuous with deep excavation below antennal tooth.

Abdomen (Figure 4.1) rounded dorsally. Second somite with transverse groove on tergum, bordered posteriorly by low ridge; posterodorsal margin of third somite produced; pleura of anterior three somites unarmed marginally, posteroventral margin rounded; fourth pleuron with posteroventral tooth (Figure 4.2C); fifth pleuron bearing moderately strong posteroventral tooth and numerous long setae on ventral margin (Figure 4.2C). Sixth somite 1.35-1.95 times longer than fifth somite; armed with small posteroventral tooth; posterolateral process terminating in acute tooth.

Telson (Figure 4.1, 4.2 D-E) length 3.10-4.31 times anterior width, 1.25-1.48 times longer than sixth abdominal somite in dorsal midline; lateral margins parallel in

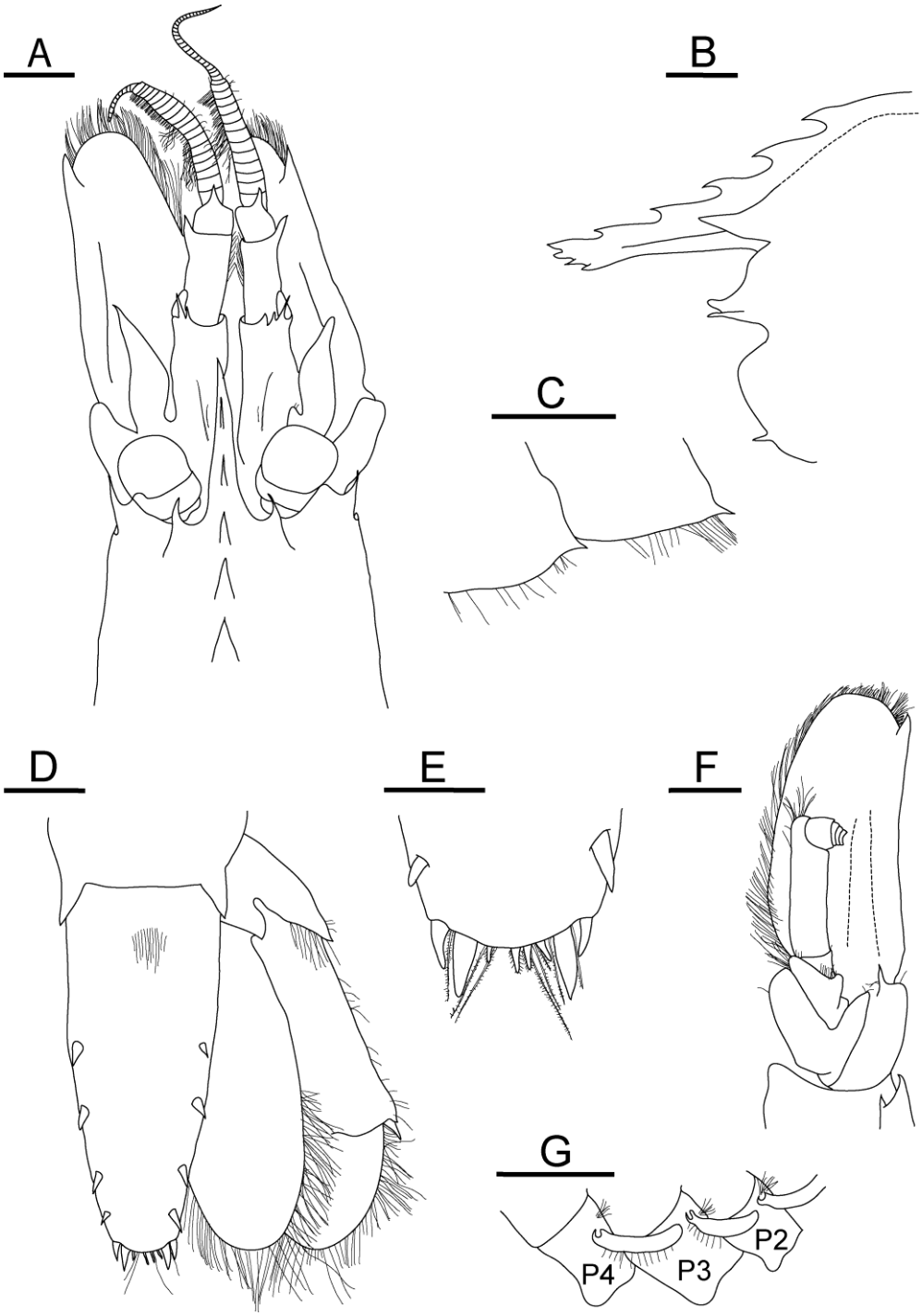

Figure 4.2. *Lebbeus virentova* sp. nov., holotype, female (CL 15.4 mm), [USNM 1183692], from the Von Damm Vent Field, Mid-Cayman Spreading Centre: A, anterior part of carapace and cephalic appendages, dorsal view; B, anterior part of carapace, lateral view; C, posterolateral margins of left pleura of fourth and fifth abdominal somites, lateral view; D, telson and right uropod, dorsal view; E, posterior part of telson, dorsal view; F, left antennal peduncle and scale, ventral view; G, coxae of right second to fourth pereopods, showing presence of epipod on third pereopod and corresponding setobranch on fourth pereopod, lateral view. Scale bars: A-D, G-H = 2 mm; E = 1mm.

anterior third, tapering posteriorly to convex posterior margin, bearing 3-6 (usually 4) dorsolateral spines on each side; posterior margin with 2 pairs of lateral spines (mesial pair longer), 4-6 median spiniform setulose setae and several longer thin plumose setae (Figure 4.2E).

Uropods (Figure 4.1, 4.2D) with broad rami exceeding distal margin of telson; exopod with distinct transverse suture and small spine at distolateral angle; endopod shorter and narrower than exopod; posterolateral projection of protopod triangular with acute tip.

Eyes (Figure 4.1, 4.2A) subpyriform with stalk narrowing proximally; cornea distinctly wider than stalk, its maximum width 0.13-0.15 times CL; ocellus absent.

Antennular peduncles (Figure 4.1, 4.2A) extending approximately to distal 0.2 of antennal scale. First segment as long or slightly longer than distal two segments combined, not quite reaching midlength of antennal scale, dorsodistal margin armed with 2 or 3 (sometimes bifid) slender teeth; stylocerite reaching or slightly exceeding dorsodistal margin of first peduncular segment, terminating in acute point, mesial margin sinuous. Second segment approximately 0.4 length of first segment; bearing strong distolateral tooth. Third segment less than half as long as second; with small dorsodistal tooth. Lateral flagellum with thickened aesthetasc-bearing portion approximately 0.3 times CL.

Antenna (Figure 4.1, 4.2F) with bascicerite bearing small, acute ventrolateral tooth; carpocerite reaching to approximately distal 0.3-0.4 of antennal scale. Antennal scale 0.64 times CL, 3 times longer than wide; lateral margin straight; distolateral tooth nearly reaching rounded distal lamella of blade.

Mouthparts (Figure 4.3) similar to those of other species of the genus. Mandible (Figure 4.3A) composed of flattened incisor, stout molar and biarticulate palp; incisor process bearing 2 acute distal teeth and 2 fine setae on mesial margin; molar process subcylindrical with obliquely truncate grinding surface and area of dense setae distally; palp curved, basal article broad with few short setae, distal article bearing many long setae.

Maxillule (first maxilla) (Figure 4.3B) with well-developed endites; coxal endite bearing numerous long setae; basial endite with row of stiff setae and row of spines along the mesial margin; palp curved weakly, slightly bilobed, bearing several distal setae.

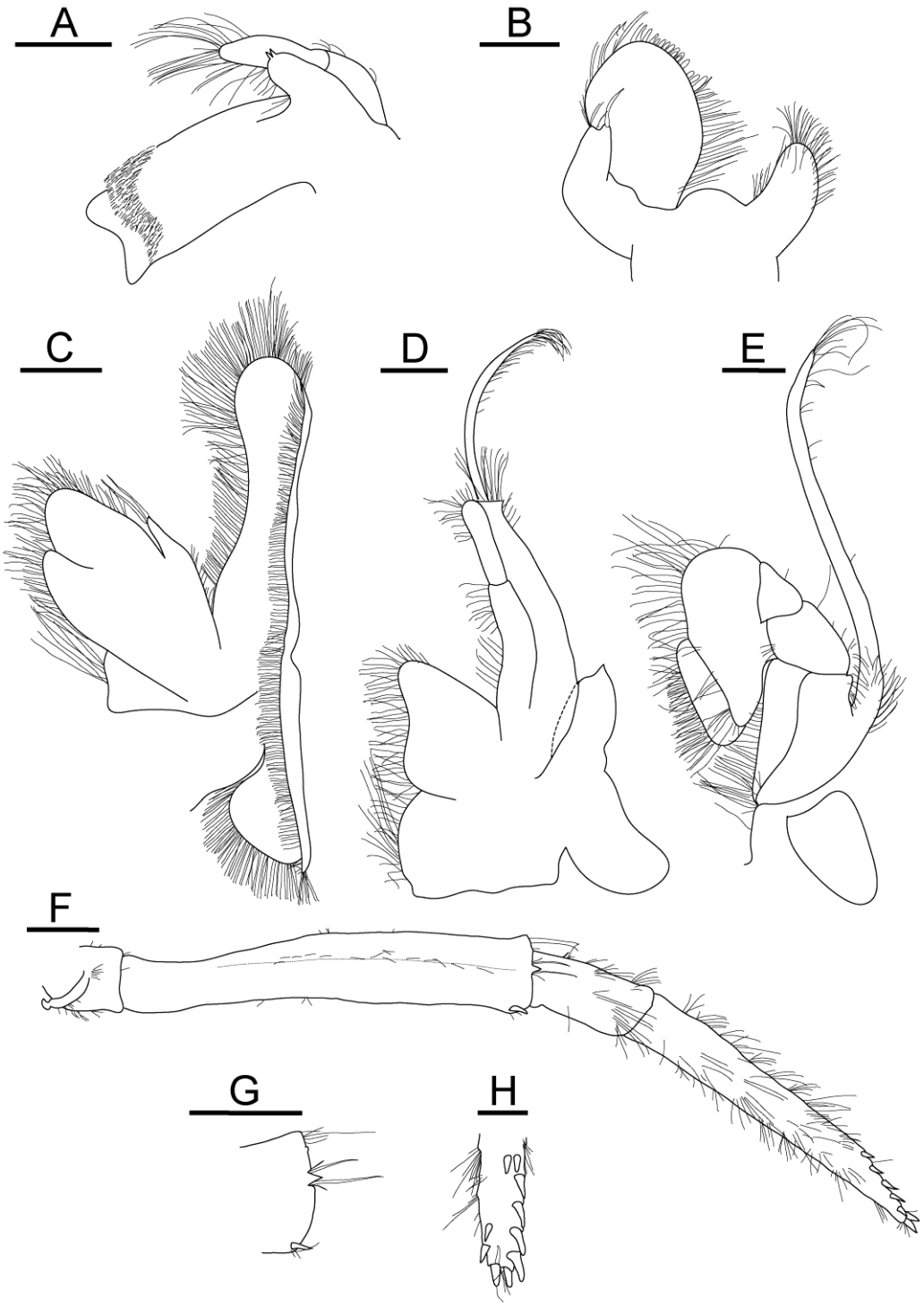

Fig. 4.3. *Lebbeus virentova* sp. nov., holotype, female (CL 15.4 mm), [USNM 1183692], from the Von Damm Vent Field, Mid-Cayman Spreading Centre: A, left mandible, ventral view; B, left maxillule (first maxilla), dorsal view; C, left maxilla (second maxilla), ventral view; D, left first maxilliped, ventral view; E, left second maxilliped, ventral view; F, right third maxilliped, lateral view; G, distal part of antepenultimate segment of right third maxilliped, dorsal (extensor) view; H, distal part of ultimate segment of right third maxilliped, dorsal view. Scale bars: A-E, H = 1mm; F-G = 2mm.

Maxilla (second maxilla) (Figure 4.3C) with bilobed upper endite, fringed with many setae and flanked by well-developed palp with two distal setae; lower endite reduced, bearing several long setae; scaphognathite well developed, with rounded posterior lobe fringed with numerous setae on all margins.

First maxilliped (Figure 4.3D) with well-developed endites fringed with setae; palp biarticulate; exopod with caridean lobe; epipod large, bilobed.

Second maxilliped (Figure 4.3E) with broad ultimate segment fringed with stiff setae; ischial segment with excavated mesial margin; exopod and epipod well-developed.

Third maxilliped (Figure 4.3F) exceeding antennal scale by half length of ultimate segment. Antepenultimate segment approximately 0.8 times as long as two distal segments combined; armed with a small tooth and two long spiniform setae on distolateral margin and a small spine at ventrodistal angle (Figure 4.3G); lateral surface bearing row of spiniform setae on blunt ridge parallel to dorsal margin. Ultimate segment approximately three times longer than penultimate segment, with dense tufts of setae; tapering distally, with short row of corneous spines distomesially and distolaterally (Figure 4.3H).

Strap-like, terminally hooked epipods present on third maxilliped to third pereopod; corresponding setobranchs on first to fourth pereopods (Figure 4.2G).

First pereopod (Figure 4.4A) moderately stout, extending to distal margin of antennal scale. Chela (Figure 4.4 B-E) approximately 1.6 as long as carpus; dactylus approximately 0.6 times as long as palm, strongly curved distally, terminating in two corneous claws with two smaller corneous claws arising inferior to terminal claws; fixed finger terminating in one corneous claw flanked by two smaller corneous claws. Carpus bearing grooming apparatus (a feature widely spread in the Hippolytidae, e.g. Bauer, 1978), comprised of a dense patch of serrate setae arising from a recessed area on mesial face.

Second pereopod (Figure 4.4F) distinctly more slender than first, overreaching antennal scale by approximately 0.33 length of carpus when extended. Chela (Figure 4.4G) small; dactlyus terminating in two corneous claws; fixed finger terminating in one corneous claw. Carpus divided into seven articles.

Third to fifth pereopods (Figure 4.4 H-L) similar in structure, long and slender, normally folded at mero-carpal articulation, decreasing in length and stoutness

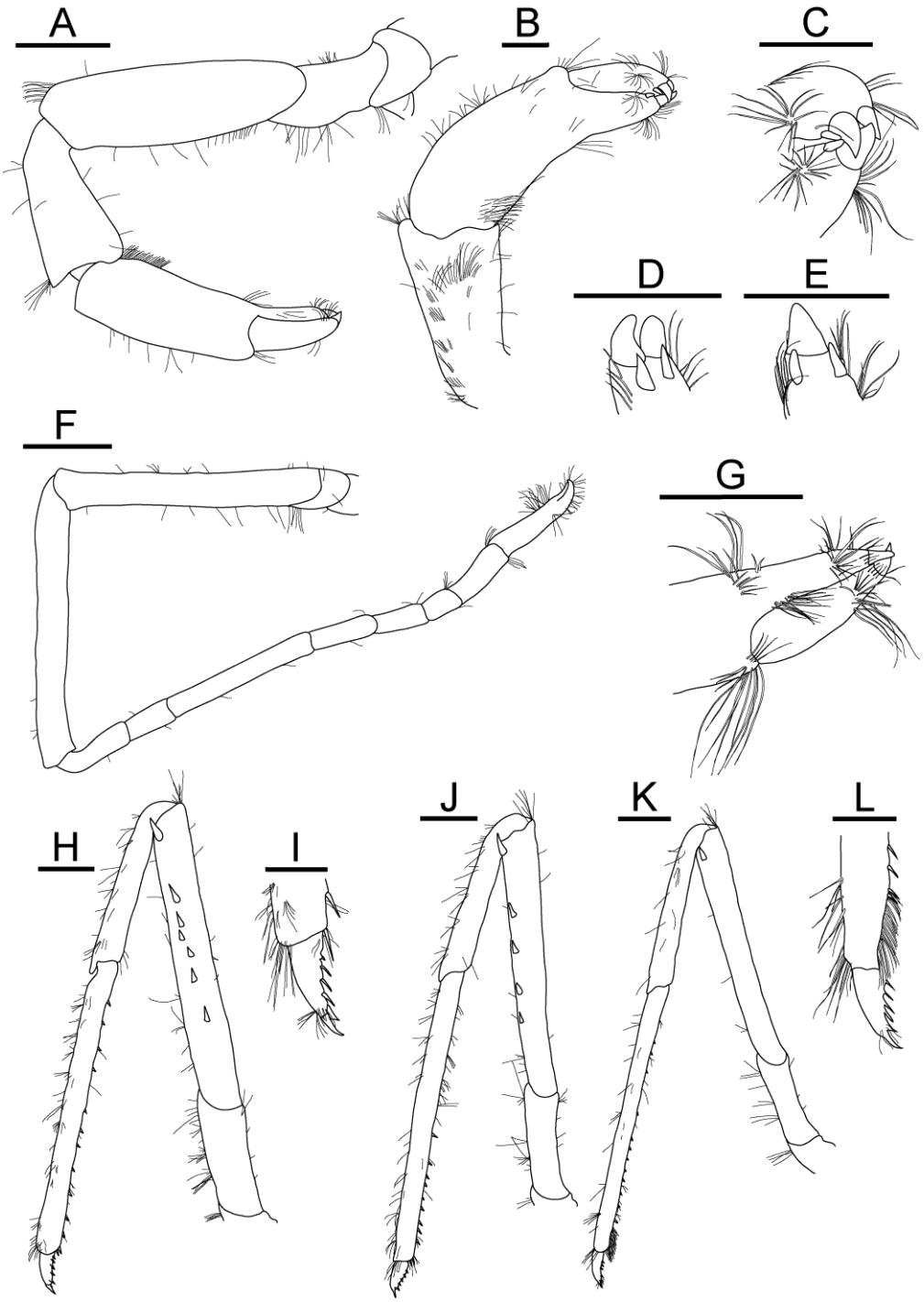

Figure 4.4. *Lebbeus virentova* sp. nov., holotype, female (CL15.4 mm), [USNM 1183692], from the Von Damm Vent Field, Mid-Cayman Spreading Centre: A, left first pereopod, lateral view; B, chela and carpus of left first pereopod, mesial view; C, distal part of chela of left first pereopod; D, same, tip of dactylus, inner view; E, tip of fixed finger, inner view; F, left second pereopod, lateral view; G, chela of left second pereopod, lateral view; H, left third pereopod, lateral view; I, same, dactlyus and distal part of propodus, lateral view; J, left fourth pereopod, lateral view; K, left fifth pereopod, lateral view; L, same, dactlyus and distal part of propodus, lateral view. Scale bars: A, F, H, J-K = 2mm; B-E, G, I, L = 1mm.

posteriorly. Third pereopod (Figure 4.4 H-I) overreaching antennal scale by approximately 0.9 length of propodus; dactylus 0.14 length propodus, terminating in acute unguis and armed with 5 or 6 accessory spinules on flexor margin, distalmost spinule distinctly larger than others, making dactylus tip appear biunguiculate; carpus approximately 0.6 as long as propodus; propodus with 2 rows of ventral accessory spinules; merus armed with 3-7 lateral spines.

Fourth pereopod (Figure 4.4J) overreaching antennal scale by approximately 0.6 length of propodus; dactlyus with 5 or 6 accessory spinules on flexor margin; propodus with two rows of ventral flexor spinules; merus armed with 3-5 lateral spines.

Fifth pereopod (Figure 4.4 K) overreaching antennal scale by approximately 0.2 length of propodus; dactlyus with 5 or 6 accessory spinules on flexor margin (Figure 4.4L); propodus with two rows of ventral flexor spinules; merus armed with 1-2 lateral spines.

Female pleopods similar to those of other species of the genus, without distinctive feature.

4.6 Colouration in life

See Figure 4.5. Carapace bright red anteriorly, becoming paler distally; gonad green and visible through carapace. Abdomen pale, scattered with red chromatophores, making abdomen appear pinkish red. Rostrum and cephalic appendages translucent. Cornea darkly pigmented. Third maxilliped red with darkly pigmented corneous pinules on ultimate article. Pereopods red with thin white bands at joints; chelae of first two pereopods terminating in darkly pigmented corneous claws; corneous spines and ungui on dactyli of third to fifth pereopods also darkly pigmented. Gills typically bright white and visible through carapace.

Eggs green.

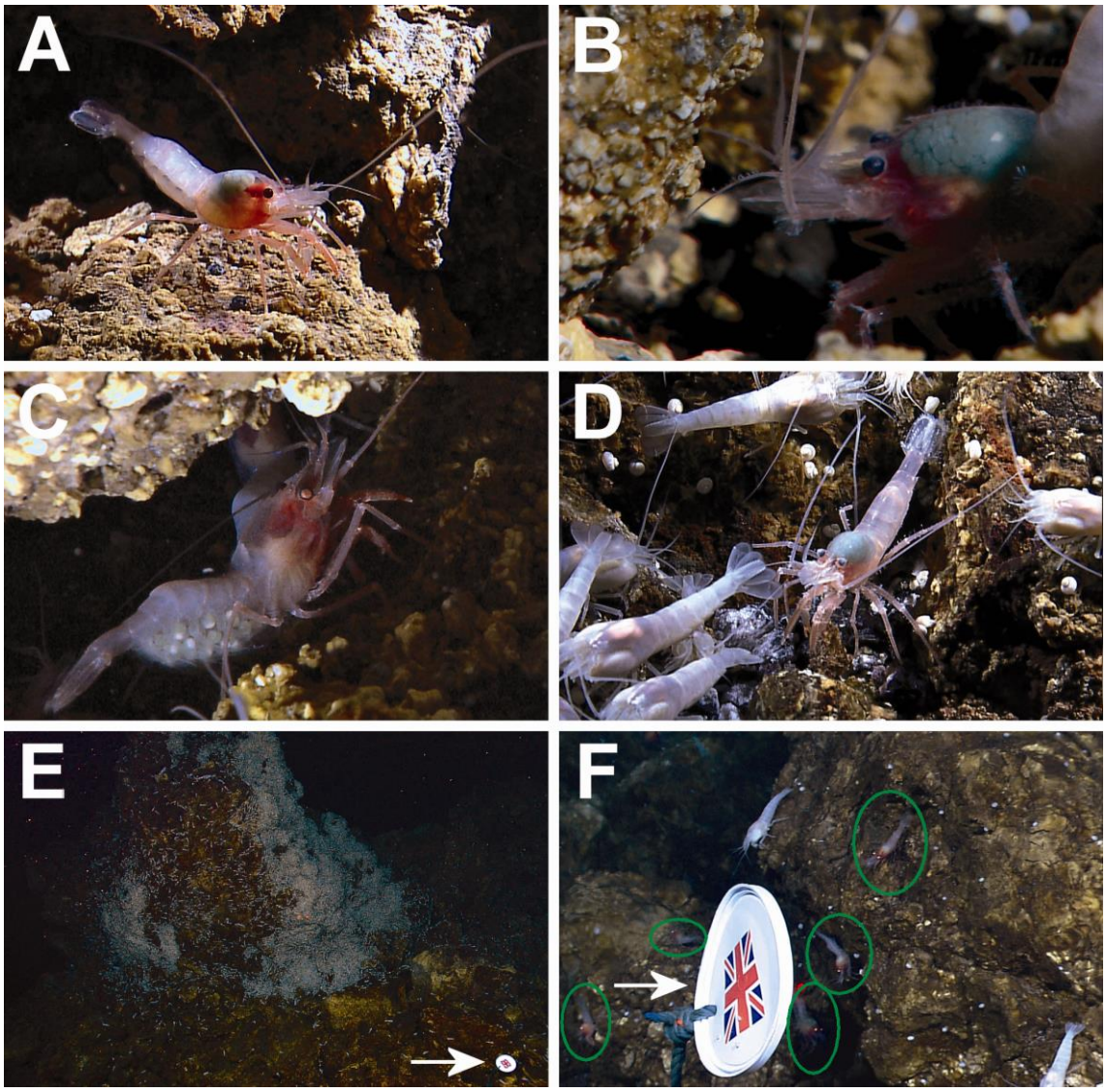

Figure 4.5. *Lebbeus virentova* sp. nov., live *in situ* at the Von Damm Vent Field, Mid-Cayman Spreading Centre, ~2300 m. A-B, female with green eggs visible through carapace; C, berried female; D, *L. virentova* sp. nov. (centre) surrounded by specimens of the alvinocaridid shrimp *Rimicaris hybisae* Nye, Copley, Plouviez, 2012 and skeneid gastropods; E, base of the spire, arrow points to JC44 navigational marker; F, base of the spire (close-up), arrow points to JC44 navigational marker, green ellipses highlight occurrences of *L. virentova* sp. nov..

4.7 Comparative remarks

Lebbeus virentova sp. nov. belongs within the group of *Lebbeus* species characterised by the presence of epipods on the anterior three pairs of pereopods and absence of armature on the anterior three abdominal pleura. It is closest in morphology to the following species: *L. antarcticus* (Hale, 1941); *L. carinatus* Zarenkov, 1976; *L. cristatus* Ahyong, 2010; *L. formosanus* Chang, Komai & Chan, 2010; *L. kuboi* Hayashi, 1992; *L. microceros* (Krøyer, 1841); *L. pacmanus* Komai, Tsuchida & Segonzac, 2012; *L. polyacanthus* Komai, Hayashi & Kohtsuka, 2004; *L. shinkaiae* Komai, Tsuchida & Segonzac, 2012; *L. similior* Komai & Komatsu, 2009; *L. thermophilus* Komai, Tsuchida & Segonzac, 2012; *L. washingtonianus* (Rathburn, 1902); *L. wera* Ahyong, 2009 (see Komai *et al.*, 2012 for updated distribution data on these species).

Characters shared between these species and *Lebbeus virentova* sp. nov. include: rostrum styliform, not reaching distal margin of second segment of antennular peduncle, armed with four of more dorsal teeth including postrostral teeth and more than one ventral tooth; distinct u- or v-shaped notch inferior to base of supraorbital tooth; sinuous anterolateral margin of carapace between antennal and pterygostomial teeth and deep excavation below antennal tooth; first segment of antennal peduncle bearing more than one tooth on dorsodistal margin; dactyli of posterior three pairs of pereopods distinctly biungulate.

Morphological differences between *Lebbeus virentova* sp. nov. and allied species are summarised below. The comparisons are limited to females because there is no information available on males of the new species.

Lebbeus virentova sp. nov. most closely resembles *L. carinatus*, *L. cristatus*, *L. formosanus*, *L. kuboi*, *L. microceros*, and *L. thermophilus* in the stylocerite reaching or slightly overreaching the dorsodistal margin of the first segment of the antennular peduncle and having relatively few dorsal rostral teeth (six or less). *Lebbeus virentova* sp. nov. is separated from *L. carinatus* by the longer carpocerite (reaching to distal 0.3-0.4 of antennal scale versus reaching its midlength), longer third maxilliped (overreaching antennal scale by half length of ultimate segment versus reaching just beyond it) and longer first pereopod (reaching distal margin of antennal scale versus falling short of it).

The new species is distinguished from *Lebbeus cristatus*, *L. formosanus* and *L. kuboi* by the longer antennular peduncle (reaching base of distolateral tooth of antennal

scale versus not reaching it), the shorter distolateral tooth of the antennal scale (not reaching the lamella versus reaching it), and number of meral spines on the posterior three pairs of pereopods. It is differentiated further from *L. cristatus* and *L. formosanus* by the shorter third maxilliped (overreaching antennal scale by half length of ultimate segment versus overreaching it by two-thirds and one-third respectively) and the presence of a posteroventral tooth on the fourth abdominal pleuron (versus absent and variable), and from *L. kuboi* by the straight (versus curving dorsally) rostrum and greater number of dorsal teeth (4-6 versus 2-4).

Lebbeus virentova sp. nov. is distinguished easily from the Atlantic species *L. microceros* by the shorter stylocerite (not reaching or slightly overreaching dorsodistal margin of second segment of antennular peduncle), the longer antennular peduncles (reaching base of distolateral tooth of antennal scale versus not reaching it), longer carpocerite reaching to distal 0.3-0.4 of antennal scale versus reaching its midlength), small tooth (versus strong, curved tooth) on the distolateral margin of the antepenultimate article of the third maxilliped, and fewer meral spines on the fifth pereopod (1-2 versus 3). The new species differs from *L. thermophilus* by the presence of a posteroventral tooth on the fourth abdominal pleuron (versus variable), the longer antennular peduncles (reaching base of distolateral tooth of antennal scale versus far falling short of it), the longer first pereopod (reaching distal margin of antennal scale versus falling short of it), and the presence of plumose setae on the posterior margin of the telson.

Lebbeus virentova sp. nov. is separated from *L. polyacanthus*, *L. shinkaiae* and *L. wera* by fewer dorsal rostral teeth (4-6, including 2-3 postrostral versus 6 or more, including 3 or more postrostral), the presence of plumose setae on the posterior margin of the telson, and number of teeth on the meri of the posterior three pereopods.

The new species is differentiated from *Lebbeus antarcticus*, *L. pacmanus*, *L. similior* and *L. washingtonianus* by the longer stylocerite (reaching or slightly overreaching the dorsodistal margin of the first segment of the antennular peduncle versus not) and the presence of plumose setae on the posterior margin of the telson. In addition, the first pereopod of *L. virentova* sp. nov. is longer than that of *L. pacmanus* and *L. similior*, and shorter than that of *L. antarcticus* (reaching distal margin of antennal scale versus falling short of it, overreaching it by length of fingers). Furthermore, *L. virentova* sp. nov. has a longer carpocerite than *L. antarcticus* and *L. similior*, and shorter carpocerite than *L. pacmanus* and *L. washingtonianus* (reaching to

distal 0.3-0.4 of antennal scale versus reaching its midlength, reaching distal 0.2 of antennal scale).

4.8 Distribution and habitat

Presently known only from the type locality, the Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean, in 2294-2375 m water depth. See Connelly *et al.* (2012) for a description of the geological, geochemical and biological setting of the Von Damm Vent Field.

Observed on the edifice spire in close proximity to actively venting orifices with high abundances of the alvinocaridid shrimp *Rimicaris hybisae* Nye, Copley & Plouviez, 2012 (see Chapter 2), and below the spire on the conical mound with *R. hybisae*, *Alvinocaris* sp., skeneid gastropods (see Chapter 3), zoarcid fish and siboglinid polychaetes.

4.9 Etymology

The species name, *virentova*, is the combination of the Latin, *vireo* (= be green), and *ova* (= eggs), in reference to the green eggs of the new species.

4.10 Molecular phylogeny

Partial sequences of the COI (683 bp) and 16S (523 bp) regions of *Lebbeus virentova* sp. nov. were consistent amongst specimens. Fixed and unique mutations were evident in the partial sequences of the COI and 16S regions in comparison with all other species in the GenBank database. The only partial sequence of the 16S region for *Lebbeus* in GenBank is from *L. virentova* sp. nov. [JQ837266].

Based on NJ and ML phylogenetic analyses for COI sequences available in GenBank, *Lebbeus virentova* sp. nov. exhibits the smallest evolutionary distance (6.5% divergence) to the species recorded therein as "*L. carinatus*" from 13° North on the East Pacific Rise (EPR) [AF125421.1, AF125422.1]. *Lebbeus carinatus* Zarenkov, 1976 was described from off Peru and has not been recorded from the EPR, whereas *L. laurentae* is known only from 13° North at the EPR. *Lebbeus laurentae* is a replacement name for

L. carinatus de Saint Laurent, 1984 (a junior homonym of *L. carinatus* Zarenkov, 1976). It is apparent therefore that "*L. carinatus*" [AF125421.1, AF125422.1] is *L. laurentae*. Based on a 588-bp alignment, NJ and ML methods produced identical topologies and place the new species in the same clade as *L. laurentae* (100% and 96% bootstrap support for NJ and ML methods respectively) (Figure 4.6).

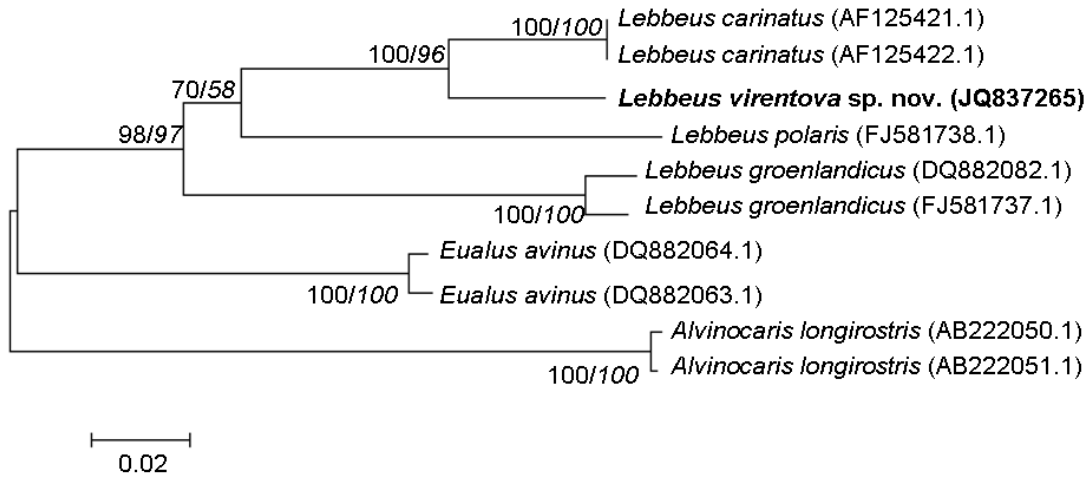

Figure 4.6. Neighbour-joining tree of *Lebbeus* based on a 588-base pair alignment of partial nucleotide sequences from the mitochondrial COI DNA region with *Eualus avinus* (Rathburn, 1899) (Hippolytidae) and *Alvinocaris longirostris* Kikuchi & Ohta, 1995 (Alvinocarididae) as outgroups. Evolutionary distance computed using the Jukes-Cantor method (Jukes & Cantor, 1969) is represented by branch length; scale bar is proportional to inferred nucleotide divergence. Bootstrap support calculated on 1000 re-sampling replicates is shown by the numbers along the branches (neighbour joining, roman text; maximum likelihood, italic text). GenBank accession numbers are given after species names.

4.11 Discussion

Morphological analysis of this hippolytid shrimp reveals it to be a new species in the genus *Lebbeus*. Based on morphology, the new species belongs to the species group characterised by the presence of epipods on the anterior three pairs of pereopods, stylocerite reaching or slightly overreaching the dorsodistal margin of the first segment of the antennular peduncle, and six or fewer dorsal rostral teeth. It is distinguished from other species by a combination of morphological features (see above). Consistency in partial sequences of the COI mtDNA and 16S rDNA genes between specimens from the Von Damm Vent Field confirms that they belong to a single species, and the presence of unique and fixed mutations in the sequences indicate that they are genetically distinct from all other species in the GenBank database.

Seven species of *Lebbeus* have been recorded previously from hydrothermal vents, six of which are only known from a vent environment (Table 4.1); they may be vent-endemic (Komai *et al.*, 2012). These six species are from vent fields on the East Pacific Rise and in western Pacific back-arc basins at 691-2640 m water depth (Table 4.1). The new species therefore appears to be the first hippolytid shrimp to be described from a vent field outside the Pacific Ocean, and may be the first record of the genus in the Caribbean.

The recent discovery of hydrothermal vents and chemosynthetic assemblages on the Mid-Cayman Spreading Centre (MCSC) has provided an opportunity to enhance existing knowledge of biodiversity in the deep sea. *Lebbeus virentova* sp. nov. is the third taxon described from the Von Damm Vent Field, where it co-occurs with the alvinocaridids *Rimicaris hybisae* and *Alvinocaris* sp.. Based on observations made from two research cruises to two vent fields of the MCSC, the new species is so far only known from the Von Damm Vent Field. In contrast, *R. hybisae* is present and abundant at the Von Damm and Beebe vent fields. The Beebe Vent Field is only 30 km from the Von Damm Vent Field, but is 2660 m deeper and has different geological and geochemical settings (Connelly *et al.*, 2012).

The genus *Rimicaris* Williams & Rona, 1986 is a deep-water (1700-4960 m) genus known exclusively from hydrothermal vents (Nye *et al.*, 2012). The genus *Lebbeus* exhibits a shallower bathymetric range, from the littoral zone to at least 2640 m, and is not endemic to hydrothermal vents (e.g., Squires, 1990; Hayashi, 1992; De Grave & Fransen, 2011). The presence of *L. virentova* sp. nov. at the Von Damm Vent

Field and its absence from the Beebe Vent Field, suggest that water depth and/or environmental conditions may determine its distribution among MCSC vent fields. Further characterisation of the faunal composition of assemblages at the vent fields at the MCSC will elucidate the vent biogeography of this region.

Approximately half of all species of *Lebbeus* have been described from the northwest Pacific (De Grave & Fransen, 2011), suggesting a possible centre of radiation for the genus in that region (e.g. Vavilov, 1926). An extensive and comprehensive molecular phylogenetic analysis of the genus *Lebbeus* and higher taxa, requiring the collection and molecular analyses of further specimens, is a prerequisite for clarifying the phylogenetic relationships, evolutionary history and geographic distribution of this genus.

4.12 Acknowledgements

Thanks to the Master and ship's company of the *RV Atlantis*, the crew of the ROV *Jason II* and fellow scientists on the 18th voyage (16th leg) of the *RV Atlantis*; C. German, chief scientist. V. Nye was supported by a UK NERC award (NE/F017774/1) to J. Copley. NSF award OCE-1031050 to C.L. Van Dover and Cliff Cunningham and NASA ASTEP subcontract through WHOI (NNX09AB75G) to C.L. Van Dover supported the biological component of the *Atlantis* Cayman cruise. The authors are grateful to T. Komai and one anonymous referee for reviewing the manuscript and for offering comments for improvements.

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5. Spatial variation in the population structure and reproductive biology of *Rimicaris hybisae* (Caridea: Alvinocarididae) at hydrothermal vents on the Mid-Cayman Spreading Centre

This work has been published as: Nye V., Copley J.T. & Tyler P.A. (2013) Spatial variation in the population structure and reproductive biology of *Rimicaris hybisae* (Caridea: Alvinocarididae) at hydrothermal vents on the Mid-Cayman Spreading Centre. *PLoS ONE* 8(3), e60319. Doi: 10.1371/journal.pone.0060319. Published 29 March 2013.

Abstract

The dynamics and microdistribution of faunal assemblages at hydrothermal vents often reflect the fine-scale spatial and temporal heterogeneity of the vent environment. This study examined the reproductive development and population structure of the caridean shrimp *Rimicaris hybisae* at the Beebe and Von Damm Vent Fields (Mid-Cayman Spreading Centre, Caribbean) using spatially discrete samples collected in January 2012. *Rimicaris hybisae* is gonochoric and exhibits iteroparous reproduction. Oocyte size-frequency distributions (21-823 μm feret diameters) varied significantly among samples. Embryo development was asynchronous among females, which may result in asynchronous larval release for the populations. Specimens of *R. hybisae* from the Von Damm Vent Field (2294 m depth) were significantly larger than specimens from the Beebe Vent Field. Brooding females at Von Damm exhibited greater size-specific fecundity, possibly as a consequence of a non-linear relationship between fecundity and body size that was consistent across both vent fields. Samples collected from several locations at the Beebe Vent Field (4944-4972 m depth) revealed spatial variability in the sex ratios, population structure, size, and development of oocytes and embryos of this mobile species. Samples from the Von Damm Vent Field and sample J2-613-24 from Beebe Woods exhibited the highest frequencies of ovigerous females and significantly female-biased sex ratios. Environmental variables within shrimp aggregations may influence the distribution of ovigerous females, resulting in a spatially heterogeneous pattern of reproductive development in *R. hybisae*, as found in other vent taxa.

5.1 Introduction

Deep-sea chemosynthetic environments supporting chemosynthesis-based faunal assemblages are distributed widely but patchily throughout the global ocean. Reproduction is therefore an essential process for the establishment and maintenance of isolated populations of specialist vent, seep and whale-fall fauna. Understanding the life histories of organisms inhabiting these insular environments is a prerequisite for understanding their ecology, population biology, dispersal, gene flow and biogeography (Young *et al.*, 1996; Tyler & Young, 1999; Ramirez-Llodra *et al.*, 2002; Van Dover *et al.*, 2002.)

More than 400 new faunal species have been described from deep-sea hydrothermal vents since the 1970s (Desbruyères *et al.*, 2006), and aspects of life-history biology have been elucidated in more than 90 species from vents, seeps, and whale falls (Copley, Nye *et al.*, unpublished data). Studies have described a variety of reproductive traits and developmental modes in species from chemosynthetic environments, and revealed spatial and temporal patterns in the reproductive development of some species (see Tyler & Young, 1999; Young, 2003 for reviews).

The Mid-Cayman Spreading Centre (MCSC) is an ultraslow-spreading and geographically isolated ridge in the Caribbean that hosts two high-temperature hydrothermal vent fields (Connelly *et al.*, 2012). The Beebe Vent Field (~4960 m) is situated on the axis of the MCSC and it consists of a sulfide mound (~80 m diameter, 50 m height) surmounted by several active black-smoker chimney complexes and areas of diffuse flow (Connelly *et al.*, 2012). The Von Damm Vent Field (~2300 m) is a conical mound (~150 m diameter, 70 m height) venting clear, buoyant fluids, located off-axis (approximately 13 km away from the Beebe Vent Field) on the upper slopes of the Mount Dent oceanic core complex (Connelly *et al.*, 2012).

Research efforts on the fauna at MCSC vents have so far focused on the taxonomy, phylogenetics and assemblage compositions. The Beebe vent assemblage includes provannid gastropods, anemones and ophiuroids, whereas the faunal assemblage at the Von Damm Vent Field includes skeneid gastropods, hippolytid shrimp, lysianssid amphipods and tubeworms (Connelly *et al.*, 2012; Nye *et al.*, 2012; Nye *et al.* 2013 a, b). The alvinocaridid shrimp *Rimicaris hybisae* (Nye *et al.*, 2012) is present and abundant at both known MCSC vent fields (Nye *et al.*, 2012).

To date, more than 125 species representing 33 families of decapods have been reported from deep-sea chemosynthetic environments (Martin & Haney, 2005), yet the reproductive traits of only ten species have been described (Van Dover *et al.*, 1985; Ramirez-Llodra *et al.*, 2000; Perovich *et al.*, 2003; Copley & Young, 2006; Ramirez-Llodra & Segonzac, 2006; Copley *et al.*, 2007; Hilário *et al.*, 2009). Reproductive patterns of decapods from chemosynthetic environments are thought to have strong phylogenetic constraints (Van Dover *et al.*, 1985; Tyler & Young, 1999).

The family Alvinocarididae (Christoffersen, 1986) is represented to date by 27 described species from eight genera and appears to be endemic to deep-sea chemosynthetic environments (Nye *et al.*, 2012). Alvinocaridid shrimp examined previously exhibit planktotrophic development and gametogenesis characteristic of carideans (Van Dover *et al.*, 1985; Ramirez-Llodra *et al.*, 2000; Copley & Young, 2006; Ramirez-Llodra & Segonzac, 2006; Copley *et al.*, 2007). Seasonal reproduction has been described in *Alvinocaris stactophila* (Williams, 1988) from the Brine Pool cold seep (650 m) in the Gulf of Mexico, where the seasonal peak in surface productivity and its export may be a cue for larvae to hatch (Copley & Young, 2006).

Zonation in the population structure and reproductive biology of *Alvinocaris stactophila* has also been revealed at the Brine Pool (Copley & Young, 2006; Nye, unpublished data). Avoidance of sulfidic extremes by female crustaceans brooding embryos has been proposed for several taxa at vents and seeps (e.g. Perovich *et al.*, 2003; Copley & Young, 2006; Hilário *et al.*, 2009; Sheader & Van Dover, 2007; Rogers *et al.*, 2012). A similar explanation has been invoked to explain the apparent scarcity of ovigerous females of *Rimicaris exoculata* (Williams & Rona, 1986) in the immediate vicinity of black smokers at deep vents on the Mid-Atlantic Ridge (MAR) (Gebruk *et al.*, 1997; Ramirez-Llodra *et al.*, 2000).

The aims of this study were therefore to: (1) examine variation in the population structure and reproductive features of *Rimicaris hybisae* between the Beebe and Von Damm vent fields; (2) assess spatial variation in the reproductive features of *R. hybisae* within the Beebe Vent Field; (3) discuss and compare the results with data available for other alvinocaridid species. This is the first study on the autecology of vent fauna from the Mid-Cayman Spreading Centre, and reveals a high degree of spatial variability in the population structure and reproductive features of this motile species in the vent environment.

5.2 Materials and methods

To assess spatial variation in population structure and reproductive features, samples of *Rimicaris hybisae* were collected from two vent fields at the Mid-Cayman Spreading Centre, Caribbean, during the 18th voyage (16th leg) of the *RV Atlantis* (see Table 5.1). Samples were collected using a suction sampler attached to the remotely operated vehicle (ROV) *Jason II*. Four samples were collected from different locations within the Beebe Vent Field (4944-4972 m; Table 5.1): J2-613-24 was collected from a large, high-density aggregation of shrimp at the base of a chimney; J2-619-15 was collected from a large, high-density aggregation of shrimp at the edge of a gulley; J2-613-19 was taken from a small, dense aggregation of shrimp, next to anemones, provannid gastropods and bacterial mats; J2-620-32 was taken in a peripheral area, dominated by anemones with sparse shrimp (Figure 5.1 A-D).

Two samples (J2-617-5 and J2-617-8) were collected from a large, high-density aggregation of shrimp at the Von Damm Vent Field (Figure 5.1 E-F). Although they were placed in two separate chambers of the multi-chamber suction sampler, these samples were collected within minutes of each other at the same location and depth (within a 1 m^2 area) and could not be discriminated spatially. Consequently these two samples were pooled (J2-617-5/8). Unfortunately constraints of expedition logistics precluded a replicate sample from this vent field. Specimens were fixed in 10% buffered seawater formalin for 48 h and stored in 70% isopropanol.

Carapace length (CL) of each shrimp was measured to the nearest 0.1 mm with Vernier callipers from the rear of the eye socket to the rear of the carapace in the mid-dorsal line. This is the standard measure of length for a shrimp and it is used herein as an indication of body size because it avoids errors associated with measuring a flexible abdomen (Clarke, 1993).

The sex of each shrimp was determined under a Leica MZ8 dissecting microscope (*sensu* Nye *et al.*, 2012). For all males, the presence/absence of a spermatophoric mass was recorded. All females were categorised as either: brooding (brooding embryos on pleopods 1-4); hatched (with a matrix of empty embryo sacs attached to the pleopods); or female (neither brooding nor hatched).

Table 5.1. *Rimicaris hybisae*. Sample and population data for 959 specimens used in this study.

Sample no.	Cruise	Sample method	Vent Field	Location	Depth (m)	Latitude (°N)	Longitude (°W)	Date (JD)	Total no. specimens	Males			Females				Sex Ratio TM:TF	χ^2 (1 df)	Significance
										Total	M	SM	Total	F	BF	HF			
J2-613-24	Atlantis 18_16	Jason II	Beebe	Beebe Woods	4971	18.546182	81.718086	12/01/2012	254	84	32	52	170	37	108	25	0.49:1	28.44	***
J2-619-15	Atlantis 18_16	Jason II	Beebe	Shrimp Gulley	4944	18.546563	81.717705	22/01/2012	118	48	36	12	70	42	28	0	0.69:1	3.74	NS
J2-613-19	Atlantis 18_16	Jason II	Beebe	Beebe Woods	4972	18.546974	81.718339	11/01/2012	96	44	22	22	52	47	5	0	0.85:1	0.51	NS
J2-620-32	Atlantis 18_16	Jason II	Beebe	Beebe Woods	4964	18.546929	81.718278	23/01/2012	94	76	48	28	18	16	0	2	4.2:1	34.56	***
			Beebe	All					562	252	138	114	310	142	141	27	0.81:1	5.78	*
J2-617-5/8	Atlantis 18_16	Jason II	Von Damm	Spire	2294	18.376630	81.798143	19/01/2012	397	141	103	38	256	147	77	32	0.55:1	32.74	***
								Total	959	393	241	152	566	289	218	59	0.69:1	30.85	***

BF, brooding female; F; female without brooding or recently hatched; H, female recently hatched (with a matrix of empty embryo sacs attached to the pleopods); JD, Julian day; M, male without spermatophore; NS = not significant; SM, male with spermatophore; TM, total males; TF, total females.

* = *P* value <0.05; ** = *P* value <0.01: *** = *P* value <0.001

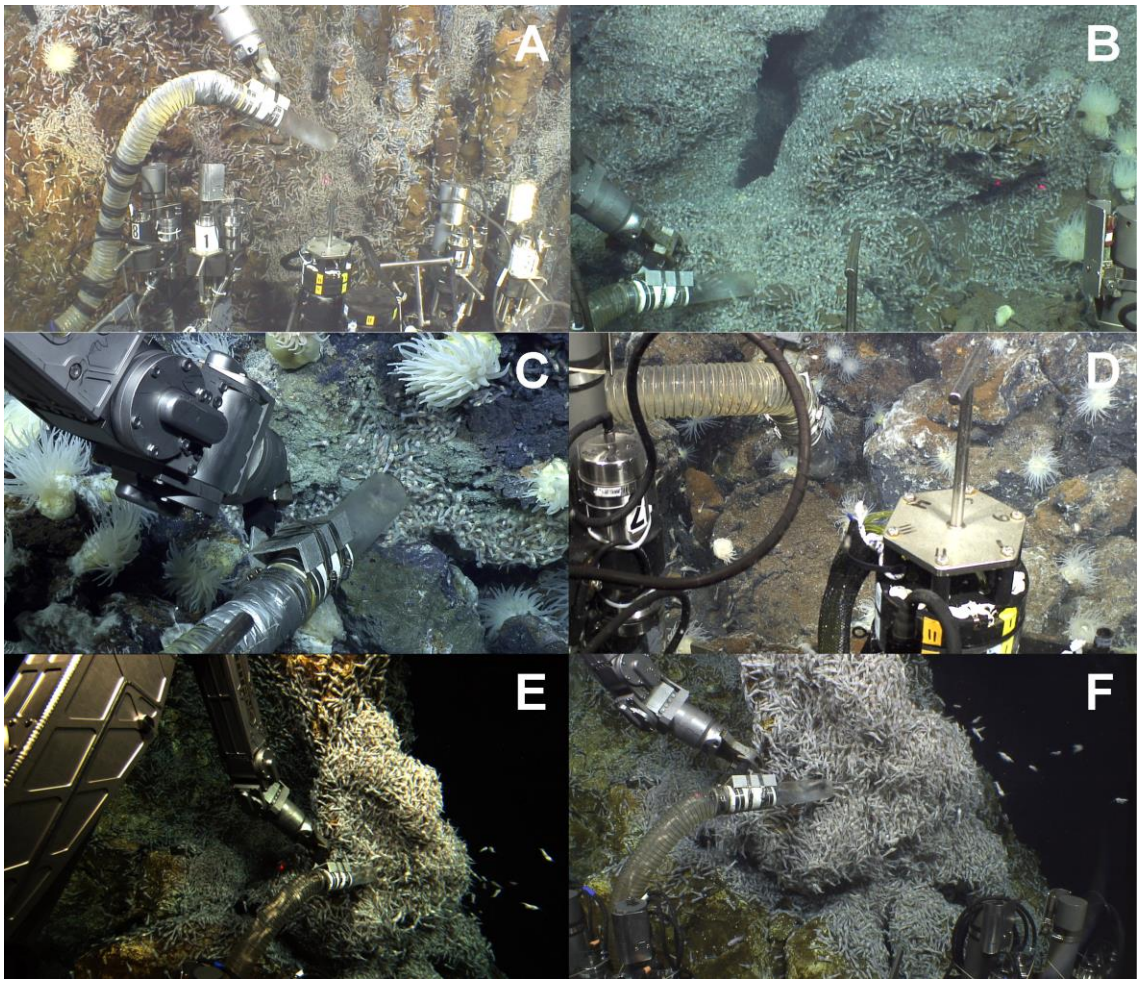

Figure 5.1. *Rimicaris hybisae*, sample locations. A-D, Beebe Vent Field; E-D, Von Damm Vent Field, Mid-Cayman Spreading Centre, Caribbean. A, J2-613-24; B, J2-619-15; C, J2-613-19; D, J2-620-32; E, J2-617-5; F, J2-617-8.

The ovaries were dissected from the female shrimp and where oocyte size allowed, individual oocytes were removed from each ovary under a Leica EZ4 HD dissecting microscope. Images of oocytes were captured using a Leica EZ4 HD dissecting microscope. Packing of oocytes in ovaries often results in irregular oocyte shapes in *Rimicaris hybisae*. Consequently oocytes were laid flat and measured directly, rather than from histological sections, to ensure maximum cross-sectional areas were recorded (*sensu* Copley & Young, 2006). Where female specimen numbers and condition allowed, the feret diameters and areas of 100 oocytes were measured in 30 females per sample (9 females for J2-620-32) using ImageJ. Feret diameter was used to standardise variations in oocyte shape. Images of oocytes were calibrated with measurements of a graticule slide at identical magnification.

Broods of embryos were removed from the pleopods of brooding females under a Leica MZ8 dissecting microscope. Within each brood, all embryos had developed synchronously and were at the same stage of development. The developmental stage of each brood was scored on the basis of morphological features (*sensu* Ramirez-Llodra *et al.*, 2006): early-stage embryos without features; mid-stage embryos with clear body differentiation; late-stage embryos with clear larval features (e.g. separation of the abdomen from the cephalothorax and developed eyes), including hatched larvae (Figure 5.2). Numbers of embryos per brood were counted to determine minimum realised fecundity (*sensu* Anger & Moreira, 1998). Although it was not possible to guarantee that embryo batches were complete, embryos were attached firmly to each other and the mothers' pleopods (within which they were enclosed) and broods remained intact post-sampling. Size-specific fecundity was calculated as number of embryos divided by carapace length (Table 5.2).

To determine mean embryo size, a sub-sample of ten broods at each developmental stage from both vent fields was selected at random. Embryos were laid flat and images of the embryos were captured using a Leica EZ4 HD dissecting microscope. The greater and lesser diameters of 100 embryos per brood were measured using ImageJ.

Frequencies of males and females in samples were tested for significant variation from a 1:1 sex ratio using χ^2 test with Yates' correction for one degree of freedom. In analyses of population structure and size-frequency distribution of females, brooding and hatched females were pooled as ovigerous females. To correct for variation in the sex ratio between samples, spatial variation in the population structure was assessed by comparing the ratio of ovigerous (brooding and hatched) females to non-ovigerous females, the ratio of brooding to hatched ovigerous females, and the ratio of males with spermatophores vs without (rather than the overall proportions). Frequencies of ovigerous females, brooding females and males with spermatophores were tested for significant variation from a 1:1 ratio between vent fields (Table 5.3) and between pairwise combinations of samples within the Beebe Vent Field (Table 5.4) using χ^2 test with Yates' correction for one degree of freedom. Population structure was examined using the size-frequency distribution of 959 individuals (Table 5.5).

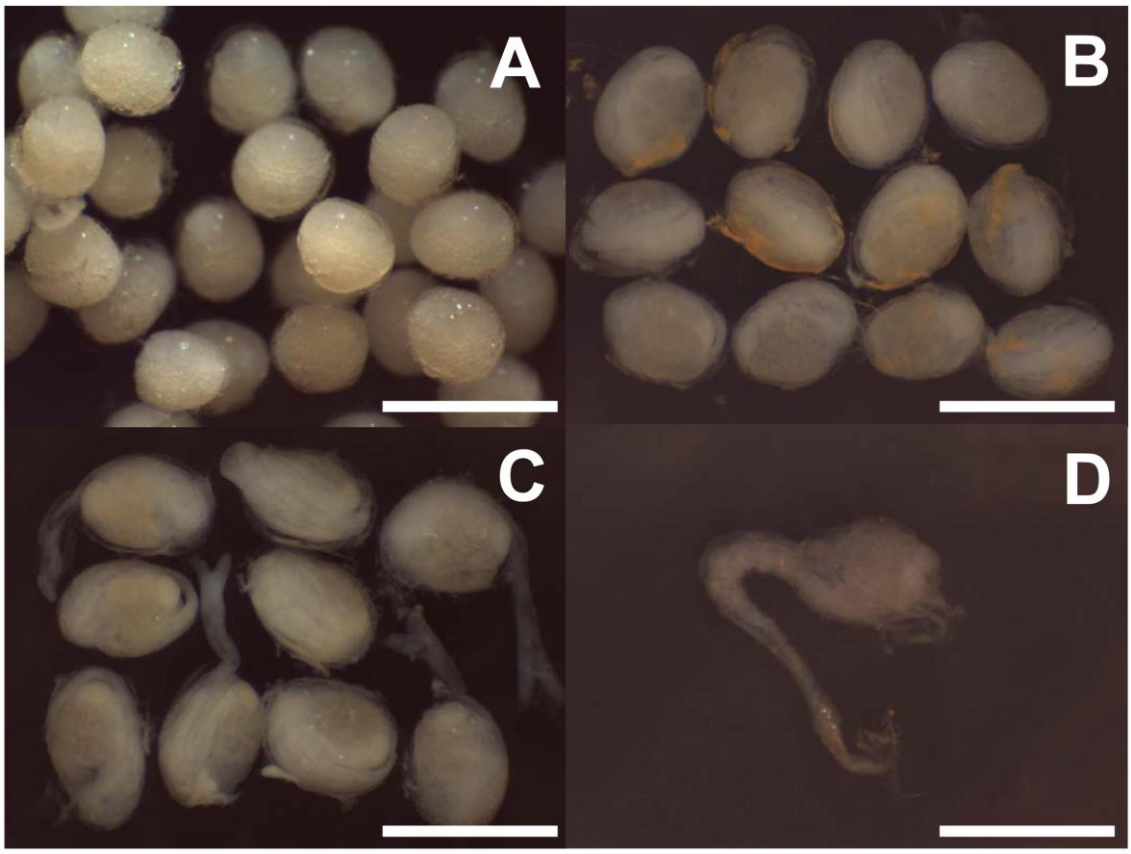

Figure 5.2. *Rimicaris hybisae*. Embryo stages and larvae. A, Early-stage embryos; B, mid-stage embryos; C, late-stage embryos with larvae ready to hatch; D, hatched larva. Scale bars = 1 mm.

5.3 Results

5.3.1 Population structure in January 2012

The gonads of *Rimicaris hybisae* are paired organs laying over the digestive gland of the cephalothorax. Of the 959 specimens of *Rimicaris hybisae* examined, 393 were identified as male (41%), resulting in an overall sex ratio that deviated significantly from 1:1 (393 males; 566 females) (Table 5.1). All males examined had only testes and all females studied had only ovaries. Of the 393 males, 152 (39%) were carrying spermatophores. Of the 566 females, nearly half (277, 49%) were either brooding embryos (218, 39%) or had hatched larvae recently (59, 10%).

The specimens ranged in body size (carapace length) from 5.2 mm (male, Von Damm) to 19.4 mm (male, Von Damm). The carapace length of the smallest male identified (5.2 mm, Von Damm) was less than that of the smallest females (6.2 mm,

Table 5.2. Average minimum realised fecundity and embryo sizes of caridean shrimp from hydrothermal vents and cold seeps.

Species	*Alvinocaris muricola* (*n* = 9)	*Alvinocaris stactophila* (*n* = 55)	*Alvinocaris stactophila* (*n* = 65)	*Alvinocaris lusca* (*n* = 1)	*Alvinocaris markensis* (*n* = 1)	*Chorocaris chacei* (*n* = 1)	*Mirocaris fortunata* (*n* = 30)	*Rimicaris exoculata* (*n* = 2)	*Rimicaris hybisae* (*n* = 562)	*Rimicaris hybisae* (*n* = 397)
Site	Congo Basin (seep)	GoM: Brine Pool IMB (seep)	GoM: Brine Pool MMB (seep)	Galapagos Rift (vent)	MAR: Lucky Strike (vent)	MAR: Lucky Strike (vent)	MAR: Lucky Strike (vent)	MAR: Snake Pit & TAG (vent)	MCSC: Beebe (vent)	MCSC: Von Damm (vent)
Depth (m)	3113-3150	500		2500	1690	1690	1690	3480-3650	4944-4972	2294
CL (mm) Mean ±SD	20.7 ±2.3	3.77	3.91	11.45	13	16.8	7.16 ±0.18	17.05	10.2 ±1.5	13.9 ±2.3
Minimum realised fecundity (embryos) Mean ±SD	3130 ±1180.9	147	98	407	2007	2510	174.7 ±22.8	912	341.7 ±146.2	1054.2 ±229.8
Size-specific fecundity Mean ±SD	149.1 ±48.0	39	25	35	154	149	24.3	53	30.6 ±11.8	68.0 ±13.2
Mean embryo size (mm)	0.66 x 0.55	0.80	0.79 x 0.57	0.50 x 0.34	0.66 x 0.52	-	0.70 x 0.49	0.72 x 0.62	0.64 x 0.48	0.63 x 0.48
Reference	Ramirez-Llodra & Segonzac 2006	Copley & Young 2006	Copley & Young 2006	Van Dover *et al.* 1985	M. Segonzac, unpublished data	Ramirez-Llodra *et al.* 2000	Ramirez-Llodra *et al.* 2000	Williams & Rona 1986; Ramirez-Llodra *et al.* 2000	This study	This study

CL, carapace length; GoM, Gulf of Mexico; IMB, inner mussel bed site; MMB, middle mussel bed site; MAR, Mid-Atlantic Ridge; MCSC, Mid-Cayman Spreading Centre; *n*, number of females analysed; Size-specific fecundity, embryos/mm CL; SD, standard deviation.

Table 5.3. *Rimicaris hybisae*. Spatial variation in population structure between the Beebe and Von Damm vent fields, January 2012.

	Beebe	Von Damm	Ratio Beebe: Von Damm	χ^2 (1 df)	Significance
Proportion of females ovigerous in samples	54.2%	42.6%	1.27:1	13.47	***
Proportion of ovigerous females brooding	83.9%	70.6%	1.19:1	13.22	***
Proportion of males with spermatophore	45.2%	30.0%	1.51:1	18.23	***

Ovigerous was defined as brooding or hatched; proportion of females ovigerous refers to the ratio of ovigerous females to all females in samples. *** = *P* value <0.001.

Table 5.4. *Rimicaris hybisae*. Spatial variation in population structure within the Beebe Vent Field, January 2012.

Sample	J2-613-19	J2-613-24	J2-620-32	J2-619-15
J2-613-19	-	**914.90 *****	**0.03 NS**	**71.08 *****
J2-613-24	*4.30 **	-	**43.67 *****	**57.70 *****
J2-620-32	*4.75 **	*19.19 ****	-	**5.11 ***
J2-619-15	*11.02 ****	*19.19 ****	*5.07 **	-

Results of χ^2 (1 df) analyses on proportions of females ovigerous (brooding or hatched; bold text) and proportions of males with spermatophores (italic text) in samples.

NS = not significant; * = *P* value <0.05; ** = *P* value <0.01: *** = *P* value <0.001.

Table 5.5. *Rimicaris hybisae*. Variation in body size (carapace length), January 2012.

	Carapace length (mm)					
	Males			Females		
Vent Field	All	Without spermatophore	With spermatophore	All	Non-ovigerous	Ovigerous
Beebe	10.1 (9.0-10.7)	9.7 (8.7-10.6)	10.2 (9.5-10.9)	10.6 (9.8-11.3)	9.9 (8.3-10.8)	10.9 (10.4-11.6)
Von Damm	14.9 (13.2-15.0)	13.8 (12.6-14.5)	14.8 (14.2-15.1)	14.7 (12.9-15.6)	13.3 (11.5-14.9)	15.3 (14.8-15.8)

Carapace length is shown as median (inter-quartile range).

Beebe Woods); this indicated that any bias in sex ratio was not the result of immature males being misidentified as females. The carapace lengths of the smallest brooding female and smallest male with spermatophores were 8.5 and 6.9 mm respectively (both Beebe Woods).

The size-frequency distribution of the specimens displayed two modal peaks and a short tail of large specimen sizes (Figure 5.3A). The carapace lengths of the largest females identified were 17.2 and 18.1 mm at the Beebe and Von Damm vent fields respectively. The carapace lengths of the largest males identified were 17.4 and 19.4 mm at the Beebe and Von Damm vent fields respectively. The size-frequency distributions of all males and females in January 2012 were significantly different (Mann-Whitney *U*-test, $T = 168261.5$, $p < 0.001$). Males were represented throughout the size-frequency distribution of the samples, but there were proportionally fewer large males resulting in a lower median body size in males at both vent fields (Table 5.5; Figure 5.3 B-C). However, the size-frequency distributions of non-ovigerous (neither brooding nor hatched) females were not significantly different from males (Mann-Whitney *U*-test, $T = 99797.5$, $p > 0.05$) and the ratio of males to non-ovigerous females did not deviate significantly from 1:1 (393 males: 289 non-ovigerous females, $\chi^2 = 0$, 1 df, $p > 0.05$). Ovigerous (brooding and hatched) females were confined to the peak and tail of large sizes and were significantly larger than non-ovigerous females at both vent fields (Table 5.5; Figure 5.4 A-B; Mann-Whitney *U*-test, Beebe: $T = 15731.0$, $p < 0.001$; Von Damm: $T = 19167.0$, $p < 0.001$). Males with spermatophores were significantly larger than males without spermatophores at both vent fields (Table 5.5; Figure 5.4 C-D; Mann-Whitney *U*-test, Beebe: $T = 168261.5$, $p < 0.001$; Von Damm: $T = 3541.0$, $p < 0.001$).

The brooded embryos of *Rimicaris hybisae* had a mean size of 0.64 x 0.48 mm in January 2012 (Table 5.2). There was no significant difference in embryo sizes between samples from the Beebe and Von Damm vent fields (Table 5.2; Mann-Whitney *U*-test, $T = 2250750.0$, $p > 0.05$). Embryos in the early developmental stage were 0.58 $\pm$0.04 mm mean greater diameter and 0.46 $\pm$0.04 mm mean lesser diameter. The mean embryo size in the medium developmental stage was 0.64 $\pm$0.07 mm greater diameter and 0.48 $\pm$0.04 mm lesser diameter. Embryos in the most advanced stage were 0.69 $\pm$0.07 mm mean greater diameter and 0.51 $\pm$ 0.05 mm lesser diameter.

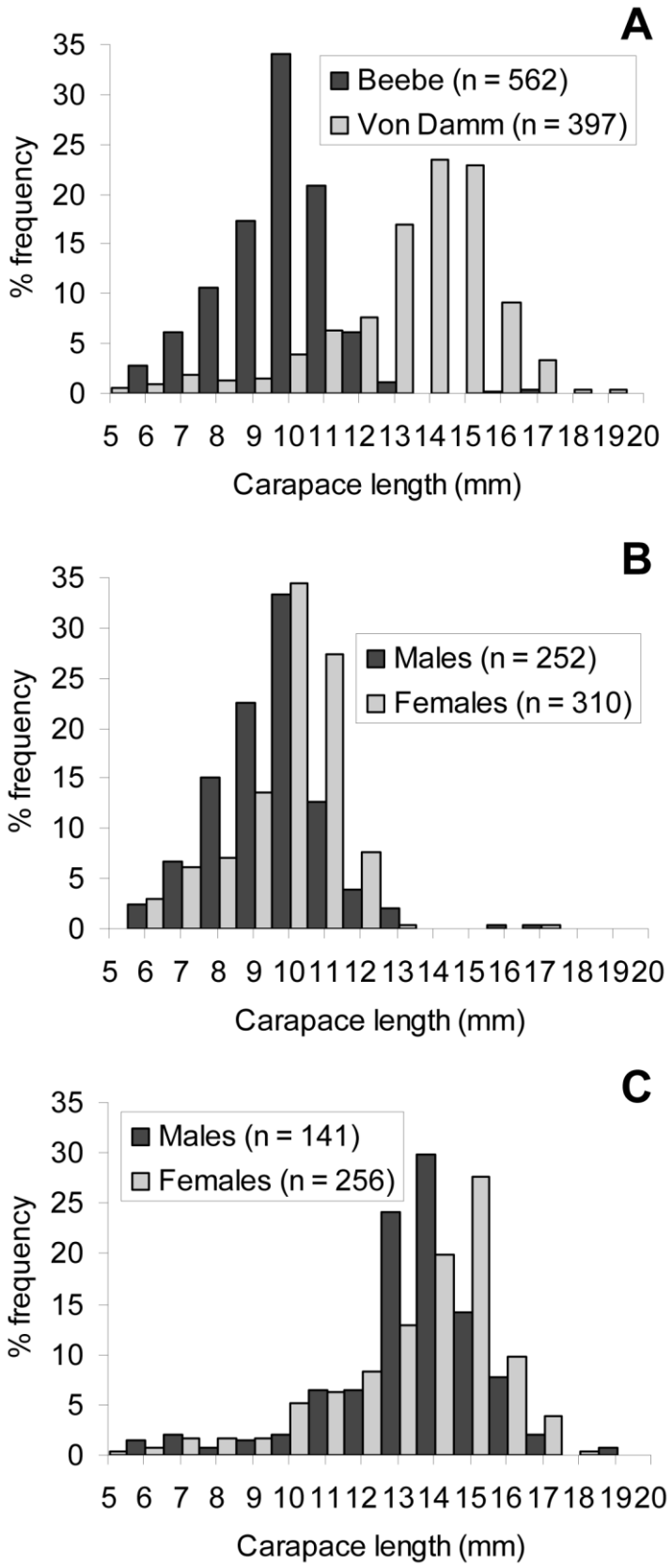

Figure 5.3. *Rimicaris hybisae*. Size-frequency distribution, January 2012. A, All specimens; B, Beebe Vent Field; C, Von Damm Vent Field. n: no. of individuals measured.

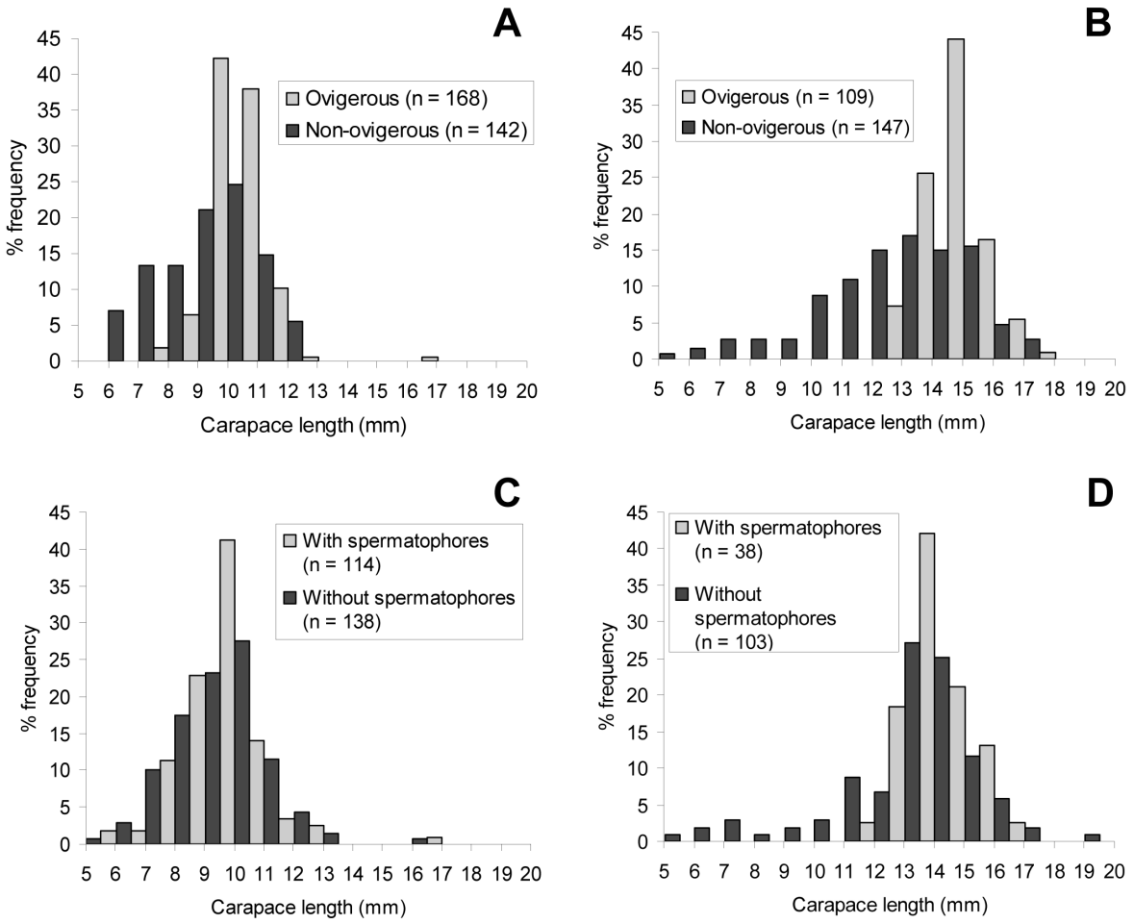

Figure 5.4. *Rimicaris hybisae*. Size-frequency distribution of females and males, January 2012. A, Females, Beebe Vent Field; B, females, Von Damm Vent Field; C, males, Beebe Vent Field; D, females, Von Damm Vent Field. n: no. of individuals measured.

5.3.2 Spatial variation in reproductive features

5.3.2.1 Sex ratio. In January 2012 at the Von Damm Vent Field, 36% of specimens were male, resulting in a sex ratio that deviated significantly from 1:1 (Table 5.1). Males represented 45% of individuals sampled from the Beebe Vent Field, resulting in an overall sex ratio that deviated significantly from 1:1 (Table 5.1). However, there was significant variation in the sex ratio exhibited in different samples from the Beebe Vent Field (Table 5.1). Specimens from samples J2-613-19 (Beebe Woods) and J2-619-15 (Shrimp Gulley) did not deviate significantly from a 1:1 sex ratio. Sample J2-613-24 (Beebe Woods) showed significant female bias (67% of specimens were females), but the ratio of males to non-ovigerous females did not differ

significantly from 1:1. A significant male bias was present in sample J2-620-32 (81% of specimens were males) (Table 5.1).

5.3.2.2 Population structure. To correct for variation in the sex ratio between samples, spatial variation in the occurrence of ovigerous females was assessed by comparing the ratio of ovigerous (brooding and hatched) females to non-ovigerous females (rather than the overall proportions). A significantly greater proportion of females were ovigerous at the Beebe Vent Field (54%) than at the Von Damm Vent Field (43%; Table 5.3). The majority of ovigerous females sampled from both vent fields were brooding, as opposed to females that showed evidence of having just hatched their brood (Table 5.3). However, brooding females represented a significantly greater proportion of ovigerous females at the Beebe Vent Field (84%) compared to the Von Damm Vent Field (71%; Table 5.3). The majority of males sampled from both vent fields were without spermatophores (Table 5.3). However, males with a spermatophoric mass accounted for a significantly greater proportion of the sampled male population at the Beebe Vent Field (45%) than at the Von Damm Vent Field (30%; Table 5.3). As a result of too few data points, it was not possible to determine the correlation between frequencies of ovigerous females, brooding females and males with spermatophores.

Within the Beebe Vent Field, the highest proportion of ovigerous females was 78% in sample J2-613-24 (Figure 5.5A). All samples were significantly different from each other in the proportion of ovigerous females with the exception of samples J2-613-19 and J2-620-32 (Table 5.4). All ovigerous females were brooding in samples J2-613-19 and J2-619-15, whereas 81% were brooding in J2-613-24 and all had hatched in J2-620-32 (Figure 5.5B). Brooding females accounted for a significantly greater proportion of ovigerous females at J2-619-15 than J2-613-24 (Figure 5.5B; $\chi^2 = 5.31$, 1 df, $p < 0.05$), and at J2-613-24 than J2-620-32 (Figure 5.5B; $\chi^2 = 4.14$, 1 df, $p < 0.05$). All other pairwise combinations of samples could not be tested for significant variation from a 1:1 ratio as a result of expected frequencies of zero.

The highest proportion of males with a spermatophoric mass was 62% in sample J2-613-24 (Figure 5.5C). All samples from the Beebe Vent Field were significantly different from each other in the proportion of males with spermatophores (Table 5.4, Figure 5.5C). As a result of too few data points, it was not possible to determine the correlation between frequencies of ovigerous females, brooding females and males with spermatophores.

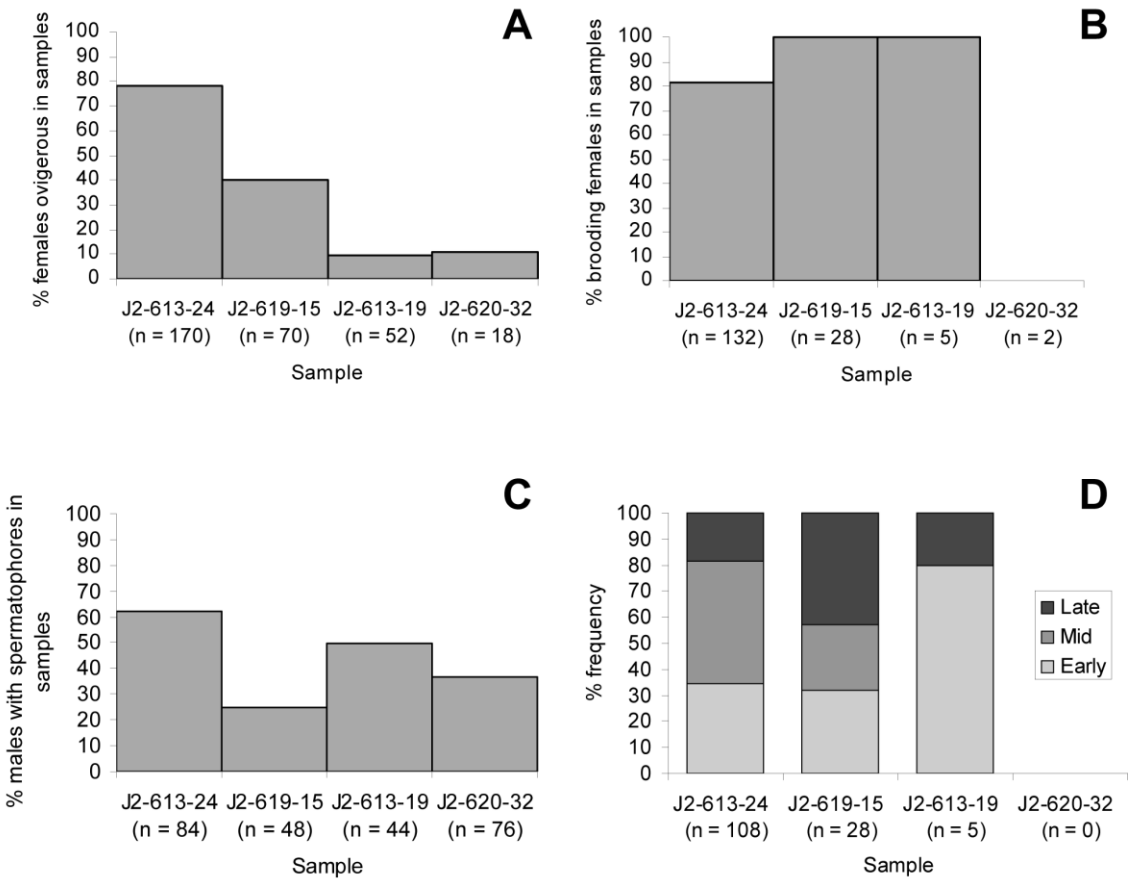

Figure 5.5. *Rimicaris hybisae*. Spatial variation in samples from the Beebe Vent Field in A, proportion of females ovigerous (defined as brooding embryos or hatched) in samples of females; B, proportion of ovigerous females brooding in samples of ovigerous females; C, proportion of males with spermatophores in samples of males; D, proportion of embryos in samples of brooding females at each developmental stage. n: sample size (100%) in each case.

5.3.2.3 Size-frequency distribution of shrimp. Overall, shrimp sampled from the Von Damm Vent Field were significantly larger than those from the Beebe Vent Field (Table 5.5; Figure 5.3; Mann-Whitney *U*-test, $T = 281949.0$, $p < 0.001$). Although the January 2012 samples as a whole displayed two modal peaks and a short tail of large sizes, the peak of larger sizes was absent among samples from the Beebe Vent Field, where smaller shrimp were sampled with proportionally greater frequency (Figure 5.3). The peak of larger sizes was prominent among the samples from the Von Damm Vent

Field, where smaller shrimp were sampled with proportionally lower frequency (Figure 5.3).

Both males and females were significantly larger at the Von Damm Vent Field than the Beebe Vent Field (Table 5.5; Figure 5.3; Mann-Whitney *U*-test, males: $T = 42656.0$, $p < 0.001$; females: $T = 104412.5$, $p < 0.001$). Females were represented throughout most of the size-frequency distribution at both vent fields, but there were proportionally greater large females at the Von Damm Vent Field (Figure 5.3; Mann-Whitney *U*-test, Beebe: $T = 61750.5$, $p < 0.001$; Von Damm: $T = 25602.5$, $p < 0.025$). Non-ovigerous females were significantly smaller at the Beebe Vent Field than the Von Damm Vent Field (Table 5.5; Figure 5.4; Mann-Whitney *U*-test, $T = 12744.5$, $p < 0.001$), as were ovigerous females (Table 5.5; Figure 5.4; Mann-Whitney *U*-test, $T = 24202.0$, $p < 0.001$). The smallest carapace length exhibited by an ovigerous female at the Von Damm Vent Field was 13.3 mm, compared with 8.5 mm at the Beebe Vent Field. Males with and without spermatophores were both significantly larger at the Von Damm Vent Field than the Beebe Vent Field (Table 5.5; Figure 5.4; Mann-Whitney *U*-test, males with spermatophores: $T = 5026.5$, $p < 0.001$; males without spermatophores: $T = 18127.0$, $p < 0.001$). The smallest carapace length shown by a male with a spermatophore was 12.9 mm at the Von Damm Vent Field (compared with 6.9 mm at the Beebe Vent Field).

The size-frequency distributions of non-ovigerous (neither brooding nor hatched) females were not significantly different to males at the Beebe Vent Field (Table 5.5; Figure 5.3B, 5.4A; Mann-Whitney *U*-test, $T = 26586.0$, $p > 0.05$) and the ratio of males to non-ovigerous females did not deviate significantly from 1:1 (138 males: 142 non-ovigerous females, $\chi^2 = 0$, 1 df, $p > 0.05$). There was a significantly lower proportion of large non-ovigerous females compared with males at the Von Damm Vent Field (Table 5.5; Figure 5.3C, 5.4B; Mann-Whitney *U*-test, $T = 22364.5$, $p < 0.001$). However, the ratio of males to non-ovigerous females did not deviate significantly from 1:1 (141 males: 147 non-ovigerous females; $\chi^2 = 0$, 1 df, $p > 0.05$).

Overall, there was significant variation in the size-frequency distributions of shrimp collected from different locations within the Beebe Vent Field (Figure 5.6; Kruskal-Wallace multisample test, $H = 150.857$, 3 df, $p < 0.001$). There was also significant variation in the size-frequency distributions of each sex between samples from the Beebe Vent Field (Kruskal-Wallace multisample test, males: $H = 42.8$, 3 df, $p < 0.001$; females $H = 107.0$, 3 df, $p < 0.001$).

Shrimp in sample J2-613-19 displayed the smallest median size for each sex (males: CL 9.1 mm, IQR 8.2-10.2; females: CL 9.5 mm, IQR 8.3-9.9). However, the median sizes of males and females in sample J2-613-19 were not significantly different from those in sample J2-620-32 (Figure 5.6; Dunn's Multiple Comparison Test, $p > 0.05$). The median sizes and size-frequency distributions of males and females were not significantly different within sample J2-613-19 (Figure 5.6; Mann-Whitney *U*-test, $T = 2388.0$, $p > 0.05$; Kolmogorov-Smirnov two-sample test, $D = 0.05$, $p > 0.05$).

The median sizes and size-frequency distributions of males and females were not significantly different within sample J2-620-32 (Figure 5.6; Mann-Whitney *U*-test, $T = 723.0$, $p > 0.05$; Kolmogorov-Smirnov two-sample test, $D = 0.12$, $p > 0.05$). However, the number of females was low. The median sizes were CL 9.2 (IQR 8.4-10.5 mm) for males and 9.3 mm (IQR 7.4-10.3) for females.

Sample J2-613-24 exhibited a peak of large females and significantly greater median sizes for females than males (Figure 5.6; males: CL 10.3 mm, IQR 9.7-10.8; females: CL 10.8 mm, IQR 10.2-11.4; Mann-Whitney *U*-test, $T = 8779.5$, $p < 0.001$). The distribution of sizes between males and females was significantly different (Kolmogorov-Smirnov two-sample test, $D = 0.25$, $p < 0.01$).

Shrimp in sample J2-619-15 exhibited the largest median size for both males (CL 10.6 mm, IQR 10.1-11.2) and females (CL 11.2 mm, IQR 10.1-11.2) but the median size of males in J2-613-24 (CL 10.3 mm, IQR 9.7-10.8) was not significantly different (Figure 5.6; Dunn's Multiple Comparison Test, $p < 0.05$). Sample J2-613-15 exhibited a peak of large females and significantly greater median sizes for females than males (Figure 5.6; Mann-Whitney *U*-test, $T = 2267.5$, $p < 0.01$). The distribution of sizes between males and females was significantly different (Kolmogorov-Smirnov two-sample test, $D = 0.30$, $p < 0.05$).

5.3.2.4 Fecundity. The embryos in broods of *Rimicaris hybisae* formed a dense mass attached to pleopods 1-4 underneath the female abdomen. The broods were orange in colour and visible in video footage from the Beebe and Von Damm vent fields in January 2012. The greatest minimum realised fecundity determined among 218 brooding females examined from January 2012 was 1707 in an individual from the Von Damm Vent Field with a carapace length of 16.6 mm.

Specimens from the Von Damm Vent Field exhibited significantly greater fecundity than those from the Beebe Vent Field (Table 2; Von Damm vs J2-613-19, vs

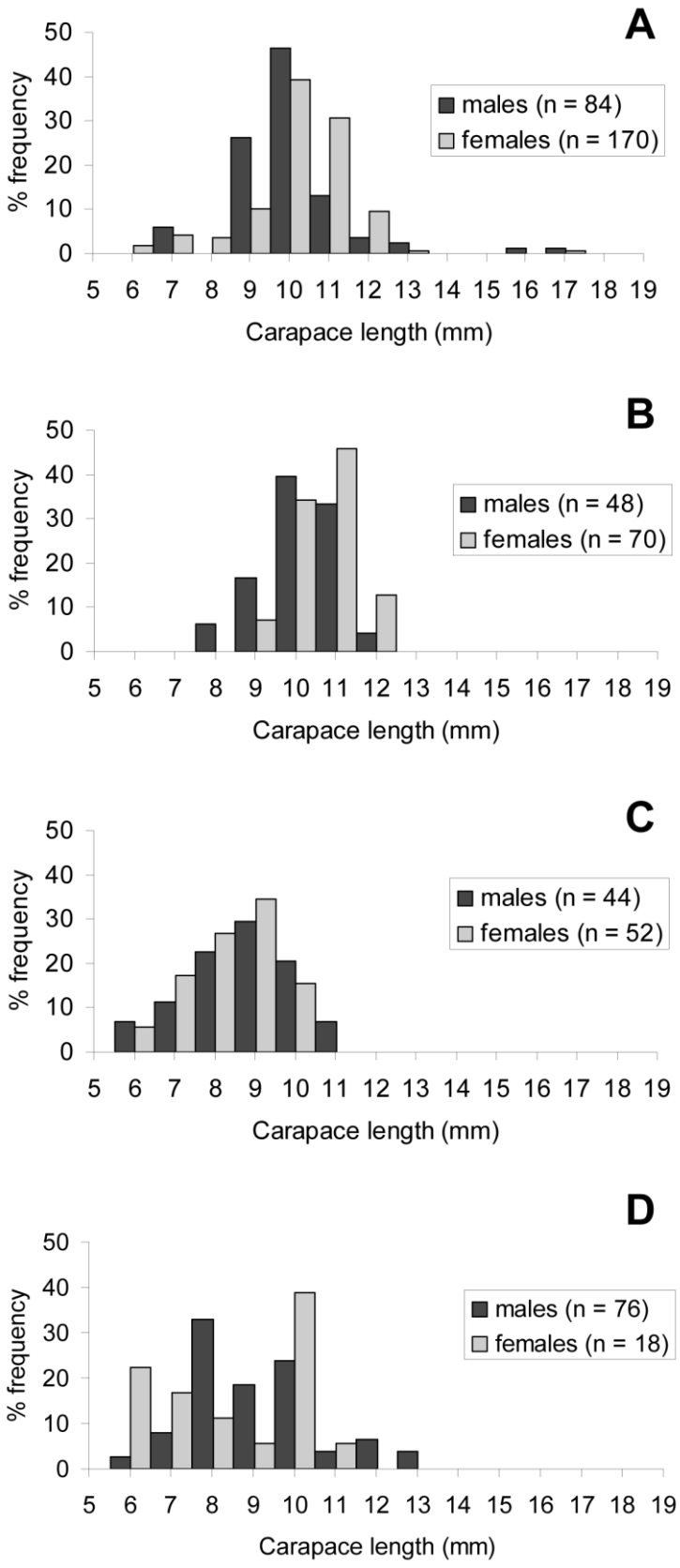

Figure 5.6. *Rimicaris hybisae*. Size-frequency distribution within the Beebe Vent Field, January 2012. A, Sample J2-613-24; B, sample J2-619-15; C, sample J2-613-19; D, sample J2-620-32. n: no. of individuals measured.

J2-613-24, vs J2-619-15, Kruskal-Wallace multisample test, $H = 139.8$, 3 df, $p < 0.001$; Dunn's Multiple Comparison Test, $p < 0.005$). The median number of embryos brooded by females from the Von Damm Vent Field was 1062 (IQR 931-1191). The median number of embryos brooded by females from the Beebe Vent Field ranged from 122 (IQR 20-304, J2-613-19, Beebe Woods) to 385 (IQR 357-439, J2-619-15, Shrimp Gulley). Differences in the fecundities of females among the samples from the Beebe Vent Field were not significant (Dunn's Multiple Comparison Test, $p > 0.05$).

Minimum realised fecundity correlated positively with carapace length (Figure 5.7A; Spearman correlation, $r = 0.77$, $p < 0.0001$). The slope of the relationship between $\log_e$-transformed fecundity and $\log_e$-transformed body size (carapace length) did not differ significantly among specimens from the Von Damm Vent Field and the Beebe Vent Field (Figure 5.7A; $F = 0.06$, $p < 0.05$). Overall, size-specific fecundity (related to carapace length) ranged from 1.2 to 102.8 embryos mm^{-1}. Although brooding females at Von Damm carried significantly more embryos per mm carapace length than those at Beebe (Figure 5.7B), this difference could therefore be attributable to the larger body sizes of brooding females at Von Damm and a non-linear relationship between fecundity and body size, which appears to be consistent across the two vent fields.

5.3.2.5 Embryo developmental stages. Within each brood of the 218 brooding females analysed, the embryos had developed synchronously and were all at the same stage of development (early, mid or late; Figure 1). However, there was no evidence of synchrony between the broods of different females. Overall, the majority of broods (48%) were in a medium developmental stage. One third (34%) exhibited an early stage of development, whereas advanced-stage broods were the minority (19%). The majority of broods at both the Von Damm and Beebe vent fields were in mid-stage development (60% and 41% respectively), and there were a greater proportion of broods at the early-developmental stage compared to the late stage (30% vs 10% Von Damm; 36% vs 24% Beebe).

In sample J2-613-19, 80% of broods were at the early stage and the remaining 20% were at the late stage (Figure 5.5D). In contrast, 47% of broods were at the mid-stage in sample J2-613-24; 34% were early-stage and 19% were late-stage (Figure 5.5D). Late-stage broods accounted for 43% of broods in sample J2-619-15, with 32% and 25% at the early and mid-stage broods respectively (Figure 5.5D). There were no brooding females in sample J2-620-32.

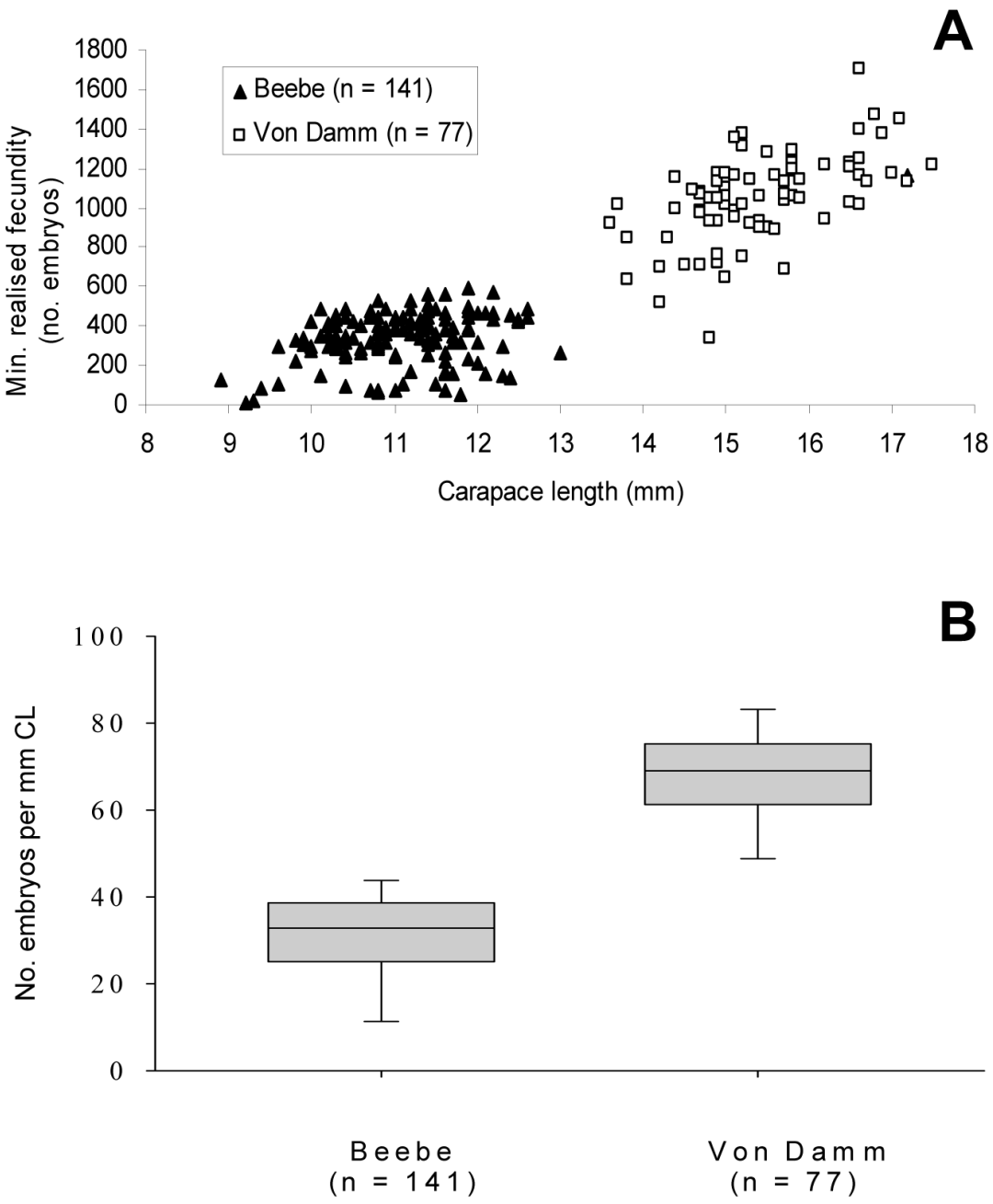

Figure 5.7. *Rimicaris hybisae*. Fecundity (no. of embryos on pleopods), January 2012. A, Variation of $\log_e$-transformed minimum realised fecundity with $\log_e$-transformed carapace length; B, corrected for body size (carapace length). n: no. of individuals measured.

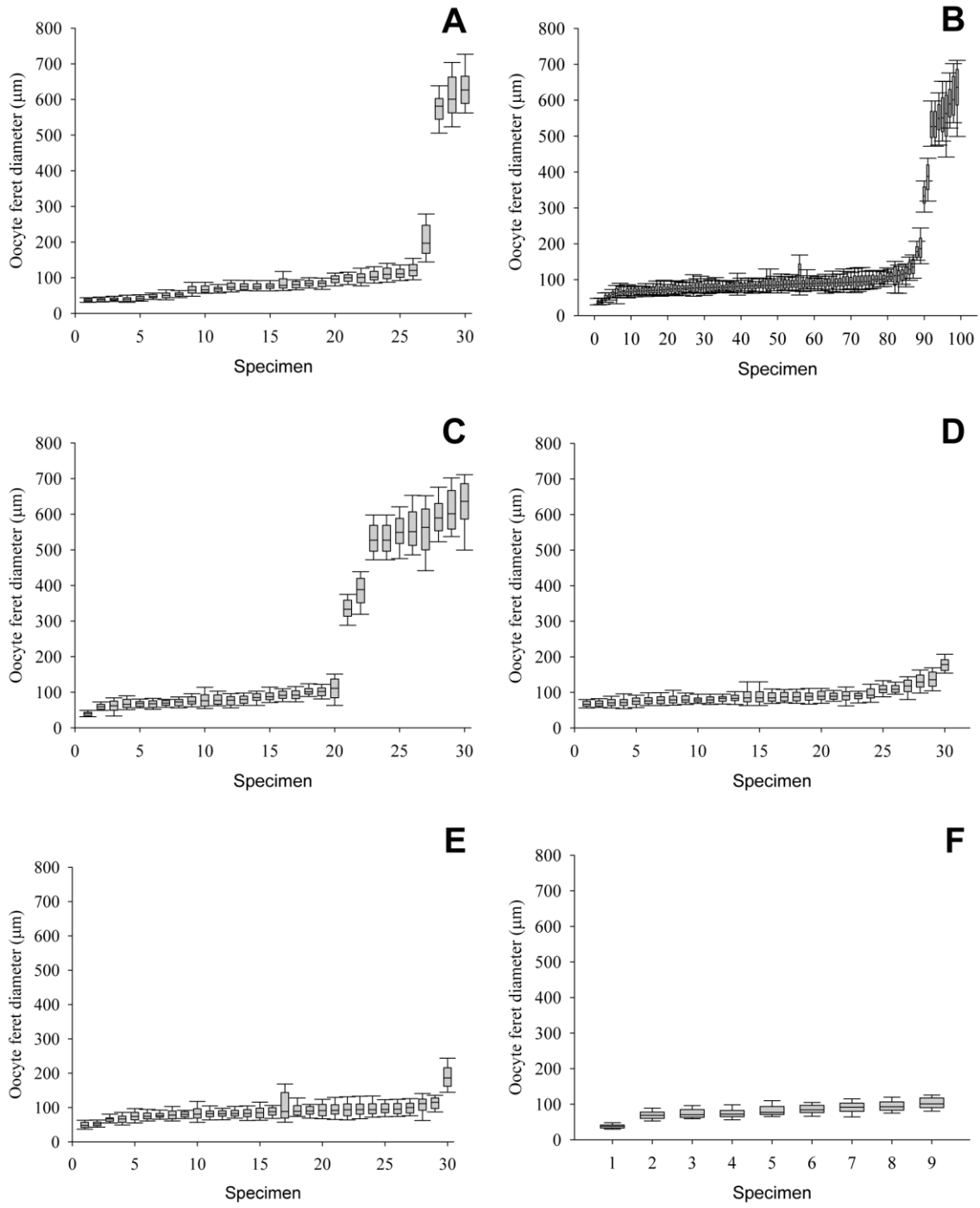

Figure 5.8. *Rimicaris hybisae*. Spatial variation in oocyte size-frequency distribution at the Beebe (B-F) and Von Damm vent fields, January 2012. A, Von Damm Vent Field; B, Beebe Vent Field (all samples); C, sample J2-613-24; D, sample J2-619-15; E, sample J2-613-19; F, sample J2-620-32.

5.3.2.6 Oocyte size-frequency distribution. Females examined from January 2012 contained oocytes with feret diameters ranging from 21 μm (non-ovigerous female, CL 12.3, Von Damm) to 823 μm (non-ovigerous female, CL 16.7, Von Damm) (Figure 5.8). Specimens examined from both vent fields included both ovigerous (brooding and hatched) and non-ovigerous females.

The median oocyte size in individuals from the Von Damm Vent Field was 82 μm (IQR 59-109). Three distinct oocyte sizes were apparent among females collected from the Von Damm Vent Field in January 2012 (Figure 5.8A). The larger oocytes belonged exclusively to large, non-ovigerous females (CL 14.4-17.5 mm). These specimens exhibited median oocyte sizes ranging from 581 μm (IQR 545-602) to 626.5 μm (IQR 589-665). The small oocytes belonged to ovigerous and non-ovigerous females (CL 11.6-17.2 mm); these specimens displayed a range of median oocyte sizes from 37 μm (IQR 33-41) to 120.5 μm (IQR 105.5-137). One individual (non-ovigerous female, CL 14.96 mm) showed a median oocyte size outside both these ranges (196.5 μm, IQR 169-246).

The median oocyte size in 99 individuals from the Beebe Vent Field was 86 μm (IQR 71-109). Three distinct oocyte sizes were apparent among females collected from the Beebe Vent Field in January 2012 (Figure 5.8B). Females examined in samples J2-619-15, J2-613-19 and J2-620-32 contained relatively small oocytes with feret diameters between 26 and 262 μm (Figure 5.8). Median oocyte sizes in individuals from sample J2-619-15 ranged from 66.5 μm (IQR 61-74.5) to 178 μm (IQR 162-191.5). Median oocyte sizes in the nine females from sample J2-613-19 ranged from 48 μm (IQR 42-57) to 186.5 μm (IQR 162-215.5). The range of median oocyte sizes in females from sample J2-620-32 was 38 μm (IQR 33-41.5) to 101 μm (IQR 90.5-117). Specimens examined from these samples included both ovigerous and non-ovigerous females. Three distinct oocyte sizes were evident among females from sample J2-613-24 (Figure 5.8C). Here the larger oocyte sizes belonged to non-ovigerous females (CL 9.2-10.3 mm). These individuals exhibited median oocyte sizes ranging from 527 μm (IQR 496-569) to 636 μm (IQR 586.5-683.5). The mid-size oocytes also belonged to non-ovigerous females (CL 9.6-10.7 mm); these specimens displayed median oocyte sizes ranging from 333 μm (IQR 315.5-358.5) to 388 μm (IQR 353-419.5). The smaller oocytes were from ovigerous and non-ovigerous females (CL 9-12.4 mm). These females revealed median oocyte sizes ranging from 40 μm (IQR 34.5-44) to 111 μm (IQR 84.5-136.5).

Overall, there was significant variation in oocyte sizes between samples (Kruskal-Wallis multi-sample test, $H = 371.8$, 4 df, $p < 0.01$) and no evidence of synchrony between samples. There were significant differences in oocyte sizes between every pairwise comparison of all five samples (Dunn's Multiple Comparison Test, $p < 0.05$).

5.4 Discussion

5.4.1 General features of reproduction in *Rimicaris hybisae*

The gonads of *Rimicaris hybisae* are similar to those of other caridean shrimp (Ramirez-Llodra *et al.*, 2000; Bauer, 2004; Ramirez-Llodra & Segonzac, 2006). *Rimicaris hybisae* exhibits sexual dimorphism and is a gonochoric species, consistent with all other alvinocaridid species studied to date.

The maximum size of males analysed was larger than the maximum size of females of *Rimicaris hybisae*. Nevertheless, in January 2012 females exhibited a significantly larger median body size and greater size range than males with proportionally greater large females at both the Beebe and Von Damm vent fields. A larger size in females has been inferred for *Alvinocaris muricola* based on the maximum size of males vs females (Ramirez-Llodra & Segonzac, 2006) and a larger size of females is a common feature of caridean shrimp (Company & Sarda, 2002). However, the variance in the size of sexes in this study appears to be the result of spatial variation in the proportion of males and females in samples, rather than sexual dimorphism.

Males with spermatophores were significantly larger in carapace length than males without spermatophores in January 2012. Ovigerous (brooding and hatched) females were significantly larger than non-ovigerous females at both vent fields, whereas the size-frequency distributions of non-ovigerous females were not significantly different from males and the ratio of males to non-ovigerous females did not deviate significantly from 1:1. The larger size of ovigerous females has been reported previously for *Alvinocaris stactophila* from the Brine Pool cold seep (Copley & Young, 2006). A greater size of ovigerous females may be advantageous for embryo production where fecundity correlates positively with body size, as in *Rimicaris hybisae*. A positive correlation between body size and fecundity has been determined for every alvinocaridid species in which these variables have been examined (*A.*

muricola and *A. stactophila*, Ramirez-Llodra *et al.*, 2000; Copley & Young, 2006; *Mirocaris fortunata*, Ramirez-Llodra & Segonzac, 2006; *R. hybisae*, this study). This feature of carideans is a result of space availability between the pleopods for attachment of embryos (Corey & Reid, 1991).

The maximum oocyte size measured in *Rimicaris hybisae* females in January 2012 was 823 μm, which is greater than maximum oocyte sizes recorded for other alvinocaridid shrimp (*R. exoculata*: 500 μm (Ramirez-Llodra *et al.*, 2000), 601 μm (Copley *et al.*, 2007); *Alvinocaris muricola*: 515 μm (Ramirez-Llodra & Segonzac, 2006)). However, the smaller mature oocytes in the range exhibited by *R. hybisae* (Figure 5.8) fall within the range recorded previously in the literature. Although it is possible that mature oocytes attain a greater size in *R. hybisae* than in other alvinocaridids for which information is available, an alternative explanation is that the true size range of mature oocytes has not been captured yet in other species, given smaller sample sizes from which data have been generated in other species.

Embryos had developed synchronously within (but not between) broods in January 2012. The developmental stages of embryos observed in *Rimicaris hybisae* were similar to those described for *Alvinocaris muricola* (Ramirez-Llodra & Segonzac, 2006). One experimental study found that the embryos of two brooding specimens of *Alvinocaris* sp. hatched after 73 and 79 days of rearing, with complete hatching of the brood over 7 and 14 days (Koyama *et al.*, 2005). Nevertheless, it remains to be determined how long alvinocaridid females incubate their young *in situ*, how long embryos take to develop from one stage to the next and whether these features are variable between different environmental conditions. Further experimental studies in the laboratory and *in situ* would be required to elucidate these knowledge gaps.

Tyler & Young (1999) reported that a complete life cycle had yet to be elucidated for a single vent or seep species. Following their template for a generalised marine invertebrate life cycle, what is now known, inferred, or unknown in gametogenesis, copulation/spawning, embryo development, larval ecology, dispersal and recruitment are summarised for *Rimicaris hybisae* in Figure 5.9.

Embryos of *Rimicaris hybisae* were variable in size but there was no spatial variation in embryo size. Embryos ranged in size from 0.42-0.76 mm (greatest diameter) with a mean size that was consistent with those reported in other alvinocaridid species to date (Table 5.2). The small size of embryos revealed in all the alvinocaridids for which data are available is indicative of planktotrophic larvae with extended

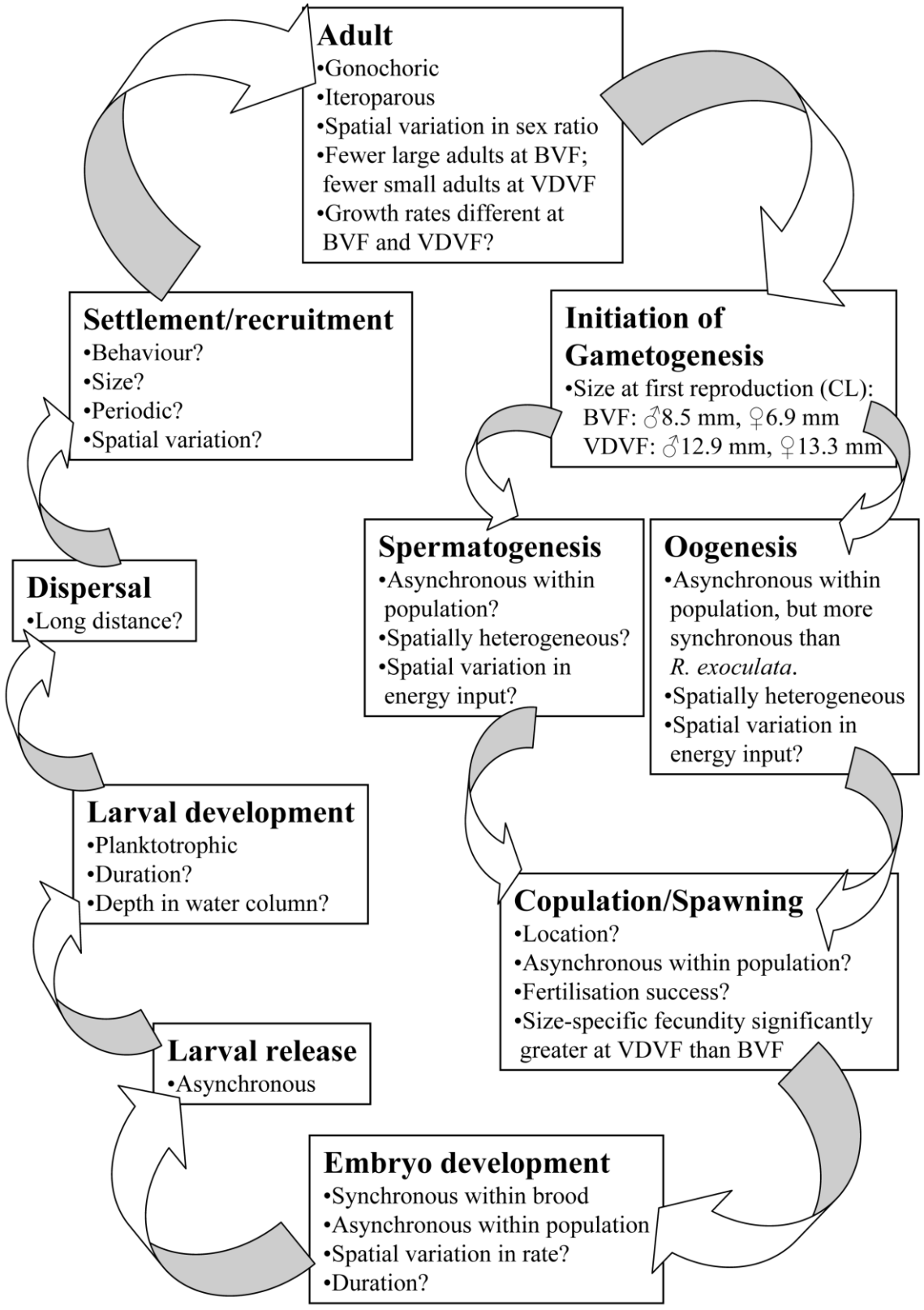

Figure 5.9. *Rimicaris hybisae*. Inferred life-history (*sensu* Tyler & Young, 1999). Stages are in bold. BVF, Beebe Vent Field; CL, carapace length; VDVF, Von Damm Vent Field.

development (Bauer, 2004). This hypothesis is supported by biochemical and experimental studies for other species (Creasey *et al*., 1996; Pond *et al*., 1997 a, b, c; Allen, 1998; Allen Copley, 1998; Gebruk *et al.*, 2000; Pond *et al.*, 2000; Koyama *et al.*, 2005; Stevens *et al.*, 2008). A long larval duration could facilitate extended dispersal between patchily distributed chemosynthetic environments and promote genetic diversity and colonisation of new vents (Shank *et al.*, 1998; Tyler & Young, 2003; Teixera *et al.*, 2012). Biochemical studies would be required to confirm the trophic ecology of *R. hybisae* during the larval (and adult) phase.

It has been suggested that upward vertical movement during the larval phase could explain the broad geographic distribution of certain species with planktotrophic larvae entrained in deep-water currents (Van Dover *et al.*, 2002). Vent shrimp postlarvae have been found to be present in midwater above MAR vents and to extend great distances laterally from known chemosynthetic sites (Herring & Dixon, 1998). However, the period and depth of planktotrophic development in the water column has not been specified for a single alvinocaridid species to date. The larval phase remains one of the least known stages in the life-cycle of most deep-sea species (Young, 2003), yet elucidating the larval ecology of a species is a prerequisite to understand patterns of reproduction and recruitment. Studies of larval tolerances, lifespan and mortality during dispersal combined with hydrographic and phylogeographic data are required to address this significant gap in the life-cycle of *Rimicaris hybisae* and other alvinocaridid species.

5.4.2 Spatial variation in population structure and reproductive features

In January 2012, the sampled population of *Rimicaris hybisae* at the Von Damm Vent Field was dominated by large females and a lesser proportion of slightly smaller males. The sex ratio was significantly female-biased but the ratio of males to non-ovigerous females did not differ significantly from 1:1. However, there was a significantly smaller proportion of large non-ovigerous females compared with males. Large ovigerous females accounted for a high proportion of the female population. Males with a spermatophore represented quite a high proportion of the male population but were only present in the larger size classes (CL > 12 mm).

Overall, the sampled population at the Beebe Vent Field was similar to that at the Von Damm Vent field (see above), but with a significantly greater proportion of

ovigerous and brooding females and males with spermatophores. The sex ratio at the Beebe Vent Field was also significantly female-biased overall, although males represented a greater proportion of the population in comparison with the Von Damm Vent Field. There was no significant difference in size between males and non-ovigerous females and the ratio of males to non-ovigerous females did not differ significantly from 1:1. However, individual samples from the Beebe Vent Field were heterogeneous. Recognition of this spatial heterogeneity is crucial to consider in any attempts to infer temporal patterns from limited spatial samples.

Ovigerous female *Rimicaris hybisae* and males with spermatophores were present with the greatest frequency in sample J2-613-24. This sample was dominated by ovigerous females and the sex ratio was significantly female-biased. However, the ratio of males to non-ovigerous females did not differ significantly from 1:1. Females were significantly larger than males and both sexes were significantly larger than those in samples J2-613-19 and J2-620-32.

Large male and female *Rimicaris hybisae* were present with the greatest frequency in sample J2-619-15. The sex ratio in this sample did not differ significantly from 1:1. Ovigerous females were present with the second greatest frequency here and females were significantly bigger than males. Males with spermatophores were present with the lowest frequency in this sample.

Ovigerous female *Rimicaris hybisae* were present with the lowest frequency in samples J2-613-19 and J2-620-32 and the size of males and females were not significantly different from each other in these samples. The sex ratio in sample J2-620-32 was significantly male-biased, whereas it did not differ significantly from 1:1 in J2-613-19.

The population of *Rimicaris hybisae* showed a significant clear bias towards females in the sample from the Von Damm Vent Field and sample J2-613-24 from Beebe Woods. These samples also contained the greatest proportion of ovigerous females. Both samples were collected from large, high-density aggregations of shrimp. Ramirez-Llodra & Segonzac (2006) described a clear bias towards females in *Alvinocaris muricola* in the cold-seep site north of Regab in the Congo Basin. Copley & Young (2006) identified a specific distribution of males and females within the Brine Pool mussel bed, with ovigerous females avoiding the sulfidic or anoxic extremes in the environment. Hydrothermal vents exhibit fine-scale heterogeneity in physico-chemical conditions (Luther *et al.*, 2001). Environmental variables within shrimp aggregations

may influence the distribution of ovigerous females, resulting in a spatially heterogeneous pattern of reproductive development in *R. hybisae*, as found in other vent taxa (Copley *et al.*, 2003; Pradillon *et al.*, 2007). Further sampling and collection of environmental data would be required to test this hypothesis.

Shrimp at the Von Damm Vent Field were significantly larger than shrimp at the Beebe Vent Field both overall and within each population category. The smallest ovigerous female (CL 6.9 mm) and male with a spermatophore (CL 8.5 mm) at the Beebe Vent Field were much smaller than the smallest ovigerous female (CL 13.3) and male with spermatophore at the Von Damm Vent Field (CL 12.9 mm). Size at the onset of maturity is considered a key life-history parameter that should also reflect the longevity and life-time investment in reproduction of a species (Anger & Moreira, 1998). Assuming these data represent the minimum size of sexual maturity in *Rimicaris hybisae*, males and females at the Beebe Vent Field may reach maturity at smaller sizes than their counterparts at the Von Damm Vent Field.

Size-specific fecundity in *Rimicaris hybisae* falls within the range of values reported in other alvinocaridid species (Table 5.2). Fecundity was significantly positively correlated with body size in *R. hybisae* and there was no significant difference in the slope of the relationship between $\log_e$-transformed fecundity and $\log_e$-transformed carapace length at the two vent fields. There is no clear relationship between the size-specific investments in reproduction in alvinocaridids and phylogeny, depth or environment (vent versus seep) (Ramirez-Llodra & Segonzac, 2006). Some variation in measured fecundity may result from females losing eggs during collection (Ramirez-Llodra, 2002) but variation in fecundity may also result from variations in reproductive success, which may be affected by environmental conditions (Copley & Young, 2006). Environmental factors such as pressure, availability of photosynthetically-derived nutrition, temperature, and/or fluid chemistry may vary between the two vent fields which are considerably different in depth. However, a non-linear relationship between fecundity and carapace length may result from volume effects either for the developing ovary or in the space around the pleopods on which embryos develop. Consequently, the greater size-specific fecundity of females at Von Damm may be attributable to their larger body size rather than environmental conditions, given the homogeneity of regression between $\log_e$-transformed carapace lengths and $\log_e$-transformed fecundities across both vent fields.

The oocyte size-frequency distributions exhibited significant variation between samples and all stages of developing oocytes were present amongst females in January 2012. Most females had gonads containing previtellogenic oocytes (<100 μm) and early vitellogenic oocytes (>100 μm). However, several large non-ovigerous females from the Von Damm Vent Field and sample J2-613-24 contained large vitellogenic oocytes. These data indicate iteroparous reproduction in *Rimicaris hybisae*. However, the variation observed was less than that recorded among individual female *R. exoculata* from TAG (Copley *et al.*, 2007), suggesting a greater degree of synchrony in the oocyte development of *R. hybisae* than reported for *R. exoculata*.

Embryo development was clearly asynchronous between females of *Rimicaris hybisae*, indicating that larval release may also be asynchronous for the population as a whole. Environmental variability may potentially affect every reproductive process, resulting in embryo development proceeding at different rates within a vent field.

Video from a previous cruise (NOAA *Okeanos Explore*r) to the Von Damm Vent Field in August 2011 showed no evidence of brooding *Rimicaris hybisae*, whilst individuals in video collected during January 2012 showed clear evidence of embryo carrying, suggesting possible periodic reproduction for *R. hybisae*. The collection and analyses of additional samples spanning several seasons of the year are a prerequisite to determine the potential periodicity or even seasonality in reproduction and possibly recruitment of *R. hybisae*.

Periodic reproduction has also been suggested for *Rimicaris exoculata* based on the almost complete absence of ovigerous females in collections from summer months (when most samples have been collected) (Herring, 1998). However, observations of oocyte size-frequency distributions of females collected in different times of the year indicate iteroparous, asynchronous reproduction and lack of seasonal reproduction in *R. exoculata* from MAR vents (Ramirez-Llodra *et al.*, 2000; Copley *et al.*, 2007). An alternative hypothesis that has been proposed is that ovigerous females stay outside the main populations (Ramirez-Llodra *et al.*, 2000). However, recent observations of ovigerous females of *R. exoculata* within the main aggregations around high temperature zones at Logatchev in March 2007 (Gebruk *et al.*, 2010) refute the latter hypothesis.

Periodic production of eggs has also been proposed for *Mirocaris fortunata* (Ramirez-Llodra *et al.*, 2000), whereas continuous egg production with periodic spawning was suggested for *Alvinocaris muricola* (Ramirez-Llodra & Segonzac, 2006).

In contrast, a seasonal pattern of reproduction has been revealed in *A. stactophila* at the Brine Pool cold seep (Copley & Young, 2006) and a few other species with planktotrophic larvae from vent environments (e.g. Perovich *et al.*, 2003; Colaço *et al.*, 2006; Dixon *et al.*, 2006). Surface productivity may therefore be important for the nutrition of planktotrophic larvae of some vent and seep species, particularly at shallower depths.

5.5 Conclusions

Reproductive features examined in *Rimicaris hybisae* at the Beebe and Von Damm vent fields are consistent with those described previously for other alvinocaridid species, consistent with phylogenetic constraint of such features in vent species. Several gaps remain, however, in understanding the life cycle of this and other alvinocaridid species.

Samples collected from the Von Damm and Beebe vent fields in January 2012 revealed spatial variation in the population structure and reproductive features of *Rimicaris hybisae*. These data highlight a high degree of spatial variability in the reproductive features of a motile species in the vent environment. The sample from the Von Damm Vent Field and sample J2-613-24 from Beebe Woods exhibited the highest frequencies of ovigerous females and significantly female-biased sex ratios. Any bias in sex ratio was not the result of immature males being misidentified as females. Nevertheless, when the generally large ovigerous females were excluded, there was no significant deviation from a 1:1 sex ratio. Environmental variables within shrimp aggregations may influence the distribution of ovigerous females, resulting in a spatially heterogeneous pattern of reproductive development in *R. hybisae*, as found in other vent taxa. This hypothesis would require testing with the collection and analysis of further samples with environmental data (temperature, fluid chemistry).

Reproduction in *Rimicaris hybisae* is iteroparous. The oocyte development of *R. hybisae* appears to exhibit a greater degree of synchrony than reported previously for *R. exoculata*. However, embryo development and larval release may be asynchronous for the population as a whole. Analysis of video from a previous cruise to the Von Damm Vent Field in a different season revealed no evidence of ovigerous females. These results suggest a lack of synchrony and possible periodicity in the reproductive

development of *R. hybisae*. Any subsequent investigation of temporal variation in the reproductive development of *R. hybisae*, however, needs to take into account the spatial variation also revealed by this study.

Specimens of *Rimicaris hybisae* from the Von Damm Vent Field were significantly larger than specimens from the Beebe Vent Field and may reach maturity at larger sizes than their counterparts at the Beebe Vent Field. Given a possible non-linear relationship between fecundity and carapace length, the larger body sizes of brooding females at Von Damm may result in a greater size-specific fecundity compared with females at the Beebe Vent Field.

5.6 Acknowledgements

Thanks to the Master and ship's company of the *RV Atlantis*, the crew of the ROV *Jason II* and fellow scientists on the 18th voyage (16th leg) of the *RV Atlantis*, especially C.L. Van Dover, S. Plouviez and J.W. Clarke; C. German, chief scientist. V. Nye was supported by a UK NERC award (NE/F017774/1) to J. Copley. NSF award OCE-1031050 to C.L. Van Dover and Cliff Cunningham and NASA ASTEP subcontract through WHOI (NNX09AB75G) to C.L. Van Dover supported the biological component of the *Atlantis* Cayman cruise. The helpful comments of two reviewers are gratefully acknowledged.

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6. Fine-scale spatial variation and time-series of reproductive development in *Rimicaris hybisae* (Caridea: Alvinocarididae) at hydrothermal vents on the Mid-Cayman Spreading Centre

This work has been submitted for publication as: Nye V., Copley J.T. & Tyler P.A. (2013) Fine-scale variation and time-series of reproductive development in *Rimicaris hybisae* (Caridea: Alvinocarididae) at hydrothermal vents on the Mid-Cayman Spreading Centre. *PLoS ONE* (submitted).

Abstract

Organisms experience spatial and temporal heterogeneity at a variety of scales in the dynamic environment of deep-sea hydrothermal vent fields. Consequently it is important to determine the influence of this variability on processes such as the reproductive development of vent species. We have investigated spatial variation in the population structure and reproductive development of the abundant alvinocaridid shrimp *Rimicaris hybisae* at the Von Damm Vent Field (Mid-Cayman Spreading Centre, Caribbean) for the first time, and extended studies of spatial variability of this species at the neighbouring Beebe Vent Field. In addition, we examined inter-annual variation in the population structure and reproductive development of *R. hybisae* between January 2012 and February 2013 at both vent fields. Specimens of *R. hybisae* from Von Damm (2301-2379 m depth) were significantly larger than specimens from Beebe (4960-4965 m depth) and there were a significantly greater proportion of females brooding at Von Damm than at Beebe. Samples collected from several locations within these vent fields exhibited significant heterogeneity in sex ratios, population structure, size-frequency, and oocyte development of this motile species. Samples from the Von Damm main mound and Beebe Chimlets displayed the highest frequencies of ovigerous females and significantly male-biased sex ratios. There was no correlation, however, between the distribution of ovigerous females with density of aggregation or sex ratio. The significantly lower proportion of ovigerous females in the sampled population in February 2013 compared to January 2012 is consistent with possible periodic or

seasonal reproduction in *R. hybisae*, superimposed upon a pattern of spatial variation within the vent fields. This first time-series study of reproduction in vent fauna from an ultraslow-spreading system has revealed previously unappreciated variation, indicating the necessity for large samples with replication to determine patterns in space and the factors that affect population structure and development at hydrothermal vents.

6.1 Introduction

Deep-sea hydrothermal vent fields are spatially and temporally heterogeneous, insular, and ephemeral environments. Ecological investigations in these environments have focused on the composition and stability of faunal assemblages, the genetic structure of populations, and how biogeographic patterns are established and maintained (e.g. Corliss *et al.*, 1979; Grassle, 1985; Tunnicliffe, 1988; Young *et al.*, 1996; Gebruk *et al.*, 1997; Jollivet *et al.*, 1995, 1999; Vrijenhoek, 1997; Shank & Halanych, 2007; Vrijenhoek, 2013; Plouviez *et al.*, 2013).

Vent organisms experience fluctuations in the temperature and chemistry of their environment, over spatial scales of centimetres to metres and temporal scales of seconds to years (e.g. Shank *et al.*, 1998; Sarrazin *et al.*, 1999; Luther *et al.*, 2001). Consequently, the responses of faunal assemblages to spatial and temporal variations are key features to elucidate and understand in vent ecology (Gebruk *et al.*, 2010). In particular, these variations may influence processes such as reproductive development, which in turn are involved in establishing and maintaining the isolated populations and biogeographic ranges of taxa endemic to hydrothermal vents (Tyler & Young, 1999).

To date, aspects of life-history biology have been elucidated in more than 90 species from hydrothermal vents, cold seeps, and organic food falls (Nye *et al.*, 2013a). Studies have revealed a variety of reproductive traits and developmental modes in species from deep-sea chemosynthetic environments, and revealed spatial and temporal patterns in the reproductive development of some species (e.g. Tyler & Young, 1999; Sheader *et al.*, 2000, 2004; Young, 2003; Copley *et al.*, 2003; Copley & Young, 2006; Nye *et al.*, 2013a).

Only recently was it recognised that the world's slowest-spreading ridges host hydrothermal activity (German *et al.*, 1998; Bach *et al.*, 2002; Edmonds *et al.*, 2003;

Connelly *et al.*, 2007; German *et al.*, 2010; Connelly *et al.*, 2012). Observations made on these ridges continue to provide insights into the tectonic process, magmatic architecture, and hydrothermal evolution of oceanic crust at all spreading rates (Snow & Edmonds, 2007; Hayman *et al.*, 2011). However, there have been few ecological investigations of hydrothermal vent fields on ultraslow-spreading ridges (Pedersen *et al.*, 2010; Schander *et al.*, 2010; Copley, 2011; Connelly *et al.*, 2012; Sweetman *et al.*, 2013), and the inter-annual dynamics of their fauna have yet to be elucidated.

There are fundamental differences in the longevity and spacing of hydrothermal vents along fast- versus slow-spreading ridges (Lalou & Brichet 1982; Lalou *et al.*, 1990, 1993; Juniper & Tunnicliffe, 1997; Van Dover *et al.*, 2002). For example, catastrophic perturbations, such as eruptions, occur on timescales of years to decades on fast-spreading ridges, whereas they appear be less frequent on slower-spreading ridges (Lalou, 1991; Desbruyères *et al.*, 2001). The loci of hydrothermal activity on slow- and ultralow-spreading ridges may also be active for greater millennial-scale periods compared with shorter, decadal-scale periods on their faster-spreading counterparts (Snow & Edmonds, 2007).

Fast-spreading ridges are found only in the Southern and Pacific oceans, whereas slow and ultraslow ridges dominate the Arctic, Atlantic and southwest Indian oceans (Sinha & Evans, 2004). Hydrothermal-vent assemblages were first discovered on the slow-spreading Mid-Atlantic Ridge (MAR) in 1985 (Rona *et al.*, 1986). Only three times-series studies at three MAR vent fields have been published to date (Copley *et al.*, 2007; Gebruk *et al.*, 2010; Cuvelier *et al.*, 2011), revealing little or no long-term ecological changes in hydrothermal assemblages, in contrast with the much faster rates of habitat turnover and succession at vent sites in the East Pacific (e.g. Juniper & Tunnicliffe, 1997; Sarrazin *et al.*, 1997; Desbruyères *et al.*, 1998; Shank & Halanych, 2007). Under conditions of continuous venting, TAG displayed constancy in assemblage structure on decadal time scales (Copley *et al.*, 2007). Over a decade at the Logatchev vent field, changes were observed in the composition and abundance of species but no successional changes were observed (Gebruk *et al.*, 2010). Cuvelier *et al.* (2011) determined that the rate of change in community dynamics at Lucky Strike was approximately 15% slower than observed previously at vents on faster-spreading ridges in the Northeast Pacific.

The ultraslow-spreading Mid-Cayman Spreading Centre (MCSC) is a geographically isolated ridge in the Caribbean that hosts two high-temperature

hydrothermal vent fields (Connelly *et al.*, 2012). The Beebe Vent Field (~4960 m depth) lies on the axis of the MCSC and consists of a sulfide mound (~80 m diameter, 50 m height) surmounted by several active black-smoker chimney complexes and areas of diffuse flow (Connelly *et al.*, 2012). The Beebe vent assemblage includes provannid gastropods, anemones and ophiuroids.

The Von Damm Vent Field (~2300 m depth) lies approximately 13 km away from the Beebe Vent Field on the upper slopes of the Mount Dent oceanic core complex (Connelly *et al.*, 2012). It is a conical mound (~150 m diameter, 70 m height) that vents clear, buoyant fluids, (Connelly *et al.*, 2012). Vent fauna at the Von Damm Vent Field includes skeneid gastropods, hippolytid shrimp, lysianssid amphipods and tubeworms (Connelly *et al.*, 2012; Nye *et al.*, 2012; Nye *et al.*, 2013b, c). The alvinocaridid shrimp *Rimicaris hybisae* is present and abundant at both known MCSC vent fields (Nye *et al.*, 2012).

A previous study described the general reproductive features of *Rimicaris hybisae* and examined the reproductive development and population structure of this species at the Beebe and Von Damm vent fields using spatially discrete samples collected in January 2012, revealing a high degree of spatial variability in the population structure and reproductive features of this motile species (Nye *et al.*, 2013a). The lack of samples from more than one spatial point within the Von Damm Vent Field, however, precluded analysis of spatial variation within that vent field at that time (Nye *et al.*, 2013).

Using samples collected from the vent fields of the MCSC in February 2013, the aims of this study are: (1) to investigate spatial variation in the population structure and reproductive development of *R. hybisae* at the Von Damm Vent Field; and (2) to examine inter-annual variation in the population structure and reproductive development of *Rimicaris hybisae* between January 2012 and February 2013, in the first time-series study of vent fauna from an ultraslow-spreading system.

6.2 Materials and methods

Samples of *Rimicaris hybisae* were collected from two vent fields at the Mid-Cayman Spreading Centre, Caribbean during the 82nd voyage of the *RSS James Cook*

(see Table 6.1). Samples were collected using a suction sampler attached to the remotely operated vehicle (ROV) *Isis* in February 2013.

Four samples were collected from different locations within the Beebe Vent Field (4943-4965 m; Table 1): JC82/173/F was collected from a large, high-density aggregation of *Rimicaris hybisae* on the surface of a chimney edifice; JC82/001/F was taken from a small, dense aggregation of *R. hybisae*, next to anemones, provannid gastropods and larger aggregations of shrimps; JC82/032/F was collected from a low-density aggregation of *R. hybisae* at the base of an active black-smoker chimney complex; JC82/109/F was taken in a peripheral area, dominated by bacterial mats and anemones with sparse shrimp.

Five samples were collected from different locations within the Von Damm Vent Field (2301-2379 m; Table 6.1): JC82/082/F was taken from a small, high-density aggregation of *Rimicaris hybisae*, next to gastropods (*Iheyaspira bathycodon*), zoarcid fish and sparse *Lebbeus virentova*; JC82/089/F was collected from a dense aggregation of *R. hybisae* with *I. bathycodon*; JC82/016/F and JC82/043/F were taken from separate, small aggregations of *R. hybisae* with zoarcid fish and *I. bathycodon*; JC82/064/F was collected in an area with sparse shrimp (*R. hybisae* and *L. virentova*) and *I. bathycodon*.

Specimens were fixed in 10% buffered seawater formalin for 48 h and subsequently transferred to 70% isopropanol. A Leica MZ8 dissection microscope was used to examine specimens, perform dissections and capture images.

The carapace length (CL) of each shrimp was measured to the nearest 0.1 mm with Vernier callipers from the rear of the eye socket to the rear of the carapace in the mid-dorsal line and is used herein as the standard indication of body size (*sensu* Clarke, 1993; Nye *et al.*, 2013a).

The sex of each shrimp was determined (Nye *et al.*, 2012). In a previous study, the carapace length of the smallest male identified was 5.2 mm (Nye *et al.*, 2013a). To avoid potential bias in analyses of sex ratio as a result of misidentification of immature males as females, specimens with a carapace length <5.2 mm were classified as juveniles. For all males, the presence/absence of spermatophores was recorded. All females were categorised as either: brooding (brooding embryos); hatched (with a matrix of empty embryo sacs attached to the pleopods); or female (neither brooding nor hatched) (*sensu* Nye *et al.*, 2013a).

Ovaries were dissected from the females. Where oocyte size allowed, individual oocytes were removed from each ovary and their images were captured. Oocytes were

Table 6.1. *Rimicaris hybisae*. Sample and population data for 1012 specimens used in this study.

Sample no.	Cruise	Sample method	Vent field	Location	Depth (m)	Latitude (N)	Longitude (W)	Date (2013)	Total no. specimens	Males			Females				Juveniles.	Sex ratio TM:TF	χ^2 (1 df)	Significance	Max. temp. (°C)
										Total	M	SM	Total	F	BF	HF	Total				
JC82/082/F	JC82	Isis	Von Damm	Main mound	2301	18°22.592	81°47.883	10/02	107	11	8	3	96	94	1	1	0	0.11:1	65.94	***	14.0
JC82/064/F	JC82	Isis	Von Damm	Main mound	2310	18°22.587	81°47.886	10/02	117	73	39	34	44	39	4	1	0	1.66:1	6.70	**	4.8
JC82/089/F	JC82	Isis	Von Damm	Main mound	2340	18°22.597	81°47.877	10/02	130	81	39	42	49	29	11	9	0	1.65:1	7.39	**	11.7
JC82/016/F	JC82	Isis	Von Damm	Fumerole	2313	18°22.502	81°47.859	10/02	307	81	73	8	225	219	6	0	1	0.36:1	65.68	***	26.1
JC82/043/F	JC82	Isis	Von Damm	Fumerole	2379	18°22.561	81°47.845	12/02	29	5	3	2	23	20	3	0	1	0.22:1	10.32	**	8.3
			Von Damm	All					690	251	162	89	437	401	25	11	2	0.57:1	49.75	***	
JC82/109/F	JC82	Isis	Beebe	Anemone Fields	4960	18°32.830	81°43.130	19/02	28	13	1	12	15	14	0	1	0	0.87:1	0.04	NS	4.8
JC82/173/F	JC82	Isis	Beebe	Beebe Woods	4943	18°32.754	81°43.088	19/02	186	93	27	66	93	91	0	2	0	1:1	0.01	NS	NA
JC82/032/F	JC82	Isis	Beebe	Hashtag Chimneys	4963	18°32.781	81°43.109	19/02	48	18	4	14	26	26	0	0	4	0.69:1	1.11	NS	8.2
JC82/001/F	JC82	Isis	Beebe	Beebe Chimlets	4965	18°32.807	81°43.109	23/02	60	47	9	38	13	6	1	6	0	3.62:1	18.15	***	4.5
			Beebe	All					322	171	41	130	147	137	1	9	4	1.16:1	1.66	NS	
								Total	1012	422	203	219	584	538	26	20	6	0.72:1	25.77	***	

BF, brooding female; F; female without brooding or recently hatched; HF, female recently hatched (with a matrix of empty embryo sacs attached to the pleopods); M, male without spermatophore; NS = available; NS = not significant; SM, male with spermatophore; TM, total males; TF, total females.

* = *P* value < 0.05; ** = *P* value < 0.01; *** = *P* value < 0.001.

laid flat and measured directly to ensure maximum cross-sectional areas were recorded (*sensu* Copley & Young, 2006; Nye *et al.*, 2013a). Where female specimen numbers and condition allowed, the feret diameters of 100 oocytes were measured in 30 females per sample (15 females for JC82/043/F; 14 for JC82/109/F; 23 for JC82/032/F; 11 for JC82/001/F) using ImageJ. Feret diameter was used to standardise variations in oocyte shape. Images of oocytes were calibrated with measurements of a graticule slide at identical magnification.

Broods of embryos were removed from the pleopods of brooding females under a Leica MZ8 dissecting microscope. The developmental stage of each brood was scored on the basis of morphological features and numbers of embryos per brood were counted to determine minimum realised fecundity (*sensu* Nye *et al.*, 2013a). Although it was not possible to guarantee that embryo batches were complete, embryos were attached firmly to each other and their mothers' pleopods (within which they were enclosed) and broods remained intact post-sampling. Size-specific fecundity was calculated as number of embryos divided by carapace length.

Frequencies of males and females in samples were tested for significant variation from a 1:1 sex ratio using χ^2 test with Yates' correction for one degree of freedom. In analyses of population structure and size-frequency distribution of females, brooding and hatched females were pooled as ovigerous females (*sensu* Nye *et al.*, 2013a). To correct for variation in the sex ratio between samples, spatial variation in the population structure was assessed by comparing the ratio of ovigerous (brooding and hatched) females to non-ovigerous females, the ratio of brooding to hatched ovigerous females, and the ratio of males with spermatophores vs without (rather than the overall proportions). Frequencies of ovigerous females, brooding females and males with spermatophores were tested for significant variation from a 1:1 ratio between vent fields and between pairwise combinations of samples within vent fields using χ^2 test with Yates' correction for one degree of freedom. Population structure was examined using the size-frequency distribution of 1012 individuals.

Population structure and sex ratios were compared with those determined from samples collected in January 2012 (Nye *et al.*, 2013a). The median sizes of specimens from the Beebe and Von Damm vent fields were tested for significant inter-annual variation with those of specimens collected in January 2012 (in Nye *et al.*, 2013a) using the Mann-Whitney *U*-test. Oocyte-size frequency distributions and median sizes of

oocytes were tested for significant inter-annual variation at both vent fields using the Mann-Whitney *U*-test and Komlogorov-Smirnov test respectively.

6.3 Results

6.3.1 Population structure and fecundity in February 2013

Of the 1012 specimens of *Rimicaris hybisae* examined, 41.7% were identified as male, resulting in an overall sex ratio that deviated significantly from 1:1 (Table 6.1). Of the 422 males, 219 (51.9%) were carrying spermatophores. Of the 538 females, 46 (7.9%) were either brooding embryos (26, 4.5%) or had hatched larvae recently (20, 3.4%) (Table 6.1).

Overall, the size-frequency distribution of the specimens displayed two modal peaks and tails of small and large sizes (Figure 6.1A). Specimens ranged in body size (carapace length) from 4.1 (juvenile, Beebe) to 19.0 mm (female, Von Damm). Males and females were represented throughout the size-frequency distribution of the samples and juveniles were present at both vent fields (Table 6.2; Figure 6.1 B-C). The carapace lengths of the largest females identified were 13.6 and 19.0 mm at the Beebe and Von Damm Vent Fields respectively. The carapace lengths of the largest males identified were 17.6 and 18.0 mm at the Beebe and Von Damm vent fields respectively. The carapace lengths of the smallest brooding female and smallest male with spermatophores were 7.4 and 7.0 mm respectively (both Von Damm).

The greatest minimum realised fecundity among the 26 brooding females sampled was 1703 in a female from the Von Damm Vent Field with a carapace length of 17.1 mm. A single brooding female was present in the samples collected at the Beebe Vent Field; this individual was carrying 312 embryos with a carapace length of 11.6 mm. The median number of embryos brooded by females from the Von Damm Vent Field was 1056 (IQR 734-1262) and minimum realised fecundity correlated positively with carapace length (Spearman correlation $r = 0.79$, $p < 0.001$). Overall, size-specific fecundity (related to carapace length) ranged from 26.9 (the Beebe brooding female) to 99.6 embryos mm^{-1}, with a median value of 68 embryos mm^{-1} at the Von Damm Vent Field (IQR 46-76).

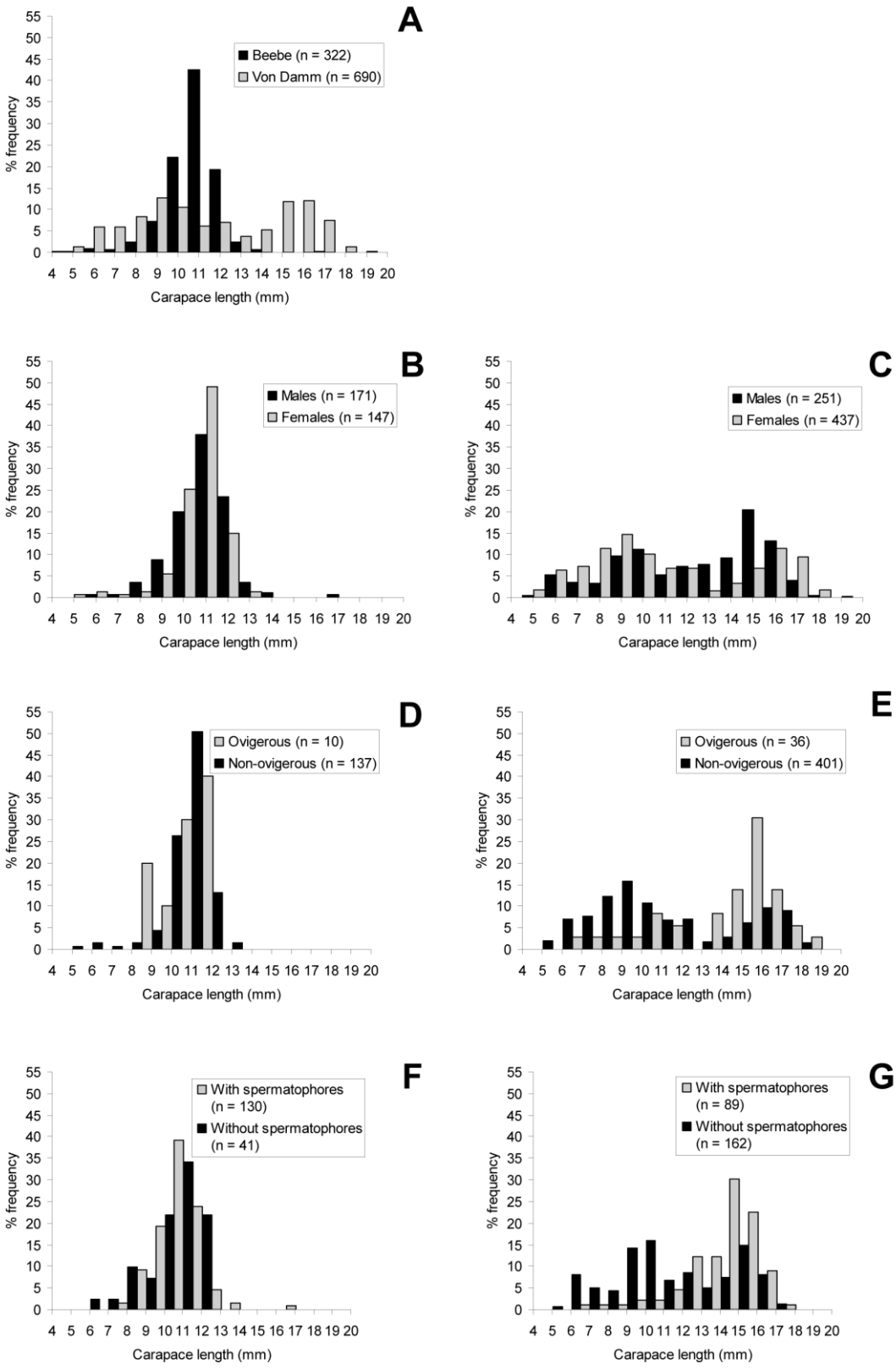

Figure 6.1. *Rimicaris hybisae*. Size-frequency distribution at the Beebe and Von Damm vent fields, February 2013. (A) All specimens; (B) Beebe Vent Field; (C) Von Damm Vent Field; (D) females, Beebe Vent Field; (E) females, Von Damm Vent Field; (F) males, Beebe Vent Field; (G) males, Von Damm Vent Field. n: no. of individuals measured.

Table 6.2. *Rimicaris hybisae*. Variation in body size (carapace length), February 2013.

	Carapace length (mm)						
	Males			Females			Juveniles
Vent Field	All	Without spermatophore	With spermatophore	All	Non-ovigerous	Ovigerous	All
Beebe	11.5 (10.5-12.0)	11.1 (10.6-12.1)	11.5 (10.6-12.1)	11.3 (10.6-11.8)	11.3 (10.6-11.7)	11.6 (10.8-12.0)	4.3 (4.2-4.5)
Von Damm	13.5 (10.1-15.6)	11.1 (9.3-14.9)	15.4 (14.0-16.3)	10.7 (8.9-15.7)	10.4 (8.8-15.2)	16.0 (13.4-16.9)	4.6 (4.4-4.7)

Carapace length is shown as median (inter-quartile range); ovigerous was defined as brooding or hatched.

Table 6.3. *Rimicaris hybisae*. Spatial variation in population structure between the Beebe and Von Damm vent fields, February 2013.

	Beebe	Von Damm	Ratio Beebe: Von Damm	χ^2 (1 df)	Significance
Proportion of females ovigerous in samples	6.8%	8.2%	0.28:1	1.08	NS
Proportion of ovigerous females brooding	10.0%	69.4%	0.04:1	134.80	***
Proportion of males with spermatophores	79.0%	35.5%	1.46:1	224.20	**

Ovigerous was defined as brooding or hatched; proportion of females ovigerous refers to the ratio of ovigerous females to all females in samples.
** = *P* value < 0.01; *** = *P* value < 0.001.

6.3.2 Spatial variation in reproductive features in February 2013

6.3.2.1 Sex ratio. In February 2013, males represented 36% of individuals sampled from the Von Damm Vent Field, resulting in an overall sex ratio that deviated significantly from 1:1 (Table 6.1). All samples from this vent field exhibited significant variation in the sex ratio (Table 6.1). Samples JC82/082/F, JC82/016/F and JC82/043/F revealed significant female bias (Table 6.1). A significant male bias was present in samples JC82/064/F and JC82/089/F (Table 6.1). Overall, the ratio of males to non-ovigerous females also differed significantly from 1:1 (0.63:1, $\chi^2 = 34.0$, 1 df, $p < 0.001$). In samples JC82/082/F, JC82/016/F and JC82/043/F the ratio of males to non-ovigerous females differed significantly from 1:1 (JC82/082/F: 0.12:1, $\chi^2 = 64.0$, 1 df, $p < 0.001$; JC82/016/F: 0.37:1, $\chi^2 = 62.6$, 1 df, $p < 0.001$; JC82/043/F: 0.25:1, $\chi^2 = 7.8$, 1 df, $p < 0.01$).

Males represented 53% of specimens sampled from the Beebe Vent Field resulting in a sex ratio that did not deviate significantly from 1:1 (Table 6.1). However, sample JC82/001/F exhibited significant male bias (78% of specimens were males) (Table 6.1).

6.3.2.2 Population structure. To correct for variation in the sex ratio between samples, spatial variation in the occurrence of ovigerous females was examined by comparing the ratio of ovigerous (brooding and hatched) females to non-ovigerous females. The proportion of females that were ovigerous at the Von Damm Vent Field (8%) was not significantly different than at the Beebe Vent Field (7%) (Table 6.3). However, a significantly greater proportion of ovigerous females were still brooding at the Von Damm Vent Field (69%) compared with the Beebe Vent Field (10%; Table 6.3). Males with spermatophores accounted for a significantly greater proportion of the sampled male population at the Beebe Vent Field (76%) than at the Von Damm Vent Field (35.5%; Table 6.3).

Significant variation in the population structure was evident between samples from the Beebe Vent Field. Within this vent field, the highest proportion of ovigerous females was 54% in sample JC82/001/F (Figure 6.2A). Pairwise combinations of samples were not tested for significant variation in the proportions of females that were ovigerous as a result of low numbers. All ovigerous females were hatched in samples

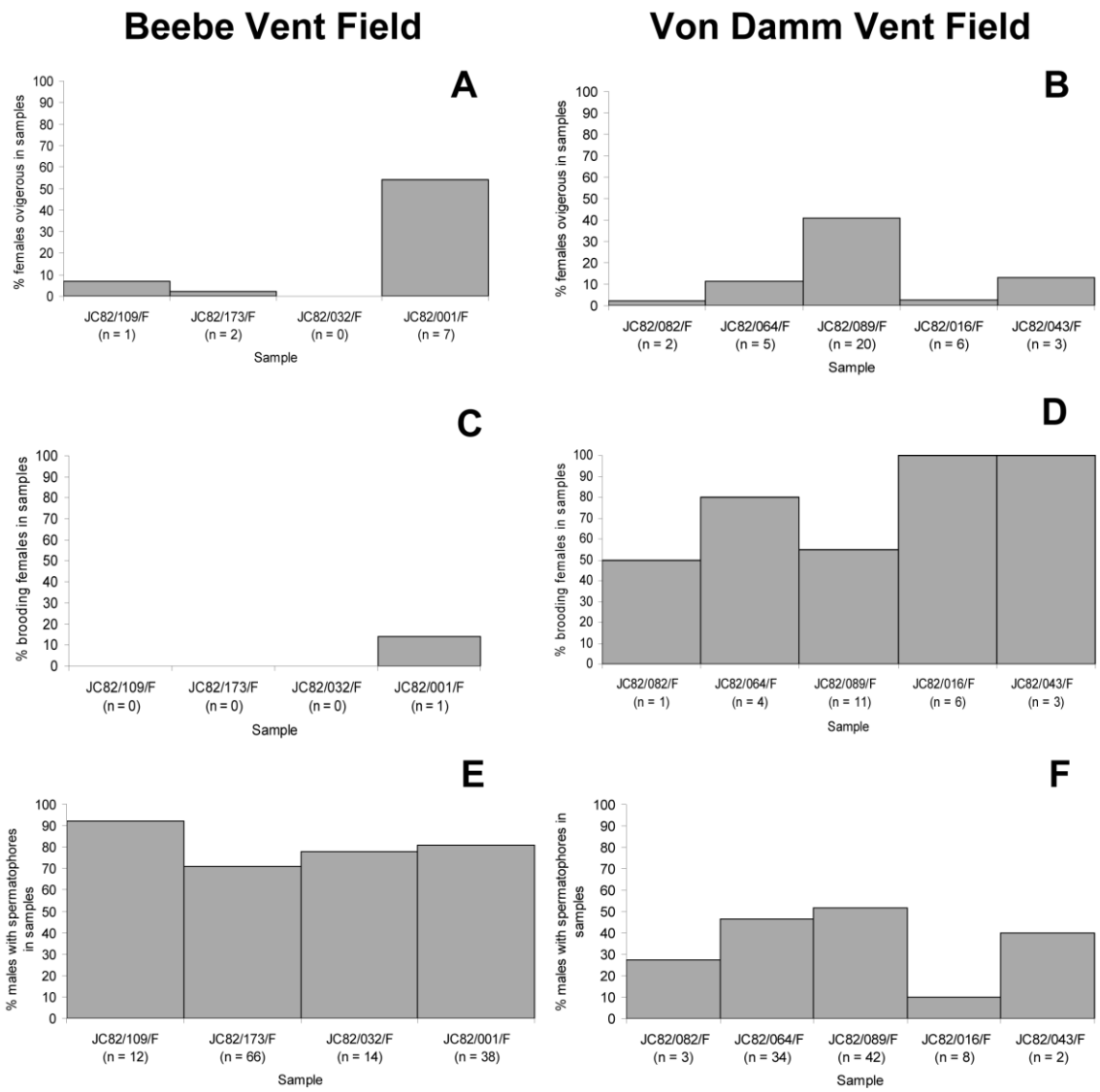

Figure 6.2. *Rimicaris hybisae*. Spatial variation in population structure at the Beebe and Von Damm vent fields, February 2013. (A, B) Proportion of females ovigerous (defined as brooding embryos or hatched) in samples of females; (C, D) proportion of ovigerous females brooding in samples of ovigerous females; (E, F) proportion of males with spermatophores in samples of males. n: sample size (100%) in each case.

JC82/109/F, JC82/173/F and JC82/032/F, whereas 14% of ovigerous females were brooding in sample JC82/001/F (Figure 6.2C). The highest proportion of males with spermatophores was 92% in sample JC82/109/F and the lowest was 71% in sample JC82/173/F (Figure 6.2E). All samples from the Beebe Vent Field were significantly different from each other in the proportion of males with spermatophores, with the exception of samples JC82/109/F and JC82/032/F (Table 6.4).

There was also evidence of significant variation in the population structure between samples within the Von Damm Vent Field. Here, sample JC82/089/F contained the greatest proportion of ovigerous females (41%; Figure 6.2B). Sample JC82/089/F was significantly different from all Von Damm samples (Table 6.5). Other pairwise combinations of samples were not tested for significant variation in the proportions of females that were ovigerous as a result of low numbers. All ovigerous females were brooding in samples JC82/016/F and JC82/043/F. Brooding females accounted for at least 50% of ovigerous females in all other samples from this vent field (Figure 6.2D). The highest proportion of males with spermatophores was 52% in sample JC82/089/F; the lowest was 10% in sample JC82/016/F (Figure 6.2F). All samples from the Von Damm Vent Field were significantly different from each other in the proportion of males with spermatophores, with the exceptions of JC82/082/F with JC82/016/F and JC82/043/F, and JC82/043/F with JC82/016/F (Table 6.5).

6.3.2.3 Size-frequency distribution between vent fields. The key difference between the size-frequency distributions at the Beebe and Von Damm vent fields in February 2013 was that both ovigerous females and males with spermatophores were significantly larger at the Von Damm Vent Field. Overall, shrimp sampled from the Von Damm Vent Field were significantly larger than those from the Beebe Vent Field (Table 6.2; Figure 6.1A; Mann-Whitney *U*-test, $T = 152309.5$, $p < 0.05$). As a whole, the February 2013 samples displayed two modal peaks and a tail of small and large sizes. The peak of larger sizes was, however, absent among samples from the Beebe Vent Field, where intermediate-size shrimp were sampled with proportionally greater frequency (Figure 6.1A). A small peak of larger sizes was revealed among the Von Damm samples, where intermediate-sized shrimp were sampled with proportionally lower frequency (Figure 6.1A).

Table 6.4. *Rimicaris hybisae*. Spatial variation in population structure within the Beebe Vent Field, February 2013.

Sample	JC82/109/F	JC82/173/F	JC82/032/F	JC82/001/F
JC82/109/F	-	-	-	-
JC82/173/F	4.30 *	-	-	-
JC82/032/F	4.75 *	19.19 ***	-	-
JC82/001/F	11.02 ***	19.19 ***	5.07 *	-

Results of χ^2 (1 df) analyses on proportions of males with spermatophores (italic text) in samples.
NS = not significant; * = *P* value < 0.05; ** = *P* value < 0.01: *** = *P* value < 0.001

Table 6.5. *Rimicaris hybisae*. Spatial variation in population structure within the Von Damm Vent Field, February 2013.

Sample	JC82/082/F	JC82/064/F	JC82/089/F	JC82/016/F	JC82/043/F
JC82/082/F	-	**NA**	**13.14 *** **	**NA**	**NA**
JC82/064/F	24.32 ***	-	**7.84 ** **	**NA**	**NA**
JC82/089/F	32.09 ***	0.65 NS	-	6.50 *	11.13 ***
JC82/016/F	1.46 NS	14.88 ***	21.78 ***	-	
JC82/043/F	0.00 NS	26.69 ***	24.57 ***	2.5 NS	-

Results of χ^2 (1 df) analyses on proportions of females ovigerous (brooding or hatched; bold text) and proportions of males with spermatophores (plain text) in samples.
NA + not applicable; NS = not significant; * = *P* value < 0.05; ** = *P* value < 0.01: *** = *P* value < 0.001.

Males were significantly larger at the Von Damm Vent Field than the Beebe Vent Field but there was no significant difference in the median size of females between the two vent fields (Table 6.2; Figure 6.1A, C; Mann-Whitney *U*-test, males: $T = 29402.0$, $p < 0.001$; females: $T = 44210.0$, $p > 0.05$). Ovigerous females were significantly larger at Von Damm compared with Beebe but there was no significant difference in the size of non-ovigerous females between vent fields (Table 6.2; Figure 6.1 D-E; Mann-Whitney *U*-test, ovigerous: $T = 114.0$, $p < 0.01$; non-ovigerous: $T = 39733.5$, $p > 0.05$). Males with spermatophores were significantly larger at Von Damm than at Beebe, although there was no significant difference in the size of males without spermatophores (Table 6.2; Figure 6.1 F-G; Mann-Whitney *U*-test, with spermatophores: $T = 14614.5$, $p < 0.001$; without spermatophores: $T = 3786.5$, $p > 0.05$).

At the Von Damm Vent Field, males were significantly larger than females and non-ovigerous females (Table 6.2; Figure 6.1C; Mann-Whitney *U*-test, females: *T* =

95522.5, $p < 0.001$; non-ovigerous females: $T = 92914.0$, $p < 0.001$), and the ratio of males to non-ovigerous females deviated significantly from 1:1 (251 males: 401 non-ovigerous females; $\chi^2 = 34.05$, 1 df, $p < 0.001$). Males with spermatophores were significantly larger than males without spermatophores at Von Damm (Table 6.2; Figure 6.1G; Mann-Whitney *U*-test, $T = 15435.5$, $p < 0.001$).

At the Beebe Vent Field, the ratio of males to non-ovigerous females did not deviate significantly from 1:1 (171 males: 137 non-ovigerous females; $\chi^2 = 3.54$, 1 df, $p > 0.05$) and there were no significant differences between the size of males and females (Table 6.2; Figure 6.1B; Mann-Whitney *U*-test, males vs females: $T = 22463.0$, $p > 0.05$; males vs non-ovigerous females: $T = 20159.0$, $p > 0.05$), or the size of males with/without spermatophores (Table 6.2; Figure 6.1D, F; Mann-Whitney *U*-test, $T = 2985.0$, $p > 0.05$).

6.3.2.4 Size-frequency distribution within vent fields. Significant differences were revealed in the size-frequency distributions of specimens from different locations within both vent fields. However, there was less variation between spatial samples from the Beebe Vent Field than exhibited between spatial samples from the Von Damm Vent Field (Figure 6.3).

There was significant variation in the size-frequency distributions of specimens collected from different locations within the Beebe Vent Field (Figure 6.3 A-D; Kruskal-Wallace multisample test, $H = 20.362$, 3 df, $p < 0.001$). Significant variation was evident in the median sizes of males between samples from Beebe but the median sizes of females were not significantly different between samples (Kruskal-Wallace multisample test, males: $H = 26.138$, 3 df, $p < 0.001$; females $H = 2.763$, 3 df, $p > 0.05$). There was no significant variation in the size-frequency distributions of specimens (overall and for each sex) between samples JC82/109/F and JC82/173/F (Dunn's Multiple Comparison Test, $p > 0.05$).

At the Beebe Vent Field, specimens in sample JC82/032/F displayed the smallest median size for each sex (males: CL 10.0 mm, IQR 8.6-10.2; females: CL 11.0 mm, IQR 10.2-11.9). In sample JC82/173/F, males exhibited a significantly greater median size than females (Figure 6.3B; males: CL 11.6 mm, IQR 11.1-12.0; females: CL 11.3 mm, IQR 10.8-11.7; Mann-Whitney *U*-test, $T = 301.0$, $p < 0.05$), whereas females revealed a significantly greater median size than males in sample JC82/032/F (Figure 6.3C; males: CL 10.0 mm, IQR 8.6-10.2; females: CL 11.0 mm, IQR 10.2-11.9; Mann-

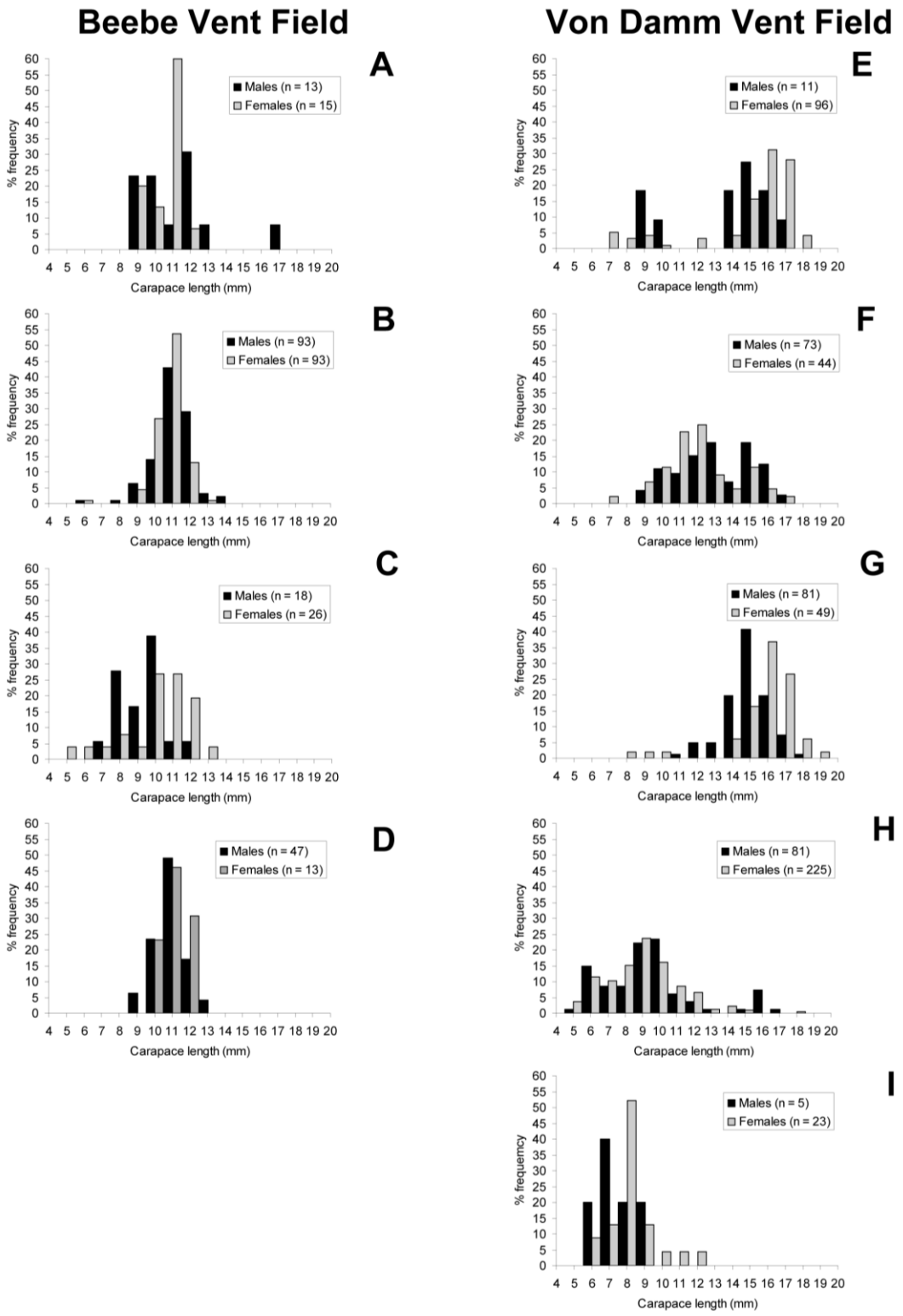

Figure 6.3. *Rimicaris hybisae*. Size-frequency distribution within the Beebe and Von Damm vent fields, February 2013. (A) Sample JC82/109/F; (B) sample JC82/173/F; (C) sample JC82/032/F; (D) sample JC82/001/F; (E) sample JC82/082/F; (F) sample JC82/064/F; (G) sample JC82/089/F; (H) sample JC82/016/F; (I) sample JC82/043/F. n: no. of individuals measured.

Whitney *U*-test, $T = 9566.5$, $p < 0.05$). The distribution of sizes between males and females, however, were not significantly different within any of the samples from the Beebe Vent Field (Kolmogorov-Smirnov two-sample test, JC82/109/F $D = 0.07$, $p > 0.05$; JC82/173/F $D = 0.09$, $p > 0.05$; JC82/032/F $D = 0.02$, $p > 0.05$; JC82/001/F $D = 0.09$, $p > 0.05$).

There was also significant variation in the size-frequency distributions of specimens collected from different locations within the Von Damm Vent Field (Figure 6.3 E-I; Kruskal-Wallace multisample test, $H = 392.449$, 4 df, $p < 0.001$). Here there was significant variation in the size-frequency distributions of each sex between samples (Kruskal-Wallace multisample test, males: $H = 121.297$, 4 df, $p < 0.001$; females $H = 235.652$, 4 df, $p < 0.001$). There was not, however, significant variation in the size-frequency distributions of specimens (overall and for each sex) between samples JC82/082/F and JC82/089/F (Dunn's Multiple Comparison Test, $p > 0.05$).

Specimens in sample JC82/043/F displayed the smallest median size for each sex (males: CL 7.7 mm, IQR 7.0-8.7; females: CL 8.5 mm, IQR 8.1-9.0), followed closely by sample JC82/016/F (males: CL 9.6 mm, IQR 8.0-10.7; females: CL 9.3 mm, IQR 7.9-10.5). In sample JC82/064/F, males exhibited a significantly greater median size than females (Figure 6.3F; males: CL 13.5 mm, IQR 12.0-15.4; females: CL 12.1 mm, IQR 11.1-13.9; Mann-Whitney *U*-test, $T = 21390.0$, $p < 0.05$), whereas females revealed a significantly greater median size than males in samples JC82/082/F and JC82/089/F (Figure 6.3E, G; Mann-Whitney *U*-test, JC82/082/F males: CL 15.2 mm, IQR 11.5-16.1; females: CL 16.3 mm, IQR 15.2-17.2; $T = 389.5$, $p < 0.05$; JC82/089/F males: CL 15.5 mm, IQR 14.8-16.1; females: CL 16.6 mm, IQR 15.7-17.2, $T = 4171.0$, $p < 0.001$). The distribution of sizes between males and females, however, were not significantly different in any of the samples from the Von Damm Vent Field (Kolmogorov-Smirnov two-sample test, JC82/082/F $D = 0.09$, $p > 0.05$; JC82/064/F $D = 0.04$, $p > 0.05$; JC82/089/F $D = 0.05$, $p > 0.05$; JC82/016/F $D = 0.05$, $p > 0.05$; JC82/043/F $D = 0.20$, $p > 0.05$).

6.3.2.5 Oocyte size-frequency distributions. Females analysed from February 2013 contained oocytes with feret diameters ranging from 14 μm (non-ovigerous female, CL 12.3 mm, sample JC82/016/F, Von Damm) to 939 μm (non-ovigerous female, CL 10.4 mm, sample JC82/089/F, Von Damm). Three distinct oocyte sizes were

apparent among females collected from both the Beebe and Von Damm Vent fields in February 2013 (Figures 6.4-5).

Females from the Beebe Vent Field exhibited a significantly greater median oocyte size than females from the Von Damm Vent Field (Figure 6.4; Mann-Whitney *U*-test, $T = 94286618.0$, $p < 0.001$). The median oocyte size in 78 individuals from the Beebe Vent Field was 92 μm (IQR 69-126), whereas the median oocyte size in 135 individuals from the Von Damm Vent Field was 81 μm (IQR 50-110). The oocyte size-frequency distributions were not significantly different, however, between the vent fields (Figures 6.4-5; Kolmogorov-Smirnov two-sample test, $D = 0.006$, $p > 0.05$).

Overall, there was significant variation in oocyte sizes between all samples (Kruskal-Wallis multi-sample test, $H = 5976.1$, 8 df, $p < 0.01$) and no evidence of synchrony between samples. There were significant differences in oocyte sizes between every pairwise comparison of all samples with the exceptions of JC82/043/F and JC82/016/F, JC82/109/F and JC82/032/F, and JC82/082F with JC82/032/F and JC82/089/F (Dunn's Multiple Comparison Test, $p < 0.05$).

6.3.2.6 Embryo developmental stages. Embryos within each brood had developed synchronously and were at the same developmental stage, but the broods had not developed synchronously within the sampled population. The majority of embryos brooded by females at the Von Damm Vent Field were at the early (48%) and mid stage (44%) and only 8% were advanced. The single brooding female from the Beebe Vent Field was carrying early-stage embryos. The only brooding female in sample JC82/082/F from the Von Damm Vent Field also exhibited early-stage embryos.

Among the other samples at the Von Damm Vent Field there was variation. Half of the brood in sample JC82/064/F were early-stage embryos and half were mid-stage. In contrast, 55% of broods were mid-stage in sample JC82/089/F; 36% were early-stage and 9% late-stage. The majority (67%) of broods were early-stage in sample JC82/016/F, with equal proportions (16.5%) of mid- and late-stage broods. Two-thirds (67%) of broods were mid-stage in sample JC82/043/F; the rest were early-stage.

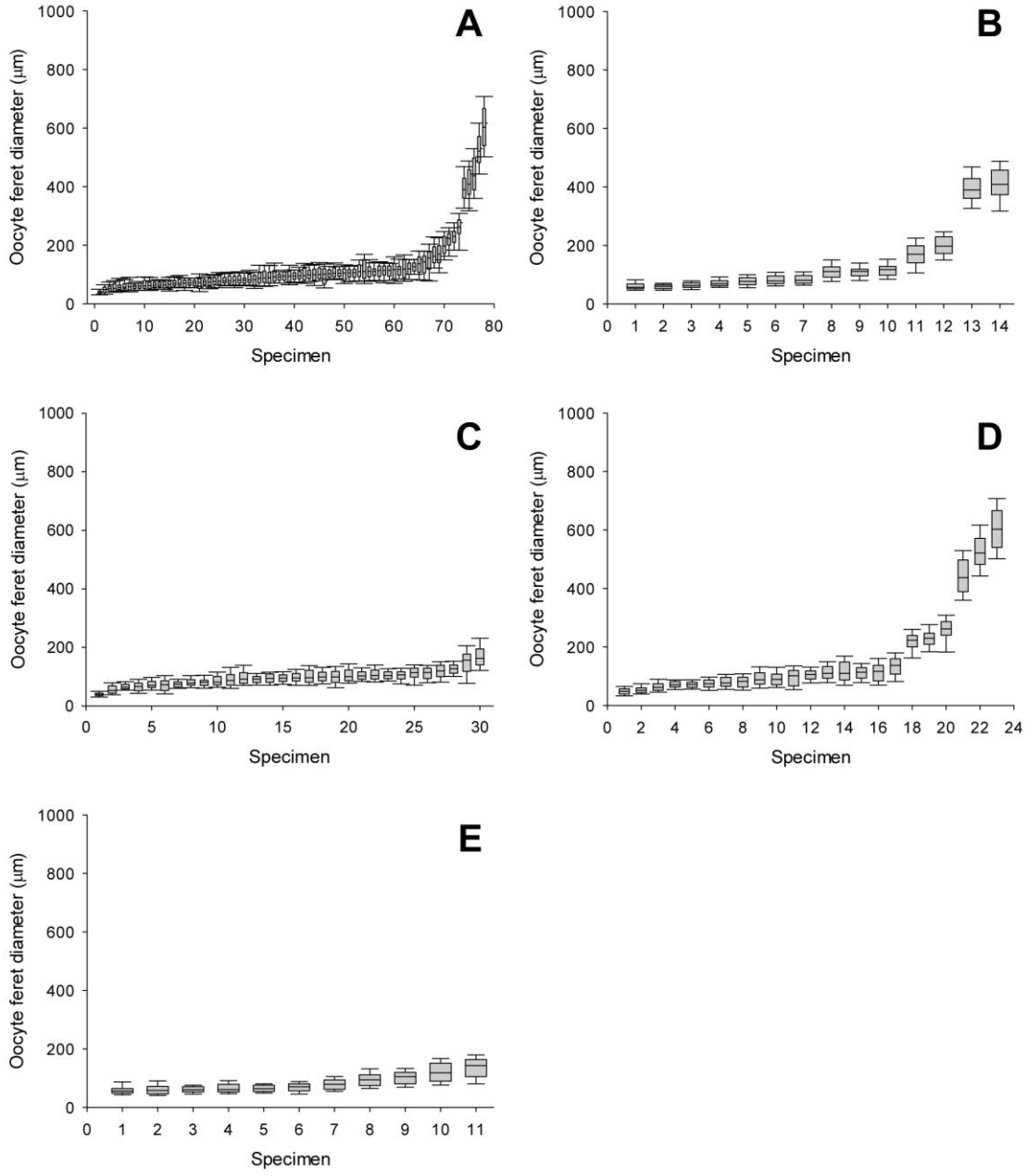

Figure 6.4. *Rimicaris hybisae*. Spatial variation in oocyte size-frequency distribution at the Beebe Vent Field, February 2013. (A) Beebe Vent Field (all samples); (B) sample JC82/109/F; (C) sample JC82/173/F; (D) sample JC82/032/F; (E) sample JC82/001/F.

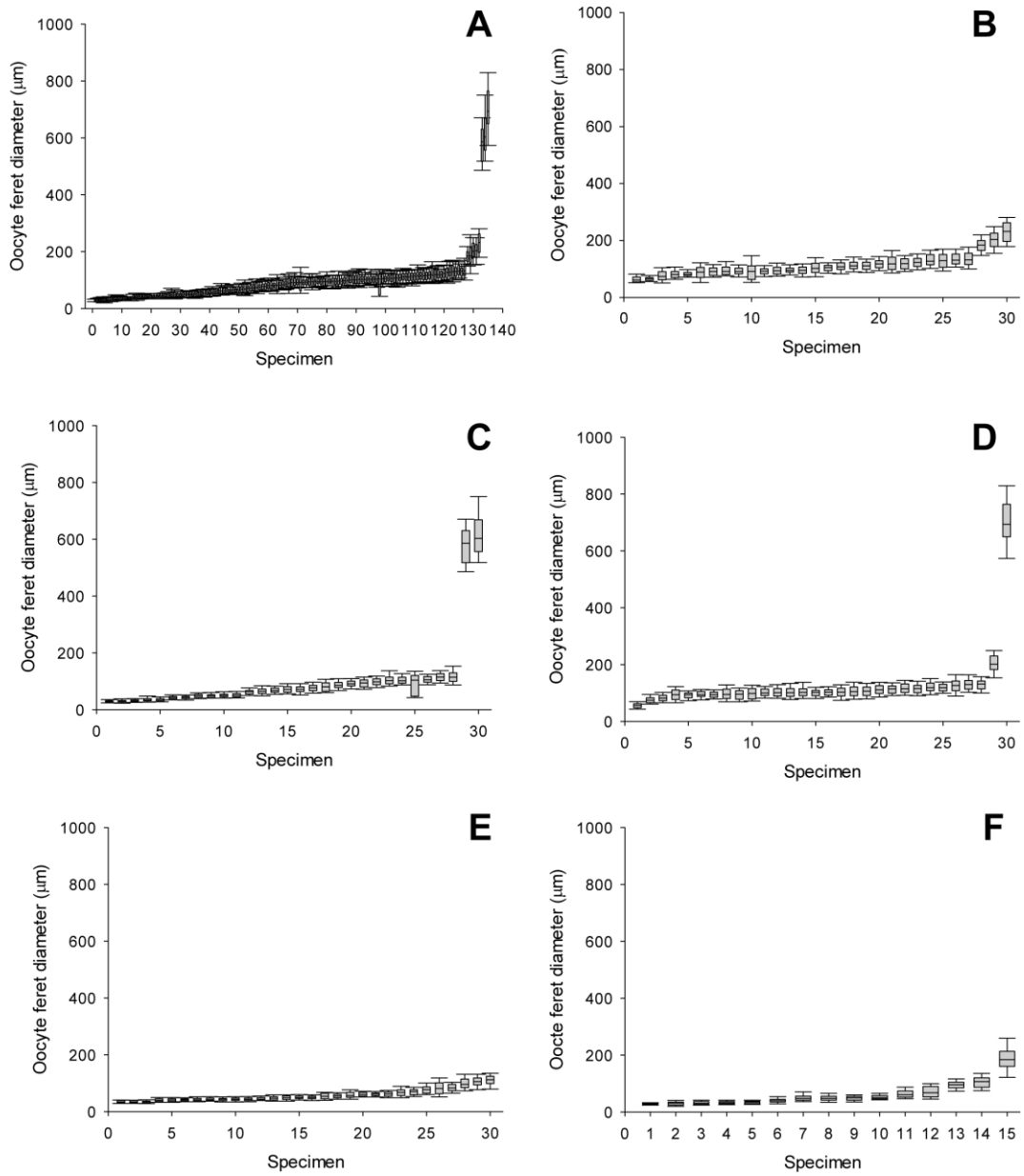

Figure 6.5. *Rimicaris hybisae*. Spatial variation in oocyte size-frequency distribution at the Von Damm Vent Field, February 2013. (A) Von Damm Vent Field (all samples); (B) sample JC82/082/F; (C) sample JC82/064/F; (D) sample JC82/089/F; (E) sample JC82/016/F; (F) sample JC82/043/F.

6.3.3 Inter-annual variation

6.3.3.1 Population structure. The key difference between the population structures in February 2013 compared with January 2012 was that overall a significantly lower proportion of females were ovigerous (7.9% versus 49%; $\chi^2 = 28.26$, 1 df, $p < 0.001$). Brooding females represented a significantly greater proportion of ovigerous females at the Beebe Vent Field (54%) than the Von Damm Vent Field in 2012 (Nye *et al.*, 2013a), whereas a significantly greater proportion of ovigerous females were brooding at the Von Damm Vent Field compared with the Beebe Vent Field in 2013 (Figure 6.2 C-D). A higher proportion of males had spermatophores in 2013 (51.9%) than in 2012 (39.0%) but this difference was not significant ($\chi^2 = 1.56$, 1 df, $p > 0.05$).

Overall, the sex ratios were similar in each year (0.72:1 in 2013; 0.69:1 in 2012). The Von Damm population was significantly female biased in both years (0.57:1 in 2013; 0.55:1 in 2012). Samples were collected from one location at Von Damm in 2012. Spatially discrete sampling in 2013 revealed two samples that were significantly male biased (Table 6.1). At Von Damm, the ratio of males to non-ovigerous females was significantly biased towards non-ovigerous females in 2013 (see above) but did not deviate significantly from 1:1 in 2012 (Nye *et al.*, 2013a). The Beebe population was significantly female biased in 2012 (0.81:1), whereas the sex ratio did not deviate significantly from 1:1 in 2013 (1.16:1; Table 6.1).

6.3.3.2 Size-frequency distribution between vent fields. Overall, specimens were significantly larger at the Von Damm Vent Field compared with the Beebe Vent Field in 2012 (Nye *et al.*, 2013a) and 2013 (see above; Figure 6.6 A-D). Females were significantly larger at Von Damm than at Beebe in 2012 (Nye *et al.*, 2013a) but there was no significant difference in the size of females between vent fields in 2013 (see above; Figure 6.6 A-D). Males with and without spermatophores were significantly larger at Von Damm than Beebe in both years (see above; Nye *et al.*, 2013a). However, there was no significant difference in the size of males without spermatophores between vent fields in 2013 (see above), whereas males without spermatophores were significantly larger at Von Damm than at Beebe in 2012 (Nye *et al.*, 2013a).

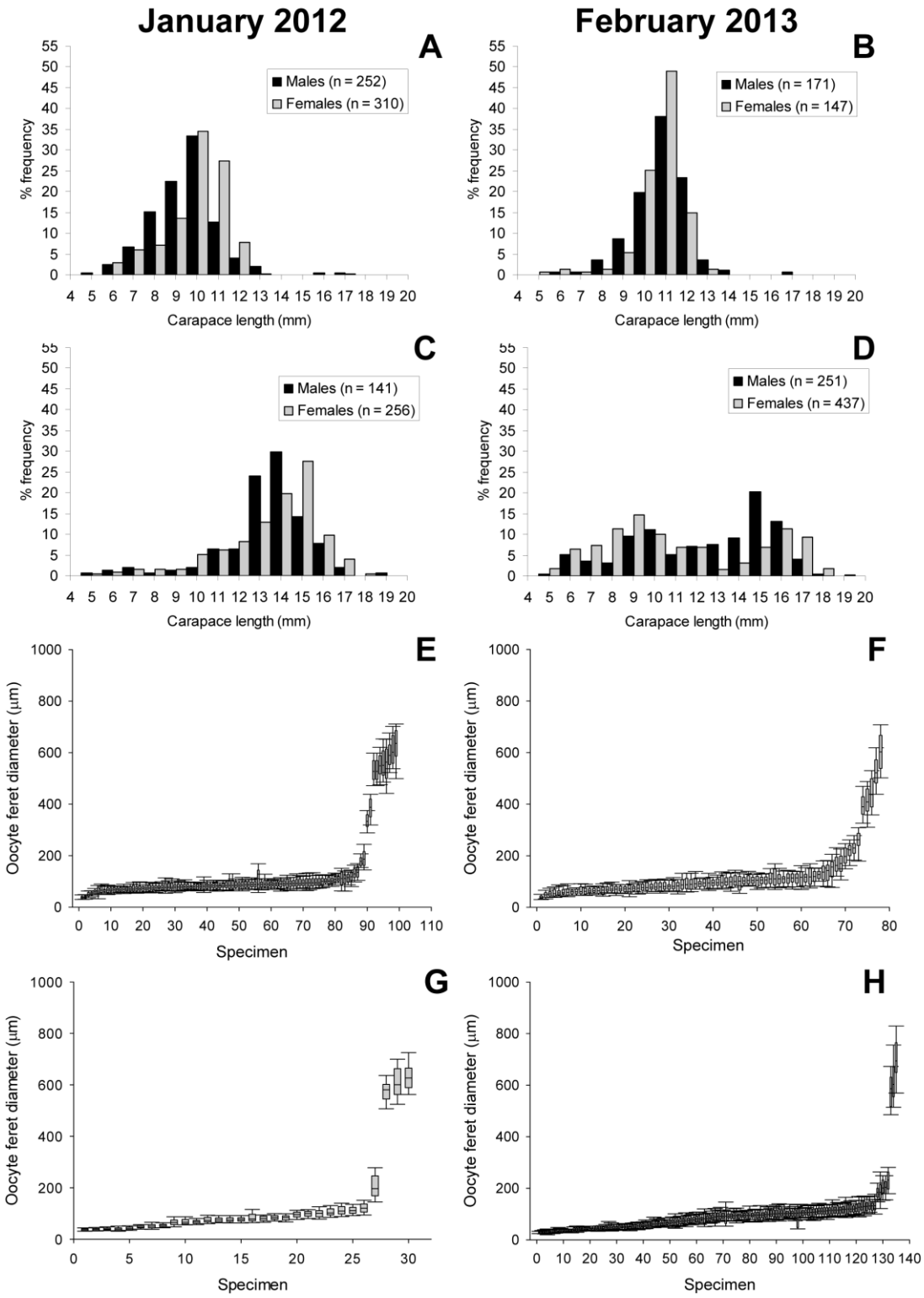

Figure 6.6. *Rimicaris hybisae*. Inter-annual variation in size-frequency (A-D) and oocyte-size frequency (E-F) distributions at the Beebe (A, B, E, F) and Von Damm (C, D, G, H) vent fields, January 2012 and February 2013.

6.3.3.3 Size-frequency distribution within vent fields. The median size of specimens sampled from the Beebe Vent Field was significantly larger in February 2013 compared with those sampled in January 2012; intermediate-size shrimp were sampled with proportionally greater frequency in 2013 (Figure 6.6 A-B; Mann-Whitney *U*-test, Beebe: $T = 182300.5$, $p < 0.001$). Significant variation was revealed in the size-frequency distribution of males and females from different locations within Beebe in 2012 (Nye *et al.*, 2013a), whereas no significant variation was exhibited by females from different Beebe locations in 2013.

The median size of specimens from the Von Damm Vent Field was significantly larger in January 2012 than in February 2013 (Mann-Whitney *U*-test, $T = 251540.0$, $p < 0.001$). Here larger-size shrimp were sampled with proportionally greater frequency in 2012, producing a single modal peak with a tail of smaller sizes, whereas there were modal peaks of intermediate and large sizes in 2013 (Figure 6.6 C-D). At the Von Damm Vent Field males were significantly larger than females and non-ovigerous females in 2013 (see above) but were not significantly different in size here in 2012 (Nye *et al.*, 2013a).

6.3.3.4 Oocyte size-frequency distributions. Females analysed in 2013 exhibited significantly greater median oocyte sizes than those studied in 2012 at both vent fields (Figure 6 E-H; Mann-Whitney *U*-test, Beebe: $T = 70899588.0$, $p < 0.001$; Von Damm: $T = 25941704.5$, $p < 0.001$). However, the oocyte size-frequency distributions were not significantly different between 2012 and 2013 at either vent field (Figure 6 E-H; Kolmogorov-Smirnov two-sample test, Beebe: $D = 0.004$, $p > 0.05$; Von Damm: $D = 0.007$, $p > 0.05$).

Females from the Beebe Vent Field exhibited a significantly greater median oocyte size than females from the Von Damm Vent Field in 2013 (see above) but the median oocyte sizes were not significantly different between vent fields in 2012 (Mann-Whitney *U*-test, $T = 17683194.0$, $p < 0.001$).

6.4 Discussion

6.4.1 Spatial variation in reproductive features between vent fields

In February 2013, the sampled population of *Rimicaris hybisae* at the Beebe Vent Field was dominated by intermediate-sized, non-ovigerous females and a lesser proportion of slightly smaller males. The small proportion of ovigerous females was confined to intermediate sizes. The sex ratio and the ratio of males to non-ovigerous females did not deviate significantly from 1:1. Males with spermatophores accounted for a high proportion of the male population and were present in most size classes. The key differences in the sampled population at the Von Damm Vent Field compared with the Beebe Vent Field were a significantly lower proportion of males with spermatophores and greater proportion of brooding females. The small proportion of ovigerous females was distributed across a wide range of sizes but was evident in greatest frequency in large sizes. In contrast with Beebe, the sex ratio at Von Damm was significantly female biased.

Specimens from Von Damm were significantly larger than those from the Beebe Vent Field as a result of a greater proportion of large-size individuals, consistent with a previous study (Nye *et al.*, 2013a). In particular, ovigerous females and males with spermatophores were significantly larger at Von Damm, also as described previously (Nye *et al.*, 2013a). The consistent observation of this difference in samples from February 2013 therefore supports the hypothesis that *Rimicaris hybisae* may reach maturity at smaller sizes in the Beebe Vent Field than in the Von Damm Vent Field.

At the Von Damm Vent Field in February 2013, males were significantly larger than non-ovigerous females and the ratio of males to non-ovigerous females deviated significantly from 1:1. Males with spermatophores were significantly larger than males without spermatophores as a result of a greater proportion of large sizes. Females from the Beebe Vent Field exhibited a significantly greater median oocyte size than females from the Von Damm Vent Field. However, comparison of overall distributions of oocyte sizes revealed that the oocyte size-frequency distributions were not significantly different between the vent fields.

6.4.2 Spatial variation in reproductive features within vent fields

Individual samples from the Beebe and Von Damm vent fields were heterogeneous. Ovigerous females were sampled with the greatest frequency in samples JC82/001/F (Beebe Chimlets, maximum temperature 4.5°C) and JC82/089/F (Von Damm, main mound, maximum temperature 11.7°C). These samples were collected from small, high-density aggregations dominated by males with spermatophores and significantly male-biased sex ratios.

Specimens from January 2012 revealed the greatest frequencies of ovigerous females in samples collected from large, high-density aggregations of *Rimicaris hybisae* with female-biased sex ratios (Nye *et al.*, 2013a). However, a considerably larger proportion of the sampled female population was ovigerous at both vent fields in January 2012. Using the data from the present study and Nye *et al.* (2013a), it is evident that samples in high-density aggregations and samples with high frequencies of males with spermatophores exhibited variations in the frequencies of ovigerous females. This evidence suggests that the distribution of ovigerous *R. hybisae* females at the Beebe and Von Damm vent fields is not correlated directly with aggregation density or sex ratio within aggregations, and emphasises the need to examine large samples with replication to determine patterns in space and the factors that determine them in vent environments.

Previous studies have revealed a clear bias towards females in the alvinocaridid shrimp *Alvinocaris muricola* Williams, 1988 at the cold-seep site north of Regab (Congo Basin) and the avoidance of sulfidic or anoxic extremes by ovigerous females in *Alvinocaris stactophila* Williams, 1988 (Ramirez-Llodra & Segonzac, 2006; Copley & Young, 2006). Fine-scale heterogeneity in physical-chemical conditions is a feature of hydrothermal-vent environments (Luther *et al.*, 2001). Environmental variables within shrimp aggregations may affect the distribution of ovigerous females, resulting in a spatially heterogeneous pattern of reproductive development in *Rimicaris hybisae* (Nye *et al.*, 2013a), as described in other vent taxa (e.g. Copley *et al.*, 2003; Pradillon & Gaill, 2007).

Temperature data were collected from each sample location in the present study (Table 1). However, samples with the highest frequencies of ovigerous females exhibited variations in temperature recorded at their collection locations. Consequently temperature does not appear to be a useful indicator here. This may be because other environmental parameters, such as concentration of dissolved oxygen and hydrogen

sulfide, may influence the distribution of ovigerous females, resulting in the observed spatially heterogeneous reproductive pattern of reproductive development in *Rimicaris hybisae*, as found in other taxa from chemosynthetic environments (Copley *et al.*, 2003; Perovich *et al.*, 2003; Copley & Young, 2006; Pradillon & Gaill, 2007; Sheader & Van Dover, 2007; Hilário *et al.*, 2009). Further sampling with the collection of additional environmental data would be required to test this hypothesis.

Despite variation in the sex ratio found within both vent fields, overall (2012 and 2013) the data suggest that there appears to be a bias in the sex ratio towards females, at least at the Von Damm Vent Field. This could be as a result of the differential distribution of the sexes, whereby sampling within the MCSC vent fields has not been sufficiently spatially comprehensive to capture the distribution of the sexes. Explanations for skewed sex ratios found in hydrothermal vents have typically focused on the environmental parameters of the vent environment (see above). However, other possible explanations are worth consideration.

Skewed sex ratios can be a result of a multitude of factors (Stouthamer *et al.*, 2002; West, 2009). Some crustaceans are prone to parasitic infection that can cause sex reversal or sterility in the host (e.g. Ironside *et al.*, 2011 and references therein). However, no parasites or evidence of sex reversal were visible in the specimens studied. Another potential factor is differential mortality of males and females (e.g. as described in copepods by Kiørboe, 2006; Hirst *et al.*, 2010). If males are distributed closer to venting fluids than females the harsher conditions could result in a greater level of mortality in males. Alternatively, aggressive competition by males for mates or for space close to venting fluids may also result in a higher degree of male mortality versus female mortality. However, there was no clear pattern found with the distribution of males/females and proximity to venting fluids.

A third possible explanation for sex ratio bias in the vent environment is selection to reduce the proportion of males in the population. The presence of relatively small habitat patches and a high number of individuals, as found at MCSC vent fields, were first propounded by Hamilton (1967) as potentially causing intense competition for mates, leading to variance in reproductive success amongst males and ultimately selection to diminish the proportion of males within a population (Hamilton, 1967). The island-like distribution of vent fields, characterised by discrete habitat patches and high levels of biomass, may be an ecological driver for intraspecific competition. For example, the common occurrence of spermatophores and internal fertilisation in vent

invertebrates (see Appendix 1) may be driven to some extent by intraspecific competition and selection to maximise reproductive output amongst populations of vent species that are distributed in fragmented by dense populations. The size of the populations of *Rimicaris hybisae* at the Beebe and Von Damm vent fields is not known; it would be challenging to produce an accurate estimate of population size given the densities of the shrimps in multiple layers in some locations within the vent fields. However, selection to reduce the proportion of males in the MCSC vent environment could potentially explain the bias in sex ratios described above.

Significant differences were revealed in the size-frequency distributions of specimens among samples at both vent fields. There was, however, greater variation between spatial samples from the Von Damm Vent Field than between samples from the Beebe Vent Field. Large female and male *Rimicaris hybisae* were present with the greatest frequencies in samples JC82/089/F and JC82/082/F from the Von Damm Vent Field. Juvenile and small specimens were sampled with the greatest frequencies in samples JC82/043/F and JC82/016/F. These two samples were collected from small, peripheral point-sources of high-temperature venting (fumeroles) at the Von Damm Vent Field and are consistent with observations of *R. hybisae* 'nurseries' at fumeroles within this vent field (Nye, Copley, Tyler, unpublished data).

Significant spatial variability was evident in the oocyte size-frequency distributions of *Rimicaris hybisae* in February 2013. All stages of oocyte development were evident amongst females and there was no synchrony between samples. Asynchronous gametogenesis has been proposed previously for this species on the basis of similar variation in oocyte size-frequency distributions (Nye *et al.*, 2013a). That putative pattern is supported here by the similarity of oocyte size-frequency distributions in this study (see below). The observed variation, however, was less than that determined among *R. exoculata* from the TAG vent field (Copley *et al.*, 2007), consistent with the suggestion that there is a greater degree of synchrony in the oocyte development of *R. hybisae* at the Beebe and Von Damm Vent Fields than recorded for *R. exoculata* at TAG (Nye *et al.*, 2013a).

Embryo development was asynchronous between females and spatially heterogeneous, consistent with an asynchronous release of larvae for *Rimicaris hybisae* at population level (Nye *et al.*, 2013a). It remains to be determined if spatial variation also occurs in the rate of embryonic development in this species, and for how long embryos are brooded. Lab-based experimental studies would be required to elucidate

the duration of development time and parameters determining rate of development in *R. hybisae*.

6.4.3 Inter-annual variation

The key difference between the population structure in February 2013 versus January 2012 was a significantly lower proportion of ovigerous females overall. This was not a result of variation in the sex ratio between samples because spatial variation in the occurrence of ovigerous females was examined by comparing the ratio of ovigerous (brooding and hatched) females to non-ovigerous females in both years. Similar numbers of each sex were sampled in both years and the overall sex ratios were similar in 2012 and 2013. Consequently it is unlikely that this is the result of under-sampling either sex.

It is possible that these results are not representative of the population because of spatial variability in the distributions of ovigerous females. However, samples in 2012 and 2013 were collected from a range of locations, spanning high and low density aggregations, in close proximity and distal to visible sources of venting. Unless the observed inter-annual variation is random, it is reasonable to consider that *Rimicaris hybisae* was at an earlier stage of reproductive development at the population level in February 2013, with a lower proportion of females brooding developing embryos, compared with January 2012.

The oocyte size-frequency distributions were not significantly different in pattern and range between January 2012 and February 2013 at either vent field and there was no synchrony between samples in either year. These findings are consistent with a non-seasonal pattern of reproductive development (see below). However, the proportion of females that were ovigerous in samples was evidently variable between years, despite sampling many spatial points, and could be interpreted as evidence of a seasonal reproductive pattern in *Rimicaris hybisae* at the Beebe and Von Damm vent fields.

Seasonal reproduction has been described in *Alvinocaris stactophila*, from the Brine Pool cold seep (650 m depth) in the Gulf of Mexico (Copley & Young, 2006). In contrast to *Rimicaris hybisae*, *A. stactophila* exhibit similar ooctye size-frequency distributions within samples from the same month but differences between months (even during the same season). This pattern of variation within and between samples provides a benchmark for determining seasonal reproduction in alvinocaridid shrimp

and *R. hybisae* does not appear to conform to the expected pattern for a seasonal alvinocaridid species, which shows similar oocyte size-frequency distributions among individuals within samples.

The presence of the same pattern and range of oocyte size-frequency distributions in November 2004 as September 1994 indicated that reproduction is not seasonal in *Rimicaris exoculata* at TAG (Copley *et al.*, 2007). Although there is greater synchrony in *R. hybisae*, this pattern is similar to that found in January 2012 and February 2013 in *R. hybisae* at the Beebe and Von Damm vent fields.

It has been suggested that crustacean species in deep-sea chemosynthetic environments must obtain the sterols required for reproduction from phytoplankton-derived sources (Pond *et al.*, 2000). Studies of alvinocaridid shrimp from chemosynthetic habitats have suggested that individuals may store a lifetime supply of these compounds during their planktonic larval stage (Pond *et al.*, 2000; Allen *et al.*, 2001). Copley & Young (2006) hypothesised that *Alvinocaris stactophila* may use a seasonal peak in the supply of phytodetrital flux to the Brine Pool (~650 m depth) as a cue to synchronise gametogenesis and thereby time the release of planktotrophic larvae to coincide with peak food availability in the water column. An alternative hypothesis is that photoperiod length at the dysphotic-zone depth of the Brine Pool may provide a zeitgeber for reproductive seasonality. The TAG (3600 m depth), Beebe (4960 m depth) and Von Damm (2300 m depth) vent fields are situated much deeper than the Brine Pool, beyond the boundary of the dysphotic zone. There is little seasonal variation in surface productivity at TAG; this trait may therefore preclude the possibility of a cue for seasonal reproduction at this vent field (Copley *et al.*, 2007). The collection and analysis of samples from a different season would be required to resolve if *Rimicaris hybisae* exhibits a seasonal pattern of reproductive development. Furthermore, seasonal variation in surface productivity above the Mid-Cayman Spreading Centre would need to be investigated to assess the possibility of a seasonal phytodetrital flux as a possible zeitgeber to synchronise gametogenic cycles, and food source for dispersing shrimp larvae.

An alternative explanation of the results is that there is a spatial pattern in the distribution of ovigerous females that has not been captured in samples of *Rimicaris hybisae* to date despite sampling numerous spatial points. Copley & Young (2006) identified a specific distribution of males and females of *Alvinocaris stactophila* at the Brine Pool where ovigerous females may avoid the sulfidic or anoxic extremes of the

cold-seep environment. Avoidance of the environmental extremes of the hydrothermal vent environments has also been proposed for several other crustacean species.

Perovich *et al.* (2003) examined the reproductive development of the vent crab *Bythograea thermydron* Williams, 1980 at 9°N on the East Pacific Rise (EPR) and found a significant difference in the mean sizes of oocytes from the vent and peripheral zones. Samples from the vent periphery were also dominated by brooding females and those whose eggs had recently hatched, consistent with females with mature gonads migrating to the vent periphery to brood and hatch their eggs. Hilário *et al.* (2009) reported no ovigerous females in samples of *B. laubieri* Guinot & Segonzac, 1997 and *B. vrijenhoeki* Guinot & Hurtado, 2003 females collected from a Pacific-Antarctic vent, which supports the segregation behaviour of ovigerous females away from the direct influence of hydrothermal activity (Hilário *et al.*, 2009). A spatial pattern was also reported for the amphipod *Ventiella sulfuris* (Barnard & Ingram, 1990) at EPR vents, where no mature individuals were found in central vent habitats (Sheader & Van Dover, 2007). These authors concluded that adults move to the periphery of vents to reproduce and brood before returning to feed at vent habitats and undergo a new phase of gonad maturation and emigration (Sheader & Van Dover, 2007). In the spatially heterogeneous environment at vents and seeps, peripheral areas may minimise exposure of brooding females and embryos to predation pressure, potentially toxic fluids and, in the case of vents, fluctuations in temperature (Perovich *et al.*, 2003; Sheader & Van Dover, 2007).

It has been proposed that ovigerous females of *Rimicaris exoculata* stay outside the main populations at MAR vents (Ramirez-Llodra *et al.*, 2000). However, ovigerous females of this species were observed within the main aggregations around high temperature zones at Logatchev in March 2007 (Gebruk *et al.*, 2010). Observations of ovigerous females of *Rimicaris hybisae* within the main aggregations around high temperature zones at the Beebe and Von Damm vent fields in January 2012 (Nye *et al.*, 2013a) are consistent with those observations. The results presented in the current study underpin the difficulties of determining the patterns and causes of spatial variation in the dynamic and heterogeneous vent environment and the complexity of teasing apart patterns in space and time.

There was no significant difference in the size of females between vent fields in 2013. However, males were significantly larger at Von Damm than at Beebe in 2012 (Nye *et al.*, 2013a). This was the result of spatial variation in the proportion of males and females in samples, rather than sexual dimorphism in *Rimicaris hybisae* (Nye *et al.*,

2012). The sex ratio at the Beebe Vent Field did not deviate significantly from 1:1 in 2013, whereas it was significantly female biased in 2012. The observed inter-annual variation in the proportion of males and females in samples may be the result of spatial variation in the proportion of males and females in samples, rather than a change in the sex ratio of the Beebe population. Furthermore, the lack of a significant difference in the size of females between vent fields in 2013 (versus the significantly larger size of females at Von Damm compared to Beebe in 2012) may be a result of the greater proportion of males present in the sampled population at Beebe in 2013.

Shrimp from the Beebe Vent Field were significantly larger in February 2013 compared with those sampled in January 2012. There was significant variation in the size-frequency distribution of males and females from different locations within Beebe in 2012 (Nye *et al.*, 2013a), whereas no significant variation was exhibited by females from different Beebe locations in 2013. Fewer females were present in samples collected from the Beebe Vent Field in February 2013, as a result of spatial variation in the proportion of males and females in samples (see above). Consequently the observed inter-annual variation in the size-frequency distribution within the Beebe Vent Field may be the result of spatial variation in the proportion of males and females in samples, rather than a change in the size-frequency distribution of the population over a year.

Shrimp from the Von Damm Vent Field were significantly larger in January 2012 than in February 2013 and males were significantly larger than females and non-ovigerous females in 2013 (see above) but were not significantly different in size here in 2012 (Nye *et al.*, 2013a). Shrimp were collected from one spatial point only in January 2012 (Nye *et al.*, 2013a) whereas samples were collected from five spatial points in February 2013 and may therefore have captured more variation within the Von Damm population.

6.5 Conclusions

Samples collected in February 2013 documented spatial variation in the population structure and reproductive features of *Rimicaris hybisae* at the Von Damm Vent Field for the first time, and illustrated further spatial variability at the Beebe Vent Field. Time-series investigations of vent species are rare, and are novel at ultraslow-

spreading ridges. This study extended the reproductive time-series of *R. hybisae* and revealed inter-annual variation in the overall population structure and reproductive development of this species, superimposed upon the pattern of spatial variation.

The key conclusions from this study are that: (1) there is no correlation between the distribution of ovigerous females at the Beebe and Von Damm vent fields with density of aggregation or sex ratio; (2) reproductive development in *Rimicaris hybisae* may be periodic or seasonal at the vent-field scale; (3) these findings reveal previously unappreciated spatial variation in the reproductive development of a motile species at hydrothermal vents.

Spatial variation in reproductive development within a vent field may be a fundamental feature of vent fauna, and potentially more complex than realised previously, highlighting the challenges of elucidating the ecological dynamics in these heterogeneous, insular, and ephemeral environments.

6.6 Acknowledgements

The authors thank the Master and ship's company of the *RRS James Cook*, the *Isis* ROV technicians, and fellow scientists on the 82nd voyage of the *RRS James Cook*; J. Copley chief scientist. V. Nye was supported by a UK NERC award (NE/F017774/1) to J. Copley.

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7. Summary and conclusions

"At a time when most think of outer space as the final frontier, we must remember that a great deal of unfinished business remains here on Earth. As robots crawl on the surface of Mars, as spacecraft exit our solar system, and as the orbiting Hubble Space Telescope pushes back the edge of the visible universe, we must remember that most of our own planet has still never been seen by human eyes." – BOB BALLARD

The preceding chapters presented investigations of the composition and life-history biology of the recently discovered faunal assemblages at the Beebe and Von Damm vent fields, Mid-Cayman Spreading Centre (MCSC), focusing on macrofaunal invertebrates. This chapter provides a general synthesis of the previous chapters, highlights the conclusions drawn, suggests directions for future work, and examines the biogeographic relationships among deep-sea vent faunas at global scale.

7.1 New species from vent fields on the Mid-Cayman Spreading Centre

7.1.1 Summary and conclusions

The deep ocean is the largest, yet least explored, ecosystem on Earth (Ramirez-Llodra *et al.*, 2010). The magnitude of marine biodiversity and proportion of marine species awaiting description have been debated in the literature (e.g., Lambshead, 1993; Lambshead & Boucher, 2003; see Bouchet, 2006 for recent review). A recent estimate is that 91% of marine species await description (Mora *et al.*, 2011). Discoveries in the last four decades of deep-sea chemosynthetic habitats have significantly enhanced understanding of the biodiversity and functioning of deep-sea ecosystems, and more than 400 new faunal species have been described from deep-sea hydrothermal vents since the 1970s (Desbruyères *et al.*, 2006).

Prior to the discovery, visualisation and sampling of two hydrothermal vent fields on the Mid-Cayman Spreading Centre (MCSC) (Connelly *et al.*, 2012), the composition of the faunal assemblages at MCSC vents remained unknown. This thesis presented three species new to science, supporting the hypothesis that the MCSC vent

fauna is distinct at species level (Chapters 2-4; Nye *et al.*, 2012; 2013 a, b). It characterised the morphology and distribution of the new species, and evaluated the phylogenetic and biogeographic relationships of the new species with those of other taxa (Chapters 2-4).

The new species of alvinocaridid shrimp *Rimicaris hybisae* is visually dominant at the Beebe and Von Damm vent fields, and is the only species known to be present and abundant at both these vent fields. The visually dominant species at MCSC and MAR vent systems are both members of the genus *Rimicaris* (Williams & Rona, 1986; Connelly *et al.*, 2012; Nye *et al.*, 2012; Gebruk & Mironov, 2006). Although the MCSC vent fauna may be distinct at the species level, the presence of shrimp-dominated faunal assemblages at the Beebe and Von Damm vent fields supports higher-level taxonomic affinities with the fauna of Mid-Atlantic vent systems (e.g. Desbruyères *et al.*, 2001; Gebruk & Mironov, 2006; see section 7.3 below). The apparent absence of the hippolytid shrimp *Lebbeus virentova* and gastropod *Iheyaspira bathycodon* from the Beebe Vent Field, which occurs at a depth ~20% deeper than any other known vent site (Fabri *et al.*, 2011), suggests that depth and/or environmental conditions may preclude the presence of these species at the Beebe Vent Field.

7.1.2 Wider implications

With the inclusion of additional data on the taxonomic composition of the Beebe and Von Damm faunal assemblages, these data provide a foundation for ecological and biogeographic investigations of the MCSC vent fauna, and contribute to the continued elucidation of the distribution, diversification and evolution among invertebrate species from deep-sea chemosynthetic environments. Taxonomic studies reveal biodiversity for potential utilisation by human societies (e.g., pharmaceuticals, biotechnology, clues to public health: Chivian & Bernstein, 2008). Furthermore, knowledge of biodiversity is crucial to understand, conserve and/or sustainably manage the global environment; this is particularly pertinent given the potential emergence of a vent-mining industry in the foreseeable future (e.g. Hoagland *et al.*, 2010; Van Dover, 2011). This work contributes to meeting several "challenges" in NERC's biodiversity and Earth system science themes and addresses NERC's science goal of "exploring ecosystems to discover novel biodiversity and increasing knowledge of the distribution of biodiversity".

7.1.3 Future directions

Further investigation of the MCSC and its vent fauna has the potential to characterise fully the faunal assemblages at the Beebe and Von Damm vent fields, and to determine whether any additional taxa are shared between the two known MCSC vent fields and/or with other biogeographic provinces. Future exploration of the MCSC and the Caribbean region may yet reveal additional areas of hydrothermal activity and chemosynthesis-associated fauna, potentially expanding the known distributions of species currently considered to be endemic to the Beebe and/or Von Damm vent fields.

Although video surveys and some sampling of the non-vent fauna of the MCSC have been executed, the non-hydrothermal fauna of this volcanic ridge remains to be characterised. The non-hydrothermal fauna of mid-ocean ridges are poorly known and have only been studied previously at shallower depths (Copley *et al.*, 1996). Investigating the biodiversity of the isolated and deep MCSC has great potential to elucidate further the distribution and diversity of deep-ocean fauna and to contribute to the NERC goal of exploring ecosystems for novel sources of biodiversity.

To comprehend the full biodiversity of fauna inhabiting chemosynthetic environments in the deep ocean requires more than changing percentages and adding figures to the literature. The discovery and description of more taxa in the future has the potential to enhance current understanding of the ecology of deep-sea chemosynthetic environments, and to test hypotheses of the biogeographic relationships and evolutionary history of deep-sea fauna.

The accuracy of ecological work and biogeographic hypotheses are dependent on correct species identification. Traditional taxonomy, which emphasises the characterisation of morphological diversity, is an essential tool but cannot always account for other biological attributes, including developmental (Shank *et al.*, 1998) and ecological adaptations (Goffredi *et al.*, 2003; Johnson *et al.*, 2006, 2008; Borda *et al.*, 2013), contributing to inflated or underestimates of diversity (Vrijenhoek, 2009, 2010). The integration of molecular data has greatly improved our knowledge of species distributions and delimitations. However, the history of a particular gene might not reflect accurately the phylogeny of the species containing that gene (see Martin & Davis, 2001 and references therein). Consequently several genes should be analysed to infer species identities and evolutionary relationships, and molecular data should be

interpreted in conjunction with morphological and ecological data to provide robust taxonomic studies.

Despite a significant effort to characterise and describe the faunal assemblages of deep-sea chemosynthetic ecosystems, many taxa await formal description, and it is likely that many species are yet to be discovered in unexplored and under-sampled localities. Consequently determining how many chemosynthetic species exist, what their distributions are, and how those distributions are controlled require further investigation and exploration. Exploration of chemosynthetic environments in the deep ocean has focused to date on easily detectable geological anomalies. However, less readily detectable habitats suitable for inhabitation by chemosynthetic faunal assemblages may remain as yet undiscovered and could prove to be crucial to elucidating patterns of dispersal, connectivity and biogeography of chemosynthetic taxa.

7.2 Reproductive biology and ecology

7.2.1 Summary and conclusions

Reproduction is an essential process for the establishment and maintenance of isolated populations of specialist vent, seep and whale-fall fauna. Aspects of life-history biology have been elucidated in more than 90 species from deep-sea chemosynthetic environments; most species examined so far display phylogenetic conservatism in life-history features (e.g. Van Dover *et al.*, 1985; Copley & Young, 2006; Tyler *et al.*, 2007). Vent species exhibit a variety of reproductive patterns and modes of larval development, refuting an early naive prediction that there would be particular life-history adaptations to these insular and ephemeral environments (Tyler & Young, 1999). Previous studies have revealed spatial and temporal patterns in the reproductive development of some vent and seep species (e.g. Sheader *et al.*, 2000, 2004; Copley *et al.*, 2003; Copley & Young, 2006). Ecological studies of vent fauna on ultraslow-spreading ridges are, however, in their infancy, and the inter-annual dynamics of their fauna remained to be elucidated. The discovery of hydrothermal vent fields on the MCSC provided the opportunity to investigate the life-history biology and the spatial and inter-annual dynamics of the taxa that have colonised it.

This thesis presented work on the reproductive biology and ecology of the visually dominant species at the Beebe and Von Damm vent fields, MCSC, providing the first studies on the autecology of vent fauna from the MCSC (Chapters 5-6; Nye *et al.*, 2013 c and submitted). These chapters described the general reproductive features of *Rimicaris hybisae*; investigated spatial variation in the population structure and reproductive development of *R. hybisae* at the Beebe and Von Damm vent fields; examined variation in the population structure and reproductive features of *R. hybisae* between the Beebe and Von Damm vent fields; revealed inter-annual variation in the population structure and reproductive development of *R. hybisae* between January 2012 and February 2013; and discussed the results in the context of the published literature.

The reproductive biology of *Rimicaris hybisae* was found to be consistent with planktotrophic development and the reproductive biology of other alvinocaridid species, and appears to be phylogenetically constrained. Spatially discrete samples collected in January 2012 and February 2013 revealed a high degree of spatial variability in the population structure and reproductive features of this motile species at the Beebe and Von Damm vent fields. There was no correlation between the distribution of ovigerous females at the Beebe and Von Damm vent fields with density of aggregation or sex ratio. There appears to be a bias in the sex ratio towards females, at least at the Von Damm Vent Field; this could be an example of mate competition, or a result of insufficient sampling, or environmental variability (see Chapter 6). Specimens of *R. hybisae* from the Von Damm Vent Field were significantly larger than specimens from the Beebe Vent Field and may reach maturity at larger sizes than their counterparts at the Beebe Vent Field. Given a possible non-linear relationship between fecundity and carapace length, the larger body sizes of brooding females at Von Damm may result in a greater size-specific fecundity compared with females at the Beebe Vent Field. The reproductive time-series of *R. hybisae* revealed inter-annual variation in the overall population structure and reproductive development of this species, superimposed upon the pattern of spatial variation. Reproductive development in *R. hybisae* may be periodic or seasonal at the vent-field scale.

These findings advance knowledge of the reproductive biology of vent species and reveal previously unappreciated spatial variation in the reproductive development of a motile species at hydrothermal vents. Spatial variation in reproductive development within a vent field may be a fundamental feature of vent fauna, and potentially more

complex than realised previously, highlighting the challenges of elucidating the ecological dynamics in these heterogeneous, insular, and ephemeral environments.

7.2.2 Wider implications

Elucidating the life-history biology of vent species is essential to ultimately reveal the influence of life-history biology on the biogeographic relationships among deep-sea vent fauna at the global scale. The determination of the reproductive patterns of the visually dominant species at vent fields on the MCSC advances knowledge of the reproductive biology of vent species and is used to examine whether taxa shared with other biogeographic provinces exhibit planktotrophic development, rather than other modes, and the influence of life-history biology on the biogeographic relationships among deep-sea hydrothermal vent faunas at the global scale (section 7.3). This work also highlights the difficulties of determining the patterns and causes of spatial variation in the dynamic and heterogeneous vent environment, and the complexity of teasing apart patterns in space and time. It emphasises the need to examine large samples with replication to determine patterns in space and the factors that determine them in heterogeneous vent environments.

7.2.3 Future directions

Rimicaris hybisae is the only species from the Beebe and Von Damm vent fields to be subjected to studies of its reproductive traits. Further investigations are currently underway (Nye *et al.*, in preparation.) and are planned to determine the reproductive traits of other species at these vent fields.

Several gaps remain in understanding the life cycle of *Rimicaris hybisae* and other alvinocaridid species (see Figure 5.9). Experimental studies in the laboratory and *in situ* are desirable to determine the duration of brooding and embryonic development in alvinocaridid shrimp and whether these features are variable between different environmental conditions. The collection of additional samples with environmental data are required to test the hypothesis that environmental variables within aggregations of *R. hybisae* affect the distribution of ovigerous females, as described in other vent taxa

(e.g. Copley *et al.*, 2003; Pradillon & Gaill, 2007). Analyses of additional samples of *R. hybisae* collected at different seasons of the year are underway to test the hypothesis that reproductive development in *R. hybisae* is periodic or seasonal at the vent-field scale (Nye *et al.*, in preparation.).

Aspects of life-history biology have been elucidated in less than 100 species from chemosynthetic environments in the deep ocean (Nye *et al.*, 2013c). This is less than a quarter of the new faunal species that have been described from deep-sea hydrothermal vents to date (Desbruyères *et al.*, 2006). A variety of reproductive patterns and modes of larval development have been described in species from chemosynthetic environments, and phylogenetic constraint appears to be prevalent (e.g. Van Dover *et al.*, 1985; Copley & Young, 2006; Tyler *et al.*, 2007). Further work is necessary, however, to gain a more complete understanding of the reproductive patterns of fauna inhabiting these environments, and to ultimately inform the influence of life-history biology on the biogeographic relationships among fauna from deep-sea chemosynthetic environments at global scale. Several key questions remain to be addressed. For example: is spatial variation in reproductive development a fundamental feature of heterogeneous chemosynthetic environments? What is the extent (geographically and taxonomically) of reproductive seasonality in fauna endemic to dynamic and ephemeral chemosynthetic environments? What are the cues that drive seasonality in reproductive processes in chemosynthetic environments in the deep ocean?

Ecological investigations of vent fauna at ultraslow-spreading centres are in their infancy and the inter-annual dynamics of their fauna remained to be elucidated. Further time-series studies at ultraslow-spreading ridges are required to determine the extent and pace of ecological changes in hydrothermal assemblages in these environments, and to elucidate if/how such changes compare to those determined previously at faster-spreading ridges.

7.3 Biogeography

7.3.2 Introduction

Biodiversity patterns among deep-sea chemosynthetic fauna have been discussed at length leading to hypotheses of various biogeographic provinces (see section 1.4 and references therein). Debate concerns whether the shallow Mid-Atlantic Ridge (MAR) vent fields (Menez Gwen, Lucky Strike, and possibly Rainbow), constitute a separate province (Van Dover *et al.*, 2002; Shank, 2004; Ramirez-Llodra *et al.*, 2007), or if they fall within the main MAR province (Ramirez-Llodra *et al.*, 2007). The number of provinces in the Pacific, and whether Indian-Ocean vents constitute a novel biogeographic province or a subset of Pacific-vent fauna, are also contentious issues. Despite thousands of publications on deep-sea chemosynthetic ecosystems in the literature, unexplored ocean regions remain critical missing pieces of the biogeographic picture, and how the biogeographic regions are separated remains a first-order question.

The MCSC was identified as a priority for investigation by the international Census of Marine Life to advance understanding of the global biogeography of chemosynthetic ecosystems. The description of novel species from the Beebe and Von Damm vent fields, MCSC, (Chapters 2-4) combined with additional data on the taxonomic composition of the Beebe and Von Damm faunal assemblages provide a foundation for examining the bioegeographic affinities of the MCSC vent fauna.

In order to examine how the fauna at the Beebe and Von Damm vent fields fit into the current understanding of the biogeography of deep-sea hydrothermal vents, an analysis of genus presence/absence of faunal assemblages at known hydrothermal vent fields (for which data on the faunal assemblage composition are available) was undertaken, incorporating morphological and molecular taxonomic data from the most recently discovered vent sites. This is the first analysis of vent biogeography to include data from the vent fields at the MCSC, Southwest Indian Ridge (SWIR) and Arctic Mid-Ocean Ridge (AMOR).

7.3.2 Materials and methods

A database for presence/absence of species and genera was constructed for 74 hydrothermal vent fields. This was based on the Bachraty *et al.* (2009) dataset for 63 hydrothermal vent fields; taxa that are clearly not vent endemic were removed. Data on the taxonomic composition of faunal assemblages at the following vent fields were added: Beebe and Von Damm, MCSC (Connelly *et al.*, 2012; Nye *et al.*, 2012, 2013 a, b; unpublished data); Broken Spur, MAR (Copley *et al.*, 1997); Dodo and Solitaire, Central Indian Ridge (CIR: Nakamura *et al.*, 2012); Dragon, SWIR (Copley, 2011; unpublished data); E2 and E9, East Scotia Ridge (ESR: Rogers *et al.*, 2012; unpublished data); Moytirra, MAR (Wheeler *et al.*, 2013); Soria Moria II, Trollveggen and Loki's Castle, AMOR (Pedersen *et al.*, 2010; Schander *et al.*, 2010).

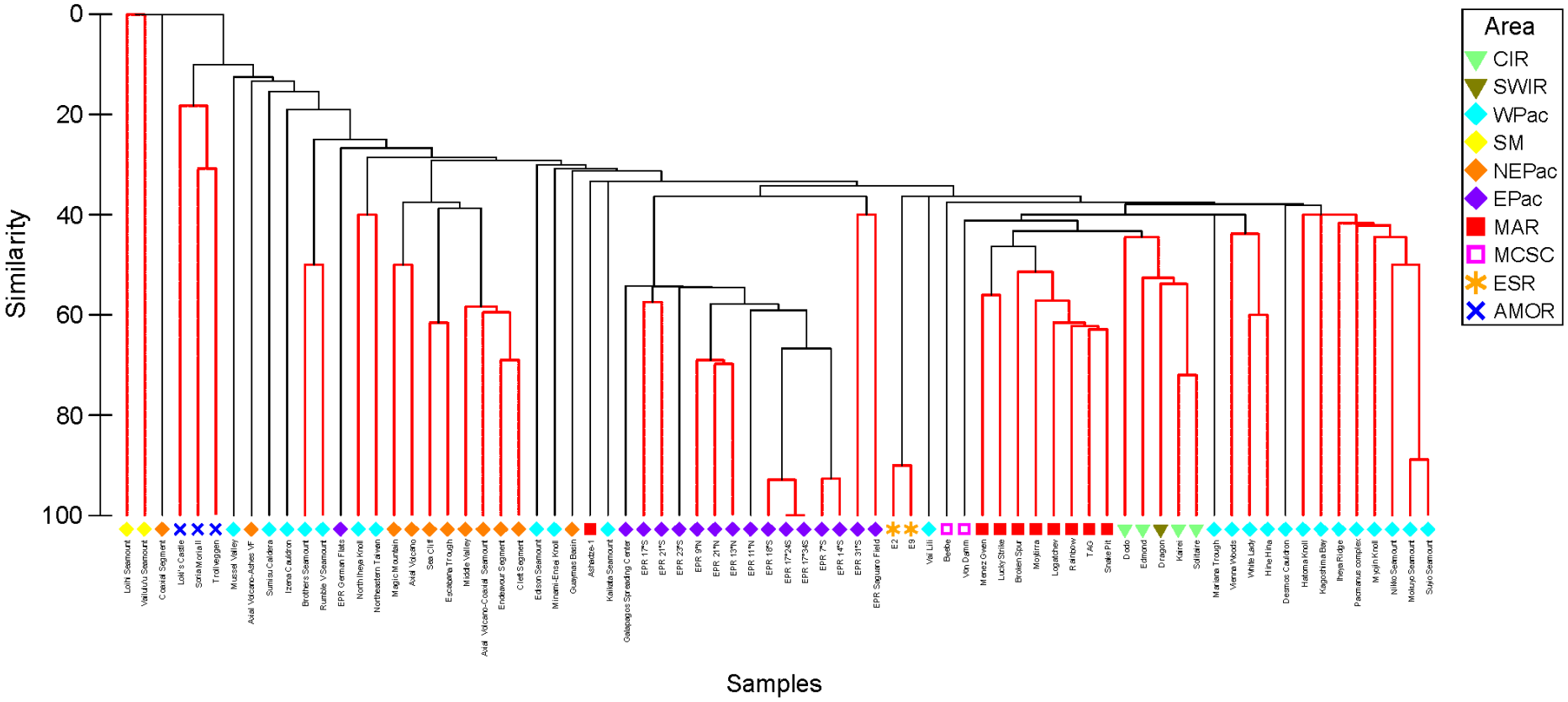
Hierarchical cluster analysis was executed using PRIMER v6 (Clarke & Warwick, 2001). The binary dataset for genus presence/absence was transformed into a similarity matrix using Sørensen's similarity coefficient. Cluster analysis was performed using single linkage and incorporating a similarity profile test (SIMPROF) to produce the dendrogram and test the null hypothesis that the vent fields did not differ significantly from each other (95% significance level). Single linkage was chosen because it allows for a small sampling effort and scant data.

7.3.3 Results

Results of the cluster analysis are presented in Figure 7.1.The cluster analysis defined 17 significant clusters (referred to hereafter as clusters). The Beebe and Von Damm vent fields did not form a cluster with each other or any other area (Figure 7.1). The MAR was split into two clusters: a cluster comprised of six vent fields and a smaller cluster containing Menez Gwen and Lucky Strike. The Ashadze vent field (MAR) did not form a cluster with any other vent field. The three AMOR vent fields formed a single cluster separate from all other areas.

Vent fields in the Pacific Ocean formed several clusters. The Northeast Pacific split into three clusters with three vent fields excluded from all clusters: Guaymas Basin (Gulf of California), Axial Volcano Ashes and Coaxial Segment (Juan de Fuca Ridge). The intra-plate mid-ocean seamounts (Loihi Seamount and Vailulu'u Seamount) formed

Figure 7.1. Cluster analysis of Sørensen's similarity for invertebrate genera from deep-sea hydrothermal vents using single linkage on presence/absence data. Clusters highlighted in red could not be significantly differentiated and are judged coherent by the SIMPROF similarity profile test.



AMOR, Arctic Mid-Ocean Ridge; CIR, Central Indian Ridge; EPac, East Pacific; ESR, East Scotia Ridge; MAR, Mid-Atlantic Ridge; MCSC, Mid-Cayman Spreading Centre; NEPac, Northeast Pacific; SWIR, Southwest-Indian Ridge; WPac; West Pacific; SM, mid-ocean seamounts.

a distinct cluster. The EPR was divided into five clusters, including a cluster of northern EPR vent fields. The German Flats (South EPR) and Galapagos Spreading Centre (East Pacific) did not cluster with any other vent field. The Western Pacific was split into four clusters. Mussel Valley (North-Fiji Back-Arc Basin), Sumisu Caldera (Izu-Ogasawara Arc), Izena Cauldron and Minami-Ensei Knoll (Okinawa Trough), Edison Seamount (Tabar-Feni Volcanic Fore Arc), Vaï Lil (Lau Back-Arc Basin) and Mariana Trough (Mariana Back-Arc Basin) did not cluster with any other vent fields.

The CIR and SWIR vent fields formed a single cluster separate from all other areas. The two ESR vent fields also formed a distinct cluster.

7.3.4 Discussion and conclusions

Overall, the results reveal significant clusters within geographic regions, highlighting similarities between the fauna of vent fields within provinces but also revealing structure within those provinces (Figure 7.1). The Beebe and Von Damm vent fields did not form a distinct biogeographic province or cluster with any other province. (Figure 7.1). These results illustrate the differences in the faunal assemblages between the two vent fields, which may be the result of bathymetry, environmental conditions and/or the life-history biology of the vent fauna. The topology reveals the faunal assemblages at MCSC vent fields to be closest to those of the MAR, Indian and Western Pacific. Biogeographic links between the East Pacific and Atlantic were proposed by Van Dover *et al.* (2002). Atlantic/East Pacific affinities have been shown for several annelid taxa (Black *et al.*, 1997; Tunnicliffe *et al.*, 1998; Borda *et al.*, 2013; Stiller *et al.*, 2013). The results of this analysis, however, do not support the hypothesis that the vent fauna at the MCSC is similar to that of the East Pacific.

Although the taxa described from the MCSC vents to date are distinct at the species level, the ophiuroid *Ophiotenella acies* (Tyler *et al.*, 1995) and gastropods of the genus *Provanna* are present at the Beebe Vent Field and vent fields on the MAR. The presence of *Rimicaris*-dominated faunal assemblages at the Beebe and Von Damm vent fields supports higher-level taxonomic affinities with the fauna of Mid-Atlantic vents (e.g. Desbruyères *et al.*, 2001; Gebruk & Mironov, 2006).

Teixeira *et al.* (2013) demonstrated low-levels of genetic diversity in COI and 18S between *Rimicaris hybisae* (MCSC) and vent shrimp at the MAR (see Chapter 2), supporting the occurrence of large-scale effective migration across the Atlantic Ocean at some point in the evolutionary history of the Alvinocaridae. High rates of gene flow and low genetic variation have been reported for *R. exoculata* along the MAR from 36°N to 4°S (Creasey *et al.*, 1996; Shank *et al.*, 1999; Teixeira *et al.*, 2010, 2012). Alvinocaridid shrimp, including *R. hybisae*, have been shown to exhibit planktotrophic larval development with hypothesised long-range dispersal (see Chapter 5 and references therein). Upward vertical movement during the larval phase could explain the broad geographic distribution of certain species with planktotrophic larvae entrained in deep-water currents (Van Dover *et al.*, 2002). Vent shrimp postlarvae have been found to be present in midwater above MAR vents and to extend great distances laterally from known chemosynthetic sites (Herring & Dixon, 1998). These data seem to support dispersal potential of alvinocarid shrimp in Atlantic water masses. Overall, the data support the hypothesis that the dominant taxa at vents on the MCSC is similar to those of the MAR, from which they migrated over evolutionary history (or vice versa). Future studies of the life-history biology of other vent taxa at the MCSC have the potential to test the hypthothesis that the isolation of the MCSC may impose a filter on the life-history biologies of the taxa that have colonised it, thereby elucidating the role of modes of larval development and dispersal in determining biogeographic distributions.

The biogeographic roles of cold seeps were not considered in this analysis. However, the presence of siboglinid tubeworms (*Escarpia* sp. and *Lamellibrachia* sp.) at the Von Damm Vent Field, and results of population-genetics studies (Plouviez *et al.*, personal communication), suggest that the vent fauna at the MCSC also exhibits affinities with that of Atlantic cold seeps. There is no evidence to date that suggest that the vent fauna at the Beebe Vent Field exhibits affinities with that of cold seeps.

Overall, vent fauna at the MCSC appears at present to be distinct at the species level but displays affinities at higher taxonomic levels with the fauna of the MAR and Atlantic cold seeps. Faunal affinities to the Pacific may also be indicated by the presence of *Iheyaspira* and *Lebbeus* at the Von Damm Vent Field (see Chapters 3 and 4). However, the dataset for these taxa is viewed as too incomplete to conclude that the presence of these taxa supports a transoceanic faunal connection across the Panamanian Isthmus.

Widespread submarine volcanic activity along the Lesser Antilles island arc, Caribbean, is well documented (e.g. Polyak *et al.*, 1992; Divine & Sigurdsson, 1995). Shallow-water hot springs are known along the coasts of some of the islands, such as Dominica, St. Lucia and Montserrat (Johnson & Cronan, 2001) and the Kick'em Jenny hydrothermal vent is located north of Grenada (Kochinsky *et al.*, 2007). However, little information is available about submarine venting of hydrothermal fluids along this arc and in deeper waters (e.g. Polyak *et al.*, 1992). Exploration of this region and the potential discovery and investigation of additional chemosynthetic sites could elucidate further the vent biogeography of the Caribbean. Further characterisation of the faunal assemblages at the Beebe and Von Damm vent fields, studies of the life-history biology and population connectivity of the species present, and investigations of the physical oceanography of the region also have the potential to inform further our current understanding of the biogeography of the MCSC at global scale.

The results support the hypothesis that shallow MAR vent fields (Menez Gwen and Lucky Strike) constitute a separate province from their deeper counterparts (Van Dover *et al.*, 2002; Shank, 2004; Ramirez-Llodra *et al.*, 2007), in contrast to the single MAR province suggested by multivariate-regression tree analyses (Bachraty *et al.*, 2009; Rogers *et al.*, 2012) and a network-theory approach (Moalic *et al.*, 2012). The Ashadze-1 vent field did not cluster with any other vent field. The Ashadze-1 is the deepest vent field on the North MAR (4080 m depth), and the faunal assemblage here appears to contain few of symbiotic species dominant in other areas on the MAR (Fabri *et al.*, 2011). Bathymetry may be a major geographical barrier to species dispersal along the MAR, as invoked previously (Van Dover *et al.*, 2002).

Indian-Ocean vents (SWIR and CIR) appear to constitute a single, distinct biogeographic province at present (Figure 7.1; Van Dover *et al.*, 2002; Shank, 2004; Ramirez-Llodra *et al.*, 2007; Moalic *et al.*, 2012; Rogers *et al.*, 2012), rather than the subset of Pacific-vent fauna proposed by Bachraty *et al.* (2009) based on data from the CIR. The topology lends support to the hypothesis that this province occupies an intermediate position between Mid-Atlantic and West-Pacific provinces (Moalic *et al.*, 2012), illustrating links between the fauna of these provinces.

The results are consistent with a separate biogeographic at the ESR (Rogers *et al.*, 2012). These authors found that the ESR vents exhibit a unique species composition and structure and suggest that the environmental conditions of the Southern Ocean may act as a barrier to some vent animals.

The topology also suggests that the Arctic forms a distinct biogeographic province. The deep-vent ecosystems described recently from the Arctic exhibit the absence of vent shrimp and vent mussels but some species are closely related to species recorded from vents in the Northern Pacific (Pedersen *et al.*, 2010). The composition of the faunal assemblage may be the result of local specialisation and migration from cold-seep environments in combination with recent migration of vent fauna into the Arctic from the Pacific (Pedersen *et al.*, 2010).

The cluster analysis divided the Pacific into six provinces: the EPR, the Northeast Pacific and three West-Pacific provinces, and the intra-plate mid-ocean seamounts (Loihi and Vailulu'u seamounts). The topology splits the EPR into five regions, including a northern region. The Galapagos Spreading Centre is closest to the EPR but without significant support. The sediment-hosted Guaymas Basin does not fall within the Northeast-Pacific province. These results suggest that the vent biogeography of the Pacific may be more complex than proposed by previous studies (Van Dover *et al.*, 2002; Ramirez-Llodra *et al.*, 2007; Bachraty *et al.*, 2009; Moalic *et al.*, 2012).

7.3.5 Wider implications

Ephemeral, insular and globally-distributed deep-ocean chemosynthetic environments with their endemic faunas provide 'natural laboratories' for studying the interactions between ecological and evolutionary processes that shape global patterns of marine life. Understanding interactions between ecological and evolutionary processes that determine global patterns of marine life are essential to inform the sustainable use of marine resources and conservation strategies. The need is especially urgent in the deep sea where the potential emergence of a vent-mining industry may be imminent (Hoagland *et al.*, 2010; Van Dover, 2011). This work advances our understanding of biogeographic patterns of chemosynthetic ecosystems, which was an objective of the international Census of Marine Life ChEss programme.

7.3.6 Future directions

The identifications of biogeographic provinces and their delimitation are important goals within biogeography but this work is not straightforward and continues to evolve with greater sampling effort and the application of different approaches. Recent explorations have filled in most of the missing gaps in the current picture of vent biogeography at large scale. This places vent biogeography in an exciting position whereby workers now have the potential to test the influence of various factors, such as life-history biology and functional traits, on the distribution of vent fauna at global scale.

7.4 References

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"…the only other place comparable to these marvellous nether regions, must surely be naked space itself, out far beyond the atmosphere, between the stars, where sunlight has no grip upon the dust and rubbish of planetary air, where the blackness of space, the shining planets, comets, suns, and stars must really be closely akin to the world of life as it appears to the eyes of an awed human being, in the open ocean, one half mile down." – WILLIAM BEEBE

Appendix 1: Phyletic overview of reproduction and developmental modes of invertebrates in deep-sea chemosynthetic environments

Phylum Mollusca

Class Aplacaphora (Solenogastres)

Data on the life-history biology of aplacophorans from deep-sea chemosynthetic environments is currently limited to *Helicoradomenia*, the only known aplacophoran genus in the vent ecosystem (Scheltema & Kuzirian, 1991; Tunnicliffe, 1991). Species of *Helicoradomenia*, apparently endemic to hydrothermal vents, have been collected widely in the East Pacific, south-west Pacific, and the Triple Junction in the Indian Ocean, but not in the Atlantic (Scheltema, 2008). Two genera, one perhaps being *Helicoradomenia*, are also found on whalefalls, but *Helicoradomenia* has not been found with the other sulfide-based fauna in seeps (Scheltema, 2008).

The aplacophoran *Helichorademia juani* is hermaphroditic, consistent with other neomenioid aplacophorans, and produces eggs (~80 µm diameter) (Tyler & Young, 1999) within the range reported (120 x 70 µm) for other Solenogastres from vents in the south-east Pacific and East Pacific Rise (EPR) (Salvini-Plawen, 2008). Savage (1997) examined the reproductive biology of *Helichorademia juani* from the High Rise vent field (Juan de Fuca Ridge, 2200 m). Samples were collected over one month from three different areas. Vitellogenesis occurs in the gonad and oocytes are stored in the pericardial cavity after vitellogenesis has occurred. All stages of gamete development were present in the gonads of males and females, suggesting continuous gametogenesis and asynchronous reproduction (Savage, 1997). It is not known how quickly *H. juani* reaches sexual maturity. Early maturing species have a higher probability of reaching maturity but a lower lifetime fecundity than species that delay maturity (Tyler *et al.*, 2008).

Oocytes are fertilised as they pass through the seminal receptacles and lower gametoducts, though it remains to be seen if the second meiotic division occurs here or after release and if spawn are released singly or in batches (Savage, 1997). Internal fertilisation may

be an advantage in the vent environment if gametes are protected from toxic waters of the vent environment before fertilisation.

Lecithotrophy is suggested for *Helichorademia juani*, based on observations of low fecundity, continuous reproduction and phylogenetics (Savage, 1997). Evidence determined from egg size in the gonads (Scheltema, 1987), the occurrence of swimming larvae (Pruvo, 1890; Baba, 1938; Okusu, 2002; Nielson *et al.*, 2007), or settlement onto panels (Mullineaux, personal communication in Sheltema, 2008) has revealed that all aplacophorans, with the exception of some brooders, have lecithotrophic larvae. Tyler & Young (1999) suggested that selective pressures in the vent environment might favour species with yolky eggs that provide nutrition to a larva during long-distance dispersal. In addition to being able to sustain populations at existing hydrothermal vents, the ephemeral nature of vents necessitates recruitment by the *Helicoradomenia* to new active vent sites for their continued survival. Although the dispersal capabilities of lecithotrophic development are not fully understood, ongoing recruitment experiments at vent sites demonstrate the ability of *Helicoradomenia* species to disperse (Scheltema, 2008).

Class Gastropoda

Gastropods are a numerically dominant taxon in deep-sea chemosynthetic environments, but compared to the larger megafauna, relatively little attention has been paid to their reproductive biology and modes of development (Tyler *et al.*, 2008). Within the Gastropoda, the majority of studies on reproduction have been on the Lepetodrilidae (Vetigastropoda). Gastropods exhibit the greatest gametogenic variation, with most species producing a small number of large eggs and a few species producing large number of small eggs (Gustafson & Lutz, 1994). Indirect methods have been used to examine the dispersal capabilities of vent gastropods, including gene flow between sites (e.g. Craddock *et al.*, 1997; Vrijenhoek, 1997) and larval shell morphology (Lutz, 1988). Lutz *et al.* (1984) suggested that the majority of vent gastropods appear to be phylogenetically constrained to a non-planktotrophic developmental mode. The evidence indicates that most vent gastropods have a similar reproductive pattern of early maturity, internal fertilisation and lecithotrophy, but exceptions are found.

Patelligastropoda, Neolepetopsidae

Eulepetopsis vitrea is a free-spawning patellid gastropod with no secondary sexual glands (Fretter, 1990). Tyler *et al.* (2008) described gametogenic biology in this species using samples from 14 vents at east Pacific vent sites and the Mid-Atlantic Ridge (MAR). Vitellogenesis begins when oocytes reach 60-70 µm in diameter. The maximum observed oocyte size was 232 µm with a fecundity of <200 oocytes per individual. This was the largest oocyte size and

smallest fecundity of all seven gastropods in Tyler *et al.*'s (2008) study, demonstrating the inverse correlation between oocyte size and fecundity found in many taxa (Thorson, 1950). Reproduction is presumably continuous as there was no indication of episodicity in oocyte production (Tyler *et al.*, 2008). The ovaries of *E. vitrea* are similar to a typical limpet ovary with the oocytes being contained within follicular chambers and provided with nutrition from the surrounding follicle cells (K. Eckelbarger, personal observation in Tyler *et al.*, 2008).

Lecithotrophic development has been suggested for *Eulepetopsis vitrea* (Craddock *et al*., 1997; Tyler *et al.*, 2008), which may limit long-distance dispersal capabilities. Theory (Wright, 1943; Kimura & Weiss, 1964) suggests that species with limited long-distance dispersal will migrate among habitat islands in a 'stepping-stone' fashion and will therefore exhibit genetic evidence of 'isolation-by-distance'. Lutz *et al.* (1986) predicted that molluscs with non-planktotrophic larvae (and potentially limited dispersal abilities) may be able to maintain widespread populations by dispersing in a stepwise manner between disjunct vent sites along a ridge axis. In studies of gene flow Craddock *et al.* (1997) and Vrijenhoek (1997) found that *E. vitrea* did not exhibit the expected decline in rates of gene flow with increasing geographic distances between localities. Instead, the results for *E. vitrea* were consistent with Wright's (1951) 'island' model of dispersal, which is expected for species with long-distance dispersal and thorough mixing with a migrant pool (Craddock *et al.*, 1997; Vrijenhoek, 1997). Evidently more work needs to be done to fully confirm this species' developmental mode and dispersal ability and to elucidate the complete life-history biology of patelligastropods from deep-sea chemosynthetic communities.

Vetigastropoda, Lepetodrilidae

The Lepetodrilidae are vent and seep endemic (Hodgson *et al.*, 2009). Species of the limpet genus *Lepetodrilus* from vents in the east Pacific and MAR exhibit early maturity with females undergoing gametogenesis at less than one third maximum body size (Kelly & Metaxas, 2007; Tyler *et al.*, 2008). Early maturity has also been documented in *L. fucensis* from northeast Pacific vents (Kelly & Metaxas, 2007). Early maturity is typical of an opportunistic species (Bridges *et al.*, 1994). Although species that mature early have a higher probability of reaching maturity than species that delay maturity, they have lower lifetime fecundity (Tyler *et al.*, 2008). Spermatogenesis in *Lepetodrilus fucensis* follows a similar pattern to that described for many shallow water gastropods (Hodgson *et al.*, 1997). Ovarian trabeculae, although unusual in gastropod molluscs, are present in *L. atlanticus, L. elevatus, L. ovalis, L. pustulosus* and *Rhynchopelta concentrica* (Peltospriridae) (Tyler *et al.*, 2008). The trabeculae allow nutrients to be delivered to the ovary and supports rapid gametogenesis (Hodgson & Eckelbarger, 2000). A

maximum unfertilised oocyte size of <90 µm and a powerful relationship between shell length and fecundity were found in *Lepetodrilus* species from MAR and east Pacific vents (Tyler *et al.*, 2008). Such an egg size might be indicative of planktotrophic larval development (Tyler *et al*., 2008). Individual fecundity was typically <1,000, but a maximum of 1,800 oocytes were found in one individual. Tyler *et al.* (2008) found no evidence of reproductive periodicity or spatial variation in reproductive traits (Tyler *et al.*, 2008), indicating that the energy available to the limpets was constant in time and space.

The vent limpet family Lepetodrilidae represents a relatively well-documented example of taxa with copulatory and sperm-storage organs (Kano, 2008). Fretter (1988) described the anatomy and physiology of five species of *Lepetodrilus* and showed that the male bears a penis and the female has a seminal receptacle (see also Warén & Bouchet, 2001; Kelly & Metaxas, 2007). The penis and receptacle are also found in the vent limpet genus *Clypeosectus* and are apparently homologous to those of *Lepetodrilus* (Haszprunar, 1989). Fretter (1988) described a different kind of male copulatory structure for the hydrothermal vent genus *Gorgoletis*, and argued that this penis evolved independently from that of *Lepetodrilus*, indicating that semi-internal fertilization is advantageous in the vent environment.

Tyler *et al.* (2008) discovered the reproductive morphologies of *Lepetodrilus elevatus*, *L. ovalis*, *L. pustulosus* (east Pacific vents) and *L. atlanticus* (MAR vents) to be similar to those previously described in the genus (Fretter, 1988; Warén & Bouchet, 2001), and noted the presence of sperm in the receptaculum seminalis of the three east Pacific species. In three individuals, sperm were present in the genital groove, but not the *receptaculum seminalis*, suggesting that sperm may be stored, rather than merely deposited in the genital groove (Tyler *et al.*, 2008). Kelly & Metaxas (2007) found sperm in the *receptaculum seminalis* in 48 out of 57 *L. fucensis* females, whereas both the *receptaculum seminalis* and genital groove contained sperm in 39 out of 40 *L. elevatus* females examined by Pendlebury (2005). The shell length at which *L. elevatus* began storing sperm was consistent with the size at which individuals first started producing vitellogenic oocytes (Pendlebury, 2005). The presence of stored sperm would facilitate fertilization as the oocytes are produced and released (Kelly & Metaxas, 2007). Sperm storage could also decrease the energy associated with copulation (Pendlebury, 2005). The stacking behaviour, close proximity and high abundances of *L. fucensis* may also facilitate sperm transfer between the sexes in this species (Kelly & Metaxas, 2007).

Finding no direct connection between the receptacle and oviduct, Fretter (1988) argues that mature ova are fertilized in the mantle cavity of the female (semi-internal fertilization). Males are believed to mount the female shell and release their gametes, which are then swept into the female receptacular tract by the inhalant current produced by the female gill (Fretter, 1989). Ultrastructural analysis of sperm morphology supports this argument. *Lepetodrilus*

produces ent-aquasperm rather than the ect-aquasperm typical of vetigastropods with external fertilization (Hodgson *et al.*, 1997). Semi-internal fertilization could be an example of mate competition.

The presence of sperm storage in other vent taxa (Zal *et al.*, 1995; Jollivet *et al.*, 2000; Copley *et al.*, 2003: Hilário *et al.*, 2005) implies that this trait provides an adaptive advantage in the turbulent and potentially toxic hydrothermal vent environment. Sperm storage could prevent the rapid dilution of gametes in the turbulent flow regime of vents (Tyler & Young, 1999). Internal fertilization may also protect gametes from the high temperature, acidic hydrothermal fluids and/or toxic sulfides and minerals emanating from vents (Warén & Bouchet, 1989; Hodgson *et al.*, 1997). Such harshness of the vent habitat explains reasonably the evolution of copulatory behaviour and sperm storage in the numerically dominant *Lepetodrilus* (Kelly & Metaxas, 2007).

Continuous reproduction is common in gastropods (Webber, 1977) and is the most common strategy for molluscs inhabiting the deep sea and hydrothermal vents (Tyler & Young, 1999; Young, 2003). Berg (1985) proposed that as the majority of vent limpets examined by Tyler *et al.* (2008) have all stages of oocytes within their gonad at any one time, reproduction is continuous with high fecundity. Kelly & Metaxas (2007) found all stages of gamete development present in the gonads of male and females of *Lepetodrilus fucensis* from northeast Pacific vents, suggesting continuous gametogenesis and asynchronous reproduction in this species. Pendelbury (2005) found no spatial or temporal differences in the oocyte frequencies of *L. ovalis*, *L. elevatus*, *L. pustulosus* and *L. atlanticus*, and concluded that their reproductive cycles were quasi-continuous within individuals and asynchronous among individuals. Size-frequency distribution of *L. elevatus* populations, however, showed a poly/bimodal distribution, which could be indicative of episodic reproduction (Mullineaux *et al.*, 1998; Sadosky *et al.*, 2002). Larval abundance data at 9°N on the EPR appears to show significant differences in abundance of lepetodrilid larvae among samples taken at different times of the year (Mullineaux et al., 2005). Metaxas (2004) also detected order-of-magnitude differences in abundances of *L. fucensis* between sampling dates. Such temporal variation may be due to variation in spawning, but could also be caused by other processes, such as differential survival or larval behaviour (Mullineaux *et al.*, 2005). Fluctuations in the currents that carry larvae to and from vents may also explain this discrepancy (Tyler *et al.*, 2008). Periodic reversals in along-ridge flows have been recorded at 9°N on the EPR suggesting currents are major factors in larval dispersal and recruitment (Mullineaux *et al.*, 2002). High abundance of early developmental stages of *L. fucensis* in the water column and in near-bottom flows over spatial and temporal scales (Metaxas, 2004) implies a constant gamete production and spawning of larvae (Kelly & Metaxas, 2007).

Appendix 1

Poor venting conditions (e.g. waning or senescent vents) and parasitic infection have been shown to limit reproductive output in *Lepetodrilus fucensis* from northeast Pacific vents (Kelly & Metaxas, 2007; Tunnicliffe *et al.*, 2008). Using a stage-based matrix model, Kelly & Metaxas (2010) explored life-history traits and population dynamics of the numerically dominant *L. fucensis* at vents on the Juan de Fuca Ridge (northeast Pacific) to determine potential mechanisms for the success of this species in the dynamic and ephemeral vent environment. They found that reducing reproductive output by up to 90% reduced population size but did not result in population extinction. Although high levels of reproductive failure in *L. fucensis* can reduce offspring production and population size, continuous reproductive output may ensure this species' success in the vent ecosystem (Kelly & Metaxas, 2010).

It has been argued that hydrothermal vent gastropods are most likely constrained to lecithotrophic development (Lutz *et al.*, 1984; Berg, 1985; Gustafson & Lutz, 1994) but egg sizes in some species (*Lepetodrilus atlanticus*, *L. elevatus*, *L. ovalis* and *L. pustulosus*) are suggestive of planktotrophy (Tyler *et al.*, 2008). Although egg size cannot be used as a perfect indicator of larval development mode, the maximum oocyte size of the four lepetodrilid species studied by Tyler *et al.* (2008) and in *L. fucensis* are small when compared with species with known lecithotrophic development (Eckelbarger, 1994; Kelly & Metaxas, 2007). It has been inferred that larval development in lepetodrilids is planktotrophic, although there is no evidence of shell growth during their time in the plankton (Mullineaux & Mills, personal observation in Tyler *et al.*, 2008). Non-planktotrophic development has been suggested for *L. elevatus*, *L. glariftensis* and *L. pustulosus* (based on larval shell characteristics), but *L. pustulosus* did not exhibit the expected decline in rates of gene flow with increasing geographic distance between localities (Craddock *et al.*, 1997; Vrijenhoek, 1997). The number of samples of *L. elevatus* and *L. glariftensis* were inadequate to reject the null hypothesis of isolation by distance ('stepping-stone' dispersal) for this species (Vrijenhoek, 1997).

Inferences about dispersal potential based on egg sizes and larval shell morphology may not accurately represent realised dispersal in the deep sea (reviewed by Young, 2003). Shallow water species can generally achieve a greater dispersal with planktotrophy than lecithotrophy, but in the cold deep sea, the metabolism of larvae is greatly reduced, allowing a wider dispersal than for shallow water counterparts (Tyler *et al.*, 2008). Where nutrients are limited, such as in the deep ocean, lecithotrophy may allow wider dispersal than planktotrophy (Shilling & Manahan, 1994). The very broad geographic distributions of echinoderms in deep sea support this concept (Young *et al.*, 1997). Away from hydrothermal habitats, cold bottom temperatures (~2°C) may lower or arrest larval development and permit dispersal over vast distances (Vrijenhoek, 1997; see also Pradillon *et al.*, 2001).

Population studies at vents have highlighted the spatial heterogeneity of the vent environment and the importance of energy availability for reproductive success. *Lepetodrilus*

fucensis is ubiquitous along the Juan de Fuca Ridge (northeast Pacific) where it is the numerically dominant species, exceeding 105 individuals m^{-2} in low-temperature diffuse venting conditions (Chase *et al.*, 1985; Tsurumi & Tunnicliffe, 2001; Bates, 2008). Nutritional availability has been implicated as a controlling factor in the reproductive output of *L. fucensis*, producing a female bias in high flux locations (Bates, 2008; Kelly & Metaxas, 2007).

Kelly & Metaxas (2007) studied *L. fucensis* populations at north-east Pacific vents and found that mean oocyte diameter and fecundity was greatest in females from actively venting sites and smallest in those from senescent habitats. Gametogenic maturity of limpets was significantly lower in males and females from senescent habitats (Kelly & Metaxas, 2007). Although the gametogenetic pattern of *L. fucensis* is phylogenetically constrained, selection of actively venting habitats by *L. fucensis* may maximise its reproductive output. Many studies of marine herbivores have demonstrated that reproductive fitness is influenced by diet, and reproductive output decreases under stress (Eckelbarger, 1994; Eckelbarger & Watling, 1995; Kennish, 1997; Foster *et al.*, 1999; Ramirez-Llodra, 2002). It is most likely that *L. fucensis* fails to reproduce in waning vent habitats because of a lack of resources available to divert to reproduction (Kelly & Metaxas, 2007). These authors suggest that the multiple feeding strategies of *L. fucensis* may allow for a constant supply of energy to be allocated to reproduction in any habitat except senescent vents.

Bates (2008) examined habit partitioning by *Lepetodrilus fucensis* and found a spatial mismatch between the sexes within a vent at decimetre scales. High-flux locations revealed female-biase, whereas the vent periphery and waning vents exhibited male-biased sex ratios. Bates (2008) hypothesized that females experience a higher cost of reproduction and outcompete males for habitats with greater food availability. Further work is required to determine if males migrate inwards to mate and if the effective male breeding population is smaller than the overall male population (Bates, 2008).

Copley *et al.* (2003) examined the reproductive development of *Paralvinella palmiformis*, an alvinellid inhabiting vents on the Juan de Fuca Ridge and found evidence of a strong spatial variation in gamete frequency and development. A bias of brooding females in low-flux locations away from sulfidic extremes has also been found in vent and seep invertebrates (see Table 3 and below). Evidence of spatial variation in gender distributions and reproductive parameters emphasizes the need to examine life history traits at environmentally relevant scales. Hydrothermal vents and cold seeps provide excellent natural systems for testing spatial ecological patterns.

The life-history traits of early reproduction, sperm storage, high fecundity and continuous gametogenesis have apparently enabled *Lepetodrilus* to successfully exploit the ephemeral and variable vent and seep ecosystems.

Appendix 1

Neomphalina, Neomphaloidea, Neomphalidae

Tyler *et al.* (2008) examined the reproductive biology of *Cyathermia naticoides* from east Pacific vents. Ovaries were found to be similar to typical limpet ovary and vitellogenesis began at ~50 µm with yolk distributed throughout the oocyte. The maximum observed oocyte size and fecundity were ~120 µm and <400 oocytes per individual respectively. No indication of episodicity in oocyte production was found (Tyler *et al.*, 2008), indicating quasi-continuous and asynchronous reproduction, typical of most vent molluscs (Tyler & Young, 1999; Young, 2003). The oocyte-frequency distributions of *C. naticoides* had a strong bimodal distribution characteristic of other gastropods (Christiansen & Fenchel, 1979; Tyler *et al.*, 2008).

The reproductive morphology of *Cyathermia naticoides* is characteristic of internal fertilisation (Warén & Bouchet, 1989). Male specimens have a large penis, a large prostrate gland and a testis, which occupies the majority of the visceral mass (Tyler *et al.*, 2008). Internal fertilisation might prevent direct exposure of gametes to the potentially toxic vent waters (Fretter, 1988; Warén & Bouchet, 1989) or maximise reproductive success where there is competition for mates. Because the site of fertilisation is different in the Neophalidae, Neolepetopsidae and Peltospiridae, some form of 'internal' fertilisation may have evolved independently in each family (Tyler *et al.*, 2008). This would imply that internal fertilization is important for successful reproduction in the hydrothermal vent environment.

Mode of larval development in *Cyathermia naticoides* is unknown and cannot be inferred from egg size because the maximum oocyte size is intermediate between planktotrophy and lecithotrophy (Tyler *et al.*, 2008). The reproductive biology of *Cyathermia naticoides* appears to be phylogenetically constrained.

Neoamphalina, Peltosprioidea, Peltospriridae

Tyler *et al.* (2008) recently studied reproduction in *Rhynchopelta concentrica* and reported a maximum oocyte size of 184 µm and a fecundity <600. In the same study *Eulepetopsis vitrea* (Family Neolepetopsidae) was found to have a maximum oocyte size of 232 µm with a fecundity of <200 oocytes per individual, illustrating the inverse relationship between fecundity and oocyte size (Thorson, 1950). The ovary, containing tightly-packed oocytes in the specimens studied, was a smaller portion of the body than that described in the Lepetodrilidae, though trabeculae were found in the ovaries of both *R. concentrica* and lepetodrilid species (Tyler *et al.*, 2008). Trabeculae allow nutrients to be delivered to the ovary and support rapid gametogenesis (Hodgson & Eckelbarger, 2000). Vitellogenesis begins when the oocytes are between 40 µm and 50 µm. Based on oocyte size (mean of 130 µm, maximum of 184 µm) and the lengthy period of oogenesis (beginning at one third of the maximum oocyte size), Tyler *et al.* (2008) suggested lecithotrophic development for this species. These authors found no indication of episodicity in oocyte production and concluded that *R. concentrica* has a quasi-

continuous, asynchronous reproductive cycle, that is common to most vent molluscs studied so far (Tyler & Young, 1999; Young, 2003; Kelly & Metaxas, 2007).

Rhynchopelta concentrica exhibits seperate sexes (Fretter, 1989). The presence of a large pallial vas deferens that acts as a prostrate, the discovery of viable sperm within the female receptacular duct and filiform introsperm confirm that *R. concentrica* has internal fertilization (Franzen, 1955; Tyler *et al.*, 2008; Hodgson *et al.*, 2009). Ripe ova have frequently been seen in the ovarian duct (Fretter, 1989) but fertilized ova have yet to be seen. Oocytes are probably fertilized during their exit from the mantle cavity (Tyler *et al.*, 2008). Internal fertilisation requires a mechanism for sperm transfer. A number of aphalic gastropods use spermatophores to transfer sperm (Robertson, 1989). High densities of *R. concentrica* (Govenar *et al.*, 2005) could facilitate a means of sperm transfer (Hodgson *et al.*, 2009). Further investigations of the distal region of the male reproductive tract are required to establish whether spermatophores are also produced by *R. concentrica* (Hodgson *et al.*, 2009).

Based on the evidence available to date, it seems that broadcast spawning by vent gastropods may be rare, with fertilisation occurring internally or within the mantle cavity. Warén & Bouchet (1989) suggested that high sulfide levels in the vent water would be poisonous to gastropod spermatozoa, precluding external fertilisation. Eckelbarger & Young (2002) suggested that the sperm of the vent orbinid *Methanoarcia dendrobranchiata* is modified to minimise exposure to hypoxic conditions. *Eulepetopsis vitrea*, however, is a free-spawning gastropod (Fretter, 1990) inhabiting the vent environment and vent bivalves that successfully live in similar conditions all appear to be spawners releasing aquasperm (Eckelbarger & Young, 1999; Tyler & Young, 1999).

Neogastropoda, Buccinidae

Whelks of the family Buccinidae are among the more conspicuous of the predatory and scavenging gastropods in hydrothermal vent assemblages (Harasewych & Kantor, 2002) data on their reproduction are limited. Martell *et al.* (2002) studied the population and some biological features of *Buccinum thermophilum* from three vents on the Endeavour Segment of the Juan de Fuca Ridge. Sexes are separate and egg masses were found to be similar to those described for other buccinids. Egg size before division was ~275 µm and egg numbers varied between 200 and 450 per capsule (Martell *et al.*, 2002). All eggs in the same mass were apparently at the same stage, although food eggs were not differentiated from embryos by Martell *et al.* (2002). Mass size, capsule number and number of eggs in *B. thermophilum* are similar to descriptions for *B. cyaneum* (Miloslavich & Dufresne, 1994, Martell *et al.*, 2002).

The only type of development known for *Buccinum* is encapsulated metamorphosis and direct development to a benthic juvenile from the capsule with nutrition provide by food eggs (Webber, 1977; Fretter & Graham, 1994). Species that deposit direct-developing egg capsules at

vents are likely to ensure a good food supply for their young but may have a limited dispersion and restricted range (Martell *et al.*, 2002). Shallow water species often form mating aggregations (Strathmann, 1987). It remains to be seen if *B. thermophilum* exhibits such behaviour.

Seasonal reproduction has been hypothesised in *Buccinum thermophilum*, based on size-frequency distribution of adults and the lack of advanced embryos in summer-collected egg masses (Martell *et al.*, 2002). However, the nature and extent of evidence for seasonality in this species is not compelling. Martell *et al.* (2002) based their hypothesis on just 5 egg masses and population data, collected over one season only. Oocyte size-frequency analysis is a more robust means of detecting seasonal gametogenic cycles (Grant & Tyler, 1983), necessitating time-series observations to determine when the proportion of mature oocytes declines (Le Pennec & Beninger, 2000). Time-series data collected over all seasons and a substantially greater sampling effort are prerequisites for determining temporal patterns and would be necessary to test the hypothesis of reproductive seasonality in *B. thermophilum*.

Neritoida, Neritidae

Bathynerita naticoidea is a bathyal gastropod endemic to hydrocarbon seeps in the Gulf of Mexico and the southern Barbados Prism at depths from 400-1700 m (Carney, 1994; Olu *et al.*, 1996; Zande & Carney, 2001). Eckelbarger & Young (1997) report maximum egg sizes of 135-145 μm in *B naticoida*, whereas a maximum egg size of 180 μm diameter is reported from histology and direct measurement of oocytes by Van Gaest (2006). Ultrastructural evidence indicates that *B. naticoidea* uses both heterosynthetic and autosynthetic pathways of vitellogenesis consistent with 'fast' egg production (Eckelbarger & Watling, 1995; Eckelbarger & Young, 1997). Vitellogenesis in this species begins at oocyte diameters of 25-30 μm and ova are 135-145 μm in diameter when deposited in egg capsules (Van Gaest, 2006).

Egg capsules of *Bathynerita naticoidea* were found to range in size from 1.2 x 0.9 mm to 2.9 x 2.15 mm and contain 25-180 embryos (Warén & Bouchet, 2001; Zande, 1994). Large germinal vesicles are present in oocytes immediately after encapsulation, which demonstrates that oocytes are deposited immediately after fertilisation before the germinal vesicle has time to break down (Webber, 1977). This is consistent with other neritids, which store sperm in the pallial oviduct; fertilization occurs as the oocytes pass by this structure (Webber, 1977). Oocytes and embryos are negatively buoyant and encapsulated development occurs for approximately four months at ambient temperature (8°C) in the laboratory (Van Gaest, 2006).

Van Gaest (2006) suggests synchronous, seasonal gametogenesis in *Bathynerita naticoidea* based on oocyte size-frequency analysis of specimens sampled in February, March, July, September and December (2002-2005) from the Brine Pool cold seep (Gulf of Mexico). Cohorts of pre-vitellogenic oocytes, however, were found in all individuals examined and large

oocytes were present in individuals from all months. Van Gaest (2006) attributes the presence of large oocytes across all months to incomplete spawning of oocytes and argues that it is common for prosobrach gastropods to have all stages of gametogenesis regardless of seasonality (Webber, 1977), concluding that gametogenesis is synchronous and seasonal in this species at the Gulf of Mexico cold seeps.

Zande (1994) argued that *Bathynerita naticoidea* undergoes direct development and hatches out of capsules as crawl-away juveniles. The limited dispersal potential inherent in direct development provides no obvious mechanism for the colonization of new seeps, nor does it explain the wide distribution of this species at isolated cold seeps (Van Gaest, 2006). The relatively large number and small size of embryos within egg capsules laid by *B. naticoidea* indicate planktotrophic development (Thorson, 1950; Bouchet & Warén, 1994; Warén & Bouchet, 2001). Laboratory observations of larvae feeding on microalgae immediately after hatching and the size range of larvae collected in MOCNESS samples also support planktotrophy in this species (Van Gaest, 2006).

It has been hypothesised that the larvae of *Bathynerita naticoidea* are released during the spring, roughly around the time of the spring phytoplankton bloom in the Gulf of Mexico (Van Gaest, 2006). This would suggest a coupling between production in surface waters and the release of larvae (Müller-Karger *et al.*, 1991). Evidence for seasonality in planktotrophic species inhabiting the Brine Pool is also reported for the mussel *"Bathymodiolus" childressi* (Tyler *et al.*, 2007) and the Alvinocaridid shrimp *Alvinocaris stactophila* (Copley & Young, 2006). Non-planktotrophic species from this cold seep appear to have continuous reproductive patterns (see table 3 and below). Van Gaest (2006) suggests that the selective pressure for seasonal reproduction in *B. naticoidea* derives from the necessity of the planktotrophic larvae to obtain an adequate amount of food in the euphotic zone during the spring phytoplankton bloom.

Based on the presence of *Bathynerita naticoidea* larvae in plankton tows from the upper 100m of the water column in February, a dispersal period of at least eight months was proposed (Van Gaest, 2006). Laboratory observed vertical swimming behaviour immediately after hatching coupled with swimming speeds suggest that the larvae of *B. naticoidea* swim to the euphotic zone soon after hatching and probably reach the photic zone before energy reserves are depleted (Van Gaest, 2006). Laboratory observations also demonstrated that *B. naticoidea* larvae can tolerate the high temperatures found in the upper water column in the Gulf of Mexico throughout the year, and the salinities in the upper water column and the adult habitat (Van Gaest, 2006).The length of larval life, swimming behaviour, and presence of larvae in the upper water column suggests the potential for long-distance dispersal of *B. naticoidea* (Van Gaest, 2006).

In summary, there appears to be no typical reproductive pattern in gastropods from deep-sea chemosynthetic communities. This has been demonstrated by evidence of continuous, quasi-

continuous and seasonal reproductive patterns; broadcast spawning and forms of internal fertilization, and development modes include brooding, lecithotrophy and planktotrophy. Evidence from this class therefore supports the paradigm that there is no reproductive pattern typical of deep-sea chemosynthetic systems.

Class Bivalvia

One of the most conspicuous taxa in vent and seep habitats are the Bivalvia (e.g. Berg, 1985; Fustec *et al.*, 1987). Vesicomyids and mytilids often dominate the molluscan fauna in these environments (Tyler & Young, 1999). The majority of reproductive studies on the Bivalvia have generally focused on mytilid and vesicomyid species.

Vesicomyidae

Vesicomyids host chemoautotrophic sulfide-oxidising bacteria and are always found associated to reducing environments, where they are consistently one of the dominant components (Parra *et al.*, 2009). Information regarding the reproductive patterns of the vesicomyidae is rather limited.

Berg (1985) reported delayed reproduction in *Calyptogena magnifica* from the Galapagos and EPR, with full maturity reached at ~44% of their maximum size. First signs of sexual maturity are estimated to occur between 1-4 yrs, with fully ripe condition at 3-15 yrs (Berg, 1985). Although most bivalves are gonochoric, external sexual dimorphism is unusual. For *C. pacifica*, variation in the shell shape was described by Dall (1981) and this was later suggested to demonstrate sexual dimorphism. Later the dimorphism was proposed for the whole genus *Calyptogena* (Kylova & Sahling, 2006) but it was not confirmed. External sexual dimorphism has been described in *C. gallardoi* (Parra et al., 2009).

Vesicomyids studied to date exhibit large gonads embedded in the posterior-dorsal part of visceral mass adjacent to the digestive gland and surrounding the reduced gut and are commonly organized in ramified tubular ancini (Parra *et al.*, 2009). Gametogenesis is intragonadal (Tyler & Young, 1999). In male *Calyptogena gallardoi* mature sperm are stored near genital openings in ancini lined with a secretor epithelium that resembles a seminal receptacle (Parra *et al.*, 2009). It is not unusual for a seminal receptacle to occur in gonochoric bivalves (Jespersen & Lützen, 2001). Such a structure would allow for the storage of large volumes of spermatozoids ready to be spawned once unpredictable appropriate environmental conditions occur (Parra *et al*., 2009). Sperm are neutrally buoyant (Fujikura *et al.*, 2007) and show inter-species variations in shape and flagellum length (see Beninger & Le Pennec, 1997).

Transovarial endosymbiont acquisition has been reported for *Calyptogena soyoae* (Endow & Ohta, 1990), *C. magnifica*, *C. phaseoliformis* and *C. pacifica* (Cary & Giovanni,

1993), underscoring the closeness of the host-symbiont relationship in these species. By comparison, in the mixotrophic species studied to date, post-spawning transmission seems to be the rule (Le Pennec & Beninger, 2000), as shown for *Bathymodiolus thermophilus* (Herry & Le Pennec, 1987).

The vesicomyids fall between the extremes of the smallest and largest oocytes reported in bivalves from deep-sea chemosynthetic environments. The largest oocyte diameter is found in the vent species *Calyptogena magnifica* (482 µm; Berg, 1985). Smaller mature oocytes (~200 and 273 µm in diameter) are reported for *Vesicomya* cf. *venusta* and *C. gallardoi* respectively (Heyl *et al.*, 2007; Parra *et al.*, 2009). In *C. gallardoi*, 1-2 eggs are enclosed in gelatinous capsules (Berg & Alatalo, 1982) and mature oocytes are driven toward the genital opening through evacuator conduits lined by 'paddle' cilia (Parra *et al.*, 2009).

The large oocytes of vesicomyids indicate lecithotrophic development (e.g. Berg, 1985; Fiala-Medioni & Le Pennec, 1989; Lisin *et al.*, 1997; Parra *et al.*, 2009). The traditional interpretation of lecithotrophic development as a short-term planktonic stage in conditions found in reducing habitats could be untrue (Beninger & Le Pennec, 1997). As these authors inferred for the solemyid *Acharax alinae*, large amounts of yolk could enable a prolonged larval stage of vesicomyids to disperse through cold, oligotrophic areas which surround the scattered reducing habitats, explaining the wide but disconnected areas inhabited by some species.

Species of *Calyptogena* studied to date all appear to spawn freely into the water column. Vesimomyids form large aggregations that are interpreted as being reproductively advantageous for broadcast spawners (Momma *et al.*, 1995). So far, information concerning temporal variations and seasonal reproductive patterns of vesicomyids is unclear. The only reproductive study to date that has examined gametogenesis at different times of the year was on the cold seep clams *Calyptogena kilmeri* and *C. pacifica* (Lisin *et al.*, 1997). Although analysis of gonadal development through the year suggest a seasonal peak in reproductive output in *C. kilmeri*, similar analyses indicated a continuous reproductive pattern in *C. pacifica*. In *C. kilmeri* the proportion of the gonad that is reproductively active varied with season but the mean oocyte size did not (Lisin *et al.*, 1997). These authors speculated that although *C. kilmeri* receives all or most of its nutrition from endosymbiotic bacteria, rich seasonal phytoplankton productivity off central California may contribute indirectly to seasonal reproduction by its possible influence on larval development and survival. However, their sample size was small, spawning periodicity in *C. kilmeri* is questionable (based on the current evidence) and larval development is thought to be non-planktotropic for this species.

In *Vesicomya* cf. *venusta* (Blake Ridge seep) five stages of reproductive condition were distinguished for the female gametes, all of which were present simultaneously (Heyl *et al.*, 2007). Most females were in the developing and ripe stages, while all males were in the ripe stage (Heyl *et al.*, 2007). Continuous reproduction was also proposed for *Calyptogena*

magnifica, *C. laubieri*, *C. phaseoliformis* and *C. gallardoi*, due to the simultaneous presence of all stages of gametes in the gonads (Berg, 1985; Fiala-Medioni & Le Pennec, 1989; Parra *et al.*, 2009). Observations of *in situ* spawning events of *C. soyoae* and *C. okutanni* made over 1 yr revealed no evidence of a seasonal pattern (Fujikura *et al.*, 2007). This supports previous suggestions of continuous reproduction in *C. soyoae*, based on *in situ* observations of spawning and TEM (transmission electron microscopy) observations of gonads in specimens collected in summer and autumn (Endow & Ohta, 1990; Momma *et al.*, 1995; Fujiwara *et al.*, 1998).

The absence of seasonal peaks in reproductive output does not mean that there is no synchrony in reproduction efforts. Fujiwara *et al.* (1998) demonstrated with *in situ* experimentation that spawning of the cold seep clam *Calyptogena soyoae* occurs in response to a 0.1-0.28°C temperature rise. Males spawned first, usually followed 7-11 minutes later by the spawning of females, but not every male spawning event was followed by the spawning of females (Fujiwara *et al.*, 1998). Although temperature change is one of the few possibilities for an environmental cue in the deep sea and is known as a cue for shallow-water bivalves (Galtsoff, 1932; Loosanoff, 1937), the cause of temperature rise of this magnitude at cold seeps is not known (Tyler *et al.*, 1999).

Fujikura *et al.* (2007) studied spawning of *Calyptogena soyoae* and *C. okutanii* using *in situ* observations over 1 yr at Japanese cold seeps. Female spawning was always preceded by male spawning and decreased near-bottom current speeds. Egg releases usually followed sperm release within 10 minutes. Fujikura *et al.* (2007) hypothesised that egg release was either triggered by the presence of sperm in the water column and a decreasing current speed, or that egg release was induced when compounds in or released with sperm exceeded a threshold concentration. This study supports the suggestion made by Fujiwara *et al.* (1998) that gamete presence in the water column may act as a secondary cue for female spawning.

Successful fertilisation is a crucial factor in the life-history of free-spawning aquatic animals (e.g. Giese & Kantani, 1987; Pechenik, 2005). One of the primary benefits of reproductive synchronisation for sperm and egg release by broadcast spawners is the increased likelihood of fertilisation (Van Dover, 2000). How this synchrony is maintained in vent and seep taxa still needs answering. Further study of vesicomyid reproductive patterns has the potential to enhance our understanding of how disconnected populations of vesicomyids are established and maintained and to differentiate traits specific to the family (Parra *et al.*, 2009).

Solemyidae

Protobranch bivalves are rare at vents (Tyler & Young, 1999). *Acharax* is one of only two genera of the order Solemyioida to have survived beyond the Palaeozoic. *Acharax alinae* have been found in soft sediments peripheral to vents of the Lau Basin (Fiji); two specimens were examined by histology and TEM by Beninger & Le Pennec (1997). The structural

characteristics of the gametogenic cells were similar to those described previously for littoral species. Oocytes reached 660 µm in diameter and all sizes were present at the same time, suggesting continuos spawning (Beninger & Le Pennec, 1997). The spermatozoa were enormous, with a head and midpiece 28 µm long and a tail up to 100 µm. These are considerably longer than sperm of protobranchs from deep-sea sediments (Tyler *et al.*, 1993) and are probably a co-evolutionary consequence of the extremely large oocyte size (Beninger & Le Pennec, 1997). Because the larva of the Solemyidae is a non-feeding stage, the extraordinarily large and vitellus-rich oocytes could be an adaptation for an extended lecithotrophic development, which would favour either long-range dispersal or protracted benthic development (Beninger & Le Pennec, 1997). The larval stage could be further extended by the low temperature of the deep-sea environment (Mullineaux & France, 1995). Extended lecithotrophic development appears characteristic of some ancient lineages (Blacknell & Ansell, 1974). However, it seems that there is no single development mode amongst taxa inhabiting deep-sea chemosynthetic environments.

Mytilidae

Little is known of reproduction in mytilids from chemosynthetically supported environments. Bathymodiolid mussels are characterised by high reproductive diversity, especially in their state of sexuality (Le Pennec & Beninger, 1997, 2000). Examinations suggest that *Bathymodioulus thermophilus* (Galapagos vents), *B. elongatus* (Fiji Basin) and *B. azoricus* (Lucky Strike, MAR) are hermaphrodites (Berg, 1985; Le Pennec & Beninger, 1997; Comtet & Desbruyères, 1998). Strong evidence of hermaphrodism has also been documented in *Idas washingtonia* from several whale skeletons (Tyler *et al.*, 2009), whereas *B. thermophilus* (EPR), *B. puteoserpentis* (Snake Pit, MAR) and *"B". childressi* (Louisiana Slope) are gonochoric (Le Pennec & Beninger, 1997; Eckelbarger & Young, 1999; Tyler & Young, 1999).

Gametogenesis is mytilid-like, beginning in the posterior gonad and continuing through the mantle (Tyler & Young, 1999), indicating that this process is phylogenetically constrained in the Mytilidae (Eckelbarger & Young, 1999). Phylogenetic conservatism is also evident from TEM observations of male gametes (Le Pennec & Beninger, 1997; Kádár *et al.*, 2006).

Vitellogenesis appears to be largely autosynthetic in modiolids (Eckelbarger & Young, 1999) and egg sizes are small. The mixotrophic Mytilidae possess the smallest oocytes of the entire vent and seep species, ranging from 40-50µm (*Bathymodioulus thermophilus*, 13°N EPR) (Le Pennec *et al.*, 1984) up to 80 µm in *"B". childressi* (Gulf of Mexico) (Eckelbarger & Young, 1999). As with other modiolid bivalves, *Idas washingtonia* has a small egg size (<50 µm) and high fecundity, indicative of planktotrophic development (Tyler *et al.*, 2009).

Whether endosymbionts contribute energy to larvae is unclear. Indirect evidence suggests that the vent mussels *Bathymodioulus. azoricus, B. puteoserpentis* and *B. thermophilus* aquire

their symbionts from the environment (horizontal transmission) rather than transferring them via the ovum (Won *et al.*, 2003; Herry & Le Pennec, 1987). This contrasts with evidence of trans-gonadal acquisition of obligate symbionts in vesicomyid clams. Cary & Giovannoni (1993) suggested that symbionts are passed directly from adults to larvae in eggs (vertical transmission) in *B. thermophilus*. The presence of bacterial symbionts in the gill tissue of postlarvae and juveniles of the seep mussel *B. heckerae* and the vent mussel *B. azoricus* is inferred from TEM (Salerno *et al.*, 2005), but the stage of infection is unknown. Vertical symbiont transmission would presumably offer larval mussels immediate access to symbiotic energy resources (Trask & Van Dover, 1999). Using isotopic analysis of post-larvae, Salerno *et al.* (2005) found no convincing evidence that the larval diet of *B. heckerae* or *B. azoricus* was of photosynthetically derived origin.

Small oocyte size, high fecundity and evidence from shell morphology suggest planktotrophic development for *Bathymodiolus* species (e.g. Lutz *et al.*, 1980; Gustafson & Lutz, 1994; Le Pennec & Beninger, 1997; Gustafson *et al.*, 1998) but larvae have not been observed feeding in culture. Arellano & Young (2009) studied development in *"Bathymodiolus" childressi*. By comparing calculated settlement times to known spawning seasons, these authors estimated a larval life duration of 5-13 months and concluded that the larvae of *"B". childressi* may be teloplanic (long-distance dispersing). This estimate shows great variability and rests on the assumption that current knowledge of the timing of spawning is correct. However, teloplanic dispersal would provide a biological explanation for the widespread distribution of *"B". childressi* throughout the Gulf of Mexico. The dispersal capabilities of *"B". childressi* may also have contributed to the trans-Atlantic distribution of closely related bathymodiolids (Olu-Le Roy *et al.*, 2007).

The mussel genus *Bathymodiolus* is one of the most speciose and widespread genera found at hydrothermal vents and cold seeps (Tyler *et al.*, 2007). Dispersal is potentially widespread (Berg, 1985; Lutz, 1988; Carney *et al.*, 2006) and conforms to the 'island' model (Vrijenhoeck, 1997). Planktotrophic larval development may account for its wide distribution and high gene flow.

Tyler *et al.* (2009) suggested that the occurrence of protandric hermaphrodism, high fecundity and planktotrophic development may be adaptations of *Idas washingtonia* to the ephemeral nature of their habitat. These authors examined gametogenesis in *I. washingtonia* using samples collected from several whale falls over a range of seasons and several years. They suggested that *I. washingtonia* has a seasonal, non-continuous reproductive cycle. Gametogenesis in *Bathymodiolus* from shallow Atlantic vents and the bathyal Gulf of Mexico cold seeps is also seasonal, whereas in the Pacific this process appears to be quasi-continuous (Tyler *et al.*, 2009). Histological evidence from time-series samples of *"Bathymodiolus" childressi* (Gulf of Mexico) demonstrates strongly periodic and synchronous gametogenesis and

periodic spawning each year over an extended period from October through February (Tyler *et al.*, 2007). There is also evidence that *"B." childressi* recruitment is seasonal at the Brine Pool (MacDonald *et al.*, 1990 a, b). Discontinuous spermatogenesis has been suggested for *B. puteoserpentis* (MAR) and *B. elongatus* (north Fiji Basin: Le Pennec & Beninger, 1997), but not for *B. thermophilus* (Galapagos Rift: Lutz *et al.*, 1980; Berg & Alatalo, 1982). Evidence from population biology has shown periodicity in recruitment for *B. azoricus* (Comtet & Desbruyères, 1998; Khripounoff *et al.*, 2001), and gametogenic development (Colaço *et al.*, 2006, Dixon *et al.*, 2006) at MAR vents. Further evidence supporting annual reproduction in *B. azoricus* was provided by observations of the onset of spermatogenesis in captivity during a 1 yr aquarium study (Kádár *et al.*, 2006)

Strong evidence of seasonal reproductive patterns in several mytilid species contrasts with the continuous reproductive activity reported in gastropods and other bivalves. Seasonality in species inhabiting chemosynthetically driven habitats may seem counter-intuitive because of the continuous energy availability (Tyler *et al.*, 2009). Although the role of heterotrophy remains poorly understood in potential mixotrophic species inhabiting these environments (Riou *et al.*, 2010), the non-continuous reproduction of some species is consistent with their non-exclusive reliance on endosymbionts (Fiala-Medioni *et al.*, 1986, 1994, 2002; Fisher *et al.*, 1988; Page *et al.*, 1991; Trask & Van Dover, 1999; Southward *et al.*, 2001). Both *Idas washingtonia* and mytilids of the genus *Bathymodiolus* from chemosynthetically driven ecosystems are capable of filter feeding (Page *et al.*, 1990, 1991; Smith & Baco, 2003). In all cases of seasonality in *Bathymodiolus* and *I. washingtonia*, the seasonal reproductive pattern appeared to be driven by food availability for larvae feeding in the water column during the period of the vertical flux of spring bloom production to the seabed (Dixon, 2006; Tyler *et al.*, 2007, 2009). The availability of phyto-detritus to these mixotrophs may influence the seasonal reproductive pattern (Tyler *et al.*, 2009).

Limidae

Jarnegren *et al.* (2007) used histology, laboratory, and *in situ* observations to examine reproduction in *Acesta* sp. (Gulf of Mexico cold seeps). Although reproductive adults were functionally gonochoric, protandric hermaphrodism was evident; males changed to females at approximately 77 and 90 mm shell height respectively (Jarnegren *et al.*, 2007). Not all individuals changed sex and the sex ratio was male-biased. *Acesta* sp. is a broadcast spawner, with individuals releasing either sperm or eggs but not both (Jarnegren *et al.*, 2007). Size of the larval shell and spawned egg size (~179 µm) suggest lecithotrophic development and the reproductive pattern was continuous (Jarnegren *et al.*, 2007).

Pectinidae

Bathypecten vulcani is considered a relict species from the Paleozoic and it is the only pectinid species reported from hydrothermal ecosystems (Le Pennec *et al.*, 2002). Histological and ultra-structural studies revealed that spermatogenesis is identical to that of littoral pectinids (Le Pennec *et al.*, 2002). Continuous gametogenesis with multi-annual spawning has been suggested (Le Pennec, unpublished in Le Pennec *et al.*, 2002) based on specimens from the EPR, although it is not clear how many specimens were studied and over what time-scale they were sampled. Le Pennec (unpublished in Le Pennec *et al.*, 2002) documented oocyte size at 100 µm. How quickly this species reaches sexual maturity, its fecundity and form of larval development remain to be determined.

Phylum Annelida

Class Polychaeta

Polychaete annelids form a significant part of deep-sea chemosynthetic assemblages. Species from hydrothermal vents, methane seeps and hydrates and whale falls have been examined to determine their reproductive biology, resulting in more studies on the life-history traits of polychaetes than for any other class.

Orbiniida, Orbiniidae

Methanoaricia dendrobranchiata is associated with dense populations of the mussel "*Bathymodiolus" childressi* at brine pools on the Louisiana slope, Gulf of Mexico (Eckelbarger & Young, 2002), where it thrives in hypoxic and highly sulfidic conditions (Smith *et al.*, 2000; Nix *et al.*, 1995). The species is gonochoric, although the sexes cannot be distinguished externally, and the reproductive morphology is similar to that of other orbiniids (Eckelbarger & Young, 2002).

In the female, synchronous, intraovarian egg development occurs with the release from the ovary of large (~280 µm), yolky eggs into the coelom at first meiotic metaphase (Eckelbarger & Young, 2002). The mature spermatozoon resembles an ent-aquasperm, unlike the ect-aquapserm described in other orbiniids (Eckelbarger & Young, 2002). Eckelbarger & Young (2002) suggested that this unusual spermatozoon morphology has evolved as a result of the hypoxic environment in which the adults live, and that fertilization biology is probably modified in some way to minimize sperm exposure to high levels of sulfide. Intracoelomic germinal vesicle breakdown may indicate that fertilisation occurs very quickly after egg release

as a means to minimise egg exposure to sulfides (Eckelbarger & Young, 2002). It is not yet known if copulation or pseudocopulation occurs in this species, or if gametes are simply spawned. No evidence for sperm storage has been described and the details of fertilisation, fecundity and sexual maturity remain unknown.

Based on the life histories of other orbiniids, Eckelbarger & Young (2002) predicted that *Methanoaricia dendrobranchiata* is an annual, iteroparous breeder that produces an egg mass or cocoon. Although its egg size may be indicative of non-planktotrophic development (Thorson, 1951) and reproductive traits in the Orbiniidae generally show strong phylogenetic constraint (see Eckelbarger & Young, 2002), there is no guarantee that these predictions equate to reality. For example, the ent-aquasperm sperm of *M. dendrobranchiata* is currently unique within the family (Eckelbarger & Young, 2002) and is not phylogenetically conservative. The collection and analysis of time-series samples are required to determine the reproductive pattern of *M. dendrobranchiata* and further study of this species is required to determine and understand its life-history biology and dispersal capabilities.

Phyllodocida, Hesionidae

Eckelbarger *et al.* (2001) described gametogenesis, spawning behaviour, and early development in the methane-seep polychaete *Hesiocaeca methanicola* from the Green Canyon site in the Gulf of Mexico. *H. methanicola* is gonochoric and has a gametogenic pattern similar to that described for the hesionid *Kerfesteina cirrata* (Olive & Pillai, 1983; Eckelbarger *et al.*, 2001). Vitellogenesis involves both autosynthetic and heterosynthetic processes and, in addition to ovarian structure, suggests a relatively slow process of egg formation (Eckelbarger & Young, 2001). Oocyte size-frequency analysis of adult females indicates a wide range of oocyte sizes and vitellogenic stages, suggesting asynchronous gametogenesis (Eckelbarger & Young, 2001).

Spermatogenesis in *Hesiocaeca methanicola* differs substantially from other hesionids and produces ect-aquasperm which is consistent with observations of external fertilization (Eckelbarger & Young, 2001). Gametes are broadcast and the males spawn exclusively through the anus, behaviour previously unknown in polychaetes (Eckelbarger & Young, 2001). The presence of posterior coelomic ducts for sperm release in the males suggests that the species is iteroparous and undergoes repeated spawning (Eckelbarger *et al.*, 2001), although breeding frequency has not been established for this species due to a lack of time-series samples.

Fertilized eggs develop into a trocophore and planktonic larval development was estimated to be at least 21 days (Eckelbrager & Young, 2001). Later stages of development have not been studied, but these authors hypothesised, on the basis of egg size and developmental rate, that dispersal distance may exceed the 21 day estimate for *Lamellibrachia* sp. (Young *et al.*, 1996).

Appendix 1

Phyllodocida, Polynoidae

Polynoids are commonly encountered at hydrothermal chimneys, diffuse venting areas and whale falls (e.g. Chevaldonné *et al.*, 1998; Jollivet *et al.*, 2000). The reproductive features of known polynoid species show conservatism in life-history patterns with the majority of species having seasonal breeding, intraovarian oogenesis, small eggs that develop planktotrophically and simple ect-aquasperm (Eckelbarger *et al.*, 2005). These reproductive traits seem to be phylogenetically constrained (Eckelbarger *et al.*, 2005), yet notable exceptions have been described from hydrothermal vents and whale falls.

To date three polynoid species from deep-sea chemosynthetic environments have had their reproductive features described. *Branchipolynoe seepensis* is a commensal living in the mantle cavity of mussels and *Opisthotrochopodus* sp. nov. is a free-living polychaete that also inhabits vent mussel beds (Van Dover *et al.*, 1999; Jollivet *et al.*, 2000). *Bathykurila guaymasensis* is the only annelid known to be shared between hot vents and whale falls (Glover *et al.*, 2005). All three species exhibit pronounced sexual dimorphism (Van Dover *et al.*, 1999; Jollivet *et al.*, 2000; Glover *et al*., 2005). A sexually dimorphic variation in size with larger females has been observed in *B. seepensis* and *B. guaymasensis*, but in *Opisthotrochopodus* sp. nov. males were significantly larger (Van Dover *et al*., 1999; Jollivet *et al.*, 2000; Glover *et al.*, 2005).

Glover *et al.* (2005) proposed asynchronous gametogenesis with continuous recruitment for *Bathykurila guaymasensis*, based on the range of oocyte sizes observed in specimens and the lack of a significant cohort of juveniles in the population. A continuous reproductive pattern has also been suggested for *Branchipolynoe seepensis* and *Opisthotrochopodus* sp. nov. based on population size structure and oocyte size-frequency distributions (Van Dover *et al*., 1999). Jollivet *et al.* (2000) found evidence for discrete breeding periods in *B. seepensis*. In contrast, seasonal trends are apparent in shallow-water polynoid species (Daly, 1972; Britayev, 1991).

The maximum mature oocyte diameter recorded for *Bathykurila guaymasensis* was ~220 µm (Glover *et al.*, 2005). This is large compared to shallow water polynoids that generally undergo planktotrophic development and have small eggs (~120 µm) (Giagrande, 1997), but similar to the large eggs (~390 µm maximum diameter) recorded for *Branchipolynoe seepensis* and *Opisthotrochopodus* sp. nov. (Van Dover *et al.*, 1999).

Evidence suggests that internal fertilisation and sperm storage occurs in *Branchipolynoe seepensis* (Van Dover *et al*., 1999; Jollivet *et al*., 2000) and *Opisthotropodus* sp. nov. (Van Dover *et al.*, 1999), requiring coordinated mating. In *B. seepensis* sperm bundles are deposited by the male onto female genital papillae, implying pseudocopulation as the males have no penis (Jollivet *et al.*, 2000). Glover *et al.* (2005) observed evidence for a spermathecae-gonoduct containing filiform sperm in *Bathykurila guaymasensis* and determined that the enlarged nephridial papillae are used as sperm transfer organs. The suggestion of internal fertilisation and

sperm storage in *B. guaymasensis* is consistent with that for *B. seepensis* and *Opisthotrochopodus* sp. nov. (Van Dover *et al.*, 1999; Jollivet *et al.*, 2000). More detailed histological study of *B. guaymasensis* and *Opisthotrochopodus* sp. nov. would be required to confirm sperm storage and internal fertilisation in these two species. The presence of spermathecae was unknown previously in the Polynoidae (Jollivet *et al.*, 2000). Shallow-water species deploy external fertilization (Daly, 1972; Britayev, 1991). Sperm storage, internal fertilisation and pairing may be an adaptive response to the unstable vent environment (Tyler & Young 1999) since it is also a general trend in alvinellid, ampharetid and siboglonid species (e.g. McHugh, 1989; Chevaldonné & Jollivet, 1993; McHugh & Tunnicliffe, 1994; Zal *et al.*, 1994, 1995; Chevaldonné *et al.*, 1997; Desbruyères *et al.*, 1998; Hilário *et al.*, 2005).

Most of the vent polychaetes studied to date produce relatively few large oocytes (Jollivet *et al.*, 2000) although there are exceptions. For example, *Riftia pachyptila* spawns numerous small fertilized oocytes (Hilário *et al.*, 2005). *Branchipolynoe seepensis*, *Opisthotrochopodus* sp. nov. and *Bathykurila guaymasensis* all produce large eggs and a hypothesised lecithotrophic mode of development (Van Dover *et al.*, 1999; Jollivet *et al.*, 2000; Glover *et al.*, 2005). Lecithotrophic development has been correlated with poor dispersal ability in some organisms (Vrijenhoek, 1997). More recent evidence suggests that lectithotrophs may actually be favoured in cold, deep-sea oligotrophic waters with little available nutrition for feeding larvae (e.g. Tyler & Young, 1999).

Genetic analyses based on ribosomal and mitochondrial sequences found no genetic differentiation over large spatial scales for either the Atlantic or Pacific *Branchipolynoe* species (Jollivet *et al.*, 1998, Hurtado *et al.*, 2004), suggesting either possible long-distance dispersal or a recent colonisation of the sites. In contrast, Daguin & Jollivet (2005) demonstrated that *B. seepensis* displayed genetic breaks along the MAR between populations ranging from 14 to 35° N, indicating that dispersal could be more limited than previously thought (Plouviez *et al.*, 2008).

Glover *et al.* (2005) hypothesised high dispersal abilities for *Bathykurila guaymasensis* based on the presence of indistinguishable morphotypes of the species at both hydrothermal vents in the Guaymas Basin and whale bones in the California basins. This hypothesis is supported by molecular evidence, with broad sharing of COI haplotypes between the Santa Cruz and Santa Catalina basin whale-fall sites (Glover *et al.*, 2005). These results indicate that vent polynoid species with (inferred) modes of lecithotrophic development can, in some cases, disperse effectively across relatively long distances (thousands of kilometres) (Glover *et al.*, 2005).

The polynoids studied to date exhibit a high degree of similarity to each other (despite different habitats), but significant differences to polynoids from other environments, in their reproductive traits. Sexual dimorphism, elongate sperm, asynchronous gametogenesis within

individuals and among individuals within a population, apparent sperm storage and specialized mode of fertilisation are attributes shared with various polychaete species in deep-sea chemosynthetic communities, suggesting that these traits may be adaptations to these environments.

Sabellida, Siboglonidae

As a result of the ecological importance of siboglonids in deep-sea chemosynthetic assemblages their reproductive traits and dispersal biology have been widely discussed. Most siboglonids are sexually dimorphic (e.g. Brooke & Young, 2009) and, with the exception of *Osedax*, appear to have males and females of equivalent size (Southward, 1999; Rouse, 2008; Brooke & Young, 2009). In *Osedax* sp., the females have dwarf males that occur in 'harems' in their tube lumens (Braby *et al.*, 2007; Rouse, 2008). These males appear to be paedomorphic, resembling siboglonid larvae (Southward, 1999; Rouse *et al.*, 2004, 2008, 2009). The similarity of *Osedax* males to the larvae of *Osedax* and other siboglonids, in addition to similarities to the neuromuscular organization seen in other annelid larvae, supports the hypothesis of paedomorphosis (Worsaae & Rouse, 2010). Rouse *et al.* (2004) hypothesized environmental sex determination in *Osedax* whereby the larvae that settle on bones mature as females and the larvae that subsequently land on females become males. Strong circumstantial evidence supports this hypothesis (Rouse *et al.*, 2008; Vrijenhoek *et al.*, 2008), although definitive experiments are still required.

Data on when siboglonids reach sexual maturity are lacking. Growth rates are poorly understood and appear to be highly variable. *Riftia pachyptila* inhabits ephemeral hydrothermal vents and has one of the fastest growth rates known for any marine invertebrate (Lutz *et al.*, 1994). Rapid growth and early maturation may be expected in species that occupy a relatively ephemeral environment (Grassle & Grassle, 1974) such as vents and whale falls, e.g. as described in *Osedax roseus* (Rouse, 2008). Conversely, the vestimentiferans *Lamellibrachia luymesi* and *Seepiophila jonesi*, which inhabit the more stable environment of cold seeps, are long-lived and extremely slow growing.

High concentrations of wax esters have been reported in the posterior region and gonads of *Ridgeia piscesae* and *Seepiophila jonesi* and wax-rich eggs have been found in *Lamellibrachia luymesi* and *Riftia pachyptila*, suggesting that vestimentiferans store energy as lipids in the gonads (Young *et al.*, 1996; Gardiner *et al.*, 2001; Marsh *et al.*, 2001; Hilário *et al.*, 2008). The proportion of the female body that is dedicated to the ovary is high in *Osedax* (Rouse *et al.*, 2004, 2008; Glover *et al.*, 2005; Fujikura *et al.*, 2006), correlating with high reproductive output (Rouse *et al.*, 2009). Eggs sizes are typically small (e.g. ~105 µm *Lamellibrachia* sp.; ~115 µm *Escarpia* sp. (Young *et al.*, 1996)) and numerous (fertilized) oocytes are spawned (Hilário *et al.*, 2005).

With the exception of *Osedax*, insemination is internal in all siboglonid species that have been examined (Hilário *et al.*, 2005; MacDonald *et al.*, 2002; Miyake *et al.*, 2006; Rouse *et al.*, 2009), via spermatophores in frenulates (Southward, 1999) or spermatozeugmata in vestimentiferans (Jones & Gardiner, 1985; Hilário *et al.*, 2005). The hypothesis of internal fertilisation in *Osedax* is supported by high fertilisation rates and the usual proximity of the dwarf males to the trunk oviduct of females (Rouse *et al.*, 2004, 2008, 2009; Vrijenhoek *et al.*, 2008). The sperm transfer mechanism and exactly where fertilisation occurs in *Osedax* species are yet to be determined. Rouse (2009) suggested that the close proximity of *Osedax* dwarf males to female *Osedax* may ensure a greater fertilisation success rate than seen in other siboglonids which, in most cases, must obtain spermatozeugmata or spermatophores spawned by males freely into the water (Bakke, 1976; Hilário *et al.*, 2005). Sperm storage may also maximise fertilisation success in some siboglonids (see below). Some species, e.g. *Ridgeia piscesae* and *Tevnia jerichonana* also have efficient sperm transfer from males to females via direct contact between the sexes (Southward & Coates, 1989; MacDonald *et al.*, 2002; Southward *et al.*, 2005).

Hilário *et al.* (2005) reported sperm storage at the posterior end of the oviduct (the spermatheca) and internal insemination in *Riftia pachyptila*, *Ridgeia piscesae*, and *Tevnia jerichonana* from Pacific vents, and *Lamellibrachia luymesi* and *Seepiophila jonesi* from cold seeps in the Gulf of Mexico. The presence of sperm within female tissues has been documented for *Osedax* (Katz *et al.*, 2008). However, the mechanism of sperm transfer and whether sperm are stored by females in this genus remain to be determined.

A continuous reproductive pattern based on analyses of time-series data has been suggested for the siboglonid species studied. Observations suggest that spawning does not appear to be synchronized across local populations of *Riftia pachyptila* (Van Dover, 1994). *Osedax* females were not synchronised in their release and only one, sometimes two females, were witnessed in an observed area to spawn at any one time (Rouse *et al.*, 2009).

Siboglonid adults rely on endosymbiotic bacteria for their nutrition (Rouse *et al.*, 2009). The question of whether symbionts are transmitted via the parent or whether they are acquired from the environment is still in debate. Cary *et al.* (1993) found no symbionts in *Riftia pachyptila* oocytes and none have been found in early-stage siboglonids. Slightly later stages with a temporary mouth and alimentary canal contained bacteria in some of their endodermal cells (Southward, 1988; Jones & Gardiner, 1988, 1989; Callsen-Cencic & Flügel, 1995). Cytological and molecular analysis of the spawned oocytes of *Osedax rubiplumius* and *Osedax* sp. revealed no evidence of bacterial endosymbionts, suggesting environmental acquisition of symbionts, as proposed in other siboglonids (Rouse *et al.*, 2009). This hypothesis is supported by the discovery that vestimentiferans of different genera from the same locality host identical

symbionts (Feldman *et al.*, 1997). However, these bacteria have not been identified from the natural environment near vents or seeps (Southward *et al.*, 2005).

Vestimentiferan tube worms at vents and seeps produce lecithotrophic embryos and larvae which can potentially disperse in water columns for up to several weeks (Young *et al.*, 1996; Marsh *et al.*, 2001; Miyake *et al.*, 2006). Spawning process does not appear to be synchronized in siboglonids (e.g. Van Dover, 1994; Rouse *et al.*, 2009). Hilário *et al.* (2005) demonstrated experimentally that zygotes are arrested at the first meiotic phase. Meiosis is completed after eggs are released from the female so the dispersal phase includes the entire embryonic and larval period (Hilário *et al.*, 2005). It has been estimated that the larvae of the vent species *Riftia pachyptila* can disperse more than 100 km over a 5-week period (Marsh *et al.*, 2001). *Osedax* species exhibit similar developmental modes and dispersal times (Rouse *et al.*, 2009). Under optimal circumstances, a dispersal time of this magnitude may be long enough to disperse between adjacent ridge segments or to provide genetic exchange among disjunct seep communities on continental slopes or widely scattered and ephemeral whale bones (Marsh *et al.*, 2001; Young *et al.*, 1996; Hilário *et al.*, 2005; Mullineaux *et al.*, 2002; Jones *et al.*, 2008: Vrijenhoek *et al.*, 2008).

Inferred high fecundity (e.g. Rouse *et al.*, 2009), leading to the release of high numbers of fertilized eggs (Hilário *et al.*, 2005) and a continuous reproductive pattern appear to have enabled the exploitation of widely spaced and ephemeral environments by siboglonids. The breeding traits of internal fertilisation followed by zygote release rather than brooding assures a high level of fertilization without sacrificing dispersal potential of lecithotrophic larvae (Hilário *et al.*, 2005).

Terebellida, Alvinellidae

Alvinellid polychaetes are endemic to hydrothermal vents in the Pacific (Chevaldonné *et al.*, 1997) and generally exhibit wide distributions (Desbruyères & Laubier, 1991). Reproductive strategies have now been investigated in several species and early development stages were obtained in *Alvinella pompejana* (Pradillon *et al.*, 2001).

All described alvinellid species are gonochoric and sexually dimorphic (Pradillon & Gaill, 2007). For example, males have paired modified peribucal tentacles, which are absent in females (Zal *et al.*, 1994; Desbruyères *et al.*, 1998; Zhadan *et al.*, 2000) and genital pore dimorphism has been observed in several species (Zal *et al.*, 1994; Zhadan *et al.*, 2000).

The reproductive anatomy of alvinellids is similar in the species studied to date (e.g. Desbruyères *et al.*, 1998; McHugh, 1989; Pradillon & Gaill, 2003; Zal *et al.*, 1994; Zhadan *et al.*, 2000). Females possess paired oviducts connected to a spermathecae (in which spermatozoids are stored) that opens to a unique genital pore (e.g. Pradillon & Gail, 2003). Alvinellids are the only terebellids known to possess spermathecae (Jouin-Toulmond *et al.*,

2002). Spermathecae have been described in other polychaetes from deep-sea chemosynthetic environments, including polynoids and siboglonids.

The earliest stages of spermatozoa development were described in the coelomic cavity of *Paralvinella palmiformis* and *P. pandorae pandorae* (McHugh, 1989). Ultra-structural investigations revealed that the spermatozoa belong to the introsperm category and displayed modified structures with variation between species (Jouin-Toulmond *et al.*, 2002). Maximum diameter in coelomic oocytes from alvinellid species range from 200-275 µm (Copley, 1998; Copley *et al.*, 2003; Desbruyères *et al.*, 1998; McHugh, 1989; Pradillon & Gaill, 2003; Zal *et al.*, 1994). Ultra-structural analyses revealed autosynthesis of yolk in *Alvinella pompejana* (Pradillon & Gaill, 2003) which, combined with evidence of extraovarian vitellogenesis, suggest slow oogenesis (Pradillon & Gaill, 2007). This contradicts the hypothesis of fast egg production and high reproductive rate for species inhabiting unstable environments (Sanders, 1979). The organization of the genital tract seems to allow storage of a pool of ripe oocytes which could then be spawned at any time, possibly in response to specific environmental or biological cues such as sperm transfer (Pradillon & Gaill, 2003, 2007). This trait would increase spawning flexibility, despite a slow egg production, in the dynamic vent environment. However, spawning cues in deep-sea vents remain poorly understood. Storing gametes may simply be a means of maximising fertilization success.

There seems to be huge variation in alvinellid fecundity. Average values range from 3,900 oocytes per female in *Paralvinella grasslei* to 80,000 in *Alvinella pompejana* (Chevaldonné *et al.*, 1993; Desbruyères *et al.*, 1998; Pradillon *et al.*, 2005a), and up to a maximum of 978,000 in *A. pompejana*, which is among the highest known in polychaetes and depends on the stage of maturity, not the size of the female (Faure *et al.*, 2007). These high fecundities are much greater than those described in other Terebellids inhabiting hydrothermal vents. For example, in the Ampharetidae brood size ranges from 2,000-25,000 coelomic oocytes for *Amathys lutzi*, whereas the range in size is smaller (9,600-12,500) in *Amphisamytha galapagensis* (Blake & Van Dover, 2005).

In several alvinellid species, spermatozoa were observed inside the spermathecae and sperm transfer from males to females via pseudocopulation has been suggested (Desbruyères *et al.*, 1998; Jouin-Toulmond *et al.*, 2002; Zal *et al.*, 1994). *In situ* observations reveal that Alvinellids often leave their tubes to enter the tubes of other individuals (Chevaldonné & Jollivet, 1993; Fustec *et al.*, 1987). Sperm transfer may occur during these events (Pradillon & Gaill, 2007). Pairing of the sexes for reproduction has been suggested for *Paralvinella pandorae irlandei* and *P. grasslei*, based on observations of paired individuals in mucus cocoons (Jollivet, 1993; Zal *et al.*, 1995). Pseudocopulatory behaviour and sperm transfer to the spermathecae is interpreted as a means to avoid gamete loss by avoiding dispersion of gametes and to increase fertilisation efficiency in the highly dynamic environment (Zal *et al.*, 1995). This is hard to

reconcile with Zal's (1994) observations of modified and degenerated sperm in the spermathecae of *P. grasslei*. Degenerated sperm are inconsistent with high fertilization efficiency. However, Zal's (1994) interpretation was based on poorly-fixed material and subsequent TEM investigation of sperm storage in *P. grasslei* has revealed spermathecae packed with well-preserved, non-degenerate spermatozoa (personal observation). The presence of a spermathecae in females enables fertilization by several males and insemination while they are still immature (Faure *et al.*, 2007).

Sperm transfer to the spermathecae does not necessarily mean that fertilization is internal (Pradillon & Gaill, 2007). Internal fertilisation has been suggested, for example, in *Alvinella pompejana* (Chevaldonné *et al.*, 1993), although it is still unknown whether fertilisation occurs in the spermathecae or at spawning when mature oocytes and spermatozoa are emitted through the female genital pore. In *A. pompejana* fertilised eggs have not been found in spermatozoa-filled spermathecae, despite the presence of oviducts packed with ripe oocytes. This has lead to the suggestion that oocytes pass through the spermathecae during spawning with external fertilisation post-spawning (Pradillon & Gaill, 2003). An alternative interpretation would be that the ripe eggs had not yet been fertilised. Internal fertilisation and/or internal insemination may enable maximum reproductive success in the dynamic vent environment by minimizing exposure of gametes to the external environment and maximising contact between gametes. Experimentation is required to determine the site of fertilisation in alvinellids.

The majority of vent species display continuous reproduction. Asynchronous reproduction and a continuous population were suggested for *Paralvinella pandorae* (McHugh, 1989) and *P. sulfincola* (Copley, 1998). In contrast, synchrononous reproduction was proposed for *P. grasslei* (Zal *et al.*, 1995), although Zal *et al.* (1995) did not indicate the spatial scale over which such synchrony may extend. Copley *et al.* (2003) suggested that *P. palmiformis* reproduced synchronously at the vent scale (High Rise vent field), in response to periodic variation linked to tidal regime in environmental factors such as temperature (Chevaldonné *et al.*, 1992; Schultz & Elderfield, 1997). McHugh (1989) proposed that some synchronisation of reproductive development may occur between female *P. palmiformis* at Northeast Pacific vents and inferred discontinuous reproduction with a possible discrete breeding period in this species. Each sample, however, was taken from a different location within the vent on each occasion, so spatial variation in reproductive development was not addressed. Consequently the temporal variation described by McHugh (1989) may have actually been spatial.

Spatial variation in the reproduction and settlement of *Alvinella pompejana* and semi-continuous reproduction with short-term synchrony were described at EPR vents by Faure *et al.* (2007). In *P. palmiformis* spatial variation in reproductive patterns was found at the vent scale, which may reflect the successional mosaic of the vent community, with immature individuals in earlier successional stages (Copley *et al.*, 2003). In *Alvinella pompejana* the dynamic

disturbance/colonisation process results in a mosaic of patches hosting individuals at different reproductive stages (Pradillon *et al.*, 2005a). It has been suggested that well-established *Alvinella* colonies form an isolating layer on chimney walls that may greatly reduce temperature gradients (Le Bris *et al.*, 2005). Only females are only found in such colonies; this may reflect their preference for a milder environment during reproduction (Pradillon & Gaill, 2007).

On the basis of oocyte size, non-planktotrophic development has been hypothesized for all known alvinellid species (Chevaldonné *et al.*, 1997; Copley, 1998; Copley *et al.*, 2003; Desbruyères *et al.*, 1998; Faure *et al.*, 2007; McHugh, 1989, 1995; Zal *et al.*, 1994, 1995). Such types of development often imply limited dispersal capabilities, although larval stages have never been collected and their dispersal abilities remain uncertain.

Genetic evidence has indicated the existence of exchanges between widely scattered populations (Jollivet *et al.*, 1995). A subsequent genetic study (Jollivet *et al.*, 1998) showed that propagules may only be capable of dispersing a few tens of kilometres per generation. Chevaldonné *et al.* (1997) modelled potential larval dispersal of alvinellid polychaetes from the EPR. According to their model, which assumed 100% fertilisation, propagule travelling time cannot exceed 15-30 days, whereas reported distances between sites inhabited by Alvinellids would require longer lasting abilities. Chevaldonné *et al.* (1997) concluded that either some aspects of alvinellid biology have been overlooked and long-distance dispersal does occur, and/or that the spatial-temporal dynamics of vent sites over geological timescales allows short-range dispersal processes to maintain gene flow despite weak dispersal ability in the alvinellidae.

In vitro experiments have shown that embryos of *Alvinella pompejana* exhibit low temperature tolerance and death above 20°C, suggesting that embryos may not develop in the adult colonies (Pradillon *et al.*, 2005b). It has been hypothesised, based on *in vitro* evidence, that early embryos disperse through cold abyssal water in a state of developmental arrest, completing their development when they encounter water warm enough for their growth and survival (~10°C) (Pradillon *et al.*, 2001). This mechanism could potentially allow wide dispersal capabilities, although the potential duration of such dormancy is unknown.

Alvinellids inhabit an ephemeral and spatially patchy environment and must reproduce, disperse and maintain genetic diversity accordingly. Morphological features, such as oviducts and spermathecae, combined with gamete storage may be advantageous in the unstable vent environment, regardless of whether these features evolved there or not. Other features of their life-history biology are not yet understood. A combination of experimentation and observational work is needed to resolve how sperm reach the females, where fertilisation occurs and how larvae develop and disperse.

Appendix 1

Terebellida, Ampharetidae

Descriptions of reproduction in the Ampharetidae are limited to the tube-dwelling detrivore *Amphisamytha galapagensis*, a species inhabiting vents of the Pacific (Reuscher, 2009), and *Amathys lutzi*, which inhabits tubes attached to mussel shells or rocks at hydrothermal vent sites along the MAR (Blake & Van Dover, 2005). Ongoing molecular studies suggest that *A. galapagensis* from Northeast Pacific ridges, as studied by McHugh & Tunnicliffe (1994), should be assigned to a new cryptic sister-species that is distinct from *A. galapagensis* but very closely related (Chevaldonné *et al.*, in preparation in Chevaldonné *et al.*, 2002).

Both species are gonochoric and a lack of external sexual dimorphism has been described in *Amathys lutzi* (Blake & Van Dover, 2005). Both of these vent ampharetids contain coelomic gametes at very small body lengths (<3.55 mm), suggestive of early sexual maturation (Blake & Van Dover, 2005). Rapid maturation and growth may be related to the relatively constant nutrition available at hydrothermal vents (Gustafson & Lutz, 1994).

Observations of sperm morphology in *Amphisamytha galapagensis* and *Amathys lutzi* revealed no modifications in sperm ultra-structure and mature sperm fit the description of ect-aquasperm, consistent with external fertilisation and leading to the conclusion that these species broadcast spawn (McHugh & Tunnicliffe, 1994; Blake & Van Dover, 2005). No evidence of sperm storage has been found in either species (Blake & Van Dover, 2005). The absence of sperm modifications and lack of spermathecae in females indicate external fertilization for these species. It remains to be determined if fertilisation occurs at or near the tube opening or elsewhere.

Based on the presence of numerous (300) small eggs (~150 µm diameter) in the body cavity of some female specimens of *Amphisamytha galapagensis*, Zottoli (1983) inferred a high reproductive rate in this species, yet vent ampharetids and alvinellids may be categorized as slow egg producers, with vitellogenesis taking place as the oocytes float freely in the coelom (Blake & Van Dover, 2005; Pradillon & Gaill, 2007). Brood size in slow egg producers is expected to vary with food availability (Eckelbarger personal communication in Blake & Van Dover, 2005), and ranged from 2,000-25,000 in *Amathys lutzi* (Blake & Van Dover, 2005).

Observations of gametes (male and female) at all stages of development within mature specimens in populations sampled at different times of each year, suggest that *Amphisamytha* sp. (NE Pacific vent sites) may reproduce continuously (McHugh & Tunnicliffe, 1994). In contrast, oocyte-frequency analysis of *Amathys lutzi* females (Logatchev vent field, MAR) suggested the presence of cohorts of maturing oocytes, consistent with periodic release of gametes (Blake & Van Dover, 2005). *A. lutzi* specimens were only collected during July. The reproductive pattern of this species cannot be confirmed without analysis of time-series samples.

Maximum oocyte diameter in *Amathys luzti* (~190 µm) (Blake & Van Dover, 2005) is similar to that of *Amphisamytha galapagensis* (~240 µm). Larvae of these species have not been collected or cultured. Based on egg size, it has been assumed that development is lecithotrophic in *A. lutzi* (Blake & Van Dover, 2005), *A. galapagensis* (Zottolli, 1983) and *Amphisamytha* sp. (McHugh & Tunnicliffe, 1994), and not specialised for long-distance dispersal. Lecithotrophy so far appears to be predominant amongst polychaetes in deep-sea chemosynthetic communities, although it is not unique amongst vent animals. Larvae capture and/or culture are required to confirm the developmental mode in these ampharetids. Experimentation and modelling have the potential to elucidate their dispersal capabilities.

Amathys lutzi and *Amphisamytha galapagensis* occupy similar habitats in the vent environment and share basic reproductive attributes (Blake & Van Dover, 2005). It has been argued (e.g. Faure *et al.*, 2007) that reproductive features, such as oviducts and spermathecae, and continuous/semi-continuous reproductive patterns could have evolved recently in response to the unstable nature of the vent habitat. This may prove to be true for species that share these traits. However, as the ampharetid (Blake & Van Dover, 2005) and hesionid (Eckelbarger *et al.*, 2001) species studied to date have revealed, such traits are not unique or common to all vent polychaetes.

Subphylum Crustacea

Order Amphipoda

Amphipods have been recorded as abundant at vent sites in the Atlantic and Pacific (Sheader *et al.*, 2000, 2004). The reproductive patterns of three species have been described (Sheader *et al.*, 2000, 2004; Sheader & Van Dover, 2007). The pardaliscid *Halice hesmonectes* forms swarms at sites on the EPR (Martin *et al.*, 1993), whereas the lysianassoid *Ventiella sulfuris* forms swarms along the east Pacific south of Guaymas Basin (Barnard & Ingram, 1990). The eusirid *Bouvierella curtirama* is associated with mussel beds on the MAR (Van Dover & Trask, 2000).

Halice hesmonectes and *Bouvierella curtirama* (Sheader *et al.*, 2000, 2004) exhibit sexual dimorphism, whereas sexual dimorphism is not marked in *Ventiella sulfuris* (Sheader & Van Dover, 2007). Mature females of *B. curtirama* were significantly larger than males (Sheader *et al.*, 2004), whereas in *H. hesmonectes*, males were slightly larger than females (Sheader *et al.*, 2000). *B. curtirama* exhibits plasticity in the growth stage/size at which maturity reached, an advantage in the dynamic and spatially heterogeneous vent environment (Sheader *et al.*, 2004).

For example, where female mortality is high (e.g. due to predation intensity), early maturation would be advantageous; if an essential food component is in short supply, egg production and development could be delayed (Sheader *et al.*, 2004).

Most vent invertebrate species studied to date have relatively high fecundity. In contrast, vent amphipods exhibit low fecundity (Sheader *et al.*, 2004). For the species studied to date, egg and brood sizes are within the normal range of their shallow-water counterparts but larger than those of typical deep-water species (Johnson *et al.*, 2001; Sheader *et al.*, 2000, 2004; Sheader & Van Dover, 2007). This may be a response to food availability and higher temperatures in the vent environment, but it may also serve to increase number of offspring available for dispersal to new vents (Sheader & Van Dover, 2007). Variations in egg and brood size are evident between species. For example, the eggs of *H. hesmonectes* are ~75% larger (by volume) than those of *B. curtirama*, an observation Sheader *et al.* (2004) attributed to the apparent lack of grazing predation upon *H. hesmonectes*. Amphipod brood size is controlled by oocyte resorption during a critical phase of vitellogenesis; this may be a mechanism to fine-tune egg output to the resources available to each female (Sheader *et al.*, 2004). Reproductive output was highest in *Ventiella sulfuris*, which exhibited an extreme degree of ovarian development (Sheader & Van Dover, 2007), a feature typical of deep-sea scavenging species (Hessler *et al.*, 1978; Ingram & Hessler, 1987).

Based on the direct measurement of oocytes, and supported by observations of population structure, a continuous pattern of reproduction has been described in all species of vent amphipod examined to date. Continuous reproduction might be expected in a vent habitat with a constant food supply (Sheader *et al.*, 2000), but this is not the only reproductive pattern in vent invertebrates. Reproductive output may show variations seasonally or periodically, if food resources (including eggs/larvae and phytoplankton-derived material) vary (Sheader *et al.*, 2004). For example, spatial variation in reproductive output (mean brood size and number of broods) and female maturity were described in *Bouvierella curtirama* (Lucky Strike, MAR) (Sheader *et al.*, 2004). Reproductive output was greater at Sintra vent, due to female maturation at a smaller body size and production of more broods of greater size. Sheader *et al.* (2004) inferred that these differences reflected differences in food availability between sites. A spatial pattern was also reported for *Ventiella sulfuris* at EPR vents, whereby no mature individuals were found in central vent habitats (Sheader & Van Dover, 2007). These authors concluded that adults move to the periphery of vents to reproduce and brood before returning to feed at vent habitats and undergo a new phase of gonad maturation and emigration (Sheader & Van Dover, 2007). In contrast, *Halice hesmonectes* males and females leave the vent at the instar prior to maturity and adults do not return to vents (Sheader & Van Dover, 2007).

The movement of brooding females to the vent/seep periphery has been documented in other crustaceans (e.g. Perovich *et al.*, 2003; Copley & Young, 2006). There may be benefits in

removing eggs and early juveniles from the direct influence of potentially toxic vent fluids (Sheader & Van Dover, 2007) and predation pressure on the adults and juveniles may be lower in peripheral regions (Perovich *et al.*, 2003). Furthermore, lower temperatures may be energetically beneficial for egg/juvenile development and adult survival in the vent environment (Sheader & Van Dover, 2007).

Whereas most invertebrates in deep-sea chemosynthetic communities studied so far have a pelagic dispersive larval stage, amphipods are phylogenetically constrained to direct development (Sheader *et al.*, 2000, 2004; Sheader & Van Dover, 2007). Fertilization is external, with a marsupium formed by oostegites on the ventral surface of the perion (Sheader *et al.*, 2000). Eggs (usually large and yolky) are brooded within the marsupium and, after hatching, are released as small juveniles (Sheader *et al.*, 2000). Direct development might not be ideal for species inhabiting short-lived and isolated habitats where the ability to disperse and colonise new vent sites is essential. Between vent sites, genetic evidence indicates that *Ventiella sulfuris* migrates along the EPR ridge axis in a stepping-stone manner, unconstrained in gene flow by distances as great as 1,200 km, but contact between disjunct ridge axes is limited (France *et al.*, 1992). On the slow-spreading MAR, *Bouvierella curtirama* populations are restricted in their movement along the ridge crest because of topography (Sheader *et al.*, 2004). Although *Halice hesmonectes* colonises vents within a few months of their formation (Sheader *et al.*, 2000), it is likely that the dispersive capability of this species is also limited because colonisation is by small juveniles (Sheader & Van Dover, 2007). For other amphipod species, brooding females, with their greater mobility may be a means of colonizing new vents, whereas reproductive output per brood is too low to make dispersal by juveniles unlikely (Sheader *et al.*, 2000).

Order Decapoda

To date, more than 125 species representing 33 families of decapods have been reported from deep-sea chemosynthetic environments (Martin & Haney, 2005) yet the reproductive traits of only 10 species have been described. Reproductive patterns of decapod crustaceans are thought to have strong phylogenetic constraints (Van Dover *et al.*, 1985; Tyler & Young, 1999), as exhibited in many aspects of their reproductive biology (see below).

Caridea, Alvinocarididae

Alvinocaridid shrimp, endemic to hydrothermal vents and/or cold seeps (Martin & Haney, 2005), exhibit the characteristic reproductive features of caridean decapods (Tyler & Young, 1999; Ramirez-Llodra *et al.*, 2000). For example, morphological and histological analyses have shown that gonad morphology and gametogenesis are phylogenetically

constrained (Ramirez-Llodra *et al.*, 2000; Ramirez-Llodra & Segonzac, 2006). Variability has, however, been found in their reproductive patterns.

Of all the alvinocaridid shrimp described from chemosynthetic sites, data on egg size is available for a few species only. In general, mature egg size is ~200-500 µm, depending on the species and the sample, and the oocytes are produced in cohorts in the ovaries (Tyler & Young, 1999; Ramirez-Llodra *et al.*, 2000). Fecundity is variable, ranging from <400 eggs in *Mirocaris* (Tyler & Young, 1999) up to 5,798 eggs in *Alvinocaris muricola* (Ramirez-Llodra *et al.*, 2006). There is a positive correlation between fecundity and body size, characteristic of crustaceans (Ramirez-Lodra *et al.*, 2000; Ramirez-Llodra & Segonzac, 2006). Of the species studied so far, *A. stactophila* and *Mirocaris fortunata* produce the larger embryos and have the lowest size-specific fecundity (Copley & Young, 2006; Ramirez-Llodra *et al.*, 2000), suggesting a higher investment per embryo and higher individual larval survival. In contrast, *A. muricola* produces a high number of embryos in the lower range (0.66 x 0.55 mm) of embryo sizes for caridean shrimp (Ramirez-Llodra *et al.*, 2006).

Eggs are released as cohorts onto the pleopods and all species are believed to copulate (Tyler & Young, 1999). Although copulatory behaviour is yet to be observed at vents and seeps, observations of non-mobile aflagellate sperm (Ramirez-Llodra & Segonzac, 2006) are characteristic of caridean shrimp and are thought to be an adaptation to fertilisation through copulation (Bauer, 2004).

Oocyte size-frequency distributions in *Alvinocaris muricola* females and observations of embryos at different stages of development in different females (Regab seep, Gulf of Guinea, 3150 m) provided evidence of continuous reproduction in this species at the Regab seep (Ramirez-Llodra & Segonzac, 2006). Ramirez-Llodra *et al.* (2000) also suggested a continuous reproductive pattern in *Rimicaris exoculata* (TAG), *Mirocaris fortunata*, and *Chorocaris chacei* (Lucky Strike), based on oocyte-frequency data from samples collected from these MAR vent sites. A lack of seasonal reproduction in *R. exoculata* at TAG is supported strongly by evidence from time-series samples, whereby no significant differences were found in the oocyte size-frequency distributions of females (Copley *et al.*, 2007). Seasonal sampling would be required to confirm the lack of seasonality in reproduction of *A. muricola*, *M. fortunata* and *C. chacei*.

Evidence indicates seasonal and iteroparous reproduction in *Alvinocaris stactophila* from the Brine Pool cold seep (Gulf of Mexico) (Copley & Young, 2006). In contrast to *Rimicaris exoculata*, *A. stactophila* exhibits similar oocyte size-frequency distributions within samples from the same month but variation between months. Histological evidence of reproductive development in seasonally collected male specimens of both these species (sample locations consistent with the females) also supports seasonal reproduction in *A. stactophila* at the Brine Pool and continuous reproduction in *R. exoculata* at TAG (E. Brown, unpublished data).

Copley *et al.* (2007) attributed the differences in the reproductive pattern of *A. stactophila* and *R. exoculata* to the fact that the TAG hydrothermal site lies much deeper (3600 m) than the Brine Pool (650 m) and is situated within an oligotrophic gyre where little seasonal variation in surface productivity occurs. Oocyte size-frequency distributions and the differences in embryo development between spring and autumn indicated that spawning occurs in November in *A. stactophila*, with females brooding embryos over winter and hatching planktotrophic larvae to coincide with the spring peak in surface productivity and its export (Copley & Young, 2006).

It has been suggested that crustacean species in chemosynthetic environments must obtain the sterols required for reproduction from phytoplankton-derived sources (Pond *et al.*, 2000a). Studies of alvinocaridid shrimp from chemosynthetic habitats suggested that individuals may store a lifetime supply of these compounds during their planktonic larval stage (Pond *et al.*, 2000a; Allen *et al.*, 2001). Adults may perceive seasonal variations in the supply of such compounds as a cue for synchronization of gametogenesis (Copley & Young, 2006). Copley & Young (2006) hypothesised that reproduction in *Alvinocaris stactophila* may rely on a seasonal peak in the supply of such compounds in phytodetrital flux to the Brine Pool, using it as a cue to synchronize gametogenesis and thereby timing the release of planktotrophic larvae to coincide with peak food availability in the water column. Collection of further seasonal samples for lipid analysis would be required to test this hypothesis. Seasonal reproductive cycles, apparently driven by the seasonal peak in photosynthetically-derived food availability for planktotrophic larvae, have also been described in mussels (Tyler *et al.*, 2007) gastropods (Van Gaest, 2006) and crabs (Perovich *et al.*, 2003) from the Brine Pool and mussels from Menez Gwen vent field (MAR, 850 m) (Dixon *et al.*, 2006).

Copley & Young (2006) observed spatial variation in samples of *Alvinocaris stactophila* collected from different locations across the Brine Pool mussel bed. The proportion of males in samples declined from the outer to the inner zone of the mussel bed, while the proportion of females carrying eggs increased. The paucity of ovigerous *Rimicaris exoculata* in samples collected from TAG in November, may also result from a spatial pattern rather than a temporal one (Copley *et al.*, 2007), but this suggestion has not been tested by collecting samples of shrimp from the periphery of the vent field for comparison. Assuming that the pattern in water chemistry observed by Smith *et al.* (2000) persisted when their samples were collected, Copley & Young (2006) suggested that the observed zonation may have resulted from the congregation of ovigerous females towards the inner zone, where the sulfide flux from underlying sediments may be absent or reduced. The same hypothesis has been used to explain the apparent scarcity of ovigerous *R. exoculata* females in the immediate vicinity of black smokers at deep MAR vents (Copley, 1998; Ramirez-Llodra *et al.*, 2000). This theory is remains to be tested experimentally, although it is supported by suggestions that other crustaceans avoid the sulfidic

extremes of chemosynthetic environments while brooding embryos (Perovich *et al.*, 2003; Sheader & Van Dover, 2007) but see to Chapter 5.

All alvinocaridid shrimp species studied to date exhibit small egg sizes and embryos, indicative of planktotrophic development (Van Dover *et al.*, 1985; Ramirez-Llodra *et al.*, 2000; Copley & Young, 2006; Ramirez-Llodra & Segonzac, 2006; Copley *et al.*, 2007). A long dispersal time between patchily distributed and dynamic vent and or seep habitats would appear to be advantageous, wherever it evolved. Feeding, planktotrophic larvae, unconstrained by yolk reserves are potentially capable of long residence times in the water column, although developmental studies are required to determine larval lifespan in alvinocaridid shrimp.

The planktotrophic larval development of alvinocaridids suggests that these species can disperse and colonize geographically distant sites (Ramirez Llodra & Segonzac, 2006). Larvae of vent shrimp from the MAR are known to disperse widely from the adult habitat (Tyler & Dixon, 2000). Morphological, genetic and biochemical data currently available on larvae and juveniles of some species indicate that the planktotrophic larvae migrate upwards in the water column and feed on organic matter produced in the photic zone (Pond *et al.*, 1997a, b; Shank *et al.*, 1998; Herring & Dixon, 1998; Tyler & Dixon, 2000). It has been suggested that this vertical movement during dispersal could explain the broad geographic distribution of certain species with feeding larvae entrained in deep-water currents (Van Dover *et al.*, 2002).

Anomura, Galatheidae

Within the Anomura, vent-associated species of the family Galatheidae (squat lobsters), are probably vagrants rather than endemics, although some species are known only from vents and may prove to be true endemics (Martin & Hayney, 2005). The galatheid genus *Munidopsis* is the most speciose and widespread of all vent-associated taxa and has been reported from hot vents and cold seeps, probably reflecting the fact that it is a widespread and speciose deep-sea genus (Martin & Hayney, 2005). Data on reproduction of species from chemosynthetic environments is sparse.

Sexes are separate and reproductive patterns appear to be phylogenetically constrained. Fecundity in the squat lobster *Munidopsis lentigo* (Galapagos Rift) is low and observations of large egg sizes indicated lecithotrophic development in this species (Van Dover *et al.*, 1985). Lecithotophic development has also been suggested in *Munidopsis* sp. 1 from the Brine Pool cold seep (Gulf of Mexico) (J. Copley, unpublished data). An aversion to the sulfidic extremes of the Brine Pool habitat while brooding, similar to that found in the shrimp *Alvinocaris stactophila* at the same site (Copley & Young, 2006), has been suggested for *Munidopsis* sp. 1, based on the distribution of brooding females across different zones of the Brine Pool mussel bed (J. Copley, unpublished data). Egg size in vent decapods varies considerably and is exceptionally large in *M. lentigo*, whereas brood size is relatively low (13 eggs per female) due

to the inverse relationship between the two features in crustaceans (Van Dover *et al.*, 1985). A phylogenetic or evolutionary constraint that precludes a shift from lecithotrophic development with large eggs to planktotrophic development with small eggs was suggested by Van Dover *et al.* (1985) for two species of *Munidopsis*. Van Dover *et al.* (1985) concluded that *M. lentigo* was phylogenetically constrained to its developmental mode and that habitat *per se* does not dictate egg size and developmental mode. A larger study within the Galatheoidae (in different environments) is consistent with this hypothesis (Van Dover & Williams, 1991).

Brachyura, Bythograidae

The crab family Bythograeidae are known only from hydrothermal vents (Martin & Hayney, 2005) where they form an important element of the higher levels in the food chain (Tyler & Young, 1999). Although at least 13 species in five genera have been recognized (Martin & Hayney, 2005), the reproductive biology of only three species, has been described to date. *Bythograea thermydron* is abundant at vent sites on the EPR (Dittel *et al.*, 2008), whereas *B. laubieri* and *B. vrijenhoeki* are amongst the dominant fauna of the Pacific-Antarctic Ridge from 31-38° south.

Histological and morphological observations made by Perovich *et al.* (2003) and Hilàrio *et al.* (2009) indicated that the ovarian structure and general pattern of vitellogenesis for *Bythograea thermydron, B. laubieri* and *B. vrijenhoeki* is characteristic of brachyuran decapods. In contrast to *Munidopsis* sp., *Bythograea thermydron* produces large numbers of small eggs, which are brooded on the ventral surface of the abdomen in typical crab fashion (Williams, 1980; Van Dover *et al.*, 1984). For *B. laubieri* and *B. vrijenhoeki*, vitellogenic oocyte size (>100 µm) and oocyte size at onset of vitellogenesis (60 µm) were significantly smaller than those reported for *B. thermydron* by Perovich *et al.* (2003) (~500 and 150 µm respectively), even when potential tissue shrinkage of 20% through histological processing was taken into consideration (Hilàrio *et al.*, 2009). Because the three species were very similar in morphology and size (Desbruyères *et al.*, 2006), morphology does not appear to have been a contributing factor to interspecific differences in egg size.

Bythograea species inhabiting chemosynthetic environments presumably demonstrate a similar pattern of copulation and fertilisation as that described in brachyuran crabs (Dittel *et al.*, 2008), although mating has not been observed and it is not known how long females brood their eggs.

Analysis of oocyte-size-frequency distributions in specimens collected over different months of the year clearly indicated group-synchronous gametogenesis in *Bythograea thermydron* (9°N, EPR) and provided strong evidence that *B. thermydron* (9°N, EPR) undergoes a seasonal cycle of reproduction, with egg-hatching peaking in April/May (Perovich *et al.*, 2003). In contrast, oocyte size-frequency distributions for females of *B. laubieri* and *B.*

vrijenhoeki collected simultaneously (Pacific-Antarctic Ridge) contained oocytes at all stages of development, suggestive of a lack of synchrony in gonad development and reproduction for the population as a whole (Hilário *et al.*, 2009). These differences in the reproductive pattern of *Bythograea* species imply that the timing of gametogenesis in this genus is not phylogenetically constrained.

Seasonal reproduction based on evidence from time-series samples has been suggested in other decapods (Copley & Young, 2006), mussels (Dixon *et al.*, 2006; Tyler *et al.*, 2007) and a gastropod (Van Gaest, 2006) from chemosynthetic environments. Although cues for synchronizing gametogenesis in vent and seep species are not fully understood, synchrony of reproduction in *B. thermydron* has been linked to seasonal variation in the supply of photosynthetically-derived nutrients (Perovich *et al.*, 2003). The geographic distribution of *B. laubieri* and *B. vrijenhoeki* falls within the boundaries of the oligotrophic South Pacific Subtropical Gyre (SPSG), where the absence of phytoplankton blooms and constant oligotrophy may preclude environmental cues for seasonal reproduction (Hilário *et al.*, 2009). It is possible that the SPSG may also function as a barrier to dispersal and therefore a determining factor in the biogeographic patterns of *Bythograea* species (Hilário *et al.*, 2009).

In addition to evidence of a seasonal pattern in the reproduction of *Bythograea thermydron* at 9°N (EPR), Perovich *et al.* (2003) found a significant difference in the mean sizes of oocytes from the vent and peripheral zones and that samples from the vent periphery were dominated by brooding females and those whose eggs had recently hatched. On the basis of this evidence, Perovich *et al.* (2003) suggested that females with mature gonads migrate to the vent periphery to brood and hatch their eggs. Hilário *et al.* (2009) reported no ovigerous females in samples of *B. laubieri* and *B. vrijenhoeki* females collected from a Pacific-Antarctic vent, which supports the segregation behaviour of ovigerous females away from the direct influence of hydrothermal activity (Hilário *et al.*, 2009). Spatial patterns in brooding females away from the physical and chemical extremes of the vent and seep habitat to peripheral regions has also been described for alvinocaridid shrimp (Ramirez-Llodra *et al.*, 2000; Copley & Young, 2006) and amphipods (Sheader & Van Dover, 2007). Although the reason for this behaviour has not been determined, it presumably provides some kind of advantage, perhaps to maximise larval survival. In the spatially heterogeneous environment of vents and seeps, peripheral areas may minimise larval exposure to predation pressure, potentially toxic fluids and, in the case of vents, high temperatures (Perovich *et al.*, 2003).

The high fecundity and small eggs observed in *Bythograea thermydron*, *B. laubieri* and *B. vrijenhoeki* suggest that development is planktotrophic in these species (Van Dover *et al.*, 1985; Hilário *et al.*, 2009). To date, little is known about zoeal development. Later development in *B. thermydron* is includes a single megalopa stage (Van Dover *et al.*, 1985; Jinks *et al.*, 2002; Perovich *et al.*, 2003) with no obvious morphological adaptations specific to

existence in vent environments (Martin & Dittel, 2007). Developmental studies have the potential to elucidate the number and duration of larval stages in other species.

If larvae are hatched in the vent periphery, as suggested by Perovich *et al.* (2003), they would not become entrained in the vent plume but would instead disperse in bottom currents or could migrate to higher positions in the water column outside of the plume (Kim & Mullineaux, 1998). Although the advantages of this type of dispersal are not clear, laboratory behavioural experiments on *Bythograea thermydron* megalopae have demonstrated that the larval stages have wide tolerance for variations in temperature and pressure, which may be an adaptation for vent existence, and possess the physiological potential to exploit a large portion of the water column above vents (Epifanio *et al.*, 1999). In contrast, laboratory experiments on the temperature tolerance of lab-reared larvae of the vent crab *Gandalfus yunohana* (Bythograeidae) suggested relatively high temperature requirements for larval survival and development, leading Hamasaki *et al.* (2010) to propose that the zoeae inhabit warm near-surface waters during their planktotrophic development. The flexibility inferred for *B. thermydron* could enhance dispersal potential and may be a special adaptation for vent existence (Epifanio *et al.*, 1999), but the actual vertical distribution of the larval forms is unknown and would require confirmation by *in situ* sampling. Dittel *et al.* (2005) used stable isotope analysis to show that the megalopal and first juvenile stages of *B. thermydron* had both carbon and nitrogen isotope signatures consistent with a phytoplankton source of primary production. Studies on species of vent shrimp have reported larvae as far as 100 km from a known vent site and 1,000 m above the seabed, suggesting that dispersal may occur in mesopelagic regions outside the vent environment (Pond *et al.*, 1997b; Pond *et al.*, 2000a, b; Herring & Dixon, 1998) where the larvae rely on photosynthetically derived food sources (Copley *et al.*, 1998; Gebruk *et al.*, 2000).

Reproduction in the *Bythograea* species studied to date appears to be generally typical of brachyuran crabs. Many reproductive and life history traits of decapod crustaceans from hydrothermal vents and cold seeps are though to have strong phylogenetic constraints (e.g. Van Dover *et al.*, 1985; Tyler & Young, 1999; Perovich *et al.*, 2003), but such constraints are not evident in every aspect of their reproductive biology. Recent evidence of seasonal and non-seasonal reproductive patterns within the same genus of vent crabs (Perovich *et al.*, 2003; Hilário *et al.*, 2009) for example, suggests that environmental parameters may also shape reproductive patterns in these organisms.

Appendix 1

Phylum Echinodermata

Ophiuroidea, Ophiuridae

To date, very little is known of the reproductive patterns of echinoderms from chemosynthetic environments. *Ophioctenella acies* appears to only inhabit chemosynthetic environments in the Atlantic and is known from vents on the MAR, MCSC and at cold seeps (Stohr & Segonzac, 2005; personal observation). Its gametogenic biology and population size structure were examined by Allen (2005), using samples collected during one month from Logatchev and Snake Pit vent sites on the MAR. The oocyte size-frequency distributions of individuals suggested asynchronous gametogenesis and continuous reproduction in *O. acies* at these sites (Allen, 2005). This reproductive pattern is consistent with observations of a lack of synchrony in male reproductive development and population size-frequency distributions indicative of continuous recruitment (Allen, 2005). Most studies of *O. acies* to date from all sites contained individuals of different ages, from early post-larvae to adults, indicating regular successful recruitment. In contrast, seasonal reproductive cycles have been described in species of non-vent deep-sea ophiuroids (Tyler & Gage, 1980; Sumida *et al.*, 2000; Gage *et al.*, 2004). This implies that the timing of gametogenic cycles is not phylogenetically constrained in ophiuroids. Allen (2005) suggested that continuous reproduction may be an adaptation to the continuously available food supply in the vent environment, allowing vitellogenesis to proceed throughout the year. Continuous reproduction is a trait of many vent species (e.g. Hilàrio *et al.*, 2005; Copley *et al.*, 2007; Tyler *et al.*, 2008), although seasonal reproductive patterns have also been described (e.g. Perovich *et al.*, 2003). Continuous reproduction in *O. acies* has not been confirmed by analyses of time-series samples. However, the waters surrounding Logatchev and Snake Pit vent sites are oligotrophic, hence there is no obvious seasonal cue (such as a seasonal peak in the flux of phytodetritus) to synchronize gametogenesis.

The small oocyte size (max. 80 μm) and limited yolk reserves within oocytes of *Ophioctenella acies* suggest planktotrophic development for this species (Allen, 2005). This is supported by observations of post-larval structures (Desbruyères *et al.*, 2006). This developmental mode seems to be common amongst confamilial non-vent deep-sea brittle stars (Young, 2003) and appears to be phylogenetically constrained.

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