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Reproductive traits of pioneer gastropod species colonizing deep-sea hydrothermal vents after an eruption

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Abstract The colonization dynamics and life histories of pioneer species determine early succession at nascent hydrothermal vents, and their reproductive ecology may provide insight into their dispersal and population connectivity. Studies on the reproductive traits of two pioneer gastropod species, *Ctenopelta porifera* and *Lepetodrilus tevnianus*, began within a year after an eruption on the East Pacific Rise (EPR) that eliminated vent communities near 9°50′N from late 2005/early 2006. Standard histology was used to examine gamete release, instantaneous female fecundity, and time to maturation. Both species exhibited two-component oocyte size–frequency distributions indicating quasi-continuous reproduction with high fecundity. In samples collected in December 2006, both *C. porifera* and *L. tevnianus* individuals were reproductively mature. The smallest reproducing *C. porifera* were 4.2 mm (males) and 5.4 mm (females) in shell length, whereas reproductive *L. tevnianus* were smaller (2.3 and 2.4 mm in males and females, respectively). Most *C. porifera* were large (>6.0 mm) compared to their size at metamorphosis and reproductively mature. In contrast, most *L. tevnianus* were small (<1.0 mm) and immature. Reproductive traits of the two species are consistent with opportunistic colonization, but are also similar to those of other *Lepetodrilus* species and peltospirids at vents and do not fully explain why these particular species were the dominant pioneers. Their larvae were probably in high supply immediately after the eruption, due to oceanographic transport processes from remote source populations.

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Introduction

Hydrothermal vent organisms are distinctive in their ability to persist in extremely unstable environments with strong chemical gradients. High concentrations of sulfides, iron, and trace metals (Co, Cu, Pb, Zn) occur in vent fluids (reviewed by Von Damm 1990). Endemic vent species populate regions of the deep sea associated with tectonic and volcanic activity that cause chemical-rich fluids up to 403°C to exit the seafloor (reviewed by Van Dover 2000). Vent fluids mix with the cooler ambient seawater creating environments highly variable in temperature and chemical composition (e.g., Johnson et al. 1986). The base of the food chain consists of chemoautotrophic microbes capable of using energy from reduced chemicals in the vent fluids to fix carbon (reviewed by Karl 1995). In addition to being chemically dynamic environments, vents are distributed discontinuously along mid-ocean ridges as dictated by underlying magma chamber and tectonic plate activity (reviewed by Fornari and Embley 1995). Vent species are bound by tens to hundreds of kilometers along mid-ocean ridges (Tunnicliffe 1992; Chevaldonné et al. 1997). Thus,

the populations are fragmented, and the dynamics and persistence of the metapopulations depend on population connectivity as mediated by larval dispersal and recruitment (e.g., Neubert et al. 2006).

At fast-spreading ridges, such as the East Pacific Rise, eruptions occur on a time scale of less than 100 years (MacDonald et al. 1980; Haymon et al. 1993). Geophysical models of fluid circulation beneath the basaltic crust reveal unsteady vent fluid convection (Watremez and Kervevan 1990), creating highly variable, transient habitats. Volcanic eruptions or tectonic quakes can obliterate entire communities and provide new substrata for colonization. Following such an event, the new basalt substratum may continue to change morphologically (Haymon et al. 1991), and vent fluids may vary in flux and chemical composition (Von Damm 1995; Butterfield et al. 1997) with associated increases or decreases in primary production (reviewed by Karl 1995). These eruptions are important disturbances that affect initial colonization patterns (reviewed in Tunnicliffe 1992) and genetic exchange among vent sites (reviewed by Jollivet 1996).

The processes influencing how hydrothermal vent invertebrate populations are connected are still poorly understood. Since vent populations are physically fragmented, hydrodynamic transport (e.g., Chevaldonné et al. 1997; Kim and Mullineaux 1998; Mullineaux et al. 2003; Adams and Mullineaux 2008), larval lifespan (Marsh et al. 2001), and distribution within the water column (Mullineaux et al. 2005) are all thought to be important in long-range dispersal and on-site retention. Thus, vent inhabitants can be considered part of greater metapopulations (Vrijenhoek 1997; Jollivet et al. 1999; Neubert et al. 2006). A first step in understanding the complex patterns of connectivity within a metapopulation is to estimate the dispersal capabilities of the species (reviewed by Levin 2006). In the context of a nascent vent, the dispersal capabilities of pioneer species (those that colonize the site first) are of particular interest in understanding early successional processes and community structure. Because adult reproductive morphology can be used to infer details of a species' life history (Eckelbarger 1994), analyses of fecundity and spawning periodicity have increased our knowledge of the life histories of several abundant vent species (e.g., McHugh and Tunnicliffe 1994; Pendlebury 2005; Tyler et al. 2008). Studying reproductive characteristics of pioneer species provides insight into their dispersal, connectivity, and colonization dynamics.

The ephemeral nature of hydrothermal vents should favor species with r-type life history strategies including fast growth, early reproduction, and widespread larval dispersal (Van Dover et al. 1985; Young 2003). While most vent species reproduce continuously, reproductive strategies include a wide variety of fertilization mechanisms, parental investment, and larval feeding (Tyler and Young 1999; Young 2003). For instance, vetigastropods (including *Lepetodrilus* spp.) typically have a non-feeding planktonic larval stage. However, some vetigastropods brood their embryos within the pallial cavity and release a crawling stage (Bouchet and Warén 1994).

An eruption in late 2005/early 2006 on the East Pacific Rise (EPR) near 9°50′N provided an opportunity to study primary succession and life histories of pioneer species. Seismic data and camera footage indicated that newly released lava from the eruption wiped out previously existing communities and created new hydrothermal vents and substrata for colonization (Tolstoy et al. 2006; Cowen et al. 2007; Soule et al. 2007). Response cruises mobilized shortly after the event (Von Damm et al. 2006) allowed biologists to study the early stages of faunal colonization. Although camera-based observations conducted after a prior (1991) eruption at this site documented successional patterns in the large, structure-forming species (Shank et al. 1998), this new eruption provided an opportunity to study a diverse group of species including those too small to observe in images.

Within 10 months of the eruption, colonization experiments (Mullineaux et al. 2010) revealed the appearance of the gastropod *Ctenopelta porifera* (Fig. 1a). This species is found at 13°N on the EPR (Warén and Bouchet 1993), but

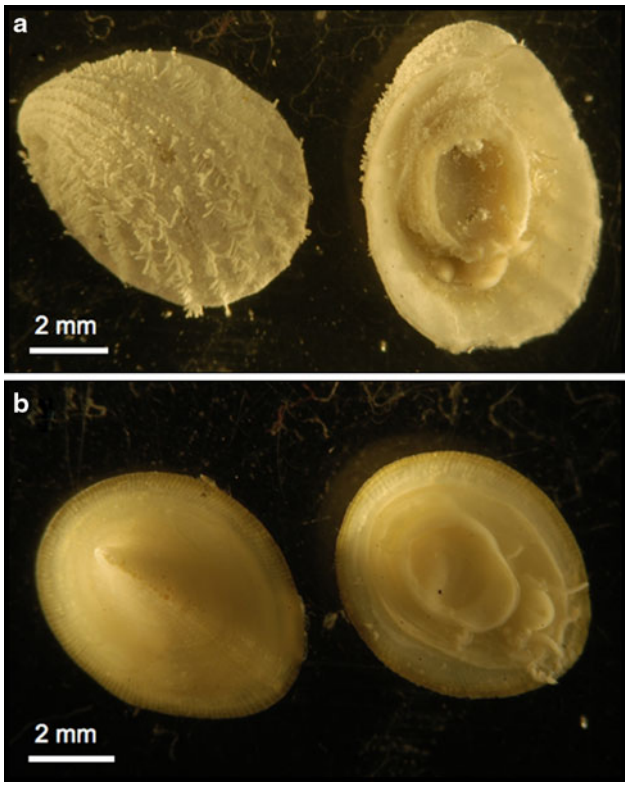

Fig. 1 Images of adult *Ctenopelta porifera* (**a**) and *Lepetodrilus tevnianus* (**b**)

had not been observed in the benthos near 9°50′N (or anywhere else in the world's oceans). Another notable change was the abundance of the gastropod *Lepetodrilus tevnianus* (Fig. 1b), which had been rare relative to congeners *L. ovalis*, *L. pustulosus*, *L. cristatus*, and *L. elevatus* prior to the eruption (McLean 1993). *Lepetodrilus tevnianus* is typically associated with the tubeworm *Tevnia jerichonana* and was first found at 11°N (McLean 1993) on the EPR, although its range is now known to span from 11° N to 23° S (Johnson et al. 2008). The appearance of these two pioneer species, *C. porifera* and *L. tevnianus*, raises the question of how their life histories might facilitate early arrival after disturbance. Although general morphology of both species has been described in detail (*C. porifera* by Warén and Bouchet 1993; *L. tevnianus* by McLean 1993), no internal examinations or specific histological studies have been conducted on either. Ecological and reproductive studies have been conducted on the other species of *Lepetodrilus* on the EPR (*Lepetodrilus ovalis, L elevatus, L. pustulosus, L. cristatus*), the Mid-Atlantic Ridge (*L. atlanticus*), the Guaymas Basin (*L. guaymasensis*: Fretter 1988; McLean 1988; Pendlebury 2005), and the Juan de Fuca Ridge (*L. fucensis*: Fretter 1988; McLean 1988; Kelly and Metaxas 2007). On the EPR, *L. elevatus* had been the dominant gastropod species in established communities since the 1991 eruption (Shank et al. 1998; Van Dover 2000; Mullineaux et al. 2003), with other *Lepetodrilus* species (*L. ovalis*, *L. pustulosus*, *L. cristatus*) found in lower densities.

The main objective of this study was to use standard histological techniques to characterize the reproductive morphology of the pioneer gastropods *C. porifera* and *L. tevnianus* to determine whether they had higher fecundity, smaller oocyte size, or more variable reproductive timing than other related gastropod species inhabiting established vents in this region. We investigated the size at first reproduction to estimate how quickly new colonists could contribute progeny to the local community. We also quantified population structure (size distribution of colonists), which, in combination with minimum size of reproduction, provides an estimate of the proportion of the population that is reproductive and potentially contributing to local population growth. The broader goal was to gain insight into processes influencing initial colonization at hydrothermal vents.

Methods

Field sampling and initial analyses

Colonization surfaces were placed at P-vent (9° 50.28′ N, 104° 17.47′ W) on the East Pacific Rise during post-eruption cruises aboard the *RV Atlantis* in October 2006 (9 months after estimated eruption date) and December 2006 (11 months after eruption). The first set was recovered in December 2006 after a 1.5 months deployment and the second set in November 2007 after 11 months. The colonization surfaces were comprised of six Lexan plates, each 10 × 10 × 0.6 cm, separated by 0.9-cm cylindrical spacers. The surfaces were roughened by 50-grit sandpaper, pitted with a metal press, and banded with 0.1-cm-deep grooves made with a wood saw. The plates and spacers were held together with cable ties to form a six-layered sandwich with a polypropylene-braided line as a handle (Fig. 2). Sandwiches were deployed and recovered from individual collection compartments on the submersible *Alvin* similarly to the blocks used by Mullineaux et al. (2000). Once aboard *RV Atlantis*, sandwiches were immediately transferred to buckets of 2°C seawater in the cold room for initial observations. The colonization surfaces, all attached colonists, and all detached individuals within the initial collection chamber that were retained in a 63-μm sieve were processed as described later. Samples from the first recovery cruise (December 2006) were used to assess the size structure and reproductive status of *C. porifera* and *L. tevnianus* populations roughly 1 year after the eruption and to describe reproductive morphology, oocyte size, and fecundity (defined as total number of vitellogenic oocytes) in these species. Samples from the subsequent cruise (November 2007) were used to compare reproductive status of those populations a year later.

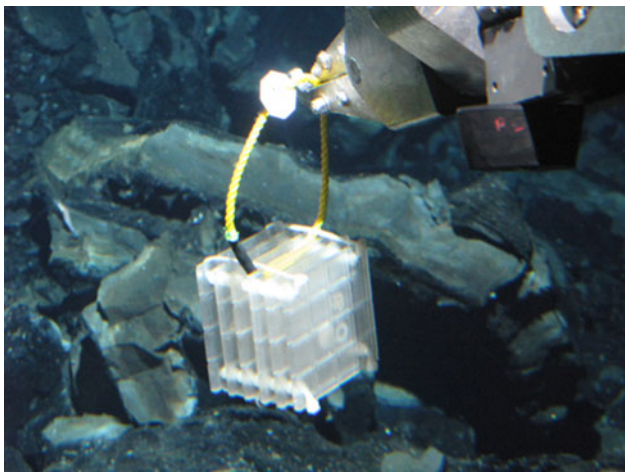

Fig. 2 Photograph of sandwich colonization surface (10 cm on a side) being deployed by *DSV Alvin*

Recovery of colonization substrates

During the first recovery cruise (December 2006), three sandwiches each were recovered and analyzed from hot (18–30°C) and cool (2–4°C) environments at P-vent. The

Table 1 *Ctenopelta porifera* and *Lepetodrilus tevnianus* individuals collected in December 2006 and November 2007 from P-vent and V-vent sites

Date	Site	Species	Total	Analysis	Selection criterion (mm)	Number selected		
						Male	Female	Unk
2006	P	*C. porifera*	48	RM	5.0–10.0	10	16	
				SFR	<5.0	2	2	
				OSF	7.0–9.0	–	8	
				F	6.0–10.0	–	10	
				SL	All	10	17	21
	P	*L. tevnianus*	2,683	RM	3.5–7.0	14	15	
				SFR	<3.0	5	5	
				OSF	3.5–7.0	–	8	
				F	3.5–7.0	–	10	
				SL	All	17	17	2649
2007	P	*C. porifera*	16	RM	6.0–10.0	4	12	
				OSF	7.0–9.0	–	4	
				F	6.0–10.0	–	6	
				SL	All	4	12	16
	V		28	RM	5.0–10.0	7	7	
				F	6.0–10.0	–	4	
				SL	All	7	7	14
	P	*L. tevnianus*	91*	RM	6.0–10.0	11	11	
				OSF	3.5–7.0	–	5	
				F	3.5–7.0	–	10	

Total is number recovered on sandwiches. A subset of individuals used for each analysis (reproductive morphology RM, size at first reproduction SFR, oocyte size–frequency OSF, fecundity F) was selected haphazardly from within a size range (Selection criterion). Shell length analyses (SL) used all individuals (All). Additional criterion for all reproductive analyses was that sections were whole and undistorted. Sex of selected individuals was categorized as Male, Female, or unknown (Unk)

* Individuals subsampled haphazardly from total on sandwiches to match SL range (2.0–7.0 mm) from 2006. This subsampling precluded analysis of size–frequency distribution (SL) for *L. tevnianus* from 2007

colonization surfaces and all attached and detached (>63 μm) individuals were preserved in 80% ethanol. In the laboratory, the individual plates were separated, and the lengths of all organisms were measured. Size was determined for *C. porifera* by measuring overall shell length (SL) on the dorsal side from the protoconch to the opposite lip of the shell. For *L. tevnianus*, the protoconch is not at the edge of the shell (Fig. 2b), so the maximum length across the dorsal side of the shell was used as shell length. Individuals were counted, identified to lowest taxonomic group possible, and stored in 95% ethanol. All *C. porifera* found were used for reproductive analysis, as well as all *L. tevnianus* >2.0 mm in shell length. Small (<2.0 mm SL) *L. tevnianus* were excluded from reproductive analyses because they were difficult to section, but were retained for analyses of population structure. Numbers of specimens used in each reproductive analysis and selection criteria are listed in Table 1.

During the second recovery cruise (November 2007), five sandwiches were recovered from the hot environment at P-vent using the same *DSV Alvin* protocol as in December 2006. Once inside the cold room, individual *L. tevnianus* adults >2.0 mm SL were haphazardly selected from the plates (Table 1). All specimens of *C. porifera* found on the plates ($n = 16$; Table 1) were used in reproductive analyses. Because so few individuals were found at P-vent, sandwiches from V-vent (9° 47.23′ N, 104° 16.95′ W), a vent ~4.8 km away, were used to supplement the numbers ($n = 28$; Table 1). For both species, all samples picked in the cold room were initially preserved for 4 days in 7% formalin and then transferred to 70% ethanol.

Reproductive morphology and analyses

All specimens examined for reproductive morphology ($n = 107$; Table 1) were removed from their shells and were serially dehydrated using graded ethanol, cleared with xylene, and embedded in molten histology paraffin wax using standard molds. Samples from December 2006

initially preserved in 80% ethanol were transferred to formalin for 4–5 days prior to standard histology fixation steps. We refer to this as the "reverse" method of initial fixation, and it produced whole, undistorted sections with internal organs approximately the same size as in the 2007 samples (no apparent shrinkage) for reproductive analyses. Using standard haematoxylin and eosin procedures (reviewed by Kiernan 2008), paraffin blocks were cut transversely at 5-μm thickness on a rotary microtome and stained. Assembling the serial sections for each individual and viewing them in sequence determined the general three-dimensional shape and location of the gonads.

From the December 2006 cruise, our selection of male and female *C. porifera* specimens ($n = 22$; Table 1) purposely spanned 5.0–10.0 mm SL for analyses of gonad morphology, oocyte size-frequency, and fecundity. Those with SL < 5.0 mm were selected for size at first reproduction analysis but were not used for oocyte size–frequency and fecundity analyses. From the November 2007 cruise, we used all individuals recovered from P-vent for the analyses listed earlier and an additional 14 selected individuals from V-vent with the same range in SL (5.0–10.0 mm) for fecundity analysis and male morphology only (Table 1). For *L. tevnianus*, males and females from each cruise were selected for analyses of gonad morphology, oocyte size-frequency, and fecundity. Individuals 2.0–7.0 mm SL were used to examine size-specific reproduction (Table 1). Female and male gonad morphologies and fecundities were determined using a Zeiss Axiovert 200 M microscope, and an AxioCam MRc5 camera, using Zeiss Axiovision version 4.4 Software.

Size at first reproduction

All specimens initially collected for reproductive morphology analysis were examined for sex, but cross-sectioning of the smaller specimens was used to assess the minimum size at reproductive maturity of each species. For *C. porifera*, all four individuals <5.0 mm from December 2006 were used (none within this size range was recovered in November 2007), whereas for *L. tevnianus*, individuals in the 2.0–3.0 mm SL range were selected from the December 2006 samples (10 specimens; Table 1). All specimens used for size at first reproduction were serially cross-sectioned from the dorsal-side down through the foot. For *L. tevnianus,* individuals of <3.0 mm, it was nearly impossible to differentiate between sexes based on exterior morphology due to their small size. Thus, both sex and reproductive maturity were determined in cross-sections. Estimating size at first reproduction was of particular interest for the specimens collected in 2006 because it was used, in combination with SL frequency data, to estimate the proportion of individuals that was reproductive within a year of the eruption.

Oocyte size–frequency analysis

Individual females were selected based on shell length (>7.0 mm, *C. porifera*; >3.5 mm, *L. tevnianus*) to provide reproductively mature specimens for analyses of oocyte size (Table 1). The gonads were observed in three transverse sections spaced roughly 100 μm apart. Both previtellogenic and vitellogenic oocytes were measured. Oocyte size–frequencies per individual were calculated prior to averaging total size–frequency ranges of oocytes from all individuals selected for these analyses. Images were captured with an AxioCam MRc5 camera using Zeiss Axiovision version 4.4 Software. The feret diameters (diameter of the oocyte if it were a perfect circle) (as in Pendlebury 2005; Kelly and Metaxas 2007) of all oocytes with visible nuclei were calculated using ImageJ software (v. 1.39, National Institute of Health, Bethesda, Maryland, USA). A standard Pearson's chi-squared test using JMP (v. 1.5.2) was conducted on oocyte frequency data from all specimens of each species to test for synchrony within populations. If the chi-squared test demonstrated that the populations were synchronous, then the oocyte size data were pooled among individuals for subsequent analyses.

To determine whether different cohorts could be distinguished in the size distributions, an Expectation–Maximization (EM) algorithm (McLachlan 1987; McLachlan and Peel 2000) was used in a mixture-model analysis to test for two normal distributions of oocyte size. As an initial step, for both *C. porifera* and *L. tevnianus* and both sampling years, we tested the null hypothesis that log oocyte diameter follows a single normal distribution against the alternative hypothesis that it follows a mixture of two normal distributions with the same variance. We then proceeded to test for both species the null hypothesis that the parameters of the two-component normal mixture distribution were the same in both years (for each species) against the alternative hypothesis that they differed (details in Online Resource 1).

Fecundity

The total number of vitellogenic oocytes in each female (instantaneous fecundity) was used as the measure of fecundity in this study. Ten females of each species from each cruise were used in the analysis (Table 1). For *C. porifera* samples from November 2007, only six females were available for fecundity analysis from P-vent, so the data were supplemented with four specimens from V-vent. Females of each species were selected to span the available range in SL (Table 1). For *L. tevnianus*, the range was

3.0–7.0 mm (as those <3.0 mm had lower quality sections), and for *C. porifera*, it was 6.0–10.0 mm. A two-step process was used to calculate fecundity, as described by Pendlebury (2005). For each species, in the first step, three specimens were serially sectioned to find vitellogenic oocytes with visible nuclei (i.e. germinal vesicles), which were used to determine average vitellogenic oocyte diameter. This average diameter was then used as the spacing for cross-sectioning of the remaining specimens. Average vitellogenic oocyte size was ~150 μm for *C. porifera* and ~100 μm for *L. tevnianus*. The full gonad volume was sectioned for each specimen, and oocytes found in each section were totaled. For both *C. porifera* and *L. tevnianus*, we used a regression model assuming a negative-binomial distribution to test for statistically significant differences in fecundity between years (details in Online Resource 2).

Population structure

Size (SL) frequency data were compiled from all *C. porifera* and *L. tevnianus* individuals found at P-vent in December 2006 samples. Species-level identification of *C. porifera* was definitive for specimens of all sizes, but identification of *L. tevnianus* was definitive only for specimens >2.0 mm SL. Smaller individuals could be distinguished as *Lepetodrilus* sp., and we assume that they were *L. tevnianus* because no other species of that genus were found in the 2006 samples (this assumption is revisited in the "Discussion"). In November 2007 samples, all *C. porifera* individuals found at P-vent and at V-vent were pooled for size–frequency analyses, because no significant difference in mean SL was detected between the sites (two-sample *t* test, *t* ratio $= -1.73$, $df = 14$, $P < 0.11$; JMP v. 1.5.2). A two-sample *t* test was also conducted to compare *C. porifera* SL between years. Size distributions of *L. tevnianus* from the December 2007 collections were not analyzed, because only a subset of these individuals was measured.

Results

Reproductive morphology and gametogenesis

Serial sections revealed that *C. porifera* is gonochoristic (for the detailed description and images of reproductive morphology, see Online Resource 3), and all analyzed individuals with the exception of one male (2.5 mm SL) had mature spermatozoa (males) or oocytes (females). The smallest male with a mature testis had a SL of 4.2 mm, and the smallest female with mature oocytes had a SL of 5.4 mm (previously recorded as 4.8 and 5.9 mm, respectively, by Warén and Bouchet 1993). Therefore, the size at first reproduction was determined to be 2.5–4.2 mm for males and <5.4 mm for females. Cross-sections of the sole immature male revealed tissue similar in appearance to that of the digestive gland (Online Resource 3) in the regions where the gonads would be in a reproductively mature adult.

All oocytes were tightly packed into the ovary. Oogonia developed in the epithelium of the ovary (Online Resource 3) and grew to 20–30 μm in diameter, when they developed into pre-vitellogenic oocytes as distinguished by their visible nuclei and dark purple staining. Vitellogenesis was generally apparent when oocytes were 60–70 μm. In most samples, vitellogenic oocytes typically reached a maximum diameter of 230–250 μm (a few of the oocytes were >300 μm, probably due to distortion).

Cross-sections revealed that *L. tevnianus* is also gonochoristic (Online Resource 3), and all adults with SL > 2.4 mm had either mature spermatozoa (males) or an ovary (females). Of the male specimens selected for size at first reproduction studies (SL 2.2–2.4 mm), only those 2.3 mm or larger were reproductively mature. Of the female specimens (SL 2.2–2.6 mm), only those 2.4 mm or larger were mature. In immature individuals, gonads were difficult to distinguish from the digestive gland (Online Resource 3). Light microscopy of histological sections of *L. tevnianus* stained with haematoxylin and eosin (Online Resource 3) illustrates that the ovary occupies a large volume of the gastropod (approximately one-third), and that oocytes are tightly packed throughout the ovary. Oogonia develop in the germinal epithelium of the ovary and grow until they are about 20 μm in diameter when they develop into pre-vitellogenic oocytes (Online Resource 3). These oocytes begin to develop into mature vitellogenic oocytes (characterized by acidophilic granular cytoplasm) at 50–60 μm, generally reaching a maximum of 130–150 μm (although a few rare oocytes, 2 of 1,497 measured, reached 210 μm). The general shape of the gonads in males, and the location relative to the stomach, digestive gland, and foot, were similar to those of the ovary in females (Online Resource 3).

Oocyte size–frequency

Oocyte size distributions did not vary significantly between individuals for either *C. porifera* ($\chi^2 = 550$, $df = 25$, $P > 0.24$) or *L. tevnianus* ($\chi^2 = 255$, $df = 17$, $P > 0.24$). Size–frequency distributions pooled over multiple individuals of *C. porifera* (Fig. 3a, b) and *L. tevnianus* (Fig. 3c, d) appeared to have more than a single normally distributed component. The mixture-model analysis identified two size-components in the oocyte size–frequency data for both species (Table 2), possibly reflecting two different cohorts of oocytes. The null hypothesis that log oocyte size follows

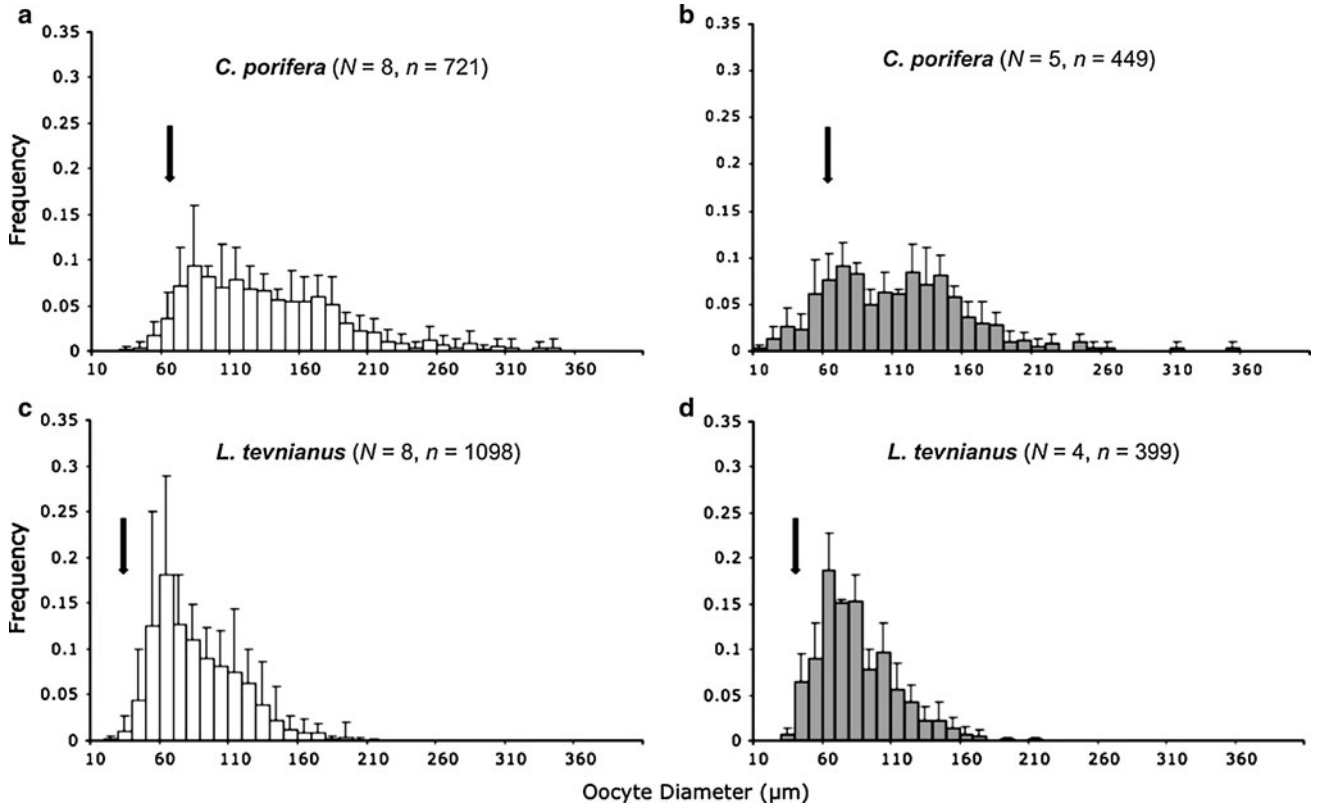

Fig. 3 Frequency of oocyte diameter (μm) for *Ctenopelta porifera* (**a**, **b**) and *Lepetodrilus tevnianus* (**c**, **d**). Samples from December 2006 (*unfilled bars*) and November 2007 (*filled bars*). Values pooled from multiple individuals; shown with SE. N = number of individuals sampled and n = total number of oocytes. *Arrows* indicate diameter at onset of vitellogenesis

Table 2 Maximum likelihood (ML) estimates of oocyte diameter parameters from the EM mixture-model analysis for *C. porifera* and *L. tevnianus*

Species	Year	n	μ	σ	log L_o	μ_1	μ_2	σ	π	log L_1
C. porifera	2006	721	4.7	0.4	234.3	4.4	5.0	0.30	0.44	242.0
	2007	448	4.8	0.4	203.8	4.4	5.0	0.27	0.35	210.3
L. tevnianus	2006	1098	4.3	0.4	470.5	4.0	4.6	0.27	0.49	484.2
	2007	399	4.3	0.4	203.1	4.0	4.4	0.30	0.49	203.4

Oocyte diameter data (log-transformed; n = total number of oocytes) pooled among individuals from 2006 to 2007 samples. Estimates are mean μ and variance σ^2 (log μm) for the null hypothesis H_o (that log oocyte diameter follows a single normal distribution), and means μ_1 and μ_2 (log μm), standard deviation σ (log μm), and probability π that an observation belongs to the first component, for the alternative hypothesis H_1 (that log oocyte diameter follows a mixture of two normal distributions with the same variance). Log L_o and log L_1 are the maximized log likelihoods under H_{o1} and H_1, respectively

Table 3 Maximum likelihood (ML) estimates of oocyte diameter parameters from the EM mixture-model analysis, fit under the null hypothesis H_o, that the parameters of the two-component normal mixture distribution were the same in both years for *C. porifera* and *L. tevnianus*

Species	μ_1	μ_2	σ	π	log L_o
C. porifera	4.4	5.0	0.29	0.39	444.0
L. tevnianus	4.0	4.6	0.27	0.50	682.4

Data transformations and parameters as in Table 2

a single normal distribution was rejected in all cases except for *L. tevnianus* from November 2007 (details in Online Resource 1). We therefore assumed conservatively that in all cases, the distribution of log size follows a two-component normal mixture. If this assumption were incorrect, it would not affect the validity of the test. For both species, the null hypothesis that the parameters of the two-component normal mixture distribution were the same in both years was tested against the alternative hypothesis of difference between years. The results of fitting the model under the second null hypothesis are given in Table 3 (detailed results in Online Resource 1). After running the EM algorithm, the second null hypothesis was rejected for both species ($P = 0.002$ for *C. porifera* and $P = 0.03$ for *L. tevnianus*). It is important to note, however, that the test is quite powerful for these sample sizes, and even small differences between the parameters may lead to the rejection of the null hypothesis that they are the same in both years.

Using the results of the mixture-model analysis, the two components of oocyte diameters in *C. porifera* had means of 81.5 and 148.4 μm for both 2006 and 2007 (log diameters shown in Table 2). In *C. porifera*, pre-vitellogenic oocytes (defined as all those <60 μm) accounted for ~12% of all oocytes from December 2006 and ~20% from November 2007. The two components of oocyte diameter in *L. tevnianus* had means of 54.6 and 99.5 μm for 2006, and 54.6 and 81.5 μm for 2007. In *L. tevnianus*, oocytes in the pre-vitellogenic stage (those <40 μm) comprised ~5% of oocytes.

Fecundity

Fecundity in both species was relatively high (more than 500 vitellogenic oocytes in the largest females) and increased roughly exponentially with size (Fig. 4). Size-specific fecundity in *L. tevnianus* was higher than in *C. porifera* because it matured at a smaller size, but fecundity in the largest individuals (509 oocytes in *C. porifera* and 596 in *L. tevnianus*) was similar. The relationship between fecundity and SL was roughly exponential for both species (Fig. 4). Regression-model analysis revealed no difference between years in this relationship for *C. porifera* (F-ratio = 0.13, $P = 0.88$), but a significant difference for *L. tevnianus* (F-ratio = 5.51, $P = 0.02$) (details in Online Resource 2). This difference was difficult to interpret given the low number of individuals sampled.

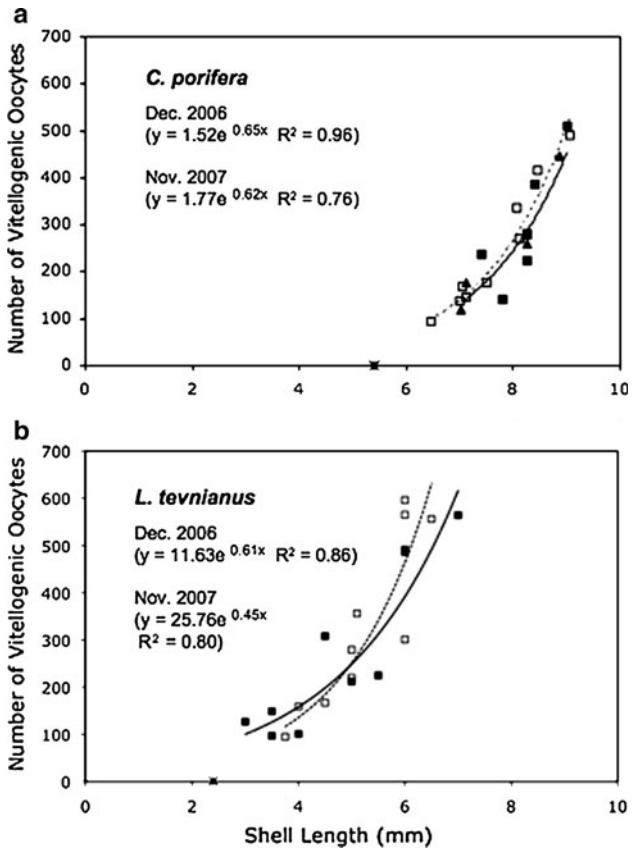

Fig. 4 Fecundity (number of vitellogenic oocytes) over a range of SL for *Ctenopelta porifera* (**a**) and *Lepetodrilus tevnianus* (**b**). Samples from December 2006 (P-vent, *unfilled squares*) and November 2007 (P-vent, *filled squares*; V-vent, *filled triangles*). $n = 10$ individuals from each date. Exponential regressions generated for December 2006 (*dashed lines*) and November 2007 (*solid lines*). Shell length at first reproduction represented as a *star*

Population structure

Distributions of SL for both species were determined from all individuals collected in December 2006 (Fig. 5). Shell lengths of *C. porifera* in December 2006 were 2.5–8.6 mm. All individuals but one (98% of population) were reproductively mature (Fig. 5b). Shell lengths of *C. porifera* at P-vent in November 2007 were 7.0–10.7 mm; SL at V-vent were 5.9–10.7 mm (Fig. 5c). All individuals in these samples were assumed to be reproductively mature based on the estimated SL at first reproduction (4.2 mm for males and 5.4 mm for females). The two-sample t test revealed no significant difference in SL distribution between samples from P-vent and V-vent (November 2007) (t-ratio = −1.73, $df = 14$, $P < 0.11$). However, there was a significant difference in the size distributions of *C. porifera* between 2006 (P-vent only) and 2007 (P-vent and V-vent pooled) (t-ratio = −5.99, $df = 42$, $P < 0.001$).

Shell lengths of *L. tevnianus* (including *Lepetodrilus* specimens too small to identify to species, but assumed to be *L. tevnianus* as no other species of this genus was found among samples collected in the area) were 0.2–7.0 mm (Fig. 5a). Using 2.4 mm as a conservative estimate of SL at first reproduction (males may reproduce at SL 2.3–2.4 mm, and sexes are difficult to distinguish at these sizes), ~22% of the population was reproductively mature and potentially contributing larvae (Fig. 5a).

Discussion

Histological analysis revealed that reproductive traits, such as quasi-continuous spawning, of both *C. porifera* and *L. tevnianus* are similar to related species within their respective families (Table 4). These observations suggest that their appearance as pioneers in the post-eruption community at 9° N was not due to unusual reproductive adaptations. Reproductive traits of these species and related *Lepetodrilus* spp. and peltospirids indicate that they are all potentially opportunistic vent colonizers.

For both *C. porifera* and *L. tevnianus*, oocyte and initial vitellogenic oocyte diameters fell within the range of other EPR species (Table 4), but values generally were greater

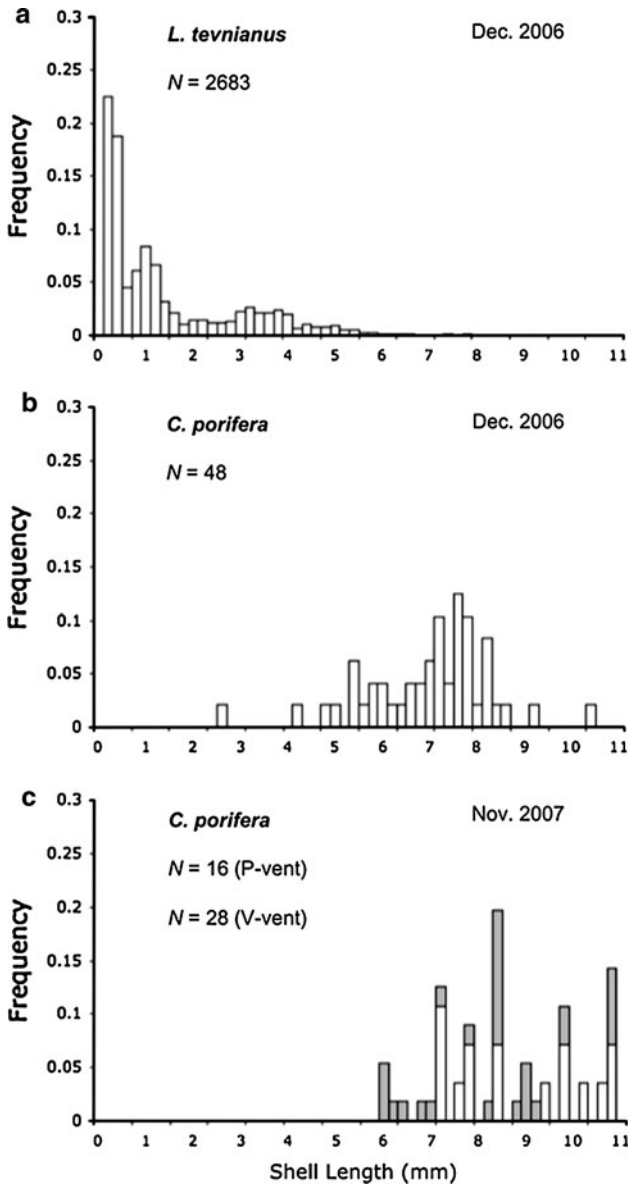

Fig. 5 Frequency of gastropod SL in populations of *Lepetodrilus tevnianus* in December 2006 (**a**), *Ctenopelta porifera* in December 2006 (**b**), and *C. porifera* in November 2007 (**c**). Individuals from P-vent (*unfilled bars*) and V-vent (*filled bars*)

than those reported in Pendlebury (2005). Gonad location in *C. porifera* was similar to that of *Rhynchopelta concentrica*. When comparing oocyte diameters among species, it is important to note that values reported for a particular species vary between different studies. For example, Fretter (1988) and Berg (1985) reported values for *R. concentrica* from 13° N and 21° N (EPR) that were considerably larger than those reported from 9° N by Pendlebury (2005).

The two-component distribution of oocyte diameters from multiple individuals indicates that gametogenesis is most likely quasi-continuous in both *C. porifera* and *L. tevnianus*. The similar oocyte diameter distributions among individuals suggest that reproduction was not asynchronous within the populations of either species. Oocyte size distributions in quasi-continuous spawners are expected to vary slightly over time, as the mature oocytes are released (reviewed by Eckelbarger and Watling 1995). The slight, but significant, difference observed between sampling dates in oocyte diameter distributions of both *C. porifera* and *L. tevnianus* may indicate responses to changes in the surrounding micro-environment, but they were very subtle when compared with the reproductive periodicity observed in discontinuous spawners that respond to large-scale changes such as detritus pulses (reviewed by Tyler et al. 1994). This synchronous, quasi-continuous, reproduction is consistent with the reproductive cycles of other vent molluscs (reviewed by Tyler et al. 1994; Tyler and Young 1999; Pendlebury 2005; Kelly and Metaxas 2007; Tyler et al. 2008).

The present study demonstrates that *L. tevnianus* matures at a smaller size than *C. porifera* and has higher fecundity at any given size. These results are consistent with Pendlebury's (2005) observed differences between other *Lepetodrilus* spp. and peltospirids on the EPR. We do not know whether *Lepetodrilus* spp. reproduce at an earlier age than peltospirids, because the size-to-age relationships in these species are unknown. Still, elevated levels of fecundity at small sizes are indicative of pioneer or weed species (Barrett 1992), leading to an expectation that *Lepetodrilus* spp., but not peltospirids, should be pioneer colonists at EPR vents. Observations following the late 2005/early 2006 eruption (Mullineaux et al. 2010) show this not to be the case, as the early colonists included a peltospirid (*C. porifera*), but not the full suite of pre-eruption *Lepetodrilus* species.

Another difference worth noting between the two species is the preponderance of small (<4.0 mm SL) individuals in *L. tevnianus*, but not *C. porifera* populations, despite a SL at recruitment of <0.4 mm for both (179 μm in *L. tevnianus* and 290–325 μm in *C. porifera*: Mills et al. 2009; Warén and Bouchet 1993). If some of the smallest (<2.0 mm) *Lepetodrilus* individuals are species other than *L. tevnianus*, then our calculations of the proportion of the population that are reproductive (22%) would be an underestimate.

The average SL of *C. porifera* individuals was significantly greater in 2007 than 2006, and this apparent absence of new recruits in 2007 could be a consequence of a high juvenile growth rate, a high juvenile mortality rate, or discontinuous recruitment. We think that discontinuous larval recruitment is the most likely explanation, because it is consistent with measures of larval supply of *C. porifera* after the eruption. Their larval numbers were high shortly after the eruption in July 2006, but had declined

Table 4 Summary of known reproductive traits of hydrothermal vent gastropods including members of the families Lepetodrilidae (*Lepetodrilus atlanticus*, *L. fucensis*, *L. cristatus*, *L. elevatus*, *L. ovalis*, *L. pustulosus*, *L. tevnianus*) and Peltospiridae (*Rhynchopelta concentrica*, *Ctenopelta porifera*)

Species	Date	Location	Oocyte size (μm)		Source
			Onset of vitellogenesis	Vitellogenic (Max.)	
Family Lepetodrilidae					
L. atlanticus	March–April 2001	MAR	35–40	92	Pendlebury (2005)
L. fucensis	July 2001	JdFR, Exp	35–45	60–110	Kelly and Metaxas (2007)
	July–Sept. 1984			100–140	Fretter (1988)
L. cristatus	March 1984	EPR	30–35	140–150	Fretter (1988)
L. elevatus	Dec. 2001	EPR	30–35	84	Pendlebury (2005)
	April–May 1979			74–95	Berg (1985)
L. ovalis	Dec. 2001	EPR	30–35	87	Pendlebury (2005)
L. pustulosus	March 1984	EPR	30–35	230	Fretter (1988)
	Dec. 2001			84	Pendlebury (2005)
	April–May 1979			83–204	Berg (1985)
L. tevnianus	Dec. 2006	EPR	35–40	130–150 (210)	This study
Family Peltospiridae					
R. concentrica	Dec. 2001	EPR	50–60	90 (184)	Pendlebury (2005)
	April–May 1979			132–152	Berg (1985)
C. porifera	Dec. 2006	EPR	60–70	230–250 (330)	This study

Oocyte diameters listed as maximums or ranges. *EPR* East Pacific Rise, *MAR* Mid-Atlantic Ridge, *JdFR* Juan de Fuca Ridge, *Exp* Explorer Ridge, *GR* Galapagos Rift

substantially by October 2006 (Mullineaux et al. 2010) and may have been low by the time of our collections. All individuals from both years were large enough (SL > 5.4 mm in females and >4.2 mm in males) to be reproductively mature, with the single exception of the 2.5-mm male collected in 2006, so the coarse resolution in our estimate of size at first reproduction did not affect calculations of reproductive maturity at the population level.

We suspect that the slight differences between years observed in oocyte diameter distributions of both *C. porifera* and *L. tevnianus* are due to variations in larval supply. Larval abundance and supply can be temporally variable at vents due to physical oceanographic transport (Metaxas 2004; Mullineaux et al. 2005; Adams and Mullineaux 2008), and pioneer colonists arriving after a major disturbance may simply represent whatever species are available in the plankton at that time. Variation in larval supply could have been caused by reversals in along-axis currents (e.g., Chevaldonné et al. 1997; Marsh et al. 2001; Adams and Mullineaux 2008) or the passage of mesoscale eddies (e.g., Adams 2007). If this type of oceanographically driven variation in larval supply determines the species' composition of pioneers, the interaction between early-arriving species' reproductive characteristics and episodic oceanographic events may set the templates for successional trajectories in nascent vent communities.

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