Journal of Shellfish Research, Vol. 27, No. 1, 107–118, 2008.

REPRODUCTION OF GASTROPODS FROM VENTS ON THE EAST PACIFIC RISE AND THE MID-ATLANTIC RIDGE

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ABSTRACT The gametogenic biology is described for seven species of gastropod from hydrothermal vents in the East Pacific and from the Mid-Atlantic Ridge. Species of the limpet genus *Lepetodrilus* (Family Lepetodrilidae) had a maximum unfertilized oocyte size of <90 μm and there was no evidence of reproductive periodicity or spatial variation in reproductive pattern. Individuals showed early maturity with females undergoing gametogenesis at less than one third maximum body size. There was a power relationship between shell length and fecundity, with a maximum of ~1,800 oocytes being found in one individual, although individual fecundity was usually <1,000. Such an egg size might be indicative of planktotrophic larval development, but there was never any indication of shell growth in larvae from species in this genus. *Cyathermia naticoides* (Family Neomphalidea) had a maximum oocyte size of ~120 μm and a fecundity of <400 oocytes per individual. *Rhynchopelta concentrica* (Family Peltospiridae) had a maximum oocyte size of 184 μm and a fecundity <600, whereas in *Eulepetopsis vitrea* (Family Neolepetopsidae) maximum oocyte size was 232 μm with a fecundity of <200 oocytes per individual. In none of these three species was there any indication of episodicity in oocyte production. From our observations we support the paradigm that there is no reproductive pattern typical of vent systems but is more related to species' phylogeny.

KEY WORDS: East Pacific Rise, gastropod, reproduction, gametogenesis, hydrothermal vent, Mid-Atlantic Ridge

INTRODUCTION

Since the discovery of hydrothermal vents in 1977 and the description of their associated ecosystems, there has been intense interest in both syn- and autecology at hydrothermal vents. Particularly prominent has been the analysis of abundance patterns of vent organisms, as related to physiological requirements. Analysis of the reproductive biology has lagged behind other aspects of vent organism ecology as, for proper analysis, individuals need to be sampled from different times of the year. Tyler & Young (1999) and Young (2003) reviewed the data, which mainly characterized the larger megafuana including siboglinid and alvinellid polychaetes, bivalve molluscs. and Crustacea. In general the reproductive cycle and larval development of an organism appears to be constrained primarily phylogenetically with few vent-specific reproductive characteristics (but see Pradillon et al. 2001). Conversely, the numerically dominant taxon, the gastropod Mollusca, has received very little attention, other than descriptions of their reproductive morphology (Wàren & Bouchet 1993, 2001) and a recent study of the limpet *Lepetodrilus elevatus* McLean 1988 from 13°N on the EPR (Matabos et al. 2007). The aim of this paper is to describe the reproductive biology of seven gastropod species from 9°N East Pacific Rise (EPR) and the Mid-Atlantic Ridge (MAR), collected at several different times of the year.

The wide diversity of structural and ecological complexity in gastropods is reflected in the wide range of reproductive and developmental modes (for reviews see Fretter & Graham 1994). Archaeogastropods (including limpets) are generally dioecious and are usually broadcast spawners with external fertilization. The zygotes develop to a trochophore and subsequently a pelagic, often planktotrophic, veliger. Among the Caenogastropoda and Vetigastropoda reproductive modes are more diverse than in the Archaeogastropoda. The more advanced gastropods are generally gonochoristic and copulate, fertilizing their oocytes internally. Subsequent embryonic development generally occurs within an externally deposited egg capsule, although viviparity and egg brooding are often found. Hatching yields either crawl-away juveniles or planktonic larvae.

Indirect methods have been used to examine the dispersal capabilities of vent gastropods, including larval shell morphology (Lutz 1988) and gene flow between vent sites (Craddock et al. 1997, Vrijenhoek et al. 1997). Adult reproductive morphology can also be used to determine larval development (Eckelbarger 1994), and other aspects of life history. From the evidence available most hydrothermal vent gastropods appear to have a similar reproductive pattern of early maturity, internal fertilization, and lecithotrophic larvae. There are exceptions, such as *Eulepetopsis vitrea* McLean 1990 and *Phymorhynchus major* Waren & Bouchet 2001, both of which broadcast spawn and the latter of which is known to produce planktotrophic larvae (Desbruyères et al. 2006).

Four species of the genus *Lepetodrilus* (Family Lepetodrilidae) investigated in this study, *Lepetodrilus elevatus, Lepetodrilus ovalis* McLean 1988, *Lepetodrilus pustulosus* McLean 1988 and *Lepetodrilus atlanticus* Waren & Bouchet 2001 have similar reproductive morphologies (Fretter 1988, Wàren & Bouchet 2001). Males have a penis, which arises from the base of the right cephalic tentacle, and a pallial *vas deferens*, which serves as a prostate. Female lepetodrilids have a receptaculum seminalis, which has been found to contain sperm in the three EPR species. Eggs are believed to be fertilized within the mantle cavity, so the term "internal" fertilization cannot strictly be used. To date ripe ova have only been found in the mantle cavity

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of *L. pustulosus* (Fretter 1988). *Lepetrodrilus pustulosus* of approximately one third of the maximum size have been found to contain ripe oocytes (Fretter 1988). Spermatogenesis in *L. fucensis* McLean 1988, a NE Pacific limpet has been described by Hodgson et al. (1997). The manner in which the lepetodrilids (and peltospirids) copulate is discussed by Fretter (1989). 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.

In addition, we examine three non-lepetodrilid genera. *Eulepetopsis vitrea* (Family Neolepetopsidae) is a free-spawning patellid gastropod with no secondary sexual glands (Fretter 1990). Males of *Rhynchopelta concentrica* McLean 1989 (Family Peltospiridae) lack a penis, but the presence of a large pallial *vas deferens* that acts as a prostate and the discovery of viable sperm within the female receptacular duct suggest that fertilization is likely to be internal. Ripe ova have been seen frequently in the ovarian duct but fertilized ova have yet to be found (Fretter 1989). *Cyathermia naticoides* Wàren & Bouchet 1989 (Family Neomphalidae), the only coiled-shell gastropod investigated here, has a reproductive morphology characteristic of internal fertilization (Waren & Bouchet 1989). Male specimens have a large penis, modified from the left cephalic tentacle, a large prostate gland and a testis, which occupies the majority of the visceral mass. The oviduct of female specimens is described as short and spacious and opens to the side of the rectum.

In the species examined, internal fertilization might be a mechanism by which gametes can avoid direct exposure to the potentially toxic vent waters (Fretter 1988, Waren & Bouchet 1989); although the sperm are exposed to the toxins, the length of exposure is surely less than that of a broadcast spawner. The site of fertilization appears to be different in each of the three families, suggesting that some form of ''internal'' fertilization has evolved independently in each family and is therefore an important requirement when reproducing at hydrothermal vent sites. Lutz et al. (1984) suggest that the majority of vent gastropods appear to be phylogenetically constrained to a non-planktotrophic mode of development.

Because of the lack of suitable time-series samples, episodicity of reproduction at vents is poorly resolved. There is evidence of seasonal reproduction in the bivalve *Bathymodiolus azoricus* Cosel & Comtet 1998 for the MAR (Colaço et al. 2006, Dixon et al. 2006). Continuous reproduction would be considered more plausible when energy availability is high and continuous, and the ecosystem is ephemeral.

Berg (1985) proposed that as the majority of vent limpets examined in this study have all stages of oocytes within their gonad at any one time, reproduction is continuous. However, size- frequency distributions of *Lepetodrilus elevatus* populations show 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).

Habitat may have an effect on reproductive output and timing (see review by Ramirez Llodra 2002). For example, the gametogenic synchronicity of *Paralvinella grasslei* Desbruyères & Laubier 1982 appears to be a function of habitat as well as time (Copley et al. 2003). Mullineaux et al. (1998) examined the magnitude of FAP (Fluorescent Aging Pigments) in *L. elevatus* from different zones within a vent field. The amount of FAP varied widely between zones and Mullineaux et al. (1998) concluded that *L. elevatus* inhabiting warmer zones have a higher metabolic rate than those individuals in cooler zones. It is likely that variations in metabolism would be reflected in the reproductive output of an individual.

The aims of the present study are to:

- Investigate the female reproductive morphology and gametogenesis of seven species of vent gastropod, with a particular emphasis on periodic and spatial variability.
- Obtain fecundity estimates for each of the seven species.
- Examine the fecundity of one abundant species, *Lepetodrilus elevatus*, for spatial and temporal variability.
- examine and describe the sperm storage mechanism of *L. elevatus*.

MATERIALS AND METHODS

Field Sampling

Most material was collected at individual vents at the 9°N East Pacific Rise vent field (Table 1). Samples were often the by-products of collections of *Riftia pachyptila*, *Alvinella pompejana* Desbruyères & Laubier 1980 or *Bathymodiolus thermophilus* Kenk & Wilson 1985. Additional samples were collected from the Rosebud and Calyfield sites of the Galapagos Rift. On board, residues from the biobox were washed in cold seawater and examined under a $\times 10$ stereomicroscope. *Lepetodrilus atlanticus* were collected from *B. azoricus* beds at Menez Gwen on the MAR. All gastropods were separated to species and fixed in 5% formaldehyde for a minimum of 24 h before being transferred to 70% propan-2-ol for long-term storage. Occasional specimens were prepared for ultrastructural analysis using standard techniques (see Eckelbarger et al. 2001).

Laboratory Methods

In all species, with the exception of *Lepetodrilus elevatus*, size was taken as the shell length. In *L. elevatus* the maximum distance from the edge of the shell to the edge of the protoconch was used, as total length is not found to be a sensitive indicator of growth in juveniles (Sadosky et al. 2002). Images of the shells were taken using a video camera and dissecting microscope, and the diameters were measured using an image analysis program (Matrox-Rainbow runner/Sigmascan Pro4). For histology, shells of limpets were removed using forceps with the exception of *Cyathermia naticoides* where rapid decalcifying solution (hydrochloric acid/EDTA) was used for approximately two hours. The entire individual was dehydrated in 100% propan-2-ol for six hours, cleared in xylene for six hours and embedded in paraffin wax in a 70°C oven for approximately 15 h. The samples were then set in wax blocks and sectioned at 7 μm. Sections for morphology, oocyte size-frequency and fecundity analysis were stained using Meyer's haemotoxylin and eosin.

Oocyte Analysis

Images from two slides of each individual were captured using Matrox Rainbow Runner, and the feret diameters of approximately 100 oocytes per individual were measured in Sigma Scan Pro 5. Only oocytes with a visible nucleus were measured. Feret diameter is used in lieu of diameter, as the tight

TABLE 1.

Samples of vent gastropods used in this study.

Site	Date	Species Sampled	Sampling Method	Community Structure
Galapagos				
Rosebud	27/05/02	L.e., L.p., E.v.	Manipulator	Small *B. thermophilus* and *C. magnifica* (<2 cm long).
Calyfield	06/02/02	E.v., L.e., L.p.	Manipulator	*C. magnifica, B thermophilus* seacucumbers,
Flo Zone	29/12/01	L.e, L.p., L.o.,E.v.	Slurp	Barnacles, *B. thermophilus* and *C. magnifica* present
9°N East Pacific Rise				
Biovent	13/12/99	L.e., L.p., L.o.,E.v., C.n., R.c.	Manipulator	*R. pachyptila* plentiful with *B. thermophilus* moving in Coffin Washings
Biovent	17/12/01	L.e., L.p., L.o.,E.v., C.n., R.c	Mussel pot	Lots of mussel/tubeworm clumps with mussels dominating. Tubeworm clumps buried in mussels Several Small individual *C magnifica*. Rusty *R. pachyptila*..
Q vent/biovent	28/11/98	L.e., L.p., L.o.,E.v., B.s., C.n., R.c.	Manipulator	Large *R. pachyptila* dominate
Mussel Bed	26/12/01	L.e., L.p., L.o.,E.v., *C.n., R.c*	Mussel pot	In certain clumps of mussels many *B. symplector* Clumps of *B thermophilus* and *C. magnifica*, clams dominating mussels in discrete patches
Dead Mussel				
Bed 3729	16/12/01	B.s., E.v.	Manipulator	Dead mussels cascading down wall, lots of serpulids, disarticulated shells, dead clams
Biovent	14/5/00	L.e., L.p., L.o.,E.v., C.n., R.c	Manipulator	*B. thermophilus* and *R.pachyptila*
East Wall	14/12/01	L.e., L.p., L.o.,E.v., C.n., R.c	Mussel pot	Many *B. thermophilus* with1 large *R. pachyptila* stand with a number of smaller
East Wall	21/12/01	L.e., L.p., L.o.,E.v., C.n., R.c	R.cSlurp	Many *B. thermophilus* with1 large *R. pachyptila* stand with a number of smaller stands some of which are rusty. Mussels dominate
Tica	12/12/01	L.e., L.p., L.o.,E.v., C.n., R.c	Bush master	Tubeworms large and impressive. Clumps 4–5 m wide and at least that high
Train Station	15/12/01	L.e., L.p., L.o.,E.v., C.n.,	Mussel pot	*B. thermophilus* dominate Temperatures taken in shimmering watermean 6.4 C
Alvinellid pillar near Train Station 4650, 77686	15/12/01	E.v., C.n., R.c,B.s.	Slurp	*A. pompejana* assemblage
Mid-Atlantic Ridge				
Menez Gwen	03/03	L.a.	Cage	Full of *Bathymodiolus azoricus*
Menez Gwen	04/03	L.a.	Cage	Full of *B. azoricus*

Species abbreviations: E.v.:*Eulepetopsis vitrea*, L.e.:*Lepetodrilus elevatus*, L.p *Lepetodrilus pustulosus*, L.o. *Lepetodrilus ovalis*, L.a. *Lepetodrilus atlanticus*, C.n *Cyathermia naticoides*, R.c. *Rhynchopelta concentrica*.

packing of oocytes within the ovary can severely distort the shape of the oocytes.

Determination of Fecundity

Instantaneous fecundity was determined as the total number of vitellogenic oocytes per individual. Two different methods to estimate the fecundity of *Lepetodrilus elevatus* were initially compared. The ovary of *L. elevatus* is not discrete. It is gradually overlain by the digestive glands, hence methods that rely on known volume of gonad (Ramirez-Llodra et al. 2000) are not suitable. The ovary was serially sectioned and each vitellogenic oocyte was counted. The overlying sections were compared with ensure that no egg was counted more than once. This method was considered to be the most accurate procedure, but it is time consuming and not always practical. A second and more practical method was to take sections approximately 56 μm apart and count the number of vitellogenic eggs on each section. Results indicate that the median vitellogenic egg diameter is 56 μm; hence this distance was used between each section under the assumption that the majority of oocytes would only be counted once. The size-distribution of vitellogenic oocytes was not found to be a function of size of the individual animal; hence the method was not likely to bias the

results in favor of larger animals with larger eggs, which would be counted more than once. The results of actual fecundity counts from the two methods showed no significant differences (paired T-test: $T = -1.62$, $P = 0.140$) (Fig. 1). As a result method two was used to estimate fecundity in *L. elevatus*. The fecundity of *L. elevatus* was determined for different locations and times of year: Biovent in November 1998, December 2001 and May 2000 and East Wall in December 2001. Fecundity counts from a minimum of nine individuals per site were obtained. Method 2 was also used to estimate the actual fecundity of four additional species: *Lepetodrilus pustulosus*, *L. ovalis*, *Rhynchopelta concentrica* and *Eulepetopsis vitrea*. Because of the difficulty in obtaining good sections of *Cyathermia naticoides* and *L. atlanticus*, method 1 was used. We estimated fecundity of three individuals of each of the six species, with sites and time of year being kept constant within each species.

RESULTS

Gametogenesis in the Genus Lepetodrilus

Lepetodrilus elevatus, L. pustulosus and *L. ovalis*

Ovarian morphology and gametogenesis of the three EPR lepetodrilids (*L. elevatus, L. pustulosus and L. ovalis*) are very similar. The ovary occupies a large volume of the animal and develops from the very posterior end. It gradually overlays the foot and the shell muscle. Ventrally it is replaced by the digestive gland on all but on the dorsal right side where it extends up to the visceral mass and mantle cavity. Oogonia develop in the germinal epithelium at the periphery of the ovary. The epithelium extends into the ovary, so oogonia are also present throughout the entire ovary. In *L. elevatus*, trabeculae (inward protrusions of connective tissue) are particularly apparent. Oogonia grow to approximately 20 μm before becoming previtellogenic oocytes. Small previtellogenic oocytes are mainly composed of a large nucleus and basophilic cytoplasm (Fig. 2A). When the oocytes have a diameter between 30–35 μm, vitellogenesis begins (Table 2). Yolk granules generally first become visible at the periphery of the oocyte. Vitellogenic oocytes are characterized by an acidophilic granular cytoplasm (Fig. 2A). The maximum feret diameters for oocytes of the three species are similar at 84–87 μm (Table 2).

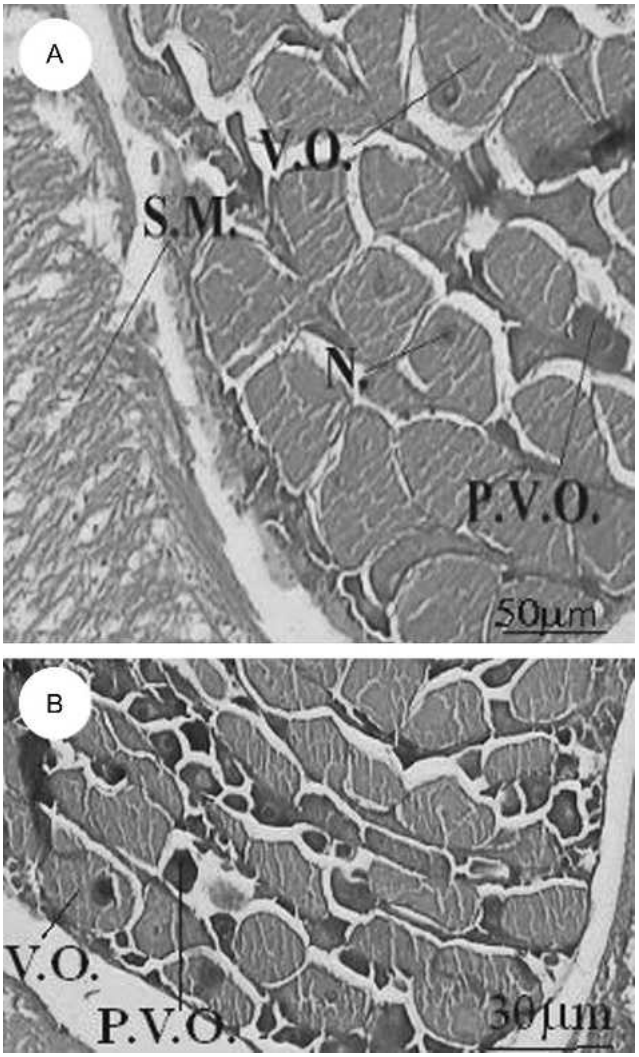

Figure 2. Ovarian structure of *Lepetodrilus*. (A) *L. elevatus*. (B) *L. atlanticus* P.V.O., previtellogenic oocyte; V.O., vitellogenic oocyte: S.M., shell muscle.

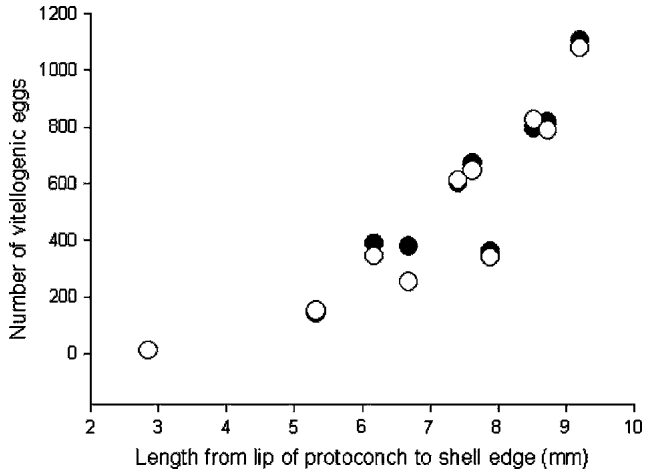

Figure 1. Comparison of two different methodologies to calculate the fecundity of *Lepetodrilus elevatus*.

Lepetodrilus atlanticus

The ovary of *L. atlanticus* is similar in terms of its position and morphology to the ovary of the EPR lepetodrilids (Fig. 2B). It lies dorsal to the foot and ventral to the visceral mass, with the digestive gland gradually over-laying it on the right side. The maximum oocyte size (92 μm) is slightly larger than the EPR lepetodrilids (Table 2).

Temporal Variability in the Genus Lepetodrilus

There were no significant differences in adult size (tested by ANOVA) among seasons for any of the species used in this study (Table 3).

The oocyte size-frequency distributions of the *L. elevatus, L. pustulosus* and *L. ovalis* taken at 9°N EPR in November 1998, December 1999, May 2000 and December 2000 show a bimodality related to previtellogenic oocytes (<35 μm) and vitellogenic

TABLE 2.

Maximum oocyte size and size of oocyte at which onset of vitellogenesis occurs for seven species of gastropod.

Species	Maximum Oocyte Size (μm)	Size of Oocyte at which Vitellogenesis Begins (μm)
L. atlanticus	92	35–40
L. elevatus	84	30–35
L. pustulosus	84	30–35
L. ovalis	87	30–35
C. naticoides	123	45–50
R. concentrica	184	50–60
E. vitrea	232	60–70

oocytes (>35 μm) (Fig. 3). There was a high proportion of previtellogenic oocytes (approximately 60%); vitellogenic oocytes generally peak (approximately 10%) at 50–60 μm in *L. pustulosus* and *L. ovalis* and at 40–50 μm in *L. elevatus*. Oocyte distribution in *L. atlanticus* was more uniform than in the Pacific species (Fig. 5 see later).

Spatial Variation in the Genus Lepetodrilus

There was limited spatial variation in the oocyte size/frequency at different vent sites (11°N EPR, Galapagos Rift and Train Station) when compared with 9°N EPR (Fig. 4). The oocyte size distributions of *L. elevatus* from 11°N, the Galapagos vent and Train Station 9°N had a high mean proportion of previtellogenic oocytes (approximately 60%) where as the oocyte size distribution from the Biovent vent site at 9°N had only 30%. There was no noticeable spatial variation between the oocyte size frequency distributions of *L. pustulosus*. In *L. ovalis*, there were no discernable differences between the two oocyte size distributions at Train Station and Biovent, both of which are very similar to the distributions described for *L. pustulosus*. To ensure that there was no size bias among samples, we compared shell diameters to ensure no size bias between the samples. All P values were >0.05 suggesting that there was no significant difference between the shell diameters at the two sites (Table 3).

The variability (standard deviations) of the size classes within a site were compared with those between the sites using a Mann-Whitney U-test. The high P values and low test statistics (U) indicate there is no significant differences between the standard deviations shown intra or inter monthly.

Although the data are limited to samples from March and April, there was no significant difference (ANOVA $F = 1.151$, $P = 0.274$) in the oocyte frequency distributions of the Atlantic populations of *L. atlanticus* (Table 3, Fig. 5).

TABLE 3.

Analysis of variance statistic (F) and associated probability between the shell diameters of the gastropods from different temporal samples used in this study.

Species	F	P	D.f.
L. atlanticus	1.151(t-statistic)	0.274	1 (16)
L. elevatus	0.163	0.921	3
L. pustulosus	0.327	0.806	3
L. ovalis	0.424	0.658	2
C. naticoides	0.984	0.407	2
R. concentrica	0.470	0.705	2
E. vitrea	1.134	0.344	2

Gametogenesis in Non-lepetodrilid Species

Eulepetopsis vitrea

The ovary of *E. vitrea* is situated at the posterior end of the body, dorsal to the foot and on the right side. At maximum size, the ovary extends to the large right kidney. On the left side, the ovary extends around the small digestive gland with its upper limit just below the pallial cavity. The ovary of *E. vitrea* was never tightly packed with oocytes (Fig. 6A, B). Vitellogenesis begins when the oocytes reach a diameter of 60–70 μm (Table 2). Yolk granules generally first become visible at the periphery of the oocyte (Fig. 6E). Vitellogenic oocytes, reaching a maximum of 232 μm diameter, have an acidophilic granular cytoplasm.

Rhynchopelta concentrica

The ovary of *R. concentrica* appears to fill a smaller proportion of the body than the lepetodrilid species. It is confined to the mantle cavity and lies dorsal to the right shell muscle. Within the ovary, the oocytes appear tightly packed. Vitellogenesis begins when the oocytes are between 40μm and 50μm, reaching a maximum size of 184 μm (Fig. 6C). The oviduct, which runs through the center of the ovary, frequently contained unfertilized vitellogenic oocytes.

Cyathermia naticoides

Because of the coiled nature of *C. naticoides* fixative penetration and histology were poor. All stages of oocyte were present (Fig. 6D). Vitellogenesis began at ~50 μm and maximum oocyte size was 123 μm, with yolk being distributed throughout the oocyte (Fig. 6F).

Temporal Variation in Eulepetopsis vitrea, Rhynchopelta concentrica, *and* Cyathermia naticoides

The oocyte size-frequency distributions of *Cyathermia naticoides*, *Rhynchopelta concentrica* and *Eulepetopsis vitrea* were obtained from samples taken in November 1998, December 1999 and 2000, May 2000 and December 2001 (Fig. 7). *Cyathermia naticoides* and *R. concentrica* both had strong bimodal distributions, with the first peak relating to previtellogenic oocytes and the second to vitellogenic oocytes. *Cyathermia naticoides* generally had a high proportion of previtellogenic oocytes (approximately 60%), with vitellogenic oocytes generally peaking at 90–100 μm. *Rhynchopelta concentrica* had a lower proportion of previtellogenic oocytes than *C. naticoides* (approximately 50%). The range of oocyte size extended much higher in December 2001 than in the two remaining time periods; this was caused by a single individual with an exceptionally large oocyte of 184 μm, with the next oocyte closest in size being 149 μm. The oocyte size-frequency distribution of *E. vitrea* was less obviously bimodal than the other two species and in May 2000, three peaks were apparent. Previtellogenic oocytes constituted approximately 60% of the total oocytes. Vitellogenic oocytes peaked at the 80–100 μm size

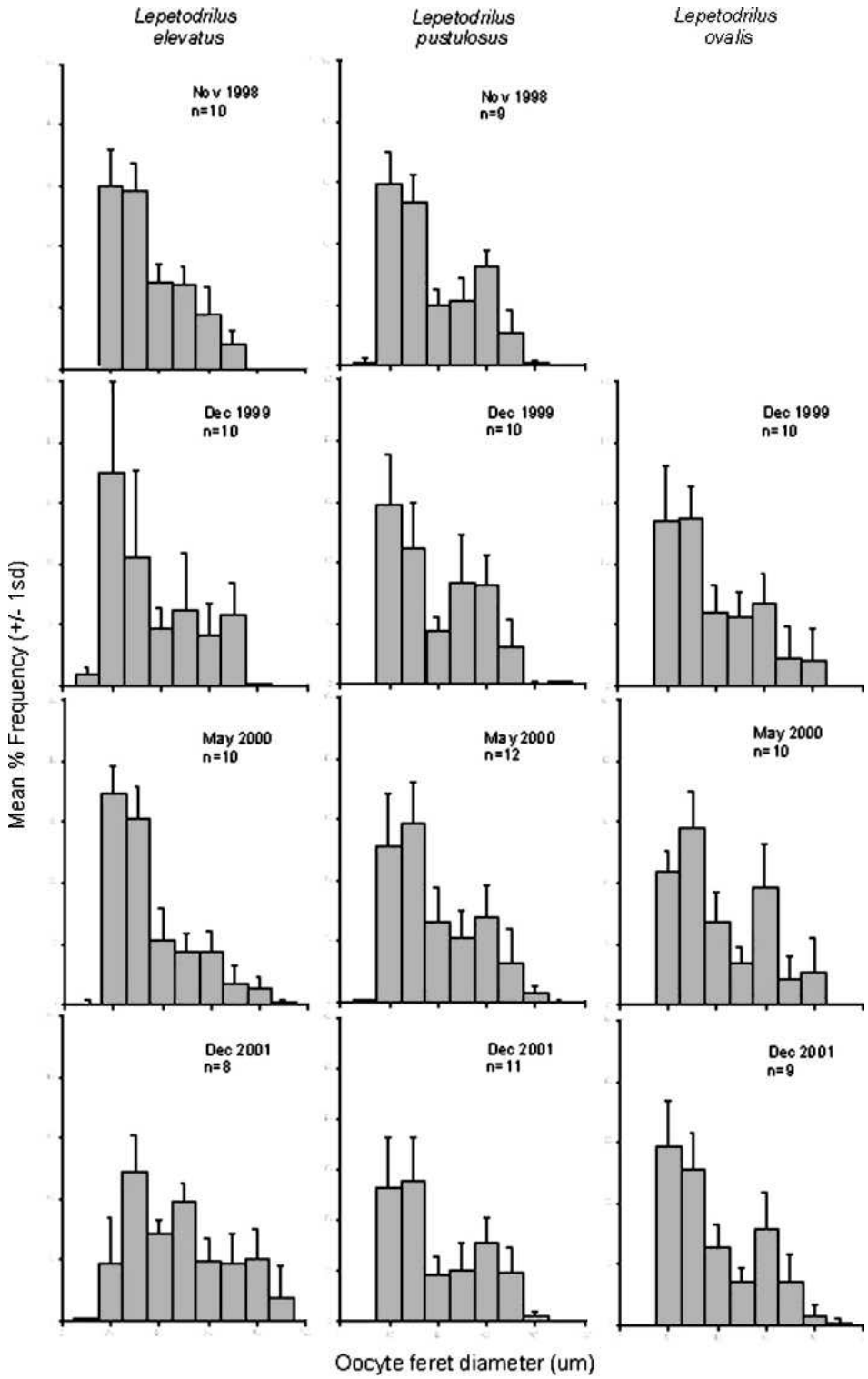

Figure 3. Mean oocyte size-frequency distribution of *Lepetodrilus elevatus*, *Lepetodrilus pustulosus* and *Lepetodrilus ovalis*. Error bars refer to standard deviation. Samples collected from Biovent, 9°N EPR. $N =$ number of individuals; $n =$ number of oocytes measured.

class although a third peak at 160–180 μm was present in May 2000.

To determine if the proportion of vitellogenic oocytes is related to adult size, Spearman's rank correlation (r_s) was applied to the oocyte size data (Table 4). The strong positive values of r indicate a strong relationship between vitellogenic oocytes and adult size. This may indicate that these vent gastropods reproduce only once in their lifetime. To compare monthly oocyte size-frequency distributions, it is therefore first necessary to ensure that the size distributions of the individuals in each month are approximately equal.

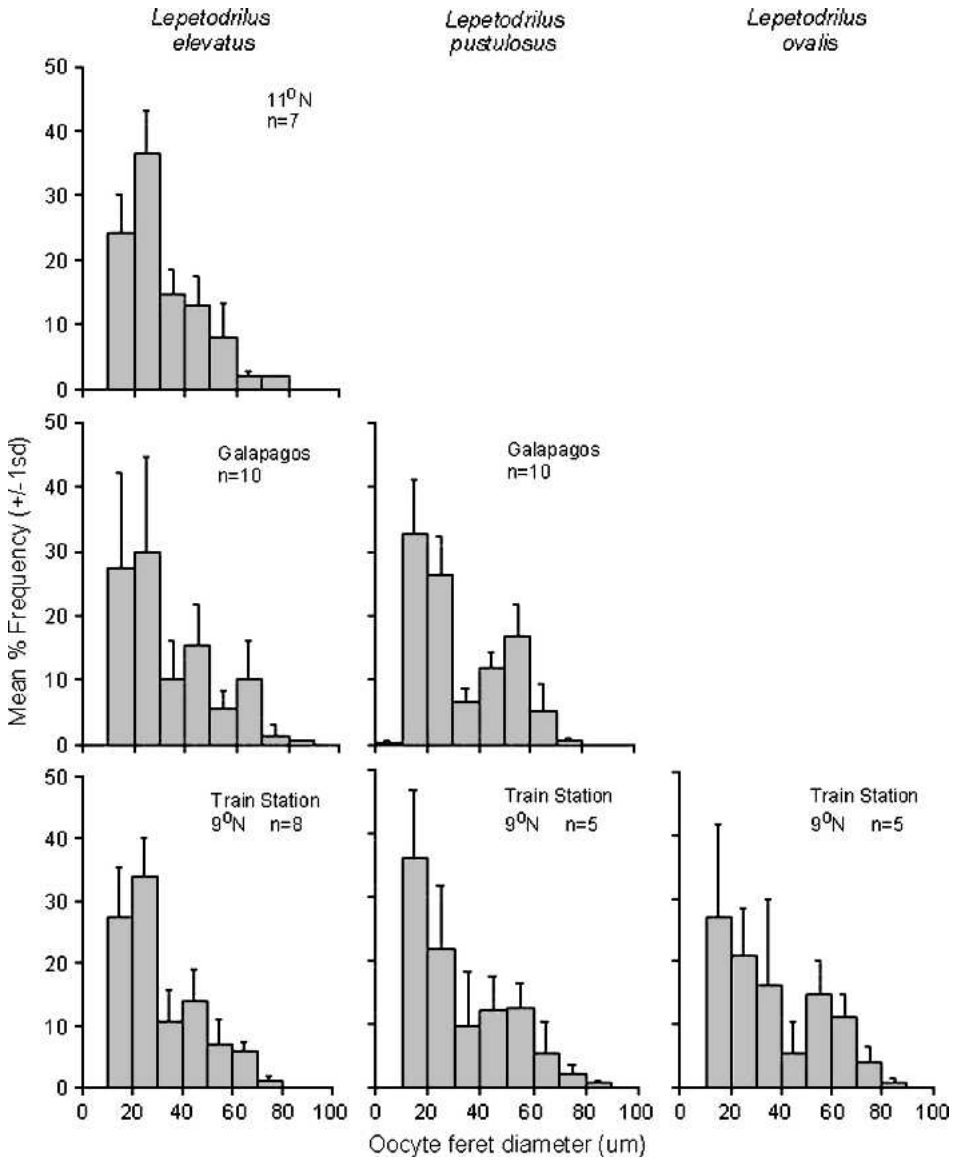

Figure 4. Mean oocyte size-frequency distribution of *Lepetodrilus elevatus*, *Lepetodrilus pustulosus and Lepetodrilus ovalis*. Error bars refer to standard deviation. Samples collected in December 2001.

Fecundity

Fecundity and size showed a curvilinear relationship in *L. elevatus* from different sites and different times (Fig. 8A). Samples were taken from Biovent in November 1998, December 2001, and May 2000 and from East Wall in December 2001.The smallest individual to possess vitellogenic oocytes had a length of 2.85 mm. The largest instantaneous fecundity was 1,142 found in an individual with a length of 8.40 mm.

To assess the effect of spatial and temporal variation upon fecundity in *L. elevatus*, a general linear model was fitted to log-transformed data. All *t*-values were significant at $P < 0.05$, indicating a significant fit. The General Linear Model gives a low *F*-value and high probability indicating that there is no significant effect of time on fecundity. The high *F*-value associated with length, together with the low probability suggests that the influence of length on actual fecundity is significant. Although data are limited for the remaining six species, there is a relationship between the number of vitellogenic eggs and size in all of them (Fig. 8C, D).

***Sperm Storage of* Lepetodrilus elevatus**

The genital groove of *L. elevatus* runs down the right side of the body within the mantle cavity, towards the gonad (Fretter 1988). It narrows considerably and opens into the receptaculum seminalis that is surrounded by columnar epithelium. Sperm within the genital groove were disorientated and in prostatic fluid. Sperm in the receptaculum seminalis were orientated with their heads penetrating the columnar epithelium. Only sperm on the dorsal side of the receptaculum seminalis were disorientated. Of the 40 individuals examined, only one individual lacked sperm in the genital groove. Three individuals had sperm in the genital groove but not the receptaculum seminalis. The smallest individual found to contain sperm had a shell diameter of 2.9 mm. Only two individuals (December 2000 and

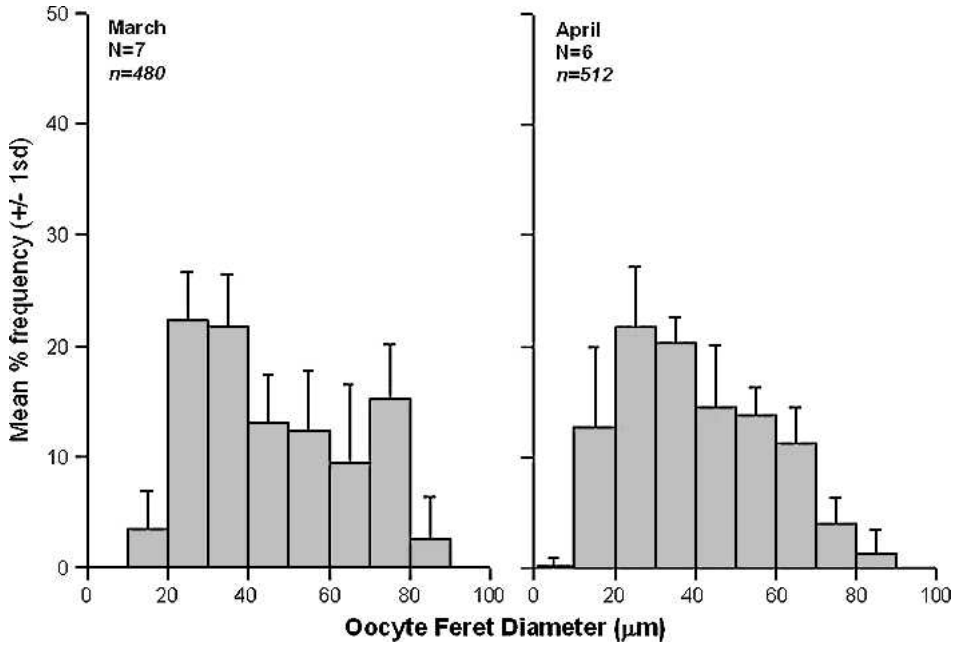

Figure 5. Mean oocyte size-frequency distribution of *Lepetodrilus atlanticus*. Error bars refer to standard deviation. *N* = number of individuals; *n* = number of oocytes measured.

December 2001) of the 40 examined, had oocytes within the mantle cavity. The eggs were positioned near the receptaculum seminalis. One individual had two oocytes within the mantle cavity, with feret diameters of 81 μm and 78 μm, and one individual had only one oocyte within the mantle cavity with a feret diameter of 85 μm. The presence of the germinal vesicle within the oocytes indicates that fertilization has not yet taken place. The 40 individuals examined were taken from four different times of year, and no trend in oocyte presence or sperm absence can be seen with time.

DISCUSSION

Although egg size cannot be used as a perfect indicator of larval development mode, the maximum oocyte sizes of the four lepetodrilid species are small when compared with species with known lecithotrophic development (Eckelbarger 1994). The shallow-water trochid *Gibbula umbilicalis* (da Costa 1778) has an oocyte size of approximately 140 μm, 50 μm larger than that of the lepetodrilids, and shows planktotrophic development (Robert 1902). Conversely, *Crepidula aculaeta* (Gmelin 1791), showing direct development, has a maximum oocyte of 488 μm (Collin 2003). The oocyte size range found for the three EPR lepetodrilids is over 30% smaller than the measurements given by Fretter (1988), but agree with Berg (1985) for *Lepetodrilus pustulosus* and *Lepetodrilus elevatus*. From this we might infer that larval development in lepetodrilids is planktotrophic but there is no evidence of shell growth during their time in the plankton (Mullineaux & Mills pers. obs.).

The maximum oocyte sizes of the remaining limpets are >100 μm with vitellogenesis occurring at 50% the maximum size. The size of the average oocyte of *Rhynchopelta concentrica* has been placed as 152 μm by 132 μm (Berg 1985), and "10% greater" (Fretter 1989). The mean oocyte size for *R. concentrica* is ca. 90 μm, although the mean *vitellogenic* oocyte is approximately 130 μm. Conversely, *R. concentrica* taken from 13°N and 21°N on the EPR (Berg 1985, Fretter 1989) have a considerably larger mean oocyte size than those collected from 9°N. The oocyte size, and the lengthy period of vitellogenesis, beginning at one third of the maximum oocyte size, are suggestive of lecithotrophic development. The maximum oocyte size of *Eulepetopsis vitrea* is less than three quarters of the size given by Fretter (1990). This could be a function of spatial variability, because the samples used by Fretter (1990) were collected from the Galapagos Rift and 13°N. However, histology can account for shrinkage of up to ~20% and could also account for the discrepancy in the oocyte sizes. Maximum oocyte size in *C. naticoides* is intermediate between planktotrophy and lecithotrophy.

The bimodal distributions of the oocyte size-frequencies found in this study are typical of other gastropods (Christiansen & Fenchel 1979). Based on such past observation of nonvent gastropods, the bimodal nature of the histograms would suggest the vent limpets are iteroparous although the curvilinear relationship between size and fecundity in lepetodrilids would support semelparity. The diversity of gametogenesis observed in the hydrothermal vent limpets, coupled with the diversity in site of sperm storage and fertilization (Fretter 1988, 1989, 1990) suggest varying reproductive patterns.

The trabeculae, present in the ovaries of the four lepetodrilid species and *R. concentrica*, allow nutrients to be delivered to the ovary and support rapid gametogenesis (Hodgson & Eckelbarger 2000). Ovarian trabeculae are unusual in gastropod molluscs. The ovaries of *E. vitrea* and *C. naticoides* are more 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 pers. obs.).

The lack of secondary reproductive organs, such as albumin glands, coupled with the absence of *L. elevatus* containing fertilized oocytes, suggest that brooding is short, if it occurs at all. It seems likely that in all six species that undergo internal

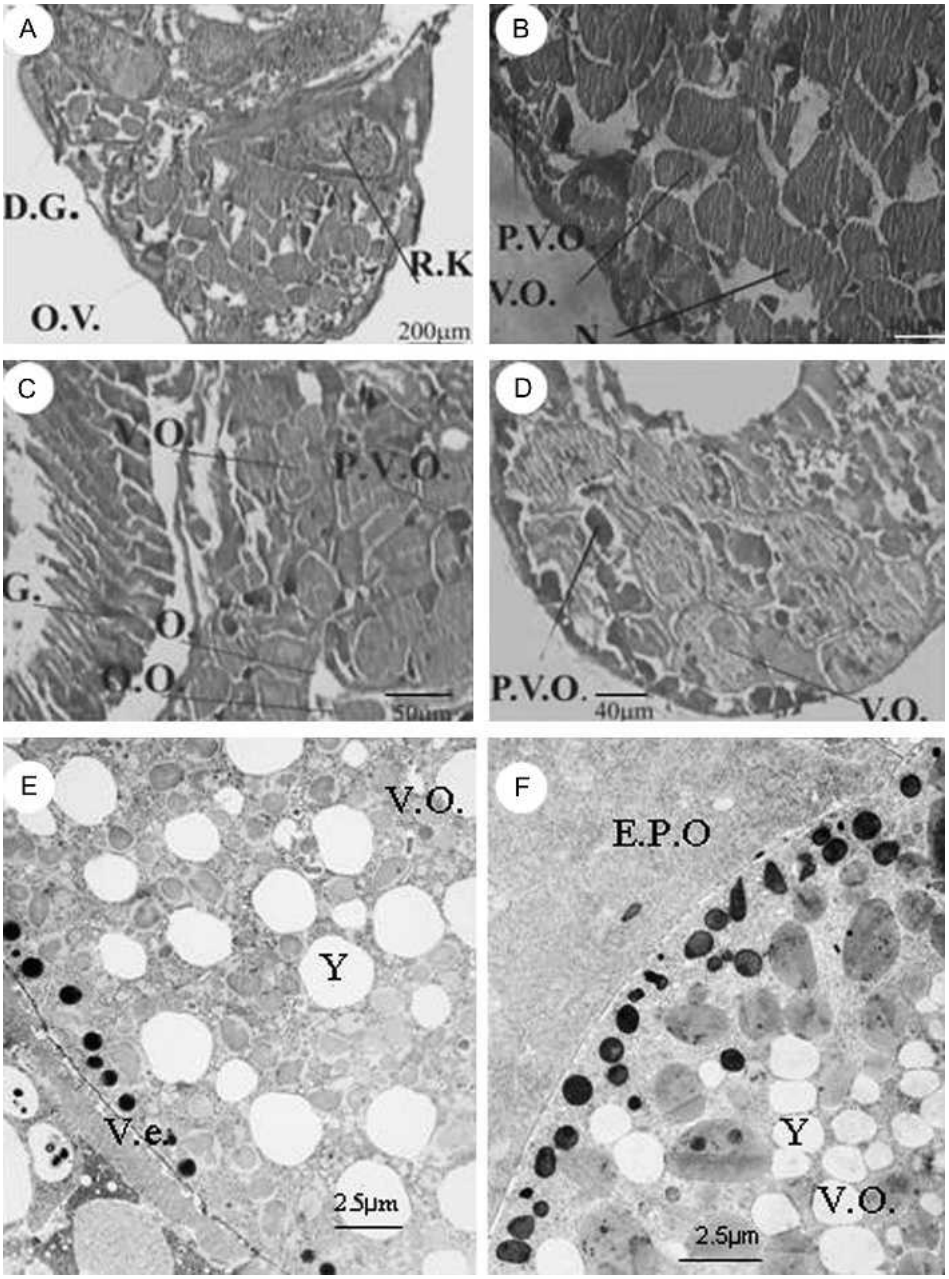

Figure 6. (A) Section of developing ovary of *Eulepetopsis vitrea*, (B) well developed ovary in *E. vitrea*, (C) section of ovary of *Rhynchopelta concentrica*, (D) Section of ovary of *Cyathermia naticoides*, (E) Ultrastructure of cytoplasm of unfertilized oocytes of *E. vitrea*, (F) ultrastucture of unfertilized oocyte of *C. naticoides*. DG, digestive gland; E.P.O Early previtellogenic oocyte; F, foot; G, gill; H, head; R.K., right kidney M.S., mantle skirt; N., nucleus; O; oviduct; O.O., oocyte; O.S., esophagus; O.V., ovary; P.V.O., previtellogenic oocyte; S.M., shell muscle; T., trabeculae; V.O., vitellogenic oocyte; Y., Yolk.

fertilization, the oocytes are fertilized during their exit from the mantle cavity. The number of individuals in a brood of *L. elevatus* and *L. pustulosus* appear to be low (maximum of four and six respectively) (Fretter 1988).

A previous study (Berg 1985) suggests that these limpets are phylogentically constrained to leicithotrophic development. The dispersal capabilities of lecithotrophic larvae are not fully understood. Shallow water species can generally achieve a greater dispersal with planktotrophy than with lecithotrophy. However, in the cold, often nutrient poor waters of the abyss, the metabolism of larvae is greatly reduced, allowing a wider dispersal than shallow water counterparts. Shilling and Manahan (1994) concluded that because the nutrients in Antarctic waters are depleted for much of the year, lecithotrophy may allow wider dispersal than planktotrophy. This idea is supported for the deep sea by the very broad geographic distributions of lecithotrophic echinoderm species (Young et al. 1999).

Episodicity

The oocyte size-frequency analysis demonstrated that gametogenesis is highly asynchronous among individuals, but

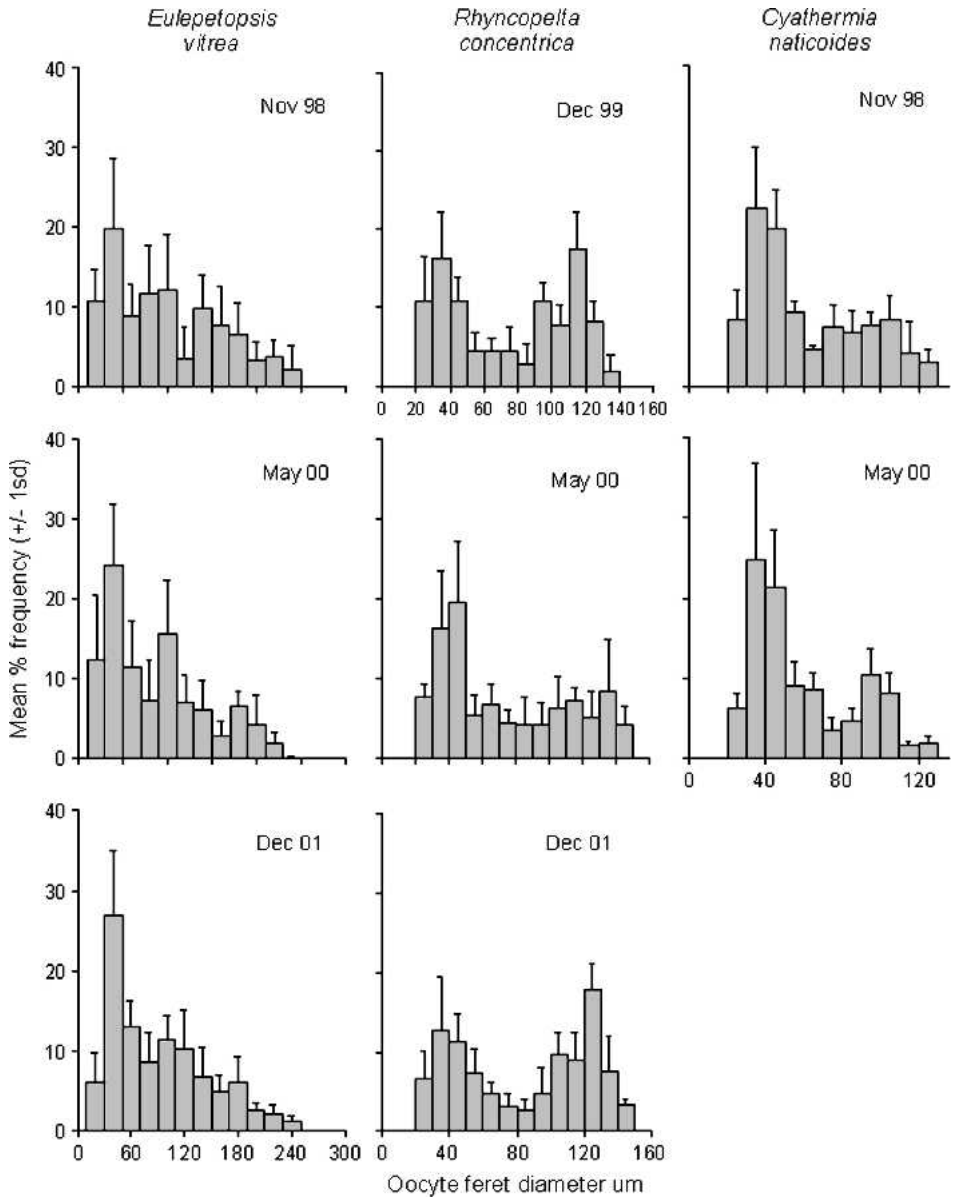

Figure 7. Mean oocyte size- frequency distribution of *Cyathermia naticoides*, *Rhynchopelta concentrica* and *Eulepetopsis vitrea*. Error bars refer to standard deviation. Samples obtained from Biovent 9°N (EPR). N = number of individuals; n = number of oocytes measured.

showed no temporal episodicity at the level of populations. The fecundity of *Lepetodrilus elevatus* and the occurrence of oocytes within the mantle cavity showed no temporal variation. The reproductive cycle of the seven limpet species examined was quasi-continuous and asynchronous, as is the cycle of the majority of vent molluscs (Tyler & Young 1999, Young 2003).

There was no evidence of spatial variation in oocyte size frequency of the three EPR lepetodrilids. Copley et al. (2003) examined the reproductive development of *Paralvinella palmiformis* Desbruyères & Laubier 1986, an alvinellid inhabiting vents on the Juan de Fuca Ridge. In contrast to the vent limpets, a strong spatial variation in gamete frequency and development was evident.

Fluctuations of juvenile recruitment of limpets on settlements blocks and seasonal differences in larval counts (Mullineaux et al. 1998) suggest episodic recruitment. However, the adult population structure of limpets tends to be unimodal (Mullineaux et al.1998, Sadosky et al. 2002). One plausible explanation for this discrepancy would be fluctuations in the currents that carry the larvae to and from hydrothermal vents. Periodic reversals in along-ridge flows have been noted at 9°N (EPR) suggesting currents are major factors in larval dispersal and recruitment (Mullineaux et al. 2002).

Fecundity

Fecundity is a major variable within a life history pattern (Ramirez Llodra 2002). The correlation of actual fecundity with size is a reflection of ovarian growth. The size at which individuals first develop vitellogenic oocytes is consistent with the size at which sperm are present within the receptaculum seminalis. *Lepetodrilus elevatus*, like *L. pustulosus* (Fretter 1988) mature very early, with females being capable of reproducing at less than one third of their maximum size. The reproductive

TABLE 4.

Spearmans rank Correlation statistic (r), associated probability and number of individuals for the relationship between shell diameter and the ratio of vitellogenic oocytes of the total number of oocytes: previtellogenic oocytes measured for the oocyte size-frequency distributions shown in Figures.

Species	Spearmans Rank Coef. (r)	P	N
L. atlanticus	0.780	0.001	20
L. elevatus	0.305	0.005	84
L. pustulosus	0.296	0.011	72
L. ovalis	0.470	0.001	67
C. naticoides	0.425	0.004	44
R. concentrica	0.454	0.005	37
E. vitrea	0.530	0.009	23

pattern of early maturity is typical of an opportunistic species (Bridges et al. 1994); early maturing species have a higher probability of reaching maturity but a lower lifetime fecundity than species that delay maturity.

The inverse correlation between oocyte size and fecundity in many taxa (Thorson 1950) is particularly noticeable in this study, with the EPR lepetodrilids having the largest fecundity and *Eulepetopsis vitrea*, the species with the largest oocyte size, having the smallest fecundity.

No temporal or spatial variation was observed in the fecundity of *L. elevatus*, although specimens from 1998 showed a higher level of variance than the remaining specimens. The lack of temporal variation suggests that the energy available to the limpets remained consistent. The abundance of nutrition present at hydrothermal vents could indicate that energy is rarely a limiting factor in the life-history of the limpet. The lack of spatial variation in fecundity between the Biovent and East Wall samples suggests copious energy supplies at both sites. At the time of sampling both East Wall and Mussel Bed had thriving vent communities (Van Dover pers. comm.). However, a comparison with a site such as "Dead Mussel Bed," where nonvent fauna was beginning to dominate could reveal a spatial variation in fecundity. Although many of the gametogenic processes are phylogenetically constrained, abiotic and biotic factors such as habitat stability and nutritional availability should affect the quantity and quality of the oocytes (Ramirez Llodra 2002).

Sperm Storage

The majority of *Lepetodrilus elevatus* examined had sperm within the receptaculum seminalis and the genital groove. The receptaculum seminalis is an organ frequently associated with the storage of sperm; by burying the heads within the epithelium, sperm within the receptaculum seminalis are thought to derive nutrition from the epithelial cells (Fretter & Graham 1994). The disorientated sperm on the dorsal side of the receptaculum are presumably those, which have recently been deposited from the genital groove, indicating that orientation occurs in the receptaculum seminalis. In three individuals, sperm were present in the genital groove, but not the receptaculum seminalis, suggesting the possibility that sperm may be stored, rather than merely deposited in the genital groove. The lack of glycogen granules within the genital groove suggests that the storage time is not lengthy; it is likely that the sialomucin present within the walls of the genital groove has some function in aiding the transport of sperm (Eckelebarger pers. obs.). The prevalence of sperm storage in polychaetes and vestimentifera at hydrothermal vents (Zal et al. 1995, Hilario et al. 2005) suggests there is some adaptive advantage in such a turbulent and potentially toxic environment.

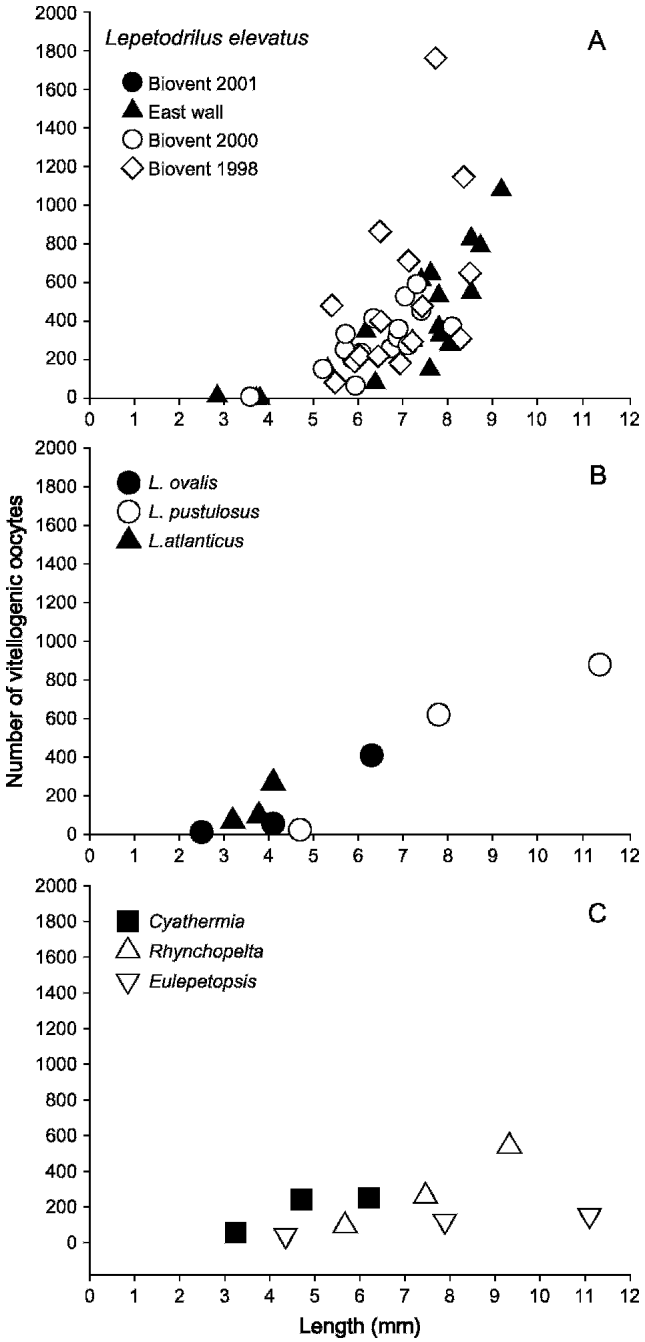

Figure 8. Fecundity in vent gastropods. (A) Fecundity in *Lepetodrilus elevatus* from different sites and times. (B) Fecundity in *L. ovalis*, *L.pustulosus* and *L. atlanticus*. (C) Fecundity in *Cyathermia naticoides*, *Rhynchopelta concentrica* and *Eulepetopsis vitrea*.

ACKNOWLEDGMENTS

The authors thank the Masters and crew, and the submersible groups that have enthusiastically supported our field program; Cindy Van Dover (Duke University Marine Laboratory) for

access to samples of vent gastropods from the East Pacific Rise; and Mary Rice for assisting with e.m. fixations at sea. This study was carried out during the tenure of NSF grants OCE-0243688, OCE -0118733 and OCE-9619606

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