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Seasonality and zonation in the reproductive biology and population structure of the shrimp *Alvinocaris stactophila* (Caridea: Alvinocarididae) at a Louisiana Slope cold seep

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ABSTRACT: Ecological patterns in deep-sea chemosynthetic environments are often considered to be independent of variations in photosynthetic primary production. This study examined spatial and temporal variation in the reproductive development and population structure of the caridean shrimp *Alvinocaris stactophila* at the Brine Pool cold seep on the Louisiana Slope. To assess spatial variation, samples were collected by submersible from different locations across the Brine Pool mussel bed in November 2003. The proportion of males in samples declined from the outer to the inner zone of the mussel bed, while the proportion of female shrimp carrying eggs increased. This zonation may have resulted from the congregation of large ovigerous females towards the inner zone, where sulphide flux from underlying sediments may be absent or reduced. To examine temporal variation in reproductive development, oocyte size-frequency distributions were compared between August 1997, March 2002, February 2003, November 2003, and July 2004. All oocytes in early spring samples were small and previtellogenic, while summer samples contained larger vitellogenic oocytes. The largest oocytes were found in large non-ovigerous females from the autumn, whereas ovigerous females from the same season contained the smallest oocytes observed in this study. Embryos in the autumn exhibited no differentiation, in contrast to well-developed embryos from early spring that hatched into zoea larvae. These features indicated seasonal and iteroparous reproduction in *A. stactophila*, with females carrying embryos from autumn to early spring, possibly timing the release of planktotrophic larvae to exploit a seasonal peak in surface productivity and its export.

KEY WORDS: Cold seeps · Alvinocaridid shrimp · Reproduction · Seasonality · Zonation

INTRODUCTION

More than 600 new morphological species of mega- and macrofauna have been described from deep-sea chemosynthetic environments in the past 25 years (Van Dover et al. 2002). Although the physiologies of several species have been studied in detail (Childress & Fisher 1992), development of ecological understanding has been slower (Tunnicliffe 1991, Sibuet & Olu 1998) and few species have been subjects of primarily reproductive investigations (Tyler & Young 1999). However, recent studies have revealed spatial and temporal variation in the reproductive development of some species at hydrothermal vents (Copley et al. 2003, Perovich et al. 2003). Similar patterns have yet to be examined at cold seeps, although seasonality has been described in some reproductive features of 1 seep species (Lisin et al. 1997). Characterising such aspects of life history biology is a prerequisite for understanding the dynamics of these insular populations.

The Louisiana Slope in the northern Gulf of Mexico hosts several cold-seep communities at depths be-

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tween 400 and 1000 m (Kennicutt et al. 1985, MacDonald et al. 1990a). Chemosynthetic primary production at these sites is supported by biogenic methane released from the seafloor by salt tectonism and diapirism (Brooks et al. 1987), with hydrogen sulphide also available from the reduction of seawater sulphate by sulphate-reducing bacteria in sediments (Sibuet & Olu 1998).

Brine Pool NR1 is located at 27°43.4′N, 91°16.5′W on the Louisiana Slope at a depth of 650 m (MacDonald et al. 1990b). The site consists of a pockmark filled with hypersaline brine (salinity 120), forming a 'lake' on the seafloor that is 22 m long by 11 m wide. Bubbles of methane issue from the centre of the pool. The brine pool is surrounded by a bed of mussels *Bathymodiolus childressi* that varies from 3 to 7 m in width and covers an area of ~540 m^2 (MacDonald et al. 1990b). In addition to mussels, the Brine Pool seep community includes orbiniid polychaetes, neritid gastropods, galatheid crabs and the caridean shrimp *Alvinocaris stactophila* (MacDonald et al. 1990a).

Alvinocaris stactophila belongs to the same family (Alvinocarididae) as shrimp from hydrothermal vents (Komai & Segonzac 2003). Adult *Rimicaris exoculata* at hydrothermal vents appear to feed on epibiotic chemosynthetic bacteria (Polz et al. 1998), but this feature cannot be generalised across the group (Gebruk et al. 1993) and there is no evidence that *A. stactophila* exhibits a similar mode of nutrition (Williams 1988). Nine species of *Alvinocaris* have been described at hydrothermal vents and cold seeps worldwide (Komai & Segonzac 2005, Komai et al. 2005), but *A. stactophila* is so far only known from the cold seeps of the Louisiana Slope (Williams 1988). Reproductive patterns of decapod crustaceans from chemosynthetic environments are thought to have strong phylogenetic constraints (Van Dover et al. 1985, Tyler & Young 1999), and previously examined alvinocaridid shrimp exhibit planktotrophic development and are not thought to reproduce seasonally (Ramirez Llodra et al. 2000).

Orton (1920) predicted that the breeding period of deep-sea species would be continuous as a consequence of the constant temperature of their environment. Although many deep-sea species undergo continuous or asynchronous reproduction, some species reproduce opportunistically or seasonally, linked to variations in food supply (Tyler 1988, Eckelbarger & Watling 1995). *In situ* primary production in deep-sea chemosynthetic environments is not known to vary seasonally and is usually orders of magnitude greater than organic input from the photic zone. Seasonal variation in phytodetrital input is therefore unlikely to influence the reproductive timing of species in these environments directly through their energetics. However, species with planktotrophic larvae that disperse away from chemosynthetic environments might still exhibit seasonal reproductive patterns. Such patterns would constitute ecological links between deep-sea chemosynthetic environments and the dynamics of surface productivity.

Seasonal reproduction has been proposed so far in 2 species from chemosynthetic environments. The clam *Calyptogena kilmeri* exhibits seasonal variation in the proportion of its gonad undergoing reproduction at Monterey Canyon cold seeps, even though oocyte size-frequency distribution does not vary between November and March (Lisin et al. 1997). At hydrothermal vents, egg hatching in the crab *Bythograea thermydron* peaks during April–May at vent sites along the East Pacific Rise (Perovich et al. 2003). Seasonal reproduction is currently being described in bathymodiolid mussels in shallow mid-Atlantic hydrothermal vents (A. Colaço pers. comm.) and Louisiana Slope cold seeps (P. Tyler pers. comm.).

Bythograea thermydron also shows a spatial pattern in reproductive behaviour at hydrothermal vents, with females migrating to the vent periphery to extrude, brood and hatch their eggs (Perovich et al. 2003). Spatial variation in reproductive development is also known for the polychaete *Paralvinella palmiformis* at Juan de Fuca hydrothermal vents, which reflects the heterogeneity of the vent community (Copley et al. 2003). Although zonation in the physiological condition of mussels is known at the Brine Pool cold seep in the Gulf of Mexico (Smith et al. 2000), spatial variation in reproduction has yet to be examined in cold seep species.

The aims of this study were to: (1) determine any spatial variation in the population structure and reproductive features of *Alvinocaris stactophila* across the Brine Pool mussel bed; (2) examine any seasonal patterns in the reproduction of this species at the Brine Pool. The null hypothesis was that if reproduction is not seasonal, variation in reproductive development within samples from different times of year should be as great or greater than the variation between them (Tyler et al. 1982).

MATERIALS AND METHODS

To assess spatial variation in population structure and reproductive features, 5 samples of *Alvinocaris stactophila* were examined from different locations across the Brine Pool mussel bed in November 2003: 2 samples were collected from the inner zone of the mussel bed, within 1 m of the Brine Pool; 2 samples were collected from the middle zone of the mussel bed; and, 1 sample was taken from the outer zone, within 1 m of the outer margin of the mussel bed. Constraints

of expedition logistics unfortunately precluded a replicate sample from this outer zone.

Samples were collected using a hydraulic scoop and suction sampler attached to the manipulator of a Johnson Sealink submersible. The proportion of ovigerous females in samples was recorded immediately aboard ship. All specimens were fixed in 10% buffered seawater formalin for 48 h and stored in 70% isopropanol.

Carapace length and total length of each shrimp were measured to the nearest 0.1 mm using Vernier calipers, and individuals were sexed under a dissecting microscope. An asymmetrial mesial extension on the endopod of pleopod 1 and the presence of the appendix masculina on pleopod 2 were used to distinguish males (Williams 1988). The egg masses of 125 ovigerous females from November 2003 were removed from the pleopods, and the eggs counted under a dissecting microscope to determine realised fecundity (sensu Anger & Moriera 1998).

To assess seasonal variation in reproductive development, oocyte size-frequency distributions were compared from females collected by submersible in August 1997, March 2002, February 2003, November 2003 and July 2004. These samples comprised pooled collections of individuals from different locations across the mussel bed, rather than spatially discrete samples. Specimens were fixed in formalin and preserved in isopropanol as for the spatial study. The ovaries were subsequently dissected from female shrimp, and individual oocytes were removed from each ovary under a Leica MZ8 dissecting microscope. Images of oocytes were captured from the microscope using a JVC TK-1280E video camera connected to PC with a Matrox Rainbow Runner video card. Where female specimen numbers allowed, the feret diameters of 80 oocytes were measured in each of 12 females per sample using Jandel Scientific's SigmaScan Pro image analysis software. Feret diameter—the diameter of a hypothetical circle of equal area to the object measured—was used to standardise variations in oocyte shape. Images of oocytes were calibrated from measurements of a graticule slide at identical magnification.

During measurement, oocytes were laid flat in a dissecting dish for measurement to ensure that maximum cross-sectional areas were recorded. Packing of oocytes in ovaries often results in irregular oocyte shapes in *Alvinocaris stactophila*, particularly among mature oocytes. Previous determinations of oocyte size-frequency distribution have measured the areas of cells in histological sections bisecting the nucleus and nucleolus (Ramirez Llodra et al. 2000, Perovich et al. 2003). Given the occurrence of irregularly shaped oocytes in *A. stactophila* and the often non-central position of the nucleus within the cell, such measurements are influenced by the orientation of sectioning in relation to the shape of the cell. Measurement of oocyte sizes from histological sections was therefore rejected in favour of direct measurement of maximum cross-sectional areas of oocytes dissected from the ovary.

Subsamples of intact ovaries from each month were examined histologically to assess development. The ovarian tissue was dehydrated in graded alcohols, cleared with Histoclear, set in paraffin wax and sectioned by microtome at 5 µm. Sections were mounted on glass slides and stained with haematoxylin and eosin.

RESULTS

Population structure in November 2003

A total of 487 specimens of *Alvinocaris stactophila* were collected and examined in the 5 samples from November 2003. Only 175 specimens were identified as male (36%), resulting in an overall sex ratio that deviated significantly from 1:1 (175 males:312 females, $\chi^2 = 19.0$, 1 df, $p < 0.0001$). Of the 312 females, 147 were ovigerous (30%). The carapace length of the smallest male identified (4.9 mm) was similar to that of the smallest female (4.85 mm), which indicated that any bias in sex ratio was not the result of immature males being misidentified as females.

The overall size-frequency distribution of the specimens measured displayed 2 clear modal peaks and a tail of large specimen sizes (Fig. 1A). Males were represented throughout the size-frequency distribution of the samples, though there were proportionately fewer large males than ovigerous females (Fig. 1B). There were few non-ovigerous females in the peak and tail of larger sizes (Fig. 1C), whereas ovigerous females were restricted to the peak and tail of larger shrimp in the samples (Fig. 1D).

The size-frequency distributions of males and all females in November 2003 were significantly different (Mann-Whitney *U*-test, $T = 30270.5$, $p < 0.0001$). However, the size-frequency distributions of males and non-ovigerous females were not not significantly different (Mann-Whitney *U*-test, $T = 26668.0$, $p > 0.05$) and the ratio of males to non-ovigerous females did not deviate significantly from 1:1 (175 males:165 non-ovigerous females, $\chi^2 = 0.0941$, 1 df, $p > 0.05$).

Spatial patterns

The proportion of males in individual samples declined from the outer to the inner zone of the mussel bed (Fig. 2A). The sex ratio in the single sample from the outer zone did not differ significantly from 1:1

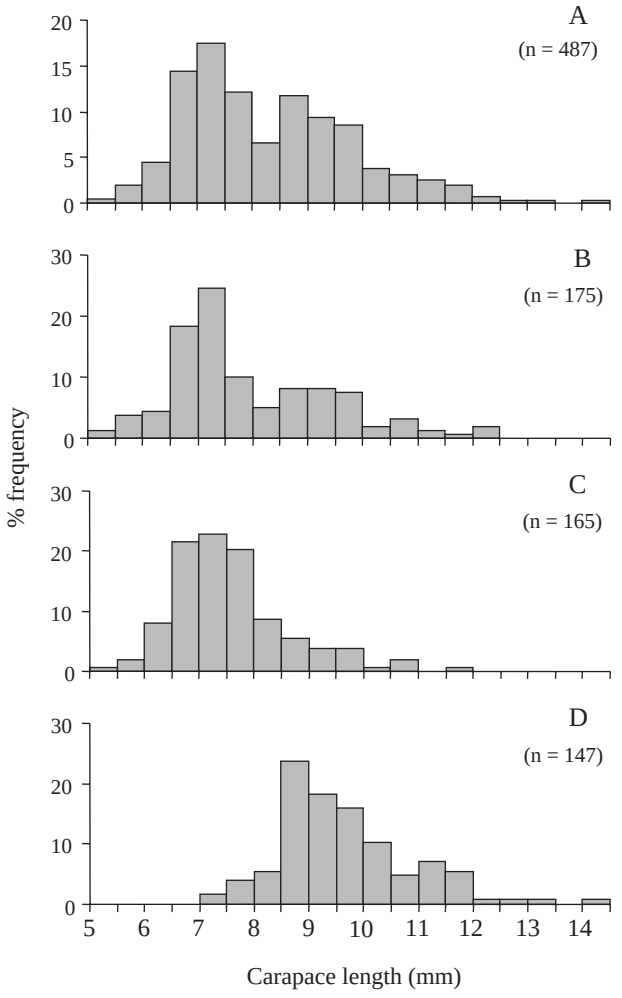

Fig. 1. *Alvinocaris stactophila*. Size-frequency distribution at the Brine Pool, November 2003. (A) All specimens, (B) males, (C) non-ovigerous females, (D) ovigerous females. n: no. of ind. measured

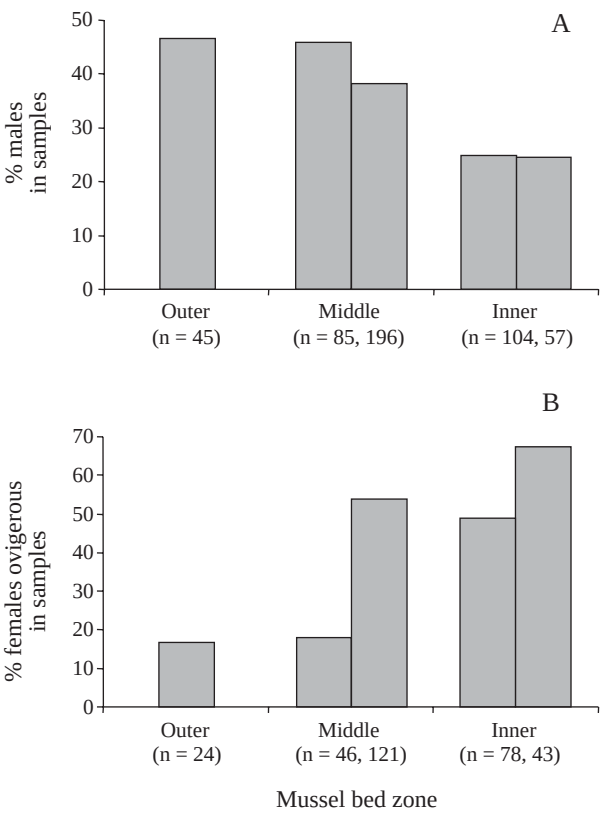

Fig. 2. *Alvinocaris stactophila*. Spatial variation in (A) sex ratio (% males in samples), and (B) proportion of females carrying eggs (% females ovigerous in samples) at the Brine Pool, November 2003. n: sample size (100%) in each case

(χ^2 = 0.023, 1 df, p > 0.05). Samples from the other zones, however, had significantly lower proportions of males than expected for a 1:1 sex ratio (middle zone: χ^2 = 13.30, 1 df, p < 0.01; inner zone: χ^2 = 35.79, 1 df, p < 0.001). There were no significant differences in sex ratio between replicate samples from the same zones, but significant differences were apparent between zones (χ^2 = 11.2, 2 df, p < 0.01).

To correct for the changing sex ratio across the mussel bed, spatial pattern in the occurrence of ovigerous females was assessed by comparing the ratio of ovigerous to non-ovigerous females, rather than overall proportions of ovigerous females in samples. The proportion of females carrying eggs in individual samples increased from the outer to the inner zone of the mussel bed (Fig. 2B), with a significant difference between zones (χ^2 = 12.4, 2 df, p < 0.01). There was no significant difference in the proportion of females carrying eggs in the 2 replicate samples from the inner zone (χ^2 = 3.21, 1 df, p > 0.05). In contrast, the proportion of females carrying eggs in the 2 middle zone samples was more variable, with a significant difference between them (χ^2 = 10.8, 1 df, p < 0.001).

Size-frequency distributions of shrimp also varied in samples across the mussel bed (Kruskal-Wallis multi-sample test, H = 31.3, 2 df, p < 0.0001). Although the November samples as a whole displayed 2 modal peaks and a tail of large sizes, the peak of larger sizes was most prominent in the inner zone (Fig. 3A). Both peaks were present in the middle zone (Fig. 3B) and the peak of larger sizes was absent among samples from the outer zone, where smaller shrimp were sampled with proportionally greater frequency (Fig. 3C).

The maximum realised fecundity determined among 125 ovigerous females examined from November 2003 was 1144 eggs from an individual with a carapace length of 13.8 mm. Realised fecundity correlated positively with carapace length of specimens (Fig. 4; Pearson correlation, r = 0.46, p < 0.0001). Correcting for

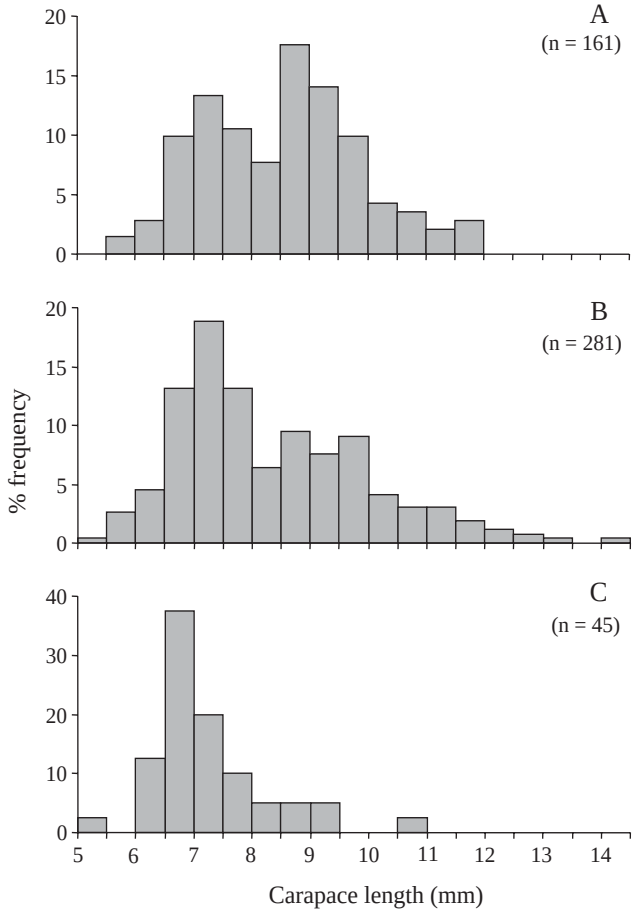

Fig. 3. *Alvinocaris stactophila*. Spatial variation in size-frequency distribution at the Brine Pool, November 2003; (A) inner zone, (B) middle zone, (C) outer zone. n: no. of ind. measured

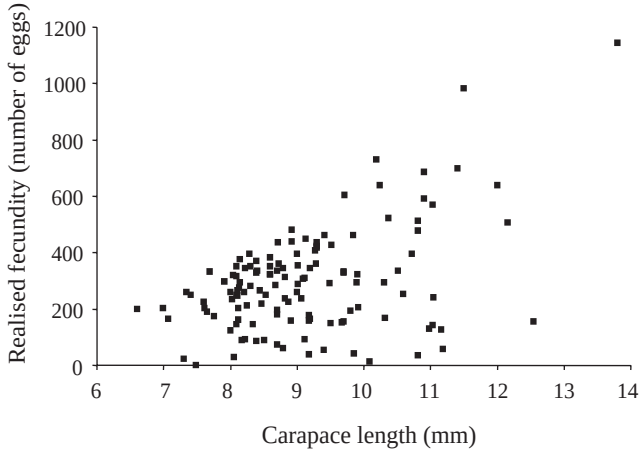

Fig. 4. *Alvinocaris stactophila.* Variation in realised fecundity (no. of eggs on pleopods) with body size (carapace length) for 125 females collected November 2003

variation in body size, ovigerous females from the inner zone carried more eggs per mm carapace length (CL) than those from the middle zone (inner zone: mean 38.6 eggs mm^{-1} CL ± 3.77 95% CI; middle zone: mean 24.9 eggs mm^{-1} CL ± 3.91 95% CI; *t*-test, $t = 4.84$, 118 df, $p < 0.0001$). The number of ovigerous females in the outer zone sample (n = 5) was too small to extend this analysis further.

The population structure in the outer zone in November 2003 therefore comprised a higher proportion of small non-ovigerous females and the generally smaller males compared to the other zones. The inner zone contained a high proportion of large ovigerous females. The population of the middle zone exhibited features that varied between these 2 extremes.

Temporal patterns

Females examined from February 2003 and March 2002 all contained relatively small oocytes with feret diameters between 50 and 225 µm (Fig. 5A). Mean oocyte sizes measured in the 12 individual shrimp from February ranged from 119 µm (±2.20 95% CI) to 186 µm (±4.46 95% CI). Mean oocyte sizes in individuals from March ranged from 126 µm (±2.59 95% CI) to 141 µm (±2.43 95% CI). Specimens examined from these months included both ovigerous and non-ovigerous females. Females from July 2004 and August 1997 all contained larger oocytes between 225 and 500 µm in feret diameter (Fig. 5B). The range of mean oocyte sizes measured in individuals collected in July was 256 µm (± 6.30 95% CI) to 372 µm (±7.61 95% CI), and the range in August was 288 µm (±5.12 95% CI) to 425 µm (± 7.65 95% CI).

Two very distinct peaks of oocyte sizes were apparent among females collected in November 2003 (Fig. 5C). The peak of larger oocytes belonged exclusively to large non-ovigerous females, specifically those few non-ovigerous individuals found in the modal peak and tail of larger sizes in the population structure at that time (Fig. 1C). These specimens exhibited mean oocyte sizes ranging from 487 µm (±7.59 95% CI) to 567 µm (±10.18 95% CI). The peak of small oocytes in November 2003 was produced by large ovigerous females and smaller non-ovigerous females from the peak of smaller-sized individuals in the population structure. These specimens showed a range of mean oocyte sizes from 93 µm (±2.81 95% CI) to 167 µm (±3.94 95% CI).

Oocytes in all specimens examined from early spring samples were previtellogenic (Fig. 6A), in contrast to larger oocytes in summer samples (Fig. 6B). Large non-ovigerous females examined from late autumn contained even larger vitellogenic oocytes (Fig. 6C), while

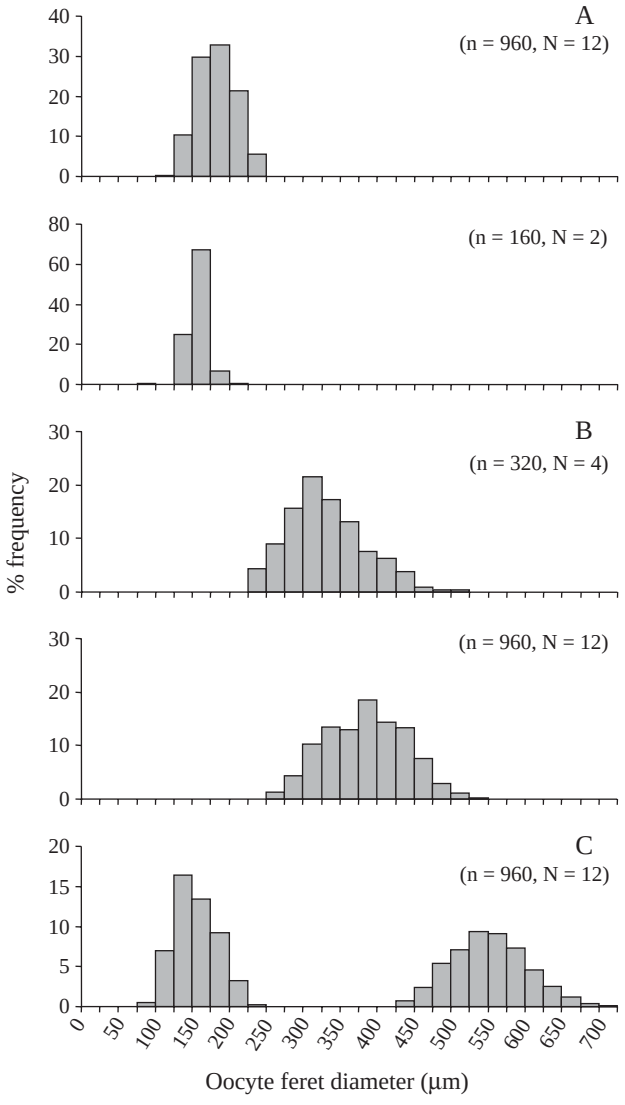

Fig. 5. *Alvinocaris stactophila*. Temporal variation in oocyte size-frequency distributions at the Brine Pool. (A) Early spring (upper plot February 2003, lower plot March 2002), (B) summer (upper plot July 2004, lower plot August 1997), (C) autumn (November 2003). n: no. of oocytes measured. N: no. of ind. examined

ovigerous females and small non-ovigerous females contained small previtellogenic oocytes (Fig. 6D).

Oocyte size-frequency distributions exhibited greater variation between different seasons than variation within them. The coefficient of variation (CV: calculated as the percentage ratio of standard deviation to the mean, thereby providing a measure of variance independent of the magnitude of the mean) of all oocytes measured in this study was 52.09. In contrast, the CV of oocytes measured from spring and summer samples were 16.80 and 16.86, respectively. The CV of the large oocytes measured in large non-ovigerous shrimp from November was 22.71, while that of the small oocytes in ovigerous and small non-ovigerous specimens from that month was 9.64. Overall, the variance in oocyte size between samples was greater than that within them (early spring vs. summer vs. autumn: Kruskal-Wallis multisample test, $H = 1144.0$, 2 df, $p < 0.0001$).

The proportion of females carrying eggs on their pleopods also varied between seasons. Ovigerous females were not found among the 156 females collected in August 1997 and July 2004. They were present, however, in samples collected in March 2002 and February 2003 (40% of 25 females collected) and November 2003 (47% of 312 females examined). The embryos carried by females in November 2003 did not show visible differentiation. In contrast, those examined in March 2002 and February 2003 were well-developed, with features such as eyes and appendages clearly visible. Several embryos hatched into zoea larvae during examination aboard ship in February 2003.

DISCUSSION

Zonation in population structure and reproductive features

In November 2003, the population of *Alvinocaris stactophila* in the outer zone of the Brine Pool mussel bed was dominated by small non-ovigerous females and a high proportion of generally smaller males. The sex ratio in this zone did not differ significantly from 1:1 and ovigerous females were comparatively scarce. In contrast, the inner zone was dominated by large ovigerous females with fewer males present. These features were coherent among the replicate samples collected from this zone.

The total proportions of males and ovigerous females collected from the middle zone were intermediate between those of the inner and outer zones. However, individual samples from the middle zone were heterogeneous, with one similar to samples from the inner zone and the other similar to the sample from the outer zone. Overall, the results indicated that ovigerous female *Alvinocaris stactophila* were present with greatest frequency in the inner zone of the Brine Pool mussel bed in November 2003, though this conclusion would be much stronger with a replicate sample from the outer zone.

In a previous study describing spatial variation in water chemistry across the mussel bed (Smith et al. 2000), water-bathing mussels in the inner zone were found to be methane-rich but oxygenated, with hydrogen sulphide either absent or only occasionally found at low concentrations in brine infusing the underlying sediments. This contrasted with the water-

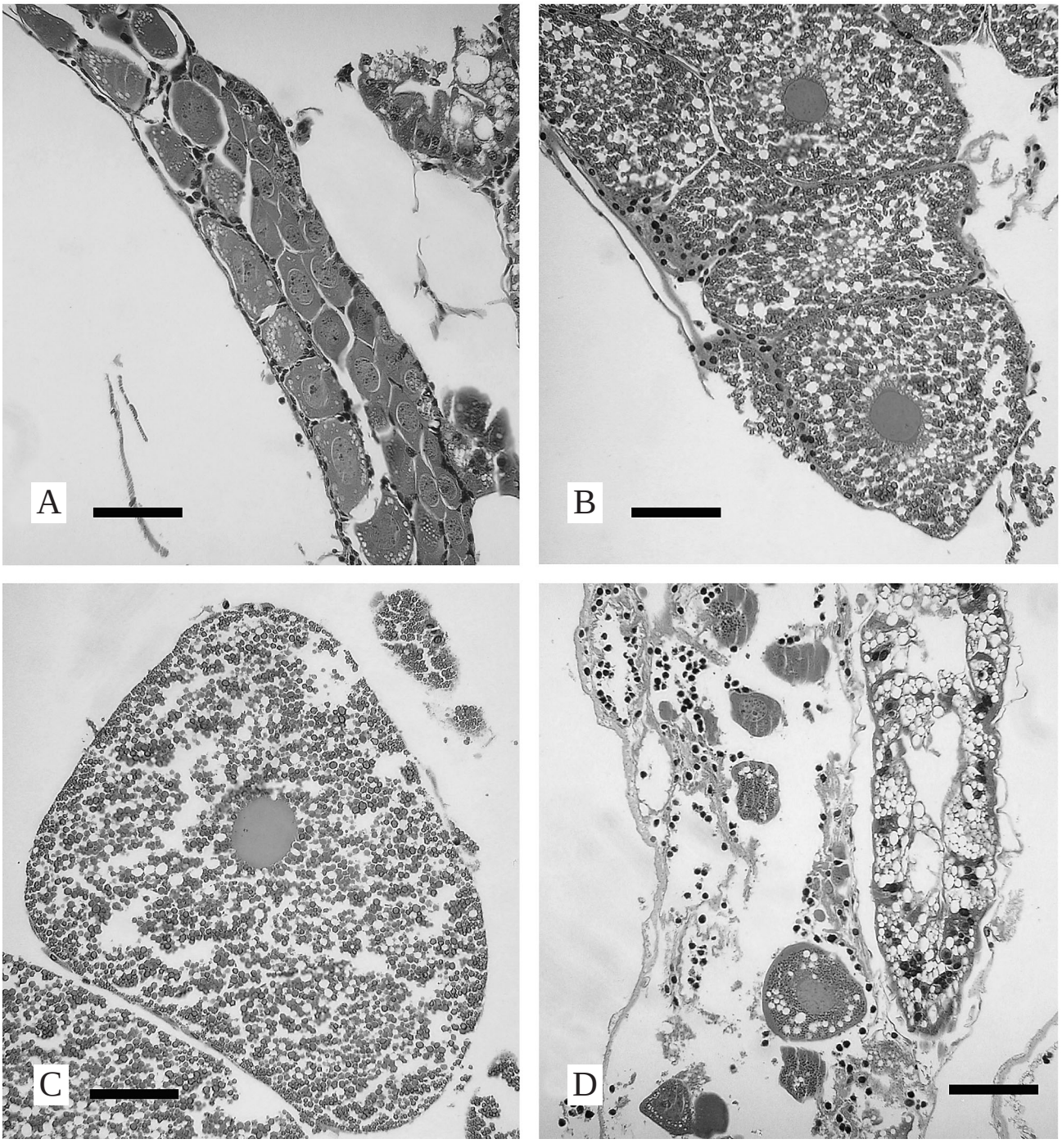

Fig. 6. *Alvinocaris stactophila*. Female reproductive development. (A) Small previtellogenic oocytes in an ovigerous female from March 2002, (B) large vitellogenic oocytes in a non-ovigerous female from July 2004, (C) large vitellogenic oocyte in a non-ovigerous female from November 2003, (D) small previtellogenic oocytes in an ovigerous female from November 2003. Scale bars = 100 µm

bathing mussels in the outer zone that consistently contained low oxygen, with very high levels of hydrogen sulphide (>1000 µmol l^{-1}) in the sediments beneath. Measurements in the middle zone were highly heterogeneous, exhibiting characteristics of both the inner and outer zones. Indices of physiological condition and growth of mussels were significantly higher in the inner zone, which also appeared to be the region of active recruitment to the population (Smith et al. 2000).

Smith et al. (2000) defined the inner zone as lying within ~1 m of the edge of the open brine pool, and the other zone as lying within ~1 m of the outer edge of the mussel bed. These zones are congruent with those used in our study. Assuming that the pattern in water chemistry recorded by Smith et al. (2000) persisted in November 2003, the distribution of ovigerous *Alvinocaris stactophila* suggests that they may avoid the sulphidic or hypoxic extremes of the chemosynthetic environment when incubating their embryos.

This hypothesis is consistent with that proposed by Perovich et al. (2003) to explain the congregation of ovigerous *Bythograea thermydron* towards the periphery of a vent field. The same hypothesis has also been evoked to account for the apparent paucity of ovigerous female *Rimicaris exoculata* in the immediate vicinity of black smokers at deep Mid-Atlantic hydrothermal vents (Copley 1998, Ramirez Llodra et al. 2000).

Although fecundity correlates with body size in *Alvinocaris stactophila*, there is considerable variation in the number of eggs carried by females of similar sizes in the samples examined here (Fig. 4). Rather than revealing a linear relationship between fecundity and body size, the data define a region of possible fecundities. However, when taking the linear upper boundary of the data as the maximum possible fecundity for particular body sizes, the fecundity of *A. stactophila* appears to be comparable to that described in limited samples of other alvinocaridid shrimp (Ramirez Llodra et al. 2000).

Some variation in measured fecundity may result from females losing eggs during collection (Ramirez Llodra 2002). Variation in fecundity may also result from variations in reproductive success, which may be influenced by environmental conditions. This factor is suggested by the higher size-corrected fecundity from the inner zone compared to the middle zone, even though ovigerous females were too scarce in the outer zone sample to explore this feature further.

Overall there were fewer males in November 2003 samples than expected for a 1:1 sex ratio. The size-frequency distributions of males and females were significantly different, with proportionately fewer large males. When ovigerous females were excluded, the sex ratio did not deviate significantly from 1:1 and there was no significant difference in size-frequency distribution of males and non-ovigerous females. The spatial variation in population structure and sex ratio across the mussel bed is therefore consistent with congregation of ovigerous females towards the inner edge of the mussel bed. Unless larger males occupy some other as yet unsampled habitat, the population structure also suggests a possible difference in either growth or age-related mortality between the sexes.

Seasonality in reproductive development

Reproduction in *Alvinocaris stactophila* at the Brine Pool appears to be seasonal and iteroparous. The ovaries of female shrimp from early spring all contained relatively small oocytes. Specimens from summer months all contained larger oocytes, and the largest oocytes were found in large non-ovigerous females collected in autumn. However, ovigerous females from the autumn contained the smallest oocytes measured in this study, along with small non-ovigerous females that may have been new recruits to the adult population that had not achieved reproductive maturity in that year. Variation in oocyte size-frequency distributions between seasons was greater than variation within them, even though some samples came from different years and from potentially different locations in the mussel bed. These features are consistent with a coherent seasonal pattern of reproduction, rather than undersampling of any interannual or spatial variation.

Eggs carried on the pleopods of females collected in November 2003 did not exhibit visible differentiation and were assumed to have been recently fertilised. In contrast, embryos carried in early spring were well-developed and hatched into zoea larvae as observed in February 2003. The prevalence of ovigerous females also varied among samples from different times of year, peaking in the autumn, even though these samples were not spatially discrete and therefore did not take account of spatial variation in the occurrence of ovigerous females indicated by the November 2003 samples.

Alvinocaris stactophila may therefore undergo the following life cycle at the Brine Pool: (1) larvae hatch in early spring and undertake an as yet unspecified period of planktotrophic development in the water column; (2) recruitment occurs sometime before autumn (females from the peak of small sizes in November 2003 were not ovigerous but exhibited synchrony of gametogenic development with larger ovigerous females presumed to be developing a new cohort of oocytes for reproduction the following year); (3) new female recruits develop oocytes in their ovaries over at least 1 yr, with vitellogenesis occurring sometime between early spring and summer; (4) mating occurs in autumn and females carry developing embryos from autumn to early spring; (5) mature females embark on at least one further cycle of reproductive development in their lifetime — a cohort of oocytes starts to develop in their ovaries while they brood embryos on their pleopods.

Assuming annual recruitment events, the population structure and indicated iteroparity of *Alvinocaris stactophila* suggest a possible adult lifespan at the Brine

Pool of 2 to 3 yr. This contrasts with the seep tubeworm *Lamellibrachia luymesi* at Louisiana Slope seeps, which may have a lifespan of around 250 yr (Bergquist et al. 2000). The question of larval lifespan remains open for *A. stactophila*. It may be that larvae hatching in early spring are recruited to the adult population by the autumn of the same year. Alternatively, larvae could spend more than 1 yr or multiple years in the plankton.

Alvinocaris stactophila shows little overlap in the range of oocyte sizes from different seasons (Fig. 5), in contrast to other species of alvinocaridid shrimp described as non-seasonal, which exhibit much wider variations in oocyte sizes between individuals from the same sample (Ramirez Llodra et al. 2000). The variation in oocyte size-frequency distributions of *A. stactophila* also contrasts with patterns of seasonal reproduction previously described in 2 other species from deep-sea chemosynthetic evnvironments. In the vent crab *Bythograea thermydron*, samples from different months exhibited similar overall ranges of oocyte sizes (Perovich et al. 2003). However, a predominance of small previtellogenic oocytes in samples from May compared to a sample from November was interpreted as evidence of seasonality. In the clam *Calyptogena kilmeri* at Monterey Canyon cold seeps, mean oocyte diameter increased from August to November, but not from November to March (Lisin et al. 1997).

Previous studies have measured oocyte sizes from histological sections, rather than by dissecting individual oocytes from ovaries for measurement. While histology provides valuable qualitative information about oocyte development, caution is required when measuring the areas of sections of irregular-shaped oocytes with non-central nuclei. Sizes of such oocytes may be misjudged when the plane of sectioning is not parallel to the major axis of the oocyte. Use of this technique could therefore obscure patterns in the oocyte size-frequency distributions of some species.

Photosynthetic primary production above the continental slope of the northern Gulf of Mexico varies seasonally, and typically exhibits a peak in early spring seawards of the shelf break (Muller-Karger et al. 1991). Although persistent hypoxic zones associated with the productivity of the Mississippi plume occur on the inner continental shelf, the plume does not usually impact waters overlying the continental slope (Wiseman et al. 1997, Chen et al. 2000) and there is no evidence of bottom water hypoxia at the Brine Pool (Smith et al. 2000). However, the early spring peak in freshwater run-off can detach the coastal buoyancy current, and push nutrient-rich water offshore, where it may provide a seasonal stimulus for primary production in addition to changes in mixed layer depth (Muller-Karger et al. 1991).

Ecklebarger & Watling (1995) proposed contrasting reproductive patterns that deep-sea species may show in response to a seasonal peak in surface productivity and its export. Species able to undertake 'fast' egg production by heterosynthetic vitellogenic pathways may undergo rapid oogenesis and spawn soon after seasonal organic input to the deep-sea benthos; however, this pattern is thought to be restricted to meiofaunal species. Alternatively, for 'slow' egg-producing species that use mixed and autosynthetic vitellogenic pathways, seasonal organic input may initiate and synchronise vitellogenesis, with spawning occurring some time later following a longer period of gametogenesis.

Rather than exhibiting a direct energetic response, *Alvinocaris stactophila* may exploit seasonal variation in phytodetrital flux to the Brine Pool as a zeitgeber to synchronise gametogenic cycles, and thereby time the release of planktotrophic larvae to coincide with peak food availability in the water column. Crustacea are not thought to be capable of de novo synthesis of sterols required for reproduction and must obtain these compounds from their diet. Bacteria may also be incapable of synthesising some of these compounds, which suggests that species in chemosynthetic environments must obtain them from phytoplankton-derived sources (Pond et al. 2000). Reproduction in *A. stactophila* might therefore rely on the supply of such compounds in phytodetrital material, which may peak seasonally. Collection of further seasonal samples for lipid analysis would be required to examine this possibility. Studies of other alvinocaridid shrimp inhabiting chemosynthetic environments suggest that individuals may sequester a lifetime supply of such phytoplankton-derived compounds during their planktotrophic larval stage (Pond et al. 2000, Allen et al. 2001). This does not preclude adults from perceiving seasonal variations in the supply of such compounds and using that signal as a zeitgeber to synchronise gametogenic cycles.

Deep-sea chemosynthetic environments are widely regarded as independent from photosynthetic ecosystems by virtue of their *in situ* chemosynthetic primary production. The seasonal reproduction of *Alvinocaris stactophila* described here indicates that some species may still be influenced by patterns of surface primary productivity and its export. The recognition of such links may be important in understanding the biogeography and evolutionary history of some groups in chemosynthetic environments.

Alvinocaridid shrimp are not represented in the emerging fossil record of vents and seeps (Little & Vrijenhoek 2003). Although this may be a result of taphonomy, molecular evidence indicates that this group colonised these environments less than 20 million years ago (Shank et al. 1999). The evidence for

relatively recent radiations or re-radiations of vent and seep taxa suggests that some evolutionary patterns in deep-sea chemosynthetic environments may have been influenced by events affecting the photic zone (Little & Vrijenhoek 2003). This may apply particularly to taxa with ecological links to patterns of surface productivity. Determining how widespread this feature may be in groups inhabiting deep-sea chemosynthetic environments therefore merits further attention.

Attempts to understand patterns of biogeography and gene flow between insular deep-sea chemosynthetic environments have tended to consider the potential role of seafloor topographic features as barriers to dispersal (Tyler et al. 2002, Van Dover et al. 2002). While these may be important, the recognition of links between surface productivity and ecological patterns in chemosynthetic environments suggests that features such as oligotrophic gyres might also influence the dispersal, gene flow and biogeography of some species.

CONCLUSIONS

Samples collected by submersible from the Brine Pool cold seep in November 2003 revealed zonation in the population structure and reproductive features of *Alvinocaris stactophila*. The proportion of males in samples declined from a 1:1 sex ratio in the outer zone of the mussel bed to 1:3 in the inner zone, while the proportion of female shrimp carrying eggs increased from 17 to 55%. The size-frequency distribution of shrimp also varied across the mussel bed, with the inner zone dominated by larger shrimp. However, when the generally large ovigerous females were excluded, there was no significant deviation from a 1:1 sex ratio, nor was there a difference in the size-frequency distribution of the sexes. The zonation in population structure in November 2003 may therefore have resulted from congregation of large ovigerous females towards the inner zone, where sulphide flux from sediments may be absent or reduced. Such behaviour would be similar to that proposed for some other crustacean species brooding embryos in chemosynthetic environments.

Reproduction in *Alvinocaris stactophila* at the Brine Pool is seasonal and iteroparous. Oocytes from early spring were small and previtellogenic, whereas those from summer months were larger and vitellogenic. The largest oocytes found in this study occurred in large non-ovigerous females sampled in the autumn. However, ovigerous females from November 2003 all contained small oocytes, which suggested that spawning was occurring in the population at that time. Females brood embryos on their pleopods from autumn to early spring, with a new cohort of oocytes developing in their ovaries for reproduction the following year. The hatching of planktotrophic larvae in early spring may coincide with a seasonal peak in the productivity of surface waters and its export. Even though adults exploit a locally abundant chemosynthetic source of nutrition, reproduction in *A. stactophila* may therefore be influenced by surface productivity. However, the nature of the cue used to synchronise the gametogenic cycle remains unkown.

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