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Assessment of decadal-scale ecological change at a deep Mid-Atlantic hydrothermal vent and
reproductive time-series in the shrimp *Rimicaris exoculata*

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RUNNING HEAD

Decadal-scale dynamics of a deep Mid-Atlantic vent community

This study presents a comparison of distribution and abundance of dominant megafaunal species at the TAG hydrothermal mound on the Mid-Atlantic Ridge from 1994 to 2004. A Geographical Information System (GIS) database was compiled from georeferenced observations of faunal abundances at 534 locations on the TAG hydrothermal mound, determined by image analysis of ROV dive footage from November 2004. These data are compared with observations from submersible dives in 1994 to assess changes in the extent and population density of aggregations of the shrimp *Rimicaris exoculata* at the central black smokers of TAG. The GIS database was also used to assess changes in abundance and distribution of the anemone *Maractis rimicarivora* by simulating the path of a biotranssect conducted in 1994 and 1995. There was no evidence of a decline in the extent of shrimp aggregations at the central black smokers of TAG between 1994 and 2004. This result indicates that occasional exposure to high-intensity submersible lighting, which took place during several scientific expeditions in the intervening period, does not pose an immediate conservation threat to populations of *R. exoculata*. Similarly, there were no significant differences in the distribution and abundance of anemones between 1994 and 2004. These results indicate a constancy in the identity, distribution and abundance of dominant species at TAG that contrasts with other vent sites where quantitative time-series have been established. The reproductive pattern of *R. exoculata* was also examined by dissection and direct measurement of oocytes from females collected in September 1994 and November 2004, providing the first comparison of reproductive development in samples from different months for this species. There was no significant difference in oocyte size-frequency distributions of females collected in these samples, indicating a lack of seasonal reproduction in *R. exoculata*.

INTRODUCTION

Hydrothermal vents are highly dynamic, heterogeneous and ultimately ephemeral deep-sea environments. Vent organisms can experience variations in the temperature and chemistry of their environment on the timescale of seconds as a result of turbulent mixing of hydrothermal fluids and ambient seawater, typically overlaid on longer-period tidal variations. On longer timescales, volcanic events at mid-ocean ridges can disturb vent communities directly, while tectonic events can disrupt the subsurface plumbing of hydrothermal systems. As a consequence of the local variations in hydrothermal activity that result from such processes, temporal variation has been noted in the composition, abundance and distribution of megafauna at vents on subannual to interannual timescales.

Hydrothermal vent communities were discovered in the eastern Pacific in the late 1970s and the longest ecological time-series are therefore available from those sites. Interannual variation has been noted in the distribution and abundance of megafauna at 13°N on the East Pacific Rise, related to an interruption in hydrothermal activity (Desbruyères, 1998).

Expeditions to 9°N have recorded the early stages of vent community development following a volcanic eruption in 1991 that destroyed part of an established community, while community composition and venting patterns at 21°N demonstrate temporal stability at a decadal scale (Desbruyères, 1998). Meanwhile on the Galapagos Rift, decadal-scale variation has been noted in the vent community at Rose Garden despite an apparent lack of fluctuations in venting over the period of visits (Desbruyères, 1998), implying ecological change mediated by biological interactions.

Hydrothermal vent communities were first observed on the Mid-Atlantic Ridge in 1985 (Rona et al., 1986) and ecological studies of Mid-Atlantic vent communities have therefore lagged behind those of their eastern Pacific counterparts. At shallow (<2000 m) vent sites close to the Azores, long-term monitoring of communities dominated by the mussel *Bathymodilus azoricus* is underway, but there have been few studies of temporal variation at deeper vent systems further south on the Mid-Atlantic Ridge. Interannual temporal variation has been examined in faunal distributions at Broken Spur vent field (Copley et al., 1997) and subannual variation investigated at the TAG hydrothermal mound before and after drilling by the Ocean Drilling Program in 1995 (Copley et al., 1999). Anecdotal observations suggest interannual stability in community composition at the TAG hydrothermal mound (Gebruk et al., 1997), but this feature has yet to be investigated using quantitative techniques.

The TAG hydrothermal mound (26° 08' N, 44° 49' W; Figure 1) is one of the largest known submarine hydrothermal deposits, comprising a mound 200 m in diameter and 60 m high principally composed of massive sulphides. The mound is situated 2.4 km east of the spreading axis of the Mid-Atlantic Ridge at a depth of ~3600 m (Rona et al., 1986).

Geochronology of mound sulphides indicate an intermittent pattern of venting over the past 50 000 years and current activity is thought to have begun ~60 years ago after ~4000 years of quiescence (Lalou et al., 1993). Focussed high-temperature (>360°C) hydrothermal discharge occurs from a central black smoker complex at the summit of the mound, on top of a cone-shaped deposit of massive anhydrite 20-30 m in diameter and 10-15 m high.

Widespread low-temperature diffuse venting and isolated black smoker venting also occurs across the upper surface of the mound.

The vent community at TAG is dominated by aggregations of the Alvinocaridid shrimp *Rimicaris exoculata* at the central black smokers (Van Dover et al., 1988), while anemones (*Maractis rimicarivora*) dominate the periphery of the mound. As its name suggests, this anemone species has been observed ingesting *R. exoculata*, but factors controlling its distribution have yet to be investigated. Other species recorded on the mound (Gebruk et al., 1997) include three other Alvinocaridid species (*Chorocharis chacei*, *Alvinocaris markensis* and *Mirocaris fortunata*), brachyuran and galatheid crabs (*Segonzacia mesatlantica* and *Munidopsis* sp.), gastropods (*Phymorhynchus moskalevi*) and ophiuroids (*Ophioctenella acies*). Undescribed chaetopterid polychaetes are also present on the periphery of the mound, in the zone most remote from active vents (Galkin, 2002). The substratum of the mound varies from exposed anhydrite and fresh sulphides at the central black smokers to oxidised sulphide rubble and regions of ponded hydrothermal sediments in the periphery.

Rimicaris exoculata is known at hydrothermal vents on the Mid-Atlantic Ridge from the Lucky Strike vent field southwards, often present in high-density aggregations (>1500 individuals m⁻²) around high-temperature vents at deeper sites (Van Dover et al. 1988, Copley et al. 1997; Gebruk et al., 1997; Gebruk et al., 2000). The shrimp derive nutrition from epibiotic bacteria (Van Dover et al., 1988; Gebruk et al., 2000), though iron-oxidising bacteria inhabit the gut of the shrimp and may also play a role in their nutrition.

Very few ovigerous females of *R. exoculata* have previously been found in samples, which have generally been collected in summer months (Gebruk et al., 1997). Oocyte size-

frequency distributions of female shrimp in a September sample from TAG and a July sample from the Rainbow hydrothermal vent field do not indicate seasonal synchrony of development (Ramirez Llodra et al., 2000), but oocyte development has yet to be examined in samples from TAG in other months or seasons for comparison. Such comparison has identified seasonal reproduction in another Alvinocaridid species at a cold seep, where ovigerous females were also absent from summer samples (Copley & Young, 2006).

R. exoculata possesses a non-imaging dorsal eye (Van Dover et al., 1989), which may serve as a photoreceptor for the faint light emitted from hydrothermal vents. Exposure to the high-intensity floodlights of submersibles may irreversibly damage the photoreceptors of *R. exoculata*, resulting in a breakdown of the rhabdom layer that is not regenerated (Herring et al., 1999). This observation has led to a recent suggestion that submersible dives pose an immediate threat to the conservation of the shrimp population at TAG (Johnson, 2005). Anthropogenic activity, specifically scientific sampling, is suspected to have modified the abundance, distribution and composition of fauna at Juan de Fuca vents compared to unsampled sites in the same area (Tunnicliffe, 1990). Whether scientific activity poses a conservation threat to shrimp populations at Mid-Atlantic Ridge vents, however, has yet to be ascertained.

The TAG hydrothermal mound was visited by several submersible expeditions between 1994 and 2004. Dives took place in July-September 1994, March 1995 (Copley et al., 1999), July 1997 (Gebruk et al., 2000), August 1998 (Chiba et al., 2001), August 2001 (Lutz et al., 2002), June 2002 (Vereshchaka et al., 2002), June 2003 and November 2004. During filming of the IMAX documentary *Volcanoes of the Deep Sea* at TAG in August 2001, 4000 W of HMI (hydrargyrum medium arc-length iodide) lights were attached to the submersible *Alvin* on boom arms to illuminate up to 50 m of seafloor at once (Lutz et al., 2002). The per-watt light output of HMI lights (~100 lumens per watt) is typically five times that of tungsten-halogen lighting and has the same colour temperature as sunlight (5600 K).

It is not possible to determine whether submersible lighting has an ecological impact on shrimp populations without a "dark control", specifically a population not exposed to lighting that can also be monitored over time. Such a control would be difficult to achieve, given that it would need to experience the same fluctuations in non-anthropogenic factors as the exposed population. But it is possible to assess whether the shrimp population at TAG has

declined over the past decade, even though it may not be possible to elucidate the factors involved. If there is no evidence of a decline in the shrimp population, however, then exposure to submersible lighting at the frequency of recent visits clearly does not pose an immediate conservation threat as suggested (Johnson, 2005). This does not imply that there is no effect, but rather that there is no immediate conservation risk from the level and nature of submersible visits in recent years.

The aims of this study are therefore: (1) to compare the distribution and abundance of the dominant megafauna on the TAG hydrothermal mound in 1994 and 2004, assessing whether the population of the *R. exoculata* is in decline and examining factors in the distribution of anemones; (2) to examine the reproductive development of female *R. exoculata* collected during autumn for the first time, to address the question of possible reproductive seasonality in this species at this site.

METHODS

Fieldwork techniques and instrumentation

Two dives using the *Jason-2* ROV were conducted at the TAG hydrothermal mound (Figure 1) in November 2004 during Cruise 180-1 of the *RV Knorr*. Dive J2-108 (1-2 November) undertook recovery of previously-deployed temperature probes from the mound, mapping of microbathymetry and biological sampling. Dive J2-109 (3-5 November) included microbathymetric mapping of the mound and collection of biological samples.

Three long baseline (LBL) transponders were deployed to facilitate acoustic navigation of *Jason-2*, in locations chosen to optimise coverage and eliminate shadowing by topography during operations on the TAG mound. Transponders were also deployed with 200 m tethers and a 2800 m baseline to improve coverage of the mound. Transponder positions on the seabed were determined by systematic acoustic interrogation from the ship, using the sound velocity profile to 1830 m from a T-5 expendable bathythermograph and Levitus tables for deeper velocities.

Video footage was obtained from three cameras on *Jason-2*. One camera was attached to a pan-and-tilt mount that was controlled by scientific observer in the control van aboard the *RV Knorr*. A fixed camera was also mounted on the upper rail of the vehicle to provide a forward view and another pan-and-tilt camera was used by the vehicle pilot. Three-chip CCD video images were simultaneously recorded onto DVD from these cameras for subsequent analysis. Video footage included a head-up display indicating vehicle heading, depth and time. Two lasers attached parallel to each other 0.2 m apart on the pan-and-tilt science camera mount were used to provide scale in images.

The distribution and abundance of shrimp aggregations in 2004 was compared with that determined from dives at TAG using the *Mir* submersibles in September 1994. An LBL navigation system was also used during these dives and known markers on the TAG mound and the commentary of dive observers were also used to refine navigational references. Video with laser scale was recorded during these dives from PAL and NTSC format betacam cameras and copied onto Hi-8 tapes for subsequent analysis.

The *Jason-2* dives in 2004 provided views of all sides of the central black smoker complex, as did the *Mir* submersible dives in 1994 (specifically BRAVEX dives 6, 9, 11, 12, 13).

The distribution and abundance of anemones in November 2004 was compared with records from a transect in June 1994, conducted by the *Argo-II* camera sled, and a transect conducted in March 1995 by *DSV Alvin* using a 3-chip CCD video system with laser-dot scale (Copley et al., 1999). In addition to obtaining video footage of the TAG mound, the *Jason-2* dives in November 2004 recovered temperature probes deployed by *DSV Alvin* in June 2003. Two types of probe were used in this deployment. Deep Sea Power and Light (DSPL; San Diego, CA) SeaLogger probes were deployed in areas of high-temperature black smoker venting, while VEMCO (VEMCO Ltd, Nova Scotia, Canada) Minilog-T probes were deployed in areas of lower-temperature diffuse venting. Complete data records were obtained from seven DSPL and nine VEMCO probes after recovery. DSPL probes recorded temperatures every 10 minutes from 1100h GMT on 27 June 2003 to 2000h GMT on 13 May 2004. VEMCO probes recorded temperatures every 8 minutes from 1100h GMT on 27 June 2003 to 1956h GMT on 24 June 2004. Background deep-sea temperatures were recorded during these deployments by a SeaBird SBE26 Wave and Tide Gauge positioned ~400 m northeast of the TAG mound. Temperature data were calibrated using laboratory-derived coefficients and

converted from absolute values to anomalies above background by subtracting the bottom-water temperature records (~ 2.7 °C) from the Seabird instrument.

Analysis of faunal microdistributions

Frames from dive footage were captured on a PC for analysis where the image met criteria of visible laser scale and simple topography at right angles to the view of the camera. A total of 534 images meeting these criteria were captured for analysis from the November 2004 dive footage. Each image was georeferenced from its time-stamp using the WHOI Virtual Van Dive Database, which provided processed navigational data for each dive.

Captured images were analysed using Jandel Scientific SigmaScan Pro software, using the laser-dot scale for spatial calibration. Where substratum did not comprise a flat surface, geometric approximations of topographic features such as cylinders, cones and hemispheres were used to estimate surface area. Image areas of ~ 1 m² were considered as separate observations for analysis to standardise sampling effort; images meeting the criteria for analysis below this area were rejected and images of areas greater than 1 m² were subdivided for separate analyses. Abundances of anemones per m² were calculated from images.

The ArcView software suite (ESRI, Redland, CA) was used to construct a Geographical Information System (GIS) database, consisting of faunal abundances recorded from dive footage layered over microbathymetry data using georeferencing information. This database was interrogated to extract a transect of anemone abundances from east to west across the mound, matching the path of transects conducted in 1994 and 1995 (Copley et al., 1999). A north-to-south transect of anemone abundances was also extracted from the 2004 GIS database. Navigational data for observations along transects were converted into along-transect distances, using bathymetry to match the origin used in the 1994 and 1995 transects. The abundance of anemones was also examined around each temperature probe during its recovery, by capturing and processing images meeting the previously-defined criteria for analysis where possible.

Locations where shrimp covered more than 50% of the available substratum were recorded to measure the extent of shrimp aggregations encountered by the 2004 and 1994 dives.

Individual locations ~ 1 m² in area where these aggregations occurred were georeferenced using their time-stamp and navigational data from the WHOI Virtual Van Dive Database. To

estimate the abundance of shrimp in aggregations, close-up frames of aggregations meeting the previously-defined criteria for analysis were examined. SigmaScan Pro software was used to estimate the image area and count the number of shrimp present. Five replicate images from different locations were analysed in this way from each of the 2004 and 1994 dive programmes. During the 2004 dives, the manipulator arm of *Jason-2* was also used to disturb shrimp aggregations to determine whether a monolayer or multiple layers of animals were present.

Analysis of reproductive development in Rimicaris exoculata

Specimens of *Rimicaris exoculata* were collected in November 2004 by *Jason-2* using a suction sampler. Specimens were also collected in crab traps deployed in the vicinity of the central black smokers. Male shrimp were distinguished from females aboard ship by the presence of an asymmetrical mesial extension on the endopod of pleopod 1. Specimens were fixed for 48 h in a buffered solution of 4% formaldehyde in filtered seawater and preserved in 70% isopropanol for subsequent determination of oocyte size-frequency distribution.

Oocyte size-frequency distributions have previously been examined in single summer samples of *R. exoculata* from the TAG and Rainbow hydrothermal sites by measurement of cross-sectional areas of oocytes in histological sections (Ramirez Llodra et al. 2000). This method assumes that histological sections bisecting the nucleolus represent the maximal cross-sectional area of the cell. Packing of crustacean oocytes in ovaries, however, often results in irregular shapes and nuclei are seldom centrally located. Differences in the position of the nucleus and angle of sectioning relative to the shape of the cell can therefore result in variation in measurements of cross-sectional area. Considering the typical major and minor axis lengths of Alvinocaridid oocytes observed in histological sections, this error can exceed 40% (Copley, unpublished). For this study, measurement of oocytes in histological sections was therefore rejected in favour of direct measurement of oocytes individually dissected from ovaries.

Ovaries were dissected from females and individual oocytes carefully removed from the ovaries under a Leica MZ8 stereomicroscope. The maximum cross-sectional area of each oocyte was measured by laying the cell flat on a petri dish. A JVC TK-1280E video camera connected to a PC via a Matrox Rainbow Runner video card was used to capture images of oocytes from the microscope. Images were analysed using Jandel Scientific SigmaScan Pro

image analysis software to calculate feret diameters of oocytes, which represent the diameter of a hypothetical circle of equal area to the object measured. Images of a graticule slide captured at identical magnification to the oocyte images were used to provide spatial calibration. The feret diameters of 80 oocytes were measured in each female *R. exoculata* in a sample of 20 females collected in November 2004. To enable direct comparison of oocyte-size frequency distributions, the same techniques were applied to twenty females collected during 1994, which had been fixed and preserved using an identical protocol.

RESULTS

Distribution and population density of shrimp aggregations

The central black smokers of the TAG mound were occupied by aggregations of the shrimp *Rimicaris exoculata* in September 1994 and November 2004. Aggregations of *Rimicaris exoculata*, defined as greater than 50% coverage of substratum by shrimp, covered an estimated area of 81.2 m² around the central black smokers in November 2004 (Figure 2A). These shrimp aggregations extended to the northeast of the central black smoker chimneys, occupying areas around black smoker orifices on the slopes of the central anhydrite cone. In contrast, shrimp aggregations occupied an area of 70.4 m² around the central black smoker chimneys in September 1994, extending to the southeast. (Figure 2B). There is therefore no evidence of a decline in the overall extent of shrimp aggregations at the central black smokers of TAG between 1994 and 2004.

Shrimp aggregations, defined as locations where shrimp covered more than 50 per cent of substratum in analysed images, were not observed in the NE quadrant of the upper terrace in 2004. Aggregations were observed in this area in March 1995, however, during recovery of a timelapse camera system (Copley et al., 1999). These aggregations had developed since November 1994, coincident with a local increase in high-temperature hydrothermal venting, and therefore appear to have been a transient feature during the decadal scale of this study.

The mean abundance of shrimp measured in aggregations in 2004 was 1191 ind m⁻² ±326 95% CI, which exhibited no significant difference to the mean value of 1120 ind m⁻² ±474 95% CI, measured using the same technique in images from 1994 (Figure 3; t-test, t =0.242, 8 d.f., p > 0.05). Disturbance of aggregations by the manipulator arm of *Jason-2* in

November 2004 revealed a layer of juvenile shrimp beneath the adults visible in video images. This layer was not counted, however, in the comparison of adult abundance as its presence or absence in 1994 could not be confirmed.

Distribution of anemones and influence of temperature

The east-west transect extracted from the GIS database of anemone abundance measurements in November 2004 revealed two peaks in abundance, east and west of the black smoker complex (Figure 4C). This pattern was also apparent in the transects previously undertaken in 1994 and 1995 (Copley et al., 1999; Figure 4A, B). The north-south transect extracted from the 2004 GIS database also shows two peaks either side of the black smoker complex (Figure 4D), consistent with the transects bisecting a ring-shaped distribution of anemones around the periphery of the upper terrace of the mound. Comparing anemones abundances in each of the eastern and western sectors of the 1994, 1995 and 2004 transects reveals no significant difference between them (Kruskal-Wallis multisample tests, eastern sectors: $H = 1.798$, 2 d.f., $p = 0.407$; western sectors: $H = 0.532$, 2 d.f., $p = 0.767$).

Anemone abundances were recorded at the sites of six DSPL temperature probes and eight VEMCO temperature probes during their recovery (Figure 5A); unsuitable camera views precluded assessments of anemone abundance at two other locations where probes collected data. In general, the temperature records of probes were variable and indicated episodic hydrothermal discharge at individual locations, while the amplitude of background bottom-water variation during temperature probe deployment was ~ 0.05 °C (Sohn, unpublished). Mean temperature anomalies recorded by each probe during their deployment, however, show a significant negative correlation with anemone abundances measured at probe locations in November 2004 (Figure 5B; Spearman rank order correlation: $r = -0.72$, $p = 0.0032$). No anemones were present in the immediate vicinity of DSPL probes 2, 6, 8 and VEMCO probe 1, which were recovered from areas of sediment cover.

Reproductive time-series of Rimicaris exoculata

Female *Rimicaris exoculata* exhibited a wide range of oocyte size-frequency distributions in the sample analysed from November 2004 (Figure 6). The size-frequency distribution of the smallest oocytes measured in a single female from that month was 76 – 108 μm , while the distribution of largest oocytes in a female was 463–619 μm . Specimens collected from TAG

in September 1994 and analysed for this study using the same technique showed a similar wide range of oocyte size-frequency distributions. The size-frequency distribution of smallest oocytes recorded from September 1994 was 93–135 μm , while that of the largest oocytes in a female was 466 – 601 μm . There was no significant difference in the median sizes of oocytes in females of *R. exoculata* collected from TAG in the different months (Mann-Whitney U-test, $U = 1736611$, $p = 0.566$).

DISCUSSION

Decadal-scale ecological dynamics of the TAG mound

This study presents the first quantitative assessment of decadal-scale ecological change at a deep Mid-Atlantic hydrothermal vent. The results indicate that the distributions and abundances of the two dominant megafaunal species at the TAG hydrothermal mound have been largely invariant between 1994 and 2004. Aggregations of the shrimp *Rimicaris exoculata* occupy the central black smokers, while peak abundances of the anemone *Maractis rimicarivora* occur in a ring around the periphery of the upper terrace of the mound. Although not quantified, similar distributions of these species were also noted in the initial dives to the TAG mound in 1985 (Rona et al., 1986).

The decadal-scale invariance in dominance, distribution and abundance of species at TAG contrasts with the dynamics established for many East Pacific hydrothermal vent communities (Desbruyères, 1998), where changes in such features have been associated with local interruptions of hydrothermal activity or disruption by volcanic events and subsequent succession. Sites where venting patterns have demonstrated temporal stability, however, have exhibited greater stability of community composition (Desbruyères, 1998). East Pacific vents may experience periods of relative stability of venting interspersed with highly unstable periods, resulting in communities dominated either by bivalves or vestimentiferans respectively (Desbruyères, 1998).

In contrast, the frequencies of tectonic and volcanic events that can disrupt the pathways for vent fluids are lower on the slow-spreading Mid-Atlantic Ridge, resulting in greater temporal stability in the location and activity of vent sites. Geochronology of sulphides indicates millennial-scale stability in the locus of hydrothermal discharge at TAG (Lalou et al., 1993),

which may be associated with a long-lived normal fault underlying the mound. The composition of high-temperature fluids at TAG (Parker et al., 2005) and rise height of the hydrothermal plume (Wichers et al., 2005), which is indicative of heat flux, appear to have been invariant over the same decadal period as the biological observations of this study.

Although the overall pattern of venting has remained constant at TAG on a decadal scale, with the high-temperature discharge focused through the central black smoker complex, there have been small-scale changes in the pattern of venting on the mound. Such changes include variation in the prevalence of black smoker venting in the NE quadrant of the upper terrace. Black smoker venting increased in this area between September 1994 and March 1995, matched by the development of aggregations of *R. exoculata* (Copley et al., 1999). In 2004, however, black smoker activity was less prevalent in the same area, indicated by bathymetry and seafloor markers, and shrimp aggregations were absent. The observed shift in the distribution of shrimp aggregations from the SW side of the central black smoker complex in 1994 to the NE side by 2004 (Figure 2) may also result from small-scale changes in the pattern of high-temperature venting, as black smoker venting appear to be more prevalent in this area in 2004.

Apart from small-scale changes related to changes in the pattern of hydrothermal activity, there is no evidence of a decline in the overall area or population density of aggregations of *R. exoculata* between 1994 and 2004. These results suggest no immediate conservation threat to the shrimp population from exposure to high-intensity wide-field lighting during the intervening period. Current levels and modes of scientific activity at TAG may therefore be considered sustainable with regard to conservation of the shrimp population, contrary to recently-raised concerns (Johnson, 2005). This does not refute the evidence that exposure to light may result in irreversible changes to the photoreceptors of *R. exoculata* (Herring et al., 1998). It also does not imply that there are no ecological consequences from such changes, as we have no "dark control".

The abundances of *R. exoculata* measured in shrimp aggregations in this study are lower than those reported by other workers. Van Dover et al. (1988) report abundances of 2000-2500 ind m⁻², while Gebruk et al. (2000) describe aggregations containing of 3000 ind m⁻². The precise techniques used to estimate abundance in these studies are uncertain, however, and Gebruk et al. (2000) acknowledge difficulties in obtaining reliable estimates from the

complex topography of vent chimneys. Use of a consistent technique to analyse footage from 1994 and 2004 ensures comparability of results within the present study, however, even if precise comparison with other studies is not possible. Previous studies also describe shrimp aggregations as comprising a monolayer of adults on sulphide surfaces (Van Dover et al., 1988). Disturbing shrimp aggregations in 2004, however, revealed a layer of juvenile shrimp beneath closely-packed adults. These juveniles were not counted in analysis, as their presence or absence could not be determined *a posteriori* in 1994 footage. The comparison of abundances presented here is therefore that of adults only. Aggregations of juveniles have previously been reported as occurring in patches distinct from adult aggregations (Gebruk et al., 2000), but not as a lower layer in adult aggregations as seen in 2004. Whether this is a transient feature of shrimp aggregations, possibly related to a recent recruitment event, is unknown.

Interannual variation in faunal distribution has been recorded at the Broken Spur vent field on the Mid-Atlantic Ridge, associated with growth of vent edifices (Copley et al., 1997). At TAG, however, chimney growth may largely be restricted to the central black smokers that are the focus of the high-temperature discharge required for mineralisation. This may constrain the growth of new surfaces exposed to hydrothermal flow, required for the development of further shrimp aggregations (Copley et al., 1997). Frequent cycles of chimney growth and collapse have been inferred and observed directly at the central black smokers of TAG and overall chimney growth appears to have occurred between June 2003 and November 2004 (Sohn, pers. obs.). Quantification of chimney growth, however, is precluded by differences in the accuracy of systems used to map the microbathymetry of the mound in 1994 and 2004.

The large and continuous nature of the TAG mound may provide resilience to the overall interruption of venting from the clogging of conduits, compared to individual vents in fields of separate sulphide edifices. Records from individual temperature probes indicate episodic variability in hydrothermal discharge in different locations on the TAG mound, but constancy in the overall vigour of venting over ~1 year (Sohn, unpublished). Subsurface events such as seismicity on the underlying normal fault and mineralisation and fracturing may therefore reorganise the pattern of flow within the TAG mound rather than modulating its overall vigour. The negative correlation between anemone abundances and mean temperature anomalies recorded over ~1 year by the VEMCO and DSPL probes suggests that the

distribution of anemones may be ultimately constrained by a maximum thermal tolerance of $>25\text{ }^{\circ}\text{C}$ (Figure 5B). Direct physiological investigation is required to establish thermal tolerance, however, as this apparent boundary could also arise from ecological interactions, such as shrimp aggregations in higher-temperature areas preventing settlement of anemones. Substratum type also appears to influence anemone distribution, as four of the zero anemone abundances were recorded where probes were deployed in sedimented locations (VEMCO probe 1; DSPL probes 2, 6, 8; Figure 4). Although temperature records of probes ceased ~ 5 months before their recovery and the measurement of anemone abundance at each location, the response of anemone distribution to changes in hydrothermal activity does not appear to occur on subannual timescales (Copley et al., 1999).

Overall, the results of this study reveal a constancy in the community structure at TAG on a decadal scale, which may be related to constancy in the activity and overall pattern of venting at the site. The relative constancy of faunal distributions and abundances corroborates the constancy in the physical dynamics of the mound determined by geophysical and geochemical techniques. These features contrast with the dynamics established for other hydrothermal sites on similar timescales and may arise from the lower frequency of disruptive events on a slow-spreading ridge and the resilience of a single large mound to total clogging of vent fluid conduits by mineralisation. The latter hypothesis may be tested by comparing the decadal-scale ecological dynamics of TAG with those of other deep Mid-Atlantic vents where venting occurs from fields of separate smaller structures, such as Broken Spur, Snake Pit or Logatchev. The decadal-scale dynamics of these sites have yet to be determined, however, and it is possible that the constancy of community structure at TAG is unique among hydrothermal systems discovered so far. The GIS database compiled for this study from georeferenced faunal abundance measurements in 2004 establishes a detailed baseline for further time-series studies at TAG.

Reproductive time-series of Rimicaris exoculata

In addition to constancy observed in faunal distributions and abundances at TAG on a decadal scale, there is similarity in the oocyte size-frequency distributions of female *Rimicaris exoculata* collected from different months of the year a decade apart (Figure 6). Within each sample, individual females exhibit contrasting oocyte size-frequency distributions. These show a continuous range from individuals with ovaries containing only small oocytes (e.g. $76 - 108\text{ }\mu\text{m}$) to those with ovaries containing much larger oocytes (e.g.

466 – 601 μm). Asynchronous gametogenesis has previously been proposed for this species on the basis of similar variation in oocyte size-frequency distributions determined by histological sectioning in single summer samples from TAG and Rainbow (Ramirez Llodra et al. 2000). That putative pattern is confirmed here by the similarity of oocyte size-frequency distributions in a sample from late autumn, determined by dissection and direct measurement of cells to provide larger sample sizes and avoid a potential source of measurement error as previously described.

The presence of the same pattern and range of oocyte size-frequency distributions in November 2004 as September 1994 indicates that reproduction is not seasonal in *R. exoculata* at TAG. Seasonal reproduction has been described in another Alvinocaridid species, *Alvinocaris stactophila*, from the Brine Pool cold seep in the Gulf of Mexico (Copley & Young, 2006). In contrast to *R. exoculata*, individual *A. stactophila* exhibit similar oocyte size-frequency distributions within samples from the same month but differences between months. This pattern of variation within and between samples of *A. stactophila* provides a touchstone for determining seasonal reproduction in Alvinocaridid shrimp and *R. exoculata* clearly does not conform to the same pattern. Spawning occurs in November in *A. stactophila*, with females brooding embryos over winter and hatching of planktotrophic larvae coinciding with the spring peak in surface productivity and its export. The TAG hydrothermal site, in contrast, lies much deeper (3600 m) than the Brine Pool cold seep (650 m) and is situated within an oligotrophic gyre where there is little seasonal variation in surface productivity. These features may therefore preclude possible cues for reproductive seasonality.

The scarcity of ovigerous *R. exoculata* noted in summer samples (Gebruk et al., 1997; Ramirez Llodra et al., 2000) also persists in the November sample examined here and may result from a spatial pattern rather than a temporal one. Other crustaceans appear to avoid the sulphidic extremes of chemosynthetic environments while brooding embryos (Copley & Young, 2006). It is therefore possible that ovigerous *R. exoculata* leave the aggregations around high-temperature vents where samples are generally collected (Ramirez Llodra et al., 2000). This hypothesis has yet to be tested by collecting samples of shrimp from the periphery of the vent field for comparison.

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FIGURE CAPTIONS

Figure 1. *Location map of study site (adapted from Desbruyères et al., 2001). TAG = TAG hydrothermal mound (26° 08' N 44° 47' W, water depth 3650 m).*

Figure 2. *Distribution of aggregations of the shrimp *Rimicaris exoculata* at the central black smokers of TAG in A: November 2004 and B: September 1994. Bathymetry is shown for the TAG mound in 2004 from Sohn (submitted); contour interval 2 m. Bathymetry is shown for 1994 from ODP Leg 158 Scientific Party (1994); contour interval 5 m. Grey shading denotes areas where shrimp occupy >50% of visible substratum.*

Figure 3. *Abundance of *Rimicaris exoculata* (ind m⁻²) in aggregations at the central black smokers of TAG in 1994 and 2004. Mean values are displayed with error bars depicting 95% confidence intervals.*

Figure 4. *Abundances of the anemone *Maractis rimicariivora* (ind m⁻²) along A: an east-west biotranssect conducted in July 1994 (data from Copley et al., 1999); B: an east-west biotranssect conducted in March 1995 (data from Copley et al., 1999); C: and east-west biotranssect extracted from the GIS database of November 2004 observations and D: a north-south biotranssect extracted from the 2004 database.*

Figure 5. *Location of temperature probes and paths of biotranssects on the TAG hydrothermal mound (A) and abundances of the anemone *Maractis rimicariivora* recorded at probe locations in November 2004 versus mean temperature anomaly recorded by probes between June 2003 and July 2004 (B). Squares denote VEMCO temperature probes and circles denote DSPL temperature probes.*

Figure 6. *Oocyte size frequency distributions of individual *Rimicaris exoculata* measured in samples from A: September 1994 and B: November 2004. Boxes show median, lower and upper quartiles and whiskers denote 5- and 95-percentiles of distributions of 80 oocytes per female. Oocyte size-frequency distributions of individual shrimp are presented in order of increasing median value from left to right for each sample.*











