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Influence of environmental conditions on early development of the hydrothermal vent polychaete *Alvinella pompejana*

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Summary

Dispersal and colonisation processes at deep-sea vents are still not fully understood, essentially because early life stages of vent species remain unknown. The polychaete worm *Alvinella pompejana* forms colonies on chimney walls at East Pacific Rise vent sites where the temperature can frequently exceed 20°C. *In vitro* studies in pressure vessels showed that the early embryos tolerate temperatures in a lower range (10–14°C), suggesting that they would have to escape the colony to develop. Pressure vessels offer the advantage that each parameter can be independently controlled, but they do not simulate the more complex and dynamic conditions naturally encountered at vent sites. Accordingly, in addition to incubations in pressure vessels, we incubated embryos directly at a vent site, in different habitats along a gradient of hydrothermal influence. Embryos incubated on an adult *A. pompejana* colony where temperature and H_2S concentrations were relatively high showed a very low survival rate and did not develop, whereas embryos incubated in a *Riftia pachyptila* clump environment with a lower hydrothermal signature, or at the base of the chimney where the influence of the hydrothermal activity was very weak, survived well and developed. Although the average temperature recorded in the *A. pompejana* colony was within the range tolerated by embryos (13°C), frequent peaks above 20°C were recorded. Estimated sulphide concentration at this site reached 200 μmol l^{-1}. Punctuated exposure to both high temperature and elevated sulphide levels probably explain the low survival of embryos within the *A. pompejana* colony. The *in situ* experiments further support the idea that embryos require conditions with moderate hydrothermal influence not generally found within an adult colony. However, as much more benign physicochemical conditions can be found within a few tens of cm of adult colonies, embryos do not necessarily have to leave their vent of origin to develop. Further analyses are needed to pinpoint the specific factors that affect the survival and development of embryos at vents.

Key words: hydrothermal vent, development, embryo, pressure vessel, polychaete, temperature tolerance, H_2S, environmental condition.

Introduction

Organisms living in the hydrothermal environment are subject to great instability on various spatial and temporal scales (Haymon et al., 1993; Hessler et al., 1988; Lalou, 1991). Catastrophic and chaotic extinctions in populations (Tunnicliffe et al., 1990) forces vent animals to disperse continuously and colonise new active sites (Shank et al., 1998; Tunnicliffe et al., 1997). Population genetics demonstrate exchanges between well-separated populations growing on distant vents along ridges (Vrijenhoek, 1997). The distances over which these genetic exchanges maintain contact between populations differ among vent species. This could be partly due to different dispersal and colonisation capabilities, which are therefore central issues in hydrothermal ecology (Mullineaux and France, 1995; Van Dover et al., 2002). Indeed, since many hydrothermal vent organisms are either sessile or highly sedentary as adults, dispersal processes must mainly be ensured by larvae. Although knowledge of larval life is essential to an understanding of dispersal and colonisation processes, we currently have only glimpses of the early life-history biology of hydrothermal vent endemic organisms (Tyler and Young, 1999).

Collection of early life stages from deep waters has presented considerable challenges. Larvae of probable vent origin have been collected in plankton tows (Berg and Van Dover, 1987; Kim et al., 1994; Mullineaux et al., 1995), pumps (Kim and Mullineaux, 1998) and sediment traps (Khripounoff

et al., 2000) deployed near vent habitats, but it was often difficult to identify them and therefore to assess their origin. As alternatives to *in situ* collection and to avoid species misidentification, methods have been developed to obtain early life stages of hydrothermal vent organisms by *in vitro* fertilisation (Marsh et al., 2001; Pradillon et al., 2001). These methods still require us to overcome several difficulties. First, adults have to be collected from vents using a submersible and brought to the surface without being damaged. Although biological sampling has now been conducted at vents for more than 25 years, this remains a difficult task. Second, pressure vessels are required to maintain many organisms retrieved from the deep sea (Jannasch and Wirsen, 1984; Shillito et al., 2004; Wilson and Smith, 1985; Yayanos, 1978, 1981). Not only adults but also embryos have to be returned to deep-sea pressure because they cannot develop normally at atmospheric pressure (Tyler and Young, 1998; Young and Tyler, 1993; Young et al., 1996). Incubations in pressure vessels are then required for vent animals early life stages. Besides pressure, temperature should also be controlled. In the case of hydrothermal vent organisms, it is *a priori* not known whether embryos would need abyssal (cold) or hydrothermal (warmer and with different chemistry) conditions to develop.

Until now, embryos of only two hydrothermal vent organisms have been obtained by *in vitro* fertilisation and reared in pressure vessels: the vestimentiferan tubeworm *Riftia pachyptila* Jones 1981 (Marsh et al., 2001) and the tubicolous polychaete *Alvinella pompejana* Desbruyères and Laubier 1980 (Pradillon et al., 2001). Embryos of *R. pachyptila* were successfully reared to the trochophore stage after 34 days of incubation in abyssal conditions at 2500 m depth (2°C and pressure of 26 MPa). Larval life-span of *R. pachyptila* was estimated to be 38 days by measuring oxygen consumption of embryos during development and energy storage within the egg. These data were coupled with measurements of local currents to estimate large dispersal distances of up to 100 km (Marsh et al., 2001).

For *A. pompejana*, as for other species of the alvinellid family, observation of young worms at vents suggested that embryos would develop directly or with a lecithotrophic non-feeding mode, without a dispersal phase (Desbruyères et al., 1985; Desbruyères and Laubier, 1986; Zal et al., 1995). However, larvae of *A. pompejana* were never identified at vent sites. This polychaete species is a pioneer in the colonisation of the hottest areas of vent chimneys on the East Pacific Rise (Fustec et al., 1987; Taylor et al., 1999). The hydrothermal fluid bathing alvinellid colonies differs greatly from abyssal seawater, both in temperature and chemical composition. Hydrothermal fluids are higher in temperature, more acidic, richer in sulphide and depleted in oxygen relative to abyssal seawater (Childress and Fisher, 1992; Johnson et al., 1986, 1994; Le Bris et al., 2003; Luther III et al., 2001; Sarradin et al., 1998). These environments are also much more variable; steep, fluctuating gradients near vents contrast markedly with the stability and homogeneity of abyssal seawater. If embryos of *A. pompejana* develop at vent sites under natural conditions, they probably require different conditions than embryos, such as those of *Riftia pachyptila*, which develop in cold abyssal waters.

Preliminary experiments with *in vitro* fertilisation of *A. pompejana* suggested that embryos arrest development at low abyssal temperature (2°C), but resume development when temperature increases to 10°C (Pradillon et al., 2001). However, they were unable to develop when the temperature is above 20°C. These results suggested that embryos must develop at intermediate temperatures, lower than in the adult habitat, where temperatures around or above 20°C have been reported (Cary et al., 1998; Chevaldonné et al., 1992; Desbruyères et al., 1985), but higher than the surrounding abyssal sea. Pradillon et al. (2001) proposed that embryos could either develop at the bottoms of chimneys where intermediate temperatures are found, or be carried in an arrested state far from their places of origin, completing development only when warmer habitats are encountered.

Conditions in pressure vessels can be tightly controlled, permitting determination of some of the environmental parameters that support early development under natural conditions. However, in pressure vessels, the complexity of the vent habitat cannot be simulated. Hydrothermal vents offer many microhabitats with a wide range of physical and chemical conditions (Le Bris et al., 2003; Sarradin et al., 1998). These environments are highly dynamic, shifting constantly in the relative influence of oceanic seawater and hydrothermal fluid. This environmental dynamic may have a strong effect on developing embryos and should be taken into account when determining the suitable conditions for early development in *A. pompejana* and its dispersal capabilities.

In this regard, development experiments initially conducted in pressure vessels (Pradillon et al., 2001) were compared to similar experiments conducted at a vent site in order to confirm or reject the hypotheses describing potential areas suitable for development. During the *PHARE* cruise in 2002, in parallel to pressure vessel experiments, embryos obtained by *in vitro* fertilisation were returned to positions at a vent chimney that experienced different levels of hydrothermal influence. This set of *in situ* experiments allowed us to compare development in fluctuating natural conditions to that observed in controlled thermal conditions of pressure vessels, and to investigate further the conditions that allow development of embryos at the vent site by considering the influence of fluctuating temperature, pH and sulphide exposure.

Materials and methods

In vitro *fertilisation*

Alvinella pompejana Desbruyères and Laubier 1980 were collected from hydrothermal vents at 9°N and 13°N on the East Pacific Rise (EPR) during cruises in May 1999, December 1999 and May 2000 at ca. 2500 m depth. Most individuals were dead or moribund upon reaching the surface, after the submersible ascent. Therefore, gametes were collected by dissection as soon as possible after collection and recovery;

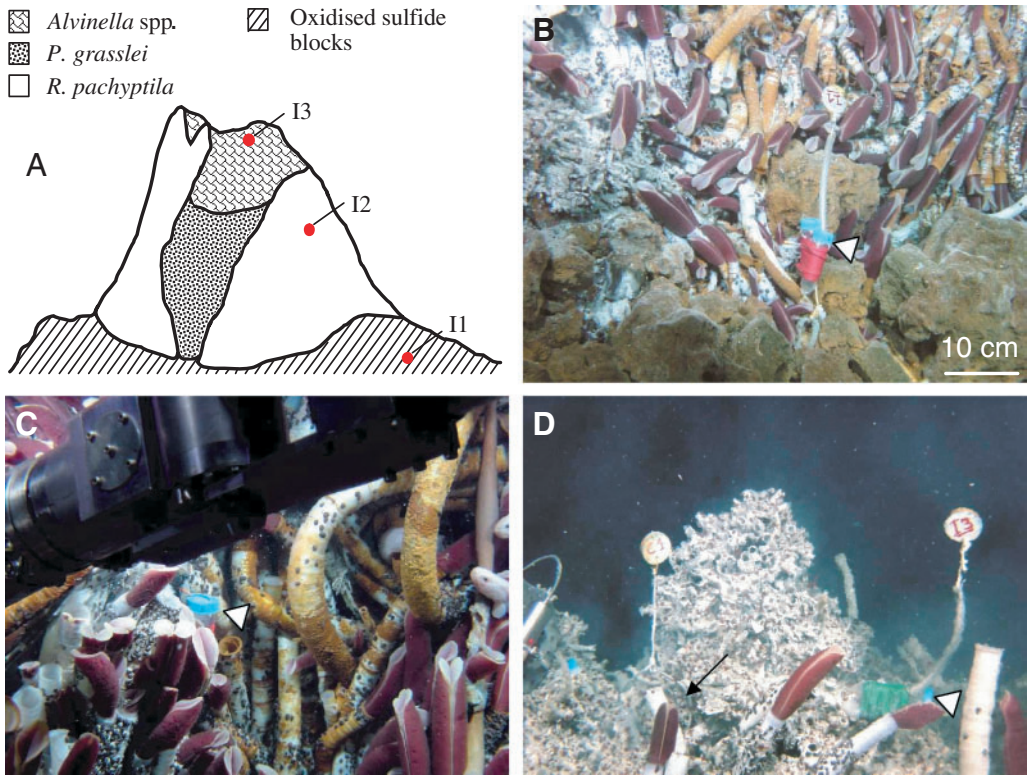

Fig. 1. ELSA (13°N/EPR), the instrumented chimney where incubators were deployed. (A) Lateral view of the ELSA chimney with its faunal coverage, described according to the dominant species observed on video recordings. Positions of incubators I1, I2 and I3 deployed on the chimney are indicated. (B) Incubator I1 (arrowhead) deployed on oxidised sulphide blocks at the base of the chimney. (C) Close-up view of incubator I2 (arrowhead) inside the *Riftia pachyptila* clump. (D) Close-up view of the upper part of the chimney with incubator I3 (arrowhead) and the autonomous probe deployment (black arrow).

adults were maintained at 4°C for no more than 4 h before dissection.

Spermatozoa collected from the spermathecae of females yielded a higher percentage of zygotes (82%, as indicated by elevation of a fertilisation envelope) than did sperm collected directly from males (45%), so the former were used for all experiments. For each culture, oocytes and spermatozoa collected from a single female were mixed in 2.5 ml of 0.2 μm filtered seawater. Adhesive clusters of spermatozoa were broken apart by vigorous pipetting. After mixing oocytes and sperm, cultures were maintained at atmospheric pressure and at room temperature (20°C) for 30 min, then sperm concentration was reduced by dilution in a larger volume of filtered seawater.

Incubations in controlled conditions

For the experiments at atmospheric pressure, zygotes were pipetted into 10 ml glass vials filled with seawater until overflowing. *In situ* pressure experiments (≈26 MPa), were conducted in stainless steel pressure vessels (Autoclave, Rantigny, France). We used two types of systems. In some experiments, embryos were incubated directly within 100 ml vessels (Pradillon et al., 2004); in others, embryos in 10 ml plastic vials were enclosed in a 20 l pressure vessel (Shillito et al., 2001).

Zygotes were exposed to five temperature treatments (2°C, 10°C, 14°C, 20°C and 27°C) at atmospheric pressure (1 atm= 1.03×10^5 Pa), and three temperature treatments (2°C, 10°C and 20°C) at *in situ* pressure (≈26 MPa). Cultures maintained at *in situ* pressure had to be decompressed for microscopic observation of embryos. Embryos subsequently recompressed did not develop further. Consequently for each observation we used a distinct batch of embryos that was decompressed only for a single observation. For each temperature treatment, we conducted two incubations of different durations: 24 h and 48 h for 20°C, 48 h and 72 h for 10°C, and 72 h and 8 days for 2°C. Each experiment was conducted on a batch of at least 100 eggs (occasionally as many as 500), depending on the number of mature oocytes available from a single female.

Developing embryos were observed and staged under a compound light microscope. Cultures at atmospheric pressure (0.1 MPa) were sampled every 12 h or more frequently, discarding each sample after it had been examined. We counted at least 20 embryos per sample. Embryos reared in pressure vessels were examined within 30 min of depressurisation. Light micrographs were taken either directly on board ship using a digital image capture system (Optronics, Gelota, CA, USA) coupled to an Olympus DIC microscope, or after fixation in a 3% glutaraldehyde seawater solution, using

a Nikon camera coupled to the microscope. A few embryos were fixed for observation by scanning electron microscopy (SEM).

Cleavage rates of embryos were estimated using the successive observations of the developing embryos reared at 1 atm. By linearly interpolating the percentage of embryos at each developmental stage between two successive observations, we estimated the time at which 50% of the embryos had reached a given stage. This time was considered to be the average time lapse required to reach this stage in a population of embryos that were not developing synchronously.

Incubations at a vent site

Incubations in the natural environment were conducted during the *PHARE* cruise on 13°N EPR in May 2002 using a simple apparatus similar to that used for *in situ* rearings of *Riftia pachyptila* (Marsh et al., 2001). Three large *Alvinella pompejana* females, collected by the ROV Victor, were dissected for oocytes and sperm collection. Gametes from all three females were mixed as described above, then held at 8°C until fertilisation membranes were observed on all oocytes (5 h after fertilisation). The culture was then diluted and split into six 50 ml plastic vials filled with filtered (0.2 µm mesh size) seawater at 8°C. About 1000 fertilised oocytes were introduced into each plastic vial to yield a final density of approximately 20 embryos ml^{-1}. The vials were covered with 30 µm nylon mesh and mounted in pairs on three weighted moorings of 25 cm long polypropylene line. These moorings were immersed in an insulated box filled with 8°C surface seawater, and the box was placed in the submersible basket just before launching. This procedure allowed us to protect the incubators and limit the temperature increase (sea surface water temperature was around 29°C) at the beginning of each dive. Once on the bottom, incubators were deployed at a vent site named ELSA on a 3 m high chimney (marker HOT 3). This white smoker harboured alvinellid colonies on its edge, and was surrounded by clumps of *Riftia pachyptila*. The seafloor just at the base of the edifice was covered by rusty oxidised sulphide blocks, with a few tubeworms between cracks. Incubators were deployed at three selected locations (Fig. 1): (I1) at the base of the chimney between oxidised sulphide blocks close to tubeworms with rusty-coloured tubes, (I2) between the tubes, within the dense *Riftia* clump covering most of the flanks of the chimney, (I3) at the surface of the alvinellid colony, near the top of the chimney. Incubators were recovered after 5 days and transported to the surface in an insulated box to minimise thermal shock during recovery. As soon as they arrived on board (5 h after recovery from the vent site, with depressurisation during the last 1.5 h, due to the ascent of the submersible) embryos were retrieved from the incubators and fixed in a 3% solution of glutaraldehyde in a buffer of 0.1 mol l^{-1} cacodylate in 0.3 mol l^{-1} NaCl, then washed in a buffer of 0.2 mol l^{-1} cacodylate in 0.35 mol l^{-1} NaCl. In the laboratory, a sub-sample of 100 embryos was drawn from each incubator to estimate the proportion of embryos attaining various developmental stages.

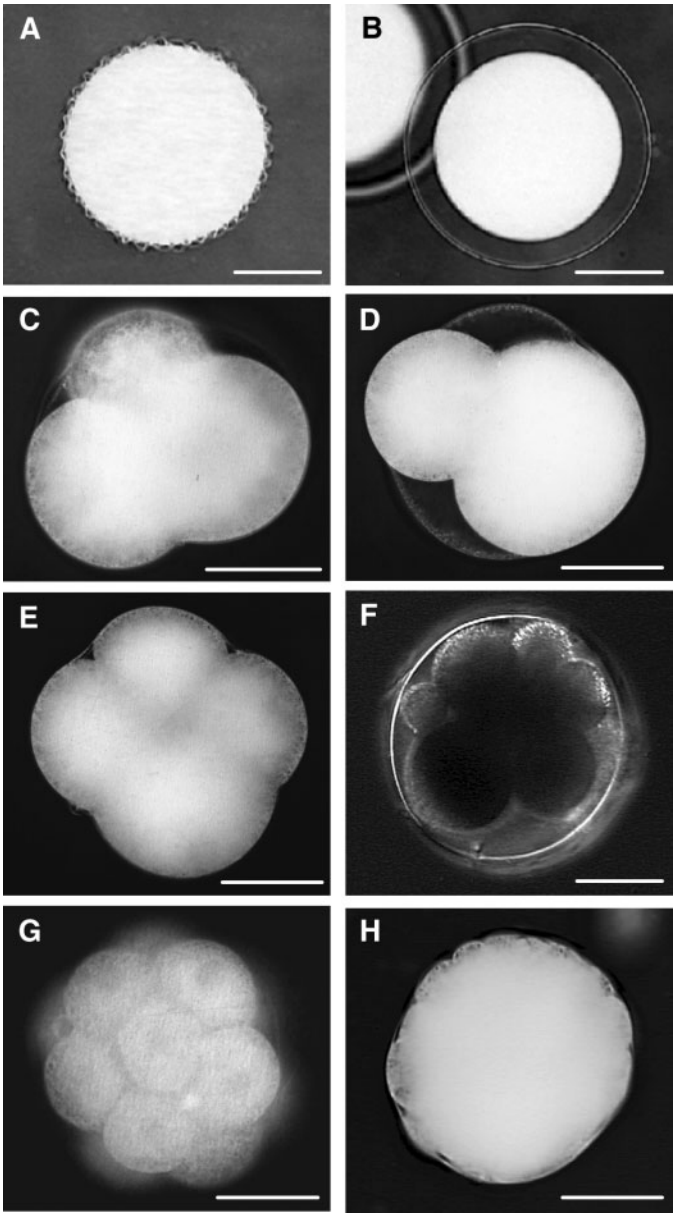

Fig. 2. Early development of *Alvinella pompejana* after *in vitro* fertilisation and incubation at 26 MPa. (A) Oocyte just after fertilisation. (B) Fertilisation envelope. (C) First polar lobe. (D) 2-cell stage. (E) 4-cell stage. (F) 8-cell stage. (G) 12-cell stage. (H) >12-cell stage. A and B are from cultures incubated at atmospheric pressure, 5 and 30 min after fertilisation, respectively. Embryos in C, D, E and G were obtained after *in situ* incubation at the bottom of the ocean at 5°C for 5 days. Embryos in F and H were obtained after incubation in pressure vessels at 10°C for 72 h and 96 h, respectively. Scale bars, 50 µm.

Temperature and chemical measurements

During deployment of the incubation experiments, we recorded ambient temperatures for about 1 min at each site, at a rate of one measurement every 5 s, by positioning the ROV

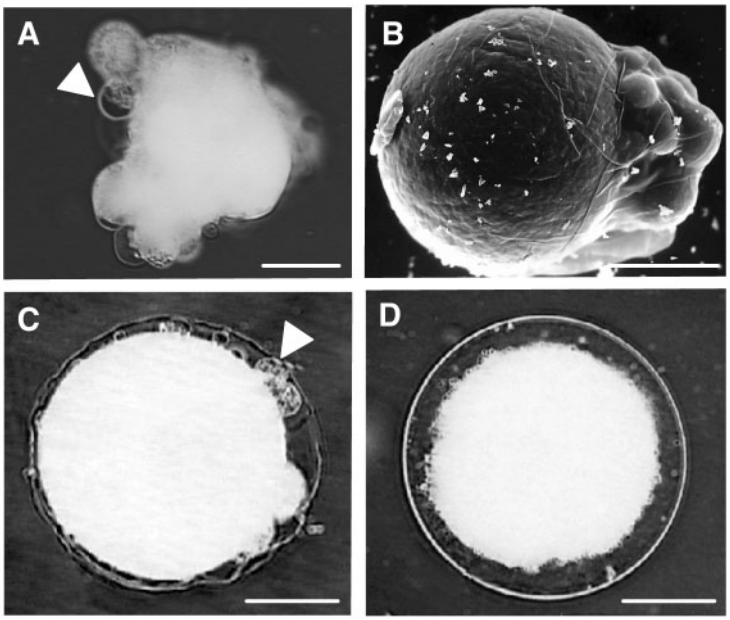

Fig. 3. Abnormal morphologies observed during *in vitro* development in *Alvinella pompejana*. Observations were made after 96 h of incubation at 10°C (A,B) and after 24 h of incubation at 20°C (C,D), at 1 atmosphere. (A) Embryo with abnormal general shape and bubbles (arrowhead). (B) Embryo with abnormal cleavage giving one large blastomere and many very small cells. (C) Bubble formation (arrowhead) at the surface of the oocyte, possibly responsible for subsequent breaking of the cell. (D) Oocyte with broken plasma membrane and cytoplasm spreading into the perivitelline space. B was obtained using SEM; other pictures of live embryos before fixation were recorded on board ship. Scale bars, 50 μm.

temperature sensor within a few centimetres below and above incubators. These measurements were repeated twice for each incubator during the time of the experiment. The mean temperature, standard deviation (S.D.) and maximum temperature for each incubator were calculated for the pooled data from each site. As part of a general monitoring of the chimney, the temperature was also recorded continuously over the whole duration of the experiment, at a rate of one measurement per 10 min, using individual autonomous probes (Micrel, Hennebont, France). Two autonomous probes were deployed in the study area: the first one at the edge of the chimney on the alvinellid colony, about 20 cm from the incubator I3 (Fig. 1), the second one at the base of the chimney on oxidised sulphide rocks, about 50 cm from the incubator I1. No probe was deployed in the tubeworm bush where was the incubator I2. PH and sulphide measurement were taken over the alvinellid colony, about 50 cm from the incubator I3, using an *in situ* probe and a submersible flow analyser as described in Le Bris et al. (2003). We correlated these chemical measurements with temperature. Assuming as a first approximation that these relationships could be extrapolated to the whole edifice, the pH and sulphide ranges in the vicinity of each incubator were inferred from temperature.

Results

Early development in Alvinella pompejana

Before focusing on developmental and survival rates of embryos incubated in different temperature conditions, we attempted to identify the successive steps of early development in normal embryos. Further detection of embryos undergoing 'abnormal' events was established by comparison with this 'normal development'.

Before fertilisation, oocytes removed from the female oviducts are flattened spheres with an undulating membrane on their surface. The germinal vesicle appears as a less dense area slightly removed from the centre of the oocyte. The nucleus is markedly lighter than the cytoplasm, which is filled with dense vitelline reserves. Oocytes become spherical within a few minutes of being diluted in seawater. Sperm are not required to mediate this shape change. The germinal vesicle disappears at the time of shape change or shortly thereafter, and the oocytes at this stage appear completely homogeneous under the light microscope (Fig. 2A). Between 30 min and several hours after fertilization, the fertilisation envelope elevates progressively, beginning at a single point on the periphery of the oocyte (Fig. 2B).

Before first cleavage, a polar lobe is formed (Fig. 2C) and this leads to the first asymmetrical cleavage (Fig. 2D). The size ratio between the two cells is relatively constant, the large blastomere being about 1.5 times larger in diameter than the small one. However, embryos with either a larger difference in blastomere size or blastomeres of virtually identical size were also observed occasionally. As in many other spiralians, 4-cell embryos typically have one blastomere that is larger than the other three (Fig. 2E). Subsequent cleavages did not always occur in perfect synchrony; it was not uncommon to observe 5- or 6-cell stages between 4-cell and 8-cell stages (Fig. 2F) of apparently normal embryos. Twelve-cell embryos were also observed between 8-cell and 16-cell stages. We were not able to confirm the exact numbers of cells in embryos with more than 16 cells, so we refer to more advanced embryos as having >16-cells.

The cleavage pattern that we observed for *Alvinella pompejana* embryos was very similar to that of other polychaetes, so we regarded embryos departing from this general pattern as abnormal. Several kinds of abnormalities were observed in the general shape of the embryo, and in the integrity of the embryonic cells. Anomalies in the spatial arrangement of blastomeres (Fig. 3A) and the size ratios of cells (Fig. 3B) were observed in about 20% of embryos reared at atmospheric pressure, but rarely in embryos incubated at 250 atm. Such abnormally shaped embryos often had 1 or 2 very large blastomeres, and many very small blastomeres at one pole of the embryo. Breakdowns in cellular integrity were evidenced by the formation of bubbles or blebs on the cell membrane (Fig. 3A,C), which ultimately caused the cytoplasm

to spill into the perivitelline space (Fig. 3D). This phenomenon did not seem to be linked to pressure conditions, and led to the complete degradation of the embryo within a few hours. Henceforth, we refer to embryos with shape anomalies as 'abnormal embryos' and to embryos with cellular blebs as 'degrading embryos'.

Developmental rates and embryonic survival

Experiments at 1 atm

Initial descriptions of *A. pompejana* embryos were based on cultures maintained at 1 atm, as this allowed us to follow the different steps of developmental processes. At regular intervals after fertilisation, we sampled embryos from the main culture to determine the proportion of embryos at various developmental stages. Abnormal embryos were discarded and not counted in the frequency calculations. Degrading embryos were counted and used to estimate survival rates.

As previously reported (Pradillon et al., 2001), no embryos survived after 24 h at a temperature of 20°C, whereas more than 80% survived at all lower temperatures (Fig. 4). Embryos developed normally at 10°C and 14°C. At 2°C, embryos survived but did not develop (Fig. 4). As shown in Fig. 4, development was not highly synchronised among embryos, and each observation showed embryos at different developmental stages. Because of this lack of synchrony, it was difficult to determine the typical cleavage rates by direct observation. At 14°C, all embryos remained uncleaved 7 h after fertilisation, but 56% attained or passed the 2-cell stage by 16 h after fertilization. The two subsequent cleavages each occurred after 3 h intervals (Fig. 5). At 10°C, the time to first cleavage was nearly twice as long as at 14°C; about half of the embryos underwent first cleavage by 26 h after fertilization, and the subsequent two cleavages occurred at 42 and 69 h after fertilization, respectively (Fig. 5).

Experiments at 250 atm

In terms of survival, results obtained under pressure were similar to those obtained with cultures conducted at 1 atm. At 20°C, embryos did not survive more than 48 h, and we

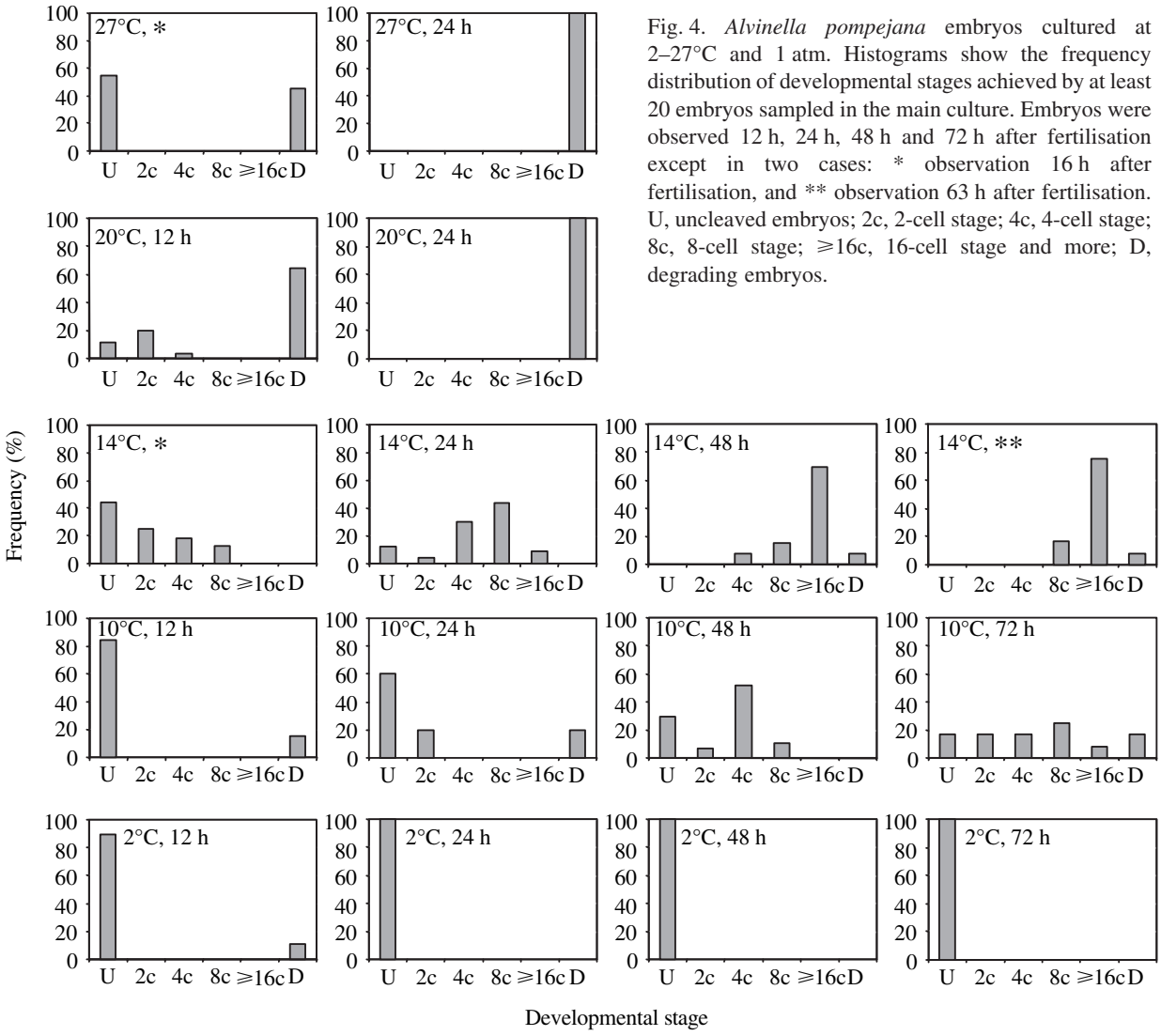

Fig. 4. *Alvinella pompejana* embryos cultured at 2–27°C and 1 atm. Histograms show the frequency distribution of developmental stages achieved by at least 20 embryos sampled in the main culture. Embryos were observed 12 h, 24 h, 48 h and 72 h after fertilisation except in two cases: * observation 16 h after fertilisation, and ** observation 63 h after fertilisation. U, uncleaved embryos; 2c, 2-cell stage; 4c, 4-cell stage; 8c, 8-cell stage; ⩾16c, 16-cell stage and more; D, degrading embryos.

obtained no cleaved embryos at this temperature (Fig. 6). However, as observations of embryos in the pressure vessel were not made at regular intervals, it is possible that embryos did actually cleave while they were under pressure, but started to degrade before they were observed. At 2°C, fertilised oocytes did not cleave, even after 8 days of incubation.

We obtained developing embryos at 10°C. As in 1 atm cultures, all the embryos in these cultures did not develop at the same rate. Cleavage rates were slightly slower under pressure than at 1 atm after 48 h; most of the cleaved embryos were at the 2-cell stage under pressure, whereas most embryos incubated without pressure were at the 4-cell stage after 48 h. However, the 72 h experiments at 10°C showed very comparable frequencies in developmental stages obtained with or without pressure.

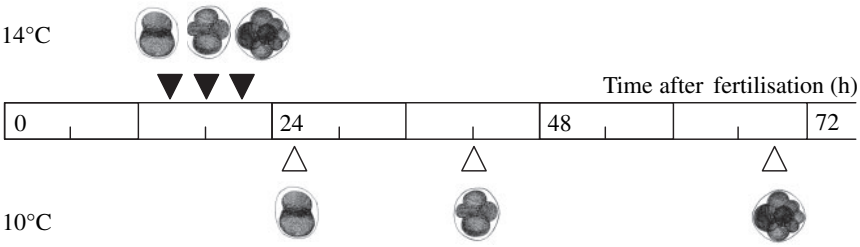

Fig. 5. Effect of temperature on cleavage rates in *Alvinella pompejana* embryos incubated at 1 atm. Cleavage times were estimated from the frequency of developmental stages presented in Fig. 4.

Experimental incubations in situ

The prevailing local conditions at vents vary not only in temperature, but also with respect to the chemical environment. Static incubations in pressure vessels give only a partial picture of the developmental potential of these embryos. In order to take into account dynamic temperature changes and the chemistry of the hydrothermal fluids, embryos were incubated *in situ*, at different locations in the vent environment.

After 5 days of incubation, only 10% of the initial input of fertilised oocytes remained in the incubator deployed on an adult alvinellid colony (I3). None of these oocytes had cleaved (Fig. 7). By contrast, embryos incubated among *Riftia pachyptila* (I2) or at the base of the chimney on oxidised sulphide blocks (I1) experienced nearly 100% survival, and 70% of them had cleaved (Fig. 7). As in the laboratory incubations, variable developmental stages indicated a lack of synchrony in developing embryos (Fig. 7). However, the distributions of the developmental stages at the I1 and I2 sites were very similar, with 2-cell and 4-cell stages representing 50% of the total number of embryos, and embryos at the 8-cell stage or greater representing 30% of the total number.

Short-term temperature records taken by the ROV temperature probe right on the incubators give an indication of the temperature ranges experienced by the embryos. Although located on oxidised rock at the bottom of the chimney where no fluid venting was visible, the temperature data at I1 displayed a weak but significant temperature increase above ambient seawater (Table 1). The environment of incubator I2 in the middle of the tubeworm clump was only slightly warmer on average than the previous one (Table 1). The mean temperature reported for I3 was a few degrees lower than 20°C. An estimation of the thermal range in the immediate environment of incubators can be defined as the difference between the maximum and mean temperatures recorded for

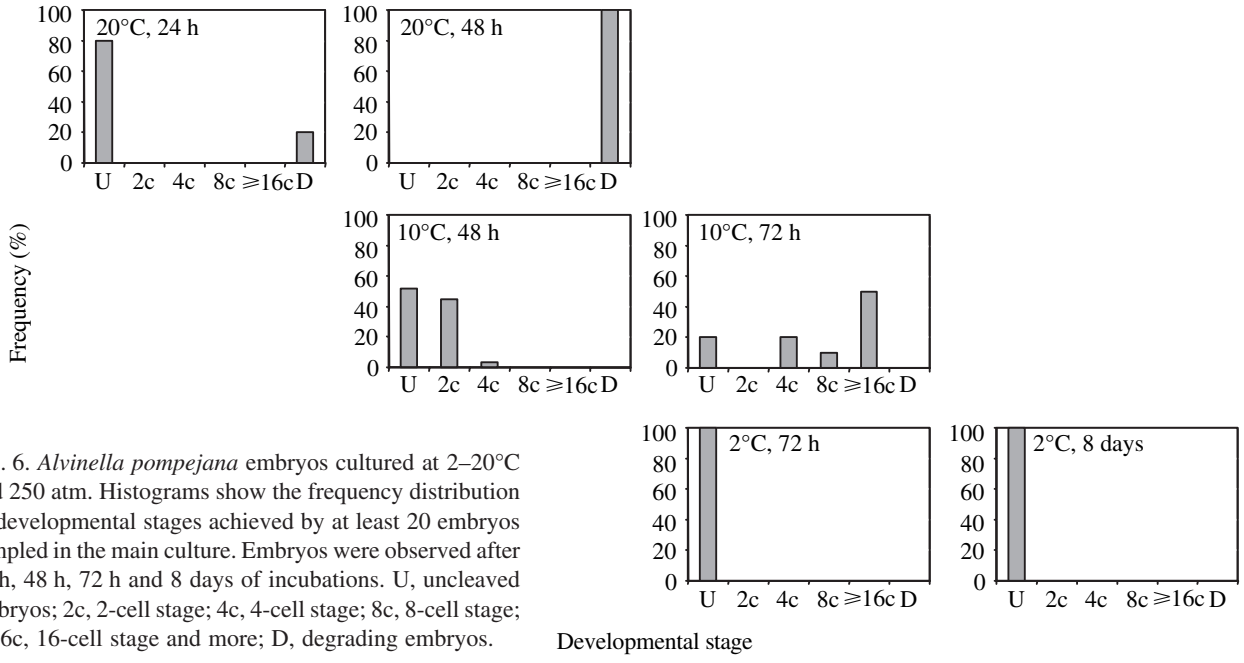

Fig. 6. *Alvinella pompejana* embryos cultured at 2–20°C and 250 atm. Histograms show the frequency distribution of developmental stages achieved by at least 20 embryos sampled in the main culture. Embryos were observed after 24 h, 48 h, 72 h and 8 days of incubations. U, uncleaved embryos; 2c, 2-cell stage; 4c, 4-cell stage; 8c, 8-cell stage; ⩾16c, 16-cell stage and more; D, degrading embryos.

each incubator (Table 1). In all three cases, this range was about 5°C.

Fig. 8 illustrates the very different thermal patterns characterising the top and the bottom of the chimney, during the experiment. High temperature fluctuations were recorded on the edge of the chimney. Such fluctuations are characteristic of alvinellid colonies (Chevaldonné et al., 1991). This dynamic pattern results from the turbulent venting of the hot fluid and its dilution into cold seawater, and from a daily scale modulation by tidal and bottom current effects (Chevaldonné et al., 1991). It can be noted here that while temperature mostly ranges between 10 and 20°C, the record exhibits temperatures as high as 25–27°C, sometimes in sharp peaks but often for a duration of several hours. In contrast, the autonomous probe at the base of the chimney did not record any significant change in temperature and stayed near the 2°C background temperature. These records are indicative of the two extremes that can be found outside the hydrothermal plume on a single edifice.

The pH and sulphide content estimated from the mean and maximum temperatures suggest that the I1 environment would remain low in sulphide with a mean value under 20 μmol l^{-1}, while the I2 environment would reach about 60 μmol l^{-1} and the I3 value would remain above 200 μmol l^{-1} on average (Table 1). The range of variation at these three sites is expected to be large, with maximum values of 115, 145 and 263 μmol l^{-1}, respectively. The pH of the three areas should be much less variable, with values lying around 7.5 (Table 1).

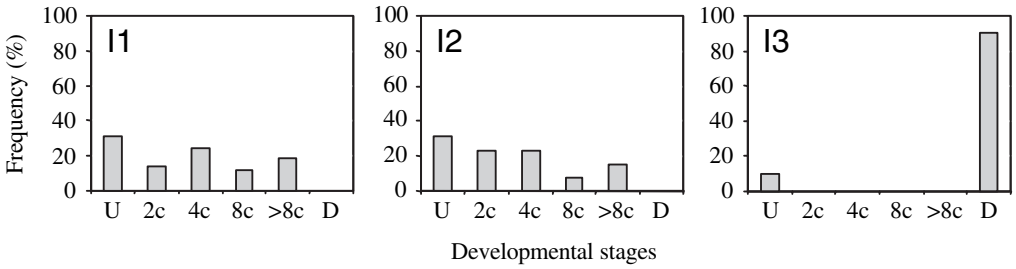

Fig. 7. *Alvinella pompejana* embryos cultured at the ELSA hydrothermal vent site, positions I1, I2 and I3 (see Fig. 1). Incubators were deployed for 5 days before recovery by the submersible. U, uncleaved embryos; 2c, 2-cell stage; 4c, 4-cell stage; 8c, 8-cell stage; >8c, above 8-cell stage; D, degrading embryos.

Discussion

Since embryos or larvae of *A. pompejana* have never been found in plankton collections or within the hydrothermal vent fields, we have no direct observations of the physical and chemical conditions experienced by early life history stages in the places where they develop. Our *in vitro* and *in situ* experimental approaches provide some preliminary indications of where larvae and embryos might develop, based on their physiological tolerances. Incubation of the embryos at different pressures and temperatures in the laboratory allowed us to assess the influence of these main environmental factors under controlled conditions. Natural conditions in vent habitats, however, involve a complex set of interrelated and dynamic environmental factors, including pressure, temperature and chemical factors (Childress and Fisher, 1992; Johnson et al., 1986, 1994; Le Bris et al., 2003; Sarradin et al., 1998). *In situ* incubations were performed to monitor the development of the embryos in different situations and compare these observations with the results of laboratory studies.

As has been observed for other embryos from these depths (e.g. Young and Tyler, 1993; Marsh et al., 2001), more embryos developed normally at the pressure of the adult colonies than at atmospheric pressure. The abnormalities observed at 1 atm included aberrant relative sizes of blastomeres and unusual arrangements of cells, both suggesting alterations of cell division. Cell arrangements during cleavage are mostly controlled by cytoskeletal dynamics, namely the assembly, movement and disassembly of cytoskeleton proteins. Polymerisation properties of proteins such as actin and tubulin are affected by pressure increases, causing alterations in cell shape and cleavage processes (Begg et al., 1983; Bourns et al., 1988; Salmon, 1975a,b). For organisms normally living at high hydrostatic pressure, atmospheric pressure might also affect cytoskeleton proteins and produce abnormal cell morphologies during development.

Although embryos of *A. pompejana* can sometimes develop at 1 atm, most embryos appear to be obligately barophilic, requiring abyssal pressure to develop normally. Similar barophilia was found in embryos of the vestimentiferan tubeworm *R. pachyptila* (Marsh et al., 2001). The existence of physiological pressure thresholds in deep-water and shallow-water echinoderms has previously been used to explain bathymetric distribution of species, as well as dispersal

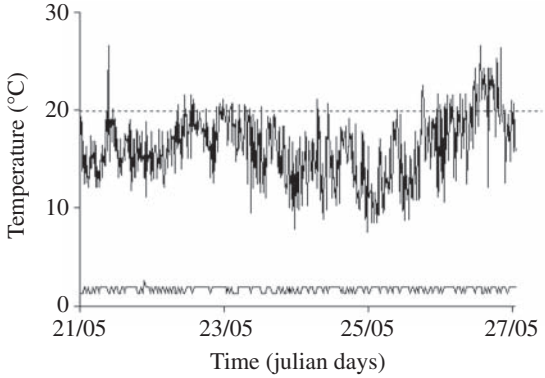

Fig. 8. Temperature recorded over the duration of the experiment by autonomous probes. The higher temperature recordings were taken at the surface of the alvinellid colony about 20 cm from incubator I3, and the lower ones at the base of the chimney a few dm from incubator I1.

Table 1. *Mean and maximum temperature determined in the vicinity of each incubator*

	Temperature (°C)			pH (estimated)		[Sulphide] (μmol l^{-1}; estimated)	
Incubator	Mean	Max	*N*	Mean	Min	Mean	Max
I1	4±2	9	23	7.7	7.3	18	115
I2	6±2	11	32	7.5	7.3	59	145
I3	13±4	17	30	7.2	7.1	194	263
Background seawater	2			7.8		0	

Temperature (*T*) values are means ± S.D., recorded by the ROV temperature sensor.

PH and sulphide concentrations were estimated from the temperature and the relationships determined in the study area using: pH=–0.43 ln(*T*)+8.29 (r^2=0.8177; *N*=36); [Sulphide] (μmol l^{-1})=19.7*T*–62.7 (r^2=0.9353; *N*=15).

capabilities (Tyler and Young, 1998; Tyler et al., 2000; Young and Tyler, 1993; Young et al., 1997, 1996).

Even though embryos of *Alvinella pompejana* require abyssal pressures to develop, they require temperatures higher than those typical of the vast abyssal water column (Pradillon et al., 2001). The only areas where temperature can rise above 2–4°C in the deep Pacific Ocean are near hydrothermal vents. If embryos are transported 200 m above the ocean floor by a buoyant hydrothermal plume, as has been suggested for other vent larvae (Baker and Massoth, 1987; Kim et al., 1994; Mullineaux et al., 1995), they will disperse in a cold environment where development cannot proceed. We suggested elsewhere that a reversible arrested development might permit very long distance dispersal in these worms (Pradillon et al., 2001), but it also seems plausible that significant numbers of alvinellid embryos might remain near the parental colonies, developing in microhabitats with the appropriate temperatures while experiencing very little dispersal. Indeed, in the vast cold-water environment of the abyss, habitats with elevated temperatures near hydrothermal vents are the only places where development of *A. pompejana* is possible. Although spatially very limited, areas of venting offer a large assortment of microhabitats even at the scale of a single hydrothermal chimney (Sarradin et al., 1998). These environments reflect variable contributions of the hydrothermal fluid to the medium, hence generating a wide range of temperature and chemical conditions (Le Bris et al., 2003; Sarradin et al., 1998). Temperatures ranging from 20°C at tube openings (Cary et al., 1998; Chevaldonné et al., 1992; Desbruyères et al., 1985), and up to 80°C inside tubes (Cary et al., 1998) have been reported for *A. pompejana* colonies. According to our results, such temperatures would preclude the development of embryos inside the maternal tubes of *A. pompejana*, as has been hypothesized for the closely related species *Paralvinella pandorae pandorae* (McHugh, 1989). In other words, embryos must probably escape the high temperature portions of the adult colonies in order to develop (Pradillon et al., 2001). Development in some colder part of adult colonies, perhaps near the openings of the tubes, could be possible in some cases; the environmental gradients in colonies are steep, and temperatures as low as 10°C have been measured at the tube openings of some colonies (Chevaldonné et al., 1991; Di Meo-Savoie et al., 2004; Le Bris et al., 2003; Sarradin et al., 1998). Thus, from *in vitro* experiments at various temperatures, we can hypothesize the places where embryos might develop: (1) in the coolest parts of some adult colonies, (2) near the bases of chimneys or in tubeworm aggregations where temperatures are moderate, or (3) dispersing in the cold abyssal water column until a warm-water vent habitat is ultimately encountered. We further tested these three hypotheses by exposing embryos *in situ* to various microhabitats in the environs of a single edifice. These experiments provide additional information on the range of thermal conditions that permit embryonic development. Results from incubators I1 and I2 indicate that embryos can develop at temperatures lower than 10°C; a slight temperature increase of just a few degrees above background abyssal temperatures is sufficient.

Analysis of the I3 incubation (in the adult colony) indicates that embryos may be killed by intermittent pulses of excessively high temperature even when the average conditions lie within the limits of thermal tolerance (Table 1). Short-term temperature records reached a maximum of 17°C close to the incubator, but longer-term continuous monitoring indicated that daily fluctuations and short spikes above 25°C are common (Fig. 8). Thus, the embryos in the colony incubators may have been exposed to temperatures exceeding 20°C during at least part of their deployment time. The effect of exposing embryos to high temperatures for brief periods has not been investigated under controlled conditions, so we cannot discount the possibility that embryos in adult colonies succumbed to physical or chemical extremes other than high temperatures.

Within the range of temperatures observed, only slight changes in pH are expected between the three incubators and the water bathing I3 should not have been significantly more acidic than that of I1 and I2 (Table 1). The estimated sulphide concentration, on the other hand, distinguishes the environments of I1 and I2 from I3. We estimated sulphide content from temperature, assuming a similar sulphide content–temperature relationship for the alvinellid colony and the other habitats of the chimney. At a given temperature, however, the siboglinid tubeworm habitat could be much lower in sulphide than alvinellid habitats, due to various subsurface

processes involving sulphide consumption and thermal exchange (Le Bris et al., 2003). In this case, the difference between the first two incubators and the third one would be even larger, with sulphide concentrations around I1 and I2 much lower than those estimated in Table 1. Sulphide is, however, likely to be present in the two lower areas. This experiment would suggest, therefore, that a moderate sulphide exposure does not prevent the embryos from developing. Second, exposure to an average sulphide content as high as 200 μmol l^{-1} could be lethal to the embryos, providing an alternative explanation for the differences in embryonic survival and development observed among the different environments.

These field experiments thus complement and support our preliminary laboratory observations on the conditions required for early development of *Alvinella pompejana.* They show that embryos exposed to the conditions prevailing in a colony of *A. pompejana* do not survive, whereas ones nestled in *Riftia pachyptila* clumps, or in other areas of weak but significant hydrothermal influence, develop successfully. Whether temperature conditions, sulphide concentration, or a combination of these factors is responsible for high mortality rates in adult colonies remains to be discovered, but it is clear that embryos can only develop in portions of the vent environment where conditions are less extreme than those tolerated by the adults.

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