Cah. Biol. Mar. (2002) 43 : 395-398

Detection of sperm transfer and synchronous fertilization in *Ridgeia piscesae* at Endeavour Segment, Juan de Fuca Ridge

Ian R. MACDONALD[1], Verena TUNNICLIFFE[2], and Eve C. SOUTHWARD[3]
(1) *Texas A&M University - Corpus Christi, 6300 Ocean Dr., Corpus Christi, TX 78412 USA. E-mail: imacdonald@falcon.tamucc.edu*
(2) *University of Victoria, Box 3020, Victoria, BC V8W 3N5, Canada*
(3) *Marine Biological Association of the U.K., Citadel Hill, Plymouth PL1 2PB, U.K.*

Introduction

Many factors affect the reproductive success of an animal, beginning with the health of adults during gametogenesis, the degree of population synchrony in gamete release and the conditions that determine fertilization success. The East Pacific Rise vestimentiferan (Annelida: Pogonophora or Polychaeta: Siboglinidae), *Riftia pachyptila* Jones 1981 appears to have a strategy that does not require adult encounters: ripe gametes are released *en masse* into the water, where fertilization apparently occurs remote from the adults (van Dover, 1994). In contrast, the Juan de Fuca Ridge vestimentiferan, *Ridgeia piscesae* Jones 1985 uses a different strategy: transfer of sperm in discrete packages from male to female, where fertilization is believed to take place (Southward & Coates, 1989). The success of fertilization will depend upon proximity of adults, efficiency of transfer and receptivity of the female.

In a detailed, in situ examination of a population of living, *Ridgeia piscesae*, we observed large numbers of sperm masses entangled in the long, dense branchial filaments. The collected specimens all appeared to be in ripe condition. In this paper, we describe sperm mass appearance, both in situ and in preserved collections, and propose a hypothetical model for the sequence of sperm transfer and fertilization in this invertebrate. We also consider the factors that could influence sperm transfer and reproductive activity. This is the first observation of multiple sperm mass transfer as seen *in situ*. The abundance of sperm bundles found in the present collection of specimens greatly exceeds that found in all previous collections.

Materials and methods

The vent-fields of Endeavour Segment (Juan de Fuca Ridge, northeast Pacific Ocean) comprise a wide variety of venting settings, which are inhabited by a single species of vestimentiferan. We investigated populations of *Ridgeia piscesae* on and around the sulphide structure called “S&M” (47°56.9’N, 129°05.9’W) with the ROV *Ropos.* Venting on S&M is variable in intensity and composition both in space and time (Sarrazin et al., 1997). Hot fluid may break through the mineralized surface, killing animals and exposing a new surface for colonization. Vestimentiferans were collected from such a surface in July 1995, a year after it was denuded. For comparison, two additional collections were taken on the side and base of the structure.

In April 2001, we examined S&M again using high-resolution photography of the vent assemblages on the structure. *Ropos* deployed a newly designed digital camera array mounted on the ROV arm. The camera, a Nikon Coolpix 990®, was modified to fit in a titanium alloy housing with a flat optical port. A pair of 250W quartz lamps co-mounted with the housing provided light. Additional illumination for extreme close-ups was supplied by a 36-element LED array built into the lens adaptor. A continuous video signal to the surface provided a preview image for frame and focus determination. Actual images were stored as 2048x1536 pixel digital files in the camera’s memory card for later download. Camera function was controlled from the surface by entering commands on a hand held computer.

Two sites on S&M are compared here: one at the top of the structure, where flow appeared vigorous near a “beehive” at 2180 m depth (Fig. 1A), and another on a flange near the base (Fig. 1B) where there was a gradient of

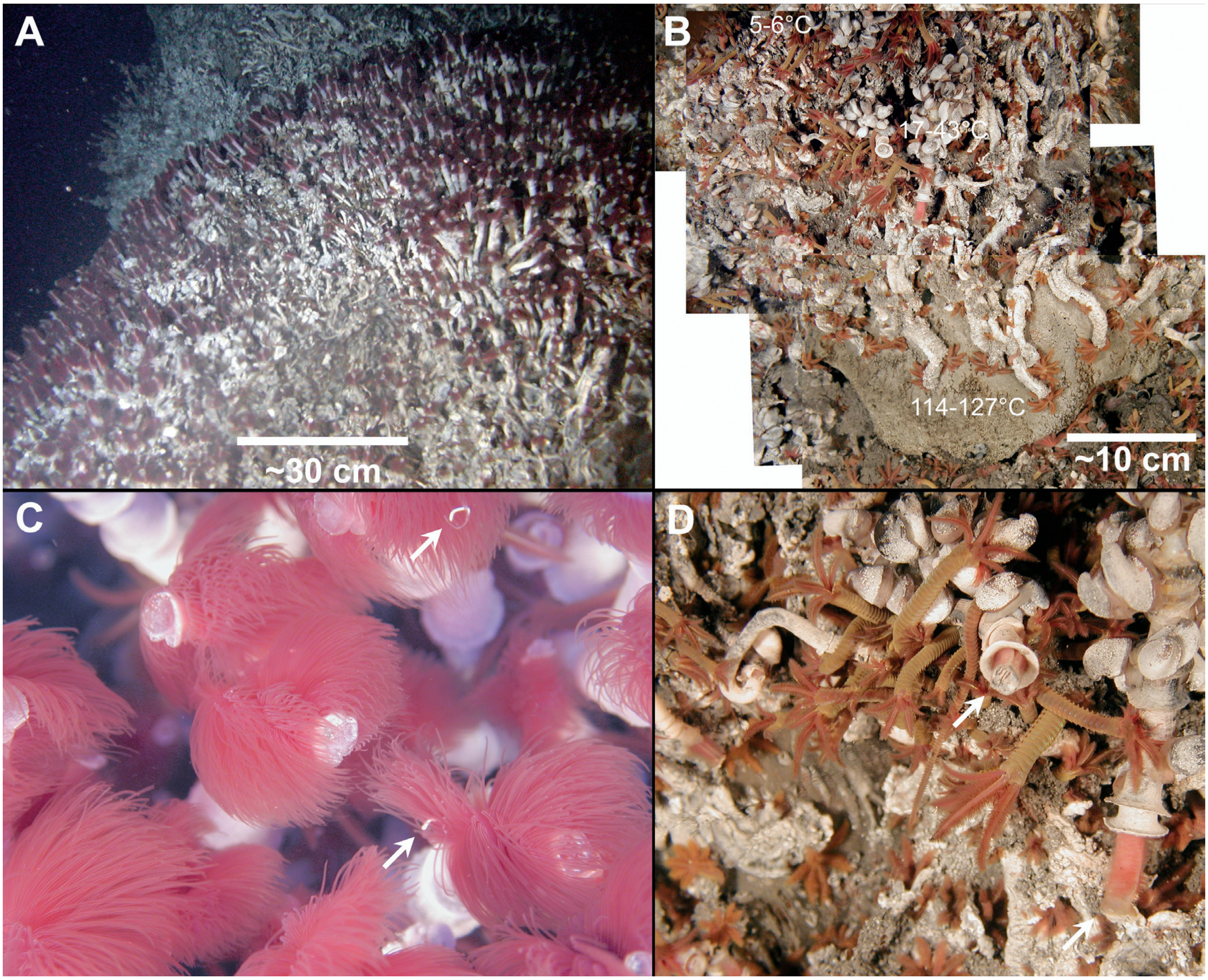

Figure 1. A. Tubeworm assemblage on top of S&M structure. Scale approximate. Abundant limpets and alvinellid polychaetes are visible. **B.** Flange at base of S&M structure. Scale approximate. Temperature ranges recorded with thermistor probe deployed by *Ropos*. **C.** Branchial plumes of *Ridgeia piscesae*. White disc on each worm is top of obturaculum. Arrows indicate two sperm masses entangled in branchiae. Tubes are about 1 cm in diameter. **D.** Close view of assemblage at base of chimney. Here, *Ridgeia* are farther apart and branchiae appear grazed down to obturaculum (arrows).

flow vigour (depth 2200 m). A temperature probe deployed by the ROV arm delivered spot measurements. A sample of vestimentiferans from the top site was returned to the surface and used for detailed observations.

Preserved animals were measured, and the position of any external sperm masses or eggs noted. The extent of the genital grooves on the vestimental region was noted. The trunk was slit open and the gonoducts and gonads examined under a stereomicroscope. Small samples of the contents were quickly examined on a slide, under a higher-power microscope. If gametes were present the sex could be determined with certainty. The ovisacs or seminal vesicles (small enlargements at the anterior ends of the gonoducts) were classified as empty or full of gametes. The reproductive state of the animals was classified as: empty, developing, mature/spawning or spent.

Results

The assemblage at the top of the S&M structure in 2001 (Fig. 1A, C) consisted predominantly of *Ridgeia piscesae*, with a smaller proportion of palm worms (Type V assemblage of Sarrazin et al. (1997)). The tubeworms in this setting were robust and upright, the majority oriented so that the dorsal grooves were uppermost and the branchiae faced outwards. Shimmering effluent discharge was evident throughout the assemblage. The long branchial filaments were very mobile in the surge created by the effluent flow and by the wake of the ROV. Thread-like, white objects were entangled in the waving filaments of numerous tubeworms (Fig. 1C). They remained firmly attached to the filaments despite frequent agitation in the surge. Tubeworms on the flange also comprised a Type V assemblage, but were less numerous, recumbent in haphazard orientation, and had branchial filaments greatly reduced by predation (Fig. 1D). Effluent discharge was restricted to overflow at the flange lip, as shown by the steep thermal gradient between the overflow point and the tubeworm assemblage (Fig. 1B). No sperm masses were seen among the flange tubeworms.

The vestimentiferans sampled from the 2001 population photographed in Figure 1A were large and filled their white tubes. The morphology is typical of the *Ridgeia* form found in vigorous water flow providing good growth conditions (Southward et al., 1995). Thread-like sperm masses (Southward & Coates, 1989) were still attached to the branchial filaments immediately after collection. Twenty specimens were dissected: 11 females and 9 males. All individuals had abundant trophosome tissue and intact branchial filaments. There was little evidence of predation on the anterior structures; only two individuals had lost the anterior-most branchial filaments. The paired genital grooves on the floor of the dorsal vestimental cavity show sexual dimorphism in mature specimens, those of the female being shorter and wider than those of the male (Fig. 2A, B). In the female the paired ovaries, gonocoels and oviducts run alongside the ventral blood vessel, close to the mid line of the trunk, but at the anterior end they turn outward and dorsally towards the gonopores. Here each oviduct expands to form a muscular ovisac that can retain and store full-sized oocytes (ca.110 μm diameter) prior to fertilization. Both sides are functional but the left is normally much smaller than the right. The male gonoducts follow much the same course as those of the female and each expands to form a muscular seminal vesicle just internal to the gonopore. The distended seminal vesicle in mature males is about the same size as the extruded sperm mass and presumably its function is to compact and shape the sperm mass. All the individuals in the 2001 sample harboured ripe gametes, except for four males, one of which was extruding a final sperm mass and three had large but empty seminal vesicles. Of the remaining males, three had a sperm mass in one seminal vesicle, another was extruding a mass and the last had a mass in the genital groove as well as a mass inside the seminal vesicle.

A full range of sperm transfer stages was evident in the females. Of the eleven specimens, only one (spent female) did not have an exterior sperm mass evident. More than one mass was present on three individuals. Locations included branchial filaments, dorsal obturacular groove and dorsal vestimental cavity. In one case, a sperm mass was firmly attached to the exterior of the trunk, 5 mm below the gonopores, which lie at the base of the vestimental grooves. In other females, the attachment was less obvious, but the masses were firmly wedged under the vestimental fold near or over one gonopore. In four individuals, a sperm mass lay over the gonopore and full-sized oocytes were just below in the ovisac. In two more individuals, part of the sperm mass was drawn into the ovisac while released oocytes were entangled on the dorsal branchiae and more oocytes were present in the oviducts.

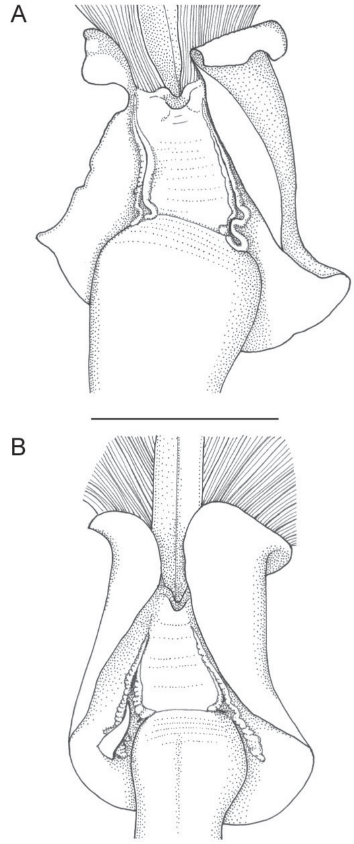

Figure 2. Vestimental region of *Ridgeia piscesae*. Scale, 10 mm. **A.** Male. **B.** Female. Lateral vestimental folds enclose dorsal vestimental cavity. Base of obturaculum and branchial filaments shown are above and anterior trunk is shown below. Two gonopores lie within genital grooves, anterior to the trunk margin in both sexes. Grooves are longer and deeper in male than in female. A spermatophore is present in left groove of female, close to gonopore.

The vestimentiferans collected in July 1995 from S&M top were similar in appearance to those seen in 2001 (Fig. 1A, C); these worms had colonized a surface bared the previous summer. Specimens were mostly at a stage just before full ripeness. Eight of ten females were mature and three carried sperm masses; six of ten males were still developing gametes while three were mature and one was spent. The second collection from an area of lower flow had individuals similar to those photographed in Figure 1A, C. Of the seven individuals large enough to dissect, none were mature and three had no gametes. Similarly, in the third collection from weak flow at S&M base, only four of 13 individuals were mature. All these animals had reduced trophosome tissue that did not fill the trunk. We have examined many collections and 100s of specimens of

Ridgeia from vents of Juan de Fuca Ridge. Only when vigorous flow is present do we find evidence of synchronous gamete development. It is more common for a few individuals to show maturation stages (also seen by Goodson (1996)).

Discussion

We confirm the supposition of Southward & Coates (1989) that fertilization of *Ridgeia piscesae* probably occurs following transfer of sperm masses from the plumes of males to females in clusters of tubeworms. Because sperm is transferred by contact - or pseudocopulation - and only gametes are (presumably) broadcast, we refer to the process as fertilization, not spawning. The hypothetical scenario is expanded in Figure 3. We think that sperm masses are transported up the ciliated genital grooves of males and become entangled in the branchiae. The mass may transfer directly to the female branchiae or be carried by water currents. Eventually it comes to rest close to the female gonopore on the floor of the cavity formed by the vestimental flaps. The ciliated grooves of the female may be involved in transport. Fertilization could occur in the ovisac before eggs are released, or at the time of release. If no mature oocytes are ready, sperm masses may be held in temporary storage in the ovisacs. There appears to be production of a limited number of mature gametes during a fertilization event: a male produces several sperm masses and a female may release discrete lots of eggs. The occurrence of 'spent' males suggests that sperm release is not continuous.

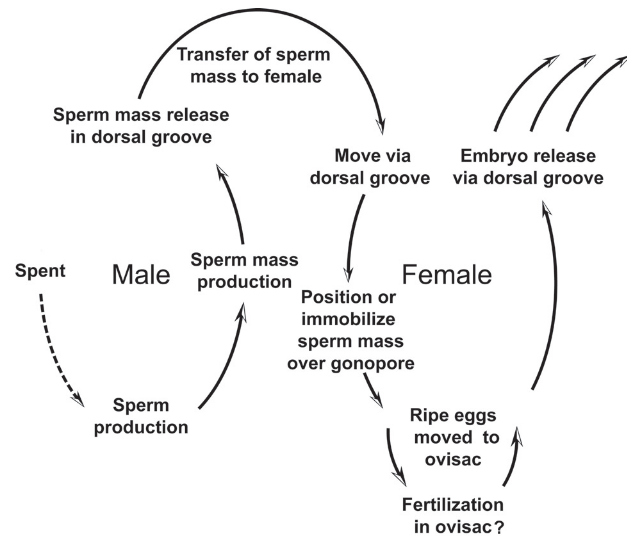

Figure 3. Hypothetical summary of fertilization process in *Ridgeia piscesae.*

Fertilization efficiency would be maximized if reproductive maturation was synchronized and if adults were densely packed. The passive movement of the branchial filaments in flowing water probably increases the chance of transfer during a fertilization event. Conversely, reduction of branchial filament length due to predation (Tunnicliffe et al., 1990) or other factors would reduce the probability of transfer. Although the two assemblages were less than 20 m apart, reproductive activity was evidently restricted to the top assemblage. We propose that concentration of reproductive activity in a few, robust assemblages within a vent-field populated by many other inactive assemblages could be a general rule for this species. Histological examinations from fresh collections should be made to test our hypothetical model. Visibility of sperm bundles can be used to select reproductively active aggregations for collection. The presence of long, healthy, branchial filaments may be an additional sign of reproductive capability.

Acknowledgements

We thank the crew of *CSS Tully*, Team *Ropos*, Kim Juniper, Jason Flores, Anna Metaxas and Amanda Bates for help at sea, A. Southward and two anonymous reviewers for helpful comments on the manuscript, and NSERC Canada and the United States MMS for programmatic support.

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