Reproductive and physiological condition and juvenile recruitment in the hydrothermal vent tubeworm *Ridgeia piscesae* Jones (Polychaeta: Siboglinidae) in the context of a highly variable habitat on Juan de Fuca Ridge

by

Candice St. Germain
B.Sc., Memorial University of Newfoundland, 2007

A Thesis Submitted in Partial Fulfillment
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Supervisory Committee

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Abstract

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The hydrothermal vent environment, in its extreme spatial and temporal variability, offers the opportunity to study habitats that are naturally fragmented and unstable. The vestimentiferan tubeworm *Ridgeia piscesae* is a foundation species inhabiting hydrothermal vent habitat in the Northeast Pacific Ocean. *R. piscesae* is a phenotypically plastic species and is arranged in a metapopulation spatial structure, with each local population displaying one of a range of morphotypes. *Ridgeia piscesae* participates in an obligate symbiosis that is dependent on hydrogen sulphide in the hydrothermal vent fluid that supplies each local population. Hydrothermal fluid flow is highly variable in the hydrothermal vent environment and hydrogen sulphide flux is a limiting nutrient for *R. piscesae*; this variability may create differences in habitat quality. The objective of this study is to determine whether local populations of *R. piscesae* centered on high and low flux hydrothermal fluid outputs are similar in body condition, reproductive condition, and juvenile recruitment. Using the submersibles ROPOS and Alvin, I collected high flux and low flux sample pairs from within meters of each other at multiple sample sites on Axial Seamount and the Endeavour segment of the Juan de Fuca Ridge. I used morphological measurements, histology and lipid analysis to assess physiological and reproductive condition. I also determined the relative abundances of new and older recruits in high and low flux local populations. I found that low flux habitat was inferior in its ability to support *Ridgeia piscesae* at all stages in the tubeworm's life cycle. In terms of body condition, local populations in low flux habitat had lower body weight, greater body length, smaller anterior tube diameter, lower trophosome volume, lower total lipid volume, and lower branchial plume condition. With respect to reproductive condition, local populations in low flux habitat had lower proportions of reproductive individuals, less sperm transfer, lower gonad volume, and fewer mature oocytes; there was no difference in sperm development stages between high and low flux habitat. From the perspective of the individual, low flux tubeworms live longer, and lifetime reproductive output may be comparable to high flux tubeworms. However, turnover is higher in the high flux habitat, so reproductive output of high flux populations is greater than that of low flux populations. Juvenile recruitment was biased toward high flux habitat, although this trend was not significant and recruitment to low flux habitat was still notable. The differences between reproductive output and juvenile recruitment between these habitats support a source-sink model of population dynamics. From the perspective of the metapopulation, low flux habitat is inferior in its ability to support *Ridgeia piscesae* at all stages in the tubeworm’s life cycle. This distribution of relative contributions to the overall population of a key species in a Marine Protected Area (MPA) should factor into management decisions affecting MPA boundaries and use.

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Acknowledgments

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My family for always encouraging me to follow my dream.

Dedication

I dedicate my thesis to my grandfather Gerald (Pinky) St. Germain who inspired in me a love for aquatic environments. He took me fishing bright and early every morning at the cottage and entertained my endless chatter and questions about life and all things fishing.

Preface

Metapopulation theory is an important concept at present because of human-driven habitat fragmentation. Levins (1969) introduced the term “metapopulation” to describe a spatially structured population that persists through re-colonization events despite localized extinction events. In metapopulations, according to the original description, all patches, or local populations, are the same, the distance between pairs of local populations does not affect recolonization, and the number of local populations must be high. In 1996, Reich and Grimm further defined “metapopulation” such that, within a metapopulation, each local population must have its own dynamics, at least some local populations are at risk of extinction because they are so small, local populations are connected by dispersing individuals, and dispersers can establish new populations on empty patches. The application of the metapopulation concept to the marine environment is controversial, as the dispersive larval stage of most marine species creates “open” populations. Larval dispersal decouples reproduction and recruitment, which means that local populations are demographically connected rather than distinct (Caley *et al.* 1996). However, Grimm (2003) points out that the success of the model is not based on its general application or empirical evidence, but in the questions that are asked when one applies the model to a study population. Grimm states that, in most cases, it is difficult to determine whether a marine population is actually a metapopulation, but if the metapopulation concept is used as a working hypothesis, the questions answered along the way can provide insights about key population processes and structures. It is for this reason that I choose to frame my study from a metapopulation perspective.

Suitable habitat for vestimentiferans in the hydrothermal vent environment is highly fragmented and quite variable. Spreading ridge segments host multiple hydrothermal fields, each containing numerous hydrothermal fluid vents classified as high temperature (80-350°C) and diffuse flow (0-30°C). Chemosynthetic bacteria are the primary producers at hydrothermal vents, and use hydrogen sulphide in the venting hydrothermal fluid to fix carbon. The chemosynthetic primary production provides the carbon source necessary for the growth and maintenance of hydrothermal vent invertebrates (Di Meo *et al.* 2000), and can support communities of incredibly high biomass, each centered on local point sources of hydrothermal fluid venting (Sarrazin and Juniper 1999).

Ridgeia piscesae is a Vestimentiferan tubeworm that inhabits hydrothermal vents in the Northeast Pacific Ocean. This sessile marine invertebrate participates in primary production through a symbiotic relationship with sulphide oxidizing bacteria; the bacteria live in an organ, called the trophosome, in the tubeworm. *R. piscesae* is a phenotypically plastic species and occurs in a wide range of morphotypes that are distributed according to hydrothermal fluid flow regime. Phenotypic variation is so great that two species were originally described in the *Ridgeia* genus, *R. piscesae* and "*R. phaeophiale"* (Jones 1985). The "short fat", *R. piscesae*-like morphotype is associated with high flow hydrothermal fluid vents while the "long skinny", *R. phaeophiale*-like morphotype aggregates on low flow vents (Southward *et al.* 1995; Sarrazin *et al.* 1999; MacDonald *et al.* 2002; Urcuyo *et al.* 2003, 2007). The different morphotypes are distributed as numerous local populations centered on point-source hydrothermal fluid vents, which offers the opportunity to ask questions about this species from a metapopulation perspective.

Availability of hydrogen sulphide in the hydrothermal vent fluid influences the amount of carbon fixation possible by the tubeworm's symbiotic bacteria. Chemoautotrophic carbon fixation in this symbiosis requires O_2, CO_2, and H_2S, and a study by Childress *et al.* (1991) with *Riftia pachyptila* showed that O_2 and CO_2 fluxes are dependent on the flux of H_2S. Chemoautotrophic carbon is the energy source for tubeworm growth and reproduction. If the formation of different morphotypes is caused, either in full or in part, by variability in energy availability stemming from hydrogen sulphide flux, then there are likely differences in the physiological and reproductive condition of the high flux and low flux *Ridgeia piscesae* morphotypes.

In 2003, Tsurumi and Tunnicliffe characterized the fauna associated with *Ridgeia piscesae* bushes on three of the four southern segments of the Juan de Fuca Ridge. They found differences in the communities associated with different tubeworm bush structure, which they thought to be caused by differences in hydrothermal fluid flux. Subsequently, Marcus *et al.* (2009) studied post-eruption succession and found that the presence of *R. piscesae* may be a pre-requisite for the colonization of some hydrothermal vent species. The results of these two studies indicate that *R. piscesae* acts as a foundation species. Marcus *et al.* (2009) make the point that *R. piscesae* is the sole ecosystem engineer on the Juan de Fuca Ridge. Preliminary evidence of differences in condition based on hydrothermal fluid flux is reported in De Burgh (1989) and MacDonald (2002). In 1989, De Burgh *et al.* found differences in the structure of the trophosome in different *R. piscesae* morphotypes. In the *R. phaeophiale*, low flow, morphotype the trophosome had distinct structure separated by the blood vascular system while in the *R. piscesae*, high flux morphotype the trophosome was amorphous and completely filled the trunk cavity. MacDonald *et al.* (2002) found that *R. piscesae* aggregations in high flow had increased sperm transfer and a higher proportion of mature individuals than those in low flow. They proposed that the major reproductive activity may come from a few isolated, robust local populations.

The results of Tsurumi and Tunnicliffe (2003), Marcus *et al.* (2009), De Burgh *et al.* (1989), and MacDonald *et al.* (2002) lay the groundwork for my study. The objective of my study is to investigate physiological and reproductive condition and juvenile recruitment in *Ridgeia piscesae*. I test the following null hypothesis: All local populations of *R. piscesae* contribute equally to future generations as inferred from measurements of body condition, reproductive potential, and juvenile recruitment. If the null hypothesis is not supported, it suggests that there may be source-sink dynamics acting in the *R. piscesae* metapopulation in the study area. In metapopulation theory, a sink population is a local population in which local reproduction is insufficient to balance local mortality, whereas in a source population, local reproduction is greater than local mortality (Pulliam 1988). Sink populations cannot persist without input from source populations. Sink populations can be considered to occupy marginal habitat. Marginal habitat falls on the boundary of the ecological niche of a species and, while it can host reproduction, marginal habitat is of low importance to the demography of the entire population (Kawecki 2008). Even though reproduction in sink populations is low, sinks are important to the metapopulation because, through dispersal, they can rescue populations in danger of extinction (Gyllenberg *et al.* 1996).

Both the metapopulation structure and phenotypic plasticity of *Ridgeia piscesae* populations make it a model organism for studying the effects of habitat on animal condition. My study sites are located on the Endeavour and Axial segments of the Juan de Fuca Ridge in the Northeast Pacific Ocean. I delineate local populations as homogenous groups of individuals centered on separate hydrothermal fluid outputs. These populations may be very close, but experience distinct fluid flow regimes, as visualized by variable shimmering and turbulence. Many of my sampling sites are located within the Endeavour Hydrothermal Vents Marine Protected Area, Canada's first MPA, designated in 2003. I will extend my findings into a short commentary on the suitability of the designated MPA for maintaining *R. piscesae* populations.

Chapter 1
Body condition

1.1 Introduction

The hydrothermal vent environment in the Northeast Pacific is highly variable in both space and time with respect to physico-chemical parameters. The tectonic processes acting in these areas are highly unpredictable in the short-term. As a result, the lifespan of individual hydrothermal vents is short; individual vents are estimated to last an average of ten years (Tunnicliffe and Juniper 1990). Also, hydrothermal fluid flow rate and chemistry can be vastly different for vents in close proximity, which causes marked differences in species abundance and distribution on meter and centimeter scales (Sarrazin *et al*. 1997; Sarrazin *et al*. 1999; Matabos *et al*. 2008; Bates *et al*. 2010). In this environment, many species have narrow habitat preferences (Bates *et al.* 2010), creating a heterogenous community distribution in space (Sarrazin *et al.* 1997; Sarrazin *et al.* 1999) and in time (Marcus *et al*. 2009). However, unlike many hydrothermal vent animals, *Ridgeia piscesae* has the ability to inhabit a wide range of conditions, which allows this tubeworm to proliferate at hydrothermal vents in the Northeast Pacific (Tunnicliffe and Juniper 1990; Sarrazin *et al*. 1997; Tunnicliffe *et al*. 1997). *R. piscesae* is dependent on hydrogen sulphide, in the form HS^-, found in hydrothermal fluid, which causes the tubeworm to aggregate around hydrothermal fluid outputs and creates a patchy distribution. Sulphide concentrations range from <0.1 µM in low flux habitat to 300 µM in typical high flux habitat (Johnson *et al*. 1988). Energy production for *R. piscesae* by symbiotic sulphide oxidizing bacteria depends on sulphide availability, so variability in flow rate and concentration of hydrothermal fluid creates an environment of differing habitat quality.

Ridgeia piscesae is a vestimentiferan tubeworm that lives in a chitinous tube with numerous growth flanges, each laid down after a separate growth period. Tube growth ranges from 0-2.5 cm/year for low flux tubeworms, to 95 cm/year for high flux, and the length of growth periods is variable between worms as well as over the lifetime of individual worms (Tunnicliffe *et al*. 1997; Urcuyo et al. 2007). Given the differing growth rates, age estimates for the two morphotypes may differ by an order of magnitude. Conservative age estimates for *R. piscesae* in low flux habitat are 10-30 years (Urcuyo et al. 2007), while rapid growth rate suggests an average lifespan of one to a few years for those in high flux. *R. piscesae* has four body regions, the most anterior of which, the obturaculum, supports the branchial plume. The branchial plume is an area of gas exchange used in the uptake of hydrogen sulphide, oxygen, and carbon dioxide, resources that are essential for carbon fixation by symbiotic bacteria. The second body region, the vestimentum, forms the vestimental fold dorsally, enclosing the genital grooves and the gonopores. The obturaculum and vestimentum represent the majority of the somatic tissue of the tubeworm body. The third region, the trunk, contains the dorsal and ventral blood vessels, the gonad, and the trophosome, the nutritive tissue. The trophosome houses sulphide oxidizing, symbiotic bacteria that use hydrogen sulphide and oxygen to fix carbon dioxide into organic molecules, the energy source for the gutless tubeworm host. The fourth region, the opisthosome, contains chetae and helps the worm anchor into the tube. Several different *R. piscesae* morphotypes have been described, the main differences being tube and body length and width, tube colour, branchial plume colour, and number of obturacular saucers (Southward *et al*. 1995). Hydrothermal fluid flux rate appears to play a major role in the development of these different morphotypes; in the redescription of the species, Southward *et al.* (1995) noted two extreme morphotypes found in high flow and low flow habitat.

A frequent problem in hydrothermal vent work is that of characterizing the environment. It is not always possible to take environmental samples, such as water samples, thus researchers must rely heavily on submersible video. In 1997, Sarrazin *et al.* characterized fluid flux based on visual cues from submersible video. They created community categories to define differences in the biotic community on high temperature sulphide edifices. Each category was associated with a characteristic hydrothermal fluid flow regime and mean hydrothermal fluid temperature. This work was ground-truthed when Sarrazin *et al*. (1999) showed that previously established gradients in visual cues and temperature matched up with gradients in concentration of many hydrothermal fluid components, including sulphide. Bates *et al.* (2005) also found good agreement between visual cues for hydrothermal fluid flow and temperature measurements. In this study, I characterize hydrothermal fluid flux based on visual cues from submersible video and limited temperature data. I assume that a higher degree of fluid shimmering and higher temperatures indicate a higher fluid flux rate, and thus a higher flux of the resource sulphide.

In this chapter, I use the term ‘condition’ to refer to the relative state of development of individual tubeworms. I assume that tubeworms of better condition have higher body weights, greater gas exchange surface area, larger amounts of lipids and more trophosome; all except for trophosome volume are widely used condition measures in other invertebrate studies (Smith 1985; Fisher *et al*. 1988; Colaco *et al*. 2011). There are few studies that have evaluated condition among the vestimentiferans. In the studies that have, most created condition indices by evaluating the relationship between ash free dry weight and tube volume in various ways (Smith 1985; Nix *et al*. 1995; Bergquist *et al*. 2003).

The patchy distribution of *Ridgeia piscesae* populations and the ability to live in a range of different habitat conditions make *R. piscesae* an interesting model organism to study the effects of habitat on physiological condition. In this chapter I test the following hypotheses:

1. *Ridgeia piscesae* in high flux habitat have better body condition than those in low flux, as determined using the following condition measures: body weight, anterior tube diameter, trophosome volume, total lipid amount, and branchial plume condition.

2. The same patterns in body condition in high and low flux occur at vent habitats over scales of meters, kilometers, and tens of kilometers.

1.2 Methods

1.2.1 Study site

Juan de Fuca Ridge is part of 3 ridge spreading system in northeast Pacific Ocean. It is bounded by Explorer Ridge to the north and Gorda Ridge to the south. Endeavour and Axial two are separate ridge segments on the Juan de Fuca Ridge (Figure 1). The Endeavour vent fields are located along the inside edge of the Endeavour rift valley. The vent fields, each hosting several large sulphide structures, are spaced about 2 km apart along the ridge (Kelley *et al*. 2001). Depth at Endeavour ranges from 2200 m to 2220 m. Axial Volcano lies across the Axial ridge segment and summits at 1500 m depth. There is a venting field at the southwest corner of the caldera which contains numerous large sulphide mounds, including Mushroom vent (Butterfield *et al*. 1990). In 1998, a volcanic eruption produced many new venting sites and invigorated old sites, including Marker 33, on the southeast side of the volcano (Embley *et al*. 1999). At both Endeavour and Axial, there is a combination of highly concentrated, point-source hydrothermal fluid flow and dilute, diffuse fluid flow venting from both chimneys and complex sulphide structures as well as cracks in the basalt seafloor (Butterfield *et al*. 1990). Exiting fluids can range from near-ambient temperatures to upwards of 350°C. Site locations and physical descriptions for samples collected in 2008 and 2009 can be found in Tables 1 and 2, respectively.

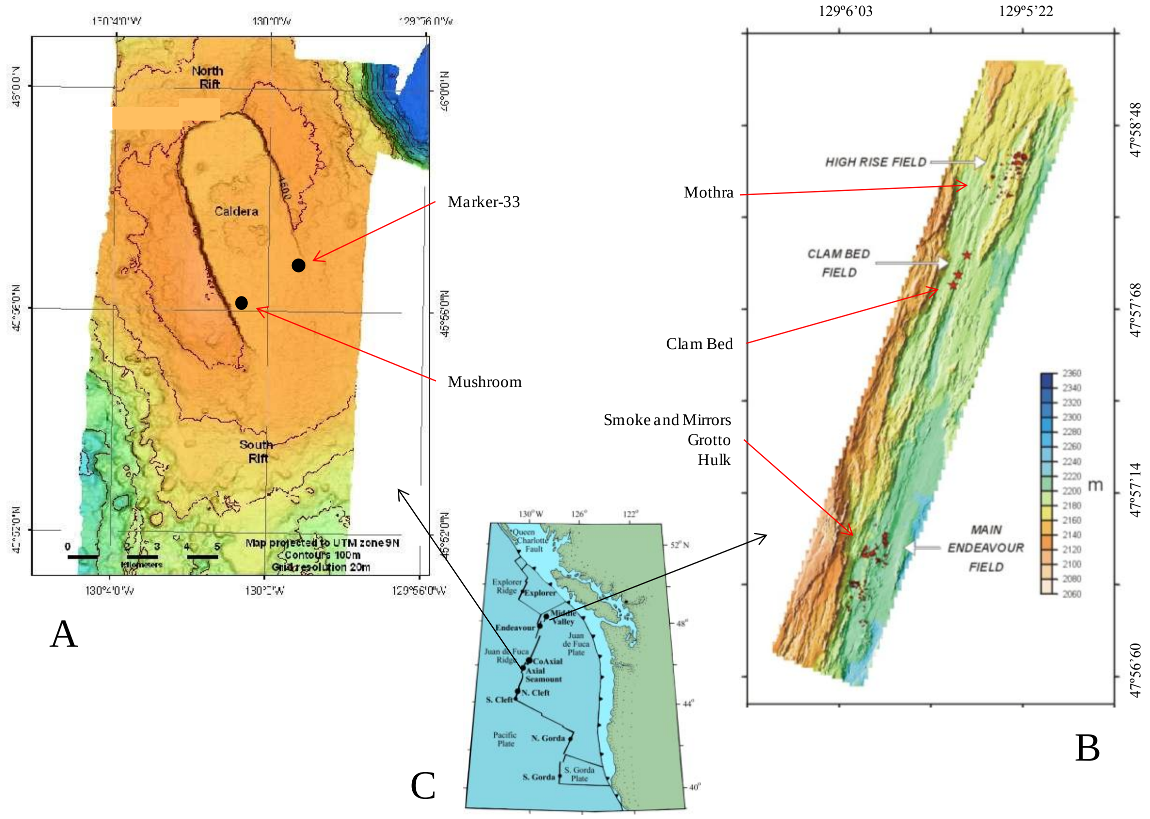

Figure 1. Location of sampling sites.

A. Axial Seamount (adapted from MBARI 2005)
B. Middle of Endeavour Segment (Adapted from Skebo *et al.* 2006)
C. Juan de Fuca Ridge, Northeast Pacific Ocean
Note: Black and red circles and red stars represent active venting sites.

Table 1. Site information for samples collected in 2008.

Ridge Segment	Vent field	Site	Site Description	Site code	Sample	Latitude	Longitude	Depth (m)
Endeavour	Main Endeavour Field	Smoke and Mirrors	Small clump of short fat tubeworms aggregated on small chimney on larger sulphide structure	SM	SMH1	N47 56.8799	W129 05.9096	2181
Endeavour	Main Endeavour Field	Smoke and Mirrors	Small clump of short small tubeworms a few meters away from HSM1	SM	SML1	N47 56.8802	W129 05.9099	2181
Endeavour	Main Endeavour Field	Grotto	Many short fat tubeworms covering rounded top of sulphide structure, hydrothermal fluid from small black smoker on side of structure reaches tubeworms	GR	GRH2	N47 56.9521	W129 05.9038	2189
Endeavour	Main Endeavour Field	Grotto	Long skinny tubeworms on small mound of sulphide between larger sulphide structures, whole mound is orange in colour	GR	GRL2	N47 56.9611	W129 05.8923	2188
Endeavour	Main Endeavour Field	Grotto	Clump of short fat tubeworms on top of flange of complex sulphide structure, many point sources of hydrothermal fluid	GR	GRH3	N47 56.9513	W129 05.8988	2189
Endeavour	Main Endeavour Field	Grotto	Many long skinny tubeworms covering smaller mound on complex sulphide structure, whole mound is orange in colour.	GR	GRL3	N47 56.9434	W129 05.8997	2191
Endeavour	Main Endeavour Field	Hulk	Large expanse of numerous long fat tubeworms underneath flange on sulphide structure with many flanges	HU	HUH4	N47 57.0051	W129 05.8238	2190
Endeavour	Main Endeavour Field	Hulk	Small mound with long skinny tubeworms at bottom of large sulphide structure	HU	HUL4	N47 56.9995	W129 05.8177	2197
Endeavour	Main Endeavour Field	Hulk	Clumps of long fat tubeworms on spires on top of large sulphide structure	HU	HUH5	N47 56.9860	W129 05.8207	2202
Endeavour	Main Endeavour Field	Hulk	Large field of long skinny tubeworms on basalt seafloor at base of sulphide structure	HU	HUL5	N47 56.9967	W129 05.8250	2199
Endeavour	Clam Bed	-	Clump of short fat tubeworms top of small sulphide structure	CB	CBH6	N47 96.2902	W129 09.1490	2196
Endeavour	Clam Bed	-	Vast field of long skinny tubeworms on basalt seafloor adjacent to small sulphide structure	CB	CBL6	N47 96.2845	W129 09.1476	2199
Endeavour	Clam Bed	-	Large clump of short fat tubeworms surrounding small chimney on small sulphide structure	CB	CBH7	N47 96.2938	W129 09.1482	2204
Endeavour	Clam Bed	-	Field of long skinny tubeworms on basalt seafloor adjacent to small sulphide structure	CB	CBL7	N47 96.2942	W129 09.1489	2206
Endeavour	Mothra	-	Clump of short fat tubeworms on top of spire on large sulphide structure	MO	MOH8	N47 56.0076	W129 10.8839	2279
Endeavour	Mothra	-	Short skinny tubeworm clump on basalt seafloor adjacent to sulphide structure	MO	MOL8	N47 56.0085	W129 10.8802	2286

Table 2. Site information for samples collected in 2009.

Ridge	Vent field	Site	Site Description	Site code	Sample	Latitude	Longitude	Depth
Endeavour	Main Endeavour Field	Hulk	Large expanse of numerous long fat tubeworms underneath flange on sulphide structure with many flanges	HU	High	N47 57.005	W129 05.8141	2190
Axial	Axial Seamount ASHES	Mushroom	Clump of short fat tubeworms on side of medium sulphide structure	MU	Moderate 1	N47 55.7345	W129 04.6055	1517
Axial	Axial Seamount Southern Rift Zone	Marker 33	Aggregation of long fat tubeworms around large crack in basalt seafloor	M33	Moderate 2	N47 55.6252	W129 04.6146	1521
Axial	Axial Seamount Southern Rift Zone	Marker 33	Aggregation of long skinny tubeworms around large crack in basalt seafloor	M33	Low	N47 55.6258	W129 04.6138	1520

1.2.2 Sample collection and preservation

Samples were collected during June 2008 using the remote operated vehicle (ROV) ROPOS and in July 2008 and June 2009 using the human occupied vehicle (HOV) Alvin. Samples taken in 2008 were collected in pairs, with one high flux and one low flux sample from each site. Sampling sites were selected based on local flow characteristics. The submersible first flew around to find sites with high flux and low flux areas within approximately 10 m of each other. High flux sampling areas were characterized by vigorously venting hydrothermal fluid, visible as a high degree of shimmering in submersible video (Figure 2). In high flux areas, shimmering hydrothermal fluid flow reached *Ridgeia piscesae* tubeworm plumes directly. Low flux sampling areas were characterized by either very light or no shimmering fluid flow, and, if present, the shimmering was adjacent to the tubeworm plumes. Of the samples taken in 2009, there was only one set of paired samples, Marker 33 moderate and Marker 33 Low; the remaining samples were independent of each other. After site selection, temperature was measured at the level of the tubeworm plumes using a temperature probe held by the submersible's hydraulic arm. We searched for the maximum plume level temperature for many minutes for most samples. Temperature was also measured at the base of the tubeworm clumps for the samples collected in 2009. After temperature measurement, clumps of at least 30 tubeworms were collected by grasping the tubeworms near the base with a claw on the hydraulic arm of the submersible and placing them into separate bioboxes with closing lids.

At the surface, *Ridgeia piscesae* tubeworms were separated from the rest of the animals and debris in the samples and sorted by anterior tube diameter. From the 2008 samples, the largest twenty-five tubeworms in each sample were preserved by slitting their tubes and placing them into 95% ethanol. From the 2009 samples, eight of the largest females and eight of the largest males were removed from their tubes. Three females and three males were placed into 10% seawater formalin for histology and five females and five males were frozen at -80°C for lipid analysis.

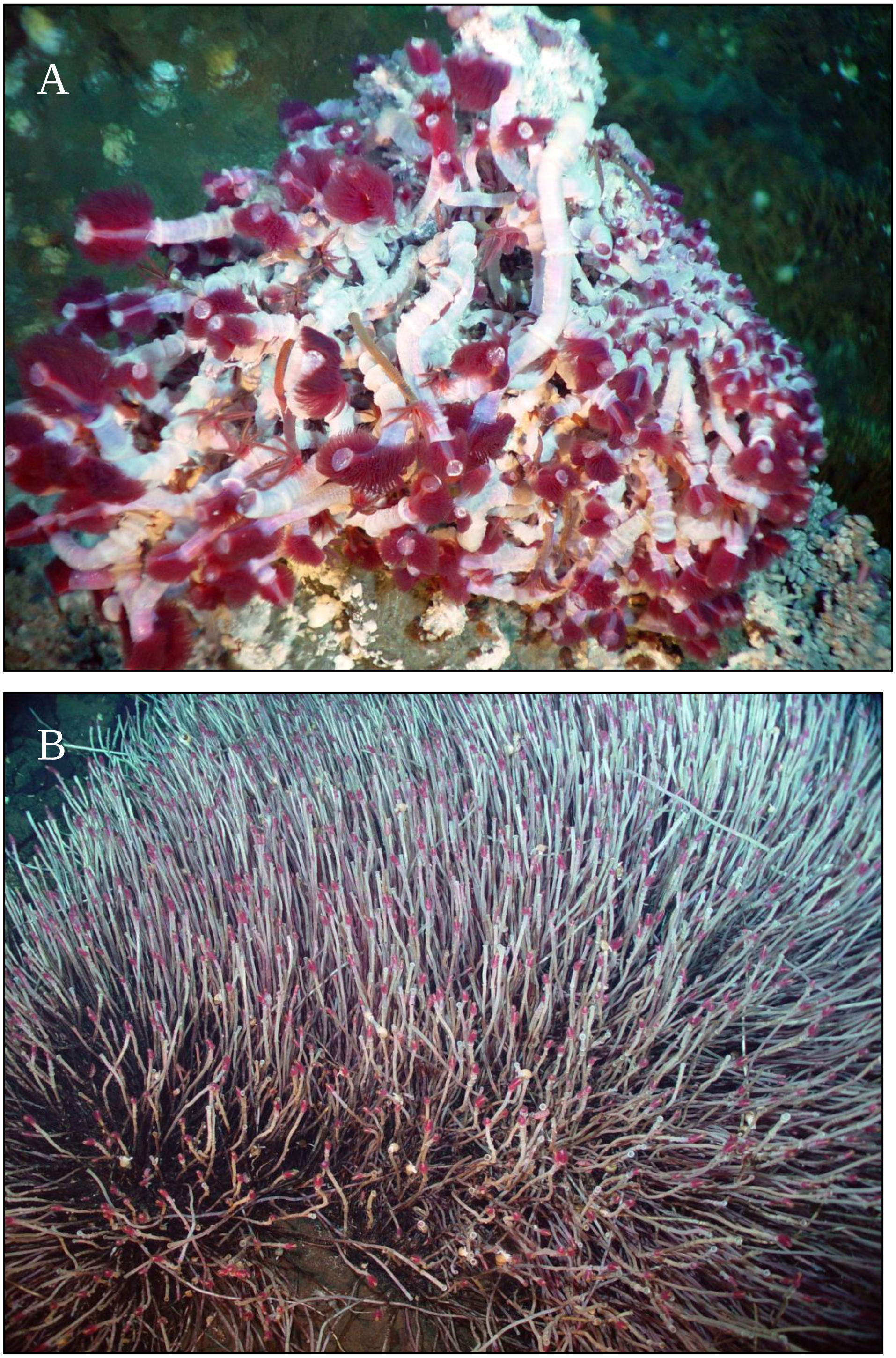

Figure 2. Representative images of sample sites.

A. High flux: vigorous hydrothermal fluid venting is visible as shimmering. Distance across photo is 60 cm
B. Low flux: Note the lack of shimmering fluid flow. Distance across photo is 200 cm.

1.2.3 Sample processing

Dissections

In the lab, measurements were made on the 2008 samples as listed in Table 3. For body measurements, the tube was cut longitudinally and removed from the body. The sex of the tubeworms was determined based on the appearance of the genital grove (MacDonald et al. 2002) and verified by the presence (female) or absence (male) of egg sacs just inside the gonopores, as well as with the presence of eggs or sperm. Egg sacs were present in immature females with no eggs.

Table 3. Quantitative and qualitative measurements performed on samples collected in 2008

Observation type (quantitative /qualitative)	Video (V) /Specimen (S)	Data type	Tube (T) /Body (B)	Measurement	Units
Qual	V/S	-	T	Tube colour/texture/dimensions	-
Quan	S	Discrete	T	Tube length	mm
Qual	V/S	-	B	Branchial plume colour/morphology	-
Quan	S	Discrete	B	Anterior tube diameter	mm
Quan	S	Discrete	B	Body length	mm
Quan	S	Discrete	B	Body width	mm
Quan	S	Discrete	B	Obturaculum-vestimentum length	mm
Quan	S	Discrete	B	Obturaculum length	mm
Quan	S	Discrete	B	Vestimentum width	mm
Quan	S	Discrete	B	Obturaculum-vestimentum wet weight	g
Quan	S	Discrete	B	Total wet weight	g
Quan	S	Categorical	B	Sex	m/f
Quan	S	Categorical	B	Branchial plume condition	0-4

Plume condition was rated on a scale from 0-3, a description for which can be found in Table 4 and Figure 3. This scale was later converted to a plume condition index for each sample by calculating the relative proportion of individuals in each plume condition category in each sample and multiplying these proportions by the weights listed in Table 4. During dissection, it became apparent that many tubeworms fitting the descriptions of categories 2 and 3 were actually juveniles. Juveniles could not be properly assessed under the rating scale I used because the short branchial filaments were the result of developmental stage, and not damage or poor adult condition. The predominance of juveniles in some samples was informative, though, so I weighted the condition index to account for their presence. Samples in which all individuals were juveniles had a condition index of 0, while samples in which all individuals were in category 0 plume condition, with no shortened branchial filaments, had a condition index of 10.

Table 4. Description of the plume condition rating scale, including weights used for determination of plume condition index.

Plume condition category	Physical description	Weight for condition index
0	Branchial filaments are long, extending away from the obturaculum	10
1	Branchial filaments are long, extending away from the obturaculum, but some are shortened	7.5
2	Branchial filaments are greatly shortened and/or large sections are missing	5
3	Obturaculum is completely devoid of branchial filaments	2.5
Juveniles	Branchial filaments are short, lie close to the obturaculum and are few in number	0

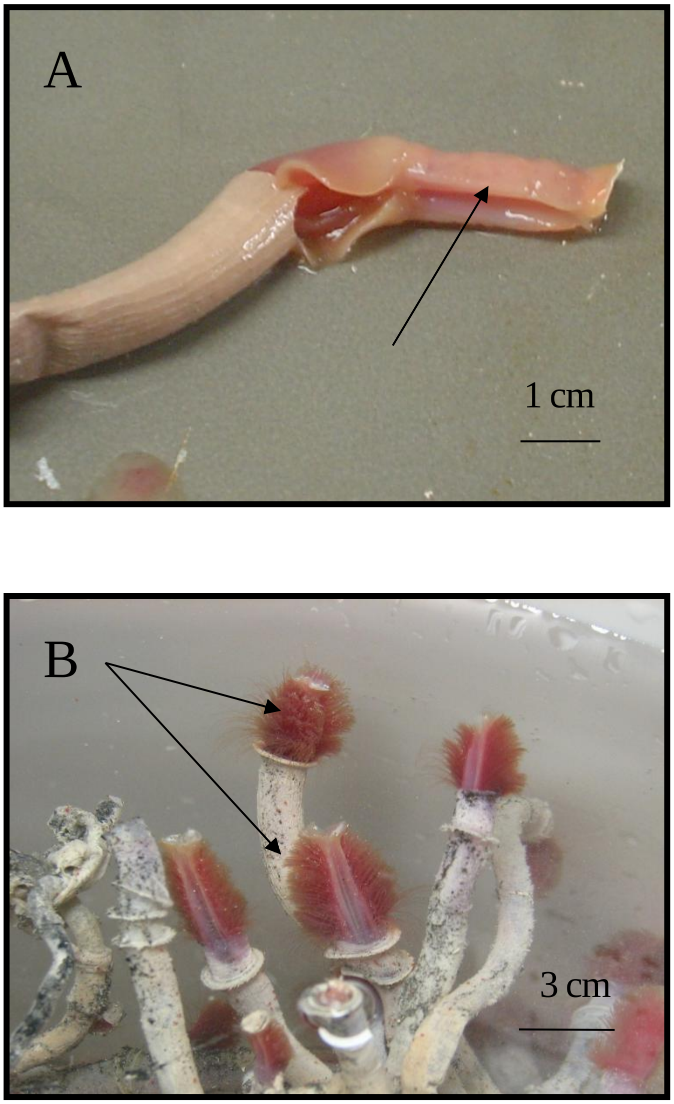

Figure 3. Representative images of *Ridgeia piscesae* branchial plumes at extreme ends of the branchial plume condition scale.

A. Branchial plume condition category 3: obturaculum is completely devoid of branchial filaments
B. Branchial plume condition category 0: branchial filaments are long, extending away from the obturaculum.

Note: Arrows point to branchial plumes

The relationship between wet and dry weight was determined using extra, ethanol-preserved, tubeworms, including five tubeworms from each of three high flux and three low flux samples. The obturaculum-vestimentum wet weight and total body wet weight were measured. The obturaculum-vestimentum and whole body were subsequently dried at 60°C for 72 hours and reweighed. The average values of this relationship were then used for high flux and low flux tubeworms (Table 5) to approximate obturaculum-vestimentum dry weight and total dry weight for each of the worms previously dissected.

Table 5. Average values of the relationship between wet and dry weight for different body regions in high flux and low flux tubeworms.

Flux	Body region	Dry weight as proportion of wet weight	Standard Deviation
High	Obturaculum-vestimentum	0.60	0.03
High	Whole body	0.30	0.08
Low	Obturaculum-vestimentum	0.60	0.17
Low	Whole body	0.55	0.07

The relationship between total body dry weight and total body ash-free dry weight was determined using extra, ethanol-preserved tubeworms; five males and five females from each of two high flux samples and one low flux sample. Total body wet weight and dry weights were measured as described in the preceding paragraph. Subsequently, the dried tubeworms in their weigh boats were combusted in a muffle furnace at 550°C for one hour and allowed to cool in a dessicator for six hours before re-weighing. The difference between dry weight (g) and ash weight (g) was recorded as ash-free dry weight (g). The ratio of ash-free dry weight to dry weight was consistent among samples with an average value of 0.925 (+/- 0.029 SD), so I chose to use dry weight (g) as the variable in all weight analyses.

Histology

Samples collected in 2009 were used for histology. Three males and three females were used from each of three samples: High, Moderate 2, and Low. The trunk length of each tubeworm was measured and then three 1 cm sections were cut from it: one beginning at the trunk top (0%), one beginning at 30% of trunk length, and one beginning at 60% of trunk length. Each of the three sections was placed into a separate vial and processed according to Table 6. After dehydration and infiltration, the tissues were embedded in JB4 plastic and allowed to harden under a slight vacuum. The complete embedding procedure can be found in Electron Microscopy Sciences JB-4 Plus Technical Data Sheet (EMS Catalogue # 14272-00). Using a glass knife microtome, 7 µm thin sections were cut from the embedding blocks and mounted on glass slides. Tissues were stained according to Table 6. After staining, the slides were cover-slipped and allowed to dry for 24 hours.

The trunk cross-sections were imaged using the SPOT RT KE digital camera and software. Image J software was then used to determine the total cross-sectional area occupied by trophosome and other trunk tissues. Using a grid overlay and the cell counter plug-in, the number of grid squares, of known area, that were at least 50% occupied by each different tissue type was counted. To determine the best grid size for speed and accuracy of analysis, five different pictures, each at three different grid square sizes, 1000, 2000, and 3000 pixels were analyzed. Chi-square goodness of fit tests showed no significant difference in total cross sectional area of different tissue types for the three different grid sizes. For speed of analysis a grid size of 3000 pixels was used. To estimate the total volume of trophosome for each tubeworm, the trophosome cross-sectional areas at 0%, 30%, and 60% of trunk length were each multiplied by one third of the trunk length and the resulting values were summed.

Table 6. Processing and staining specifications for histological thin sections.

Procedure	Chemical	Concentration	Time (min)
Dehydrate	Ethanol	50%	30
Dehydrate	Ethanol	70%	30
Dehydrate	Ethanol	80%	30
Dehydrate	Ethanol	90%	30
Dehydrate	Ethanol	95%	30
Dehydrate	Ethanol	100%	30
Dehydrate	Ethanol	100%	30
Infiltrate	JB4	-	60
Infiltrate	JB4	-	Overnight
Infiltrate	JB4	-	5 hours
Stain	Hematoxylin	-	10
Wash	Running tap water	-	3-5
Rinse	Ethanol	70%	10 seconds
Differentiate	Acid alcohol	1% HCL in 70% ethanol	3 seconds
Rinse	Ethanol	70%	10 seconds
Wash	Running tap water	-	3-5
Counterstain	Eosin	0.50%	1
Wash	Running tap water	-	3-5

Lipids

For lipid analysis, three males and three females from each of four samples collected in 2009 were used. The wet weight of each worm was measured and the water content determined based on the dry weight of two extra worms from each sample. Lipids were extracted and weighed according to the methods reported in Bligh and Dyer (1959). Briefly, tubeworm bodies were homogenized with a mixture of chloroform and methanol using a Potter-Elvehjem type tissue homogenizer. Water in the tissues created a ratio of chloroform:methanol:water of 1:2:0.8. This mixture was filtered and diluted so that a final ratio of 2:2:1.8 (C:M:W) was reached. The filtrate formed two layers, and the top, inorganic, layer was discarded. The bottom, lipid-containing, layer was washed several times with a stock inorganic layer solution and then dried under nitrogen in a hot water bath at 50°C. After subsequent drying in a desiccator over silica gel crystals, the lipid extract was weighed and checked for the presence of inorganic substances. Tubeworm dry weights were estimated based on the relationship between wet and dry weight in the two extra worms from each sample. Total lipid amounts were calculated as milligrams lipid per gram tissue dry weight.

1.2.4 Data analysis

The statistical packages R (The R Foundation for Statistical Computing, 2007), SPSS 13.0 for Windows Student version (SPSS Inc. 1989-2004), and Primer 5 (Primer-E Ltd., 2002) were used for all statistical analyses. Wilcoxon rank-sum tests were used for all pair-wise comparisons because the data were not normally distributed. In analyses where all high flux and all low flux samples were compared, sample averages were counted as one data point to avoid pseudoreplication. However, when comparing only one sample to another, all data points were used, as the use of sample averages was not possible. Standard deviations around averaged values were still included in graphical representations to give an idea of the within-sample variability. Principal components analyses (PCA) were run using normalized data and a maximum of five PC axes.

1.3 Results

1.3.1 Sample site characteristics

Tables 7 and 8 contain lists of the sites sampled along with their relative temperature and hydrothermal fluid flow characteristics where sampled, for samples collected in 2008 and 2009, respectively. At each high flux site tubeworms were directly in the path of vigorous, shimmering fluid flow. At low flux sites, when fluid flow was visible, it was only lightly shimmering and adjacent to the tubeworms. Temperatures at the high flux sites were consistently higher than those at the low flux sites, but this difference was not significant (p=0.059, Wilcoxon rank-sum test on pooled high and pooled low flux temperatures) at the five sites measured.

Table 7. Sample site characteristics for each *Ridgeia piscesae* sample collected in 2008.

Sample	Vent field	Structure	Maximum Temperature at *Ridgeia* Plume (ºC)	Fluid flux characteristics
HSM1	Main Endeavour Field	Smoke and Mirrors	10	Point-source of shimmering, vigorous fluid flow, tubeworms directly in flow
LSM1	Main Endeavour Field	Smoke and Mirrors	5	Point-source of vigorous, shimmering fluid flow 1 m away from tubeworms, tubeworms not in flow
HGR2	Main Endeavour Field	Grotto	30	Numerous point-sources of vigorous, shimmering fluid flow, tubeworms directly in flow
LGR2	Main Endeavour Field	Grotto	3.6	No sign of fluid point-sources, no shimmering flow, waters still except for ambient current
HGR3	Main Endeavour Field	Grotto	30	Numerous point-sources of vigorous, shimmering fluid flow, tubeworms directly in flow
LGR3	Main Endeavour Field	Grotto	11.4	No sign of fluid point-sources, no shimmering flow, waters still except for ambient current
HHU4	Main Endeavour Field	Hulk	-	Numerous point-sources of shimmering fluid flow, tubeworms directly in flow and plumes jostling due to turbulence of flow
LHU4	Main Endeavour Field	Hulk	-	No sign of fluid point-sources, no shimmering flow, waters still except for ambient current
HHU5	Main Endeavour Field	Hulk	-	Numerous point-sources of vigorous, shimmering fluid flow, tubeworms directly in flow
LHU5	Main Endeavour Field	Hulk	-	Slight shimmering of slow-moving, diffuse fluid flow from cracks in basalt
HCB6	Clam Bed	-	27	Point-source of shimmering, vigorous fluid flow, tubeworms directly in flow
LCB6	Clam Bed	-	2.4	Slight shimmering of slow-moving, diffuse fluid flow from crack in basalt
HCB7	Clam Bed	-	-	Point-source of shimmering, vigorous fluid flow, tubeworms directly in flow
LCB7	Clam Bed	-	-	No sign of fluid point-sources, no shimmering flow, waters still except for ambient current
HMO8	Mothra	-	-	Point-source of shimmering, vigorous fluid flow, tubeworms directly in flow
LMO8	Mothra	-	-	No sign of fluid point-sources, no shimmering flow, waters still except for ambient current

Table 8. Sample site characteristics for each *Ridgeia piscesae* sample collected in 2009.

Note: Moderate 2 sample classified as moderate because of evidence of recent change in hydrothermal fluid flux

Sample	Vent field	Structure	Maximum Temperature at *Ridgeia* Base (ºC)	Fluid flux characteristics
High	Main Endeavour Field	Hulk	27.7	Numerous point-sources of vigorous, shimmering fluid flow, tubeworms directly in flow
Moderate 1	Axial Volcano	Mushroom	-	Numerous point-sources of vigorous, shimmering fluid flow, tubeworms adjacent, but in flow
Moderate 2	Axial Volcano	Marker 33	26	Point source of vigorous, shimmering fluid flow, tubeworms directly in flow
Low	Axial Volcano	Marker 33	4.4	Fluid lightly shimmering throughout tubeworm aggregation

1.3.2 Overall morphological differences among samples collected in 2008

Tubeworms in high flux and low flux samples were notably different based on qualitative observations made on whole samples before dissection (Tables 9 and 10, Figure 4), but represented a range of morphotypes rather than just two. In general, unpreserved tubeworms in high flux samples had dark red obturacula with long branchial filaments extending outward, and their tubes were short, wide, and white in colour. In contrast, tubeworms in low flux samples generally had light pink obturacula with short branchial filaments lying close to the obturaculum, and their tubes were long, thin, and beige to orange in colour. There were some exceptions to this distinction. In the high flux samples, the tubes of worms in Hulk sample HUH4 were long and wide rather than short and wide. This was not seen in the other Hulk high flux sample, HUH5. At the Clam Bed site, worm tubes in sample CBH7 had many growth flanges (tube thickening indicating growth stop-starts), something that was not seen in worms from sample CBH6, the other Clam Bed high flux sample. However, worms in both Clam Bed high flux samples did have chitinous tubes that were thicker than in any other high flux sample. Worms from the Grotto high flux sample GRH3 had longer branchial filaments than in any other sample, including the other Grotto high flux sample GRH2. This produced “bushier-looking” obturacula.

Table 9. Qualitative characteristics of *Ridgeia piscesae* from each sample collected in 2008

Sample	Site	Number	Physical appearance of *Ridgeia piscesae* individuals		
			Branchial plumes	Branchial filaments	Tubes
HSM1	Smoke and Mirrors	25	Dark red	Long filaments extend away from obturaculum	Short, very wide, white
LSM1	Smoke and Mirrors	24	Small, red	Short filaments lie close to obturaculum	Short, skinny, beige, crumbly
HGR2	Grotto	25	Dark red	Long filaments extend away from obturaculum	Short, wide, translucent, white, thin but not papery
LGR2	Grotto	24	Very small, light pink	Short filaments lie close to obturaculum	Short, very wide
HGR3	Grotto	20	Dark red	Very long filaments extend away from obturaculum	Short, very wide
LGR3	Grotto	25	Light pink	Short filaments lie close to obturaculum	Medium length, skinny, yellow-brown, very skinny, translucent, papery
HHU4	Hulk	25	Dark red	Long filaments extend away from obturaculum	Long, wide, opaque, white
LHU4	Hulk	25	Light pink	Short filaments lie close to obturaculum	Long, skinny, cream-orange, translucent, papery
HHU5	Hulk	24	Dark red	Long filaments extend away from obturaculum	Short, wide, white
LHU5	Hulk	25	Red	Only a few filaments, if any	Beige-orange, long, skinny, translucent, papery
HCB6	Clam Bed	25	Dark red	Long filaments extend away from obturaculum	Short, very wide
LCB6	Clam Bed	18	Light pink	Medium-length filaments lie close to obturaculum	Long, skinny, beige, opaque, brittle
HCB7	Clam Bed	25	Dark red	Long filaments extend away from obturaculum	Short, wide, chitinous, translucent, white, numerous flanges
LCB7	Clam Bed	20	Light pink	Short filaments lie close to obturaculum	Long, skinny, beige, opaque, brittle
HMO8	Mothra	25	Dark red	Long filaments extend away from obturaculum	Short, wide, firm, chitinous, translucent, white
LMO8	Mothra	25	Small, light pink	Short filaments lie close to obturaculum	Short, skinny, beige, stiff

Table 10. Qualitative characteristics of *Ridgeia piscesae* from each sample collected in 2009.

Sample	Site	Number	Physical appearance of *Ridgeia piscesae* individuals		
			Branchial plumes	Branchial filaments	Tubes
High	Hulk	12	Dark red	Long filaments extend away from obturaculum	Long, wide, opaque, white
Moderate 1	Mushroom	12	Dark red	Long filaments extend away from obturaculum	Short, wide, chitinous, translucent, white
Moderate 2	Marker 33	12	Dark red	Long filaments extend away from obturaculum	Long, wide, opaque, brittle, beige-white, thick coating of white bacteria
Low	Marker 33	12	Red	Medium filaments extend away from obturaculum	Long, skinny, beige, opaque, brittle

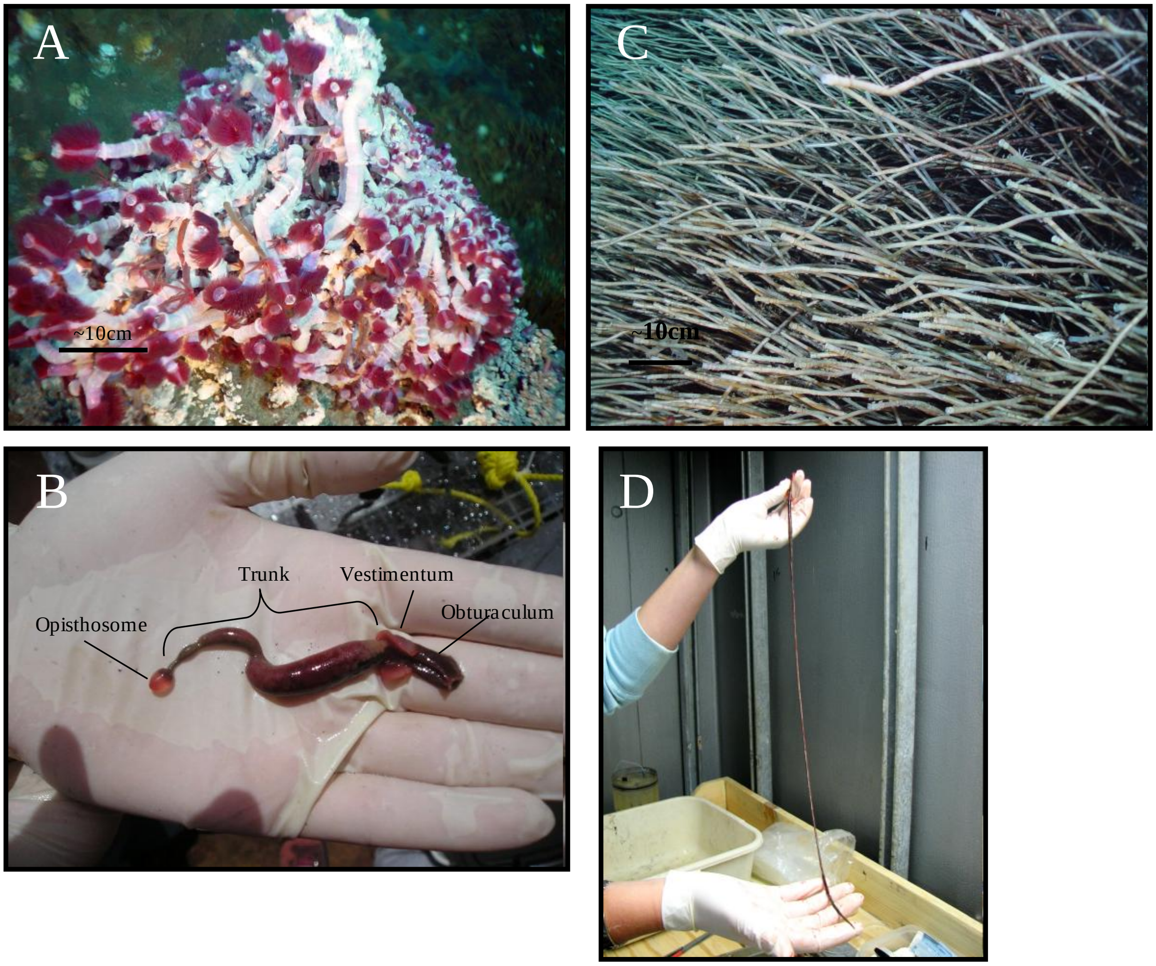

Figure 4. Images depicting *Ridgeia piscesae* extreme morphotypes.

A. High flux morphotype, *in situ*. Distance across image is ~60 cm
B. High flux morphotype, body removed from tube. Vestimentiferan body regions are labelled.
C. Low flux morphotype *in situ*. Distance across image is ~90 cm
D. Low flux morphotype, body removed from tube

In the low flux samples, worms from Smoke and Mirrors sample SML1 and Hulk sample HUL5 had red, instead of light pink, obturacula. In the Clam Bed low flux sample CBL6, worms had medium-length branchial filaments, longer than those of worms in any other low flux sample. Tubeworms from the samples SML1, GRL2, and the Mothra low flux sample MOL8 had short, thin tubes rather than long, thin tubes. Worms from both of the Clam Bed low flux samples as well as the Mothra low flux sample had thick, brittle tubes, while worms from the rest of the low flux samples had papery or crumbly tubes.

While there were differences in appearance among tubeworms from different samples, all tubeworms within the same sample had the same morphotype. This was true for both high and low flux samples.

1.3.3 Determination of body condition

Obturaculum-vestimentum weight

The average dry weight of the obturaculum-vestimentum (OV) region of tubeworms in high flux samples was significantly greater than that of tubeworms in low flux samples ($p<0.005$, Wilcoxon rank-sum test on pooled averages) (Figure 5). Average OV dry weights ranged from four to ten times higher in high flux samples. The only exception was at the site CB7, where the OV dry weights of high and low flux samples were not significantly different. The tubeworms in sample CBH6 had the highest OV dry weights, while tubeworms in samples SML1, GRL2, and MOL8 had the lowest. At the scale of vent field, four of the five high flux samples from Main Endeavour Field (MEF) were not significantly different from each other with respect to OV dry weights. The two Clam Bed high flux samples were significantly different from each other as well as from every other sample ($p<0.05$, Wilcoxon rank-sum tests). However, the Mothra high flux sample was not significantly different from one of the MEF high flux samples. At the scale of vent field in the low flux samples, multiple samples from MEF were significantly different from each other with respect to OV dry weights ($p<0.05$, Wilcoxon rank-sum tests). The Clam Bed samples were also significantly different from each other ($p<0.05$, Wilcoxon rank-sum test), but not from two MEF samples. The Mothra sample had significantly different OV dry weights than any other sample ($p<0.05$, Wilcoxon rank-sum tests).

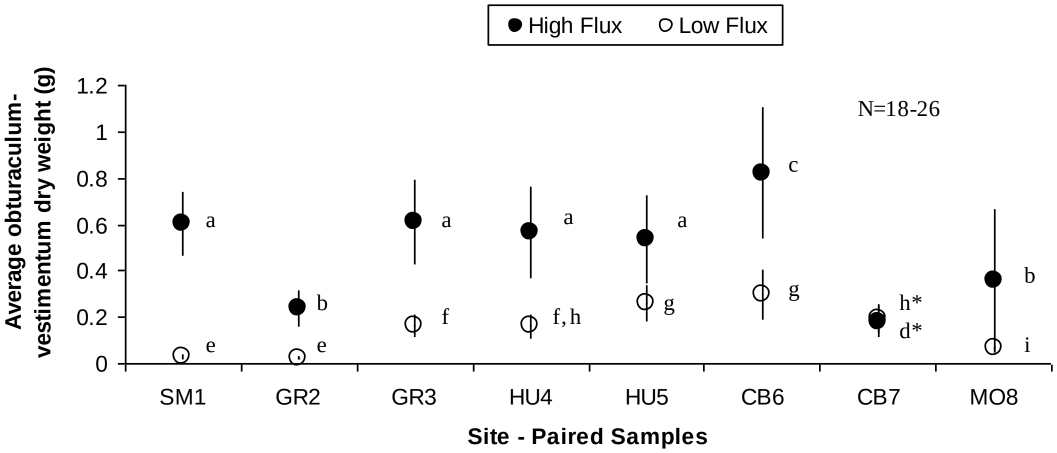

Figure 5. Average obturaculum-vestimentum dry weight (g) for high flux (black) and low flux (open) samples, with standard error bars for each sample.

N ranged from 18-26 for each sample. Letters a through i show where differences among samples lie (pairwise Wilcoxon rank-sum tests). Asterisks (*) indicate only high flux-low flux sample pair where there was no significant difference in obturaculum-vestimentum dry weight. All samples collected from Endeavour.

Total body weight

The average total body dry weight for tubeworms from high flux samples was not significantly different from that of tubeworms from low flux samples when I pooled high flux and low flux sample averages and compared them (Figure 6). However, when I made pair-wise comparisons between high flux and low flux samples from each site, there were significant differences in six of the eight sample sites ($p<0.05$, Wilxocon rank-sum tests). Again, as with the OV dry weights, these differences in total body dry weight were substantial. At the scale of individual vents, none of the samples taken from the same sites showed similarity in total body dry weights. At the scale of vent field, three of five Main Endeavour Field (MEF) high flux samples were not significantly different from each other in terms of total body dry weight, while this was the case with two of five low flux MEF samples. The two Clam Bed high flux samples had significantly different total body dry weights ($p<0.05$, Wilcoxon rank-sum test), but one was not significantly different from the Mothra high flux sample. The two Clam Bed low flux samples also had significantly different body dry weights ($p<0.05$, Wilcoxon rank-sum test). The Mothra low flux sample had tubeworms with significantly different total body dry weights than any other sample ($p<0.05$, Wilcoxon rank-sum tests).

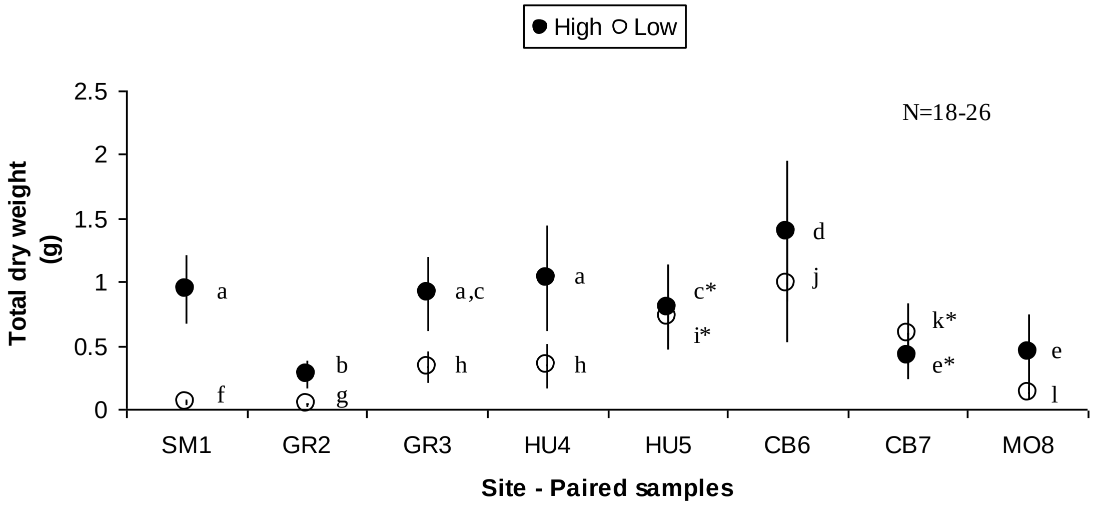

Figure 6. Average total body dry weight (g) for high flux (black) and low flux (open) samples, with standard error bars for each sample.

N ranged from 18-26 for each sample. Letters a through l show where differences among samples lie (pairwise Wilcoxon rank-sum tests). Asterisks (*) indicate high flux-low flux sample pairs where there was no significant difference in total body dry weight. All samples collected from Endeavour.

Anterior tube diameter

The average anterior tube diameter (ATD) of tubeworms in high flux samples was significantly greater than that of tubeworms in low flux samples ($p<0.001$, Wilcoxon rank-sum test on pooled averages)(Figure 7). Average ATDs ranged from one and a half to almost three times higher in high flux samples, with the exception of site CB7, where ATDs of high and low flux samples were not significantly different. The tubeworms in sample CBH6 had the highest ATDs, while tubeworms in samples SML1, GRL2, and MOL8 had the lowest. At the scale of individual vents, HUH4 and HUH5 were the only two samples from the same vent structure that showed no significant difference in ATD between the two samples. At the scale of vent field, multiple high flux samples from

Main Endeavour Field (MEF) were significantly different from each other with respect to ATD ($p<0.05$, Wilcoxon rank-sum tests). The two Clam Bed high flux samples were significantly different from each other ($p<0.05$, Wilcoxon rank-sum tests) but CBH6 was not significantly different from some MEF high flux samples, while CBH7 was not significantly different from CBL7 or one MEF low flux sample. The Mothra high flux sample was not significantly different from one of the MEF high flux samples or one of the Clam Bed low flux samples. At the scale of vent field in the low flux samples, multiple samples from MEF were significantly different from each other with respect to ATDs ($p<0.05$, Wilcoxon rank-sum tests). The Clam Bed samples were also significantly different from each other ($p<0.05$, Wilcoxon rank-sum test), but CBL6 was not significantly different from a MEF and a Mothra high flux sample while CBL7 was not significantly different from CBH7. The Mothra low flux sample had significantly different ATDs than any other sample ($p<0.05$, Wilcoxon rank-sum tests). Average anterior tube diameter and average vestimentum width were directly proportional for both high flux ($R^2 = 0.96$) and low flux ($R^2 = 0.98$) tubeworms (Figure 8).

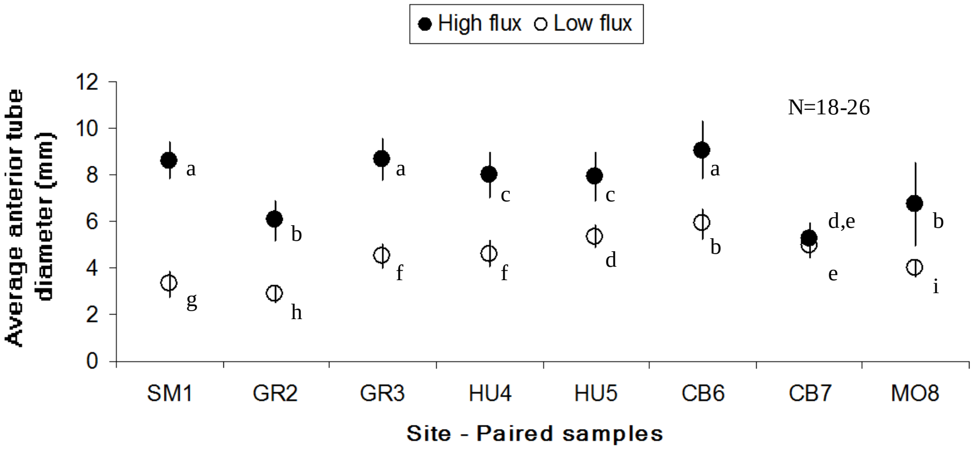

Figure 7. Average anterior tube diameter of high flux (black) and low flux (open) samples, with standard error bars for each sample.

N ranged from 18-26 for each sample. Letters a through i show where differences among samples lie (pairwise Wilcoxon rank-sum tests). Asterisks (*) indicate only high flux-low flux sample pair where there was no significant difference in anterior tube diameter. All samples collected from Endeavour.

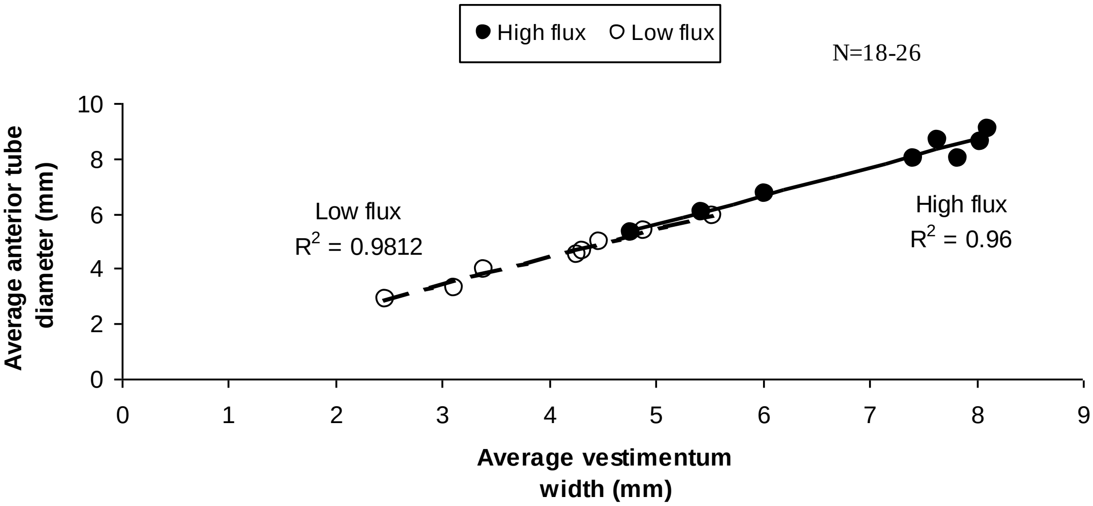

Figure 8. Linear regression of average anterior tube diameter (mm) on average vestimentum width (mm) for both high flux (black) and low flux (open) tubeworms.

R^2 values for the regressions are displayed.

Trophosome volume

A comparison of the relative cross-sectional area occupied by trophosome in tubeworms from high flux and low flux habitat can be seen in Figure 9. One notable difference in the appearance of trophosome tissue was the presence of green-coloured granules in many tubeworms from low flux habitat. These granules were not seen in tubeworms from high flux habitat. Tubeworms collected from high flux habitat had significantly higher trophosome volumes than tubeworms collected from moderate and low flux habitat ($p<0.01$ and $p<0.05$, respectively, Wilcoxon rank-sum tests)(Figure 10). There was no significant difference in trophosome volume between tubeworms from moderate and low flux habitat. Also, there was no significant difference in trophosome volume between male and female tubeworms when high, moderate, and low sample data were pooled together. Also worth noting, although not exclusive to the trophosome, there was a greater proportion of water in the trunk tissues of high flux tubeworms than in low, as measured by difference between wet and dry weights.

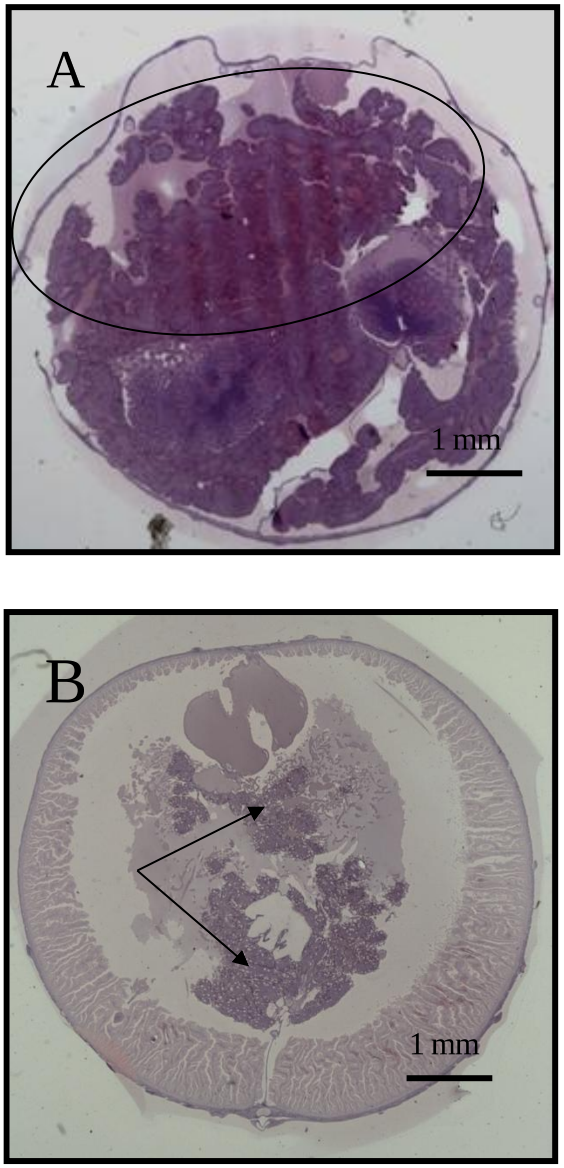

Figure 9. Cross-sections of *Ridgeia piscesae* trunk region.

A. High flux tubeworm

B. Low flux tubeworm

Note: Circle and arrows show trophosome area.

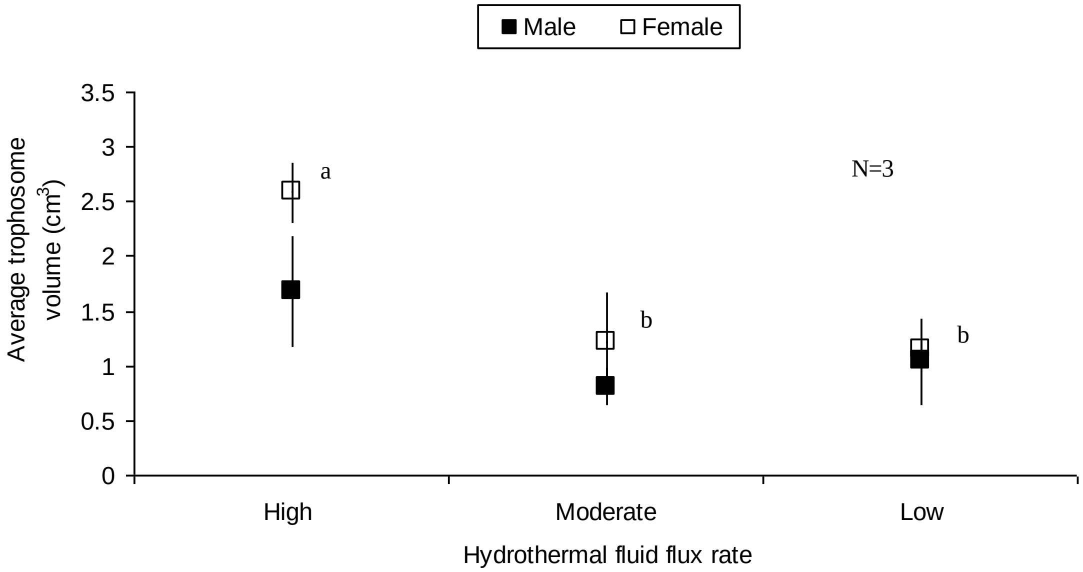

Figure 10. Average trophosome volume (cm^3) with standard error bars for male (black) and female (open) *Ridgeia piscesae* in high (Endeavour), moderate (Axial), and low (Axial) hydrothermal fluid flux rates.

Letters a and b show where differences among samples lie (pairwise Wilcoxon rank-sum tests)

Total lipid amount

There was no significant effect of flux type on lipid amount (Figure 11). Female *Ridgeia piscesae* had significantly higher lipid amounts than males ($p<0.05$, Wilcoxon rank-sum test, data from all flux rates pooled together). Statistical power was likely constrained by sample size but variability was very low and the graph does depict a relationship of higher lipid expected in higher flow worms.

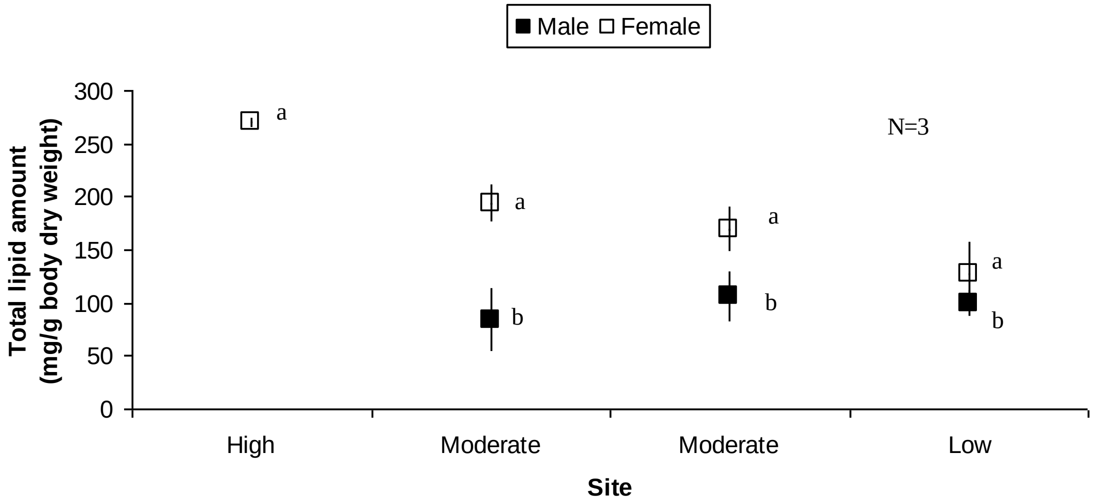

Figure 11. Average total lipid amount (mg/g body dry weight) with standard error bars for male (black) and female (open) *Ridgeia piscesae* in high (Endeavour), moderate (Axial), and low (Axial) hydrothermal fluid flux rates.

Letters a and b show where differences among samples lie (pairwise Wilcoxon rank-sum tests)

Branchial plume condition

In almost all cases, tubeworms from high flux samples had long branchial filaments extending out from the obturacula, causing the obturacula to appear “bushy”. In contrast, almost all worms from the low flux samples had shortened branchial filaments, causing the obturacula to appear “shaved” (Figure 3). High flux samples had significantly higher branchial plume condition indices than low flux samples ($p<0.001$, Wilcoxon rank-sum test on pooled high and pooled low indices)(Figure 12). In the high flux samples, four samples had long, untouched branchial filaments. Of the remainder, only one sample, CBH7 had a condition index below 9.8. In the low flux samples, only three samples, GRL3, HUL5, and CBL6, had any worms with untouched branchial filaments, and the plume condition index was below 7 for all low flux samples. In samples SML1, GRL2, and MOL8 the branchial filaments of all tubeworms were not yet fully developed. In sample CBL7, forty percent of the worms had immature obturacula, while fifty percent of the obturacula had patches missing and ten percent had no filaments whatsoever.

Samples CBH7 and CBL7 had the lowest plume condition indices of all high flux and all low flux samples with developed plumes, respectively. These were also the only two samples with any tubeworms completely devoid of branchial filaments.

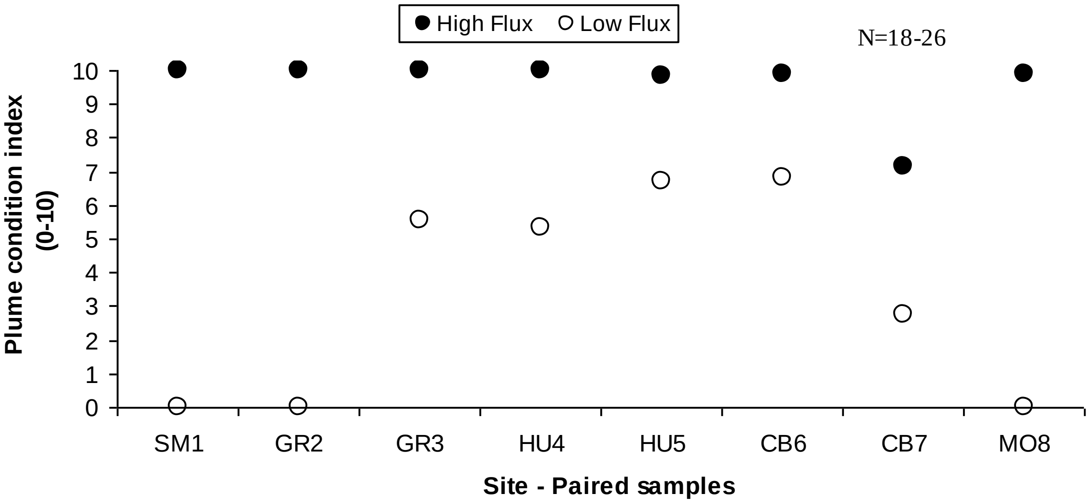

Figure 12. Branchial plume condition index for tubeworms in high flux (black) and low flux (open) samples.

Index ranges from 0 (juveniles/cropped filaments) to 10 (all individuals in sample have uncropped filaments). N ranges from 18-26 for each sample. All samples collected from Endeavour

Body length

The average body length of all tubeworms from high flux samples was not significantly different than that of tubeworms in low flux samples ($p=0.19$, Wilcoxon rank-sum test on pooled averages)(Figure 13). However, within all sites but two, SM1 and GR2, the low flux tubeworms were longer than the high flux tubeworms, significantly so in most sites. The tubeworms in sample HUL5 had the highest body lengths, while SML1 hosted the lowest. There were no patterns either at the scale of individual vents or at the scale of vent field.

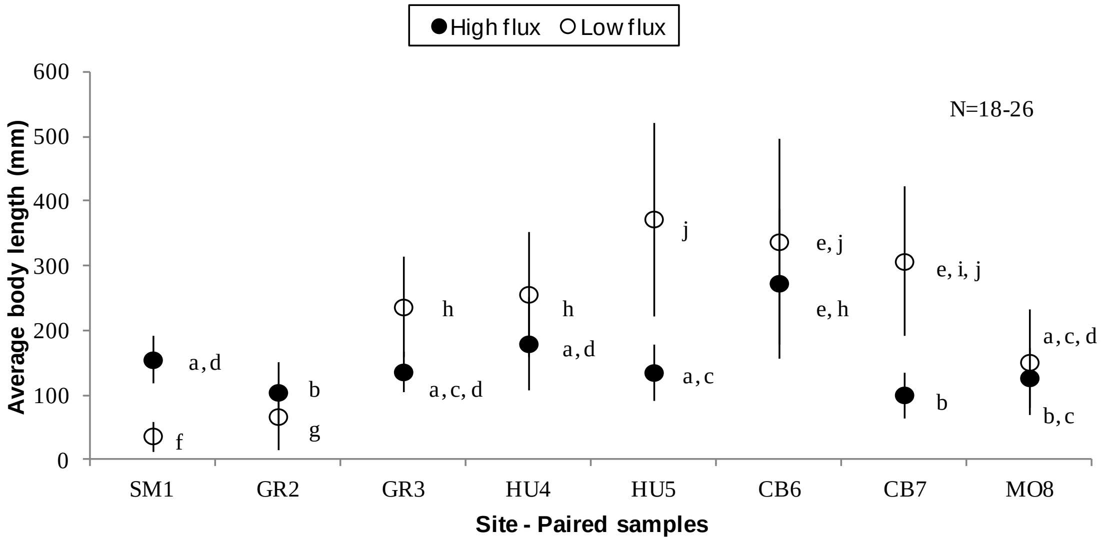

Figure 13. Average body length (mm) for high flux (black) and low flux (open) samples, with standard error bars for each sample.

N ranged from 18-26 for each sample. Letters a through j show where differences among samples lie (pairwise Wilcoxon rank-sum tests). All samples collected from Endeavour

Within-site variability in body measurements

The distribution of obturaculum-vestimentum dry weight values and total body dry weight values can be seen in Figures 14 and 15, respectively. Obturaculum-vestimentum dry weight values had a range of 0.312 g for tubeworms within sample CBH7 as compared to a range of 1.716 g for all tubeworms sampled. Total dry weight values had a range of 0.831 g within sample CBH7 as compared to a range of 2.482 g for all tubeworms sampled. The distributions of both obturaculum-vestimentum dry weight values and total dry weight values for tubeworms within sample CBH7 were somewhat normal, with some evidence of bimodality (Figures 14A and 15A). The distributions of both obturaculum-vestimentum dry weight values and total dry weight values for all tubeworms sampled were right skewed, with the majority of tubeworms in smaller weight categories.

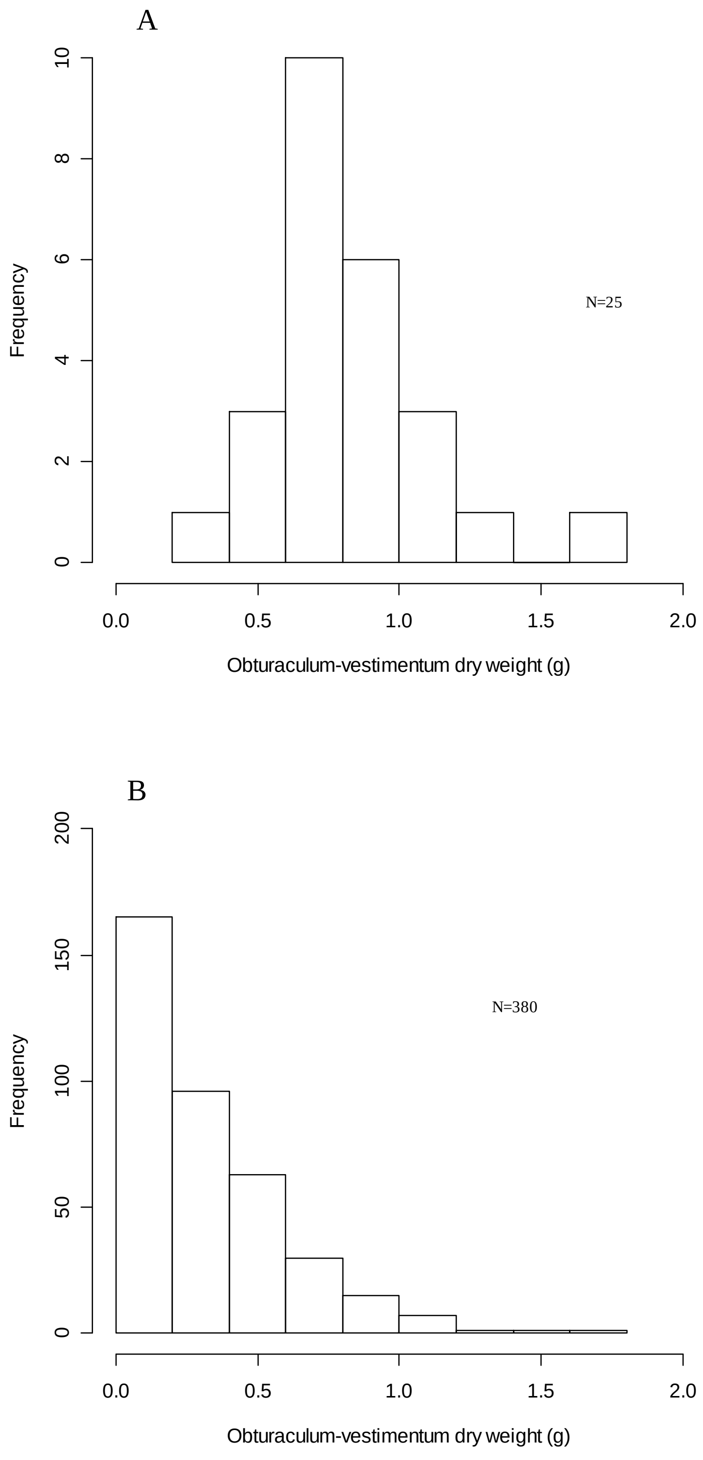

Figure 14. Histograms of obturaculum-vestimentum dry weight values.

A. All tubeworms in Clam Bed high flux sample CBH7
B. All tubeworms sampled in 2008

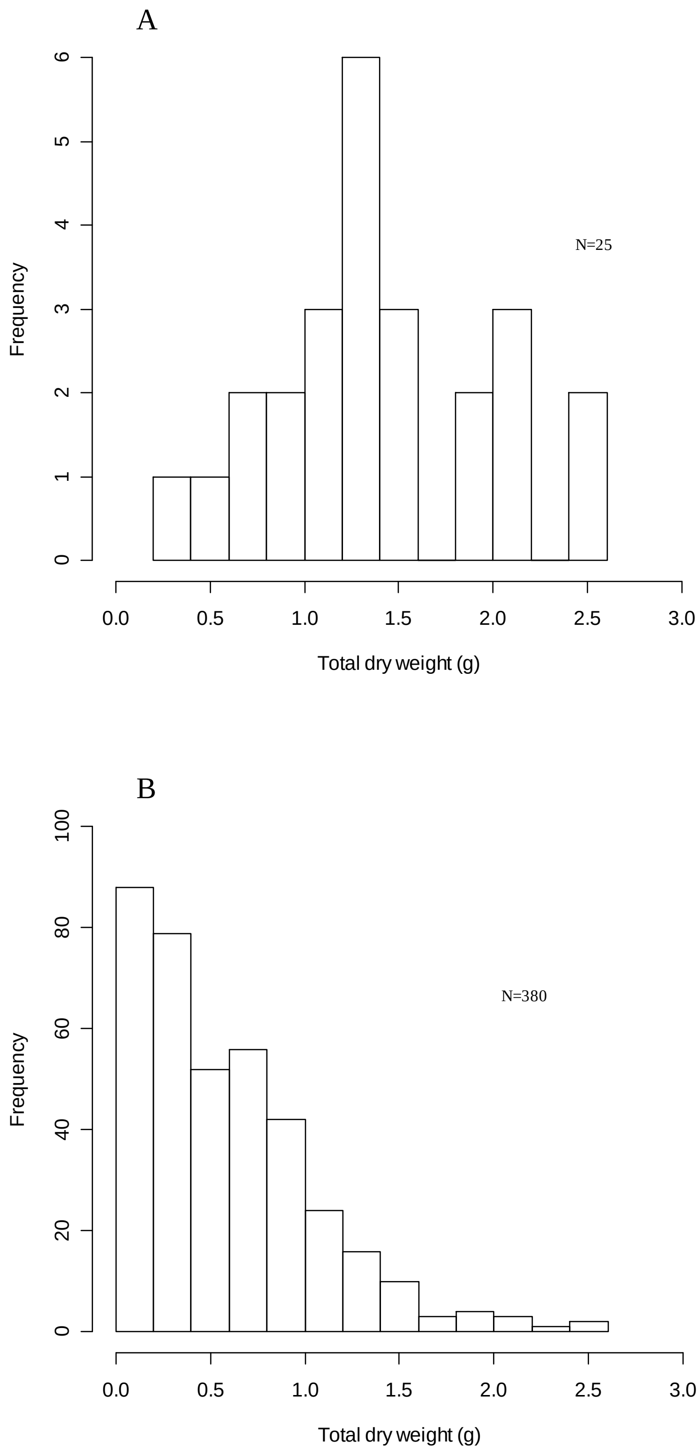

Figure 15. Histograms of total body dry weight values

A. All tubeworms in the Clam Bed high flux sample CBH7
B. All worms sampled in 2008

Summary analysis of body measurements

I used four variables to quantify similarity between sites in a principal components analysis (PCA): body width (mm), body length (mm), total body dry weight (g), and obturaculum-vestimentum length (mm). High flux and low flux sites clustered reasonably well with no interspersion (Figure 16). PC axes 1 and 2 accounted for 96.7% of the variation in the data. In the high flux samples, at the scale of individual vents, HUH4 and HUH5 clustered reasonably well, while GRH2 and GRH3 were separated, as were CBH6 and CBH7. In fact, CBH6 was an outlier separated from all of the other samples, both high flux and low flux. At the scale of vent field, four of the five high flux samples from the Main Endeavour Field (MEF) clustered together while the fifth, GRH2, clustered with Clam Bed and Mothra high flux samples.

In the low flux samples, two loose clusters formed. The samples SML1, GRL2, and MOL8 clustered together and separately from the rest of the low flux samples. At the scale of individual vents, none of the samples taken from the same vents at Grotto, Hulk, or Clam Bed clustered together. Samples CBL6 and HUL5 were separate from the main low flux cluster, as well as from each other. At the scale of vent field, Clam Bed and Mothra samples were interspersed with MEF samples.

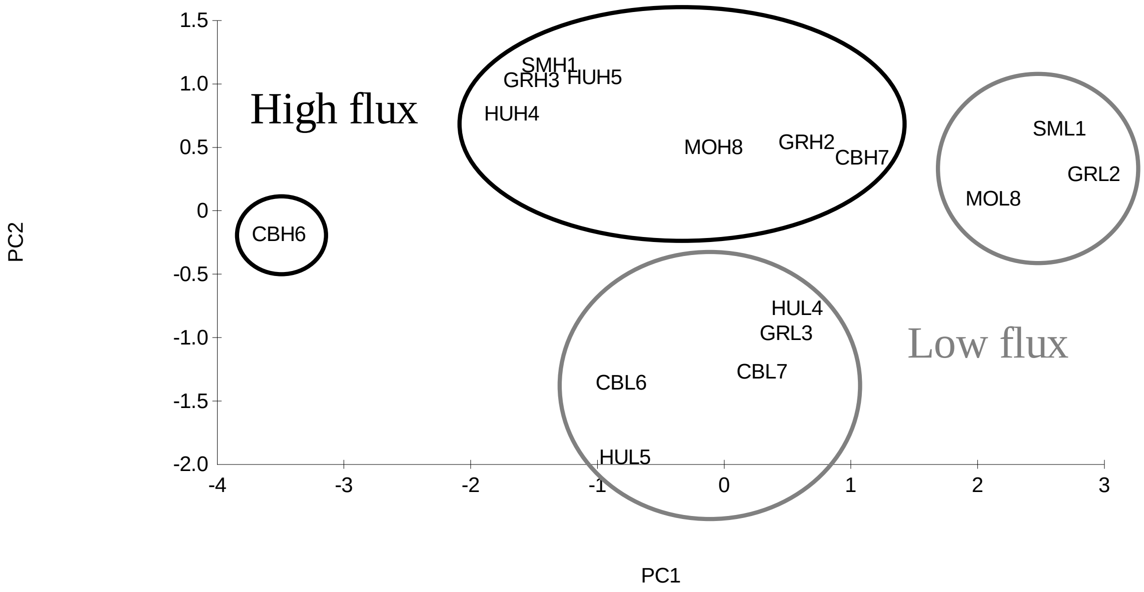

Figure 16. Principal components analysis of all samples collected in 2008. Calculated using average values of the variables body width (mm), body length (mm), total body dry weight (g) and obturaculum-vestimentum length (mm). High flux samples in black circles, low flux samples in grey circles. N=16 for each variable.

1.4 Discussion

1.4.1 Sample site characteristics

At high flux sites, there was a high degree of shimmering in video imagery and the rapid movement of hydrothermal fluid jostled tubeworms. Conversely, at low flux sites with a low degree of shimmering, if any, tubeworms were not disturbed by flowing hydrothermal fluid. Fluid temperatures were highly variable at the branchial plume level, especially within high flux sites because of the rate and unpredictability of mixing with ambient seawater. Often, we hunted for the maximum temperature among the plumes for many minutes. Bates et al. (2010) document the notable variability of temperatures around vent animals in high fluid fluxes. Plume level temperature at the low flux sites was more stable, and it was easier to obtain consistent temperature measurements.

Similar variability was observed at high and low flux sites among limpets at Endeavour, although Bates *et al* (2005) were able to establish a relationship between visual cues and temperature measurements. Sarrazin *et al.* (1999) also found a positive correlation between visible flow index and physico-chemical measurements, including temperature and sulphide. Subsequently, visual characterization of fluid flow, among other parameters, was used to distinguish community types in other studies (Andersen et al. 2006). It is likely that, at some high flux sites, maximum temperature was underestimated because of the high variability. Tunnicliffe *et al.*(1985) found that fluid temperature varied by more than 20ºC from the base to the branchial plumes within tubeworm aggregations. Likewise, using temperature as a proxy, Urcuyo *et al.* (2003) found that sulphide concentrations were as much as 1000 times greater at the base than at plume level in low flux tubeworm aggregations. Using data from the 2009 sample sites, I found similar variability between base and plume temperature measurements. Visual flow index has been used successfully in other studies and is a reliable proxy for sulphide delivery. Thus, while operational constraints limited the extent of temperature data for this study, there is enough corroboration from other work and the few comparative measurements here to designate high and low flux habitats and make a reasonable assumption that dissolved sulphide availability is similarly different.

1.4.2 Differences among tubeworms from high and low flux habitat

The main morphological differences between unpreserved high flux and low flux *Ridgeia piscesae* tubeworms were in tube length (qualitative observation), width, and colour, and body length and width, as well as branchial filament length and branchial plume colour. The high flux and low flux morphotypes are so different that they were originally described as two different species, *Ridgeia piscesae* and "*Ridgeia phaeophiale"* (Jones 1985). The "species" were later re-described by Southward *et al. (1995)* as different morphotypes of one species, (collapsed to *R. piscesae)* collected from high flux and low flux habitat, respectively. Aside from tube length, the current specimens match the original descriptions. The differences in tube colour may be a result of the relative ages of the tubeworms. The low flux tubeworms are longer-lived and have more time to accumulate staining on the outside of the tube. The orange colour of the tubes in the low flux environment is likely iron oxide that is more likely to accumulate in low environments. Differences in the other morphological characteristics will be discussed below. Phenotypic plasticity occurs in other hydrothermal vent organisms and *R. piscesae* is not alone in this trait among the Vestimentifera; *Riftia pachyptila* also displays different morphotypes that were once proposed to be different species (Jones 1984) and phenotypic plasticity is thought to be characteristic of the Vestimentifera (Black *et al*. 1994). However, *R. piscesae* does appear to be a more extreme case. *R. piscesae* displays a larger range of morphotypes and can tolerate a broader range of habitats than other vestimentiferans (example *Riftia pachyptila* - Black *et al.* 1994). During sampling, I made every effort to choose samples representing the two extremes in a continuum of habitat types and morphologies. However, the morphologies of specimens in some samples fell near the middle of the continuum.

Weight of the obturaculum-vestimentum region is a good measure of long-term growth in *Ridgeia piscesae*. The weight of the obturaculum-vestimentum region should not fluctuate in response to animal condition and reflects the maximum growth that each tubeworm achieved over its lifetime. Conversely, total body weight includes the trunk region, containing the trophosome and germinal tissues, whose weight does fluctuate in the short term, in response to animal condition; trophosome tissues are degenerated in the periphery of the trophosome, possibly as a source of nutrients for the tubeworm hosts (Bright and Sorgo 2003; Nyholm et al. 2008). Selecting the largest worms from each sample, I found a fourfold to tenfold difference in obturaculum-vestimentum weight between tubeworms from high flux and low flux sample pairs. Similar differences were found with total body weight, as all but two of eight sites showed significant differences in pair-wise comparisons. The similar pattern shown by obturaculum-vestimentum weight and total body weight indicates that tubeworms with higher mass over the short term also grow larger in the long term. However, no significant difference was found in total body weight when high flux samples were compared, as a group, to low flux samples; this variability shows that site to site differences do exist and may, in part, be a result of sample selection from a continuum of morphotypes. Both short term and long term growth is made possible by carbon fixation by the tubeworm’s symbiotic bacteria (1981). With increased hydrothermal fluid flow, there is an increased supply of reduced inorganic sulphide to the symbiotic bacteria to provide energy for carbon fixation. The limiting factors in this symbiosis are currently unknown. However, if sulphide is a limiting nutrient, increased sulphide supply would allow for the production of more energy for the tubeworm, available for growth and maintenance over the short and long term. Smith *et al.* (2000) found differences in body condition in methane-oxidizing mussels from habitats of different quality. Mussels in high quality habitat, with ample methane and moderate oxygen available for methane oxidation had higher body weight to shell volume ratios than did mussels from low quality habitat. Obturaculum-vestimentum weight and total body weight appear to be good measures of body condition in *R. piscesae,* but quantifying the ratio of somatic tissue to gonad tissue may be more precise. The gonado-somatic index is widely used, but extraction of the gonad from *R. piscesae* is difficult and time consuming (Hilario *et al.* 2008) and would not have been efficient for a large-scale study such as this.

Anterior tube diameter was significantly higher in high flux sites than in low. With increased diameter, tubeworms are able to take up more space laterally, which may be one tactic in the competition for access to hydrothermal vent fluid in densely packed tubeworm communities; growth in length may take the plumes beyond the optimal mixing zone. Also, ATD was directly proportional to anterior vestimentum diameter (AVD); as the vestimentum is the muscular ‘plug’ that wedges the animal in its tube, this relationship is not surprising. A wider body can likely support a larger branchial plume for increased gas exchange, further increasing access to the dissolved gasses in the hydrothermal vent fluid. Schoener (1983) showed that, with interspecific competition, larger body size increases exploitative competitive ability. Schoener also found that preemptive competition, where an individual passively grows into a space before another can use it, was the most common form of competition among sessile marine invertebrates.

The trophosome of *Ridgeia piscesae*, houses symbiotic bacteria and is the location of carbon fixation for the tubeworm. I found that tubeworms from high flux habitat had significantly more trophosome than those from moderate or low flux habitat. Tubeworms in high flux habitat experience a greater supply of hydrogen sulphide over time, thus can support a larger trophosome, which I interpret as a contributing factor to better body condition. It is common practice to determine rates of carbon fixation in terms of the amount of carbon fixed per gram of trophosome (Girguis and Childress 2006; Nyholm *et al*. 2008). Although there have been no studies on whether high flux tubeworms have higher carbon fixation rates, they do support larger populations of bacteria (deBurgh *et al*. 1989) in larger trophosomes, so higher total amounts of carbon fixation seem probable. It is also possible that this creates a positive feedback loop, where more carbon fixation allows the worm to grow larger and increase trophosome volume, and the worm is, thus, capable of still greater amounts of carbon fixation.

There was no difference in amount of trophosome tissue between male and female tubeworms despite the likelihood that females require more energy to produce their lipid rich oocytes than males require to produce their sperm bundles. However, I only examined nine tubeworms for this part of the study, so the results are inconclusive: trophosome volumes in females may be significantly higher than in males if the sample sizes were larger. In many of the cross sections taken from low flux tubeworms there were green and brown granules deposited in the trophosome tissue. These granules were not present in high flux tubeworm cross-sections. Granule deposition in the trophosome was noted by de Burgh (1986) and is likely caused by the accumulation of precipitated metals within the worms' body tissues. Given the relatively longer lifespan of the low flux tubeworms (Urcuyo *et al*. 2007), there is more time for the accumulation of metals by deposition. Also, deposits occurred mostly in the posterior end of the trophosome (personal observation), supporting the supposition that this is the oldest part of the trophosome (1986). The effect of these deposits is not known. In the calculation of water content for conversion from wet to dry body weights, I found that the trunk region of high flux tubeworms contained a higher proportion of water than the trunk region of the low flux tubeworms. While this trend was for the whole trunk and not the trophosome exclusively, the trophosome is a large component of the trunk, and may drive the trend. *Ridgeia piscesae* in high flux environments have more trophosome and likely have higher carbon fixation rates, creating a greater demand for dissolved inorganic carbon (DIC). To buffer the demand for DIC, it is concentrated in the tubeworm coelomic fluid for easy exchange with the hemolymph (Scott *et al.* 1999). In 1999, Scott *et al.* showed that *Riftia pachyptila*, a tubeworm that experiences higher fluid flux than *R. piscesae,* had higher concentrations of DIC in the coelomic fluids. It is possible that a similar dynamic occurs between high flux and low flux *R. piscesae*, but that the greater amount of DIC manifests as a larger volume of coelomic fluid rather than coelomic fluid with a higher DIC concentration.

Little is known about the exact mechanism of carbon translocation from symbiont to host; translocation may occur by way of diffusion of fixed carbon species out of bacterial cells or by host digestion of symbiont cells (Felbeck *et al*. 1985). Regardless of the mechanism, all host tissues are derived from bacterially-fixed carbon. Evidence for the bacterial origin of carbon in tubeworm tissues can be seen in tubeworm lipids; all tubeworm lipids either contain symbiotic biomarkers, or are modified from lipids that do (Fullarton *et al*. 1995). The total amount of lipid in a tubeworm can act as one indicator of energy production by bacterial symbionts for the growth and maintenance of both symbiotic partners. I found that tubeworms from high flux environments had more lipid than those in low flux environments, further supporting a model that related body condition to fluid flux. With increased sample sizes it is likely that this relationship would become significant. The average values for total lipid that I found were comparable to previous findings for *Seepiophila jonesi* (Hilario *et al*. 2008), but about three to five times greater than in *Riftia pachyptila* (Phleger *et al.* 2005) (Table 11).

Table 11. Comparison of average lipid amounts for *Ridgeia piscesae, Seepiophila jonesi* (Hilario *et al. 2008*), *and Riftia pachyptila* (Phleger *et al.* 2005).

*No value for male tubeworms, **note – authors used wet weight, ***mg/g wet weight converted to mg/g dry weight using average ratio of wet:dry weight for *R. piscesae.*

Sex	*Ridgeia piscesae* Average (mg/g dry weight)	*Seepiophila jonesi* Female Average* (mg/g dry weight)	*Riftia pachyptilla Average** (mg/g wet weight)*	*Riftia pachyptilla Average*** (mg/g dry weight)*
Female	190.24 +/- 60.37	224 +/- 50	11.5 +/- 4.9	~38.33
Male	96.88 +/- 11.22	-		

Investment into different types of lipid can give information about nutritional strategies. Polar lipids and sterols are primarily used in membrane structure, whereas triacylglycerols (fats), and wax esters (found in oocytes) are used for short-term and long-term energy storage, respectively. In *Ridgeia piscesae* (Pond *et al*. 2002; Fullarton *et al.* 1995) and *Riftia pachyptila (*Phleger *et al.* 2005*)*, phospholipid and sterol amounts were high and wax ester amounts were moderate relative to triaclyglycerol, suggesting a stable food source with little need for investment into energy storage, at least in the short-term. *Seepiophila jonesi* showed the opposite trend (Hilario *et al*. 2005) indicating variation within the vestimentiferans, possibly because of differences in resource availability in the hot-vent and cold-seep environments that these tubeworms inhabit. The low flux tubeworms appear to have a lower body condition, which supports the theory of variability or instability in their energy supply. I predict that low flux tubeworms would have a greater proportion of wax esters in their lipids as an investment into long-term energy storage to buffer a variable energy supply. I did not quantify separate lipid classes, but I did find that females had more total lipid than males in all habitat types, supporting a likely substantial contribution by wax esters from oocytes, and indicating female investment into long-term energy storage. Females in the high flux environment had higher total lipid amounts than those in the moderate and low flux environments. Again, this difference would likely be significant with increased sample sizes, and would indicate a larger surplus of energy available for storage and later use in high flux females. However, results should be interpreted cautiously, as energy may be utilized differently by different morphotypes. Other energetic costs such as the elimination of protons, one by-product of sulphide oxidation, may account for up to 60% of the oxygen consumed for ATP production in *Riftia pachyptila (*Girguis et al. 2002*)*, decreasing the amount of energy available to the tubeworm for growth and energy storage. Also, studies with *R. pachyptila* show that tubeworms are able to use glycogen for energy production through anaerobic pathways (Sorgo *et al*. 2002), adding another layer of complexity to an already complicated picture. Little is known about energy partitioning in *R. piscesae*. Hilario *et al. (2005)* found that lipid class composition, wax ester content in particular, was a good indicator of female reproductive condition in *Seepiophila jonesi* and suggested that this may apply to other vestimentiferans as well. With further replicates, total lipid content could be developed as a condition index, especially if lipid class composition was also determined.

The tubeworm branchial plume is an area of gas exchange used in the uptake of hydrogen sulphide, oxygen, and carbon dioxide, resources that are essential for carbon fixation by symbiotic bacteria. Andersen *et al.* (2006) found that the high flux morphotype has a thinner branchial epithelium, which decreases diffusion distance and increases the rate of uptake of essential resources, possibly an adaptation to the lower oxygen concentrations in the high flux habitat. The branchial epithelium thickness likely produces the differences in branchial plume colour observed between high and low flux tubeworms. The bright red, hemoglobin-rich blood would be more visible through the thinner epithelium of the high flux morphotype. It is also possible that blood has a darker red colour because haemoglobin is more concentrated in the blood of high flux tubeworms. Predation on *Ridgeia piscesae* branchial plumes, primarily by polynoid polychaetes, or scale worms, is common (Tunnicliffe *et al*. 1990; Juniper *et al*. 1992). Predation is detrimental to the tubeworms because the scale worms clip off large portions of the branchial plume, effectively decreasing the uptake surface area. In my study, samples from high flux habitat had significantly higher branchial plume condition indices (BPCI) than those from low flux habitat. In many of the tubeworms from low flux habitat, branchial filaments were completely removed from the obturaculum, and there were “pits” left in the obturacular tissue. This evidence of predation supports the theory that the differences in branchial plume condition are caused by grazing and not by differential development of the plume to suit different habitat conditions. The poor condition of branchial plumes in the low flux habitat likely exacerbates an already sub-optimal situation by further restricting resource uptake. However, some cold-seep vestimentiferans are capable of sulphide uptake directly from the sediment across the body wall (Freytag *et al*. 2001). Urcuyo (1998) suggested the low flux morphotype of *Ridgeia piscesae* is also capable of sulphide uptake across the posterior body wall, which would decrease the demand for uptake on a branchial plume in poor condition. The difference in sulphide uptake method may be the reason for the trend toward higher body length in low flux environments. If low flux tubeworms are acquiring sulphide near the substrate, the posterior end of the body would need to reach down to the posterior end of the tube. Conversely, as observed for most high flux individuals, the high flux tubeworm would only need to be in the very top of its tube to reach the hydrothermal vent fluid with its branchial plume. Nonetheless, regardless of plume condition and body length, higher densities of scale worms occurred in the high flux habitat (personal observation). However, since there are five known species of scale worm on the Juan de Fuca Ridge, and four are indistinguishable in video imagery, it is possible that the scale worms in the high flux habitat are a different species than those in the low flux habitat and they may not feed on *R. piscesae*. In this study, I measured branchial plume condition by rating the condition of the branchial plume on a scale from 0-3 for each tubeworm and then creating an index for the entire sample. This method was fast and efficient, which allowed me to examine 373 tubeworms. A more accurate, but also more time consuming method would be to determine the actual surface area of the branchial plume, so that no assumptions as to actual gas exchange surface would be necessary.

High flux and low flux sites separated well in a PCA analysis, which supports that I did, in fact, sample different morphotypes. However, within each of the high flux and low flux clusters, there were two sub-clusters, indicating that there was some variability in the morphotypes sampled. Low flux samples SML1, GRL2, and MOL8 formed a cluster with the lowest obturaculum-vestimentum weights and the lowest total body weights of all samples. Also, all tubeworms in these samples displayed branchial filaments that had only just begun development. Taken together, these results suggest that tubeworms in samples SML1, GRL2, and MOL8 were juveniles and were still developing at the time of collection. This juveniles example illustrates that the study actually benefits from the collection of intermediate morphotypes because they offer a glimpse at a more complete picture. Some of the “intermediate” morphotypes may be juveniles of the two extreme morphotypes in this study, and further study of the continuum of morphotypes is necessary.

1.4.3 Spatial variability

Many studies have described the high variability in both time and space inherent in a myriad of variables at hydrothermal vents (Hessler *et al*. 1985; Hessler *et al*. 1988; Johnson *et al*. 1988; Sarrazin *et al*. 1997). Tectonic and volcanic events cause constant change, both creating new vent habitat available for colonization and cutting off hydrothermal fluid flow to older, established sites (Marcus *et al*. 2009). As well, temperature and pressure of fluid formation, composition of the substrate surrounding fluid during formation, amount of dilution with seawater before fluid exits the seafloor (Butterfield *et al*. 1994), and age of the vent (Marcus *et al*. 2009) can all cause variability in the concentration and composition of the vent fluid ultimately available to the animals at hydrothermal vents. However, while this is a highly variable environment, spatial variability tends to be on the meter and centimetre scale, and depends on the local hydrothermal fluid chemistry and flow regime (Sarrazin *et al.* 1997, 1999, Bates *et al*. 2010, Matabos 2009). I sampled from Endeavour Segment and Axial Seamount, located ~200 km apart on the Juan de Fuca Ridge. On the Endeavour Segment, I sampled from High Rise, Clam Bed, and Main Endeavour Fields, located at 2 km intervals along the ridge. At each location, there were samples taken from multiple pairs of high and low flux sample sites within meters and tens of meters of each other. I found that the within sample variability and between site variability were low relative to the variability between samples from the same site. Between vent sites separated by hundreds of meters to several kilometres, encompassing both Endeavour Segment and Axial Seamount, the range in fluid flow regimes, and thus, in morphotypes and body conditions, were similar. With few exceptions, each site hosted numerous hydrothermal fluid vents that spanned the full range from near-ambient, diffuse venting from cracks in the basalt to vigorous, point-source venting from the sides of sulphide structures. As a result, there was no difference in the range of morphotypes and body conditions between vent sites, at least within the parameters used for this study. Similarly, tubeworms within local aggregations, centered on one point source of venting, experienced the same fluctuations in fluid concentration and chemistry over time, and developed the same morphotype and body conditions as a result. In contrast, tubeworms in samples taken from different aggregations within meters or tens of meters of each other experienced vastly different fluctuations in fluid chemistry, causing high variability in both morphotype and body condition. For example, within the Clam Bed site, the two paired samples often represented opposite extremes in analyses including samples from all vents.

There was some variability in the high flux morphotype from site to site. Worms from sites HUH4, CBH7, and GRH2 were notably different from other high flux tubeworms, with longer tubes, more flanged tubes, and longer branchial filaments, respectively. These three sites separated from other high flux sites in most of the analyses. I only collected the high flux morphotype from areas where vigorous fluid flow reached the branchial plumes directly, but is possible that the different high flux morphotypes were a result of changes in venting, so that the conditions at the time of sampling were not representative of the pre-collection conditions. For example, in the case of CBH7, an increased number of flanges would be indicative of higher variability in vent fluid supply. Flanges are laid down at the end of each growth period, so the worms in CBH7 likely experienced more individual growth periods relative to tubeworms from other high flux samples. These differences, even within one morphotype, support that there are many factors affecting morphotype, and thus, the spatial variability of morphotypes.

In their paper about sulphide edifice community composition, Sarrazin and Juniper (1999) refer to the “rarer high flow *Ridgeia piscesae* community”. Although my study didn’t quantify relative proportions of each morphotype, it was clear from video imagery and from the amount of time we spent “searching” for the high flux morphotype, that it was much rarer. In the majority of habitat available to *Ridgeia piscesae*, conditions produce animals that appear to have sub-optimal characteristics that may be limiting for reproductive output.

Chapter 2
Reproductive condition and juvenile recruitment

2.1 Introduction

Because future generations of any population depend on reproduction, it is important to understand the factors affecting reproduction on an individual level and at local population levels so that one can make inferences about reproduction in the population as a whole. Where metapopulation structure exists, variations in habitat quality experienced by local populations can lead to modifications to many different life history traits, including those associated with reproductive output and dispersal. Studies with other species show that habitat condition can modify intraspecies reproductive effort in characters such as the fecundity-egg size tradeoff (Qian and Chia 1991), age at maturity (Levin and Creed 1986; Levin et al. 1987), reproductive output (Cruz-Rivera and Hay 2000), and frequency of spawning events (Spight and Emlen 1976), among others. These modifications can create a situation where offspring supplied by source populations with high reproductive output settle in sink populations where local reproductive output is not able to support the local population (Cowen and Sponaugle 2009). *Ridgeia piscesae* is a phenotypically plastic species that lives as a metapopulation in a highly fragmented habitat. The habitat is also highly variable in time and space, thus *R. piscesae* is a good model species for the study of the effects of habitat condition on life history traits associated with reproduction and dispersal.

Our understanding of reproduction in hydrothermal vent animals is limited because of the inaccessibility of the hydrothermal vent environment and also the difficulty of rearing hydrothermal vent larvae in the lab. However, the vestimentiferan life history has been pieced together with findings from many different species (Tyler and Young 1999). *Ridgeia piscesae* larvae settle from the water column at around 60 µm in length (Jones and Gardiner 1989) and feed on free living bacteria on the benthic substratum until, at a length of about 250 µm, they acquire their symbiotic bacteria (Southward 1988; Nussbaumer et al. 2006). Tube secretion begins at this time in the form of a mucous sheath secreted by pyriform glands (Nussbaumer et al. 2006). The mucous sheath subsequently hardens and the juvenile anchors to the substratum, usually the tubes of adult tubeworms when they are present.

In *Ridgeia piscesae*, spermatids develop attached to generative cells, called cytophores, produced by the germinal epithelium that is located adjacent to the ventral blood vessel and runs almost the entire length of the trunk (Gardiner and Jones 1985). Mature spermatids break away from the cytophore and form spermatozeugmata, condensed aggregations of mature spermatids, that are spawned from paired gonopores located at the base of the vestimentum near the junction between the trunk and vestimentum (Southward and Coates 1989). In *R. piscesae*, spermatozeugmata are transferred by entanglement in the branchial filaments of adjacent females and move toward the paired female gonopores, found in the same location as in males, presumably by ciliary action (MacDonald et al. 2002). Spermatids travel down the ascending tubules of the paired, U-shaped, female gonoducts and are stored in the spermatheca (Hilario et al. 2005).

Primary oocytes develop in the gonocoel of the female gonad, and radiate outward from a germinal epithelium that is closely associated with the ventral blood vessel (Malakhov *et al*. 1996). Fully developed primary oocytes travel down the descending gonocoel and past the spermatheca, where they pick up spermatids that attach to receptors on the oocyte outer membrane (Hilario *et al*. 2005). The oocytes travel up the ascending gonoducts, are spawned from paired anterior gonopores, and fertilization occurs once the oocytes reach the seawater (Hilario *et al*. 2005).

Reproductive output is high (Tyler and Young 1999); however, exact estimates are not available. Vestimentiferan gametogenesis, fertilization, and spawning are thought to be continuous (Tyler and Young 1999) with a constant output of gametes and embryos, rather than discreet seasons or periods of reproductive activity. However, *Ridgeia piscesae* lives in a highly variable environment, and limited resource availability, such as in low flux conditions, likely limit gamete production, and may confine reproductive output to discreet periods of sufficient resource availability.

After spawning, lipid-rich embryos develop into lecithotrophic, trochophore larvae that, as estimates with *Riftia pachyptila* show, can disperse in the water column for up to 38 days before settlement (Marsh *et al*. 2001). Multi-species larval studies on the

East Pacific Rise show that larvae are transported mostly in bottom currents, with some entrainment into the hydrothermal plume (Kim and Mullineaux 1998). Within the axial valley of a vigorously venting segment like Endeavour Segment, rising hydrothermal plumes drive the influx of water from the north and, because cross-currents are constrained by the valley topography, a dominant north-south water movement can transport larvae from field to field (Thomson *et al*. 2003). On the larger scale, however, molecular research with *Ridgeia piscesae* shows that there is gene flow among the three segments of the Juan de Fuca Ridge, with a dominant north to south migration from Juan de Fuca and Gorda Ridges over the Blanco Transform Fault (Black et al. 1998; Young et al. 2008).

On Axial Volcano (Juan de Fuca Ridge), Metaxas (2004) showed that vent larvae (mostly gastropods) settle from a homogenous larval pool and survive where environmental conditions meet the individual requirements of each species. Using basalt colonization plates on the East Pacific Rise, Mullineaux *et al.* (1998) showed that vestimentiferan juveniles only colonize zones inhabited by adults. Smith *et al.* (2000) provide further evidence for an environmental effect on juvenile recruitment in the vent mussel, *Bathymodiolus childressi,* where there was increased recruitment in areas with adults of better physiological condition. It is possible that, since there is such a difference in habitat conditions where *Ridgeia piscesae* occur that mechanisms have developed to either retain *R. piscesae* larvae within, or to attract larvae to, superior habitat.

While the basic life history in vestimentiferans is mostly elucidated, there are few studies that look at higher order questions, such as the effect of local habitat condition on vestimentiferan reproductive condition and strategy. In *Ridgeia piscesae*, high flux and low flux habitats host different morphotypes, with differences in growth rate and habitat turnover of at least one order of magnitude; for example, Tunnicliffe *et al.* (1997) estimated growth rates of 95 cm per year for tubeworms in high flux habitat, while Urcuyo (2007) estimated growth rates of 0 to 2.5 cm per year, and decadal longevity for tubeworms in low flux habitat. In Chapter 1, I showed that *R. piscesae* in high flux habitat have a better physiological condition than those in low flux habitat, most likely because increased sulphide flux provides more energy for growth and maintenance. This chapter focuses on whether differences in apparent fluid flux create differences in reproductive condition, reproductive strategy, and juvenile recruitment. In this study, I define reproductive condition as a combination of the relative proportion of reproductively mature individuals and the relative reproductive output of those individuals.

I test the following hypotheses:

1. Tubeworms in high flux habitat have better reproductive condition than in low flux habitat.
2. Tubeworms in high flux habitat have continuous reproduction while those in low are periodic in output.
3. Juvenile recruitment is higher in high flux habitat.
4. Reproductive condition can be represented by a parameter measurable from video imagery.

2.2 Materials and Methods

2.2.1 Study site

Refer to section 1.2.1 for a detailed description of the study sites, including maps.

2.2.2 Sample collection and preservation

All samples collected in 2008 and 2009 were used in data collection for Chapter 2. Refer to section 1.2.2 for detailed sample collection and preservation procedure.

2.2.3 Sample processing and data analysis

Dissections

During the dissections outlined in section 1.2.3, tubeworms collected in 2008 were checked for the presence of spermatozeugmata (Figure 17), sperm bundles, in the vestimental fold. Proportions of females with spermatozeugmata present were calculated and plotted for high and low flux habitat. Reproductive condition of each tubeworm was also rated on a scale from 0-3, a description for which can be found in Table 1. This scale was later collapsed into two categories: reproductively immature and reproductively mature (Table 12). Proportions of reproductively mature males and females in each sample were plotted for high and low flux habitat.

Figure 17. In-situ photograph of high flux *Ridgeia piscesae* tubeworms with spermatozeugmata, sperm bundles, caught in their branchial plumes. Arrows point to sperm bundles. Distance across photo is approximately 50 cm.

Table 12. Description of the reproductive condition rating scale including the collapse of the scale into reproductive maturity.

Reproductive condition category	Physical description	Reproductively mature/ immature (I/M)
0	No gametes visible in gonad	I
1	Few gametes visible in gonad tubules, egg and sperm sacs empty	I
2	Gonad tubules, egg and sperm sacs moderately full of gametes	M
3	Gonad tubules, egg and sperm sacs packed full of gametes	M

Histology

Samples collected in 2009 on Axial Volcano were used for histology. Three males and three females were used from each of three samples: High, Moderate 2, and Low. Refer to section 1.2.3 for the complete histological procedure.

To estimate the total volume of gonad for each tubeworm, the gonad cross-sectional areas (Figure 18) at 0%, 30%, and 60% of trunk length were each multiplied by one third of the trunk length and the resulting values were summed. Averages and standard deviations of gonad volume (cm^3) were calculated and plotted using three males and three females from each of three samples.

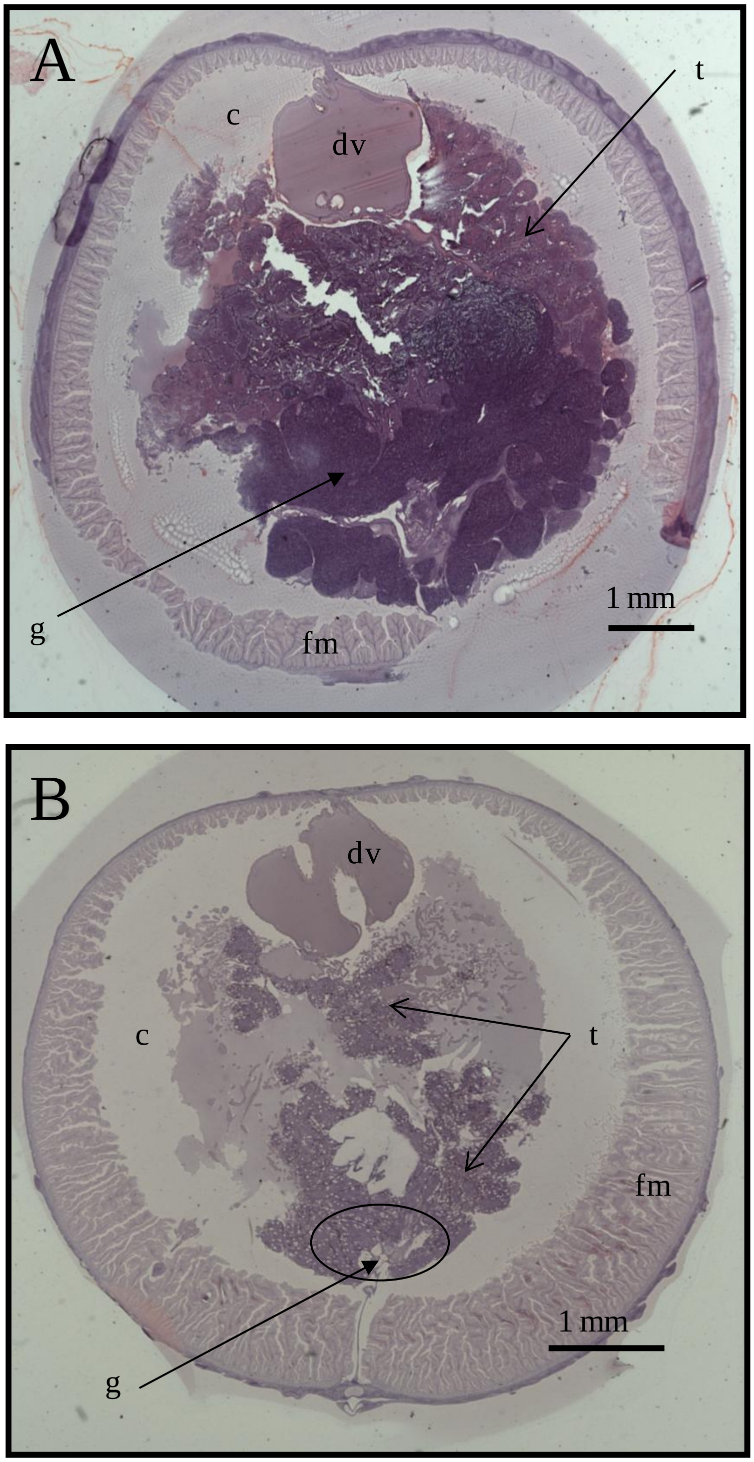

Figure 18. Cross-sections through *Ridgeia piscesae* anterior trunk region; gonad (g), trophosome (t), dorsal blood vessel (dv), coelom (c), feather muscle (fm).

A. High flux habitat
B. Low flux habitat

Oocyte cross sectional area (Figure 19) was measured using Image J software calibrated using a stage micrometer. Only those oocytes within the ascending gonad tubule and sectioned through the nucleus were measured. Oocyte cross-sectional area was measured instead of oocyte diameter because the irregular shape of tightly packed oocytes would cause inconsistency in measurement. Average oocyte cross-sectional area was calculated for each of three females from each site and these values were used to calculate averages and standard deviations for each site.

Figure 19. Cross-section through gonad in trunk region of *Ridgeia piscesae* female

Cross section shows mature primary oocytes (mo) inside the ascending gonoduct coelom (gdc) and developing primary oocytes (do) inside the descending gonocoel coelom (gc) as well as the dorsal blood vessel (dv) and trophosome tissue (t). Germinal epithelium is located beyond the bottom right corner of the image

To estimate the number of primary oocytes in the ascending tubule of the gonad for each female (Figure 19), I counted the number of oocytes sectioned through the nucleus at 0%, 30%, and 60% of trunk length and multiplied each by the following: [trunk length (mm) ÷ 0.1 (mm) ÷ 3] to get the number of eggs in each of three body sections. I then summed the number of eggs in each of the three body sections to obtain an estimate of the total number of oocytes in the ascending tubules of the gonad for each female. The trunk length was divided into 0.1 mm increments to account for restrictions in vertical packing of oocytes that average 80-120 μm in maximum diameter. Averages and standard deviations were calculated and graphed for three females from each of three sites.

At 0%, 30%, and 60% of trunk length, male tubeworms were examined for the presence of sperm in four development categories, pictures and descriptions for which can be found in Figure 20.

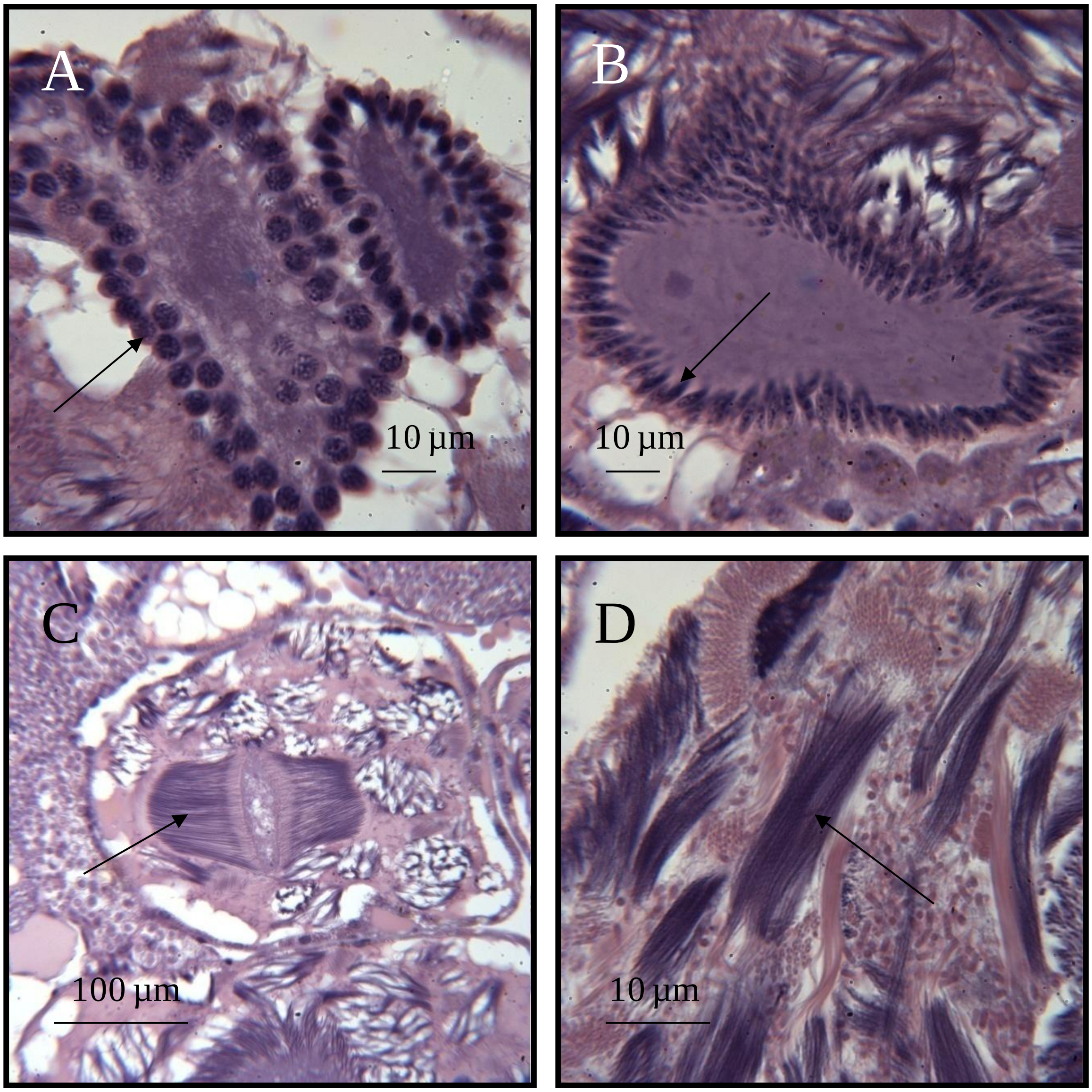

Figure 20.Cross-sections of *Ridgeia piscesae* through gonad in anterior trunk region depicting sperm development categories.

Arrows point to sperm nuclei.

A) category 0 – nuclei surrounding cytophore are spherical
B) category 1 – nuclei surrounding cytophore are elliptical
C) category 2 – nuclei surrounding cytophore are elongate and spiral-shaped
D) category 3 – bundles of sperm have broken away from cytophore

Recruitment

Samples collected in 2008 from Endeavour were used to quantify recruit length and density. In this study, the term ‘recruit’ refers to any *Ridgeia piscesae* individual that settled onto the collected adult tubes and survived to the time of collection. Individuals that subsequently settled onto recruit tubes were not counted, as this would confound density estimates. Because of mortality between settlement and collection, recruitment estimates are lower than larval supply and settlement estimates (Keough and Downes 1982); I did not quantify larval supply or settlement.

During the dissections outlined in section 1.2.3, all recruits attached to each adult tubeworm (Figure 21) were counted and measured for tube length. Little is known about the relative growth rates of *Ridgeia piscesae* juveniles in variable hydrothermal habitats, but recruits up to 2 mm in length were classified as new recruits, assuming most recent settlement. All recruits greater than 2 mm (3-293 mm) in length were classified as old recruits as there is currently no method to determine juvenile age, and growth rates in different habitats likely diverge very shortly after settlement and metamorphosis from feeding larva to symbiotic juvenile. Histograms of the relative proportions of juveniles at each length in all high flux and all low flux samples were constructed.

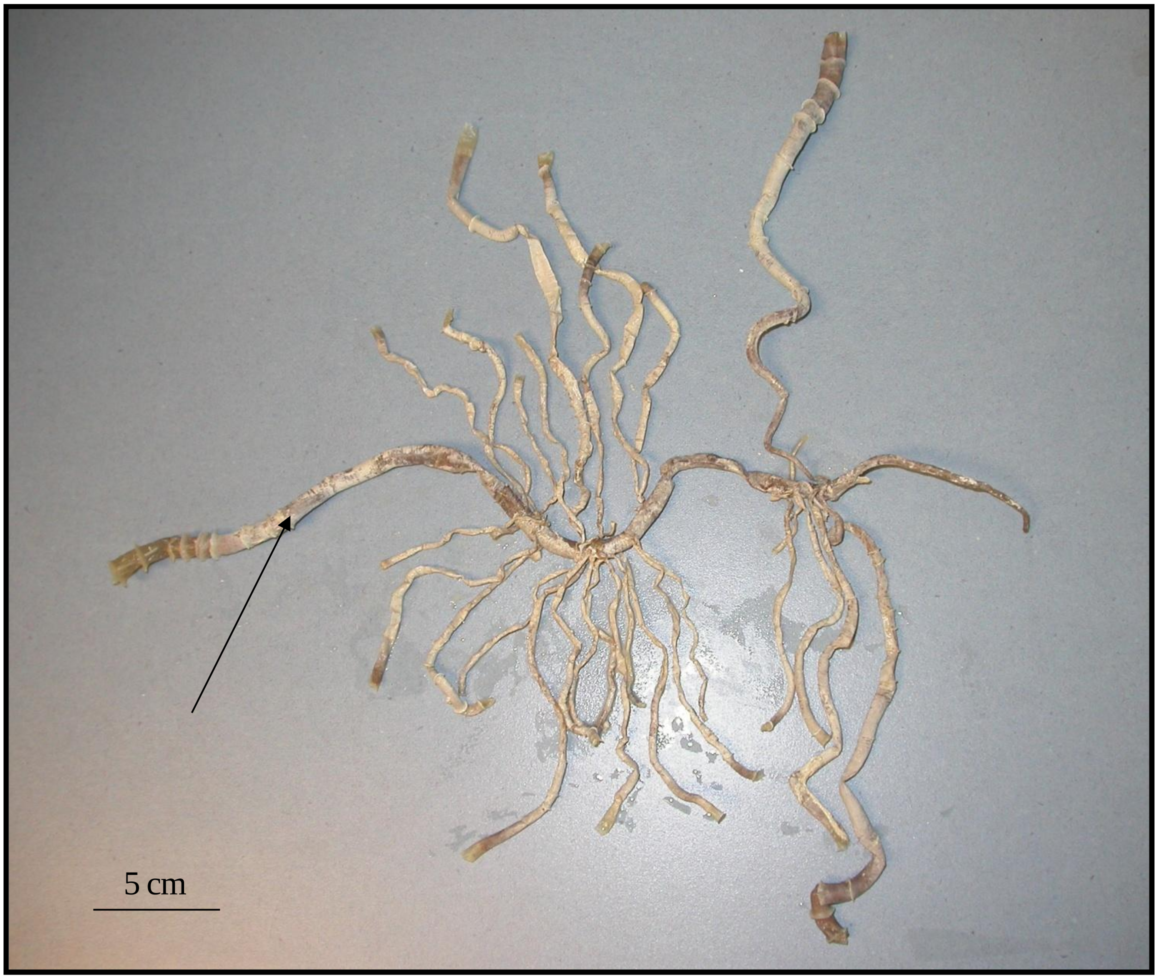

Figure 21. Adult *Ridgeia piscesae* tubeworm (arrow) with juvenile *R. piscesae* recruited directly onto the adult tube.

Recruit density was measured by dividing the total number of recruits in one sample by the summed adult tube surface area (cm^2) for that same sample. Adult tube surface area was determined for each adult using the following equation for the surface area of a cylinder:

$$2\pi r^2 + 2\pi rh$$

Where r = ½ anterior tube diameter (cm)

and h = tube length (cm)

Surface areas for all adults in each sample were summed to give the total surface area available for recruitment in each sample. Densities of both new and old recruits were plotted by site and by flux type.

Predictor variable

Morphological variables visible, and likely measureable, in video imagery include anterior tube diameter and obturaculum length. Searching for a suitable variable to predict reproductive condition from video imagery, I used logistic regression to show relationships between the independent variables, anterior tube diameter and obturaculum length, and the dependent variable, proportion reproductive for each of the 2008 samples. I used average values of continuous variables for each sample to avoid pseudoreplication and a quasi-binomial error structure to account for overdispersion in the models.

Statistical packages

SPSS 13.0 for Windows Student version (SPSS Inc. 1989-2004) was used to perform Wilcoxon rank-sum tests for all pair-wise comparisons because the data were not normally distributed. In analyses where all high flux and all low flux samples were compared, sample averages were counted as one data point to avoid pseudoreplication. However, when comparing only one sample to another, all data points were used, as the use of sample averages was not possible. Standard deviations around averaged values were still included in graphical representations to give an idea of the within-sample variability.

Logistic regression was performed using the statistical package R (The R Foundation for Statistical Computing, 2007).

2.3 Results

2.3.1 General qualitative characteristics of the gonad

In most high flux tubeworms, the gonad was cream-coloured, took up a large proportion of the trunk area, and was full of eggs or sperm. In most low flux tubeworms, the gonad took up only a small proportion of the trunk area. However, in some low flux tubeworms, the top 1-2 cm of the gonad was large and full of eggs or sperm, while the remaining gonad was small and empty. In the low flux tubeworms, the gonad was frequently difficult to find during dissection and was stained green, likely a result of metal deposits inside the tubeworm trunk.

2.3.2 Reproductive condition

Proportion reproductive

High flux samples had a significantly higher proportion of reproductive individuals than low flux samples when all males were pooled, all females were pooled, and all worms were pooled together (Wilcoxon rank-sum tests, $p<0.001$,0.05, and 0.05, respectively) (Figure 22). In high flux samples, the proportion of reproductive males and females was greater than 0.87 for all sites but CB7, where it was 0.60 for males and 0.20 for females. In low flux samples, the proportion of both reproductive males and reproductive females was 0 for sites SM1, GR2, and MO8. The proportion of reproductive males was similar to that of reproductive females in all samples except for in the low flux sample at site CB6. Here, the proportion of reproductive females was twice the proportion of reproductive males.

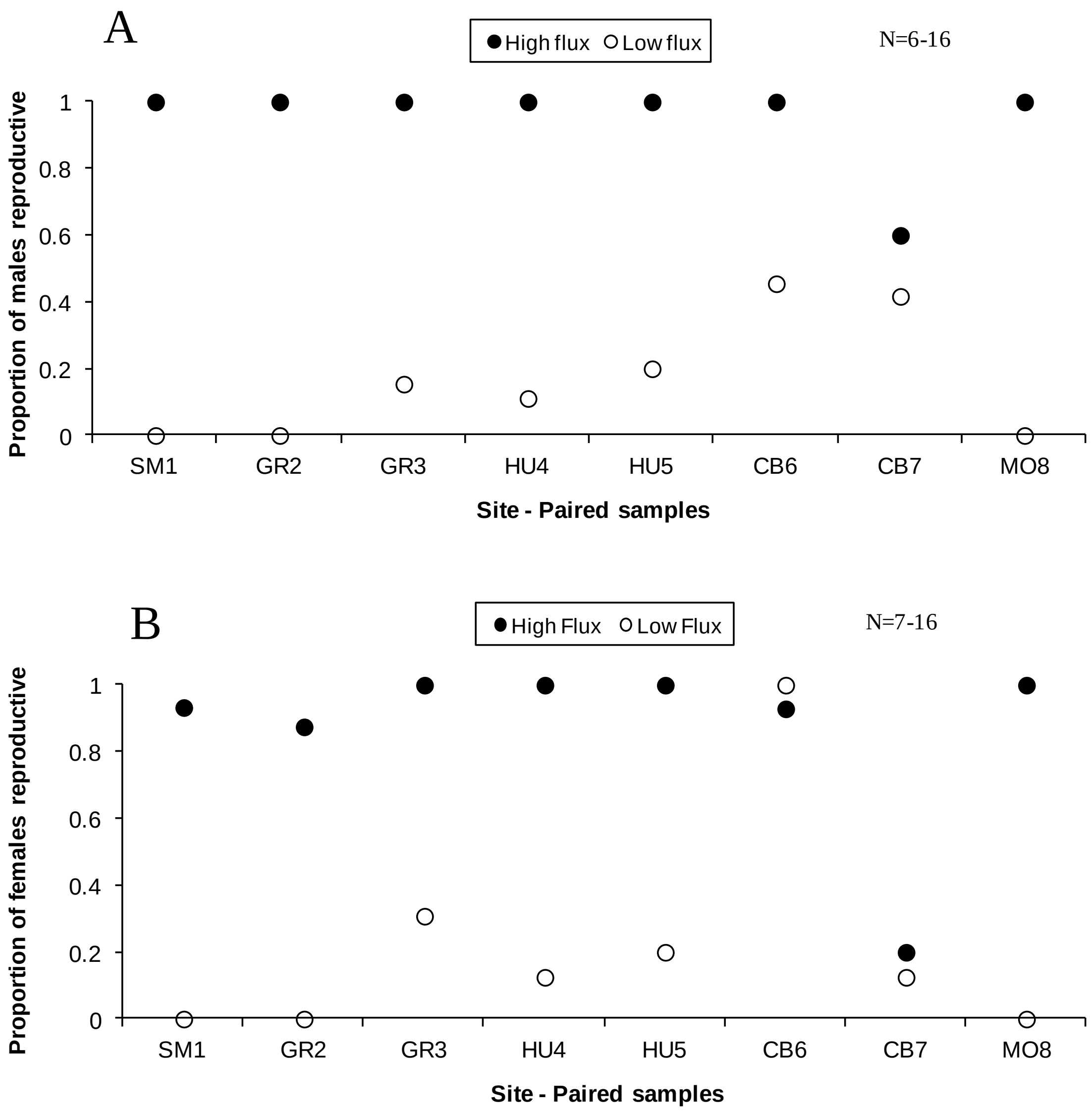

Figure 22.Proportion of tubeworms in a reproductive state in each high flux (black) and low flux (open) sample.

All samples taken from Endeavour. Sample sizes are unequal and N ranges from 7-16 for individual samples.

A. Male tubeworms
B. Female tubeworms

Sperm transfer to females

Figure 17 depicts spermatozeugmata caught in *Ridgeia piscesae* branchial plumes during transfer from male to female. Although not clearly visible in Figure 17, video imagery showed spermatozeugmata attached to male gonopores, each by one long, thin filament. There was a significantly higher proportion of females with spermatozeugmata in the vestimental fold in high flux samples than in low flux (Wilcoxon rank-sum test, $p<0.005$) (Figure 23). All high flux samples had at least some females with sperm in the vestimental fold. Among the low flux samples, five of eight had no females with sperm bundles in the vestimental fold. Overall, 36.9% of high flux females and 36.1% of high flux males held spermatozeugmata compared with only 5.2% of low flux females and 0% of low flux males.

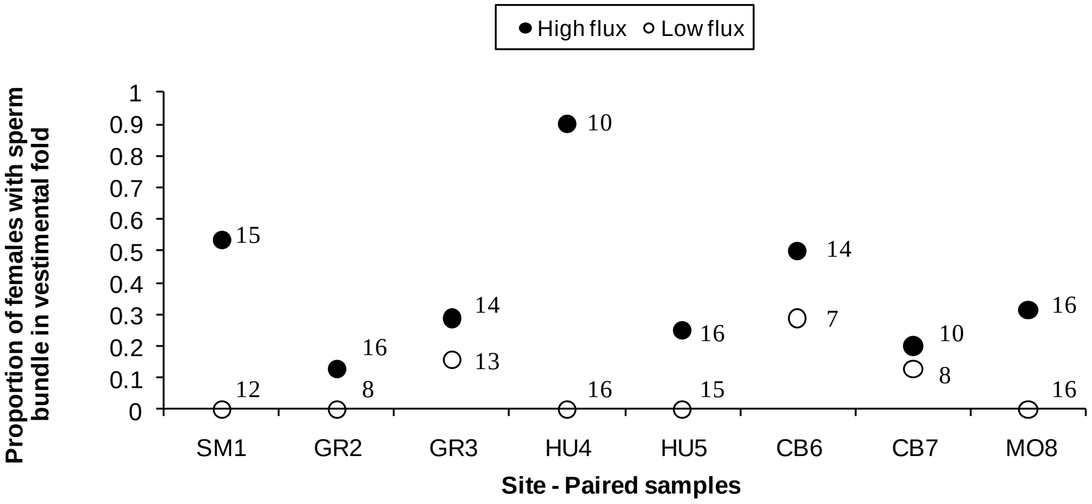

Figure 23. Proportion of females with sperm bundles inside the vestimental fold* in each high flux (black) and low flux (open) sample.

Sample size is beside each data point. *At the time of dissection.

Gonad volume

Representative photographs of the gonad in tubeworms from high flux and low flux habitats can be seen in Figure 18. Tubeworms from high flux samples from Axial had a significantly higher volume of gonad than those from moderate ($p<0.005$) or low

($p<0.005$) flux samples (Wilcoxon rank-sum test on pooled high, moderate, and low flux samples) (Figure 24). There was no significant difference in gonad volume beween tubeworms from moderate and low flux samples. There was no significant difference in gonad volume between male and female tubeworms. However, the average for high flux males was three times that of high flux females. If sample size were increased there would likely be a significant difference, especially if more high flux samples were included in the analysis.

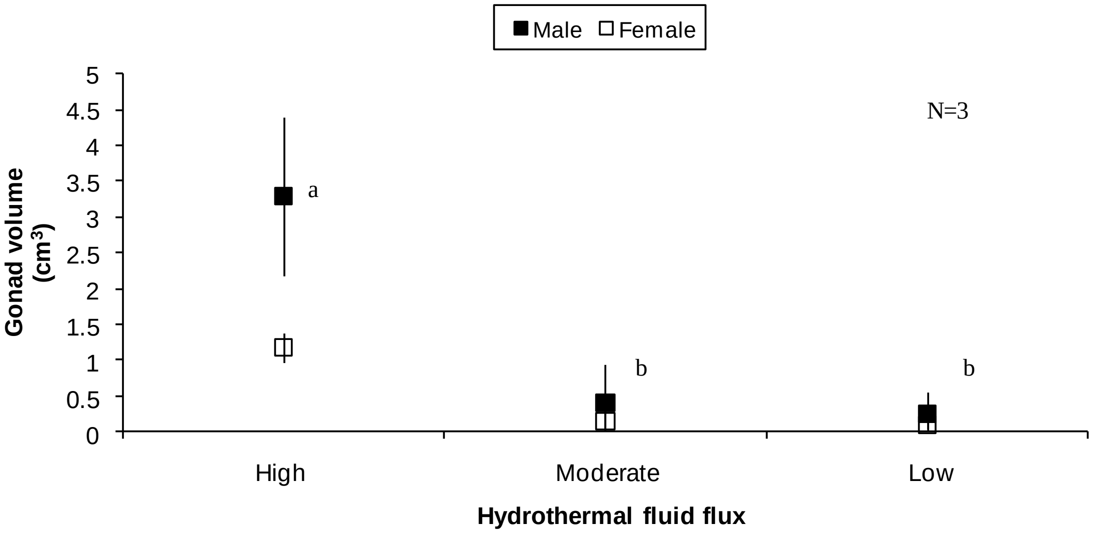

Figure 24. Average gonad volume (cm^3) for male (black) and female (open) *Ridgeia piscesae* in high (Endeavour), moderate (Axial), and low (Axial) hydrothermal fluid flux rates

Error bars represent standard deviation. Letters a and b show where differences among samples lie (pairwise Wilcoxon rank-sum tests)

Oocytes

Figure 19 is a representative photo of primary oocytes in one of the two ascending tubules of the female gonad; there were no consistent differences between the paired tubules within females. Developing oocytes in one of the two descending tubules are also visible. In areas where oocytes were not tightly packed, they were oval in shape with an off-center, circular nucleus and nucleolus. In areas of tight packing, the oocytes were compressed into irregular shapes. Oocytes were filled with granules that resembled lipid droplets. There was no significant difference in the average total number of oocytes in the ascending tubule of the gonad among female tubeworms from high, moderate, and low flux habitats (Figure 25). However, with larger sample sizes, the average total number of oocytes in high flux females would likely be significantly higher, as the average is three times greater than that in moderate and low flux samples. There was no significant difference in the average cross-sectional area of oocytes in the ascending gonad tubule among female tubeworms from high, moderate, and low flux habitats (Figure 26). Continuous development of oocytes was evident, as oocytes of increasing size were seen radiating away from the germinal epithelium.

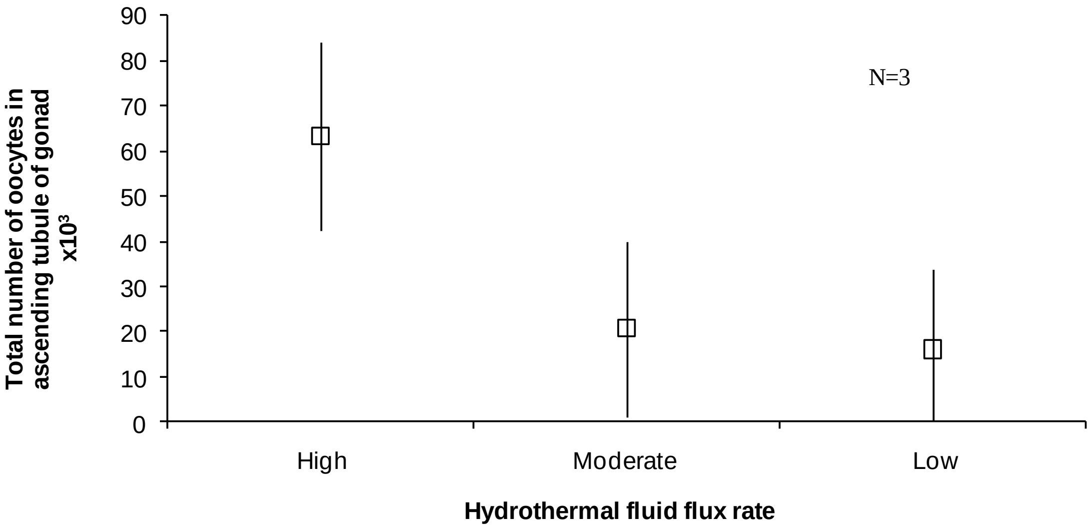

Figure 25. Average total number of oocytes in both ascending gonoducts of gonad in female *Ridgeia piscesae* from high (Endeavour), moderate (Axial) and low (Axial) flux habitat.

Error bars represent standard deviation.

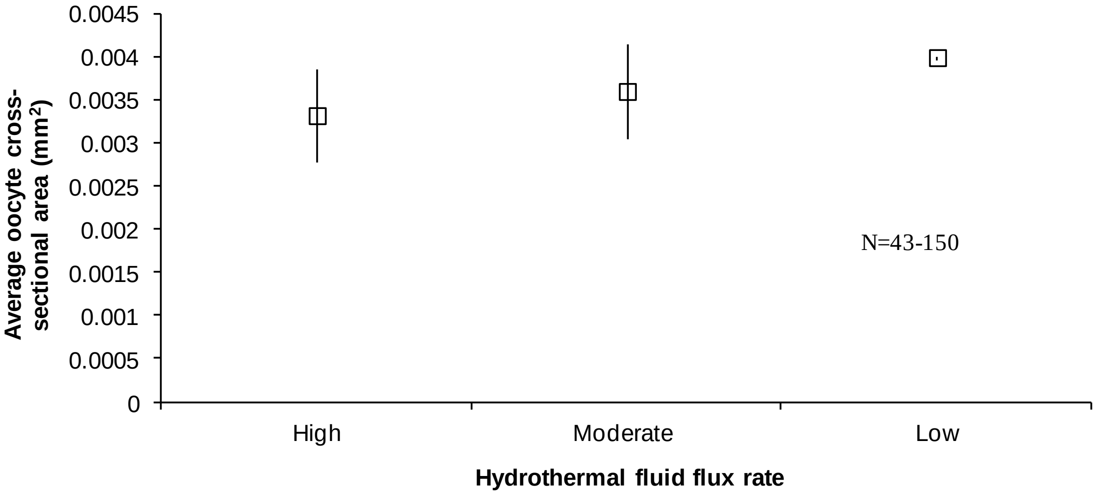

Figure 26. Average cross-sectional area of primary oocytes sectioned through the nucleus in ascending gonad tubule of female *Ridgeia piscesae* from high (Endeavour), moderate (Axial), and low (Axial) flux habitat.

Error bars represent standard deviation. N=150 (high), 43 (moderate), and 43 (low)

Sperm development

Representative photos of sperm development categories can be seen in Figure 20. In sections from the top of the male gonad, within the top ten percent of the trunk length, only sperm in development category 3 were present (Table 13). In sections from the bottom of the male gonad, taken at sixty percent trunk length, six of eight worms had sperm in categories 0-2 present, but not category 3. Sections from the middle of the gonad, taken at thirty percent trunk length, displayed a variation of these two combinations, but category 3 sperm were never found with category 0-2 sperm. There was no difference among tubeworms from high, medium and low flux samples in the spatial distribution of sperm development stage within the trunk.

Table 13. Sperm development categories present in cross-sections taken at 10% (top), 30% (middle), and 60% (bottom) of trunk length in male *Ridgeia piscesae* from high, moderate, and low flux habitat at Endeavour (high) and Axial (moderate and low).

NG – no gonad visible
category 0 - nuclei surrounding cytophore are spherical
category 1 – nuclei surrounding cytophore are elliptical
category 2 – nuclei surrounding cytophore are elongate and spiral-shaped
category 3 - bundles of sperm have broken away from cytophore

Site	Fluid Flux Rate	Worm	Sperm development categories present (0-3)		
			10%	30%	60%
Hulk	High	1	3	0-2	0-2
		2	3	0-2	0-2
		3	3	0-2	0-2
Marker 33	Moderate	1	NG	-	-
		2	3	0-2	NG
		3	3	3	3
Marker 33	Low	1	3	3	0-2
		2	3	3	0-2
		3	3	0-2	0-2

2.3.3 Juvenile Recruitment

Figure 21 is a representative photo of *Ridgeia piscesae* juvenile recruits on adult *R. piscesae* tubes. There was no significant difference in the density (individuals/cm^2) of new (0-2 mm) or older (3-293 mm) recruits among high and low flux samples (Wilcoxon rank-sum test on pooled high and pooled low flux samples) (Figure 27). However, six of eight low flux samples had no new recruits compared with only three of eight high flux samples. Only two samples had no recruits at all, both CB low flux samples. The highest densities of new recruits occurred at SM1 in both high and low flux samples, GR3 in the high flux sample, and CB6 in the high flux sample. The density of older recruits was significantly higher than the density of new recruits for both high flux and low flux samples (p<0.01 and 0.05 for high and low, respectively).

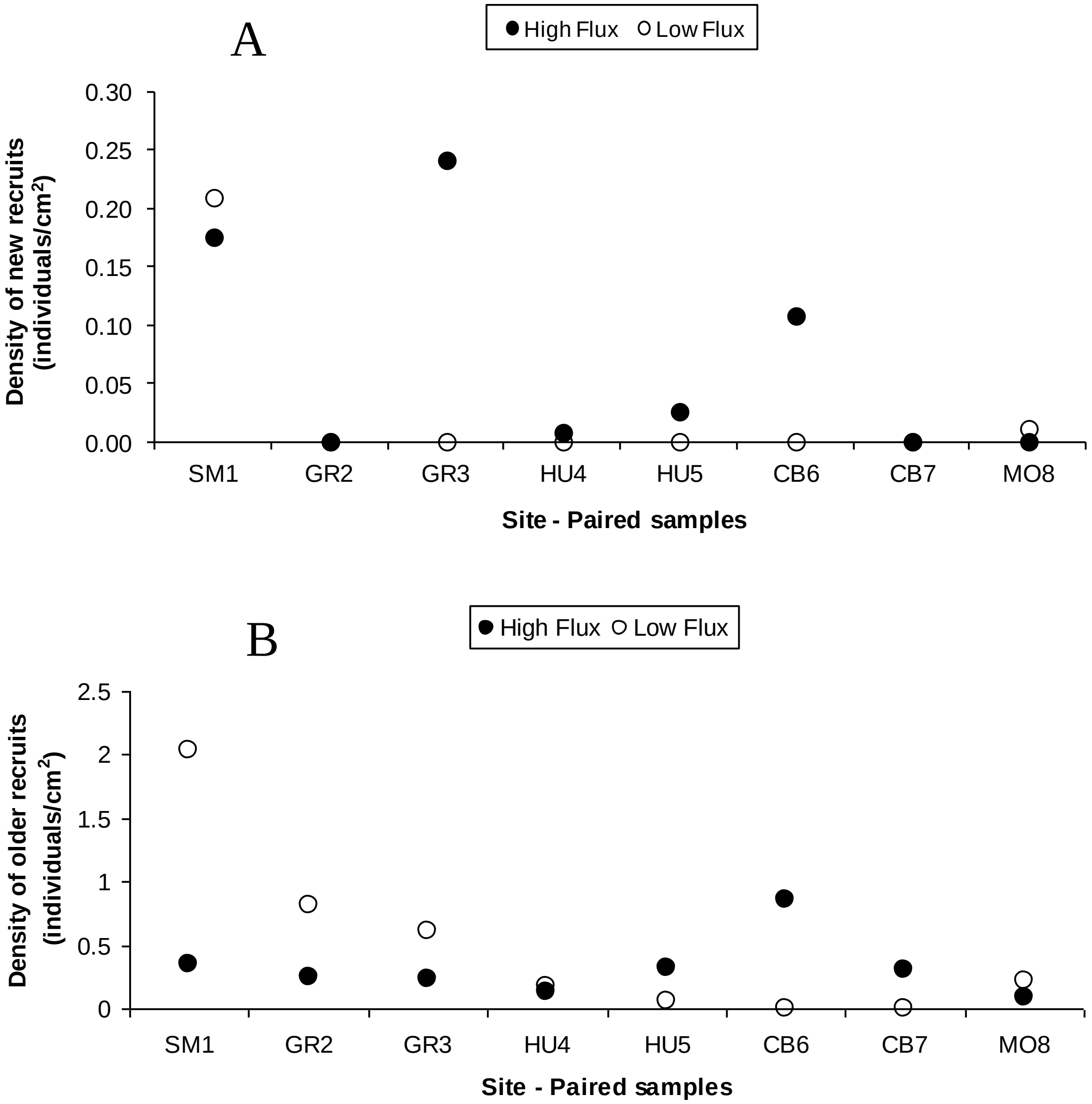

Figure 27. Density (individuals/cm^2 adult tube surface area) of recruits onto adult tubes in each sample calculated using sum of surface area for all adult tubes.

A. New recruits (0-2 mm)
B. Older recruits (2-x mm, where x is the maximum recruit size in a sample)

In the 2009 Endeavour samples, over smaller juvenile lengths, the high flux habitat showed less variability in the proportion of juveniles in each length class from 0-5 mm than did the low flux habitat (Figure 28). In the high flux habitat, there were high proportions of juveniles in all length classes from 0-5 mm. In the low flux habitat, proportions were low in the 1 mm length class, and increased to a maximum in the 5 mm length class. In both high and low flux habitat, length classes from 6-20 mm comprised smaller proportions of the pool of juveniles under 20 mm in length. When juveniles of all lengths from all 2009 Endeavour samples were combined, the majority of juveniles were in the 0-25 mm length class for both high and low flux habitat (Figure 29). The low flux habitat had slightly higher proportions of juveniles than high flux in the length classes above 25 mm. The longest juveniles in high flux habitat were in the 175-200 mm length class (Figure 29A), while low flux habitat had juveniles reached into the 300-325 mm length class (Figure 29B).

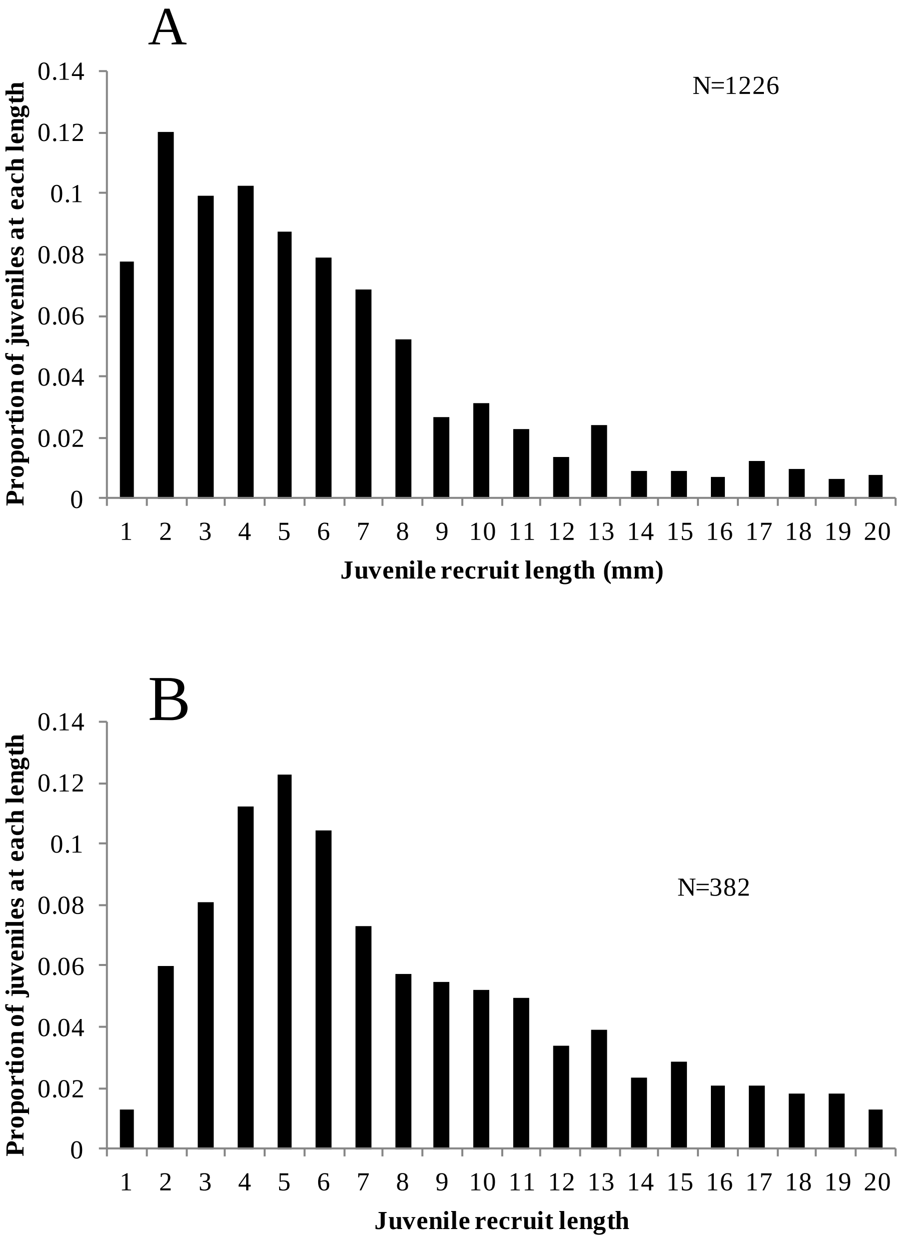

Figure 28. Histograms of the proportion of juveniles in 1 mm size intervals from 0-20 mm.

A. All high flux samples combined
B. All low flux samples combined

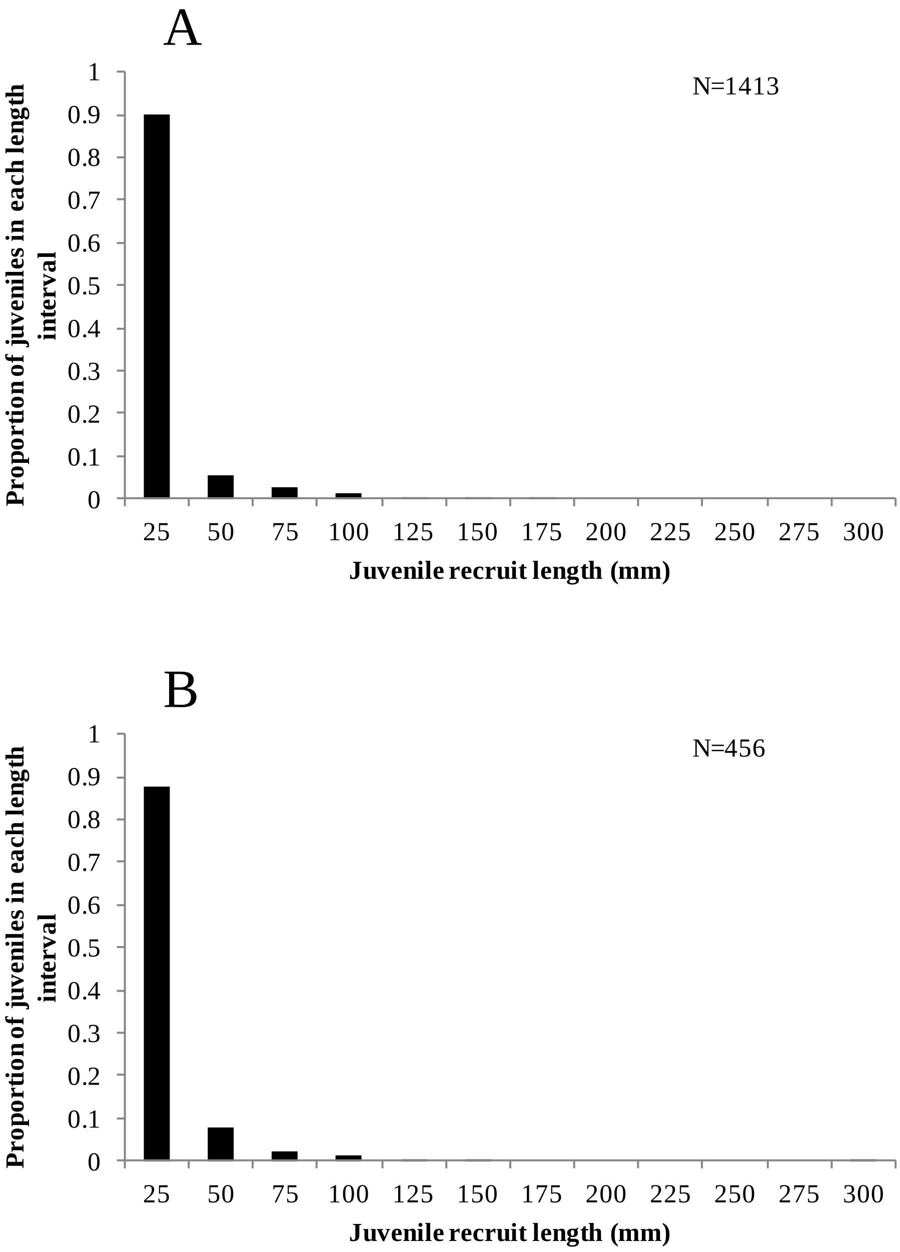

Figure 29. Histograms of the proportion of juveniles in 25 mm length intervals

A. All high flux samples combined
B. All low flux samples combined

2.3.4 Predictor variable for reproductive condition

There was a logistic relationship between proportion reproductive and average anterior tube diameter when high and low flux samples were plotted together (Figures 30 and 31). At anterior tube diameters (ATD) of 4mm and below, worms were not reproductive, while between 4.5 and 6 mm there was some variability around a linear increase in proportion reproductive. When ATD was greater than 6 mm, the proportion of reproductive individuals in any sample was greater than 0.9. The regression equation was:

$$\mathrm{Logit}(p) = -10.99 + 1.99 * \mathrm{ATD}$$

After exponentiation of the parameter estimates, the relationship between ATD and reproductive maturity was that the likelihood of being mature increases by a factor of 7.32 (95%C.I.=4.87-12.02, n=16) with every 1 mm increase in ATD. I also tested obturacular length for predictive ability, but there was no consistent relationship with ATD.

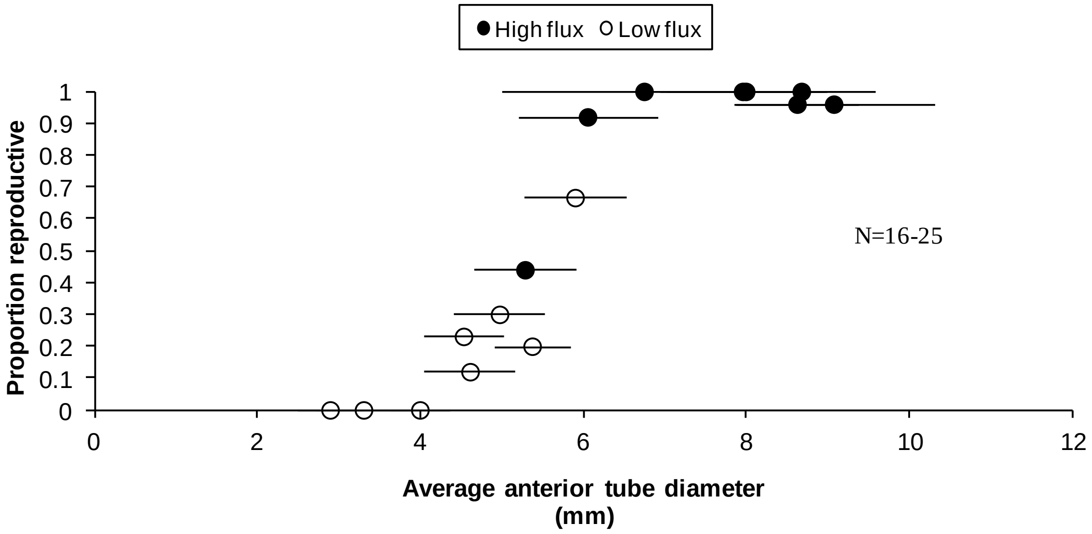

Figure 30. Relationship between the proportion of reproductive individuals and average anterior tube diameter (mm) using average values from tubeworms in high flux (black) and low flux (open) samples.

N ranges from 18-26 for each sample. Error bars represent standard deviation.

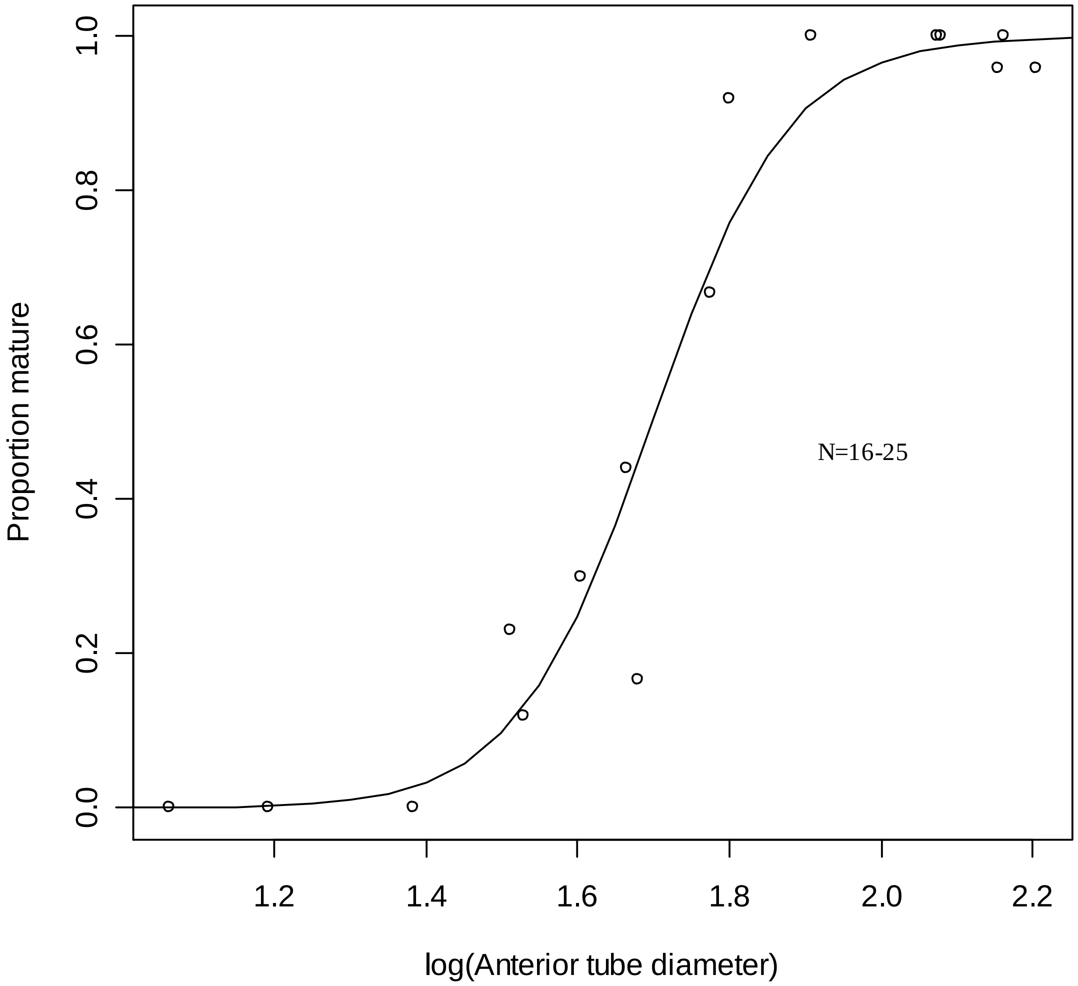

Figure 31. Non-linear regression of proportion reproductive on log average anterior tube diameter using pooled average values from tubeworms in high flux and low flux samples.

N ranges from 18-26 for each sample.

2.4 Discussion

2.4.1 Reproductive condition

Of the five parameters used to determine *Ridgeia piscesae* reproductive condition, four showed notable differences between high flux and low flux environments. First, there was a higher proportion of reproductively mature tubeworms in high flux habitat, and males and females were equally (nearly) reproductive within each sample. A positive relationship between food quantity/quality and reproductive output exists for different marine invertebrates, spanning numerous taxa (Ramirez Llodra 2002) and can be expanded to *R. piscesae* if dissolved sulphide species are considered as food. With more sulphide available for carbon fixation in the high flux environment, growth occurs more rapidly (Tunnicliffe et al. 1997; Urcuyo et al. 2007), and more individuals attain mature reproductive state. The sex ratio in *R. piscesae* is 1:1 (Urcuyo *et al*. 2003) as is the case for many vestimentiferans (Bright and Lallier 2010). To maximize reproductive success, there is usually co-ordination in the timing of reproductive maturity of males and females via environmental, internal or social cues (Ims 1990). A timed reproductive cycle is unknown in *R. piscesae*, but an equal ratio of reproductively mature males and females within populations increases the chances that when one individual is ready to reproduce, there is another ready to supply the gametes for fertilization. Coordination in reproductive maturity between populations would not be as important, as mating only occurs between closely spaced individuals from the same population.

There was a higher percentage of females with spermatozeugmata in their vestimental fold in high flux than in low. An increase in females with spermatozeugmata indicates an increase in the success of sperm transfer in high flux habitat. The proportion of females with spermatozeugmata did not reflect the proportion of reproductive males so this is not likely an artifact of the higher proportion of reproductive individuals in the high flux habitat. One possible factor in the success of sperm transfer is the proximity of individuals. Since the *Ridgeia piscesae* sperm bundle does not “swim”, unlike that of *Riftia pachyptila* (Cary *et al*. 1989), individuals must be tightly aggregated for sperm transfer to occur. Although not quantified, density of individuals was greater in high flux habitat than in low (personal observation). In a study with acorn barnacles, *Semibalanus balanoides,* which also use direct sperm transfer via an elongate penis, Hoch (2008) found that the rate of fertilization decreased as neighbor distance increased. During video observation, we saw spawned spermatozeugmata, each still attached to the male gonopores via a long thin filament, waving in the turbulent fluid flow; our study is the first known observation of this behavior. This method of “fishing” for female branchial plumes is likely supported by increased turbulence, as seen in high flux environments, and likely further increases the success of sperm transfer in high flux habitat. There did not appear to be a minimum age for capture of spermatozeugmata; in populations where mature males were present, many immature females had spermatozeugmata in their vestimental folds. It is unknown whether females can store sperm before maturity or the period of time for which sperm are viable, but sperm storage would allow for immediate fertilization once females reached maturity.

Another likely explanation for decreased sperm transfer in low flux habitat is decreased branchial plume condition (Chapter 1, 1.4.2). Sperm transfer occurs by entanglement of the spermatozeugmata in the female branchial plume and subsequent transfer to the gonopores (Southward and Coates 1989; MacDonald et al. 2002), a process that may be inhibited by the shortening of branchial filaments by predators. However, regardless of apparent sperm transfer, Hilario *et al.* (2005) reported generally high rates of fertilization across the vestimentiferans, ranging from 40-91% of spawned oocytes fertilized, in *Riftia pachyptila, Tevnia jerichonana,* and *Lamellibrachia lumesi*; they did not determine *Ridgeia piscesae* fertilization rates. *R. piscesae,* along with many other vestimentiferan species, is capable of sperm storage (Hilario *et al.* 2005), which may buffer low fertilization rates in areas where sperm transfer is limited. Sperm storage occurs in many marine invertebrate species *(Brante et al*. 2011) and is an important strategy in the hydrothermal vent environment, where gametogenic cues and spawning synchrony are limited (Tyler and Young 1999).

There was a higher volume of gonad in high flux tubeworms than there was in moderate and low flux tubeworms. Larger gonad volume likely has an additive effect with increased number of reproductive individuals and increased sperm transfer to increase reproductive output in high flux areas. There was a higher volume of gonad in males than in females, although this difference was not significant, likely because of small sample sizes. If the difference between males and females were significant, it would indicate that a larger volume of sperm relative to eggs may be necessary to counteract loss during sperm transfer. However it is unlikely that males would require more energy to produce a larger volume of gonad, as eggs, being full of lipid droplets and much larger than sperm, are more energetically costly to produce (Trivers 1972). In the female gonad, the average number of primary oocytes in the ascending tubules of the gonad, on their way to being spawned, was greater in high flux than in low. This difference was not significant, but I only counted a fraction of the eggs in each female. The high flux female gonad was packed full of eggs, while in low flux, eggs were loosely aggregated within the gonad (personal observation). Given that the high flux female gonad is significantly larger, and eggs are packed more closely, it is likely that fecundity estimates, based on total number of eggs per female, would be significantly greater in high flux females. In a study with the hydrothermal vent limpet, *Lepetodrilus fucensis*, Kelly and Metaxas (2007) found that reproductive output, as measured by fecundity, was maximized in vigorous flow environments in comparison with lower flow environments.

I found no difference in the cross-sectional area of oocytes in the ascending tubule of the female gonad between tubeworms from high and low flux habitat. For the most part, oocyte size is conserved among invertebrate taxa, and would not be affected by adaptive pressure from the surrounding environment (Van Dover *et al*. 1985). However, Kelly and Metaxas (2007) did find significant differences in egg sizes among *Lepetodrilus fucensis* from environments with differing hydrothermal fluid flow. *Ridgeia piscesae* have a non-feeding, lecithotrophic larval development and oocytes are typically 80-100 µm in diameter (Young *et al*. 1996). Oocytes are somewhat on the small side for this developmental strategy (Young et al. 1996; Tyler and Young 1999), as the larval lifespan depends on large yolk supplies within the oocyte (Lucas *et al*. 1979). Regardless, consistent oocyte size means that, once spawned and fertilized, probability of survival should be similar, barring differences in oocyte lipid stores. Consistent oocyte size simplifies fecundity estimates, as there is no need to account for the differences in survival probability that different oocyte sizes would create.

Reproduction is difficult from the perspective of a low flux tubeworm. Not only are there fewer potential mates ready to contribute gametes for reproduction, they are spaced more widely, making successful sperm transfer less likely. If an individual is close enough for sperm transfer, the branchial plume may already be rendered incapable of trapping spermatozeugmata through predation by polychaete scale worms. When sperm transfer does succeed, females have smaller gonads, containing fewer gametes ready for fertilization, resulting in a relatively lower reproductive output overall. It seems that the only adaptation to counter the difficult task of reproduction facing low flux tubeworms is the ability for sperm storage in females that allows them to take full advantage of any sperm that do enter the reproductive tract.

2.4.2 Reproductive Strategy

There are many different reproductive strategies in the hydrothermal vent environment and reproductive strategies seem to be taxonomically constrained (Van Dover *et al*. 1985). However, through adaptation, reproductive strategy can be modified for success in a particular environment (Tyler and Young 1999). A good example is with the hot vent polychaetes *Paralvinella pandorae* and *Paralvinella palmiformis*, where both are in the same genus, but the former displays continuous reproduction while the latter is periodic in reproductive output (McHugh 1989). *Ridgeia piscesae* offers the opportunity to separate environment from taxonomy within one species to see if there is any adaptation in reproductive strategy between habitats of apparently different quality. During dissection of low flux tubeworms, I found that the top two centimeters of gonad was frequently packed full of gametes while the rest was mostly empty. In contrast, high flux tubeworms were always packed full of gametes throughout the length of the gonad, which led me to question whether high flux tubeworms were continuous spawners, while low flux tubeworms were periodic.

I found no support for a difference in reproductive timing. There was no difference in the sperm developmental stages present in high and low flux tubeworms, with all four stages present in each male examined. Also, although not quantified, there appeared to be the full range of oocyte development in all females with oocytes. The presence of all stages of gamete development, both within individual tubeworms and among tubeworms in each sample, indicates continuous production of gametes and, likely, continuous reproductive output. When reproduction is continuous, mechanisms for the separation of mature gametes are needed (McHugh 1987), and these were apparent in the tubeworms in my study. In male tubeworms, I found only the earliest developmental stages in the posterior gonad and only the final developmental stage in the anterior gonad, closest to the gonopore. In female tubeworms, although not quantified, oocyte size obviously increased from the germinal epithelia, at the center of oocyte clusters, to the margins of the oocyte clusters where apparently mature primary oocytes broke free from the clusters (Figure 3). The presence of these mechanisms for separation of mature gametes is further support for continuous reproduction. However, this was a static measurement of a dynamic process, and would not identify seasonal trends. Although difficult to show, seasonal trends have been seen in the bivalve, *Calyptogena kilmeri*, where there was no difference in oocyte size frequency throughout the year, but the proportion of gonad engaged in gametogenesis differed seasonally (Lisin *et al*. 1997). Studies to date show no seasonality in vestimentiferans (Tyler and Young 1999). However, I did find differences in gonad volume between high and low flux, which I interpreted as differences in reproductive condition. It may be that *Ridgeia piscesae* only fully engage the gonad when habitat conditions are optimal, creating some level of periodicity and, again, an increased reproductive output in high flux habitat.

2.4.3 Juvenile recruitment

Ridgeia piscesae is well known for its rapid, widespread recruitment at Northeast Pacific hydrothermal vents. On CoAxial Segment, Juan de Fuca Ridge, only seven months after an eruption in 1996, Tunnicliffe *et al.* (1997) found *R. piscesae* at every new vent site created by the eruption. While recruitment is widespread, it is unknown whether habitat quality has an effect on *R. piscesae* recruitment. I found no significant difference in the overall density of either new (0-2 mm) or older (>2 mm) recruits between high and low flux habitat. However, tubeworms from six of eight low flux sites but only three of eight high flux sites were completely devoid of new recruits. Also, the highest densities of new recruits occurred at site SM1, in both high and low flux samples, and in the high flux samples at sites GR3 and CB6. The higher new recruit densities showed some tendency toward increased recruitment to the superior, high flux, habitat. Smith *et al.* (2000) found similar results with *Bathymodiolus childressi,* with higher recruitment in areas of higher adult physiological condition. In a colonization study using basalt colonization plates, Mullineaux *et al.* (1998) found that vestimentiferans only colonized zones at the East Pacific Rise inhabited by adults. However, since it was a 3-year study, colonization likely reflected survival of recruits rather than initial recruitment patterns. The *R. piscesae* trocophore larvae have limited swimming ability while dispersing, as well as limited mobility once settled in the sediment (Young *et al*. 1996), so it is unlikely that larvae "select" superior habitat. It is more likely that the larvae settle at random and survive only in areas suitable for growth and survival.

Density of older recruits was fairly even across high flux sites, which suggests that recruit survival may be density dependent, at least in the high flux habitat. In contrast, density of older recruits was variable across low flux sites. Older recruits were found in all three of the high flux samples, and four of the six low flux samples, that had no new recruits. The presence of older recruits in these samples suggests that, even though there had been no recent recruitment, the habitats were able to support recruitment in the past. There were some sites where both new and old recruit densities were high relative to other samples, but others where new and old recruit densities were contrasting. This variability likely stems from the fact that recruitment and post-recruitment survival depend on a myriad of biotic and abiotic factors (Caley *et al*. 1996).

New recruits represent those juveniles that have recently settled and have not yet been influenced by conditions in the surrounding habitat. Older recruits have been in their respective habitats for an indeterminate amount of time and have likely adjusted growth rates and other life processes according to the local biotic and abiotic conditions. If the size structure shown in Figure 28 is a representative snapshot of recruit sizes at any time, then there is some evidence for an adjustment in growth rate according to habitat. In the low flux habitat, there was a build-up of juveniles around 5 mm in length, which was not seen in the high flux habitat. It is likely that, upon settlement, all juveniles are growing at the same rate, still relying on embryonic lipid reserves. When metamorphosis occurs and juveniles begin to rely on the reduced sulphide in vent fluid for growth, growth likely slows in the low flux environment because of decreased hydrothermal fluid flux. With decreased growth rate, it would take a longer time for each juvenile to grow beyond 5 mm, thus causing a build up of juveniles at this length. It is unlikely that the peak in abundance of 5 mm juveniles was caused by a recruitment event. Many of the high flux and low flux samples were taken from within meters of each other, and if there had been a recruitment event, it would have been apparent across the high flux sites as well.

Size frequency histograms for all juvenile size classes showed greater proportions of longer juveniles in low flux habitat than in high flux. In adult *Ridgeia piscesae*, tubeworms from low flux habitat have longer tube lengths than those from high flux (Chapter 1), so it is not surprising to find similar differences in the juveniles. Since it likely takes longer for low flux tubeworms to grow a given amount in length, the low flux juveniles are probably much older on average than the high flux juveniles. This is supported by studies with adult *Ridgeia piscesae*, in which tubeworms in the low flux habitat are much older, by an order of magnitude (Tunnicliffe et al. 1997; Urcuyo et al. 2007). Differences in tubeworm growth rates between high and low flux environments mean that, beyond lengths of a few millimeters, it is impossible to use size as a basis for comparing tubeworm age.

Based on trends in the older recruits, this mortality is likely density-dependent. According to Caley *et al.* (1996), in a population where recruitment is density dependent, any initial differences in cohort strength will disappear over time. When length classes of new and older recruits are looked at together, there is high variability in cohort strength over the initial stages, but this variability evens out over the older cohorts. Also, based on Roughgarden *et al.* (1985), *Ridgeia piscesae* likely has a low settlement rate. Roughgarden *et al.* state that in space limited populations with low settlement, all ages are mixed together rather than arranged as a mosaic of cohorts, the condition in populations with high settlement.

2.4.4 Condition measure

The preceding work shows that high and low flux habitats support tubeworms of differing reproductive ability. It is possible to distinguish high from low flux habitat, and thus high from low flux tubeworms in video imagery, as was done for sample selection in this study. However, these are two extremes in a continuum of habitat types and it is difficult to distinguish habitats quantitatively in the middle of this continuum. In order to obtain estimates of the proportion of reproductive to non-reproductive populations, there is a need for some form of fast, non-invasive, universal indicator of reproductive condition.

Video imagery is the primary means by which hydrothermal vent organisms are viewed, and several studies use video survey as a non-invasive technique to characterize hydrothermal vent biodiversity and colonization patterns, among many other variables (Sarrazin et al. 1997). However, most studies to date have examined species presence-absence or individual counts rather than attempting to infer individual or population condition from video surveys. Anterior tube diameter (ATD) is one of the few variables that is consistently visible and measureable in video imagery for *Ridgeia piscesae*. I found that ATD did show some capacity to predict reproductive condition. On average, tubeworms had to reach a minimum ATD of 4 mm before they became reproductive, and beyond an average ATD of 6 mm, at least 90% of individuals in each sample were reproductive, regardless of their host habitat. It appears that the transition to a reproductive state occurred between ATDs of 4 and 6 mm, as there was a rapid increase in the proportion of reproductive individuals in each sample over this interval. A limitation to this model is that no low flux tubeworms over 6 mm ATD, and no high flux tubeworms under 5 mm ATD were examined. Also, *R. piscesae* exists in a continuum of morphotypes, the center of which is not represented in my samples. This model represents a beginning in the development of a universal predictor, but more samples, from all levels in the continuum of morphotypes, need to be assessed before anterior tube diameter can be used as a reliable, universal predictor of reproductive condition.

2.4.5 Conclusions

Overall, high flux habitat was superior to low in the context of reproductive condition and, likely, recruitment in *Ridgeia piscesae*. High flux habitat supported a greater number of reproductive individuals, with increased sperm transfer and larger gonads, which appeared to support increased gamete production. On an individual level, even though high flux tubeworms have increased reproductive output, they have shorter life cycles than low flux individuals (Tunnicliffe et al. 1997; Urcuyo et al. 2007). It may be that high and low flux individuals are similar in lifetime reproductive output, but low flux individuals spread it out over a longer timeframe. Regardless, there is higher turnover in high flux habitat, so even if the lifetime reproductive output of individual tubeworms is similar, the high flux habitat supports many more individuals per unit time, and the reproductive output of the population would be higher.

Although there was initially some evidence of periodic spawning, histological examination showed all populations to be continuous spawners with mechanisms for separating mature gametes from immature. MacDonald *et al. (2002)* suggested that reproductive success would be maximized if individuals were closely aggregated and reproductive maturation was synchronized. In my study, tubeworms in high flux environments were more tightly packed than those in low (personal observation), and, as Macdonald *et al*. saw, in both habitat types maturation was synchronized within aggregations but not always between aggregations. However, aggregation density, or carrying capacity, likely depends on a tradeoff between resource acquisition and reproductive success; tubeworms must maintain enough space to allow access to hydrothermal vent fluid, while packing closely enough to allow for sperm transfer. MacDonald *et al. (2002)* also proposed that the concentration of reproductive activity in *Ridgeia piscesae* may be limited to a few, robust aggregations. While reproductive activity does exist in low flux assemblages, my study agrees with Macdonald *et al.* in that the concentration of reproductive activity occurs in rare, robust, high flux assemblages.

Juvenile recruitment was highly variable, but new juveniles were found in a higher proportion of high flux sites than low. Also, there was some evidence for increased growth rate in the high flux juveniles, which agrees with previous findings for adult tubeworms (Tunnicliffe et al. 1997; Urcuyo et al. 2007). While the exact mechanisms of juvenile recruitment are unknown, the increased number of juveniles likely acts as a mechanism to keep the superior habitat at carrying capacity to maximize reproductive output and population success.

It is evident, from general observation of video imagery, that high flux habitat is much rarer than low; there is a need for quantification of the relative proportions represented by each. In general, mapping studies at hydrothermal vents have focused either on geological and hydrothermal fluid characteristics, or on animal distribution, with little connection between the two. Recently, some studies have tried to bridge this divide by looking at community composition in differing habitat (Sarrazin et al. 1997; Sarrazin et al. 1999; Sarrazin and Juniper 1999) for example. There is a need for more multiple-approach mapping studies of this type. I have presented a conceptual model of increased reproductive potential and increased juvenile recruitment in superior, high flux habitat and proposed a method for the fast, non-invasive identification of highly reproductive populations. While the model needs further investigation, it provides a framework for broadscale, cross-vent mapping of habitat suitability for *Ridgeia piscesae*.

Summary

In the assessment of population connectivity, there are many processes that can be studied to evaluate spatial connections between populations (Cowan 2009 Figure 2). For *Ridgeia piscesae*, as for most marine invertebrates, many of these processes occur in the water column, away from the adult population; this makes a full characterization and model very difficult. My study investigated two processes, potential spawning output and post-settlement abundance and survival. I measured physiological and reproductive condition to estimate the potential for spawning output and to determine whether the highly variable habitat produces individuals of similar condition that make similar contributions to future populations. I then measured juvenile settlement to determine whether juveniles recruit equally to high and low flux local populations. I also assessed the ability of certain external morphological features, visible and possibly measureable in video imagery, to predict the reproductive potential of local populations.

Before spawning, adults must have access to sufficient nutrition for proper growth and maintenance and the production of offspring. In this study I found that, indeed, there are differences in both the physiological condition and the reproductive condition of *Ridgeia piscesae* populations based on the habitat type that the tubeworms occupy. In terms of physiological condition, tubeworms from high flux habitat had higher body weight, anterior tube diameter, total lipid volume, trophosome volume, and branchial plume condition than did those from low flux habitat. In terms of reproductive condition, high flux habitat had a greater number of reproductive individuals, and high flux individuals had increased sperm transfer and larger gonads, which appeared to support increased gamete production. The two extreme morphologies that I studied were not equal in physiological condition or reproductive potential, which makes a strong case for local sources and sinks in the *R. piscesae* metapopulation in the Northeast Pacific Ocean. The high flux habitat type likely produces many more propagules and would have a greater contribution to future populations than low flux. However, this does not mean that the low flux populations have no value to the *R. piscesae* metapopulation. Through dispersal, marginal, or sink, populations can rescue source populations at risk of extinction (Gyllenberg *et al.* 1996) and dispersal can bring alleles selected for in the marginal habitat back to the source habitat (Kawecki 2008), which may make the species, as a whole, better adapted for survival in such a variable environment. Also, from a community perspective, *R piscesae* provides habitat for a number of other hydrothermal vent organisms. While the number of species supported by low flux *R. piscesae* populations is relatively low, it is still important; low flux populations represent a transition between the fauna of hydrothermal vents and that of the deep sea, and there are species in the low flux habitat that aren't found in high flux (Tsurumi and Tunnicliffe 2003).

Although I did not measure the relative proportions of each habitat type, it was apparent, in video imagery and limited video transect surveys, that the high flux habitat and populations were rare in comparison to low flux. As a result, much of the population inhabits marginal habitat, and input to future populations comes from relatively few, highly productive local populations. On an individual level, lifetime reproductive output in high and low flux habitat may be similar, as low flux tubeworms live longer than those in high flux (Tunnicliffe *et al.* 1997; Urcuyo *et al.* 2007). However, at the population level, there is a higher turnover of individuals in high flux habitat, so reproductive output would still be much higher than in low flux. For a more accurate picture of the relative contributions of high and low flux habitat, video transect mapping of the different habitat types is needed. I found that Anterior Tube Diameter (ATD), visible in video imagery, did have some power to predict reproductive maturity. However, this model requires much more development and testing before reliable predictions could be made from video imagery.

In terms of post-settlement abundance, I found that the distribution of new juvenile recruits among populations did show some bias toward higher recruitment in the high flux habitat; however the trend was not significant. Since larval supply to high and low flux pairs within meters of each other should be similar, if this trend does reflect the true conditions of the population, it means that either larvae are able to settle preferentially into superior habitat, which is unlikely based on low motility, or that mortality acts on the larvae before they reach 1 mm, the smallest size that I was able to measure. Regardless, recruitment into low flux habitats is significant while reproductive output is likely low. The unequal ratio of juvenile recruitment to reproductive output further supports source-sink dynamics in the *Ridgeia piscesae* metapopulation.

In the local populations I studied, even though all individuals were of the same morphotype, most populations hosted the full range of development, from new recruit to spawning adult. However, there were some notable exceptions. Two of the eight low flux sites I sampled were completely devoid of both new and older recruits. It is likely that, at the time of sampling, conditions in these two habitats were not suitable for juvenile settlement and survival. Also, there were no adults in three of the samples from low flux habitat and adults comprised less than half of the sampled population in all but one of the other low flux samples. In contrast, in the high flux habitat, all samples but one had an adult component of over 80%. This shortage of adults in the low flux environment, even though I selected the largest worms from each local population, may be because of the growth dynamics of the low flux tubeworms. It may take longer for low flux tubeworms to mature, resulting in a larger proportion of juveniles in the low flux habitat than in high flux. It is unlikely that the shortage of adults is the result of variation in larval supply because, given the proximity of the pairs of high and low flux samples, any variation in would be reflected in the corresponding high flux samples as well.

From the perspective of the metapopulation, low flux habitat is inferior in its ability to support *Ridgeia piscesae* at all stages in the tubeworm's life cycle (Figure 32). Local populations in low flux habitat have lower physiological and reproductive condition, with lower potential reproductive output, as well as lower juvenile recruitment and survival, and the limited numbers of recruits that are supported take much longer to develop into reproductively contributing adults.

Canada's first Marine Protected Area (MPA) the Endeavour Hydrothermal Vents MPA (EHVMPA) was created in 2003 to protect a novel ecosystem that we are only just beginning to understand. With the habitat fragmentation inherent in the hydrothermal environment, many of the species protected by this MPA can likely be classified as metapopulations. When designing MPAs, spatial scale is one of the most important variables to consider, especially when working with metapopulations. My spatial comparisons showed little difference in the measured variables at the vent field scale, as a wide range of fluid flow regimes and morphotypes was represented in each vent field.

This means that the EHVMPA would likely protect not only the full range of *Ridgeia piscesae* morphotypes, but also a high proportion of the habitats important to other species as well. Also, with metapopulation dynamics acting, MPAs can rescue local populations outside of the protected area, in addition to preserving populations within the protected area. In the highly variable hydrothermal vent environment, management also needs to consider time scale when designing MPAs. Hydrothermal vents at the East Pacific Rise persist for only a few decades before flow wanes and animals die off (Vrijenhoek 1997), while some vents on the Juan de Fuca Ridge may be up to 200 years old (Kim and McMurtry 1991). High variability in hydrothermal vent longevity means that an MPA protecting only geographical area may fail in the long term if the hydrothermal vents in that area become extinct. As the field of metapopulation biology develops, the methods of environmental protection need to develop with it. For the hydrothermal vent environment, when source-sink dynamics are shown to be occuring, the relative contributions of each local population should be taken into consideration. I suggest protecting habitat types rather than a particular area of habitat. With *Ridgeia piscesae*, despite differences in the reproductive output of the two habitat types studied, both are important and should be protected. The high flux tubeworms are the major source of reproductive output for the *R. piscesae* metapopulation, and the low flux tubeworms host distinct communities, including species that are not found anywhere else (Tsurumi and Tunnicliffe 2003).

This study showed that the high flux habitat is much more productive for *Ridgeia piscesae*, and that the majority of input for future populations likely comes from local populations in high flux habitat. However, in order to strengthen the results of this study, I suggest that population genetic studies be undertaken with a similar goal in mind: to tease out differences between *R. piscesae* individuals from high flux and low flux habitat. Population genetics are indispensable in the analysis of spatial population structures in the marine environment (ex. Jolly *et al.* 2003). In 2008, Young *et al.* did study the broadscale population genetics to determine range-wide gene flow patterns for *R. piscesae*, but a more fine-scale analysis is required to show differences with respect to habitat and the ultimate importance of each habitat type to future generations.

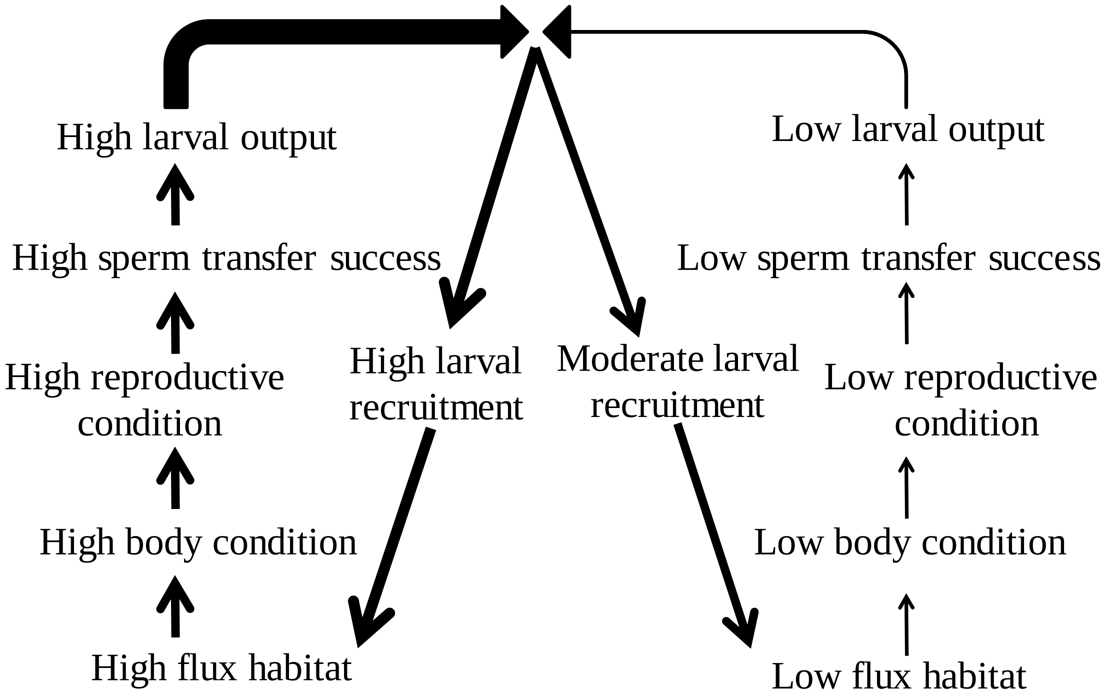

Figure 32. Study summary figure.

References

Andersen AC, Flores JF, Hourdez S (2006) Comparative branchial plume biometry between two extreme ecotypes of the hydrothermal vent tubeworm *Ridgeia piscesae*. Canadian Journal of Zoology 84: 1810-1822

Bates AE, Lee RW, Tunnicliffe V, Lamare MD (2010) Deep-sea hydrothermal vent animals seek cool fluids in a highly variable thermal environment. Nature Communications 1: Article 14 DOI: 10.1038/ncomms1014

Bates AE, Tunnicliffe V, Lee RW (2005) Role of thermal conditions in habitat selection by hydrothermal vent gastropods. Marine Ecology Progress Series 305: 1-15

Bergquist DC, Ward T, Cordes EE, McNelis T, Howlett S, Kosoff R, Hourdez S, Carney R, Fisher CR (2003) Community structure of vestimentiferan-generated habitat islands from Gulf of Mexico cold seeps. Journal of Experimental Marine Biology and Ecology 289: 197-222

Black MB, Lutz RA, Vrijenhoek RC (1994) Gene flow among vestimentiferan tube worm (*Riftia pachyptila*) populations from hydrothermal vents of the eastern Pacific Marine Biology. Marine Biology 120: 33-39

Black MB, Trivedi A, Maas PAY, Lutz RA, Vrijenhoek RC (1998) Population genetics and biogeography of vestimentiferan tube worms. Deep Sea Research Part II: Topical Studies in Oceanography 45: 365-382

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917

Brante A, Fernandez M, Viard Fdr (2011) Microsatellite evidence for sperm storage and multiple paternity in the marine gastropod *Crepidula coquimbensis*. Journal of Experimental Marine Biology and Ecology 396: 83-88

Bright M, Lallier FH (2010) The biology of vestimentiferan tubeworms. Oceanography and Marine Biology 48: 213-266

Bright M, Sorgo A (2003) Ultrastructural reinvestigation of the trophosome in adults of *Riftia pachyptila* (Annelida, Siboglinidae). Invertebrate Biology 122: 347-368

Butterfield DA, Massoth GJ, McDuff RE, Lupton JE, Lilley MD (1990) Geochemistry of hydrothermal fluids from Axial Seamount hydrothermal emissions study vent field, Juan de Fuca Ridge: subseafloor boiling and subsequent fluid-rock interaction. Journal of Geophysical Research 95: 12895-12921

Butterfield DA, McDuff RE, Mottl MJ, Lilley MD, Lupton JE, Massoth G (1994) Gradients in the composition of hydrothermal fluids from the Endeavour segment vent field: Phase separation and brine loss. Journal of Geophysical Research 99: 9561-9583

Caley MJ, Carr MH, Hixon MA, Hughes TP, Jones GP, Menge BA (1996) Recruitment and the local dynamics of open marine populations doi:10.1146/annurev.ecolsys.27.1.477. Annual Review of Ecology and Systematics 27: 477-500

Carney SL, Flores JF, Orobona KM, Butterfield DA, Fisher CR, Schaeffer SW (2007) Environmental differences in hemoglobin gene expression in the hydrothermal vent tubeworm, *Ridgeia piscesae*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 146: 326-337

Cary SC, Felbeck H, Holland ND (1989) Observations on the reproductive biology of the hydrothermal vent tube worm *Riftia pachyptila*. Marine Ecology Progress Series 52: 89-94

Cavanaugh CM, Gardiner SL, Jones ML, Jannasch HW, Waterbury JB (1981) Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: Possible chemoautotrophic symbionts 10.1126/science.213.4505.340. Science 213: 340-342

Childress JJ, C. R. Fisher, J. A. Favuzzi, R. E. Kochelvar, Sanders NK, Alayse AM (1991) Sulfide-driven autotrophic balance in the bacterial symbiont-containing hydrothermal vent tubeworm, *Riftia pachyptila* Jones. Biological Bulletin 180: 135-153

Colaco A, Bettencourt R, Costa V, Lino S, Lopes H, Martins I, Pires L, Prieto C, Santos RS (2011) LabHorta: a controlled aquarium system for monitoring physiological characteristics of the hydrothermal vent mussel Bathymodiolus azoricus. Ices Journal of Marine Science 68: 349-356

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity doi:10.1146/annurev.marine.010908.163757. Annual Review of Marine Science 1: 443-466

Cruz-Rivera E, Hay ME (2000) Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. Ecology 81: 201-219

De Burgh ME (1986) Evidence for a physiological gradient in the vestimentiferan trophosome: size-frequency analysis of bacterial populations and trophosome chemistry. Canadian Journal of Zoology 64: 1095-1103

De Burgh ME, Juniper SK, Singla CL (1989) Bacterial symbiosis in northeast Pacific Vestimentifera: a TEM survey. Marine Biology 101: 97-105.

Di Meo CA, Wilbur AE, Holben WE, Feldman RA, Vrijenhoek RC, Cary SC (2000) Genetic variation among endosymbionts of widely distributed vestimentiferan tubeworms 10.1128/AEM.66.2.651-658.2000. Applied Environmental Microbiology 66: 651-658

Embley RW, Chadwick WW, Jr., Clague D, Stakes D (1999) 1998 Eruption of Axial Volcano: Multibeam anomalies and sea-floor observations. Geophysical Research Letters 26

Felbeck H, Powell MA, Hand SC, Somero GN (1985) Metabolic adaptations of hydrothermal vent animals. Bulletin of the Biological Society of Washington 6: 261-272

Fisher CR, Childress JJ, Arp AJ, Brooks JM, Distel D, Dugan JA, Felbeck H, Fritz LW, Hessler R, Johnson KS, Kennicutt II MC, Lutz RA, Macko SA, Newton A, Powell MA, Somero GN, Soto T (1988) Variation in the hydrothermal vent clam, *Calyptogena magnifica*, at the Rose Garden vent on the Galapagos spreading center. Deep-Sea Research 35: 1811-1831

Freytag JK, Girguis PR, Bergquist DC, Andras JP, Childress JJ, Fisher CR (2001) A paradox resolved: Sulfide acquisition by roots of seep tubeworms sustains net chemoautotrophy. 10.1073/pnas.231589498 Proceedings of the National Academy of Sciences of the United States of America 98 13408-13413

Fullarton JG, Dando PR, Sargent JR, Southward AJ, Southward EC (1995) Fatty acids of hydrothermal vent *Ridgeia piscesae* and inshore bivalves containing symbiotic bacteria. Journal of the Marine Biological Association of the United Kingdom 75: 455-468

Gardiner SL, Jones ML (1985) Ultrastructure of spermiogenesis in the vestimentiferan tube worm *Riftia pachyptila* (Pogonophora: Obturata). Transactions of the American Microscopical Society 104: 19-44

Girguis PR, Childress JJ (2006) Metabolite uptake, stoichiometry and chemoautotrophic function of the hydrothermal vent tubeworm *Riftia pachyptila*: responses to environmental variations in substrate concentrations and temperature 10.1242/jeb.02404. J Exp Biol 209: 3516-3528

Girguis PR, Childress JJ, Freytag JK, Klose K, Stuber R (2002) Effects of metabolite uptake on proton-equivalent elimination by two species of deep-sea vestimentiferan tubeworm, *Riftia pachyptila* and *Lamellibrachia* cf *luymesi*: proton elimination is a necessary adaptation to sulfide-oxidizing chemoautotrophic symbionts. The Journal of Experimental Marine Biology 205: 3055-3066

Grimm V, Reise K, Strasser M (2003) Marine metapopulations: A useful concept? Helgoland Marine Research 56: 222-228

Gyllenberg M, Osipov A, Soderbacka G (1996) Bifurcation analysis of a metapopulation model with sources and sinks. Journal of Nonlinear Science 6: 329-366

Hessler RR, Lonsdale P, Hawkins J (1988) Patterns on the ocean floor. New Scientist 1605: 47-48

Hessler RR, Smithey WM, Keller CH (1985) Spatial and temporal variation of giant clams, tube worms and mussels at deep-sea hydrothermal vents. Bulletin of the Biological Society of Washington 6: 411-428

Hilario A, Tyler PA, Pond DW (2008) A new method to determine the reproductive condition in female tubeworms tested in *Seepiophila jonesi* (Polychaeta: Siboglinidae: Vestimentifera) doi:10.1017/S0025315408001586. Journal of the Marine Biological Association of the United Kingdom 88: 909-912

Hilario A, Young C, Tyler PA (2005) Sperm storage, internal fertilization, and embryonic dispersal in vent and seep tubeworms (Polychaeta: Siboglinidae: Vestimentifera). Biological Bulletin 208: 20-28

Hoch JM (2008) Variation in penis morphology and mating ability in the acorn barnacle, *Semibalanus balanoides*. Journal of Experimental Marine Biology and Ecology 359: 126-130

Ims RA (1990) The ecology and evolution of reproductive synchrony. Trends in Ecology & Evolution 5: 135-140

Johnson KS, Childress JJ, Hessler RR, Sakamotoarnold CM, Beehler CL (1988) Chemical and biological interactions in the Rose Garden hydrothermal vent field, Galapagos spreading center. Deep-Sea Research Part a-Oceanographic Research Papers 35: 1723-1744

Jolly MT, Viard F, Weinmayer G, Gentil F, Thiebaut E, Jollibet D (2003) Does the genetic structure of *Pectinaria koreni (*Polychaeta: Pectinariidae) conform to a source-sink metapopulation model at the scale of the Baie de Seine? Helgoland Marine Research 56: 238-246

Jones ML (1984) The giant tube worms. Oceanus 27: 47-53.

Jones ML (1985) On the Vestimentifera, new phylum: six new species, and other taxa, from hydrothermal vents and elsewhere. Bulletin of the Biological Society of Washington 6: 117-158

Jones ML, Gardiner SL (1989) On the early development of the vestimentiferan tube worm *Ridgeia sp*. and observations on the nervous system and trophosome of *Ridgeia sp*. and *Riftia pachyptila*. Biol Bull 177: 254-276

Juniper SK, Jonasson IR, Tunnicliffe V, Southward AJ (1992) Influence of a tube-building polychaete on hydrothermal chimney mineralization. Geology 20: 895-898

Kawecki TJ (2008) Adaptation to marginal habitats. Annual Review of Ecology, Evolution, and Systematics 39: 321-342

Kelley D, Delaney J, Yoerger D (2001) Geology and venting characteristics of the Mothra hydrothermal field, Endeavour segment, Juan de Fuca Ridge. Geology 29: 959-962

Kelly NE, Metaxas A (2007) Influence of habitat in the reproductive biology of the deep-sea hydrothermal vent limpet *Lepetodrilus fucensis* (Vetigastripoda: Mollusca) from the Northeast Pacific. Marine Biology 151: 649-662

Keough MJ, Downes BJ (1982) Recruitment of marine invertebrates: The role of active larval choices and early mortality Oecologia. Springer Berlin / Heidelberg, pp 348-352

Kim KH, McMurtry G (1991) Radial growth rates and 210Pb ages of hydrothermal massive sulfides from the Juan de Fuca Ridge. Earth and Planetary Science Letters 104: 299-314

Kim SL, Mullineaux LS (1998) Distribution and near-bottom transport of larvae and other plankton at hydrothermal vents. Deep Sea Research Part II: Topical Studies in Oceanography 45: 423-440

Levin LA, Caswell H, DePatra KD, Creed EL (1987) Demographic consequences of larval development mode: Planktotrophy vs. lecithotrophy in *Streblospio benedicti*. Ecology 68: 1877-1886

Levin LA, Creed EL (1986) Effect of temperature and food availability on reproductive responses of *Streblospio benedicti* (Polychaeta: Spionidae) with planktotrophic or lecithotrophic development. Marine Biology 92: 103-113

Levins R (1969) Effect of random variations of different types on population growth. Proceedings of the National Academy of Sciences of the United States of America 62: 1061-1065

Lisin SE, Hannan EE, Kochevar RE, Harrold C, Barry JP (1997) Temporal variation in gametogenic cycles of vesicomyid clams. Invertebrate Reproduction and Development 31: 307-318

Lucas MI, Walker G, Holland DL, Crisp DJ (1979) An energy budget for the free-swimming and metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia). Marine Biology 55: 221-229

MacDonald IR, Tunnicliffe V, Southward EC (2002) Detection of sperm transfer and synchronous fertilization in *Ridgeia piscesae* Jones at Endeavour Segment, Juan de Fuca Ridge. Cahiers de Biologie Marine 43: 395-398

Malakhov VV, Popelyaev IS, Galkin SV (1996) Microscopic anatomy of *Ridgeia phaeophiale* Jones, 1985 (Pogonophora, Vestimentifera) and the problem of the position of Vestimentifera in the system of the Animal Kingdom. IV. Excretory and reproductive systems and coelom. Riussian Journal of Marine Biology 22: 249-260

Marcus J, Tunnicliffe V, Butterfield DA (2009) Post-eruption succession of macrofaunal communities at diffuse flow hydrothermal vents on Axial Volcano, Juan de Fuca Ridge, Northeast Pacific. Deep Sea Research Part II: Topical Studies in Oceanography Marine Benthic Ecology and Biodiversity: A Compilation of Recent Advances in Honor of J. Frederick Grassle 56: 1586-1598

Marsh AG, Mullineaux LS, Young CM, Manahan DT (2001) Larval dispersal potential of the tubeworm *Riftia pachyptila* at deep-sea hydrothermal vents 411: 77-80

Matabos M, Thiebaut E, Le Guen D, Sadosky F, Jollivet D, Bonhomme F (2008) Geographic clines and stepping-stone patterns detected along the East Pacific Rise in the vetigastropod *Lepetodrilus elevatus* reflect species crypticism. Marine Biology 153: 545-563

McHugh D (1987) The life-history patterns of two hydrothermal vent polychaetes, *Paralvinella pandorae* Desbruyeres and Laubier and *Paralvinella palmiformis* Desbruyeres and Laubier. Masters thesis. University of Victoria, BC, Canada

McHugh D (1989) Population structure and reproductive biology of two sympatric hydrothermal vent polychaetes, *Paralvinella pandorae* and *P. palmiformis*. Marine Biology 103: 95-106

Metaxas A (2004) Spatial and temporal patterns in larval supply at hydrothermal vents in the Northeast Pacific Ocean. Limnology and Oceanography 49: 1949-1956

Mullineaux LS, Mills SW, Goldman E (1998) Recruitment variation during a pilot colonization study of hydrothermal vents (9°50'N, East Pacific Rise). Deep Sea Research Part II: Topical Studies in Oceanography 45: 441-464

Nix ER, Fisher CR, Vodenichar J, Scott KM (1995) Physiological ecology of a mussel with methanotrophic endosymbionts at three hydrocarbon seep sites in the Gulf of Mexico. Marine Biology 122: 605-617

Nussbaumer AD, Fisher CR, Bright M (2006) Horizontal endosymbiont transmission in hydrothermal vent tubeworms. Nature 441: 345-348

Nyholm SV, Robidart J, Girguis PR (2008) Coupling metabolite flux to transcriptomics: Insights into the molecular mechanisms underlying primary productivity by the hydrothermal vent tubeworm *Ridgeia piscesae*. Biological Bulletin 214: 255-265

Phleger CF, Nelson MM, Groce AK, Cary SC, Coyne KJ, Nichols PD (2005) Lipid composition of deep-sea hydrothermal vent tubeworm *Riftia pachyptila*, crabs *Munidopsis subsquamosa* and *Bythograea thermydron*, mussels *Bathymodiolus sp.* and limpets *Lepetodrilus spp*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 141: 196-210

Pond DW, Allen CE, Bell MV, Van Dover CL, Fallick AE, Dixon DR, Sargent JR (2002) Origins of long-chain polyunsaturated fatty acids in the hydrothermal vent worms *Ridgea piscesae* and *Protis hydrothermica*. Marine Ecology-Progress Series 225: 219-226

Pulliam HR (1988) Sources, sinks, and population regulation doi:10.1086/284880. The American Naturalist 132: 652

Qian P-Y, Chia F-S (1991) Fecundity and egg size are mediated by food quality in the polychaete worm *Capitella sp*. Journal of Experimental Marine Biology and Ecology 148: 11-25

Ramirez Llodra E (2002) Fecundity and life-history strategies in marine invertebrates. Advances in Marine Biology 43: 87-170

Reich M, Grimm V (1996) Das metapopulationskonzept in okologie und naturschutz: Eine kritische Bestandsaufnahme. Z Okologie und Natursch 5: 123-139

Roughgarden J, Iwasa Y, Baxter C (1985) Demographic theory for an open marine population with space-limited recruitment. Ecology 66: 54-67

Sarrazin J, Juniper SK (1999) Biological characteristics of a hydrothermal edifice mosaic community. Marine Ecology Progress Series 185: 1-19

Sarrazin J, Juniper SK, Massoth G, Legendre P (1999) Physical and chemical factors influencing species distributions on hydrothermal sulfide edifices of the Juan de Fuca Ridge, northeast Pacific. Marine Ecology Progress Series 190: 89-112

Sarrazin J, Robigou V, Juniper SK, Delaney JR (1997) Biological and geological dynamics over four years on a high-temperature sulfide structure at the Juan de Fuca Ridge hydrothermal observatory. Marine Ecology Progress Series 153: 5-24

Schoener TW (1983) Field experiments on interspecific competition. The American Naturalist 122: 240-285

Smith EB, Scott KM, Nix ER, Korte C, Fisher CR (2000) Growth and condition of seep mussels (*Bathymodiolus childressi*) at a Gulf of Mexico brine pool. doi:10.1890/0012-9658(2000)081[2392:GACOSM]2.0.CO;2. Ecology 81: 2392-2403

Smith KL (1985) Deep-sea hydrothermal vent mussels: Nutritional state and distribution at the Galapagos Rift. Ecology 66: 1067-1080

Sorgo A, Gaill F, Lechaire J-P, Arndt C, Bright M (2002) Glycogen storage in the *Riftia pachyptila* trophosome: Contribution of host and symbionts. Marine Ecology Progress Series 231: 115-120

Southward EC (1988) Development of the gut and segmentation of newly settled stages of *Ridgeia* (Vestimentifera): Implications for relationship between Vestimentifera and Pogonophora doi:10.1017/S0025315400043344. Journal of the Marine Biological Association of the United Kingdom 68: 465-487

Southward EC, Coates KA (1989) Sperm masses and sperm transfer in a vestimentiferan, *Ridgeia piscesae* Jones, 1985 (Pognophora: Obturata). Canadian Journal of Zoology 67: 2776-2781

Southward EC, Tunnicliffe V, Black M (1995) Revision of the species of *Ridgeia* from northeast Pacific hydrothermal vents, with a redescription of *Ridgeia piscesae* Jones (Pognophora: Obturata = Vestimentifera). Canadian Journal of Zoology 73: 282-295

Spight TM, Emlen J (1976) Clutch sizes of two marine snails with a changing food supply. Ecology 57: 1162-1178

Thomson RE, Mihaly SF, Rabinovich AB, McDuff RE, Veirs SR, Stahr FR (2003) Constrained circulation at Endeavour ridge facilitates colonization by vent larvae 424: 545-549

Trivers RL (1972) Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (Ed. by B. Campbell). London:Heinemann

Tsurumi M, Tunnicliffe V (2003) Tubeworm-associated communities at hydrothermal vents on the Juan de Fuce Ridge, northeast Pacific. Deep Sea Research Part I: Oceanographic Research Papers 50: 611-629

Tunnicliffe V, Embley RW, Holden JF, Butterfield DA, Massoth GJ, Juniper SK (1997) Biological colonization of new hydrothermal vents following an eruption on Juan de Fuca Ridge. Deep Sea Research Part I: Oceanographic Research Papers 44: 1627-1644

Tunnicliffe V, Garrett JF, Johnson HP (1990) Physical and biological factors affecting the behaviour and mortality of hydrothermal vent tubeworms (vestimentiferans). Deep Sea Research Part A. Oceanographic Research Papers 37: 103-125

Tunnicliffe V, Juniper SK (1990) Dynamic character of the hydrothermal vent habitat and the nature of sulphide chimney fauna. Progress in Oceanography. Deep-Sea Biology 24: 1-13

Tunnicliffe V, Juniper SK, de Burgh ME (1985) The hydrothermal vent community on Axial Seamount, Juan de Fuca Ridge. In: Jones M (ed) The Hydrothermal Vents of the Eastern Pacific Ocean: An Overview. Bulletin of the Biological Society of Washington, pp 453-464

Tyler PA, Young CM (1999) Reproduction and dispersal at vents and cold seeps doi:10.1017/S0025315499000235. Journal of the Marine Biological Association of the United Kingdom 79: 193-208

Urcuyo IA, Bergquist DC, MacDonald IR, VanHorn M, Fisher CR (2007) Growth and longevity of the tubeworm *Ridgeia piscesae* in the variable diffuse flow habitats of the Juan de Fuca Ridge. Marine Ecology Progress Series 344: 143-157

Urcuyo IA, Massoth GJ, Julian D, Fisher CR (2003) Habitat, growth and physiological ecology of a basaltic community of *Ridgeia piscesae* from the Juan de Fuca Ridge. Deep Sea Research Part I: Oceanographic Research Papers 50: 763-780

Urcuyo IA, Massoth GJ, MacDonald IR, Fisher CR (1998) In situ growth of the vestimentiferan *Ridgeia piscesae* living in highly diffuse flow environments in the main Endeavour Segment of the Juan de Fuca Ridge. Cahiers de Biologie Marine 39: 267-270

Van Dover CL, Factor JR, Williams AB, Berg CJ (1985) Reproductive patterns of decapod crustaceans from hydrothermal vents. Proceedings of the Biological Society of Washington 6: 223-227

Vrijenhoek RC (1997) Gene flow and genetic diversity in naturally fragmented metapopulations of deep-sea hydrothermal vent animals. Journal of Heredity 88: 285-293

Young CM, Vazquez E, Metaxas A, Tyler PA (1996) Embryology of vestimentiferan tube worms from deep-sea methane/sulphide seeps. Nature, London 381: 514-516

Young CR, Fujio S, Vrijenhoek RC (2008) Directional dispersal between mid-ocean ridges: deep-ocean circulation and gene flow in *Ridgeia piscesae*. Molecular Ecology 17: 1718-1731

Appendix A
Dissection Data

The tables in Appendix A present the original raw dissection data for samples collected in 2008.

Table 14. Raw dissection data for sample SMH1

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	400	10.00	165	10.30	5.72	1.39	m	2		0
2	277	8.40	150	7.20	2.95	1.13	f	3		0
3	267	8.70	121	8.50	3.67	0.92	f	1		0
4	263	9.00	129	8.30	3.34	0.97	f	2	*	0
5	258	8.10	136	7.60	2.86	0.95	f	3	*	0
6	300	8.40	151	8.30	4.10	1.08	f	2		0
7	296	10.00	200	9.20	3.22	1.13	f	3		0
8	275	8.00	121	7.10	2.20	0.85	f	3	*	0
9	272	7.70	207	7.90	2.71	0.86	m	3	*	0
10	232	7.70	180	7.50	2.30	0.90	f	2		0
11	244	7.20	144	6.70	1.66	0.71	f	2		0
12	236	7.70	168	6.90	2.17	0.60	m	3	*	0
13	341	8.65	123	8.35	3.27	1.41	f	3	*	0
14	336	8.65	266	8.20	4.85	1.12	m	3	*	0
15	304	8.60	186	7.30	3.31	0.77	m	3	*	0
16	332	10.00	100	10.10	3.58	1.48	f	3		0
17	272	8.65	150	7.70	3.66	0.95	m	3	*	0
18	170	9.95	144	9.30	3.30	1.37	f	3	*	0
19	261	8.80	115	8.10	3.23	0.95	m	3	*	0
20	284	8.65	199	9.35	4.16	1.11	m	3	*	0
21	202	8.10	133	7.15	2.33	0.83	f	3	*	0
22	249	9.15	148	8.05	3.18	1.23	f	3	*	0
23	218	8.55	188	6.75	2.62	0.81	m	3	*	0
24	270	8.30	137	7.15	2.30	0.66	m	3	*	0
25	210	8.60	126	7.95	2.19	0.97	f	3	*	0

Table 15. Raw dissection data for sample SML1

**Plume condition of "i" represents immature tubeworms

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	236	4.15	29	4.10	0.11	0.07	f	0		i**
2	128	3.50	56	3.10	0.12	0.07	m	1		i
3	185	4.10	125	4.15	0.24	0.13	f	0		i
4	102	4.60	60	4.25	0.24	0.11	f	1		i
5	111	4.10	29	3.75	0.11	0.05	m	1		i
6	88	3.00	35	2.60	0.08	0.04	m	1		i
7	71	2.80	54	2.95	0.05	0.02	m	1		i
8	124	3.80	28	3.60	0.08	0.05	f	0		i
9	126	3.30	61	3.10	0.12	0.06	f	0		i
10	125	3.25	18	2.85	0.07	0.03	m	0		i
11	128	3.25	33	3.30	0.11	0.06	f	0		i
12	123	3.40	19	3.40	0.08	0.04	m	1		i
13	152	3.75	28	3.65	0.09	0.06	f	0		i
14	96	3.80	24	3.75	0.09	0.05	f	0		i
15	98	2.70	31	2.70	0.06	0.04	m	0		i
16	78	3.20	42	2.50	0.09	0.04	m	1		i
17	66	2.70	31	2.10	0.04	0.02	f	0		i
18	43	3.10	31	3.10	0.12	0.06	m	1		i
19	56	2.55	37	2.40	0.09	0.05	f	0		i
20	80	2.70	37	2.25	0.09	0.05	f	0		i
21	85	3.00	21	2.50	0.05	0.03	f	0		i
22	48	3.00	17	2.60	0.06	0.03	m	0		i
23	55	3.25	22	3.05	0.06	0.05	m	1		i
24	66	2.20	14	-	-	-	-	0		-

Table 16. Raw dissection data for sample GRH2

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	301	7.90	71	7.80	1.54	0.62	f	3		0
2	239	6.10	136	5.50	1.07	0.47	f	3	*	0
3	385	6.65	116	6.65	1.66	0.54	m	2		0
4	274	5.10	129	4.60	1.15	0.38	m	2		0
5	324	6.75	150	7.10	1.58	0.63	m	2		0
6	231	5.95	211	4.60	1.55	0.43	m	2		0
7	284	6.25	103	5.60	0.86	0.49	f	2		0
8	350	5.20	73	5.50	0.86	0.38	f	2		0
9	229	6.00	91	5.25	0.76	0.39	f	2		0
10	359	6.65	108	1.20	0.61	0.28	f	1		0
11	350	5.15	228	4.80	0.62	0.24	m	2		0
12	102	6.00	83	5.50	0.80	0.34	f	2		0
13	447	5.85	75	6.00	0.83	0.39	f	2		0
14	433	7.40	75	7.15	1.48	0.67	f	2		0
15	190	5.70	97	4.75	0.43	0.20	f	3		0
16	263	5.00	91	4.95	0.56	0.22	m	2		0
17	179	6.90	33	6.30	0.46	0.23	m	2		0
18	245	6.25	185	5.10	1.12	0.42	f	3	*	0
19	302	5.80	115	5.20	0.92	0.46	f	3		0
20	286	6.40	69	6.00	0.67	0.37	f	2		0
21	129	4.35	78	4.35	0.33	0.17	f	1		0
22	79	6.60	106	5.10	1.24	0.53	f	3		0
23	168	4.90	65	5.25	0.67	0.27	m	2		0
24	183	5.21	69	5.25	0.84	0.31	m	2		0
25	74	7.20	70	6.40	0.90	0.41	f	3		0

Table 17. Raw dissection data for sample GRL2

**Plume condition "i" represents immature tubeworms

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	182	3.30	54	3.10	0.13	0.08	m	0		i**
2	168	2.40	42	2.60	0.04	0.03	f	0		i
3	132	3.00	67	2.25	0.08	0.04	m	1		i
4	122	3.00	54	2.55	0.09	0.05	m	1		i
5	148	3.65	46	3.00	0.12	0.08	f	1		i
6	216	3.20	67	3.10	0.10	0.04	f	0		i
7	119	2.65	52	2.65	0.04	0.02	f	0		i
8	251	3.05	241	2.50	0.10	0.06	m	0		i
9	216	3.60	211	3.00	0.08	0.05	f	0		i
10	141	3.05	59	2.65	0.05	0.04	f	0		i
11	148	3.10	58	2.11	0.09	0.05	m	0		i
12	83	2.95	32	2.25	0.06	0.04	f	0		i
13	104	3.20	37	2.30	0.06	0.04	f	0		i
14	111	3.25	39	2.65	0.11	0.07	m	0		i
15	136	2.70	54	2.85	0.07	0.04	m	0		i
16	115	2.20	67	2.20	0.06	0.03	m	0		i
17	125	3.00	76	2.30	0.07	0.04	m	1		i
18	87	2.30	50	2.10	0.04	0.02	m	1		i
19	71	3.05	82	2.40	0.06	0.03	m	0		i
20	116	2.60	34	2.20	0.05	0.03	m	1		i
21	65	2.50	35	2.10	0.05	0.04	m	0		i
22	115	2.40	53	2.25	0.05	0.04	m	0		i
23	109	2.60	67	2.20	0.03	0.02	m	0		i
24	90	2.60	47	2.05	0.01	0.01	m	0		i

Table 18. Raw dissection data for sample GRH3

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	287	10.50	161	9.20	5.25	1.85	f	3	*	0
2	254	9.65	176	8.60	3.90	1.20	f	3	*	0
3	252	8.80	180	6.25	2.33	0.94	f	3		0
4	325	8.50	179	8.05	4.63	1.21	m	3	*	0
5	242	8.00	156	7.00	3.43	0.76	m	2		0
6	289	8.15	144	7.65	3.58	0.95	m	3	*	0
7	280	9.05	98	7.60	3.10	1.16	f	2		0
8	252	9.05	110	8.00	3.64	1.45	f	2		0
9	267	8.55	117	7.80	2.66	0.97	f	3		0
10	270	7.20	131	7.00	2.74	0.70	m	2		0
11	279	8.50	103	7.65	2.10	0.80	f	3		0
12	310	9.00	191	7.10	3.74	0.93	m	2		0
13	256	9.50	111	8.65	2.79	1.04	f	2		0
14	267	8.55	114	7.70	2.31	0.85	f	3		0
15	246	7.70	127	6.60	1.73	0.73	f	3	*	0
16	158	8.60	132	6.40	1.82	0.68	f	2	*	0
17	324	10.65	176	9.40	4.33	1.57	f	3		0
18	277	7.70	110	7.20	1.90	0.84	f	2		0
19	286	8.20	85	7.20	2.23	0.95	f	3		0
20	304	7.65	133	7.65	2.57	0.85	m	2		0

Table 19. Raw dissection data for sample GRL3

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	587	5.20	344	5.10	1.44	0.32	m	2		1
2	332	4.05	402	4.35	0.57	0.22	m	1		2
3	303	4.35	185	4.20	0.38	0.21	f	0		2
4	481	4.60	230	4.30	0.56	0.26	f	1		2
5	369	4.10	257	3.90	0.47	0.21	m	1		2
6	449	4.70	286	4.10	0.87	0.43	f	2		2
7	298	4.10	128	4.00	0.30	0.17	f	1		2
8	369	4.00	172	4.75	0.45	0.23	m	1		2
9	401	4.60	318	3.25	0.68	0.30	f	1		2
10	321	4.25	128	3.65	0.32	0.16	f	3		2
11	413	3.70	167	3.65	0.26	0.14	f	0		2
12	459	4.25	276	4.15	0.58	0.24	f	3	*	1
13	419	4.65	253	3.85	0.49	0.20	m	1		2
14	566	5.00	299	4.75	0.97	0.35	m	1		2
15	360	4.10	126	4.10	0.43	0.21	m	1		2
16	441	4.95	163	5.10	0.51	0.27	f	0		2
17	512	4.55	279	4.40	0.75	0.30	m	1		2
18	367	4.65	131	4.60	0.59	0.32	f	1		1
19	504	4.95	300	4.65	0.66	0.29	f	3		2
20	346	4.10	136	3.30	0.48	0.25	m	1		2
21	573	4.30	275	4.60	0.71	0.25	m	1		2
22	489	4.65	236	4.65	0.99	0.40	f	1	*	1
23	559	6.00	247	6.20	1.25	0.52	m	2		2
24	750	4.75	313	4.95	0.81	0.29	m	1		2
25	460	4.35	275	3.30	0.49	0.22	f	1		0
26	491	5.50	223	3.95	0.57	0.27	m	1		2

Table 20. Raw dissection data for sample HUH4

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	471	8.00	93	8.70	2.98	1.29	f	3		0
2	738	7.80	336	7.95	5.12	1.02	m	2		0
3	499	7.40	248	8.80	5.23	1.29	m	3	*	0
4	454	8.25	109	7.70	2.48	0.74	m	3	*	0
5	491	7.60	118	7.20	3.26	0.87	m	3	*	0
6	621	8.40	257	8.35	4.78	1.30	m	3	*	0
7	512	9.65	134	9.65	4.54	1.58	f	3	*	0
8	739	9.25	313	9.10	7.56	1.71	m	3	*	0
9	501	7.65	129	7.65	3.49	0.90	m	3	*	0
10	660	9.60	223	9.55	5.86	1.41	m	3	*	0
11	607	8.65	189	7.65	3.00	0.78	m	3	*	0
12	303	7.20	234	9.10	3.56	0.86	m	2		0
13	377	8.15	108	8.05	2.58	0.87	f	3	*	0
14	467	10.20	160	7.80	3.70	0.45	f	3	*	-
15	395	7.95	107	7.80	2.12	0.85	f	3	*	0
16	631	7.20	226	7.65	2.64	1.02	f	3	*	0
17	363	7.10	94	7.20	2.20	0.92	f	3	*	0
18	570	7.25	300	7.10	3.02	0.75	m	3	*	0
19	518	7.70	156	7.15	2.67	0.90	m	3	*	0
20	576	8.20	171	7.20	3.21	0.39	m	2		-
21	551	8.45	242	7.20	4.09	1.01	m	3	*	0
22	303	8.10	161	7.35	2.01	0.81	f	3	*	0
23	295	7.10	141	7.15	2.40	0.85	f	3	*	0
24	330	7.20	115	6.70	2.00	0.75	f	3	*	0
25	399	5.75	140	6.10	1.44	0.37	m	2		0

Table 21. Raw dissection data for sample HUL4

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	646	4.35	201	4.10	0.51	0.19	f	0		2
2	683	5.25	194	4.60	0.93	0.38	f	0		2
3	631	4.70	237	4.35	0.69	0.30	m	0		2
4	592	5.15	228	5.00	0.63	0.29	f	1		2
5	382	3.95	200	-	0.15	-	m	1		-
6	433	5.10	224	4.30	0.39	0.21	f	1		2
7	507	4.65	239	4.10	0.52	0.26	f	1		2
8	702	4.65	258	4.15	0.50	0.22	m	0		2
9	724	5.25	336	5.30	1.02	0.38	f	0		2
10	392	4.10	117	3.80	0.33	0.18	m	0		2
11	591	4.90	298	5.05	0.47	0.24	f	1		2
12	651	5.20	435	4.85	1.36	0.41	f	0		1
13	1322	5.25	588	5.10	1.40	0.42	f	1		2
14	595	4.65	276	4.75	0.97	0.35	m	2		2
15	589	4.65	229	4.00	0.57	0.26	f	3		2
16	390	4.80	141	4.15	0.36	0.21	f	2		1
17	466	4.35	178	3.60	0.50	0.23	f	1		2
18	772	5.60	343	5.70	1.21	0.49	f	1		2
19	519	3.80	319	3.65	0.49	0.22	f	1		2
20	336	4.35	205	4.10	0.60	0.24	m	1		1
21	313	3.80	234	3.25	0.27	0.13	m	1		2
22	457	4.30	204	4.10	0.46	0.21	f	1		2
23	365	5.05	249	4.30	0.49	0.26	m	1		2
24	372	3.80	270	3.40	0.46	0.22	f	1		2
25	299	3.55	213	3.85	0.28	0.11	m	1		2

Table 22. Raw dissection data for sample HUH5

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	247	7.95	174	7.60	3.60	0.83	m	2		0
2	310	7.10	191	6.40	2.92	0.64	m	3		1
3	336	7.20	120	7.00	2.11	0.53	m	2		1
4	476	10.60	196	10.00	6.04	1.97	f	2		0
5	278	8.50	97	8.10	2.88	1.05	f	2		0
6	246	7.20	122	6.75	1.92	0.77	f	2		0
7	260	8.10	92	8.00	2.31	0.87	f	2	*	0
8	232	6.40	100	7.60	1.43	0.65	f	3		0
9	345	7.60	138	6.20	2.28	0.88	f	3	*	0
10	296	7.30	187	7.20	2.22	0.91	f	3		0
11	243	7.15	106	6.60	1.31	0.51	f	3	*	0
12	350	8.00	142	6.85	2.21	0.65	m	3	*	0
13	306	9.05	169	7.00	4.36	0.98	m	3	*	0
14	390	7.60	153	6.40	3.98	0.90	m	3	*	0
15	222	6.30	78	6.50	1.23	0.63	f	3		0
16	389	8.40	189	7.40	3.64	1.00	m	3	*	0
17	110	10.00	101	8.85	3.68	1.56	f	3		0
18	227	8.05	110	7.95	2.11	0.79	f	3		0
19	316	9.15	128	8.20	2.90	1.20	f	2		0
20	233	8.65	160	9.10	2.78	1.02	f	3		0
21	340	7.25	227	7.70	3.46	0.87	m	2		0
22	347	8.10	93	7.20	1.67	0.72	f	3		0
23	171	7.15	76	6.90	1.55	0.70	f	3		0
24	246	8.10	109	6.35	1.75	0.76	f	3		0

Table 23. Raw dissection data for sample HUL5

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	845	5.30	318	4.70	1.28	0.34	f	3		1
2	880	5.60	318	4.00	1.54	0.56	f	1		1
3	885	6.05	260	5.30	1.24	0.30	f	1		1
4	744	5.25	316	5.00	1.43	0.42	f	1		2
5	841	5.00	405	4.60	1.03	0.26	m	3		2
6	726	6.10	335	5.40	2.11	0.89	f	1		1
7	912	5.00	376	4.20	1.44	0.40	m	2		1
8	977	6.20	947	6.25	1.75	0.55	f	3		1
9	604	5.50	252	4.60	1.14	0.41	m	1		1
10	779	5.15	371	4.90	0.96	0.32	f	3		2
11	825	5.70	314	5.50	1.32	0.37	f	1		1
12	739	5.25	295	4.35	0.86	0.29	f	1		1
13	1007	5.00	534	5.60	1.82	0.63	f	1		1
14	545	4.25	232	3.65	0.88	0.32	m	1		2
15	822	5.05	243	4.40	1.06	0.38	m	1		2
16	588	5.25	183	4.65	1.09	0.42	f	1		1
17	929	5.75	478	5.20	1.14	0.48	f	1		2
18	949	5.95	385	5.20	1.64	0.64	f	0		2
19	641	5.50	400	5.30	1.52	0.51	f	1		1
20	891	5.60	462	5.60	1.55	0.43	f	1		0
21	818	4.90	543	4.75	1.40	0.35	m	1		0
22	382	4.65	343	5.00	1.14	0.38	m	1		2
23	900	5.75	387	4.75	1.16	0.47	m	1		2
24	762	4.90	319	4.15	0.68	0.31	m	1		2
25	715	5.60	310	5.05	1.53	0.50	m	1		1

Table 24. Raw dissection data for sample CBH6

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	553	10.10	350	9.70	5.13	2.04	f	3		0
2	683	9.05	560	7.60	7.06	1.57	m	2		0
3	415	8.20	314	8.00	4.26	1.28	m	2		0
4	316	10.55	187	9.35	3.72	1.08	f	2	*	0
5	360	9.50	325	8.65	4.77	1.71	f	3	*	0
6	445	7.65	136	7.10	2.70	0.84	m	3	*	0
7	594	11.00	466	10.20	5.22	1.32	f	3		0
8	529	9.40	409	8.50	7.28	1.72	m	2		0
9	326	8.15	120	8.00	2.64	0.78	m	2		1
10	411	8.30	183	6.85	2.62	1.12	f	1	*	0
11	420	9.00	275	8.15	6.12	1.33	m	2		0
12	425	8.25	301	7.90	4.62	1.32	m	2		0
13	503	9.20	381	8.15	8.13	1.71	m	3	*	0
14	386	8.70	268	7.15	4.17	1.59	f	2	*	0
15	623	8.10	365	7.80	3.72	1.18	m	3	*	0
16	285	8.70	66	7.15	1.32	0.39	f	2		-
17	302	8.35	288	7.00	4.33	1.35	f	3		0
18	467	8.40	279	8.10	6.06	1.51	m	2		0
19	397	8.60	222	8.55	4.10	1.61	f	2	*	0
20	378	11.65	211	8.60	6.94	1.23	f	3		-
21	386	9.05	215	7.60	3.31	1.20	f	3	*	0
22	340	8.10	320	6.75	4.00	1.07	m	2		0
23	384	12.40	272	10.10	8.29	2.87	f	3	*	0
24	367	9.05	256	8.30	4.15	1.57	f	3		0
25	289	7.35	75	7.40	1.93	0.86	f	3		0

Table 25. Raw dissection data for sample CBL6

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	345	6.20	209	6.40	2.30	0.79	m	1		1
2	434	6.10	185	5.70	1.73	0.60	m	1		2
3	834	6.30	526	5.60	3.45	0.65	m	1		1
4	301	4.60	447	4.50	1.24	0.28	m	1		2
5	376	5.70	138	6.00	1.33	0.57	f	3		2
6	475	6.00	216	6.00	1.12	0.41	m	3		1
7	1017	7.00	466	6.00	3.24	0.82	f	3	*	1
8	361	6.00	197	6.40	1.41	0.57	m	2		1
9	316	5.30	384	5.00	1.67	0.47	f	2	*	1
10	317	6.30	238	6.10	1.53	0.54	f	2		2
11	526	6.05	583	5.70	1.84	0.49	m	2		2
12	487	5.10	256	3.20	0.36	0.12	m	1		2
13	436	5.70	175	5.60	0.77	0.27	m	1		1
14	749	6.30	361	5.95	2.67	0.71	f	2		1
15	796	5.10	579	4.90	2.43	0.43	f	2		0
16	826	6.70	297	5.60	1.60	0.50	m	2		1
17	434	5.30	220	5.30	0.89	0.26	m	2		0
18	1245	6.40	605	5.80	2.75	0.54	f	2		2

Table 26. Raw dissection data for sample CBH7

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	357	5.60	105	5.30	0.88	0.40	f	1		1
2	252	5.60	164	4.25	1.02	0.06	f	2		-
3	402	5.70	138	5.25	1.13	0.41	m	2		2
4	252	5.60	114	4.30	0.74	0.30	m	2		2
5	339	6.00	116	5.20	0.94	0.31	m	3		1
6	339	5.20	75	5.10	0.78	0.35	f	1		0
7	244	5.10	55	4.35	0.39	0.20	m	2		0
8	205	4.10	83	3.10	0.49	0.25	m	3	*	1
9	232	6.60	87	5.20	1.02	0.38	m	2		1
10	189	5.10	81	4.10	0.37	0.18	f	0		2
11	267	5.20	96	4.45	0.64	0.31	f	1		2
12	272	6.10	166	5.80	1.23	0.48	m	1		1
13	366	6.15	87	6.20	1.78	0.58	m	2		1
14	189	5.60	87	4.90	0.89	0.37	m	2		2
15	170	5.10	58	5.25	0.76	0.35	f	3		2
16	272	4.90	122	4.10	0.60	0.25	m	1		1
17	273	5.20	99	5.35	0.73	0.26	m	2		3
18	298	5.70	195	4.70	1.15	0.43	m	1		0
19	175	5.30	51	4.65	0.44	0.21	f	1		2
20	287	5.00	122	5.20	0.98	0.38	m	1		2
21	229	3.80	72	3.90	0.27	0.15	f	0		2
22	289	5.30	86	4.90	0.65	0.32	f	1	*	1
23	306	4.90	78	4.55	0.57	0.23	m	1		2
24	223	4.40	76	4.25	0.31	0.16	f	0	*	1
25	289	4.80	117	5.00	0.44	0.23	m	1	*	2

Table 27. Raw dissection data for sample CBL7

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	1180	5.60	439	4.25	0.89	0.20	f	0		2
2	682	5.70	248	4.75	1.22	0.41	f	0	*	2
3	478	4.60	169	4.15	0.68	0.28	f	1		2
4	678	5.70	355	4.70	1.25	0.42	m	1		2
5	612	5.30	292	4.40	1.17	0.36	f	1		2
6	646	5.10	527	4.40	1.71	0.32	m	1		0
7	686	4.90	362	4.25	1.28	0.32	m	1		1
8	410	4.95	164	4.30	0.71	0.29	m	2		2
9	568	4.40	185	4.25	0.56	0.19	m	2		1
10	598	4.35	260	4.10	0.74	0.30	m	1		1
11	570	4.10	257	4.60	0.61	0.21	m	1		2
12	822	5.70	357	5.00	1.51	0.52	f	1		2
13	913	5.30	463	5.20	1.78	0.50	f	2		1
14	746	5.60	469	5.10	1.62	0.48	f	1		1
15	644	5.30	353	5.20	1.75	0.39	m	2		0
16	259	4.00	123	3.60	0.46	0.16	m	0		3
17	679	4.30	333	4.40	0.88	0.25	m	2		1
18	587	4.90	207	4.70	0.95	0.35	m	1		2
19	671	5.00	391	4.10	1.32	0.31	m	2		2
20	527	4.60	194	4.10	0.50	0.17	f	0		3

Table 28. Raw dissection data for sample MOH8

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	383	12.10	68	11.00	3.58	2.51	f	2		0
2	288	10.20	220	8.60	4.62	1.73	f	3	*	0
3	316	7.00	213	5.20	1.31	0.53	f	2		0
4	266	8.20	175	7.80	2.80	0.79	m	2		0
5	344	5.85	79	5.30	0.78	0.30	m	3		0
6	287	8.90	163	7.55	2.70	1.08	f	3		0
7	286	6.75	128	7.00	1.92	0.63	m	2		0
8	266	7.25	118	7.15	1.85	0.71	f	3		0
9	180	6.10	99	5.70	0.95	0.34	m	2		0
10	192	4.20	147	4.70	0.72	0.30	f	3		0
11	217	7.60	147	6.70	2.25	0.82	f	3		0
12	194	5.70	157	5.60	0.87	0.32	f	2		0
13	192	6.95	151	5.60	1.35	0.53	f	2	*	0
14	285	5.70	122	5.00	0.96	0.42	f	3		0
15	219	6.30	142	5.25	1.13	0.41	f	3	*	0
16	243	6.60	69	5.55	0.98	0.37	f	3		0
17	165	6.85	147	5.75	1.51	0.58	f	3	*	0
18	261	7.90	197	6.50	1.67	0.48	f	2		-
19	189	4.35	50	4.20	0.43	0.16	m	2		0
20	158	5.75	109	5.20	0.81	0.40	f	3	*	0
21	149	5.70	92	4.65	0.87	0.29	m	2		1
22	250	5.10	67	4.90	0.61	0.26	f	2		0
23	122	5.00	107	4.40	0.81	0.25	m	2		0
24	180	5.95	142	4.90	0.69	0.27	m	2		0
25	255	6.60	78	6.30	1.17	0.36	m	2		0

Table 29. Raw dissection data for sample MOL8

****Plume condition of "i" represents immature tubeworms**

Tubeworm number	Tube Length (mm)	Anterior tube diameter (mm)	Body Length (mm)	Obturaculum-vestimentum diameter (mm)	Total Weight (g)	Obturaculum-vestimentum weight (g)	Sex (m/f)	Reproductive condition (0-3)	Sperm bundle in vestimental fold (*)	Plume condition (0-3)
1	403	4.15	95	3.25	0.21	0.11	f	0		i**
2	236	4.00	102	3.55	0.23	0.10	f	0		i
3	337	4.25	208	3.75	0.36	0.14	m	0		i
4	231	4.25	54	3.05	0.13	0.08	m	0		i
5	385	4.55	191	3.10	0.51	0.15	f	0		i
6	281	3.35	114	3.15	0.25	0.09	m	0		i
7	306	4.30	44	3.30	0.25	0.16	m	0		i
8	404	3.80	187	3.40	0.27	0.09	f	0		i
9	275	4.25	188	3.25	0.21	0.09	f	0		i
10	289	4.00	66	3.60	0.20	0.12	m	0		i
11	328	4.10	107	4.10	0.20	0.10	f	1		i
12	204	3.90	165	3.05	0.14	0.10	f	0		i
13	277	4.90	173	4.30	0.41	0.19	f	0		i
14	326	4.00	83	3.10	0.13	0.08	m	0		i
15	272	3.65	139	3.40	0.23	0.09	m	0		i
16	419	3.75	269	3.30	0.32	0.13	m	1		i
17	488	4.10	402	3.85	0.28	0.10	m	0		i
18	294	3.90	70	2.60	0.11	0.08	f	0		i
19	399	4.20	214	3.65	0.42	0.15	f	1		i
20	243	4.05	183	3.30	0.28	0.13	f	0		i
21	196	4.00	62	3.40	0.17	0.10	f	0		i
22	224	3.20	213	3.55	0.13	0.07	f	0		i
23	375	3.75	240	3.25	0.34	0.08	f	0		i
24	226	3.75	97	3.25	0.15	0.07	f	0		i
25	180	3.65	125	3.30	0.21	0.11	f	0		i

Appendix B
Juvenile data

The tables in Appendix B present the original juvenile data for samples collected in 2008.

Table 30. Juvenile data for samples SMH1 (A) and SML1 (B)

A

Juvenile length (mm)	Frequency (#)
1	24
2	48
3	26
4	26
5	12
6	10
7	13
8	12
9	8
10	5
11	3
12	3
13	3
14	2
15	1
16	1
17	2
18	1
20	1
23	2
25	1
28	2
32	1
33	1
34	1
36	1
50	1
52	1
79	1
83	1
89	1
117	1
144	1
163	1

B

Juvenile length (mm)	Frequency (#)
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	0
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
21	0
22	0
23	0
24	0
25	0
26	0
27	0
28	0
34	0
38	0
41	0
43	0
44	0
45	0
49	0
50	0

Table 31. Juvenile data for samples GRH2 (A) and GRL2 (B)

A

Juvenile length (mm)	Frequency (#)
5	3
6	1
7	1
8	1
9	2
10	1
17	1
28	1
30	1
35	1

B

Juvenile length (mm)	Frequency (#)
3	1
6	2
8	2
9	2
10	1
11	2
12	2
16	1
19	1
20	1
26	1
29	1
109	1

Table 32. Juvenile data for samples GRH3 (A) and GRL3 (B)

A

Juvenile length (mm)	Frequency (#)
<1	1
1	53
2	55
3	22
4	16
5	16
6	12
7	12
8	3
9	5
10	4
11	5
12	1
13	1
14	1
15	2
18	3
19	1
20	1
40	1

B

Juvenile length (mm)	Frequency (#)
3	1
4	1
5	6
6	4
7	2
8	3
9	3
10	2
11	3
12	1
13	4
14	1
15	2
17	1
18	1
39	1
40	1
58	1
66	1

Table 33. Juvenile data for samples HUH4 (A) and HUL4 (B)

A

Juvenile length (mm)	Frequency (#)
2	4
3	8
4	6
5	9
6	6
7	5
8	4
9	2
10	1
11	3
12	3
13	3
16	1
17	2
19	3
20	2
21	1
22	2
24	1
26	2
27	1
35	1
49	1
55	1
81	1

B

Juvenile length (mm)	Frequency (#)
3	1
4	5
5	2
6	7
7	2
8	2
9	3
10	6
11	3
12	2
13	5
14	1
15	3
16	2
17	1
18	2
20	2
21	1
22	1
23	2
24	1
26	1
28	1
29	1
30	1
32	1
33	2
35	1
38	1
39	3
41	2
48	1
49	1
51	1
52	1
55	1
61	1
63	1
68	1
93	1
94	1
96	1
97	1
102	1
144	1
293	1

Table 34. Juvenile data for samples HUH5 (A) and HUL5 (B)

A

Juvenile length (mm)	Frequency (#)
2	7
3	7
4	10
5	7
6	8
7	9
8	15
9	1
10	2
11	1
12	2
13	3
14	1
15	1
16	2
17	1
18	1
21	2
22	1
24	1
25	1
26	2
27	1
28	1
30	1
37	1
38	1
58	1
69	1
79	1

B

Juvenile length (mm)	Frequency (#)
3	1
5	1
6	1
9	1
13	1
18	1

Table 35. Juvenile data for sample CBH6

Juvenile length (mm)	Frequency (#)
1	32
2	57
3	76
4	86
5	76
6	73
7	56
8	38
9	20
10	31
11	20
12	10
13	24
14	8
15	9
16	6
17	11
18	9
19	5
20	7
21	7
22	9
23	4
24	7
25	4
26	6
27	4
28	4
29	5
30	3
31	4
32	2
34	2
36	1
37	2
38	1
40	3
41	2
42	2
43	3
46	6
47	2
50	2
52	2
53	6
55	1
58	4
59	1
60	2
61	1
63	3
64	1
65	1
66	1
67	2
68	2
70	2
71	1
73	1
74	1
76	2
78	1
81	2
84	2
86	1
87	2
88	1
91	1
95	2
96	1
97	1
101	1
111	1
123	1
126	1
132	1
133	1

Table 36. Juvenile data for sample CBH7

Juvenile length (mm)	Frequency (#)
5	1
6	2
7	1
8	1

Table 37. Juvenile data for samples MOH8 (A) and MOL8 (B)

A

Juvenile length (mm)	Frequency (#)
3	1
4	1
14	1

B

Juvenile length (mm)	Frequency (#)
2	1
4	2
5	1
6	2
7	1
9	2
10	1
11	1
12	1
15	1
24	1
34	1
42	1
45	1
67	1
68	1
95	1

Appendix C
Lipid data

The table in Appendix C presents the raw lipid data for samples collected in 2009.

Table 38. Raw lipid data for samples collected in 2009

Tubeworm	Total lipid amount (mg/g body dry weight)			
	High	Moderate 1	Moderate 2	Low
Female 1	54.59	21.91	23.18	18.87
Female 2	56.43	9.95	15.93	14.61
Female 3	54.94	16.94	25.39	17.99
Male 1	NA	39.22	34.38	24.75
Male 2	NA	33.29	38.70	25.00
Male 3	NA	39.59	30.07	15.39

Appendix D
Histology data

The tables in Appendix D present the raw histology data for samples collected in 2009.

Table 39. Cross-sectional area (mm^2) of trophosome in male and female tubeworms at 10, 30, and 60% trunk length

		Trophosome cross-sectional area (mm^2)		
		High	Moderate	Low
Female 1	10%	-	3.67	3.13
	30%	9.93	5.32	3.08
	60%	10.98	21.22	3.25
Female 2	10%	-	3.95	3.48
	30%	19.70	2.63	3.62
	60%	6.62	4.17	2.44
Female 3	10%	6.98	3.32	7.27
	30%	8.83	6.22	8.30
	60%	14.37	3.48	4.38
Male 1	10%	2.81	3.27	3.04
	30%	3.52	-	6.08
	60%	7.80	-	3.29
Male 2	10%	8.11	5.49	2.94
	30%	5.58	4.31	3.44
	60%	4.39	0.66	2.23
Male 3	10%	9.89	1.16	6.45
	30%	10.14	4.67	4.26
	60%	1.96	2.73	1.39

Table 40. Cross-sectional area of gonad (mm^2) in male and female tubeworms at 10, 30, and 60% trunk length

		Gonad cross-sectional area (mm^2)		
		High	Moderate	Low
Female 1	10%	-	0.44	0.14
	30%	5.78	0.51	0.08
	60%	3.76	0.00	0.86
Female 2	10%		0.12	0.11
	30%	2.69	1.42	0.12
	60%	9.92	0.19	0.14
Female 3	10%	5.27	0.16	0.13
	30%	3.53	2.25	0.69
	60%	3.93	0.19	1.16
Male 1	10%	7.78	0.16	0.51
	30%	23.84	-	0.09
	60%	21.51	-	0.24
Male 2	10%	7.13	2.12	0.25
	30%	13.32	14.67	0.04
	60%	16.21	0.01	0.24
Male 3	10%	2.56	0.61	1.43
	30%	6.65	0.19	8.38
	60%	12.00	0.26	0.03

Table 41. Cross-sectional area (um^2) of mature oocytes in ascending female gonad tubule that were sectioned through the nucleus

*total number of mature oocytes for each female tubeworm tallied at bottom

Oocyte cross-sectional area (µm^2)									
High				Moderate			Low		
1	2	2(Cont'd)	3	1	2	3	1	2	3
2540.1	3478.6	3515.9	2721.6	-	3824.8	4231.5	4366.2	-	4469.8
3311.6	4003.1	2813.0	3724.8		3077.5	3411.7	3852.0		3779.7
4994.1	3198.8	4713.6	3556.2		3752.8	3512.9	3734.2		3630.7
5074.7	3437.1	3066.8	2759.1		3321.4	3897.2	4165.2		3603.1
2410.8	3225.8	3520.1	3745.1		3436.5	4079.5	4552.5		4193.3
3716.4	4320.5	3456.2	3159.5		2712.1	3223.3	2905.7		4246.3
3552.8	5447.0	2953.2	4805.6		3716.3	3684.0	4220.0		3721.8
4428.2	3929.6	4102.2	2685.5		3508.3	3592.5			4034.6
5414.8	3051.3	2838.1	3224.3		2278.1	4469.8			4642.1
3465.4	2489.9	3774.5	3208.5		3667.4	3779.7			4101.3
2214.1	1774.9	5010.4	3548.0		3837.8	3630.7			4206.9
3122.6	2669.8	3410.5	4078.5		3129.1	3603.1			4513.7
6038.0	2434.5	3535.0	2498.1		3130.9	4193.3			4495.5
4761.8	2376.3	3499.2	3323.9		3532.4	4246.3			4302.9
7715.9	2329.4	1274.3	2340.0		3509.5	3721.8			2839.1
4864.5	2039.6	2948.1	2268.8		3422.4	4034.6			5237.8
5128.0	2613.1	3011.4	2662.2		3224.9	4642.1			7344.6
3899.6	2912.3	4826.2	2170.4		2778.8	4101.3			2219.2
3189.4	2105.3	2690.7	3429.6		3246.6	4206.9			2460.2
3635.5	2441.1	4541.9	2590.1		3031.1	4513.7			5204.2
3323.0	2569.8	2968.1	2717.9		1293.8	4495.5			4417.4
3042.6	2029.6	3735.1	2829.1			4302.9			3692.7
3941.3	2145.9	2757.0	3516.5						4425.6
4266.7	2393.3	2984.7	2519.0						4160.9
3385.5	2274.9	3766.0	3278.5						3032.9
3396.8	3158.8	4130.0	3258.8						3097.7
3690.3	2665.5	2634.6	2116.8						4963.4
3993.7	3155.8	4516.7	2963.8						3743.0
2573.8	2546.8	2869.6	4013.1						4500.1
2803.9	2631.3		3091.9						4940.7
3163.0	2901.1		2118.0						2699.8
3671.0	2895.4		1003.0						4330.2
4334.1	2657.1		3795.1						3261.8
3994.0	2375.7		2257.3						3721.2
4380.1	2478.4		2722.8						2494.7
	2185.5		2655.8						3440.5
	2299.7		2410.8						
	1822.4		2565.9						
	3002.6		2259.1						
	3029.8		2989.6						
	3382.0		2312.1						
	4721.5		2721.3						
	4361.4		2593.2						
26		72	34	0	12	13	0	0	27