

# **Myxozoan Fish Disease Research and Monitoring**

## **Final Project Report**

January 30, 2013

Humboldt State University, Sponsored Programs Foundation

Project #2010-0501-001

*Prepared for:*

National Fish and Wildlife Foundation

1133 Fifteenth Street N.W., Suite 1100

Washington D.C. 20005

by

Margaret A. Wilzbach

California Cooperative Fish Research Unit

Humboldt State University

The following is a draft manuscript that is in review for publication by the journal *Freshwater Science* (as MS #12-140). The manuscript describes the results of the flume experiment we conducted to evaluate the impact of flow variation and substrate type on the dislodgement and survival of the freshwater polychaete, *Manayunkia speciosa*. The manuscript represents the final report for this project. It is co-authored with David M. Malakauskas (Michigan State University), Sarah J. Willson (California Cooperative Fish Research Unit), and Nicholas Som (U.S. Fish and Wildlife Service).

## Abstract

We quantified micro-scale flow forces and their ability to entrain the freshwater polychaete, *Manayunkia speciosa*, which is the intermediate host for two myxozoan parasites (*Ceratomyxa shasta* and *Parvicapsula minibicornis*) that cause substantial mortalities in salmonid fishes in the Pacific Northwest. In a laboratory flume, we measured the shear stress associated with two flow levels and three substrates and quantified associated dislodgement of polychaetes, evaluated survivorship of dislodged worms, and observed behavioral responses of the worms in response to increased flow. Logistic regression modeling was used to evaluate the probability of worm dislodgement for treatment combinations of velocity (mean flow velocity of 55 cm/s with a shear velocity of 3 cm/s, and mean flow velocity of 140 cm/s with a shear velocity of 5 cm/s) and substrate type (depositional sediments and analogues of rock faces and the filamentous alga, *Cladophora*). Few worms were dislodged at shear velocities below 3 cm/s on any substrate. Above this level of shear, probability of dislodgement was strongly affected by both substrate type and velocity. After accounting for substrate, odds of dislodgement were 6× greater at the higher flow. After accounting for velocity effects, probability of dislodgement was greatest from fine sediments, intermediate from rock faces, and negligible for *Cladophora*. Survivorship of dislodged polychaetes was high. Observations revealed that polychaetes exhibited a variety of behaviors for avoiding increases in flow, including extrusion of mucus, burrowing into sediments, and movement to lower flow microhabitats. Taken together, our findings suggest that polychaete populations likely exhibit high resiliency to flow-mediated disturbance events.

**Key words:** *Ceratomyxa*, *Parvicapsula*, shear, invertebrate drift, flow, Klamath

## Introduction

The dynamics of flow play an important role in the lives of lotic aquatic organisms (e.g., Craig 1990, Hart et al. 1996) and their community structure (e.g., Lake 1990, Giller 1991, Resh et al. 1988, Nelson and Lieberman 2002). Flows affect larval and adult dispersal, as well as movement of gametes, nutrients, food particles, and waste removal (Wotton 1988, Rudek et al. 1991, Gaylord and Gaines 2000), and flow variation greatly influences the morphology, physiology and behavior of lotic organisms (e.g., Statzner 1988, Craig 1990, Lancaster et al. 2006). High flows such as spates and floods, or fluctuating flows in the context of managed rivers, may lead to reorganization of river substrates and the associated benthos due to increased, and often variable, velocities and hydraulic forces (Troelstrup and Hergenrader 1990, Lake 2000). Resultant scour and fill patterns may redistribute organisms or may reduce populations (e.g., Giller et al. 1991, Cobb et al. 1992, Death 1996, Matthaei et al. 1997, Mosisch and Bunn 1997). As flow is a defining feature of riverine environments, invertebrates utilize a number of methods to cope with flow-mediated disturbance, including movements to low flow microhabitats (Lancaster 1999), where hydraulic forces are less severe and lower levels of disturbance are experienced. Examples of this behavior include seeking refuge in the hyporheic zone (Dole-Olivier et al. 1997), and entering the drift and swimming down toward the substrate in areas with reduced flow (Lancaster 1999). Selection of habitats with stable substrates or macrophytes may also be used as long-term strategies to reduce exposure to the effects of high flows (Richardson and Mackay 1991). Invertebrates have also developed specific morphological and behavioral adaptations including a streamlined body shape, physical attachment mechanisms (e.g., claws, suckers, hooks and mucus), formation of drag-minimizing colonies, the addition of ballast materials (e.g., mineral cases of some Trichoptera), and rheotaxis (Schnauder et al. 2010).

Most studies of invertebrate response to flow have quantified large scale flow and habitat descriptors such as mean velocity and mean depth (Davis and Barmuta 1989), along with substrate particle size and general ecological classifications of the preferences of species (Schnauder et al. 2010). While these coarse-scale measurements have proven useful in habitat models that are used for purposes such as river restoration (Gore et al. 1998), they offer limited insight into the near-bed flow microhabitat in which benthic invertebrates are found, which

usually differs significantly from macrohabitat flows. For example, flows near the substrate are typically slower as a result of interactions with substrate roughness or the presence of a boundary layer (Muschenheim et al. 1986, Vogel 1994). This necessitates fine-scale measurement of flows which can be difficult to obtain in the field. Therefore, one approach to measuring these fine-scale flow patterns is to use a laboratory flume which provides a controlled environment in which to quantify flow effects on substrates and aquatic biota (Muschenheim et al. 1986).

One way in which flows may affect invertebrate distributions is through direct dislodgement of the animal from the substrate. To date, relatively few studies have sought to directly quantify micro-level flow forces and their ability to dislodge invertebrates from substrates (e.g., Dorier and Vaillant 1953/1954, Dussart 1987, Schnauder et al. 2010). Subsequently, a large body of data concerning species-specific behaviors of benthic invertebrates relating to changes in near-bed hydraulics is lacking despite a need for such information (Lancaster 1999, Palmer et al. 1996). Studies which have sought to quantify these forces have typically concentrated on relatively large, common taxa such as *Ecdyonurus* (Dorier and Vaillant 1953/1954) and *Calopteryx* (Schnauder et al. 2010), but only coarse flow measures have been related to less-studied organisms such as Bryozoa (e.g., Mason et al. 1970), Oligochaeta (e.g., Marchese 1987), and Polychaeta (e.g., Stocking and Bartholomew 2007) which can be abundant in aquatic systems.

*Manayunkia speciosa* Leidy (Canalipalpa: Sabellidae) is a small freshwater polychaete that occurs in the Great Lakes and coastal areas of North America (Pettibone 1953, Hiltunen 1965, Mackie and Qadri 1971, Holmquist 1973, Rolan 1974, Spencer 1976, Brehm 1978, Croskery 1978, Carlton 1979). It is a habitat generalist, tolerating a broad range of environmental conditions in water chemistry (e.g., pH, dissolved oxygen), temperature, depth and mean velocity (Hiltunen 1965, Holmquist 1973, Poe and Stefan 1974, Rolan 1974). Within the Klamath River, the worm has been found to occupy a variety of substrates including depositional sediments, bedrock, woody debris, and macrophyte beds (Stocking and Bartholomew 2007, Willson et al. 2010). This benthic polychaete usually occupies a tube (Leidy 1883) except in cases of physical disturbance (Willson et al. 2010), and can be locally abundant with reported densities of over 40,000 individuals per m<sup>2</sup> (Pettibone 1953, Stocking and Bartholomew 2007). It has become an organism of increasing interest because it is an intermediate host for 2 salmonid parasites,

*Ceratomyxa shasta* Noble and *Parvicapsula minibicornis* Kent, which have recently caused substantial mortalities in wild and stocked juvenile salmonids within the Pacific Northwest, particularly within the lower Klamath River basin (Foott et al. 1999, 2004).

Herein we quantified shear stresses needed to dislodge the polychaete, *M. speciosa*, in a laboratory flume, from substrates similar to those on which the worm is found in the Klamath River basin of California and Oregon (USA), to facilitate future predictions of polychaete response to altered flows. We quantified *M. speciosa* substrate tenacity similar to the approach of Schnauder et al. (2010) to facilitate comparisons with values obtained for other freshwater lotic taxa, and we modeled probability of dislodging the polychaete as a function of velocity and substrate type. We also describe the behavioral responses of *M. speciosa* to varying flows and substrate types.

## **Methods**

### *Collection and culture of the polychaete*

Polychaetes attached to rocks from a location where *Manayunkia speciosa* (Fig. 1) had been found in previous years were collected from the Klamath River, CA, upstream of the Interstate 5 Rest Area (41° 51' 35.8" N, 122° 34' 00.1" W). Rocks were placed in coolers with ice and river water and transported back to the Humboldt State University fish hatchery. The polychaetes were maintained in culture facilities described in Willson et al. (2010). Collections occurred in late June and late October of 2011. Prior to the start of a trial, rocks were brought into the lab and visually scanned under a dissection microscope to locate and remove individual polychaetes. The polychaetes were then placed in small crystallizing dishes in an aerated 5 gallon aquarium until they were needed for use. The aquarium was kept at an ambient temperature of 20 °C, and weekly water changes were made using water from the fish hatchery.

### *Flume description*

An experimental flume modified from Vogel and LaBarbera (1978), measuring 2.6 m in length, 1.35 m in height and 0.44 m in width, was constructed in-house at Humboldt State University for use in experimental trials (Fig. 2). The flume was a closed-circuit, recirculating system, with the channel measuring 43 cm wide, by 26 cm high, by 175 cm long internally. Water was returned through a loop of 20.3 cm PVC pipe of approximately 5.3 m in length. The water was moved by 2 propellers on a shaft that was powered by a 2 HP, 3 phase inverter duty AC induction motor. In order to decrease large-scale turbulence, a collimator 35 mm in length was made from parallel sheets of 1.3 cm egg crate light diffuser and placed upstream of the center of the flume. The flume was filled with dechlorinated tap water and kept at ambient indoor temperatures of 18 °C for all trials.

### *Experimental trials*

Polychaete dislodgement and behavioral responses to increases in 2 mean flow velocities were evaluated in separate trials using 3 different substrates. For trials, worms were placed on a substrate which was then placed in standing water in the flume and water was gradually increased to a mean velocity of 55 cm/s or 140 cm/s. Three substrate types were tested: fine sediments, and analogues of rock faces and the filamentous green alga *Cladophora*. Five trials were conducted for each of the 6 combinations of substrate and velocity level. We also conducted 5 additional trials for each velocity—substrate combination to control for the effects of polychaete handling and laboratory conditions. In control trials, polychaetes were left in standing water for the entire duration of the experiment.

Twenty polychaetes, ranging in length from 2-3 mm, were used once per trial. A trial included a 30 min period in which the open channel velocity was increased to its terminal level, followed by a 45 min period of polychaete exposure to the test velocity. Trials were conducted during daylight hours, with overhead fluorescent lighting. Polychaete dislodgment was measured as the percentage of individuals remaining on the substrate at the end of a trial. Polychaete behaviors, including tube-building, feeding, movements on or off of substrates, direction of movements, and response to collisions of entrained sediments were observed and recorded throughout each trial.

Test velocities and substrate types were chosen to simulate polychaete habitats in the Klamath River, and were also influenced by results of pilot trials. The low flow level was set after observing that polychaetes were not dislodged from any substrate type in the flume at a mean velocity below 55 cm/s. The high flow level was the maximum working mean velocity that we were able to produce in the channel. Both velocities would likely only be able to occur in swift runs or riffles. Three of the most common substrates in which polychaetes have been collected in the Klamath River include beds of *Cladophora* or vascular macrophytes, organically enriched fine sediments, and large, stable substrates such as boulders or large woody debris (Stocking and Bartholomew 2007, Willson et al. 2010, Malakauskas and Wilzbach 2012). Establishing live plants in the flumes would have been difficult and the flumes were not large enough to accommodate boulders, thus we developed analogues of these to use for the trials. In preliminary trials we also experimented with the use of aquarium gravel (D50 = 4.5 mm) as a substrate, and discarded this as a treatment choice after finding that the polychaetes immediately abandoned it.

Rock faces were mimicked with unglazed ceramic tiles measuring 15 cm × 15 cm. *Cladophora* was mimicked with square finishing pads of synthetic fibers (3M, St. Paul, Minnesota), hereafter referred to as “pseudophora” (Fig. 3). Pseudophora squares were cut to be 6.25 cm<sup>2</sup> and 2 mm high and were affixed to the center of a tile with Deftane polyurethane (Deft, Inc., Irvine, California). Depositional sediments and fine benthic organic matter (FBOM) used in the trials were obtained from Fern Lake (40° 52' 29.4" N, 124° 04' 25.9" W), on the Humboldt State University campus. Sediments were filtered through a 500 µm screen and stored dry until use (Willson et al. 2010).

Preliminary trials established that *M. speciosa* would not affix to any substrate if some fine particulate organic matter was not also present, and we thus added approximately 0.5 cm<sup>3</sup> of FBOM to the center of each of the substrate types. After preparation, all substrates were placed in plastic containers measuring 25 cm × 20 cm and 8 cm in height filled with hatchery water. Worms were removed from their tubes, as preliminary trials showed that this facilitated attachment to substrates, and then placed on the center of each substrate. After 1 h had elapsed, an air bubbler was added to each container. Polychaetes then acclimated for 24 h prior to the start of a trial, during which time all tubes were reconstructed. Before substrates were placed in the flume, worms were again counted to ensure that all 20 individuals were present.



For trials conducted on tile and pseudophora substrates, the substrates were removed from their containers and were placed into a 15 cm × 15 cm trench cut into a 1 cm thick sheet of foam that was placed on the bottom of the flume. This prevented separation of flow around an isolated tile. For sediment trials, 2 pieces of foam, painted with polyurethane and coated with sediment, were placed flat on the bottom of the channel in series, with a 15 cm gap between them. An acrylic tray of sediment, measuring 15 cm × 15 cm × 1 cm, was placed in the space between the foam sheets, and aquarium gravel and sediment were used to fill the spaces between the tray and the channel walls. This was done to reduce the amount of sediment and gravel entrained in the flow.

Following an experimental trial, the substrate was removed from the flume and returned to a plastic container with air bubblers for 48 h at 20 °C, after which worms on the substrates were counted and mortalities were assessed. Additionally, sediment that was scoured during the course of the sediment trials was collected from the flume, using a turkey baster and plastic spoon. Sediment was examined to locate worms, and these polychaetes were also set aside for 48 h before mortalities were evaluated.

Velocity measurements were made during a trial to allow us to characterize and compare open channel and near-bed flows at the test velocities. Following the gradual increase in channel velocity over 30 min to the terminal velocity, we waited 10 min for flows to stabilize before taking velocity readings. All velocity measurements were made using a SonTek (SP-AV10M01) Acoustic Doppler Velocimeter (ADV) (SonTek/YSI, San Diego, California) which was mounted above the center of the flume, directly over the polychaetes. Seventeen measurements were taken at 5 mm intervals above the bed for each trial to obtain a velocity profile and

Reynolds stress ( $\overline{u'w'}$ ) for use in calculations such as determining shear velocity. At each height, 3-dimensional flow velocity measurements were sampled at a rate of 10 Hz over a period of 45 s. The high flow setting resulted in a surface wave and flow separation around the probe, leading to encapsulation of the probe head by air. As a result, measurements could not be obtained above a height of about 4.5 cm. However, sufficient data points were obtained to perform a regression and to make calculations with respect to flows experienced by *M. speciosa*.

A Reynolds stress profile was obtained using temporally averaged velocity fluctuations in the streamwise ( $u'$ ) and vertical directions ( $w'$ ) at 9 heights (5 runs at each velocity-substrate combination) above the bed. A regression was then used to estimate Reynolds stress at the bed, and the shear velocity was calculated:

$$U_* = \sqrt{-\overline{u'w'}}$$

where  $U_*$  is shear velocity. Authors have used multiple methods to express velocities experienced by invertebrates, including measurements at a height of 5 mm above the bed. Therefore, we also calculated velocities at 5 mm above the bed to facilitate comparison with other published values. A least squares regression of  $\ln z$  against  $U$  was used to calculate  $U$  at a height of 5 mm.

We used the Reynolds number ( $Re$ ) to characterize the mean flow. This dimensionless value is a ratio of inertial to viscous forces (Vogel 1994) and is calculated as:

$$Re = \frac{UR_h}{\nu}$$

where  $U$  is the mean velocity,  $R_h$  is the hydraulic radius of the flume, and  $\nu$  is the kinematic viscosity of water. Reynolds numbers greater than 2000 indicate turbulent flows while values of less than 500 indicate laminar flows. A regression of velocity against height, using data from velocity profiles obtained at nine mean velocities (ranging from 14 cm/s to 125 cm/s) was used to estimate a mean velocity for calculation of a Reynolds number at the high flow setting.

In addition, we also characterized the effective drift velocity and the body length Reynolds numbers at the low and high velocity settings. Effective drift velocity represents the

average flow experienced by invertebrates (Schnauder et al. 2010) and has a direct relationship with drag forces acting upon the body of the organisms:

$$U_{\perp,d} = \frac{\int_0^h U(z) dz}{h}$$

where  $h$  is the average height of the invertebrate. The effective drift velocity in turn was used to calculate the body length Reynolds number:

$$Re_l = \frac{U_{\perp,d} l}{\nu}$$

where  $l$  is the length of the invertebrate. Here, this represented the average tube width of an individual worm. As some worms formed aggregates of tubes, the length the tube mass was also used as a length. Body length Reynolds numbers are typically below 10 for lotic invertebrate larvae and over 1000 for adult forms (Statzner 1988). As  $Re_l$  decreases, drag forces become proportionately greater than lift forces (Vogel 1994), with drag forces being typically more important for benthic, lotic invertebrates (Statzner 1988).

### *Statistical analyses*

We modeled worm dislodgement as a binomial random variable,  $y_i \sim Bin(m_i, \pi_i)$ , where  $y_i$  is the number of worms dislodged given  $m_i$  worms available, and  $\pi_i$  is the probability of dislodgement. To assess the effects of treatments on dislodgement probability, we used a binomial generalized linear model with a logit link function (Myers 1990), where

$$\text{logit}(\pi_i) = \boldsymbol{\chi}\boldsymbol{\beta}$$

and  $\boldsymbol{\chi}$  is a design matrix of treatment levels and  $\boldsymbol{\beta}$  is a vector of regression coefficients. We assessed the goodness of fit for our model via deviance  $\chi^2$  tests, and assessed the significance of treatment effects via drop-in-deviance  $\chi^2$  tests. Analyses were conducted using R statistical software, version 2.15 (R Development Core Team, Vienna, Austria).

## Results

### *Probability of dislodgement*

Observed variation in polychaete dislodgement, within combinations of the velocity and substrate treatments, could not be attributed to random polychaete behavior or laboratory conditions, as all control trials for both the low and high velocity trials exhibited no polychaete dislodgement, in contrast to observed dislodgement in all non-control trials at both velocities. The odds of dislodgement were greater in non-control trials than in controls ( $p = 0.0004$ ) on tiles. The odds of dislodgement in the non-control trials were estimated to be 22.6 (95% CI: 5.6 to 90.6× higher) times higher than control trials, after accounting for the effects of velocity. On pseudophora substrate, observed polychaete dislodgement probabilities among the control and non-control trials were nearly identical (Fig. 4) at each velocity level. Though this condition precluded the separation of observed dislodgement on the pseudophora substrate from random chance or laboratory conditions in the strictest sense, the low levels of dislodgement on pseudophora substrates overall warranted their inclusion in a broader analysis of substrate and velocity effects on the probability of dislodgement.

Lack of variation at the 0% and 100% levels for the low velocity pseudophora and high velocity sediment trials, respectively, precluded their inclusion in standard logistic regression models and our ability to assign standard errors to their estimates. We proceeded by reporting their observed

estimates without standard error estimates, and fit the logistic regression models on the remaining data. Odds of dislodgement were associated with substrate type ( $p < 0.0001$ ) and velocity ( $p < 0.0001$ ). After accounting for substrate, the odds of dislodgement at high velocity were estimated to be 6 times higher than at low velocity (95% CI: 3 to 11.6× higher). Relative to pseudophora substrates, which showed low probabilities of dislodgement comparable to chance alone as described above, the odds of dislodgement on tile substrates were estimated to be 167× higher (95%CI: 22 to 1257× higher), and the odds of dislodgement on sediment substrates were estimated to be 2452× higher (95% CI: 299 to over 20,000× higher), after accounting for velocity effects. These stark odds differences reflect the gradient in proportion of dislodgement from Fig. 4, and were further bolstered by the observed 0% dislodgement and 100% dislodgement at the low-velocity/pseudophora and high-velocity/sediment treatment combinations, respectively (Table 1).

#### *Flows and polychaete response*

Shear velocities averaged 2.3 cm/s at the low flow level and 4.7 cm/s at the high flow level. Flows at both velocities exceeded the threshold value of 2000, and were turbulent, with Reynolds numbers of  $6 \times 10^5$  and  $1.6 \times 10^6$  for low and high velocities respectively. Shear velocities were higher for trials conducted on fine sediments compared to other substrates which may reflect the influence of the gravel sublayer (Table 2). Actual shear stress for a homogeneous layer of sand would likely be similar to those found for the other substrates at the low velocity trials. Average velocity of the flow field around the polychaetes (i.e., the effective drift velocity) was 38.3 cm/s at the low flow level and 103.7 cm/s at the high flow level. Body-length Reynolds numbers were all greater than 100, which combined with high mean flow Reynolds numbers, indicated that the worms were affected to a greater extent by pressure drag than by lift forces.

Isolated worms were observed to experience the least dislodgement within tile trials, while worms that were in ball-shaped masses experienced the greatest dislodgement as would be expected due to increased drag. Additionally, some worms were lost immediately after velocity increases despite efforts to slowly increase velocities. Worms within the pseudophora remained affixed to the substrate even when fine sand and FBOM was scoured out from around them.

Polychaetes from the sediment trials were typically scoured as a group of worms as the mucus trails that they made while burrowing formed a matrix that stabilized the substrate around the colony. This stabilization was evident as sediment was scoured from around the upstream and lateral sides of the mucus matrix before the actual worm colony was dislodged. Worms that were not scoured from the sediment at the low velocity were often affixed to the heavier gravel under the sediment.

#### *Survivorship of dislodged polychaetes*

No mortalities occurred among worms that remained on substrates after velocity trials (total  $n = 198$ ). Forty-nine percent ( $n = 56$ ) of worms were recovered from scoured sediments and 98% survived. Most worms recovered were no longer in tubes, though it could not be determined if this was a direct result of dislodgement, or a result of handling. Additionally, 3 tubes without worms were recovered from scratches in the acrylic channel sides at the end of trials.

#### *General behavioral observations*

Polychaetes in the lab constructed tubes that were similar to those constructed by worms found in depositional areas in the Klamath River. Materials used in tube construction were most coarse near the proximal end (the base), and often had particles of reduced or very fine size diameters in the distal section. Three distinct sections were thus observed in polychaete tubes: a loose base of mucus and relatively more coarse organic and inorganic particles (generally  $<500 \mu\text{m}$ ), a middle section with relatively intermediate organic particles ( $<250 \mu\text{m}$ ), and a distal section with very fine ( $<5 \mu\text{m}$ ), regular particles. Individual particles from the very fine, distal section were individually distinguishable only under  $400\times$  magnification.

*M. speciosa* did not colonize any substrate that lacked FBOM. Polychaetes that were deposited on the tile without FBOM generally responded by rolling on the tile for a short time, after which the worms either elevated their posterior ends away from the substrate, while moving them in a circular or back and forth direction, or immediately began to move away from the

center of the tile. Those that moved away from the center of the tile often ended up off of the tile or underneath it. This basic set of behaviors was observed on other substrates as well, including gravel in preliminary trials that lacked FBOM. When deposited on a tile containing a thin layer of FBOM, worms rolled through it, coating their bodies. If too little FBOM was available, worms generally rolled into each other and formed a ball-shaped mass. If sufficient FBOM was present, worms built individual tubes. Worms added to pseudophora substrates without FBOM burrowed down into the substrate until they reached the tile, and then moved away from the center of the tile. Worms that were added to the sediment substrate immediately burrowed into the substrate, and often immediately made a tube. A portion of the worms also moved through the sediment leaving tracks of mucus before constructing a tube.

Extension of tentacles into the water column was not observed to be directly affected by flow at the low velocity. However, when worm tubes were subject to collision from inorganic or organic particles in the water, or buffeted sufficiently by the flow at the high velocity, worms retreated into their tubes. If collisions ceased, worms once again extended their tentacles into the water column after a lapse of several minutes. In trials on tiles, worms exhibited a variety of behaviors in response to increasing flows. These included retreat into their tubes, tube evacuation, and movement away from the center of the tile, and eventually underneath it. This movement was either in a direction perpendicular to the mean direction of flow or in the downstream direction. Worms were never observed moving upstream in response to flow. A small number (estimated at 10 to 20%) of worms that evacuated their tubes were also observed to move downstream on extruded strands of mucus, the length of which increased over time. The extrusion of mucus allowed some individuals to move in suspension to the end of the tile where they reattached themselves and then moved underneath it. In some cases, worms reattached themselves to the tile multiple times as they proceeded downstream. A few worms were lost to the current while hanging from mucus strands.

## **Discussion**

Our findings suggest that the high flows in this study influence *M. speciosa* distribution directly by restricting habitable substrates to those with greater stability and texture and by preventing

attachment of the polychaete to substrates in extreme flow environments. However, the worm exhibits attachment abilities comparable to taxa found in higher gradient lotic reaches, and are therefore likely to tolerate a wide range of velocities, provided sufficiently stable substrates for attachment. This polychaete also exhibits behaviors in response to changes in flow including movement to areas of lower velocity which may minimize effects on individuals during times of higher flows. As polychaetes on highly mobile substrates are more likely to be displaced than polychaetes on stable substrates, predictions of *M. speciosa* response to altered flows must therefore consider the relative proportions of the population which occupy each type of habitat, which may differ among systems depending upon such potential factors as food availability, competition, predation, and patterns of dispersal.

Shear velocities faced by the benthic polychaetes under test velocities were comparable to other near-bed values reported in the literature. Our low flow trials produced shear velocities that were comparable to the higher shear velocities recorded by Schnauder et al. (2010), who tested tenacity of a collection of invertebrates from a regulated, lowland river with a sandy bed in an experimental flume. Specifically, *M. speciosa* that were dislodged at the lower flow had a similar tenacity to that of *Gomphus vulgatissimus* (Odonata: Gomphidae) and *Bithynia tentacula* (Neotaenioglossa: Bithyniidae), which are both typically found in slower waters with depositional substrates. *M. speciosa* that remained affixed in the higher flow trials had a comparable tenacity to the Tricladida and *Goera pilosa* (Trichoptera: Goeridae), and a greater tenacity than many snails and dragonflies collected from streams with relatively steep grades and coarse substrates (Statzner et al. 1988). These findings suggest that this polychaete species is able to tolerate lotic waters with all but the swiftest flows, and that it has evolved to tolerate conditions found in the Klamath River. A low body-length Reynolds number for *M. speciosa* individuals fell within the typical range of small, larval invertebrates (Statzner 1988) due to its tube morphology. This indicates drag forces acting on the polychaete dominate over lift forces, and may explain why polychaete tubes appear to be longer and more conspicuous only later in the season in the Klamath River, when flows are lower. Tube length may also be related to size of the polychaetes, with larger worms building larger tubes, however we observed that variation in tube sizes among individuals of different size classes was less pronounced than variation throughout the year. The tube shape of this polychaete may also be an adaptation to the worm's low mobility while in its tube and to the multidirectional, swirling flows in which it is often



found. A cylindrical shape offers a consistent profile to flow regardless of direction within the transverse flow plane.

The proportion of polychaetes dislodged was a function of the interaction between flows and substrate type. The greatest detachment occurred in the sediment at both flow levels while little detachment occurred in the pseudophora substrate at either flow level. This supports the hypothesis that stable substrates form the primary habitat of *M. speciosa* (Stocking and Bartholomew 2007, Malakauskas and Wilzbach 2012). This is also consistent with findings of habitat preferences for suspension feeding lotic invertebrates in general (Richardson and Mackay 1991), which must live in flows sufficiently high to deliver food items, but which must avoid being detached from the substrate. Macrophytes and *Cladophora* housing polychaetes likely serve to buffer the worms against catastrophic flooding due to the reduced current velocities contained within (sensu Nikora et al. 1998). Macrophytes and *Cladophora* also trap fine benthic organic matter (FBOM) (Madsen et al. 2001) which worms feed on and use for construction of their tubes. Polychaete colonies themselves may also serve to alter local near-bed flows resulting in increased stabilization of substrates and deposition of sediments, including fine particulate organic matter (FPOM) (e.g., Daro and Polk 1973, Jumars and Nowell 1984, Frithsen and Doering 1986). Aggregates of closely-spaced tubes have been observed to lead to skimming flows (Friedrichs et al. 2000), a condition in which the maximum turbulent kinetic energy and shear stress occurs above the bed (Nowell and Church 1979). Taken together with the mucus-related sediment stabilization we observed, this may suggest that polychaete resistance to dislodgement may be higher in the river than our laboratory findings when polychaetes occur in higher numbers. This flow alteration also likely leads to increased deposition of sediments, bringing additional FPOM to the polychaetes.

Estimated probability of dislodgement of polychaetes from the tile substrate was 28% and 70% under low and high velocities, respectively, and was intermediate to associated probabilities of detachment from the sediment and pseudophora substrates. Tiles probably most accurately tested actual tenacity of individual worms without the influence of the highly moveable sediments and flow buffering of the pseudophora. However, the tile substrate proved to be the most challenging when attempting to induce polychaete attachment and some worms were likely unable to fully attach before our trials. Aggregates of polychaetes exhibited greater surface area,

and higher body-length Reynolds numbers, and therefore experienced greater drag and lift forces. When considering an unglazed tile as a representation of a rock substrate, it must also be considered that the tile has less roughness than many natural rocks on which *M. speciosa* is found, and therefore provides less surface area for mucus attachment. Surfaces of greater roughness have been shown to allow for greater invertebrate tenacity (Richardson and Mackay 1991, Lau and Martinez 2003). The lack of a biofilm on the tiles may have also been a factor since some invertebrates have enhanced attachment abilities in the presence of a biofilm (Zardus et al. 2008). In addition to potentially greater attachment abilities on natural rocks, *M. speciosa* were commonly observed to occur within rock crevices or interstices in the river. Flow over these depressions can be classified as skimming when individual roughness elements are of sufficient height and density (Morris 1955). We commonly observed polychaetes to be locally distributed around boulders in the river. Boulders experience significantly different hydraulic forces over the entirety of their surfaces based on boulder morphology, and orientation, and this effect has been shown to influence invertebrate community structure (Bouckaert and Davis 1998). Combined, these factors suggest the dislodgement we observed on this substrate may underestimate polychaete tenacity in a natural setting.

Polychaetes exhibited unexpected behavioral plasticity in response to increased flows as tube-dwelling polychaetes are typically considered to be sessile; invertebrates which have that have a greater ability to resist dislodgement often do so at the expense of mobility (Martinez 2001). In addition to retreating deeper into their tubes and burrowing into sediments in response to flow-mediated disturbance, *M. speciosa* exhibited more active flow-avoidance behaviors. Worms were observed to evacuate their tubes at higher flows while on the tile substrate, and relocated under the tile. The burrowing into sediments and retreat under the tiles suggests that these worms may actively seek out low flow microhabitats during periods of high flows in nature, similarly to other lotic invertebrates (e.g., Marmonier and Cruzé des Châtelliers 1991, Dole-Olivier et al. 1997, Lancaster 1999). If true, it is then also possible that *M. speciosa* actively chooses appropriate micro-flow habitats in which to build a tube for initial settlement during more stable conditions. However, it is unlikely that relocation would be frequent during the low flow season as females brood young in their tubes, and worms experience increased predation when outside of their tubes (Willson et al. 2010). Polychaetes were also able to cope with flows by trailing downstream on extruded strands of mucus. A number of lotic invertebrate larvae,

including hydropsychid and polycentropid caddisflies, and simuliid blackflies, trail downstream on silk threads in a similar manner in order to enhance their settling abilities (Rader 1997).

Mortalities among worms that had experienced high flows or had been dislodged from substrates were extremely limited indicating that *M. speciosa* is unlikely to suffer significant mortalities as a direct result of flow alterations in a natural system. It is therefore likely that potential flow-mediated mortalities are a result of settlement in suboptimal habitats as has been observed in other polychaetes (Zamorano et al. 1995). Worms that were collected after being scoured from the sediment substrate were often found outside of their tubes making them more susceptible to predation. *M. speciosa* in the Klamath River have not been found in anoxic substrates (Stocking and Bartholomew 2007) and it is likely that worms settling in these habitats would also experience greater mortalities. Conversely, it is also possible that flow-mediated dislodgement may offer a means of dispersal, as we observed several polychaete tubes stuck to scratches in the acrylic walls of our flume channel after high flow trials, although none of these tubes contained worms.

## References

- Bouckaert, F. W., and J. Davis. 1998. Microflow regimes and the distribution of macroinvertebrates around stream boulders. *Freshwater Biology* 40:77-86.
- Brehm, W. T. 1978. First Gulf of Mexico coast record of *Manayunkia speciosa* (Polychaeta: Sabellidae). *Northeast Gulf Science* 2:73-75.
- Carlton, J. T. 1979 (issued). Introduced invertebrates of San Francisco Bay. Fifty-eighth annual meeting of the Pacific Division of the American Association for the Advancement of Science, San Francisco State University, San Francisco, California, June 12-16, 1977. Pacific Division of the American Association for the Advancement of Science c/o California Academy of Sciences Golden Gate Park San Francisco.
- Cobb, D. G., T. D. Galloway, and J. F. Flanagan. 1992. Effects of discharge and substrate stability on density and species composition of stream insects. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1788-1795.

- Craig, D. A. 1990. Behavioral hydrodynamics of *Cloeon dipterum* larvae (Ephemeroptera: Baetidae). *Journal of the North American Benthological Society* 9:346-357.
- Croskery, P. 1978. The freshwater co-occurrence of *Eurytemora affinis* (Copepoda: Calanoida) and *Manayunkia speciosa* (Annelida: Polychaeta): possible relicts of a marine incursion. *Hydrobiologia* 59:237-241.
- Daro, M. H., and P. Polk. 1973. The autoecology of *Polydora ciliate* along the Belgian coast. *Netherlands Journal of Sea Research* 6:130-140.
- Davis, J. A., and L. A. Barmuta. 1989. An ecologically useful classification of mean and near-bed flows in streams and river. *Freshwater Biology* 21:271-282.
- Death, R. G. 1996. The effect of patch disturbance on stream invertebrate community structure: the influence of disturbance history. *Oecologia* 108:567-576.
- Dole-Olivier, M. -J., P. Marmonier, and J. -L. Beffy. 1997. Response of invertebrates to lotic disturbance: is the hyporheic zone a patchy refugium? *Freshwater Biology* 37:257-276.
- Dorier, A., and F. Vaillant. 1953/1954. Observations et experiences relatives à la résistance au courant de divers invertébrés aquatiques. Extrait de la *Laboratoire de Hydrobiologie et Pisciculture Grenoble* 45/46:9-31.
- Dussart, G. B. J. 1987. Effects of water flow on the detachment of some aquatic pulmonate gastropods. *American Malacological Bulletin* 5:65-72.
- Foott, J. S., J. D. Williamson, and K. C. True. 1999. Health, physiology, and migrational characteristics of Iron Gate Hatchery Chinook, 1995 Releases. U.S. Fish and Wildlife Service, California-Nevada Fish Health Center. Anderson, California.
- Foott, J. S., R. Harmon, and R. Stone. 2004. Effect of water temperature on non-specific immune function and Ceratomyxosis in juvenile Chinook salmon and steelhead from the Klamath River. *California Fish and Game* 90:71-84.
- Friedrichs, M., G. Graf, and B. Springer. 2000. Skimming flow induced over a simulated polychaete tube lawn at low populations densities. *Marine Ecology Progress Series* 192:219-228.

Frithsen, J. B., and P. H. Doering. 1986. Active enhancement of particle removal from the water column by tentaculate benthic polychaetes. *25*:169-182.

Gaylord, B., and S. D. Gaines. 2000. Temperature or transport? Range limits in marine species mediated solely by flow. *The American Naturalist* *155*:769-789.

Giller, P. S., N. Sangpraduh, and H. Towney. 1991. Catastrophic flooding and macroinvertebrate community structure. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* *24*:1724-1729.

Gore, J. A., D. J. Crawford, and D. S. Addison. 1998. An analysis of artificial riffles and enhancement of benthic community diversity by physical habitat simulation (PHABSIM) and direct observation. *Regulated Rivers: Research and Management* *14*:69-77.

Hart, D. D., B. D. Clark, and A. Jasentuliyana. 1996. Fine-scale field measurement of benthic flow environments inhabited by stream invertebrates. *Limnology and Oceanography* *41*:297-308.

Hiltunen J. K. 1965. Distribution and abundance of the polychaete, *Manayunkia speciosa* Leidy, in western Lake Erie. *Ohio Journal of Science* *65*:183-185.

Holmquist, C. H. 1973. Fresh-water polychaete worms of Alaska with notes on the anatomy of *Manayunkia speciosa* Leidy. *Zoologische Jahrbücher Abteilung für Systematik* *100*:497-516.

Jumars, P. A., and A. R. M. Nowell. 1984. Effects of benthos on sediment transport: difficulties with functional grouping. *Continental Shelf Research* *3*:115-130.

Lake, P. S. 1990. Disturbing hard and soft bottom communities: a comparison of marine and freshwater environments. *Australian Journal of Ecology* *15*:477-488.

Lake, P. S. 2000. Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society* *19*:573-592.

Lancaster, J. 1999. Small-scale movements of lotic macroinvertebrates with variations in flow. *Freshwater Biology* *41*:605-619.

Lancaster, J., T. Buffin-Belanger, I. Reid, and S. Rice. 2006. Flow- and substratum-mediated movement by a stream insect. *Freshwater Biology* *51*:1053-1069.

- Lau, W. W. Y., and M. M. Martinez. 2003. Getting a grip on the intertidal: flow microhabitat and substratum type determine the dislodgement of the crab *Pachygrapsus crassipes* (Randall) on rocky shores and in estuaries. *Journal of Experimental Marine Biology and Ecology* 295:1-21.
- Leidy, J. 1883. *Manayunkia speciosa*. *Proceedings of the Academy of Natural Sciences in Philadelphia* 35:204-212, Pl.IX.
- Mackie, G. L., and S. U. Qadri. 1971. A polychaete, *Manayunkia speciosa*, from the Ottawa River, and its North American distribution. *Canadian Journal of Zoology* 49:780-782.
- Madsen, J. D., P. A. Chambers, W. F. James, E. W. Koch, and D. F. Westlake. 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia* 444:71-84.
- Malakauskas, D.M. and M.A. Wilzbach. 2012. Invertebrate assemblages in the lower Klamath River, with reference to *Manayunkia speciosa*. *California Fish and Game* 98(4), *in press*.
- Marchese, M. R. 1987. The ecology of some benthic Oligochaeta from the Paraná River, Argentina. *Hydrobiologia* 155:209-214.
- Marmonier, P., and M. Cruézé des Châtelliers. 1991. Effects of spates on interstitial assemblages of the Rhône River. Importance of spatial heterogeneity. *Hydrobiologia* 210:243-251.
- Martinez, M. M. 2001. Running in the surf: hydrodynamics of the shore crab *Grapsus tenuicrustatus*. *Journal of Experimental Biology* 201:3097-3112.
- Mason, W. T., J. B. Anderson, R. D. Kreis, and W. C. Johnson. 1970. Artificial substrate sampling, macroinvertebrates in a polluted reach of the Klamath River, Oregon. *Journal (Water Pollution Control Federation)* 42:R315-R328.
- Matthaei, C. D., U. Uehlinger, and A. Frutiger. 1997. Response of benthic invertebrates to natural versus experimental disturbance in a Swiss prealpine river. *Freshwater Biology* 37:61-77.
- Morris, W. M. 1955. A new concept of flow in rough conduits. *Transactions of the American Society of Civil Engineers* 120:373-98.

- Mosisch, T. D., and S. E. Bunn. 1997. Temporal patterns of rainforest epilithic algae in relation to flow-related disturbance. *Aquatic Botany* 58:181-193.
- Muschenheim, D. K., J. Grant and E. L. Mills. 1986. Flumes for benthic ecologists: theory, construction and practice. *Marine Ecology Progress Series* 28:185-196.
- Myers, R. H. 1990. *Classical and modern regression with applications*. Duxbury: Pacific Grove, CA.
- Nelson, S. M., and D. M. Lieberman. 2002. The influence of flow and other environmental factors on benthic invertebrates in the Sacramento River, U.S.A. *Hydrobiologia* 489:117-129.
- Nikora, V. I., A. M. Suren, S. L. R. Brown, and B. J. F. Biggs. 1998. The effects of the moss *Fissidens rigidulus* (Fissidentaceae: Musci) on near-bed flow structure in an experimental cobble bed flume. *Limnology and Oceanography* 43:1321-1331.
- Nowell, A. R. M., and M. Church. 1979. Turbulent flow in a depth-limited boundary layer. *Journal of Geophysical Research* 84:4816-4824.
- Palmer, M. A., J. D. Allan, and C. A. Butman. 1996. Dispersal as a regional process affecting the local dynamics of marine and stream benthic invertebrates. *Trends in Ecology and Evolution* 11:322-326.
- Pettibone, M. H. 1953. Fresh-water polychaetous annelid, *Manayunkia speciosa* Leidy, from Lake Erie. *Biological Bulletin* 105:149-153.
- Poe, T. P., and D. C. Stefan. 1974. Several environmental factors influencing the distribution of the freshwater polychaete, *Manayunkia speciosa* Leidy. *Chesapeake Science* 15:235-237.
- Rader, R. B. 1997. A functional classification of the drift: traits that influence invertebrate availability to salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* 54:1211-1234.
- Resh, V. H., A. V. Brown, A. P. Covich, M. E. Gurtz, H. W. Li, G. W. Minshall, S. R. Reice, A. L. Sheldon, J. B. Wallace, and R. Wissmar. 1988. The role of disturbance in stream ecology. *Journal of the North American Benthological Society* 7:433-455.

- Richardson, J. S., and R. J. Mackay. 1991. Lake outlets and the distribution of filter feeders: an assessment of hypotheses. *Oikos* 62:370-380.
- Rolan, R. G. 1974. The fresh-water polychaete, *Manayunkia speciosa*, in a thermal discharge channel, Cleveland Harbor, Lake Erie. *American Midland Naturalist* 92:207-213.
- Rudek, J., H. W. Paerl, M. A. Mallin, and P. W. Bates. 1991. Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. *Marine Ecology Progress Series* 75:133-142.
- Schnauder, I., S. Rudnick, and X. F. Garcia. 2010. Incipient motion and drift of benthic invertebrates in boundary shear layers. Pages 1453-1461 in Dittrich, A., Ka. Koll, J. Aberle and P. Geisenhainer (editors). *River Flow 2010 - Proceedings of the International Conference on Fluvial Hydraulics*. Braunschweig, Germany.
- Spencer, D. R. 1976. Occurrence of *Manayunkia speciosa* (Polychaeta: Sabellidae) in Cayuga Lake, New York, with additional notes on its North American distribution. *Transaction of the American Microscopical Society* 95:127-128.
- Statzner, B. 1988. Growth and Reynolds number of lotic macroinvertebrates: a problem for adaptation of shape to drag. *Oikos* 51:84-87.
- Statzner, B., J. A. Gore, and V. H. Resh. 1988. Hydraulic stream ecology: observed patterns and potential applications. *Journal of the North American Benthological Society* 7:307-360.
- Stocking, R. W., and J. L. Bartholomew. 2007. Distribution and habitat characteristics of *Manayunkia speciosa* and infection prevalence with the parasite *Ceratomyxa shasta* in the Klamath River, Oregon–California. *Journal of Parasitology* 93:78–88.
- Troelstrup, N. H., and G. L. Hergenrader. 1990. Effect of hydropower peaking flow fluctuations on community structure and feeding guilds of invertebrates colonizing artificial substrates in a large impounded river. *Hydrobiologia* 199:217-228.
- Vogel, S. 1994. *Life in moving fluids*. Princeton University Press, Princeton, New Jersey.



Vogel, S., and M. LaBarbera. 1978. Simple flow tanks for research and teaching. *BioScience* 28:638-643.

Wotton, R. S. 1988. Dissolved organic material and trophic dynamics. *BioScience* 38:172-178.

Willson, S. J., M. A. Wilzbach, D. M. Malakauskas, and K. W. Cummins. 2010. Lab rearing of a freshwater polychaete (*Manayunkia speciosa*, Sabellidae) host for salmon pathogens. *Northwest Science* 84:183-191.

Zamorano, J. H., C. A. Moreno, and W. E. Duarte. 1995. Post-settlement mortality in *Phragatopoma virgini* (Polychaeta: Sabellariidae) at the Mehuin Marine Reserve, Chile. *Marine Ecology Progress Series* 127:149-155.

## Tables

Table 1. Estimated probabilities (95% Confidence Intervals) of dislodgement for combinations of velocity and substrate analogues.

Velocity	Substrate		
	<i>Cladophora</i>	Rockface	Fines
Low	0 (-) <sup>a</sup>	0.28 (0.19, 0.38)	0.85 (0.76, 0.91)
High	0.014 (0.002, 0.090)	0.70 (0.59, 0.79)	1 (-) <sup>a</sup>

<sup>a</sup> (-) for confidence intervals reflects the lack of observed variation at the 0% and 100% levels for all 5 trials of the Low/*Cladophora* and High/fine sediment treatment combinations, which precluded the estimation of standard errors on these estimates. Estimated probabilities of dislodgement on *Cladophora* analogue substrates are comparable to randomness from control trials.

Table 2. Hydraulic parameters (95% Confidence Intervals) and polychaete body measurements exposed to flow under differing experimental conditions of velocities and substrate type.  $U_*$  is shear velocity,  $U_{\perp,d}$  is the effective drift velocity,  $U_{z=5}$  is the velocity at a reference height of 5 mm above the bed,  $l$  is the average length of a polychaete tube,  $h$  is the height of a polychaete tube above the bed, and  $Re_l$  is the body-length Reynolds number.

Velocity	Lab Substrate	Substrate Represented	$U_*$ cm/s	$U_{\perp,d}$ cm/s	$U_{z=5}$ cm/s	$l$ mm	$h$ mm	$Re_l$ x10 <sup>2</sup>
Low	Tile	Rock Face	2.9 (2.8, 3.1)	40 (37, 43)	51 (49, 53)	0.5-3 <sup>a</sup>	1-3	1.9-13
High	Tile	Rock Face	4.9 (3.9, 5.8)	110 (108, 112)	116 (113, 119)	0.5-3 <sup>a</sup>	1-3	5.4-34
Low	Pseudophora	<i>Cladophora</i>	3.2 (2.9, 3.5)	40 (33, 47)	52 (46, 58)	0.5	1-3	1.7-2.3
High	Pseudophora	<i>Cladophora</i>	3.3 (2.5, 4.1)	108 (103, 113)	122 (117, 127)	0.5	1-3	5.3-5.8
Low	Fines	Fines	4.3 (4.2, 4.4)	35 (33, 37)	50 (48, 52)	0.5	1-3	1.6-1.8
High	Fines	Fines	7.4 (6.9, 7.9)	93 (91, 95)	125 (122, 128)	0.5	1-3	4.6-4.7

<sup>a</sup>Lengths over 0.5 mm represent aggregates of clumped individuals

## Figure Legends

Figure 1. *Manayunkia speciosa*. Inset photo shows the anterior end of a polychaete inside of its tube, with antennae extended. *M. speciosa* rarely leave their tubes in nature.

Figure 2. Experimental flume setup. The flume measured 2.6 m in length, 1.35 m in height and 0.44 m in width. The water was moved with two propellers on a shaft, powered by a 2 HP AC induction motor. Velocity readings were taken using an ADV in the center of the flume channel, and directly over the polychaetes.

Figure 3. Comparison of *Cladophora* and an experimental analogue. A “pseudophora” analogue (left) was used as a substitute for actual *Cladophora* (right) as a substrate in flume trials for *Manayunkia speciosa*. Vertical lines at bottom are 1 mm apart.

Figure 4. Observed proportions of dislodgement among the 5 trials at each combination of control (Con), velocity (low: 55 cm/s; high: 140 cm/s), and substrate. Observed proportions are jittered (shifted horizontally to show overlapping trials). Bars represent medians and circles represent individuals.

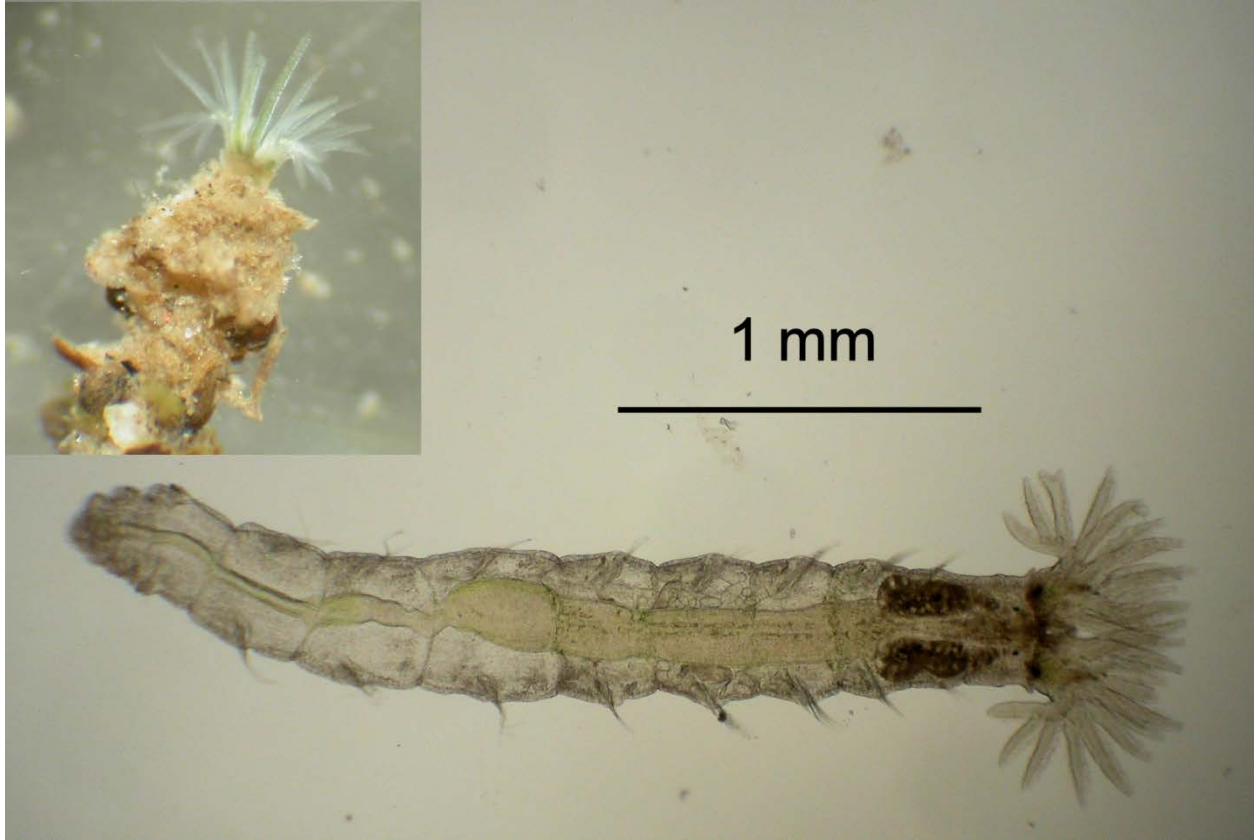


Figure 1.

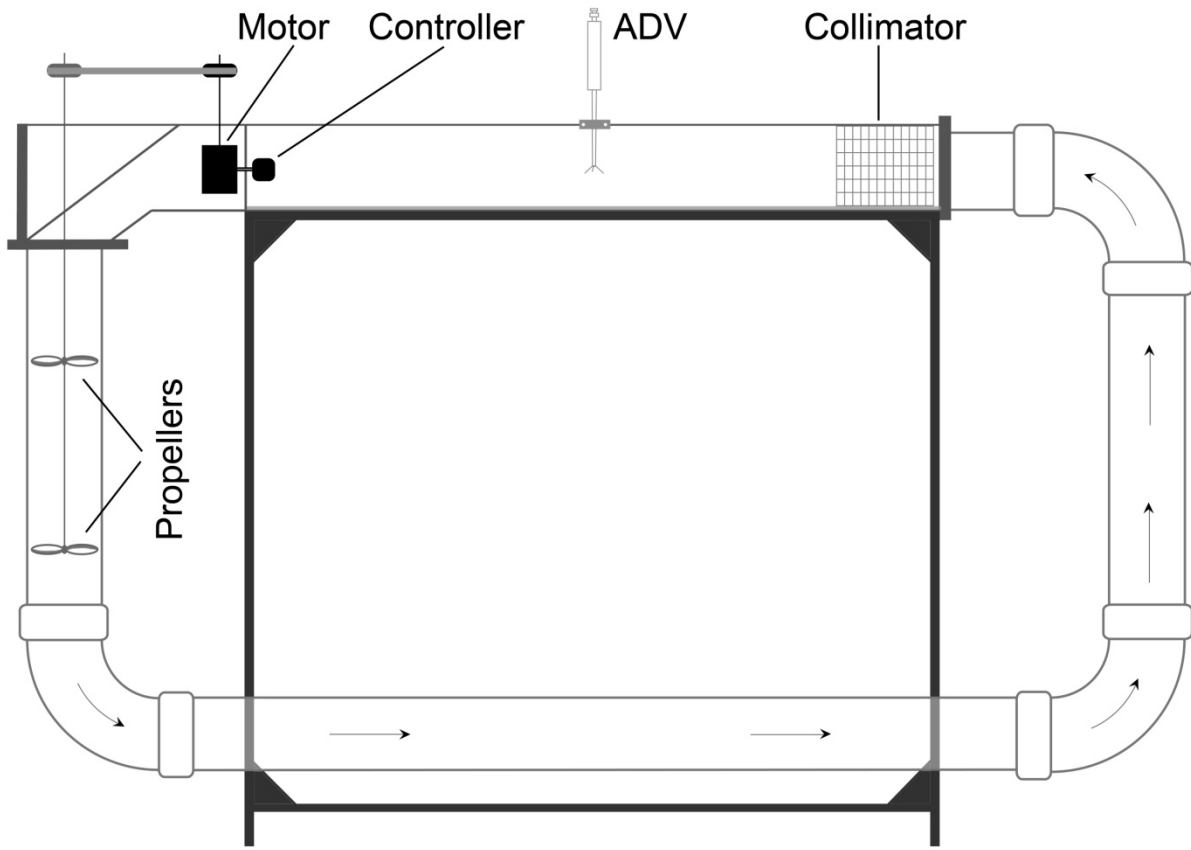


Figure 2.

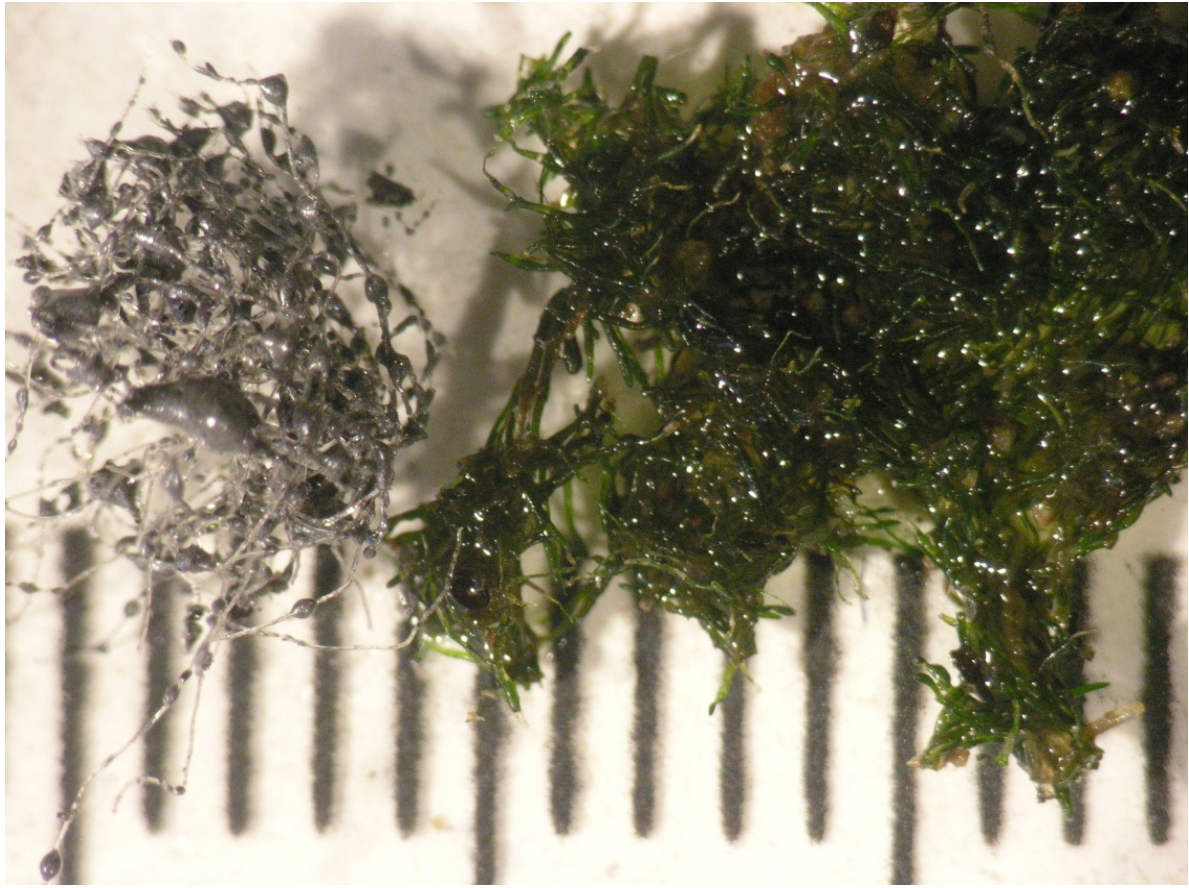


Figure 3.

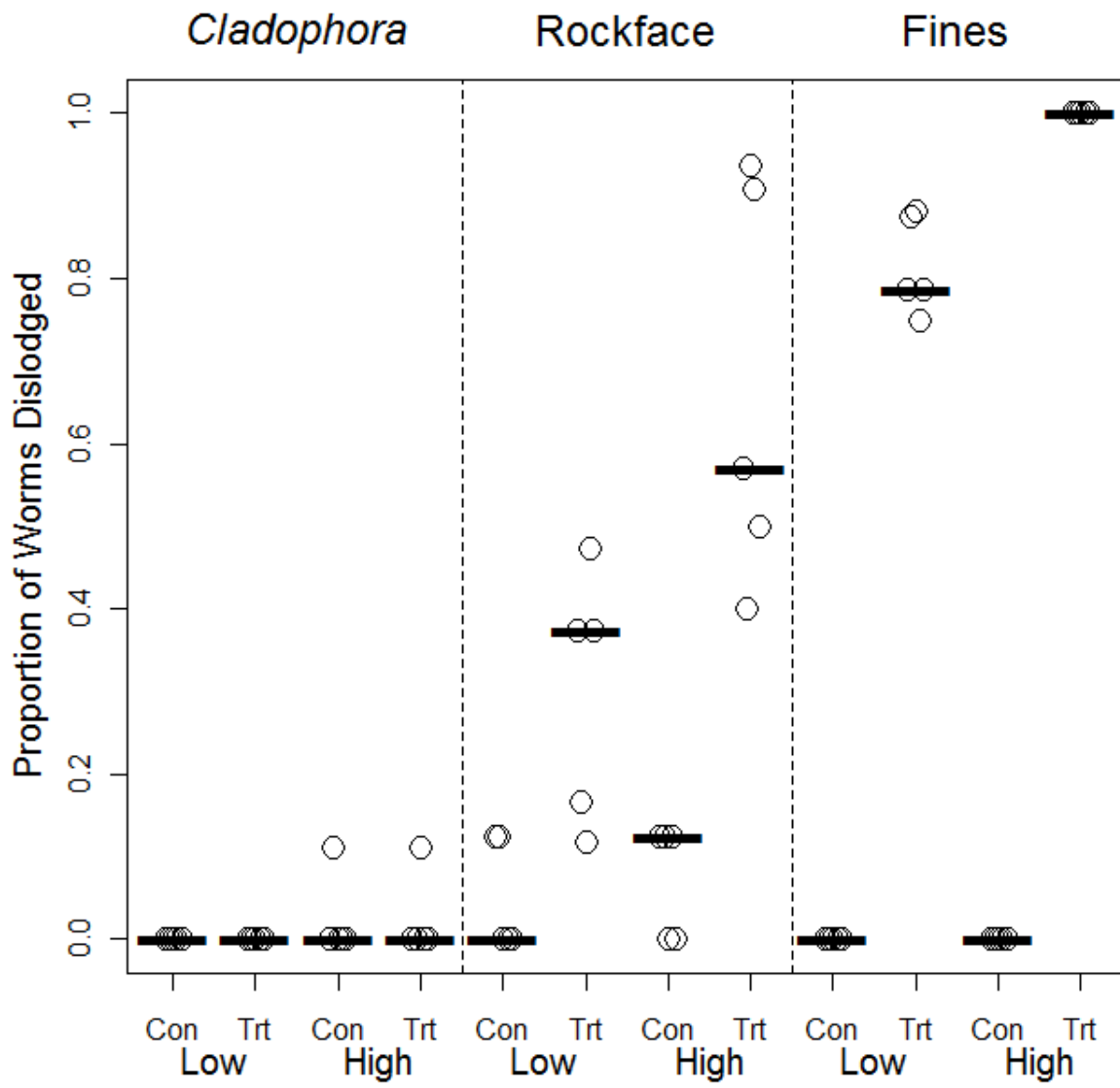


Figure 4.