AN ABSTRACT OF THE THESIS OF

<u>Michelle S. Jordan</u> for the degree of <u>Master of Science</u> in <u>Water Resources Science</u> and <u>Microbiology</u> presented on <u>November 9, 2012</u>
Title: <u>Hydraulic predictors and seasonal distribution of *Manayunkia speciosa* density in the Klamath River, CA, with implications for ceratomyxosis, a disease of salmon and trout.</u>

Abstract approved:

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The freshwater polychaete *Manyaunkia speciosa* was identified as an obligate host of

the salmonid parasite *Ceratomyxa shasta* in 1997, prompting increased research on the

small benthic invertebrate. *Ceratomyxa shasta* infection in fish can cause mortality,

and presents a disease risk for both hatchery and wild salmon and trout. *Ceratomyxa

shasta* is endemic to rivers of the Pacific Northwest, and its effects have been

particularly well documented in the Klamath River, Oregon and California. One

option for managing *C. shasta* impacts is by decreasing densities of *M. speciosa*

through habitat manipulation, thus decreasing amplification of the parasite. The

Klamath River is regulated by irrigation and hydropower dams, thus manipulating the

hydrograph to destabilize habitat is a possibility. Decreasing habitat through flow

manipulation requires a thorough understanding of the hydraulic environment of

polychaete habitat, and how that environment changes with discharge. This thesis

proposes an influence diagram of physical variables driving *M. speciosa* density, and

investigates several of them. Samples were collected for enumerating *M. speciosa*

density from nine sites in the Klamath River over 15 months, and seasonal density changes were examined, as were the relationships between density and hydraulic variables (depth, average velocity, substrate size, Reynolds number, Froude number). Density increased directly with depth and inversely with velocity, and was greater on small (silt, sand) and large (boulder, bedrock) substrate relative to medium substrate (gravel, cobble). Density was highest in the summer (July, August, September), and there was evidence that summer densities were influenced by spring discharges through the mechanism of substrate mobilization. Differences in infection prevalence among seasons and habitats were also investigated; however, very low overall incidence of infection limited any conclusions. Based on these results, it is recommended that habitat modeling for management of *M. speciosa* populations include a habitat stability component that incorporates s whether peak discharge the previous year surpassed a stability threshold.

Hydraulic predictors and seasonal distribution of *Manayunkia speciosa* density in the Klamath River, CA, with implications for ceratomyxosis, a disease of salmon and trout

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Michelle S. Jordan, Author

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CONTRIBUTION OF AUTHORS

Dr. Bartholomew and Dr. Grant served as co-major advisors and contributed to experimental design and manuscript preparation. Dr. Alexander contributed to experimental design and manuscript preparation, and participated in sample collection, processing, and data analysis.

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CHAPTER 1: INTRODUCTION

DAMS, AQUATIC PARASITES, AND CONCEPTUAL MODELS

Anthropogenic alteration of aquatic ecosystems can disrupt host-parasite dynamics of parasites with complex (multiple obligate hosts) life cycles, and result in disease outbreaks in people and animals. Dams in particular have been associated with increased parasite burden to humans (Jobin, 1999). Although dams provide important human services such as flood protection and irrigation, they may also alter the environment and human and animal behavior in a manner that exacerbates existing diseases, or allows new diseases to become established. Constructed reservoirs and canals may create breeding grounds for disease vectors and habitat for aquatic invertebrate hosts of parasites with complex life cycles, and may increase human population density and subsequently the potential for contamination of, and contact with, the water (Fenwick, 2012; Lafferty & Armand, 1999). Parasites of fish and other aquatic animals may be similarly influenced by dams (Morley, 2007). In addition, fish may be subjected to environmental stressors associated with dam construction (Baxter, 1977) which can decrease their ability to resist parasites. These include impaired water quality (temperature, DO, turbidity), altered flow regime, and a decrease in available habitat.

Methods for managing parasites with complex life cycles in reservoirs and the rivers downstream are varied and dependent on the ecology of each disease (Jobin, 1999). Diseases of humans are often managed by drug treatment of individual patients or by decreasing contact with the parasite (Fenwick, 2012). Hatchery fish can be treated for select diseases during rearing, but once they are released to the wild the ability to influence their exposure to environmental parasites is limited. Another option for managing parasites with complex life cycles is to target an intermediate host, rather than the parasite itself. Decreasing the density of one obligate host may decrease or disrupt parasite replication, lessening the disease burden to the obligate host of interest. Methods for decreasing host abundance include chemical application (e.g. molluscicides for schistosomiasis; McCullough et al., 1980), physical separation

of hosts and vectors (e.g. mosquito nets for malaria; Alonso et al., 1991), and habitat manipulation (e.g. changing earthen ponds to concrete raceways in hatcheries for whirling disease in fish; Hoffman, 1990). Manipulating host habitat in open systems like lakes and rivers is more complicated than in closed systems like hatcheries. For example, attempts to mitigate whirling disease impacts to trout by manipulating habitat for the oligochaete host of the parasite have had limited success (Thompson, 2011).

One of the difficulties of disease management through habitat manipulation is that it requires a thorough understanding of the ecology of the target host, the hydraulics of its aquatic physical habitat, and the linkages between them. Two- and three-dimensional hydraulic models are a common tool in fisheries management for predicting the amount and quality of physical habitat for fish under varied flow regimes (Conallin et al., 2010; Dunbar et al., 2011). Predicting changes in benthic invertebrate habitat is a more recent application of hydraulic models (Gore et al., 2001) . In the habitat modeling process, the physical flow model is coupled with statistical models of habitat preference to assess the amount of habitat available at each discharge value. Applying this approach to disease management utilizes the same methods, but with an opposite objective: determining a flow regime with the least available habitat for the invertebrate host. Modeling ecological responses to flow manipulations requires the linkage of causal abiotic factors (e.g. water depth and velocity, extreme flow) with dependent biotic variables (e.g., invertebrate density and size). These linkages may be inter-dependent, or have intermediate steps that are influenced by factors not included in the original hypothesis (Stewardson & Webb, 2010).

An influence diagram or conceptual model is a useful tool for identifying potential intermediates, as well as researcher biases. This thesis proposes a conceptual model of the factors influencing high densities of *Manayunkia speciosa*, the invertebrate host of a parasite of salmon and trout, investigates several hypothesized linkages through field studies, and then evaluates the model based on the results.

THE KLAMATH RIVER AND CERATOMYXOSIS

The first dam on the Klamath River, which runs from Upper Klamath Lake in southern Oregon to the Pacific Ocean in northern California (Figure 1.1), became operational in 1922. By 1962 there were five dams, the most downstream of which was located at river kilometer (rkm) 306 of the approximately 423 rkm river, cutting off all passage of fish into the upper river. In 2012, these dams remain in operation, but their futures are uncertain. The Federal Energy Regulatory Commission (FERC) license for the four dams owned by the electric company PacifiCorp expired in 2006. Relicensing the project would be expensive, requiring the construction of fish passage and improvement of water quality and resulting in a loss in hydropower. Therefore, negotiations on the removal of the four dams have been undertaken; removal is not certain, but could occur as early as 2020. The decommissioning discussion has prompted research and debate on how the dams have influenced the Klamath system, whether mitigating fish disease impacts with the dams still in place – altering the hydrograph, for example – could be useful, and what the impacts of dam removal will be.

It is generally accepted that the dams have contributed to the drastic declines in Klamath River stocks of Chinook and coho salmon, steelhead, and Pacific lamprey, by decreasing available habitat and impairing water quality (Hamilton et al., 2011). However, the effects of the dams on disease is less understood. One parasite that has been hypothesized to contribute to the declines is the myxozoan *Ceratomyxa shasta,* the cause of ceratomyxosis. In 2001, a survey of juvenile Chinook and coho salmon in the Klamath mainstem and estuary documented high prevalence of infection, pathology, and presumed *C. shasta* mortality (Foott et al., 2002). Monitoring in subsequent years documented up to 45 percent prevalence of infection in out-migrating juvenile salmon (Nichols & Foott, 2005). Sentinel exposures of non-native juvenile rainbow trout and salmon at several locations in the Klamath River also resulted in high mortality (Hallett et al., 2012). Non-native rainbow trout are known to be highly susceptible to *C. shasta*; however Chinook and coho salmon from rivers

where *C. shasta* is endemic have lower susceptibility (Bartholomew, 1998). The high infection prevalence in these relatively resistant fish has therefore led to the hypothesis that the dams may be exacerbating or amplifying disease in the Klamath system. It has also raised the question of how *C. shasta* will respond to removal of the dams, and whether manipulating the timing and magnitude of flows from the dams might decrease disease impacts.

Ceratomyxa shasta has a complex life cycle, requiring a benthic invertebrate host, the 3 – 5 mm freshwater polychaete *Manayunkia speciosa,* to reach reproductive maturity. Infection with *C. shasta* occurs when *M. speciosa* contacts and consumes the 5-10 µm myxospore stage of the parasite. Inside *M. speciosa*, the parasite develops into the actinospore stage that is infective to the fish. The actinospore is released through the polychaete's epidermis (Bartholomew et al., 1997; Meaders & Hendrickson, 2009) and enters the water where it may contact a susceptible fish and perpetuate the life cycle. The completion of the Klamath dams, and the resulting more stable hydrograph, may have created more habitat for the polychaete host, or decreased the frequency and magnitude of disturbance to which it is subjected, thereby allowing it to proliferate. There is the potential to create disturbance by releasing pulsed flows from the dams to dislodge the worms themselves or to mobilize the bed they are attached to. Before such an attempt is made, it is first necessary to understand how hydraulics and geomorphology influence the distribution and density of *M. speciosa* and its infection prevalence with *C. shasta*.

A CONCEPTUAL MODEL FOR *MANAYUNKIA SPECIOSA* DENSITY AND *CERATOMYXA SHASTA* INFECTION PREVALENCE

Figure 1.1 shows a hypothesized conceptual model of the physical environmental variables that influence the density of *M. speciosa* at a given location. They are divided into two categories: a) single-point hydraulic variables, and b) large-scale hydrologic/geomorphologic parameters. Single-point hydraulic variables are flow variables that may be measured at one point in space and time (and from which more complex variables may be calculated) and used to predict whether polychaetes

will be found at that location. Those included in the model are: 1) substrate, 2) depth, and 3) velocity. Large-scale hydrologic variables are those that occur over a larger spatial or temporal scale: 4) geomorphology, 5) hydrograph, 6) temperature degree days (Figure 1.1). These large-scale parameters may influence the single-point variables over time or space. For example, a hydrograph is a graphical depiction of streamflow in a river over time, and the depth and velocity at a given point varies with streamflow. This section details the justification for each factor and linkage in the conceptual model, beginning with the single-point hydraulic variables. Although biological factors (e.g., nutrient availability, species-species interactions) are understood to be important in population dynamics, this thesis is limited to investigating physical habitat variables.

Velocity, depth, substrate and polychaetes

Benthic invertebrates are adapted to life in fluid flow. Body shape, structures for attachment, and behavior are mechanisms by which aquatic invertebrates are able to establish, feed and reproduce in a constantly moving environment (Vogel, 1996). *Manayunkia speciosa* constructs a flexible tube of mucus, sand and silt that it attaches perpendicularly to the substrate. A series of hooks on the worm's posterior end aid the polychaete in attaching to the inside of the tube. In a field survey for *M. speciosa*, Stocking & Bartholomew (2007) found the highest densities where velocity (at 20cm above the bed) was less than 0.05m/s. This suggests a limit to the tenacity of the tube, or another means by which velocity is density limiting. Four mechanisms through which velocity may be related to polychaete density are i) feeding limitation, ii) oxygen availability, iii) substrate mobilization, and iv) dislodgement.

Manayunkia speciosa is a filter feeder. It protrudes its tentacular crown from the end of its tube and extracts food particles from the water column. For benthic filter feeders, higher velocities may be associated with higher rates of contact with food particles and increased growth rate (Hentschel & Larson, 2005). However, a threshold may be reached above which the organism is mechanically unable to feed (Ackerman, 1999). It is possible that *M. speciosa* is able to remain attached in velocity conditions

under which it is unable to feed. In this case it would be feeding and not attachment that is habitat limiting. Water velocity also influences dissolved oxygen concentration (DO), and benthic macroinvertebrates have varying degrees of sensitivity to fluctuations in DO (Connolly et al., 2004). Observations by Stocking & Bartholomew (2007) suggested that velocities close to zero may be limiting for *M. speciosa*, through promotion of anoxia. However, the salt-water relative *Manayunkia aesturania* dominated the species assemblage in an estuary where pulp and paper mill wastes caused a reduction in DO (Millner, 1980). *Manayunkia speciosa* DO requirements could have a profound effect on its distribution, either excluding it from certain habitats, or allowing it to outcompete more DO sensitive species in oxygen poor habitats.

Stream hydraulics are an important determinant of habitat suitability for benthic invertebrates (Statzner & Higler, 1986). Variables like velocity, depth and substrate size can be good predictors of species abundance and diversity (Jowett, 2003). The combination of these three variables determines whether the bed will mobilize, and substrate instability has been associated with decreased benthic invertebrate abundance (Death & Winterbourn, 1995). The initiation of substrate mobilization, called incipient motion, occurs when the destabilizing forces (drag, lift, buoyancy) acting on a particle become greater than the stabilizing force of the particle's weight (Chanson, 2004). Incipient motion is related to velocity, depth and substrate size through shear stress. A polychaete attached to cobble could be considered as a small sediment particle, acted upon by this shear force. Shear could therefore influence polychaete abundance through both substrate mobilization, and the ability of the animal to remain attached to the substrate. The polychaete's small size also suggests that small-scale velocity patterns like those caused by the roughness of a boulder may be important for determining habitat, particularly if the polychaete is able to escape to small refugia like pits and cracks in the boulder (Lancaster & Hildrew, 1993). Unfortunately, animals in the benthic meiofaunal size class like *M. speciosa* are often overlooked in studies of aquatic invertebrates, and whether the established

patterns of hydraulic constraints on benthic invertebrate habitat are true for *M. speciosa* remains uncertain.

Geomorphology, flow regime and temperature

Changes in velocity, depth, and substrate over time are influenced by river geomorphology and flow regime. Disturbance, or changes in these variables outside of the predicted range, is an important control in stream ecology (Resh et al., 1988). For example, periodic disturbance may displace monopolistic competitors and allow less successful competitors to become established (Hemphill, 1991; McAuliffe, 1984). The suitability of a particular location as habitat for *M. speciosa* will depend not just on whether the current velocity, depth, and substrate conditions are within its tolerance levels, but also whether those levels were exceeded in the past.

River features (eddys, runs, pools, riffles) respond differently to changes in discharge. The rate of change of velocity and sediment transport with increasingdischarge differs depending on the feature type and site-specific characteristics. For example, the velocity reversal hypothesis suggests that the bed of pools are more subject to disturbance during flooding than runs (Keller, 1971). Velocity in recirculation zones, or eddies, tends to be slower than the main stem, and increase at a slower rate than main stem velocities as discharge increases (Thompson et al., 1999). There also may be variation in response within a feature class. Eddies form due to a decrease in pressure caused by a channel constriction or bend, or an obstruction like a boulder. If discharge increases enough to overtop the obstruction, then the eddy will lose strength: the direction of flow will change to downstream; velocities will increase and potentially cause major disturbance. Reversing upstream flow is less likely if the eddy is caused by a channel constriction or bend. These differences in velocity response to discharge changes translate to differences in disturbance (substrate mobilization, dislodgement) potential. Two locations may have the same values of velocity, depth, and substrate when they are sampled during summer low flow, but have very different densities of *M. speciosa* based on how they responded to a high flow event the previous winter or spring. Similarly, a particular

feature may provide high quality habitat during years with a stable hydrograph, but be highly subject to disturbance during a flood.

The hydrologic cycle also influences water temperature, which is important to the growth and reproduction of aquatic animals. Degree day (cumulative number of degrees) is often used to describe temperature thresholds for biological function. For example, an animal may require a particular number of degree days before it becomes reproductively mature or breaks diapause (Verberk et al., 2008). A particular location in the river may have hydraulic variables conducive to high polychaete densities, but if temperatures are cooler than average, they may be absent.

Infection prevalence

The components of the conceptual model for *M. speciosa* density may also be driving factors of high infection prevalence of *C. shasta* in *M. speciosa*. Although particular conditions may support high densities of *M. speciosa,* if these populations are not infected, then their relevance to disease dynamics is low. The size of the waterborne spore stage of *C. shasta* is on the same order of magnitude as silt and clay particles, and is subjected to the same flow forces. As with food particles, *M. speciosa* must extract *C. shasta* spores from the flow to become infected. The rate at which the polychaetes encounter spores is important, as is their ability to mechanically remove them from the flow; this may be a function of velocity. Areas of low velocity where sedimentation occurs may lead to settling of spores and higher rates of encounter and infection. Depth and substrate type are likely not as important for infection prevalence. Temperature has been shown to influence the progression of *C. shasta* development in the fish host (Udey et al., 1975), and may also influence development in the polychaete host.

STUDY OBJECTIVES

The goal of this thesis was to develop a more rigorous definition of physical habitat for *Manayunkia speciosa*, and investigate how habitat influences infection prevalence with *Ceratomyxa shasta*, to aid in informing management of ceratomyxosis

in the Klamath River. The specific objectives addressed in chapters two and three are to:

I.   Identify seasonal changes in *M. speciosa* population density, physical habitat, and *C. shasta* infection.

II.   Investigate whether hydraulic variables can be used to predict *M. speciosa* distribution and density.
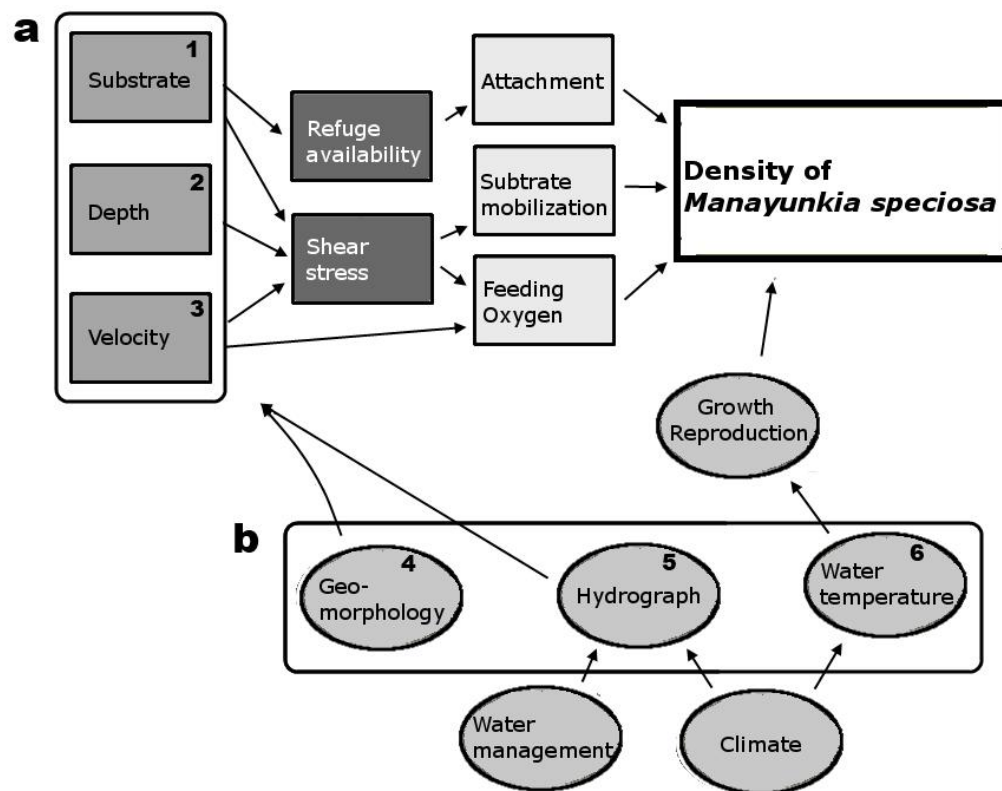

Figure 1.1 Influence diagram of physical habitat influences on *Manayunkia speciosa* density including, a) single-point hydraulic variables, b) large-scale parameters, and mechanisms through which they might act.

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CHAPTER 2: SEASONAL TRENDS IN *MANAYUNKIA* SPECIOSA DENSITY AND INFECTION WITH *CERATOMYXA SHASTA* IN THE KLAMATH RIVER, CA

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ABSTRACT

Manayunkia speciosa is a freshwater benthic polychaete, and the alternate host of *Ceratomyxa shasta*, a parasite of salmon and trout. There is interest in mitigating *C. shasta* disease impacts in the Klamath River, OR, and strategies for decreasing *M. speciosa* population densities have been proposed. One strategy is manipulation of water releases from dams to cause disturbance (e.g. substrate mobilization, dislodgement of the polychaete, inhospitable flow conditions) and decrease *M. speciosa* habitat. To be biologically relevant, the magnitude and timing of flow manipulations are crucial, but the habitat requirements and seasonal dynamics of *M. speciosa* populations are not well understood. In this study, we repeatedly sampled nine sites in the Klamath River over fifteen months. We examined differences in *M. speciosa* densities and prevalence of infection with *C. shasta* among seasons, river features (runs, eddies and pools) and reaches, and assessed relationships among these factors and site-scale habitat parameters. Density was not significantly higher in any one feature class over time, and principal components analysis of physical parameters (depth, velocity, dissolved oxygen, temperature, proportion nonfines, silt, sand, and clay, and percent organics) showed considerable overlap among the features (runs vs. eddies vs. pools). Generally, density was highest in deep, low velocity environments, and fluctuated seasonally, with the highest values in the summer. Density decreased from the first to the second summer for three of the sites, possibly due to disturbance caused by high discharge during the winter and spring. Infection prevalence was low (average 1.8%) and only six samples contained infected polychaetes, limiting our ability to compare among seasons or reaches. The yearly fluctuation in density supports the hypothesis that *M. speciosa* has an annual life cycle.

INTRODUCTION

      Manayunkia speciosa is a small (3-5mm) freshwater polychaete and an obligate host in the complex life cycle of *Ceratomyxa shasta,* a myxozoan parasite of salmon and trout (Bartholomew et al., 1997). The parasite alternates between *M. speciosa* and a salmonid host, and two spore stages, myxospores and actinospores (Bartholomew et al., 1997). The myxospore stage is ingested by the polychaete and develops into the actinospore stage (Bartholomew et al., 1997; Meaders & Hendrickson, 2009), which is released into the water column and infects a susceptible salmonid through attachment to the gill (Bjork & Bartholomew, 2010). In the fish host, the parasite develops into the myxospore, and is subsequently released upon the death of the fish, or occasionally through the intestine prior to death (Richard Holt, Oregon State University, pers. comm.).

      When managing parasites with complex life-cycles it is often more feasible to target one of the alternate hosts, rather than the parasite itself (Jobin, 1999). In the Klamath River, California, prevalence of *C. shasta* infection in out-migrating juvenile Chinook salmon has been estimated at up to 45 percent in some years (True et al., 2010), and juvenile Chinook and coho salmon placed in the river at highly infectious locations for three days may experience 100 percent mortality due to *C. shasta* (Hallett et al.,2012). Klamath River salmonids have economic and cultural significance, and there are several stakeholder groups invested in their conservation; they include Native American tribes, commercial and sport fisheries, and local communities (Most, 2006). These interests have prompted research into factors influencing the *C. shasta* life cycle to facilitate mitigation of disease impacts on salmon and trout.

      Manayunkia speciosa is a benthic filter feeder that inhabits a flexible tube composed of mucus, sand, and organic matter, which it constructs and attaches perpendicularly to the substrate. It protrudes its crown of tentacles from the tube for feeding and respiration (Lewis, 1968). Reproduction is sexual and young are brooded in the maternal tube, after which they emerge and construct their own tubes (Hazel, 1966; Willson et al., 2010). *Manayunkia speciosa* was first identified in the Schuylkill

River in southeast Pennsylvania, U.S.A. (Leidy, 1858), and has since been described from the Great Lakes, the Pacific Northwest, California and Alaska (Hiltunen, 1964). The specimens described from the west coast are likely to be genetically distinct from the east coast specimens (Stephen Atkinson, Oregon State University, pers. comm.); however, their biology and ecological preferences appear to be similar.

The first documentation of the polychaete in the Klamath River was by Hazel (1966) who observed specimens in Sevenmile Canal, which feeds into Agency Lake. There were no subsequent reportings of the polychaete until Stocking & Bartholomew (2007) surveyed *M. speciosa* densities from Upper Klamath Lake to the mouth of the river. The polychaete was observed in a variety of river features including runs, eddies, pools, and riffles, and on two primary substrates: sand/silt, and the mat-forming alga *Cladophora sp.* attached to boulders. The anecdotal observation of a dense population of polychaetes on sand/silt, and the absence of that population after a high flow event, led Stocking & Bartholomew (2007) to hypothesize that microhabitat (described as whether the polychaetes were on sand/silt or algal mat) stability influences *M. speciosa* distribution.

Physical disturbance is important for structuring benthic macroinvertebrate communities in rivers (Lake, 2000; Resh et al., 1988). For example, periodic disturbance in the form of flow alterations may displace monopolistic competitors and allow less successful competitors to become established (Hemphill, 1991; McAuliffe, 1984). Different river features (runs, eddies, pools) have variable stability and potential for disturbance; as river discharge increases or decreases, the physical characteristics of each feature class respond differently. For example, the velocity reversal hypothesis of pool-riffle maintenance proposes that velocities in pools increase at a faster rate than those in riffles in response to increasing discharge (Thompson et al., 1999; Pasternack et al., 2010). Similarly, velocities in the main stem increase faster than velocities within recirculation zones, or lateral separation eddies (Schmidt, 1990). These differences translate into differences in the potential for feature-scale substrate mobilization, and associated physical disturbance. If feature-

scale habitat stability is a determinant of *M. speciosa* distribution, then disease management related to decreasing polychaete densities could be focused on those features with the greatest disturbance potential.

In this study, we investigated the hypotheses that *M. speciosa* densities are higher in eddies than pools and runs over time, and that differences in density can be explained by differences in the environmental characteristics of these features. We repeatedly sampled nine sites from three habitat types with different feature-scale stability (runs, eddies, pools) in three reaches over 15 months. We examined differences in polychaete density among features, reaches, and sampling periods, and investigated whether they could be explained by differences in feature-scale characteristics (average depth, velocity, proportion silt, sand, and clay, percent organics, and substrate size). Identifying the physical habitat characteristics that support high densities of *M. speciosa* over time could aid in focusing management on areas that have the greatest potential for decreasing polychaete populations.

Sampling over a year also allowed us to examine seasonal dynamics in *M. speciosa* density in the Klamath River. Work by Willson et. al. (2010) suggested that in a laboratory setting, *M. speciosa* has an annual life cycle. However, environmental sampling of *M. speciosa* in the Klamath has been sporadic (Stocking & Bartholomew, 2007; Willson et al., 2010), and no studies have monitored populations through time to investigate seasonal changes in density. Understanding when densities are at their peak and minimum would aid in timing management strategies to have the greatest impact on polychaete populations.

We also investigated differences in infection prevalence among river features and seasons, because the presence of polychaetes is not sufficient to assess disease risk to fish. To be important from a disease management perspective, the polychaetes must also be infected with *C. shasta*. Velocity is generally slower in eddies and pools relative to runs, and we therefore hypothesized that infection prevalence would be higher in eddies and pools, due to settling of myxospores and an increased rate of contact with *M. speciosa.*

MATERIALS AND METHODS

Study area

Samples were collected from three reaches of the Klamath River between the Shasta (river kilometer (rkm) 288) and Scott (rkm 234) River tributaries (Figure 2.1). This section of river has previously been identified as an area of elevated infectivity (Hallett et al., 2012). Reaches were selected based on accessibility and biological relevance. The biological relevance criteria included either prior identification of *M. speciosa* in the reach (e.g. Stocking & Bartholomew, 2007; Alexander et al., submitted), or high *C. shasta* induced mortality in sentinel fish (naïve hatchery fish held in situ; Stocking et al., 2006; Hallett et al., 2012). To test the hypothesis that density is higher in eddies over time, one eddy, run, and pool, were selected for sampling from each reach. Descriptions of reaches and features are from the first sampling trip in 2010, and are considered the baseline conditions.

Reach 1 (Figure 2.1, R1) is the most upstream reach: at rkm 277, it is 29 rkm below the most downstream dam on the Klamath, Iron Gate (rkm 306). Reach 1 is a location of long-term polychaete monitoring (Bartholomew, unpublished data), and is near the Tree of Heaven campground, which has been referred to in previous studies (Stocking & Bartholomew, 2007). An eddy is formed at a bend and constriction in the river, and the substrate in the eddy is dominated by large boulder rip-rap used to stabilize the road. The pool is formed at the same bend and constriction, and is also influenced by a reed bed just upstream that slows velocity along the rivers edge. Although all flow in the pool was downstream during summer low flow, a recirculation current developed and was maintained during the higher winter and spring flows. The run is the most down-stream feature, and was shallow (<1.5m), with macrophytes as a dominant feature.

Reach 2 (Figure 2.1, R2) is located just upstream of the confluence of Beaver Creek with the Klamath River (Rkm 258). This is a location where sentinel fish exposures have been conducted since 2003 (Stocking et al., 2006) and where high parasite levels in the water have been consistently identified (Hallet et al., 2012). The

eddy is formed by a large gravel bar constriction. It had the highest velocities of the three eddies, and lacked an area of zero velocity in the center. The run is located along stabilizing rip-rap, and was the deepest of the three features. The pool is the furthest downstream site in this reach, and the bed is dominated by silt and macrophytes.

Reach 3 (Figure 2.1, R3) is the furthest reach downstream (Rkm 253). It is also the longest reach (0.8 km) due to the distance between the lateral separation eddy and the nearest pool. The eddy was the largest of the three eddies, and was formed by a constriction. The run is located directly downstream of the eddy and was shallow (<1m). The pool was the most shallow of the three pools (<0.5m), and was stagnant in places.

Sampling regime and flow conditions

The nine sites were sampled up to 12 times between July 2010 and September 2011 (Table 2.1). Some sites were not sampled in February, March and April 2011 because of dangerous flow conditions. In mid-February 2011 discharge rose from 51 cubic meters per second (cms) to peak at 170 cms (~2-year flood), fell to 42 cms for a brief period, and then rose again to ~170 cms, and was sustained above 85 cms until the beginning of July 2011, when it returned to baseline summer flow of approximately 30 cms (Figure 2.2). Sampling trips were categorized by season (Summer vs Non-Summer), and by their timing in relation to peak flows (Pre vs Post) as follows: Summer Pre Flow (SPr) - July, August, and September 2010; Non Summer Pre Flow (NSPr) - October and December 2010 and January and February 2011; Non Summer Post Flow (NSPo) - May and June 2011; Summer Post Flow (SPo) - July, August, and September 2011 (Figure 2.2). Each sampling trip lasted from four to eight days depending on conditions. Sampling trips were four to five weeks apart, and dates varied between years (Table 2.1).

Sample collection

Pools and runs were randomly sampled four times on each sampling occasion. Runs were divided into 100 transects and a sample was taken at each of four transects selected from a random numbers table. At that transect the sampler closed her eyes,

turned in several circles and tossed a colored marker rock in the air. The sample was taken at the marker rock. Pools were divided into 20 transects and sampled similarly. Eddies were sampled up to seven times through a stratified random sampling method designed to capture the flow heterogeneity inherent in eddies. The eddy was broken into seven sections based on flow patterns: a) separation point, b) reattachment point, c) primary upstream flow current, d) dead zone, e) bank (vertical), f) bank (horizontal), g) eddy fence (Figure 2.3). Each section was divided into 20 transects and a randomly selected transect was sampled. In deep areas, an anchor was dropped and the sample was taken where the anchor fell, otherwise a marker rock was dropped. A combination of snorkel and SCUBA diving was used to sample in non-wadeable areas.

Biological sampling: At each sampling point benthic material was collected using a sampling device similar to that used by Stocking & Bartholomew (2007). The sampler (Figure 2.4) was constructed of a lightweight PVC joint with a 17cm diameter aperture and 83µm mesh. The following deviations from the Stocking & Bartholomew (2007) model were made: 1) a 0.5L removable collection bottle was added to the end of the collection bag; 2) a ring of neoprene was attached to the bottom of the device to improve the seal between the sampler and substrate; and 3) a neoprene cap with a slit was attached to the top to decrease sample loss (Figure 2.4).

To collect a sample, the sampler was held against the substrate and the researcher inserted her arm into the slit in the top. The entire surface area enclosed by the sampling device was agitated using a common kitchen scouring pad (e.g. Brillo$^®$ Pad) and the water flowing through the mesh screen transported the sample into the collection bag and cup. In low-flow areas, the researcher removed the sampling device from the surface using a forward swooping movement while plugging the slit in the top of the device, creating an artificial flow of water that aided in moving the sample into the collection bag. On bedrock and boulder, the surface was scraped clean and as much periphyton as possible was removed and collected. On sand and silt,

approximately the first five centimeters were disturbed and collected. Gravel and cobble falling completely within the sampler were turned over during the agitation.

After the sample was washed into the collection bottle, it was removed and capped for transport. Samples were kept on ice until they could be strained through 83μm mesh and preserved in laboratory grade 95% ethanol. If samples could not be strained within 24 hours, 95% ethanol was added directly to the collection bottles, resulting in a 50-75% solution, and the bottles were kept at 4 ℃ until they were strained. All samples were fully preserved (water strained off, 95% ethanol added) within 48 hours of collection.

Water chemistry: Temperature was measured for each site using a 6600 v2 multimeter (Yellowsprings Instruments, Yellow Springs, OH) in 2010 and a Pro Plus (Yellowsprings Instruments) in 2011. Conductivity and pH were also measured; however, probe malfunction resulted in an incomplete dataset, so these variables were excluded from the analyses. Malfunction also affected dissolved oxygen (DO) readings and these could only be included for NSPr.

Depth and Velocity: Four depth and velocity (at 0.6 depth) measurements were taken in each pool and run on each sampling occasion; up to seven measurements were taken in each eddy. Measurements were taken at the same locations as the biological samples.

Fines and organics: Substrate was characterized by classification of fines and non-fines (surface fines grid, <2 mm = fine; (Overton et al., 1997; Krueger et al., 2006). Sediment cores of the top 5 cm of the substrate were also collected for use in determining proportion silt, sand, and clay, and percent organics. Fines classification and substrate sample collection were carried out at the same locations as the biological samples.

Pebble counts: Pebble counts were conducted in December and January 2010, and in September 2011. At wadeable sites Wolman pebble counts with n=100 pebbles were performed (Wolman, 1954). At non-wadeable sites, modified Wolman pebble counts using SCUBA were conducted. SCUBA divers positioned themselves on the

bottom of the river and pulled themselves along hand over hand with their eyes closed. At every 4th hand-span, the index finger of the free hand was touched to the substrate at the end of the anchoring hand. The first pebble touched was picked up and the b-axis measured.

Sample processing

Biological samples: Biological samples were processed for enumeration of *M. speciosa* using the methods of Stocking & Bartholomew (2007) with modification. Each sample was emptied into a gridded sampling tray (WILDCO, Yulee, FL: gridded liner and tray 182-E50). Two ten-sided dice were used to select three of the 15 squares in the grid. Using a transfer pipette and tweezers, the material in each square was transferred to a 5 ml scintillation vial and dyed with a 1:4 addition of Rose Bengal (stock solution = 0.05g/L 95% ethanol). Each subsample was examined under a dissecting microscope at 250X magnification and all *M. speciosa* were counted and placed in a 1.5ml microcentrifuge tube with 95% ethanol. If a subsample had a high density of polychaetes, it was quartered. If one sample quarter had more than 200 polychaetes, the count was multiplied by four, and the rest of the subsample was not counted.

DNA Extraction: The polychaetes counted in the three subsamples were pooled in a gridded petri dish. A square was randomly selected and all polychaetes within it were moved into individual 200µl wells in a 96-well plate filled with 95% ethanol. Prior to placement in a well, each polychaete was touched to a paper towel, rinsed in clean ethanol, and then touched to a paper towel again to remove any parasite that might be surface-associated. This process was repeated until either the entire sample, or 200 polychaetes were transferred. A power calculation based on the infection prevalence observed by Stocking & Bartholomew (2007) showed that n=200 polychaetes would give ~90% (p = 0.05) confidence of detecting a difference between 1% and 8% (highest documented) infection prevalence (Statistica© software). At 80% confidence of detecting, samples with at least 137 individuals were suitable for statistical analyses.

The following extraction methods are modified from Stocking & Bartholomew, (2007).The ethanol was evaporated off the samples, and 95µl ATL buffer and 5µl Proteinase K were added to each well. The plate was sealed with pierce-able storage foil and placed in a flatbed incubator at either 55º C for 3-4 hours with vortexing each hour, or on a flatbed at 37º C overnight. RNase A (10µl of 5mg/ml) was then added to each well. The plate was recapped, vortexed, and incubated at 37º C for one hour and then heated at 85º C for 15min.

qPCR: DNA from six polychaetes (2 µl/polychaete) was pooled and diluted with 188 µl molecular grade H_2O prior to qPCR. One µl of the pooled DNA was used in a qPCR assay developed for the detection of *C. shasta* (Hallett & Bartholomew, 2006; Stocking & Bartholomew, 2007). Each pool was run in two wells and if either well of a pool fluoresced in the first qPCR, all 6 samples were run individually, using a 1:100 dilution. A positive control of extracted actinospores, and a negative control of molecular grade water were included on each plate. Inhibition was tested by running two pools from each extraction plate a second time, but spiked with the positive control. If neither spiked pool fluoresced, all samples from the corresponding extraction plate were considered to be inhibited and were additionally diluted and re-assayed. To determine the quantification cycle (Cq) value that corresponded to a positive infection, positive controls were designed using individual polychaetes and one or eight actinospores, with six replicates of each. A positive *C. shasta* infection was defined as a sample with a Cq value equal to or greater than the value of eight actinospores added to one polychaete. Actinospores develop in the polychaete in 8-spore packets. Therefore, the DNA from 8 spores should approximate a polychaete with an infection that had produced at least one mature spore packet.

Fines and organics: Substrate samples were assessed by the hydrometer gravimetric method (Day, 1968), and apportioned into proportions of sand, silt, and clay by multiplying the proportion of fines (determined using the surface fines method; Overton et al., 1997; Kruger et al., 2006) with the relative proportions of sand, silt, and clay (determined by the hydrometer method; Day, 1968). Percent

organic material was determined using the ash-free dry mass method (Steinman, Lamberti, & Leavitt, 2007).

Statistical analyses

A principal components analysis (PCA) was used to compare environmental data among sites during the four sampling periods (SPr, NSPr, NSPo, SPo). Variables that loaded on a principal component axis with a value > |0.3| were considered influential for that axis. Density data were analyzed by 2-way ANOVA for the main effect of feature and the minor effect of reach for each sampling period. Differences in density between summer periods for individual sites were tested for using 2-sided t-tests. Linear regression was used to examine relationships between density and individual principal components. Density data were log transformed for normality. PCA and linear regression were performed using SAS® Software, and ANOVA and t-tests were performed using R: A language and environment for statistical computing (R development core team, 2011).

 RESULTS

Density of *Manayunkia speciosa*

Seasonal differences: Polychaete density differed among seasons (1-way ANOVA, $p \ll 0$) (Figure 2.5b). The general pattern was for high density during the summer, and low density the rest of the year, mimicking the rise and fall in water temperature (Figure 2.5c), and opposite the change in discharge (Figure 2.5a). Density was highest the first summer (SPr) with an average 21,244 (sd = 29,869) individuals m^{-2}, dropped off in NSPr to 1,459 (sd = 3,260) individuals m^{-2}, stayed low in NSPo at 332 (sd = 8,57) individuals m^{-2}, and then increased the second summer (SPo) to an average of 13,955 (sd = 57,150) (Table 2.2). However, density was significantly lower in the second summer (SPo) than in the first summer (SPr) (TukeyHSD, p 0.007), and low enough in SPo that it did not differ significantly from density in the NSPr period (TukeyHSD, p = 0.08).

Feature and reach differences: Comparing among features and reaches for each sampling period identified significant differences in density; however, no feature

(runs, eddies, pools) or reach (R1, R2, R3) had consistently higher densities than the others among seasons (Table 2.2; Figure 2.6a,b). There were higher densities in the eddies than the pools during the first summer (SPr), and in the eddies than the runs in NSPr (TukeyHSD, p = 0.017; TukeyHSD, p =0.014). However, density did not differ among features during the two post-flow periods (NSPo, SPo).

Differences in density among reaches were only detected in the two summer seasons (Figure 2.6b). In SPr, densities were higher in Reach 1 and Reach 3 than Reach 2 (TukeyHSD, p = 0.0001 and p = 0.012 respectively). In SPo, the only significant difference was higher densities in Reach 1 than Reach 3 (TukeyHSD, p = 0.038).

Individual site differences: The overall difference in density between summers was further investigated by comparing density at each individual site in the first summer, with density at that site in the second summer. Density was lower the second summer than the first summer in R1Run (Reach 1 run) and R1Pool, and in R2Eddy (Two Sample t-test, p = 0.004; p = 0.021; p = 0.011) Density was not higher the second summer than the first at any site (Figure 2.7).

Environmental data

*Principal components analysis:*The three feature types (runs, eddies, pools) did not cluster tightly for any combination of principal component axes (PCA) in any season (Figure 2.8). On all PCAs, depth and velocity loaded on opposite ends, differentiating features with fast, shallow flow from those with slow, deep flow. In general, eddies and pools were characterized as slow, deep flowing environments (Figure 2.8). In all seasons, the environmental variables that were most influential for PC1 were: i) non fines (-0.51 to -0.47) and the proportions of ii) sand, iii) silt, and iv) clay (0.3-0.51), thus distinguishing features with a high proportion of fines from those with a low proportion.

For SPr, the first four of the eight principal components (PC) explained 90% of the variance, for NSPr they explained 83%; for NSPo 89%; and SPo 84% (Table 2.3). For NSPo and SPo, depth and velocity were the most influential variables for PC2

(|0.64-0.72| and |0.42-0.7|, respectively). For SPr, depth and velocity were the most influential for PC3 (|0.67| and |0.61|). For NSPr, depth was influential for PC3, and velocity for PC4.

Regressing density against PCs showed significant relationships with PC2 in NSPr and SPo, with PC3 in SPr, and PC4 in NPr. In each case, the dominant variables were depth and velocity, and density was higher in slower, deeper environments.

Pebble counts: Median grain size (d50), and 85th percentile (d85) differed among sites and before and after the high flow (Table 2.4). Plotting grain size distribution shows variability among sites in how curves differed between pre and post flow periods (Figure 2.9). Median grain size from the pre flow collections in December 2010 and January 2011 ranged from 8.6 to161.8 mm. Median grain size from the post flow collections in September 2011 ranged from > 2 to < 256 mm. The largest change in median grain size occurred at R3Run and R3Pool; the smallest change occurred at R1Eddy. A pebble count was not conducted at R2Pool in 2010 due to time constraints.

Infection Prevalence

The Cq threshold value for infection was set at 37.5, corresponding to 95% confidence intervals around the positive controls. Of 254 samples tested, infected polychaetes were detected in only 14. Six of these samples, and 10 samples that did not have any infected individuals, had sufficient polychaetes ($n \geq 137$) to represent a statistically valid estimate of infection prevalence (Figure 2.10). In the six samples with at least 137 individuals, prevalence of infection ranged from 0.5 to 6.3 percent, with an average of 1.8 percent. These samples were collected from July 2010, August 2011, and September 2011. Infected polychaetes were found in an additional eight samples with fewer than 137 individuals from July 2010, June 2011, and September 2011. Infection prevalence was not calculated for these samples. Samples with $n \geq 137$ were too few to statistically compare among habitats, reaches or seasons.

DISCUSSION

The fluctuation in *M. speciosa* density over the year in which we sampled supports the hypothesis of an annual life cycle and suggests a habitat stability threshold. We observed a strong seasonal component to density, with the highest values during the warm summer months, a subsequent decrease during the fall, sustained low densities during the winter and spring, and an increase the next summer. The increase in density from June to July in the second study year indicates these populations have the potential for high fecundity. Laboratory observations (Willson et al., 2010) suggested that *M. speciosa* has a one-year life cycle, thus the decrease in density in the fall may be the result of adult senescence and mortality, with a subset of the progeny produced late in the summer surviving through the winter to reproduce the following spring.

The seasonal fluctuation in density was also influenced by the high flows during the winter and spring, which may explain the lower densities observed in the second summer relative to the first. The decrease in density from summer 2010 to summer 2011 was not identical among individual sites, which could be explained by a differential effect of flow. For example, sites with greater potential for bed mobilization (e.g. with smaller substrate) had a greater change in density. Also, differences in density by feature class did not support our original hypothesis: we expected to observe consistently higher densities in eddies and pools over runs, but instead observed high heterogeneity in density among features. For example, eddies had significantly higher densities than pools in the first summer, but there was no difference detected the second summer. A possible explanation for the lack of pattern in density by feature class is that grouping sites by feature class is too general and masks physical habitat heterogeneity.

Indeed, principal components analysis of temperature, depth, velocity, and substrate characteristics showed overlap among the feature classes, and no tight clustering of any class. Also, the principal components that had a significant relationship to density only accounted for a small amount of the overall variability in the physical habitat characteristics (at most 23%). These results indicate both that the

surface flow characteristics that distinguish runs, eddies, and pools may not be good indicators of *M. speciosa* habitat, and that a variable not included in the PCA may be driving density patterns.

Substrate size may be an important factor for polychaete habitat. Two of the three sites where density was significantly higher (R1Pool, R3Eddy) had similar d50 (9 mm and 14 mm, respectively). The third site (R3Eddy) had a relatively larger d50 (162 mm). Density decreased from the first summer to the second at the two sites with small d50, but was statistically the same both summers at the site with large d50. Also, when d50 was large (R1Eddy) there was no apparent change in substrate size distribution between summers, while the distributions for the sites with small substrate showed evidence of change. Substrate size may therefore be important in its relation to habitat stability: *M. speciosa* may colonize and persist on features with small substrate during years with stable discharge and then become displaced during mobilizing flows. Thus, management aimed at reducing *M. speciosa* numbers in the Klamath might be best targeted at these feature types: pools and eddies with small substrate size, requiring only moderate flows to mobilize the bed. In contrast, features like R1Eddy (large substrate, formed by a bend in the river rather than an obstruction) would require much higher flows to disturb the bed.

2010 and 2011 were low disease years, with low levels of parasite detected in the water, and in sentinel fish (Hallett et al., 2012). We also documented low occurrence of infection among polychaete populations. This, combined with the small number of samples with sufficient polychaetes to statistically confirm infection prevalence, limited our ability to compare among features, reaches and seasons. However, there are still several interesting observations that can be made. The highest detected infection prevalence of 6.3% (September 2011) is consistent with previous work by Stocking & Bartholomew (2007) who found one population with 8.3% infection. In that study the infected population was collected in March, a period that we were unable to sample due to dangerous high-water conditions. The highest infection prevalence was observed in R2Eddy, a site where velocity was high year-

round and polychaete densities were low. This is evidence against our hypothesis that low velocity areas promote infection, and it is possible that instead high velocity facilitates infection through increased transportation rate of myxospores to the polychaetes. For example, feeding studies observed a positive correlation between velocity and growth rate in some spionid polychaetes, attributed to increased transport as well as re-suspension (Hentschel & Larson, 2005). However, we had few samples with statistically significant numbers of polychaetes from high velocity environments and therefore can only hypothesize.

The sites where density was high the first summer and low the second summer (R1Pool, R3Eddy), also each had a population of polychaetes with 0.5% infection prevalence the first summer (July 2010) and not the second. Using the estimate of Meaders & Hendrickson (2009) that an infected polychaete can produce 340 spores per day, R1Pool would have produced over 316,000 spores per day for each square meter of infected polychaetes. R3Eddy would have produced just under 500,000. The absence of these populations the second summer therefore represents a decrease in spore contribution to the river, lending further support to the conclusion that these habitat types (slow velocity, small substrate, high disturbance potential) may be appropriate targets for management.
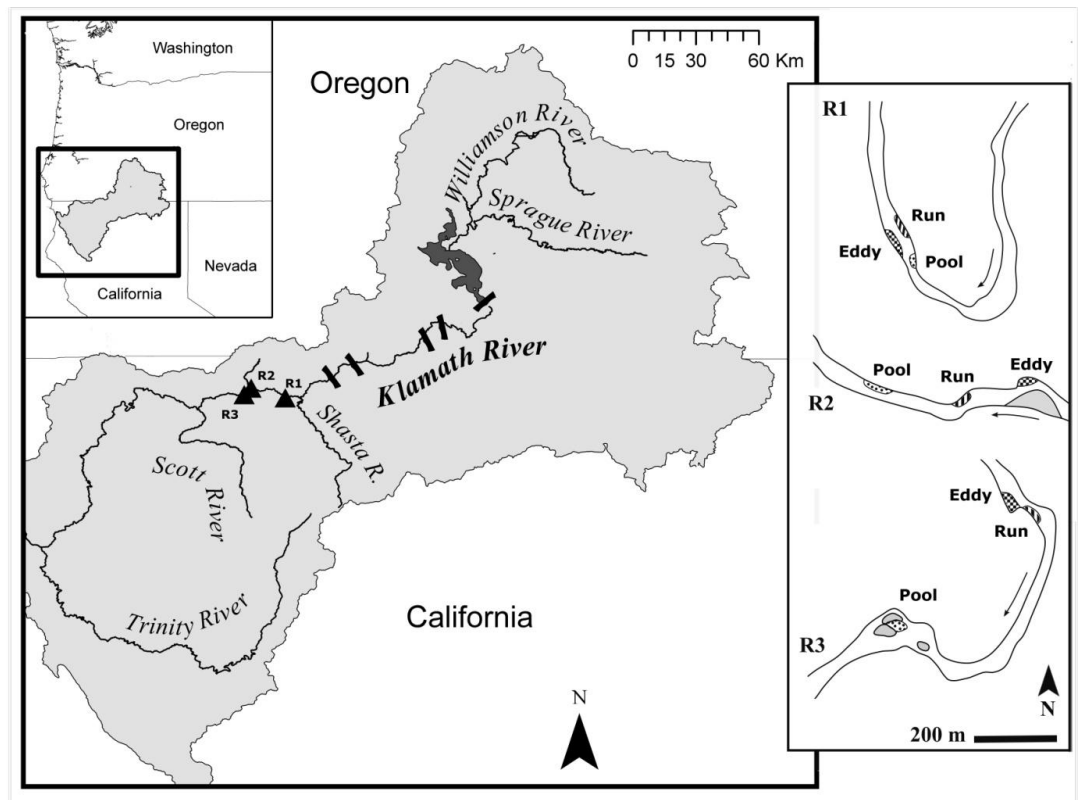
Figure 2.1. Sampling sites on the Klamath River, Ca. Triangles represent sampling reaches: Reach 1 (R1) = 277 rkm , Reach 2 (R2) = 258 rkm , Reach 3 (R3) = 253 rkm. Solid bars mark locations of dams, the most downstream of which is Iron Gate Date (rkm 306). The inset shows the location of features within each reach.

Table 2.1. Sampling regime. Numbers in cells indicate sampling date. NS = Not sampled.

Reach	Site	Year	January	February	May	June	SPr — July	August	September	NSPr — October	December
1	Run	2010					6	18	11	19	11
1	Eddy	2010					6	18	11	19	11
1	Pool	2010					6	18	11	19	11
2	Run	2010					9	19	12	20	13
2	Eddy	2010					9	19	12	20	13
2	Pool	2010					9	19	12	20	13
3	Run	2010					7	17	12	19	10
3	Eddy	2010					7	17	12	19	13
3	Pool	2010					7	17	12	19	10

Reach	Site	Year	NSPr — January	February	NSPo — May	June	SPo — July	August	September
1	Run	2011	28	27	19	15	13	19	16
1	Eddy	2011	28	26	19	15	13	19	16
1	Pool	2011	28	26	19	15	13	19	16
2	Run	2011	29	NS	NS	15	11	18	14
2	Eddy	2011	29	NS	NS	15	11	18	14
2	Pool	2011	29	NS	20	14	11	18	14
3	Run	2011	29	27	NS	14	12	18	16
3	Eddy	2011	28	27	20	14	12	18	16
3	Pool	2011	30	NS	NS	NS	12	18	16
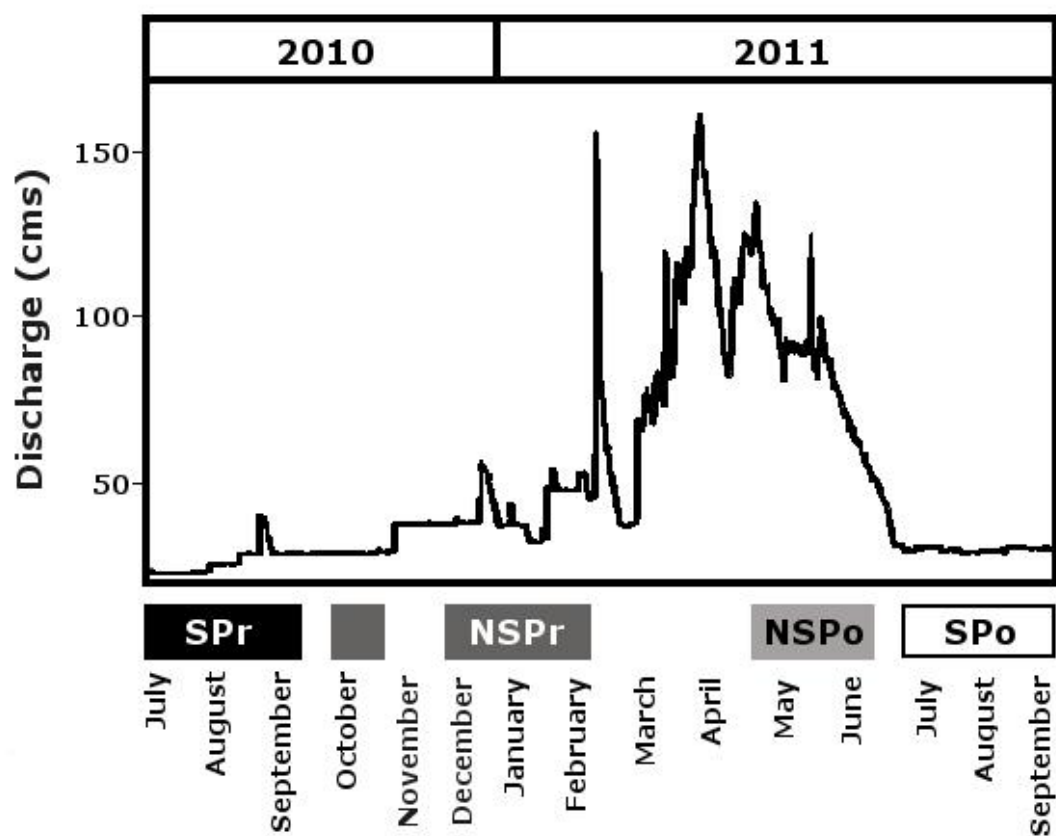
Figure 2.2. Discharge and sampling periods. Discharge is in cubic feet per second (cfs) Data are from the closest USGS gaging station (#11516530), located below Iron Gate Dam. Sampling periods are noted in colored rectangles: SPr =Summer Pre-Flow, NSPr = Non-Summer Pre-Flow, NSPo = Non-Summer Post-Flow, SPo = Summer Post-Flow.
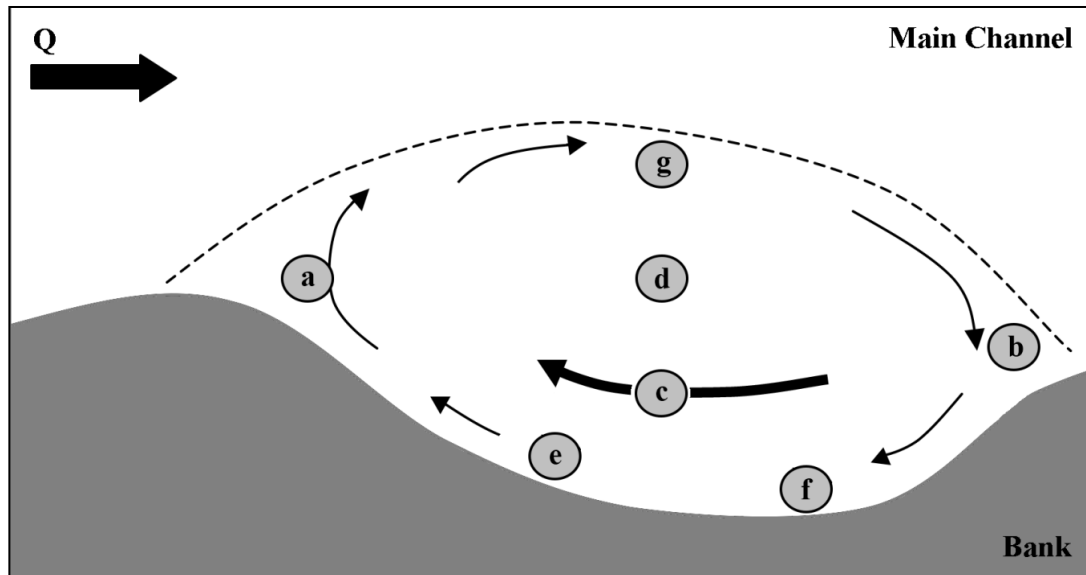
Figure 2.3. Stratified sampling within the lateral separation eddies. Sample locations are: a) separation point, b) reattachment point, c) primary upstream flow current, d) dead zone, e) bank(vertical), f) bank(horizontal), g)eddy fence.
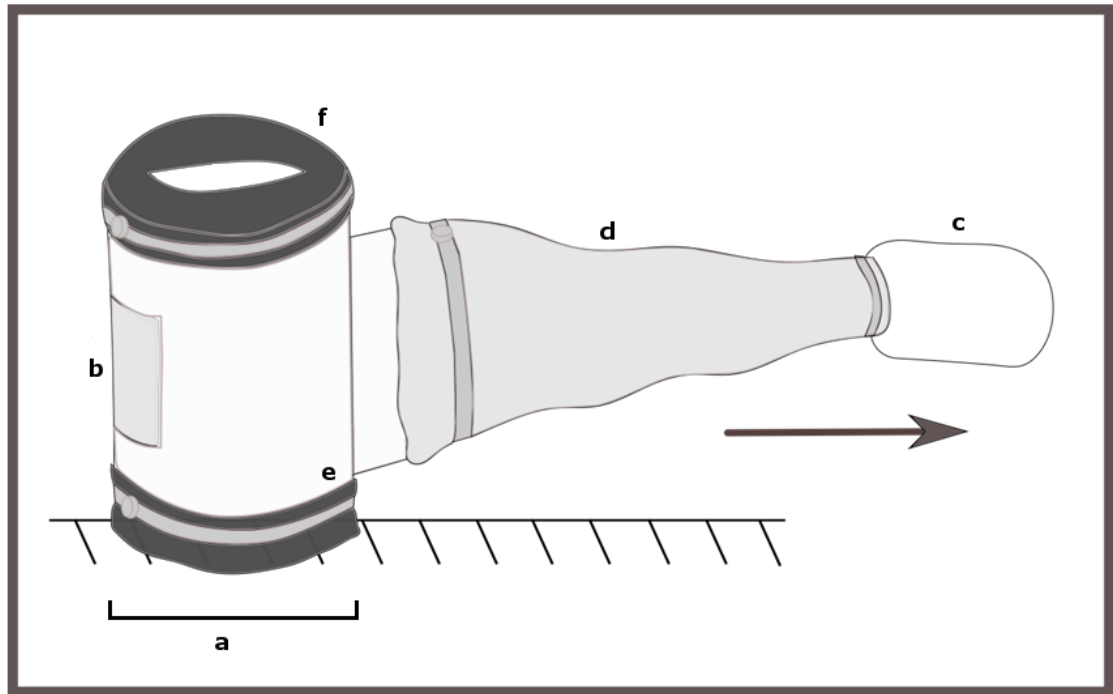
Figure 2.4. Schematic of the device used for biological sampling: a) PVC joint with a 17 cm diameter aperture, b) mesh-covered opening to allow water to flow into the sampler, c) collection cup, d) mesh bag, e) neoprene skirt, f) neoprene cover with slit. Mesh size = 83µm. The arrow denotes flow direction.
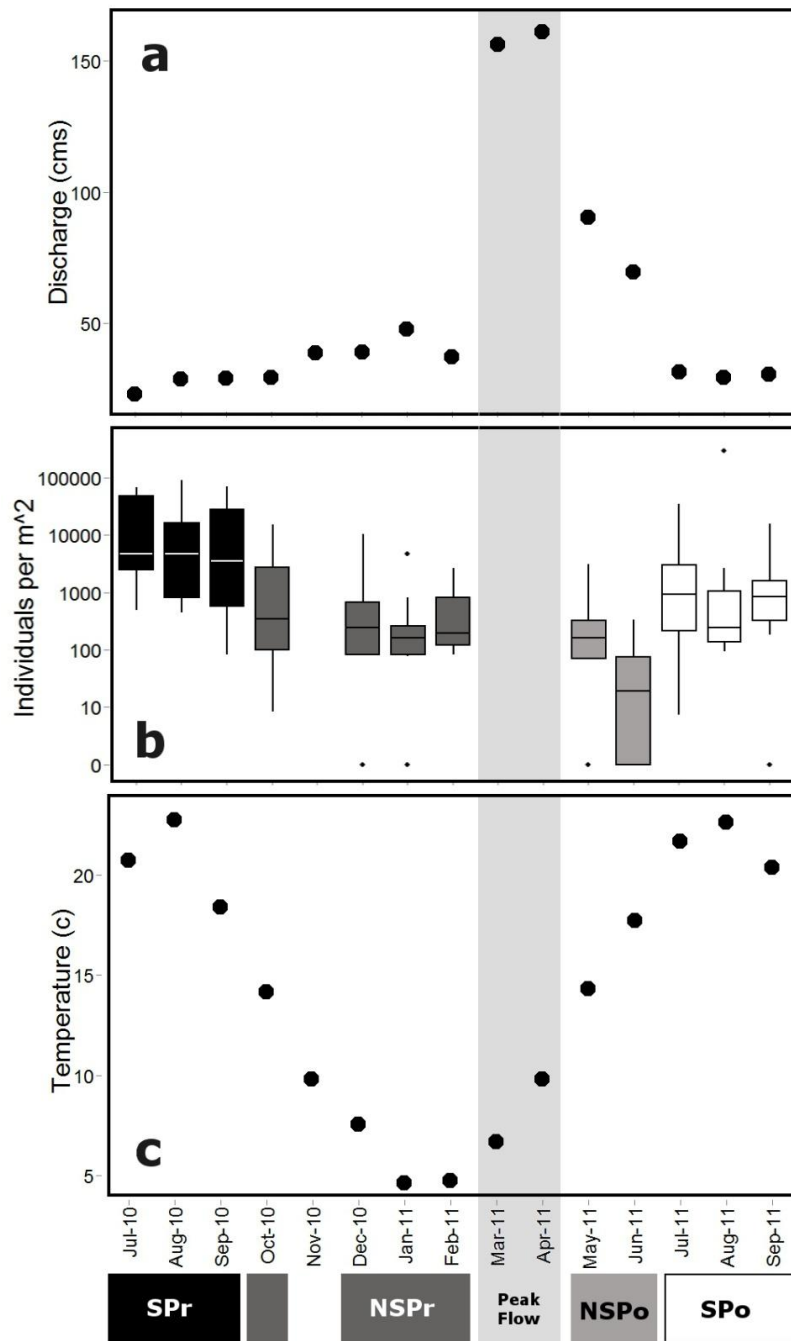
Figure 2.5. a) Discharge, b) density, and c) temperature by month and season. Discharge is presented as the average discharge during sampling trips for months that were sampled. For months that were not sampled (November, March, April) the peak monthly discharge is presented. Discharge taken from USGS gage Klamath River Below Iron Gate Dam.

Table 2.2. Summary statistics for *Manayunkia speciosa* density measurements. 'n' is equal to the number of months a site was sampled. On each sampling occasion (month) the runs and pools were sampled four times, and the eddies were sampled up to seven time.

| Reach	Feature	n	Site means poly/m2	sd	Reach and Feature means	poly/m2	sd	Season means poly/m2	sd
Summer Pre									
1	Run	3	4720	3029	**Reach**	**poly/m2**	**sd**	**poly/m2**	**sd**
1	Eddy	3	29723	33239	1	34331	33763	21244	29869
1	Pool	3	68549	18505	2	25013	33840		
2	Run	3	10610	15799	3	4388	9276		
2	Eddy	3	2056	2342					
2	Pool	3	499	447	**Feature**	**poly/m2**	**sd**		
3	Run	3	6657	5959	Run	7329	8963		
3	Eddy	3	67761	20151	Eddy	33180	34573		
3	Pool	3	620	169	Pool	23223	35232		
Non Summer Pre									
1	Run	4	45	52	**Reach**	**poly/m2**	**sd**	**poly/m2**	**sd**
1	Eddy	4	1559	1539	1	1492	1623	1459	3260
1	Pool	4	2871	1397	2	165	156		
2	Run	3	247	254	3	2481	5232		
2	Eddy	3	84	77					
2	Pool	3	164	81	**Feature**	**poly/m2**	**sd**		
3	Run	4	218	99	Run	163	160		
3	Eddy	4	6544	7527	Eddy	2969	5110		
3	Pool	3	81	81	Pool	1222	1634		
Non Summer Post									
1	Run	2	81	114	**Reach**	**poly/m2**	**sd**	**poly/m2**	**sd**
1	Eddy	2	202	188	1	121	128	331	857
1	Pool	2	81	115	2	806	1566		
2	Run	1	59	NA	3	120	183		
2	Eddy	1	11	NA					
2	Pool	2	1578	2231	**Feature**	**poly/m2**	**sd**		
3	Run	1	0	NA	Run	55	76		
3	Eddy	2	180	213	Eddy	155	164		
3	Pool	0	ND	ND	Pool	829	1553		
Summer Post									
1	Run	3	283	107	**Reach**	**poly/m2**	**sd**	**poly/m2**	**sd**
1	Eddy	3	107249	164976	1	36254	98184	13955	57150
1	Pool	3	1230	1247	2	5089	11119		
2	Run	3	1855	1131	3	522	713		
2	Eddy	3	603	921					
2	Pool	3	12809	18898	**Feature**	**poly/m2**	**sd**		
3	Run	3	1052	1052	Run	1063	1031		
3	Eddy	3	405	463	Eddy	36086	98251		
3	Pool	3	108	124	Pool	4716	11259		
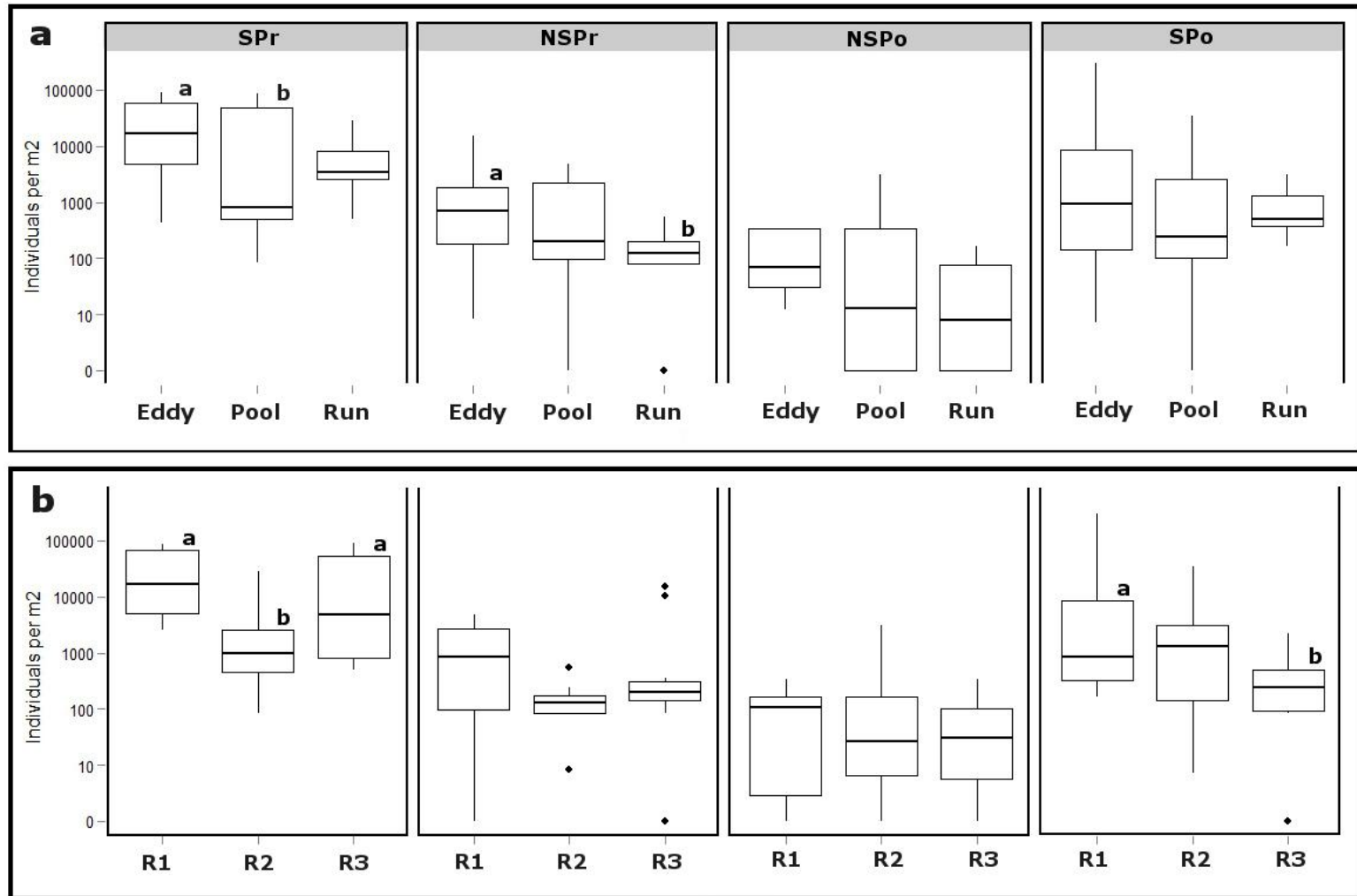
Figure 2.6. Differences in *Manayunkia speciosa* density among river features a), and river reaches b), for the four sampling periods. Each period was analyzed individually: different letters indicate a significant difference in density (2-way ANOVA, $p < 0.05$). R = reach.
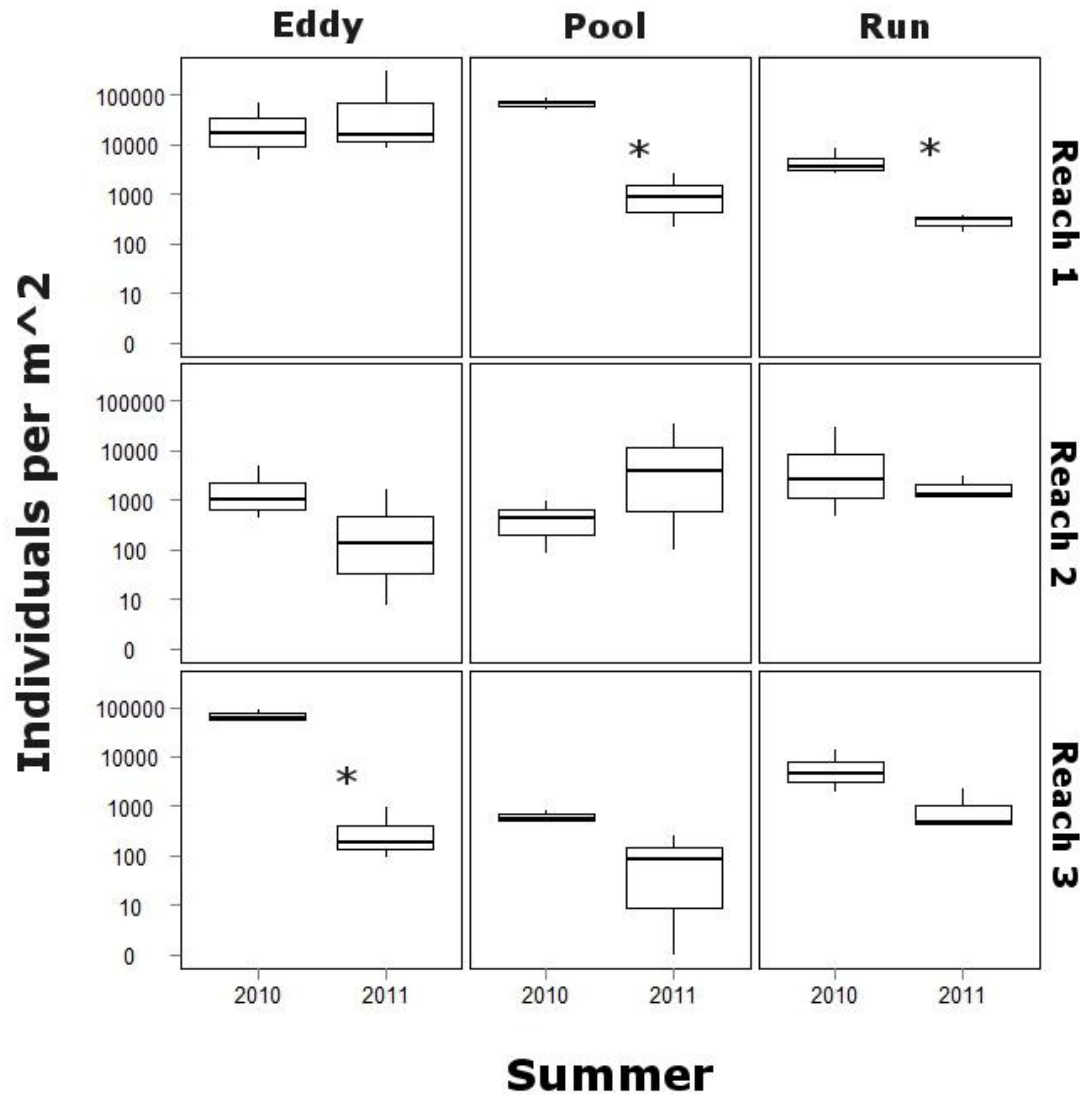
Figure 2.7. Comparison of *Manayunkia speciosa* density between summer 2010 (SPr) and summer 2011 (SPo) for individual sites. Asterisk denotes a significant difference between summers (2-tailed t-test, $p < 0.05$).
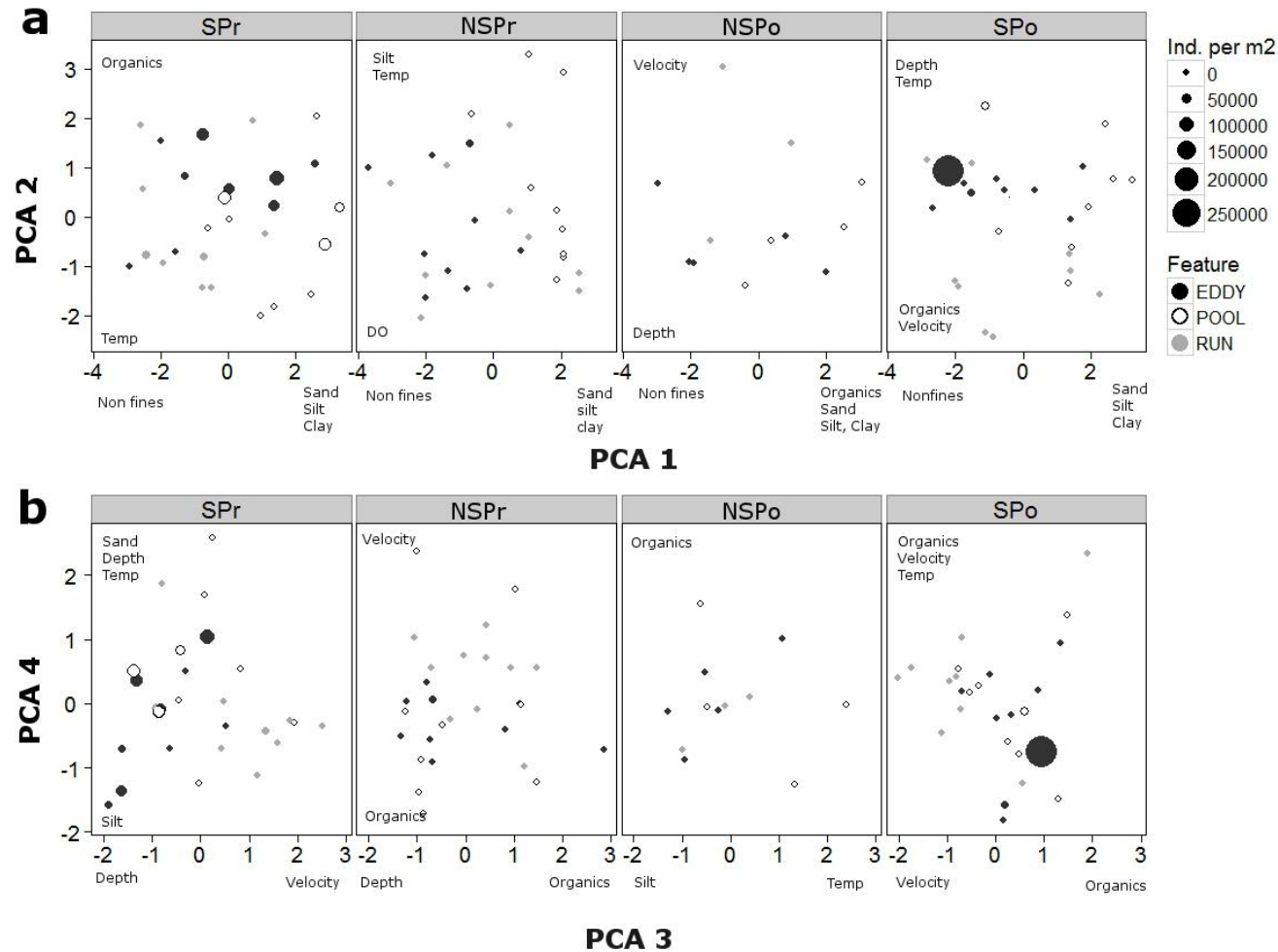
Figure 2.8. Principal component analysis of environmental data by feature class and season for a) the PC1 vs PC2 and b) PC3 vs PC4. Size of circles corresponds to density of *Manayunkia speciosa*. Text on the plots indicate which variables loaded high or low on each axis. Organics refers to the proportion of the sediment sample that was organic material.

Table 2.3. The first four principal components for each of the four seasons: Summer Pre Flow (SPr), Non Summer Pre Flow (NSPr), Non Summer Post Flow (NSPo), Summer Post Flow (SPo).

		Eigenvectors						Eigenvectors			
Season	Environmental Variable	PC1 (42%)	PC2 (18%)	PC3 (18%)	PC4 (12%)	Season	Environmental Variable	PC1 (47%)	PC2 (20%)	PC3 (15%)	PC4 (7%)
SPr	Organics	0.11	**0.69**	0.20	-0.28	NSPo	Organics	**0.38**	-0.03	0.16	**0.81**
SPr	Non fines	**-0.51**	0.22	-0.11	-0.19	NSPo	Depth	0.20	**-0.64**	0.16	0.17
SPr	Sand	**0.45**	-0.30	0.17	**0.30**	NSPo	Velocity	0.05	**0.70**	-0.20	0.27
SPr	Clay	**0.51**	0.05	-0.01	-0.11	NSPo	Temperature	-0.16	0.11	**0.76**	-0.18
SPr	Silt	**0.41**	0.26	-0.11	**-0.41**	NSPo	Non fines	**-0.47**	-0.18	-0.23	0.24
SPr	Depth	-0.07	0.25	**-0.67**	**0.41**	NSPo	Sand	**0.46**	0.19	0.26	-0.23
SPr	Velocity	-0.29	-0.02	**0.61**	0.04	NSPo	Silt	**0.34**	-0.17	**-0.46**	-0.29
SPr	Temperature	0.10	**0.50**	0.28	**0.67**	NSPo	Clay	**0.50**	0.03	-0.05	-0.14

|		PC1 (37%)	PC2 (23%)	PC3 (13%)	PC4 (9%)			PC1 (43%)	PC2 (19%)	PC3 (12%)	PC4 (11%)
Season	Environmental variable					Season	Environmental variable				
NSPr	Organics	-0.24	0.02	**0.56**	**-0.38**	SPo	Organics	0.13	**-0.37**	**0.71**	**0.39**
NSPr	Depth	-0.14	0.00	**-0.74**	0.02	SPo	Depth	-0.06	**0.72**	0.07	-0.04
NSPr	Velocity	0.19	-0.24	0.26	**0.84**	SPo	Velocity	-0.24	**-0.42**	**-0.50**	0.33
NSPr	Temperature	-0.18	**0.62**	-0.02	0.23	SPo	Temperature	-0.18	**0.38**	-0.04	**0.83**
NSPr	DO	0.24	**-0.60**	-0.05	-0.11	SPo	Non fines	**-0.51**	0.01	0.26	-0.15
NSPr	Non fines	**-0.50**	-0.16	0.11	0.10	SPo	Sand	**0.49**	-0.03	-0.29	0.16
NSPr	Sand	**0.49**	0.14	-0.14	-0.10	SPo	Silt	**0.38**	0.04	0.28	0.03
NSPr	Silt	**0.30**	**0.39**	0.15	0.11	SPo	Clay	**0.50**	0.12	-0.08	0.02
NSPr	Clay	**0.47**	0.08	0.14	-0.24						
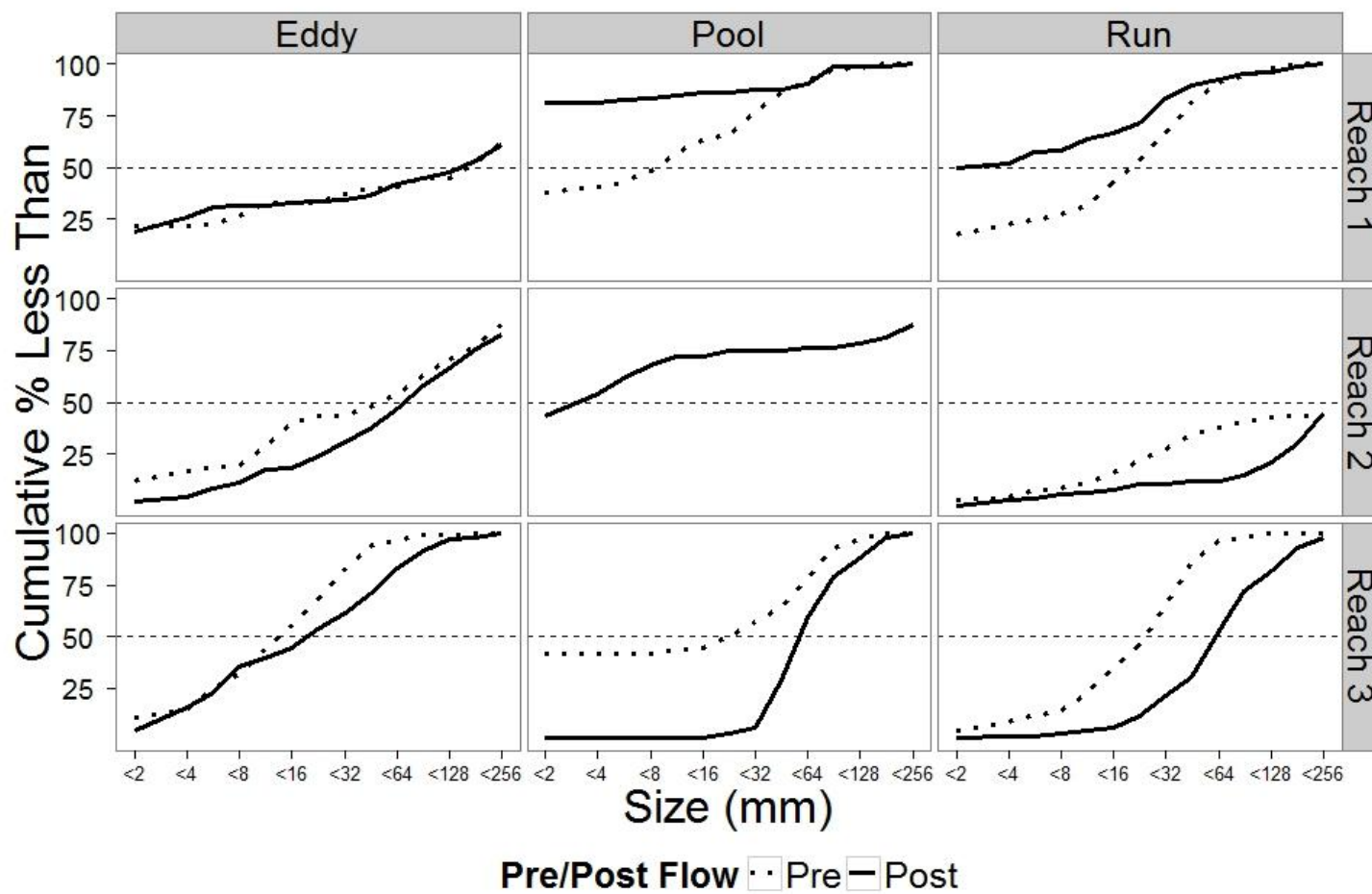
Figure 2.9. Grain size distribution. Each curve represents n ≥ 100 pebbles. 'Pre' counts were collected in December 2010 and January 2011, prior to the pulsed flow and subsequent high spring flows. 'Post' counts were collected in September 2011. There are no 'Pre' data for the pool in reach 2.

Table 2.4. Median grain size (d50) and d84 for all sites, pre and post spring high-flows. n = the number of pebbles measured.

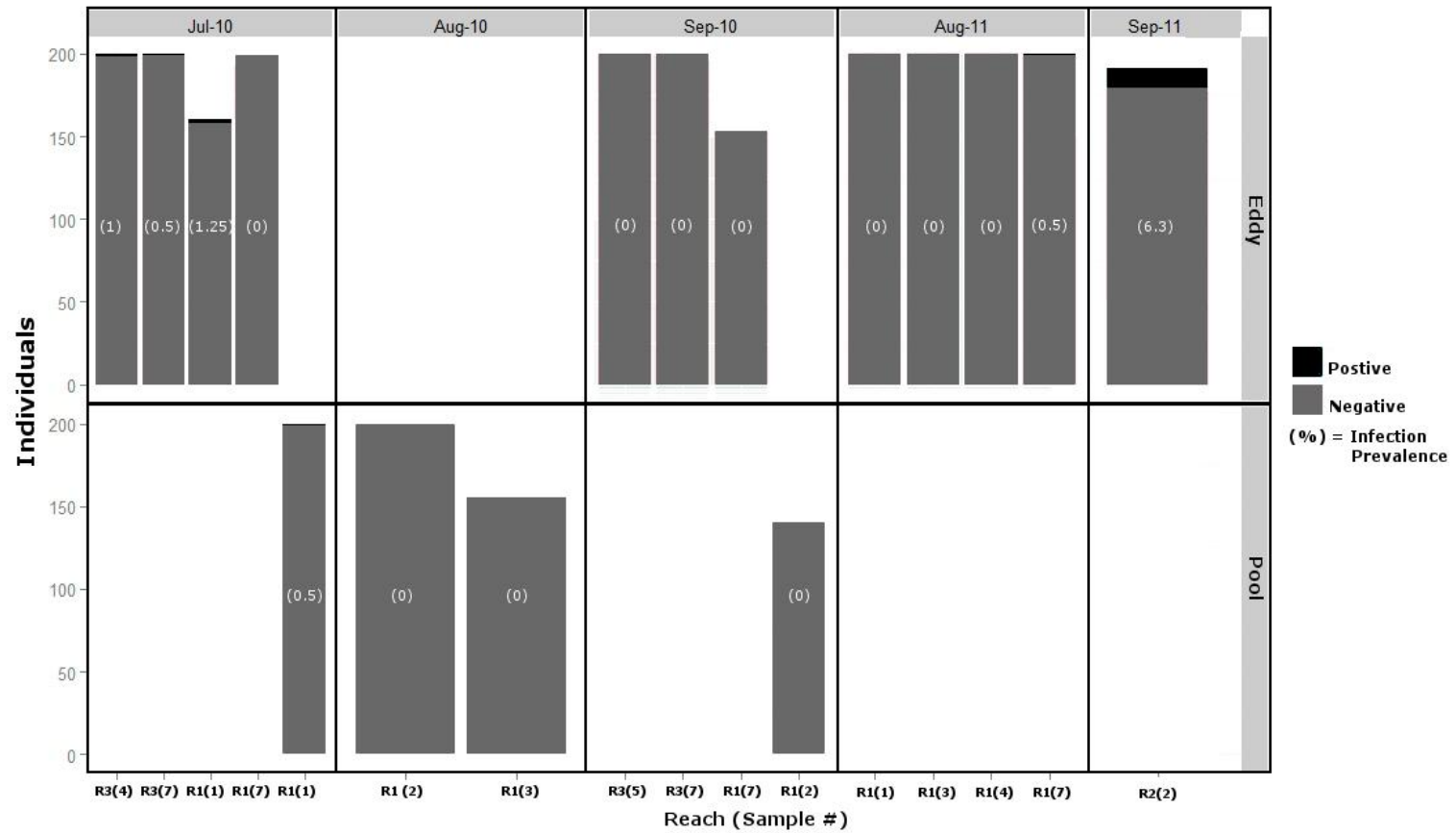| Reach	Habitat	n Pre	n Post	d50			d84		
				Pre	*Post*	*Δ*	*Pre*	*Post*	*Δ*
1	Run	101	108	20	2	-18	50	33	-17
1	Eddy	115	107	162	148	-14	>256	>256	NA
1	Pool	108	114	9	<2	- >7	42	9	-33
2	Run	106	116	>256	>256	NA	>256	>256	NA
2	Eddy	113	105	51	71	+20	227	>256	>29
2	Pool	ND	110	ND	3	NA	ND	214	NA
3	Run	104	104	24	61	+37	44	137	+93
3	Eddy	112	108	14	20	+6	34	66	+32
3	Pool	101	103	22	58	+36	73	109	+36

Figure 2.10. Proportion of polychaetes that tested positive for *Ceratomyxa shasta*. Only samples with at least 137 individuals (statistically reliable samples) are shown.

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CHAPTER 3: HYDRAULIC PREDICTORS OF *MANAYUNKIA SPECIOSA* DENSITY IN THE KLAMATH RIVER, CA

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ABSTRACT

The freshwater polychaete *Manayunkia speciosa* is the obligate alternate host of *Ceratomyxa shasta,* a salmonid parasite that causes mortality in Klamath River salmon and trout. Management strategies for parasites with multi-host life cycles may target one of the alternate hosts rather than the parasite itself, and reducing the impacts of *C. shasta* through decreasing *M. speciosa* habitat in the Klamath has been proposed. Assessing the feasibility of such a management strategy requires a thorough understanding of the variables that define habitat for the polychaete. In this study we examined the relationships between *M. speciosa* density and several hydraulic variables by developing a predictive density model. Samples (*M. speciosa* density, depth, velocity, substrate size) were collected, and hydraulic variables calculated (Reynolds and Froude number), in three reaches of the Klamath River on a monthly basis from July to September of 2010, and a zero adjusted negative binomial model (a two-part hurdle model) was fit to the data. Samples collected from July to September of 2011 were then used to assess the predictive ability of the model. In 2010 the best fitting model included depth, velocity, substrate, and a velocity-substrate interaction term in the count model, and only depth in the zeros model; density increased with depth, decreased with velocity, and was higher on small and large substrate than medium; the probability of a zero decreased with depth. The model over predicted the 2011 samples on small and medium substrate, and under predicted on large substrate. Overall model prediction had low precision, but this varied by substrate, and precision was highest on large substrate. An extended period of high discharge during the winter and spring between the 2010 and 2011 sampling may explain the over prediction on small and medium substrate, as these substrates may have been mobilized, causing disturbance. Subsequent modeling efforts should include a stability factor to account for whether flows the previous winter were high enough to cause disturbance.

INTRODUCTION

      Ceratomyxa shasta is a parasite of salmon and trout and requires a small (3-5mm) freshwater polychaete, *Manayunkia speciosa*, to complete its life cycle (Bartholomew et al., 1997). The parasite has two waterborne spore stages: the actinospore, which is infective to the fish, and the myxospore, which is infective to the polychaete (Bartholomew et al., 1997). Salmon are exposed to the actinospore stage in the river as juveniles migrating to the ocean, and as adults returning to their spawning grounds. Although high prevalence of infection of *C. shasta* occurs in both life stages of salmon in the Klamath River, OR (Foott et al., 2009), migration through a highly infectious zone of the river has been linked to reduced survival of juvenile salmon (Fujiwara et al., 2011). Opportunities for decreasing disease in the wild are limited, as it is not feasible to treat either the large volume of river water containing neither the parasite, or the wild and hatchery fish that may by exposed to the parasite at multiple stages in their life histories.

      Management strategies for parasites with complex life cycles commonly target not the parasite itself, but an intermediate host (Jobin, 1999). Decreasing densities of an intermediate host below a threshold may lessen the disease burden to the host of interest, or even disrupt the parasite life cycle completely. Approaches include directly manipulating the host or vector (Lardans & Dissous, 1998), reducing available habitat for the host (Gu et al., 2006), and decreasing spatial or temporal overlap between the hosts and the parasite. These examples are for diseases of humans; however, examples of successful management exist: management of whirling disease in hatchery trout has included the replacement of earthen ponds with concrete raceways, thus removing habitat for *Tubifex tubifex*, the invertebrate host of the causative agent, *Myxobolus cerebralis* (Wagner, 2002). Unfortunately, attempts to decrease *T. tubifex* numbers in the wild through habitat reduction are few, and have attained limited success (Thompson et al., 2011).

      One option for controlling polychaete populations in the Klamath River is manipulation of the hydrograph. The Klamath is regulated by a series of irrigation and

hydropower dams, and the discharge schedule may be altered to increase overall heterogeneity or create pulsed flows. Changes to the flow regime after the construction of dams (Poff et al., 1997) may alter invertebrate community dynamics (Growns & Growns, 2001) and it has been hypothesized that decreased flow heterogeneity in the Klamath River has allowed the proliferation of *M. speciosa* above historical densities, and that altering the hydrograph to create pulsed releases of water would decrease densities, by either decreasing habitat for the polychaete or directly increasing mortality. Evaluating this hypothesis requires an understanding of the relationship between the hydraulic environment and *M. speciosa* density.

Stream hydraulics influence the distribution and density of benthic macroinvertebrates (Growns & Davis, 1994; Brooks et al., 2005), and have been used in predictive models of presence and abundance. Hydraulic variables, including those that can be directly measured (depth, velocity, and substrate size), as well as calculated variables like Froude (Fr) and Reynolds (Re) number, have been correlated with macroinvertebrate distribution and density. These relationships have not been well established for *M. speciosa*, and information on its distribution and habitat requirements are sparse, or inferred from related species. Data suggest that *M. speciosa* has a broad tolerance for depth and substrate: individuals have been observed at locations with water depth of less than 1.5 m (Holmquist, 1967; Leidy, 1858) to over 16 m (Pettibone, 1953), and in both lotic (flowing; Hazel, 1966; Leidy, 1858; Stocking & Bartholomew, 2007) and lentic (non-flowing; Holmquist, 1967; Pettibone, 1953; Stocking & Bartholomew, 2007) environments. In lotic environments, an inverse relationship between velocity and density has been observed (Stocking & Bartholomew, 2007). Similar to the related *Manayunkia spp.* from Lake Baikal in Russia (Kozhov et al., 1998), *M. speciosa* has been observed on both soft (e.g. silt) and hard (e.g. cobble & boulder) substrate, at times embedded in periphyton covering the hard substrate (Stocking & Bartholomew, 2007). The current understanding of how these variables influence polychaete density is insufficient to inform management and a more quantitative investigation is required.

In this study, we investigated the relationship between polychaete density and several physical habitat variables by developing a predictive model. We collected random biological samples at nine sites within the Klamath River in July – September of 2010, recorded depth, velocity and substrate class, and calculated Re and Fr at each sample location. We developed a predictive model of density based on these variables, and then tested the predictive ability of the model using an independent dataset collected the following year.

MATERIALS AND METHODS

Site description

The Klamath River originates from Upper Klamath Lake in southern Oregon, and runs 423 rkm to the Pacific Ocean in Northern California (Figure 3.1). Discharge near the mouth averages a yearly low of 85 m^3/s and high of 850 m^3/s (USGS gage11530500). Flow is regulated by five irrigation and hydropower dams, the most up-stream of which controls water levels in Upper Klamath Lake. Samples were collected from nine sites in a reach of the Klamath River between the Shasta and Scott River tributaries (Figure 3.1). This reach has been termed the 'infection zone' due to the observation of higher parasite levels in the water relative to the rest of the river (Hallett & Bartholomew, 2006; Fujiwara et al., 2011). Flow in this section is typically lowest in August and peaks in April, coinciding with spring rains and melting snowpack. Discharge from Iron Gate Dam, the most downstream dam and 29 rkm upstream of the sampling sites, peaked at approximately 170 cms during the spring of 2011 (USGS station 11516530, Klamath River below Iron Gate Dam Gage). Although 170 cms is approximately equal to the magnitude of a 2-year flood, discharge had not reached this level in five years (Figure 3.2a). Water temperature was highest during August 2010, at over 20 C, and lowest in January 2011, falling below 5 C. Temperatures were similar during both sampling periods, and the springs leading up to them (Figure 3.2b).

The nine sampling locations included three each of runs, eddies, and pools. Stocking & Bartholomew (2007) observed *M. speciosa* in each of these three feature

classes at frequencies near 50%, which suggested that we would obtain both presence and absence samples. The inclusion of samples at locations where the organism of interest is absent increases the accuracy of predictive models (Brotons et al., 2004).

Sample collection

The nine features were sampled in July, August and September of 2010 and 2011. Sampling trips were four to five weeks apart, and each trip lasted three to five days. The following samples were collected using the methods described in Chapter 2.

Biological sampling: At each sampling point *M. speciosa* were collected using the sampling device described in Chapter 2). In brief, the sampler was placed on the river bed, the substrate was agitated or scraped and the suspended material collected in a cup at the end of a mesh bag attached to the sampler. Samples were stored on ice for transport, strained through 83µm mesh and preserved in laboratory grade 95% ethanol.

Environmental data collection: Depth and velocity were measured at each biological sampling point. Mean column velocity was measured at 0.6 of the depth using a Marsh McBirney Flowmate 5000, with the probe attached to an extendable painters pole. The pole was marked in 0.1m increments and used for depth measurements. Substrate at the sampling point was classified by visual estimation into three substrate categories: Small ($\leq$ 4mm; sand & silt), Medium (4-264mm; gravel & cobble), and Large (>264mm; boulder & bedrock).

Sample processing

Biological samples: Biological samples were processed for enumeration of *M. speciosa* using the methods of Stocking & Bartholomew (2007) with modification. Each sample was emptied into a gridded sampling tray (WILDCO, model 182-D10). Two 10-sided dice were used to select three of the 15 squares in the grid. Using a transfer pipette and tweezers, the material in each square was transferred to a 15 ml scintillation vial and dyed with a 1:4 addition of Rose Bengal (stock solution = 0.05g/L 95% ethanol). Each subsample was examined under a dissecting microscope at 250 x magnification, and all polychaetes were counted and placed in a 1.5ml microcentrifuge tube with 95% ethanol. If the density of polychaetes in a subsample

was high, it was quartered. If >200 polychaetes were counted in one sample quarter, the count was multiplied by four and the rest of the subsample was not counted.

Calculated variables: Reynolds number and Froude number were calculated as in Quinn and Hickey (1994).

The equations used were:

$$Re = \frac{V * D}{\nu}$$

$$Fr = \frac{V}{(g * D)^{0.5}}$$

where V = mean column velocity, D = depth,

ν = kinematic viscosity = 1.00 x 10^{-6} m^2/s ,

and g = acceleration due to gravity.

Statistical analyses

Zero adjusted negative binomial: Multivariate zero adjusted negative binomial (ZANB) regressions of *M. speciosa* against the measured and calculated predictors (depth, velocity, substrate, Re, Fr) were performed. ZANB is a hurdle model with two parts: a negative binomial general linear model that models count data, and a binomial model that models the zeros (Zuur, 2009). The two model parts can have the same, or different predictor variables. Hurdle models are employed when the number of zeros is greater than what would be predicted by the count distribution (in this case, a negative binomial distribution). The negative binomial distribution was selected over a poisson, distribution because it accounts for over-dispersion (variance > mean) in the data. Analyses were performed using R (R development core team, 2011), and the PSCL package (Jackman, 2012) with the 'zeroinfl' and 'hurdle' functions (Zeileis et al., 2008).

Model fitting: A ZANB model was fit to the 2010 data by starting with the full model equation, incorporating velocity, depth, substrate, and their interactions into both parts of the model (count, and zero). Non-significant terms were removed and AIC (Akaike Information Criterion) values compared to evaluate the relative fit of the

models. AIC is a common method of comparing the relative fit of statistical models. Models with velocity and depth replaced by first Re, and then Fr, were also run and compared to determine the relative fit of the model.

Model validation: The best fitting model was used to predict density values in the 2011 dataset. Sample predictor variables (depth, velocity, etc) were supplied to the model, and the predicted values compared to the observed. A perfect prediction would result in a slope of 1, an intercept of 0, and an R^2 of 1.

RESULTS

Density

A total of 258 samples were collected: 127 from 2010, and 131 from 2011. The number of samples in which polychaetes were detected was greater in 2010 than in 2011 ($\chi^2 = 17.6$, p $= 2.66 \times 10^{-5}$): polychaetes were observed in 80% (102) of the samples from 2010 and 56% (74) of the samples from 2011 (Figure 3.3a). Frequency of occurrence of polychaetes by depth varied between years: in 2010 polychaetes were detected in 96% (48/50) of samples at depths over 1.5m, but in 2011 they were only detected in 58% (32/55) ($\chi^2 = 20.6$, p $= 5.52 \times 10^{-6}$; Figure 3.4). In both years, the data were over-dispersed (variance > mean). In 2010, density ranged from 0 to 529,801 individuals/m^2, with a mean of 24,505 individuals/m^2 (variance $= 4.39 \times 10^9$)(Figure 3.3b). In 2011, density ranged from 0 to 728,517 individuals/m^2, with a mean of 18,965 individuals/m^2 (variance $= 8.77 \times 10^9$) (Figure 3.3c). The presence of zero-inflation and over-dispersion in the data indicated that a hurdle model (to account for excess zeros) with a negative binomial count regression (to account for over-dispersion) were appropriate.

Explanatory variable distributions

Samples (positive and negative) were collected from locations with mean column velocities of 0 to 0.59 m/s in 2010, and 0 to 0.93 m/s in 2011 (Figure 3.5a). The average velocity sampled did not differ between years (two-sample t-test, p $= 0.29$). The average depth sampled also did not differ between years (two-sample t-test, p $= 0.55$) and ranged from 0 to 3.75 m in 2010 and 0 to 3.4 m in 2011 (Figure 3.5b).

Medium substrate was the most commonly sampled size class both years (Figure 3.5c), and there was no difference in the sizes sampled between years ($\chi^2 = 0.10$). Calculated Reynolds number ranged from 0 to 9.4 x 10^5 with a mean of 1.7 x 10^5 (variance = 3.8 x 10^{10}). Calculated Froude number ranged from 0 to 0.27, with a mean of 0.05 (variance = 0.003). Reynolds number was natural log transformed before being used in the model, and Froude number was square root transformed.

Model fitting with 2010 data

The best-fitting ZANB model included depth, velocity, substrate and a substrate:velocity interaction in the negative binomial portion of the model, and only depth in the zeros model (Table 3.1). In 2010, density was directly related to depth (p = 2.18x10^{-12}, coef = 1.04, std error = 0.15), and inversely related to velocity (p = 5.94x10^{-9}, coef = -11.81, std error = 2.03) (Figure 3.6a). Density increased on small substrate relative to large substrate (p = 0.04, coef = 1.13, std error = 0.55) and decreased on medium substrate relative to large (p = 0.01, coef = -1.37, 0.54). This is not immediately apparent from the boxplots in Figure 3.6a because the relationship was influenced by a significant interaction between substrate and velocity. The interaction was driven by differences in how density responded to velocity by substrate class: as velocity increased, density decreased similarly on both small and large substrate, but on medium substrate there was more variability and higher relative densities (p = 2.43x10^{-7}, coef = 14.42, std error = 2.80) (Figure 3.6).

The only significant term for the zeros model was depth (p = 0.0007, coef = 1.32, std error = 0.39), highlighting the decreasing proportion of zeros (samples without polychaetes) with increasing depth (Figure 3.4, 2010 only). Although relationships between density and Froude number and Reynolds number were detected, they were weaker than the relationships between density and depth and velocity (Table 3.1).

Model predictions for 2011

The model built with the 2010 dataset had poor predictive power for the 2011 data set, highlighting differences in the density-environmental variable relationships

between years (Figure 3.6). Plotting predicted versus observed densities for 2011 resulted in an intercept of -5689, a slope of 1.62, and an R-squared value of 0.163 (Figure 3.8a,b). The model over-predicted density on small and medium substrate (Figure 3.8c, d) and had very poor precision (R-squared = 0.027 and 0.0388 respectively). On large substrate the model under predicted density (Figure 3.8e) but had better precision (R-squared = 0.2079). The model also under-predicted the number of zeros in the 2011 data set: predicted = 28, actual = 57.

DISCUSSION

We found that in 2010, *M. speciosa* density was significantly related to depth, velocity, and substrate size: density increased with increasing depth, decreased with increasing velocity, and was lowest on medium substrate. In general, high densities of polychaetes were found in slow deep water, on large and small substrate. Density also increased with decreasing Re and Fr, but with a weaker fit. We also found evidence of a change in population size and habitat use from 2010 to 2011: the overall number of samples in which polychaetes were detected decreased, as did the number of polychaetes detected at depth (>1.5 m), and the average polychaete densities on small and medium substrates. These changes in density are reflected in the model predictions: more zeros occurred in 2011 than predicted, and density was over-predicted primarily on small substrate. One possible explanation for these changes is the occurrence of a perturbation between sampling years; specifically, the high discharge observed in winter and spring of 2011.

Low flow in the years preceding 2010 may have allowed *M. speciosa* to colonize less stable habitat (e.g. smaller substrate in unprotected areas), which was subsequently disturbed during the higher discharge of winter and spring 2011. The over-prediction of summer densities on small substrate would therefore be a legacy effect of flow-mediated disturbance. An improved predictive model would include a term to account for flow history; for example, whether a discharge maximum (e.g. 2-year flood) was exceeded in the previous year. Adequately assessing this hypothesis will require additional density data from years with varying flow regimes. If the

hypothesis is supported, in addition to improving the predictive density model, it would also suggest that there is potential for managing *M. speciosa* density by increasing hydrograph heterogeneity and destabilizing habitat that would otherwise be suitable during low flow years.

Predictive models of species abundance based on environmental variables may also be imprecise due to variance inherent in the data (our data were extremely over-dispersed) and the potential for missing important explanatory variables. Factors not analyzed in our modeling included biotic (e.g. food availability, inter-species competition), non-hydraulic abiotic (temperature), and alternative hydraulic (near-bed hydraulics). Including a subset of these variables in future modeling work could prove beneficial.

Biotic interactions are important for determining species distribution and abundance: the physical template might be hospitable for the organism of interest, however if food is limited, or there is competition for resources, the organism may not colonize or persist (Richardson, 1992; Rowe & Richardson, 2001). Unfortunately, little is known about the feeding habits of *M. speciosa* or its role in community dynamics, and these topics are in need of further investigation. However, while food availability may contribute to polychaete distribution and abundance overall, it is likely not a primary influence on the observed distribution as the Klamath River originates in Upper Klamath Lake, a hypereutrophic lake with common summer algal blooms (Kann & Welch, 2005). In addition to biotic factors, abiotic variables like water temperature are important to population dynamics: water temperature influences species richness (Jacobsen et al., 1997) community composition (Gore, 1977), growth, reproduction, and development of aquatic organisms (Merritt et al., 1982). However, temperature likely does not explain the density differences we observed because temperature differences among sampling locations and between sampling years were negligible, and the warming pattern during the springs leading up to sampling periods were similar.

Although velocity, depth, substrate size, Fr and Re have been correlated with aquatic invertebrate density, estimates of near-bed hydraulics may provide better prediction of species abundance. *Manayunkia speciosa* is a benthic organism with low mobility, and an entirely aquatic life cycle. Like all aquatic organisms, it is subject to the hydraulic environment, which determines its ability to remain in place, feed, and reproduce (Vogel, 1994). Large-scale hydraulic variables like water depth, mean column velocity, and average substrate size do not precisely describe the forces acting on a polychaete and its tube, which exist on the scale of millimeters. Instead, they estimate the average conditions of the flow, which may not be representative of the hydraulic environment around a polychaete clinging to a boulder. Therefore there may be hydraulic parameters that are more relevant for describing physical habitat for *M. speciosa* than those we selected. For example, Brooks et al. (2005) found that Roughness Reynolds number was most strongly associated with density, and Merigoux & Doledec (2004) observed a decrease in taxon richness, and in the proportion of filter feeders, with increasing shear stress. Therefore we suggest that future predictive modeling work also explore the relationship between *M. speciosa* density and these and other near-bed hydraulic variables.
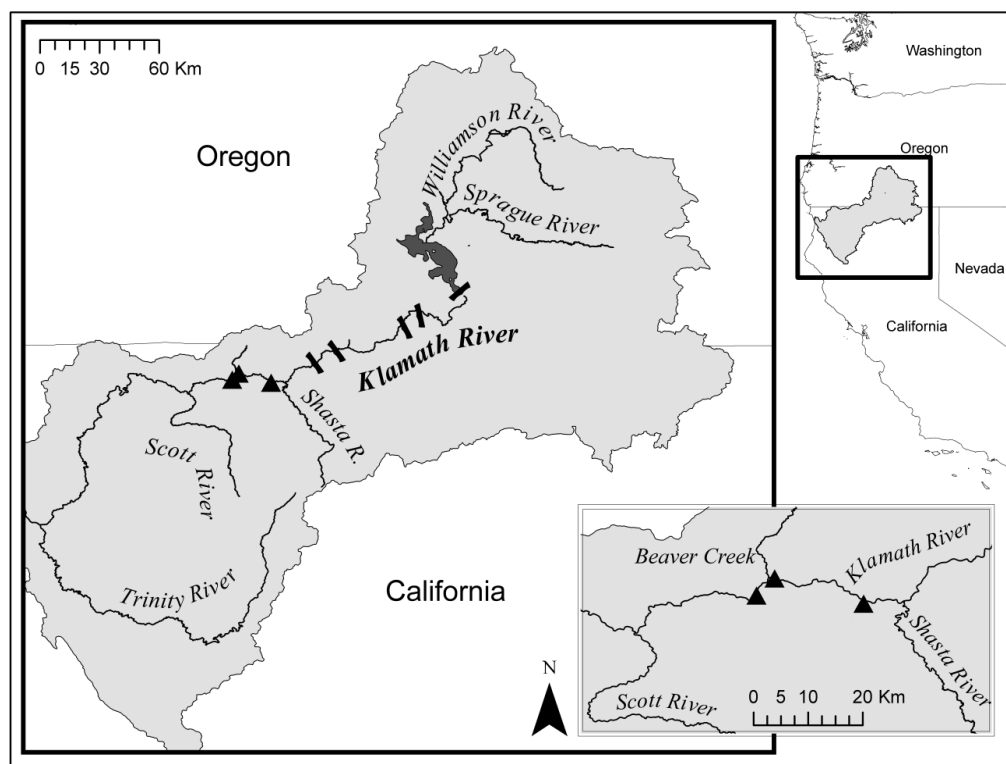
Figure 3.1 Sampling sites on the Klamath River, CA. Solid bars mark locations of dams, the most downstream of which is Iron Gate Dam (Rkm 306).
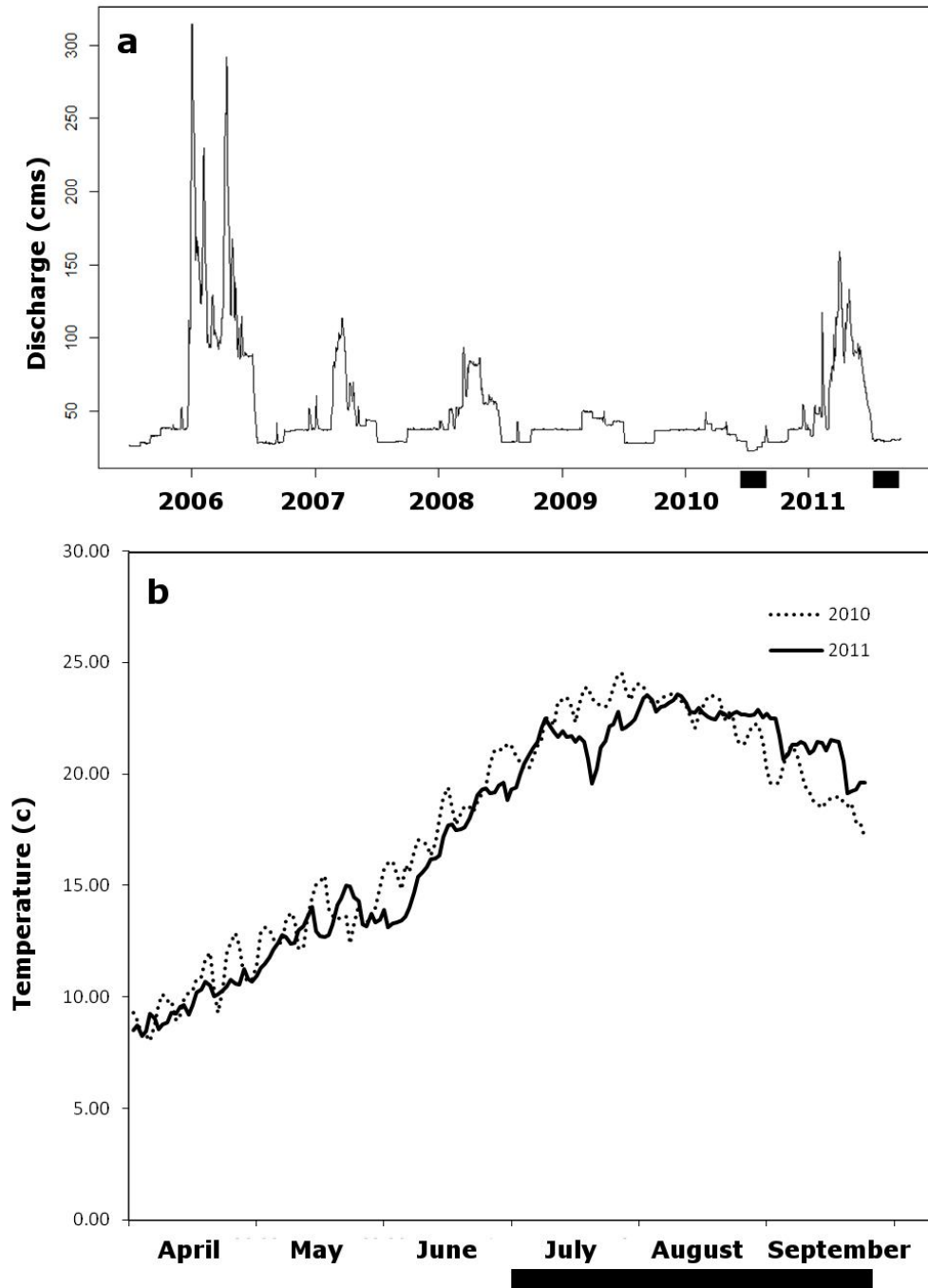
Figure 3.2. a) Discharge in m^3/s at USGS gage 11516530, Klamath River below Iron Gate Dam, and b) temperature in degrees Celsius, at the middle sampling reach. Black rectangles denote sampling periods.
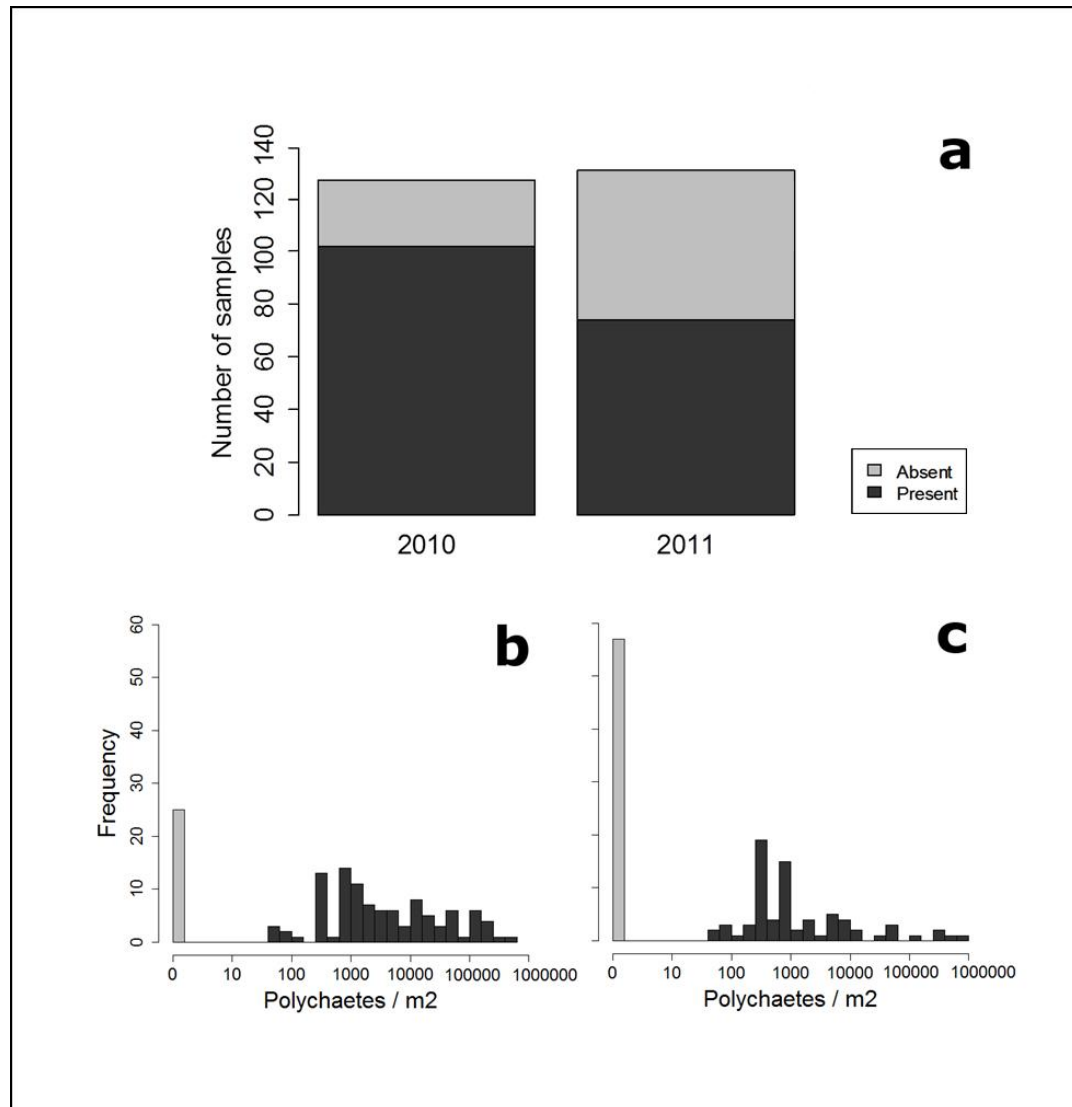
Figure 3.3. a) Number of samples with *Manyunkia speciosa* present versus absent in 2010 and 2011 and frequency distribution of density for b) 2010, and c) 2011. The spike at zero (b, c) is an indication of zero inflation.
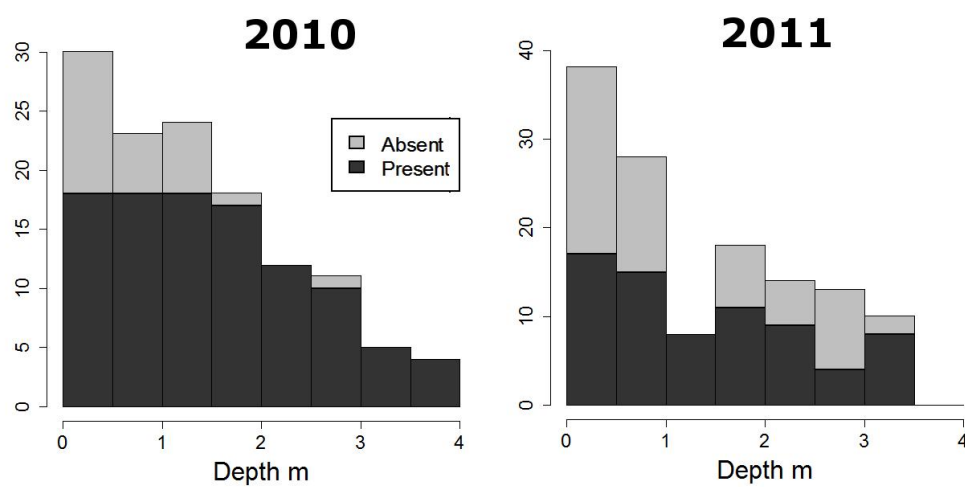
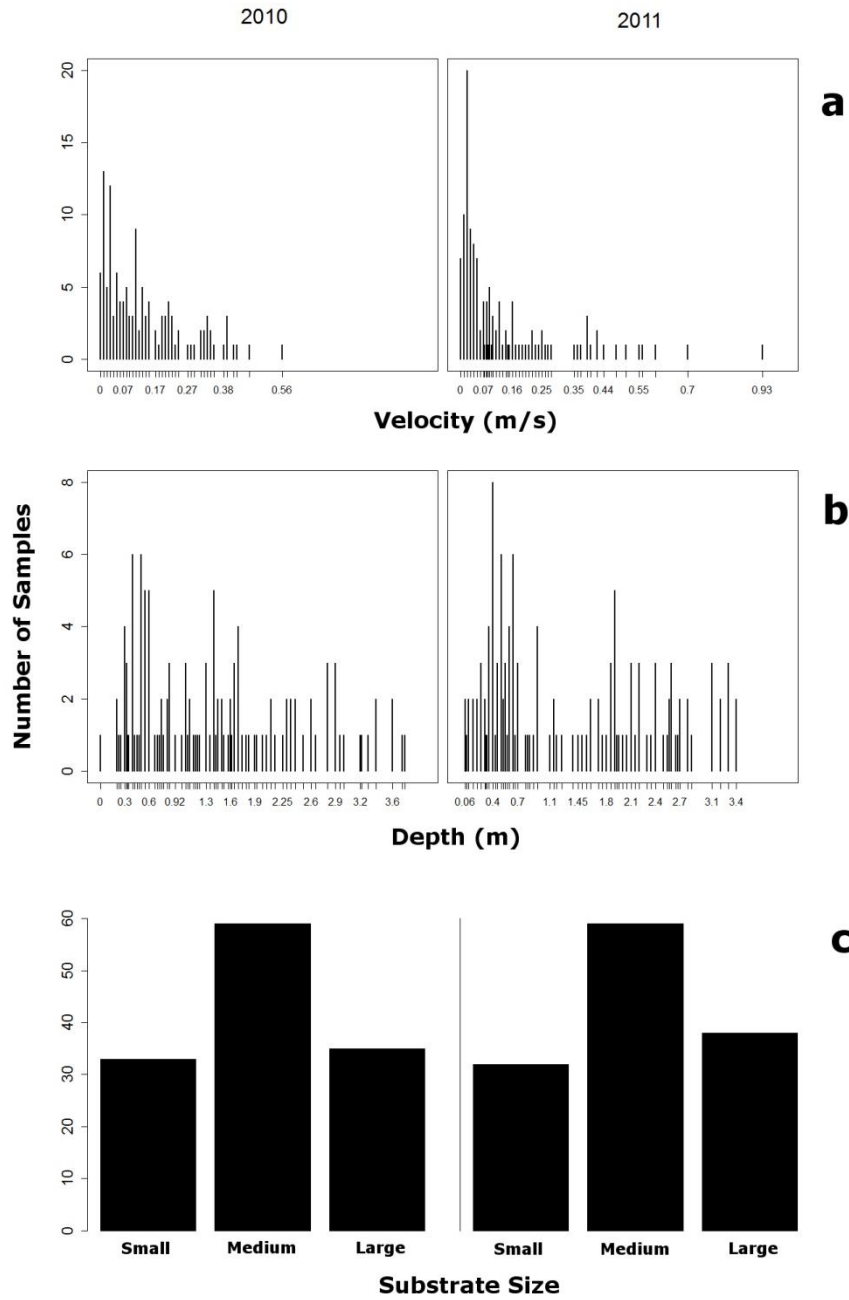Figure 3.4. Presence/absence of *Manayunkia speciosa* by depth for 2010 and 2011.

Figure 3.5. Frequency distribution of the a) velocities, b) depths, and c) substrate classes that samples were collected from for 2010 and 2011. Plots include samples both positive and negative for *Manayunkia speciosa*.

Table 3.1. Comparison of fits for zero adjusted negative binomial models. Count = formula for the count model and Zero = formula for the zeros model. V = velocity, D = depth, S = substrate type, Fr = Froude number, Re = Reynolds.

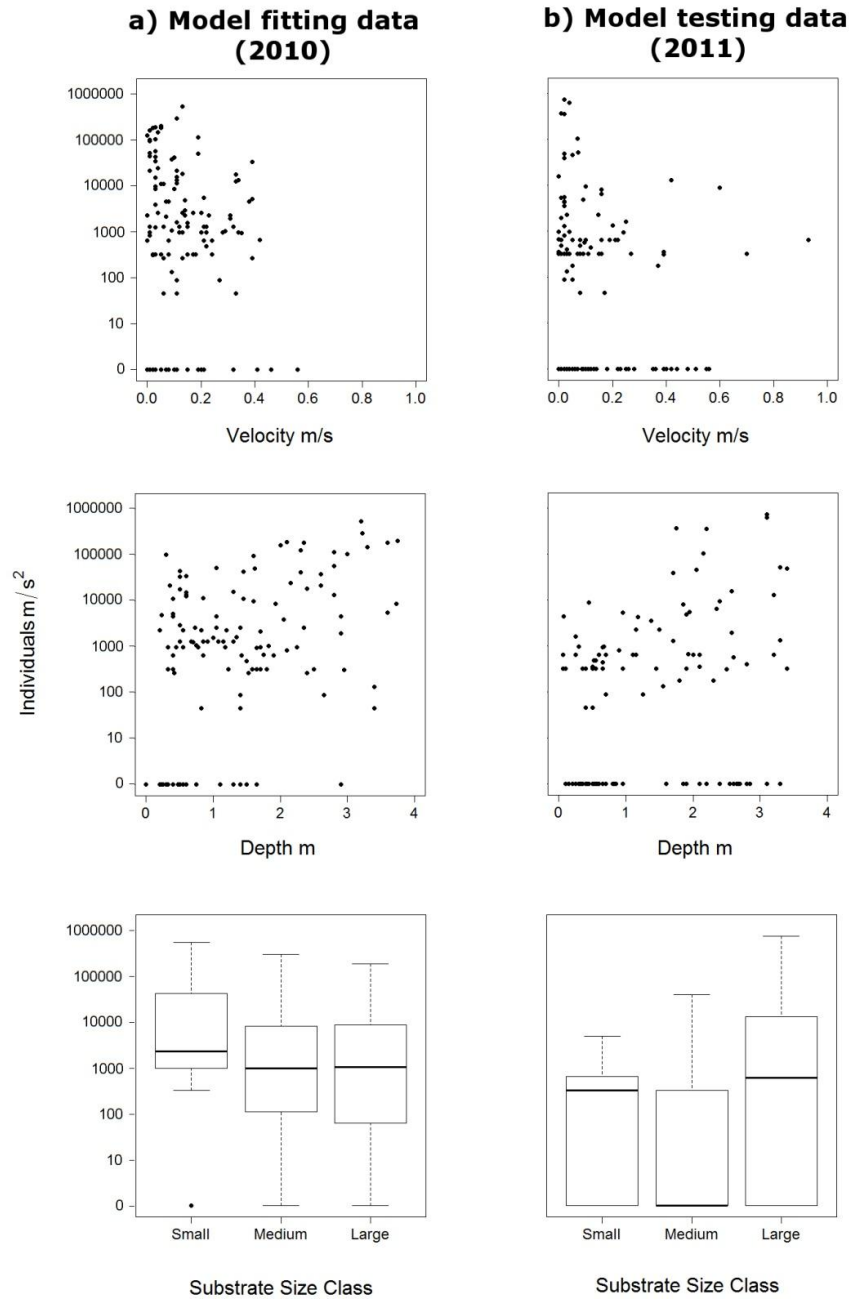| Formula				
Count	*Zero*	*Log-likelihood*	*Df*	*AIC*
V * D * S	V * D * S	-1094	25	2239
V * S + D	D	-1100	10	2220
Fr * S	Fr * S	-1129	13	2270
Re * S	Re * S	-1122	13	2283

Figure 3.6. Natural log scale of *Manayunkia speciosa* density versus the measured hydraulic variables a) velocity, b) depth, and c) substrate size class, for 2010 (modeling fitting) and 2011 (model testing) datasets.
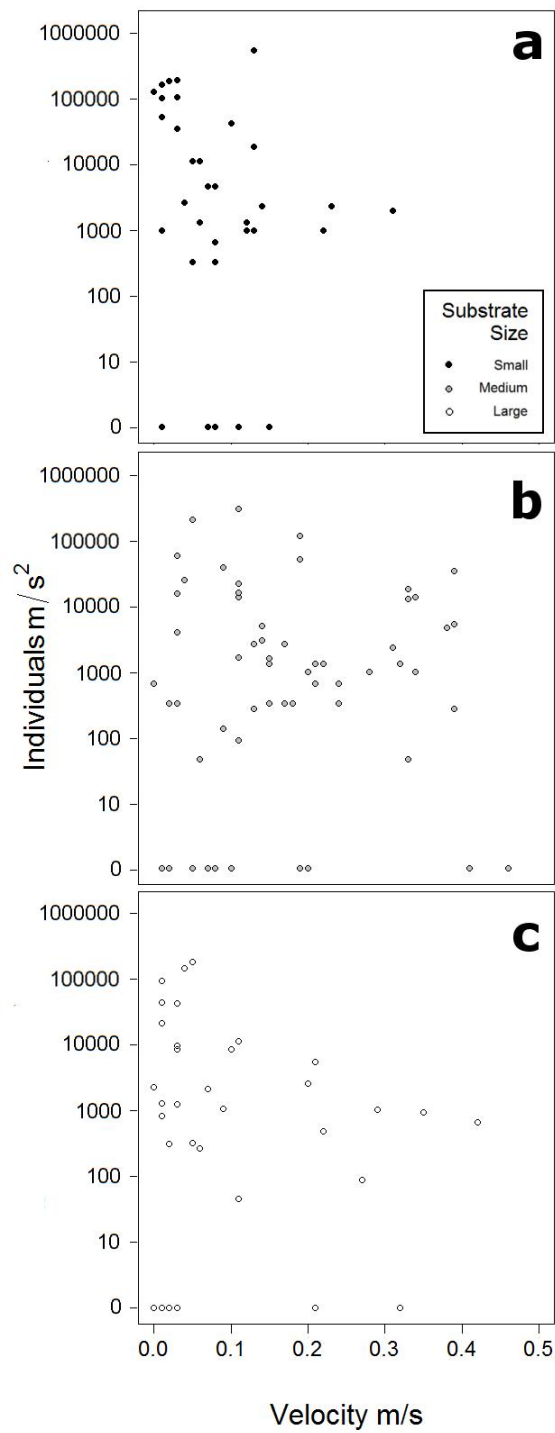
Figure 3.7. *Manayunkia speciosa* density versus velocity by substrate size class (a = small, b = medium, c = large) for model fitting data from 2010.

Figure 3.8. a) Observed versus predicted polychaete densities for the 2011 dataset, b) close up of observed versus predicted, excluding the four extreme observed values, and natural log of observed versus predicted polychaete density for c) small, d) medium, and e) large substrate size classes. Predicted values were generated using a model built on the 2010 dataset. Trend lines are linear regressions.

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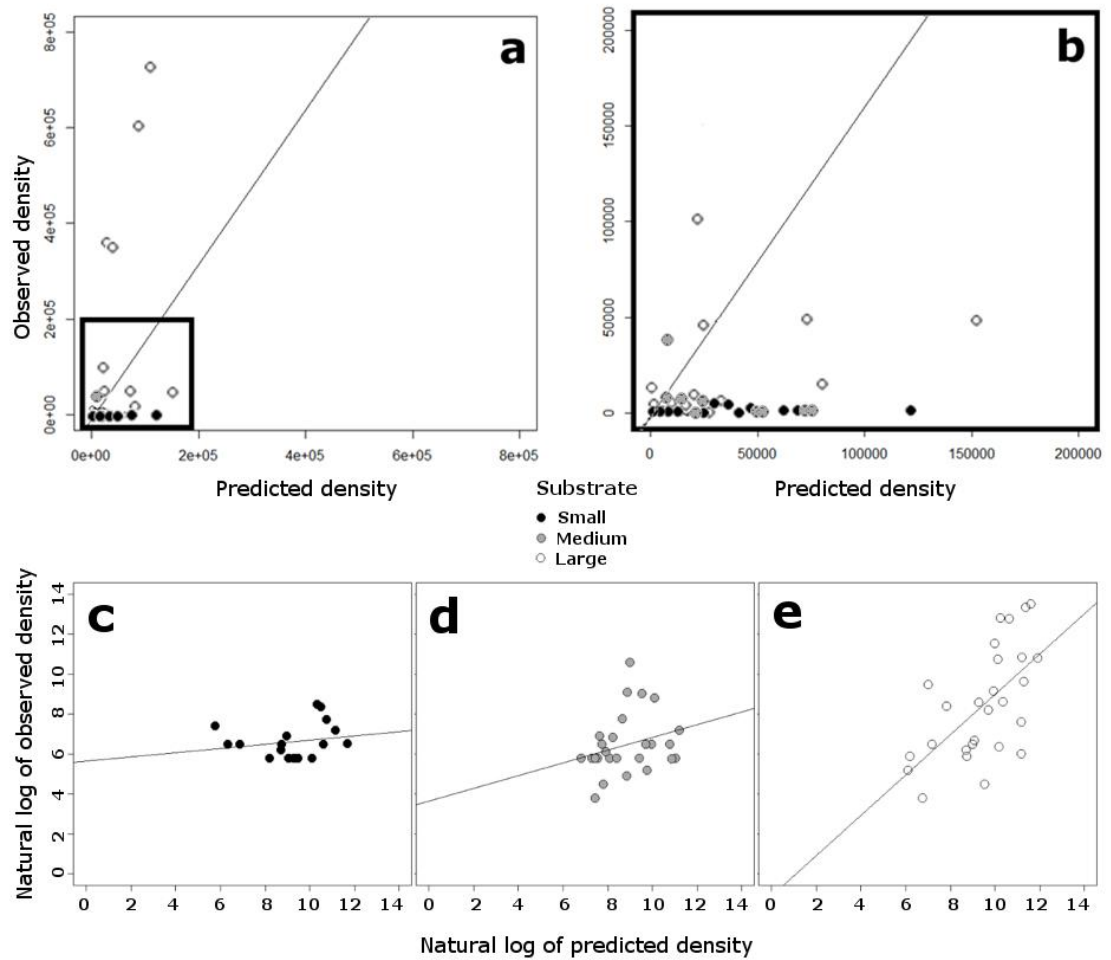Bartholomew, J., Whipple, M., Stevens, D., & Fryer, J. (1997). The life cycle of Ceratomyxa shasta, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *Journal of Parasitology, 83*(5), 859-868.

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CHAPTER 4: SUMMARY

This thesis investigated factors driving the distribution and abundance of the freshwater polychaete, *Manayunkia speciosa*, and infection of the polychaete with *Ceratomyxa shasta*, a parasite of salmon and trout. I found that *M. speciosa* density and distribution is influenced by both local-level hydraulic variables (depth, velocity, substrate size), and large-scale hydrologic factors (hydrograph). Density is directly related to depth, and inversely related to average velocity. Substrate size also determines density, but it appears the relationship is influenced by broader trends in discharge. Specifically, *M. speciosa* is able to colonize and reach high densities on small substrate in the Klamath River in sheltered areas with low velocity; however, because these areas are more susceptible to disturbance than locations with larger substrate, the probability of finding *M. speciosa* on small substrate is influenced by prior discharge patterns. The magnitude of a discharge event necessary to remove *M. speciosa* from small substrate habitats was not investigated in this study, but is likely similar to the flow necessary to mobilize the substrate itself. Additionally, the duration of the flow event may be important: a short event may only move the polychaetes a short distance and not cause mortality, allowing them to re-colonize. The fluctuation in density over a year followed a similar pattern to water temperature (highest in the summer, lowest in the winter), indicating that there may be a degree-day threshold for reproduction, and a tolerance limit. Unfortunately, infection prevalence was very low overall, and we were unable to draw any conclusions about the influence of habitat on infection.

Hydraulic models are currently being developed at the three study reaches from this thesis. The purpose of these models is to predict the discharge necessary to decrease the amount of available polychaete habitat, or to cause disturbance. The polychaete density predictive model developed in Chapter 3 did not perform well enough to be used in conjunction with the hydrodynamic models to predict habitat. However, it does provide an important baseline of data to be used in further investigation: in the summer of 2012, an additional polychaete density dataset was

collected to refine the predictive model, and in 2013 a validation set will be collected. The methods and locations for this sampling were based off of the data from Chapter 3. Ultimately all four years of data may be combined to create a model that includes yearly differences in high flow. This could account for whether discharge the previous year had reached a threshold for destabilizing habitats with small substrate. If the threshold was exceeded, then the model would predict lower densities in those habitats.

Revisiting the influence diagram that was introduced in Chapter 1, the work in Chapters 2 and 3 supported the hypothesized importance of substrate, depth, and velocity in determining polychaete density. The work also provided evidence that the hydrograph impacts density through the potential for disturbance; however this dataset only represents two water years and sampling during additional water years is necessary to discriminate between the influence of discharge, and of natural fluctuations in population size from one year to the next. The seasonal cycle of density mimicked that of water temperature and investigation into this correlation could include laboratory studies to determine if degree day thresholds for development and reproduction exist. The influence of geomorphology on density remains unclear, as the feature types (runs, eddies, pools) tested did not yield categorical differences in density. Further work would benefit from a less coarse description of geomorphology, focusing on the specific characteristics of each site. This improved understanding of the factors influencing *M. speciosa* density will aid in developing management strategies for decreasing disease impacts of *C. shasta* on salmon and trout in the Klamath River.

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