Ecology of aquatic insects in monsoonal temperate glacier streams of Southeast Tibet: A departure from the conceptual model

DISSERTATION

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Abstract

The cryosphere is shrinking as a result of climate change. Mountain glaciers, a key component of the cryosphere, serve as headwaters to glacier meltwater streams which support communities of stenothermic organisms. The Tibetan plateau is known as "the Third Pole" for its high number of glaciers, yet very few scientific papers have been published on aquatic invertebrate ecology of glacier-fed streams in the region. On the edges of the Tibetan Plateau in Southeast Tibet's Hengduan mountains, monsoonal temperate glaciers extend well below the treeline as valley glaciers, and are perhaps the most endangered cryosphere-dominated streams in the world due to their low latitudes and altitudes, which makes them sensitive to atmospheric temperature changes. The glaciated headwaters of the Mekong and Yangtze Rivers comprise a small fraction of the annual river discharge, yet at a local scale provide glacial meltwater that supports endemic and potentially rare species.

Water temperature and channel stability differ between seasons due to the torrential flow from glacial meltwater during the summer melt season. The Milner & Petts (M&P) model of macroinvertebrate presence in glacier streams was based on the environmental factors of water temperature and channel stability during the summer melt season. In low temperature water close to the glacier, the macroinvertebrate communities are generally limited to Diamesinae chironomids, and further downstream more taxa are

found where water temperature and channel stability increase. Therefore, temperature and channel stability are examined as potential limiting factors on the distribution of invertebrate communities, with the goal to compare the insect communities in Southeastern Tibet's glacier-fed streams with the widely-accepted M&P model of invertebrate community structure.

Since discharge and hydrology may influence invertebrate distribution in glacier streams, hydraulic characteristics and invertebrate communities in six microhabitats (pool, riffle, run, step-run, rapid, and step-rapid) were examined over three seasons in the Mingyong Glacier stream. This is the first known study to examine hydraulics at the microhabitat level in glacier streams, and to compare microhabitats by season. Certain hydrologic characteristics have been found to trap and retain leaf litter at different rates, with backwater areas having the greatest trapping efficiency even in high discharge. Moreover, microhabitats with turbulent water entrain oxygen from the atmosphere which can lead to greater biomass growth. Microhabitats with larger substrate create hydrologic refugia for insects, and higher velocity microhabitats tend to have larger boulder substrate. Therefore it was hypothesized that in the Mingyong Glacier mainstem rapids and step-rapids with greater water velocities and velocity ranges would support greater aquatic insect species richness and abundance.

In addition to hydraulics and environmental variables, the salinity tolerance of glacier stoneflies was examined to partially study the research question, "will metakryal zone insects from glacier streams adapt to groundwater stream salinity levels once the glaciers are gone"? Moreover, the morphological changes of their chloride cells following exposure to waters of differing salinity were quantified by using scanning electron microscopy images. The survivorship of stoneflies from upstream glacial meltwater sites was compared with stoneflies from downstream glacial meltwater sites to salinity treatments.

The ecological result of this research is a clear departure from the accepted M&P glacier stream model where temperature and channel stability are suggested to determine aquatic invertebrate presence in glacier streams. Temperature and channel stability did not exhibit a relationship with species presence, richness, or abundance in Southeast Tibet's temperate glacier streams during the summer melt season. There were fourteen taxa in addition to Diamesinae in the metakryal zones where the T_{max} temperature remains $<2^{o}$C all year. This is contrary to almost all other studies of glacier streams. The results suggest that other factors such as the mass elevation effect (MEE) of the mountain range, glacier and stream size, winter water temperatures, elevational position of the glacier, ice/snow coverage, and position of the glacier tongue beneath the treeline could be as important in these systems as water temperature and channel stability. In addition, the fact that water is perennially flowing from beneath the glaciers could be a result of the position of the snout beneath the mountain range's atmospheric zero degree isotherm which results in the monsoonal temperate glaciers falling in the categories of warm-based/wet-based glaciers. The fact that the stream does not freeze could be a major reason for the departure from the model, as many of the streams that were in agreement with the model were located at higher latitudes and in alpine zones (above the treeline) therefore more likely to be above the respective mountain range atmospheric zero degree isotherm where the glacier beds and streams are more likely to freeze during the winter. However, very little is published regarding the conditions of glacier metakryal zones during the winter season.

The results of the study on hydraulic characteristics suggest that rapids, as microhabitats with the highest water velocities and water velocity ranges, trap and retain more organic carbon than pools and riffles, with runs also containing significantly greater organic carbon than riffles. Overall taxa abundance was greatest in runs, which were significantly greater than riffles and pools. This suggests that in glacier-fed streams, the higher velocity microhabitats may be important habitats to examine invertebrate ecology in order to attain representative abundance of taxa in streams.

Chloroperlidae were located furthest from the glacier source, and survived for the longest time in the higher salinity experimental treatments (0.95% and 1.2% NaCl). However, the lethal toxicity of salinity (LC_{50}) to Taeniopterygidae, Nemouridae, and Chloroperlidae were not different, suggesting that naturally-occurring salinities in these mountain stream waters may be in a range tolerable to metakryal stoneflies. Changes in morphology of chloride and caviform cells on Chloroperlidae and caviform cells on Taeniopterygidae occurred as predicted in an inverse manner with salinity. This is the first study that has observed a morphological response from caviform cells, connecting them with an osmoregulatory function.

The results of this research indicate a major departure from the established M&P model of glacier macroinvertebrate community structure. The results suggest that a modified model may be appropriate for glacier-fed streams in the monsoonal temperate

region where glacier tongues extend far beneath the extremely high treeline in v-shaped valleys at relatively low altitudes. One reason for the departure may be the perennial nature of the streams under temperate wet-based glaciers which have water present throughout the ice mass. Therefore it would be appropriate to examine winter metakryal conditions in glacier-fed streams in agreement with the M&P model to understand if the streams are intermittent, impacted by surface ice, or freeze solid. Hydraulic and substrate characteristics in this study have an influence on the presence of carbon and invertebrate abundance. Habitats with higher water velocity and potential hidden backwaters support a greater abundance of certain taxa. Hydraulic characteristics and ice presence during the winter could be the two of the major reasons for the difference between the M&P model and this study, so it is suggested that year round hydraulic characteristics be examined in other glacier streams.

I dedicate this dissertation to four of the most important people in my life: my wonderful late parents John David Fair and Jennifer Mast Fair, and dearest late Aunt Carol Keith, Emeritus Professor of Nursing, OSU. Thank you to my loving and supportive husband, Eddy Chi Chung Wu. I also thank Tao Tao, who has been by my side for the past 17 and a half years through it all.

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Vita

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Chapter 1: Introduction to Stream Ecology

Introduction

By volume, headwater streams are the smallest component of river systems, yet they represent 70 percent of the length of rivers (Leopold et al., 1964). Serving a function similar to capillaries of the human body, headwater streams exchange nutrients with the valleys in which they reside and provide ecosystem services by transforming and transporting nutrients downstream. The hydraulic exchange with terrestrial components in the geomorphological setting of the stream determines the chemical and physical characteristics of the water, and therein sets the baseline for which species inhabit the streams. H.B. Hynes (1975) provided perhaps the most universally applicable statement about headwater streams "In every respect the valley rules the stream".

Monitoring the health of headwaters can serve as an indicator of the overall vitality of the surrounding landscape. Macroinvertebrates as the primary inhabitants of headwater streams are optimal indicator organisms as their communities reflect physico-chemical changes to instream water quality due to landscape alterations, point source, and non-point source pollutants (Deshon 1995; Karr & Chu 1999; Moore 2009). The lethal and sublethal stressor responses of disturbance will vary by individual aquatic taxon. This has led to the development of invertebrate and habitat indices (e.g., Invertebrate Community Index (ICI) and Index of Biotic Integrity (IBI)) that are used to determine human impacts to stream habitat and enforce legal standards of the Clean Water Act (CWA) in several states in the U.S. (Karr et al., 1986; Deshon, 1995; Yoder & Rankin, 1998). In order to expand indicator organisms as a tool reflecting global scale changes such as climate change, the biogeographical distribution of aquatic insects in a historical, spatial, and physiological context should be considered to understand the potential pool of insects available to colonize particular streams.

1.1. Biogeographical Patterns of Aquatic Insect Dispersal

The global distribution of adult aquatic insects has occurred over evolutionary time scales across geographical spatial influences of latitude, altitude, land-area, and range barriers. Latitudinal gradients of terrestrial biodiversity follow a distinct pattern of decreasing biodiversity from the tropics to the high latitudes, reflecting land-size and plate tectonics (Terborgh 1977, Rosenzweig 1992). The large range space in the tropics allows for a higher probability of allopatric speciation and lessened chance of extinction due to land and climate disasters (Rosenzweig 1992). The number of niche habitats increases with land size area. As the number of species increases, population pressures force specialization that split habitats by niche partitioning. For example, in Puerto Rico and Panama, similar forest canopies are found but the number of birds differs due to Puerto Rico being an island with a smaller area supporting fewer species. In Puerto Rico, birds utilize two habitat layers in the canopy, but due to the heightened diversity and natural selection pressures in Panama, the birds are forced to recognize four habitat layers (Rosenzweig 1992). From this terrestrial example it is clear that habitats are not just physical areas of space, but they have coevolved with the interplay of biotic forces as functional spaces for organisms. Principles from terrestrial examples can be applied to water bodies, with larger streams providing more microhabitats for colonization. While larger range sizes on land and in water increase available habitats, the global pattern of aquatic insect dispersal is not so clear (Pianka 1966).

As mentioned, terrestrial animals display a distinct decrease in species richness with increasing latitudes, but non-fish aquatic fauna are the exception to this rule (Patrick, 1949). The ability of adult aquatic insects to migrate through flight is a key variable determining the distribution of aquatic insects. As nymphs, dragonflies remain in a particular water body, but as adults they can traverse mountain ranges and continents. Others, such as certain Plecoptera have weak flight muscles (Marden and Kramer 1994) and smaller dispersal ranges. Likewise, the latitudinal trends in aquatic insect biodiversity tend to be group-specific. Diptera increase in species richness with higher latitudes (Scott et al 2011). One possibility for this trend could be due to the physiological adaptations of chironomids to extreme low temperatures (Danks 1971) and the lack of predators in >60N latitudes (Scott et al 2011). Peak diversity of Plecoptera has been observed at 40ºN, with ephemeropteran genera richness exhibiting peaks at several latitudes: 40ºN, 10ºN, 10ºS, and 30ºS (Vinson & Hawkins 1998; Vinson & Hawkins 2003). Below 30ºN, plecopteran richness was observed to be low in comparison with temperate latitudes, with the exception of a spike in species richness in a Malaysian tropical stream near the equator (Bishop 1973). In attempts to find clear patterns of diversity paralleling terrestrial biota, aquatic insect diversity has yet to produce a definitive latitudinal trend for all groups (Covich 1988; Allan 1995). Altitude, on the other hand, has been hypothesized to have a strong influence on aquatic insect distribution (Lomolino 2001).

Altitudinal influences on aquatic biota dispersal in mountain ranges have been hypothesized to result in distinct patterns. Four biogeographical reasons have been proposed for elevational gradients in species richness: 1) gradients in land area, 2) climate gradients, 3) geographic isolation, and 4) zonal community feedback (Lomolino 2001). One study found that overall taxa richness differed across biomes related to altitude, with the greatest number of taxa found in streams from high montane zones (n=52), intermediate richness in subalpine regions (n=35), and lowest taxa richness in valley streams (n=18) (Donald & Anderson 1977; Bishop 1973). Most studies concluded that the highest taxa richness occurred in montane and transition zones between mountains and valleys (Carter et al. 1996; Tate & Heiny 1995). Plecoptera showed a decrease in species richness with higher latitudes when elevation decreased, but an increase in species richness with latitude where the elevation increased (Heino et al. 2003b). This example shows that certain taxa may be less sensitive to latitude, and more physiologically fit to ecological conditions based on elevation.

Landform area not only influences latitudinal patterns of diversity, but it also influences species richness in mountain ranges. Although the general pattern of reduced species richness with increasing elevation due to land area reduction towards the mountain peak is a widely accepted concept, the relationship is not always linear. Terborgh (1977) studied Ecuadorian bird species through mist netting, and initially found a straight linear relationship between elevation and species richness. However, in a reanalysis of his data, he standardized the time he spent capturing birds, adjusted the number of species captured/time and found a hump-shaped curve with a species richness peak at 1400 m a.s.l. (Rosenzweig 1992). This mid-elevation diversity hotspot is not uncommon, with one explanatory variable being climate differences with elevation. Most mountains have a condensation altitude that creates microhabitats not otherwise found in drier climates. This "humidity peak" creates conditions for bryophytes and other plants to grow and support the formation of additional habitats (Rahbek 1995). A humidity peak is found with latitudinal gradients which makes the direct comparison of mountain elevation with latitude unrealistic.

The highest treelines are found in mountains in the tropics near the equator, with decreasing treeline altitudes northward toward the poles (Ward 1994). However, the Massenerhebung, or mass elevation effect (MEE) partially explains the reasons for treeline position differences within the same latitude (Grubb 1971). The MEE describes higher treelines in the interiors of large mountain ranges due to thermodynamic retention, and lower elevation treelines on the outer perimeters of the ranges (Zhang & Bai 2016). Large mountain masses in close proximity with other large mountain ranges (e.g., Himalayas) have higher treelines than similar-sized isolated mountains due to higher heat retention and wind shadow effect of the surrounding topography allowing for extended tree growth (Schröter 1908). Zhang & Bai (2016) found that the intra-basin (e.g. river altitude) elevation in between mountain ranges is the most important aspect of the MEE formation. Treelines above 4,500 m a.s.l. are only found in two areas of the world, the Southeastern Tibetan Plateau at 29 and 30°N, and the Central Andes at 18°S. These mountainous regions have intra-basin elevations of 3,800 m a.s.l. and 4,200 m a.s.l. The southern Rocky Mountains in the United States have several tree stands at 4,000 m a.s.l., which also reflects the effect of the MEE. The MEE has not been discussed in the glacier stream ecology literature, most likely because studies have been focused on the mid latitudes where glacier-fed streams typically arise above the treeline in the alpine zone in European mountain ranges.

A definitive trend in the latitudinal dispersal of aquatic insects has thus far proven somewhat elusive; nonetheless it is important as a backdrop for comparative studies in global aquatic ecology. Altitudinal gradients of insect dispersal may have a clearer trend for certain taxa (e.g., stoneflies) but the exact reasons based on physiological tolerance levels has not been described in detail. Stream ecology theory had its beginnings by analyzing insects at the fine patch scale and has progressively linked stream ecology at larger spatial scales. At the same time, stream ecology has borrowed theoretical approaches from terrestrial ecology, which are interconnected fields due to the tight connectivity of headwater streams to the terrestrial realm.

1.2. Theoretical Background of Stream Ecology

August Thienemann proposed three broad principles of instream invertebrate ecology in 1954, which included: 1) Increased habitat heterogeneity increases aquatic insect diversity, 2) Species richness will decline as habitat conditions are degraded, but the abundance of prevailing larvae will increase, and 3) A stable stream will result in a stable community of insects. Eugene and Howard T. Odum advanced stream theory by injecting ideas from system energetics (e.g., Odum 2002), which led to the examination of energy budgets in streams, and recognition of streams as ecosystems in their own right.

The examination of the broad principles of stream ecology let to the integration of general ecology theories such as the disturbance theory, in attempts to describe the reasons for observed differences in stream communities.

Ecological principles such as biotic interaction theory and disturbance theory were incorporated into stream ecology through the works of Connell (1978) and Huston (1979) to understand how disturbance spatially and temporally altered stream communities. The general ecological definition of disturbance (Pickett & White 1985) stated that "disturbance is a distinct event that disrupts community structure, changes availability of resources, and alters the surrounding physical environment". In stream ecology, a spate is considered a disturbance due to substrate movement and enhanced invertebrate drift that causes temporary changes to the habitat and invertebrate communities. Resh et al. (1988) clarified the definition of disturbance for streams by emphasizing the need for the frequency and intensity of the disturbance to fall outside of the predicted range. For example, a 100-year flood rather than a seasonal spate would be considered a disturbance due to the life cycle adaptations of insects falling into the time scale of seasonal spates. The intermediate disturbance hypothesis contends that ecological communities rarely reach an equilibrium state. When disturbances occur, patchy and dynamic habitats are reset as individuals are killed and less competitive individuals move into newly opened ecological space (Townsend and Scarsbrook 1997). For the intermediate disturbance hypothesis (Connell 1978) to be generally accepted into stream ecology, a stream ecosystem devoid of disturbance with biotic interactions that are in equilibrium would have to be established as the null hypothesis (Minshall 1985). To date, there are discussions as to how the intermediate disturbance hypothesis fits into stream ecology as a driver of biodiversity (e.g., Resh et al. 1988, Stanley et al. 1994, Lake 2000).

Classification systems have been developed to link habitat with biota in order to predict communities of organisms with habitat conditions. Functional feeding groups (FFGs) were developed as a classification system based on the morphology of mouth parts which describe foraging strategies of different groups of aquatic insects (Cummins 1973). The ability to characterize insect communities based on the partitioning of food resources allowed for the analysis of communities on a functional ecological basis. In a further development to link the reason for the presence of species to their immediate environment, the habitat template model (Southwood 1977) was developed that linked organism traits to habitat filters. Habitat filters function similar to nested sieves separating sediment into size classes. Sediment progressively sifts through the nested mesh sizes until the smallest grains are filtered out at the bottom. In this analogy, the smallest grains represent insects with the most similar traits adapted to unique conditions. In the habitat template each successive habitat filter represents a smaller spatial scale condition that filters out insects not possessing a trait for the condition (Poff 1997; Statzner 2004). Filters can begin at the ecoregion level and move progressively through the mountain range, watershed, stream, and microhabitat levels. The spatial scales of interest are specified in each individual study. The habitat template model is a method to link habitat and traits of insects; however, the myriad of ways that traits can be assigned to biota and the numerous spatial scales leaves the model at the discretion of the individual researcher which can lead to misinterpretations.

Traits are assigned to species based on the inferred knowledge of the insect's biology and its functional relevancy to the habitat. For example, *Polypedilum* v*anderplanki,* the sleeping midge found in arid African vernal pools, has the trait for anhydrobiosis (Watanabe 2002; Cornette et al. 2010; Cornette & Kikawada 2011). This trait allows for the sleeping midges to desiccate for long periods of time in order to exploit the temporary vernal pool habitat. The species with the anhydrobiosis trait would possess the evolutionary fitness to pass through the vernal pool microhabitat filter.

Traits for a particular physical characteristic may be assigned to link the insect to multiple habitats depending on the usage of the microhabitat. For example, dorso-ventral flattened body morphology can be a trait for withstanding shear stress in high flow velocity, but it can also be a be a trait to slip into narrow crevices to escape predators (Poff 1997). As well, habitat filters can be specific to the microhabitat level, such as moss on a boulder, or applicable at multiple filter levels, such as temperature (Poff 1997). In essence, filters are a selective force connecting the evolved trait of an insect with a habitat that supports the life needs of the insect. Statistically describing how insects with particular traits respond to gradients in the environment has given momentum to the traits-based method of analyzing insect community responses to environmental gradients (Poff et al. 2006). The number of habitat spatial scales and ways of assigning traits can lead to convoluted studies, so it is important for the author to explicitly state the reasons and objectives for the traits, habitat filters, and spatial scale in each study.

Vannote et al. (1980) expanded the spatial scale focus of stream ecology from the stream reach to the riverscape by linking streams into longitudinal gradients of food supply type and corresponding biotic adjustments with the River Continuum Concept (RCC). The RCC detailed how organic matter and invertebrate communities are connected through the partitioning of resources (FFGs) in a downstream direction. Organic leaf matter entering the stream at the forested headwater is initially processed by microbes and leaf-shredder macroinvertebrates and transformed into particulate organic matter (POM) that is then consumed by downstream filter feeders (e.g., Simuliidae). The RCC was based on streams originating in forest-canopied, first-order, temperate streams that joined with other first-order streams to become second–order streams, and so on, following the Strahler hierarchical stream classification system (Strahler 1952). The RCC was enhanced with a geomorphological perspective by Luna Leopold, who noted that streams change in a predictable manner with width, depth, water velocity, and temperature following a distinctive downstream pattern. This linkage of geomorphological features with FFG communities was the first comprehensive hypothesis of how streams and their watersheds functioned in the riverscape.

Making predictions of community structure based on habitat characteristics has many challenges. The multicollinearity of physico-chemical variables and uncertainties of species interactions has made it difficult to hone in on the most important determinant of species richness, density, and diversity. Still, single variables are sought out as explanatory reasons for invertebrate community distribution, such as water temperature and channel morphology (Hawkins et al. 1993; Poole and Berman 2001). Stream hydraulics has been proposed as being more important than temperature as a determinant of aquatic insect diversity (Statzner et al., 1988). Statzner and Higler (1986) suggested that stream hydraulic heterogeneity determines how macroinvertebrates are distributed throughout the stream catchment and on a longitudinal basis within a stream. Headwater streams often begin as trickles with low hydraulic stress, and transition into steeper-gradient, higher hydraulic stress zones, and eventually into lower hydraulic stress zones as the slope levels off. Of course, this pattern will vary by climate zone, geomorphology, and water source (e.g., lake outlet, groundwater spring). Hydraulic zones shift either upstream or downstream as river discharge fluctuates. Statzner & Higler (1986) suggest that hydraulics is more globally applicable than the RCC, which overlooks stream patterns outside of temperate zones. For example, the RCC does not incorporate differences in high elevation and high latitude headwaters, arid region headwaters, and streams set in deeply incised valleys (Statzner & Higler 1985); nor does it incorporate different sources of water such as snow melt, different spring sources (helocrene and rheocrene), lakes, and glaciers as modifiers to the conceptual model. In summation, the complexity of species interactions, geomorphology, climate regime, disturbances, feeding habits, and hydraulics are just a few of the factors that stream ecologists are working to understand how each as an individual factor influences invertebrate patterns, and how each fits as a piece in the larger puzzle of biogeographical ecological patterns.

The preceding introduction to general stream ecology and biogeographical concepts sets the groundwork for glacier meltwater stream ecology. The following section provides a brief literature review of the development of the glacier-fed stream conceptual model and how the patterns of glacier stream ecology compare and contrasts with basic stream ecology. This section will segue into an introduction to my dissertation research and hypotheses.

1.3 Glacier Stream Ecology: The Milner & Petts Model

Two decades ago, the nascent field of glacier stream ecology, while incorporating the basic tenets of general stream ecology, recognized that glacier streams are unique in that they arise in harsh and remote environments that alter the benthic invertebrate community structure. The first conceptual longitudinal model of community diversity in glacier streams was formulated by A.M. Milner and Geoff Petts by describing aquatic insect communities in Alaska and the Swiss Alps. They detailed unique attributes of glacier rivers, such as seasonal and diurnal discharge patterns, low water temperature, sediment load, and channel instability (Milner & Petts 1994). They hypothesized that water temperature, time since deglaciation, and channel stability would be the main driving factors of changes in community structure with distance from the glacier (Figure 1.1).

In the metakryal zone (T_{max} <2°C), only *Diamesa* spp. were found in the original model study sites, and downstream in the hypokryal zone (2-4°C), other Diamesinae, Orthocladiinae, and Simuliidae appeared, and yet further downstream, in the (4-6°C) zone, Baetidae, Nemouridae, and Chloroperlidae made their first appearance (Milner & Petts 1994). The M&P model of longitudinal appearance of macroinvertebrate species in glacier streams has been used by other glacier stream ecologists as a frame of reference for comparisons.

The original M&P model hypothesized that channel stability, represented by the lower bed score section of the Pfankuch Stability Index (PSI) (Pfankuch 1975), and water temperature (Milner & Petts 1994), were the two main factors determining longitudinal first-appearance of macroinvertebrate taxa in streams during the summer melt season. Channel stability appears to have a large impact on aquatic invertebrate communities, with stable stream channels correlated with higher taxonomic richness and abundance (Collier et al. 1993, Winterbourn 1994, Death & Winterbourn 1995). The PSI streambed bottom score is comprised of five questions covering proxies of channel stability. The answers are assigned to one of four categories, ranking the stream as excellent, good, fair, or poor. Excellent (lowest total point score) represents the most stable stream and poor (highest point score) represents the most unstable stream channel. Rock angularity, substrate surface brightness, substrate consolidation/packing, scouring and deposition, and presence/absence of moss or vegetation growth are questions representing the degree of channel stability. Although subjective, the Pfankuch index has served to compare channel stability of glacier-fed streams from different regions of the world.

The M&P model was tested at alpine stream locations in glacial-melt streams at a range of latitudes from 43°S to 79°N and from 1996-1999 as a part of the Arctic and Alpine Stream Ecosystem Research (AASER) (Environment and Climate Programme; European Union) program. The streams represented glaciers in Europe – French Pyrenees, Svalbard Norway, Iceland, Greenland, and New Zealand (Brittain et al. 2001, Gislason et al. 2001, Lods-Crozet et al. 2001, Maiolini & Lencioni 2001, and Snook & Milner 2001). As a result, the M&P model conceptual diagram was revised by the addition of Oligochaeta and Tipulidae with Orthocladiinae in the hypokryal zone and the addition of Perlodidae, Taeniopterygidae, and Empididae to the model in the (4-6°C) zone (Milner et al. 2001) (Figure 1.2). As mentioned earlier, there were a few deviations from the longitudinal colonization patterns. Miaolini & Lencioni (2001) examined a smaller-scale (0.185 km^2) Italian Alps glacier stream (Conca) with a low summer melt discharge (0.015-0.115 m^3/s) and found that the channel stability was much higher than the M&P model streams, and consequently found additional taxa in the hypokryal zone (Limoniidae, Empididae, Nematoda, Oligochaeta, and Crustacea). Lods-Crozet et al. (2001) also found that the hypokryal zone in the Swiss Alps 200 m from the glacier contained Baetidae, Taeniopterygidae, and Limnephiliidae. The hypokryal zone being located so close to the glacier indicates that the Mutt Glacier is small (0.6 km^2) and therefore the temperature rapidly warms at a downstream gradient from the glacier due to solar radiation. Jacobsen et. al (2010) found that high elevation glacier streams in the Ecuadorian Andes, such as the Crespo Glacier (1.7 km^2) does not possess a true metakryal zone due to rapid solar radiative heating of the surface water even close to the glacier. However, in the evenings, the streams freeze through (Jacobsen et al. 2010). The majority of the AASER studies were in general agreement with the M&P model that temperature and channel stability are the main habitat factors determining the species richness and abundance of insects colonizing the metakryal sites. Most of the sites were in agreement that the metakryal zone closest to the glacier is chiefly colonized by *Diamesa* spp. (Figure 1.3). Although most of the comparisons found the reasons for colonization to be met by the criteria of the M&P model, there were variations in the presence of insects.

In order to explain departures from the model, one hypothesis was that different sources of water were combining beneath the glacier to form conditions of different stream types by water source. The Alpine River and Stream Ecosystem classification (ARISE) model of water source classification developed by Brown et al. (2003) used geochemical tracing methods to determine water percent breakdown by source, and aimed to classify streams as mixtures of kryal (ice), krenal (groundwater), and nival (snowmelt). These were originally proposed as single water source categories (Steffan 1971; Ward 1994). The categories proposed by Brown (2003) were A) Krenal, B) Kreno-nival, C) Kreno-kryal, D) Nivo-krenal, E) Kryo-krenal, F) Nival, G) Nivo-kryal, H) Kryo-nival, and I) Kryal. Concentrations of Si and SO_4^{2-} were taken from groundwater, snow, and glacial meltwater throughout the year in order to identify the proportion of water type from quickly routed snow and ice, slower transported subglacial meltwater, and groundwater. ARISE was able to classify streams based on temporal shifts of predominant water sources, and also to identify the englacial, supraglacial and subglacial flows close to the glacier snout. This model identified groundwater-dominated segments of three French Pyrénées glacier streams (kreno-nival and kreno-kryal) as having the highest total abundance of insects, whereas taxonomic richness was significantly highest at kreno-nival sites (Brown et al. 2009). With a decline in meltwater contribution, Brown predicted that alpha diversity within the glacier stream would increase, but beta diversity (between sites) would decrease due to the increasing similarity between groundwater streams and non-melt season glacier streams. There have only been a couple of studies performed year-round to examine the seasonal communities in glacier-fed streams, so this prediction is outstanding in most parts of the world (Schütz et al. 2001, Füreder 2007).

The size of the glacier and the glacier's state of retreat or expansion are hypothesized to impact the longitudinal water temperature of the stream and therefore the biological gradients (Jacobsen & Dangles 2012). The metakryal, hypokryal, nival, and krenal sections of streams vary by longitudinal distance from the glacier due to the size and physical state of the glacier. A small stable glacier will have a lower impact on downstream temperature than a large receding glacier. Jacobsen & Dangles (2012) applied a Glacial Index (GI) to predict the impacts of glacial meltwater on downstream biota based on glacier size. The GI embodied physical factors such as turbidity and water temperature as a function of glacier scale (Jacobsen & Dangles 2012). The results of applying the GI to the European AASER sites and additional studies in Ecuador and New Zealand were that the GI modeled invertebrate species richness better than just the Pfankuch Index and temperature. The authors modeled what will happen to beta diversity with climate change, and found that the GI method predicted a decrease in beta diversity (Jacobsen & Dangles 2012). The model also predicts that 11-38% of regional species pools would become locally extinct with the loss of glaciers. The GI index recognizes that with an increase in size and increased recession rate, glaciers will have more noticeable effects at longer distances from the snout during the melt season, which was not built in to the original M&P model.

Glacier snouts have been compared with islands and connected with the theory of island biogeography (MacArthur & Wilson 1967; Finn et al. 2013). MacArthur & Wilson (1967) stated that species richness is a function of the size of an island (area) and isolation, with colonization and extinction being impacted by both of these factors. The larger the islands and closer they are to a mainland, the higher the species richness will be due to ease of immigration for colonization. The presence of more species however means that the colonization rate will be lower, and the extinction rate will be higher (MacArthur & Wilson 1967). Glacier termini are becoming more isolated with climate change due to the fragmentation of larger glaciers into smaller glaciers at higher altitudes (Yao et al. 2007), so in essence they are becoming similar to remote islands. These high elevation habitats are smaller in area and more isolated from surrounding mountains and from areas of the same mountain, therefore reducing immigration of new species and increasing the possibility of endemic speciation. This, coupled with poor dispersal abilities of many insects inhabiting glacier streams (e.g., brachyptery – reduced wing size), has meant that over geological time scales, endemic species have evolved as specialists based on the isolation and physical environment close to glacier snouts. The high elevation habitats also leave endemic species with fewer chances to exchange genetic material with other populations (Finn et al. 2006).

Although temperature and channel stability in general predict longitudinal gradients of insect biodiversity from glaciers, to Milner's point (2016), we are perhaps at a point where subjective indices such as the Pfankuch Stability Index could be developed quantitatively for glacier systems. Key concepts learned from glacier stream studies include, 1) glacier lake outlets modify invertebrate community structures in the metakryal zone, as certain taxa (e.g., Nematoda) are found in high abundance downstream from lake outlets (Maiolini and Lencioni 2001). Outlet lakes not only modify the temperature of the stream but also stabilize discharge and channel substrates that allow for growth of periphyton and accumulation of organic materials (Friberg et al. 2001); 2) the island-dispersal effect is evident, with fewer taxa being able to disperse to islands and with previous glaciations limiting taxa to these regions (e.g., in Norway, Greenland, and Iceland) (Blaen et al. 2014); and 3) glacier size will impact the relative distance of the harshness impact on insects (Jacobsen and Dangles 2012).

Researchers in the field of glacier stream ecology have supported the hypothesis that the main drivers of longitudinal distribution of aquatic insects are temperature and channel stability. Insect colonization variations have been found in geographic regions with different regionally-found taxa or modified diversity due to channel stabilization effects (i.e. glacier lake outlet origin), smaller sized glaciers producing a lessened discharge at the headwaters (e.g., Miaolini and Lencioni 2001), and the lack of a true metakryal zone in tropical Ecuadorian glacier streams (Jacobsen et al. 2010). My aim is to understand the reasons for higher metakryal zone diversity in the Hengduan mountain glacier streams, where the glaciers are larger, set in the subalpine zone, produce low temperature melt water, and create hydrological conditions that are extreme, which seems counter-intuitive to allowing more species to colonize the metakryal zone.

1.4 Thesis Overview

The Milner & Petts conceptual model of glacier stream ecology hypothesizes that temperature and channel stability determines invertebrate community distribution in glacier-fed streams. Although regional invertebrate community differences have been found, studies have been in general agreement that temperature and channel stability are the main factors determining invertebrate community gradients in alpine glacier streams. I have observed a departure in the structure of communities at the base of glaciers in southeast Tibet, even in metakryal temperatures ($T_{max}<2^{o}C$) and unstable, aggrading stream channels. Therefore, in Chapter 2, entitled "Invertebrate Community Structure of Monsoonal Temperate Glacier Streams: The Valley Rules the Stream in a Departure from the Model", I summarize findings of my glacier-fed stream ecological research during the peak discharge summer melt seasons of 2009, 2011, 2013, and 2015 in the Meilixueshan and Daxueshan ranges of Southeast Tibet. I hypothesize that the main drivers of longitudinal macroinvertebrate first appearance are different than temperature and channel stability, and discuss reasons for the differences.

Chapter 3, entitled "Hydraulic Microhabitats in a Glacier Headwater of the Mekong River: Hidden Backwaters beneath the Torrents.," I examine the role of hydraulic microhabitats (riffles, runs, step-runs, rapids, step-rapids, and pools) to understand how glacier discharge impacts microhabitat velocity, depth, velocity range, and substrate stability, which may affect macroinvertebrate community structure at the hydraulic microhabitat level. Since most studies focus on a riffle-run sampling strategy, I consider that by including depositional and erosional habitats, a more inclusive community structure will be attained. I hypothesize that the microhabitats with the most turbulent water flow (rapids and step-rapids) will trap and retain a greater organic carbon mass which in turn will attract more invertebrates. The aim of this chapter is to examine the structure of invertebrate communities by hydraulic habitats to understand if stream flow characteristics may be playing a role in the determination of macroinvertebrate community distribution in monsoonal temperate glacier streams.

In Chapter 4, entitled, “Glacial meltwater stonefly response to water salinity”, I outline my preliminary observations of the distribution of stoneflies based on type of gill structure and presence of tracheation in relation to water source (Figure 1.4). I also examine the survival of stonefly larvae and the morphological changes in chloride cells in response to varying salinity exposures. My hypothesis was that stoneflies living downstream from the glaciers in $T_{max} > 6^{o}C$ and in groundwater streams would be able to survive longer in higher salinities. In doing so, I aim to provide preliminary data to understand if metakryal insects may be able to colonize groundwater streams once the glaciers have melted as a result of climate change. I describe field experiments and scanning electron microscopy imagery analysis detailing how morphology of chloride and caviform cells on stoneflies changes in response to salinity. I hypothesized that the chloride and caviform cell count, area (μm^2), and density (μm^2) would have an inverse relationship with water salinity levels. The reasons for the experiments were threefold: 1) to calculate the LC_{50} of insects exposed to different salinities; 2) to examine the survival duration of stoneflies in the different salinities to select individuals to perform chloride cell analysis; 3) to understand if there is a change in the chloride cell morphology to varying levels of salinity; and 4) to understand whether or not the insects may be physiologically capable of colonizing in salinity levels found in groundwater streams, as the loss of glaciers will leave groundwater and snow melt waters as locations to colonize.

Figures

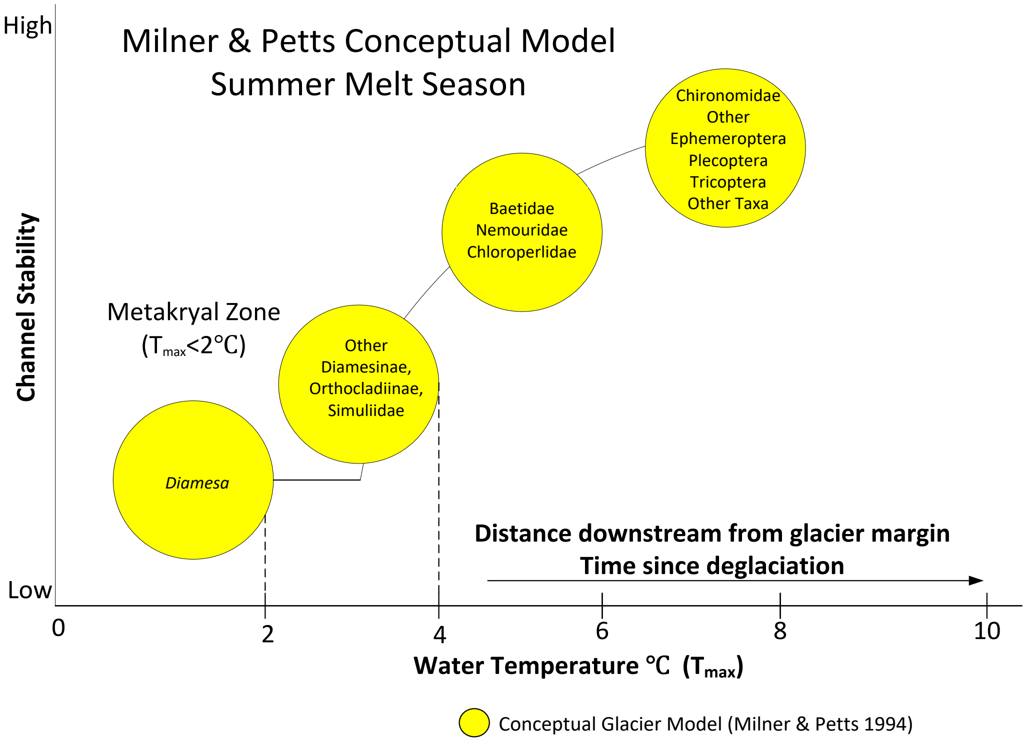


Figure 1.1. The original Milner & Petts model. Channel stability and water temperature are the hypothesized drivers of longitudinal first appearance in glacier streams (reproduced after Milner & Petts 1994).

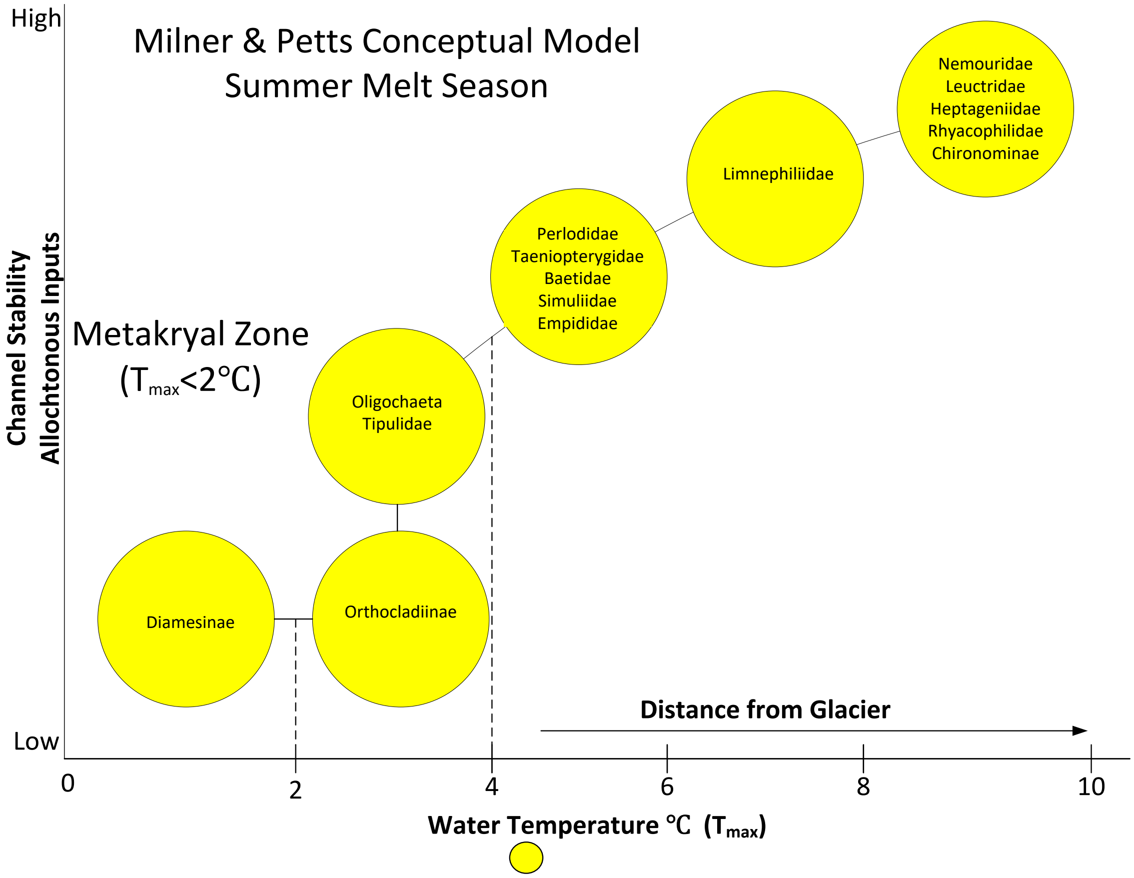


Figure 1.2. Revised Milner and Pett's conceptual model. Revised after the Alpine Stream Ecosystem Research (AASER) project (1996-1999). Oligochaetes and Tipulidae were added to the hypokryal zone (T_{max} 4^{o}C), and Perlodidae and Taeniopterygidae were added in the 4-6^{o}C temperature range. Allochtonous inputs were added to the y-axis. (reproduced after Hannah et al. 2007).

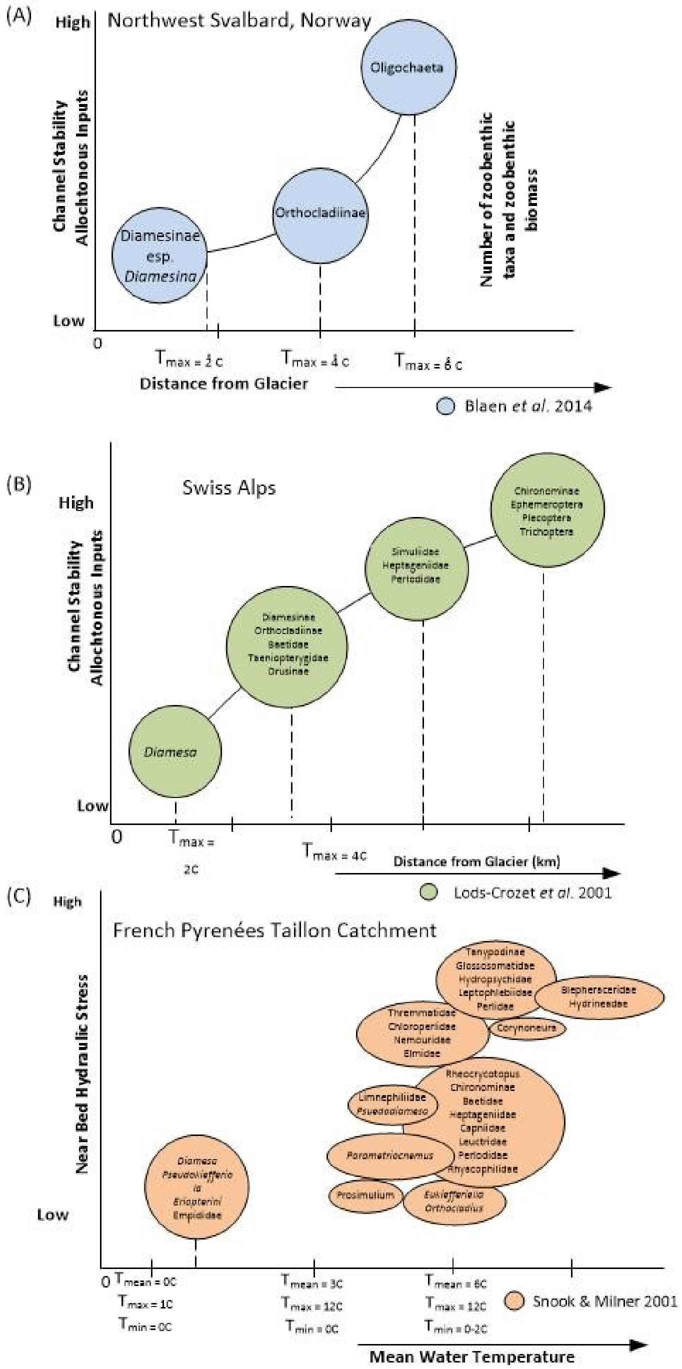


Figure 1.3. Select results from Arctic and Alpine Stream Ecosystem Research (AASER) studies. Select study results are from (A) Norway, (B) the Swiss Alps, and (C) the French Pyrenées. The studies compare respective sites with the M&P model of longitudinal first presence of invertebrates based on temperature and channel stability changes with distance from the glacier.

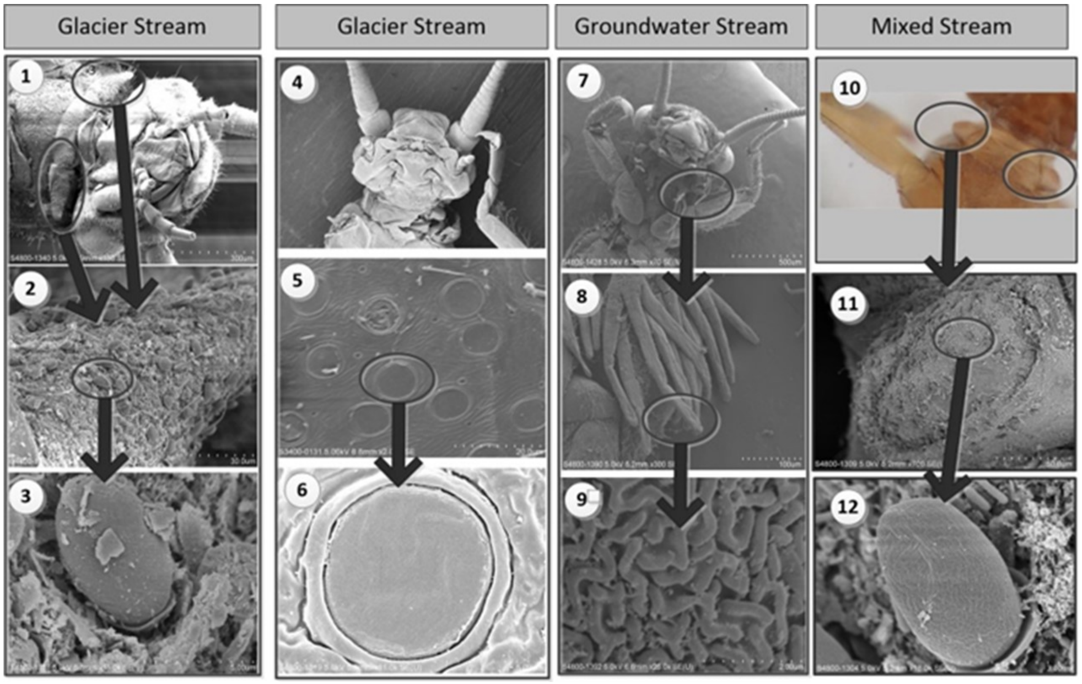


Figure 1.4. Nemouridae and Taeniopterygidae gill structures and chloride cell morphology. Nemouridae and Taeniopterygidae from glacier streams (1-6 & 10-12) and groundwater streams (7-9). The morphological structure of the osmobranchiae are single-fingered in the glacierized catchments (1-3 and 10-12), and tracheated in *Amphinemura* in groundwater streams (7-9). Taeniopterygidae have coniform chloride cells scattered over the entire ventral body surface (5-6). Nemouridae have bulbiform chloride cells covering their osmobranchiae (1-3 and 10-12) and ventral thorax region whereas *Amphinemura* tracheal gills are tracheated (8-9) and ventral thorax region has bulbiform cells.

Chapter 2: Invertebrates in Monsoonal Temperate Glacier Streams: The Valley Rules the Stream in a Departure from the Model

(formatted for *Freshwater Biology*)

Abstract

1. Invertebrate communities in four subalpine glacier-fed rivers in southeast Tibet were examined during summer melt seasons in 2009, 2011, 2013, and 2015.
2. The main habitat variables of channel stability and water temperature were examined in order to compare the longitudinal communities with the widely-accepted Milner & Petts (M&P) conceptual model of glacier invertebrate distribution.
3. A presence/absence model by water temperature zones was built to compare with the M&P model. The taxa composition, taxa richness, abundance and density (m^2) were examined using linear mixed effect models to evaluate whether or not there was a relationship among each of the response variables to temperature and channel stability.
4. The results of univariate and multivariate linear regression models resulted in no significant relationships among temperature, channel stability, and the invertebrate response variables.
5. Evaluation of presence/absence patterns indicated fifteen out of nineteen total taxa were present in the metakryal $T_{max} < 2^{o}C$ zones where the M&P model has only *Diamesa* spp. present.
6. The results support the hypothesis that the environmental influencing factors on longitudinal presence of invertebrates in monsoonal temperate glacier systems during the summer glacier melt season are those other than T_{max} water temperature and channel stability.

Introduction

By volume, headwater streams are the smallest component of river systems, yet they represent 70 percent of the length of rivers (Leopold et al., 1964). Temperate headwaters, which historically emanated from forested catchments, were described, along with their invertebrate communities, as an integral part of the entire riverscape in the River Continuum Concept (Vannote et al. 1980). Two decades ago, glacier stream ecologists outlined a glacier stream conceptual model similar to the River Continuum Concept, but depicted longitudinal invertebrate community changes as influenced mainly by water temperature and channel stability, rather than stream size, allochtonous organic material input, and riparian habitat (Milner & Petts 1994; Ward 1994a). The model recognized the importance of glacier streams originating in the alpine zone with no tree canopy coverage, pronounced seasonal and diurnal discharge patterns, low water temperature, high sediment loads, and low channel stability in determining longitudinal colonization of invertebrate communities with distance from the glacier (Milner & Petts 1994). The summer melt season, when kryal conditions are present (T_{max} <4°C), is also when reduced invertebrate richness and abundance is found in glacier streams (Steffan 1971; Ward 1994a; Milner et al. 2001; Brittain & Milner 2001). This is also the season upon which the Milner & Petts (M&P) conceptual model is based. From autumn through early spring, glacial meltwater is reduced as a result of decreasing air temperatures, and windows of opportunity are opened allowing invertebrate diversity and abundance to reach peak levels as the glacial-melt discharge decreases (Füreder 2007, Burgherr & Ward, 2001; Robinson et al. 2001; Schütz et al. 2001).

Water temperature and channel stability were hypothesized by Milner & Petts (1994) as the main factors influencing changes in benthic invertebrate community structure longitudinally from the glacier during the melt season. Invertebrate diversity increases downstream as multiple water sources and distance from the glacier ameliorate kryal conditions (Ward 1994a; Milner et al. 2001; Brown et al. 2007). In general, the metakryal zone ($T_{max} < 2^{o}C$), consists of communities with only the genus *Diamesa* spp. and in the hypokryal zone ($2^{o}C$ to $4^{o}C$), other Diamesinae, Orthocladiinae, and Simuliidae colonize, and yet further downstream in the ($4^{o}C$ to $6^{o}C$) zone, Baetidae, Nemouridae, and Chloroperlidae are found (Milner & Petts 1994). The Milner & Petts (M&P) model has been used as a frame of reference by glacier stream ecologists for comparisons since its inception.

There has been general support that temperature and channel stability are major factors influencing the longitudinal distribution of aquatic invertebrates in glacier streams (Milner 2016). Variations in taxa presence/absence trends have been found in certain study sites, with Leptophlebiidae mayfly dominating kryal zone colonization in New Zealand rather than Diamesinae (Winterbourn 2008). Greater densities of invertebrates are found in streams originating from glacier outlet lakes rather than glacier streams originating from underneath glaciers in Greenland (Friberg et al. 2001). Small glaciers ($<2km^2$) resulted in lower discharge volume, higher water temperatures, greater channel stability, and greater taxa richness closer to the glacier in the Italian and Swiss Alps than sites farther away from larger glaciers (Miaolini & Lencioni 2001; Lods-Crozet et al. 2001; Schütz et al. 2001). The European Alps examples highlight the importance of glacier size and the relative distance of its impact, with larger, receding glaciers having a greater impact on temperature and channel stability further from the snout than small, stable glaciers (e.g. Jacobsen et al. 2012). The lack of a true metakryal zone due to higher daytime water temperature and diurnal freezing highlights the importance of the position of the glacier terminus in relation to the zero-degree isotherm in tropical Ecuadorian glacier streams, which resulted in low taxa densities closer to the glacier (Jacobsen et al. 2010).

At temperate latitudes, monsoonal temperate glaciers with subalpine termini are perhaps the rarest glacier habitat remaining in the shrinking cryosphere, where the most rapid glacial retreat is occurring around the southeastern edges of the Tibetan Plateau (He et al. 2003; Yao et al. 2012). Glacier mass loss of 30±8 percent has occurred over the past 40 years in the temperate monsoonal region (Aizen & Aizen 1994; Liu et al. 2015). Monsoonal temperate glaciers are summer accumulation types receiving more snow through avalanches or snow fall during the summer, and are characterized by heavy melting and high flow velocities (Ageta & Higuchi 1984; Pan et al. 2012). The mountain ranges in southeast Tibet contain the upper reaches of the Three Parallel Rivers (Yangtze, Mekong, and Salween) (He et al. 2003; Baker 2007; Yao et al., 2012). The topographic relief of the area is tremendous, with gorges at 1,500 m a.s.l. found within 20 km of peaks topping 6,700 m a.s.l., resulting in arid valleys, subtropical, temperate, and boreal climates, as well as permanent snow fields in one of the most biodiverse temperate regions of the world (Mittermeier et al. 1998). On the eastern side of Mt. Gongga, the vertical drop of > 6,300 m within 11 km is one of the greatest on earth, just slightly larger than the Hunza Valley drop in the Karakorum (Thomas 1999). In Yunnan province's Hengduan mountain range, animal biodiversity is high, with 52% of China's high plant species and 73% of China's protected wild animal species, all within 4.1% of China's total land area (Yang et al, 2004).

Despite the tremendous biological diversity in the region, only a few aquatic invertebrate studies have been reported from Tibet (Hamerlik & Jacobsen 2012; Murakami et al. 2012; Jiang et al. 2013; Laursen et al. 2015). Most of these studies took place at sites located >1,500 km from glacier termini and have daytime temperatures reported well above 2°C, which is above the temperature of metakryal zones closest to the glaciers. Therefore, metakryal invertebrates are not well studied in the Tibetan region. In general, studies on the Tibetan plateau are above the treeline with reduced stream gradient, which are in contrast with steep gradient streams below the treeline in deeply incised valleys of the Hengduan mountain range of Southeast Tibet, which experience frequent landslides and avalanches (Korup & Montgomery 2008). My research was conducted in subalpine glacier streams of this region where my preliminary sampling in the metakryal zones suggested that invertebrate communities did not fit the M&P predictions. I hypothesized that the invertebrate communities in the Hengduan mountain glacial streams would not correspond with habitat relationship predictions of the M&P model. My objective was to understand if water temperature and channel stability have a strong relationship with invertebrate presence in monsoonal temperate glacier streams.

Methods

Study Sites

The Mingyong, Sinong, and Hailuogou Glaciers are sources of the Mekong and Yangtze Rivers in the Three Parallel Rivers region (Figure 2.1). The Mingyong Glacier (13 km^2) on Mt. Kawagebo, Meilixueshan (6,740 m a.s.l.) is located in the monsoonal temperate region of Yunnan Province (28°26′14″N 98°41′04″E) and is the southernmost and one of the lowest elevation (2,750 m a.s.l.) glaciers in China (He et al. 2003). The Mingyong Glacier River originates at 2,750 m a.s.l. and flows into the Mekong River within six km (Figure 2.2). The neighboring Sinong Glacier (10.01 km^2) River originates at 3,550 m a.s.l., and flows into the Mekong within approximately 7,000 m (Figure 2.2). The Mingyong Glacier has been retreating at approximately 50 m per year since 2010 and the Sinong Glacier has also been retreating, but at a slower rate (H. Fair, unpublished data). The monsoonal temperate Hailuogou Glacier originates at 2,900 m a.s.l. on Mt. Gongga (7,556 m a.s.l.), in the Daxueshan mountain range in Sichuan province (29°35′48″N 101°52′43″E). The Hailuogou Glacier with an area of 24.83 km^2 (Liu et al. 2010) is just under double the size of the Mingyong Glacier (13 km^2). The main Hailuogou Glacier has retreated 181 ± 23 m from 1994 to 2007 (Liu et al. 2010). Hailuogou Glacier #3 is a small glacier (1.5 km^2) above the main Hailuogou Glacier with a stream that originates at 3,850 m a.s.l. and flows underneath the Hailuogou Glacier at approximately 3,100 m a.s.l. (Figure 2.3). The Mt. Gongga glaciers are headwaters of the Dadu River, which is a tributary of the Yangtze River.

I sampled aquatic invertebrates and measured water temperature and evaluated channel stability during the summer peak glacier discharge season from June 1 to September 9 in 2009, June-July 2011, August 2013, and June-August 2015. Three sites were sampled in Mingyong in 2009, seven sites were sampled in Mingyong in 2011, one site was sampled in Mingyong in 2013, and twelve sites were sampled in Meilixueshan and Mt. Gongga in 2015. Sample sites were located from 15 m to 7,000 m from a glacier snout.

Temperature

In-situ water temperature was measured with invertebrate sampling at each site with a YSI Professional Plus hand-held meter (YSI Incorporated, Yellowsprings, OH). Hobo temperature loggers (Onset Corporation, Bourne MA) were installed in the three watersheds to verify the presence of zones with T_{max} ranges as outlined by the M&P model. From August 2013 – February 2015, temperature data loggers were installed at the stream sites at 95, 800, 2,500, 3,000, and 5,500 m from the Mingyong Glacier in the mainstem and programmed to download temperature in 15-minute increments. In 2015, temperature data loggers were re-installed on May 24-August 10 at 300, 800, 2,500, and 5,500 m from the glacier. The loggers were covered with tidbit boots to protect from sediment abrasion, and tied securely to boulders with stainless steel cable. Two air temperature loggers were installed at sites in the watershed to record 15-minute increment temperature data from August 2013-November 2014 and April - August 2015, with data extracted from June 1 – August 31 from both years to calculate water and air summer temperature measurements. Air temperatures were recorded by placing a temperature logger in a forest one km from the Mingyong Glacier in a tree nook covered with leaves, and another was installed in the village 3,500 m from the glacier in a bush. The influence of sunlight on the temperature readings was avoided by placing the loggers within shade provided by foliage. At Hailuogou, temperature loggers were installed from June 2015-August 2015 in the glacier water at 200 m from the Hailuogou 1 glacier, 150 from GL3, and 900 m downstream from Glacier 3. The Glacier 3 downstream logger was placed below the sampling site to avoid detection and tampering by tourists. Temperature logger data from 2013 was not available for Hailuogou and Sinong due to logistical constraints. T_{max} water temperatures were determined by taking the absolute maximum temperature from each respective site between June – August 31.

A Pearson product-moment correlation coefficient was computed to assess the relationship between the sites with long-term temperature logger data and in-situ water temperature data points. This was done in order to substantiate the classification of sites with only in-situ water temperature readings in the metakryal (T_{max}<2^{o}C), hypokryal (2-4^{o}C), rithral (4-6^{o}C) and downstream (>6^{o}C) temperature classification. Air temperature logger data in the village of Mingyong was used to conduct Pearson correlation tests with Mingyong Glacier water temperature in the summer months (June-August) and winter months (December 15-February 20) to assess the relationship between seasonal air temperature and glacier stream water temperature. Pearson correlation coefficients were also computed to assess the relationship between, 1) distance from the glacier and in-situ water temperature measurements; 2) distance from the glacier and T_{max} water temperature

(June-August); 3) distance from the glacier and T_{min} water temperature (June-August); and 4) size of glacier and T_{max} water temperature (June-August).

Channel and Substrate Stability

The Pfankuch Index of Channel Stability (PSI) lower bed score section was used as a visual assessment to represent channel stability, and was completed at the beginning of each sampling event (Pfankuch 1975). Rock angularity, substrate surface brightness, substrate consolidation/packing, percent of the streambed affected by scouring and deposition, and presence/absence of moss or vegetation growth were assessed to evaluate the channel and calculate the PSI lower bed score. High scores (40-60) represent unstable channels and low scores (0-20) represent stable channels. Pearson correlation coefficient was computed to assess the relationship between distance from the glacier and the Pfankuch Index score.

Invertebrate Collection

At each of the 14 stream sites on each sampling day, ten representative microhabitats were sampled (five combined riffles and runs, three combined rapids/step-rapids, one pool, and an additional representative habitat) with a Surber sampler ($0.09m^2$, mesh size 250 µm) and composited into one site sample. Invertebrate larvae other than Ephemeroptera, Plecoptera, Tricoptera (EPT), and Chironomidae were identified to the family level. Ephemeroptera, Plecoptera, and Tricoptera (EPT) were identified to genus, with Nemouridae identified to morphospecies due to limited taxonomic resolution in the region. Morphospecies identifications were based on Nemouridae gill structure and femur spine patterns (Baumann 1975). Chironomidae were identified to sub-family (Diamesinae and Orthocladiinae), tribe (Tanytarsini) or genus (*Boreoheptagyia* spp.) (Merritt and Cummins 1996; Morse & Yang 1994; Epler 2001). Hirudinea (leeches), Oligochaetes, Collembola, and water mites (Hydrachnidia) were identified at the subclass level and aquatic isopods were identified to the family level (Asellidae). Only samples from Hailuogou in 2015 with >125 Chironomidae individuals were subsampled by identifying 125 individuals and extrapolating by proportion subfamily or genus identified. No other chironomids or other invertebrates were sub sampled. Chironomids were cleared, dehydrated, mounted in Euporal (Epler 2001), and identified under a compound microscope (40x -400x). Other invertebrates, including early instar larvae, were identified using a dissecting microscope (7x - 40x) magnification.

Statistical Analysis

A presence/absence table of invertebrate taxa was constructed for each summer water temperature range (metakryal <2^{o}C, hypokryal 2-4^{o}C, rithral 4-6^{o}C, and downstream >6^{o}C) for the three watersheds (Mingyong, Sinong, and Hailuogou). If a taxon was present at a site, then a value of 1 was assigned, and if absent a value of 0 was assigned. The total number of sites within each temperature range where the taxon was present was summed. This number was divided by the total number of sites within the temperature range to calculate the percentage of sites where each taxon was present. If the taxa were present in >10% of the sites within a temperature range, then results in the chart were color coded with a black cell and grey if the taxa were in <10% of the sites. If the taxa were absent, the cell was left white.

A second table was constructed to examine the proportion of each taxon by temperature range in the Mingyong glacier mainstem from June-August. The aim was to elucidate which taxa populate in the metakryal, hypokryal, rithral, and downstream zones as a proportion of the total number of individuals of each taxon in the Mingyong Glacier stream. The individuals of each taxon present in the streams from June-August were counted and totaled for all sites and sampling dates, and the number of each individual taxon by temperature zone was divided by the total abundance of the taxon in the Mingyong Glacier stream. The table was constructed similar to the presence/absence table but the breakdown of percent abundance by temperature zone was as follows: 1) >50%; 2) 30-49%; 3) 10-30%; and 4) <10%.

Detrended correspondence analysis (DCA) was performed on the invertebrate communities in order to obtain an index that describes trends in taxa composition among sites, with DCA axes, invertebrate abundance, richness, and density (m^2) as response variables. Any taxon present in <10% (or n=1) of the sites were removed to reduce the impact of rare species on ordination. The top two DCA axes scores (AX1 and AX2) were used as dependent variables in linear mixed effect model analyses (PCORD v.6). Two sites closest to the Mingyong Glacier sampled in July 2011 (UV1 and UV2) did not have invertebrates present and so the data for these two sites were removed from the DCA ordination. Linear mixed effect models were used to address pseudoreplication as a result of repeatedly sampling selected sites. Univariate and multivariate linear mixed effect regression models were used to determine if there were significant relationships between the fixed factors temperature and channel stability and the response variables: 1) DCA axes 1 and 2, 2) species richness, 3) invertebrate abundance, and 4) density of invertebrates. Stream site was the random factor in the analyses. Linear mixed effect models in R (Ri386 3.3.1) were used to analyze the data with the lme function from the nlme package (Pinheiro et al. 2013).

Residuals of the models were examined for normal distribution with qq plots (qqPlot function, car package; Fox and Weisberg 2011) and Shapiro tests (Shapiro test function package; Shapiro & Wilk 1965). Homogeneity of variance was examined by plotting residuals from the model with the fitted values from the model (R Core Team 2013) Prior to linear mixed effect model analyses logarithmic log(x+1) or arcsine square root transformations were applied to the response variables that did not meet normality and homogeneity assumptions (AX1, abundance, and density) (Zar 1996). The level of significance for all analyses was $\alpha=0.05$.

Results

Water Temperature

There was a strong and positive correlation between summer T_{max} temperature from logger data and in-situ water temperature readings, ($r = 0.91$, $n = 15$, $p < 0.0001$) (Figure 2.4). In-situ water temperatures at all sites ranged from a minimum of 0.05°C at Glacier 3 to 11.0°C at Sinong 2. T_{max} and T_{min} temperatures were positively correlated with distance from the glaciers (Figure 2.4). The size of the glacier was not related with

T_{max} temperature (r=-0.31, n=19, p = 0.177). The air temperature in the woods 1,000 m from the glacier had a significant but weak positive correlation with water temperature at 2,500 m from the glacier (r=0.21, n=8831, p<2.2e-16), and the temperature in the village (3,200 m from the glacier) had a positive correlation with water temperature 2,500 m from the glacier (r=0.67, n=8830, p<2.2e-16). Air-water temperature relationships show increasing positive correlation of air temperature with water temperature as distances from the glacier increase.

In Mingyong, the summer T_{max} water temperature 300 m from the glacier in the mainstem was 1.81°C, which verified that the uppermost site 95 m from the glacier was well within the metakryal zone (T_{max}<2°C). The sampling site within the hypokryal zone (2-4°C) was verified with readings at 800 m from the glacier at T_{max} 2.66 °C. The T_{max} temperature at 2,500 m was 4.92°C in summer 2013-2014 and 5.02°C in summer 2015, which verified the third and fourth downstream sites as being within the rithral temperature zone (4-6°C). The furthest downstream site (5,500 m from glacier) experienced a T_{max} temperature of 6.54°C in summer 2013-2014, and 7.57 °C in 2015 which placed the furthest downstream site within the 6-8°C temperature range.

At Mt. Gongga, the T_{max} temperature at 200 m from the Hailuogou Glacier was 0.99°C between June 7 – July 4 (Table 2.1). However, the logger was removed from the river by a passerby on July 4, so water temperature was not recorded from July 4 – August 28. The uppermost Glacier 3 site (3,850 m a.s.l.) in the Hailuogou catchment was installed on June 11, at 100 m from the glacier. When we retrieved the monitor in August, the stream channel had braided and shifted by 10 m, so that the temperature monitor was entirely exposed to air. The T_{max} temperature while it was submerged in water between June 11-June 30 was 1.91°C. The T_{max} temperature of the Glacier 3 downstream site, which was placed 900 m from Glacier 3, was 6.38°C. The actual sample site was 800 m from the glacier, but in order to avoid tampering by tourists, the logger was installed further downstream. In the four glacier streams, seven samples occurred in metakryal sites (T_{max} <2°C), six in hypokryal sites (2°C to 4°C), five in rithral sites (4°C to 6°C) and three sites with T_{max} > 6°C. The sites ranged between 15 m from the glacier to 7,000 m downstream (Table 2.1).

Channel Stability

Channel stability scores (mean±SD) were high at all sites (Table 1.2), with no relationship found between the Pfankuch Stability Index and distance from the glacier (Figure 2.4). The high instability of the channel is reinforced by the attempts to retrieve water temperature logger data for the Mingyong mainstem sites. In February 2015 after the loggers had been installed in the Mingyong mainstem for 1.5 years, the uppermost site streambed had aggraded by 1.5 m from landslide debris which buried the data logger. The logger at 800 m from the Mingyong Glacier was deeply buried in glacier flour and the stainless steel wire broke when attempting to retrieve it. The logger at 3,000 m washed away. Therefore, long-term temperature data from 2013-2014 was recovered from only two out of five site loggers at 2,500 and 5,500 m from the glacier (Figure 2.5).

In the Sinong Glacier mainstem, temperature loggers were installed 1,000 m and 2,700 m from the Sinong Glacier on May 6, 2015 at locations thought to be protected from landslide, debris flow, and boulder movement. When retrieving the loggers on August 6, the upper temperature logger was buried underneath boulders and was irretrievable. The temperature logger installed 2,700 m from the Sinong Glacier recorded a T_{max} temperature of 7.12°C between Jun 1 – August 15, 2015.

Invertebrates

A total of 2,537 invertebrates and 19 taxa were collected with the top five most abundant taxa in the three watersheds including: 1) Diamesinae (1,283); 2) Nemouridae (364); 3) Orthocladiinae (364); 4) Taeniopterygidae (176); and 5) Oligochaeta (60). Fifteen out of nineteen taxa were present in > 10% of the metakryal (T_{max} <2°C) sites in Meilixueshan and Mt. Gongga (Figures 2.6 & 2.7). Besides *Diamesina* spp, other Diamesinae, Orthocladiinae, Oligochaeta, Tipulidae, Taeniopterygidae, Empididae, Baetidae, Nemouridae, Limnephilidae, Chironominae, Heptageniidae, Rhyacophilidae, Acari, Collembola, and Capniidae were present in > 10% of all stream sites in the metakryal zone. The two taxa that were not present in the metakryal zone include Chloroperlidae which were present in sites with water temperature > 4°C in the Sinong Glacier stream, and aquatic isopods which were present in water temperature >6°C.

Abundance of Invertebrates in the Mingyong Glacier stream

In the Mingyong mainstem metakryal zone, >50% of Diamesinae, Oligochaetes, *Ormosia* spp. (Tipulidae), and Nemouridae were collected (Figure 2.8). Between 30-50% of Orthocladiinae and Taeniopterygidae were collected in the metakryal zone.

Baetidae, which were also collected in the 30-50% abundance range, are found in low numbers in the summer in the metakryal zone. Between 10-30% of *Epeorus* spp., Limnephilidae, *Rhyacophila* spp, and Capniidae were collected in the metakryal zone. The taxa that did not occur in the Mingyong metakryal zone include *Rithrogena* spp., *Himalopsyche* spp. (Tricoptera), Tanytarsini (Chironomidae, Diptera), Isopods, Collembola, *Boreoheptagyia* spp. (Chironomidae, Diptera), Hydrachnidia (water mites), Empididae (Diptera), and Chloroperlidae. Several of these taxa did colonize in the metakryal zones in Hailuogou and Sinong, which included *Himalopsyche* spp, Tanytarsini, Collembola, *Boreoheptagyia* spp., Hydrachnidia, and Empididae. The taxa that were collected in >50% abundance in the hypokryal zone included Taeniopterygidae, Capniidae, *Himalopsyche* spp., Collembola, Hydrachnidia, and Empididae (Figure 2.8). The invertebrates in the >50% abundance category in the rithral zone were *Boreoheptagyia* spp. and Chloroperlidae. There were only two individuals of *Ormosia* spp. collected, with one present in the metakryal and one present in the rithral zone. The taxa that had abundances >50% in the downstream sites included *Epeorus*, Limnephilidae, Tanytarsini, Isopods, and Chloroperlidae. There were only two Empididae collected in Mingyong, one in the hypokryal and one in the downstream site. On the other hand, Empididae were collected in the metakryal zone in Hailuogou (Figures 2.6 and 2.7).

Detrended Correspondence Analysis (DCA) Ordination of Invertebrates

The DCA of invertebrate communities resulted in two axes depicting the change in taxa composition among sites. The greatest site scores on the first DCA axis were associated with increasing relative abundance of Chloroperlidae and lowest site scores were associated with increasing abundance of Diamesinae (Figure 2.9). The greatest site scores on the second axis were associated with increasing relative abundance of Oligochaetes and lowest site scores with relative abundance of Limnephilidae and *Rithrogena* spp. (Figure 2.9).

Invertebrate Communities in Relation to Temperature and Channel Stability

There was no significant relationship between temperature and channel stability and invertebrate communities in the Mingyong, Sinong, and Hailuogou streams (Table 2.2). None of the univariate models produced significant relationships between temperature or channel stability and the dependent variables of DCA axes, richness, abundance, and taxa density (Table 2.2). The multivariate model also resulted in no significant relationships between the explanatory variables temperature and channel stability and the DCA axes, richness, abundance, and density (Table 2.2).

Discussion

The results of this study are clearly different from the M&P Conceptual model both in presence/absence and in percent taxa abundance (Figures 2.7 & 2.8). The percent abundance figure indicates that some taxa are present in greater numbers and percentages than are expected to be found in metakryal zones as predicted by Milner & Petts (Figure 2.8). Nemouridae are the most abundant invertebrates in the summer in Mingyong (42%), in Sinong the most abundant are Taeniopterygidae (40%), and in Hailuogou the most abundant are Diamesinae (77%) (Figure 2.10). In the M&P model, Nemouridae colonize habitats with T_{max} temperatures >4°C (Milner & Petts 1994; Milner et al. 2001), but in the Mingyong mainstem, they are clearly suited to low temperatures with >50% abundance of Nemouridae in the summer metakryal zone. Miaolini & Lencioni (2001) collected Nemouridae and Taeniopterygidae in upper stream sites approximately one km from the Conca Glacier, a 0.185 km^2 glacier in the Italian Alps. Sites close to the glacier reached T_{max} temperatures >6°C (Miaolini & Lencioni 2001). The influence of the glacier is less extreme than glaciers such as the Hailuogou Glacier 1 which is >24x larger in area. The locations of the sites where Nemouridae were collected in Italy suggest that the sites may be below or near the treeline (Miaolini & Lencioni 2001). A few trends reported from the Conca Glacier study (Miaolini & Lencioni 2001) match the data in the Eastern Tibetan streams. For example, Chloroperlidae were present at the midstream and downstream sites in the Sinong Glacier stream 2,700 and 7,000 m from the Sinong Glacier, and Heptageniidae were much more abundant than Baetidae in all of the Southeast Tibetan streams. These same Chloroperlidae and Heptageniidae dispersal trends were found in the Conca Glacier study (Miaolini & Lencioni 2001). However, Capniidae stoneflies are the most abundant taxon over all four seasons in the Mingyong Glacier mainstem (Chapter 3), which is different from the Conca Glacier which is populated by Leuctridae rather than Capniidae. Capniidae are winter stoneflies, which are confirmed by the dramatic drop in abundance of the taxon of Chironomidae during the summer melt season (e.g., only 10 Capniidae or 0.005% of the annual number were found in summer 2015 in Mingyong versus 2,033 individuals collected during winter sampling in 2015) (Figure 2.10).

A few taxa were present in habitats in the hypokryal zone (2°C to 4°C), including Taeniopterygidae, Capniidae, *Himalopsyche* spp., Collembola, Hydrachnidia, and Empididae which is also different from the M&P model, where typically only dipterams such as Simuliidae are found in addition to Diamesinae and Orthocladiinae (Snook & Milner 2001). In July 2011 at 800 m from the Mingyong glacier, sampling took place in the hypokryal zone in a secondary flood channel immediately beside torrential rapids, which is the reason that the site is scored with a lower Pfankuch score of 32 which represents a more stable site. The results from this site suggest that Taeniopterygidae and semi-aquatic Collembola may be sensitive to channel stability. Capniidae also tend to colonize sites with higher channel stability, which could explain why their stage of growth is adapted to the winter time when the glacier streams are more stable due to reduced glacier melt. *Himalopsyche* spp. also appear to colonize in more stable reaches, although the microhabitats where I have collected *Himalopsyche* spp. tend to be high velocity rapids and step-rapids. Rapids and step-rapids are turbulent, contain large boulders, and hide backwaters beneath the surface, which could create ideal refugia for invertebrates like *Himalospyche* spp. (Chapter 3). Moreover, *Himalopsyche* spp. are found in high abundance in the Glacier 3 downstream site during August, which is a high gradient site where rapids and step rapids are dominant hydraulic microhabitats and Chironomidae are also very abundant (Chapter 4). In the Mingyong mainstem, *Tanytarsini* spp. make a first appearance in the >6^{o}C temperature range, which is in agreement with increasing Chironomidae taxa with distance from the glacier (Milner et al. 2001).

Although having similar species richness as Meilixueshan, the metakryal zone in Mt. Gongga's Hailuogou glacier contained the greatest abundance of Diamesinae of all sites. Nemouridae were present, but Taeniopterygidae were the dominant stonefly in Hailuogou during the summer, and third in abundance to Orthocladiinae in the metakryal zone. I hypothesize that the open canopy and wider glacier valley at Hailuogou may be allowing for greater periphyton growth which provides colonization space and food for chironomids (e.g., Gislason et al. 2001; Zah & Uelinger 2001; Rott et al. 2006). Even though the analysis of the periphyton at the microhabitat level (Chapter 3) did not detect a difference in periphyton dry mass between the mountain ranges, it is thought that by sampling specifically for periphyton rather than relying on periphyton by-catch from samples, the result may show a significant difference in periphyton mass between watersheds. There is a seasonal difference in periphyton loss-on-ignition in the Meilixueshan watersheds, with the loss-on-ignition (LOI) (g) being significantly greater during the summer season, which suggests the nutritional value of periphyton may be greater during the summer and result in invertebrate life cycles that favor growth stages by resource availability during the summer.

Water temperatures were validated between long-term logger temperature data and in-situ water temperature correlations. Except for one site, summer T_{max} water temperatures fall within the scope of glacier-fed rivers at T_{max} temperature <10°C (Milner et al. 2001). At Sinong 2 near the confluence of the Mekong River, the in-situ temperature was recorded as 11.0°C in August 2015, which could be due to the distance from the glacier (7,000 m) and groundwater contributions from tributaries. However, in the Mingyong, Sinong, and Hailuogou glacier streams, low water temperature is not a factor limiting invertebrate colonization. The two taxa out of twenty taxa that appear to have a sensitivity to water temperature are Chloroperlidae and aquatic isopods (Figure 2.6). Chloroperlidae were not collected over several years during high discharge sampling in water temperatures <4°C. This fits the M&P prediction and the results of the Miaolini and Lencioni (2001) study that Chloroperlidae colonize in habitats with water temperature above 4°C. Aquatic isopods were only found in temperatures > 6°C in the glacier mainstem (Figure 2.6).

Based on the correlation analysis of glacier size and water temperature, it is estimated that the Hailuogou Glacier 1 metakryal temperatures extend far downstream of the 200 m site where the logger was installed. The metakryal site in the Mingyong Glacier mainstem extends beyond 300 m from the glacier and the glacier is much smaller in area than Hailuogou Glacier 1. Invertebrate and temperature samples were not taken from downstream sites of Hailuogou Glacier 1 due to difficult logistics during the summer. Even with solar insolation from the cloudy monsoonal climate, the small size of the Hailuogou Glacier 3 (1.5 km^2) and open canopy resulted in a T_{max} temperature much greater closer to the glacier than the larger glaciers. The Glacier 3 downstream site 900 m from the glacier reached a T_{max} temperature of 6.38°C at, whereas in the Mingyong Glacier (13 km^2) mainstem, the T_{max} temperature in the 2013-2015 melt seasons was 7.57 °C at 5,500 m from the glacier.

Although there was a range in water temperature (0.50°C to 11.0°C) from upstream to downstream, the torrential water flow in monsoonal temperate streams may have resulted in low numbers of taxa and abundance in the Sinong downstream site close to the Mekong River due to a road culvert frequently blocking floodwaters, resulting in periodic deep waters which could result in invertebrate drift. Simuliidae, which are present in the M&P model hypokryal zone, are absent from these glacial-melt streams in the summer. Leuctridae, Perlodidae, and Limoniidae were not present in Mingyong, Sinong, or Hailuogou glacier-fed streams. However, I have collected Perlodidae at Mt. Gongga spring streams during the summertime (Chapter 4).

A major lesson from installing temperature loggers was that the channel stability of the stream should influence the strategy of temperature monitor placement and duration of installment. Constant monitoring should have taken place frequently throughout the year to ensure that the loggers remain in the water, are not buried deeply in glacier flour, beneath boulders, and are not exposed to air due to shifting channels. Although it would have been ideal to log temperatures at all sites, at sites like UV1 and UV2 (Figure 2.11), it would have been impossible to maintain loggers in the channel for a long period due to the proximity to the ice and unstable substrate and shifting channel.

The fact that the temperature loggers were difficult to maintain in the glacier rivers supports the classification of the streams with high Pfankuch scores.

Allochtonous Carbon in Subalpine Glacier Streams

The mass elevation effect (MEE) describes the heat retention capacity of large mountain ranges, in which the interior of ranges have higher treeline elevations than the perimeters of the range (Zhang & Bai 2016). The latitude and MEE are what places the monsoonal temperate glaciers studied far below the treeline (Ward 1994). In boreal latitudes, pine needles are the predominant allochtonous carbon source, which are not as palatable to invertebrates as deciduous leaves (Danks 2007; Robinson et al. 2000). In Southeast Tibet, limited broadleaf trees are present above some of the glacier stream sites. Even though there is no direct canopy coverage in the upper reaches of these glacier streams, avalanches, debris flows, and landslides may be seeding the mainstem channel with allochtonous material from high altitude tree stands. For example, the uppermost Mingyong Glacier sites were found to have mountain slope tree canopy coverage when performing densiometer canopy measurements (Table 2.1). However, the mountain slope tree coverage recorded from the densiometer mirror was from tree stands that were 50-1,000 m above the sample site. In the 2015 study of hydrologic microhabitats and allochtonous carbon, it was found that the greater velocity microhabitats of runs, rapids, and step-rapids contained the greatest mass of particulate organic matter (Chapter 3). These microhabitats also happen to be where Nemouridae stoneflies are found in the highest abundance. It may seem counter intuitive that greater water velocity microhabitats retain more organic matter, but the significantly greater turbulence in rapids and step-rapids could be an indication of backwaters, which are depositional zones (Gordon et al. 1992; Lancaster & Hildrew 1993). In addition, substrate in microhabitats with greater velocities tends to be larger and more stable, and boulders are often compacted together, which create spaces to trap and retain organic matter (Gomez 1983). All of these factors suggest that the presence of Nemouridae in the metakryal zone could be due to the presence of food in microhabitats, regardless of the near-freezing temperatures. Even though the original M&P model did not have allochtonous matter as an influencing factor on invertebrate presence, it was added in a revised model based on results of studies that took place during the AASER project (Milner et al. 2001; Zah & Uehlinger 2001). What I have found is that quantification of allochtonous matter trapped and retained in the channel, whether derived from aeolian sources, avalanches, or landslides may be an important aspect of glacier invertebrate population studies.

Other potential influences of higher metakryal species richness

Conductivity in alpine glacier streams is typically <50 μS/cm during the glacier melt season (Milner & Petts 1994). The streams in southeast Tibet have slightly higher conductivity during the summer (Table 2.3), which is related to higher amounts of total dissolved solids in the water. This could be due to the relative distance that melting ice is in contact with sediments at the base of the glaciers below the equilibrium line altitude (ELA), which is the point where snow accumulation equals ablation over the time span of a year (Hoinkes, 1970). Glaciers in the Himalayas are also known to have periodic surging movements in the summer (Zhang 1992), which can release sediments. In the tropical Ecuadorian Andes, a high elevation glacier terminus at 4,730 m a.s.l. is 1,030 m below the peak of the mountain (5,760 m a.s.l.) (Jacobsen et al. 2012). The Hailuogou Glacier 1 terminus at 2,900 m a.s.l. is 4,656 m below the mountain peak (7,556 m a.s.l.), and the Mingyong Glacier is 3,990 m below the peak of Mt. Kawagebo (6,750 m a.s.l.). The glacier bed lengths in these two southeast Tibetan ranges have fourfold the distance as higher altitude glaciers in the Andes. In the Andes, diurnal freezing of the glacier stream (Jacobsen et al. 2012) may be reducing basal glacier grinding caused by glacier movement. This could be resulting in decreased sediment resuspension from beneath the glacier and lower salinity meltwater emerging at the snout (e.g., 11 µS/cm, Jacobsen et al. 2012) during the daytime.

It is well known that seasonal water sources beneath glaciers consist of varying percentages of glacial meltwater, groundwater, and snow meltwater (Brown et al. 2006; Brown et al. 2007; Mark & Seltzer 2009; Schütz et al. 2001). This is likely part of the reason for the higher salinity in the southeastern Tibetan region due to the large mountain ranges and low elevation of the glacier snouts and the potential for mixed water source presence; however, there are other physical glacier characteristics that might contribute to higher salinity levels. Liu (2011) found that the Hailuogou glacier subglacial drainage system is highly impacted by ice thickness overburden that creates pressure on subglacial drainage systems. Increased glacier ice thickness expands the subglacial drainage system and thinning of the glacier causes contraction which could reduce the area of the glacier that is in contact with bedrock. With recent climate warming, the Hailuogou Glacier has thinned and the surface melt has increased. Basal sliding of the glacier has been enhanced by transport of ice from higher elevations, which causes supraglacially-forced basal motion of glaciers (Liu 2011). This enhanced basal glacier movement would increase bedrock grinding and potentially influence salinity levels. The potential of total ion concentration as a factor influencing the species richness at sites close to the glacier was not mentioned in the original M&P model, but nonetheless is a factor to consider when examining ecological differences between glacier sites.

The absence of vertebrate predators could be a factor in these streams. In nine years of sampling in the region, no fish were observed in primary headwater streams and glacier streams. The absence of fish predators may offer protection and allow greater abundances of taxa such as Plecoptera and Tricoptera to thrive. Anecdotal observations of Taeniopterygidae gut contents indicate that they are predatory as late instars and may influence the abundance of early instar Diamesinae in the metakryal and hypokryal zones. Because Taeniopterygidae inhabit the metakryal and hypokryal sites in Mingyong, this could be one reason that chironomid densities are low in Mingyong upper sites. Moreover, in the downstream GL3 site in Hailuogou, Taeniopterygidae and Diamesinae co-occur in high abundances, which could be a further indication of the predator-prey relationship. A more in depth study of food chains in subalpine glacier streams would be helpful in understanding the feeding habits of the invertebrates. This could help understand whether or not predatory behavior is influencing Diamesinae abundance in the metakryal zone and causing the noted departure from the M&P model of insect presence.

Perhaps the most important reason for high metakryal species presence in monsoonal temperate glaciers is the position of the glacier termini far beneath the zero-degree isotherm altitude, which also places the glaciers in the wet-based/warm-based category of glacier classification. In combination with the large size of the glaciers, this allows for perennial flowing water at the base of the glacier and a greater number of microhabitats due to the increased stream size and favorable ecological conditions provided by perennial flow. Due to studies comparing results to the M&P model taking place during the summer melt season (e.g., Lodz-Crozet et al. 2001), the winter conditions at the snouts of most glaciers are not known. If water is flowing from under the glaciers during the winter, this could indicate a more favorable condition for non-Chironomidae species on a year-round basis. However, in alpine zones, there is a high likelihood that the position of glacier termini in the European mountain ranges is above the zero-degree isotherm altitude and the glacier size is smaller than those in the Himalayan region. Chances are that alpine streams freeze-through or are covered with snow or ice (e.g., Schütz et. al. 2001). Globally, high altitude treelines are associated with a mean annual ground temperature of 6.7°C (±0.8 SD), with temperate zone treelines slightly higher (7-8°C), equatorial treelines slightly lower (5-6°C), and boreal and subarctic in between (5-6°C) (Körner 2004). Because the glaciers in this study are far beneath the treeline, the mean surrounding ground temperatures are most likely > 6°C which could be supporting conditions for above freezing basal temperatures and perennial flow from under the glaciers. Moreover, the mean±SD air temperature in the forest 1,000 m from the Mingyong Glacier from December 15, 2013-February 20, 2014 was 2.46±1.66°C. The air temperature is above freezing during the coldest part of the year close to the glacier snout which indicates that the glacier may be releasing ice melt.

The reason for the singular presence of *Diamesa* spp. at most metakryal sites in the M&P model comparisons could be due to winter ice either on the surface of the stream, or the presence of solid ice/intermittent stream conditions. Streams that become ice bound are a particularly harsh environment for aquatic invertebrates to tolerate (Danks 2007). Ice is a key element impacting the survival of invertebrates in the winter, and hence is a factor in structuring year-round communities of freshwater invertebrates that cannot be exposed to ice and survive (Oswood et al. 1991). However, the degree of the threat to invertebrate larvae depends on the type and extent of the ice. There are different ecological impacts of ice freeze-through, surface ice, frazil ice, and anchor ice. Frazil ice is an accumulation of ice crystals in the stream column, where waters are too turbulent to allow the water to freeze through. Frazil ice presents a more benign environment to invertebrates, but surface ice is more of a threat to insect survival. Encasement in surface ice requires some sort of physiological mechanism such as cryoprotectants to prevent inoculative freezing in invertebrates (Danks 1971). Invertebrates such as *Nemoura arctica* produce glycerol and antifreeze proteins to protect them from ice encasement in arctic and subarctic Alaskan streams (Walters et al. 2009). However, most Plecoptera likely use behavioral adaptations to avoid ice by moving to deeper waters that do not freeze (Olsson 1983).

Chironomids exhibit high tolerance to being frozen and have high rates of survival after being thawed (Danks 1971; Olsson 1981; Lee & Denlinger 1991; Denlinger & Lee 2010). Some chironomids overwinter as a particular instar which may indicate instar-level protective mechanisms or diapause strategies (Lee & Denlinger 1991; Denlinger & Lee 2010). Some arctic chironomid species evacuate their guts and overwinter in a curled position in hard, protective cocoons that are sealed on both ends and freeze into pond mud (Danks 1971). Only when the cocoon is opened do the larvae begin to move (Danks 1971).

The experimental values of supercooling points in insects may provide insight on the ability of taxa to live in metakryal zones that freeze during the winter. Experimental supercooling points tend to be lower in an experimental environment (Danks 2007), but give an indication of the natural supercooling ranges of insects. Experimental values of Empididae supercooling points (-22.6°C) are much lower than Chironomidae (-11.0°C) and EPT taxa (-3 to -7°C) (Danks 1971). The physiological tolerances of Chironomidae and other Diptera to being frozen may hold the key to explaining the differences in metakryal community structure between most alpine glacier metakryal zones and monsoonal temperate glaciers that border freezing conditions but do not freeze through. The fact that the metakryal zones in Mingyong and Hailuogou 1 do not freeze through during the winter may be the reason that non-Chironomidae taxa are able to persist year round in these streams.

The reasons for the departure from the M&P model at first glance seems to be due to the position of monsoonal temperate glaciers beneath the treeline, which could be seeding the channels with landslide-derived allochtonous tree matter. Indeed, Nemouridae have been found in greater abundance in hydraulic microhabitats that retain significantly greater amounts of leaf matter which supports the treeline hypothesis (Chapter 3). However, the fact that the base of the monsoonal glaciers do not freeze through during the winter may enable invertebrates without physiological freeze tolerance mechanisms and longer maturation life cycles (e.g., semi-voltine) to colonize and persist in habitats close to the glacier. Alpine glacier streams above the zero-degree isotherm are likely to be bound in ice during the winter season due to altitudinal position and relatively smaller sized glaciers, making them tolerable only to larvae such as freeze-tolerant *Diamesa* spp. species. Moreover, rapid colonizers are able to take advantage of short windows of ice-free habitats (Danks 1971 & 1991; Steffan 1971; Milner et al. 2001). The fact that multiple non-freeze tolerant invertebrates living close to the glacier snouts were found in Mingyong all year suggests that year-round T_{min} temperatures might be an important factor structuring metakryal communities.

Conclusion

In future studies, the T_{min} water and mean winter air temperatures, glacier size, and stream size at the metakryal zone may be the most important independent variables to compare between sites, as they influence the extent of ice during the winter, and the presence or absence of perennial flowing water. Furthermore, the consideration of the type of ice and amount of ice coverage may be important, as surface ice and solid ice have different ecological impacts than frazil and anchor ice (e.g., depleted oxygen in streams with surface ice coverage). Therefore, understanding year round conditions in glacier streams is important to understand the communities present in the summer melt season.

The fact that perennial flowing water exists beneath wet-based glaciers has important implications for the establishment of ecological permanence for insects that are not freeze tolerant, and insects that are semi-voltine and require greater than a year to mature. Whether or not the water source is from ice melt or groundwater may not make a difference, as long as the stream does not freeze through or is not covered by ice. In the wintertime, low turbidity is normal in glacial-melt streams, as the basal grinding from glacier movement during the summer months is not as pronounced during the winter.

Even though the discharge of the monsoonal temperate glaciers is torrential during the summer, depositional and hyporheic habitats may be hidden in the depths of rapids where insects can avoid hydraulic stress and feed on particulate organic matter that has been deposited and retained. Landslide-derived allochtonous matter provides a food source for invertebrates that thrive in well-oxygenated low temperature water, but yet avoid ice. It is thought that the tremendous valley relief in the region, which drives landslides, combines with the size of the glaciers and position of the glaciers beneath the zero-degree isotherm to ameliorate harsh conditions of this region's alpine glaciers. This may be allowing for the presence of greater species richness at the glacier termini with permanent flow in subalpine glaciers in the monsoonal region of Tibet.

The results of this study suggest that mountain size, glacier size, regional climate, altitudinal and latitudinal position, air temperature and T_{min} water temperatures are important, as these factors drive the presence and type of ice during the winter time which may be factors determining colonization of the year round metakryal zone by particular invertebrates. The development of an index using latitude, glacier and stream size, zero-degree isotherm, and T_{mean} and T_{min} air and water temperatures may enable prediction of metakryal zone invertebrate communities. On the other hand, the freeze tolerance and life cycle length of the metakryal invertebrates present in the summer may enable prediction of the type and extent of ice during the winter season. Constant monitoring of air temperatures to track the position of the zero-degree isotherm will be important due to the upward shift of zero-degree isotherms as a result of climate change, which in turn will influence the presence of invertebrate communities.

Table 2.1. Stream characteristics (mean±SD) from Mingyong, Sinong and Hailuogou during the summer melt season (June-August) of 2009, 2011, 2013, and 2015. Discharge is estimated based on year-round surface velocity measurements and in-channel measurements during low discharge.

Site	Mtn. Range	Dist. beneath treeline (m) Approx.	Dist. from glacier (m)	Mean Temp (^{o}C)	T_{max}	Channel Stability	Channel width (m)	Slope (%)	Disch. Range (m^3/s)	Direct Canopy	Mtn Canopy Trees Upslope	Mtn Scree Slope Canopy
Glacier 3	Gongga	-350	70	0.7±0.3	1.91	54±9	3.1±1.6	4	0.15-0.57	0.0	0.0	0.0
Glacier 3 Down	Gongga	-550	800	2.5±1.0	6.38	57±5	3.3±0.3	45	0.12-0.63	0.0	0.0	0.0
Hailuogou1	Gongga	-1,120	25	0.4±0.1	0.99	52±9	24.9±7.1	3	5.98-45.0	0.0	0.01	0.0
Mingyong UV1	Meili	-1439	15	1.5±0.5	1.79	60±0	1.9±0.4	30	0.08-0.25	0.0	0.09	0.0
Mingyong UV2	Meili	-1486	100	1.9±0.16	2.00	59±1	0.9±0.5	20	0.02-0.25	0.0	0.14	0.0
Mingyong 1	Meili	-1647	95	1.5±0.1	1.81	50±15	8.6±3.2	4	1.02-12*	0.0	0.11	0.0
Mingyong 2	Meili	-1737	800	1.9±0.2	2.66	57	8.9±3.7	6	1.12-12*	0.0	0.18	0.0
Mingyong 3	Meili	-1,914	2,500	3.4±0.5	5.08	60	9.1±2.6	8	1.47-12*	0.22	0.01	0.0
Mingyong 4	Meili	-1,929	3,000	4.3±0.1	5.08	50±1	10.3±6.9	4	1.35-12*	0.0	0.01	0.0
Mingyong 5	Meili	-2,201	5,500	4.4±0.8	7.57	60±1	20.6±6.5	5	1.99-12*	0.0	0.02	0.29
Sinong 1	Meili	-700	15	1.9	1.93	60	5.0±0.0	3	0.55-5*	0.0	0.04	0.0
Sinong 2	Meili	-2,035	7,000	11.0	11.0	60	11.6±6.7	4	1.66-6*	0.0	0.0	0.35

Table 2.2. Linear mixed effect model results for water temperature and channel stability. Linear mixed effect univariate model results (top) and multivariate results (bottom). The response variables, explanatory variable and p-values are given for each of the two types of linear mixed effect test.

Univariate Model

Response Variable	Explanatory Variable	p-value
AX1	Temp	0.9309
AX2	Temp	0.4704
Richness	Temp	0.1411
Abundance	Temp	0.7898
Density	Temp	0.7996
AX1	Pfankuch	0.6199
AX2	Pfankuch	0.4519
Richness	Pfankuch	0.2191
Abundance	Pfankuch	0.8679
Density	Pfankuch	0.8596

Multivariate Model (Temp + Channel Stability)

Response Variable	Explanatory Variable	p-value
AX1	Temp	0.9295
AX2	Temp	0.5954
Richness	Temp	0.1527
Abundance	Temp	0.7940
Density	Temp	0.8032
AX1	Pfankuch	0.6707
AX2	Pfankuch	0.5512
Richness	Pfankuch	0.2969
Abundance	Pfankuch	0.8670
Density	Pfankuch	0.8593

Table 2.3. Water quality characteristics (mean±SD) for fourteen stream sites in Mingyong, Sinong, and Hailuogou streams during the summer melt season (June-August) of 2009, 2011, 2013, and 2015.

Site	Cond (µS/cm)	Turbidity (NTU)	pH	Ca (mg/L)	K (mg/L)	Mg (mg/L)	Na (mg/L)
Glacier 3	70±3	1062±295	8.42	15.9±2.3	8.0±6.0	4.20±4.9	1.0±0.4
Glacier 3 Downstream	58±15	794±88	8.26	10.7±4.2	5.1±2.1	4.0±3.1	0.6±0.3
Hailuogou 1	81±20	603±296	8.58	8.1±2.1	3.8±3.7	3.1±3.5	0.7±0.3
Mingyong UV1	47	673±260	8.38	-	-	-	-
Mingyong UV2	70	1003±183	8.32	-	-	-	-
Mingyong 1	62±5	828±155	8.56	9.4±2.0	0.6±0.3	2.0±0.9	0.7±0.8
Mingyong 2	58	1023±137	8.72	-	-	-	-
Mingyong 3	62	734±66	8.52	-	-	-	-
Mingyong 4	63	818±111	8.64	-	-	-	-
Mingyong 5	65	731±142	8.56	12.6±4.4	0.7±0.3	3.4±1.9	0.9±0.4
Sinong 1	50	378	8.84	15.0±13.7	5.6±7.7	2.8±2.6	4.8±6.6
Sinong 2	62	546	8.43	9.4±7.1	0.30±0.1	2.6±2.1	0.6±0.4

Figures

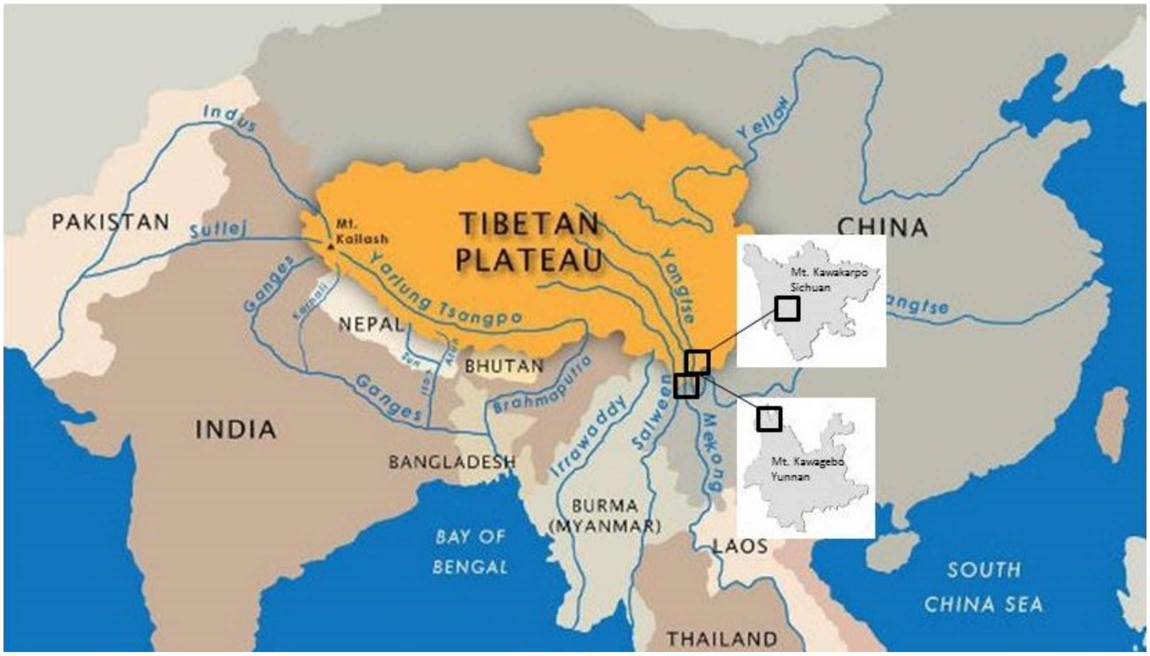


Figure 2.1. Study regions. Regions are highlighted in black boxes. The site along the Mekong River is located in Meilixueshan in the Three Parallel Rivers region, and the site in the Yangtze River headwaters is in the Daxueshan mountain range. (Tibetan plateau river map by Buckley 2009).

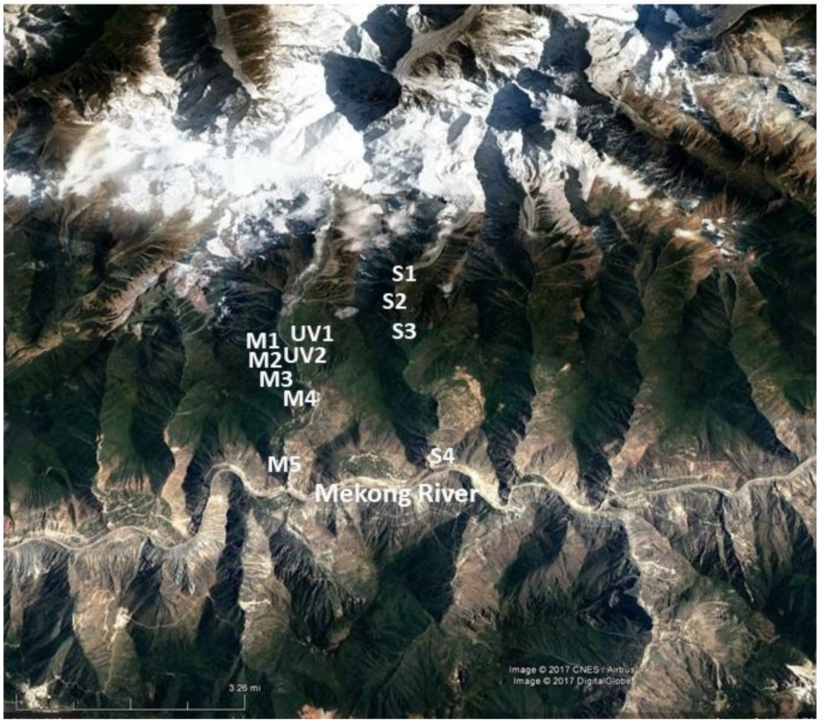


Figure 2.2. The Melixueshan mountain range. Headwaters flow into the Mekong River in Yunnan Province. M1-M5 are sites in the Mingyong Glacier mainstem and UV1 and UV2 are melt streams in the upper valley. The neighboring watershed of Sinong had a longitudinal gradient of sites sampled with S1 and S4 sampled in the summer melt season (*Source*: "Meilixueshan" 28°26′14″N 98°41′04″E Google Earth. December 18, 2014. June 15, 2017).

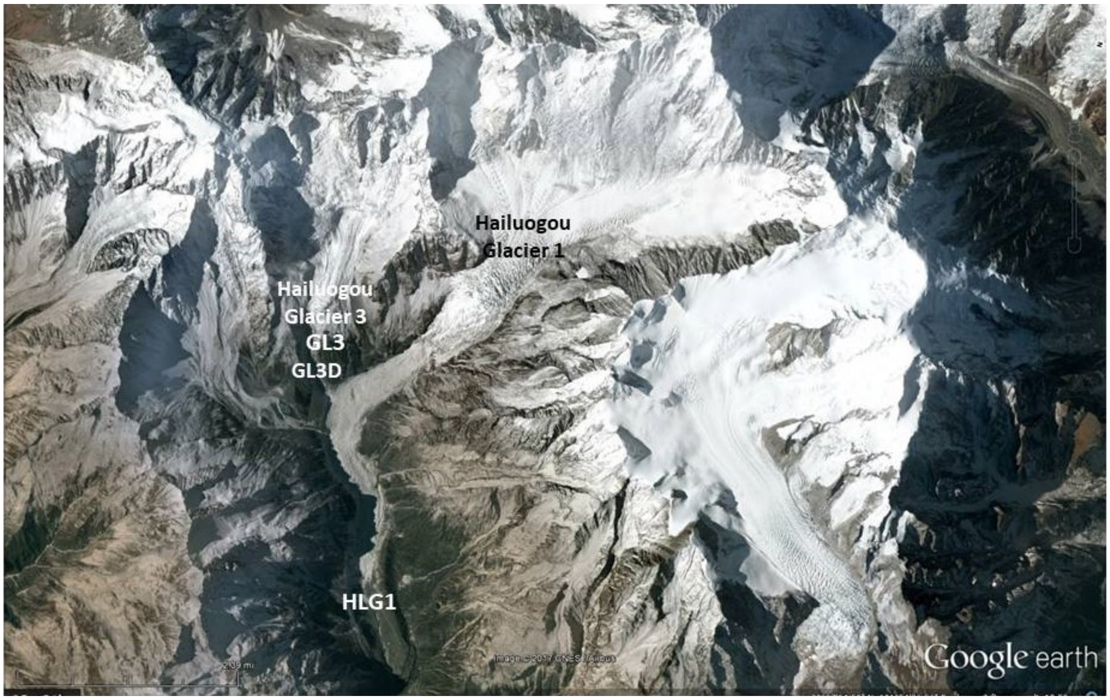


Figure 2.3. Mt. Gongga's Hailuogou Watershed. Hailuogou Glacier 1 (HLG1) and the upper Glacier number 3 (GL3) and downstream site (GL3D) in the Daxueshan range where communities of insects were collected. GL3 site is located 800 m downstream from Glacier 3 above the Chenmendong Glacier at 3,650 m a.s.l.(*Source*: "Hailuogou" 29°34'36.33"N N101 °57'57.04"E. Google Earth. December 18, 2014. June 15, 2017).

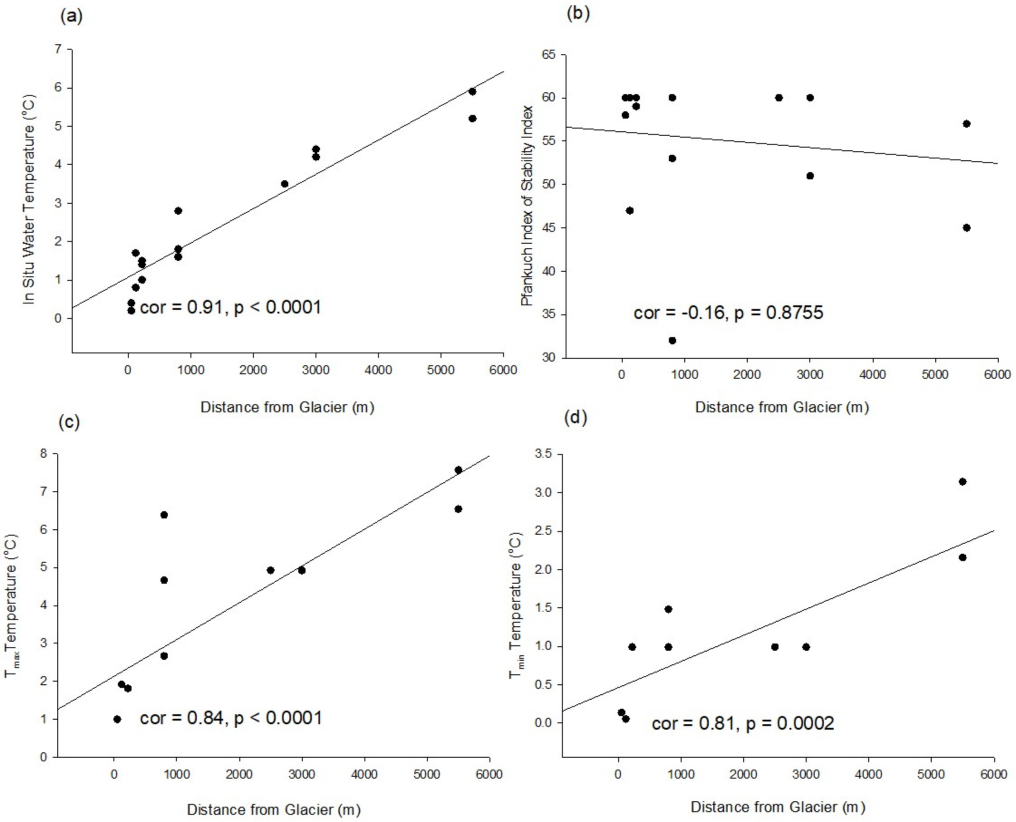


Figure 2.4. Water temperature and Pfankuch Stability Index Pearson's product-moment correlations. (a) In-situ water temperature, (b) Pfankuch Stability Index, (c) T_{max} and (d) T_{min} water temperatures with distance from glacier. In-situ water temperature, T_{max} and T_{min} were positively correlated with distance from the glacier and Pfankuch Stability Index was not related with distance from the glacier.

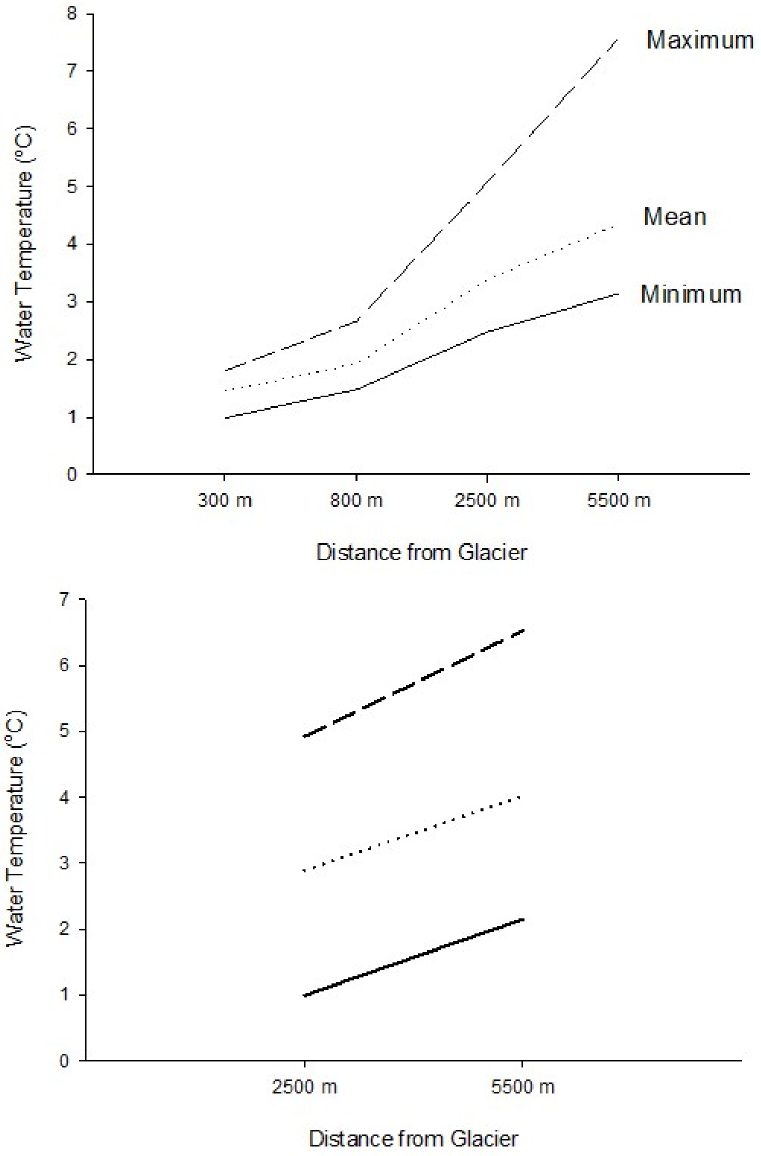


Figure 2.5. Mingyong Glacier mainstem summer water temperature readings in 2013, 2014, and 2015. June 1-August 10, 2015 (top) and August 15-31, 2013 and June 1 – August 31, 2014 (bottom). The temperatures (min, mean, and max) for the sites in 2015 are: 300 m (M1) – 0.99, 1.46, and 1.81°C; 800 m (M2) – 1.48, 1.93, and 2.66°C; 2,500 m (M3) – 1.48, 3.39, and 5.08°C; and 5,500 m (M5) – 3.14, 4.35, and 7.57°C. In the bottom figure, the temperatures (min, mean, and max) at 2500 m (M3) are 0.99, 2.87, and 4.92°C and at 5,500 m are 2.16, 3.87, and 6.54 °C.

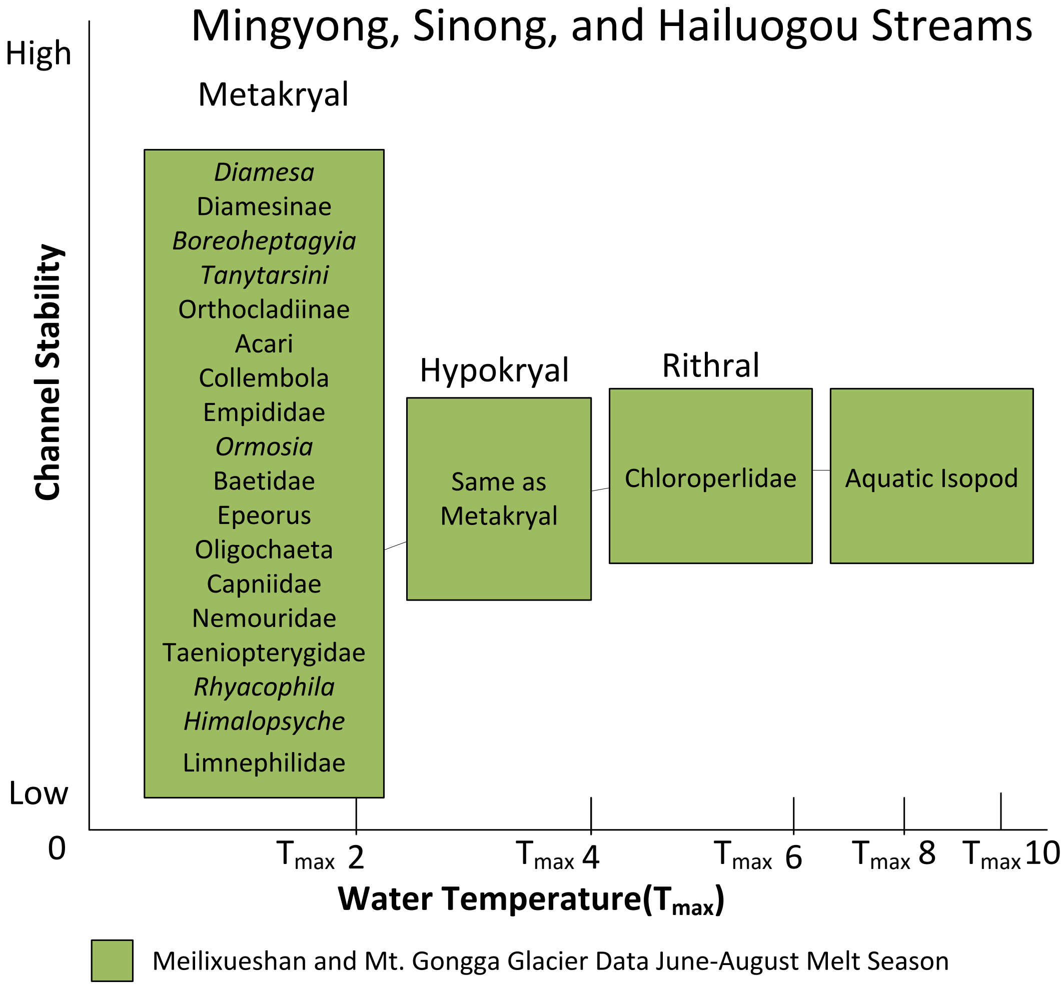


Figure 2.6. Invertebrate communities in Meilixueshan and Mt. Gongga during the summer melt season. The original M&P Model has one taxon (*Diamesa* spp.) representing the metakryal zone community. There were 15 families in Southeast Tibet's subalpine metakryal zones (T_{max} 2°C) from sampling that took place from summer 2011, summer 2013, and summer 2015.

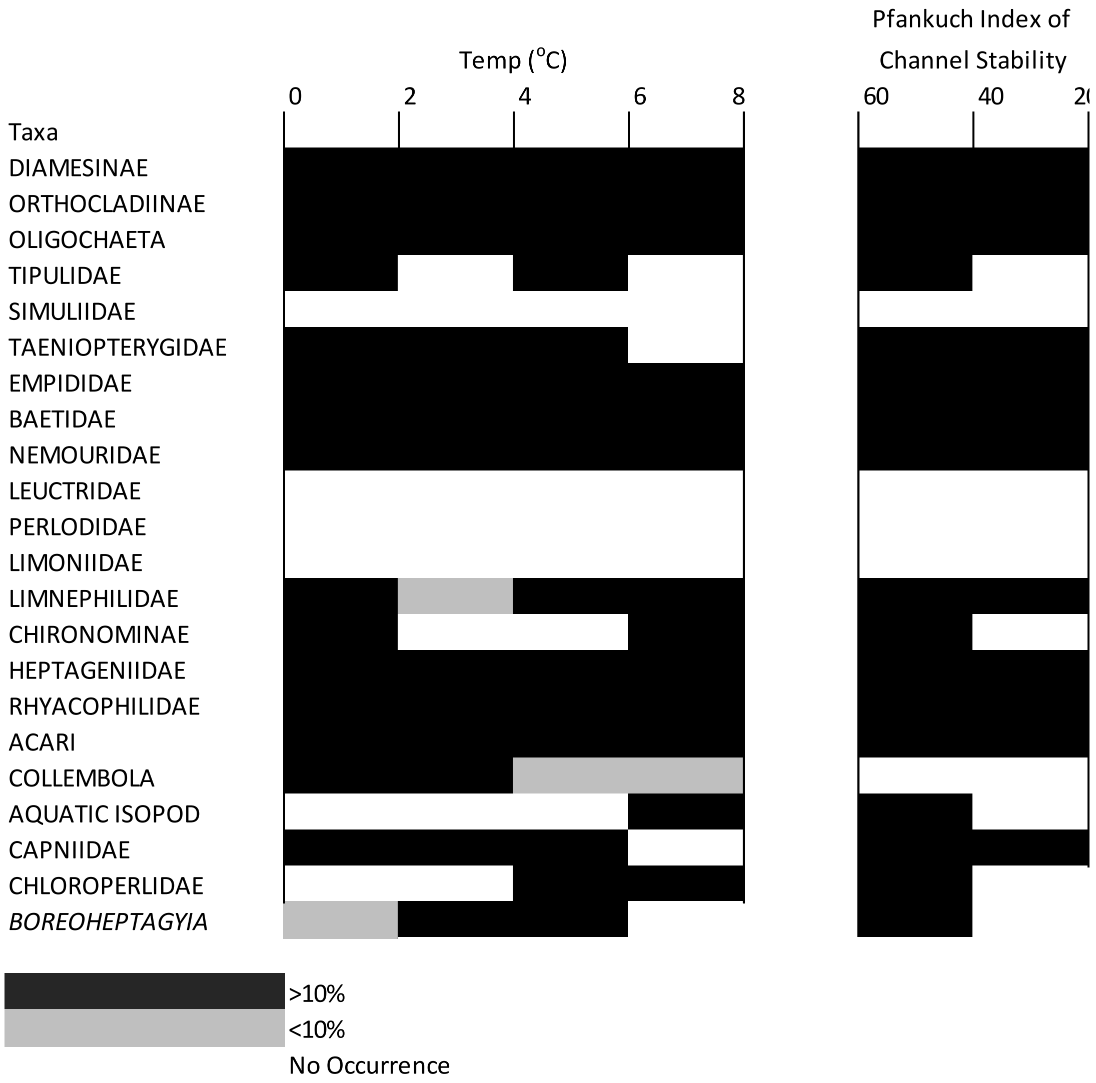
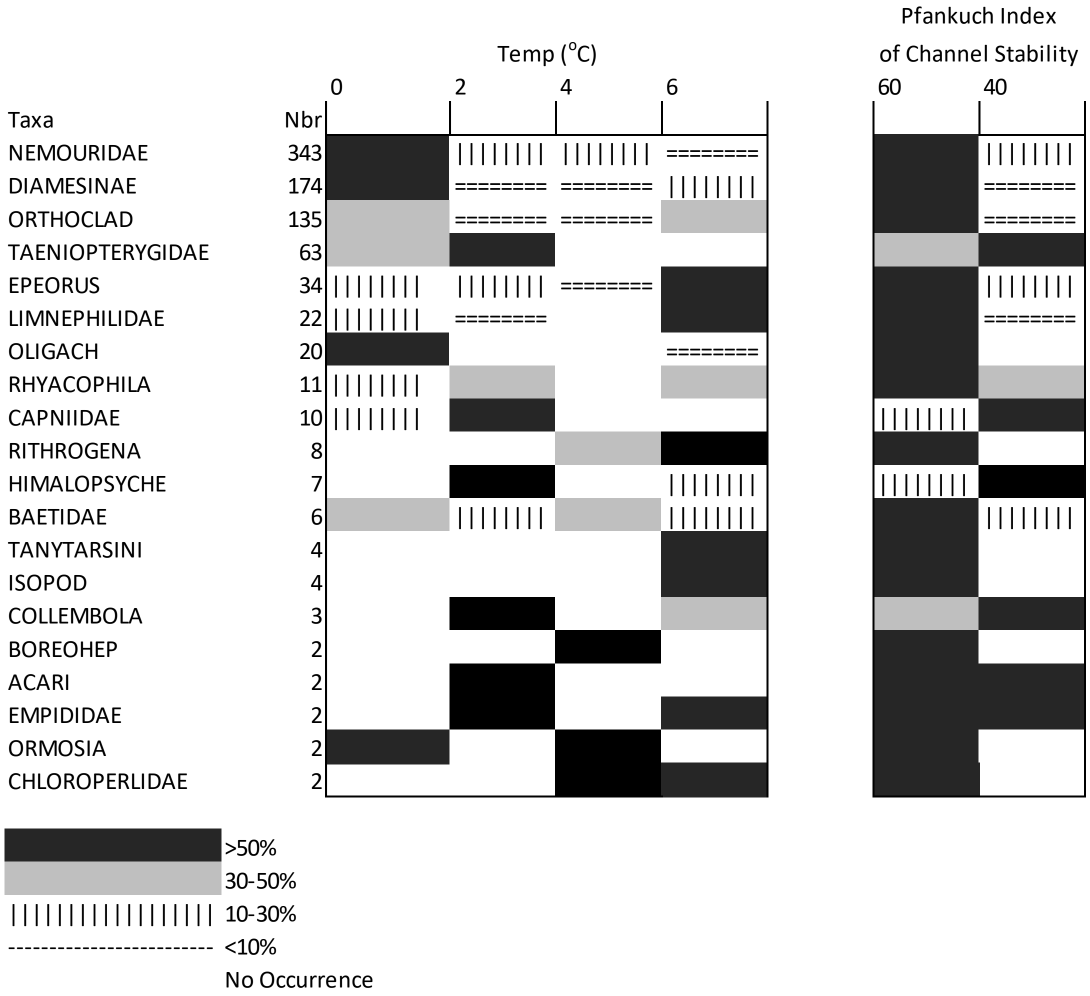


Figure 2.7. Percent of stream reaches with taxa present during summer melt season by maximum temperature (°C) and channel stability. Data is from fourteen stream sites in Mingyong, Sinong, and Hailuogou in Southeast Tibet. Black=>10% of sites have taxa present, grey = <10% of sites have taxa present, and white = no occurrence.



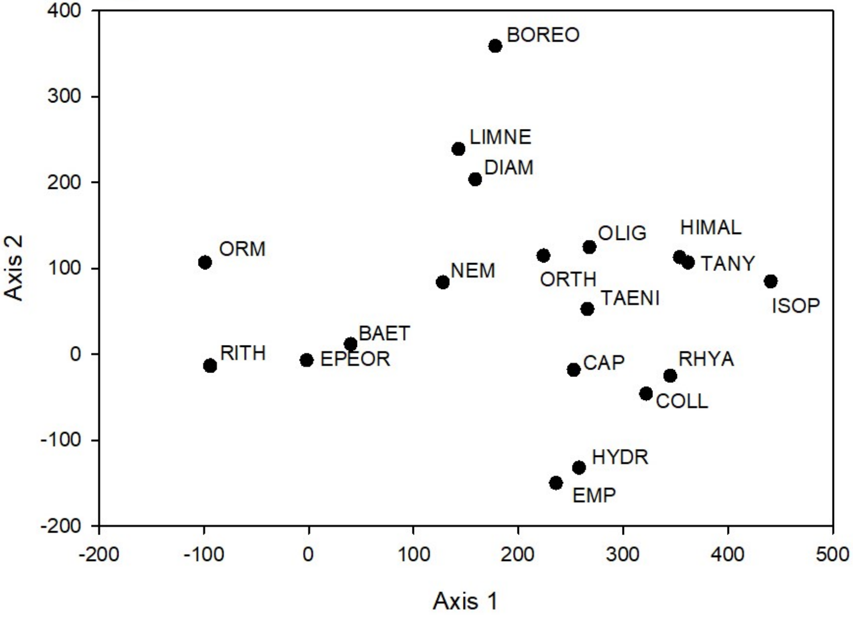
Taxa	Nbr	Temp (°C) 0	2	4	6	Pfankuch Index of Channel Stability 60	40
NEMOURIDAE	343	■	││││││││	││││││││	========	■	││││││││
DIAMESINAE	174	■	========	========	││││││││	■	========
ORTHOCLAD	135	▒	========	========	▒	■	========
TAENIOPTERYGIDAE	63	▒	■			▒	■
EPEORUS	34	││││││││	││││││││	========	■	■	││││││││
LIMNEPHILIDAE	22	││││││││	========		■	■	========
OLIGACH	20	■			========	■	
RHYACOPHILA	11	││││││││	▒		▒	■	▒
CAPNIIDAE	10	││││││││	■			││││││││	■
RITHROGENA	8			▒	■	■	
HIMALOPSYCHE	7		■		││││││││	││││││││	■
BAETIDAE	6	▒	││││││││	▒	││││││││	■	││││││││
TANYTARSINI	4				■	■	
ISOPOD	4				■	■	
COLLEMBOLA	3		■		▒	▒	■
BOREOHEP	2			■		■	
ACARI	2		■			■	■
EMPIDIDAE	2		■		■	■	■
ORMOSIA	2	■		■		■	
CHLOROPERLIDAE	2			■	■	■	

■ >50%
▒ 30-50%
│││││││││││││││││ 10-30%
------------------------- <10%
No Occurrence

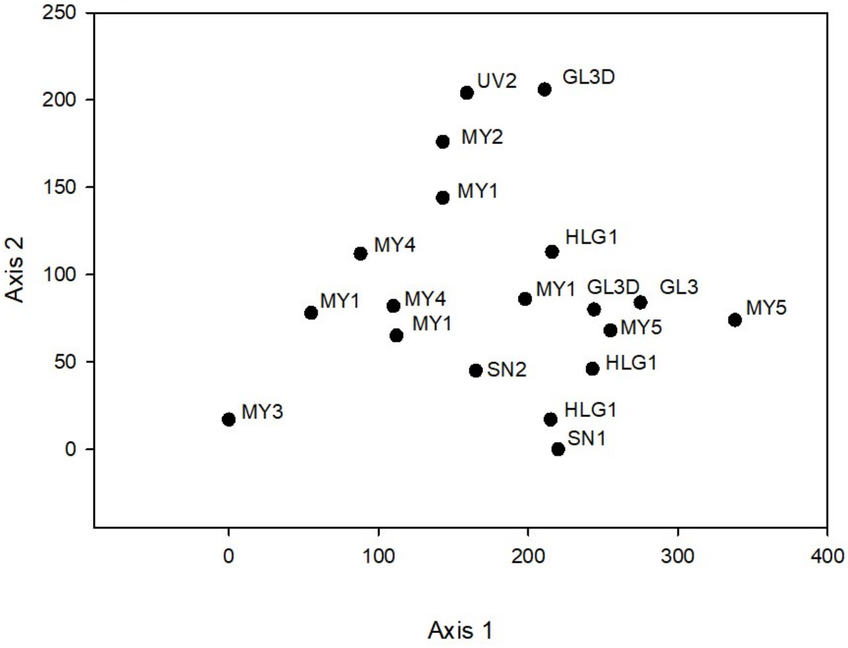
Figure 2.8. Taxa present in the Mingyong (13 km^2) and Sinong Glacier (10.01 km^2) streams by maximum temperature (°C) and channel stability. All data is from the summer melt seasons of 2009, 2011, 2013, and 2015 in Southeast Tibet. Black = > 50% of all occurrences in the temperature range, grey = <30-50% of all occurrences, vertical bars = 10-30% of all occurrences, vertical bars = <10% of occurrences, and white = no occurrences. The same color scheme and percentages apply to the Pfankuch channel stability ranges.

Figure 2.9. Detrended correspondence analysis (DCA) species and site scores. Species scores (a) and site scores (b). Data is comprised of summer season invertebrate communities from Mingyong, Yunnan; Sinong, Yunnan; and Hailuogou, Sichuan watersheds. Species codes are: BAET – Baetidae; BOR – *Borheoheptagyia*; CAP – Capniidae; CHLORO – Chloroperlidae; COLL – Collembola; DIAM – Diamesinae; EMP – Empididae; EPEOR – *Epeorus*, spp.; HIMAL – *Himalopsyche*, spp.; HYDR – Hydrachnidia; ISO – Isopod; LIMNE – Limnephilidae; NEM – Nemouridae; OLIG – Oligochaeta; ORM – *Ormosia*; ORTH – Orthocladiinae; RHYA – *Rhyacophila*, spp.; RITH – *Rithrogena*, spp.; TAEN – Taeniopterygidae; TANY – *Tanytarsini*. Site codes are as follows: GL3 – Hailuogou Glacier 3; GL3D – Hailuogou Glacier 3 downstream; HLG1 – Hailuogou Glacier 1; MY1 – Mingyong mainstem 1; MY2 – Mingyong mainstem 2; MY3 – Mingyong mainstem 3; MY4 – Mingyong mainstem 4; MY5 Mingyong mainstem 5; UV1 – Mingyong upper valley 1; UV2 – Mingyong upper valley 2; SN1 – Sinong Glacier 1; SN2 – Sinong Glacier downstream by Mekong River.

(a)


BOREO
LIMNE
DIAM
ORM
NEM
OLIG
ORTH
HIMAL
TANY
ISOP
TAENI
BAET
RITH
EPEOR
CAP
RHYA
COLL
HYDR
EMP
400
300
200
100
0
-100
-200
-200
-100
0
100
200
300
400
500
Axis 2
Axis 1

(b)


UV2
GL3D
MY2
MY1
MY4
HLG1
MY1
MY4
MY1
MY1
GL3D
GL3
MY5
MY5
SN2
HLG1
MY3
HLG1
SN1
250
200
150
100
50
0
0
100
200
300
400
Axis 2
Axis 1

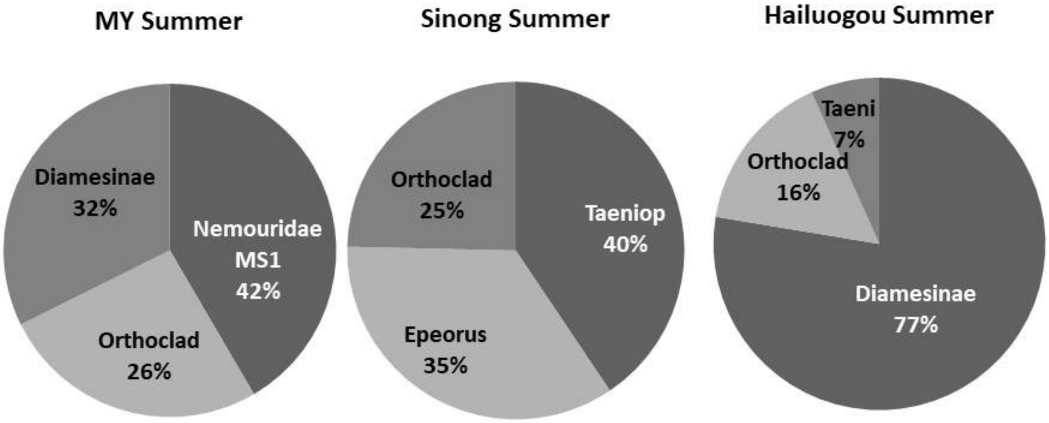


Figure 2.10. Top three abundant invertebrates by watershed during the summer glacier melt season (June-August). The percentage is calculated by dividing the total number of individuals in each taxon divided by the total number of invertebrates in each watershed during the summer. In the Mingyong Glacier stream, Nemouridae are the most abundant (42%), Diamesinae are second (32%) and Orthocladiinae are third (25%); in the Sinong Glacier stream Taeniopterygidae are most abundant (40%), Epeorus are second (35%), and Orthocladiinae are third (25%); and in Hailuogou Diamesinae are the most abundant (77%), Orthocladiinae are second (16%), and Taeniopterygidae are third (7%).

Chapter 3: Hydraulic Microhabitats in a Glacier Headwater of the Mekong River: Hidden Backwaters beneath the Torrents

(formatted for *Freshwater Biology*)

Abstract

1. Hydraulic characteristics and aquatic invertebrate communities were examined in 204 microhabitats (pool, riffle, run, step-run, rapid, and step-rapids) during three seasons (winter, spring, and summer) in the Mingyong Glacier stream on Mt. Kawagebo, Yunnan Province.

2. Measurements of water velocity (m/s), velocity range (high-low velocity), depth, Froude number, and velocity/depth ratio were used to identify hydraulic characteristics of glacier stream microhabitats.

3. The hypothesis that rapids and step-rapids would retain more particulate organic matter was supported. Overall, the abundance of invertebrates was greater in microhabitats with higher water velocities than pools and riffles, and was similar in runs, step-runs, rapids, and step-rapids.

4. Data suggests that non-riffle habitats such as runs and rapids may be important to examine for species richness and abundance in glacier streams.

5. Water velocity range (high-low velocity m/s) is a useful parameter in identifying turbulent microhabitats with backwater areas. This measure could indicate habitats that retain POM in subalpine glacier rivers with landslide and avalanche inputs, and perhaps in alpine glacier streams with aeolian-source POM.

6. Incorporating seasonal sampling strategies, even in difficult-to-sample glacier streams would aid in our understanding of these endangered cryosphere-driven stream systems.

Introduction

The hydrology and geomorphology of fluvial systems is shaped by regional atmospheric circulation patterns, climate, topography, land use, vegetation, and geology (Hynes 1970; Sklar and Dietrich 1998; Hart & Finelli 1999; Fetter 2001; Wohl 2007). In Southeast Tibet, deep river gorges are byproducts of mountain uplift and denudation processes, compounded by high unit stream power (Yang and Stall 1974; Korup & Montgomery 2008). Temperate monsoonal glaciers in Southeast Tibet have equilibrium line altitudes (i.e., snow accumulation equals ablation) with relatively high summer temperatures of 1-5^{o}C, and are located at low altitudes and latitudes, making them extremely sensitive to temperature changes (Li and Su, 1996; Shi et al. 1998; Baker & Moseley 2007). As a result, they are located in the zone of most pronounced glacier recession in Tibet (He et al. 2003; Yao et al. 2012). During the four-month summer monsoon season, greater than 6o% of river discharge and >85% of suspended river sediment is transported in Southeast Tibet (Henck et al. 2010). Due to the responsive nature of glaciers to increasing temperatures, it is possible to sample at newly-exposed glacier terminus sites on an annual basis, which creates an ideal condition to study impacts of hydraulic characteristics and climate change on aquatic invertebrate communities. Moreover, the extreme differences in hydrological conditions between the summer monsoon season and winter season allow for contrasting seasonal observations in glacier-melt streams, which are more similar to a groundwater stream in the winter and a torrential standpipe during peak melt (Gurnell & Fenn 1985; Milner & Petts 1994; Ward 1994, Death & Winterbourn 1994, Tockner et al. 1997; Füreder et al. 2001; Brown et al. 2003). Due to oscillating hydrologic conditions in glacier streams, different invertebrate species share the same ecological space by splitting colonization by season (Bretschko 1969; Füreder et al. 1998; Schütz et al. 2001).

Hydrologic patterns in streams are a complex amalgamation of vertical, lateral, and longitudinal movement, with near bed-flow patterns playing a major part in determining the distribution, abundance, and community composition of invertebrates (Hynes 1970; Statzner & Higler 1986; Hart & Jasentuliya 1996; Death 2008). Hydrologic patterns change in space and time depending on discharge, streambed roughness, and turbidity of the water (Statzner & Resh 1988). Invertebrates have evolved to survive in extreme velocity and turbulence in glacier-fed rivers, which have led to specialized adaptations and hydraulic-related traits (Poff 1997; Füreder 2007). For example, Deuterophlebiidae, also known as mountain midges, have streamlined bodies and eversible prolegs with hook-shaped claws to grip on to substrate in torrential water flow (Courtney 1994). In glacier streams with a long flood season, the hydrologic adaption that allows *Diamesa lindrothi* to live on loose moraine substrate is that of pupal case attachment in boulder crevices, which protects the insect from rolling substrate and dislodgement by high discharge (Milner & Petts 1994; Steffan 1971).

Hydraulic microhabitats are well-studied in temperate groundwater streams (e.g., Statzner & Higler 1986; Jowett 1993; Poff 1997), and the impacts of hydrologic conditions on fish is well-understood (Tennant 1976; Poff & Allan 1995; Lamouroux & Souchon 2002). The impacts of riffles on macroinvertebrate communities and individual taxon is well studied in temperate regions (e.g., Pridmore & Roper 1985; Brown &

Brussock 1991; Brooks et al. 2005), and the relationship between leaf litter and stream organisms is a mature area of study in stream ecology (e.g., Wallace et al. 1997; Angermeier & Karr 2011). Only a couple of known studies have examined allochtonous matter inputs into glacier-fed streams from the flood zones but not within the channel (e.g., Zah & Uelinger 2001; Zah et al. 2001). Beyond mentioning stream discharge as a descriptive element, most glacier stream studies hydraulic characteristics have not been explored in glacier-melt streams. This study is the first to my knowledge to examine ecological impacts of hydraulic characteristics in glacier-fed streams and the potential of hydraulic microhabitats to trap and retain particulate organic matter (POM), which in turn could increase the abundance of invertebrate taxa populations by providing a food source.

In microhabitats with high water velocity, large substrate (e.g., cobble and boulders) able to withstand the shear stress of water flow creates hydrologic refugia for insects (Lancaster & Hildrew 1993; Death & Winterbourn 1995). Sheltering in spaces behind substrate reduces the energy requirements of living in elevated water velocity (Adams et al. 1987). The importance of stable substrate is reflected by river reaches dominated by unstable substrate which contain low insect diversity (Cobb et al. 1992; Death & Winterbourn 1994). As well, aquatic insects respond to cues from changing hydrologic patterns to seek refuge either behind stable boulders, in flood zones, or in the hyporheic interstices (Gordon et al. 1992; Lancaster & Hildrew 1993; Death 2008). Although abrupt flood events can cause severe losses to invertebrate communities (Resh et al. 1988), certain insects are able to persist through long-term flood events (Townsend & Schofield 1987), and others are able to colonize rapidly after disturbance events

(Townsend & Hildrew 1976; Fisher 1983; Mackay 1992; Lake 2000). In swift-flowing currents, stable substrate serves an important ecological function for invertebrates during both base flow and elevated flow conditions.

Microhabitats with greater water velocity and substrate obstructers have elevated turbulence. Turbulence is defined as irregular movements due to eddies within a moving fluid (Tennekes & Lumley 1972). A proxy used to measure turbulence in this study was the range of water velocity around the mean velocity (Bouckaert & Davis 1998; Gordon et al. 1992). Chaotic flow caused by turbulence from substratum deflection presents a significantly different ecological habitat for insects than smooth, laminar flow (Boukaert & Davis 1998; Coleman 1986). The Froude number is the dimensionless ratio describing how gravity influences the motion of fluids (Statzner & Higler 1986; Statzner et al. 1988; Gore 1978; and Jowett 1993), and is used as a means to delineate laminar and turbulent flow. Froude numbers < 1 are defined as subcritical flows, a Froude number = 1 is when there is balance between gravity and inertia and is defined as critical flow, and supercritical flow is > 1. Subcritical flow is characterized by deep, slow flow, and supercritical flow is shallow, very fast flow that extends only a short distance downstream (Magirl et al. 2009).

$\text{Froude} = U/(g \cdot D)^{0.5}$

U – mean current velocity, g – gravity acceleration (9.8 m/s), D – mean water depth.

The Froude number has been used in conjunction with water depth and substrate size to separate hydraulic habitats, with a Froude number <0.20 delineating pools from riffles and a Froude number 0.40 delineating runs from races (Jowett 1993, Panfil & Jacobsen 1999). The Froude number is also used in this study to examine hydraulic characteristics of microhabitats.

Periphyton has growth patterns related to depth of the water, substrate type, UVB penetration, and hydrologic characteristics (Zah & Uelinger 2001; Rott et al. 2006). At high altitude in open-canopy reaches, streams potentially receive a greater UVB input (Körner 2007), but confounding factors of cloud cover, turbid water and unstable channels, and winter freezing may limit periphyton growth (Zah & Uelinger 2001; Uelinger et al. 2010; Rott et al. 2006). Certain epilithic algae such as *Lemanea* spp. can withstand hydraulic stresses like those found in glacier streams, and could be an important food source for invertebrates (Sheath & Hambrook 1988; Rott et al. 2006; Xie et al. 2004).

Aeolian-sourced organic matter may be an important source of allochtonous materials in upper reaches of alpine glacier-melt streams (Ward 1994; Zah & Uelinger 2001). Even in glacier catchments beneath the treeline, allochtonous input remains low until the end of the flood plain in glacier catchments (Zah & Uelinger 2001). In v-notch glacier valleys such as those found in Southeast Tibet, landslides, avalanches, and debris flows may deliver allochtonous carbon from higher elevation tree stands. However, even when mountain slopes deliver organic carbon via landslides and debris flows, these events frequently occur in the rainy season when flood events are likely to wash organic material downstream. Certain hydrologic characteristics have been shown to trap and retain leaf litter at different rates, with backwater areas exhibiting the greatest trapping efficiency in low, moderate, and high discharges (Hildrew et al. 1991; Snadden et al. 1992). As viewed from above the stream bed, turbulent habitats may look uninhabitable, but near-bed velocities tend to be low due to the no-slip condition at the fluid-solid boundary at the stream bed (Boukaert & Davis 1998). Leaf litter that is retained in backwaters of turbulent microhabitats, in turn may attract increased numbers of predators seeking prey (Lancaster & Hildrew 1993).

Sampling strategy examining communities of invertebrates in most glacier stream studies has been focused on riffle-run habitats or by taking kick samples at unspecified microhabitats in the reach (e.g., Castella et al. 2001; Burgherr & Ward 2001). Limiting sampling to one or two hydraulic microhabitats may enable standardization and comparisons among sites or categories of interest, but it may mask the true biological richness of streams composed of multiple microhabitat types (Minshall et al. 1985). Eliminating non-riffle habitats from sampling protocol may exclude certain insects such as column diving invertebrates and others residing in glacier stream pools (e.g., Oligochaetes and Dytiscidae), torrenticulous insect larvae (e.g., Deuterophlebiidae and Blepheraceridae), and rare taxa (e.g., Osmylidae).

In bedrock-controlled streams, rapids are omnipresent where coarse sediment has collected (Webb 1996 and 2004), and drops in bed elevation cause water velocity to increase to near critical levels (Magirl et al. 2009). Rapids control geomorphology by forcing the deposition of sand along the outer banks of the stream channel (Schmidt & Rubin 1995; Hazel et al. 2006), and deposit coarse sand bars downstream where they form further rapids (Webb et al. 1989). Rapids increase dissolved oxygen in streams by entraining air, which enhances biomass growth on sediments underneath rapids (Stevens et al. 1997). In higher elevation streams where the partial pressure of the atmosphere is lower, the presence of higher-velocity microhabitats may increase the dissolved oxygen level by entraining oxygen from the atmosphere. Rapids and step-rapids influence the formation and transformation of the stream bed, channel form, and biological conditions in mountain streams. My hypothesis was developed on the characteristics of rapids and step-rapids in steep-gradient glacier streams. If glacier streams are in v-shaped valleys, then it is hypothesized that landslides, avalanches, and debris flows will seed the glacier-fed stream with allochtonous carbon. Rapids and step-rapids with greater turbulence and large, stable, boulders, will then trap and retain greater particulate organic matter from landslides, and in turn attract a greater richness and abundance of invertebrates than slower velocity habitats of riffles and pools. In addition, it is thought that seasonal discharge will influence the composition of the invertebrate community and abundance of taxa by microhabitat.

Methods

Site Description

The subalpine glacier-fed streams where this study takes place in southeast Tibet are in high-relief mountain ranges. Many of the temperate glaciers in the region are larger than European temperate glaciers (Zhang & Bai 2016). In this study, the size of the Mingyong Glacier is 12 km^2 and in several studies testing the applicability of the Milner & Petts model with European glaciers were 0.186 km^2 (Conca Glacier, Italy), 0.2

km^2 (Taillon Glacier, France), and 0.6 km^2 (Mutt, Switzerland). The larger size of the glaciers in Southeast Tibet is most likely a result of the monsoonal climate dynamics, mass elevation effect (MEE) and higher prominence of the mountain ranges (e.g., 3,099 m a.s.l. in E. Alps versus 6,740 m a.s.l. in Meilixueshan). The glaciers in the region are summer accumulation types, have torrential summer discharge characteristics, and are receding rapidly with a glacier mass loss of 30±8 percent over the past 40 years (Ageta & Higuchi 1984; Aizen & Aizen 1994; Pan et al. 2012; Liu et al. 2015). Hydraulic microhabitat sampling took place in the Mingyong Glacier catchment, a temperate monsoonal glacier headwater of the Mekong River in Yunnan Province's Meilixueshan range (28°26′14″N 98°41′04″E). From measurements I have taken since 2009, the Mingyong Glacier has been receding on average, 50 m per year. Seven sites and 204 microhabitats (pool, riffle, run, step-run, rapid, step-rapid) were sampled in Mingyong during pre-melt (January-March), increasing discharge (April-May), and peak discharge (June-August) in 2015 (Figure 3.1). Study sites were 30-50m in length. In Mingyong, two sites flow over a bedrock outcrop 15 and 100 m from the glacier on the upper valley above the mainstem, and five mainstem sites at 90 m and 5,500 m from the Mingyong Glacier were sampled (Figure 3.2).

Six microhabitat types were first visually identified based on surface flow patterns and surface velocity, in order to quantify hydraulic and substrate characteristics within the microhabitats. Four of the microhabitats (pools, riffles, runs, and rapids) are defined in the literature (e.g., Hawkins et al. 1993; Statzner et al. 1988), and two have been used in this study (step-run and step-rapid) to further delineate greater water velocity microhabitats with a drop in channel/substrate gradient within the microhabitat, which occur in stream types with higher slope profiles (Rosgen 1994). Microhabitat types are as follows: 1) pools contain deep water with slow flow movement at the surface, and predominantly sand substrate, 2) riffles have shallow water depth, moderate water velocity, and small waves on the surface due to flow turbulence over gravel and cobble substrate; 3) runs exhibit water depths greater than riffles and swift flow with a laminar surface flow over gravel and cobble; 4) step-runs are similar to runs, but flow over groups of cobble having a slight drop in streambed elevation giving the appearance of a step; 5) rapids exhibit the greatest water velocity and white water turbulence over cobble and boulder substrate; and 6) step-rapids consist of white water flowing over large cobble/boulders with a drop in elevation giving the appearance of a step. Step runs and step rapids are different from cascades or plunge pools, as there is no free-falling water column similar to a waterfall, but rather tightly connected flow with the substrate as the streambed changes elevation (Figure 3.4).

Microhabitat Hydrologic Measurements

The Surber net was placed in the middle of each hydrologic microhabitat in order to sample macroinvertebrates. Water depth and velocity measurements were taken after invertebrate sampling was conducted at the same location where the Surber net was placed in the microhabitat. Water velocity was measured with an FP111 Flow Probe (Global Water, College Station, TX) by moving the velocity meter probe slowly up and down the stream column with the probe fan facing directly into the flow for a minimum of 45 seconds. The water velocity was recorded by the meter every second, with the running average being displayed. The minimum, average, and maximum water velocities were recorded after at least 45 seconds and three round-trip movements through the water column. The water velocity range (maximum velocity – minimum velocity), was used as an indicator of turbulence (Tennekes & Lumley 1972).

Slope was measured at the site level by using a Bushnell Golf Pinseeker 1500 Tournament Edition Laser Rangefinder (Overland Park, KS). Two operators stood mid-stream approximately 20 m apart. The downstream operator pointed the pinseeker laser at a point on the upstream operator that was the same height as the downstream operator's eyes. The distance and degree of the slope were recorded.

Substrate Size, Embeddedness, and Stability

Microhabitat substrate type (sand, gravel, cobble, boulder) were quantified by conducting Wolman 100 pebble counts at each of a selected 2-4 microhabitats at each site using a gravelometer in order to characterize general substrate types by hydraulic microhabitat (Wolman 1954; Ben Meadows Co., Janesville, WI). Substrate types consisted of: 1) sand: 0.062 – 2 mm; 2) gravel: 2 – 64 mm; 3) cobble: 64 – 256 mm; and 4) boulder: 256 – 2048 mm (Rosgen 1996). Substrate embeddedness was measured for three different hydraulic microhabitat types during each sampling event at a site. Ten substrate samples were randomly selected from a hydraulic microhabitat, removed from the bed if possible, and visually assessed for percent embeddedness by examining the sediment stain line on the substrate. If unable to move the substrate, embeddedness was estimated by feeling the entire boulder surface area and the bottom of the boulder where it was embedded into the substrate to estimate the percent of the boulder that was beneath the substrate. In order to control for experimental error, one person performed all embeddedness and substrate stability measurements. During high discharge in monsoonal temperate glacier streams, it is difficult to measure substrate stability and movement due to suspended glacier flour and zero visibility of substrate beneath the water surface. The FST hemispheres method (Statzner & Muller 1989) and mark and track method (Death and Winterbourn 1994) are not feasible to conduct during peak discharge, as only the largest boulders protruding from the water column are visible. Moreover, large boulders are typically located in the thalweg where water velocity and depth are too high to sample for invertebrate communities. A proxy used for substrate stability was whether or not the substrate was movable by human force. If the substrate could not be moved by a normal whole-body human pushing force (e.g., 225 Newtons (23 kgf)) then it was considered as stable. Again, in order to control for experimental error, one person performed all of the substrate stability evaluations.

Invertebrate Collection and Identification

At each of the sites ten microhabitats (combination of five riffles/runs, combination of three 2-3 rapids/step-rapids, one pool and an additional representative habitat of the site) were sampled with a Surber sampler ($0.09m^2$, mesh size 250 µm). Because the goal was to compare riffles with other microhabitats, an additional six riffles were sampled during the low melt season (January – February), bringing the total microhabitats sampled per stream site to sixteen during the winter. Collection of the additional six riffle samples was eliminated during spring and summer. During increased discharge in the summer, stream discharge overflows the channel banks and flow patterns such as riffles and runs appear in the flood plains while torrents dominate in the mainstem. Therefore, most of the summer microhabitat sampling took place along the edges of the thalweg in the flood zone.

Invertebrates were preserved in the field with 99 percent ethanol (Thermo Fisher Scientific, Hampton, NH). The microhabitat samples were then elutriated in a small container to remove sand, gravel, and cobble from invertebrates, POM, and filamentous algae. The larger POM was removed, air dried, and placed in a Ziploc bag for transport. The invertebrates, POM, and periphyton were placed in vials for transport.

Invertebrates were identified in the laboratory with a dissecting microscope (40 x magnification). Ephemeroptera, Plecoptera, and Tricoptera were identified to genus with Nemouridae identified to morphospecies due to limited taxonomic resolution in the region. Morphospecies identifications were based on Nemouridae gill structure and femur spine patterns (Baumann 1975). Diptera were identified to the family level except for Chironomidae which were identified to sub-family (Diamesinae and Orthocladiinae), tribe (Tanytarsini), or genus (*Boreoheptagyia* spp.) (Merritt and Cummins 1996; Morse & Yang 1994; Epler 2001). Chironomids were cleared, dehydrated, mounted in Euporal (Bioquip, Rancho Dominguez, CA) (Epler 2001), and identified with a compound microscope (40x - 400x magnification). Hirudinea (leeches), Oligochaetes, Collembola, and water mites (Acari) were identified at the subclass level and aquatic isopods were identified to the family level (Asellidae).

Carbon and Periphyton Collection

Particulate organic matter (POM), periphyton, and invertebrates were sorted from the vials transported from the field, and placed into separate vials for identification or further processing. Periphyton analysis was performed for the winter and summer seasons. The winter and summer periphyton and winter, spring, and summer POM samples were individually dried at 68°C for 48 hours, weighed, ashed in a muffle furnace at 450°C for 4 hours, and re-weighed to obtain loss-on-ignition weight.

Statistical analysis

Response variables were evaluated to determine if they met the assumptions of normal distribution with the Shapiro-Wilks test (Shapiro & Wilk 1965) and equal variance with the Levene test (Levene 1960). Twelve response variables did not meet assumptions of normality or equal variance and were either log(x+1) transformed (richness, abundance, dried periphyton, ash-free dry mass (AFDM), water depth, water velocity range, mean water velocity, Froude number, TOM dry weight, and TOM loss on ignition) or arcsine square root transformed (embeddedness and substrate stability) (Zar 1984). The level of significance for all statistical tests was $\alpha=0.05$.

Linear mixed effect models were used to address pseudoreplication as a result of repeatedly sampling selected sites. Linear mixed effect model analyses with fixed factors of microhabitat type and season, interaction effect of microhabitat and season, and random factor of site were conducted for biological response variables: 1) total taxa richness by microhabitat; 2) abundance of all invertebrates by microhabitats; and abundance for the following taxa: 3) Chironomids; 4) Diamesinae; 5) Orthocladiinae; 6) Tanytarsini; 7) *Boreoheptagyia, spp.*; 8) Ephemeroptera; 9) *Epeorus* spp.; 10) Baetidae; 11) Plecoptera; 12) Nemouridae; 13) Taeniopterygidae; 14) Capniidae; 15) Tricoptera; 16) Rhyacophilidae; 17) Diptera; 18) Deuterophlebiidae; 19) Simuliidae; and 20) Oligochaetes. Season was analyzed as a single effect variable on invertebrate abundance and as interaction effect variable with microhabitat. Invertebrate food sources were examined by conducting linear mixed effect models for the response variables: 1) dried total organic matter; 2) organic matter loss-on-ignition; 3) percent carbon; 4) dried periphyton; and 5) periphyton loss-on-ignition. Periphyton was analyzed for winter and summer seasons and TOM was measured for all three seasons.

The same linear mixed effect model with single factors microhabitat and season, and interaction effect between microhabitat and season, with random factor of site was used for microhabitat hydraulic characteristics, including: 1) water velocity; 2) velocity range; 3) depth; 4) Froude number; and 5) velocity/depth ratio. Linear mixed effect model analyses with fixed factor of microhabitat type and random factor of site were conducted for substrate types, embeddedness, and stability because substrate was not measured in each microhabitat and in all seasons. Response variables for substrate types by microhabitat included: 1) percent sand; 2) percent gravel; 3) percent cobble; and 4) percent boulders. Linear mixed effect models were conducted in R (Ri386 3.3.1(R Core

Team 2013)) using the lme function from the nlme package (Pinheiro et al. 2013). The lsmeans function from the lsmeans package was used to conduct Tukey tests (Lenth 2016).

Results

Hydrologic Characteristics of Microhabitats

Water velocity, range of water velocity, Froude number, and velocity/depth ratio differed among microhabitat types (Table 3.1). Water velocity was the lowest in pools and riffles, the greatest in step-rapids and rapids, and intermediate in runs and step runs. Water velocity range (i.e., turbulence) increased with rising velocity in microhabitats, with the greatest velocity range in step-rapids and rapids, intermediate ranges in runs and step-runs, and the lowest range in pools and riffles (Table 3.1). Froude number was greatest in runs, step-rapids, and rapids, the lowest in pools, and intermediate in riffles and step-runs. The velocity/depth ratio of pools was lower than all other microhabitats (Table 3.1).

The hydraulic characteristics that differed by season, but with no interaction effect between microhabitat and season were water velocity and water velocity range (Table 3.3). Water velocity was the lowest in the winter, and higher in the spring and summer (Table 3.4). Water velocity range was similar in winter and spring but greater in the summer (Table 3.4).

The hydraulic characteristic that exhibited an interaction effect with season and microhabitat was water depth (Figure 3.5). In the winter, pool depth was greater than riffles and similar to runs, step-runs, rapids, and step-rapids (Figure 3.5). As glacial meltwater increased in the spring, pools became deeper than runs and riffles, but were similar in depth to step-runs, rapids, and step-rapids (Figure 3.5). As glacial meltwater further increased in the summer, pools and rapids became similar in depth and were both deeper than runs and riffles (Figure 3.5). Step-rapids and step-runs were similar to pool depth in all seasons (Figure 3.5).

Substrate Size, Embeddedness, and Stability

Percent sand was the greatest in pools and lowest in rapids and step-rapids (Figure 3.6). Percent gravel was more evenly distributed among microhabitats, but was greater in riffle and run habitats than step-rapids (Figure 3.6). Percent cobble was the greatest in rapids and lowest in pools, riffles, and step-rapids, but was not significantly different from runs. Percent boulders were the greatest in step-rapids, the lowest in pools, riffles, and runs, and intermediate in rapids (Figure 3.6).

Substrate embeddedness was greatest in pools and rapids, lowest in riffles and runs, and intermediate in step-runs and step-rapids (Figure 3.7). Substrate stability was greatest in rapids and step-rapids, lowest in riffles and run, and intermediate in pools and step-runs (Figure 3.7).

Invertebrate Abundance in Microhabitats

Invertebrate abundance differed by microhabitat (Figure 3.8). The greatest invertebrate abundance occurred in runs, and the lowest in pools and riffles (Figure 3.8).

Intermediate invertebrate abundance occurred in step-runs, rapids, and step-rapids (Figure 3.8). Taxa richness did not differ by microhabitat (Figure 3.8). Ephemeropteran abundance differed by microhabitat (p=0.0296), but the post hoc test did not detect differences in abundance by microhabitat (Figure 3.8). *Epeorus* spp. mayflies were found in greatest abundance in riffles and runs, with intermediate abundance levels in step-runs, rapids, and step-rapids, and lowest abundance in pools (Figure 3.8). Plecopteran abundance was greatest in riffles and runs and lowest in pools, and intermediate in all other microhabitats (Figure 3.8). Nemouridae abundance was the greatest in runs, rapids, and step-rapids, and was the lowest in pools (Figure 3.8). Riffles and step-runs contained intermediate abundance of Nemouridae. Diptera, Chironomidae, Tricoptera, *Himalopsyche* spp., Capniidae, Diamesinae, Orthocladiinae, Taeniopterygidae, and *Boreoheptagyia* spp., did not differ in abundance by microhabitat (Table 3.5).

Fourteen of 20 invertebrate response variables differed seasonally (Table 3.4). Season had a strong effect on the abundance of most of the invertebrates analyzed in this study, with most invertebrates occurring in greatest abundance during the winter season (Table 3.4). Taxa richness, and abundance of Ephemeroptera, *Epeorus*, spp. (Ephemeropteran), Plecoptera, and Capniidae (Plecoptera) were greatest in winter and lowest in spring and summer (Table 3.4). Taeniopterygidae, Trichoptera, Rhyacophilidae, Diptera, Chironomidae, and Orthocladiinae abundance was greatest in winter, lowest in spring, and intermediate in summer (Table 3.1). *Tanytarsini* abundance increased in spring and was lowest in winter and summer (Table 3.4). Diamesinae abundance was greatest in winter and summer and lowest in spring (Table 3.4). Nemouridae and *Boreoheptagyia* abundance did not differ seasonally (Table 3.4 and Table 3.3).

Interaction effects between season and microhabitat were significant for the abundance of three taxa: 1) Simuliidae; 2) Deuterophlebiidae; and 3) Oligochaeta. Simuliidae (Diptera), which are only present in winter and spring in the mainstem, are most abundant in the step-rapid and run habitats during the winter (Figure 3.9). Intermediate levels of Simuliidae during the winter were found in riffles, step-runs, and rapids (Figure 3.9). During the spring and summer seasons, only a few Simuliidae were collected in the riffle habitat (Figure 3.9). Deuterophlebiidae were also winter colonizers in the Mingyong Glacier mainstem, with greatest abundance found in step rapids and runs, intermediate abundance in riffles, step-runs and rapids, and lowest abundance in pools. There were no Deuterophlibiidae larvae present in the streams during spring and summer at the sites where collection took place (Figure 3.9). Oligochaeta were detected as having greater abundance in rapids during the winter, with intermediate abundance in pools, runs, and step-runs, and lowest abundance in riffles (Figure 3.9). No differences were detected in the abundance of Oligochaetes in spring and summer (Figure 3.9).

Periphyton and Particulate Organic Matter

Particulate organic matter (POM) dry weight was greatest in rapids, lowest in riffles, and intermediate in other microhabitats (Figure 3.10). POM loss-on-ignition weight was greatest in rapids, lowest in pools and riffles, and intermediate in runs, step-runs, and step-rapids (Figure 3.10). Seasonally, POM dry weight was greatest in the summer, lowest in the winter, and intermediate in the spring (Table 3.4). POM loss-on-ignition was greatest in the spring, intermediate in the summer, and lowest in the winter (Table 3.4).

Periphyton from the Surber samples consisted mostly of *Lemanea*, a tubular algae that is resistant to high discharge velocities up to 4.5 m/s (Sheath & Hambrook 1988; Rott et al. 2006; Xie et al. 2004). Periphyton dry weight was different between winter and summer (Table 3.4) but not by microhabitat. There was also no interaction effect of periphyton dry weight and season (Table 3.3). However, Periphyton loss-on-ignition had an interaction effect between microhabitat and season, with differences by microhabitat occurring during the summer season where it was greatest in rapids, lowest in riffles, and intermediate in all other microhabitats (Figure 3.10).

Discussion

The hypothesis that rapids and step-rapids would retain more particulate organic matter was supported (Figure 3.10). Overall, the abundance of invertebrates was greater in runs which had a higher mean water velocity than pools and riffles, and was similar in runs, step-runs, rapids, and step-rapids (Figure 3.8). Taxa richness between microhabitats did not differ (Figure 3.8). The unexpected finding was that runs contained greater invertebrate abundance than in riffles and pools (Figure 3.8). This could be due to the high numbers of Capniidae individuals, which were present mainly in the winter in high numbers. Due to the high number of Capniidae stoneflies, stoneflies dominated the year-round abundance of taxa. This is clearly seen by comparing the mean ± SE of the top four invertebrates by abundance in all microhabitats: 1) Capniidae (9.97 ± 1.69); 2) Nemouridae morphospecies 1 (3.92 ± 0.59); 3) Orthocladiinae (3.72 ± 0.69); and 4) Diamesinae (2.50 ± 0.40).

Again, runs had a mean velocity that was higher than pools and riffles (Table 3.1), which supports the hypothesis that higher velocity microhabitats contain greater invertebrate abundance. However, my classification of runs as having a higher water velocity is different from Jowett (1993). Jowett (1993) examined hydrologic characteristics of pools, riffles, and runs in a braided gravel-bed river in the foothills of the New Zealand Southern Alps. Jowett (1993) described runs as having an intermediate velocity between pools and riffles. The mean ± SD of the runs in the Jowett (1993) study were 0.56 ± 0.27 (m/s) and riffles had a very close velocity to runs at 0.62 ± 0.34 m/s. In my study, the riffles had a mean velocity ± SD of 0.30 ± 0.19 m/s and runs were 0.66 ± 0.23 m/s. Although Jowett (1993) did not report the bankfull width, it is assumed that the stream is larger than the Mingyong mainstem, as the slope is low in that study (0.0032 – 0.0066), and the discharge was between 1.9-3.9 m^3/s. Mosley (1982) described runs as being the “average” current velocity of a particular stream, so if this is the case, then indeed the runs in my study are intermediate in velocity between riffles and rapids/step-rapids (Table 3.1). However, most studies tend to classify riffles and runs as overlapping continuums that are more different from pools than between themselves, so oftentimes even differences between invertebrate communities are indistinguishable between the two microhabitats (Pridmore & Roper 1985; Jowett 1993).

The data also supports the presence of greater turbulence in habitats with greater water velocity, as represented by the velocity range values (Table 3.1). Step-rapids and rapids have a higher velocity range than pools, riffles, and runs (Table 3.1). Moreover, the stability of the substrate in rapids and step-rapids was greater than in riffles and runs (Figure 3.7), which may be providing refugia for insects in the wake regions of cobble and boulders. One would expect the embeddedness of rapids and step-rapids to be higher due to the more stable substrate (Figure 3.7). However, the embeddedness of rapids and step-rapids was no different from riffles and runs (Figure 3.7). Therefore, it is likely that cobble and boulders in rapids and step-rapids are impacted together and provide interstitial spaces that trap and retain organic matter and provide stability for invertebrates in high flow velocities.

Jowett (1993) describes velocity/depth ratio as the measure that best distinguished between pools, riffles, and runs (Jowett 1993). In comparing values of pools, riffles, and runs of my study with (Jowett 1993), the pool velocity/depth ratio in my study was the only microhabitat that was similar to pools in (Jowett 1993). The runs and riffle values differ between our studies, which once again could be due to potential visual classification differences, the geomorphology of the channels (e.g., water slope), the riverscape position of the reaches (e.g., erosional or depositional stream zone), size of the rivers, or because riffles and runs are not discreet units and encompass a wide continuum, and thus are at least partially subjective in selection. The velocity/depth ratio in runs and riffles were 2.60±1.74 and 4.69±3.98 in Jowett (1993) and 8.13±0.72 and 5.42±0.41 in my study. The hydraulic characteristic that I found to be the most different between microhabitats was velocity (Table 3.1). So the question is what causes the results to differ? Water surface slope determines velocity and water depth (Henderson 1966), and although I measured site-level stream bed slope (Table 3.2) which was higher than the Jowett (1993) study, water surface slope was not measured at the microhabitat level. However, by comparing my stream site gradient data with Jowett's water slope measurements, it appears that the stream segments are in different Rosgen classifications, as Jowett (1993) water slope measurements are very low (Rosgen 1994). Stream location (e.g., headwater erosional stream location versus floodplain depositional location), seasons sampled, size of the watersheds, and the origin of the river (glacier versus surface water) could be elements driving differences between our results. It may be possible that a run habitat following a lowland definition is very rare in glacier stream headwaters due to the steep gradient of the channel. What I identified as runs may be an intermediate habitat between riffles and rapids. Moreover, all of the collection sites in this glacier study were predominantly classified in the Rosgen (1994) type A channel forms with steep cascading water patterns, but without the deep entrenchment as described by Rosgen (1994). This could be due to active glacier recession and incised V-shape valley mechanics, including avalanches, landslides, and bedform processes that continually aggrade and modify the channel bed.

POM and periphyton presence in microhabitats with greater water velocity, range of velocity, and Froude numbers supports the hypothesis that turbulent microhabitats may contain backwaters that retain POM (e.g., Magirl et al. 2009). Moreover, the periphyton LOI was greatest in rapids, and lowest in riffles. Complex flow patterns around larger substrate include shallow supercritical flow over the tops of large boulders in rapids and step-rapids, which would allow sunlight to penetrate and periphyton to grow (Gordon et al. 1992). In particular, rapids and step-rapids contain greatest percentage of boulders (Figure 3.6) which have large surface areas for periphyton growth. Oligochaetes, which tend to colonize lentic habitats and habitats with detritus and organic matter (e.g., Verdonschot 2001) were found in greater numbers in rapids during the winter (Figure 3.9). This supports the hypothesis that backwaters are present in rapids and step-rapids and enable lentic taxa such as Oligochaetes to colonize in the wake behind boulders in high-velocity microhabitats.

The fact that runs and rapids retain greater POM mass than riffles is an initial finding that suggests higher water velocity microhabitats in glacier streams may play an important role in invertebrate distribution by food source. Moreover, the periphyton LOI was greatest in rapids, and lowest in riffles, which shows that the quality of autochthonous growth may be higher in microhabitats with greater entrained oxygen due to turbulence. The streams in this study originate in the subalpine zone, which is different than the glacier streams studies originating in the alpine zone (e.g., Füreder et al. 2001; Lods-Crozet et al. 2001; Miaolini & Lencioni 2001; Snook & Milner 2001; Winterbourn et al. 2008; Hamerlik & Jacobsen 2012; Murakami et al. 2012). Since many of the monsoonal temperate glacier streams in this study originate in the sub-alpine zone, contributions from allochtonous matter may dominate the carbon source, even without direct canopy coverage (Table 3.2). Moreover, being located in v-shaped valleys places the Mingyong Glacier mainstem in the direct line of avalanches, landslides, and debris flow paths, which could be seeding microhabitats with pulses of POM. The examination of greater velocity microhabitats in alpine regions would allow a comparison with sub-alpine stream microhabitats. It is possible that alpine runs, rapids, and step-rapids are retaining wind-blown organic materials and attracting invertebrates.

Plecoptera had the highest year-round abundance levels of all taxa. The greater abundance of Nemouridae in runs and rapids is in line with the food that they typically consume as shredders and detritivores (Merritt & Cummins 1996) and size of substrate (cobble and boulders) that they colonize (Franken et al. 2008). In an experiment examining the effect of substrate size on *Nemoura,* spp. growth, Franken et al. (2008) found that *Nemoura*, spp. stonefly growth was impacted by substrate size and water velocity. Reduced growth occurred when *Nemoura*, spp. were placed in a flume chamber with sand substrate devoid of larger substrate refugia. The importance of substrate refuge was underlined by this experiment, as larger substrates provide interstitial refuge for stoneflies, reducing energy budgets required of living in greater water velocities (Adams et al 1987). Although my hypothesis focused on presence of boulders in greater velocity microhabitats, cobble is also found in runs and rapids in greater abundance than lower velocity habitats, which may be providing interstitial space and refuge for Nemouridae nymphs.

The greatest number of *Epeorus* spp. mayflies was collected in riffles and runs, but with intermediate abundances in step runs, rapids, and step-rapids, which comes as no surprise considering their dorso-ventrally flattened body and ventral gills that are adaptations to greater water velocities (Dodds & Hisaw 1925) (Figure 3.8). It is known that *Epeorus* spp. mayflies are rheophilic insects found on larger substrates where they graze on periphyton (Merrit & Cummins 2008; Winterbourn 1990). *Epeorus* spp. were found in greatest abundance during the winter season, which could relate to the increased clarity of the water due to reduced glacier sediment release. During the spring season there is a dramatic drop in *Epeorus* spp. abundance. This is the season that suspended glacier sediments increase, which may be a cue to *Epeorus spp.* and other mayflies with delicate structures (e.g., gills) to emerge from the stream as adults to avoid physical damage from sediment abrasion. This would especially be pertinent to rheophilic taxa living in hydraulic microhabitats with greater velocities where glacier flour abrasion impact would be more likely due to greater water velocity. Yet another reason could be that in riffles and runs, the substrate is less stable (Figure 3.7), and as the glacial meltwater increases in the beginning of the spring, these microhabitats may become less habitable as a result of rolling cobble.

Diamesinae are found in greater abundances in the summer than winter and spring (Table 3.4), which is expected due to affinity of Diamesinae to low water temperatures (Milner & Petts 1994; Ward 1994; Füreder et al. 2001; Lods-Crozet et al. 2001; Robinson et al. 2001; Milner et al. 2001; Bouchard et al. 2006). Due to greater glacial meltwater during the summer, the average water temperature during the summer is lower than the winter (Chapter 2). The mean water temperature ± SD in the Mingyong glacier stream in the winter (December 1, 2013 – February 28, 2014) at 2,500 m from the glacier was 4.26 ± 1.12^{o}C and the summer water temperature (June 1, 2014 – August 31, 2014) was $2.84 \pm 0.40\,^{o}$C. Nemouridae morphospecies 1, which are present year round in similar abundance by season suggests that they are semi-voltine, requiring greater than one year to mature. This also suggests that they are adapted to low temperature water and summer water hydraulics, as they are found in the metakryal and hypokryal zones in the greatest abundances during all seasons.

The role of substrate stability in microhabitats becomes increasingly important as water velocities increase, as the likelihood of substrate movement increases with greater velocities. Step-rapids and rapids had significantly greater stability (lower % movable substrate) than riffles and runs (Figure 3.7). This may make the greater-velocity microhabitats ideal colonization spaces as the glacial meltwater volume increases during the summer, with refuge at the wake region of boulders. At reduced water velocities in riffles and runs (table 3.1), loose substrate would allow for invertebrates to live on all sides of the gravel/cobble without the threat of tumbling gravel and cobble as might be found in rapids and step-rapids. However, in riffles with a lower percent of cobble and boulders, a large proportion of POM may move through the microhabitat without being trapped and retained for consumption purposes (Figure 3.10).

Seasonal water velocity is significantly greater in spring/summer than in winter (Table 3.4). As glacial meltwater increases, flood zones are inundated and create microhabitats that can be sampled outside of the main channel. By sampling microhabitats in the flood zone during the summer, the hydraulic characteristics are similar to spring samples taken from the mainstem. Thalweg velocities were periodically tested at peak discharge and a few measurements at the surface of hydraulic jumps resulted in null readings because they were greater than the probe was able to register (6.1 m/s).

There was significantly more total organic matter and loss on ignition in runs/rapids than in riffles (Figure 3.10). Although all three of these microhabitats are erosional in nature, the water depth of the rapids during all seasons is significantly greater than riffles (Figure 3.5). This may be playing a role in the formation of backwater areas and eddies in the wake behind the boulders (Gordon et al. 1992) that trap POM. Shear stress, which is force per unit area parallel to an object, is proportional to flow depth and slope of a stream (Gordon et al. 1992). The overall streambed gradient of the Mingyong stream is moderately high with low incision, which may be reducing the overall depth of the channel, in turn reducing tractive force on boulders (Cobb et al. 1992). This could be allowing the presence of stable boulders in greater velocity microhabitats because the reduced channel depth is reducing tractive force on boulders as the banks overflow to the flood zones.

Conclusion

Physical hydraulic characteristics, substrate size and stability, and retention of particulate organic matter in runs and rapids supported the hypothesis that microhabitats in glacier streams with greater water velocity and range of water velocity may be amenable to conditions that trap and retain organic material. The combination of substrate stability, partial embeddedness, and impaction of multiple boulders may be creating ideal traps for organic material and facilitating colonization conditions for invertebrates in high velocity microhabitats. Moreover, the range of water velocity, which I used as an indicator of turbulence, may be important in identifying backflow areas and dead zones around larger substrate. Dead zones in the wake of boulders may be creating conditions so that certain taxa such as Oligochaetes and Nemouridae are able to thrive during the extended glacier meltwater flood period in the summer.

Invertebrate abundance in Meilixueshan streams is driven by stonefly populations, even in the metakryal zone where year round temperatures remain below 2.0^{o}C. Diamesinae populations have greater abundance during the summer melt season when overall stream water temperatures are reduced (Figure 3.7). Epeorus mayflies are found in greater numbers in higher velocity erosional microhabitats. Moreover, the dynamics of POM in glacier streams in microhabitats with higher velocity ranges may be attracting invertebrates to food sources in backwater refuge at the wake of rapid and step-rapid substrates. It would be helpful to know if glacier streams originating above the treeline may be trapping aeolian carbon in rapid and step-rapid microhabitats. Sampling by hydraulic microhabitat rather than by combining microhabitats into site samples, would deepen the understanding of glacier stream hydraulic characteristics and organic matter dynamics on the distribution of invertebrates within glacier stream channels. Although not a novel concept in temperate groundwater streams, the fact that carbon dynamics vary by hydraulic microhabitat in glacier streams where carbon is limited may suggest that it is important to consider organic carbon at the microhabitat level. It is suggested that future studies consider examining higher velocity microhabitats and measuring these habitats for velocity range. Velocity range may be an indication of the presence of backwaters, which are traps for organic material that attract shredders and grazers such as stoneflies and lentic organisms such as Oligochaetes.

Tables

Table 3.1. Mean ± SD of hydraulic characteristics in Mingyong Glacier stream microhabitats for all seasons. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using Tukey's post-hoc tests.

Measurement	Pool (n=22)	Riffle (n=72)	Run (n=53)	Step-Run (n=12)	Step-Rapid (n=29)	Rapid (n=27)	p-value
Velocity (m/s)	0.08 ± 0.03^{a}	0.30 ± 0.02^{a}	0.66 ± 0.02^{b}	0.66 ± 0.07^{bc}	0.91 ± 0.06^{c}	1.01 ± 0.05^{c}	<0.0001
Vel. Range	0.17 ± 0.04^{a}	0.37 ± 0.02^{a}	0.76 ± 0.04^{b}	0.97 ± 0.12^{bc}	1.22 ± 0.08^{c}	1.36 ± 0.10^{c}	<0.0001
Depth (m)	0.16 ± 0.01^{c}	0.07 ± 0.00^{a}	0.10 ± 0.00^{b}	0.10 ± 0.01^{abc}	0.12 ± 0.01^{bc}	0.16 ± 0.01^{c}	<0.0001
Froude Nbr	0.08 ± 0.03^{a}	0.39 ± 0.03^{b}	0.73 ± 0.04^{c}	0.73 ± 0.09^{bc}	0.90 ± 0.09^{c}	0.89 ± 0.08^{c}	<0.0001
Vel./Dep. Rat.	0.69 ± 0.27^{a}	5.42 ± 0.41^{b}	8.13 ± 0.72^{b}	8.14 ± 0.98^{b}	9.59 ± 1.68^{b}	8.83 ± 1.53^{b}	<0.0001

Table 3.2. Stream characteristics (mean±SD) in Mingyong from Jan-August 2015.

Site	Dist. beneath treeline (m)	Dist from glacier (m)	Mean Temp (^{o}C)	Channel Stability	Channel width (m)	Slope (%)	Canopy Coverage	Mtn Canopy Coverage	Mtn Scree Slope Cov.
UV1	-1439	15	1.47 ± 0.49	60 ± 0	1.9 ± 0.4	30	0.00	0.09	0.00
UV2	-1486	100	1.98 ± 0.16	59 ± 1	0.9 ± 0.5	20	0.00	0.14	0.00
M1	-1647	95	1.46 ± 0.11	50 ± 15	8.6 ± 3.2	4	0.00	0.11	0.00
M2	-1737	800	1.93 ± 0.18	57	8.9 ± 3.7	6	0.00	0.18	0.00
M3	-1,914	2,500	3.39 ± 0.47	60	9.1 ± 2.6	8	0.22	0.005	0.00
M4	-1,929	3,000	4.3 ± 0.14	50 ± 1	10.3 ± 6.9	4.1	0.00	0.01	0.00
M5	-2,201	5,500	4.35 ± 0.78	60 ± 1	20.6 ± 6.5	5	0.00	0.02	0.29

Table 3.3. P-values of biological and hydraulic response variables in the Mingyong Glacier mainstem in winter, spring, and summer of 2015. *P*-values ≤ 0.05 indicate a significant effect of microhabitat or season, or a significant microhabitat by season interaction effect.

Table of p-values

Response Variable	**Microhabitat**	**Season**	**MH and Season Interaction**
Richness	p = 0.1968	p < 0.0001	p = 0.4041
Abundance	p = 0.0209	p < 0.0001	p = 0.2745
Ephemeroptera	p = 0.0296	p < 0.0001	p = 0.6126
Epeorus, spp.	p = 0.0065	p < 0.0001	p = 0.6709
Plecoptera	p = 0.0037	p < 0.0001	p = 0.3869
Capniidae	p = 0.0696	p < 0.0001	p = 0.6941
Nemouridae	p = 0.0005	p = 0.1387	p = 0.9414
Taeniopterygidae	p = 0.1091	p < 0.0001	p = 0.5296
Tricoptera	p = 0.8801	p = 0.0003	p = 0.9864
Himalopsyche, spp.	p = 0.7319	p = 0.0008	p = 0.9167
Diptera	p = 0.2214	p < 0.0001	p = 02712
Chironomid Abundance	p = 0.2522	p < 0.0001	p = 0.2766
Diamesinae	p = 0.5808	p < 0.0001	p = 0.0529
Orthocladiinae	p = 0.3391	p = 0.0002	p = 0.3765
Tanytarsini	p = 0.0138	p = 0.0333	p = 0.0111
Boreoheptagyia, spp.	p = 0.5698	p = 0.7825	p = 0.4084
Simuliidae	p = 0.0057	p = 0.0331	p = 0.0042
Deuterophlibiidae	p = 0.0057	p = 0.0331	p = 0.0042
Oligochaetes	p = 0.0057	p = 0.0331	p = 0.0042
Hydraulic Characteristics			
Velocity (m/s)	p < 0.0001	p = 0.0342	p = 0.4748
Vel. Range	p < 0.0001	p = 0.0060	p = 0.1389
Depth (m)	p < 0.0001	p = 0.3132	p = 0.0486
Froude Nbr	p < 0.0001	p = 0.2100	p = 0.7699
Vel./Dep. Rat.	p = 0.0001	p = 0.1303	p = 0.8906

Table 3.4. Mean ± SE of invertebrate abundance, hydraulic characteristics, periphyton, and particulate organic matter that had significant single factor effect by season. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using Tukey's post-hoc tests.

Seasonal Table

	Winter	Spring	Summer	p-value
Richness	5.00 ± 0.31^{b}	2.30 ± 0.29^{a}	3.15 ± 0.41^{a}	<0.0001
Abundance	46.58 ± 5.14^{b}	6.35 ± 1.03^{a}	24.90 ± 6.38^{a}	<0.0001
Ephemeroptera	4.22 ± 0.69^{b}	0.44 ± 0.10^{a}	0.20 ± 0.20^{a}	<0.0001
Epeorus spp.	3.91 ± 0.66^{b}	0.27 ± 0.08^{a}	0.20 ± 0.20^{a}	<0.0001
Plecoptera	27.21 ± 4.07^{b}	4.29 ± 0.80^{a}	10.65 ± 3.91^{a}	<0.0001
Capniidae	18.93 ± 2.96^{b}	0.08 ± 0.05^{a}	0.05 ± 0.05^{a}	<0.0001
Taeniopterygidae	2.72 ± 0.74^{b}	0.15 ± 0.07^{a}	1.45 ± 0.54^{ab}	<0.0001
Nemouridae	5.54 ± 0.96^{a}	4.05 ± 0.77^{a}	9.15 ± 2.86^{a}	0.1840
Tricoptera	0.44 ± 0.09^{b}	0.04 ± 0.02^{a}	0.25 ± 0.12^{ab}	0.0003
Rhyacophilidae	0.27 ± 0.07^{b}	0.013 ± 0.013^{a}	0.00 ± 0.00^{ab}	0.0008
Diptera	10.75 ± 1.64^{b}	1.38 ± 0.31^{a}	12.70 ± 3.88^{ab}	<0.0001
Chironomidae	8.66 ± 1.64^{b}	1.26 ± 0.31^{a}	12.65 ± 3.86^{ab}	<0.0001
Diamesinae	3.21 ± 0.57^{b}	0.39 ± 0.15^{a}	7.00 ± 2.31^{b}	<0.0001
Orthocladiinae	5.46 ± 1.23^{b}	0.81 ± 0.23^{a}	5.65 ± 1.94^{ab}	0.0002
Tanytarsini	0.009 ± 0.009^{a}	0.08 ± 0.04^{b}	0.00 ± 0.00^{a}	0.033
Hydraulic Characteristics				
Water Velocity (m/s)	0.45 ± 0.04^{a}	0.62 ± 0.04^{b}	0.74 ± 0.08^{ab}	0.034
Water Velocity Range	0.68 ± 0.05^{a}	0.67 ± 0.05^{a}	1.07 ± 0.13^{b}	0.006
Organic Matter				
Periphyton Dry Weight (g)	0.015 ± 0.009^{a}	-	0.07 ± 0.02^{a}	0.046
Particulate Organic Matter Dry Weight (g)	0.45 ± 0.09^{a}	1.16 0.30^{b}	1.29 ± 0.28^{ab}	0.005
Particulate Organic Matter LOI (g)	0.31 ± 0.07^{a}	1.02 ± 0.26^{b}	0.84 ± 0.24^{ab}	0.003

Table 3.5. Invertebrates with no difference in abundance (mean ± SE) by microhabitat.

Taxa	**Microhabitat p-value**	**Mean abundance ± SE**
Chironomids	p = 0.2522	6.26 ± 0.99
Diamesinae	p = 0.5858	2.51 ± 0.40
Boreoheptagyia, spp.	p = 0.5698	0.01 ± 0.01
Orthocladiinae	p = 0.3391	3.72 ± 0.69
Tricoptera	p = 0.8801	0.27 ± 0.05
Himalopsyche	p = 0.7319	0.04 ± 0.02
Taeniopterygidae	p = 0.1091	1.63 ± 0.40
Capniidae	p = 0.0696	9.97 ± 1.69

Figures

Figure 3.1. Study site in Southeastern Tibet's Three Parallel Rivers region on Mt. Kawagebo (Meilixueshan), Yunnan Province. (Credit: Buckley 2009).

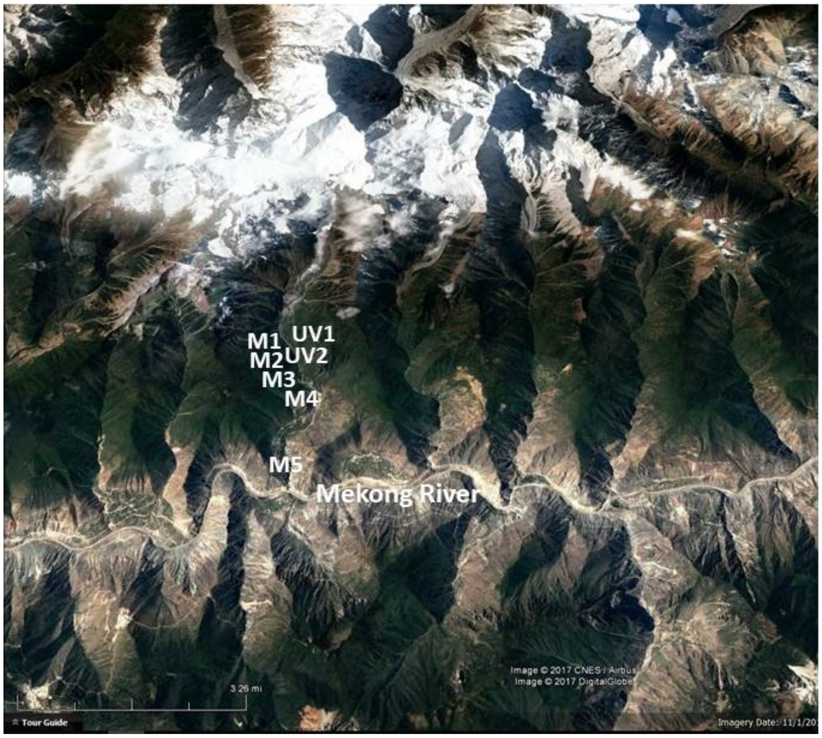


Figure 3.2. Longitudinal sampling sites in the Mingyong Glacier stream. Mt. Kawagebo, Meilixueshan, Yunnan Province. The Mingyong headwater is a source of the Mekong River in the Three Parallel River region, China (Source: Google Earth).

Figure 3.3. Visual representations of hydraulic microhabitats. In the glacier streams, the habitats include: 1) pool, 2) riffle, 3) run, 4) step-run, 5) rapid, and 6) step-rapid. Water velocity (m/s), depth (m), water velocity range (high velocity – low velocity m/s), Froude number, and the velocity/depth ratio were measured and calculated for all microhabitats (n=204).

Figure 3.4. Typical increased discharge observed in summer. This picture is taken of the mainstem site of Mingyong Glacier stream 800 m from the glacier.

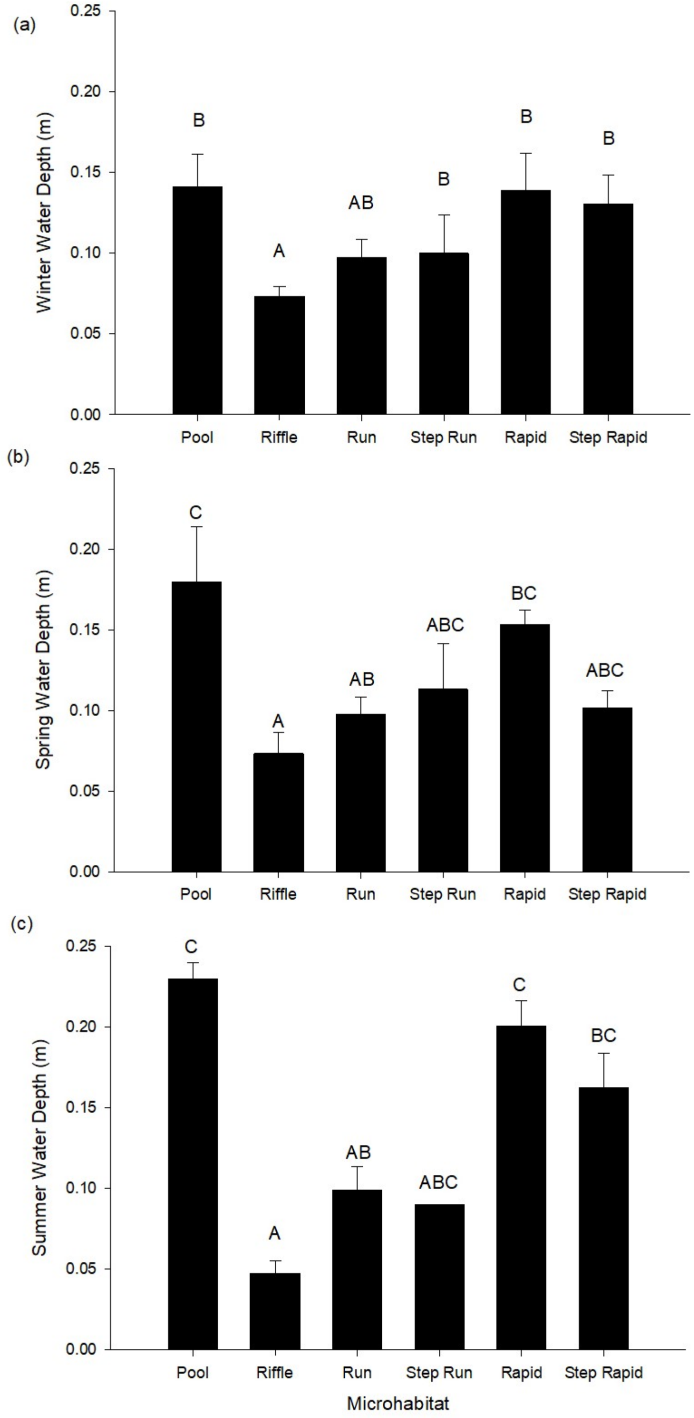


Figure 3.5. Mean ± SE microhabitat depth by season. Winter (a), spring (b), and summer (c). Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using a Tukey's post-hoc test.

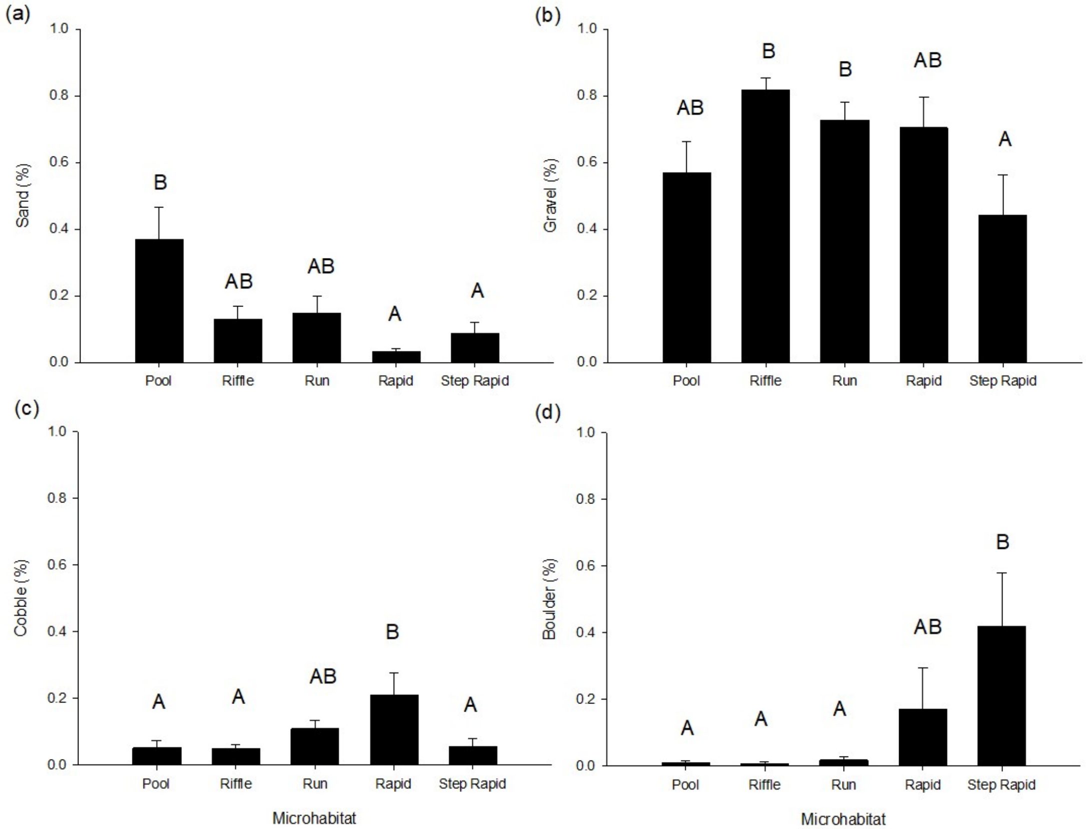


Figure 3.6. Mean substrate size ± SE by microhabitat. Data is from the Mingyong Glacier stream sites during winter, spring, and summer 2010-2011 and 2015. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using a Tukey's post-hoc test.

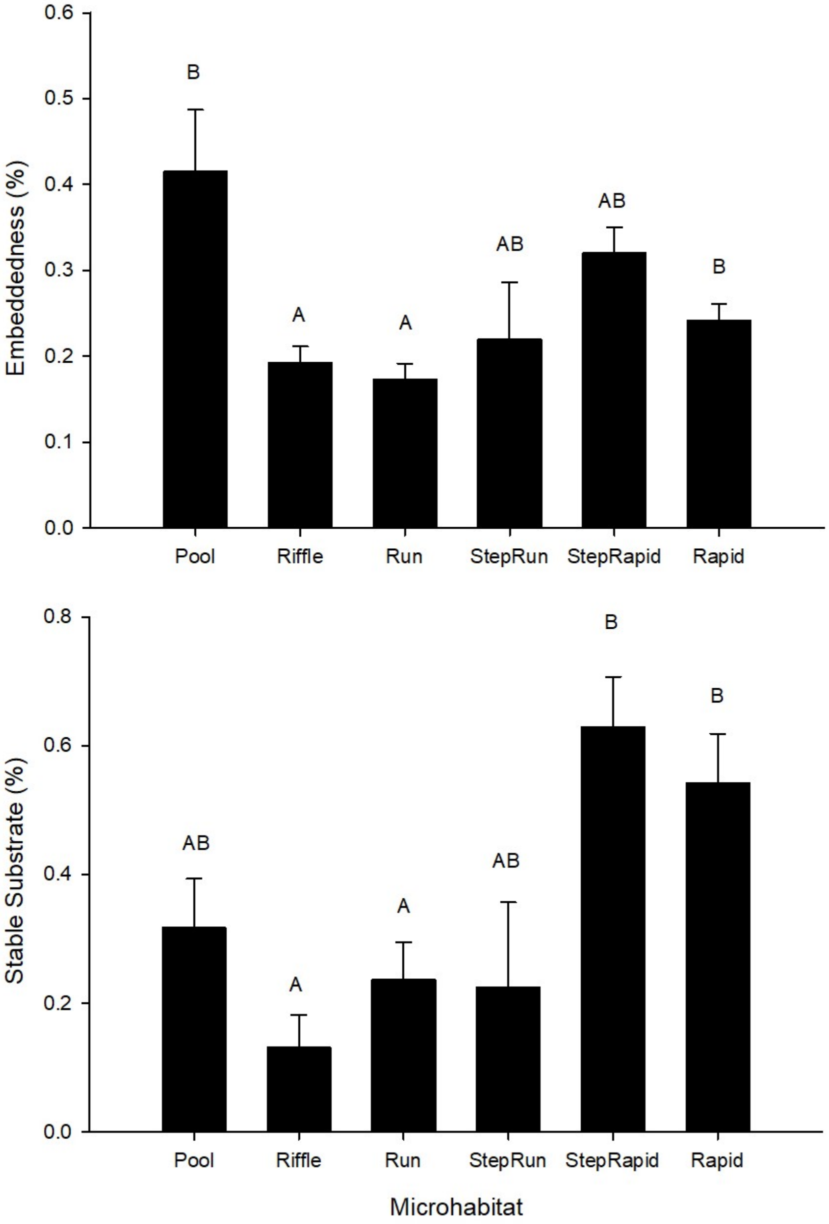


Figure 3.7. Mingyong mean substrate embeddedness ± SE and mean substrate stability ± SE by microhabitat. Data from winter, spring, and summer 2010-2011 and 2015. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using a Tukey's post-hoc test.

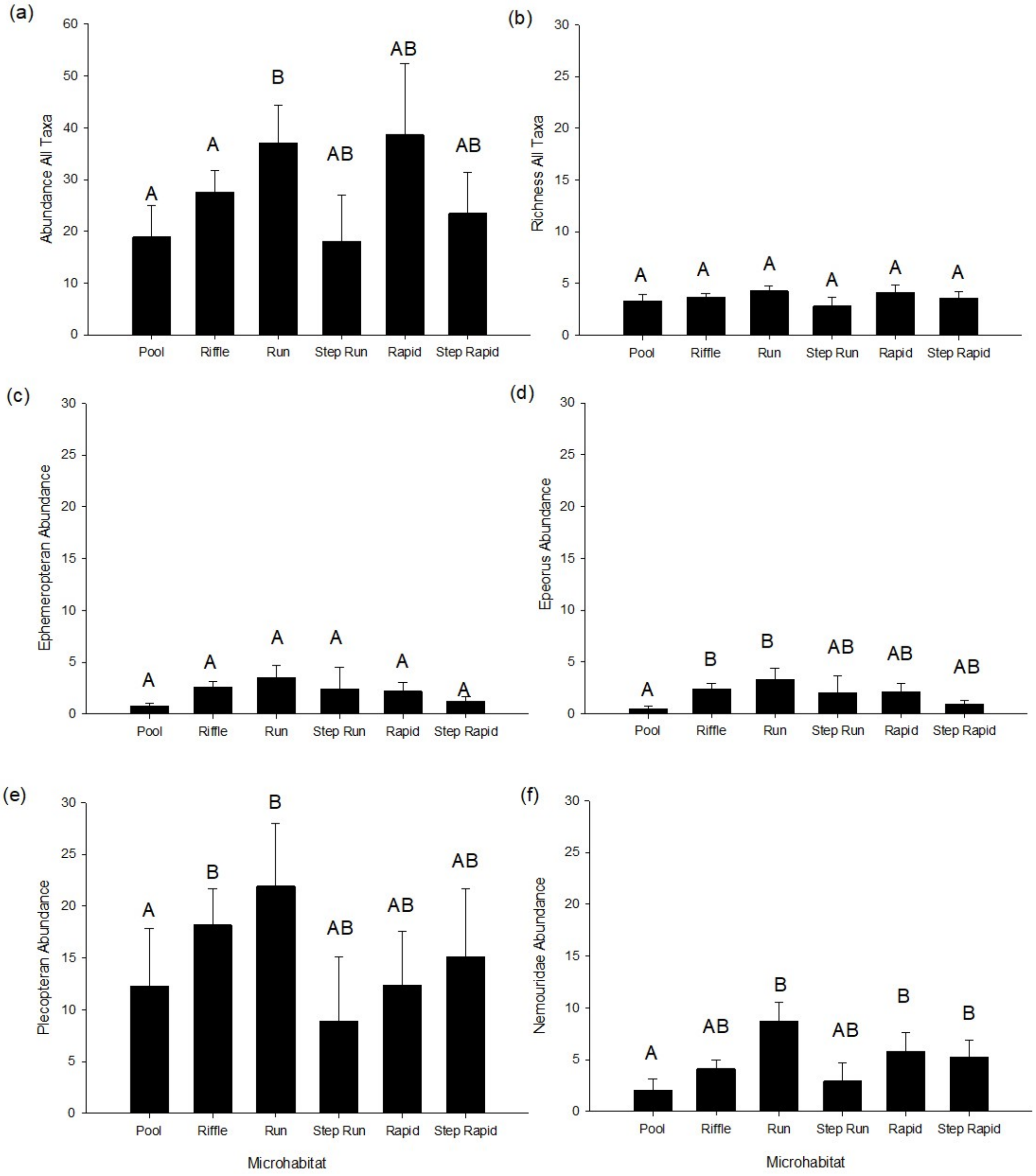


Figure 3.8. Mean overall taxa abundance and taxa richness ± SE (a and b), Ephemeropteran abundance (c), Epeorus abundance (d), Plecopteran abundance (e), and Nemouridae abundance (f) in the Mingyong Glacier stream. Data from winter, spring, and summer 2015. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using a Tukey's post-hoc test.

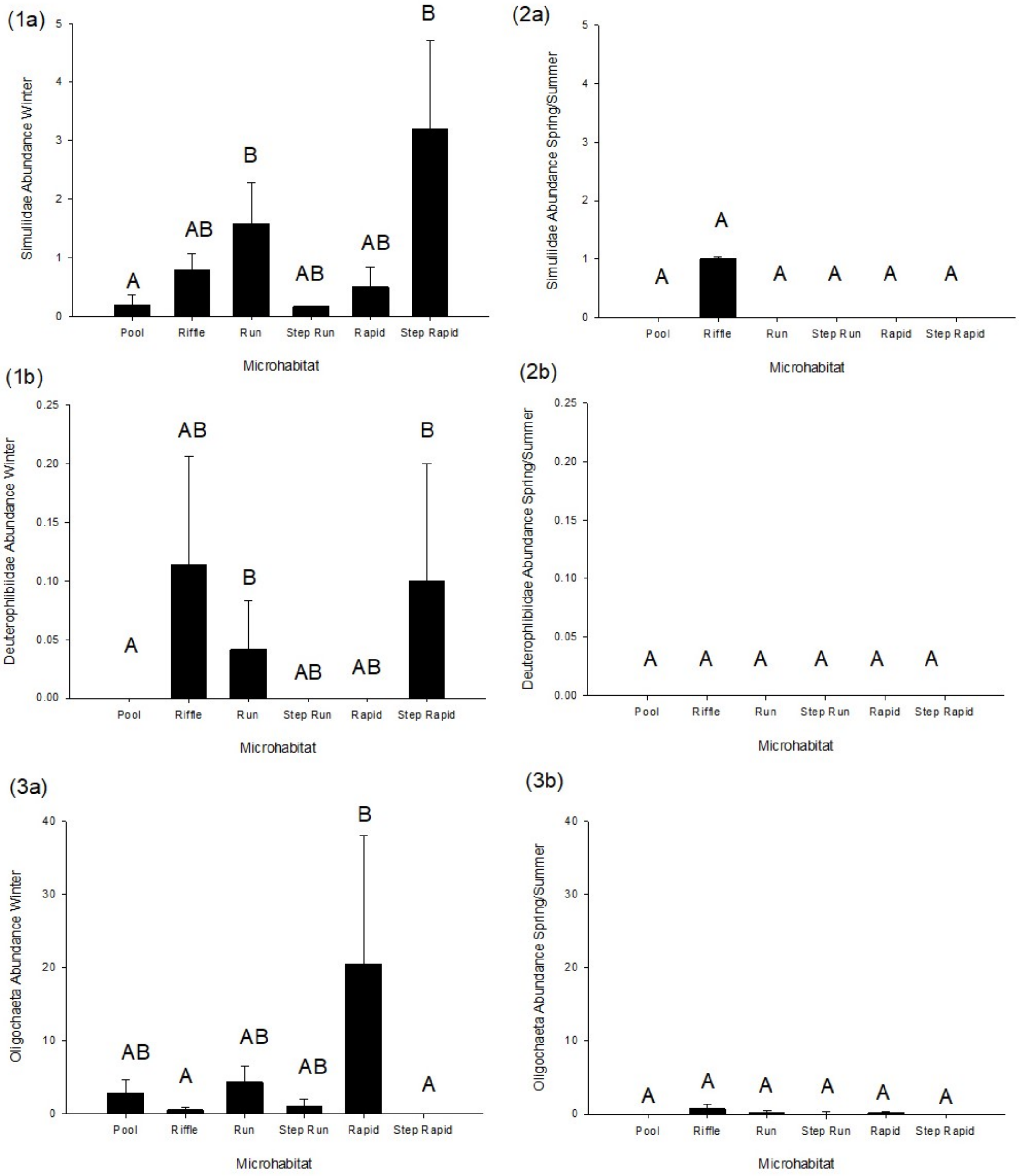


Figure 3.9. Mean abundance ± SE of Simuliidae (1a and 2a), Deuterophlebiidae (1b and 2b), and Oligochaeta (3a and 3b). Data is from the Mingyong Glacier stream sites in winter (a figures) and mean ±SE of spring/summer (b figures) of 2015. Deuterophlebiidae were not present during the spring and summer. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using a Tukey's post-hoc test.

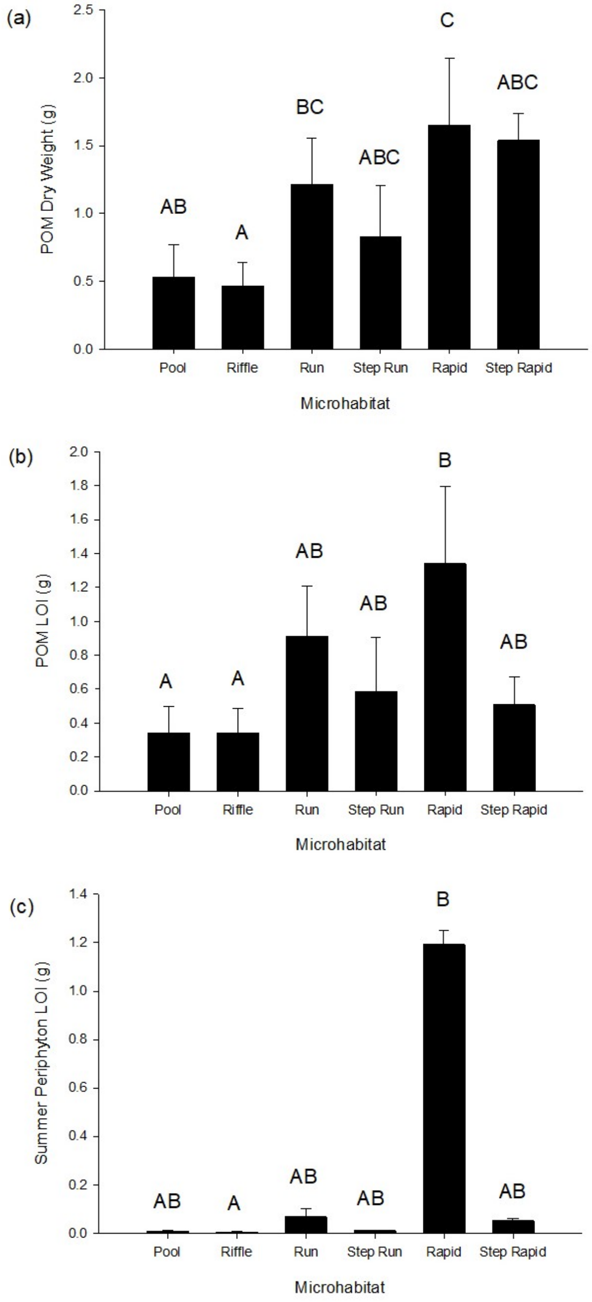


Figure 3.10. (a) Mean Particulate organic matter (POM) dry weight (g) ± SE during all seasons, (b) POM loss-on-ignition (LOI) (g) ± SE during all seasons, and (c) Periphyton LOI (g) ± SE by microhabitat during the summer. Data from the Mingyong Glacier stream sites in 2015. Periphyton LOI differences were detected in the summer season between microhabitats, whereas POM data is from winter, spring, and summer. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using a Tukey's post-hoc test.

Chapter 4: Glacial meltwater stonefly response to water salinity

(formatted for *Freshwater Biology*)

Abstract

1. Salinity tolerance of glacial meltwater stoneflies was examined to partially answer the research question “will glacial meltwater insects be able to colonize different water sources with higher salinities once the glaciers are gone?”
2. Aquatic insects from metakryal (T_{max}<2°C), hypokryal (2–4°C), and rithral (4–6°C) glacier-fed stream sites, and groundwater streams were collected during peak glacier discharge in August 2015 at Meilixueshan, Yunnan, and Mt. Gongga, Sichuan and exposed to a range of salinities.
3. The median lethal concentration (120-h LC_{50}), and 95% confidence intervals for metakryal Nemouridae 3249(1677-6296) and hypokryal Taeniopterygidae 5676(1982-16249) were similar to Chloroperlidae 9359(2765-31677) at 7 km from a glacier.
4. Survival duration for Chloroperlidae was longer than for Taeniopterygidae and Nemouridae in higher salinities (0.95% NaCl and 1.2% NaCl).
5. Morphological changes to cell area (μm^2), and density (μm^2) of Chloroperlidae chloride cells, and cell area, cell density, and cell count of Chloroperlidae caviform cells changed in an inverse manner to the distilled control as predicted. Cell count and cell density of Taeniopterygidae caviform cells changed in an inverse manner to the high salinity treatment in the predicted direction.
6. Future field tests examining physiological responses of metakryal insects to environmental factors, including stream chemistry, water temperature, and hydraulic characteristics approximating groundwater streams will enable better

predictions regarding the fate of glacial meltwater insects to disappearing glaciers.

Introduction

The Tibetan plateau is experiencing temperature warming at double the 100-year global rate of 1.0°C (Pu et al. 2008; Yao et al. 2007). It is projected that the plateau's 1995 glacier coverage of 500,000 km^2 will dissipate to one-fifth of this area by 2035 (Cruz et al. 2007; Ye & Yao 2008). Due to the combination of low latitude and altitude, the monsoonal temperate glaciers on the southeastern edge of the plateau are experiencing the greatest glacier retreat in the region (He et al. 2003; Yao et al. 2012). In the Yangtze River basin (1,722,193 km^2), the discharge of upper reach glaciers is predicted to increase by 28.5% resulting in a decrease of glacier coverage of 11.6% by 2050 (Su et al. 2000; Wang et al. 2005; Zhang et al. 2015). Glaciers in the Tibetan region have switched from a mixture of advancing and retreating during the 1990s, to that of mostly retreat (Yao 2012; Pu et al. 2008). Long-term concerns of temperature warming in the region include loss of water resources from the Third Pole glacier "ice bank account" (Thompson 2008), and the long-term viability of river biota adapted to physico-chemical conditions of glacial meltwater.

Nowhere are the ecological impacts of increasing global temperatures more evident than at the forefront of receding glaciers. A small number of insects are physiologically adapted to low temperatures and hydrological extremes in close proximity to glacier ice (Brundin 1966; Danks 1971; Denlinger & Lee 2010). Often restricted to the upper reaches of glacier and snowmelt streams, metakryal ($T_{max}<2°C$) insects exhibit a longitudinal trend of increasing abundance with greater distance from ice headwaters (Ward 1994; Milner & Petts 1994; Milner et al. 2001). Metakryal insects, along with the glaciers, are progressively retreating to higher and more remote metakryal islands that are reducing the possibility of long-term population viability (Finn et al. 2013; Brown et al. 2007; Milner et al. 2009; Jacobsen & Dangles 2012). Many large valley glaciers have retreated to such an extent that they have split, forming separate tributary streams at higher elevations (Yao et al. 2007). The worldwide loss of glaciers is so severe that in Glacier National Park, United States, the snow and glacial meltwater stoneflies *Lednia tumana* and *Zapada glacier* have been proposed as endangered species under the U.S. Endangered Species Act (Muhlfeld et al. 2011; Giersch et al. 2016). The loss of glacial meltwater habitats and extirpation of endemic metakryal insects is a potential reality under the current climate change scenario. The distribution of insects within streams in the Tibetan region is not fully understood, so it is possible that in the future multiple insects may fall into the same endangered category as *L. tumana* and *Z. glacier*.

There are two areas of research that could help predict what might happen to metakryal insects in the Tibetan region when the glaciers disappear. The first is illuminating the wider distribution of metakryal species in glacier streams. Beyond a few aquatic community studies in Tibet (Hamerlik & Jacobsen 2012; Murakami et al. 2012; Jiang et al. 2013; Laursen et al. 2015), the knowledge of metakryal insect distribution in the region is little known. Knowledge of metakryal insect distribution would elucidate basic requirements of insects to particular stream characteristics such as water temperature, flow characteristics (e.g., permanency, water velocity), pH, DO (mg/L) and dissolved ions (Berezina 2003; Zinchenko & Golovatyuk 2013; Griffith 2014). The second area of research is to understand physiological responses of insects to groundwater and snowmelt stream conditions. Insects will only have groundwater and snow-melt streams to colonize once the ice is gone, so understanding their ability to survive in groundwater conditions could help in developing climate change risk assessments (Wooton 1988; Kefford et al. 2007; Clements 2012; Poteat et al. 2012; Clements & Kotalik 2016).

The adaptations of insects living in metakryal zones ($T_{max}<2^oC$) is largely based upon glacier ice, a key modifier of water temperature, discharge volume, sediment load, and total dissolved ions (i.e., salinity as measured by electrical conductivity (EC)). Water flow and sediment resuspension originate from subglacial, englacial, and supraglacial ice melt, groundwater, and surface water runoff that oscillate seasonally and diurnally (Gorham 1960, Gurnell 1985; Mark & Seltzer 2003; Brown et al. 2006b). Ice itself is ion-depleted due to exclusion of solutes during the freezing process, but the concentration of ions in melt water depends on the origin of the water, the passage taken through the glacier, and the amount of time in contact with glacier moraine (Lemmins 1978; Gurnell 1985; Fountain 1996; Liu & Liu 2010). EC variations have been found to be the greatest close to the glacier terminus and at tributary confluences, and stabilize further downstream where tributary waters are well mixed (Gurnell 1985). In conjunction with ice, the geology and geomorphology of each catchment determines variability in physicochemical conditions (Collins 1979; Anderson et al. 1997; Fetter 2000; Gooseff et al. 2002). Non-polar temperate glaciers have beds above freezing which can result in enhanced glacier movement during the summer melt season. Bedrock grinding disrupts the bedrock mineral lattice and enhances dissolution of glacier sediments which could increase ions in meltwater (Stallard 1995; Anderson et al. 1997),

Aquatic insects have developed unique physiological adaptations for respiration, locomotion, and osmoregulation in lotic systems (Lancaster 2008). Osmoregulation is a physiological trait determining the distribution of aquatic insects in streams based on the total ion concentration of water (Wichard et al. 1973; Komnick 1977; Wooton 1988; Kefford et al. 2007; Clements & Kotalik 2016; Griffith 2017). Hyper-regulating freshwater insects sustain hemolymph ion concentration at a much higher level than the surrounding water and are adept at adjusting to freshwater of low ionic concentrations (Griffith 2017). But little is understood of the ability of hyper-regulating (i.e., living in low ionic concentration water) insects moving to moderate or high conductivity waters of different natural mineral content (Griffith 2012; Clements 2012 & 2016). Low salinity sites in glacier streams have been found to be strongly associated with reduced invertebrate taxa richness and certain traits allowing for survival in low temperatures (e.g., fast life cycles, freeze tolerance) (Milner & Petts 1994; Poff 2006). Temperature and conductivity have been found to be important variables ordinating glacier stream insect communities in upstream-downstream gradients (Danks 1971; Gislason et al. 2001; Ilg & Castella 2006; Walters et al. 2009). Low temperature water has also been examined as a potential factor reducing osmoregulatory stress in insects with larger exchange surface areas (Buchwalter 2003). Understanding the relationship of temperature and ion concentration on osmoregulatory function will help to predict if insects will be able to colonize in non-glacier environments with higher salinity.

Aquatic insects with cutaneous respiration are adept at hyper-osmoregulation (Buchwalter 2003). Widespread chloride cells and epithelia on insect cuticles have porous plate membranes which allow passive and active exchange of ions, in particularly the dominant osmoregulatory ions Na^+ and Cl^- (Wichard et al. 1972; Kapoor 1979). Maintaining homeostasis in freshwater requires insects to regulate absorption of water and loss of ions through the apical chloride cell membranes, in order to maintain a higher hemolymph ion concentration. If hemolymph ion levels drop, insects excrete dilute urine (Choe & Strange 2009; Griffith 2017). The osmoregulation pump is downregulated when the external medium becomes isotonic with the hemolymph, which may elicit a stressor response in hyper osmoregulating insects that are adapted to low ionic concentration waters (Wichard & Komnick 1974; Riedl et al. 2016).

Chloride cell morphology was hypothesized to be linked with ion absorptive function, with coniform cells thought to be more effective at absorbing ions than bulbiform or filiform cells due to their large surface area and singular presence (i.e., without caviform cells) (Wichard et al. 1972; Komnick 1977; Filshie and Campbell 1984). I have observed co-occurring coniform and caviform cells on Chloroperlidae and Taeniopterygidae stoneflies which have not been previously reported for these taxa. One possibility for co-occurring cells is that they are absorbing different ions such as Mg^{2+} and Ca^{2+} (Griffith 2017). Yet another possibility is that chloride cells exhibit changing morphology (number of cells, density, and area) after molting to different instar stages based on changing osmoregulatory needs and external salinity. The density of coniform cells on lateral abdominal gills of *Callibaetis coloradensis* mayflies was shown to change inversely with external ion concentrations (Wichard et al. 1973). In another study, the apical surface area of rainbow trout branchial chloride cells increased within 24 hours in response to decreases in external ion concentration (Perry & Laurent 1989). Based on assumptions that chloride cell function is similar in all aquatic organisms, it is expected that if insects are exposed to higher salinity, the area, density, and number of chloride cells will decrease, and the opposite will occur if the insect is exposed to salinities lower than their colonizing stream. However, what is not known is the duration of time until a molt occurs is unknown for the taxa in this study. But, environmental stressors such as water temperature have been shown to speed up the molting process (Buchwalter et al. 2014). Therefore it is anticipated that higher salinity may also speed up the molting process. Due to the aforementioned uncertainties, it was unknown if there would be a detectable difference in the number of the chloride cells in response to salinity during the 120-h experiment. In the study performed on the mayfly *Callibaetis coloradensis*, molting was observed to occur in some individuals within a 15-day experiment, however individual mayfly molt verification was not possible due to the experimental design (Wichard et al. 1973). Moreover, the experiment focused on observation of the lateral abdominal gills rather than the abdominal integument, so it is possible that lateral abdominal gill chloride cells function differently than chloride cells on the thorax and abdominal integument.

I predicted that nymphs acclimated to low salinity conditions close to glaciers would have higher and earlier mortality when exposed to high salinity than nymphs originating from groundwater and downstream (> 5 km) glacier sites due to acclimation to higher salinity than at metakryal sites close to the glaciers. The objectives of this research were 1) Conduct acute lethality tests to calculate and compare 120-h lethal concentration (LC_{50}) salinity values (i.e., the salinity to which exposure leaves 50 percent of the population dead after 120 h; 2) Investigate the survival duration of nymphs from different ambient salinity conditions (glacial meltwater, mixed water, and groundwater streams) to different salinity treatments; and 3) Study effects of exposure to low and high salinity on chloride cell characteristics.

The aim of this preliminary research was not to develop a predictive framework for climate mitigation but to launch discussions on how to develop causal relationships based on physiological traits for insects in remote and threatened habitats due to climate change. Understanding physiological tolerance levels through an ecotoxicological research approach (Griffith 2017) is one way of enhancing our understanding of what might happen to aquatic insects with the loss of glacial meltwater habitats. The disappearance of glaciers may leave enriched ionic concentration groundwater and snowmelt streams with different flow regimes, turbidity, and water temperatures as the only colonization options.

Methods

4.2.1 Study Sites and Environmental Characteristics

Two glaciated mountain ranges of Southeastern Tibet's Hengduan mountains were selected as study sites for the experiments (Figure 4.1). The Meilixueshan range (6,740 m a.s.l.) and Daxueshan (Mt. Gongga) (7,556 m a.s.l.) ranges have the highest mountain prominence in the Hengduan mountains of the Three Parallel Rivers region with some of the highest treelines in the world (Miehe et al. 2007). Glacial maximum occurred during the Little Ice Age, with temperate monsoonal glaciers decreasing in area since the 16th to 19th centuries (Su & Shi 2002). The Mingyong Glacier is the southernmost and one of the lowest elevation (2,750 m a.s.l.) glaciers in China (He et al. 2003) and is located in the monsoonal region of Yunnan Province (28°26′14″N 98°41′04″E). The Hailuogou Glacier (2,900 m a.s.l.) on Mt. Gongga in Sichuan Province is also a monsoonal temperate glacier located in the Daxueshan range (29°35′48″N 101°52′43″E). The Hailuogou Glacier with an area of 24.83 km^2 (Liu et al. 2010) is just under double the size of the Mingyong Glacier (13 km^2). The Mingyong Glacier terminus has been retreating on average 50 m per year since 2010 (Fair-Wu, field data) with the Hailuogou Glacier retreating 181 ± 23 m from 1994-2007 (Liu et al. 2010).

Conductivity, stream temperature, pH, and dissolved oxygen measurements used to provide mean water characteristic variables were measured in the Mingyong and Mt. Gongga Glacier streams from 2008-2015 with a YSI Professional Plus hand-held meter (YSI Incorporated, Yellowsprings, OH) each time invertebrate community sampling took place. Water samples were taken from the experimental stream sites in pre acid-washed vials and filtered through 0.45 μm Whatman filters (GE Healthcare Sciences, Pittsburg, PA) for metals analysis to understand ionic composition of the streams.

4.2.1 Collection of Insects

Insects were collected for six acute lethality experiments from five watersheds in Meilixueshan and Mt. Gongga. The melt season with torrential and turbid conditions was the most difficult to collect sufficient numbers of same-taxon insects, as insect densities drop dramatically in the spring and summer seasons (Steffan 1971; Ward 1994; Milner et al. 2001; Brittain & Milner 2001). In Meilixueshan, insects were collected for the experiments from three streams in three parallel watersheds flowing into the Mekong River. Three stream sites were glaciated and one stream site was from a groundwater stream. The Meilixueshan sites included: 1) M1 - 800 m downstream from the Mingyong Glacier at 2,533 m a.s.l.; 2) M2 - 2,500 m from the Mingyong Glacier at 2,336 m a.s.l.; 3) N1 - 8,000 m downstream from the Niuba Glacier at 2,137 m a.s.l.; and 4) L1 - Longtoujiang groundwater site at 2,043 m a.s.l. (Figure 4.2). At Mt. Gongga, larvae collection took place in 1) GL3, 800 m downstream from Glacier 3 at 3,680 m a.s.l. and at H1, a groundwater stream in the Huangbengliu catchment at 3,000 m a.s.l. (Figure 4.3). The experiments in Meilixueshan were conducted from August 5-14, 2015 and experiments at Mt. Gongga were conducted from August 23-28, 2015. Due to logistical constraints, the experiments were conducted at two sites. At Meilixueshan the experiments took place in M2 and in H1 at Mt. Gongga. The mean water temperatures (±SD) during the experimental runs were M2 (3.21±0.34°C) and H1 (8.12±0.30 °C). The water temperature readings while collecting from the other streams (either mean±SD over several days during the experimental period or spot reading at time of collection) were as follows: 1) M1 - 1.89±0.20 ^{o}C; 2) N1 – 7.7 ^{o}C; 3) L1 - 12.5^{o}C; and 4) GL3 - 2.36±0.93 ^{o}C. *Note: All specimens are stoneflies (Plecoptera) unless otherwise indicated.

4.2.2 Acute Lethality Testing Procedure (LC_{50}) and Statistical Analysis

The original aim of the acute lethality study was to compare survival of glacier stream Nemouridae morphospecies 1 and groundwater *Amphinemura* spp. to different salinity treatments (Figure 4.4). Nemouridae morphospecies 1 have unbranched horn-shaped osmobranchiae (Shepard and Stewart 1983) and were found to be strongly associated with glacier stream colonization in Meilixueshan. *Amphinemura* spp. with tracheated gills are found mainly in groundwater streams (Figure 4.4). It was hypothesized that the groundwater *Amphinemura* spp. would survive for a longer duration in high salinities due to adaptation to groundwater stream conditions with higher salinity. Due to the uncertainty of collection success, replacement species for Nemouridae morphospecies 1 and groundwater *Amphinemura* spp. were considered prior to collection. Replacements for Nemouridae morphospecies 1 included Nemouridae morphospecies 2 and *Epeorus* spp. (Ephemeroptera) in glacier streams, and replacements for *Amphinemura spp.* were either *Rithrogena* spp. (Ephemeroptera) or *Heptagenia* spp. (Ephemeroptera).

Taxa were collected for 2-3 hours at each site by turning over boulders and collecting in a Surber net and washing nymphs into a pan. Stream water in the pan was replaced frequently in order to maintain a low temperature. In order to avoid a stress response by handling the nymphs, the nymphs were placed directly into the vials from the collection pans. Due to the small size of Nemouridae and Taeniopterygidae stoneflies in the Mingyong sites, it was difficult to identify them in the field without handling them, and therefore some of the Nemouridae morphospecies 1 and Taeniopterygidae were placed in the same experimental vials. At the end of the experiment the stoneflies were identified as either Taeniopterygidae or Nemouridae when they could be examined under a dissecting microscope (10-40x magnification). Out of a total of 87 nymphs, 23 were Taeniopterygidae and 64 were Nemouridae morphospecies 1. Three extra nymphs were placed into an additional replicate 0.25% NaCl vial. The Taeniopterygidae that were placed with Nemouridae in vials were as follows: 3/12 in stream water, 2/12 in distilled water, 1/12 in 0.01% NaCl, 3/12 in 0.10% NaCl, 5/12 in 0.25% NaCl, 4/12 in 0.95% NaCl, and 2/12 in 1.20% NaCl. The Chloroperlidae, Taeniopterygidae, and Diamesinae (n=86) (Chironomidae, Diptera) all were placed in same-taxon vials as they were possible to identify, or as in the case of Taeniopterygidae at GL3 in Mt. Gongga, were the only taxon of stonefly at that stream site. Four taxa had sufficient collection numbers to conduct the experiment and estimate LC_{50} values: Nemouridae morphospecies 1 from M1, Chloroperlidae from N1, Taeniopterygidae from GL3, and Diamesinae (Chironomidae, Diptera) from GL3. All other insects were either of low collection numbers or had high mortality before the experiment could commence (Appendix Table 4.1).

The nymphs were placed into groups of four of the same taxa nymphs when possible in three replicate 20-mL vials for each NaCl concentration. The target collection number was 84 nymphs of the same family of insects for each experiment. The experimental insect vials were grouped by stream site in mesh bags and placed in M2 and H1 by attaching securely to a boulder on the river banks. By placing the vials at the same location in each of the two mountain ranges, temperature regime and sunlight were controlled. The nymphs were monitored every 24 h for mortality by placing them gently into a white container with experimental water, prodding them with a probe to see if there was a stimulatory reaction, removing and recording dead nymphs, and replacing the vial water with fresh solution from its respective treatment. The experiments were conducted for 216 h in Meilixueshan and 120 h in Daxueshan and LC_{50} values were estimated for both 120 h and 216 h exposure times for Nemouridae morphospecies 1 and Chloroperlidae, and 120 h for Taeniopterygidae from Mt. Gongga.

The acute lethality tests conducted in the field were performed using a distilled water (control) and five NaCl-distilled water solutions (0.01, 0.10, 0.25, 0.95, and 1.2% NaCl) representing a range of conductivities (11, 243, 1,500, 4,690, 16,350, and 20,486 µS/cm). Conductivity of NaCl solutions was determined in the laboratory prior to fieldwork with a YSI Professional Plus (Yellow Springs Instrument Co., Yellow Springs, OH). Stream water was used as a field reference control.

The lethal concentration (LC_{50}) representing the salinity at which 50 percent of the population died after 120 h of exposure was calculated. Acute toxicity dose-response curves for the distilled water control and NaCl treatments were fitted to a log-logistic model (Doelman and Haanstra 1989) using the Newton optimization method (Solver, Microsoft Excel, 2007). The log-logistic model was used to estimate lethal toxicity thresholds (LC_x), by minimizing sums of unweighted squared residuals (maximum likelihood), using $\log_{10}$ transformed data (Doelman and Haanstra 1989). LC_{50} values were considered significantly different if there was no overlap of 95% confidence intervals.

4.2.3 Survivorship and Statistical Analysis

In order to select nymphs for examination of chloride cell morphology changes, it was first necessary to know which individuals survived for the longest duration in the different salinities. Two survivorship results were noted as a result of ANOVA post hoc tests. The lowest observed effective concentration (LOEC), the concentration at which a difference in mortality occurs that is significantly different from the control, and the no observed effect concentration (NOEC), the concentration closest to the LOEC at which no statistical difference is observed. Percent survivorship analysis was conducted using one-way analysis of variance (ANOVA) in Sigmaplot (Systat Software 2014). Response variables were examined for normal distribution with the Shapiro-Wilks test (Shapiro & Wilk 1965). Homogeneity of variance was examined with the Brown-Forsythe test (modified Levene test) (Levene 1960). If the response variables did not meet the assumptions of normal distribution and homogeneity of variance, the Kruskal-Wallis One-way analysis of variance on ranks was conducted (Kruskal 1952). Tukey post-hoc tests were conducted to determine significant differences between treatment survival ratios. The level of significance for all analyses was $\alpha=0.05$.

4.2.4 Chloride Cell Procedure and Scanning Electron Microscopy (SEM) Imagery

Before performing experimental procedures on stonefly nymphs, the presence of chloride and caviform cells were verified by using a histochemical silver nitrate ($AgNO_3$) precipitation technique (Koch 1938). Precipitated $AgNO_3$ (99.8%, Tianjin Damao Chemical Reagant Factory, Tianjin, China) is known to stain and highlight cells that absorb chloride, which is a function of osmoregulation (Wichard et al. 1973). The histochemical precipitation of $AgNO_3$ also makes the cells that absorb chloride visible under a microscope and when imaging with variable pressure scanning electron microscopy (SEM) imaging (Wichard & Abel 1972). Therefore it was assumed that any cells that exhibited histochemical precipitation in response to the $AgNO_3$ treatment were chloride cells or cells related to osmoregulation. The presence of both coniform and caviform chloride cells was verified by imaging Taeniopterygidae from Mt. Gongga. Both types of cells cover the ventral surfaces of the anterior thoracic area, prothorax, mesothorax, metathorax, abdominal segments, mandible, and mentum (Figure 4.5). The smaller coniform cells and cells with a plug of dense material in the porous plate region are strikingly visible in the 20,000x image (Figure 4.5).

Specimens were selected for imaging (n=4 per treatment) from the highest exposure concentration at which insects survived for the longest duration (e.g., 1.2% NaCl), distilled water (control) and stream water reference. If survival was < 48 h in the highest concentration (1.2% NaCl), nymphs were selected from the next lowest concentration (e.g., 0.95% NaCl). Similar-sized nymphs were selected (late mid to early late instars) to control for developmental differences that could potentially impact the chloride cell analysis (Table 4.2).

Specimens were dehydrated using a graded ethanol series (30% to 100%) and then critical point dried using a Pelco CPD2 dryer (Ted Pella Inc., Redding CA) with liquid carbon dioxide as a transitional fluid. The dried specimens were mounted on scanning electron microscopy (SEM) stubs using carbon tape and sputter coated with a thin layer (5-10 nm) of gold-palladium 60:40. The specimens were stored in a desiccator until SEM image analysis took place at either Hong Kong University (Hitachi S-4800 field emission SEM, Hitachi High Technologies, Corp. Tokyo) or at The Ohio State University (FEI Nova NanoSEM 400 SEM, Thermo Fisher Scientific Inc., Waltham, MA).

4.2.5 Image Processing of Chloride Cells

Scanning electron microscopy (SEM) images were processed using Fiji software by generating ventral region-of-interest (ROI) image layers to quantify chloride cell measurements. The images were standardized to a magnification of 1,000x. Images were obtained from the osmobranchiae, upper, mid, and lower thorax, pronotum (Nemouridae), first abdominal segment pleural fold (Nemouridae and Chloroperlidae), and abdominal segments 1, 4, and 7 (Taeniopterygidae) (Table 4.3 and Figure 4.6). The osmobranchiae were verified following identification of sclerites on the prothoracic region, which give rise to chloride-cell covered membranous structures (Baumann 1975).

The porous plates of chloride cells were hand-traced due to the similarity of image grayscale color intensity. Porous plates that were partially visible in the image were not included in the analysis. Body parts (e.g., osmobranchiae) that were smaller than the image area were traced, and measurements based on the body part area were analyzed based on the number of cells per body part. Two chloride cell datasets per body part were generated for Taeniopterygidae and Chloroperlidae by analyzing ROIs for 1) coniform chloride cells, and 2) smaller caviform/generating/degenerating cells (Figure 4.7). Even though coniform cells were co-present with bulbiform cells on Nemouridae (Figure 4.4), many of the smaller caviform cells were not visible due to the presence of preserved debris on the integument, so caviform analysis on Nemouridae did not take place. Traced ROI layers enabled calculation of the following response variables for coniform, caviform, and bulbiform cells: 1) cell count, 2) average porous plate area (μm^2), 3) percent area coverage, and 4) cell density (μm^2). Chloride cell statistical analysis was performed using one-way ANOVA (Systat Software 2014) to test for responses of cells to salinity treatments.

Results

4.3 Collection of Insects

The targeted insects were Nemouridae morphospecies 1 from glacier sites and *Amphinemura* spp. from groundwater sites. Sufficient Nemouridae morphospecies 1 were collected, but a total of only six *Amphinemura* spp. were obtained from N1 (Appendix Table 4.1). Six experiments were conducted, but LC_{50} for three taxa

(Nemouridae morphospecies 1, Taeniopterygidae, and Chloroperlidae) were calculated. An experimental run with Diamesinae (n=84) (Chironomidae, Diptera) was conducted, but their mortality response was non-logistic, therefore an LC_{50} could not be calculated.

4.3.1 Acute Lethality (LC_{50}) Test Results

There was no statistical difference in 120-h LC_{50} values between the three insects tested, as evidenced by overlapping confidence intervals (Table 4.4). The median lethal concentration for salinity (expressed as electrical conductivity, µS/cm) at 120-h for Nemouridae morphospecies 1 was the lowest 120-h LC_{50} (3249 µS/cm) relative to Taeniopterygidae and Chloroperlidae (5676 and 9359 µS/cm, respectively).

4.3.2 Survivorship Results

The LOEC for Nemouridae was 16350 µS/cm (Table 4.3). At the downstream M2 site, either low numbers of individual taxon were collected or high mortality occurred within the first two days of the experiment. Sixty-six Nemouridae were collected, but the morphospecies 2 had high mortality within the first 2 days and therefore LC_{50} results were not calculated for the Nemouridae morphospecies 2 from the Mingyong Glacier stream downstream site (Appendix, Table 4.1).

In the mixed glacier-groundwater and groundwater sites at Meilixueshan only one experiment was conducted due to high insect mortality. Chloroperlidae collected from the downstream mixed glacier-groundwater site had no significant difference in the median survivorship values among treatment groups ($p = 0.174$), and therefore no LOEC is calculated (Table 4.3). Other taxa collected at the downstream mixed glacier-groundwater site included *Amphinemura* spp. (6), Baetidae (Ephemeroptera) (4), Heptageniidae (Ephemeroptera) (3), and Nemouridae morphospecies 2 (1) (Appendix Table 4.1). Eighty-four Heptageniidae (Ephemeroptera) were collected from the groundwater site but all of them died within 24 hours, even in the stream and distilled controls. Other mayflies (Baetidae, Leptophlebiidae, and Ephemerellidae) were collected from the same groundwater stream but also died within 24 hours in the controls and treatments. Perlidae were collected from the groundwater site in low numbers (Appendix Table 4.1).

Taeniopterygidae from GL3 at Mt. Gongga had a LOEC of 16350 µS/cm which was similar to Nemouridae (Table 4.3). *Himalopsyche* spp. (Tricoptera) (n=51) were collected with 98% of the insects expiring within 24 hours, and one small nymph surviving through the end of the experiment (>120 hours) in distilled water. Lethal toxicity thresholds for Diamesinae at the Mt. Gongga downstream site (GL3) could not be calculated using the log-logistic solver model in excel due to a non-logistic mortality with high mortality at the high and low salinity levels (Appendix Table 4.1).

4.3.3 Chloride Cell Imaging Results

The coniform and caviform cells of Chloroperlidae had significant responses to the 1.2% NaCl treatment and distilled water control. Coniform chloride cell density was significantly lower in the distilled water control (p=0.038) than the stream control, and intermediate in the 1.2% NaCl treatment (Figure 4.8). The mean chloride cell area (μm^2)

was significantly larger in response to the distilled water control and intermediate in response to the 1.2% NaCl treatment (Figure 4.8). The response of caviform and chloride cells of Chloroperlidae support the hypothesis that the cells respond in an inverse manner to external salinity (Figure 4.9). The mean number of caviform cells per insect body part were significantly higher in the distilled water control than both the stream control and 1.2% NaCl treatment (Figure 4.10). The areas of the caviform porous plates were higher in the distilled water control than the 1.2% NaCl treatment (Figure 4.10), and the mean density of cells (µm^2) was significantly higher in the distilled water control than both the stream control and 1.2% NaCl treatment (Figure 4.10).

There was no significant effect of salinity treatment on the number of Taeniopterygidae coniform cells, the mean density (µm^2), and the mean surface area of the porous plates (Table 4.5). However, Taeniopterygidae caviform cells exhibited significant responses to salinity treatments (Figure 4.11). The number of caviform cells exposed to the 0.95% NaCl treatment were significantly lower than the stream control (Figure 4.11). The density of coniform cells was significantly lower on Taeniopterygidae for both the distilled water control and 0.95% NaCl treatment than the field stream control (Figure 4.11). There was no significant effect of salinity (distilled water control and 16350 µS/cm treatment) on Nemouridae bulbiform chloride cell count (Table 4.5), mean porous plate area, and mean density of cells (Table 4.5).

Discussion

The null hypothesis for the acute lethality hypothesis could not be rejected, as the three LC_{50} responses for stoneflies colonizing the metakryal zone and downstream location had overlapping confidence intervals, and therefore were not significantly different (Table 4.3). This result suggests that if salinity was the only factor limiting stoneflies from colonizing groundwater streams, then it would be possible for these insects to colonize groundwater streams. However, in reality, the ability to colonize groundwater streams is dependent on multiple factors such as water temperature, food source, substrate type and size, hydraulic differences between glacier and groundwater streams, colonization space, and competition. For example, groundwater streams in Meilixueshan and Mt. Gongga contain robust and carnivorous Perlidae which would be a formidable predator to the smaller Nemouridae and Taeniopterygidae nymphs if they happen to colonize groundwater streams in the future.

The survivorship results support the hypothesis that mixed groundwater/glacier streams insects may be able to tolerate higher salinities due to adaptations to natural conductivities in the streams they colonize (Table 4.3). Chloroperlidae survived longer in higher concentration salinities than Nemouridae and Taeniopterygidae, and were collected at the downstream glacier site (7 km from Sinong Glacier). The h-values (Kruskal-Wallis) were high in the statistical tests, which suggest that collecting greater numbers of stoneflies may result in the detection of differences among survivorship results. The Chloroperlidae had a significantly longer survival time in 1.20% NaCl (died within 120 hours) than the Nemouridae morphospecies 1 and Taeniopterygidae (Table

4.3). Most of the Nemouridae and Taeniopterygidae died within 48 hours, which could be the main reason for undetectable morphological differences between salinity treatments for the Nemouridae bulbiform cell response variables and Taeniopterygidae chloride cell response variables. Perhaps if the Nemouridae and Taeniopterygidae had survived in the treatments as long as the Chloroperlidae, we may have seen statistically detectable responses in caviform, coniform, and bulbiform cell morphology. Although larvae exuviae were not noted as present/absent nor collected during the experiment, a few dead nymphs were observed to be in the middle of the process of molting, with exuviae partially attached to the insect body. If the Nemouridae and Taeniopterygidae had survived longer through the entire molting process, a detectable difference in chloride cell morphology may have been detected.

In high salinity treatments (16350 and 20486 µS/cm), mortality for most of the Taeniopterygidae and Nemouridae nymphs occurred within 48 hours, which is in line with the results of *Paragnetina media* (Plecoptera: Perlidae) found in an earlier experiment (Kapoor 1978). *Paragnetina media,* which are accustomed to hyper osmoregulating in low salinity freshwater, were exposed to an external salinity that was isotonic to its hemolymph ion concentration at 16350 µS/cm (0.95% NaCl), which led to a fatal stressor response (Kapoor 1978). Only 20 percent of the nymphs in the Kapoor (1978) experiment survived for 72 hours in solutions hypertonic to their hemolymph (1.1% and 1.2% NaCl). In my study, Nemouridae exposed to 0.95% NaCl and 1.2% NaCl treatments all died within 48 hours except for one Nemouridae that died within 72 hours. Nemouridae specimens treated in the 1.2% NaCl (20486 (µS/cm)) treatment were desiccated, mounted, and sputter coated in preparation for imaging, however an accident occurred in the vacuum chamber and the stub of insects was lost. Therefore Nemouridae from the 0.95% NaCl (16350 (µS/cm)) treatment were imaged. Taeniopterygidae specimens from the 16350 µS/cm treatment died within 48 hours (n=10) or 72 hours (n=2) and therefore the imaged insects consisted of 50 percent that died within 72 hours and the other within 48 hours. Therefore the exposure duration of both insects to the salinity treatments may have been too brief to elicit morphological changes to the bulbiform and coniform cells. Moreover, the flattened positions of the bulbiform cells at different angles after the critical point drying procedure make it impractical to standardize the angle from which cells are imaged. It was assumed that any differences in post-desiccation cell angle would average out when analyzing a high numbers of cells. As hypothesized by earlier researchers (Wichard & Komnick 1974; Komnick 1977), the change in bulbiform cell morphology may occur at a slower rate than coniform cells, and therefore insects with bulbiform cells that died within 48 hours during the experiment may have had undetectable bulbiform cell morphology responses due to the lack of a molt or enough time for a change to the chloride cells to be observed.

The mean caviform cell counts and cell density (μm^2) of Taeniopterygidae were significantly lower in the high salinity treatment [16350 (µS/cm)] than the stream control (Table 4.5). Although this supports the hypothesis that cells respond in an inverse manner to external salinity, it is uncertain as to whether the difference in caviform cell counts and density was due to the experimental treatment or natural variation between insects. The number of caviform cells between the distilled water control and stream control were not statistically different, which could be due to the low natural stream salinity of the glacier-fed stream and low salinity of distilled water. Cell density and caviform cell count was significantly higher in the distilled water and high salinity treatment than in the stream control Taeniopterygidae. Even though both cell density and cell counts occurred in an unexpected direction for the distilled water control, this may have been due to an initial stress reaction by the insect.

Chloroperlidae survived for up to 5 days in the 20486 (µS/cm) treatment and their coniform and caviform cell responses had an inverse response to external salinity as hypothesized. Mean chloride cell area and density were significantly higher ($p<0.001$) in response to the distilled water control than the stream control. The number of coniform cells was not significantly different from the control. The most striking result was the response of the caviform cells to the stream water control and 20486 (µS/cm) treatment. The number of cells, mean density, and mean area were higher in the distilled water control than the stream water field control and 20486 (µS/cm) treatment (Figure 4.9). The response of the caviform cells to salinity treatments is a novel finding, and suggests that the smaller cells may play a role in responding to external changes in salinity (Figure 4.9). Wichard et al. (1972) mentioned that co-occurring cells exist on insects with bulbiform cells, which led to the hypothesis that the bulbiform cells were less efficient than coniform cells at responding to salinity changes (Wichard & Komnick 1974; Komnick 1977). However, my results indicate that coniform cells co-occur with caviform cells, and perhaps caviform cells are immature coniform cells that are able to respond with morphological changes to external salinity conditions. In a study of the flower shaped sensillum of *Thaumatoperla alpine* (Plecoptera:Eusteniidae), the progression of cell growth is documented, with the immature cells containing a knob of dense material that eventually transforms into a flower shaped sensillum (Kapoor & Zachariah 1978). The caviform cells could be similar immature versions of coniform cells exhibiting morphological changes to external environmental conditions.

One of the original aims of the study was to compare the upstream glacier stream Nemouridae morphospecies 1 with groundwater/downstream glacier site *Amphinemura* spp., as the Nemouridae morphospecies 1 has hemolymph-filled osmobranchiae (Shepard and Stewart 1983) and *Amphinemura* spp. has filamentous tracheated gills. My aim was to compare the performance of these two taxa to different salinities with the hypothesis that their responses would be based on conditioning to ambient conditions and gill structure. Even though *Amphinemura* spp. stoneflies were not collected in sufficient numbers, Chloroperlidae were used as downstream nymphs in the salinity lethality test. Substantial numbers of *Epeorus* spp. and *Rhithrogena* spp. were collected at the groundwater site, but they died soon after they were removed from their natural flow regime (Appendix Table 4.1). The importance of understanding the natural hydraulic flow microhabitat should be considered when selecting insects for field experiments. If a stream flow chamber is unavailable at streamside, then it is better to select non-rheophilic insects or stoneflies, as stoneflies appear to be more tolerant to experimental conditions than mayflies.

A similar sampling result occurred at GL3 where 54 late-instar *Himalopsyche* spp. (Tricoptera) were collected, which was unanticipated. Upon removal from the stream and placement into the collection bucket/tray, the *Himalopsyche* spp. (Tricoptera) larvae clung to the aeration ball and by the time they were placed in experimental vials after being transported to the 3,000 m a.s.l. stream below the glacier within an hour, they were dorso-ventrally undulating. None of the late instar larvae survived 24 hours, and their dorso-ventral undulation suggested death was due to hypoxia. One young instar *Himalopsyche* spp. (Tricoptera) survived through day 5 in the stream control. Its small body size and physiological requirements as a young nymph may have enabled it to survive in the experimental conditions. The stream where the *Himalopsyche* spp. (Tricoptera) were collected has is a high gradient channel (>30%), with low water temperatures (2.89±0.64 between 8/5-8/10/2015), and a step-rapid pattern of turbulent flow over cobble and boulders. When using *Himalopsyche* spp. (Tricoptera) for any experiment, it may be necessary to develop an in-stream experimental chamber to run a successful experiment meeting their oxygen demand.

High mortality of Diamesinae from Mt. Gongga was observed in stream water within the first couple of days, which was an unusual response. The Diamesinae stream water control may have been turbid, and by placing the insects into the same water from the stream control at a higher temperature in the groundwater stream may have elicited oxygen-related stress. Due to the logistical constraints that would have been posed by carrying out the experiment in two separate streams (one above the glacier and one below) the groundwater stream near the experimental station was selected to conduct the two experiments within the 5-day time frame. The average difference of 5.76^{o}C higher in the groundwater stream may have prompted a stressor response in Diamesinae, as they were from a stream that had much lower temperatures. The Diamesinae were not pre-exposed to short-duration higher temperatures which could have hardened them and given them protection against higher temperatures (Chen et al. 1991). The pupation rates of the surviving Diamesinae in the moderate salinity conditions at day 5 were high, with 17/31 larvae pupating (55%) and alive on day 5. Pupation may be a stressor response to salinity changes or perhaps a response to combined higher temperature and salinity. Moreover, the high mortality of mayflies collected in the groundwater stream at higher temperatures in Meilixueshan, but placed in lower water temperatures in the Mingyong glacier mainstem may have elicited cold-shock responses that led to high mortality.

Conclusion

The coniform and caviform chloride cells of Chloroperlidae responded to the salinity treatments as predicted in the hypothesis, as there was an inverse relationship of chloride cell number, area, and density of cells to salinity. The exuviae were not collected but it is assumed that the significant morphological results between salinities were due to undergoing a molt after exposure to the treatment(s). The Chloroperlidae were from similar instar levels and they survived for five days in the highest salinities which could have given them sufficient time to undergo a molt. The Taeniopterygidae caviform cell responses also support the hypothesis. The results of the Taeniopterygidae coniform cells and Nemouridae bulbiform and caviform cells did not clearly support the predictions, but the low survival duration (<48 hours) in higher salinity treatments may not have been enough time for the chloride cells to morphologically respond to the stressor. Moreover, the analysis of Nemouridae caviform cells could not take place due to debris preserved on the integument that covered a majority of the caviform cells on some of the insects. Overall, the results support caviform cells as potential rapid responders to changes in external salinity, as both the Taeniopterygidae and Chloroperlidae caviform cells exhibited morphological changes to salinity. It is unknown which Chloroperlidae or Taeniopterygidae underwent a molt during the experiment, as shedded exuviae were not tracked. The response to chloride treatments by caviform cells was a novel finding. In the past, the role of caviform cells was unspecified, and the results of this research indicate that they may respond morphologically in an inverse manner to changes in external salinity. In order to verify these findings, it is suggested to repeat and increase the length of the experiment, and to track the molting of the individual insects to verify if this is key to the response of the chloride and caviform cells. If molts are not observed, then perhaps a new mechanism or biological pathway should be examined as a potential factor in changes to chloride cell morphology as a result of salinity changes.

Even though the LC_{50} values with overlapping confidence intervals did not allow the null hypothesis to be rejected, the cell responses and duration of survival may indicate that Chloroperlidae are able to tolerate higher salinities for a longer duration. Because of the longer survival duration, the morphological changes to the chloride and caviform cells may have occurred due to sufficient time for a molt to occur, which supported the hypothesis that morphological cell changes would occur in an inverse manner to salinity. This could be due to the fast response of the caviform cells to different salinities, but also may indicate prior acclimatization to fluctuating conductivities. Multiple water sources in downstream sites may present insects with exposure to higher salinity conditions, much as heat shock exposure prepares insects for temperatures outside of their normal range (Denlinger & Lee 2010). If insects are adapted to changing salinity, the response to and greater survival in different salinities may be a standard response, and exhibited by longer survival duration of Chloroperlidae. Yet another contributing factor may be the presence of sclerotization on the body segments of Chloroperlidae, which are reduced or absent on Nemouridae and Taeniopterygidae. This reduces the porous surface area exposed to external ion concentrations of water which may reduce stressor responses to changing salinity (Buchwalter 2003). A study comparing groundwater stream stoneflies with glacier stream stoneflies of the same family would be ideal in understanding physiological mechanisms that are potential adaptations to external salinity.

Table 4.1. Water quality characteristics (mean ± SD) for the experimental streams in the Meilixueshan and Daxueshan ranges from June-August 2015).

Site	Mtn. Range	Dist. beneath treeline (m) Approx.	Dist. from glacier (m)	Mean Temp (°C)	T_{max} Temp °C	Channel Stability	Channel width (m)	Slope (%)	Disch. Range (m^3/s)	Direct Canopy	Mtn Canopy Trees Upslope	Mtn Scree Slope Canopy
Glacier 3 Down	Gongga	-550	800	2.5±1.0	6.38	57±5	3.3±0.3	45	0.12-0.63	0.0	0.0	0.0
Mingyong 2	Meili	-1737	800	1.9±0.2	2.66	57	8.9±3.7	6	1.12-12*	0.0	0.18	0.0
Mingyong 3	Meili	-1,914	2,500	3.4±0.5	5.08	60	9.1±2.6	8	1.47-12*	0.22	0.01	0.0
Sinong 1	Meili	-700	15	1.9	1.93	60	5.0±0.0	3	0.55-5*	0.0	0.04	0.0
Sinong 2	Meili	-2,035	7,000	11.0	11.0	60	11.6±6.7	4	1.66-6*	0.0	0.0	0.35

Table 4.2. Head width ± SD and body length ± SD of specimens chosen for chloride cell analysis for distilled water, 1.2% or 0.95% NaCl and stream water.

Taxa	Head Width (mm)	Body Length (mm)
Chloroperlidae	1.10±0.04	9.18±0.52
Taeniopterygidae	0.70±0.12	3.79±0.33
Nemouridae	0.96±0.12	4.37±0.69

Table 4.3. Survivorship of stoneflies to treatment/controls. Lowest observed effective concentration is bolded (LOEC). Mean (± standard deviation) percent survival is presented for 120 h and 216 h. Lowest Observed Effect Concentrations (LOEC) are presented in bold. Nemouridae and Chloroperlidae experiments were conducted for nine days, therefore the data for 216-hour survivorship is also presented for comparison. Means in columns with a different superscript are significantly different ($p \leq 0.05$, one-way ANOVA), using a Tukey's post-hoc test.

		120-hour Survivorship (%)			**216-hour Survivorship (%)**	
Treatment	**Conductivity (µS/cm)**	**Taeniopterygidae**	**Nemouridae**	**Chloroperlidae**	**Nemouridae**	**Chloroperlidae**
Stream Reference	112	^{a}0.92±0.14	^{a}0.67±0.29	^{a}0.50±0.0	^{a}0.56±0.10	^{a}0.50±0.0
Distilled Water	11	^{a}0.75±0.25	^{a}0.72±0.05	^{a}1.00±0.0	^{a}0.64±0.13	^{a}1.00±0.0
0.01% NaCl	243	^{a}0.77±0.25	^{a}0.67±0.38	^{a}0.88±0.18	^{a}0.47±0.21	^{a}0.88±0.18
0.10% NaCl	1500	^{ab}0.58±0.38	^{ab}0.58±0.14	^{a}0.84±0.23	^{a}0.42±0.14	^{a}0.84±0.23
0.25% NaCl	4690	^{ab}0.50±0.25	^{ab}0.25±0.25	^{a}0.75±0.35	^{a}0.17±0.14	^{a}0.75±0.35
0.95% NaCl	16350	**^{b}0.08±0.14**	**^{b}0.00±0.00**	^{a}0.34±0.47	**^{b}0.00±0.00**	^{a}0.00±0.47
1.2% NaCl	20486	^{b}0.0±0.0	^{b}0.00±0.00	^{a}0.25±0.35	^{b}0.00±0.00	^{a}0.00±0.35

Table 4.4. Lethal toxicity thresholds (120-h LC_x) for stoneflies from Mingyong and Hailuogou Glacier streams exposed to varying salinity, expressed by conductivity (µS/cm). LCx values are presented with associated 95% confidence limits in parentheses.

Nymph	Stream Site	Stream Type	LC_{50}	LC_{20}	LC_{10}
Nemouridae morphospecies1	M1	Glacier upstream	3249(1677-6296)	1108(230-5342)	1649(505-5385)
Taeniopterygidae	GL3	Glacier downstream	5676(1982-16249)	2691(349-20725)	1739(117-25886)
Chloroperlidae	N1	Mixed	9359(2765-31677)	4253(463-39050)	2681(144-50041)

Table 4.5. Mean ± SE of cell count, area (μm^2), and density (μm^2) of chloride cells. Bulbiform (Nemouridae), caviform (Chloroperlidae and Taeniopterygidae), and coniform (Chloroperlidae and Taeniopterygidae) cells responded to exposure to controls and high salinity treatments (stream water, distilled water, 0.95% NaCl, and 1.2% NaCl). NA = not applicable. Note: Nemouridae caviform cells were present but not analyzed due to presence of integument debris that covered the caviform cells. A p-value ≤ 0.05 indicates a response that was statistically significant, using a Tukey's post-hoc test.

Taxa	Cell Type	Response Variable	Stream	Distilled Water	1.2% NaCl	0.95% NaCl	p-value
Nemouridae	Bulbiform	Count	47.00±7.11	55.35±7.60	NA	47.00±7.11	$p = 0.820$
Nemouridae	Bulbiform	Area	13.94±0.44	14.23±0.33	NA	13.81±0.41	$p = 0.336$
Nemouridae	Bulbiform	Density	0.004±0.00	0.005±0.00	NA	0.004±0.00	$p = 0.448$
Chloroperlidae	Caviform	Count	3.35±1.01	12.60±2.24	3.35±1.07	NA	$p < 0.001$
Chloroperlidae	Caviform	Area	0.72±0.06	1.41±0.10	0.60±0.13	NA	$p < 0.001$
Chloroperlidae	Caviform	Density	0.0002±0.00	0.0006±03.00	0.0002±0.00	NA	$p = 0.002$
Taeniopterygidae	Caviform	Count	34.46±3.87	22.5±2.81	NA	19.29±2.75	$p = 0.007$
Taeniopterygidae	Caviform	Density	0.003±0.00	0.002±0.00	NA	0.001±0.00	$p < 0.001$
Taeniopterygidae	Caviform	Area	1.78±0.12	1.89±0.18	NA	2.36±0.27	$p = 0.357$
Chloroperlidae	Coniform	Count	49.57±3.65	52.95±3.33	55.5±4.17	NA	$p = 0.498$
Chloroperlidae	Coniform	Density	0.005±0.00	0.004±0.00	0.005±0.00	NA	$p = 0.038$
Chloroperlidae	Coniform	Area	33.59±0.94	37.59±1.33	34.02±1.07	NA	$p = 0.019$
Taeniopterygidae	Coniform	Count	48.00±12.76	44.37±3.69	NA	41.32±3.44	$p = 0.697$
Taeniopterygidae	Coniform	Density	0.003±0.00	0.002±0.00	NA	0.002±0.00	$p = 0.126$
Taeniopterygidae	Coniform	Area	37.61±2.56	43.72±0.92	NA	44.42±1.16	$p = 0.226$

Figures

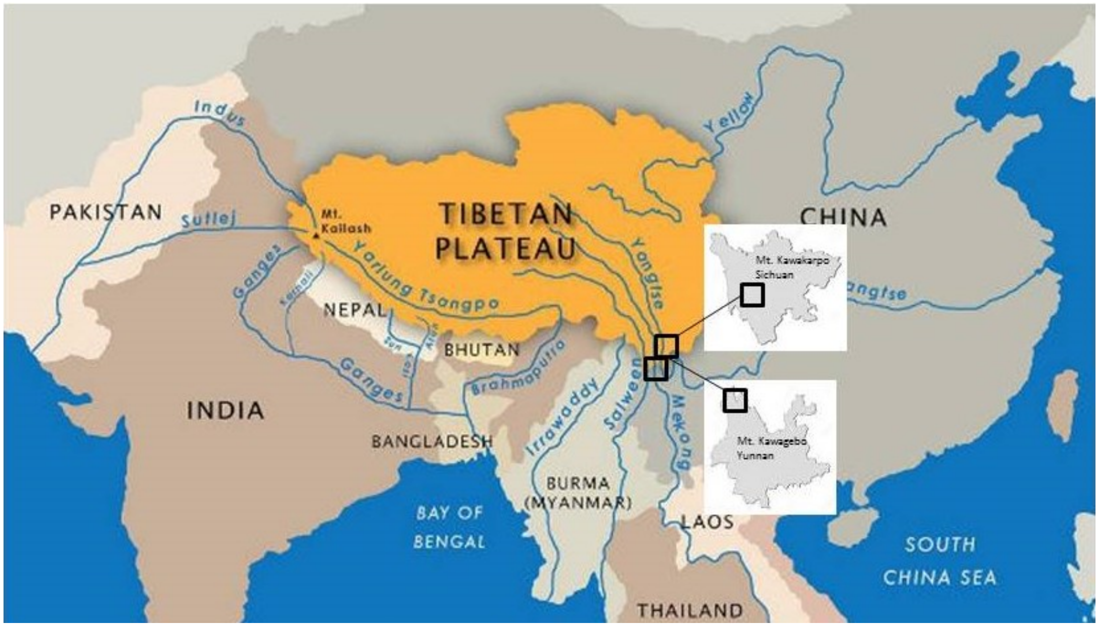


Figure 4.1. Study regions. The site along the Mekong River is located in Meilixueshan in the Three Parallel Rivers region, and the site in the Yangtze River headwaters is in the Daxueshan mountain range. (Map Credit: Buckley 2009).

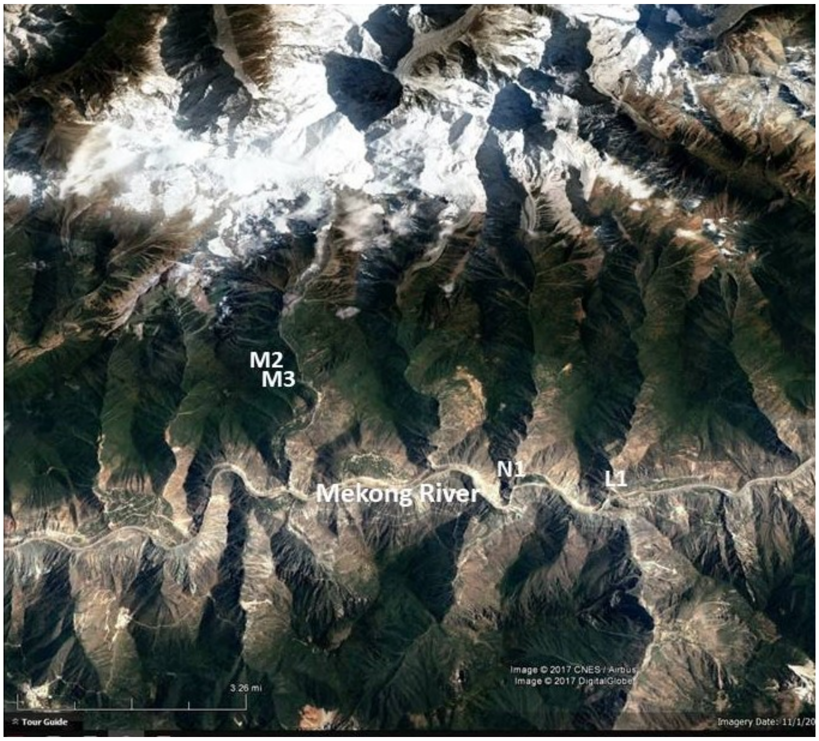


Figure 4.2. The Melixueshan mountain range. Headwaters flow into the Mekong River, Yunnan. Insects were collected for four salinity tests from the Mingyong Glacier mainstem (M1 & M2), the Niuba Glacier downstream site (N1), and the Longtoujiang groundwater site (L1). MY1 and MY2 are located 800 and 2,500m downstream from the Mingyong Glacier; N1 is 6,500m downstream from the Niuba Glacier; and L1 is at the same elevation as N1 approximately 300 m upstream from the Mekong River (*Source*: Google Earth).

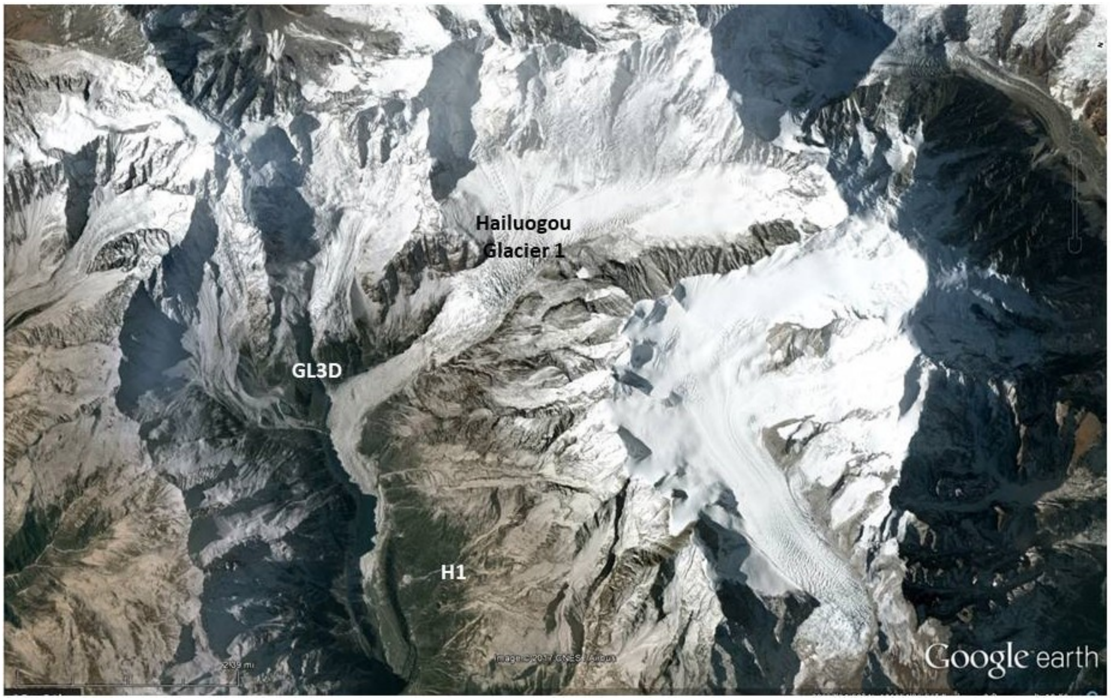


Figure 4.3. Mt. Gongga's Hailuogou Glacier and Huangbengliu watersheds. Experimental insects were collected from these sites. GL3 site is located 800m downstream from Glacier 3 above the Hailuogou Glacier at 3,650 m a.s.l., and H1 is the site of the groundwater stream in the non-glacierized Huangbengliu catchment at 3,000m a.s.l. (*Source*: Google Earth).

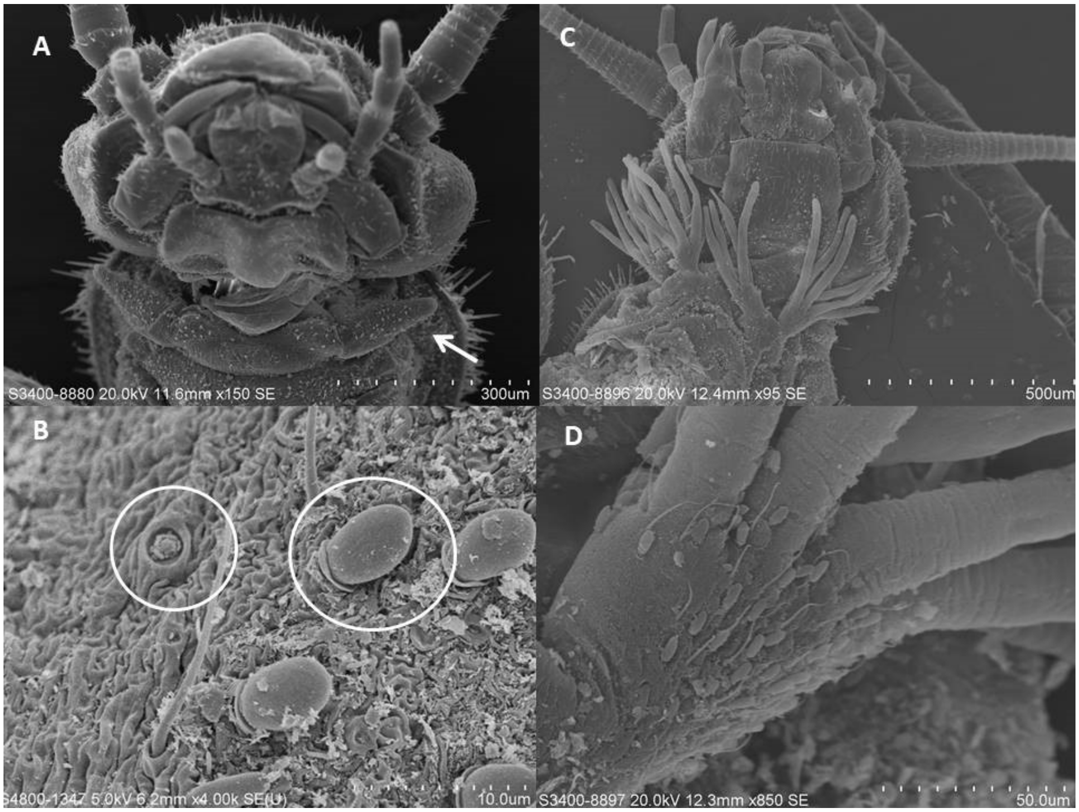


Figure 4.4. Nemouridae morphospecies 1 and Amphinemura spp. A) Nemouridae with single-finger osmobranchiae. A Caviform cell (B-left circle) and bulbiform chloride cell (B-right circle) at a 4,000x magnification from the cervical region. *Amphinemura* spp. is shown (C) with tracheal cervical gills. Bulbiform cells are apparent at the bases of the tracheated gills (D) of *Amphinemura* spp.

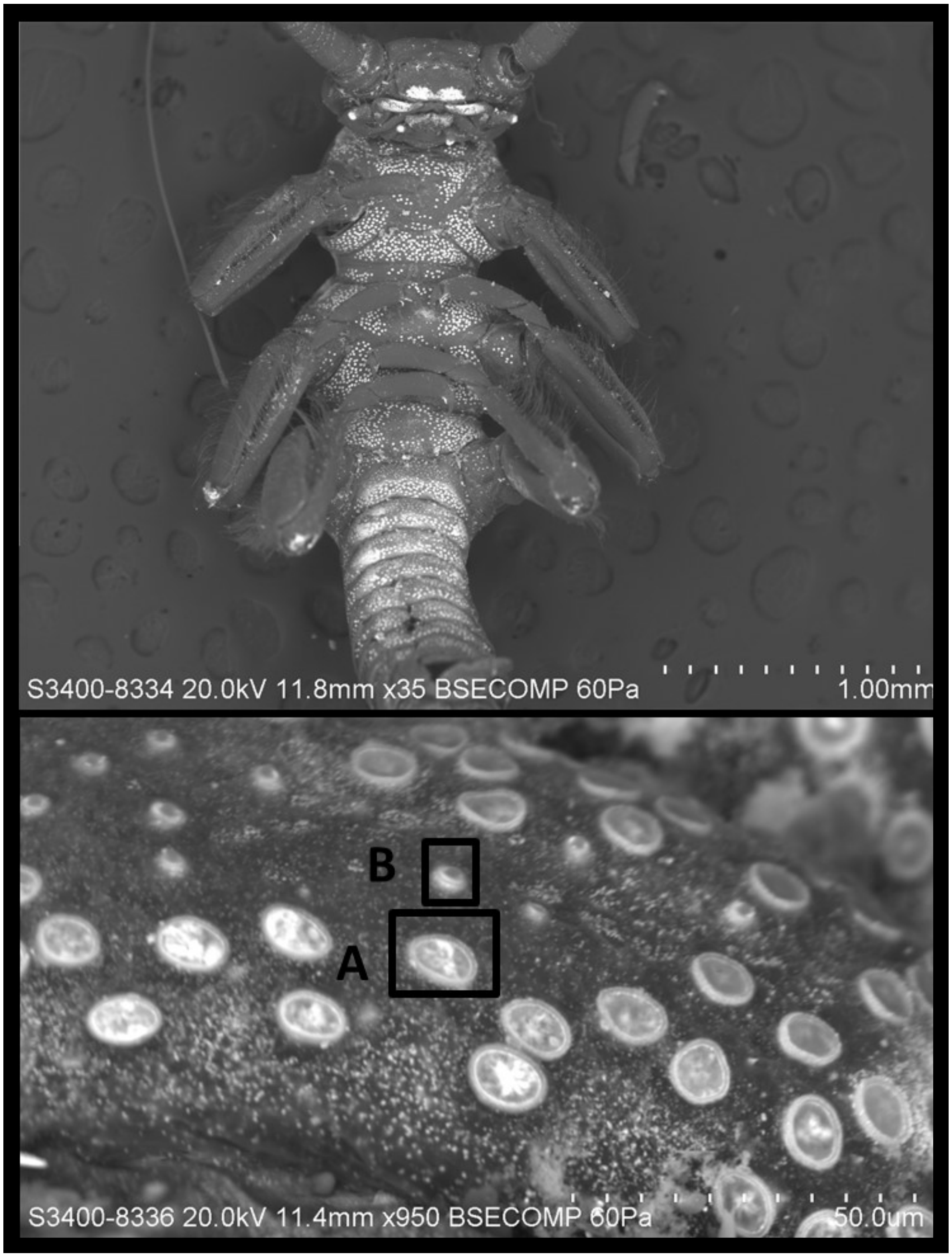


Figure 4.5. Taeniopterygidae from Mt. Gongga Hailuogou stream treated with $AgNO_3$ and imaged with variable pressure SEM (top) with abdominal segment close-up (bottom). Both the coniform (A) and smaller forming/caviform chloride cell (B) have precipitated $AgNO_3$ which indicates that the cells were absorbing chloride and sodium ions.

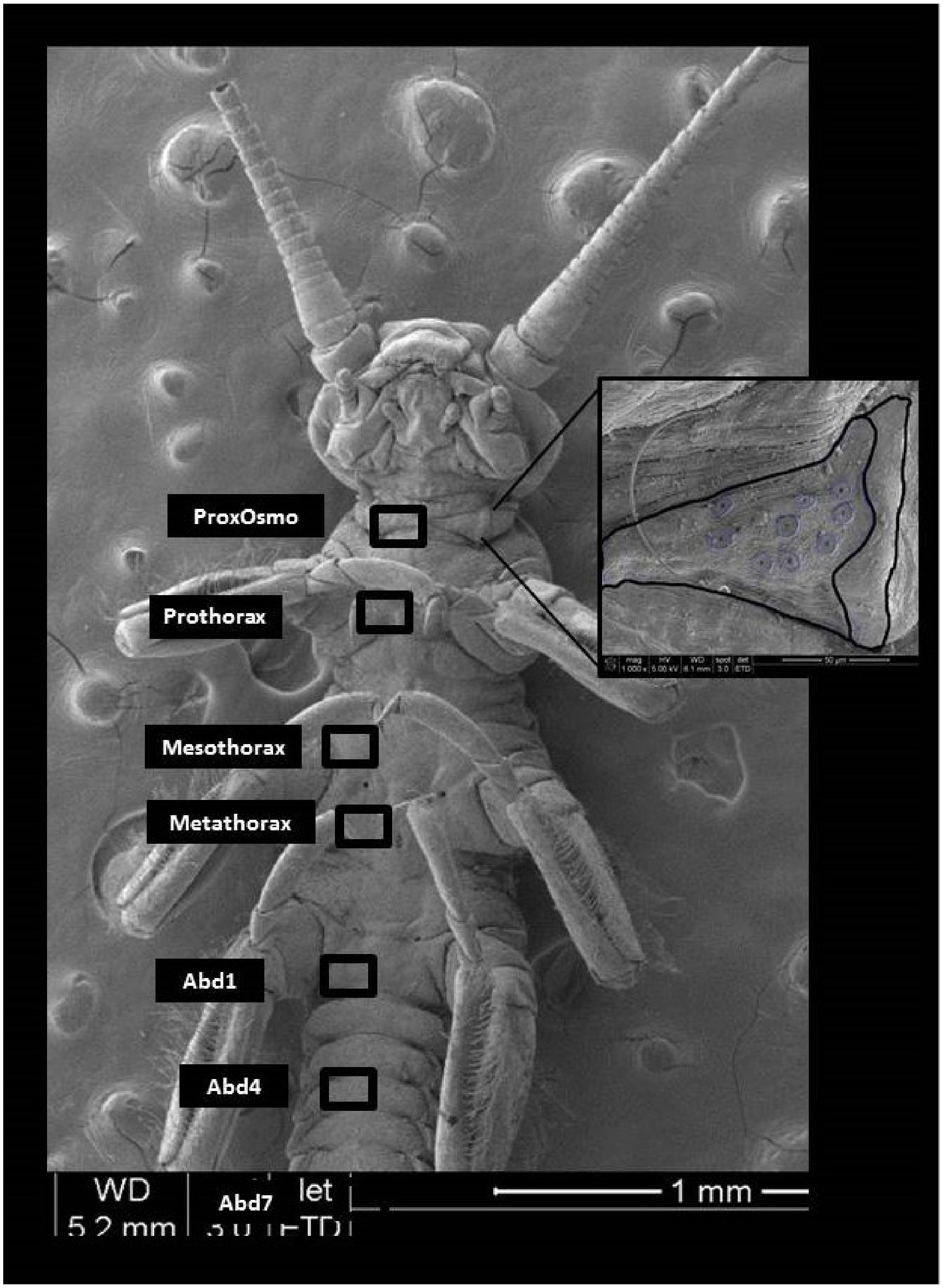


Figure 4.6. Taeniopterygidae body sections imaged for chloride cell analysis. 1) proximal segment of the osmobranchiae (inset with sclerotization and proximal segment outlined), prothorax, mesothorax, metathorax, abdomen 1, abdomen 4, and abdomen 7. Images were standardized at 1,000x magnification. Inset shows dark outline of the osmobranchiae as well as highlighted chloride cells.

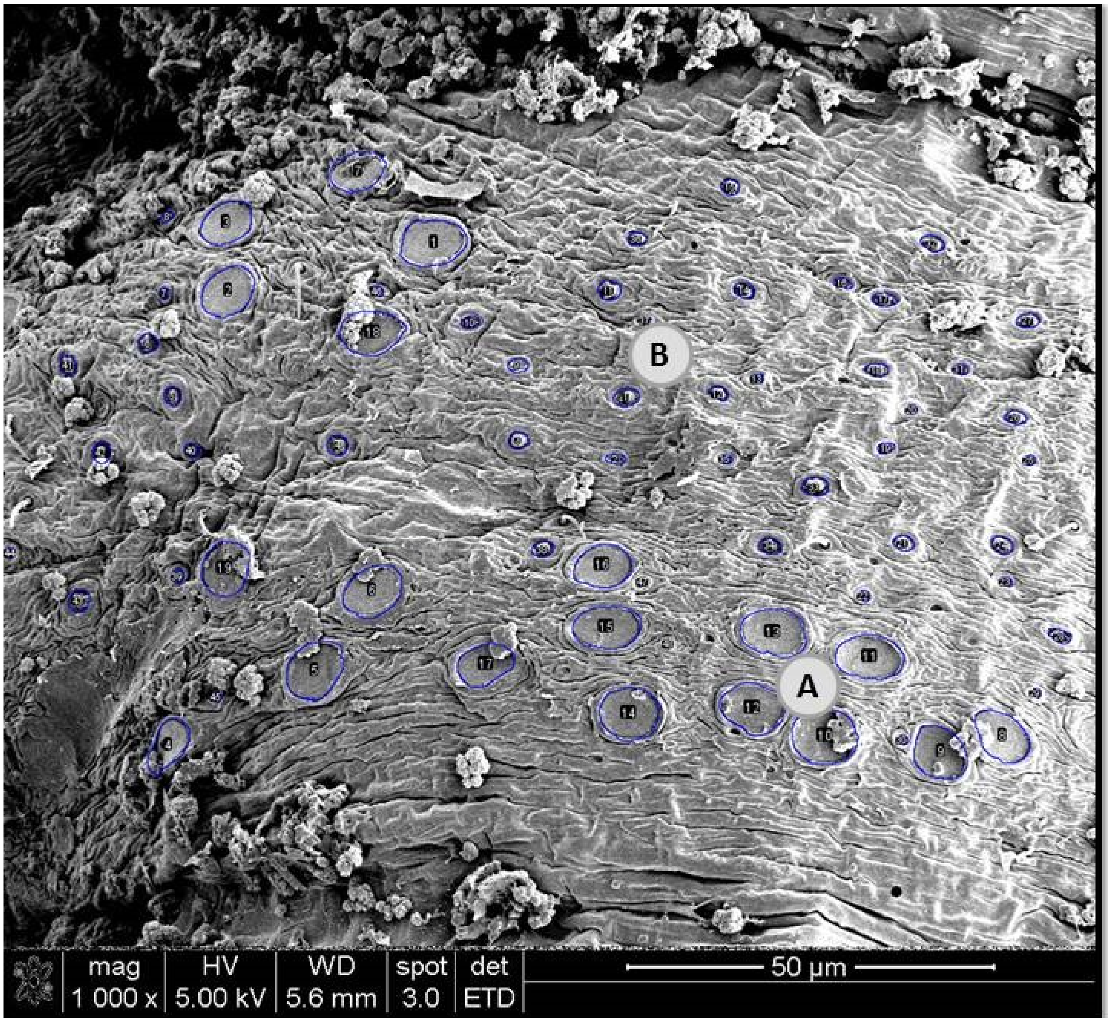


Figure 4.7. Coniform and caviform cells on Taeniopterygidae abdominal segment (refer to Figure 4.5) on Taeniopterygidae abdominal segment that was not treated with $AgNO_3$. Coniform (A) and caviform (B). Six response variables were generated from coniform and caviform cells for cell count, porous plate area (μm^2) and density (μm^2).

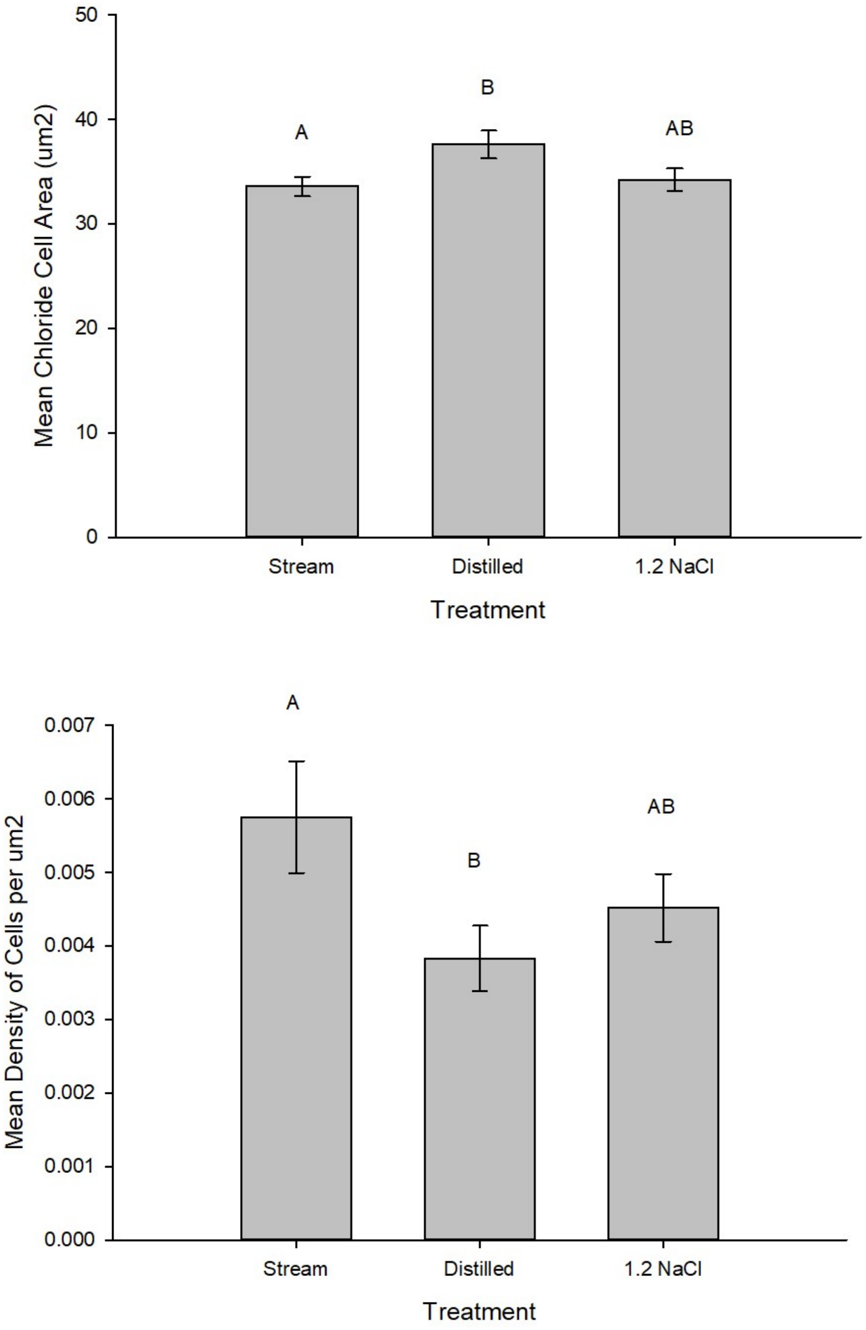


Figure 4.8. Chloroperlidae coniform cell response to EC. The mean chloride cell porous plate area (μm^2) ± SE (upper) and mean density of chloride cells (μm^2) ±SE (bottom). Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using Tukey's post-hoc tests.

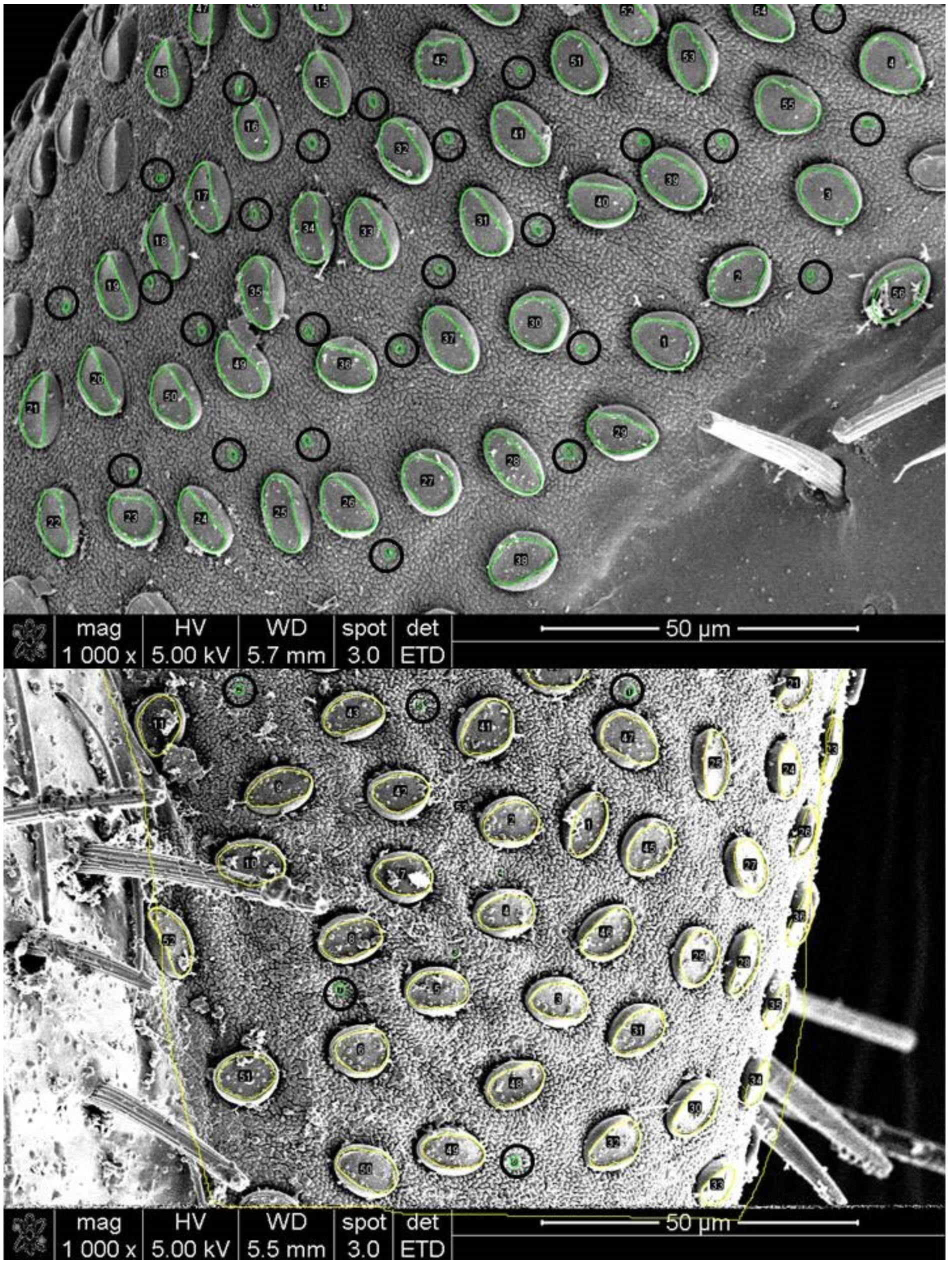


Figure 4.9. Caviform cells on Chloroperlidae pleural fold on abdominal segment 1. Caviform cells (circled in black) after exposure to distilled water control (top) and 20486 µS/cm treatment. The distilled water control image (top) has 25 caviform cells versus 5 caviform cells in the 1.2% NaCl treatment image.

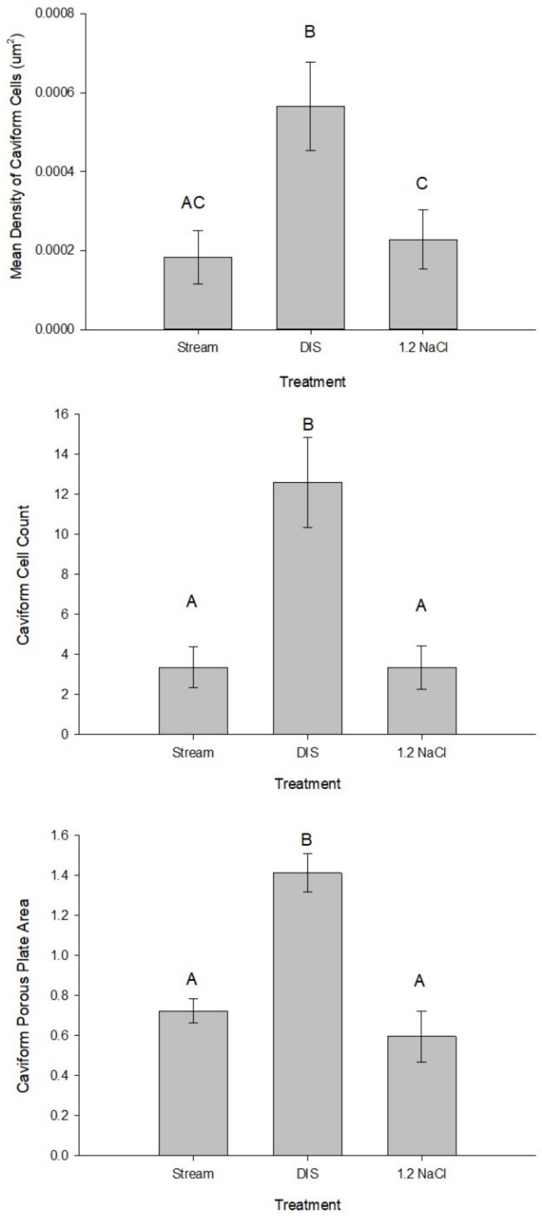


Figure 4.10. Response of Chloroperlidae caviform cells to salinity. The four imaged Chloroperlidae from 1.2% NaCl treatment all died within 96 hours, of the five imaged from the stream water control, two died within 96 hours and three were alive after 168 hours, and four were alive in distilled water after 168 hours. Mean density of caviform cells ± SE (top); mean count of caviform cells ± SE (middle); and mean area of caviform porous plates (µm^2) ± SE. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using Tukey's post-hoc tests.

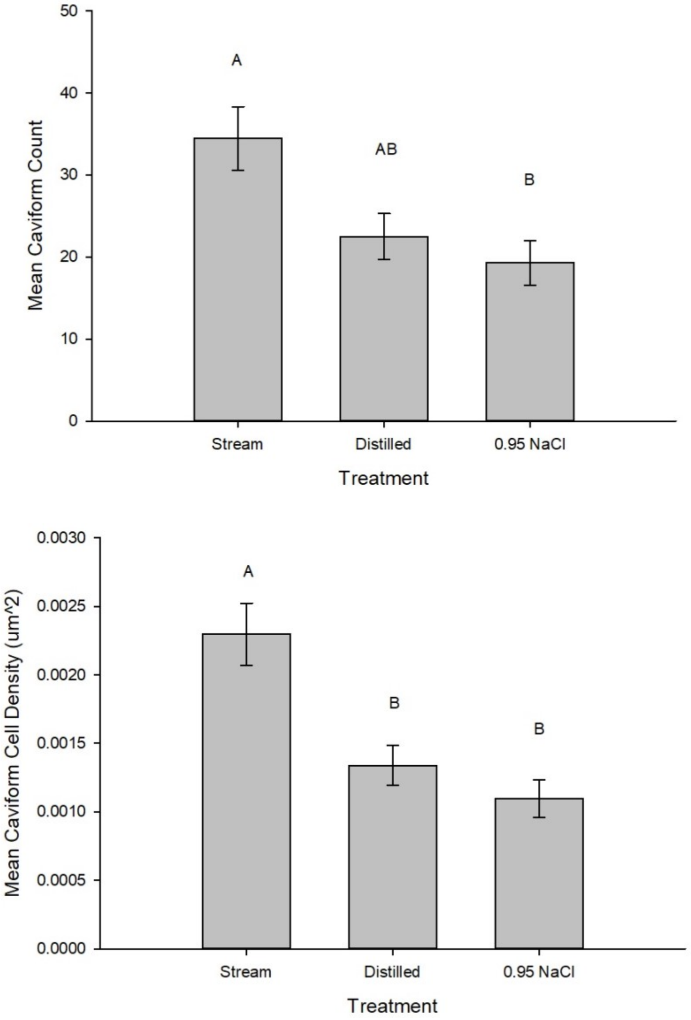


Figure 4.11. Taeniopterygidae caviform cell responses to salinity treatments. In 0.95% NaCl two imaged nymphs died within 48 hours and two died within 72 hours and in both distilled and stream water four imaged nymphs were alive at 128 hours. Significant differences in mean caviform count ±SE by body part (top) and mean density of cells (µm2) ±SE (bottom) were found. Means in columns with a different superscript are significantly different ($p \leq 0.05$, linear mixed effect model), using Tukey's post-hoc tests.

Chapter 5: Dissertation Conclusions

One of the pressing questions of our time is, “which species will survive a warming climate”? From polar bears to penguins and monarch butterflies, many of the vulnerable species are well-known due to their terrestrial habitats and tight connectivity with human cultures. However, cryptic insect larvae inhabiting streams are incredibly important to human society. Aquatic insects process detritus that assists with downstream nutrient cycling, are a food source for aquatic organisms, and serve as indicator organisms for stream health monitoring programs. Their status as water quality and environmental change indicators places aquatic insects on par with the importance of top predators such as polar bears, as benthic insects too will impact the extended food chain with their disappearance. The loss of cryptic aquatic insects will not be immediately felt by humans, but will have a cascading effect on ecosystems linked to aquatic ecosystems. In addition, their disappearance will impact our ability to detect environmental changes through aquatic biomonitoring.

The cryosphere is shrinking at a rapid pace due to anthropogenic climate warming. Aquatic insects colonizing metakryal zones ($T_{max} < 2^{o}C$) of glacier-fed streams are in the cross hairs of climate change due to their reliance on low temperature water and the sensitivity of glacier ice to radiative forcing, air temperature, and precipitation patterns. With the eventual loss of ice, the specialist insect communities living in the metakryal zones will most likely be replaced by generalist taxa that ubiquitously colonize groundwater streams with higher water temperatures and lower discharge volumes. Regardless of latitude or altitude, the species richness in glacier streams is independent of the regional species richness, as glaciers act as a filter limiting the number of adapted taxa able to colonize glacier streams. Even though the mountain range where this research takes place is in a global hotspot of biodiversity, taxa in the glacier streams are limited to only a few that are physiologically adapted to kryal conditions.

One of the first observations made during the winter of 2010 in the Mingyong Glacier mainstem was that there were populations of stoneflies and mayflies found close to the glacier, but the question was, "are these taxa present during the summer in kryal conditions at the same location?". The literature describing insect communities in glacier streams is largely based on the summer glacial melt season (e.g., Milner et al. 2001 and 2016), and the winter season is known to transform glacier streams into habitats similar to groundwater streams. During the summer, Diamesinae chironomids are the sole colonizers in the metakryal zones in the proposed Milner & Petts model of invertebrate community structure. Therefore, it was critical to sample during the summer season when the glacier discharge is torrential in order to compare Southeastern Tibet's glacier-fed stream communities with the Milner & Petts model.

Following the established Milner & Petts (M&P) model, water temperature and channel stability were examined as potential factors influencing patterns of invertebrate presence during the summer melt season in four glacier streams within two mountain ranges. The presence of invertebrates was then compared with the Milner & Petts model at a longitudinal distance from the glacier by temperature zones. The ***results of my research were a resounding departure from the M&P model***. The taxa richness in the metakryal zone in the Mingyong, Sinong, and Hailuogou Glacier mainstems was far greater than the M&P model, with fourteen taxa in addition to Diamesinae present (Chapter 2).

Several factors were considered as influencing biodiversity in the Mingyong metakryal zone. The latitude of the glaciers falls in the temperate latitude (29-30°N), but it is closer to the tropical zone than European glaciers. At a regional scale, the mass elevation effect (MEE) increases the atmospheric temperature of the interior of large mountain ranges and provides conditions for the treeline to move upslope to extremely high elevations above 4,500 m a.s.l. The MEE is prominent in two locations around the globe: 1) in the Central Andes at 18°S, and 2) in Southeast Tibet at 29-30°N. The mass elevation effect of the Tibetan Plateau and surrounding mountain ranges are undoubtedly playing a role in placing the glacier termini below the treeline, which is the highest in the world in the Southeast Tibetan region at 4,900 m a.s.l. Moreover, the collision of the Indian-Australian and Eurasian plate gave rise to the high prominence of the Southeast Tibetan mountains, which is reflected in the large glacier and stream sizes which could be creating a greater number of stream microhabitats and colonization niches for invertebrates. Due to the position beneath the treeline, the ability of Southeast Tibet's glacier streams to trap and retain particulate organic matter at the microhabitat level, and cascading effects on the invertebrate community structure was examined. During the microhabitat sampling in Mingyong during the winter, only anchor ice was observed in the mainstem. In fact, the perennial flow of water from ice-melt due to the position of some glaciers beneath the zero-degree isotherm may be allowing for greater insect diversity, including univoltine and semi-voltine insects that require a full year or longer to reach maturity.

Diamesinae are ice tolerant even when they become ice-bound, and are rapid colonizers of low temperature streams (Danks 1971). This may be a reason that only Diamesinae are found in metakryal sites of alpine glacier streams and glacier streams in higher latitudes following the M&P community structure in glacier streams. In the case of the Southeast Tibet glacier streams, the relatively low altitudinal position of the glaciers provides conditions for higher year-round basal temperatures than in alpine streams. This provides low temperature, perennial stream water which is necessary for the life cycles of semi-voltine taxa such as Nemouridae and Taeniopterygidae, which may not be able to colonize in streams that are frozen during the winter.

Lastly, because the salinity of groundwater streams is greater than glacier streams in general, the research question "will stoneflies living in metakryal zones in glacier streams be able to colonize groundwater stream conditions once the glaciers are gone?" was partially examined by conducting salinity lethality tests on stoneflies. Additionally, the response of chloride cells to the salinity concentrations was examined. Even though the results of the LC_{50} tests had overlapping confidence intervals and therefore the null hypothesis was not rejected, the survival of the insects did differ in the higher salinities (0.95% NaCl and 1.2% NaCl). Chloroperlidae survival was greater in both of these concentrations than Taeniopterygidae and Nemouridae. This result suggests that the downstream stoneflies may indeed be better adapted to higher salinity water. Moreover, both chloride cells and caviform cells on Chloroperlidae changed morphologically as a result of the treatments, which could be a result of the longer survival duration in higher salinity treatments.

The hydraulic characteristics, POM and periphyton, and invertebrate community structures were examined at the microhabitat level. Although the richness of invertebrates did not differ by microhabitat, greater abundance of insects were found in habitats with higher water velocities (runs, rapids, and step-rapids), which suggest that torrential glacial discharge during the summer in perennial streams may be less of a hindrance for colonization by insects than ephemeral water flow, which may limit colonization to those insects that tolerate ice and seasonally-dry conditions. Ice-free water flow in the metakryal zone is a potential reason for the difference in taxa richness between Southeast Tibet and European/North American glacier streams, which allows semi and univoltine invertebrates to colonize in the zone closest to the glacier. The basal temperature of the glaciers in Southeast Tibet are above freezing due to the low elevation beneath the zero degree isotherm of the atmosphere, therefore allowing glacial meltwater to contribute to stream flow year-round.

As a result of this research, the importance of understanding winter conditions in the metakryal zone of glacier streams was determined as paramount in predicting the summer taxa richness in the metakryal and hypokryal zones. The M&P model appears to be suitable for temperate and higher latitude alpine glacier streams, but most streams have not been examined or reported for winter conditions. Understanding which of the streams fitting the M&P model have frozen metakryal zones would be important in validating that this is the differentiating characteristic of Southeast Tibet monsoonal glacier streams that are warm-based glaciers. Schütz et al. (2001) performed a year-round study in Swiss Alps glacier-fed streams, which had snow coverage in the winter. Several of the upper reach sites were intermittent during the winter. The presence of frazil, surface, anchor, or solid ice, the size of the glacier and stream, and the mean air temperature near the metakryal zone during the winter may all be important variables to consider. Other parameters may help to answer the winter question and place the glaciers into different categories: 1) year-round glacier bed conditions (frozen or wet); 2) size of the glacier; 3) altitude of the glacier relative to the zero degree isotherm; 4) state of recession (e.g., stable, slightly receding, highly receding); and 5) specific glacier type (continental, mountain valley, cirque). Certain factors such as precipitation freezing altitude, accumulation type (winter or summer), and equilibrium line altitude of the glacier can support the question regarding the glacier's state of recession.

The results of this research suggest that the addition of a new conceptual model is in order to account for the distinct departure of Southeast Tibet's glacier streams from the M&P model. The high diversity in the metakryal zone may be due to the characteristics such as the sub-alpine position of the glacier termini, perennially-flowing water, large size of the glacier and stream, the wet-base of the glaciers, the constricted v-shaped valley where the Mingyong glacier-fed stream is located, and the monsoonal climate in the region. In order to bridge the gap and explain reasons for departures from the M&P model, winter data is seen as a potential defining feature of glacier streams. This will not be substantiated until data is collected during the winter season in temperate and high latitude glacial meltwater streams. The information could be as simple as "does the stream freeze through or have ice coverage during the winter?" The categories that glaciers are placed into today (e.g., warm-based glacier) will change with climate warming, so continuous monitoring of glaciers and glacial meltwater ecology is important in understanding which organisms will ultimately be impacted by disappearing glaciers.

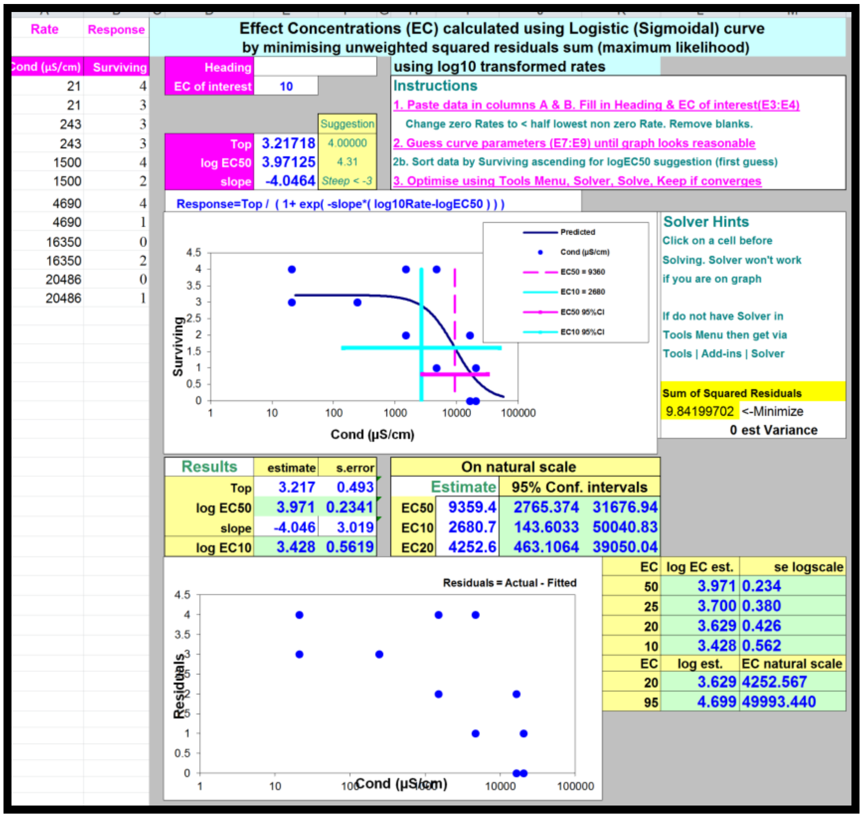
Appendix A

Table A.1. Insects that had early mortality or low collection numbers. The numbers in the columns represent the percent mortality at 120 hours, with numbers used to calculate the mortality % in parentheses. ms = morphospecies.

			Mortality (%) @ 120 hours by Treatment						
Stream	**Taxa**	**Nbr**	**Stream**	**Distilled Water**	**243 (µS/cm)**	**1500 (µS/cm)**	**4690 (µS/cm)**	**16350 (µS/cm)**	**20486 (µS/cm)**
M2	Nemouridae ms 1	34	33(2/6)	100(1/1)	67(4/6)	43(3/7)	33(2/6)	100(6/6)	100(2/2)
M2	Nemouridae ms 2	30	100(3/3)	100(4/4)	100(2/2)	100(9/9)	83(5/6)	100(4/4)	100(2/2)
M2	Nemouridae ms 3	2	-	-	-	100(1/1)	0(0/1)	-	-
M2	*Rhyacophila* spp.	4	-	-	0(0/1)	0(0/1)	0(0/1)	100(1/1)	-
M2	*Himalopsyche* spp.	1	-	100(1/1)	-	-	-	-	-
M2	Chironomidae	4	100(1/1)	-	100(1/1)	100(1/1)	0(0/1)	-	-
M1	Taeniopterygidae	22	100(3/3)	0(0/1)	67(2/3)	50(2/4)	80(4/5)	75(3/4)	50(1/2)
M1	*Rithrogena* spp.	1	0(0/1)	-	-	-	-	-	-
M1	*Ryacophila* spp.	1	-	-	-	-	-	100(1/1)	-
M1	*Epeorus* spp.	6	0(0/1)	100(3/3)	100(2/2)	-	-	-	-
N1	*Amphinemura* spp.	6	100(2/2)	0(0/1)	100(1/1)	-	0(0/1)	-	100(1/1)
N1	Baetidae	4	-	-	100(1/1)	100(1/1)	100(1/1)	100(1/1)	-
N1	Heptageniidae	3	0(0/2)	100(1/1)	-	-	-	-	-
N1	Nemouridae ms2	1	-	-	-	100(1/1)	-	-	-
L1	*Epeorus* spp.	43	100(43/43)	-	-	-	-	-	-
L1	*Rithrogena* spp.	35	97(30/31)	0(0/1)	-	-	-	100(1/1)	100(1/1)
L1	*Heptagenia* spp.	3	-	-	-	-	-	-	100(3/3)
L1	Baetidae	14	100(12/12)	-	-	-	-	-	100(2/2)
L1	Leptophlebiidae	1	100(1/1)	-	-	-	-	-	-
L1	Ephemerillidae	2	100(2/2)	-	-	-	-	-	-
L1	Chironomidae	1	100(1/1)	-	-	-	-	-	-
L1	Perlidae	10	50(3/6)	0(0/1)	0(0.1)	-	0(0.1)	-	-
GL3	Diamesinae	84	91(10/11)	15(2/13)	55(6/11)	33(4/12)	9(1/11)	100(12/12)	100(14/14)
GL3	*Himalopsyche* spp.	51	100(51/51)	100	100	100	100	100	100
H1	*Epeorus* spp.	58	100(8/8)	100(9/9)	100(8/8)	100(5/5)	100(9/9)	100(13/13)	100(7/7)
H1	*Rithrogena* spp.	6	67(2/3)	100(5/5)	-	-	-	-	-

Table A.1 continued

H1	*Amphinemura* spp.	1	100(1/1)	-	-	-	-	-	-
H1	Nemouridae ms 2	15	100(2/2)	100(3/3)	100(3/3)	100(2/2)	100(2/2)	100(1/1)	100(2/2)
H1	*Protonemura* spp.	2	100(1/1)	-	-	-	100(1/1)	-	-
H1	Chloroperlidae	7	-	50(2/4)	-	0(0/2)	-	-	0(0/1)
H1	Perlodidae	5	0(0/1)	100(1/1)	100(1/1)	-	100(1/1)	100(1/1)	-



Effect Concentrations (EC) calculated using Logistic (Sigmoidal) curve
by minimising unweighted squared residuals sum (maximum likelihood)
using log10 transformed rates

Rate	Response
Cond (µS/cm)	Surviving
21	4
21	3
243	3
243	3
1500	4
1500	2
4690	4
4690	1
16350	0
16350	2
20486	0
20486	1

Heading:
EC of interest: 10

		Suggestion
Top	3.21718	4.00000
log EC50	3.97125	4.31
slope	-4.0464	Steep < -3

Instructions

1. Paste data in columns A & B. Fill in Heading & EC of interest(E3:E4)
   Change zero Rates to < half lowest non zero Rate. Remove blanks.
2. Guess curve parameters (E7:E9) until graph looks reasonable
2b. Sort data by Surviving ascending for logEC50 suggestion (first guess)
3. Optimise using Tools Menu, Solver, Solve, Keep if converges

Response=Top / (1+ exp(-slope*(log10Rate-logEC50)))

Solver Hints

Click on a cell before Solving. Solver won't work if you are on graph

If do not have Solver in Tools Menu then get via Tools | Add-ins | Solver

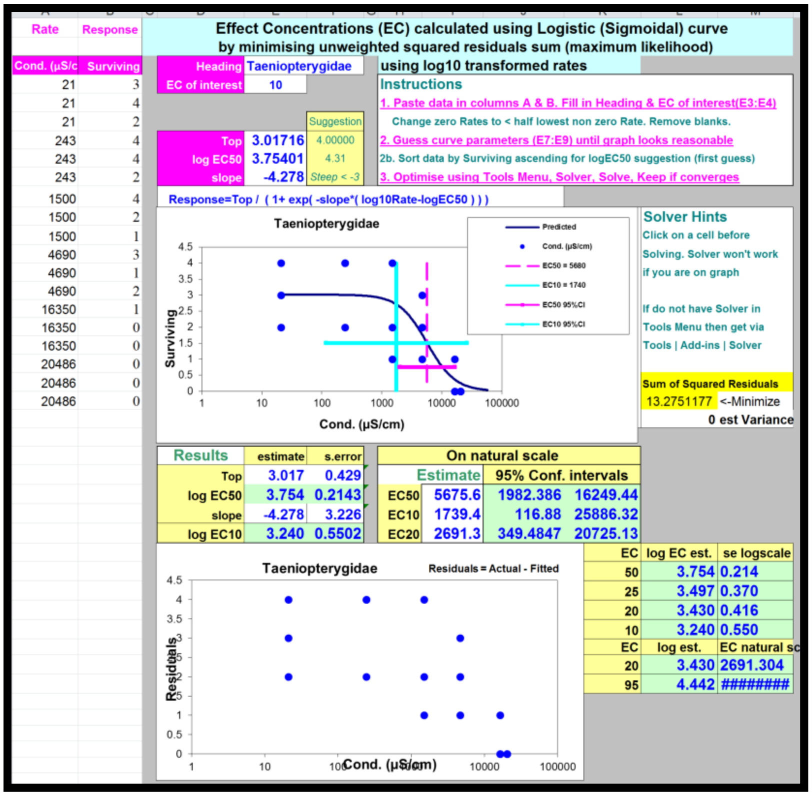
Sum of Squared Residuals
9.84199702 <-Minimize
0 est Variance

Results	estimate	s.error
Top	3.217	0.493
log EC50	3.971	0.2341
slope	-4.046	3.019
log EC10	3.428	0.5619

On natural scale			
	Estimate	95% Conf. intervals	
EC50	9359.4	2765.374	31676.94
EC10	2680.7	143.6033	50040.83
EC20	4252.6	463.1064	39050.04

EC	log EC est.	se logscale
50	3.971	0.234
25	3.700	0.380
20	3.629	0.426
10	3.428	0.562
EC	log est.	EC natural scale
20	3.629	4252.567
95	4.699	49993.440

Figure A.1. 120-hour LC_{50} salinity curve for Chloroperlidae as represented by conductivity (µS/cm) and survival on day 5 of the experiment.



Rate	Response
Cond. (µS/c	Surviving
21	3
21	4
21	2
243	4
243	4
243	2
1500	4
1500	2
1500	1
4690	3
4690	1
4690	2
16350	1
16350	0
16350	0
20486	0
20486	0
20486	0

Effect Concentrations (EC) calculated using Logistic (Sigmoidal) curve by minimising unweighted squared residuals sum (maximum likelihood) using log10 transformed rates

Heading	Taeniopterygidae	
EC of interest	10	
		Suggestion
Top	3.01716	4.00000
log EC50	3.75401	4.31
slope	-4.278	*Steep < -3*

Instructions

1. Paste data in columns A & B. Fill in Heading & EC of interest(E3:E4)

Change zero Rates to < half lowest non zero Rate. Remove blanks.

2. Guess curve parameters (E7:E9) until graph looks reasonable

2b. Sort data by Surviving ascending for logEC50 suggestion (first guess)

3. Optimise using Tools Menu, Solver, Solve, Keep if converges

Response=Top / (1+ exp(-slope*(log10Rate-logEC50)))

Solver Hints

Click on a cell before Solving. Solver won't work if you are on graph

If do not have Solver in Tools Menu then get via Tools | Add-ins | Solver

Sum of Squared Residuals

13.2751177 <-Minimize

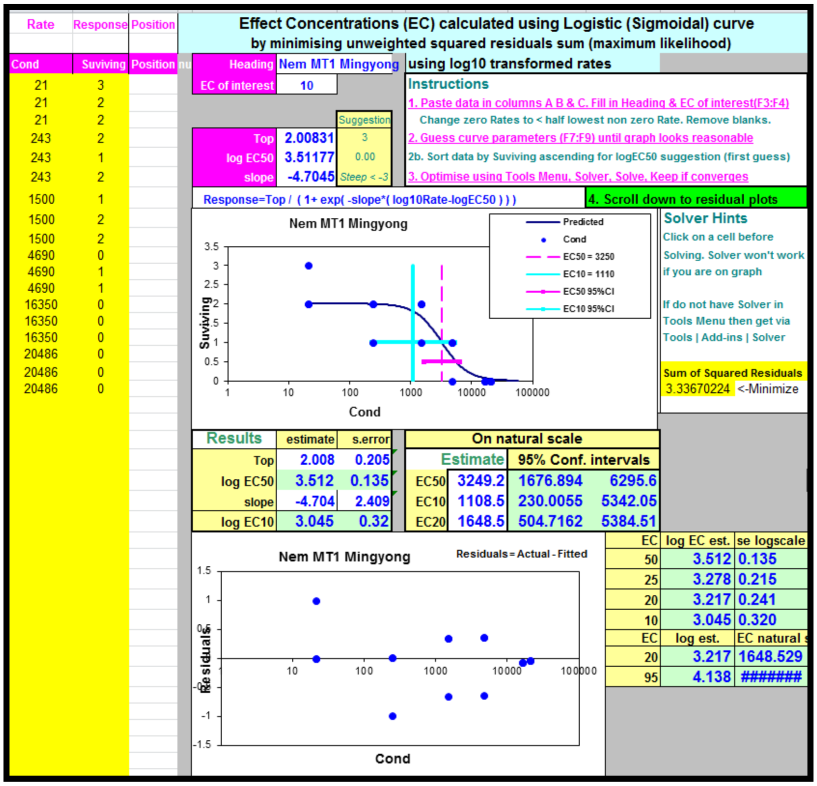
0 est Variance

Results	estimate	s.error
Top	3.017	0.429
log EC50	3.754	0.2143
slope	-4.278	3.226
log EC10	3.240	0.5502

On natural scale			
	Estimate	95% Conf. intervals	
EC50	5675.6	1982.386	16249.44
EC10	1739.4	116.88	25886.32
EC20	2691.3	349.4847	20725.13

EC	log EC est.	se logscale
50	3.754	0.214
25	3.497	0.370
20	3.430	0.416
10	3.240	0.550
EC	log est.	EC natural sc
20	3.430	2691.304
95	4.442	########

Figure A.2. 120-hour LC_{50} curve of salinity concentrations for Taeniopterygidae as represented by conductivity (µS/cm).



Effect Concentrations (EC) calculated using Logistic (Sigmoidal) curve
by minimising unweighted squared residuals sum (maximum likelihood)
using log10 transformed rates

Rate	Response	Position
Cond	Suviving	Position
21	3	
21	2	
21	2	
243	2	
243	1	
243	2	
1500	1	
1500	2	
1500	2	
4690	0	
4690	1	
4690	1	
16350	0	
16350	0	
16350	0	
20486	0	
20486	0	
20486	0	

Heading: Nem MT1 Mingyong

EC of interest: 10

		Suggestion
Top	2.00831	3
log EC50	3.51177	0.00
slope	-4.7045	Steep < -3

Response=Top / (1+ exp(-slope*(log10Rate-logEC50)))

Instructions

1. Paste data in columns A B & C. Fill in Heading & EC of interest(F3:F4)
Change zero Rates to < half lowest non zero Rate. Remove blanks.
2. Guess curve parameters (F7:F9) until graph looks reasonable
2b. Sort data by Suviving ascending for logEC50 suggestion (first guess)
3. Optimise using Tools Menu, Solver, Solve, Keep if converges
4. Scroll down to residual plots

Solver Hints
Click on a cell before Solving. Solver won't work if you are on graph

If do not have Solver in Tools Menu then get via Tools | Add-ins | Solver

Sum of Squared Residuals
3.33670224 <-Minimize

Results	estimate	s.error
Top	2.008	0.205
log EC50	3.512	0.135
slope	-4.704	2.409
log EC10	3.045	0.32

On natural scale			
	Estimate	95% Conf. intervals	
EC50	3249.2	1676.894	6295.6
EC10	1108.5	230.0055	5342.05
EC20	1648.5	504.7162	5384.51

EC	log EC est.	se logscale
50	3.512	0.135
25	3.278	0.215
20	3.217	0.241
10	3.045	0.320
EC	log est.	EC natural
20	3.217	1648.529
95	4.138	#######

Figure A.3. 120-hour LC_{50} curve of salinity concentrations for Nemouridae morphospecies 1 as represented by conductivity (µS/cm).

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