BENEFITS AND COSTS OF *LEPTOPHLEBIA* (EPHEMEROPTERA) MOVEMENTS BETWEEN RIVER CHANNELS AND FLOODPLAIN WETLANDS

by

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(Under the Direction of Darold P. Batzer)

ABSTRACT

Linkages between river channels and floodplain wetlands are important for organisms in each habitat, but especially for species that use both habitats. Nymphs of certain mayflies undergo seasonal movements into floodplains. High abundances of leptophlebid nymphs have been observed in Southeastern US floodplains, but how and why they colonize and develop in these temporary habitats has not been established. The benefits and costs of *Leptophlebia* mayfly movements into temporary floodplain wetlands were studied through descriptive observations and field experiments. While mayflies actively migrated into floodplains, few environmental (i.e. temperature, predation, food quality or abundance) advantages were apparent in wetland compared to river habitats. Despite this, mayflies had higher wetland growth rates and were adapted to tolerate short-term drying typical of floodplain habitats. The reasons why mayflies moved into floodplains remain ambiguous and may be attributed to avoiding swift river flows or an evolutionary relic behavior from more northerly climate conditions.

INDEX WORDS: Floodplains, Ecotones, Migration, Aquatic Invertebrates, *Leptophlebia*, Wetlands

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INTRODUCTION

Transitional zones between habitats, or ecotones, can be very important foci of ecological interactions and biological diversity (Risser 1995, Huftkens et al. 2009). Ecotones are found in a variety of environments, from elevation changes in mountain-lowland (Lingua et al. 2008) and estuarine-marine habitats (Ray 1991, Martino and Able 2003) to lateral shifts along forest-grassland (Coop and Givnish 2007) and river-floodplain edges. Floodplain wetlands are ecotonal habitats that connect river channels and the adjacent floodplains through seasonal flooding (Junk and Wantzen 2006). In the Southeastern U.S., the majority of freshwater wetlands are located on floodplains (Smock 1999, Sharitz and Pennings 2006). These wetlands can be highly productive because of the exchange of water, nutrients, and organic matter between the river and the floodplain during high water periods. Due to this unique hydrology, vegetation patterns are distinct between floodplains and the adjacent upland habitat (Sharitz and Pennings 2006). Fish movements between river channels and floodplains for spawning, nurseries and foraging have been extensively studied (Wellcomme 1979, Ross and Baker 1983, Winemiller and Jepsen 1998). Floodplain wetlands are also an important habitat for a variety of macroinvertebrates that have adapted their behavior and life histories to exploit these temporary habitats (Smock 1999, Wissinger 1999, Huryn 2002).

Interactions in floodplain ecotones can be disrupted if either of the adjacent habitats or the floodplains themselves are significantly altered by natural or human impacts (Galat et al. 1998, Wissinger 1999, Junk and Wantzen 2006). Mechanisms to regulate rivers, such as dams, levees, and channelization, alter natural flood pulses and interrupt biotic and abiotic exchange among the river, upland, and wetland (Henry and Amoros 1995). If wetland-river connectivity is disrupted, organisms adapted to exploit floodplains could have impaired dispersal, foraging, reproduction, and growth. At particular risk are organisms, such as some macroinvertebrates and fish, that seasonally move from rivers into the floodplains (Galat et al. 1998, Winemiller and Jepsen 1998, Huryn 2002).

The seasonal nature of floodplain wetlands has led to a range of colonization strategies by macroinvertebrates including: aerial dispersal, desiccation tolerance, aestivating eggs, and drifting or migrating into floodplains during flood events (Smock 1999, Tronstad et al. 2007). Movements from river channels into floodplains might be the only option for some aquatic macroinvertebrates to exploit the wetlands if they cannot withstand long periods of desiccation. Smock (1999) found that the majority of macroinvertebrate individuals (88-99%) moving from stream channels colonized via passive drift, but actively crawling species accounted for 73% of the total biomass. Active migration into floodplains has been observed in a variety of insect orders such as dobsonflies (Megaloptera) and caddisflies (Trichoptera; Smock 1994), however, the practice has been observed most commonly with mayflies (Ephemeroptera; Smock 1999). Nymphs of some mayfly species have been observed in mass migrations up outflows of floodplains (Neave 1930, Olsson and Soderstrom 1978, Gibbs and Mingo 1986, Huryn and Gibbs 1999). In one Maine floodplain wetland, actively migrating mayflies contributed 90% of the total macroinvertebrate biomass (Huryn and Gibbs 1999).

The movements of various mayfly species (but especially the genus *Leptophlebia*) between river channels and floodplains have been studied almost exclusively in northern latitudes including: Manitoba (Neave 1930), Sweden (Olsson and Soderstrom 1978), and Maine (Huryn and Gibbs 1999). High *Leptophlebia* abundances in more southerly latitude floodplain wetlands have been reported from Virginia (Smock 1999) and Georgia (Reese and Batzer 2007), suggesting this phenomenon might be more widespread. However, details of *Leptophlebia* life histories have not been examined in southern locations, and many of the factors that are believed to affect northern populations (temperature, ice scour) may not apply.

The most extensively studied example of mayfly movements has been in Tomah Stream, Maine. Huryn (2002) found that mayfly nymphs begin slow development in the river channel and migrate in during spring during seasonal flooding following snow melt. While in the wetland they undergo rapid development, emerge as adults and oviposit in the river channel. However, mayflies migrating into the floodplain risk desiccation if they cannot develop and emerge before the wetlands dry (Smock 1999, Huryn and Gibbs 1999).

Despite this risk there are various hypotheses for why it is more beneficial to move into the floodplain than stay in the river channel. One proposed hypothesis is that floodplain water temperatures are optimal for growth (Olsson and Soderstrom 1978, Huryn 2002). Temperature seems to be the driving force for high growth rates despite extremely high nymph densities in floodplain wetlands (Olsson and Soderstrom 1978, Sweeney et al. 1986, Gibbs and Siebenmann 1996, Huryn 2002). A second possible benefit of colonizing the floodplains is that food resources are more abundant (Neave 1930, Huryn and Gibbs 1999, Huryn 2002). While this is a common hypothesis, food resources have not been quantified for *Leptophlebia*. However, Huryn (2002) found they were depleted in ^{13}C after reaching a Maine floodplain suggesting that they were not feeding on the abundant grass detritus or periphyton. Under experimental conditions leaves with higher protein content have led to faster growth and higher fecundity in *Leptophlebia* (Sweeney et al. 1986). Third, by moving into the temporary habitats the mayflies might avoid predation by fish (Neave 1930, Olsson and Soderstrom 1978). However, while Soderstrom and Nilsson (1987) found that migrating mayflies had less risk of fish predation, they also determined that the threat from invertebrate predators increased in the wetlands. Fourth, it has been suggested that mayflies migrate to avoid high water velocities and ice scour in the rivers during spring floods (Neave 1930, Hayden and Clifford 1974, Olsson and Soderstrom 1978). While ice scour may be a factor in the northern temperate regions, river ice formation is rare in the Southeastern U.S.A. A fifth proposed hypothesis is that rivers lack adequate adult emergence sites (Soderstrom and Nilsson 1987). However, most *Leptophlebia* colonizing the floodplains are small and require a significant period of growth in the floodplains before emerging (Huryn 2002, M. Galatowitsch unpubl. data). Finally, inter-specific competition in the river may also drive particular species of mayflies to seek alternative resources in the floodplains (S. Burian, Southern Connecticut State University, person. commun.), but this hypothesis has never been tested.

I assessed the potential benefits and costs for *Leptophlebia* mayfly population movements across an ecotone in mid-sized floodplains through descriptive observations and performing field and laboratory experiments. I documented and described mayfly migrations onto the floodplain along with testing the following hypotheses that Leptophlebia mayflies migrate into floodplains because: 1) temperatures are higher, 2) mayfly food resources are more abundant and of higher quality, 3) mayflies have higher growth rates, 4) few *Leptophlebia* predators are present, 5) competition in the river limits mayfly growth rates, and 6) mayflies have sufficient desiccation resistance to withstand periods of wetland drying.

CHAPTER 2

METHODS

Study sites

I studied seasonal migrations of *Leptophlebia* mayflies from 2008 to 2009 in one floodplain wetland adjacent to the North Oconee River and a second along the Middle Oconee River, both located in the Piedmont ecoregion near Athens, GA, U.S.A. (33°59’15”N 83°23’10”W). The wetlands used in this study generally flood during the winter and spring when there is less evapotranspiration and the soils are more saturated (Reese and Batzer 2007). Although I focused on two locations, I have observed *Leptophlebia* in numerous floodplain wetlands of the Georgia Piedmont region.

North Oconee site

The North Oconee’s mean annual discharge is 14.35 m^3/s near my study site, but during flooding can increase to > 65 m^3/s and inundate much of the floodplain. The floodplain chosen for this study was 150 m wide and had frequent seasonal connections with the river channel. During moderate flood events at the site, floodwaters initially back-flow onto the floodplain through a single levee break (1–4 m wide), and then as floodwaters recede water flows back toward the river through the same gap. For small to moderate floods, the site operates much like a tidal channel. A constricted floodwater access channel (200 m long) connecting the levee gap and the main floodplain was a useful place to monitor mayfly movements between the river and floodplain. During large floods, river levels overtop the levee and the floodplain essentially becomes an extension of the river channel with water flowing through the wetland in parallel with the river. The size of the wetted area on the floodplain varies depending on river flooding. Between flood events many low areas (50–200 m^2) remain consistently inundated throughout the winter and early spring.

Middle Oconee site

The Middle Oconee floodplain had a much different flooding pattern than the North Oconee site. The channel of the Middle Oconee River is deeply incised and overflow from the river onto the floodplain occurs rarely. Depression wetlands on the floodplain instead fill from precipitation and runoff from the forested upland rather than with water from the river. Hydrologic connection between the floodplain and river occurs when the depression wetlands fill and then overflow into the river through ephemeral channels. At my study site, water infiltration from the wetland kept the short connection channel (65 m long) through the levee break inundated for much of the winter. The depression wetland itself was more ephemeral in terms of surface water and the size of the wetted area shift depending on precipitation. During 2009, when most research occurred, the depression wetland was only consistently flooded during April.

Mayfly Migrations

I established the timing and mode of *Leptophlebia* mayfly nymph colonization of the two wetlands through field collections and behavioral observations. At the North Oconee site, following major rain events two drift nets (30 cm x 45 cm with 363 µm mesh), one set against the water flow and another with, were installed in the levee breach where that floodplain connected with the river channel. Relative numbers of mayflies passively drifting with flow or actively moving against flow into or out of the floodplain were determined. During each flooding event mayflies were sampled every 12 hours (9:00 hrs and 21:00 hrs) for one-hour periods. Our preliminary studies showed that *Leptophlebia* exhibited no diurnal temporal patterns in movement, which has also been observed by Huryn (2002). Sampling was conducted twice daily for the duration of each flood event (ending when the connection between the floodplain and river was broken). Besides *Leptophlebia*, I also measured the movements of other abundant aquatic macroinvertebrate taxa during the flood events to determine if *Leptophlebia* behavior was unique. During each collection the connection channel width, depth, and water velocity was measured.

At the Middle Oconee site the ephemeral connection channel between the river and the wetland was too narrow to install the paired drift nets. However, the water draining from the wetland was relatively clear and shallow, permitting visual observations of mayfly movements. Here all mayfly movement into the floodplain was active because flow was always from the wetland towards the river.

Temperature

As ectotherms, mayflies migrating into floodplains could benefit from higher temperatures. Over the inundated period in both study sites, temperature was recorded continuously (every 10 min.) using HOBO Temperature Data Loggers. Two temperature loggers were each submerged in the wetland and river at the North Oconee site. One pair of loggers was transferred from the North Oconee to the Middle Oconee site when that wetland filled in early-April and they were maintained until the wetland dried in late-April. The remaining two temperature loggers at the North Oconee site were maintained until that wetland dried in early May.

Food Resources and Mayfly Diets

Another potential benefit of migrating into floodplains is more abundant food resources that might also be higher in quality. To assess quantity, I collected benthic organic matter in the North Oconee river channel and the floodplain wetland using a Wildco Ekman Grab (15 cm^2 bottom area; $N = 10$) in each habitat. Benthic material was dried at 50^{o}C for 24 h, weighed, combusted at 500^{o}C for 4 h, and reweighed to calculate AFDM.

To assess food ingestion, gut contents from *Leptophlebia* mayflies collected from the river (N = 10) and wetland (N = 15) at the North Oconee sites were analyzed for the types of material consumed using techniques in Eggert and Wallace (2007). Gut contents were identified on slide mounted membrane filters (0.45 μm) with an ocular grid at 200X magnification with a Leica DM-RXE microscope (Leica Microsystems, Inc., Bannockburn, IL, U.S.A.). For each slide, digital photographs were taken from 20 random fields of view. The area of all of the food items within each field of view was measured using Adobe Acrobat 7.0 Professional software.

To assess whether *Leptophlebia* nymphs were assimilating different foods in the river and wetland I collected nymphs from both habitat types at the North Oconee site and preserved them on ice until their tissues could be analyzed for C:N ratios, %N, %C, and δ^{13}C and δ^{15}N stable isotopes using a Thermo-Finnigan Delta^{plus} Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer (analysis conducted by

Colorado Plateau Stable Isotope Laboratory, Flagstaff, AZ, U.S.A.). Gut contents of nymphs were removed prior to analysis and mayfly tissues were pooled into samples of 20–40 individuals (0.6–1.2 mg total dry mass), dried at 50^{o}C for eight hours, and ground using a mortar and pestle (river: $N = 3$ pools, wetland: $N = 8$ pools).

Growth rates

Migrating mayflies could benefit from the combination of environmental and food advantages in the floodplain through faster growth rates. Relative differences in *Leptophlebia* mayfly growth rates in the river channel versus wetlands were determined using a cage experiment. Adapting methods used by Huryn (2002), cages were constructed from 10.2 cm internal diameter PVC, 7.5 cm high with two evenly spaced 4 by 8 cm side openings to permit water and dissolved oxygen flow. I used 500 μm nitex mesh to cover the openings and ends of the cages. Two weeks prior to each experimental run, leaf packs of air-dried red maple leaves (*Acer rubrum*), one of the dominant floodplain trees, were submersed in the wetland and others in the river to allow microbial conditioning. For the experiments I added 15 gm of wetted leaf mass to each cage, half with wetland litter and half with river litter.

The initial lengths of 40 mayflies collected from the North Oconee wetland population were measured and nymphs were placed individually in their own cage. To ensure growth would be detectable, I targeted the smaller individuals in the population (initial sizes 4.5–7.5 mm). Twenty of the chambers (10 with wetland leaves and 10 with river leaves) were individually anchored at random locations along the riverbank and allowed to sink to the channel bottom. The other 20 were individually submerged in the wetland at random locations but maintained near the water surface with Styrofoam floats (early experimentation indicated that submerging cages on the wetland bottom caused high mortalities).

Thus I created four treatments: 1) wetland environment-wetland leaves (floodplain control), 2) river environment-wetland leaves, 3) wetland environment-river leaves, 4) river environment-river leaves (river control). This full factorial design concurrently tested effects of habitat (wetland vs. river) and food (wetland conditioned vs. river conditioned).

After a two-week period the lengths of the mayflies were re-measured. The initial and final body lengths were converted to dry mass (DM) using length-mass regressions for *Leptophlebia* spp. (Benke et al. 1999),

$$\text{mg DM} = 0.0054L^{2.836}$$

where L is length in millimeters. I used AFDM values to estimate instantaneous growth rates (IGR) from changes in biomass over a given time interval (T):

$$IGR = \ln\,(B_2/B_1)\,/\,T$$

where B_1 and B_2 are the initial and final biomasses (Huryn 2002, Eggert and Wallace 2003). I repeated this experiment four times with new mayflies and conditioned leaves while the wetland remained inundated and mayflies were available (January–April 2009).

In the Middle Oconee site, I used similar experimental methods but due to the short duration of wetland inundation I conducted only a single run in April. In that site, I preconditioned red maple leaves in the river as before, but in the wetland I used preconditioned sedge (*Carex* sp.) because the wetland area was a sedge marsh rather than a forested wetland. Initial mayfly lengths ranged between 7 and 9.5 mm and were randomly assigned to the chambers to account for possible size-dependent factors. The four factorial treatments created were: wetland environment-wetland sedge leaves, river environment-wetland sedge leaves, wetland environment-river maple leave, and river environment-river maple leaves.

Predators

One benefit of migrating into floodplains might be to escape predation. I used a Smith-Root 12-B POW Backpack-Electrofisher equipment (Smith-Root, Inc., Vancouver, WA U.S.A.) to sample for fish along the length of the North Oconee floodplain connection channel and the interior of the floodplain wetland. Fish captured were euthanized using MS-222 buffered with calcium carbonate and then preserved in 1% formalin. The gut contents were removed and analyzed for invertebrate prey. Fish have never been observed in the Middle Oconee floodplain site under normal flood conditions.

A cost of migrating into floodplains might be increased predation from floodplain amphibians. I collected marbled salamander larvae (*Ambystoma opacum*; *N* = 50) at various stages of development from the North Oconee site and wood frog tadpoles (*Rana sylvatica*; *N* = 30) from the Middle Oconee site using D-nets (1.2 mm mesh). At each site these were the most common floodplain amphibians. Amphibians were euthanized using MS-222 buffered with calcium carbonate and preserved in 100% ethyl alcohol. The gut contents were removed and analyzed for invertebrate prey.

Competition

Another benefit of migrating into the floodplains might be escaping from intense competition in the river. Based on samples from the North Oconee River channel I determined that *Maccaffertium* (Heptageniidae) mayflies comprised 40% of the mayfly community, while *Leptophlebia* comprised < 2%; thus, I suspected that *Maccaffertium* would be the most likely competitor for *Leptophlebia*. Using the same cages and procedures as previously described for the growth rate experiment, I caged: 1) one *Maccaffertium* nymph with one *Leptophlebia* nymph ($N = 10$), 2) two *Leptophlebia* nymphs ($N = 10$), and 3) two *Maccaffertium* nymphs ($N = 10$). Nymphs of both genera were similar lengths. Each cage had 15 gm of red maple litter conditioned in the river. All 30 cages were submersed in the North Oconee River channel for three weeks, after which the lengths of all the mayflies were re-measured. The initial and final body lengths were converted to ash-free biomass using length-mass regressions (Benke et al. 1999) for *Leptophlebia* ($\text{mg DM} = 0.0054L^{2.836}$), and *Maccaffertium* ($\text{mg DM} = 0.0128L^{2.616}$), where L is length in millimeters. I used AFDM values for each mayfly to estimate instantaneous growth rates (*IGR*) from changes in biomass.

Desiccation tolerance

A cost of migrating into floodplains is the danger of being stranded in a drying habitat, and thus I hypothesize that *Leptophlebia* are adapted to tolerate drying. Desiccation resistance was tested with techniques adapted from Wissinger et al. (2003) using microcosms under controlled conditions in an environmental chamber. Thirty containers (2 L; 300 cm^2 bottom area) with a layer of substrate collected from the floodplain wetland (2 cm), preconditioned leaves (approx. 15 gm, wet mass), filtered floodplain water (250 µm mesh, 5 cm depth), and an individual

Leptophlebia mayfly from the floodplain wetland. The original water depth was maintained in six of the microcosms and in the 24 remaining containers water was gradually removed over seven days until they dried (no surface water). After 72 hours, six of the dried microcosms were rehydrated and survival was assessed over the next 48 hours. The remaining microcosms were separately rehydrated in groups after 144 hours, 216 hours, and 288 hours. At the end of the experiment the survival of nymphs in the six non-desiccation microcosms was rechecked. I hypothesized that mayflies that remain in the river would lack desiccation tolerance, so I used the same procedure to test *Maccaffertium* mayflies collected from the North Oconee River.

Statistical Analyses

Seasonality of the *Leptophlebia* movements into the North Oconee floodplain during each of the six major floods between December 2008 and April 2009 was tested using a one-way analysis of variance (ANOVA) with log (x+ 1) transformed numbers of *Leptoplebia* as the dependent variable. For each individual event, I assessed the directionality and timing of the *Leptophlebia* movements by analyzing the relative numbers of *Leptophlebia* [log (x+ 1) transformed] moving during the three stages of an individual flood (as water initially back-flowed in the wetland, when high water levels flowed through the wetland, and as receding water flowed out of the wetland). Separate one-way ANOVA tests were used to assess temporal patterns for active migration (against flow) and passive drift (with flow). Seasonality and directionality was also assessed for each of the other major aquatic macroinvertebrates co-occurring with *Leptophlebia* in the floodplain net samples. I used ANOVA tests (habitat x date) to test differences in daily mean and daily maximum temperatures between the wetland and river channel, at both the North Oconee and Middle Oconee sites.

I used paired t-tests to compare the mean AFDM of benthic organic matter, and mayfly C:N, %N, %C, $\delta^{13}C$, and $\delta^{15}N$ between the river and wetland at the North Oconee site. To contrast proportions of the different types of food found in the *Leptophlebia* gut contents I arcsin square root transformed data and then applied a two-factor ANOVA (habitat x food type).

To compare the mayfly growth rates between habitats in the four experimental periods at the North Oconee site I applied a three-factor ANOVA (habitat x leaves x date). Because I anticipated an effect of seasonality, mayfly growth rates from each monthly experimental period were analyzed individually using a two-factor ANOVA (habitat x leaves). The single experimental period at the Middle Oconee site was similarly assessed by a two-factor ANOVA. Growth rates of *Leptophlebia* or *Maccaffertium* mayflies under different competition regimes were compared with separate t-tests for inter- and intra-specific competition. I related *Leptophlebia* and *Maccaffertium* mortality rates independently to desiccation duration using Pearson's correlations.

Post-hoc pairwise comparisons for significant ANOVAs were conducted using the Tukey procedure (Zar 1999). All statistical tests used $\alpha = 0.05$, and were completed using Statistica version 6.0.

CHAPTER 3

RESULTS

Mayfly Migration

North Oconee Site

In the North Oconee site *Leptophlebia* spp. (*L. cupida* and *L. johnsoni*) and five other aquatic macroinvertebrate taxa (Ephemeroptera: Siphlonuridae - *Siphlonurus* and Ephemerellidae - *Eurylophella*; Plecoptera: Nemouridae - *Prostoia*; Diptera: Chaoboridae - *Chaoborus*, and Chironomidae) represented 98.1% by abundance of the total aquatic macroinvertebrate assemblage collected in the drift net samples. Over the season, six separate flood events were recorded (each separated when low water broke the surface connection between the river and the floodplain; Figure 1). For each of the six taxa highlighted, the second flood event of 2008–2009 had higher abundances (Tukey's $p < 0.05$) than all the other floods except for the first (Figure 2).

I used the second flood event to assess the directionality and timing of the aquatic insect movements because that was clearly when most of the aquatic insects were moving into or through the floodplain (Figure 2). Of the six dominant taxa only *Leptophlebia* and *Siphlonurus* had significant active migration (i.e., against the water flow) and this occurred primarily late in the flood event when water was flowing from the floodplain towards the river (ANOVAs, $F_{2,13} = 25.5$, $p < 0.0001$ and $F_{2,13} = 17.4$, $p = 0.0002$, respectively). Passive drift with the flow occurred for all six taxa (Figure 3). Passive drift was significantly higher during the outflow stage for *Siphlonurus* (Tukey's $p < 0.02$) and *Prostoia* (Tukey's $p < 0.01$) than the other flood stages

(Figure 3). However, relative levels in passive drift should be considered cautiously; water volumes were declining as the flow transitioned from through-flow to out-flow, so temporal differences might reflect insect concentration.

Middle Oconee Site

Leptophlebia were the only macroinvertebrates observed colonizing the floodplain from the river at the Middle Oconee site, possibly due to cascading flows from the connection channel as it entered the river. While some *Leptophlebia* managed to colonize the wetland during early flooding events in December and January, the wetland subsequently dried. Others remained in the flooded parts of the connection channel during that dry period. Once the upstream depression wetland refilled in April, *Leptophlebia* recolonized the habitat.

When migrating, *Leptophlebia* were primarily observed moving against the flow toward the wetland. Through cascading and more turbulent regions of the floodplain/river connection channel I observed hundreds of mayflies climbing along the channel edges through thin laminar flows of water. At one location a naturally occurring sediment/debris dam obstructed the connection channel where water percolated downstream subterraneously. Here, I observed mayflies crawling through narrow micro-channels (2-4 mm; 10–15 mayflies/min/pore) to continue their movement upstream towards the wetland.

Temperature

To assess potential ecological effects of temperature on *Leptophlebia* I compared temperature between the floodplain and river during different aspects of their life cycles: 1) when they were colonizing the floodplain, 2) when they were actively developing on the floodplain, and 3) when they were emerging. Colonization (mid-December 2008–mid-January 2009) was defined when 99.1% of the nymphs completed their movements into and through the floodplain (Figure 2). Growth (mid-January–mid-April 2009) was the period when nymphs remained in the wetland, until few nymphs were observed. Emergence (mid-April–May) was the period when predominantly final instar mayflies remained until the wetland dried. North Oconee water temperatures (both daily averages and daily maximums) in the wetland and river were similar ($p > 0.05$) when *Leptophlebia* were colonizing the floodplain and while they developed. Once the tree canopy closed in April (when the mayflies were emerging) temperatures were significantly lower in the wetland than the river (*t*-test, $t_{66} = 2.5$, $p = 0.01$; Figure 4).

At the Middle Oconee site, which was only fully filled in April, average daily temperature in the wetland and river were also similar. However, daily maximum water temperature was significantly higher in the wetland than the river **(***t*-test, $t_{24} = 5.59$, $p < 0.0001$), probably because here the wetland was open marsh rather than closed canopy forest as at the North Oconee site.

Food Resources and Mayfly Diets

Benthic organic matter (AFDM/m^2) in the wetland habitat was marginally higher than in the river ($p = 0.051$). During large flood events, however, I observed large quantities of leaves and woody debris being exported from the floodplain to the river. Substantial scour of leaves was obvious in the floodplain.

Gut analysis indicated that *Leptophlebia* nymphs were consuming similar food types in both the wetland and river floodplain (amorphous detritus 94.9%, diatoms 4.1%, fungi 1.0%). Carbon and nitrogen stable isotope signatures of the *Leptophlebia* nymphs from the floodplain and river were similar. However, mayflies from the river had higher %N (t-test, $t_9 = 2.4$, $p = 0.03$).

Growth Rates

Leptophlebia grew most rapidly late in the season (ANOVA, $F_{3,125} = 22.2$, $p < 0.0001$) and grew more rapidly in the floodplain than in the river ($F_{1,125} = 10.7$, $p = 0.0013$; Figure 5). Growth was independent of food source (river vs. wetland leaves), and no interaction was detected between the habitat and food source. Accelerated mayfly growth in the floodplain occurred only in February (49.2%, $F_{1,34} = 4.6$, $p = 0.039$) and April (46.8% faster; $F_{1,35} = 14.5$, $p = 0.0005$). In April, the treatment combination of the wetland leaves in the wetland habitat had faster growth than the other three treatments ($p < 0.05$). At the Middle Oconee site growth rates were not significantly different between either habitat types ($F_{1,30} = 0.44$, $p = 0.51$) or food sources ($F_{1,30} = 0.73$, $p = 0.39$).

Predators

In the North Oconee floodplain fish were commonly collected only in the area where the river connected with the floodplain, with Ocmulgee shiners (*Cyprinella callisema*) being the most frequently caught fish. In the floodplain interior only *Gambusia* spp. mosquitofish were collected. Of the fish collected only a single green sunfish (*Lepomis cyanellus*) had consumed *Leptophlebia*. In the floodplain interior, marbled salamander larvae (*Ambystoma opacum)* occurred commonly. Once the salamanders were sufficiently large (≥ 15 mm), most (approximately 80%) consumed *Leptophlebia* (1 to 6 individuals), at least until the mayflies had emerged. At the Middle Oconee site, the wood frog tadpoles (*Rana sylvatica*) examined had only consumed algae.

Competition

Overall *Leptophlebia* grew faster than *Maccaffertium* nymphs regardless of competition regime (t-test, $t_{24} = 2.48$, $p = 0.016$; Figure 6). However, within each taxon growth rates differences were similar between intra- and inter-specific conditions.

Mayfly desiccation tolerance

Every *Leptophlebia* nymph tested tolerated three days of drying but mortality increased linearly ($r^2 = 94.2$) to 83% after 12 days of desiccation. Only 40% of the *Maccaffertium* nymphs tested survived three days of drying, and thereafter I only recovered a single nymph (Figure 7). However, in the controls that remained flooded through the end of the experiments, all the *Leptophlebia* survived while all *Maccaffertium* perished. Thus, higher *Maccaffertium* mortality probably occurred from both desiccation and water stagnation.

CHAPTER 4

DISCUSSION

Leptophlebia and *Siphlonurus* movements into the floodplains was an active, rather than a passive phenomenon. *Leptophlebia* and *Siphlonurus* mayfly nymphs accounted for 45% and 37% of the active movements toward the North Oconee floodplain. There was definite seasonality to movements with dispersal occurring early in the flood season even though high floods occurred later in the season (Figure 2). Similar results were observed in Maine where *L. cupida* actively migrated into the floodplain early in the flood season, however there *Siphlonurus* entered later in the season (Huryn 2002).

The active nature of leptophlebid migration behavior might have developed to take advantage of historically widespread beaver-built impoundments along floodplains. As I observed at the Middle Oconee site, navigating debris dams and water cascades were not major obstacles for *Leptophlebia* and has also been seen in other studies of *Leptophlebia* (Neave 1930, Hayden and Clifford 1974). Regardless of the cause, it is clear that leptophlebids are strongly motivated to move from rivers into floodplains.

Migratory behavior has frequently developed as a response to large-scale environmental gradients (e.g., seasonal climate and elevation) and/or smaller scale influences (e.g., local predation pressure and food limitations). Harsh environmental conditions during winter months drive southerly migrations of birds (Visser et al 2009) and monarch butterflies (Urquhart and

Urquhart 1978). Elk in mountainous areas seek refuge in valleys during winter to enable foraging (Frair et al. 2005). Other species, such as salmon (Hoffnagle et al. 2008) undergo large-scale movements to use optimal reproductive habitats. While mechanisms of these migratory behaviors are easily understood, reasons why *Leptophlebia* nymphs migrate onto seasonal floodplains are not so obvious.

Of the hypotheses proposed for explaining benefits of seasonal mayfly nymph movements between the river channel and floodplain wetlands, I can immediately eliminate two for this system, the avoidance of river ice scour (Neave 1930, Olsson and Sodestrom 1978) and wetlands being better adult emergence sites (Soderstrom and Nilsson 1987). In northern areas avoidance of crushing ice flows in the river channels has been proposed as a possible benefit for their movements onto the floodplain (Neave 1930, Olsson and Soderstrom 1978). However, river ice formation in my study area is minimal, and in fact ice formation in the floodplains is much more common. While some aquatic insects migrate to shallows or uplands when ready to emerge, I found, as supported by Neave (1930) and Huryn (2002), that mayfly nymphs colonizing the floodplains were various sizes (3–10 mm) that still require a significant period of growth before emerging. They were migrating into the floodplains weeks or months before emergence occurred.

Intuitively I assumed there would be higher temperatures in the floodplains than the river channel, as observed by Olsson and Soderstrom (1978), but this was not necessarily true. At the North Oconee site, which is typical of forested floodplain wetlands of the Southeastern US wetlands (Smock 1994), temperatures (average or daily maximums) were the same (or lower) in the floodplain than the river. The Middle Oconee site had higher daily maximum temperatures. There the wetland was a sedge meadow with an open canopy, as in Hayden and Clifford (1974), Olsson and Soderstrom (1978), and Huryn (2002). But despite higher temperatures, mayfly growth rates were not more rapid. Overall, I found no compelling evidence that higher temperatures were inducing mayflies to migrate into the floodplains, or were stimulating higher growth rates.

I also found that floodplains provided few benefits in terms of food quantity or quality, in comparison to the river. While standing crop biomass of benthic organic material was somewhat greater in the North Oconee wetland than the river, I observed that over the course of the season there was substantial export of leaves and other organic material from the floodplain to the river during high flood events. During the largest flood events, leaf scour exported a large amount of floodplain leaves into the river (as evidenced in my drift net samples). Mayflies were migrating from the river to the floodplain at the same time leaf litter volume on the floodplain was decreasing and volume was increasing in the river. If they were focused on exploiting leaf litter, it would seem more prudent to remain in the river and use the material being delivered by floods. Thus, it seems unlikely that leaf litter quantity would induce migration.

In terms of food quality I found that mayflies were generally consuming or assimilating similar food sources in floodplains and river. Only tissue percent nitrogen levels differed, and they were higher in the river. If anything, food quality was actually higher in the river. In other temporary wetlands, Inkley et al. (2008) found that food quality can be low because fungal and bacterial biomass is reduced. In that study a caddisfly shredder preferred litter from a permanent wetland than a temporary wetland.

One clear finding from this study, at least at the North Oconee site, was the environmental benefit to colonizing the floodplains through faster growth rates. Overall at the North Oconee sites there was 36% greater growth in the wetland treatments than in the river. In Maine floodplain wetlands Huryn (2002) measured Leptophlebid growth rates in a similar manner to this study, which were attributed to higher seasonal water temperatures. However, in our system, temperature differences between the river and floodplain either were not substantial enough to influence their growth rates (North Oconee site) or did not elicit differences in growth (Middle Oconee site).

Potentially floodplain characteristics other than temperature or food quality or quantity are regulating growth. Possibly simply avoiding flow provides energetic benefits. The differences in growth rates between the river and wetland habitats could have been a result of the energetic costs of remaining in the river, as growth could not be attributed to differences in temperature or food quality. In studies of lateral distributions in rivers, leptophlebids have been typically associated with slow-moving shallow water or river margins (Olsson 1983, Burian 2001). However, *Leptophlebia* are defined as inhabiting erosional, not depositional habitats (Waltz and Burian 2008), and at the Middle Oconee site, migrating mayflies endured harsh flow environments in order to reach the floodplain. Leaving the high flow conditions of the river for the often stagnant conditions on the floodplain may introduce hypoxia as a threat.

Whether migrating into the floodplain would reduce predation pressure on mayflies was ambiguous. While fish numbers were low on the floodplains mayflies were actively migrating during daylight when any threat from fish would be greatest. When Hayden and Clifford (1974) analyzed fish gut contents for *L. cupida*, they found few. Soderstrom and Nilsson (1987) examined fish guts between river and floodplain and found fish predation on *Parameletus* mayflies was greater in the river. They suggested fish on the floodplain were spawning rather than actively feeding.

While the threat from fish may be diminished, migration into floodplain increased the threat from salamander predation. *Leptophlebia* were a significant portion of the salamander larval diets at the North Oconee site, and amphibians are common predators in many floodplain wetlands of Georgia (Schultheis 2005). Other studies have suggested insect predators pose an additional threat to mayflies that migrate into the floodplains (Hayden and Clifford 1974, Soderstrom and Nilsson 1978, Huryn and Gibbs 1999). Migrating into floodplains may just trade one threat (fish) for another (amphibians and predaceous insects).

One advantage to moving from the river into the floodplain could have been to avoid competitive pressures from other mayflies. Yet I found that *Leptophlebia* growth rates were not hindered by the presence of the dominant river mayfly, *Maccaffertium*. This suggests competition is not an energetic cost to *Leptophlebia* that remain in the river and is an unlikely driver of migration.

Besides the threat from floodplain predators, migrating to floodplain wetlands introduces the additional threat of being stranded and drying. I observed numerous mayflies that had perished in dry pools on the floodplain. However, I found *Leptophlebia* nymphs could tolerate short-term desiccation, which perhaps allows them to better withstand short lapses in rain and flooding. The river mayfly, *Maccaffertium*, did not have this capability. Neave (1930) noted that temporarily dry habitats replenished by rain supported leptophlebid nymphs, also suggesting desiccation tolerance. Kosnicki (2005) found that *Siphlonurus typicus* mayflies were able to survive 70 hours without water, and Nolte et al. (1996) found *Cloeodes hydation* (Baetidae) was able to survive 9 hours.

Conclusions

In figure 8, I summarize my findings on the relative benefits and costs of mayflies moving from river channels into floodplains. While numerous hypotheses have been proposed for why leptophlebid mayflies move between rivers and floodplains, my data suggest that for populations in Georgia mechanisms remain ambiguous. Other mechanisms may be involved or could be a combination of costs and benefits.

Perhaps despite the dramatic nature of the behavior, there is no pronounced benefit from migrating, migratory behavior may be an evolutionary relic. The Georgia Piedmont is towards the southern edge of *Leptophlebia cupida* and *L. johnsoni* ranges (Burian 2001). Those aspects of floodplains that benefit northern populations may not be relevant in Georgia, but there may be no selective pressure to abandon the behavior.

While the mechanisms driving leptophlebid migration into floodplain wetlands may be uncertain, linkages between the river and floodplain remain important for the life history of these mayflies.

In terms of ecotonal interaction, these mayflies represent an important ecological connection between the river and floodplain (Huryn 2002). In mid-sized river systems the floodplains are narrow and have significant exchanges of biota with the river during flooding (Reese and Batzer 2007). With the importance of ecotonal interactions, small rivers may be at particular risk from river regulation or changes in regional climate patterns that could restrict river-floodplain connection.

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FIGURES

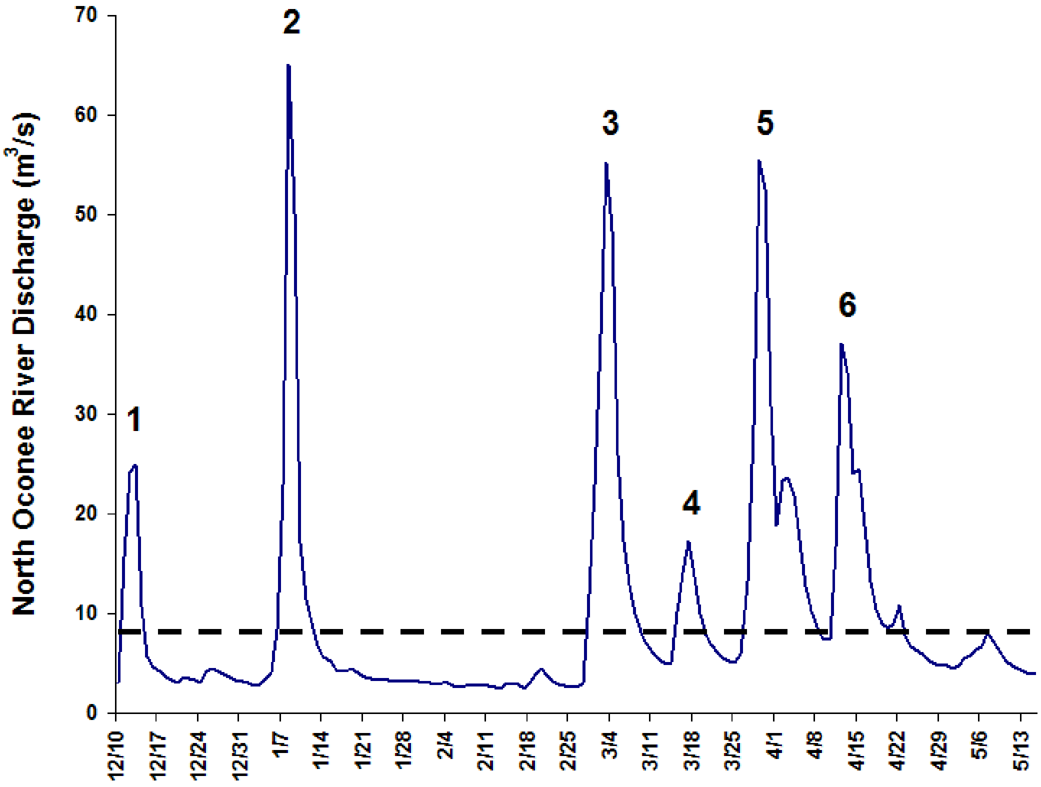

Figure 1. Hydrograph of the North Oconee River immediately downstream from the study site showing discharge through the study period from December 2008 through May 2009. Major flooding events are denoted by numbers. Line indicates when the floodplain disconnects from the river channel at the North Oconee study site. Daily measurements were taken by the U.S. Geological Survey (http://waterdata.usgs.gov/nwis/uv?02217770).

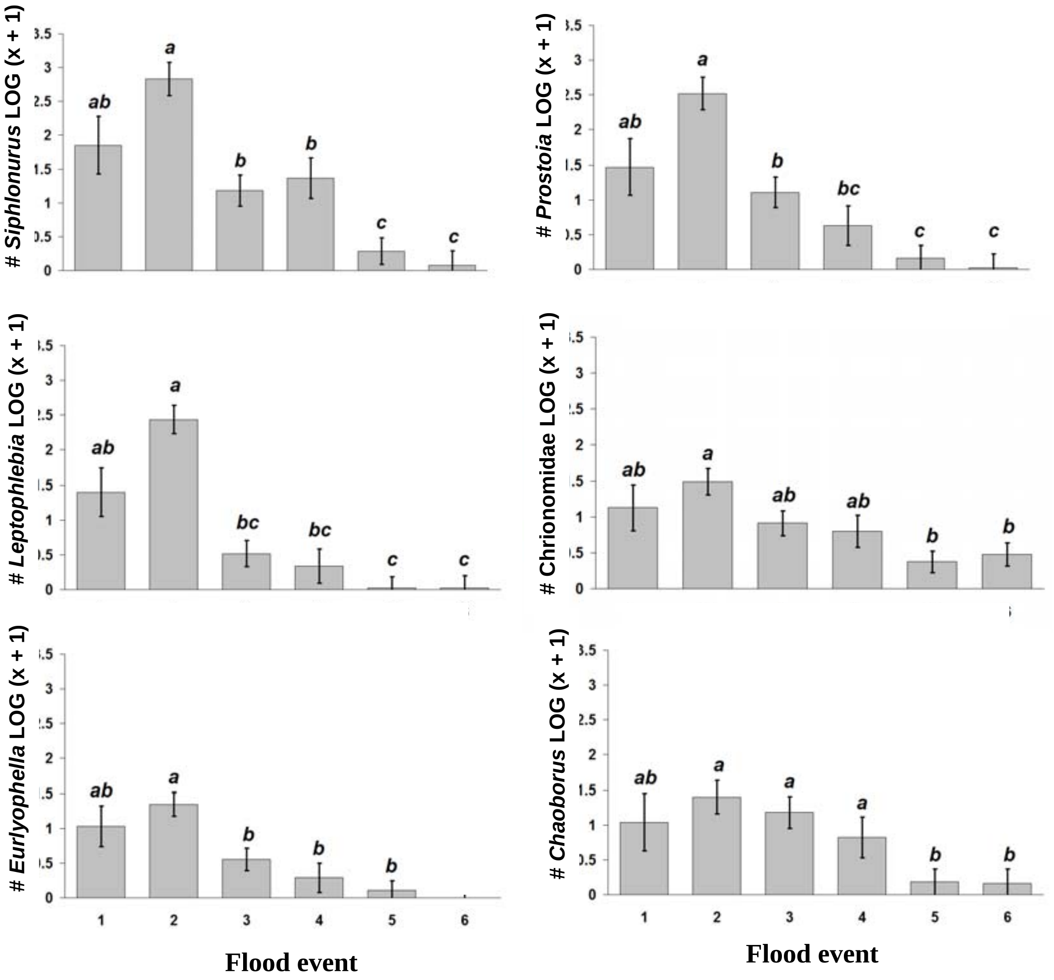

Figure 2. Mean number of six dominant nymph and larval aquatic insects caught in drift nets placed in the North Oconee river-floodplain levee breaks for the duration of six flooding periods depicted in Figure 1. Letters indicate variation among events for each taxon (*Leptophlebia* (ANOVA, $F_{5,48} = 22.8$, $p < 0.0001$), *Siphlonurus* ($F_{5,48} = 18.8$, $p < 0.0001$), *Prostoia* ($F_{5,48} = 17.4$, $p < 0.0001$), *Euryophella* ($F_{5,48} = 9.59$, $p < 0.0001$), Chironomidae ($F_{5,48} = 5.67$, $p = 0.0003$), *Chaoborus* ($F_{5,48} = 5.67$, $p = 0.0003$)) and for every organism peak movements occurred during the second event. Error bars represent ± 1 SE.

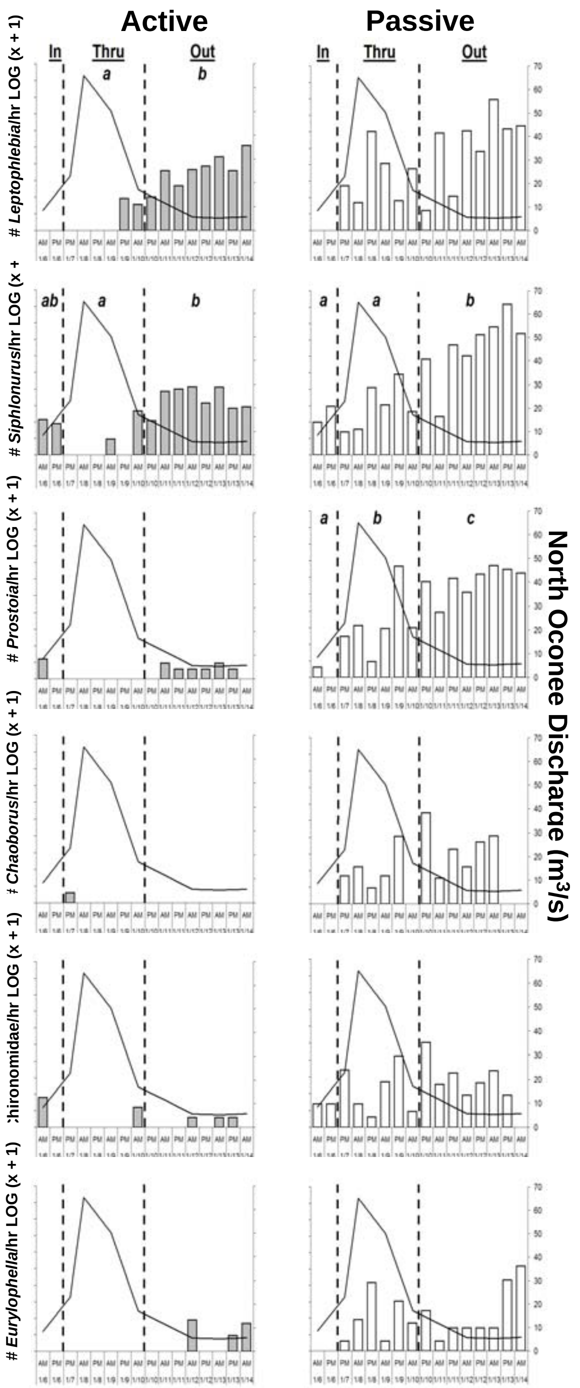

Figure 3. Number of aquatic insect nymphs or larvae dispersing actively (against water flow) and passively (with water flow), as measured every 12 hours during the second flood event (see Figure 1). Migration was divided into three periods: 1) floodwater inflow (In) and 2) outflow (Out) within the natural levee break, while 3) the river flowed through the floodplain (Thru) during the period of highest flooding. Flood stages with no insects were not included in the statistical analyses. Letters represent where significant differences were found, and values with the same letter are not significantly different ($p > 0.05$) among flood stages. Solid line shows North Oconee River discharge near the study site.

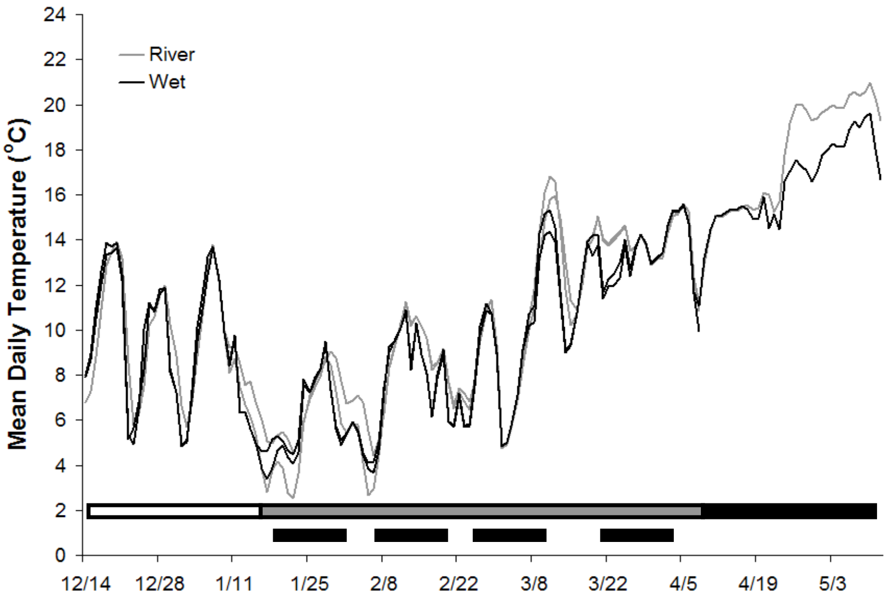

Figure 4. Mean daily temperatures measured by HOBO temperature recorders in the North Oconee River and floodplain wetland over the study period and near the growth chambers. Bar indicates when leptophlebid nymphs 1) colonized the floodplain (open), 2) grew on the floodplain (shaded), and 3) were emerging (filled). Individual black bars represent periods when the growth rate experiments were conducted.

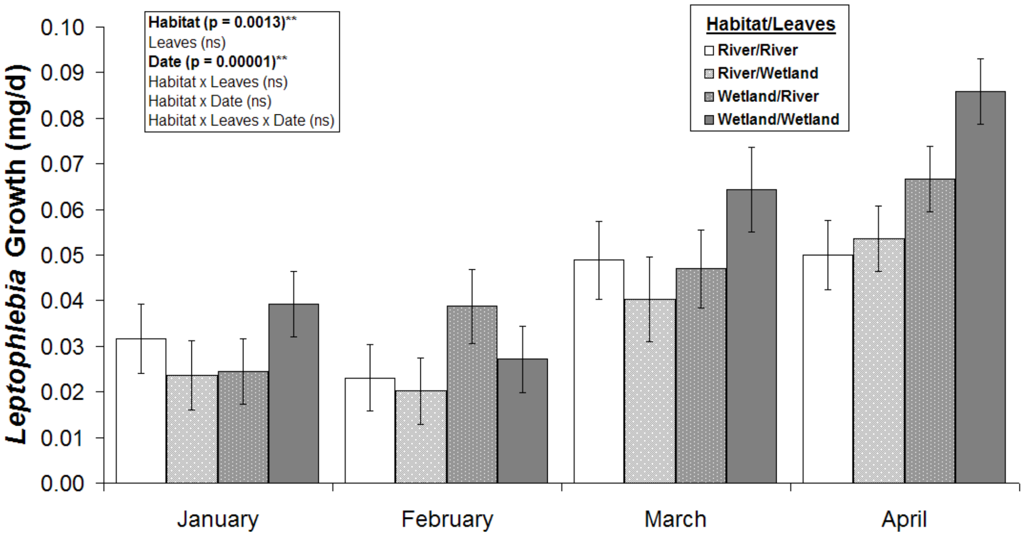

Figure 5. Mean instantaneous growth rates for *Leptophlebia* nymphs confined in growth chambers in the North Oconee river and floodplain wetland with leaves pre-conditioned in the different habitats. Growth rates were measured for two weeks each month through the *Leptophlebia* growth period in the floodplain. Error bars represent ± 1 SE.

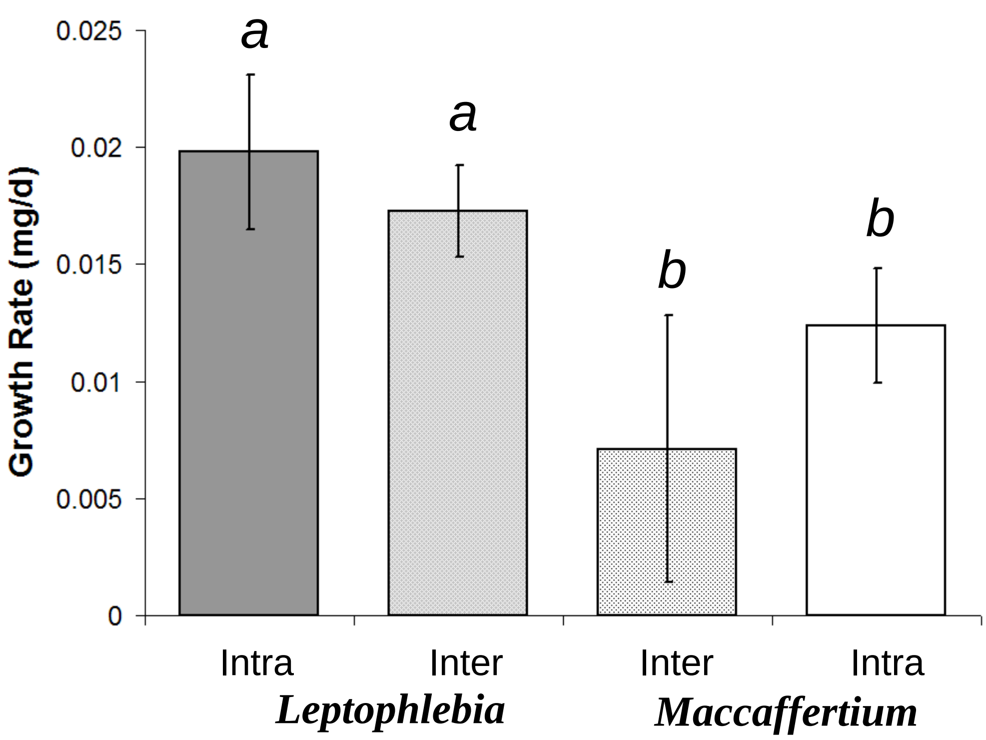

Figure 6. Mean instantaneous growth rates for *Leptophlebia* and *Maccaffertium* nymphs under intra- and inter-specific interference competition while confined in growth chambers in the North Oconee river under with leaves pre-conditioned in the river. Error bars represent ± 1 SE.

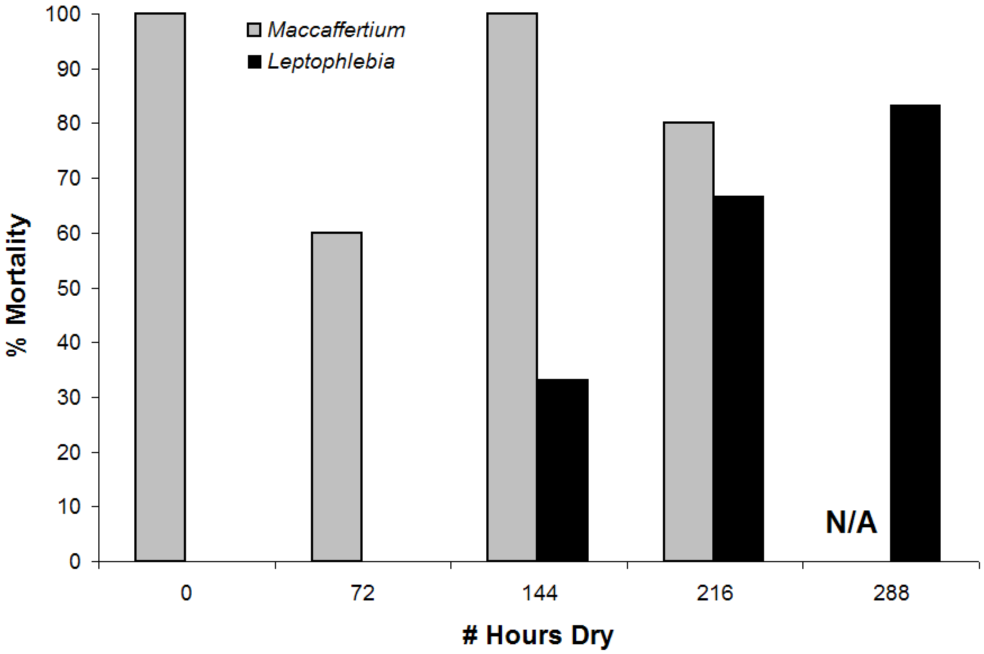

Figure 7. Percent mortality for *Leptophlebia* and *Maccaffertium* nymphs exposed to different periods of dry conditions in microcosms (2 L; 300 cm^2 bottom area). After each 72 hour drying period the microcosms were re-hydrated for 48 hours and mortality was determined. Mortality for *Leptophlebia* was initially low and increased temporally ($r^2 = 0.94$). Mortality for *Maccaffertium* was consistently high at all time periods ($r^2 = 0.01$).

Floodplain Environmental Factors	Benefit	Cost
Temperature	—	—
Water Flow	—	—
Food Quantity	—	—
Food Quality	—	—
Faster Growth Rate	✓	
Fish Predation	✓	
Amphibian Predation		✓
River Competition	—	—
Desiccation		✓

Figure 8. Relative benefit or cost to *Leptophlebia* that moved into the floodplain compared to remaining in the river environmental factors. Dashes indicate no clear net benefit to the mayflies.