

Vertical migration of adult Plecoptera (stoneflies) above forested
headwater streams

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by

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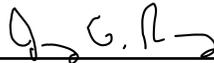
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ABSTRACT

2 Stream insects perform vital ecosystem functions and are important components of
stream and terrestrial ecosystems. Movement by adults in terrestrial environments has important
4 implications for population persistence and supporting terrestrial food webs. Larval stream
insects have limited dispersal potential, but flight-capable adults can disperse horizontally into
6 riparian forest vegetation or vertically into riparian forest canopies. Given the general lack of
studies examining vertical migrations into forest canopies, I examined differences in adult
8 Ephemeroptera, Plecoptera, and Trichoptera abundance at ground level versus in the canopy and
among summer and fall sample periods. I also examined differences in relative abundance of
10 larval and adult plecopteran families to determine if adult insects remain at their natal streams.
To examine stream insect activity, malaise and canopy traps were placed at four sites on three
12 different streams in the Mosquito Creek Valley Watershed (Pennsylvania). Larval assemblages
were collected with a d-net from the stream benthos at each site. Significantly more stream
14 insects were captured at ground-level than in the forest canopy for Ephemeroptera, Trichoptera,
and Plecoptera and for all plecopteran families. Relative canopy trap abundance revealed taxon-
16 specific differences of canopy habitat use among plecopteran families. Similarly, sites-specific
environmental characteristics likely encouraged vertical migrations. As expected, abundance of
18 adults was greater in summer than fall for all orders and five of six plecopteran families.
Leuctridae abundance was not different among summer and fall samples possibly due to multiple
20 cohorts or that our samples contained multiple species with different phenologies. Comparisons
of larval and adult relative abundance for plecopteran taxa within each site indicated that adult
22 taxa are likely migrating to or away from their natal streams. Demonstrating adult stream insects
utilize riparian forest canopies has important applications for management and restoration of

24 riparian forests. Future studies are needed to determine evolutionary drivers for why certain taxa
use the canopy such as avoiding riparian predators or for mating strategies.

26

INTRODUCTION

28 Stream insects play important roles in maintaining ecosystem structure and function.
Grazers remove algae from rocks in streams, which reduces algal biomass and increases nutrient
30 cycling (Wallace and Webster, 1996). Shredders break down leaf-litter into fine particulate
organic matter, which provides food for other stream organisms promoting nutrient cycling in
32 streams (Wallace and Webster, 1996; Graça, 2001). Stream insects are also important
components of aquatic food webs and are essential prey for fish, salamanders, and other aquatic
34 and terrestrial species (Baxter et. al., 2005). Given the prominent role of insects in stream
ecosystems, they are important indicators of stream health through bioassessments of
36 presence/absence, functional feeding groups, diversity and other measures of macroinvertebrate
assemblages or populations (McDonald et. al., 1991; Graça, 2001; Herman and Nejadhashemi,
38 2015; Bonada et. al., 2006). Understanding the biological basis for population distributions and
community composition is important for maintaining healthy stream ecosystems.

40 Stream insects have a complex life cycle with an aquatic larval stage and an adult
terrestrial stage. The larval stage is confined to the stream and more dispersal limited than the
42 adult stage. Larvae can disperse within streams by drifting, crawling, or swimming (Brewin et.
al., 1994; Merritt and Cummins, 2008). Adults emerge from streams and are typically flight
44 capable, which allows them to disperse farther distances than larvae. The abundance of flying
adults is typically greatest close to the stream (Peterson et. al., 1999). Adult stream insects can
46 live anywhere from one day up to one month (Lancaster and Downes, 2014). Stream insects are

attracted to polarized light reflected off the surface of the stream, which is likely an evolutionary
48 adaptation to remain near essential stream habitats (Kriska et. al., 1998; Kriska et. al., 2009) and
a potential driver for movement between canopy and ground-level habitats. Female insects that
50 remain near streams have an easier time finding suitable habitat to lay eggs, and males likely
aggregate near females to improve reproductive success (Kriska et. al., 1998; Kriska et. al.,
52 2009). Adult stream insects generally remain above the stream or in riparian vegetation (Peterson
et. al., 1999), and empirical evidence suggests that migration into upland areas at ground level by
54 adults is limited (Didham et. al., 2012). Didham et. al., (2012) found that stream insects were
more frequently caught in forest canopies than at ground-level when sampling in upland areas
56 where adult stream insects typically have low abundance. However, Didham et. al., (2012) did
not sample for adult stream insects near stream habitats. Little evidence exists about the use of
58 forest canopies by adult stream insects near streams where they are typically most abundant.

Several factors may influence stream insects use of forest canopy habitats as adults. Adult
60 insects may avoid riparian predators at ground level (e.g., web building spiders, amphibians, etc.)
by dispersing into riparian forest canopies. Adult stream insects may also use riparian forest
62 canopies to complete their life cycles (Winterbourn et. al., 2007). Moving into the riparian forest
canopy could provide a means for passive dispersal by accessing wind currents above the
64 canopy, which would be especially beneficial for stream insects with high a wing-aspect ratio
suited for gliding over large distances (Bilton et. al., 2001). Little is known if adult stream insects
66 are accessing riparian canopy habitat or if movement occurs between ground-level and canopy
habitats.

68 Examining vertical movement of adult stream insects can provide insight into stream
insect life histories and dispersal patterns in human dominated landscapes. Obstacles such as cars

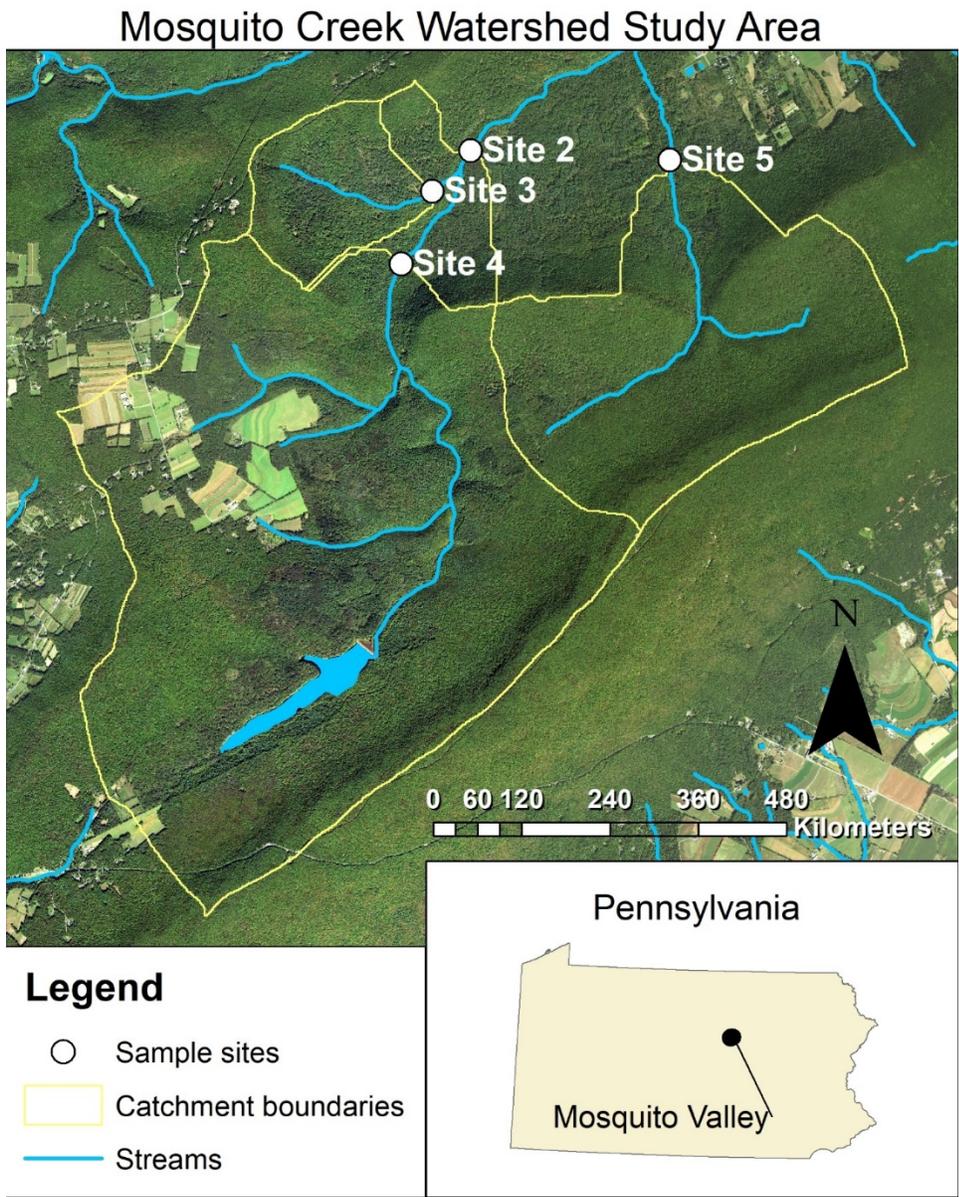
70 (that reflect polarized light) and culverts (that result in increased predation by spiders) can hinder
dispersal of adult stream insects (Smith et. al., 2009). Species evolved to disperse vertically into
72 the canopy may avoid these obstacles, which could provide an advantage in urban and suburban
landscapes. Yet, urban and agricultural landscapes commonly lack mature riparian forests along
74 streams (Walsh et. al., 2005), and the lack of riparian vegetation may discourage vertical
movements by certain organisms that typically utilize riparian forest canopies or encourage
76 migration by certain species most interested in accessing wind currents above the forest canopy.

This study examined adult stream insect activity to understand if adult stream insects
78 utilize forest canopies. Specifically, I examined differences in stream insect abundance measured
at ground level and in forest canopies and among summer and fall sampling events. I
80 hypothesized that taxa have a preference to remain at ground-level versus moving into riparian
forest canopies even though a meaningful number of adults utilize canopy habitat and that
82 movement patterns and seasonal abundance patterns will be taxon-specific. From these
hypotheses I predict that (1) abundances for all taxa are greater at ground level than in the
84 canopy, (2) relative abundances of individuals caught at ground versus canopy habitats will
differ among taxa, (3) abundances for all taxa are greater in summer than fall, (4) relative
86 abundances of individuals caught in the summer versus the fall will differ among taxa, and (5)
taxa of larvae found in the stream reaches where adult sampling took place will match adult taxa
88 found at the same site.

90 **METHODS**

All field work was conducted within the Mosquito Creek Watershed in Pennsylvania
92 (41.199977, -77.050678), located in the piedmont physiographic province (Figure 1). Samples

were collected at four stream reaches from three different streams (Figure 1). Stream reaches
94 sampled were 1st, 2nd, and 3rd order but had similar characteristics. Catchment size ranged from
1.6km² (site 3) to 19.1km² (site 2). The overall catchment for Mosquito Creek is primarily
96 forested but also contained agricultural land use upstream of sites 2 and 4 (Figure 1). The forest
was composed of a mix of conifers and hardwoods. Table 1 includes detailed information on
98 each sample site.



100 Figure 1. Map of study sites in the Mosquito Creek watershed.

Malaise and canopy traps were used to measure adult stream insect activity at ground
102 level and in the forest canopy (Figure 2). The traps used collected all terrestrial and aquatic
flying insects, but this study focused on Ephemeroptera (mayflies), Plecoptera (stoneflies), and
104 Trichoptera (caddisflies) with an additional focus on plecopteran families. The study focused on
stoneflies because they are associated with healthy stream ecosystems, are common indicators of
106 stream health, and were known to use riparian vegetation (see Hynes 1976 for more information
on Plecoptera biology and life history). Additionally, plecopteran families have a known
108 taxonomy, and only a limited number of taxa were found at the study sites. Previous research
demonstrated that flight intercept traps (i.e., malaise and canopy traps) were efficient for trapping
110 adult mayflies, caddisflies, and stoneflies (e.g., Smith, 2012). Flight intercept traps are passive
sampling devices, which means they can be left out to collect over extended periods of time.
112 Adult insects fly into a mesh barrier in the middle of the trap and most insect species will
naturally move up to a collecting jar at the top of the trap (Figure 2). Abundance of individuals
114 captured per 14-day period was used as the measure representing activity.

116 *Study location characteristics*

Habitat assessments were conducted in the summer of 2017 during a pilot study with
118 similar objectives. Measurements of habitat and physiochemical properties for each site were
conducted within a 40m reach centered at the malaise trap for upstream and downstream
120 locations and were not expected to change from 2017 to 2018. Substrate type, embeddedness,
flow type, thalweg depth (deepest part of the channel where current is the greatest), habitat, and
122 wetted stream width were recorded at 11 transects spaced 4m apart within the 40m reach. Visual
assessments were conducted to estimate the benthic substrate type(s) that comprised over 50% of

124 the stream bottom. Substrates included silt, sand (<2mm and granular), gravel (2-10mm), pebble
(1-6.4cm), cobble (6.4-25.6cm), boulder (>25cm), and bedrock. Visual assessments were used to
126 determine which flow type, riffle, run, or pool, comprised at least 50% of the habitat at each
transect. A visual assessment determined the presence or absence of habitat types at each
128 transect. Habitat types included logs, woody debris, aquatic vegetation, root balls, debris dams,
muck, leaf packs, backwater areas, and undercut banks. A Swoffer Model 3000 velocity meter
130 was used to measure discharge at the downstream reach on a single transect using the USGS
Midsection method (Turnipseed and Sauer, 2010).



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Figure 2. (Left) Image of a canopy trap and (Right) Image of a malaise trap.

134

Sampling Protocol

136 Malaise traps were placed directly above the stream channel at ground level to capture
insects flying at or near the surface of the stream. Malaise traps are open on two sides and
138 collected insects flying up and downstream (Figure 2). Malaise traps also have an open bottom to
collect insects emerging from the stream (Figure 2). Traps were 1.9m wide, 1.1m tall on the front

140 side with collecting jar, and .75m tall on the back side. Frames were constructed of PVC pipe
and attached to trees near the stream using ropes, and the traps were attached to frames using
142 bungee cords. Malaise traps were adjusted using the ropes to sit approximately one inch above
the stream surface, but the actual distance above the stream changed as stream levels fluctuated.
144 Traps were checked when samples were collected and adjusted to prevent any part of the trap
from becoming submerged, but the bottom of traps touched the water or were submerged a few
146 inches during high flow events. Individual adult insects were collected in 500ml sampling jars
attached to the top corner of the traps filled with approximately 250ml of 70% ethanol. Bottles
148 were unscrewed, the specimens removed, and the sample bottles replaced after being refilled
with 250ml ethanol during each collection period.

150 Canopy traps were open on all four sides and on the bottom. Each trap had four open
sides with a single opening being .9m wide and 1.2m tall. Canopy traps were placed 8-10m
152 directly above stream channel within the canopy and collected insects from all directions
laterally and insects moving up from below (Figure 2). The sample bottle was at the apex of the
154 trap and filled with approximately 250ml of 70% ethanol. Each trap was attached to 100lb test
braided fishing line using a 150lb snap-swivel. One end of the line was attached to a fishing
156 weight and tossed over a tree branch at a height that would allow the trap to be suspended
approximately 8-10m above the stream. The swivel and attached canopy traps on the lead end of
158 the line were raised to the appropriate height and another 150lb swivel was attached to the
trailing end of the fishing line. The trailing end swivel was attached to two zip ties around a tree
160 at ground level to hold the canopy trap in place at the right height. A kite spool was used to wrap
up the remaining line on the trailing end to keep the line from tangling and allow the trap to be
162 lowered and raised repeatedly to collect specimens. To retrieve samples, canopy traps were

lowered to the ground and sample bottles were removed. The contents were emptied into another
164 500ml jar and the trap's sample jar was refilled with ethanol and replaced.

Malaise and canopy traps were left out for 14 consecutive days during two sampling
166 periods (summer from June 18th to July 2nd and fall from August 30th to September 13th). Traps
were checked for damage and all spiders were removed during each collection. Collections were
168 made and traps were reset every three to four days during the summer sample period (emptied
four times) and every five to six days during the fall sample period (emptied three times). A total
170 of four malaise and four canopy samples were collected at each site for a total of 32 samples
during summer. A total of three malaise and three canopy samples were collected at each site for
172 a total of 24 samples during fall, but the final canopy trap sample from sites 2 and 4 were
unusable (i.e., only 22 usable samples from the fall period). Following a single strong storm, the
174 tree the canopy trap was attached to fell over at site 2, and the canopy trap at site 4 was tangled
in a branch which caused the collecting jar to be filled with water at the time of collection.

176 A D-net with a 12in opening and 500 μ m mesh net was used to collect larval aquatic
insects from the stream to characterize the in-stream assemblage. A 10-kick composite sampling
178 method was used to collect larvae from multiple habitats. A random number table was used to
select 10 sample locations at random from each habitat type in the proportion that the habitat
180 types existed within the stream. The benthic substrate was disturbed using a kicking motion and
the dislodged debris and organisms were swept downstream into the net. The benthos was
182 disturbed for 10 seconds per individual subsample (i.e., per individual kick). A multiple habitat
sampling method was employed to ensure the largest diversity was found (Allison Roy et. al.,
184 2003). One to three subsamples were collected together and then transferred to a 355 μ m sieve
where large debris (e.g., leaves, sticks, etc.) were rinsed and removed from the sample. The

186 sample was then transferred to a 500ml sampling bottle. All samples were preserved with 70%
ethanol in the field and returned to the lab for sorting. Benthic samples were taken once on July
188 2, 2018 during the summer sampling period.

190 *Sample processing*

The contents of adult samples were sorted using long sorting trays (Figure 3). Either the
192 entire sample or a portion of the sample was added to the trays and sorted under magnification.
All Ephemeroptera, Plecoptera, and Trichoptera were separated from all other organisms and
194 placed in glass vials filled with 70% ethanol for future analysis. Plecoptera were then identified
to family using Merritt et al. (2008).

196



198 Figure 3. (Left) Image of the sorting tray used to process adult and benthic insect samples, and
(Right) a similar tray shown under a microscope.

200

The contents of the benthic samples were placed in the same trays described above
202 (Figure 2), and all larval aquatic insects were separated from debris under magnification. During
separation, all Trichoptera and Ephemeroptera were enumerated. After separation, all Plecoptera

204 were identified to family and enumerated. All larval organisms were stored in glass vials filled
with 70% ethanol and were identified using Merritt et al. (2008).

206

Analysis

208 The abundance of adults in malaise traps was compared to the abundance in canopy traps
to determine if taxa preferred ground or canopy habitats. All comparisons were done using a
210 Wilcoxon sign rank test, which is used for paired data that are not normally distributed. All
analyses were run using count data. Samples were paired based on season and site (i.e. the
212 canopy trap sample at site 5 in the summer was paired with the malaise trap sample at site 5 in
the summer). A total of eight pairs and 16 total observational units were used. A paired test was
214 used to keep daily weather (that may discourage activity) and the overall reduced abundance in
the fall from biasing our results. A lack of significant difference in abundance among trap types
216 would indicate no preference for ground versus canopy habitat.

 Comparisons of abundance among seasons was also done using a Wilcoxon sign rank
218 test. Samples were paired based on trap type and site (i.e., the summer canopy trap at site 5 was
paired with the fall canopy trap at site 5). Lack of a significant difference indicated no difference
220 in abundance between seasons.

 All adult subsamples taken over the 14-day period for each site and season were
222 combined for analysis (i.e., an observational unit was the total abundance caught over a 14-day
period for a single site in a single season). During the fall sampling period, however, one
224 subsample from the canopy trap was lost at sites 2 and 4. The total abundance collected over 9
days of sampling was standardized to 14 days for analysis. Additionally, we found certain
226 plecopteran families had zero abundance in both canopy and malaise traps for a full 14-day

228 period (see results below). The fall samples were removed from the analysis comparing malaise
 230 and canopy traps to prevent biasing the results since the paired zero values would have been
 interpreted as no-difference and no preference for ground level or canopy habitats. All data were
 analyzed using the R-Statistical Program (v 3.6.1; The R Foundation for Statistical Computing).

232 **RESULTS**

Stream width ranged from 3.9m-5.7m and depth ranged from 13.4cm-28.1cm. Habitat
 234 was dominated by boulder (70-90%) or cobble substrates (60-80%; Table 1). Large woody debris
 and undercut banks were present at all sites and aquatic vegetation and leaf packs were present at
 236 two and one sites respectively (Table 1, Figure 1).

238 Table 1. Catchment characteristics and stream habitat conditions at the four sample sites
 (V=aquatic vegetation; B=undercut bank; LW= loose woody debris; LP= leaf pack).

Site	Catchment size (km ²)	Catchment %forest	Average depth (cm)	Average width (m)	Dominant benthic substrate	Dominant habitat
2	19.1	89%	27.6	5.2	Boulder (70%)	V, B, LW
3	1.6	100%	13.4	3.9	Cobble (80%)	B, LW, LP
4	16.4	88%	28.1	5.7	Boulder (90%)	B, LW
5	6.3	100%	20.5	4.1	Cobble (60%)	V, B, LW

240 Overall, 15,997 adult stream insects were identified. Trichoptera was the most abundant
 242 order (n=11,331.1), followed by Plecoptera (n=3,490.7), and Ephemeroptera (n=1,245.1
 individuals; Table 2). Qualitative analysis examining each trap type revealed that Trichoptera

244 was the most abundant in both malaise traps (n=9,191) and canopy traps (n=2,140.1) among all
orders. Trichoptera was the most abundant order in all samples except for the summer malaise
246 trap at site 3 and fall malaise at site 4. Plecoptera were at least twice as abundant in malaise traps
at site 3 than the other three sites in the summer, but the malaise trap at site 4 had the greatest
248 number of Plecoptera in the fall.

For plecopteran families, Perlodidae was the most abundant (n=1,251.1) and
250 Peltoperlidae was the least abundant (n=27) for all samples combined (Table 3). Perlodidae was
the most abundant in all malaise traps combined (n=1,148), and Leuctridae was the most
252 abundant in all canopy traps combined (n=208.1). Perlodidae was also the most abundant in all
summer malaise traps combined (n=1,031), and Nemouridae was the most abundant in all
254 summer canopy traps combined (n=111). Leuctridae was the most abundant in fall malaise
(n=374) and fall canopy (n=116.1) traps combined. The highest abundances in a single malaise
256 and canopy trap sample were 487 (Perlodidae) and 49 (Nemouridae) individuals respectively.

Total abundance of adults aggregated across the full sample period (14 days) during the
258 summer sampling period in malaise traps ranged from 48 to 524, from 300 to 1092, and from
583 to 4087 for Ephemeroptera, Plecoptera, and Trichoptera respectively (Table 2). Total
260 abundance of adults aggregated across the full sample period (14 days) during the summer
sampling period in canopy traps ranged from 4 to 63, from 41 to 156, and from 146 to 733 for
262 Ephemeroptera, Plecoptera, and Trichoptera respectively (Table 2). Total abundance of adults
aggregated across the full sample period (14 days) during fall sampling period in malaise traps
264 ranged from 5 to 31, from 84 to 238, and from 101 to 299 for Ephemeroptera, Plecoptera, and
Trichoptera respectively (Table 2). Total abundance aggregated across the full sample period (14
266 days) of adults during fall sampling period in canopy traps ranged from 0 to 4, from 13 to 48.22,

and from 41 to 92 for Ephemeroptera, Plecoptera, and Trichoptera respectively (Table 2). Zero
268 abundance values were more common for plecopteran families in the fall (27) than summer (2)
and were more common in canopy (16) than malaise (13) traps (Table 3).

270 Perlodidae was the most abundant family in all summer malaise traps except at site 4.
Leuctridae was the most abundant family in all fall malaise and canopy traps. Perlodidae were
272 generally less abundant in canopy than malaise traps. The largest abundance of Chloroperlidae
was found during the summer sampling period in the malaise trap at site 3. Zero individuals were
274 captured for Chloroperlidae, Peltoperlidae, and Perlidae during the fall sampling period for both
trap types. Nemouridae had zero individuals captured in the malaise trap at site 5 and in canopy
276 traps at sites 3 and 5 during the fall sampling period. Peltoperlidae had zero individuals captured
in canopy traps at sites 2 and 3 during the summer sampling period.

278

Analysis of ground level versus canopy preference

280 A significantly greater number of individuals for Ephemeroptera, Plecoptera, and
Trichoptera were captured in malaise than canopy traps ($p=0.008$ for all three orders; Figure 3).
282 The abundance of individuals per 14-day period was greater in malaise than canopy traps for
every paired sample (same season and site) for all three orders.

284 The relative canopy trap abundance ratio (Table 4) ranged from 0.036 to 1.333, and 16
values were not calculated due to a value of 0 for the abundance for canopy or malaise trap
286 samples (Table 4). All plecopteran taxa had the highest relative canopy trap abundance ratio at
site 5 in the summer, but the highest values differed among sites in the fall. Peltoperlidae had the
288 smallest range of relative canopy trap abundance ratio values in the summer (0.400 to 0.500) and
Leuctridae had the greatest range (0.135 to 1.033). Leuctridae also had the largest range of

290 relative canopy trap abundance ratio values in the fall (0.143 to 0.704), and Nemouridae had the
 smallest range (0.130 to 0.419).

292

Table 2. Abundances of Ephemeroptera, Plecoptera, and Trichoptera between trap type and
 294 sampling periods per 14 days. Fall canopy traps at sites 2 and 4 sampled for 9 days due to trap
 failures, and abundances were rescaled to abundance per 14 days.

Period	Trap	Site	Ephemeroptera	Plecoptera	Trichoptera
Summer	Canopy	2	4	41	332
Summer	Canopy	3	63	135	146
Summer	Canopy	4	26	87	733
Summer	Canopy	5	11	156	681
Summer	Malaise	2	258	460	2730
Summer	Malaise	3	524	1092	583
Summer	Malaise	4	48	540	978
Summer	Malaise	5	219	300	4087
Fall	Canopy	2	0	40.44	43.56
Fall	Canopy	3	4	13	41
Fall	Canopy	4	3.11	48.22	71.56
Fall	Canopy	5	1	36	92
Fall	Malaise	2	28	87	299
Fall	Malaise	3	5	133	236
Fall	Malaise	4	20	238	101
Fall	Malaise	5	31	84	177

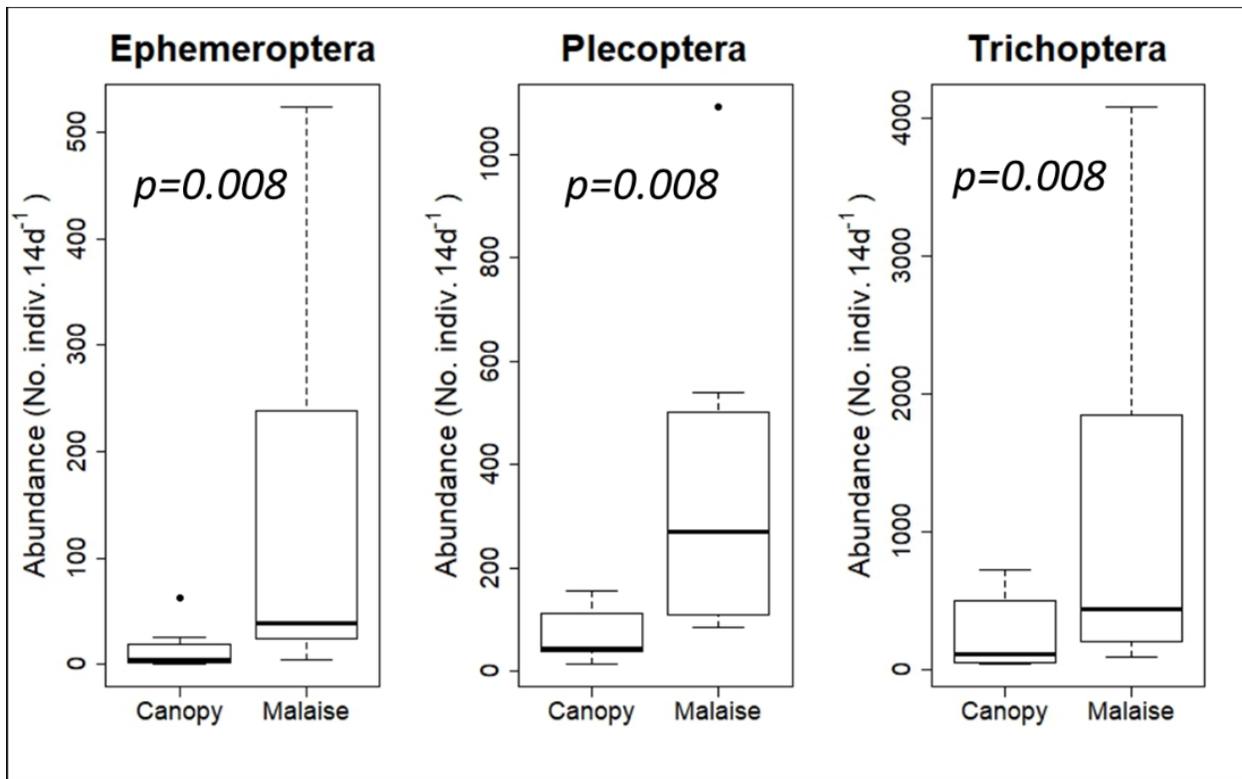
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Table 3. Abundances of the six plecopteran families between trap types and sampling periods.

Period	Trap	Site	Chloroperlidae	Leuctridae	Nemouridae	Peltoperlidae	Perlidae	Perlodidae
Summer	Canopy	2	8	6	16	0	2	9
Summer	Canopy	3	54	26	22	0	6	27
Summer	Canopy	4	8	29	24	2	3	21
Summer	Canopy	5	34	31	49	1	4	37
Summer	Malaise	2	86	31	74	8	9	252
Summer	Malaise	3	292	81	200	9	23	487
Summer	Malaise	4	32	215	130	5	10	148
Summer	Malaise	5	61	30	60	2	3	144
Fall	Canopy	2	0	37.33	1.56	0	0	1.56
Fall	Canopy	3	0	11	0	0	0	2
Fall	Canopy	4	0	35.78	10.89	0	0	1.56
Fall	Canopy	5	0	32	0	0	0	4
Fall	Malaise	2	0	53	12	0	0	22
Fall	Malaise	3	0	77	13	0	0	43

Fall	Malaise	4	0	171	26	0	0	41
Fall	Malaise	5	0	73	0	0	0	11

298



300

Figure 3: Box plots comparing abundances (No. individuals per 14-day period) of

302

Ephemeroptera, Plecoptera, and Trichoptera among canopy and malaise traps.

304

The family-level analysis of Plecoptera found a significantly greater number of adults in malaise than canopy traps for Perlodidae ($p=0.008$), Leuctridae ($p=0.017$), and Nemouridae

306

($p=0.022$; Figure 4). Abundance of Chloroperlidae, Perlidae, and Peltoperlidae were 0 in all 4

308

canopy and malaise traps in the fall and were not analyzed using the Wilcoxon sign rank test for comparing abundances among trap types since the test would fail to find a significant difference with eight samples (four pairs) regardless of the numerical difference among samples.

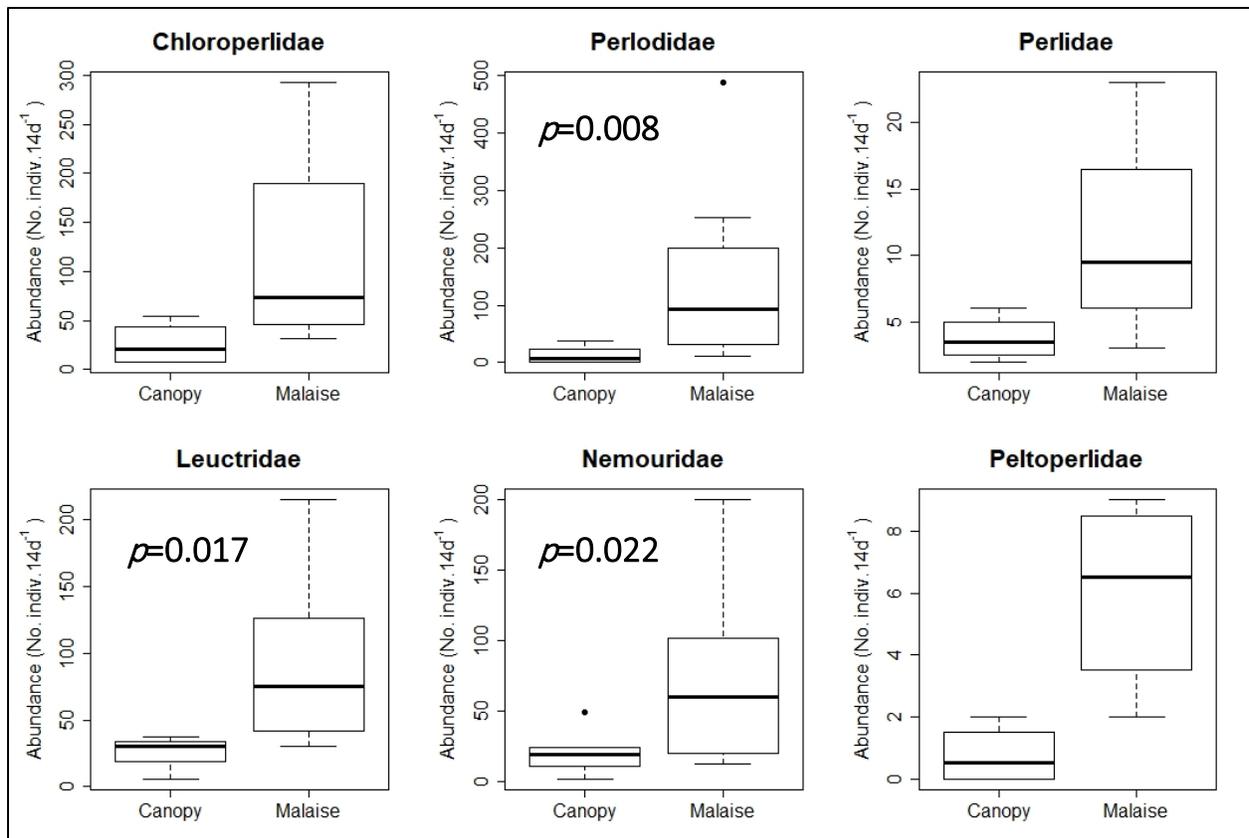
310 Table 4. Table of relative number of individuals caught at canopy versus malaise traps calculated as the abundance in canopy traps
 divided by abundance in malaise traps (i.e., relative canopy trap abundance). Low values indicate abundances in malaise traps were
 312 much higher than canopy traps. Values >1 indicate more individuals caught in canopy than malaise traps. NA indicates an abundance
 of 0 for either the canopy or malaise trap (see footnote).

Period	Site	Chloroperlidae	Leuctridae	Nemouridae	Peltoperlidae	Perlidae	Perlodidae
Summer	2	0.093	0.194	0.216	N/A ¹	0.222	0.036
Summer	3	0.185	0.321	0.110	N/A ¹	0.261	0.055
Summer	4	0.250	0.135	0.185	0.400	0.300	0.142
Summer	5	0.557	1.033	0.817	0.500	1.333	0.257
Fall	2	N/A ²	0.704	0.130	N/A ²	N/A ¹	0.071
Fall	3	N/A ²	0.143	N/A ¹	N/A ²	N/A ¹	0.047
Fall	4	N/A ²	0.209	0.419	N/A ²	N/A ¹	0.038
Fall	5	N/A ²	0.438	N/A ²	N/A ²	N/A ¹	0.364

314 ¹ zero abundance in the canopy trap (i.e., the numerator)

² zero abundance in the malaise and canopy trap

316



318 Figure 4: Box plots comparing abundances (No. individuals per 14-day period) of plecopteran
 families among canopy and malaise traps. Box plots excludes the fall samples for
 320 Chloroperlidae, Peltoperlidae, and Perlidae due to having zero abundance in both canopy and
 malaise traps during that sampling period. *p*-values were calculated for Wilcoxon sign rank tests,
 322 but no statistical analysis was done for Chloroperlidae, Peltoperlidae, and Perlidae due to the
 zero abundance in both trap types during the fall sampling period.

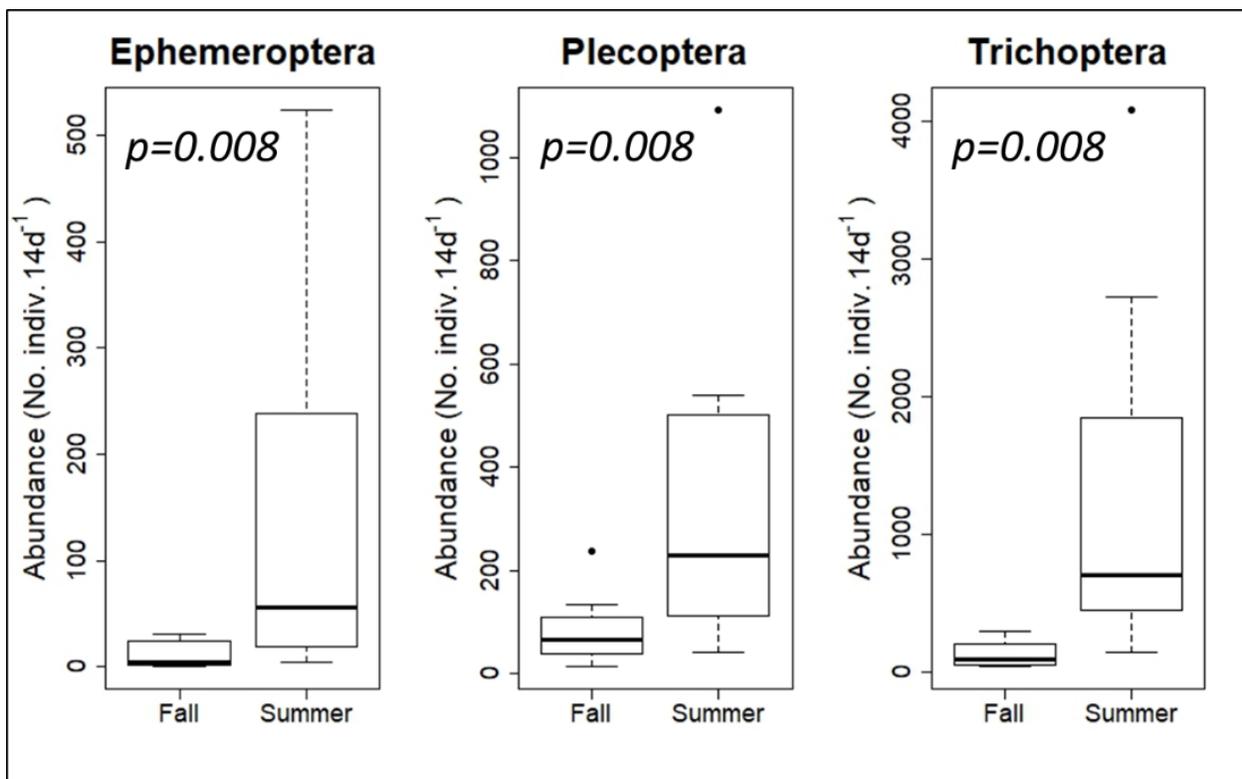
324

Analysis of seasonal differences in abundance

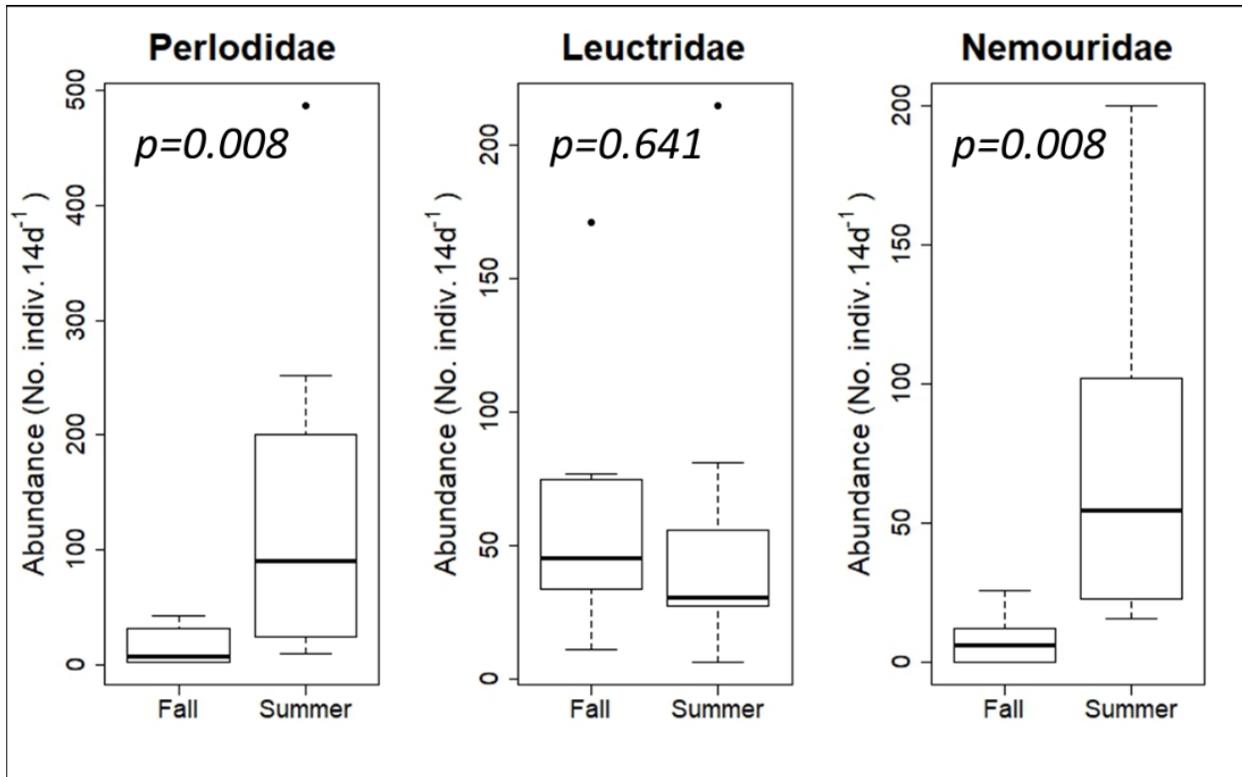
326 A significantly greater number of individuals for Ephemeroptera, Plecoptera, and
 Trichoptera, were captured during the summer sampling period than the fall sampling period
 328 (*p*=0.008 for all three orders; Figure 5). The abundance of individuals per 14-day period was

greater in the summer sampling period than the fall sampling period for every paired sample
330 (same trap type and site) for all three orders.

No individuals were caught during the fall sampling period for Choloroperlidae,
332 Peltoperlidae, and Perlidae (Table 3), which demonstrated an obvious difference in abundance
among seasons without the use of a statistical test. Perlodidae ($p=0.008$) and Nemouridae
334 ($p=0.008$) had a significantly greater number of individuals caught during the summer sampling
period than the fall sampling period. Leuctridae abundance among seasons was not significantly
336 different ($p=0.641$; Figure 6).



338 Figure 5: Box plots comparing abundances (No. individuals per 14-day period) of
340 Ephemeroptera, Plecoptera, and Trichoptera among sampling period.



342 Figure 6: Box plots comparing abundances (No. individuals per 14-day period) of plecopteran
 families among sampling period.

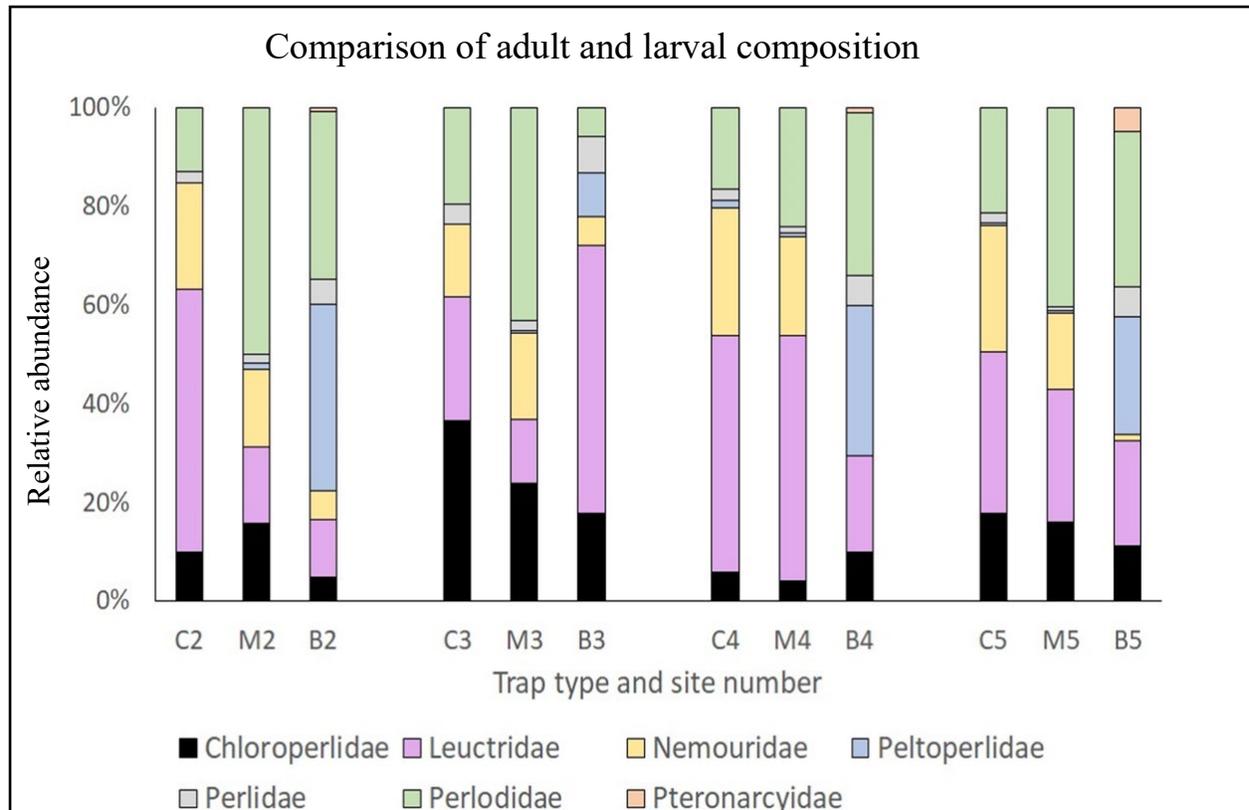
344

Analysis comparing adult and larval assemblages

346 Relative abundance of larvae in benthic samples generally did not match the relative
 abundance of adults in malaise or canopy traps (Figure 7). Larval Peltoperlidae commonly had
 348 high relative abundance at sites 2, 3, and 5 but had some of the lowest relative adult abundances
 in malaise and canopy traps. Similarly, adult Nemouridae relative abundance was generally
 350 greater for malaise and canopy traps than larval relative abundance. Relative abundance of
 Leuctridae was larger in the canopy trap at site 2 than the malaise trap and benthic sample.
 352 Leuctridae also had a higher relative abundance in the benthos sample than in the malaise or
 canopy trap samples at site 3, and Leuctridae relative abundance in the benthos sample at site 4

354 was lower than the adult and canopy sample. Perlodidae had a larger relative abundance in the
malaise trap than the canopy trap or benthic sample at sites 2 and 3, but overall Perlodidae had
356 consistent relative abundance across sites. Chloroperlidae had higher relative abundance for all
sample types at site 3 than the other three sites.

358



360 Figure 7: Stacked bar chart representing relative abundance and assemblage composition at each
site for adult plecopteran taxa in malaise and canopy samples and larval plecopteran taxa in
362 benthic samples.

364 **DISCUSSION**

Research on adult stream insect activity has focused on analyzing abundance at ground
366 level with minimal research being conducted on vertical migration of adult stream insects into

368 riparian forest canopies (Sode and Wiberg-Larsen, 2003; Petersen et. al., 2004). My study was
used to determine if adult stream insects are migrating vertically into the riparian forest canopy,
how abundance of adults differed seasonally, and how the relative abundance of taxa in the
370 plecopteran adult assemblage compared to the relative abundance of larval taxa in the benthos.
While adult stream insects generally preferred staying at ground level, this study demonstrated
372 biologically meaningful abundances of adults in forest canopies not documented previously. As
expected, adults were more abundant in the summer than fall. Additionally, the relative
374 abundance of adult Plecoptera varied among sites and did not consistently match relative
abundances of larvae in the benthos.

376

Ground level versus canopy abundance

378 Didham et. al. (2012) previously found a larger abundance of Ephemeroptera and
Trichoptera adult stream insects in forest canopies than at ground level and more Plecoptera at
380 ground level than in the canopy. This study found a significantly greater number of
Ephemeroptera, Plecoptera, and Trichoptera in malaise than canopy traps and a significantly
382 greater number of individuals of plecopteran families in malaise than canopy traps. Didham et.
al., (2012), however, sampled in upland areas and found a substantially lower overall abundance
384 than this study (e.g., 428 Ephemeroptera, Plecoptera, and Trichoptera individuals in the canopy
caught over 70 days using 317 60cmx23cm sticky traps versus 2,736 individuals in the canopy
386 from 3 orders over 28 days of sampling). While the results of this study may indicate a
preference to stay at ground level, the overall results conclusively indicated that a portion of the
388 adult assemblage is using forest canopy habitats near streams. Staying near the stream is likely
an adaptation to remain near locations for egg laying and to aggregate near potential mates

390 (Petersen et. al., 2006). A small portion of the stream insect populations may express a
phenotype for high dispersal, which leads to only a few individuals migrating vertically into the
392 forest canopy. Maintaining genetic diversity and a ‘high dispersal trait’ is important for
population persistence given that stream habitats may infrequently go dry (Boulding and Hay,
394 2001). Additionally, individuals may be using the forest canopy only for short-duration activities
related to life-history processes such as roosting and mating. In this case, only a small portion of
396 the population may be in the canopy at one time, but most or all individuals would migrate to the
canopy and back to the stream at some point during their life cycle (Petersen et. al., 2006).

398 The observed difference in abundance among trap types may be the result of
discrepancies in trap capture efficiencies. Malaise traps are likely more efficient at capturing
400 adult stream insects than canopy traps due to their placement and size. Malaise traps are placed
directly along the stream corridor where most stream insects travel and are designed to catch
402 insects moving up and down the stream channel (Smith, 2012; Sode and Wiberg-Larsen, 2003).
Adult stream insects likely do not aggregate around any features in the forest canopy like they do
404 above the stream in response to polarized reflected light. Even though canopy traps were placed
directly over the stream channel, adults 8-10m above the stream in the canopy are likely more
406 dispersed than individuals that are aggregated directly above the stream at ground level. Future
work could use canopy traps at ground level to account for differences in trap efficiency.

408 Additionally, abundance in malaise traps may represent adult activity through dispersal
and emergence patterns from the stream. Additional research using malaise traps at these streams
410 suggested that adult abundance in malaise traps is a function of emergence rather than adults
moving up and down the stream channel (i.e., not just a result of adult “activity”; Tasker 2019,
412 unpublished data). Abundance of adults caught in malaise traps is commonly used as a proxy for

adult activity including studies suggesting adults rarely move into upland areas (Sode and
414 Wiberg-Larsen, 2003; but see contradicting research by MacNeale et al., 2005 who found upland
dispersal by labelling insects with ¹⁵N). Newly emerged individuals may cling to malaise traps
416 instead of natural substrates to allow their wings to dry. Once on the traps, adults may follow the
netting upward to the collecting jar. This type of behavior could inflate the abundance of
418 individuals caught in malaise traps. Testing the relative contribution of flying adults that
intercept malaise traps and emerging adults from the stream to abundance in malaise traps is an
420 important research question given the common use of malaise traps to measure adult stream
insect assemblages.

422

Taxon-specific differences among plecopteran families

424 This was the first study to demonstrate any biologically relevant use of the forest canopy
by Plecoptera. Measures of relative canopy trap abundance showed taxon-specific differences in
426 the use of the canopy among plecopteran families. Perlodidae had small relative canopy trap
abundance ratios even though ground level abundance was high, and Perlidae and Peltoperlidae
428 had low ground level abundances but high relative canopy trap abundance ratios. The relative
abundance of individuals in the canopy could be a function of the abundance at ground level, but
430 a post-hoc analysis of canopy abundance compared to malaise abundance for each site and
season suggested that density dependent factors were not driving vertical migration (R-squared
432 values calculated by Microsoft Excel; Figure 8). While not a correlation analysis, this post-hoc
analysis examined if adult abundance in the canopy was a function of the abundance at ground
434 level (presumably driven by movement vertically by a predictable portion of the population).

High ground-level abundance was not consistently related to high canopy abundance, which
436 suggested that dispersal into the canopy was not density-dependent.

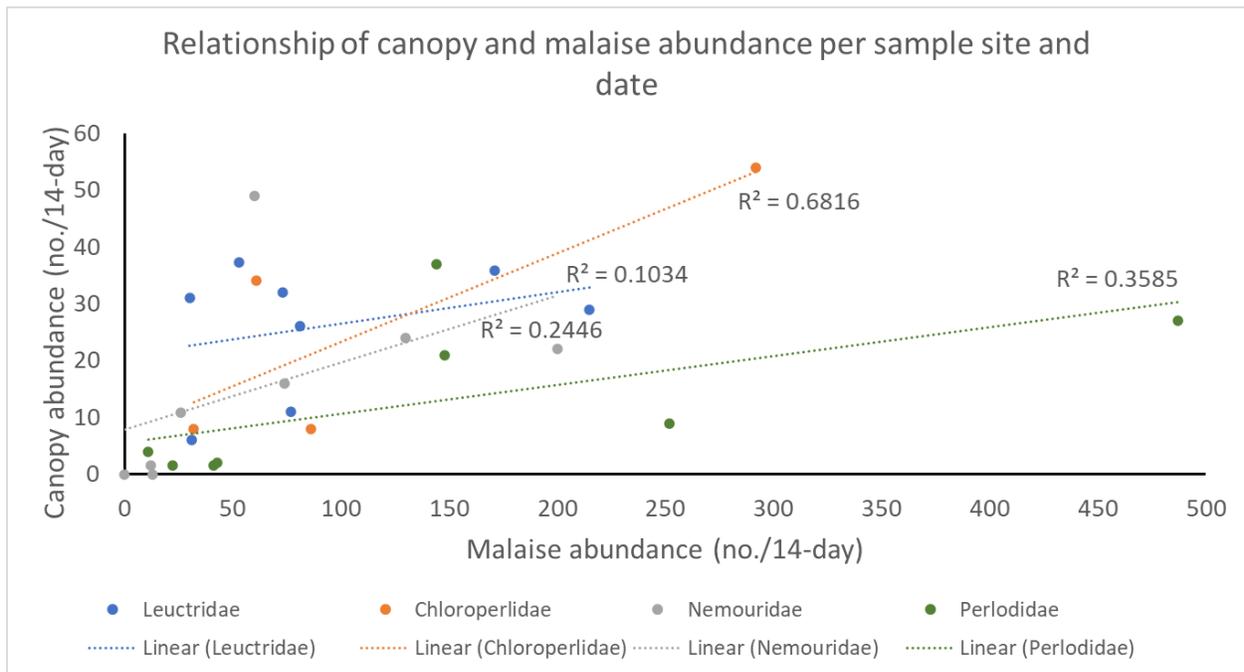
Site 5 consistently had the largest relative canopy trap abundance ratio and commonly
438 had low abundance at ground level. This result suggested site-specific factors may encourage
movement into the canopy. Factors such as riparian tree species and age of the forest may
440 directly alter canopy use or indirectly alter canopy use by changing riparian predators.

Additionally, ground level riparian vegetation sometimes covered the stream channel at site 5 but
442 not the reaches at the other sample sites. A dense understory growing over the stream channel
may reduce the amount of polarized reflected light observed by adults and eliminate a cue for
444 aggregating above the channel at ground level. Additionally, Leuctridae had the greatest relative
canopy trap abundance ratio range, which could indicate species and site specific factors interact
446 to determine vertical migrations.

448 *Seasonal difference in abundance*

Many stream insects use fall, winter, and spring to grow and store enough resources to
450 complete metamorphosis (Lancaster and Downes, 2014). During late spring and early summer,
these stream insects emerge to complete their life cycles by mating and laying eggs. Thus, the
452 greatest abundance of emerging adults occurs at similar times among taxa. Seasonal abundance
data for insect orders and five plecopteran families conformed to expectations that summer
454 abundances are greatest. The lack of a significant difference among seasons for Leuctridae,
however, could be due to multiple species emerging at different times (i.e., different summer and
456 fall emerging species). Detecting species-specific phenological differences requires species-level
identifications, which was beyond the scope of this project. The lack of difference for Leuctridae

458 could also be due to multiple, asynchronous cohorts emerging at different times of the year or
 due to multiple clutches. Clutches that emerge during the summer would likely be larger in size
 460 than clutches that emerge during the fall (Sweeney and Vannote, 1986). Further study to
 document timing of life history processes is needed to inform conservation of stream insect
 462 biodiversity (Cheney et. al., 2019).



464 Figure 8. Scatter plot and R-squared values for the four most abundant plecopteran families to
 466 determine if canopy abundance is a function of the abundance at ground level.

468 *Adult and larval assemblage comparison*

Lastly, we examined whether the composition of the larval assemblage collected in
 470 benthic samples matched the adult assemblages collected in malaise and canopy traps at each
 site. The lack of congruence among larval and adult assemblages at a single site is likely the
 472 result of immigration and emigration by adults. Adults moving away from or to a site may be in

search of a) better habitat to lay their eggs, b) food (for the few species that eat as adults), or c)
474 mates (Lancaster and Downes, 2014). Site 3 had a greater relative abundance of Leuctridae
larvae, which could indicate that site 3 had different in-stream habitat than the other sites. Site 3
476 had the smallest catchment and was the shallowest stream. Additionally, site 3 had more fine
sediment than other sites even though it was dominated by cobble substrates. Adults of certain
478 taxa emerging from surrounding reaches may avoid site 3, or adults may emerge from site 3 and
immediately leave in search of better habitat such as faster moving water or reaches with low
480 sediment in the benthos. Both processes would result in the observed differences between larval
and adult relative abundance.

482

Applications

484 Riparian areas are commonly deforested in landscapes with urban and agricultural land
use (Poff et. al., 2011). Riparian deforestation increases stream bank erosion, decreases benthic
486 habitat quality, decreases riparian habitat quality, and decreases riparian and stream biodiversity
(Sweeney et. al., 2004). Preserving riparian forests is essential to protect the ecological integrity
488 of streams and aquatic organisms that rely on riparian vegetation (Pollock and Beechie, 2014).
Stream insects are essential for terrestrial and aquatic food webs (Baxter et. al., 2005), and
490 benefits to insect assemblages from riparian forest conservation will have secondary benefits for
aquatic and terrestrial species.

492 The results of this study demonstrated the importance of riparian restoration and
conservation for adult stream insects that use the riparian canopy. Planting trees along stream
494 banks can benefit a stream by reducing nutrient inputs, liming bank erosion, and improving
aquatic habitat (Sweeney et. al., 2004), and is a common approach to stream restoration. Riparian

496 reforestation may also benefit adult insect survival for species that utilize the riparian canopy.
Restoration projects that plant trees, however, may need to wait until riparian forests grow
498 mature canopies to benefit populations and support biodiversity. Additionally, understory density
may also affect vertical migrations. While replanting trees has overall benefits to streams and
500 direct benefits for stream insect adults, the extended time until a benefit is realized for adult
insects demonstrates the importance of preserving mature riparian forests and activity patterns of
502 adults may change as forests mature through time.

504 *Conclusions*

Previous studies have concluded that adult stream insect abundance is highest at ground-
506 level above the stream, but the use of surrounding terrestrial habitats can be important for
population persistence and for supporting terrestrial foodwebs. The significantly higher
508 abundance of adult stream insects captured at ground level versus in the canopy confirms that
stream insect activity is high at ground level, but this study demonstrated that adult stream
510 insects migrate into riparian forest canopies. The use of the canopy differs among plecopteran
families, and a combination of site-specific and taxon-specific preferences are controlling
512 vertical migrations by Plecoptera. Movement into riparian forest canopies may offer an
evolutionary advantage to avoid predators, find mates, or access wind currents above canopies
514 for dispersing. Larval assemblages differed from adult assemblages at the same sites, which
indicated that migration by adult plecopteran away from or to specific sites is likely occurring
516 due to site-specific factors. Future studies are needed to understand the evolutionary drivers of
vertical migration by adults, and knowledge of the mechanisms determining vertical migrations
518 can provide insight about conserving and restoring riparian forests and stream ecosystems.

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