

Does Species Diversity of Caddisflies Enhance Detritus Breakdown and Nutrient Release in Temporary Ponds?

Student: Dianna Krejsa
Mentor: Scott Wissinger

Advanced Independent Research
Summer 2011

Abstract:

In small temporary ponds (“Kettle Ponds”) near the Rocky Mountain Biological Laboratory in Gothic, Colorado, several species of detritivorous caddisflies coexist as detritus processors. Three congeneric caddisflies, *Limnephilus externus*, *Limnephilus sublunatus*, and *Limnephilus tarsalis* play an important role in aquatic trophic cascades by: 1) liberating N and P stored in detritus and 2) stimulating algal growth, as well as being a direct food source for carnivores. To test whether these species have the same trophic position and ecosystem function, and therefore are functionally redundant, I assembled single-species and multi-species assemblages with the same total biomass in microcosms for 30 days and compared water nutrient levels (NH₄⁺ and P) and algal growth as the experiment progressed. I predicted that multi-species caddisfly treatments would process more detritus to support the hypothesis that each species occupies a specific niche and so are not functionally redundant. I also predicted that higher detritus processing would lead to more nitrogen and phosphorus in the water and thus higher algal growth. Experimental results did not support that hypothesis, but other confounding factors like intraguild predation within the multi-species microcosms may have biased outcomes. In a broader context, these results provide evidence that detritivores have significant impacts on greater algal growth and the release of nutrients back up aquatic trophic cascades.

Keywords: ecosystem function, functional redundancy, interspecific competition, intraguild predation, detritus processing, nutrient cycling, diversity, caddisfly, *Limnephilus* spp.

Introduction:

Diversity is an important element of ecology and is usually an indicator of ecosystem health because of its link to productivity and community stability (Loreau et al. 2001, Giller et al. 2004, Riess et al. 2009). An underlying assumption of the biodiversity-ecosystem function (BEF) concept is that even closely related species have different effects on biophysical processes (nutrient transfers, energy flows); i.e., species are not “functionally redundant.” If a species were functionally redundant, the loss of that species might have little impact on ecosystem function.

The BEF concept and research in functional redundancy is based on the general observation that closely related species can coexist because of partitioning along one or more niche dimensions. Because of resource partitioning, functional redundancy may not exist to a high enough degree to completely recover if a species deletion occurs. Thus, a decline in the population of even one species has the potential to lead to a decline in ecosystem function despite the presence of other closely-related species that play a similar, but slightly different role in an ecosystem (Cardinale et al. 2002).

Most research on how species diversity affects ecosystem function has focused on green pathways (plants → herbivores → carnivores) in food webs. However, primary consumers like herbivores harvest only a small fraction of primary production of photosynthesizing plants; most plant nutrients and energy enter terrestrial and aquatic food webs as detritus, or dead organic matter (Oertli 1993). Because detritivores mobilize detritus-bound nutrients and energy, they have the potential to affect many ecosystem functions (Moore et al. 2004). Thus, quantifying the pathways by which detrital energy and nutrients are released is critical for understanding ecosystem function. Despite this appreciation for the importance of detritus to ecosystems, not much research has addressed the degree to which a decline in diversity of macro-detritivores—in this case, caddisflies—affects an ecosystem (Cardinale et al. 2002, Creed et al. 2009).

Previous studies indicate that detritivorous caddisflies play a pivotal role in the remobilization of nutrients and energy to the consumer food web of high-elevation ponds. Because these habitats are oligotrophic (and thus have low levels of nutrients, making sustaining life difficult) they are dependent on detritivores that can free-up nutrients from the surrounding environment (Klemmer et al. 2011.; Thornton and Wissinger ms). In both of these studies, detritus breakdown rates, conversion of detritus to animal tissue, the release of plant nutrients (nitrogen and phosphorus), and algal growth all increase 2-3 fold across a range of caddisfly densities that reflect natural fluctuation in abundance. Those studies focused on the caddisfly, *Limnephilus externus*, which is the only species that occurs in subalpine (>3000 m elev.) permanent ponds with paedomorphic tiger salamanders. These previous investigators argue that because there is no functional redundancy for *L. externus* (i.e., no other detritivores in those communities), this one species should play an inordinately important role in permanent pond ecosystem function (Klemmer et al. 2011; Thornton and Wissinger, ms).

The purpose of my study was to determine the degree to which species-level diversity enhances detritus processing by detritivorous caddisflies in montane temporary ponds. In the Kettle Ponds (~2800 m elev.) south of Gothic, long-duration (dry late in summer or early fall) temporary ponds contain 3-6 species of detritivorous caddisflies. Survey data collected during the ten days prior to the start of the experiment indicated that multi-species assemblages in the Kettle Ponds include 3-4 species in the genus *Limnephilus*. To test the hypothesis that mixed-species assemblages process more detritus, and therefore mobilize more detrital nutrients and energy, I compared detritus processing in single-species vs. mixed species assemblages with the

same total biomass of caddisflies. I used three abundant caddisflies (*Limnephilus externus* = Le; *L. sublunatus* = Ls, and *L. tarsalis* = Lt) that co-occur in many of the Kettle Ponds and Marshes (K1, K4, K5, K6, M1, M2) to the south of Gothic. I predicted that more detritus would be processed in the presence of these three caddisfly species than in the presence of a single species of caddisfly at the same total-biomass density. I also predicted that higher detritus processing would lead to higher concentrations of nitrogen and phosphorus in the water and thus higher algal growth.

Methods:

General Experimental Design

The experiment was conducted in a temporary field laboratory (WeatherPORT, Hansen WeatherPORT, Delta, CO) in front of Willey Lab using the same microcosms used by Thornton and Wissinger (ms). We set up five treatments with five replicates of each treatment in microcosms with a resulting 25 total microcosms (Table 1).

Table 1: Experimental treatments and replication

Treatment	Control	<i>L. externus</i> <i>alone</i>	<i>L. sublunatus</i> <i>alone</i>	<i>L. tarsalis</i> <i>alone</i>	Three species
Microcosm replication	<i>n</i> = 5	<i>n</i> = 5	<i>n</i> = 5	<i>n</i> = 5	<i>n</i> = 5

Caddisflies used in the experiment were collected from the Kettle Ponds and Marshes (K1, K5, K6, M1) located at Rocky Mountain Biological Laboratory. The 0.25 m² microcosms (except the no-caddisfly controls) were stocked with 24 large (*Le* and *Ls*) or 48 small (*Lt*) caddisflies to hold the total biomass of detritus processors approximately constant. This stocking density corresponds to the inflection point (100m⁻²) of asymptotic responses in a previous experiment in which densities of *L. externus* were manipulated—this density had the highest detrital process in microcosms of this size (Thornton and Wissinger, ms). The treatments were randomized in their arrangement in the WeatherPORT to prevent a bias of sunlight exposure.

On days 10, 20, and 30 of the experiment, I measured nutrients (N and P) in the water column and algal biomass estimated from chlorophyll-a. At the end of the experiment (30 days) I measured the amount of detritus remaining and caddisfly developmental stage (larval instar, pupa, or adult if an empty case was present).

Microcosm Setup

Twenty-five microcosms were filled with 9000ml of well water, 20g of sedge detritus, and 5g of willow detritus (after Thornton and Wissinger, ms). This ratio reflects the shoreline coverage (4/5 sedge and 1/5 willow growth) of the detritus sources in the Kettle Pond habitats (K1, K4, K5, K6, M1, M2) where *L. externus*, *L. sublunatus*, and *L. tarsalis* coexist (Wissinger et al. 1999). Sedge (*Carex aquatilis*) and riparian willow (*Salix brachycarpa*) detritus were collected and air-dried for 96 hours. The dried detritus was weighed and divided into 0.25m² microcosms (51x36x13cm). The microcosms were allowed to incubate for five days to encourage microbial colonization before introduction of caddisflies (after Klemmer et al. 2011).

Caddisfly foraging on algae can obscure effects of increased nutrient levels on algal growth. Thus, a mesh screen with holes small enough to keep out caddisflies but large enough to

allow flow of water and some detrital material was secured in one corner of each microcosm. Six periphyton tiles (area = 6.5cm² for each) were placed behind this screen and six in the adjacent corner without a screen. A comparison of algal growth on grazed vs. ungrazed tiles provided an estimate of caddisfly grazing on benthic algae. The tiles were inoculated by scraping algae from the sides of the cattle tanks in the research meadow and washing the algal solution into a shallow tub of water with the tiles. After 5 days of inoculation, the tiles were placed in the microcosms.

Detritus Analysis

On day 30 of the experiment, remaining detritus was separated into coarse particulate organic matter (>1mm) and fine particulate organic matter (<1mm) using 1 mm mesh screen. The coarse detritus (>1mm) was dried at 60°C for 24 hours and weighed. Aliquots (0.25g ± 0.0005 g) of dried sample were ashed at 550°C for two hours to confirm uniformity in end-detrital composition (all had a loss of ~96% mass). The mean coarse detritus mass for each treatment was compared with a one-way ANOVA test.

Estimating Nutrient Levels (N and P)

Ammonium (NH₄⁺) - nitrogen and total phosphorus (P) concentrations in the water were measured on days 10, 20, and 30 in each microcosm during the experiment. To estimate total phosphorus (P), 50 mL of filtered water was collected from each microcosm. The samples were oxidized (to push all P to PO₄) by adding 0.4 g of potassium persulfate and were boiled in a water bath for 1 hour. After cooling, 8 ml of mixed reagent (Strickland and Parsons 1968) was added to each sample and the absorbance at 885 nm was recorded using a spectrophotometer (Milton Roy Spectronic 401, Rochester, NY, 14625). Total soluble P was interpreted from a standard curve of known concentrations analyzed parallel to the samples (Ostrofsky and Rigler 1987).

To measure NH₄⁺, 40 mL of filtered water was extracted from the center of each cage and mixed with 10 mL of OPA (phthaldialdehyde) reagent in amber Nalgene® bottles after Taylor et al. (2007). The water samples were processed with a Turner Designs Trilogy Laboratory Fluorometer (model # 7200-000; Sunnyvale, CA 94085) and analyzed using standard addition protocol I in Taylor et al. (2007). A two-way ANOVA was used to test for the date effects and caddisfly treatment effects on NH₄⁺- N and total soluble phosphorus (TDP).

Chlorophyll-a Analysis

To estimate algal responses to detritus processing, twelve periphyton tiles (area = 6.5cm²) were placed in each microcosm. Six tiles were in an unprotected area of the microcosm and could therefore be grazed by caddisflies, and six were behind a mesh screen to protect them from grazing but expose them to the same water and nutrient conditions (after Klemmer et al. 2011). Every ten days, two protected and two unprotected tiles were collected for sampling. Each pair of tiles was placed in a small plastic bag (Whirlpak Bag, Nasco, Modesto CA). Ten milliliters of buffered ethanol (90%) was added to each bag of tiles to lyse the algal cells and release photosynthetic pigments (Nusch 1980). After lysing for 12 to 24 hours, a 3mL aliquot of the ethanol extract was analyzed for chlorophyll-a concentration with a Turner Designs Trilogy Laboratory Fluorometer (model # 7200; Sunnyvale, CA 94085), treated with 4 drops 0.1 N hydrochloric acid, and then re-analyzed to account for phaeophytin.

A 3-way analysis of variance (ANOVA) was used to test for date effects, caddisfly treatment effects, and grazing effects on the periphyton tiles. We did not use repeated measures

because new periphyton tiles and new water samples were measured on each date (10, 20, and 30 days; Sokal and Rohlf 1981). A subsequent two-way ANOVA was used to analyze effects of caddisfly treatments and grazing on final chlorophyll- α concentration on ungrazed tiles.

Statistical Analysis

For each of the response variables above, I calculated an expected value for the three-caddisfly treatments assuming additive effect [$\sum$ (mean from single-species treatments / 3)] and then re-ran the analyses with this additional value to compare the expected and observed values of the three-species treatments using a post-hoc multiple comparisons test in the context of the overall variation in treatment means.

Results:

Detritus Decay

At the end of the experiment, there were significant differences between original mass and remaining mass of detritus across caddisfly-occupied treatments (one-way ANOVA, $F_{3,15} = 13.25$; $p = 0.0002$; Fig. 1). Post-hoc multiple comparisons tests (Fisher's PLSD) revealed that the remaining detritus was significantly less ($p < 0.05$) in caddisfly treatments than that in the no caddisfly controls except for the *L. tarsalis* treatment ($p > 0.05$). When the analysis was conducted with the predicted value for the three-species treatment, I found no difference between observed and predicted (additive) values ($p > 0.05$; Fig. 1).

Phosphorus Analysis

Results of phosphorus analysis show that total phosphorus concentration significantly increased ($F_{2,60} = 2.210$, $p = 0.0391$) in all caddisfly-occupied treatments in the first ten days then dropped off in the two subsequent sampling dates. After the first sampling, the controls were no longer significantly different in phosphorus concentration compared to all other caddisfly treatments (Fig. 2).

Ammonium Analysis

Because of analysis issues early on, results for ammonium analysis were only possible for the final sampling date. These data show that nitrogen concentrations on the final date of sampling were nearly identical (were not significantly different) across caddisfly-occupied and caddisfly-free treatments ($F_{4,15} = .253$, $p = 0.9033$; Fig. 3).

Chlorophyll-a Analysis

Chlorophyll-a accumulation on periphyton tiles increased over the course of the experiment. Grazed tiles (those accessible to caddisflies) had significantly smaller amounts of chlorophyll-a than the ungrazed (inaccessible) tiles ($F_{4,118} = 5.478$, $p = 0.0004$; Fig. 4), suggesting that caddisflies exploited algae as a food source and so can mask their own stimulatory effect on algal biomass growth.

When just the ungrazed tiles were examined (Fig. 5), the chlorophyll-a stimulatory effect of the caddisflies in all treatments was significant, especially as time went on ($F_{4,59} = 8.760$, $p < 0.0001$; Fig. 5). Figure 6 shows that the observed three-species treatment effects were not significantly different from the additive predictions of a null hypothesis (that the caddisflies are not more efficient when in multi-species assemblages) ($F_{2,22} = 0.080$, $p = 0.9232$; Fig. 6).

Differential Survival Rates

Counts of the caddisflies remaining in the microcosms at the end of the experiment showed a differential survival rate among the treatments ($F_{3,61}=4.46$, $p = 0.018$). Multiple comparison tests indicated that survival was significantly lower in the mixed-species treatments compared to the single-species treatments (between single species, $p = 0.5859$, $p = 0.2546$, and $p = 0.5404$; and between single species and multi-species, $p = 0.0036$, $p = 0.0116$, and $p = 0.0409$), especially for the two smallest species when they were in multi-species microcosms (Fig. 7).

Discussion:

Evidence for a Diversity Effect on Detritus Processing and Nutrient Feedbacks on Algae

As major ecosystem service providers, detritivorous caddisflies are key in linking nutrients between the brown and green trophic paths in aquatic habitats—especially when those habitats are oligotrophic, and hence nutrient limited. Mobilization of detrital nutrients and their re-entry into the consumer foodweb is an alternative to their burial and subsequent sequestration in anaerobic peats and other organic sediments (Oertli 1993). Because of their role in ecosystems, further investigation into niche partitioning and species-specific function is important. In this experiment, the importance of diversity was investigated to determine whether the three species (*L. externus*, *L. sublunatus*, and *L. tarsalis*) are functionally redundant or if they are distinctly important in their functions.

With the exception of *L. tarsalis*, caddisflies clearly enhanced detritus decay rates compared to that in the control (in which detritus was lost by microbial processing only), a result that is consistent with previous results (Klemmer et al. 2011, Thornton and Wissinger, ms). Detritus processing led to increased water column phosphorus concentrations and increased levels of algal biomass, at least initially, suggesting that caddisfly detritus processing provides a nutrient feedback to the algal-grazers of pond food webs. However, for all of the response variables, there was no evidence that this ecosystem function feedback was enhanced by the presence of multiple species of caddisflies as compared to the same biomass of single species.

This result differs from Cardinale et al. (2002) who found that species diversity in stream caddisflies that filter fine particulate organic matter produced the highest rates of ecosystem function (filtering) because of niche partitioning within the water column. One explanation for the absence of a diversity effect in my experiment is that the caddisflies have comparable per-biomass detritus foraging rates and prefer the same detrital resources, and thus are functionally redundant. An alternative explanation is that they are actually not functionally redundant, but that aggressive interactions (interference competition, intraguild predation) in the mixed species treatment masks a greater than additive effect on detritus processing. In a study on aphid parasitoids, intraguild effects had a primary effect in population fluctuations (Brodeur et al. 2000). They found that when more than one species of parasitoid was exploiting a host resource, species in the same guild (members of the same trophic position) would come into greater contact and, coupled with lower density of host and higher density of parasite, have an adverse effect on parasite populations. Brodeur et al. (2000) also found these effects to be amplified in the laboratory compared to field experiments.

Such intraguild predation and interference have been observed in some detritivorous caddisflies as well (Wissinger et al. 1996, 2004, 2006a,b), but the particular species

combinations in my experiment have not been explored. The survival data in mixed species treatments for *L. tarsalis* suggest this small species might be especially vulnerable to intraguild predation. Such effects have been considered both empirically and theoretically for impacts on shared prey (Vance-Chalcraft et al. 2007), but not to my knowledge for impacts on an ecosystem function such as detritus processing.

Algal Grazing by Caddisflies

Ungrazed periphyton tiles had consistently higher levels of chlorophyll-a than the grazed tiles—this provides evidence that caddisflies initially benefit from detrital resources when they first consume dead plant material, and then benefit a second time when they can harvest the algal stimulated by their own excretion. This double-harvest has not been previously reported and would be an interesting area of future research that compares caddisfly growth and development with (light) and without (dark) the added effects of stimulated algae. It also provides a caveat for demonstrating the stimulatory effect of detritus processing in open ponds – i.e., periphyton tiles placed in open pond systems will need to be protected from caddisfly grazing so that it does not mask the effects of detritus processing on nutrient release and subsequent stimulation of algal growth (Wissinger and Thornton, ms).

Algal uptake of nutrients can potentially mask nutrient feedbacks

When water nutrient levels were analyzed, phosphorus concentrations spiked at the beginning of the experiment and decreased in concentration as time went on. One possible explanation for this is that once algae became established there was greater uptake of phosphorus. The lack of any treatment effects on ammonium concentrations is consistent with this interpretation. This could also serve as evidence that the algae was strongly established and was taking in these excreted nutrients.

The chlorophyll-a data supports this algal uptake theory: as time progressed and water nutrient levels decreased, chlorophyll-a levels increased. With greater algal biomass there would be lower water nutrient concentration. The date effect of increased algae was most clear on the ungrazed tiles because they represented the stimulatory effect of caddisfly excretion on algal growth unaffected by caddisflies re-grazing on the algae. However, observed multi-species treatment levels of phosphorus, ammonium, and chlorophyll-a were not significantly different from calculated additive effects, therefore failing to support the hypothesis of greater ecosystem function in multi-species assemblages.

Summary, Conclusions, & Future Studies

Additive vs. observed comparisons in the experiment suggest the caddisflies are functionally redundant; i.e., that they fill the same niches and so have approximately the same additive effects. Although this is the most parsimonious interpretation, it is also possible that they do have different functional roles, but they interfere with each other when placed in the experimental conditions of this study. In this study, percent survival for the multi-species assemblages was significantly lower when compared to the single-species counts (Fig. 7). Because of this higher mortality rate, the expected increased level of ecosystem functions in multi-species assemblages may have been masked.

According to experimental results, it appears that multiple vs. single-species assemblages of detritivorous caddisflies: 1) process similar amounts of detritus, 2) excrete similar amounts of plant nutrients (N & P), and 3) similarly stimulate algal growth, at least in the three species

studied. The data suggests the congeners in this experiment are functionally redundant, but the gut contents of several preserved individuals from my experiment will be examined to determine their diet preferences. Niche partitioning is still possible, but based on the experimental results thus far I expect similar diets.

Though the experimental results on functional redundancy in this experiment are somewhat ambiguous, a key finding was documented in the double-harvesting phenomenon of caddisfly algal grazing. The significant difference between grazed and ungrazed tiles in the same treatment conditions (Fig. 8) shows significant impact of caddisflies on algal growth not only in the positive (provision of algal nutrients in the form of N & P) but also in the negative (the depletion of algae as grazing occurs). This finding is important first in that it informs researchers that protecting algal growth surfaces from grazing in the field is important if accurate chlorophyll-a readings are to be expected, and second in that caddisflies are having a double effect on their environment in this way. They reap the benefits of detritus processing twice over, once when dead plant matter is being chewed for its caloric value and once when the algae, stimulated by the caddisflies' own excretion, is harvested. This "double-dipping" into the aquatic trophic ladder would be an excellent area of future study.

Acknowledgements

I thank Scott Wissinger for guidance throughout the project's inception, execution, and analysis, Brad Taylor for advice and equipment for water nutrient and chlorophyll-a analysis, and Marieke Perchik and Jason Drake for countless hours of help and comic relief. I thank the National Science Foundation for funding through my REU grant, and I thank the Rocky Mountain Biological Laboratory for access to the Kettle Ponds, other research sites, research materials, and the sheer opportunity of being there.

Literature Cited:

- Brodeur, Jacques and Jay A. Rosenheim 2000. *Intraguild interactions in aphid parasitoids*. Entomologia Experimentalis et Applicata (97): 93-108.
- Cardinale, B., M. Palmer, S. Collins 2002. *Species diversity enhances ecosystem functioning through interspecific facilitation*. Nature (415): 426-29.
- Creed, R., R. Cherry, J. Pflaum, C. Wood 2009. *Dominant species can produce a negative relationship between species diversity and ecosystem function*. Oikos (118): 723-32.
- Giller, P. S., H. Hillebrand, U. G. Berninger, M. O. Gessner, S. Hawkins, P. Inchausti, C. Inglis, H. Leslie, B. Malmqvist, M. T. Monaghan, P. J. Morin, and G. O'Mullan 2004. *Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments*. Oikos (104):423-436.
- Klemmer, A., S. Wissinger, H. Greig, M. Ostrofsky 2011. *Strong Single-Species Effects of a Detritivore on Multiple Ecosystem Processes*. (Accepted to the Journal of Animal Ecology)
- Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, J.P. Grime, A. Hector, D.U. Hooper, M.A. Huston, D. Raffaelli, B. Schmid, D. Tilman, and D.A. Wardle 2001. *Biodiversity and ecosystem functioning: current knowledge and future challenges*. Science (294): 804-808.
- Moore, J.C., E.L. Berlow, D.C. Coleman, P.C. de Ruiter, Q. Dong, A. Hastings, N.C. Johnson, K.S. McCann, K. Melville, P.J. Morin, K. Nadelhoffer, A.D. Rosemond, D.M. Post, J.L.

- Sabo, K.M. Scow, M.J. Vanni, and D.H. Wall 2004. *Detritus, Trophic Dynamics and Biodiversity*. Ecology Letters (7): 584-600.
- Nusch, E.A. 1980. *Comparison of different methods for chlorophyll and phaeopigment determination*. In: H. Ria (ed.), *The measurement of photosynthetic pigments in fresh waters and the standardization of methods*. Archiv für Hydrobiologie (14): 14-36.
- Oertli, B. 1993. *Leaf litter processing and energy flow through macroinvertebrates in a woodland pond (Switzerland)*. Oecologia (96): 466-477
- Ostrofsky, M.L. and F.H. Rigler 1987. *Chlorophyll-phosphorus relationships for subarctic lakes in western Canada*. Canadian Journal of Fisheries and Aquatic Sciences (44): 775-781.
- Riess, J., J.R. Bridle, J.M. Montoya, and G. Woodward 2009. *Emerging horizons in biodiversity and ecosystem functioning research*. Trends in Ecology and Evolution (24): 505-514.
- Sokal, R.R. and F.J. Rohlf 1981. *Biometry*, 2nd edition. Freeman, New York, NY, USA.
- Steinman, F. Hauer, G. Lamberti 2006. In F. Hauer, G. Lamberti (Eds.) *Methods in Stream Ecology* (p. 362-76). Burlington, MA, USA.
- Taylor, B.W., C.F. Keep, R.O. Hall, Jr., B.J. Koch, L.M. Tronstad, A.S. Flecker, and A.J. Ulseth 2007. *Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions*. Journal of the North American Benthological Society (26): 167-177.
- Thornton, E., S. Wissinger 2011. *Nutrients Released During Detritus Processing by Caddisflies Stimulate Benthic Algal Biomass*. [unpublished]
- Vance-Chalcraft, H.D., J.A. Rosenheim, J.R. Vonesh, C.W. Osenberg, and A. Sih 2007. *The influence of intraguild predation on prey suppression and prey release: a meta-analysis*. Ecology (88): 2689-2696.
- Wissinger, S., A. Bohonak, H. Whiteman, W. Brown 1999. *Subalpine Wetlands in Colorado: Habitat Permanence, Salamander Predation, and Invertebrate Communities*. In D. Batzer, R. Rader, S. Wissinger (Eds.), *Invertebrates in Freshwater Wetlands of North America: Ecology and Management* (p. 757-790). Willey, New York, USA.
- Wissinger, S., S. Brown, J. Jannot 2003. *Caddisfly life histories along permanence gradients in high-altitude wetlands in Colorado (U.S.A.)*. Freshwater Biology (48): 255-70.
- Wissinger, S.A., *C. Eldermire, and *J.C. Whissel 2004. *The role of larval cases in reducing aggression and cannibalism among caddisflies in temporary wetlands*. Wetlands 24(4): 777-783.
- Wissinger, S.A., Grace B. Sparks, Gretchen L. Rouse, Wendy S. Brown, Heidemarie Steltzer 1996. *Intraguild Predation and Cannibalism among Larvae of Detritivorous Caddisflies in Subalpine Wetlands*. Ecology (77): 2421-2430.

Appendix: Figures, tables, and full statistical data

Bars and letters in all Figures indicate groupings of treatments that do not differ based on a post-hoc multiple comparison test (Fisher's PLSD).

Figure 1: *Detritus Analysis*.

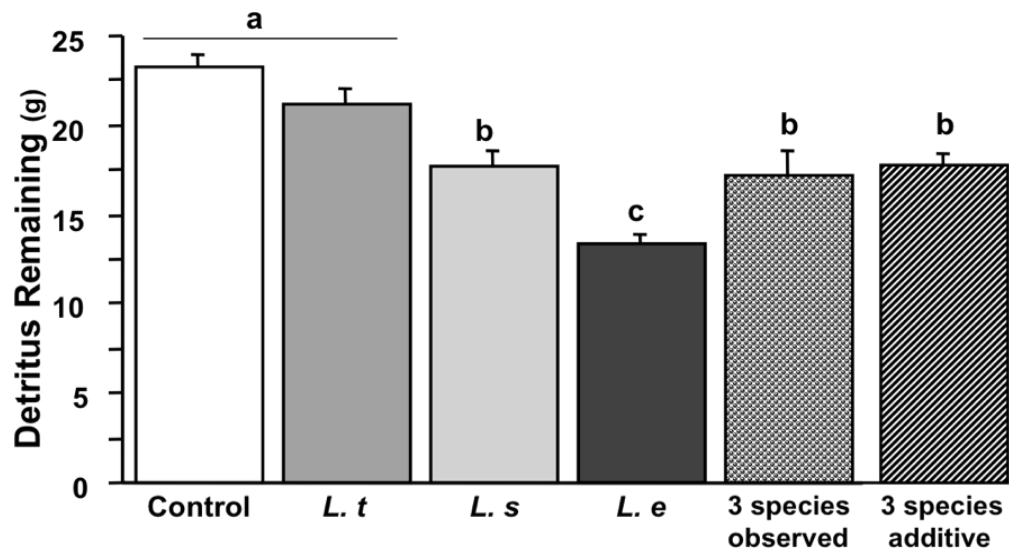


Figure 1: Consumed detritus (g) as a function of caddisfly treatments.

Figure 2: *Phosphorus Analysis*

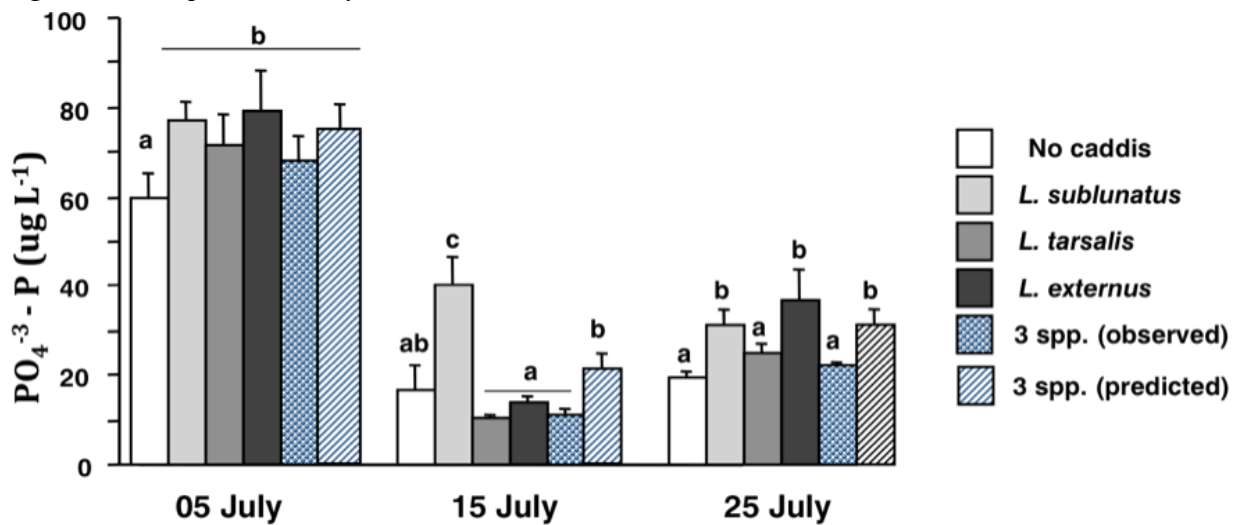


Figure 2: Phosphorus concentration as a function of caddisfly treatment and time.

Figure 3: Ammonium Analysis

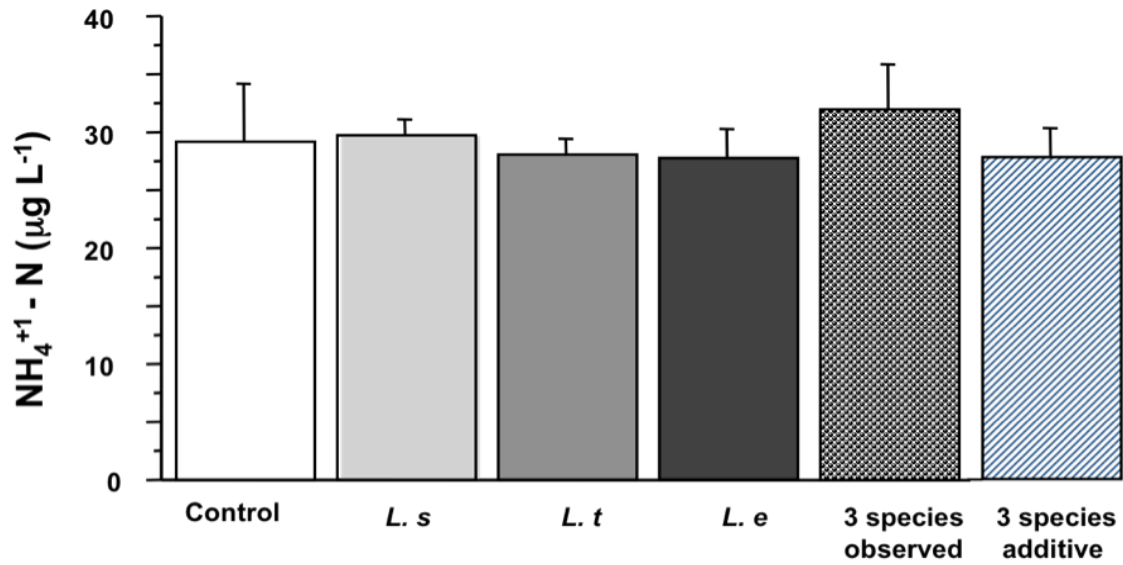


Figure 3: Ammonium concentration as a function of caddisfly treatments at the end of the experiment (25 July).

Figure 4: Chlorophyll-a Analysis

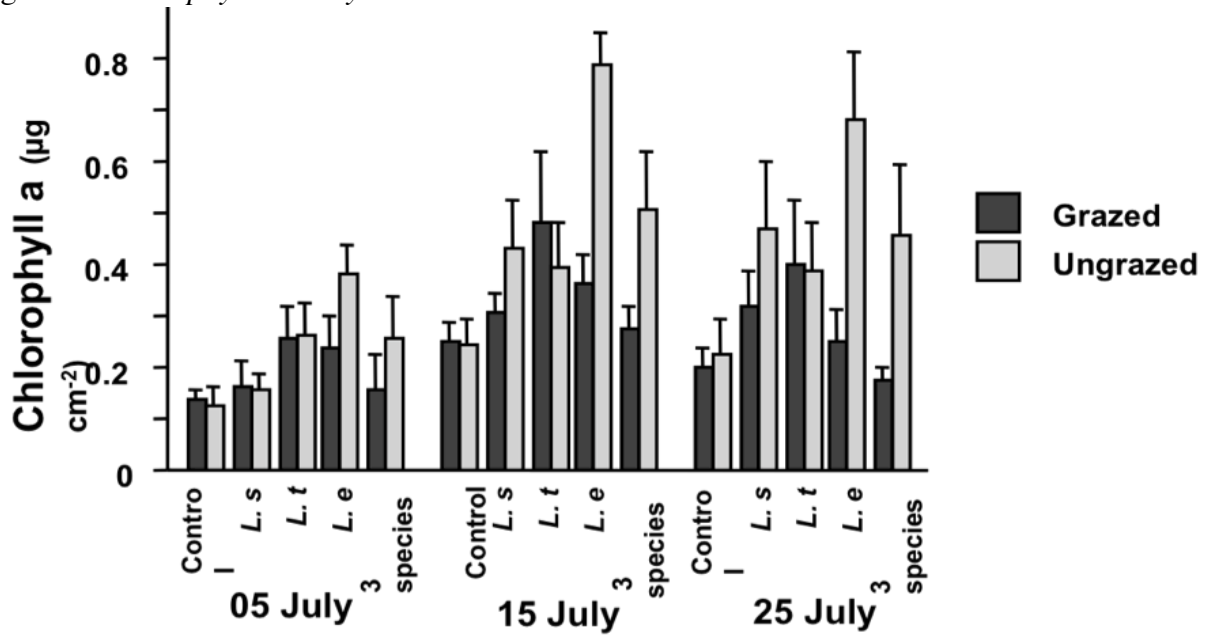


Figure 4: Chlorophyll-a concentration as a function of caddisfly treatment, date, and grazed/ungrazed surfaces.

Figures 5, 6: *Chlorophyll-a Analysis*

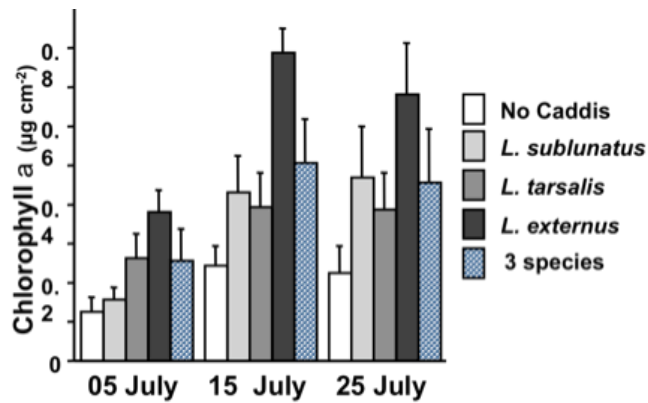


Figure 5: Chlorophyll-a concentration as a function of caddisfly treatment, date, and ungrazed surfaces only.

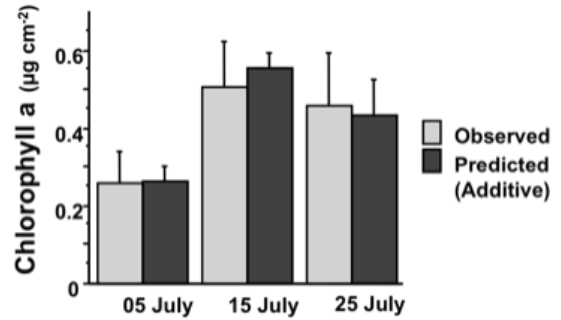


Figure 6: Chlorophyll-a concentration as a function of date and observed vs. predicted (additive) results.

Figure 7: *Percent Survival*

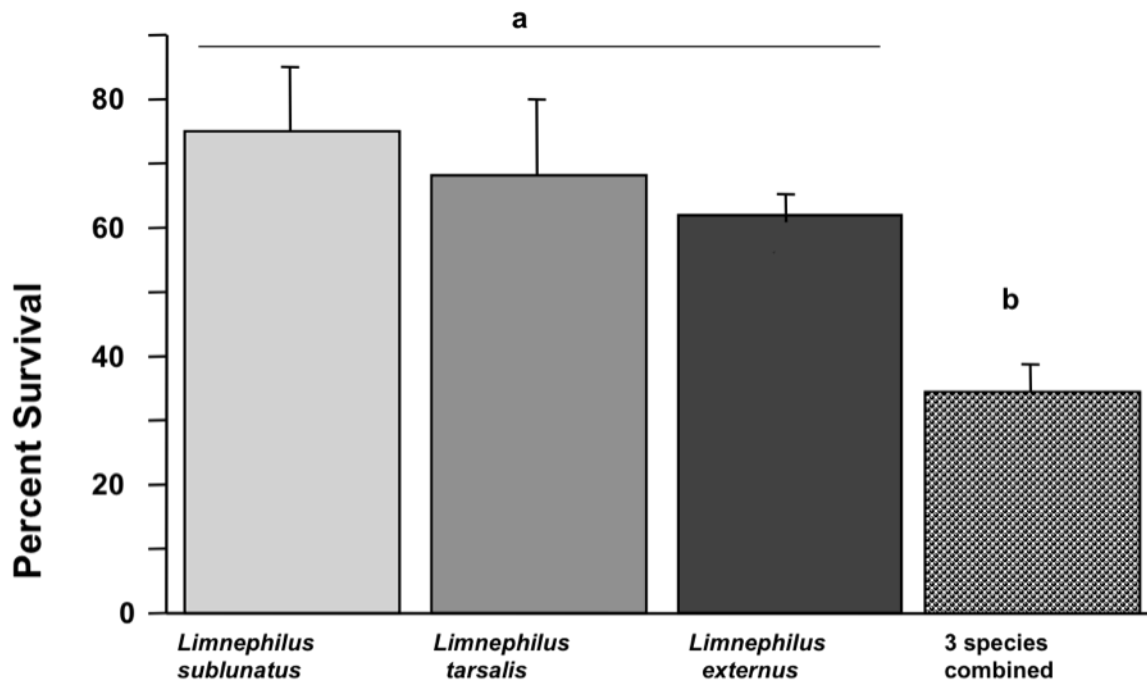


Figure 7: Percent survival as a function of caddisfly treatments.

Figure 8: *Chlorophyll-a analysis*: difference between grazed and ungrazed shows the need to cage periphyton-gathering surfaces in the field for more accurate measurements of chl-a.

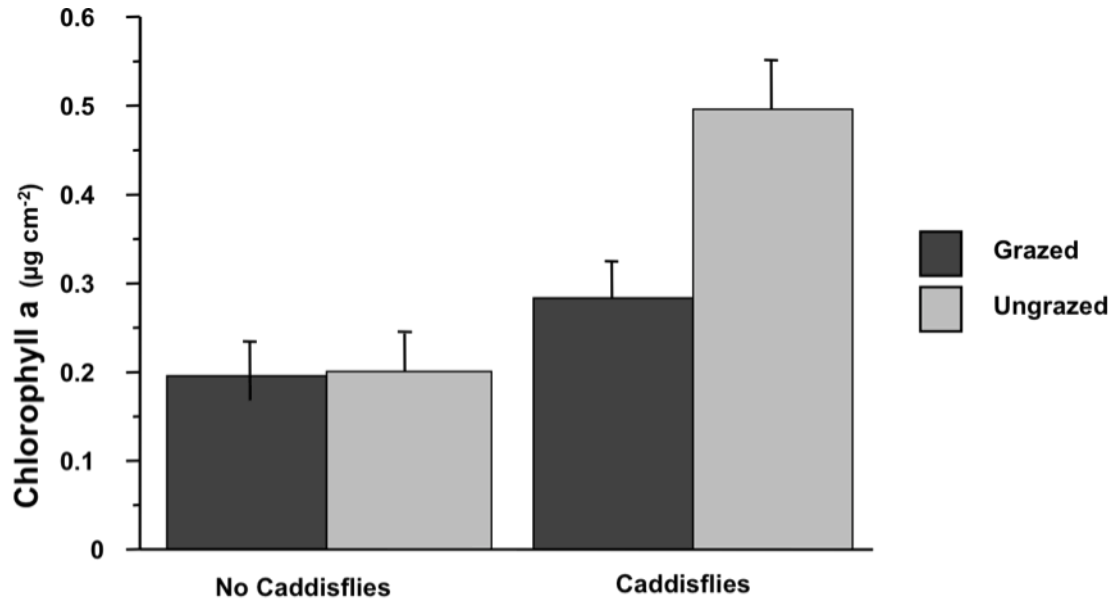


Figure 8: Chlorophyll-a as a function of caddisfly-treatments and caddisfly-free treatments comparing grazed and ungrazed periphyton tiles.

Table 2: Statistical data for Figure 1, Detritus Analysis

ANOVA Table for Final Detritus (g)

Inclusion criteria: Criteria 2 from LastDateOnly

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Some of Treatment	3	153.482	51.161	13.245	.0002	39.735	.999
Residual	15	57.939	3.863				

Table 3: Statistical data for Figure 2, Phosphorus Analysis

ANOVA Table for PO4 (ug /L)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sample Date	2	40236.962	20118.481	172.459	<.0001	344.918	1.000
Treatment	4	3294.173	823.543	7.060	.0001	28.238	.995
Sample Date * Treatment	8	2062.092	257.761	2.210	.0391	17.677	.820
Residual	60	6999.405	116.657				

Table 4: Statistical data for Figure 3, Ammonium Analysis

ANOVA Table for NH4 -Nitrogen

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	4	42.066	10.516	.253	.9033	1.012	.091
Residual	15	623.209	41.547				

Table 5: Statistical data for Figure 4, Chl-a analysis for all dates, all treatments, and all dates

ANOVA Table for Chlorophyll a

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Date	2	.962	.481	15.643	<.0001	31.287	1.000
Treatment	4	1.029	.257	8.368	<.0001	33.474	.999
Grazed?	1	.531	.531	17.272	<.0001	17.272	.993
Date * Treatment	8	.111	.014	.450	.8886	3.598	.201
Date * Grazed?	2	.106	.053	1.728	.1821	3.456	.344
Treatment * Grazed?	4	.673	.168	5.478	.0004	21.910	.979
Date * Treatment * Grazed?	8	.116	.015	.473	.8731	3.785	.210
Residual	118	3.627	.031				

Table 6: Statistical data for Figure 5, Ungrazed Tiles Only

ANOVA Table for Chlorophyll a

Inclusion criteria: Ungrazed Only2 from DiannaData

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Date	2	0.798	0.399	10.220	0.0002	20.440	0.990
Treatment	4	1.368	0.342	8.760	<0.0001	35.041	0.999
Date * Treatment	8	0.170	0.021	0.544	0.8185	4.353	0.226
Residual	59	2.303	0.039				

Table 7: Statistical data for Figure 6, Three Species Combined and compared to additive effects

ANOVA Table for Chlorophyll a Ungrazed

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Date	2	.331	.166	3.888	.0358	7.776	.637
Actual or Realized	1	.001	.001	.018	.8957	.018	.052
Date * Actual or Realized	2	.007	.003	.080	.9232	.160	.061
Residual	22	.937	.043				

Table 8: Statistical data for Figure 7, Percent Survival

Fisher's PLSD for % Survival

Effect: Species

Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
L. sublunatus, L. tarsalis	6.667	25.417	.5859	
L. sublunatus, L. externus	14.167	25.417	.2546	
L. sublunatus, 3 species	40.833		.0036	S
L. tarsalis, L. externus	7.500		.5404	
L. tarsalis, 3 species	34.167		.0116	S
L. externus, 3 species	26.667		.0409	S

Table 9: Statistical data for Figure 8, Chlorophyll-a analysis comparing caddisfly treatments and caddisfly-free treatments

ANOVA Table for Some of Chlorophyll a

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Caddisflies	1	.253	.253	5.138	.0283	5.138	.596
Some of Grazed?	1	.117	.117	2.364	.1311	2.364	.309
Caddisflies * Some of Grazed?	1	.068	.068	1.373	.2474	1.373	.198
Residual	45	2.220	.049				