

Comparing Decomposition Rates and Detritivore Preferences for
Caddisfly (Trichoptera) Cases versus Ambient Detritus
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Abstract

Humans manage microbes in their built environments by lowering humidity, filtering outside air, and applying antimicrobials that together fosters the selective dominance of microorganisms that can tolerate these harsh conditions and selects for antimicrobial resistance. Other species, however, regulate decomposition of their built environments in warm, moist conditions with diverse microbial communities. For example, larval caddisflies that live in streams and ponds use their labial silk glands to weave detritus, pebbles, and other materials into portable cases. Premature decomposition of cases is detrimental to these invertebrates, as cases serve as protection for vulnerable larvae against predation, aid in respiratory efficiency, and ensure reproductive success of adults. Plant-based cases remain in wetland ponds in Colorado's East River valley for up to nine months after caddisfly pupation and case abandonment, while the same detritus not associated with cases is absent from the pond bottom. Insect silk, which often contains antimicrobial peptides or proteins, may be a contributing factor in potential antimicrobial properties and decreased detritivore consumption of cases. In this study, I first quantified decomposition of caddisfly cases compared to that of the same non-case associated detritus, expecting caddisfly cases to possess slower rates of decomposition. Second, I explored the feeding preferences of a common invertebrate detritivore for the cases of different caddisfly species and the non-case associated detritus. Lastly, I quantified the fungal communities of cases and detritus as well as the feeding preference of detritivores. I hypothesized that cases contain less fungi, correlating to lower food value and a potential mechanism for detritivore avoidance of cases over non-case associated detritus. While *A. bimaculata* cases and *C. aquatilis* had similar slow rates of mass loss, *L. externus* and *H. designatus* cases rapidly decomposed over the course of four weeks. The latter cases with finer pieces of detritus also lost organic mass fastest, suggesting higher fungal activity and a strong detritivore feeding preference. *L. externus* and *A. bimaculata* larvae avoided *A. bimaculata* cases and preferred *L. externus* cases due to the potentially aggressive behavior of larger *A. bimaculata* larvae and physically easier to eat small pieces of detritus in *L. externus* cases. With the ideal conditions of agar plates, fungi on *C. aquatilis* cases possessed the highest colony morphotype diversity, but percent of fungal cover was similar across *C. aquatilis*, *L. externus* cases, and *A. bimaculata* cases. The behavior and case construction of caddisfly cases give insight into the diverse strategies these invertebrates employ to maintain their built environments in wetland ponds conducive to vigorous microorganism proliferation and decomposition of organic matter, informing future design of human-built environments.

Introduction

For thousands of years, humans have manipulated the temperature (<20° C) and humidity (<55%) of their built environments to allow for both physical comfort and suppression of microbial colonization. While these conditions are unsuitable for the growth and survival of many microorganisms, the selectivity of built environments as well as the frequent use of

antibiotics promote the dominance of a few species and often those with antimicrobial resistance (Lax et al. 2019). This closed-door approach has led to decreased exposure to environmental microbes and increased exposure to bodily microbes (Dunn et al. 2013), amplifying the prevalence of allergies and autoimmune disorders (Hanski et al. 2012). On the other hand, many other species thrive in warm, high humidity environments that facilitate the proliferation of diverse, healthy microbial communities. Additionally, organisms living in non-human-built environments are also more likely to produce diverse antimicrobial compounds, further limiting the dominance of one microbe. This selective management of microbes can also be important in controlling the decomposition of built environments, especially in warm, moist, oxygen-rich environments. Although there is some merit to limiting one's microbe exposure, the dominance of resistant species and frequency of autoimmune disorders demonstrate a motivation behind rethinking current architectural designs through investigation into the success of built environments of other organisms.

One such group of organisms that thrive in environments conducive to vast microbial colonization and rapid decomposition are caddisflies (*Trichoptera*), which live in warm, high humidity ponds, streams, and wetlands. Many caddisfly larvae construct portable cases made of detritus, pebbles, and other materials weaved together with silk using their labial silk glands (Frandsen et al. 2024). As caddisflies progress through five instars, or larval developmental stages, their cases serve as visual (camouflage) and physical protection against predation and cannibalism as well as aiding in respiratory efficiency (Boyero et al. 2006, Williams et al. 1987). Given the inter- and intraspecific consumption of cases at high densities (Klemmer et al. 2012), as well as the 35% loss of larval protein when forced to rebuild 5th instar *Limnephilus rhombicus* cases (Mondy et al. 2010), the maintenance of structural integrity, or resistance to decomposition and detritivore consumption, is essential to the survival and reproductive success of caddisflies. After their final, 5th instar, caddisflies pupate, and then transform into winged, sexually capable adults, eventually laying egg masses and leaving their cases empty in the water (Wiggins 2004).

Despite the wet, microbe-rich environment, caddisfly cases have been found in wetland ponds in the East River valley of Colorado long after pupation, leading researchers to hypothesize that plant-based cases may be more resistant to microbial decomposition and detritivore consumption than the same plant-based materials not involved with cases. For example, sedge (*Carex aquatilis*) and willow (*Salix sp.*) leaf-based cases of the caddisflies *Limnephilus externus*, *Anabolia bimaculata*, *Limnephilus picturatus*, and *Nemotaulius hostilis* have been found intact as long as nine months after their larvae have pupated and emerged as terrestrial adults (B.W. Taylor *personal observation* at Mexican Cut and Island Pond by Crested Butte Community School). Pieces of dead sedge from the previous season not associated with cases, however, were absent from the Island Pond, indicating that cases made of plant-based materials should decompose within the nine-month span, unless they possess antimicrobial properties or decreased detritivore consumption (B.W. Taylor *personal observation* at Island Pond).

Caddisfly silk may be one component that contributes to potentially antimicrobial and decomposition-resistant cases, as insect silk often contains antimicrobial peptides or proteins (AMPs) that can eliminate microorganisms (Wang et al. 2025). By limiting fungal growth, caddisflies both slow rates of decomposition and reduce the nutritional quality of cases for

detritivore consumption (Bärlocher and Kendrick 1975). Exploring the decomposition-resistance of silk and cases of caddisflies may have applications for developing microbial-resistant materials, approaches to managing microbes, drug development, and building design choices, especially in warm, humid environments, in the future.

My primary objective in this study was to determine if plant-based caddisfly cases decompose, or lose mass, at a different rate than non-case associated detritus of the same plant taxa but not associated cases (hereafter referred to as ambient detritus). I expected that the decomposition rates, or mass loss, of plant-based caddisfly cases are less than those of ambient plant detritus due to potential antimicrobial compounds in cases, such as the silk fibers used to construct cases. Additionally, I aimed to discover whether potential differences in decomposition rates were due to decreased fungal proliferation on caddisfly cases and detritivore feeding preference for ambient detritus. I predicted that cases contain fewer microbes, especially fungi, than ambient detritus, causing detritivores to prefer ambient detritus over cases.

Methods

Study Sites

I collected caddisfly cases from various ponds and streams near Gothic, Colorado, USA, including riparian ponds within the East River valley (e.g., Fish Hook pond, the Bellevue Beaver Ponds, the Upper East River Beaver Pond, and the Kettle Ponds).

Testing for Differences in Mass Loss between Cases and Ambient Detritus

I collected forty-five plant-based cases for each of three caddisfly species (*Limnephilus externus*, *Anabolia bimaculata*, and *Hesperophylax designatus*) and pieces of plant-based detritus of the same material (*Carex aquatilis*) and quality (e.g., dead plant material) used in *L. externus* and *A. bimaculata* cases from Fish Hook, Bellevue Beaver, and K7 ponds. *H. designatus*, which make cases of *Picea Engelmannii* cones, were used as a comparison to other cases, not the corresponding non-case associated detritus. After freezing the cases and detritus to remove microinvertebrates, I removed caddisfly larvae from their cases. I cut forty-five pieces of detritus material that were similar in wet weight to each respective case (34.6-143.2 mg: *L. externus*, 7.1-16.6 mg: *H. designatus*, 47.0-218.4 mg: *A. bimaculata*). Both the cases and detritus were air dried for thirty-six hours, then weighed on an analytical balance to the nearest 1000th of a gram. I air dried rather than oven dried to avoid destroying the microbial community and altering its structural integrity. I measured mass loss of individual cases by placing each case and ambient detritus into open-topped containers with mesh (0.3 mm: *L. externus*, 1.5 mm: *H. designatus*, *A. bimaculata*) sides and bottoms, which allowed all microbial and animal consumers access. To prevent cases or ambient detritus from floating away or being swept away by water currents, I used a single strand of fishing line or paper clip placed through a case or around the detritus pieces to secure cases and detritus to the mesh bottom. Two open-topped containers, one containing a case and one containing detritus, were attached to each of thirty-six bamboo stakes and submerged to the bottom of the pond for one to four weeks. I accounted for mass lost during transportation of containers to field sites by randomly selecting nine cases to be brought back to the lab for mass measurements immediately (*L. externus*: 9.0%, *A. bimaculata*: 1.2%, *C. aquatilis*: 3.7%, *H. designatus*: 20%; ANOVA, Tukey's post-hoc tests, $p < 0.05$ for *A.*

bimaculata-*H. designatus*). Each week I removed nine stakes. Open-topped containers were visually inspected for invertebrates and the inner contents were rinsed into sample containers for further inspection with a microscope. Cases and ambient detritus were dried in a force air oven at 60°C for twenty-four hours, weighed, burned in a muffle furnace at 500°C, and weighed again to determine both the dry and organic mass remaining at each collection time interval.

Determining Feeding Preference of L. externus and A. bimaculata

I collected twenty similar sized *L. externus* cases, twenty similar sized *A. bimaculata* cases, and pieces of *C. aquatilis* of the same quality from the Bellevue beaver pond. I removed ten of the *L. externus* and *A. bimaculata* larvae from their cases. The other ten *L. externus* and *A. bimaculata* larvae remained in their cases. I cut pieces of *C. aquatilis* that were similar in wet weight and size of the cases. I randomly placed each feeding option (*L. externus* case, *A. bimaculata* case, and two *C. aquatilis*) in each corner of ten 13 by 13 cm pond water-filled plastic containers. The cases and detritus were placed in paper clips to weigh them down. I placed one *L. externus* larva in the center of each of ten containers and one *A. bimaculata* larva in the other ten containers. After five minutes of acclimation, I performed instantaneous counts once every fifteen minutes for the next thirty minutes and then waited one hour fifteen minutes. I performed the above procedure five times, gathering fifteen counts for each container over the course of 7 hours 30 minutes.

Comparing Fungal Growth on Caddisfly and Non-case Associated Detritus

I collected ten *A. bimaculata* and *L. externus* larvae, which make cases of *C. aquatilis*, and pieces of *C. aquatilis* not associated with cases of the same quality from the Upper East River Beaver Pond. Larvae were removed from their cases. I cut ten pieces of *C. aquatilis* that were similar in wet weight to the cases. I rinsed the cases and detritus with distilled water to remove microinvertebrates. The cases and detritus were sonicated for ten minutes to suspend the bacterial and fungal cells in 10 ml of distilled water. I performed dilutions of the stock solution (1:10, 1:100, 1:1000) and pipetted 100 μ l of the concentrations for each material onto agar plates. The inoculation material contained chloramphenicol that inhibited bacterial growth, which allowed me to selectively culture fungi and yeast. After a nine-day incubation at 21°C, I counted the number and calculated the area of colonies on each plate.

Statistical Analyses

To test for differences in mass loss rates, I used the homogeneity of slopes test of the analysis of covariance (ANCOVA) model, where $mass\ at\ time(t) = material\ type + time + material\ type \times time$, and a significant interaction term ($material\ type \times time$) indicates different slopes, with a higher slope value indicating the material that loses mass, or decays, faster. To test for feeding preferences I used a PERMANOVA, and I performed individual pairwise comparisons to determine which food types were consumed preferentially when the null hypothesis of no preference was rejected (significant PERMANOVA p-value). As recommended by Lockwood (1998), I used proportional responses (e.g., percent of time feeding or percent of visits) rather than absolute responses (e.g., total time or total visits) to avoid potential bias caused by random or individual variation in absolute response across replicates. Finally, I do not have replicates to analyze fungal growth on agar plates. All analyses were performed using RStudio (2024.12.1).

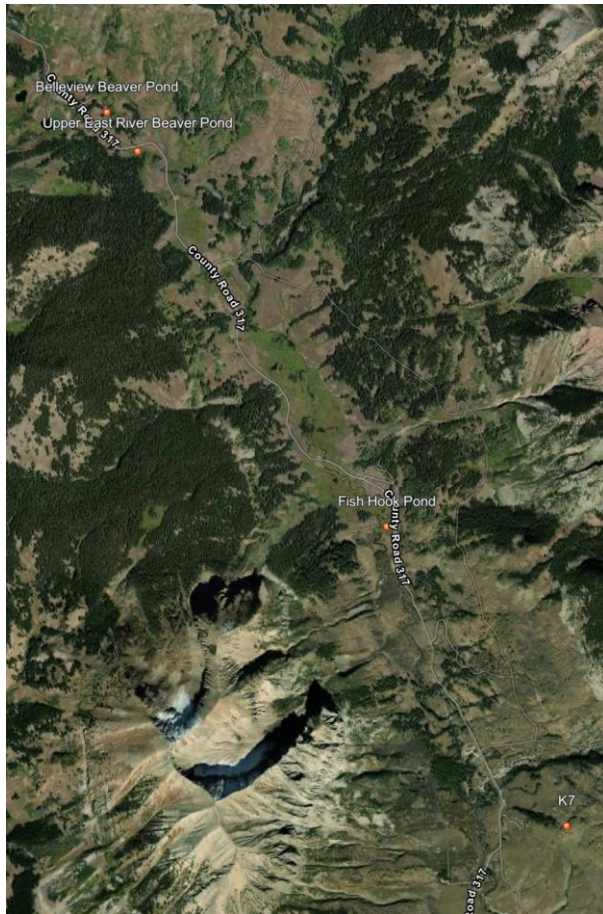


Figure 1. Map of research sites (Belleview Beaver Pond, Upper East River Beaver Pond, Fish Hook Pond, K7) across East River valley of Colorado.



Figure 2. Open-topped containers in Fish Hook Pond containing *C. aquatilis* and an *L. externus* case testing for mass loss differences.



Figure 3. *L. externus* larvae in container with *L. externus* case, *A. bimaculata* case, and *C. aquatilis* food choices.

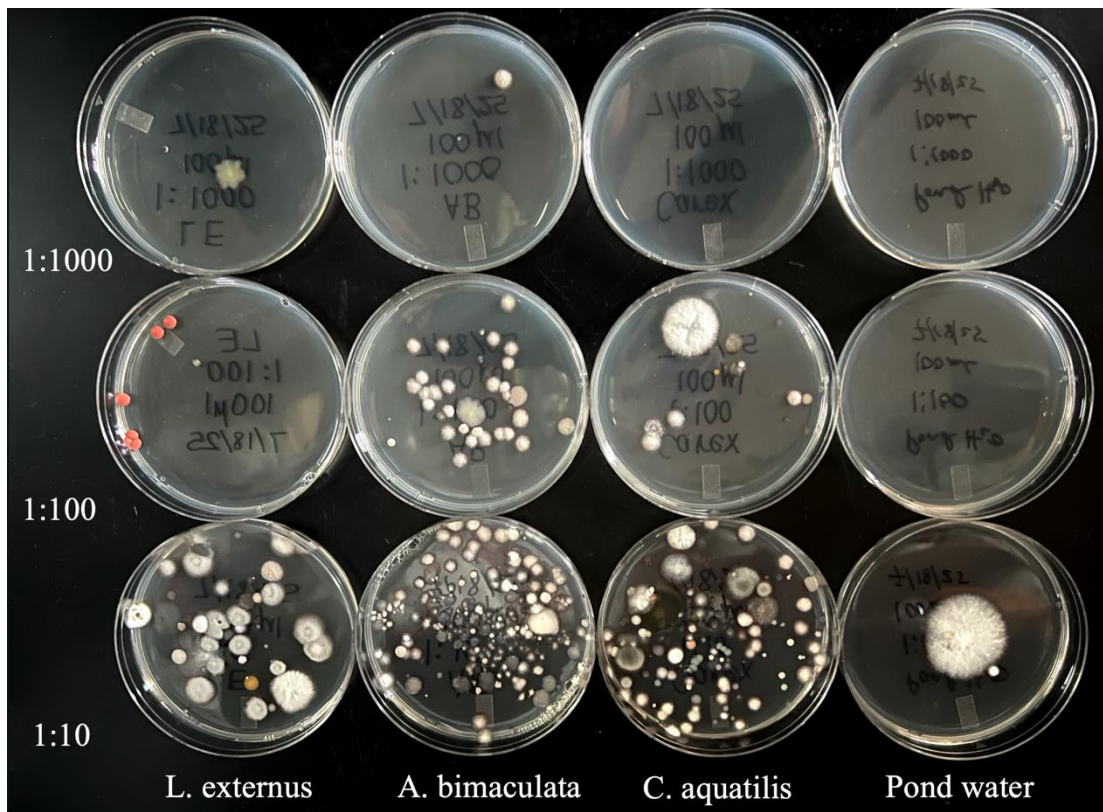


Figure 4. Agar plates cultured by different concentrations (1:1000, 1:100, 1:10) of bacteria and fungi on *L. externus* cases, *A. bimaculata* cases, *C. aquatilis*, and in pond water.

Results

A. bimaculata cases and *C. aquatilis* presented no differences in dry mass loss rate (around 5.5% per week) over the course of four weeks (Figure 5, ANCOVA, Tukey's post-hoc test, $p>0.05$), while decomposition/detritivore feeding rates of *H. designatus* and *L. externus* cases (around 11% and 13% per week) were significantly faster (ANCOVA, Tukey's post-hoc test, $p<0.001$). The initial percent of organic content, however, was 12% lower for *L. externus* cases (Figure 6, ANOVA, Tukey's post-hoc test, $p<0.001$). After time in Fish Hook Pond, the decrease in percent organic content of *L. externus* and *H. designatus* cases was 5 times greater than the other materials (ANCOVA, Tukey's post-hoc test, $p<0.001$). *A. bimaculata* cases and *C. aquatilis* had similar 2.5% per week decline in percent organic content (ANCOVA, Tukey's post-hoc test, $p<0.001$).

In the feeding choice experiment, *L. externus* larvae avoided feeding on *A. bimaculata* cases compared to *L. externus* cases and *C. aquatilis* (Figure 7, PERMANOVA, followed by individual ANOVAs and Tukey's post-hoc tests, $p=0.019$, 0.049). Though nonsignificant, *A. bimaculata* larvae spent 230% more time at *C. aquatilis* and 450% at the *L. externus* cases than *A. bimaculata* cases (PERMANOVA, $p>0.05$). *L. externus* cases were fed on the most by both detritivore species.

When looking at fungal growth of various inoculation materials, pond water fungi colonized the smallest amount of area (15.0% at most) on the agar plates and possessed only two colony morphotypes (Figure 8, Figure 9). The 1:10 *C. aquatilis*-treated plate yielded double the fungal morphotypes as the *A. bimaculata* case and the pond water plate, but the 1:10 *A. bimaculata* plate had 65% more colonies than the other plates. Moreover, the 1:10 *L. externus* plate grew 1/3 the number of colonies as the *C. aquatilis* plate.

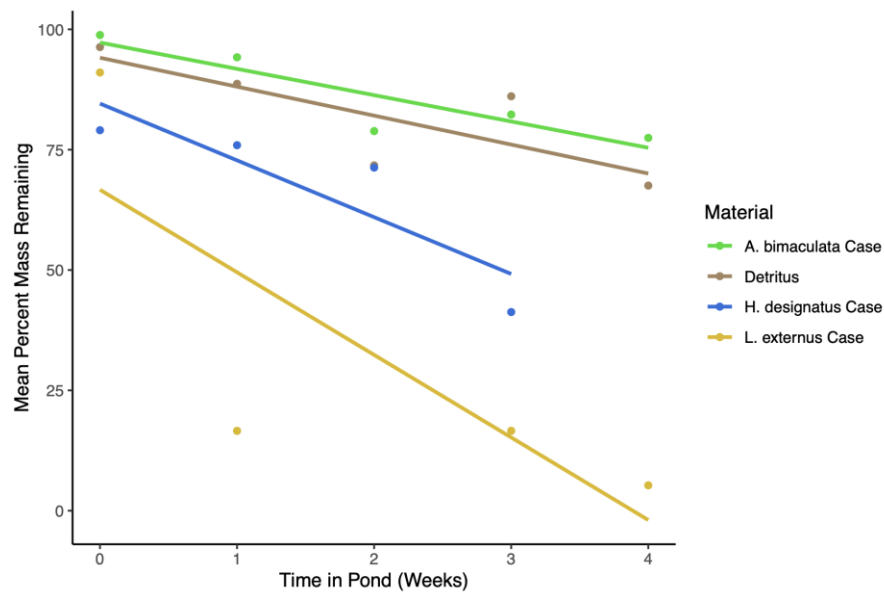


Figure 5. Mean percent mass remaining of materials (*A. bimaculata* case, detritus, *H. designatus* case, and *L. externus* case) after time in Fish Hook Pond. *A. bimaculata* cases and detritus (*C. aquatilis*) had no difference in decomposition/detritivore feeding rate (ANCOVA, Tukey's post-hoc test, $p > 0.05$). *L. externus* cases lost mass over 1.7 times faster than all other materials (ANCOVA, Tukey's post-hoc test, $p < 0.001$).

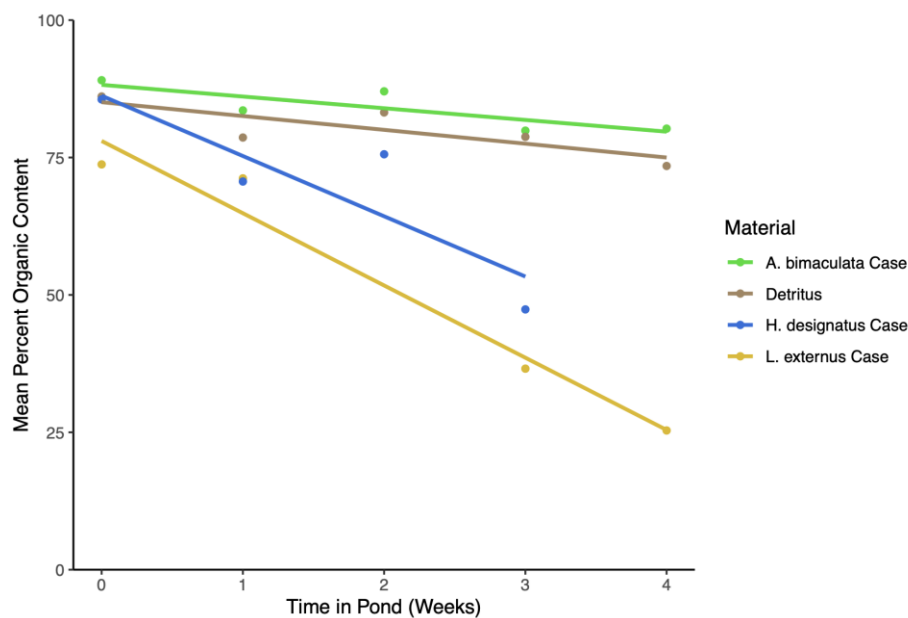


Figure 6. Mean organic content [(organic mass/dry mass) x 100] of materials (*A. bimaculata* case, detritus, *H. designatus* case, and *L. externus* case) over time in Fish Hook Pond. *L. externus* cases possessed lowest initial percent of organic content compared to all other materials (ANOVA, Tukey's post-hoc test, $p < 0.001$). Percent organic content of *H. designatus* and *L. externus* cases declined 5 times faster than those of *A. bimaculata* cases and *C. aquatilis* (ANCOVA, Tukey's post-hoc test, $p < 0.001$).

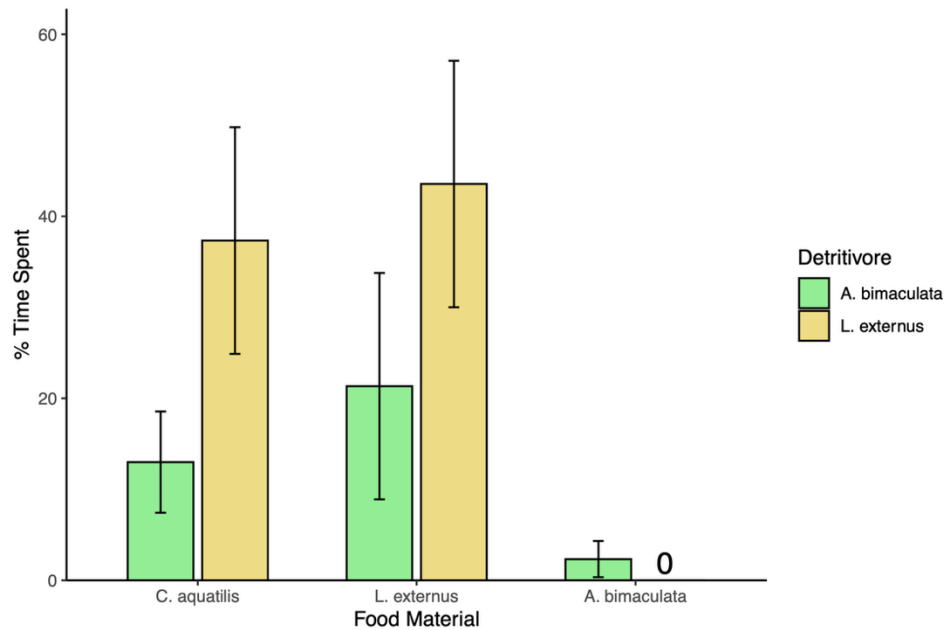


Figure 7. Percent of time ten *L. externus* and ten *A. bimaculata* larvae spent at three food materials: *C. aquatilis*, *L. externus* case, and *A. bimaculata* case. *L. externus* larvae spent more time at the *L. externus* cases and *C. aquatilis* rather than the *A. bimaculata* cases (PERMANOVA, ANOVA, Tukey's post-hoc test, $p=0.019$, 0.049). *A. bimaculata* had no significant food material preference but spent 230% more time at *C. aquatilis* and 450% at the *L. externus* cases than *A. bimaculata* cases (PERMANOVA, $p>0.05$). Error bars represent standard error.

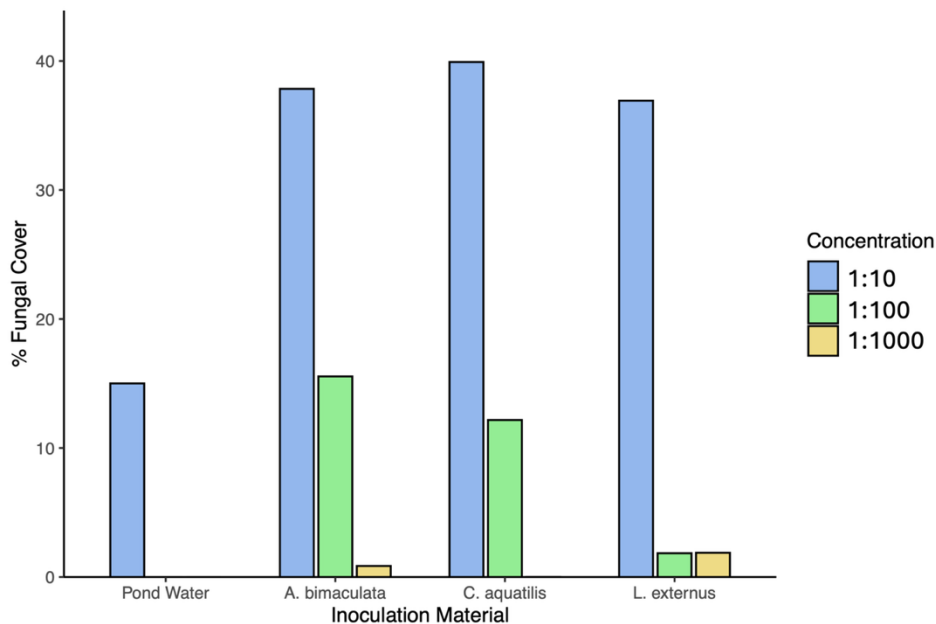


Figure 8. Percent fungal cover of agar plates by inoculation materials (pond water, *A. bimaculata* case, *C. aquatilis*, *L. externus* case) of different concentrations. 1:10 concentrated pond water-inoculated plates exhibited less than half the growth of the other 1:10 plates.

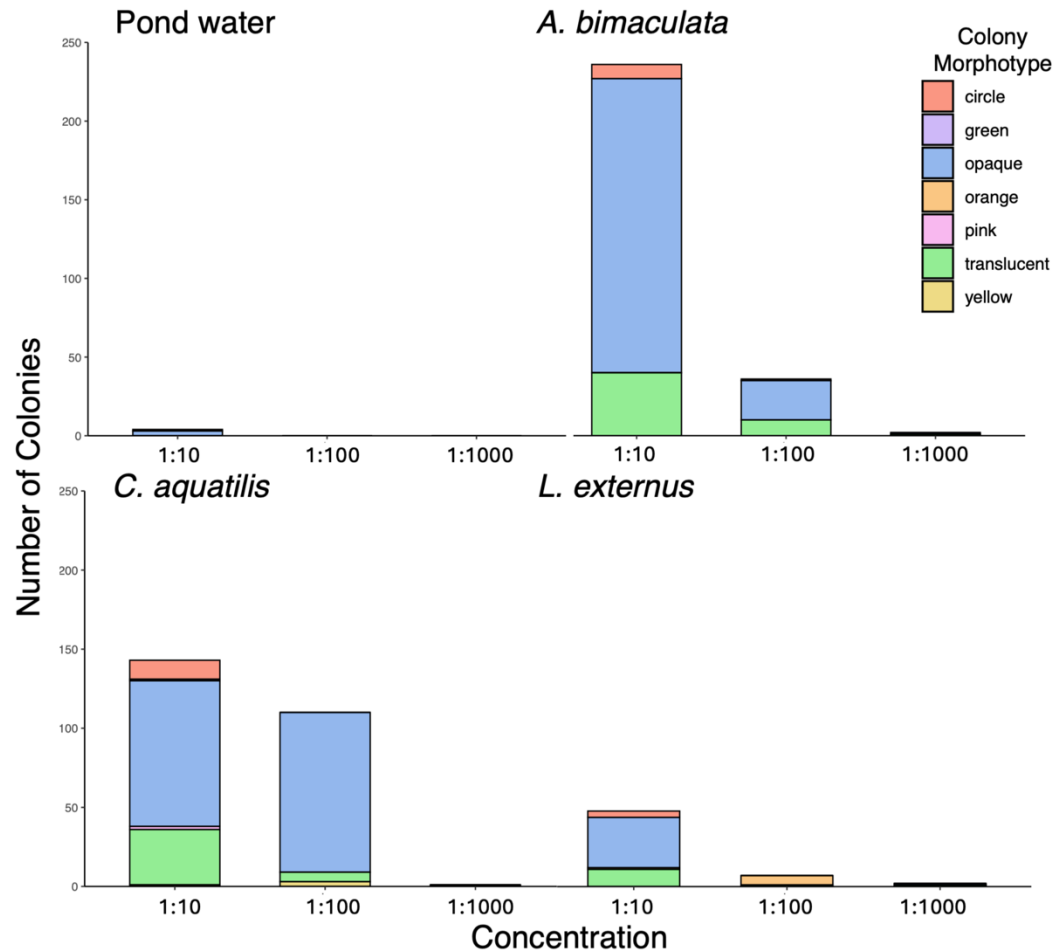


Figure 9. Number of colonies by morphotype on agar plates inoculated with materials (1=pond water, 2=*A. bimaculata* case, 3=*C. aquatilis*, 4=*L. externus* case) of different concentrations (a=1:10, b=1:100, c=1:1000). The 1:10 *C. aquatilis* plate possessed double the morphotypes as the *A. bimaculata* case and pond water plates, but the 1:10 *A. bimaculata* case plate had the highest number of colonies.

Discussion

Through mass loss, detritivore feeding choice, and fungal plate colonization experiments, I hypothesized that caddisfly (*A. bimaculata*, *H. designatus*, and *L. externus*) cases would decompose slower and be avoided by detritivores when compared to the dominant detritus (*C. aquatilis*) used to make their cases due to the potential presence of antimicrobial properties, such as silk. Cases, however, decomposed and were fed on by detritivores in the pond at the same (*A. bimaculata*) or a faster rate (*H. designatus*, *L. externus*) than the most common ambient detritus used to make cases (except for *H. designatus*). Furthermore, both *A. bimaculata* and *L. externus* larvae preferentially consumed *L. externus* cases despite their low 74% organic content while avoiding *A. bimaculata* cases. Fungi from *A. bimaculata* cases grew the largest number of colonies and ambient detritus had the highest morphotype diversity. The decomposition rates, feeding preferences, and fungal interactions of cases do not support my hypotheses.

Although *A. bimaculata* cases lost mass at the same rate as ambient detritus and were avoided by caddisfly larvae detritivores, the mean percent of organic content, which is somewhat dependent on the level of processing by case-colonizing fungi, was not statistically different from that of ambient detritus, discrediting antimicrobial silk as the cause. Alternatively, the larger body size and potential aggression of *A. bimaculata* larvae may have prompted slow mass loss and detritivore feeding aversion to their cases that caddisflies may recognize through visual or olfactory cues. Size plays a large role in aggressive behavior and interspecific predation in caddisflies, specifically in temporary ponds where expedient pupation is essential (Wissinger et al. 2003). In fact, cannibalism increases significantly when larvae differ in instar size, with smaller invertebrates as the victims. On the other hand, *L. externus* larvae are inactive and less aggressive when compared to *Asynarchus nigriculus*, likely making their cases more favorable to feed on (Wissinger et al. 2004).

Particle size, such as the size of *C. aquatilis* or *P. engelmannii* pieces used to make cases, may also affect mass loss and food preference of caddisfly larvae as well. For example, *A. bimaculata* cases contain around 1 cm wide and 3.5 cm long pieces of *C. aquatilis*, *L. externus* cases utilize finer and shorter 1 cm pieces, and *H. designatus* cases have small <0.5 cm scales of *P. engelmannii* cones. While coarse detritus (>1 mm) makes up over 70% of the diet of *L. externus* larvae in the Kettle Ponds, a more specific size preference of these larger particles (i.e. 3.5 vs. 1 vs. <0.5 cm) is unknown (Wissinger et al. 2018). The high consumption rate of *L. externus* cases and rapid mass loss of both *L. externus* and *H. designatus* cases with the smaller *C. aquatilis* and *P. engelmannii* pieces may suggest these cases are easier to consume than *A. bimaculata* cases. While the rapid mass loss of cases with finer materials could be partially explained by physical loss of small pieces in the pond or during collection, particularly for *H. designatus* with 20% mass loss during transportation, the similarly steep decline in percent organic content for these cases indicates detritivore feeding or decomposer breakdown as the primary cause.

Additionally, smaller particles equate to more surface area that fungi and other decomposers may colonize, which would manifest as a lower organic content and lead to faster decomposition and consumption by other detritivores. *L. externus* cases both lost mass quickly and possessed the lowest percent organic content out of the other cases and detritus, meaning their case-building silk may not have antimicrobial properties. Lower organic content may also be explained by more mineral sand that accumulates in the crevices of *L. externus* cases or less silk present. Quantifying the mass of silk and fungi on cases will allow potential antimicrobial properties to be evaluated more clearly in the future.

Agar plates inoculated with case or ambient detritus materials provide information on their potential fungal communities. The percent fungal cover of the 1:10 *A. bimaculata*, *C. aquatilis*, and *L. externus*-treated plates were comparable, but the number of colonies and diversity in morphotypes varied among the materials. *C. aquatilis* fungi exhibited the highest number of morphotypes, but *A. bimaculata* fungi grew into the highest number of colonies, leading to the question: does having a high diversity of fungal morphotypes on cases inhibit or facilitate decomposition? To answer this question, more replicates of these plates are needed and

DNA sequencing of morphotypes to determine which are potentially harmful or rapid decomposers.

Given that the *L. externus* case had a lower number of fungal colonies but was preferentially consumed by detritivores and the *A. bimaculata* case had the highest number of fungal colonies but was avoided by detritivores, there may be different explanations for why each species' case remains in wetland ponds up to nine months after emergence. The *L. externus* cases seen in the early spring may have been the last to pupate the previous fall, persisting in the environment free of other large, invertebrate detritivores as well as having decomposition-resistant silk. On the other hand, the colony counts of *A. bimaculata* case fungi may not indicate antimicrobial properties of the case or realized fungal growth or biomass on cases, but rather the potential growth on agar plates. Nonetheless, *A. bimaculata* cases were avoided by detritivores, allowing them to also remain in ponds longer or as long as *C. aquatilis* with detritivores still present.

The results of the feeding choice and mass loss experiments deliver insights into antimicrobial properties of cases and caddisfly life histories as detritivores in wetland ponds in the East River valley of Colorado that should be further explored in supplemental studies. For example, quantifying the amount of silk present in cases, especially those of *L. externus*, and the presence of antimicrobial peptides or proteins (AMPs) may inform future design of human-built environments. Additionally, exploring the role of food particle size preference may be vital to detritus-nutrient cycling breakdown and ecosystem health. Each of these complex caddisfly interactions sheds light on the diverse evolved strategies these invertebrates use to survive in ponds full of cannibalism and decomposition.

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