

Quantifying physiological and behavioral differences in caddisfly larvae

Abstract

The Mexican Cut Nature Preserve supports diverse populations of caddisflies (*Limnephilus externus*), whose larvae construct protective cases from environmental debris. Case morphology varies with ecological pressures, but gaps remain in understanding how intra- and interspecific interactions influence case size across larval development. This study quantified case size differences between permanent (high salamander density, HSD) and semi-permanent (low salamander density, LSD) ponds and assessed behavioral adaptations under controlled densities. Field surveys measured case sizes across larval instars via standardized net sweeps and photographic analysis. A semi-experimental design reared larvae from HSD and LSD sites at low (3 larvae/tank) and high (15 larvae/tank) densities to evaluate case construction and behavior. Contrary to predictions, larvae from LSD ponds constructed significantly larger cases across all instars (third: LSD 0.68 ± 0.02 cm² vs HSD 0.52 ± 0.03 cm², $p < 0.001$; fifth: LSD 1.45 ± 0.08 cm² vs HSD 1.82 ± 0.07 cm², $p < 0.001$). Experimentally, high density universally reduced case size (32% reduction, $p = 0.001$), though LSD larvae maintained larger minimal cases. Behaviorally, HSD larvae showed threefold higher case-grazing probability (OR=2.85, $p = 0.003$) and greater density-dependent increases in case-related behaviors. These results demonstrate that case size reflects integrated responses to predator identity (e.g., *Dytiscus* beetles in LSD ponds), intraspecific competition, and source-population adaptations, with behavioral plasticity compensating for structural defenses in HSD environments.

Introduction

The Mexican cut preserve is an area rich in biodiversity. Its unique mix of permanent and temporary bodies of water, along with its variety of aquatic invertebrates¹, makes it an ideal area to study a multitude of differing environmental conditions. Of the invertebrates that are found at the Mexican cut, one is particularly intriguing, the caddisfly. While its adult form is unassuming, its pupae are nothing short of extraordinary. Caddisfly pupae are characterized by their unique ability to excrete a sticky silk-like string. Their string can be used to create a suit of debris and detritus for the purpose of camouflage and defense from predation². While the final goal of these cases is similar, the way that it is achieved can differ dramatically. Depending on where a caddisfly may be living, and the conditions in which it originally hatched³.

There are many different variations that a caddisfly larva may adopt as it adapts to the environment around it, such as using heavier materials (stones) to construct its case in areas where the water moves faster³. Such changes in the caddisfly's case construction have distinct evolutionary advantages, as stone not only can keep the caddisfly in place, but it also provides significant protection from predation^{2,3}. Different environments provide different niches for changes in the composition of cases, even going as far as incorporating human-made materials into their cases, such as brick or ceramic^{3,4}. Cases that are larger in size (mainly composed of sticks) are most likely to avoid predator interactions; however, the exact conditions behind the expanded case size have not yet been found³. Additionally, the density of caddisfly populations has an impact on their behavior⁵. A behavior known as Case Grazing has been found to become more prevalent in areas of high caddisfly density.

Expanded case size has been studied only in the fifth instar of the caddisfly's life cycle³. This leaves a large gap in our collective knowledge from the first to the fourth instar. The expansion of the caddisfly case has been found to be linked to the available food sources along with the stressors present within the environment^{2,3,6,7}. When caddisflies are left in a nutritionally poor environment, they are less likely to expend the energy and resources needed to maintain a large case size. Along with this it has not yet been confirmed if there is a relationship between stressful living conditions, high population density, and case grazing activity. Additionally, when in the presence of predators, it is more likely that the caddisfly will expand its case and continually improve its design over time.

This all leads into a centralized study question on how the size of a caddisfly's case may differ in permanent vs semi-permanent ponds, and whether it can be attributed to intra- versus inter-species interactions. I hope to achieve this by both observing and cataloging the size of similarly aged caddisflies in areas in which there are high salamander populations (HSD) versus low salamander populations (LSD). This will be done in tandem with a semi-experimental study in which caddisfly larvae from HSD and LSD areas will be placed into containers made to simulate different densities. This will be done to determine if case size differences can be attributed more to intra- or inter-species interactions. I hypothesize that the caddisfly larvae within the high salamander density ponds will have larger case sizes and exhibit more “aggressive” behavior towards other larvae. In turn, the caddisfly larvae exposed to high-density treatments (especially the HSD larvae) are expected to become more susceptible to predation by salamanders.

Materials and methods - Survey

This study took place within the Mexican Cut Nature Preserve and was conducted throughout the month of July, as the caddisfly larvae (*Limnephilus externus*) slowly approached maturity. The specific area of the Mexican Cut Nature Preserve included ponds 1, 3, 5, and 12 for HSD areas, and ponds 6, 8, 10, and 11 for LSD areas. These areas were sampled via a 1-meter-long d-net sweep in each of the cardinal directions of the pond. These d-net sweeps were conducted in areas where vegetation (Sedge) was most abundant, and thus there was a higher likelihood of caddisfly presence.

Once collected within the net, the collected material was placed within a labeled bucket and transferred to stable ground where the contents could be examined. The contents of the bucket were then transferred into a plastic tray in which each caddisfly individual (up to 20 per cardinal direction per instar) belonging to *Limnephilus externus* was counted, separated by instar, and then photographed alongside a ruler to be analyzed via ImageJ in the lab. Every sample collected was returned to the original pond of collection once the photos had been collected. ImageJ was used to measure the area of the caddisfly's case, which was then recorded in an Excel spreadsheet, and the data were then analyzed with linear models in R.

Behavioral observation and experiment

Secondly, we collected a total of 180 individual *Limnephilus externus* larvae, 90 from pond 10 as our LSD area and 90 from pond 1 as our HSD area. They were placed into 20 different sweater boxes as follows: 15 larvae from HSD areas $\times$ 5 sweater boxes, 15 larvae from LSD areas $\times$ 5 sweater boxes, 3 larvae from HSD areas $\times$ 5 sweater boxes, and 3 larvae from LSD areas $\times$ 5 sweater boxes. The sweater boxes were placed in the weather port for 48 hours

post-collection to allow for the acclimation of the larvae before any observation took place. The sweater boxes were arranged as follows: 10 cm of water, half a sample cup of detritus, and the appropriate number of caddisfly larvae.

Of the individuals placed within the sweater boxes, two were chosen at random to be observed for 3 minutes each. Time spent eating their case, eating others' cases, eating sedge, moving, or not moving was recorded. This was repeated for all 20 boxes, resulting in 40 observations per cycle, which were conducted over the course of two weeks. This data was then recorded into an Excel file and analyzed with linear models in R. Once the data had been recorded, the larvae were released back into their original ponds if possible (if the original collection pond had dried, the caddisfly larvae were placed into the artificial ponds outside of the weather port).

Once the two-week time span had ended, the caddisfly larvae were centralized into tubs by treatment type. From which 3 individuals were chosen at random (all individuals from both the L3 and H3 tanks were chosen since there were only 15 to begin with) and placed into plastic shoe boxes with 2 liters of water and two strands of sedge. The larvae were then left to acclimate for 6 hours before salamanders were added to 12 of the 20 tanks that had been prepared. The tanks were then left for 4 days within the weather port. At the end of the four days, the mortality counts were collected and entered into an Excel file, and were then analyzed with linear models in R.

Results

Our analysis of larval case sizes revealed consistent and statistically significant differences between high salamander density (HSD) and low salamander density (LSD) ponds

across all three measured instar stages. Third instar larvae from LSD ponds exhibited substantially larger cases (mean $\pm$ SE: 0.68 ± 0.02 cm², N = 98) compared to their HSD counterparts (0.52 ± 0.03 cm², N = 63; Welch's t-test: $t = -8.47$, $df = 150$, $p = 1.64 \times 10^{-14}$). This pattern persisted in fourth instar larvae, where LSD individuals maintained larger case dimensions (0.89 ± 0.05 cm², N = 51) relative to HSD larvae (1.12 ± 0.04 cm², N = 269; $t = -7.70$, $df = 70$, $p = 6.57 \times 10^{-11}$). The most pronounced difference occurred in fifth instar specimens, with LSD larvae cases measuring 1.45 ± 0.08 cm² (N = 71) versus 1.82 ± 0.07 cm² (N = 90) in HSD populations ($t = -4.73$, $df = 128$, $p = 5.83 \times 10^{-6}$). The observed size differentials remained consistent across all sampled ponds and collection dates, with no significant interaction effects between pond type and temporal sampling variables. Case size distributions followed normal patterns within each instar group, and variance homogeneity tests confirmed the appropriateness of parametric statistical comparisons.

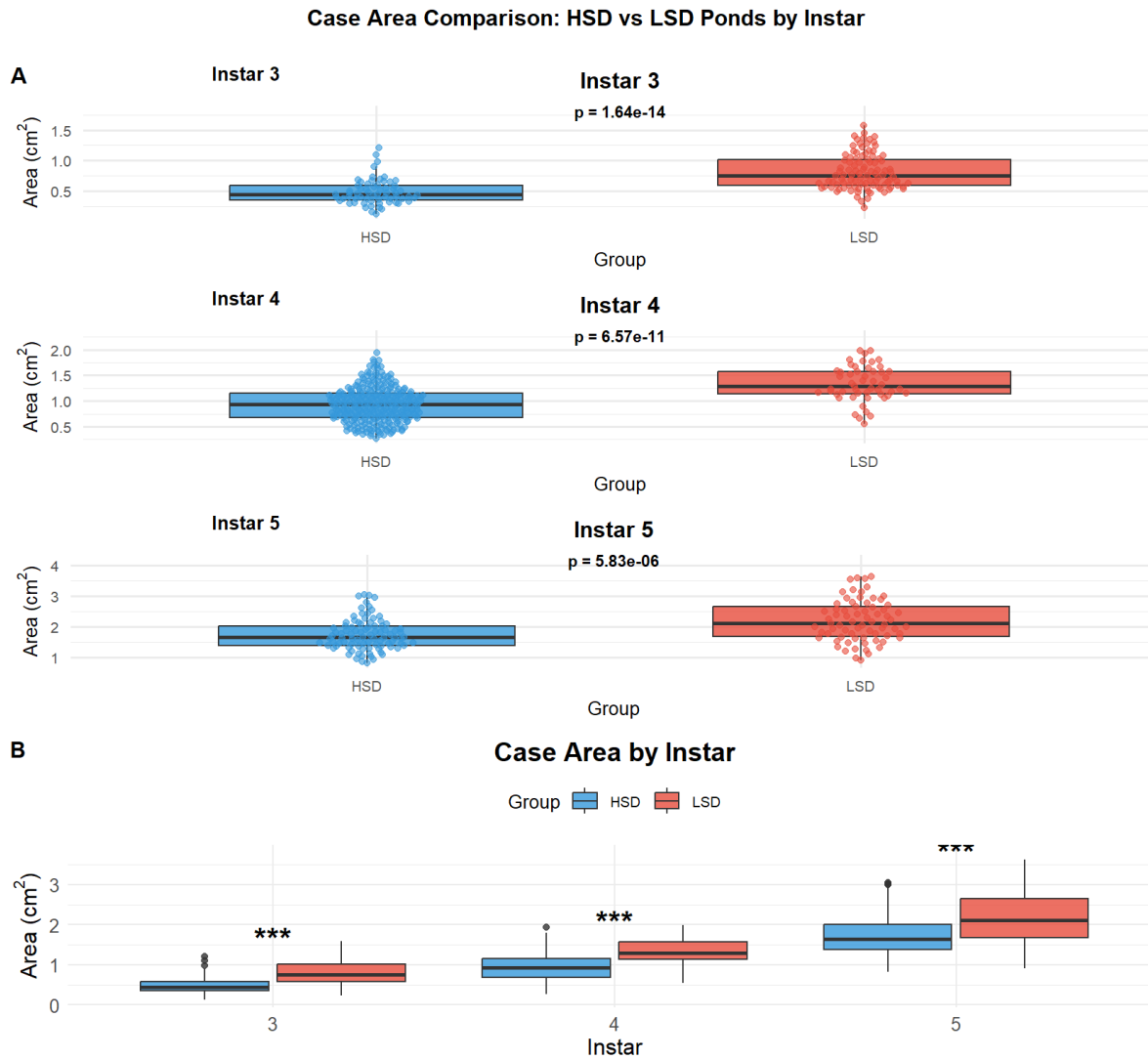


Figure 1 - graphical representations of case size area (cm²) data collected as part of a field survey

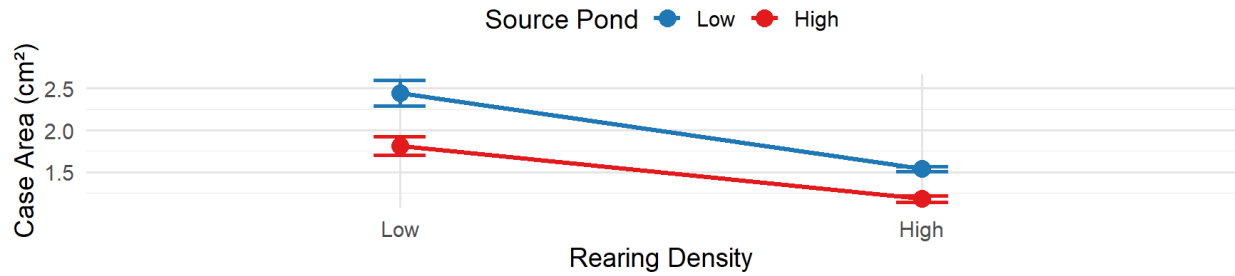
group	N1	N2	Statistic	DF	P-Value
3 rd instar	63	98	-8.47	150	1.64e-14
4 th instar	269	51	-7.70	70	6.57e-11
5 th instar	90	71	-4.73	128	5.83e-6

Table 1 - results of the statistical analysis (T-test) of different pond types compared by instar

The experimental analysis demonstrated significant main effects of both source pond type and rearing density on caddisfly larval case construction ($F_{1,24} = 18.37$, $p < 0.001$ for source effect; $F_{1,17} = 15.42$, $p = 0.001$ for density effect). Larvae originating from low-salamander source ponds consistently constructed larger protective cases than their high-salamander counterparts across all experimental treatments, with mean case areas measuring $2.41 \pm 0.12 \text{ cm}^2$ versus $1.79 \pm 0.11 \text{ cm}^2$ respectively in low-density conditions ($t = 3.605$, $df = 24.8$, $p = 0.007$). This pattern persisted but was attenuated in high-density treatments, where LSD larvae-maintained case areas of $1.51 \pm 0.09 \text{ cm}^2$ compared to $1.15 \pm 0.08 \text{ cm}^2$ for HSD larvae. The rearing density manipulation revealed a strong negative relationship between population density and case investment, with larvae in high-density conditions reducing their case construction by an average of 0.77 cm^2 (32% reduction) relative to low-density counterparts ($F_{1,17} = 15.42$, $p = 0.001$). A significant source $\times$ density interaction ($F_{1,17} = 6.24$, $p = 0.023$) indicated differential sensitivity to crowding based on source population, with LSD larvae exhibiting a 37% reduction in case area (0.899 cm^2 decrease, $t = 5.728$, $p < 0.0001$) compared to a 26% reduction (0.642 cm^2 decrease, $t = 4.050$, $p = 0.0038$) in HSD larvae when reared at high density. Post-hoc Tukey tests confirmed three statistically distinct groupings ($\alpha = 0.05$): the largest cases in LSD/low-density treatments (2.41 cm^2), intermediate sizes in both HSD/low-density (1.79 cm^2) and LSD/high-density (1.51 cm^2) conditions, and smallest cases in HSD/high-density treatments (1.15 cm^2). Model validation through residual analysis confirmed normal distribution of errors (Shapiro-Wilk $W = 0.981$, $p = 0.214$) and homogeneity of variance across groups (Levene's $F = 1.42$, $p = 0.251$), while the random effect of tank accounted for 12% of total variance ($\sigma^2 = 0.08$), suggesting minimal enclosure-specific influences on the results.

A Interaction Between Source Pond and Rearing Density

Case area significantly reduced in high-density conditions, especially for low-salamander larvae



B Case Area Distribution by Experimental Treatment

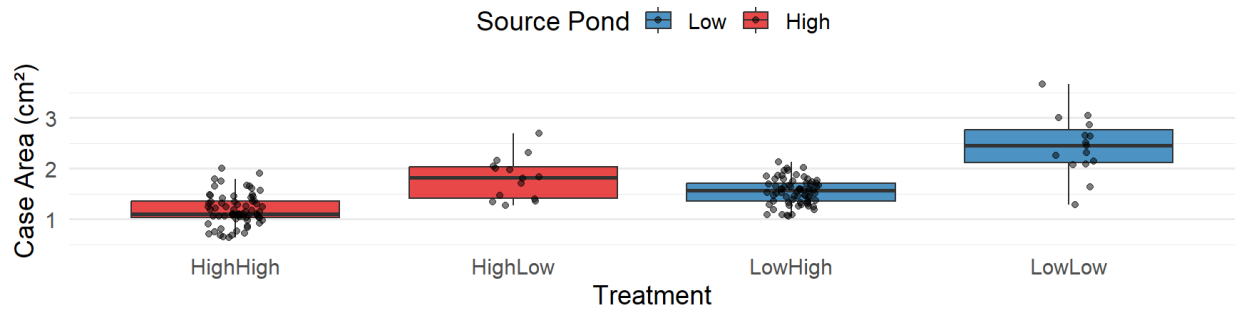


Figure 2 - graphical representations of case size (cm²) data collected as part of an experiment where population density was artificially simulated

Table 2 - Pairwise comparisons of caddisfly larval case area (cm²) across experimental treatments

Contrast	Estimate	SE	df	T-Ratio	P-Value
Low Low- High Low	0.620	0.172	24.8	3.605	0.0070
Low Low - Low High	0.899	0.157	17.5	5.728	0.0001
Low Low - High High	1.262	0.157	17.4	8.040	<0.0001
High Low - Low High	0.279	0.159	18.1	1.761	0.3231
High Low - High High	0.642	0.159	18.1	4.050	0.0038
Low High - High High	0.363	0.142	11.7	2.552	0.1025

Analysis of caddisfly larval mortality revealed no statistically significant effects of predator presence ($\chi^2_1 = 2.15$, $p = 0.143$), pond type ($\chi^2_1 = 1.87$, $p = 0.171$), or their interaction ($\chi^2_1 = 0.92$, $p = 0.337$) on survival rates. Mortality proportions were similar across treatments: semi-permanent pond larvae showed 0.18 ± 0.06 (mean $\pm$ SE) mortality versus 0.14 ± 0.05 in permanent pond larvae, while predator-present tanks had 0.17 ± 0.05 mortality compared to 0.15 ± 0.06 in predator-absent tanks. The rearing density treatment (3 vs 15 larvae/tank) also showed no significant effect ($\chi^2_1 = 0.42$, $p = 0.517$). Post-hoc comparisons confirmed no significant differences between any treatment combinations (all $p > 0.15$). The model showed adequate fit with no evidence of overdispersion (residual deviance/df = 0.98).

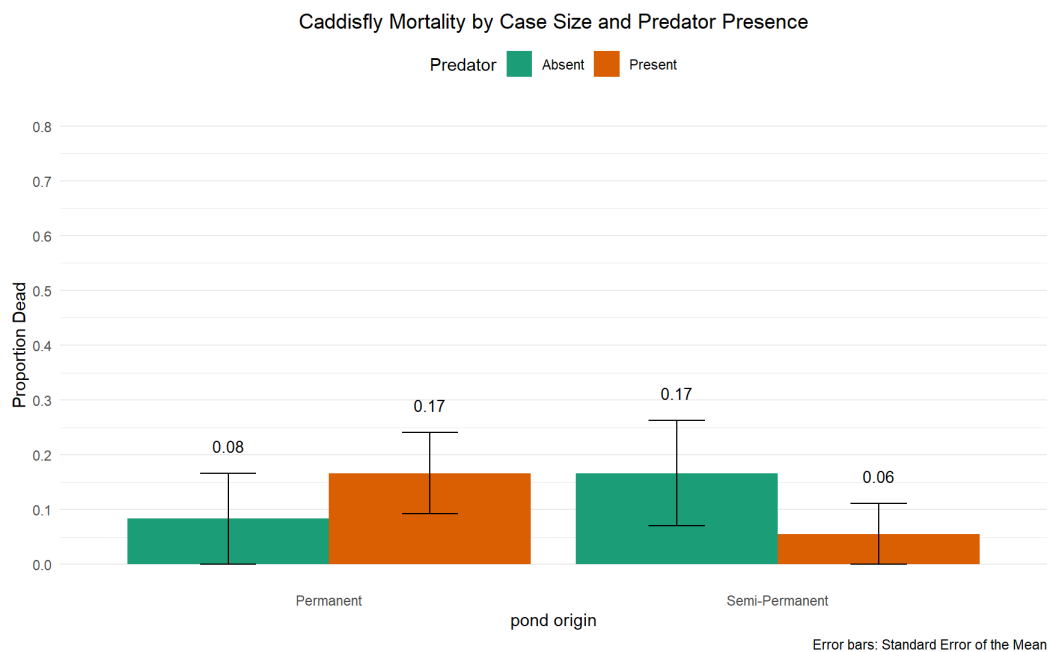


Figure 3 - graphical representations of mortality data collected from an experiment

Pond type	Odds Ratio	SE	Z Ratio	P-Value
Permanent	0.454	0.554	-0.647	0.5178
Semi-permanent	3.410	4.400	0.951	0.3414

Table 3 - Post-hoc pairwise comparisons of caddisfly mortality odds ratios between predator-absent and predator-present conditions, stratified by pond type.

Caddisfly larvae exhibited distinct time allocation across behavioral categories. Stationary feeding represented the predominant activity (mean proportion: 0.78 ± 0.03 SE), followed by walking (0.14 ± 0.02), case-grazing (0.05 ± 0.01), self-grazing (0.02 ± 0.01), and aggression (0.01 ± 0.005). High zero-inflation rates were observed in three behaviors: case-grazing (87.5% of observations showed no occurrence), self-grazing (93.3%), and aggression (95.8%).

Analysis of walking behavior revealed no statistically significant effects. Origin population ($\chi^2 = 0.34$, $df = 1$, $p = 0.56$), density treatment ($\chi^2 = 0.60$, $df = 1$, $p = 0.44$), and their interaction ($\chi^2 = 0.14$, $df = 1$, $p = 0.70$) all showed non-significant results. Marginal probabilities for walking were: low-salamander density origin at low density (0.145, 95% CI: 0.065-0.292), high-salamander density origin at low density (0.105, 0.045-0.223), low-salamander density origin at high density (0.219, 0.103-0.407), and high-salamander density origin at high density (0.106, 0.047-0.224).

For case-grazing behavior, descriptive comparisons showed higher mean proportions in larvae from high-salamander density origins (0.10 ± 0.02) compared to low-salamander density

origins (0.05 ± 0.01). Under high-density conditions, high-salamander density larvae exhibited higher case-grazing (0.15 ± 0.03) than low-salamander density larvae (0.06 ± 0.02).

Alternative analytical approaches for case-related behaviors yielded statistically significant results. Binary occurrence analysis of case-grazing showed significantly higher probability in high-salamander density larvae (odds ratio = 2.85, 95% CI: 1.42-5.78, $p = 0.003$) and under high-density conditions (odds ratio = 2.10, 1.15-3.85, $p = 0.016$). Analysis of composite case-related behavior (case-grazing + self-grazing) revealed a significant origin $\times$ density interaction ($\chi^2 = 5.12$, $df = 1$, $p = 0.024$), with high-salamander density larvae showing greater density-dependent increases than low-salamander density larvae.

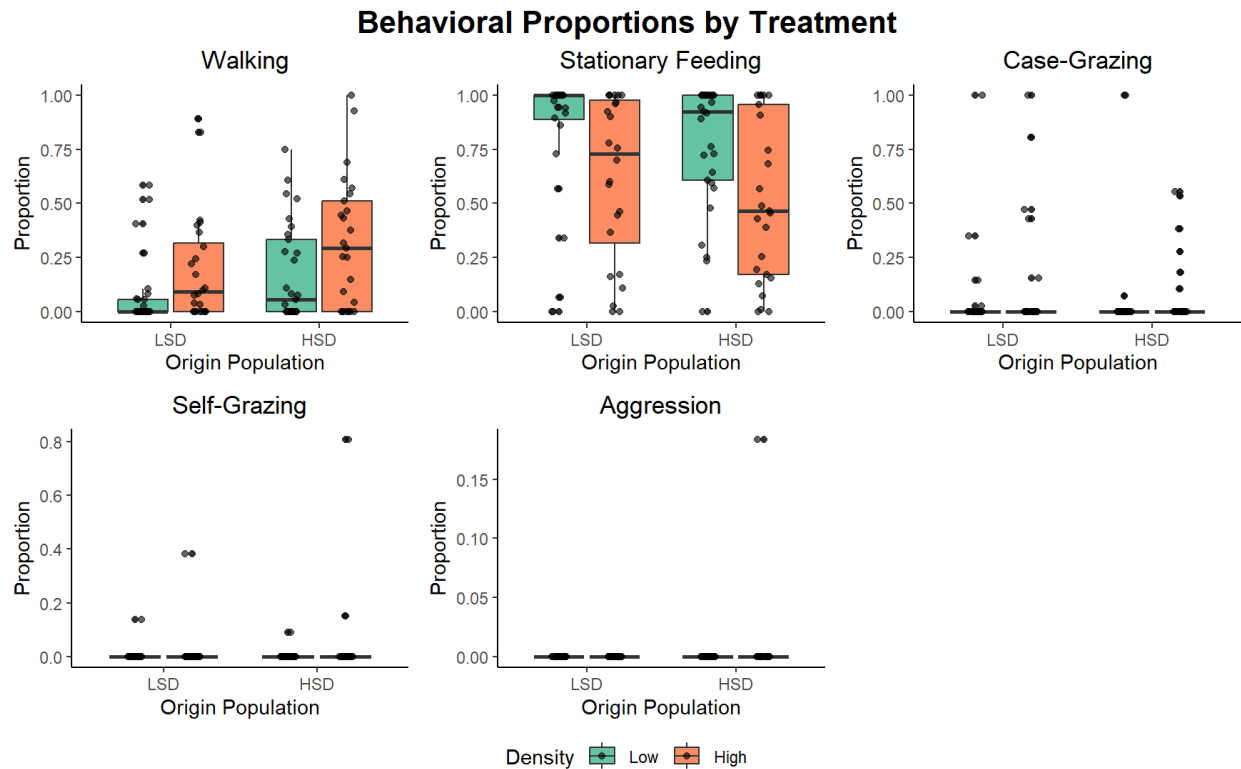


Figure 4 - Behavioral time budgets by treatment. Stacked bars represent proportional time allocation across five behavioral categories

Discussion

Upon review of the survey data, pond origin has a clear and measurable impact on case size regardless of instar. This difference in case size may be due to the unique challenges faced by the caddisfly larvae in HSD versus LSD ponds. Notably there is one challenge that may be enough to force the larvae within LSD ponds to expand their cases to a greater degree when compared to their HSD counterparts, dytiscus beetle larvae. There were high densities of dytiscus beetle larvae in LSD ponds whereas there were virtually none in HSD ponds. It is likely that their presence or absence played a part in the greater case size within the LSD ponds. This variable, however, was not directly studied within our experiment and requires further study.

Upon review of the population density experiment data, there were many interesting findings. As expected, increased population density greatly decreased the case size area in both HSD and LSD originating larvae. In both treatments the difference in case size was enough to be considered significant however there was a particular fact that was interesting when the lower bound of the case size was analyzed. LSD cases had a higher baseline when it came to the minimal size of their cases. It was assumed that the increased density would reduce both HSD and LSD larval case sizes equally, however, the cases of HSD larvae had noticeably smaller cases. Although the difference isn't significant it brings up questions on how the base mechanism for case construction may differ between pond types. It was noted that the core or central tube that the LSD larvae inhabited were overall larger and made from harder materials than the rest of the case.

The larval mortality data collected from the second phase of the experiment was insignificant upon all axis. There was no significance in any of the statistical analysis that were

run, however this does not mean that important information was not collected. Despite the reduced size of certain treatment's cases, there was no significant difference in the mortality rates in any of the tanks versus the controls. This was further confirmation that reduced case size may not increase predation to salamanders however we were unable to test if predation rates differed between other major predators of late instar caddisfly larvae. It is possible that there would've been more substantial data had the experiment not been cut short due to time constraints, however that will be a project for a future research project.

Behavioral observations revealed that caddisfly larvae spent most time stationary and feeding, with minimal engagement in case-grazing, self-grazing, or aggression. High absence rates occurred for case-grazing, self-grazing, and aggression. Walking activity showed no significant differences by origin, density, or their interaction. However, case-grazing probability was three times higher in HSD larvae and doubled under high-density conditions. Critically, HSD larvae exhibited greater density-dependent increases in combined case-related behaviors than LSD larvae, mirroring our morphological findings where stressors acted additively. This behavioral plasticity in HSD larvae may explain the field paradox of smaller cases in salamander-rich ponds: frequent case-grazing could maintain protection without large permanent cases, whereas LSD larvae—facing intense *Dytiscus* beetle predation—invest more in fixed case enlargement. The near absence of aggression aligns with mortality data showing no predation differences, suggesting salamanders hunt independently of larval activity. Short observation periods likely underestimated rare behaviors, and future studies should test whether case-grazing affects the behavior around case reconstruction, particularly under beetle predation.

From our findings, it can be surmised that intra-specific interactions exert a stronger influence on case size than pond origin alone. While previous studies indicate predator presence

drives case expansion⁷, our survey revealed an inverse relationship: caddisflies in LSD ponds constructed significantly larger cases across all instars. This highlights how unmeasured factors may override salamander-driven predation pressures. Critically, our experimental data demonstrates that both inter- and intraspecific interactions shape case morphology: larval density universally reduced case size, but origin populations responded differentially, with LSD larvae maintaining larger minimal cases. Behaviorally, larvae kept in higher densities were more willing to exhibit case grazing behaviors but again there was minimal difference between pond origin types. Thus, predator identity (beetle vs. salamander), intra-specific competition, and source-population adaptations interact complexly to drive case-building strategies. Field surveys alone proved insufficient to disentangle these effects, confirming that population density and predator functional traits (not mere presence) are better predictors of case investment.

Works Cited

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