

Understanding Aquatic Insect Oviposition to Increase Aquatic Insect Recruitment Rates

By: Charlie Baker

Abstract

Aquatic insects play many critical ecological roles in their environments. Anthropogenic changes in these environments, particularly those already under stress, leave the insect community more prone to stochastic disturbances. Despite widespread efforts by conservationists, many projects fail to increase insect recruitment to pre-disturbance levels as much of the focus is centered on the larval stage. It is currently unknown how aquatic insects select sites for laying egg masses, a process known as selective oviposition. We aimed to understand the characteristics of emergent rocks favorable for insect-selective oviposition. We hypothesized that rock size would have the most significant impact on favorability and insect success rates. Data was collected in the East River in the Colorado Rockies, on the RMBL campus. These pairs of emergent rocks exhibit various qualities, including rock embedment, water flow rate, and rock size. We observe insect behavior using a time-lapse camera. Egg masses were counted before and after the trial. After reviewing the footage, we found that 1) insects are landing and submerging on embedded and unembedded rocks equally. 2) Insects are laying egg masses more on unembedded, but using a zero-inflated model, they are not laying egg masses equally across each treatment. I suggest that aquatic insects are unable to recognize embedded and unembedded rocks, and embedded rocks may act as an ecological trap.

Introduction

Streams play a crucial role in many aspects of our world. A healthy stream with a high level of biodiversity indicates a successful and productive ecosystem, as streams play a vital role in the food web and nutrient cycle. In addition to having key ecological functions, streams also possess economic importance, as they support a variety of lucrative industries, including fishing and tourism. Some such roles include providing food for fish, amphibians, and birds, as well as processing carbon in the form of detritus and facilitating nutrient cycling. With the rise of the Anthropocene, streams are increasingly under stress, resulting in a loss of insect biodiversity of up to 51% (Fairchild et al., 2025). As a result of this loss, many researchers and practitioners have turned to restoration. However, studies have shown that insects often do not return to their original habitats once rivers are restored. Restoration processes frequently focus on benthic

substrates, an idealized meander shape, and the larval stage of insects, but overlook the adult stage, which is crucial for recruitment in restored streams (Dilworth & Taylor, 2024). The current approach to restoration for invertebrates is often summed up as “If you build it, they will come.” However, this approach only benefits insects that can adapt to what has been built, which is often based on the views of engineers and geomorphologists, rather than biologists (Shuey, 2013). It is thought that many of these insects are unintentionally excluded from restoration efforts because they are not generalist enough to adapt to these rapid changes. Aquatic insects are not as charismatic as other species and, consequently, receive less public attention.

As insects progress through their life cycle (e.g., egg, larva, pupa, adult), they eventually emerge as mature aerial insects. When it comes time to reproduce, insect communities must return to the river, and they employ different reproductive strategies. Oviposition is one such strategy, which involves deliberately laying eggs on specific substrates. Many female aquatic insects oviposit their eggs among emergent stones, rocks that protrude from the water, providing a source of dry land to access the water (Lancaster et al., 2010). It is thought that they crawl under the rock to lay eggs, offering protection from parasites, predators, and environmental stressors, such as near-zero shear stress and water velocity, which lowers the probability of egg drying (Encalada & Peckarsky, 2006). It has been observed that many taxa (e.g., caddisflies, mayflies, midges) use the same emergent rocks for oviposition, with little interspecific competition (Lancaster et al., 2010). Additionally, some studies have found that the number of substrates, such as rocks, can limit insect recruitment rates (Macqueen & Downes, 2015). However, it is not as simple as placing more rocks into the river. It remains unknown why insects select specific emergent rocks, as studies have found that only a minority of rocks are used for egg laying, leaving the rest with little to no use (Macqueen & Downes, 2015; Dilworth & Taylor, 2024).

Female insects have been observed landing on rocks and evaluating whether they are suitable for oviposition (Peckarsky et al., 2000). This suggests that not all assessment is done while flying, with any potential emerging rocks having to be examined further for oviposition. To increase insect recruitment rates, ecologists must understand the selection process of these aquatic insects and apply this knowledge to more effective restoration practices.

This study investigated how female insects select specific rocks for laying their eggs. Asking the question of whether female insects physically need to land on emergent rocks to assess their suitability, or can they assess emergent rocks from the air, and what characteristics make these rocks preferred? To answer these questions, we conducted a manipulative experiment in which we manipulated the availability of emergent rocks with different characteristics. One such characteristic is whether the rock is embedded, which occurs when the rock is cemented in the ground, often due to sediment building up around it. We also examined rocks of different sizes and observed them in both fast-flowing and slow-flowing water.

This study aims to investigate whether aquatic insects can distinguish between embedded rocks. I hypothesize that aquatic female insects use a combination of both landing on the substrate and visual stimulation to test stability. In the study by Peckarsky et al. 2000, rocks were coated with Tanglefoot, a glue that prevented the insects from leaving once they landed. Although many insects were caught on the rock, we do not know if they would have laid eggs or flown away because they were stuck in the glue. The results of this study and other studies using Tanglefoot glue are ambiguous as to whether female insects select emergent rocks solely during flight or also use post-landing characteristics for egg-laying habitat selection. Additionally, aquatic insects are known to detect horizontally polarized light (Kriska et al., 2009). Complex polarization patterns form in turbulent and heterogeneous water patterns around rocks, which might provide insects with a visual indication of suitable rocks (Xu et al., 2011). Therefore, I predict that female insects would want to avoid ovipositing on unembedded rocks because they are inaccessible and may be dislodged from the rock, potentially drowning. This may involve post-landing information, such as walking around, submerging, and flying away if the substrate is not favorable for oviposition.

Methods

Site Description

The location of our research was on the East River in (38.958937, -106.991698), Western Colorado, at the Rocky Mountain Biological Laboratory (RMBL). At the start of the study, most of the rocks were still submerged, providing limited places to set up the treatment group. However, as the season progressed, more emergent rocks became viable. Many insects have

been found to lay eggs in parts of the stream where there is sufficient water during the drier season (i.e., the middle of the stream), but locations closer to the bank were also tested. We found more success when the rocks were located in the riffles, or fast-moving areas characterized by lots of rocks that create turbulence. Different types of rocks were tested to account for the geological differences that affect the selectivity of oviposition.



Image 1: Image of study sites along the East River on RMBL campus

Sampling Design

To test the above hypotheses, eight pairs of emergent rocks were selected. One being embedded, the other unembedded, of roughly similar size and composition. Other rocks were moved further away from the pair or were submerged under the water to maximize the number of female insects' interaction with the treatment pair. We examined the visitation and behavior of female insects on embedded and non-embedded emergent rocks. To achieve this, rocks of the same size and with a similar water flow rate were selected. It is essential to inspect the embedded rocks to

ensure they are fully embedded, as they may have a lip that creates a space for insects to lay eggs. Having this space would defeat the purpose of testing embedded rocks, as aquatic insects could have spaces to lay their eggs. Next, an unembedded rock was either placed to the left or right, upstream, or downstream of the embedded rock. The unembedded rock was of similar quality and aspects to the embedded rock (i.e., similar size, shape, height, and rock composition). Next, the pair was measured in terms of the distance from the river bank and the height of the rock emerging from the water. We also measured the 2D area of the rock by categorizing it into simple 2D polygons (square, trapezoid, circle, oval, rectangle). After all the measurements were taken, each rock was marked with a paint pen with the treatment code.

The time-lapse cameras used were *Aphidius 230*, which observed each emergent rock in a pair. Rebar was secured into the riverbed downstream, approximately 1 meter away from the stones, to serve as a pole for attaching the cameras using a clamp. It is important to record downstream, as that is typically where the insects will climb onto rocks, taking advantage of the safety of eddy currents. The cameras were initially set to record at an interval of 1 second at 30 frames per second (fps), but were later changed to a 3-second interval to conserve battery power. This interval ensured the precise observation of insects landing and/or crawling underwater, resulting in approximately 35 minutes of video recording over a 10-hour period. The cameras were deployed daily around 0900, then checked at 1200 to ensure that the batteries had sufficient charge, and then taken down for the night around 1900. The camera settings were set to maximize resolution and quality (Automatic ISO and aperture adjustment, high HDR, auto white balance).

It is also vital to count the egg masses on the rock both before the trial and after to determine how many female insects that crawled underwater laid eggs. If both egg census and footage of the insect are used in tandem, the success of the female insect's egg laying can be determined. We also examined the egg mass morphotype to aid in further identifying female insects. Following the conclusion of the observation, we unembed the embedded rocks to check for egg masses. This is done to check for the unlikely instance that an insect lays eggs underneath them.

Data Processing

Once the footage was collected, we started processing the data. This involved viewing the videos to look for insects that land on rocks and then noting their behavior, which we categorized as: landed, flew away, walked, water edge, dislodged, submerged, and resurfaced. Whenever an insect moved or flew to a different part of the rock, I classified it as walking. Landed was recorded whenever an insect landed on a rock, and flew away was when an insect flew away from the rock. Water edge was when an insect walked, flew, or landed right at the waterline. Submerged means that an insect has completely submerged itself under the water. Dislodged was recorded when an insect was swept off the rock by the current or fell off the rock. Lastly, resurfacing occurred when an insect was seen climbing out from the waterline and back onto dry rock. These were the most common behaviors, and when combined, would accurately describe what the insect's actions were. Along with the time of day the insect was found, the date, the camera that recorded it, the video file name, and, when possible, identification of the insect, were recorded. Many of the taxa we were able to identify to the family and genus, others only to the order. We found that for some of the smaller insects, the camera resolution was not clear enough to ID many of the species, so we left them as unknown

Statistical Analyses

We used a generalized linear model to test for differences in the number of *Baetis* mayflies landing, the number submerged, and the number of egg masses attached. We included the rock pair as a random effect to account for variation among rocks. The data were most closely distributed according to a negative binomial distribution. However, something interesting emerged when examining the egg masses found at each rock. Approximately 68% of the data consisted of 0, which meant that these rocks had no egg masses attached to them at the end of the recording period. Because the number of zeros was so abundant, we had to use a zero-inflated count model. This model consists of two separate components: the first is a conditional model that matches the data to a specific distribution, and the second examines whether the probability of each zero is derived from a constant distribution. The probability distribution function for the zero-inflated Poisson (ZIP) random variable (Y) can be defined as,

$$\Pr(Y = y) = \begin{cases} \omega + (1 - \omega)e^{-\lambda} & \text{for } y = 0 \\ (1 - \omega)\frac{\lambda^y e^{-\lambda}}{y!} & \text{for } y = 1, 2, \dots \end{cases}$$

Where ω is the probability that a zero observation is drawn from a constant distribution, and λ is the mean of an underlying distribution

Results

At least eight insect taxa landed on the emergent rocks (Table 1). The orders Blephariceridae and Diptera were the most common insects to land on emergent rocks. The most frequent stream insect that landed on emergent rocks was the Diptera family *Blephariceridae*, with 503 visits, followed by Diptera, with 238 visits (Table 1). Blephariceridae and Diptera behavior included landing on the rockwalk, walking around, and then flying away. The mayfly, *Baetis* spp., was the only taxon that attached egg masses underneath the treatment rocks. There was a seasonal pattern in the *Blephariceridae* in which they became much more abundant around June 30th.

Table 1: Table of insect behaviors categorized by taxa. Many of the taxa were identified to the family level, but some remained at the order level.

X	Taxa	dislodged	Flew Away	landed	resurface	submerged	walk	Water Edge
1	Baetis	11	7	64	5	50	12	66
2	Diptera	0	233	239	0	0	162	16
3	Ephemeroptera	0	1	1	0	0	1	0
4	Midge	0	12	12	0	0	3	0
5	Plecoptera	1	7	9	0	1	6	3
6	Blephariceridae	0	514	514	0	0	46	13
7	Trichoptera	0	3	3	0	0	3	0
8	Unknown	1	22	29	0	3	23	8

An interesting observation I made was one emergent rock U4, where a parasitized mayfly was present. It found a place to rest and remained there until a few small worms crawled out of it. The worms then made their way to the water, abandoning their host above the waterline. Then, later, what appears to be *Blephariceridae* *Plecoptera* successfully predated the mayfly. There were five observations where *Baetis* may have resurfaced, but the camera angle made it unclear.

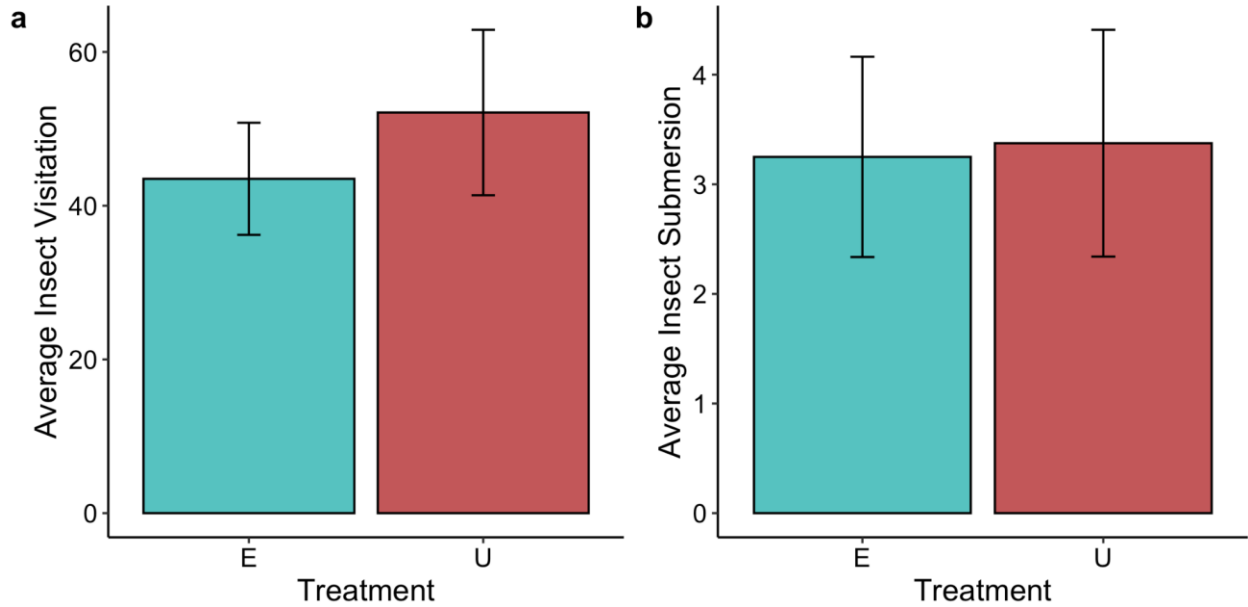


Figure 1: Bar graph with mean and standard error of (a) insect visitation and (b) insect submersion compared to treatment, embedded and unembedded. P-values for insect visitation ($p = 0.51$, $z\text{-value} = 0.655$) and insect submersion ($p = 0.75$, $z\text{-value} = 0.317$)

The number of *Baetis* that landed on the embedded rock was 120% higher than those that landed on the unembedded rock ($p > 0.51$). This indicates that *Baetis* are likely not landing on treatment more than another. Examining *Baetis* submersion rates, there is only a 0.125 difference between them ($p > 0.75$). Again, this suggests that *Baetis* are equally submerging on both embedded and unembedded rocks.

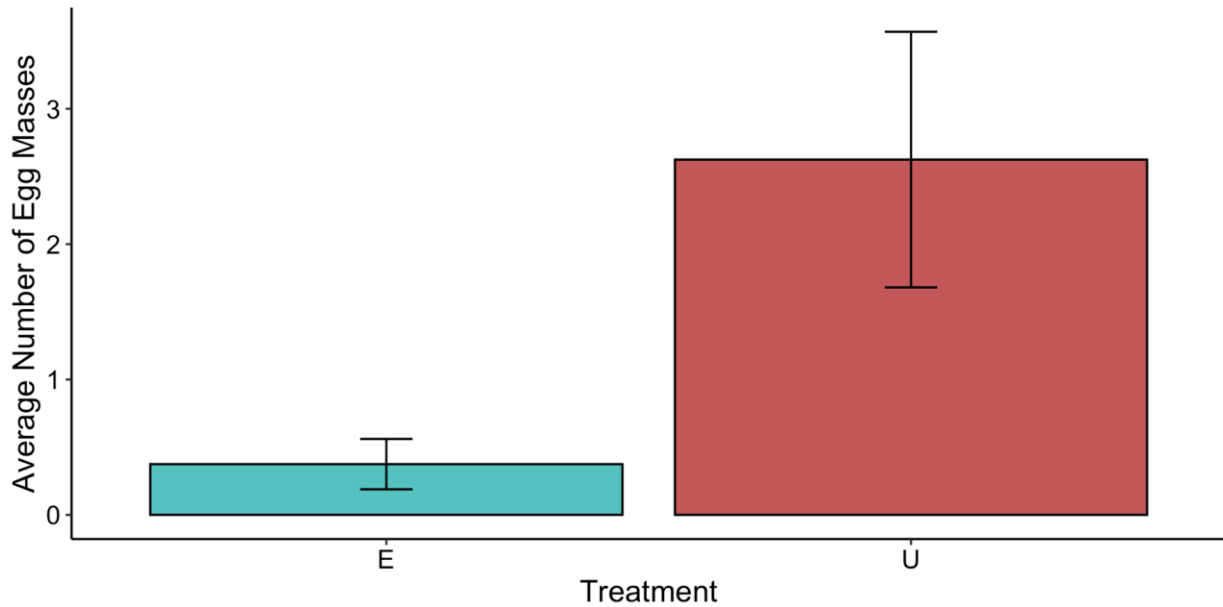


Figure 2: Bar graph of mean and standard error of egg masses found after the day was done. Mean of Unembedded (mean = 2.625, SE = 0.186) and mean of Embedded (mean = 0.375, SE = 0.944) P-value of zero-inflated Poisson ($p = 0.89$, $z\text{-value} = 0.135$)

The number of *Baetis* egg masses attached to unembedded rocks (mean = 2.625, SE = 0.186) was seven times higher than the number attached to embedded rocks ($p < 0.0319$). Based on the zero-count model, there was no significant difference in the number of zeros between embedded and unembedded rocks ($p > 0.89$), indicating that *Baetis* are equally not laying egg masses between the two treatments.

Discussion

There could be a multitude of reasons that the female insects landed and submerged on both embedded and unembedded rocks. Looking at most of the *Baetis* interactions, 71 % of them involved submerging in an attempt at oviposition. The remaining 29% were split between flying away and being dislodged, both of which were unsuccessful patterns of oviposition. According to Figures 1 and 2, both the landing behaviors and submersion behaviors proved to be statistically insignificant. This may indicate that female insects are unable to distinguish between embedded and unembedded rocks using visual cues. The treatment pairs were as close together and roughly of the same characteristics, like height and 2D surface area, so that the visual cues might be similar. This is similar to the egg masses found at the end, where the ZIP model found

no significant difference between the embedded and unembedded rocks. This is further evidence that the insects are unable to differentiate between embedded and unembedded, as laying egg masses along the vertical wall of the unembedded rock is suboptimal for egg survival (citation). Despite this, the female insects still landed on embedded rocks just as frequently, but were much less successful when it came to laying egg masses on them.

Similarly, they could be making their decision in the air, using visual cues. *Baetis* tend to select faster-moving areas with the water, such as riffles and larger rocks (Encalada & Peckarsky, 2006). Because they are likely to receive this information through a visual signal, likely using horizontally polarized light. However, I believe that either there is no visual cue that the insects are picking up on, or both visual cues from each treatment are comparable. Because most of the rocks were the same size and had similar water flow, the visual cues may not have been drastic enough to

The results also indicate that no post-landing information changes the outcome. We would expect to see much more flying away from the *Baetis* and maybe more walking around, especially on the embedded rocks, if there were any post-information decisions. I found that most of the mayflies landed and then immediately made their way to the waterline, where they submerged.

The parasite interaction previously mentioned (Table 1) is an interesting interaction because the parasite often alters the behavior of both females and, particularly, males. The parasite *Gasteromermis* sp. (Nemontoda: Mermithidae) has been found to infect male *Baetis* when they are still in the larval state and cause them to "oviposition" on emergent rocks (Vance, 1996). Once the parasite is present, it leaves its host, killing it, and then returns to the river. That being said, there was no way to identify the parasite or determine the sex of the *Baetis*, but this would slightly alter the success rate of U4 if the mayfly were male, as it would be unable to lay egg masses but still submerge under the water.

Knowing that insects are likely unable to distinguish between embedded and unembedded rocks, embedded rocks may act as an ecological trap, specifically as an equal preference trap. These

unequal traps occur when species are unable to recognize the disadvantages of an ecological process or environment and perceive it as equal to another more advantageous environment (Robertson and Hutto, 2006). An embedded rock may act as an equal preference trap, as female insects are unable to differentiate between an unembedded rock and expect to be able to crawl to the bottom, only to find that the rock is embedded. *Baetis* only have one egg mass to lay in their life, containing about 200-3,000 individual eggs, and then usually don't survive after they oviposit (Encalada and Peckarsky, 2006). Therefore, being unable to successfully lay eggs immediately results in the death of hundreds of individuals. If there are enough embedded rocks, this ecological trap may start to affect the population birth rates, which would lower the population mean.

Future research could assess the mortality rate of aquatic insects landing on embedded rocks. Encalada and Peckarsky (2007) set up drift nets to capture mayflies exhibiting varied oviposition behavior, but mentioned nothing about embedded and unembedded rocks. They found that many of the mayflies captured were drowned when found in the drift nets, but were even more prevalently found to be predated by insectivores, such as fish. Approximately one-third of the drifting female mayflies were eaten by fish; however, this number also included other types of mayflies that employ different oviposition methods (Encalada and Peckarsky, 2007). They found that 97% of mature female mayflies found in drift nets had not laid their eggs yet. This suggests that female insects are dislodged from rocks; they are unlikely to find other suitable substrates, such as submerged rocks, on which to lay their eggs. Another direction future research could take is examining the size of each rock or the flow rate of the water.

Finally, with all this in mind, when considering improvements to stream restoration, I recommend avoiding the creation of embedded rocks. However, there is a fine line to walk when adding these emergent rocks back into the water, and that is the size of the rock. Rocks that are too small risk getting flipped over during weather events, causing the egg masses to dry up (Jordt and Taylor, 2021). It has also been found that rocks that are too large will allow for the build-up of sediment, creating embedded rocks that decrease egg-laying success. Therefore, finding a “Goldilocks” size of emergent rocks, where the size is just right, is crucial for furthering aquatic insect restoration efforts.

References

- Dilworth, S., & Taylor, B. W. (2024). Facilitating the recovery of insect communities in restored streams by increasing oviposition habitat. *Ecological Applications*, 34(2), e2939. <https://doi.org/10.1002/eap.2939>
- Encalada, A. C., & Peckarsky, B. L. (2006). Selective oviposition of the mayfly *Baetis bicaudatus*. *Oecologia*, 148(3), 526–537. <https://doi.org/10.1007/s00442-006-0376-5>
- Fairchild, M. P., Swigle, B. D., Adams, A., Murray, J., Baggett, L. S., & Carroll, C. (2025). Off-Highway Vehicle Use Degrades a Mountain Stream: A Case Study Evaluating Recreation Impacts, Restoration Efficacy, and Assessing Vulnerability of Headwater Streams in the Central Rocky Mountains. *River Research and Applications*, 41(5), 1044–1057. <https://doi.org/10.1002/rra.4433>
- Kriska, G., Bernáth, B., Farkas, R., & Horváth, G. (2009). Degrees of polarization of reflected light eliciting polarotaxis in dragonflies (Odonata), mayflies (Ephemeroptera) and tabanid flies (Tabanidae). *Journal of Insect Physiology*, 55(12), 1167–1173. <https://doi.org/10.1016/j.jinsphys.2009.08.013>
- Lancaster, J., Downes, B. J., & Arnold, A. (2010). Oviposition site selectivity of some stream-dwelling caddisflies. *Hydrobiologia*, 652(1), 165–178. <https://doi.org/10.1007/s10750-010-0328-2>
- Macqueen, A., & Downes, B. J. (2015). Large-scale manipulation of oviposition substrata affects egg supply to populations of some stream-dwelling caddisflies. *Freshwater Biology*, 60(4), 802–812. <https://doi.org/10.1111/fwb.12535>
- Peckarsky, B. L., Taylor, B. W., & Caudill, C. C. (2000). Hydrologic and behavioral constraints on oviposition of stream insects: Implications for adult dispersal. *Oecologia*, 125(2), 186–200. <https://doi.org/10.1007/s004420000446>
- Shuey, J. A. (2013). Habitat Re-Creation (Ecological Restoration) as a Strategy for

Conserving Insect Communities in Highly Fragmented Landscapes. *Insects*, 4(4), Article 4. <https://doi.org/10.3390/insects4040761>

Xu, Z., Yue, D. K. P., Shen, L., & Voss, K. J. (2011). Patterns and statistics of in-water polarization under conditions of linear and nonlinear ocean surface waves. *Journal of Geophysical Research: Oceans*, 116(C7). <https://doi.org/10.1029/2011JC007350>

Encalada, Andrea C., and Barbara L. Peckarsky. 2006. "Selective Oviposition of the Mayfly *Baetis Bicaudatus*." *Oecologia* 148(3):526–37. doi:10.1007/s00442-006-0376-5.

Encalada, Andrea C., and Barbara L. Peckarsky. 2007. "A Comparative Study of the Costs of Alternative Mayfly Oviposition Behaviors." *Behavioral Ecology and Sociobiology* 61(9):1437–48. doi:10.1007/s00265-007-0376-4.

Jordt, Samantha, and Brad W. Taylor. 2021. "A Rolling Stone Gathers No Eggs: The Importance of Stream Insect Egg Laying Natural History for Stream Restoration." *Ecology* 102(7):e03331. doi:10.1002/ecy.3331.

Morphological and behavioural sex reversal in mermithid-infected mayflies. 1996. *Proceedings of the Royal Society of London. Series B: Biological Sciences*. doi:10.1098/rspb.1996.0134.

Robertson, Bruce A., and Richard L. Hutto. 2006. "A Framework for Understanding Ecological Traps and an Evaluation of Existing Evidence." *Ecology* 87(5):1075–85. doi:10.1890/0012-9658(2006)87[1075:AFFUET]2.0.CO;2.