

# Benthic boom: Understanding larval insect colonization in Moorea, French Polynesia

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Insects are important in assessing ecosystem quality and health. Current climate change models predict that in the next one hundred years, intense storms separated by long periods of drought will frequent French Polynesia. Variation in water availability may be difficult for many stream insects to cope with. Studying insect response after a disturbance as well as assessing their current distribution and abundance can help us understand greater ecological interactions and allow us to make predictions about future assemblages. The recolonization rate and habitat preference of Simuliidae and Chironomidae larvae were measured in a high elevation stream on Moorea, French Polynesia. Insect recolonization was measured 1, 3, and 6 days after an artificial disturbance event, and habitat preference was determined through the use of 3 introduced substrates: streamside moss, synthetic moss, and an empty control. Habitat preference was also evaluated through the comparison of larval densities across both experiments. Ultimately both Simuliidae and Chironomidae larvae were shown to return to baseline abundance 3 days after a disturbance event. Furthermore, chironomids preferred the synthetic moss substrate to all other habitats, while simuliids preferred the empty control compared to the moss treatment. This likely indicates that the chironomids live within the submerged moss while simuliids live on a rocky substrate. It also suggests that both species can live in a variety of environments and can adapt well to changing conditions.



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#### Introduction

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47 Nutrient cycling and energy flow and are two crucial elements responsible for maintaining a healthy ecosystem (Covich et al. 1999). Benthic macroinvertebrates, bottom dwelling 48 49 invertebrates visible to the naked eye, play the important role of primary consumer, eating bacteria, diatoms and detritus (Pinder 1986). These invertebrates act as the bridge between 50 51 primary producer and secondary consumer as they provide both aquatic and terrestrial predators 52 with nutrients that would otherwise be unavailable. In streams, fish and crustaceans scavenge 53 leaves, macrophytes, and rocks, looking for macroinvertebrates (Berk and Hellenthal 1992, 54 Pringle et al. 1993). Some of these macroinvertebrates are insects and go through a drastic life 55 cycle change when transitioning from egg to adult form. Almost all stream insects start life as an egg and hatch into a larval form that uses the stream as their primary habitat (Taylor and Karban 56 1986, Pinder 1986). After their final larval instar, the insects begin to pupate and emerge with the 57 58 ability to fly (Taylor and Karban 1986, Hill and Macdonald 2008). These insects can then 59 disperse over short distances and are often consumed by many terrestrial predators including 60 birds, reptiles, and arachnids (Pinder 1986, Tokeshi 1995).

High elevation streams on Moorea are over 100 meters in altitude and contain an assortment of insects (Resh et al. 1990). Two major fly families, Simuliidae and Chironomidae, dominate the riffles and glides within these streams (Resh et al. 1990). Adult simuliids feed on blood while in their larval form they are filter feeders that collect floating detrital matter from fast moving water (Hill and Macdonald 2008, Craig and Chance 1986). Alternatively adult chironomids do no feed, but their larval form has a variety of feeding strategies including filter feeding, scavenging and predation (Henriques-Oliveira et al. 2003, Frouz et al. 2003, Pinder 1986). Both insect families are highly dependent on water for their food as well as shelter and breeding (Pinder 1986, Ya'cob et al. 2016). Because of their broad impact and ecological importance, it is valuable to understand how changes in water availability can affect the distribution of insects. For island habitats, this knowledge is especially significant because islands are very isolated, closed systems with little influx of biota or nutrients (Love 1964). Shifts within such a system, especially at base trophic levels, can have immense impacts on higher-level trophic dynamics (Covich et al. 1988, Tokeshi 1995). Losing a large population of simuliids or chironomids could potentially starve both their aquatic and terrestrial predators. Inversely, an influx of either family could provide food for larger population of predators but also may have the potential to disturb human activities (Hill and Macdonald 2008). Fortunately, it is possible to predict changes in insect abundance with close monitoring of environmental conditions (Frouz et al. 2003, Resh et al. 1988. Brooks and Boulton 1991).

Island streams are notoriously variable habitats that regularly cycle between drought and flood conditions (Covich *et al.* 1988). Current climate models predict this pattern will become more dramatic as temperatures steadily rise (Griffiths et al. 2003, Dore 2005). Over the last 50 years, global temperature changes have produced more extreme weather systems across the South Pacific, causing both severe drought and heavy flooding (Griffiths et al. 2003, Dore 2005). Harsh environmental fluctuations can easily affect insects within temporary habitats such as ponds, pools and streams (Frouz *et al.* 2003). In small streams, larval insects tend to reside on rocks and submerged macrophytes in high flow environments where nutrients and shelter are concentrated (Ya'cob *et al.* 2016). During a drought, decreased water levels can limit available niche space and increase water temperatures, which can have varying effects on insect ecology (Dewson et al. 2007). For example, in low flow conditions some simuliid larvae have been shown to increase active drift patterns where individuals willingly release from the substrates in



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search of better habitat (Dewson et al. 2007). This drift can decrease the insect population in a specific area as well as alter low-level trophic dynamics and can potentially impact overall stream ecology (Dewson et al. 2007).

When water levels drop, the riverbed and submerged macrophytes become exposed to air and the inhabiting insect larvae leave their shelter to seek better refuge (Dewson et al. 2007). Many of these exposed macrophytes can survive using water from scattered showers frequently seen in tropical forests and continue to thrive without constant water flow. When a large weather system produces heavy precipitation, the streams can rapidly refill. As water levels start to rise, the vacated habitats become submerged once again and new opportunity for colonization becomes apparent (Strayer 2007). Although the insect richness is rather low in the Opunohu Valley compared to mainland streams, there may still be competition for this high flow environment (Resh et al. 1990, Everaert et al. 2014). It is also unclear how disturbance will affect the insects of Moorea and which microhabitat each insect family resides in. To answer these questions, field experiments were designed to test each family's habitat preferences as well as identify a clear pattern of simuliid and chironomid colonization following a disturbance event.

Methods

108 109 This study was conducted at sites within the Opunohu Valley of Moorea, French Polynesia during austral spring (Fig 1). The island of Moorea receives approximately 300cm of rainfall per 110 111 year. Water flow in rivers on Moorea is highly seasonal, peaking between November to April 112 and slowing to low flow between May and October (Resh et al. 1990). Sampling sites in this study ranged from 160 to 180 meters in elevation and were within the same 200m stretch of 113 114 stream in the Opunohu Valley. (Fig 2). All sites were accessed from the Three Coconut Trail, 115 southwest of the Belvidere outlook (Fig 1). During the first two weeks of trials, temperature extremes were recorded at 24.7°C and 21.9°C (using remote Hobo Trackers, Fig 3). Different 116 117 sites had varied surface velocities ranging from 0.4 to 0.75 meters per second (measured using 118 Float Method). For the recolonization experiment, depth fluctuated between sites and grids. 119 Depth for individual grids ranged from 0.1 to 4cm and the average depth for all grids was 120 1.65cm. All experiments were set up between 9:30-11am and samples were collected within the 121 same time frame, except for the final collection for the recolonization experiment which was 122 taken one day late.

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Experiment 1: Habitat Preference

Three new habitats, streamside moss, synthetic moss replicate, and an empty control, were placed within a glide to identify simuliid and chironomid habitat preferences (Fig. 4,5,6). Each habitat was encased in a 6x6cm mesh pouch made of 5mm mesh. Nine pouches, three replicates of each habitat, were placed in a three by three fashion and secured between two 25cm pieces of rebar (Fig 7). The experiment was then positioned in a glide and tied to an upstream cinderblock to keep it in place. After 3 days each pouch was individually removed and placed in a separate plastic bag to be analyzed in the lab.

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133 Experiment 2: Recolonization

- 134 Gridded transects were set up across five glides to test primary colonization of an empty habitat.
- 135 Each glide was partitioned into 15 sections (3 columns and 5 rows) 10 x 10cm in size (Fig 8). A
- 136 fine mesh net (<100 micron) was held downstream of each section while the area was vigorously
- 137 scraped with a wire brush for 10s to collect samples. The net was then rinsed into a tub and the



138 rinse water was transferred into a 50ml falcon tube. The bottom two rows were used as controls 139 and sampled on the initial day of set up. The bottom-most row was sampled before any 140 manipulation took place and used to assess the baseline abundance of invertebrates within the 141 glide. Afterwards, the remaining four rows were wiped down with a soft nylon brush to remove 142 invertebrates. During this step special care was taken to keep as much of the moss habitat intact 143

as possible. The second row from the bottom was then sampled and used as a reference for the 144 lowest present abundance. For the remaining three rows within the glide, samples were taken at 1, 3, and 6 day intervals. On each day, the next unsampled, downstream row was scraped and

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#### Sample Analysis

Samples from the field were individually sorted into two separate petri dishes and rinsed with ethanol to dislodge organisms and expedite handling. A dissecting microscope was used to survey all of the samples and identify each specimen. Samples from the recolonization experiment were analyzed via 15 min visual surveys, while the habitat preference samples were examined for 10 min. When an invertebrate was located, it was removed and sorted into a smaller petri dish that corresponded to its taxonomic family. After time was up on visual surveys, the specimens were counted and placed in small vials for further taxonomic identification on a later date.

There are currently 12 species of Simuliidae known from Moorea (Resh et al. 1990, Craig et al. 1995). While some of these taxa have distinct larval morphologies and can be identified to species, most show only minor variation in the shape of the larval mandibles, hypostoma, and head capsules. Reliable identification to species was therefore not possible. Chironomidae is a smaller lineage, with 12 species in French Polynesia and none officially reported from Moorea (Nishida 2002). However, Resh et al. (1990) reported five taxa from Moorea, four of which were identified only to genus and another that was identified to subfamily. Unfortunately, all chironomid species known from French Polynesia were described based on adult material. There are no associated larval characters so these specimens were not identified beyond the level of family.

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#### Data Analysis

- 169 R (R Core Team 2013) was used to analyze all of the collected data. To assess primary
- 170 colonization over the 6 day period, a separate ANOVA was run for both simuliid and chironomid
- 171 larvae and a Tukey Posthoc test was applied to measure which days were the most different.
- 172 Another two ANOVAs were run to evaluate which habitat simuliid and chironomid larvae
- 173 preferred and a Tukev Posthoc test was applied to measure which habitats showed the most 174 significant difference.

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#### 176 **Results**

- 177 Habitat Preference Experiment
- 178 Chironomidae larvae preferred the synthetic habitat while Simuliidae larvae showed no
- 179 significant preference for the moss, control or synthetic habitat (Fig 9, Table 1). Chironomidae
- 180 larvae showed a significant preference for the synthetic moss habitat over both the streamside
- moss, and control habitats (p < 0.001, Fig 9, Table 1). Simuliidae larvae showed some preference 181
- 182 for the control habitat over the moss habitat (p = 0.06, Fig 9, Table 1) but no other significant
- 183 preference was detected between any two habitats.

185 Recolonization Experiment

Both Simuliidae and Chironomidae larvae returned to normal abundance 3 days after experimental manipulation (Fig 10, Table 2, Table 3). 1 day after the disturbance, abundances were still significantly greater than Day 0 (Table 2). After 3 days, larval abundance was not significantly different than Day 0 (Table 2). Abundance on Day 6 also showed no difference than Day 0 (Table 2).

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Density Comparison

Densities of Simuliidae larvae were no different across all four treatments (real moss, introduced moss, synthetic moss, control) while Chironomidae larvae were no different across three treatments: introduced moss, real habitat, and control. (Fig 11, Table 4) Chironomids had the greatest density within the synthetic habitat almost doubling the abundance found naturally (Figure 11, Table 4).

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#### **Discussion**

Habitat Preference Experiment: Chironomidae

Chironomids of a high elevation, Moorean stream preferred the synthetic moss substrate to any other tested habitat (Table 1). This result was initially surprising because the synthetic substrate is inorganic and rigid. In streams around the world Chironomids can usually be found residing in submerged macrophytes (Power 1991, Herren *et al.* 2016, Kukuryk 2014) For example, chironomid larvae found in California have historically been referred to as tuft weaving midges because of their ability to interlace submerged macrophytes into small pouches that are used as shelter (Power 1991). The synthetic substrate was neither filamentous nor flexible enough to weave into a tuft. This observation may rule out shelter as the primary reason for the chironomids preference. Looking closer at the properties of each introduced habitat will help to identify alternative reasons for the preference toward the synthetic habitat.

There are a few reasons why chironomids may prefer the synthetic substrate over the naturally occurring moss. The first reason why the chironomids preferred the synthetic substrate to the moss substrate could be attributed to the amount of flow and available surface area to collect particulate matter. The introduced moss habitat seemed to have the lowest flow of the three treatments and was unlikely to collect any detrital matter. Indicators that lead me to believe this were the moss's unaltered, compacted state and the sediment retention from initial placement. Although the introduced moss may have been filamentous and pliable enough to weave shelter, the food availability may have been minimal. The control habitat probably had the most consistent flow of any treatment, but its lack of inner contents made it almost impossible to collect any suspended material. The synthetic treatment was spongier and the pouch itself was thicker. The synthetic habitat probably stretched across several different flow velocities and would have allowed individuals to feed and seek shelter by transferring between areas of high and low flow. The interlaced synthetic material combined with the high flow also created a large build up of detrital matter within the crevices. The chironomids attracted to the synthetic habitat may be detritivores or scavengers that preferred the synthetic treatment because of its food availability, though without species level identifications feeding modes can only be speculated.

Comparing densities of chironomids between the habitat preference and the recolonization experiment also helps to deepen understanding of the given trends. To equate densities between the two experiments, the abundance of each family on the third day of the recolonization



experiment was compared to the final results of the habitat preference experiment. The experiments were also standardized to a 10x10cm area and corrected for differences in sorting times. A significant difference was found between the densities of chironomids in the synthetic habitat versus the real stream habitat (p<0.001, Table 4). No other relation showed any type of significant difference, indicating that abundances were relatively similar between real conditions and the other introduced habitats. This also means that the variation seen in the data is most likely due to some type of preference and not because of predator exclusion. The moss within sampling sites was very thin and most likely shared similar hydrodynamic properties as the introduced moss substrate. The moss may collect some detrital particles that the chironomids scavenge for, but the habitat is not optimal. This data may suggest that the chironomids only inhabit this niche because it is available not because it is ideal. Other microhabitats similar to the synthetic treatment may be more suitable for chironomids and could reveal a similar preference.

Another reason why chironomids might prefer the synthetic treatment may relate to the habitat preference of their ancestors. A likely possibility for the chironomid's origin on Moorea is that adult insects from another island were swept away by a large storm and happened to land on a new island (Peck 2008). Although these insects have a relatively short life span, it is possible that individuals from the mainland were able to transfer from island to island and make their way into French Polynesia over the course many generations. This method of dispersal is also a good explanation for the smaller size class of chironomid present on Moorea as smaller insects have favorable dispersal properties. (Resh *et al.*1990, Peck 2008). In their previous environment, chironomids may have preferred a habitat with similar conditions as the synthetic treatment. That habitat may not have existed on Moorea and over time they could have adapted to live within the mossy habitat as we observe today.

### Habitat Preference Experiment: Simuliidae

The data from this experiment suggests that Simuliidae larvae may prefer the control habitat over the moss habitat (Table 2) but not over the synthetic habitat. Further understanding of Simuliidae feeding habits provides some explanation for the results. Most of the simuliids on Moorea have been described as filter feeders while one species has been recorded as a browser (Resh *et al.* 1990). In conjunction, larvae of the Simuliidae family tend live on hard substrates in areas of high flow where nutrients are easy to obtain (Das *et al.* 1981, Resh *et al.* 1990). Because they are filter feeders, flow within the introduced habitats was the most likely the ultimate predictor for the simuliid's preference.

As stated previously, the introduced moss habitat probably had the lowest flow of all three environments. The moss was compact and perhaps did not provide favorable conditions for simuliids to filter feed. The control habitat possibly had the most even flow throughout, as there was nothing inside the pouch that could greatly disturb movement of the water. This unobstructed, continuous flow is important when large groups of simuliids select a habitat. Chance and Craig (1986) analyzed the hydrodynamic behavior of Simuliidae larvae and found that clusters of simuliids use currents created by other individuals to enhance their own feeding abilities. This finding helps to explain why large assemblages of simuliids found in continental streams can be seen in dense, evenly spaced rows. Because of its grid like nature, it can be hypothesized that a similar pattern was forming within the control habitat. The simuliids were most likely sitting in rows along the mesh and using each other's wake to enhance their own feeding ability but there was not enough surface area to show a significant preference. If further studies are conducted, use of a tile or other hard substrate may reveal this pattern.

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Another reason for the lack of a discernable difference between the synthetic and control habitats could relate to the amount of usable surface area within the synthetic substrate. Between the two treatments there may have been a comparable amount of surface area exposed to optimal flow. Simuliids are able to position their bodies within an area of low flow and extend their labral fans into fast moving water to collect food (Craig and Chance 1986). The synthetic moss may not have allowed the simuliids to group and evenly space themselves, but individuals may still have found small pockets of favorable flow to filter feed. Also, some of the simuliids found in the synthetic habitat may have been browsers feeding on the leaf matter that built up within the synthetic pouch but further taxonomic identification is needed to confirm this theory.

#### Recolonization Experiment

Simuliidae and Chironomidae were able to return to baseline abundance within three days of experimental manipulation. According to the data, both families were in equal abundance during initial sampling. A possible explanation for the equal rate of recolonization is that a lack of competition between each family allows them to coexist in within the same glide. This absence of competition may be due to both families occupying a different microhabitat, the niche they share is large enough for both of them, they have some type of mutualistic relationship, or there exists some type of equilibrium between families. Without further sampling it will be hard to determine the exact size of their designated niche space, predict if there is some type of mutualism, or determine if equilibrium is present. However with the data, and the insight of past research, understanding the relative distribution of these families may be possible.

In order to investigate local preferences of simuliid and chironomid larvae, data from both studies was used to compare average densities across different habitats. Analysis revealed that chironomids showed a significant preference for the synthetic habitat over any other habitat while the Simuliidae larvae displayed a slight preference for the control habitat compared to the moss habitat (Table 2, Table 4). For this experiment only two types of microhabitats were sampled from, moss and bedrock. Because of their intermixed nature it is hard to accurately pinpoint the origin of every insect, however comparing similar qualities between the introduced habitats and real conditions allows us to make speculations. In terms of flow, the control habitat is most comparable to a bedrock substrate as it provides the least amount of obstruction to oncoming currents. In terms of its ability to capture detritus, the synthetic habitat is most comparable to the natural stream moss. Previous studies from numerous locations around the globe have denoted that chironomids tend to reside in submerged macrophytes or sediment while simuliids typically anchor to hard substrates (Power 1991, Kondo and Hamashima 1992, Das et al. 1981, McCreadie and Alder2012). Based on the data, in conjunction with past literature, it is likely that the moss was dominated by chironomids and the bedrock mostly consisted of simuliids. This distinction would explain the lack of observable competition and aligns with interpretations of previous studies.

#### 315 Stream Health on Moorea

Assessing the relative distribution of insects can be insightful information for determining the condition of an environment. Stream health is often determined by measuring the abundance and diversity of the inhabiting insect population (Morley 2002). In the streams of Moorea, insect diversity is very low compared to typical mainland streams, but this is most likely due to naturally occurring environmental conditions (Resh *et al.* 1990, aNIWA 2016). The National Institute of Water and Atmospheric Research states that the temperature, flow, substrate, and

water clarity of the study sites would not support many of the usual insects that indicate good stream health. Typically presence or absence of Ephemerellids, Plecopterans and Tricopterans are used to assess stream health, but none have been reported on the island of Moorea (Resh *et al.* 1990, Wright & Ryan 2016). However, this does not necessarily mean that the stream is unhealthy or polluted and even without the usual indicator insects it is still possible to gauge the health of the study stream.

While simuliids and most chironomids are very resistant to pollution and not typically used as indicator species, other collected taxa are less resistant (bNIWA 2016). Although this study focused on simuliids and chironomids, many other invertebrates were observed during sampling. Dragonfly nymphs, assorted gastropods, ceratopogonids, shrimp, nematodes and a few unidentified taxa were found while surveying samples. Dragonflies, gastropods, and shrimp are not typically found in polluted streams (bNIWA 2016). In conjunction, the study stream was far from any city center and only visited by hiking tourists. These factors help to minimize anthropogenic influence and keep much of the habitat undisturbed. Lastly, the expedient recolonization after a disturbance event suggests that there is a large abundance of breeding flies in the surrounding ecosystem. The combination of these factors suggests that the study stream was in good health. Further studies in Moorea's high elevation streams may want to analyze additional factors including dissolved oxygen, pH, and general diversity to reaffirm the health of the stream.

From these findings it is also possible to speculate about the future health of this specific stream. As climate change progresses and global temperature rises, tropical islands like Moorea are expected to see more intense weather patterns meaning that storms may be more severe and droughts will last for longer (Griffiths et al. 2003, Dore 2005). For chironomids and simuliids this weather will likely have some impact on their abundance however both families are well adapted to changes in water availability. Chironomids and simuliids will actively seek shelter in damp or pooled areas when water levels become low (Dewson et al. 2007, Frouz 2003). This shelter is not an ideal habitat, but it will allow the insect to avoid desiccation until the stream refills. Because of their large abundance, quick recolonize and keen adaptability, they may only be affected if drought conditions become severe. Furthermore, abundances of chironomids may even increase if storms become more intense. As the habitat preference study showed, the chironomids preferred the synthetic substrate. A naturally occurring, comparable habitat to the synthetic treatment could resemble a leaf pack in an area of high flow. If a leaf pack is shown to reveal a similar preference in chironomids, severe storms could generate more stream debris and ultimately result in a greater amount of habitat. This preference could also indicate that chironomids are able to abandon moss as a habitat and colonize a different substrate if necessary.

#### **Conclusions**

Overall this study aimed to track the recolonization rate of Simuliidae and Chironomidae larvae as well as test each family's habitat preference. The study revealed that both families could repopulate a disturbed habitat to its original abundance within a period of 3 days. Chironomids were also shown to have specific preference for the synthetic moss treatment while the simuliids may have preferred the control habitat. This trend suggests that like mainland streams, the Moorean simuliids probably live on hard substrates while the chironomids live within a macrophytic habitat. This pattern may only apply to Moorea's high elevation streams and further studies across a larger gradient may help to solidify this pattern. The data suggests that the study stream is in good health and that future insect populations are largely dependent on global



weather patterns. Ultimately, recognizing these trends can help scientists better understand interactions between dipterans and their environment as well as provide context for others to make comparisons between continental and island streams in the future.

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#### Acknowledgements

I would like to thank all of the professors and graduate students for their guidance and friendship during this course. Special thanks to William Kumler for his assistance using R and performing data analyses. I would also like to thank Zoe Klein for the help and support she provided as my field buddy. Lastly I would like to thank the whole Gump Station staff for running and maintaining the great facilities.

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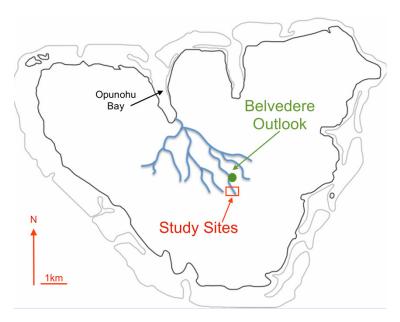
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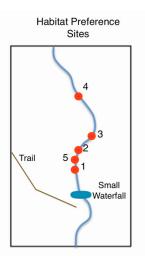
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Figure 1: Aerial view of the Opunohu Valley of Moorea, French Polynesia. Sites were south of the Belvedere outlook point, within the red box. Map based on V.H. Resh diagram 1990.



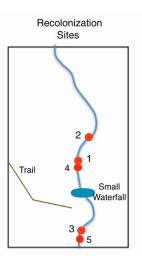


Figure 2: Expanded view of study sites **Habitat Preference Sites Between**: S17°32′645″ W149°49′663″ (site 4) and S17°31′339″ W149°51′343″ (site 1).

#### Recolonization Sites Between:

S17°31'339" W149°51'343" (site 2) and S17°32'625" W149°49'685" (site 5).

WGS84 format used. Picture is not to scale

## **River Temperature**

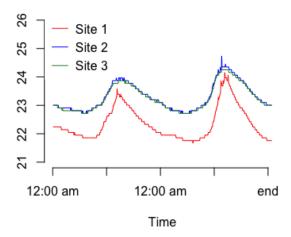


Figure 3: Temperature readings taken every 10min 6 days of trials. Site 1, 2, and 3 refer to the habitat preference experiment

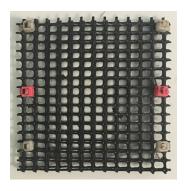


Figure 4: Empty control pouch



Figure 5: Moss filled pouch



Figure 6: Synthetic moss pouch



Figure 7: In water habitat preference experiment



 Figure 8: The in water transect used for the recolonization study labeled by grid. Grids 1-3 were sampled as the positive control. Grids 4-6 were sampled as the negative control. The remaining 3 rows were sampled in order at 1, 3, and 6 day intervals.

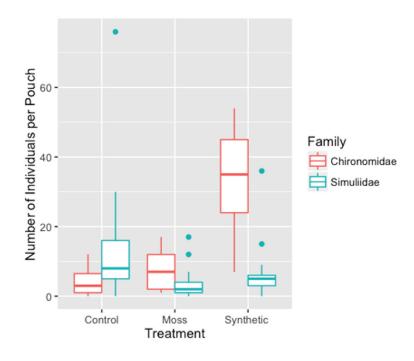


Figure 9: Changes in Simuliidae and Chironomidae larval abundance across 3 treatments (A) Control (B) Streamside Moss (C) Synthetic Moss. Medians of 5 trials are represented as dark bands. Outliers are represented by single dots.

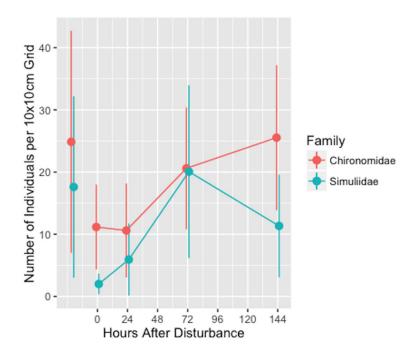


Figure 10: Changes in abundance of Chironomidae and Simuliidae larvae over a 6 day period. Medians of 5 trials are represented as dark bands. Outliers are represented as single dots.

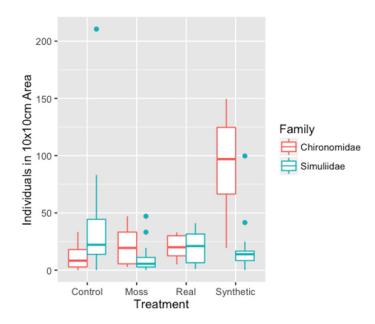


Figure 11: Changes in Simuliidae and Chironomidae larval density across 4 treatments (A) Control (B) Streamside Moss (C) Real in stream moss (D) Synthetic Moss. Medians of 5 trials are represented as dark bands. Outliers are represented by single dots.

	Simuliidae	Chironomidae	
	F = 0.39	F=1.78	
Treatments	р	р	
Moss-Synthetic	0.749	0	
Moss-Control	0.062	0.586	
Synthetic-Control	0.256	0	

Table 1 Habitat Preference Experiment: The effect size and P values of the abundance comparisons between treatments is shown in this table.

	Simuliidae	Chironomidae	
	F = 0.53	F=0.48	
Day	р	р	
0-0.5	0.002	0.026	
0-1	0.023	0.011	
0-3	0.965	0.851	
0-6	0.463	0.999	

Table 2 Recolonization Comparison: The effect size and P values of a Tukey test comparing abundance between each sampling day.

	Simuliidae		Chironomidae	
	F = 0.53		F=0.48	
Day	Mean	sd	Mean	sd
0	17.6	14.1	24.8	17.2
0.5	2	1.6	11.2	6.5
1	5.9	5.6	10.6	7.3
3	20.1	13.4	20.6	9.4
6	11.3	7.9	25.5	11.2

Table 3 Recolonization Means: Shown are the means and standard deviations of simuliid and chironomid abundance listed by day.

	Simuliidae	Chironomidae
Treatments	р	р
Control - Synthetic	0.46	0
Moss - Synthetic	0.98	0
Real - Synthetic	1	0
Moss - Real	0.99	0.99

 $\begin{array}{c} 1007 \\ 1008 \end{array}$ 

 $\begin{array}{c} 1026 \\ 1027 \end{array}$ 

 Table 4: Density Comparison Table. Shown are the P values of the only 3 significant comparisons between both experiments. Chironomids were significantly denser within the synthetic treatment compared to any other habitat. The introduced moss and real moss substrates show no difference in abundances for either family.



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