

ESTIMATING FLYING INSECT BIOMASS AND ITS SPATIOTEMPORAL
DISTRIBUTION IN METCHOSIN: BIODIVERSITY ON SOUTHERN VANCOUVER
ISLAND

by

Kennedy Nickel

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Abstract

A spatial and temporal baseline of the major flying insects (Diptera, Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, and Other arthropods) was established in Metchosin, British Columbia, Canada, by measuring the biomass (g), number of individuals, and calculating the average weight of an individual (g/indiv) from July to end of October, 2019. Malaise traps ($n = 15$) were placed on Metchosin residents' property and monitored, with trap contents collected every two weeks. Spatiotemporal differences occurred between sites for biomass (g) and number of individuals. A one-way ANOVA showed that at least one site significantly differs for biomass (g) ($F = 5.285, p < 0.001$) and number of individuals ($F = 12.859, p < 0.001$). The average weight per individual (g/indiv) did not differ between sites ($F = 1.684, p = 0.0833$). The seasonal biomass trend peaked at mid-growing season with Diptera, Hymenoptera, and Lepidoptera being the main contributors to this pattern. The principal component analysis showed Hymenoptera and Diptera were the main contributors to variation in biomass between sites, and Lepidoptera explained the least amount of biomass variation between sites over time. Dipterans and hymenopterans consisted of smaller individuals and were the most abundant throughout the season. In comparison, lepidopterans were generally larger individuals and lower in number. There were visible differences between low elevation sites (0-100 m) and high elevation sites (100-200 m) in orders with the greatest biomass (Diptera, Hymenoptera, Lepidoptera). The principal component analysis illustrates that elevation explains some of the variation between sites. In comparison to all sites, site 6 was identified as a biodiversity hot spot due to the high amount of biomass and number of individuals.

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Introduction

Insect biodiversity

Biodiversity can broadly be defined as a state or attribute that refers to the variety of life and its interactions in a given space, which can vary greatly in scale (DeLong 2013). There has been a recent demand for biodiversity data as the climate changes; however, it is essential to define the space and time at which it is measured, since patterns and interpretations can differ at different scales (Kim and Byrne 2006; DeLong 2013).

Most of the ecosystem services that humans benefit from are due to insects, especially flying insects (Losey and Vaughan 2006); therefore, it is important to understand the biodiversity trends of flying insects. Wilson (1987), expressed the importance of insects when he stated that it is “the little things that run the world”. Even though this was stated in 1987, it still remains true today; insects can persist without humans but humans cannot persist without insects (Losey and Vaughan 2006; Wilson 1987). Losey and Vaughan (2006), estimated that the ecosystem services (*e.g.*, dung burial, pest control, pollination, and wildlife nutrition) provided by native insects are worth more than \$57 billion annually in the United States alone. In contrast, not all native insects are considered economically beneficial. For example, in British Columbia the mountain pine beetle (*Dendroctonus ponderosae*) is a native beetle that has impacted millions of hectares of pine forest in British Columbia in just over a decade (Taylor et al. 2006). Recently, investment has gone into making the deteriorated pine trees into a source of biofuel, mainly cellulosic ethanol (Luo et al. 2010). In addition, *D. ponderosae* and the infected trees alter the forest ecosystem community structure by increasing the available light as well as nutrients (Goheen and Hansen 1993). Over a longer time period, the soil fertility may improve (Goheen and Hansen 1993). It is a complex process, to fully evaluate the benefits and consequences of native insects—economically and ecologically. Understanding insect biodiversity trends can help to understand this complexity. Mace et al. (2012), proposes that biodiversity itself can be regarded as an ecosystem service, by humans placing an intrinsic value on the diversity of species.

Recently researchers have documented a significant decline in insect species around the globe but the reasons for such decline have yet to be clearly identified (Hallmann et al. 2017; Sánchez-Bayo and Wyckhuys 2019). A recent meta-analysis found a decline in insect

populations and discovered that in the next few decades there may be up to a 40% extinction of the world's insect species (Sánchez-bayo and Wyckhuys 2019). Of the insect orders, the most susceptible to decline were found to be Lepidoptera, Hymenoptera, and dung beetles (Coleoptera) whereas the aquatic insect populations (Odonata, Plecoptera, Trichoptera, and Ephemeroptera) have already suffered a detrimental loss in numbers (Sánchez-bayo and Wyckhuys 2019). Sánchez-Bayo and Wyckhuys (2019), quantified the main factors associated with insect declines and attributed the loss to intensive agriculture (23.9%), pesticides (12.6%), ecological traits (12.6%), urbanization (10.7%). Hallmann et al. (2017) noted a 76% decline in flying insects over 25 years, in a protected area in Germany. In their analysis, they considered the major factors causing inter-annual variability including weather, habitat and land use variables; however, these variables only explained 20% of the variation. The other 80% is still unknown and the factors associated with intensifying agricultural practices were not included (Hallmann et al. 2017). Since we are currently in the Anthropocene where humans are the dominant influence on the environment, the major factors that are influencing the decline are likely anthropogenic (*e.g.*, intensive agriculture, pesticide use, urbanization, deforestation) (Dirzo et al. 2014; Sánchez-bayo and Wyckhuys 2019). The factors influencing insect decline will differ and are dependent on spatiotemporal scales.

Insect populations should be monitored because they are ecologically essential and if certain species are suddenly lost, a cascading effect could be the result. Insects are a key link in energy transfer between the base of the food web and higher trophic levels (Mattson and Addy 1975; Kaspari and Joern 1993). If many phytophagous insect species are lost a cascade effect could occur where insectivorous animals decrease due to depleted food source. Hallmann et al. (2014) experimentally show this cascading effect. Their results show a significant decrease in insectivorous bird populations with pesticide use, this trend is the most apparent in barn swallows (Hallmann et al. 2014).

Insect biodiversity at a spatial scale

Terrestrial arthropods represent a majority of organismic diversity, yet some of the global distribution of relative insect taxa and its abundance is largely based on percentage distribution of the world's plant species (Stork 2018). Stork (2018), used this estimation and noted that North America had the lowest number of insect species (115,503 insects) compared to the Neotropics

where the highest number of insects are found (1,620,348 insects). It is possible that Arthropod abundance is highest in low latitudes and as latitudes increase there is less biodiversity due to harsher environmental conditions (Block 1980, 1990; Danks 2004). Basset et al. (2015), explains that to fully analyze a global arthropod trend, it must first be understood at smaller scales. Basset et al. (2015), focused on the spatiotemporal arthropod trends in San Lorenzo forest in Panama, using this region as a smaller scale representative of the tropics. In their study they found that effects related to seasonal and the vertical dimensions were key (Basset et al. 2015).

Insects operate at small microclimatic and microhabitat scales (Danks 1979). Few studies have compiled insect data from across Canada to understand their spatial distribution. However, Danks (1975), analyzed the spatial distribution of insects across Canada and found that environmental heterogeneity lead to heterogenous distributions of insects. He then categorized environmental heterogeneity into three categories: (1) topography and other local influences, (2) seral stages, and (3) microhabitat stratification (Danks 1975). Each of these levels of environmental heterogeneity can influence whether or not certain insects are prevalent, by regulating the environmental conditions and species interactions. The ranges of insects could be geographically restricted, habitat restricted, or both depending on their life requirements (Danks 1975); either requiring a specific habitat for part of their life history strategy or confined to a certain geographical region. In addition, the distributions of insects could vary from transcontinental to amphicontinental depending on a few factors (Danks 1975). Northern species, such as genera of dragonflies *Aeshna* and *Leucorrhinia*, tend to be more transcontinental due to the persistence of the boreal forest and tundra across northern Canada. While southern species tend to be more confined to smaller regions such as the prairies or the west coast (Danks 1975). Amphiconintenal phytophagous species could have their ranges limited by food plants or hosts (Danks 1975); however, though many species are restricted by food, there are also species that are an exception to this generalization (Danks 1975). Whether or not insects are generalists or specialists can also greatly influence their distribution (Danks 1975). Generalists tend to be more widely dispersed compared to specialists (Danks 1975). Wolfenbarger (1946) considered insects' ability to disperse and found that some groups seem to disperse very little, and most insects do not readily use long-distance dispersal. Certain insects have a wider range and capability to disperse does not necessarily mean that they are less susceptible to decline. In 2009, Odonata became the first insect order to have a global assessment completed (Clausnitzer et al. 2009).

Though dragonflies and damselflies (Odonata) have a relatively wide distribution, about one of 10 species were threatened with extinction according to ICUN's Red List criteria (Clausnitzer et al. 2009).

Insect biodiversity at a temporal scale

Biodiversity trends can vary over years and throughout seasons; therefore, it is equally important to understand the seasonal and annual biodiversity trends of flying insects. Reproduction, growth and development, dormancy, movement and migration are the typical phases in insect life cycles (Tauber and Tauber 1981); but the occurrence and time spent in each phase can differ greatly between taxa (Danks 1994). Seasonality—including factors such as photoperiod, temperature, and moisture – greatly impacts the timing of these four phases, which could be why there are different life histories in different climates (Tauber and Tauber 1981; Wolda 1988; Danks 1994). For example, in temperate areas the winter is typically a hibernation-like period for many insects, where they undergo diapause, and as temperatures increase the growing season begins, but not necessarily within the same year (Wolda 1988). For example, the life cycle of *Sphex vesparum* (Hymenoptera: *Ichneumonidae*) includes one to four winters spent in a cocoon within a wasp nest then they emerge from spring to summer for mating and feeding purposes (Donovan 1991). Although this is a generalization and it can differ between species as well as between individuals, and the ability and timing of diapause is susceptible to change depending on environmental conditions (Tauber and Tauber 1981; Wipking and Kurtz 2000). For example, the burnet moth, *Zygaena trifolii*, has shown phenotypic variability in diapause length and timing between individuals in response to environmental conditions (Wipking and Kurtz 2000). As latitudes increase, so does the tendency for diapause in insects, as one possible strategy to combat with the cooler temperatures (Block 1990; Danks 1991, 2004). In contrast, the tropics generally lack this period of hibernation or have a significantly shorter diapause due to the year-round stable temperatures but the fluctuations of high rainfall and dry weather, that are not as prominent in temperate areas, greatly affects the temporal distribution flying insects (Wolda 1988).

All insects are poikilotherms, therefore temperature extremes can critically influence their development and survival (Ikemoto 2003; Damos and Savopoulou-Soultani 2012). In temperate areas, it is energetically unfavorable to develop in colder months such as fall and winter, where

temperatures are too cold (Ikemoto 2003; Damos and Savopoulou-Soultani 2012). In contrast, if temperatures are too high, it can effect development and survival (Ikemoto 2003; Damos and Savopoulou-Soultani 2012; Khaliq et al. 2014).

Since insects are sensitive to fluctuations in the weather the abiotic conditions can greatly impact the temporal distribution of flying insects, seasonally and diurnally. Temperature, wind speed, and time of day were found to be some of the more prominent factors affecting the temporal distribution of flying insects by regulating how much time is spent flying (Peng et al. 1992; Polatto et al. 2014). In addition, the extent of influence of these conditions can vary between flying insect taxa as well as within taxa due to size differences (Harrison and Roberts 2000; Møller 2013; Polatto et al. 2014). Møller (2013), found that flying insect abundance decreases under increased wind conditions. Moreover, Peng et al. (1992), noted that wind speed impacted the flight of smaller dipterans greater than larger ones. For example, Diptera, tended to fly primarily in the afternoon versus the evening. Temperature was the most important factor for afternoon flying dipterans while wind speed was the most important factor for evening flying dipterans (Peng et al. 1992). In summary, the taxa, body size, and time of day can regulate how abiotic conditions affect the amount of time spent flying. Although, these are likely not the only factors influencing the time insects spend flying.

Determining biodiversity using biomass

There are several approaches to determine biodiversity trends. Some of the more common approaches consider species richness and evenness, which define species as the single unit of life and focus on the diversity of single species in an area (DeLong 2013). However, this can easily become a problematic and tedious process for those groups that are not well defined, such as insects. Insects are often susceptible to cryptic biodiversity and have high species richness; it is estimated that 5.5-7 million insect species exist but barely a million are identified and described (Stork 2018). DNA barcoding techniques are revealing more cryptic species; therefore, the estimate of 5.5-7 million insects may be even greater than predicted (Stork 2018). Therefore, traditional biodiversity measurements, such as species richness and evenness, may not be the most efficient method for studying insect biodiversity. As an alternative, the biomass of selected taxa is being used to estimate biodiversity in an area (DeLong 2013; Hallmann et al. 2017; Heleno et al. 2009). This process involves determining the mass of organisms in a given area or

volume. This approach focuses more on the amount of ‘bio’ in biodiversity; ultimately, determining the standing stock and when monitored over time can explain more about the biodiversity trends that are occurring in an ecosystem or area. Measuring the biomass of insects is a feasible process that provides key biodiversity data.

Research objectives

Before addressing major questions of how much biomass is being lost or how fast is it declining, a baseline of biomass data and trends must be established (Cardinale et al. 2018). The objective of this research is to establish a baseline of flying insect biomass and distribution of the main flying arthropod taxa (Diptera, Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, and Other arthropods), over the growing season (summer to fall) in Metchosin, British Columbia, Canada, using Malaise traps at 15 different sites. This study location provides an opportunity to analyze local temperate arthropod trends from the coastline to altitudinal peaks (~180 m) of a rural municipality where there is a limited pesticide use (currently there is a ban on cosmetic pesticide use on public properties and discouragement of pesticide use in general) and mild densities of urbanization.

The municipality of Metchosin, British Columbia, is an ideal study site to further examine the spatiotemporal distribution of southern insect species in the west coast of Canada. Metchosin is a rural municipality with mild levels of urbanization and a diverse landscape from the coast to altitudinal peaks (~180 m). Scanlon and Petit (2008), note the lack of information about the spatiotemporal variation of insects, in urban areas. Therefore, more research is required to further analyze temperate flying insect distributions and the impact of urbanization should be considered in combination with the known abiotic factors. A large-scale project that includes quantifying the spatiotemporal distributions of insects can be problematic; however, the implementation of citizen scientists is making the process more feasible. By employing citizen scientists, a larger scale study area, such as Metchosin, can be analyzed efficiently.

For this project, the spatiotemporal distributions will be analyzed in the municipality of Metchosin. The recent publicity illustrating insect declines has sparked headlines that included alarming phrases like “Insect Apocalypse” and “Insect Armageddon” (Bennet et al. 2017; Jarvis 2018). This has inspired the citizens of Metchosin to partake in our investigation. Each citizen

scientist, that is selectively chosen, will ‘adopt’ a Malaise trap and house it on their own property. This allows for continuous monitoring of the traps and for more efficient collection.

It is hypothesized that the biomass will vary greatly between sites because of the different microhabitats but all sites will follow the same general trend since they are in the same general climate—having the total biomass peaking in the mid-summer then declining as the growing season comes to an end (Tauber and Tauber 1981; Hallmann et al. 2017). Sánchez-Bayo and Wyckhuys (2019), argue that in general there is a greater amount of smaller generalist insects that are replacing the vacant niches from the declined insects. I hypothesize that if a general decline of insect biomass is occurring in Vancouver Island, there will be a greater number of smaller generalist species. Since many insects have different life history strategies, the maximum biomass of each major order is hypothesized to be slightly staggered in the mid-summer, to possibly minimize competition between the taxa. This project will ultimately establish a baseline of local flying insect biomass data that can be further used to determine the main factors that are influencing the biodiversity trends as well as provide data that can be used for determining the most efficient conservation strategies.

Methods

Site determination and Malaise trap placement

The study sites were located in the District of Metchosin, British Columbia, Canada (48.38°N, 123.54°W). This rural municipality offers a diverse landscape ranging from the coast to higher altitudes of roughly 180 m, with mild urbanization and limited pesticide use (currently there is a ban on cosmetic pesticide use on public properties and discouragement of pesticide use in general). Malaise traps (n = 15) were placed on Metchosin residents’ property to be continuously monitored (Fig. 1). Prior to trap implementation, the properties were assessed to ensure that there was sufficient space and no interactions with bee farms. The Malaise traps were then placed to encompass a variety of geographical features and to the greatest amount of biotic and abiotic diversity. Traps were separated as best as they could to minimize the possibility of overlap; the two closest traps (traps 3 and 8) are roughly 400 m apart (Fig. 1).

All Malaise traps were identical in design (ez-Malaise Trap (BT1002) (height 180 cm by 165 cm)) (Appendix, Fig. 9). The traps are non-attractant and passively intercept the insects’

flight path. Using a Garmin GPSmap 60Cx, each of the Malaise traps were set up facing the same direction, with the catch head bottle aligned to the north. The elevation, latitude and longitude were then recorded at each trap location. The traps were secured tightly to the ground, to withstand heavy wind and to ensure that maximum surface area was used to catch insects. Trap 2 had fallen over in the July 20, 2018 to August 3, 2018 collection period as well as trap 13 in the collection period of August 17, 2018 to August 31, 2018.

Sample collection

The samples were collected every two weeks, starting July 6th, 2018 and ending on October 26th, 2018. The samples were caught and stored in 75% ethanol solution for preservation. Sorting and analysis of the samples (n = 118) were conducted in the laboratory at University of Victoria, British Columbia, Canada.

Determining abundance

Each of the samples (n = 118) were sorted into major flying insect orders (Hymenoptera, Diptera, Coleoptera, Lepidoptera, Hemiptera, and Other arthropods) using a dissecting microscope, Bogorov sorting tray, and fine tweezers. If an arthropod did not fit into a specified order, it was placed into the 'Other' category and this included non-insects, such as Araneae, Collembola, and Isopoda. While sorting, the number of individuals were recorded.

Measuring biomass

The wet biomass was determined for each of the samples, following the protocol originally outlined in Schwan et al. (1993) and used by Hallmann et al. (2017) and Sorg et al. (2013). The sample contents were poured into a 10 μ m nitex mesh sieve, that was 10 cm diameter. Samples were sieved at a 30-degree angle to increase the initial ethanol solution runoff speed. The size of mesh was chosen to ensure that all the caught individuals were accounted for in the total weight. With a stopwatch, the drops off the sieve were observed. Once the drops were greater than 10 seconds apart, the contents were transferred to a weighing boat. The contents were then measured to the nearest 0.001 g on an Adventurertm Ohaus analytical scale.

Statistical analysis

Principal component analysis was computed on R version 3.4.3.0 using the packages FactoMineR and factoextra. All other statistical analyses were computed on Python version 3.6.6. The packages used for statistics and data frames include: statsmodels version 0.9.0, scipy version 1.1.0, scikit-learn version 0.20.0, pandas 0.23.4, numpy 1.15.2. For graph visualizations seaborn version 0.9.0 and matplotlib version 3.0.0 packages were used. The folium version 0.7.0 was used to visualize the maps.

Assuming all sites were independent of each other, the means and 95% confidence intervals were computed for biomass (g) and average weight per individual (g/indiv). A one-way ANOVA was used to determine if there were differences between sites with regards to biomass (g), number of individuals, and average weight per individual (g/indiv). A principal component analysis (PCA) was then used to determine which insect orders contributed the most variation to biomass between sites. Elevation and time were considered as explanatory variables in the PCA. Trap 2 contents from July 20, 2018 to August 3, 2018 and trap 13 contents from August 17, 2018 to August 31, 2018 were excluded due to trap failure. Before running the PCA, the data was scaled to unit variance. The PCA results were then grouped based on time, with each group as a two-week interval starting at July 6, 2018 to illustrate biomass variation of each site with time. To further analyze elevation with regards to biomass (g), the sites were first categorically separated into low elevation (0-100 m) and high elevation (100-200 m) based on the GPS readings. To determine the differences in means between low elevation (0-100 m) and high elevation (100-200 m) sites.

Results

General spatiotemporal seasonal trends

Fifteen Malaise traps were placed on resident's properties throughout the municipality of Metchosin, British Columbia, Canada, with various elevations and some microhabitat differences (Fig. 1; Table 1).

The overall seasonal biomass (g), number of individuals, and average weight per individual (g/indiv) peak at the initial collection (July 20th, 2018) but show different seasonal trends after this initial collection date (Fig. 2A, C, E). The biomass (g) decreases over the season

(July to October) (Fig. 2A, B). The number of individuals also peaks at the start of the collection period but has a secondary peak after September 14, 2018 (Fig. 2C, D). The biomass and number of individuals varies greatly between sites but when the two parameters are considered to find the seasonal trend of average weight per individual (g/indiv) there are minimal outliers (Fig. 2). The biomass variation between sites can be seen in Figure 3, with site 6 as the largest. Site 6 also had the greatest number of arthropods (33,752 individuals) and site 15 had the least number of arthropods (1,729 individuals). The average weight per individual (g/indiv) decreases at a more linear rate compared to the seasonal biomass (g) trend (Fig. 2A, B, E, F). A one-way ANOVA showed that at least one site differs for biomass (g) ($F = 5.285, p < 0.001$) and number of individuals ($F = 12.859, p < 0.001$). There was no difference between sites for average weight per individual (g/indiv) ($F = 1.684, p = 0.0833$) (Fig. 2).

The total biomass from July 6, 2018 to October 26, 2018 varied between each trap location (Fig. 3). Site 6 had the greatest total biomass (133.012g) (Fig. 3; Table 1). Sites 3, 11, and 15 had the lowest total biomass (<10 g) but were in different areas of Metchosin, and not next to each other (Fig. 3; Table 1). The average total biomass of all 15 traps was 36.643 g with a median of 27.942 g (Table 1). The total biomass for each insect order for all Malaise traps, from the greatest to least is as follows: Diptera (153.808 g), Lepidoptera (135.560 g), Hymenoptera (115.541 g), Other arthropods (67.624 g), Hemiptera (46.814 g), and Coleoptera (30.300 g) (Table 2). Diptera, Lepidoptera, and Hymenoptera were the overall main contributors to the biomass trend.

In comparison, the principal component analysis (PCA) showed that Hymenoptera has the highest contribution to biomass variation between sites followed by Diptera and Hemiptera. Whereas, Lepidoptera explained the least amount of biomass variation between sites (Fig. 6). The PCA further shows that time was more strongly correlated with biomass variation between sites compared to elevation; however, both do account for a lot of the variation between sites.

Diptera, Hymenoptera, and Lepidoptera seasonality

The main orders contributing to the biomass (Diptera, Hymenoptera, Lepidoptera) followed similar seasonal mean biomass trends and are driving the biomass seasonal trend (Fig. 4); however, the seasonal trend of the mean average weight of individual (g/indiv) differs in Lepidoptera (Fig. 5). The seasonal mean biomass peaks at the first collection date and then

decreases quickly with time (Fig. 4). The 95% confidence intervals are larger at the start of the collection period compared to the later dates, suggesting that there is more variation in the mid-summer biomass (Fig. 4). The PCA further shows that there is high variation of biomass between sites in the early collection periods that is illustrated by the large 95% confidence ellipses (Fig. 7). Hymenoptera and Diptera are smaller sized individuals throughout the season (never exceeding 0.0015 g/indiv) compared to Lepidoptera, where the size of individual ranges roughly between 0.010-0.030 g/indiv (Fig. 5). However, the PCA shows that the biomass of Hymenoptera and Diptera explain the greatest amount of variation between sites, where Lepidoptera biomass explains the least amount of variation between sites (Fig. 6).

Coleoptera, Hemiptera, and Other arthropods seasonality

Total biomass for Coleoptera, Hemiptera, and Other arthropods throughout the season was low (30.300 g, 46.814 g, 67.624 g) compared to Diptera (153.808 g), Hymenoptera (115.541 g), and Lepidoptera (135.560 g) (Table 2). Coleoptera and Other arthropod biomass explained some of the variation between sites, but Hemiptera explained more of the variation between sites (Fig. 6). Coleoptera mean biomass also peaks at the first collection time (~1 g) and decreases with time, similar to Hymenoptera, Diptera, and Lepidoptera (Fig. 4). The 95% confidence intervals of Hemipteran mean biomass increase slightly after August 31, 2018 but the overall mean biomass remains low (<1.5 g) (Fig. 4). The Other arthropods mean biomass is also low and ranges between 0.1 to 1 g (Fig. 4).

The mean average weight of individual (g/indiv), Hemiptera had the lowest over the whole season (< 0.005 g/indiv) (Fig. 5). The mean average weight of individual (g/indiv) varied over the season in Coleoptera and Other arthropods with no apparent trend observed (Fig. 5). On September 14, 2018 and October 26, 2018 for Coleoptera, there are large confidence intervals with overlapping means, which indicate no difference and a lot of variation (Fig. 5).

Seasonal biomass and elevation

The sites were categorically separated into low elevation (0-100 m) and high elevation (100-200 m) based on the GPS readings (Fig. 7). Sites 5, 6, 8, 9, 10, 11, 12, and 13 (n = 8) were categorized as low elevation sites, while sites 1, 2, 3, 4, 7, 14, and 15 (n = 7) were categorized as high elevation sites (Table 2). In orders that had relatively high biomass (Hymenoptera, Diptera, Lepidoptera), there are visible differences between the low elevation and high elevation sites

(Fig. 8). The greatest differences between the two elevation types occurs at the initial measurements for Hymenoptera, Diptera, and Lepidoptera and as the season continues there is less noticeable of a difference between sites at high and low elevations (Fig. 8). Across all orders where there are visible differences between the two elevation types, the lower elevations appear to have more biomass than the sites at higher elevations (Fig. 8). In Hemiptera, there seems to be the greatest difference between the two elevations near the end of the season; however, the overall biomass (g) is quite low throughout the season (Fig. 8). Coleoptera and the Other arthropods also had very low biomass (g) over the season. Although, the Other arthropods shows fluctuations in biomass (g) and has one time period where there is a significant difference (August 17, 2018). The PCA results further indicate that elevation explained some of the biomass variation between sites (Fig. 6).

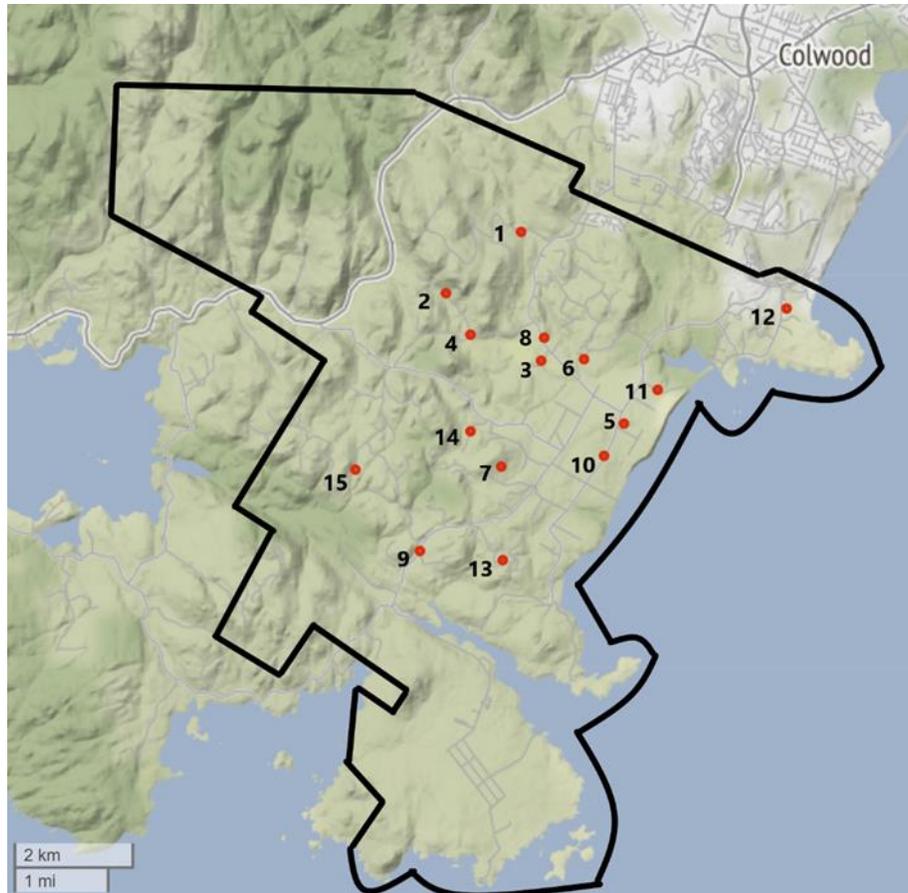


Figure 1. The municipality of Metchosin, British Columbia, Canada, is outlined with each Malaise trap location ($n = 15$) marked with the corresponding site number next to it. Each trap is on a different Metchosin residents' property.

Table 1. The Malaise trap (and site) number with corresponding latitude and longitude coordinates and elevation, taken with Garmin GPSmap 60Cx on August 3, 2018. All Malaise traps are within the municipality of Metchosin, British Columbia, Canada. The total biomass of Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, and Other arthropods from July 6, 2018 to October 26, 2018 is also included. The Other arthropods includes other members of Arthropoda that were collected in the Malaise trap. Trap 2 had fallen over in the July 20, 2018 to August 3, 2018 collection period as well as trap 13 in the collection period of August 17, 2018 to August 31, 2018.

Trap Number	Latitude	Longitude	Elevation (m)	Total Biomass (g)
1	48.406	-123.554	135	18.244
2	48.397	-123.571	182	15.228
3	48.387	-123.550	124	7.720
4	48.391	-123.565	190	21.453
5	48.378	-123.531	41	70.813
6	48.388	-123.540	48	133.012
7	48.371	-123.559	102	36.192
8	48.391	-123.549	77	27.942
9	48.359	-123.577	82	45.001
10	48.373	-123.535	51	32.549
11	48.383	-123.523	38	6.453
12	48.395	-123.495	64	18.064
13	48.358	-123.558	83	48.293
14	48.377	-123.565	138	58.955
15	48.371	-123.591	139	9.728

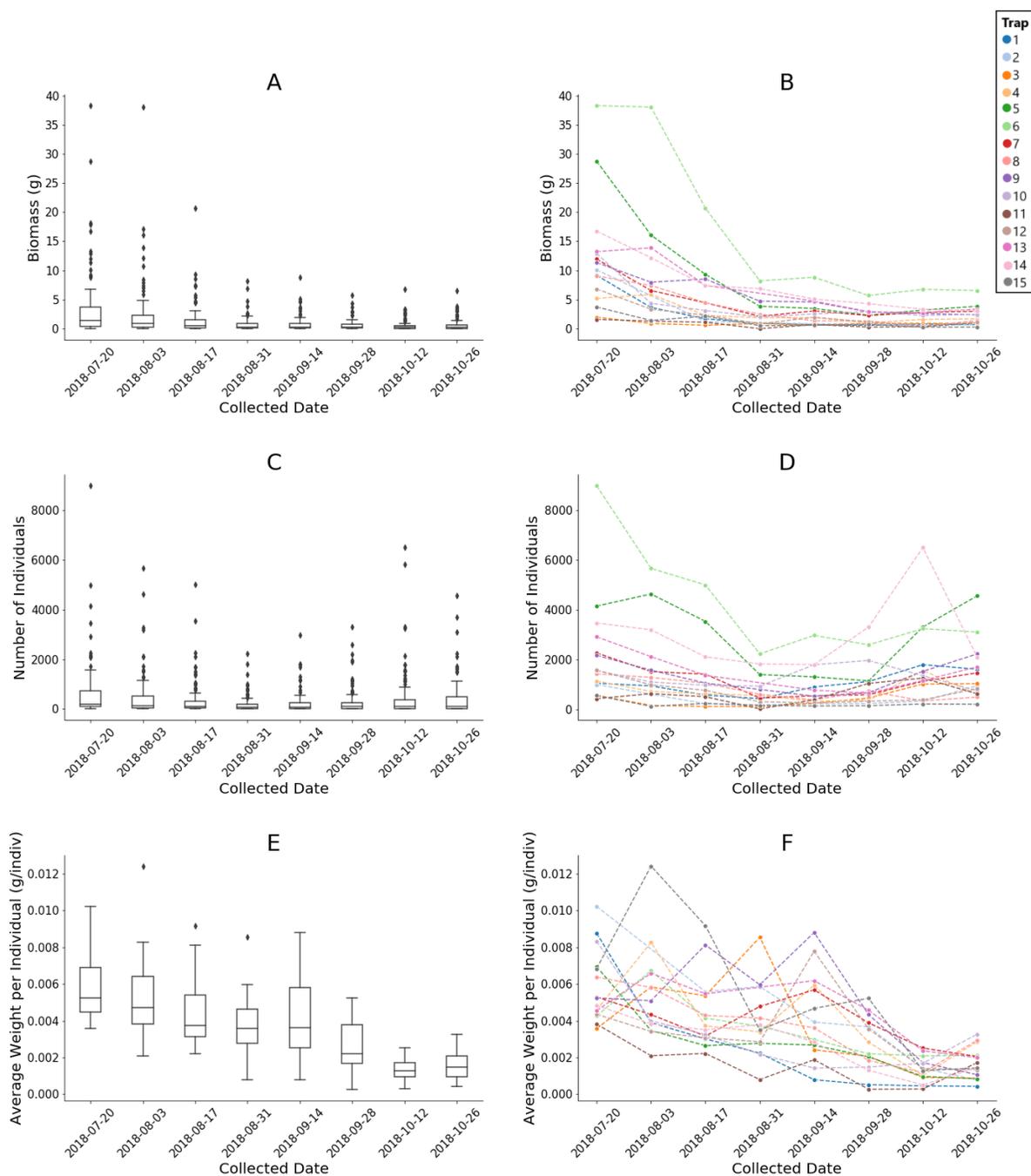


Figure 2. The biomass (g) (A, B), number of arthropod individuals (C, D), and average weight per individual (g/indiv) (E, F) that were captured in the Malaise traps ($n = 15$) in Metchosin, British Columbia, Canada, from July 6, 2018 to October 26, 2018. Collection times were every two weeks. (B, D, F) illustrate the variation for every trap for each of the parameters, with each different color representing a different trap. (A, C, E) is a box and whisker plot for each of the Malaise traps, illustrating the overall trends of the three parameters. A one-way ANOVA showed that at least one trap differs for biomass (g) ($F = 5.285$, $p < 0.001$), number of individuals ($F = 12.859$, $p < 0.001$), but it did not differ for average weight per individual (g/indiv) ($F = 1.684$, $p = 0.0833$).

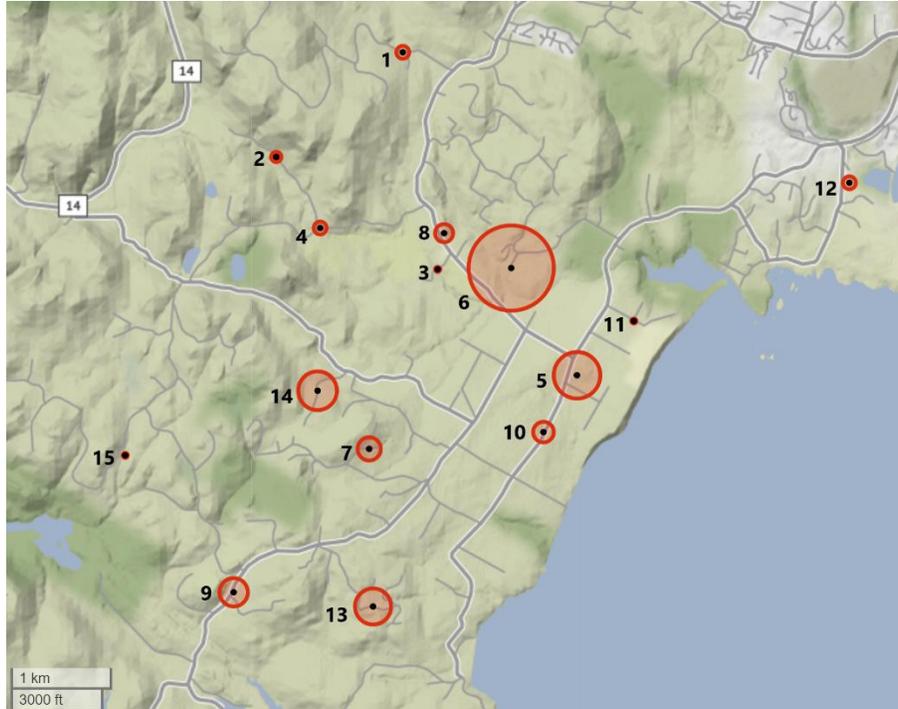


Figure 3. Each Malaise trap location ($n = 15$), in Metchosin, British Columbia, Canada, marked with the corresponding trap number next to it. The size of circle is the total biomass (g) for a site divided by number of collections. Site 6 is the largest, followed by few intermediate sized circles (5, 7, 9, 13, 14), and the smallest circles are sites 1, 2, 3, 4, 8, 10, 11, 12, 15.

Table 2. The Malaise trap (and site) number with corresponding total biomass (g) for each of the measured insect orders (Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, and Other arthropods) from July 6, 2018 to October 26, 2018. The Other arthropods includes other members of Arthropoda that were collected in the Malaise trap. All traps were in the municipality of Metchosin, British Columbia, Canada. Trap 2 had fallen over in the July 20, 2018 to August 3, 2018 collection period as well as trap 13 in the collection period of August 17, 2018 to August 31, 2018.

Trap Number	Total Biomass (g)					
	Hymenoptera	Diptera	Lepidoptera	Hemiptera	Coleoptera	Other
1	2.673	4.206	8.311	0.56	1.003	1.491
2	3.273	3.505	6.334	0.763	0.466	0.887
3	1.856	0.887	1.787	0.598	1.118	1.474
4	5.777	4.601	2.684	0.907	2.807	4.677
5	8.638	15.358	34.846	3.983	1.756	6.232
6	27.954	50.119	18.644	19.92	4.384	11.991
7	11.564	11.401	4.138	2.256	2.719	4.114
8	5.331	8.057	6.789	2.791	2.991	1.983
9	10.973	11.608	9.981	1.829	3.169	7.441
10	7.739	6.96	5.459	3.609	4.052	4.73
11	0.171	1.09	1.852	0.393	0.942	2.005
12	4.95	4.368	4.556	0.755	0.429	3.006
13	9.117	12.179	18.005	2.039	1.464	5.489
14	14.24	18.031	9.771	5.696	2.052	9.165
15	1.285	1.438	2.403	0.715	0.948	2.939
Total	115.51	153.808	135.56	46.814	30.3	67.624

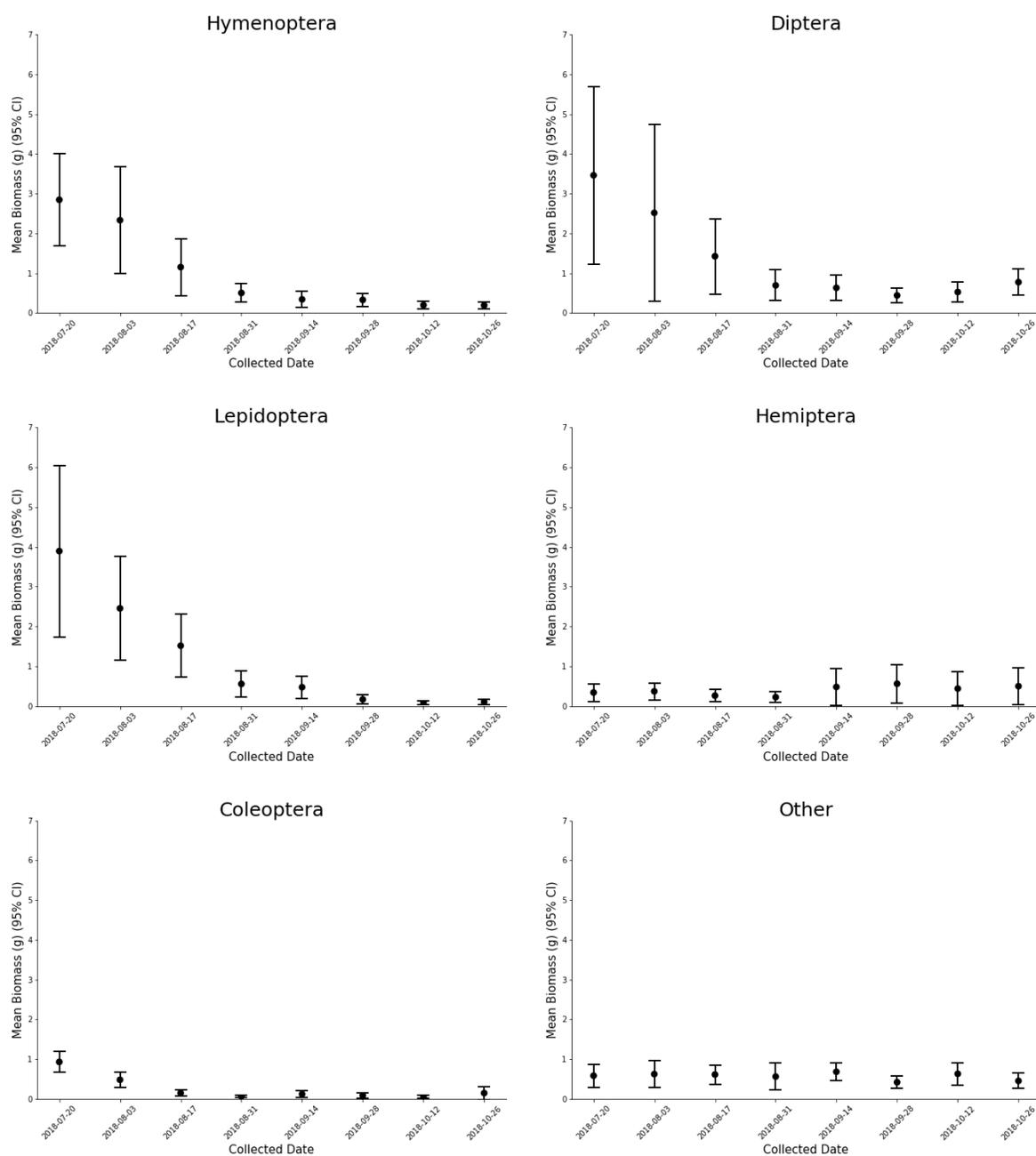


Figure 4. The mean biomass (g) with 95% confidence intervals for each of the major flying insect orders (Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, Other arthropods). The means were calculated from the Malaise traps ($n = 15$) in Metchosin, British Columbia, Canada, from July 6, 2018 to October 26, 2018 with collection times every two weeks.

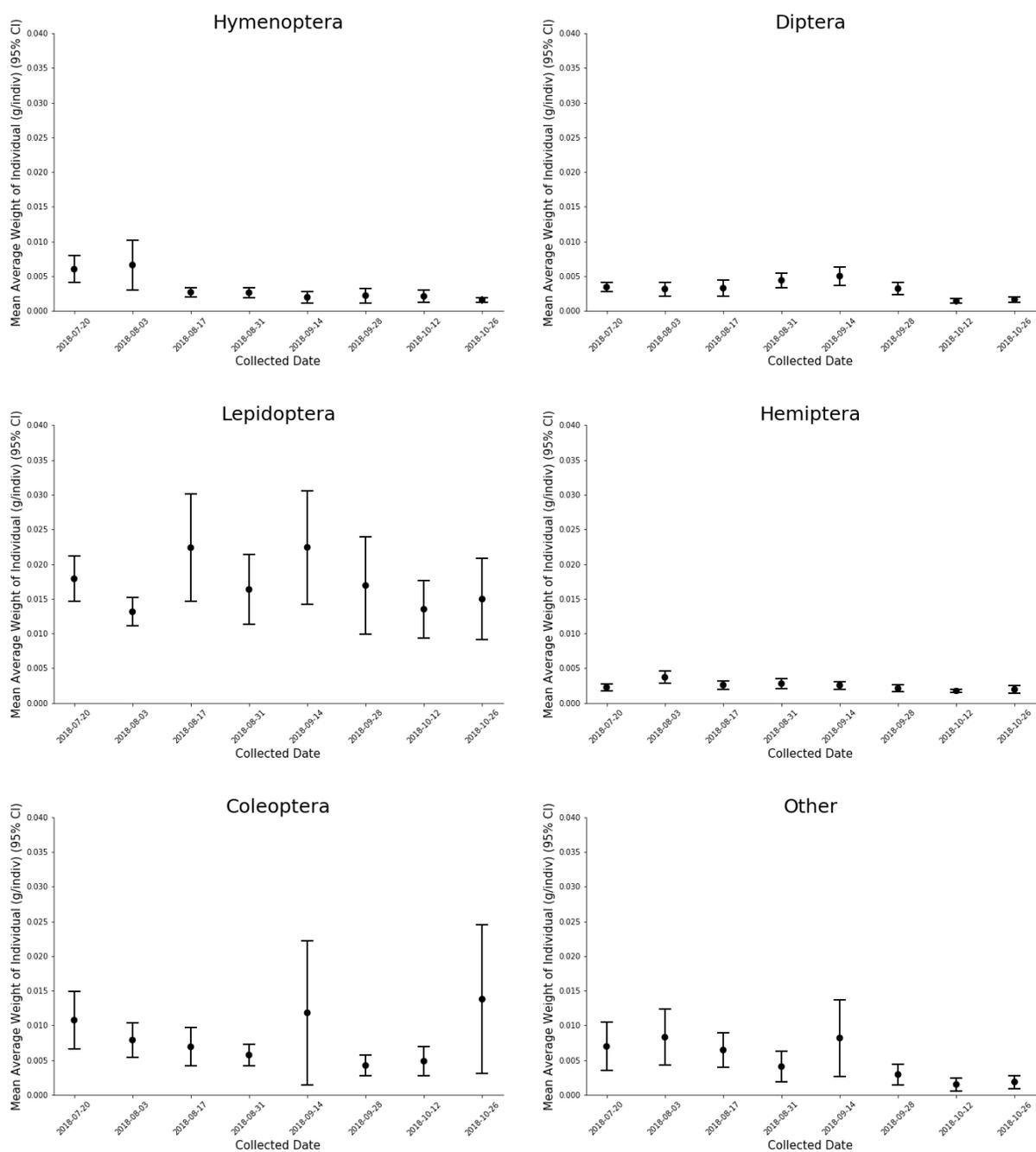


Figure 5. The mean average weight of individual (g/indiv) with 95% confidence intervals for each of the major flying insect orders (Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, Other arthropods). The means were calculated from the Malaise traps ($n = 15$) in Metchosin, British Columbia, Canada during July 20, 2018 to October 26, 2018 with collection times every two weeks.

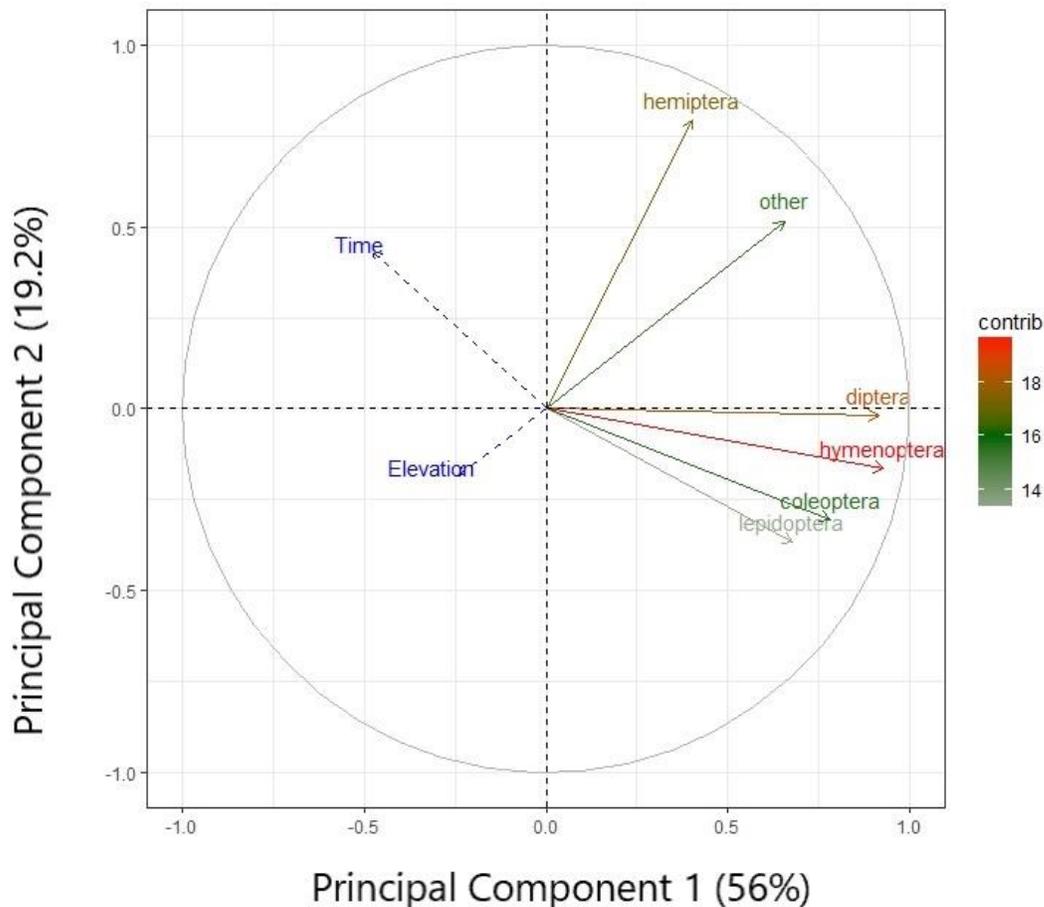


Figure 6. Principal component analysis (PCA) composition graph of the biomass (g) of the major flying insect orders (Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, and Other arthropods) for each site with time and elevation as explanatory variables. Trap 2 contents from July 20, 2018 to August 3, 2018 and trap 13 contents from August 17, 2018 to August 31, 2018 were excluded due to trap failure. The data was scaled to unit variance before running the PCA. The contribution to overall variance is indicated by the color scale; Hymenoptera has the highest contribution to site biomass variation followed by Diptera and Hemiptera. Lepidoptera has the least contribution to the site biomass variation. Time was run quantitatively rather than qualitatively and was correlated highly with insect biomass variation between sites compared to elevation. Principal component 1 and 2 were both high (56% and 19.2%) thus illustrating that the factors considered explain the spatiotemporal distribution variation of biomass between traps.

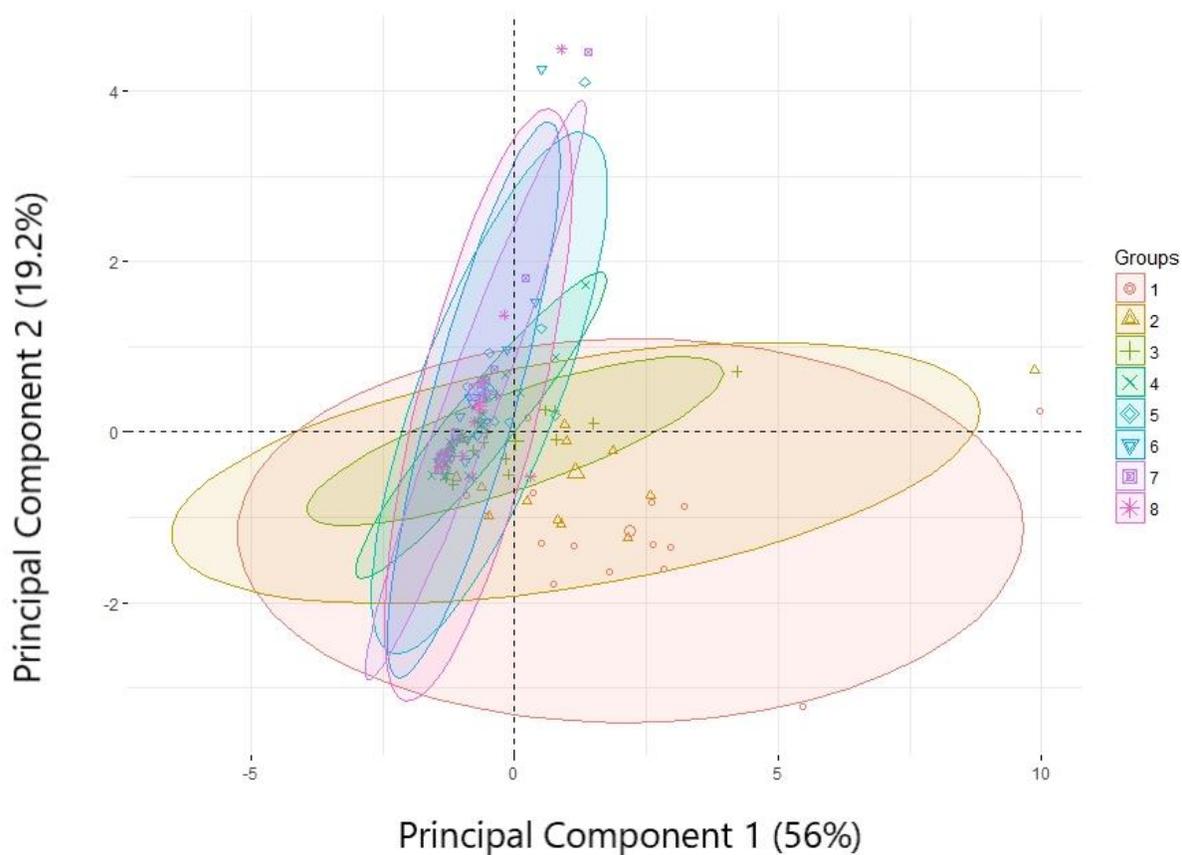


Figure 7. Principal component analysis (PCA) of the biomass (g) of the major flying insect orders (Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, and Other arthropods) for each site that are grouped by each time period ($n = 8$). Each group is two weeks, starting on July 6, 2018. The groups are 95% confidence ellipses. Trap 2 contents from July 20, 2018 to August 3, 2018 and trap 13 contents from August 17, 2018 to August 31, 2018 were excluded due to trap failure. The data was scaled to unit variance before running the PCA. Group 1 shows the most variation, and as time increases the variation between flying insect biomass for each site decreases.

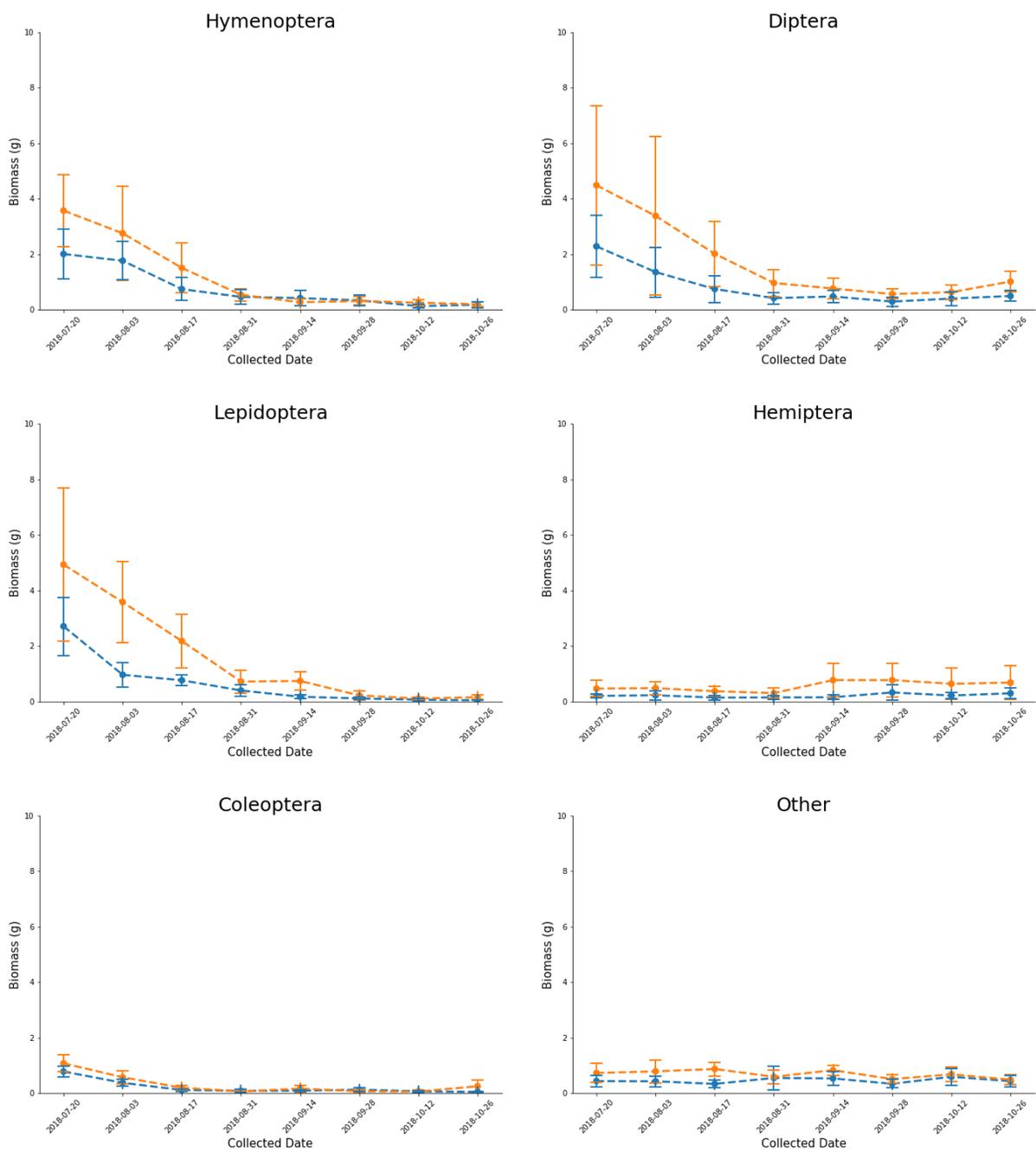


Figure 8. The mean biomass (g) with 95% confidence intervals for the two elevation types (low (0-100 m); high (100-200 m)) for each of the major flying insect orders (Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, Other arthropods). The high elevation sites are in blue and the low elevation sites are orange. The means were calculated from the Malaise traps ($n = 15$) in Metchosin, British Columbia, Canada, from July 6, 2018 to October 26, 2018 with collection times every two weeks.

Discussion

General spatial trends

I observed no apparent trends along a spatial gradient in the distribution of the biomass of major flying insect orders (*e.g.*, with distance from the coastline and/or the nearest urban city). The spatial distribution could be explained by exogenous factors, such as environmental heterogeneity, and endogenous factors, such as developmental traits, behaviour, and dispersal capabilities (Danks 1979; Vinatier et al. 2011; Yitbarek et al. 2011). Danks (1975), suggested that heterogenous distributions of insects are explained by three environmental factors: (1) local influences, (2) seral stages, and (3) microhabitat stratification. Each of the sites could show differences at any of these factors resulting in a heterogenous distribution of insect biomass. The sites with similar relative total biomass have comparable levels or combination of levels of the three environmental heterogeneity factors proposed by Danks (1975). Dole (2012), supports the importance of both exogenous and endogenous factors as an explanation of arthropod distribution since they analyzed these factors for invertebrate distributions in a Mediterranean climate. Dole (2012) found that rainfall, temperature, and elevation are correlated with the spatial distribution. Since flying insects do not typically fly in harsh conditions due to the energetic expense (*e.g.* in heavy rainfall and prevailing winds) (Abrol 2005), these factors may influence the spatial distribution of flying insects in Metchosin. Yitbarek et al. (2011), note that the mosaic ant patches, found in a forested site in Michigan, are driven more so by endogenous factors, such as the ability to forage in extreme temperatures. Although Yitbarek et al. (2011) does not discredit the influence of exogenous factors– they explain that topographical variation and light availability are also contributing factors to the spatial patchiness. Some of the factors that Yitbarek et al. (2011) considered could have influenced insect distribution in Metchosin but further sampling is needed to test the extent of the influence of exogenous and endogenous. It is difficult to fully evaluate the influence of endogenous factors in our experiment due to species specificity (Yitbarek et al. 2011). The heterogenous distribution of flying insects seen in Metchosin may be due to a combination of exogenous factors, more specifically microclimatic and microhabitat, and endogenous factors.

Elevation as an exogenous factor showed small differences between high and low elevation sites; however, PCA results confirm elevation as an explanatory factor for biomass

variation between sites as well as subjective visual differences are seen. The greatest differences between high and low elevation sites are seen in Hymenoptera, Diptera, and Lepidoptera. Several factors could have attributed to this trend. (1) These orders are the greatest contributors to biomass and thus have greater variation due to individual differences. (2) The orders could be generally more sensitive to factors involved with increasing altitude. (3) Or the orders could also be better adapted to environments associated with lower altitudes. For example Régnière and Nealis (2002), found that the probability of persistence of an invasive moth in British Columbia, *Lymantria dispar*, decreases exponentially with increasing elevation. Other studies have shown that biodiversity and species richness are typically low at high elevations due to more extreme conditions (*e.g.* high temperatures and wind exposure) (Theurillat et al. 2003; Kluge et al. 2006; Khaliq et al. 2014). Additionally Dennis et al. (1998), found that a greater plant diversity has an asymptotic relationship with insect diversity. Perhaps in Metchosin, if plant biodiversity decreases with increasing elevation so would insect biomass. Elevation is not the only exogenous factor influencing flying insect biomass trends but could be used to predict biomass trends due to the associated abiotic factors, such as wind and sun exposure (Khaliq et al. 2014).

Site 6 was identified as a biomass hotspot compared to the other 14 sites. Therefore, the microclimate and microhabitat of this area should be further investigated to understand what makes this site a biomass hot spot. It is likely that site 6 had the greatest biomass due to the greatest environmental heterogeneity. More environmental heterogeneity provides insects with more niche spaces to occupy and thus increase their biomass. It was noted that many other sites had their habitat fragmented by paving near the area, and the biomass was consequently low.

General temporal trends

My results support the prediction that the biomass of insects will peak in the middle of the growing season, which is similar to Hallmann et al. (2017) where they found the temporal distribution, from April to November. They found that the peak insect biomass was also in the mid-summer (July), in a similar climate environment to Metchosin, and they further discussed how biomass was positively related with temperature and negatively with precipitation (Hallmann et al. 2017). Time was strongly correlated with biomass variation between sites due to the environmental factors associated with different months, such as temperature and precipitation. In the middle of the growing season, there are essential resources that are available,

although not all insects will emerge at the same time and develop at the same rate (Tauber and Tauber 1981; Hallmann et al. 2017). Which could account for the high amount of biomass variation seen between sites at earlier time periods. This seasonal pattern of flying insects is further supported by Forrest and Thomson (2011), who experimentally demonstrated that temperature is an important factor for the timing of emergence. The similar emergence and growth seen in Hymenoptera, Diptera, and Lepidoptera could be due to an increasing temperature cue. Although, Khaliq et al. (2014) illustrates how extreme temperatures and fluctuating humidity can influence certain insects more than others with regards to ovulation, rate of fecundity, development, and survival. Since all insects are poikilotherms, temperature extremes greatly influence their life-history strategies. In temperate areas, like Metchosin, the summer months are the most extreme heat temperatures in the year. Thus, insects that were found to peak in the middle of the summer could have adapted for environmental preference or to withstand high temperatures. The high insect biomass found in the summer illustrates that the summer is advantageous for growth and development.

Ideally, Malaise traps would have been set out earlier in the summer. Therefore, the biomass could have peaked earlier. Although when comparing results to Hallmann et al. (2017), the peak biomass is unlikely to be significantly different. Biomass decreased the quickest of the three parameters with respect to time, which could be due to the change of season at the end of August. The PCA further indicated that time is highly correlated with biomass variation between sites and time as a factor encompasses the different seasonal environmental cues. These different environmental cues produced when the season changes, such as less light and colder temperatures, can cause a developmental change in the insects which results in the biomass to suddenly drop (Danks 1979; Wolda 1980; Tauber and Tauber 1981). The secondary peak in the number of insects at the end of the season could be explained by the high number of Collembola and Hemiptera. The Collembola and Hemiptera that were caught were small in size and thus did not contribute much to the overall biomass trend, yet their numbers increased exponentially near the end of the collection period. Therefore, Collembola may have a different life-history strategy than Diptera, Hymenoptera, and Lepidoptera. Collembola were particularly surprising to catch in the Malaise traps since the traps are designed to catch flying insects. When biomass and number of individuals are both considered, there are minimal outliers; however, there was no difference of average weight per individual found between the traps over the season. This indicates that in

all sites across Metchosin, the average weight per individual is decreasing at a similar rate. Given that there is a similar rate of decline between all sites and whether the microhabitat differs for each site, then the overall climate factors are causing the decline rather than the different microhabitat factors within Metchosin.

Diptera, Hymenoptera, and Lepidoptera seasonality

Diptera, Hymenoptera, and Lepidoptera are the main flying insect orders driving the overall spatiotemporal biomass trends seen. Hymenoptera and Diptera also explain most of the biomass variation between sites while Lepidoptera explains the least amount of biomass variation between sites. Lepidoptera are the largest individuals of all six orders and could be driving the biomass trend based on size rather than number caught. The low biomass variation explained by Lepidoptera could be due to the low number of individuals captured in all traps. The few individuals captured of Lepidoptera could then be explained by the Malaise trap exclusion mesh (for Lepidoptera) near the catch head. The mesh was to exclude Lepidoptera for conservation reasons; some lepidopteran populations are already sensitive. In our traps, only moths were captured, and all butterflies were successfully excluded. Moreover, many moths are attracted to artificial light (van Langevelde et al. 2011). Thus, traps that have artificial light nearby, especially at night, are more susceptible to catching certain Lepidoptera. However, many of the moths captured were noted as grass moths (*Pyralidae*) and thus the amount of grass would affect the number caught. The Diptera and Hymenoptera collected in the Malaise traps tended to be very small individuals compared to Lepidoptera, yet they are still main contributors to the overall seasonal biomass and explain the most biomass variation between sites. It was hypothesized that if there is a decline in insects then there are a greater number of smaller generalist insects occupying the now vacant niches from the declined insects (Sánchez-bayo and Wyckhuys 2019). Many dipteran and hymenopteran are generalists, but many dipteran and hymenopteran are also specialists, more specifically parasitic specialists (Cox 1994; Stireman III 2002). Our results found that there was a blend of generalists and specialists in Hymenoptera and Diptera. Some of the specialists within Hymenoptera include *Ichneumonidae* and micro-hymenopteran parasitoid wasps. Although, *Ichneumonidae* can also be generalists depending on the species. Within Diptera, *Tachinidae* were some of the common specialists found. Many of the generalists found in Diptera were *Muscidae*. Although, insects can have part of their life histories be specialized and others generalized. Therefore, determining whether or not insect

populations are shifting to be more generalist proves to be a more complex process. Future studies should monitor the relative amount of generalists and specialists as well as consider the life histories to determine whether insect populations are shifting.

It was hypothesized that the maximum biomass of each major order would be slightly staggered in the mid-summer to minimize competition between taxa in and as a result of the many different life-history strategies. However, the seasonal biomass between Diptera, Hymenoptera, and Lepidoptera does not illustrate staggering peak biomass in the mid-summer, as they all peak at the initial measurement on July 20th, 2018. Since food availability is essential for growth and survival at a young developmental age (Agnew et al. 2002); these three orders can have their maximum biomass at the same time if they are not utilizing the same nutritional resources, thus avoiding competition. If many of the species within Diptera and Hymenoptera are generalists, they would be more adapted to exploiting multiple resources based on resource availability for optimal growth and survival. The specialized parasitic dipterans and hymenopterans would have a strong relationship with a host and would result in little competition for that host. Lepidopterans have somewhat of a flexible diet composed of liquid (nectar, rotting fruits and vegetables, feces) which has no shortage. Therefore, all three orders can co-exist with their maximum abundance occurring at the same time during the growing season because they are not competing for similar nutritional resources that are necessary for growth and survival. Life-history strategies can differ between and within taxa, but our results show that even if there are differences in life-history strategies there is still a maximum biomass found in the middle of the summer. Although, there could be some very slight staggering of peak biomass since the biomass for each of the traps is highly variable in the earlier time periods compared to the end of the season. These results suggest that it must be advantageous to have a high abundance and growth during the middle of the summer (July). Chippendale (1982) further illustrates that insects that undergo diapause will synchronize their cycles so that they emerge with their food supply and favorable conditions, which could be occurring in Metchosin.

Coleoptera, Hemiptera, and Other arthropod seasonality

Coleoptera, Hemiptera, and the Other arthropods captured in the Malaise traps did not significantly contribute to the overall seasonal biomass trend due to their small size and low abundance. Wind can influence smaller sized insects flying ability compared to larger ones (Peng

et al. 1992). Although, this was ruled out as a factor for the low abundance of these orders because both dipterans and hymenopterans were also small individuals but were still high in biomass.

The Coleoptera captured were more diverse in size over the collection period, compared to Hemiptera species captured, where most of the species were same sized individuals within Hemiptera. Although, Hemiptera explained more of the biomass variation between sites compared to Coleoptera and Other arthropods and thus some sites are more characteristic of Hemiptera than others. The size diversity and low biomass seen in Coleoptera could be a result of employing Malaise traps rather than traps specifically used for capturing Coleoptera, such as multiple funnel traps or pitfall traps (Greenslade 1964; Lindgren 1983). Therefore, there could be more Coleoptera than estimated in Metchosin, since Malaise traps were not the most efficient trap for capturing Coleoptera. Similarly, Other arthropods were also diverse in size, over the collection period, which could have resulted from a combination of large Araneae, intermediate-sized Dermaptera, and an abundance of small Collembola, that were noted to be captured. The overall low contribution of Other arthropods to biomass further supports that the selected orders (Hymenoptera, Diptera, Lepidoptera, Coleoptera, Hemiptera) are the main flying insects captured in Malaise traps in Metchosin.

Evaluation of methodology

There are two main protocols for measuring biomass with each having their own benefits and drawbacks. The two protocols are measuring the wet biomass or the dry biomass. In our experiment, wet biomass was determined. The main benefit of wet biomass is that, by retaining the insects in the 75% ethanol solution, the samples are preserved for future research and taxonomic identification. When measuring dry biomass, as seen in Hallmann et al. (2017), the samples cannot be preserved for future research. Although a benefit of dry biomass would be time efficiency and possibly more resolving power of the biomass results. Theoretically for wet biomass, the if the 75% ethanol solution is dripping at the same rate for all measured samples there should be the same relative amount of ethanol solution in each sample. Although, Sefiane et al. (2003) demonstrates how the higher the ethanol concentration is, the faster it evaporates. In addition, whether insects have a soft or hard exterior cuticle composition could influence the amount of solution taken up (Fraenkel and Rudall 1947). Although, through observations, the

wet biomass did not fluctuate when placed on the scale and the evaporation of ethanol was likely not an issue. Therefore, dry biomass may not have better resolving power for determining biomass compared to wet biomass. The benefits and drawbacks of each protocol should be considered when determining a research project with insect biomass.

Citizen scientists were a key component of the field collection procedure for this project. The implementation of citizen scientists presents a myriad of benefits for large-scale ecology studies, but it also presents new challenges for research protocols. Bonney et al. (2009) outlines possible procedures for implementing citizen scientists effectively as well as the benefits with regards to biodiversity monitoring, biological research, and science education. One of the potential problems mentioned is the ability to recruit participants (Bonney et al. 2009). Due to the amount of enthusiasm of Metchosin residents, this was never an issue for our project. This enthusiasm for wanting to understand local biodiversity highlights the need for biodiversity data at a local scale. The main challenges that arose, including what the citizens can expect as a participant and what is expected of them, were easily overcome through effective communication.

Future directions

The purpose of this study is to establish a spatial and temporal baseline of the major flying insects (Diptera, Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, and Other arthropods) in Metchosin, British Columbia, Canada. An extension of this study would involve repeating the sampling techniques and trap placements used to monitor the biodiversity over time to determine if there is a decline and where it is most significant, both spatially and temporally. Additionally, other exogenous and endogenous factors, such as climate data and the development stage the insects are in at time of capture, should be measured. Knowledge regarding the spatiotemporal distribution of insects provides further understanding to the functionality of insects in key ecological processes.

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Appendix



Figure 9. The ez-Malaise Trap (BT1002) used in our study, overall height 180 cm by 165 cm. The Malaise traps used are non-attractant and passively intercept the insects' flight path. All traps were set up facing the same direction, with the catch head bottle aligned to the north. The traps were secured tightly to the ground, to withstand heavy wind and to ensure that maximum surface area was used to catch insects.