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The Relative Abundance and Diversity of Parasitoids of the Browntail Moth (Euproctis chrysorrhoea L.) and Factors that Influence Their Population Dynamics

Karla S. Boyd
University of Maine, karla.boyd@maine.edu

THE RELATIVE ABUNDANCE AND DIVERSITY OF PARASITOIDS OF THE BROWNTAIL MOTH (*EUPROCTIS CHRYSORRHOEA* L.) AND FACTORS THAT INFLUENCE THEIR POPULATION DYNAMICS

By

Karla Stryker Boyd

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Advisory Committee:

Eleanor Groden, Professor Emerita of Entomology, School of Biology and Ecology, Advisor

Francis Drummond, Professor Emeritus of Insect Ecology and Insect Pest Management

Charlene Donahue, Forest Entomologist, Emerita, Maine Forest Service

William Livingston, Associate Professor of Forest Resources, School of Forest Resources

Seanna Annis, Associate Professor of Mycology, School of Biology and Ecology

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Thesis Advisor: Dr. Eleanor Groden

An Abstract of the Thesis Presented
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The browntail moth (*Euproctis chrysorrhea*) is an invasive forest pest that has been present in the Northeast since it was first introduced from Europe in 1897. Originally, its range expanded very rapidly until it reached its peak invasion area of 150,000 km^2, which included most of New England and parts of Southern Canada and Long Island, NY, in 1915. After this point, its range retracted until only relic populations remained on islands in the Casco Bay Region of Maine and outer Cape Cod in Massachusetts. In 2016, a population outbreak occurred that expanded its range into inland Central Maine and appears to be continuing to expand north and east today. Our research aims to assess the relative abundance and diversity of parasitoid natural enemies present within the browntail moth population and the factors that influence browntail moth population dynamics over this outbreak period.

We sampled browntail moth in infested areas across mid-coast and central areas of Maine to assess overwintering survival, larval and pupal density, healthy moth emergence, and

parasitoid diversity and abundance. We estimated densities using timed 10-minute density counts, while survival and emergence was estimated by laboratory rearing of samples. Statistical models were constructed to determine important factors for both hosts and parasitoids, where year, distance to coast, age of infestation, habitat, and annual climate variables were tested.

Nine parasitoid species were recovered from browntail moth pupation nests, three of which were hyperparasitoids. The highest parasitism rate occurred from *Townsendiellomyia nidicola*, a primary parasitoid accounting for 24 percent and *Monodontomerus aerus*, a hyperparasitoid accounting for 36 percent parasitism across all years. Between 2016 and 2018, hyperparasitoids increased in percent parasitism while primary percent parasitism decreased. Negative binomial generalized models indicated that habitat, year, and total annual precipitation were the most significant factors determining the abundance of parasitoids, distance to coast and age of the infestation were not significant.

The mean number of pupation nests per 10-minute density count increased slightly in 2017 (28 ± 6) compared to 2016, but decreased in 2018 (20 ± 4). In comparison, the mean rank of Maine Department of Agriculture, Conservation and Forestry (MDACF) winter hibernacula per tree decreased across all years (2016 - 2018). Browntail moth emerged overwintered larvae, late-stage larvae, and pupation nests decreased at coastal sites in 2017 while inland sites increased in 2018. Moth survival, however, increased between 2017 and 2018. Negative binomial generalized models indicated that habitat was an important factor predicting estimates of overwintered post-diapausing larvae, late-stage larvae, and pupation nests while estimates of emerged overwintered larvae was the only significant factor predicting subsequent moth abundance. Abundance decreased in 2017 across all browntail moth life stages, likely due to an epizootic outbreak of the entomopathogenic fungus, *Entomophaga aulicae*.

This study presents analysis of data that indicates a high incidence of hyperparasitoids may negatively impact primary parasitism, which in turn can positively impact browntail moth survival. Both parasitoids and hosts were negatively impacted in 2017, due to the epizootic outbreak, another factor that may determine population dynamics. The data presented gives new insight into population dynamics of browntail moth and their parasitoids.

DEDICATION

I dedicate this thesis to my mother, Jane Louise (Snyder) Boyd (March 7th, 1957 - February 7th, 2020) whose love and encouragement gave me the platform to succeed throughout my life and career. She was excited for me to be pursuing a degree in something I was passionate about and reminded me of how proud she was as often as she could. As a two-time cancer survivor, she struggled with her own physical challenges while always keeping a smile on her face with a positive attitude, I was reminded to keep pushing forward with that same positivity.

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CHAPTER 1:

THE BIOLOGY, ECOLOGY, AND HISTORICAL SIGNIFICANCE OF THE BROWNTAIL MOTH (*EUPROCTIS CHRYSORRHOEA*)

Biology and Ecology

The browntail moth (*Euproctis chrysorrhoea* L., Lepidoptera: Erebidae) is an invasive forest pest in the northeastern United States that has a significant impact on forest and human health. Larvae have dark brown bodies with white spots running laterally along their dorsum and two bright red to orange Verson's glands on their 6th and 7th abdominal segments. Characteristic to their subfamily (Lymantriinae) they have dense setae covering their bodies, usually orange to brown in color. When they first hatch, larva can lack their characteristic orange spots, instead being entirely green to yellow in color.

Egg masses are laid with a protective layer of hairs that are produced from the anal tufts of the female, giving the appearance of a "brown felt lump" on the undersides of leaves. The eggs hatch after two to three weeks (Patch 1904). According to the life history of browntail moth as described by Shaefer (1974), larvae hatch in the summer usually between late-July to mid-August. Immediately after hatching, larvae begin to feed and construct "winter webs" or winter hibernacula that consist of whole or skeletonized leaves tightly wound together and coated with silk, providing the insulating structure for diapause. Nests are typically communal and constructed directly where egg masses are laid, at the terminal ends of the host branches in the upper canopy. During this time, larvae remain in the same area as their webs, feed gregariously, skeletonize leaves, and at high density, result in bronzing of foliage. In September – October,

nests are finalized and larvae molt to overwinter as 2nd and 3rd instars. Larvae emerge from these webs in late-April or early-May depending on temperature and food availability. Typically, the larvae are outside of the nest on warm sunny days, waiting for the buds to break as they can chew directly into bud tissue and begin feeding. Larvae continue to feed for eight to nine weeks, moving down the canopy of their host trees as they defoliate it.

Once mature, larvae will aggregate on remaining leaves in trees or search for new foliage and begin constructing communal pupation nests. Unlike winter webs, these are loosely woven leaf packets that can contain anywhere from 1 – 100 pupae. In cases of heavy infestation, larvae can spin these cocoons without leaves, often on man-made structures such as buildings or vehicles. Pupae require approximately two weeks to complete development, usually occurring from late-June to mid-July, before emerging as adult moths.

Adult browntail moths are white with characteristic "brown tails" that consist of brown to orange tufts of hair along the posterior portion of the abdomen. Adults are strong flyers, usually able to disperse long distances if picked up by strong winds (Patch 1904, Shaefer 1974). Once they have mated, adult females search for a suitable host for oviposition. This behavior is understudied, but there is evidence that females will seek host trees that have not been previously stressed by larval feeding (Schaefer 1974).

Browntail moth is a polyphagous species that prefers oak (*Quercus* spp.), apple (*Malus* spp.), cherry (*Prunus* spp.), pear (*Pyrus* spp.), and hawthorn (*Crataegus* spp.), but can also be found on a wide range of other deciduous trees and shrubs (Fernald and Kirkland 1903). Although they are highly polyphagous, the type and quality of host can have significant affects at a population level either through nutrition, plant resistance, or increased parasitism or predation (Schaefer 1974). Unlike most forest defoliators, they defoliate trees twice within one summer

growing season. This occurs once when they first emerge from their winter webs in April, and again in August after hatching as they are constructing their winter webs and preparing for diapause. Although this process can stress trees, it typically does not cause tree death unless several years of intense defoliation occurs on the same tree (MDACF 2020).

In addition to differences in quality of food, Schaefer (1974) suggests that the insulation properties of host trees vary and influence browntail moth overwintering mortality. He describes the tradeoff between food quality, insulative properties of hosts, and mortality suggesting that it is possible that larvae compensate for hosts that are high in nutrition but poor insulators by producing more insulative silk, incorporating more foliage, and feeding in greater amounts to prepare for diapause. He plotted web weight per larva by web volume per larva on hosts, and found *Quercus ilicifolia*, *Amelanchier arborea*, and *Quercus rubra* have the greatest amount of insulative material but failed to relate this with winter survival. Although this could greatly influence the ability for larvae to survive diapause, only Schaefer (1974) has really described winter web properties in depth, and there is no literature that describes the relationship between browntail moth survival and silk chemistry, host suitability, and the insulative properties of different tree species.

Throughout parts of its life cycle, browntail moth can pose a serious risk to human health and comfort due to their toxic urticating hairs. It has been disputed exactly when during their life cycle they produce the toxin, but sometime between their 2^{nd} to 4^{th} instar, they begin producing small, microscopic hollow setae on their 2^{nd} and 3^{rd} abdominal segments (Tyzzer 1907, Kephart 1914, Schaefer 1974). These setae are barbed and contain venom that is a mixture of esterlytic enzymes, proteases, and phospholipase A that are 50 – 100 times the concentration of similar species (Bleumink et al. 1982). When exposed to human skin the venom reacts with epithelial

cells and causes a histamine reaction which usually develops into a rash (Blair 1979). Setae can be actively or passively dislodged from the caterpillars, shed exuvia, cocoons, pupal nests, or winter webs and become airborne. Airborne setae that are inhaled can enter the bronchioles and cause respiratory distress and in severe cases anaphylaxis (Blair 1979, Diaz 2005).

In their native range, browntail moth are also considered a pest and have periodic outbreaks. This pest occurs in Europe with a range that extends as far north as Great Britain and south to Spain on its western edge, through Finland and into the Ural Mountains in Russia and south through parts of Northern Africa and into Bulgaria into Turkey and Iran in the east (Sterling and Speight 1989, EPPO 2020) (Fig 1). Similar to gypsy moth in North America, browntail outbreaks in Europe occur rapidly and for several years then decline to a point where they vanish (Sterling and Speight 1989). Although they are considered a pest, their populations are not regularly monitored in many of these areas.

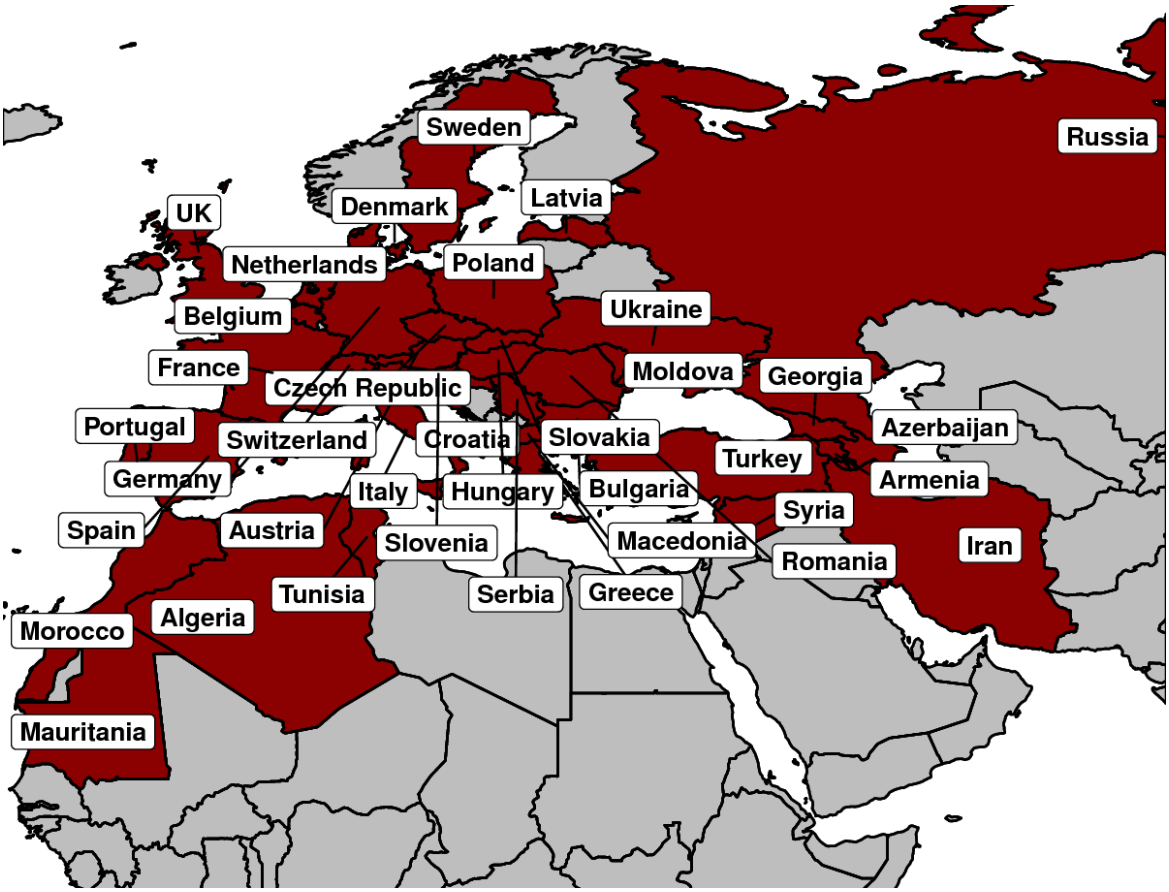

Figure 1. Countries with reports of browntail moth populations, red = positive for browntail moth. It is unclear if all this area encompasses the native range of this insect or if there are introduced populations in some of these areas. Reports of BTM in Albania, Japan, South Korea, and Norway were invalid and not included (EPPO Globral Database 2020).

The focus for research in their native range includes work on genetic differences in populations, developmental assays, and natural enemy assessments. There is evidence that genetically distinct populations exist in Siberia, France, UK, US, and Romania, with low genetic diversity in the recently split US population (Marques et al 2014). The populations selected for inclusion in this study were influenced by populations in Spain that displayed different phylogenies as they fed on non-deciduous hosts (Frago et al 2010). Frago et al (2009) found that larva have between six and eight larval instars, with an average of six instars under optimal laboratory conditions. They also found that age at maturity and size at maturity influenced female fecundity and reproductive output. Numerous assessments of natural enemies have also

been published, which will be discussed in the "Introduction and Assessments of Natural Enemies" section of this chapter.

Historical Invasion

The browntail moth (BTM) introduction in North America was first documented in Somerville and Cambridge, Massachusetts from Europe in 1897 (Fernald and Kirkland 1903). These initial few individuals were likely from ornamental nursery stocks originating from Holland, France, and Belgium (Merlatt 1911). Once established, the browntail moth populations increased rapidly spreading throughout northeastern U.S. and Canada (Table 1).

Table 1. Distribution estimates for BTM based on area defoliated, more recent estimates (1999, 2015, 2016, and 2017) do not include the small area outside of Maine in Cape Cod, Massachusetts. Initial estimates recorded in kilometers (km^2).

Year	Area (Ha)	Source(s) of Estimate
1896	7,500	Fernald and Kirkland 1903
1897	40,900	Ibid
1898	115,000	Ibid
1899	375,000	Marlatt 1911
1902	388,500	Fernald and Kirkland 1903
1905	2,173,600	Marlatt 1911
1909	5,691,700	Ibid
1914	13,062,600	Burgess 1923, Tothill 1916
1915	14,727,800	Tothill 1916
1922	4,110,300	Burgess 1923, Tothill 1916
1938	5,577,700	Metcalf and Flint 1939
1966	1,300	Schneider 1966, Pratt 1972, Schaefer 1974
1969	1,300	USDA, ARS 1969
1973	500	Schaefer 1974
1999	2,072	DCAF, MFS 1999
2015	5,281	DCAF, MFS 2015
2016	25,856	DCAF, MFS 2016
2017	22,180	DCAF, MFS 2017
2018	50,990	DCAF, MFS 2018

Although this insect was first formally identified in the United States in 1897, accounts of the presence of caterpillars can be traced back to property owners who noticed heavy defoliation of pear trees in 1892 with an estimated initial infestation of around 1890 (Fernald and Kirkland 1903). This could be because this insect can be difficult to detect at low densities. It has been suggested that the rapid spread of the moths between 1900 – 1915 could be attributed to a combination of prevailing southwesterly winds carrying females, the shipment of household goods, and railroad cars and tramp schooners carrying pupating caterpillars (Harvey 1901, Fernald and Kirkland 1903, Hitchings 1910, Matheson 1913, Schaefer 1974). In Maine, the moths were first reported on cherry in Kittery, likely attracted to strong electric lights at the Portsmouth Naval Shipyard (Patch 1904). Patch also described the incidence of moth webs and caterpillars along lighted areas, parking lots, ports, and train stations in the years following the Kittery infestation.

At their maximum distribution in 1915, browntail moth occupied around 150,000 km^2 (15,000,000 Ha) of the northeastern United States and southeastern Canada (Schaefer 1974). A combination of efforts by towns, states, and the federal government were employed to mitigate the foliar destruction and public health hazards that the moths imposed. This included destroying heavily infested host trees, cutting winter webs from trees and burning them, imposing a bounty of $0.05 per dozen to incentivize the removal of winter webs, a federal quarantine enacted in 1896, spraying arsenical insecticides in the early spring, and an effort to introduce natural enemies collected from their native range overseas (Patch 1904, Burgess and Crossman 1929, Schaefer 1974). Although there was much interest and research at the time, there is little information on the efficacy of any of these control measures and if they contributed to the subsequent collapse of the population after the initial 1915 peak distribution.

Introduction and Assessments of Natural Enemies

Throughout the early infestation period, managers from the U.S. Department of Agriculture started importing natural enemies to combat the problems associated with three introduced caterpillar pests including the browntail moth, gypsy moth (*Lymantria dispar*: L, Erebidae), and the satin moth (*Leucoma salicis*: L, Erebidae) that were rapidly defoliating forests and threatening economically viable trees (Schaefer 1974). In a technical bulletin from Burgess and Crossman (1929), a detailed account of the preparation and release of these natural enemies was reported. Over the course of this effort, 93,084,679 individual natural enemies distributed over 47 parasitoids species were released. Of these, 15 were noted as becoming established and only seven are considered of importance as enemies of browntail moth (Clausen 1956) (Table 2).

Table 2. Table adapted from Burgess and Crossman (1929) of the "positively established" natural enemies of browntail moth that are of the most importance, those bolded are the "most effective" (Clausen 1956).

Species	Family	Total number of individuals released in US by 1929
Trichomalopsis hemiptera Walk.	Ptermalidae	530,000
Apanteles laticolor Berk.*	**Braconidae**	**310,245**
Compsilura concinnata Meig.*	Tachinidae	147,759
Monodontomerus aereus Walk.*	Torymidae	15,541
Meteorus versicolor Wes.*	Braconidae	11,000
Carcelia laxifrons Ville.*	Tachinidae	9,742
Townsendiellmyia nidicola Twns.*	**Tachinidae**	**3,500**

*Reported in Maine and/or Massachusetts by Schaefer (1974).

Since this initial report, there has been one other assessment of the browntail moth natural enemies in Maine. Schaefer (1974) found all but one of the seven species reported by Clausen (1956), and an additional five species, *Trichogramma minutum, Eusisyropa blanda, Horismenus fraternus, Brachymeria compsilurae, Exorista* spp., plus several undetermined dipterans at various life stages (Table 2). His comparison between Massachusetts and Maine

populations, when browntail moth was restricted to islands and coastal areas, showed differences in the two regions' parasitoid species assemblages. An assessment of natural enemies in the Massachusetts population was conducted by Elkinton et al. (2006). They found that *C. concinnata* contributed the most to browntail moth mortality at inland sites where they artificially seeded browntail moth caterpillars. This led to the authors implicating the generalist parasitoid in the original collapse of the browntail moth population in the 1920's and 1930's. Based on current population expansion of browntail moth in the mid-coast, inland, and southern regions of Maine and lack of reports outside of Cape Cod, Massachusetts, it is likely that the current expansion originated from the Casco Bay area.

In the native range of browntail moth, there has been a wide range of literature exploring the natural enemies of this forest pest. Frago et al. (2012) described the parasitoid complex of 17 primary parasitoids and 10 hyperparasitoids for the Spain population of browntail moth that occurs on evergreen strawberry trees (*Arbutus unedo* L.). Turkey has an extensive report on the Tachinidae parasitoids of browntail moth which includes *Exorista larvarum* L., *Exorista rossica* Mesn., *Compsilura concinnata*, *Townsendiellomyia nidicola*, *Palesisa nudioculata* Ville., and *Tachinia praeceps* Zett. (Kara et al. 2016). New host records in Turkey found the parasitoid *Masicera sphingivora* Robi., a Tachinidae usually found on Sphingidae moths and *Telenomus euproctidis* Wilc., a Scelionidae egg parasitoid causing an average of 41 percent mortality of egg masses. (Kondur and Simsek 2016, Atay et al 2018).

In England, Sterling and Speight (1989) found a different complex of parasitoids than in Spain, with parasitism accounting for between 3.9 and 28.7 percent of overall mortality. However, they report that a microsporidian (spp. undertermined) was responsible for the highest amount of mortality amongst the identified agents. Several years later, Hyliš et al (2006)

described a microsporidian specific to browntail moth, *Nosema chrysorrhoeae* n. sp., in Bulgarian browntail moth populations, which could have been the undetermined species that caused the high mortality that Sterling and Speight (1989) described. Other investigations into Bulgarian populations of browntail moth showed that the microsporidian *Nosema* spp. and *Endoreticulatus* spp. had a wide prevalence throughout the region (Pilarska et al 2018).

Cory et al (2000) investigated the host range of EcNPV to discern the risk to non-targets as NPV has been considered for commercial use. They found that of the seventy-three species of Lepidoptera and two species of Hymenoptera tested, none were susceptible to EcNPV. Sterling and Speight (1989) found CPV and NPV in browntail populations in England, but concluded that they did not contribute to major mortality. Slavicek et al. (2004) investigated EcNPV in 2002 and 2003, by conducting field trials of applications of occlusion bodies (OBs) on both the spring and fall browntail moth larvae in Maine. Results showed 85% mortality of Spring larvae and 40% mortality in the Fall larvae, although there was some uncertainty due to cross-contamination with another browntail moth pathogen, *Entomophaga aulicae* Reich. In India, where browntail moth are also invasive, EcNPV has also been recently isolated and described as a potential control method in the absence of other natural enemies (Hussain et al. 2019).

Early researchers in Massachusetts also found the naturally occurring fungus, *E. aulicae,* which they refer to as the "brown-tail fungus" (Hitchings 1908, Speare and Colley 1912). This entomopathogen was first reported in Maine in 1902 (Patch 1904). Speare and Colley (1912) conducted artificial inoculations and found success in disseminating the fungus to infect caterpillars in heavily infested trees. Although this seemed promising, there is little information on what happened with these inoculations, and *E. aulicae* is often mentioned in the historical

literature but never explored further on browntail moth (Patch 1904, Burgess and Crossman 1929, Clausen 1956, Schaefer 1974, MDACF 2020).

Entomophaga aulicae was reported in Europe in 2000, infecting populations of browntail moth in Bulgaria (Pilarska et al 2018). An inoculate from these fungal outbreaks was released on healthy populations in the village of Zhenda in Bulgaria in 2016, which resulted 19 percent mortality from the fungi the following year (Annual Report of Forest Protection Station Plovdiv 2017). In 2015, new records of the fungus were found in Serbia (Tabaković-tošić et al. 2018), and Tabaković-tošić and Milosavljević (2017) observed that cadavers infected with both dipteran parasitoids and *E. aulicae,* fungal fragments were not found on internal tissues of Dipteran puparia and could not relate high parasitoid mortality to the fungus.

The fungus, *E. aulicae* has been studied in organisms other than browntail moth, although a composite list of all Lepidopteran hosts is yet to be created. Some of the major hosts that *E. aulicae* has been recorded on include eastern hemlock looper (*Lambdina fiscellaria* Guen.) and eastern spruce budworm (*Choristoneura fumiferana* Clem.) with at least 12 other hosts (McDonald and Nolan 1994, Hajek 1999). Several studies have examined the effectiveness of *E. aulicae* as biocontrol, optimal conditions for lab rearing, conidia development and spore discharge, and understanding its genetics and phylogeny (Hajek et al.1991, Nolan 1993, Hajek 1999, Lopez Lastra et al. 2001, Yamazaki et al. 2004, Choi et al. 2016). This species is thought to be a complex of cryptic species which include *Entomophaga maimaiga* and at least 3 other species groups all classified under *E. aulicae* which can only be distinguished through molecular and biochemical assays (Hajek 1999).

In 2016, browntail moth populations expanded back into parts of its historical range in Maine. Today, winter webs can be found as far north and east as Crawford, ME and as far south

as Scarborough, ME (DCAF, MFS 2019). With the movement of this pest, it brings the serious forest and human health hazards along with it. Although this invasive pest has been in North America for over 100 years, it remains understudied in terms of its current natural enemy assemblage, drivers of the population expansion, abiotic contributors to mortality, and the role that pathogens play in their population ecology.

CHAPTER 2:

ABIOTIC AND BIOTIC FACTORS INFLUENCING THE RELATIVE ABUNDANCE AND DIVERSITY OF BROWNTAIL MOTH PARASITOIDS ACROSS THREE YEARS IN MAINE

Abstract

The browntail moth (*Euproctis chrysorrhoea* L.) is an invasive forest pest that was accidentally introduced to Cambridge, MA in 1897 and caused widespread damage to forests in the early part of the 20th Century. During its peak range expansion in 1915 in the northeastern United States, this insect encompassed an area of 150,000 km^2. During this time, biological control efforts brought forth by the United States Department of Agriculture introduced 47 species of natural enemies to combat the browntail moth and gypsy moth (*Lymantria dispar* L.) invasive outbreaks. A population decline of browntail moth soon followed until only small relic populations existed on Cape Cod, Massachusetts, and in the Casco Bay Region in Maine with small outbreaks typically < 4,000 ha^2. Recently, an outbreak of browntail moth originating from the Casco Bay Region and encompassing > 50,000 ha^2 in parts of mid-coast and inland Maine became cause for concern due to both the public health and forest health issues associated with this invasive insect. This study investigates the abundance of natural enemies that were thought to previously control populations, and their distribution throughout the current outbreak areas.

The main parasitoids found in browntail populations during the present study were *Townsendiellomyia nidicola* Twns. and *Monodontomerus aerus* Walk. We found that the percent parasitism and proportions of hyperparasitoids attacking both browntail moth pupae and their primary parasitoids increased over time. Negative binomial generalized linear models indicated that there were no singular factors driving abundance of all parasitoids and distance to coast nor

age of the infestation were significant predictors of parasitoid abundance. Habitat features and annual climate variables may play significant roles in the abundance of Ichneumonidae. Abundance of all parasitoids differed significantly between years with the highest abundance in 2016 and decreasing abundance in 2017 and 2018, likely a result of an epizootic caused by the entomopathogen, *Entomophaga aulicae* E. Reich. Overall, our results indicated that hyperparasitism may be exacerbating the effects of the current outbreak by releasing browntail moth from their primary parasitoids. Parasitism in browntail moth does not seem to be regulating the outbreak, as populations with a high diversity of parasitoids continue to persist. Further research is needed to understand exactly what factors caused this rapid expansion of browntail moth into mainland parts of Maine and to what extent *E. aulicae* naturally regulates populations of this insect.

Introduction

Introduced woody plant and forest pests have been priority targets for classical biological control (Eilenberg et al. 2001). Biological control is often utilized in forestry situations where other types of human intervention are restricted or impractical. The use of pesticides and other cultural practices may be banned, and/or short-term solutions may not be economical for long-lived forest stands (Kenis et al 2016). Parasitoids are often selected from natural enemies in biological control of herbivorous insect pests because unlike other natural enemies, they can be easily studied and cultured under laboratory conditions, and many have considerable dispersal capabilities (Hassell 2001, Jervis 2007).

An extensive attempt at classical biological control using parasitoids and predators was implemented for browntail moth (*Euproctis chrysorrhoea* L, Erebidae, BTM) and gypsy moth (*Lymantria dispar*: L, Erebidae) at the turn of the twentieth century (Howard and Fiske 1911,

Burgess 1923, Burgess and Crossman 1929). Over 47 different species of parasitoids were introduced into North America from their native ranges in Europe and Asia over a course of seventeen years (Burgess and Crossman 1929) (Fig 2). Although extensive, the effort had varying levels of success with some of the introductions posing threats to native species even today (Elkinton and Boettner 2012).

The browntail moth was first recorded in North America in Somerville and Cambridge, Massachusetts in 1897 (Fernald and Kirkland 1903). The moth is native to Europe and can pose serious risks to forest stands and human health in both its introduced and native ranges, though the scale of their outbreaks in their native range may be more localized (Sterling and Speight 1989). Browntail moth is a highly polyphagous feeder of deciduous tree foliage and can defoliate the same trees twice within one year. In addition, the mid to large stage larvae are covered in venomous urticating hairs that cause a mild to severe rashes in dermally exposed people and can cause respiratory distress or anaphylaxis if inhaled (Blair 1979, Diaz 2005). The distribution of browntail moth in North America reached its maximum in 1915, when it occupied 150,000 km^2 (14,727,800 Ha) of the northeastern United States and southern Canada (Schaefer 1974). After this point, populations of browntail moth declined and eventually disappeared throughout most of the affected areas with populations remaining by the early 1970s on islands and coastal areas in the Casco Bay Region of Maine and in isolated pockets along Cape Cod, Massachusetts (Schaefer 1974). Before their resurgence, browntail moth populations were limited to this coastal region of Maine with occasional outbreaks of < 5,000 ha. The cause of this range decline is unknown as there is little data or documentation of population trends over this time. It has been suggested that the population recession was caused by a combination of winter web destruction,

natural enemies, insecticides, and climatic factors. (Schaefer 1974, Elkinton et al 2006, Frago et al 2011).

When browntail moth and gypsy moth were declared a public nuisance, the first annual report of the State Superintendent for Suppressing the Gypsy and Brown-tail Moths in Massachusetts, published in 1905, recommended natural enemies for the suppression of the gypsy and browntail moths, and both state and federal entomologists began work with the natural enemies of these insects (Kirkland 1906). The work was carried out at the Gypsy Moth Parasite Laboratory in Melrose Highlands, Massachusetts where various life stages of both insects were imported from Europe and reared for parasitoids (Howard and Fiske 1911). This work continued until Burgess and Crossman (1929) summarized the findings on natural enemies that were released throughout the infested areas of both browntail and gypsy moth (Fig 3). This early assessment of parasitism found fifteen positively established natural enemies of gypsy and browntail moth in North America, ten of which were considered of importance.

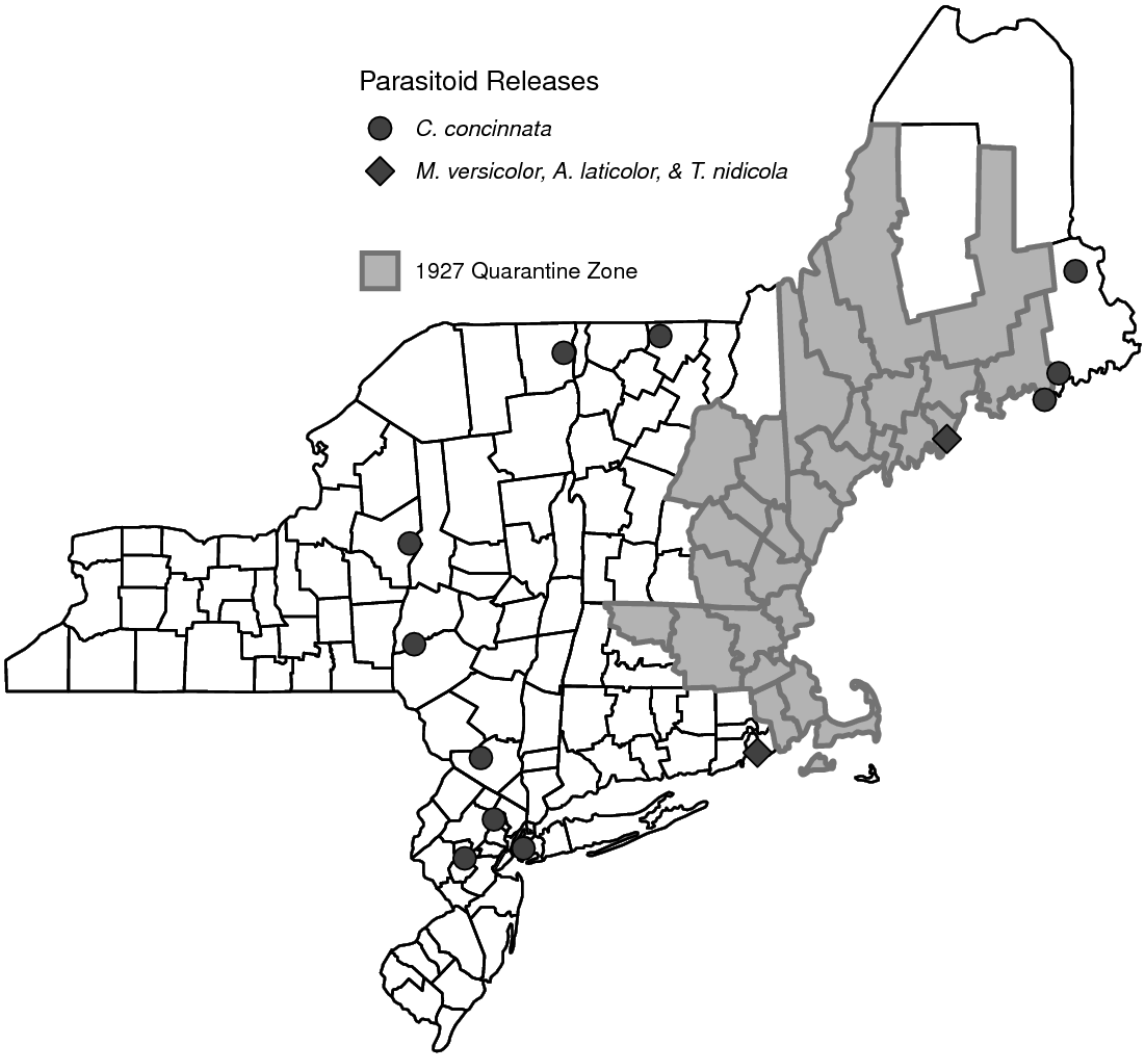

Figure 2. Dispersion of the introduced natural enemies of the browntail and gypsy moth (Data from Burgess and Crossman 1929 replotted).

After the collapse of the browntail moth population in the 1920's and 1930's, there was less demand for on-going parasitoid efficacy research. A multi-species assessment of effective parasitoids by Clausen (1956) described *Apanteles lacteicolor* Vier. and *Townsendiellomyia nidicola* Twns. as the two major parasitoids that caused between 10 to 30 percent mortality in browntail moth populations, based on reports before and after the outbreak (Howard and Fiske

1911, Burgess and Crossman 1929). Schaefer (1974) described the population dynamics of browntail moth in their isolated relic populations in Maine and Massachusetts and gave a detailed assessment of parasitism across different life stages from 1970 to 1973. He found seven parasitoids in total, four of which caused considerable mortality (Table 2).

During small outbreaks throughout the early 2000's, Elkinton et al (2006) assessed the Massachusetts browntail moth population for parasitism at natural and artificially inoculated coastal and inland sites. They found that the generalist parasitoid, *Compsilura concinnata* Meigen, had the greatest impact on artificial browntail moth infestations they established at inland sites, with almost no individuals found in the artificial and natural infestations at coastal sites. This was similar to what they observed for the incidence of tachinid *Carcelia laxifrons* Aubert and Ichneumonid parasitoids attacking browntail moths. Although these parasitoids were present at all sites, parasitoid densities were lower at the artificial and natural coastal sites. The authors concluded that coastal dune habitat with low plant density and diversity does not support a diversity of lepidopteran hosts needed by the multivoltine, generalist tachinid, *C. concinnata*, and that the lack of the suppressing pressure of this parasitoid could be the reason why small relic populations of browntail moth have been able to persist in coastal habitats in both Massachusetts and Maine. Elkinton et al (2008) also conducted studies on the influence of host and temperature on browntail moth larvae but found that these factors did not significantly change larval survival.

After the long period of browntail moth populations being limited to a few islands off the coast of Maine, outbreaks were observed in isolated pockets along mid-coast Maine between 1992 and 2014, with the largest outbreak impacting just under 4,047 ha. However, in 2015, browntail moth populations defoliated an estimated 5,281 ha, and over 25,856 ha by 2016 (Ch1,

Table 1). Over this time populations were detected as far north as Crawford, Maine, and as far south as Scarborough, Maine, with nine townships along the New Hampshire boarder on alert for the presence of browntail moth (Maine DACF 2018). Although there have been previous outbreaks of browntail moth in recent history, none have been as widely distributed throughout the state of Maine, nor have they occurred over a period greater than two years (Elkinton et al 2006).

This study describes the current diversity, abundance, and distribution of browntail moth parasitoids across Maine over three years (2016 through 2018) of the current outbreak. The primary focus was on the assessment of pupal parasitoids, but our study also reports on some parasitism in winter hibernacula and late-stage larvae. We hypothesize that parasitoid communities will be considerably different from those observed by Schaefer (1974) and Elkinton (2006) when browntail moth densities were low, and more aligned with those observed by Burgess and Crossman (1929) who analyzed parasitoid assemblages during an outbreak (Table 3). To address this hypothesis we pursued the following objectives: (1) assess the abundance, diversity, and impact of parasitoids across different browntail moth life stages, and (2) determine if there are differences in parasitoid species assemblages across geographically distributed sites, and whether abiotic and biotic factors contribute differentially to parasitism across the range of its outbreak in Maine.

Table 3. Summary of published assessments of parasitoid species attacking browntail moth. Table adapted from Schaefer (1974) to include more recent assessments of percent parasitism from Elkinton et al (2006). The abbreviation "NA" is used when percent parasitism for that species was not assessed or recovered.

Study	**Year**	***T. nidicola***	***C. conncinata***	***C. laxifrons***	***A. lacteicolor****	***M. versicolor****	***M. aerus****	**Total**
Burgess and Crossman	1927	13	14	4.1	5.2	2.1	NA	38.4
Schaefer	1974	11	3.1	0.3	4.1	0.2	4	22.7
Elkinton et al, inland	2000	13.5	40	18	NA	NA	NA	71.5
Elkinton et al, inland	2001	13.5	28	15	NA	NA	NA	56.5
Elkinton et al coastal	2000	13.5	5	40	NA	NA	NA	58.5
Elkinton et al coastal	2001	13.5	8	45	NA	NA	NA	66.5

*Hyperparasitoids

Methods

Samples were collected over a three-year period (2016 through 2018) at 21 - 40 sites per year across central and mid-coast Maine (Fig 3). New sites were added through time as new infested sites of browntail moth were discovered. The life stages sampled included diapausing larvae in winter hibernacula, feeding late-stage larvae post overwintering, and pupating larvae and pupae in individual and group pupation nests. All collected samples were reared in the laboratory and assessed for survival and parasitoids.

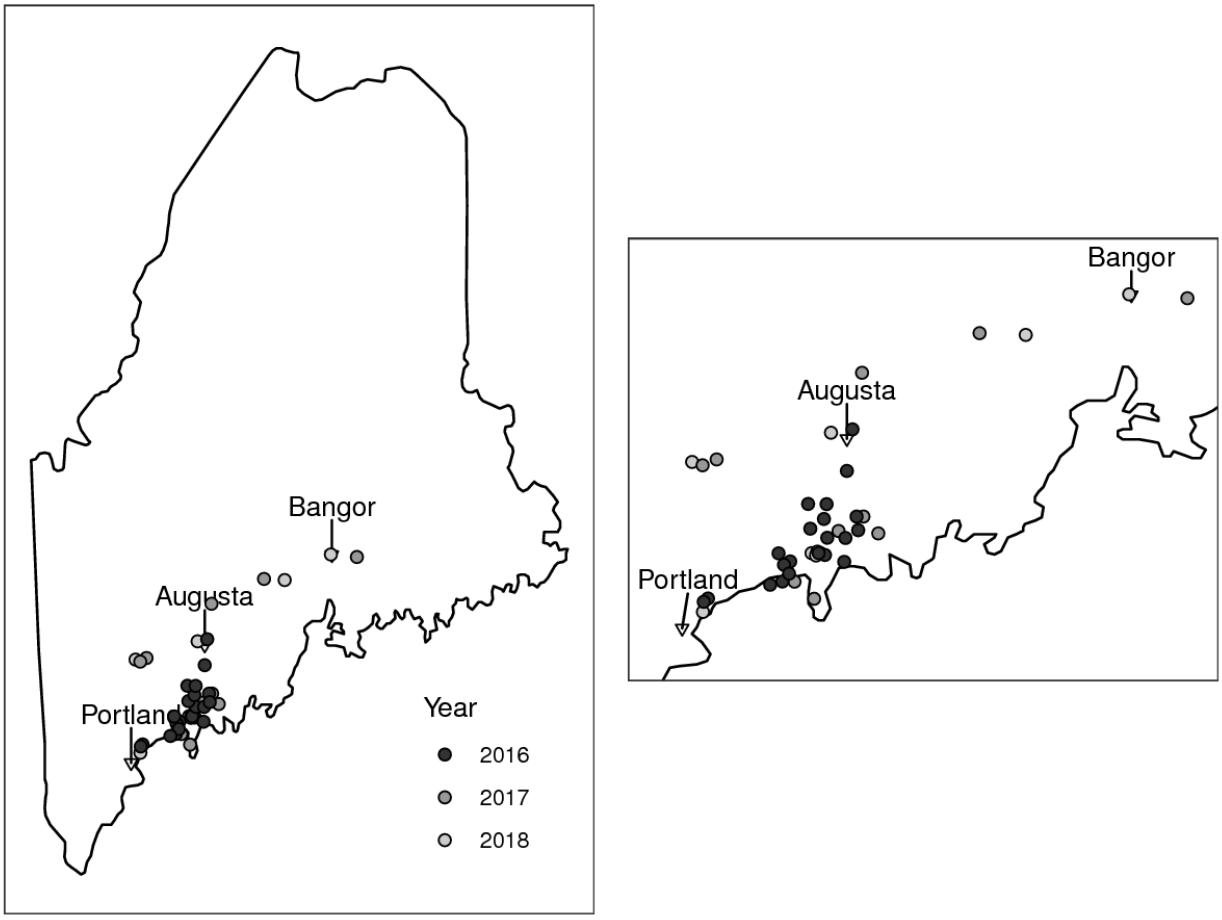

Figure 3. Map of *E. chrysorrhoea* sites sampled across mid-coast and inland Maine, indicated by year added.

Collection and Rearing of Samples

Winter hibernacula that were accessible by hand or by pole pruners (maximum of 6 m) were collected. Collections of 1 - 5 winter hibernaculae occured in early spring (late March-early April) after most winter mortality would have occurred. They were then held in cold storage at 10°C until all collections were complete (2 - 3 weeks). After these subsamples of webs from each site was removed from cold storage, and webs were placed individually in urine cups inside of plastic sandwich bags and held at an ambient laboratory temperature ~21°C. This process was repeated over a period of two weeks until a subsample of three individual nests were reared from

each site. A total of 68 winter webs were collected in 2017 and 115 winter webs were collected in 2018. All parasitoids that emerged from winter webs were collected and stored in 70% ethanol until they were identified. All parasitoid identifications of Tachinidae and Ichneumonidae were completed by the author using the appropriate identification keys (Townes 1920, McAlpine et al. 1981, Goulet and Huber 1993, Tschorsnig and Herting 1994) with help from George Boettner (Entomologist, University of Massachusetts) and entomologists at the Maine Forest Service (DACF 2016). Identifications of Chalcidoidea were made and confirmed by Michael W. Gates from the Agricultural Research Service of the USDA at the National Museum of Natural History in Washington D.C.

Late-stage larvae were collected at study sites in early to mid-June before larvae began pupating. Collections were made, either by hand if larvae were at accessible heights or by clipping branches with pole pruners if they were beyond reach. Samples were transported in coolers with ice packs and stored at 10°C for 1 to 7 days before they were set up for rearing. Up to 50 individuals were collected from each site, with one third of those individuals placed in 2mL Eppendorf snap-cap tubes and stored at -80°C for molecular analyses of fungi, another third stored in 70% ethanol for tissue smears under light microscopy, and the rest were reared. Reared larvae were placed in groups of 10 - 12 individuals per 9 cm diameter petri dish and provided with fresh foliage on a moistened filter paper. Dishes were sealed with parafilm and kept in an environmental chamber at 20°C and a 12 hr daylight cycle. Host foliage provided to rearing larvae was either oak (*Quercus ruba*) or apple (*Malus* spp.). Dishes were monitored daily for larval survival and natural enemy emergence. Dead larvae were removed and placed in individual wells of multi-well-plates (Thermo scientific, 48 wells) to monitor for pathogens. All parasitoids that emerged from reared dishes were placed in 70% ethanol for later identification.

If parasitoids could not be identified, they were categorized as “unknown”. A total of 170 larvae were collected in 2017 and 70 larvae were collected in 2018.

Pupation nest samples were collected after density counts were conducted in late-June to mid-July (See Ch. 3). Between 1 and 3 observers would walk along roadsides or within forests and count all visible browntail moth pupation nests within trees during a 10-minute timed interval. Counts were averaged across all observers and a subsample of pupation nests were collected. Pupation nest collection and storage methods were the same as that for diapausing and late stage larvae, with a goal of collecting up to 50 nests per site (See Ch. 3). Once collected, pupation nests were immediately set up in 473 mL Fabri-Kal® (Kalamazoo, MI) clear plastic cups with Fabri-Kal clear plastic dome lids that were covered with cloth or loose mesh to allow air and moisture exchange. In 2016, individual pupae were removed from nests and reared in individual 60mL Fabri-Kal clear plastic condiment containers. In 2017 and 2018, entire nests were reared after they were examined for pupal number. This method was adopted to reduce handling and our processing time and exposure to the high number of toxic setae incorporated into these nests. Nests were kept at ambient laboratory temperatures (~ 17.3 °C) in a room with open windows such that normal fluctuating diurnal conditions were experienced. All containers were checked daily for emergence of moths, parasitoids, and fungi. After emergence was complete, nests were dissected to confirm moth sex ratios, parasitoid species, and survival of pupae. A total of 592, 440, and 494 pupation nests were collected in 2016, 2017, and 2018, respectively.

All handling of browntail moth post overwintering larvae, pupae and (particularly) pupal nests were conducted in fumehoods to minimize our exposure to toxic setae. Additional

protective clothing for the laboratory included gloves, lab coats and neck scarves. Similarly, Tyvek suits, gloves, hats and scarves were required for field sampling.

Abiotic and Biotic Data

Abiotic data was obtained from Climate Data Online through the National Oceanic and Atmospheric Administration (NOAA 2019). Sampling sites were linked to data from the climate stations nearest to them, with, in some cases, multiple sites in proximity being linked to the same climate station, with a distance from sites no greater than 30 km (Appendix A, Table 10). Yearly climate variables were calculated to coincide with the annual browntail moth life cycle from $July_{t0}$ - $June_{t1}$. Due to collinearity between climate variables, the two that were hypothesized to have the greatest influence on browntail moth parasitoids were selected: average annual temperature (TAVG) and total annual precipitation (TPRCP). Distance from each site to the nearest coastal point and distance from Peaks Island, Portland, Maine were also measured for all sites using the measurement tool in google maps (Google Maps 2019). Peak's Island is the epicenter of the small relic populations that persisted during the non-outbreak period of browntail moth. Distance from this point was hypothesized to reflect the timeframe of the moth's, geographic spread during the current outbreak, and hence was used as a relative measure of the age of the infestation at each site.

Habitat vegetation data was obtained through the National Land Cover Dataset (NLCD 2016). Due to collinearity between habitat types, the three habitat types hypothesized to be most relevant were used for analyses: high intensity developed land, deciduous forest, and evergreen forest. Vegetation types present at each site were determined by identifying a 1.5 km radius around the center of each site using ArcGIS (ESRI 2011). Total area (m^2) occupied by each habitat type within this 7.0 km^2 area was calculated for each site.

Due to the year-to-year variation in diversity and abundance of parasitoid species, taxonomic groups were created to give more power to negative binomial models exploring abiotic and biotic factors correlated with parasitoid presence. These groupings included: 1) all parasitoids, 2) all fly parasitoids (Tachinidae), 3) all wasps (Ichneumonidae), and 4) all hyperparasitoids (Chalcidoidea: Ptermalidae, Torymidae). Individual parasitoid species or groups that occurred at more than twelve sites were also included in separate models. These included: 1) unemerged tachinid puparia, 2) *Townsendiellomyia nidicola* Twns., 3) *Pimpla disparis* Vier., 4) *Itoplectis conquistidor* Say, 5) *Monodontomerus aerus* Walk. The total number of pupae per nest was recorded during dissections after all parasitoids had emerged. Due to hazardous conditions of host material, hyperparasitoids were not examined for their level of trophic parasitism. Hyperparasitoids were estimated to average three individuals emerging per pupae based on reports of superparasitism, parthenogenesis, and polyembryonic cohorts in the literature (Muesebeck 1931) and counts of browntail moth pupae attacked by hyperparasites were adjusted to 0.33 * # hyperparasitoids for each sample. Tachinidae flies that did not emerge from pupa, and were likely species that overwinter in this stage, were also included in the analysis under "Tachinid puparia".

Data Analysis

Negative binomial models were used to explore correlations between abiotic and biotic variables on pupation nest parasitoid counts. Because sample sizes of pupae reared for parasitoid emergence varied between samples, counts of each of the parasitoids were standardized by adjusting parsitoid density by the number of proportional to the average sample size of 63.8 pupae per site. Variable selection for abiotic and biotic variables included those that were not correlated and hypothesized to have the most impact on browntail moth and their associated

parasitoids. Models were constructed for each of the four taxonomic parasitoids groups and each species individually utilizing annual climate variables and habitat vegetation variables. Separate models were also conducted for the distance to nearest marine coastline and distance to Peaks Island, Portland, Maine. Year was also added to each model, to account for any unaccounted between year variation. A total of two models were run for each of the four cumulative parasitoid taxa and five parasitoid species, resulting in a total of 18 models. Parasitoids that were observed emerging from winter hibernacula and mid-stage larvae were recorded, but the limited data from these stages did not warrant separate analyses.

Relative abundance and diversity were also calculated across all townships for all sites across all years. Relative abundance was calculated by taking the number of individuals within a species and dividing by the total number of individuals across all species within a site. Diversity was calculated using the Shannon-Weiner diversity index. These measures were tested for their association between site and year using a generalized linear model where year was treated as a stratum or statistical block. All analyses were run in RStudio (RStudio 2019, version 1.1.414), all negative binomial models were created using the package MASS.

Results

Overall, nine parasitoid species spanning six families were identified emerging from browntail moth winter hibernacula, mid-stage larvae, and pupal nests between 2016 - 2018. Of these species, three were hyperparasitoids, potentially attacking browntail moth as either primary, secondary, or tertiary parasitoids. Throughout the duration of the study, *M. aerus* was the only parasitoid observed emerging from winter hibernacula. Similarly, *M. versicolor* Vier. adults and several undetermined dipteran puparia were the only parasitoids observed emerging from mid-stage BTM larvae. *Townsendiellomyia nidicola* was the most abundant primary

parasitoid, accounting for 24 percent of total parasitiods across all years on BTM pupal nests. In addition, the most abundant hyperparasitoid was *M. aerus*, which accounted for 36 percent of total parasitoids recovered across all years on browntail moth pupal nests.

Emergence Observations and Percent Parasitism

Host and parasitoid emergence appeared to occur later and over a longer period in 2016 than 2017 and 2018, but this is due to differences in processing times (Fig 4 and Fig 5). In all years, emergence of moths began one to seven days prior to parasitoids, and peaked approximately, five days before emergence of Tachinidae and Ichneumonidae. In 2017 and 2018, when emergence timing in the laboratory more reflects that which occurred in the field, most moths emerged between the first and second weeks in July, whereas most primary parasitoids emerged during the second and third weeks in July. The emergence of hyperparasitoids occurred zero to twenty days after primary parasitoids.

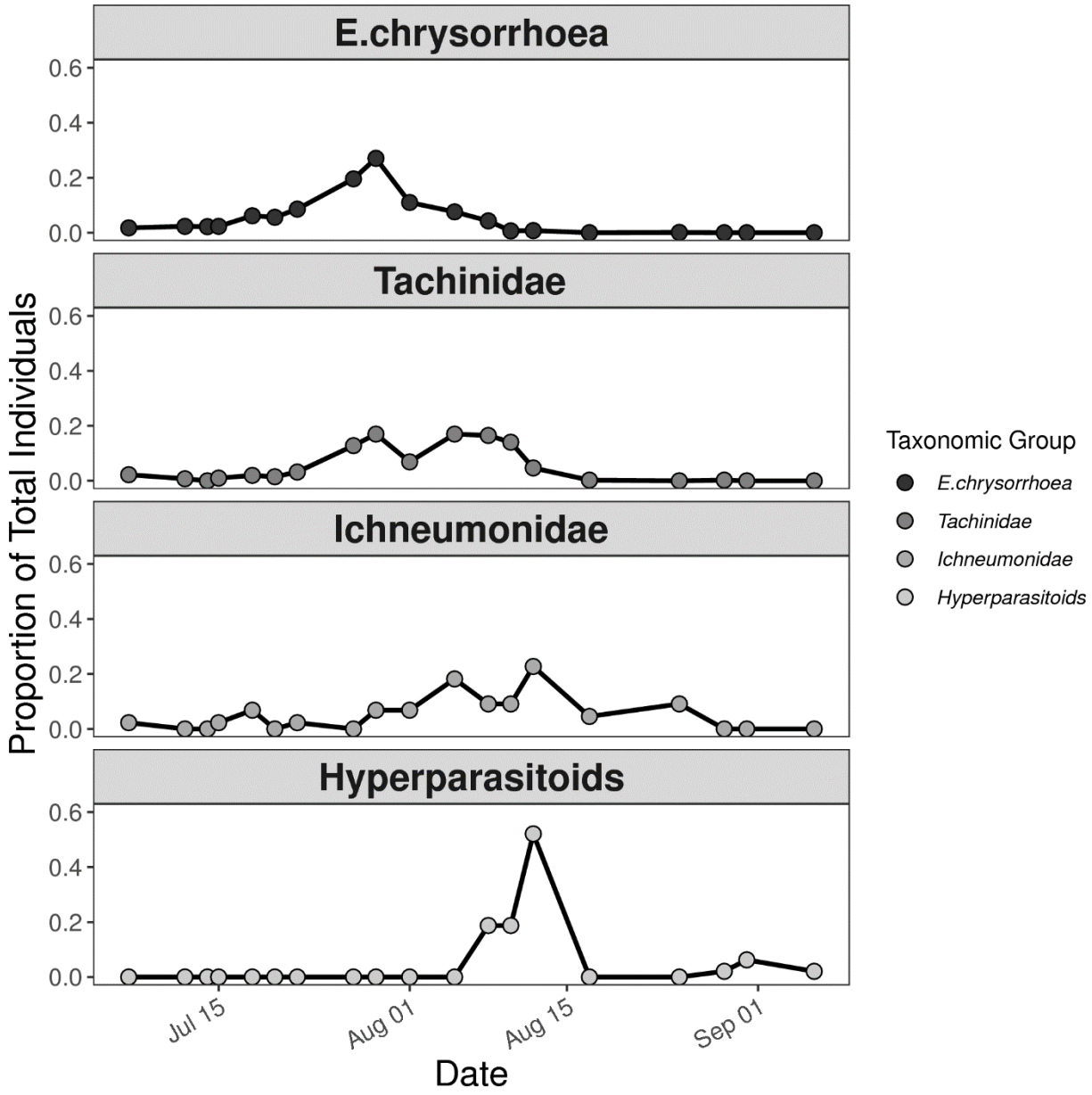

Figure 4. Emergence of *E. chrysorrhoea* and parasitoids from pupation nests in 2016. Note: Emergence rates are delayed due to different methods holding nests, most nests were delayed in their setup between 2 - 10 days.

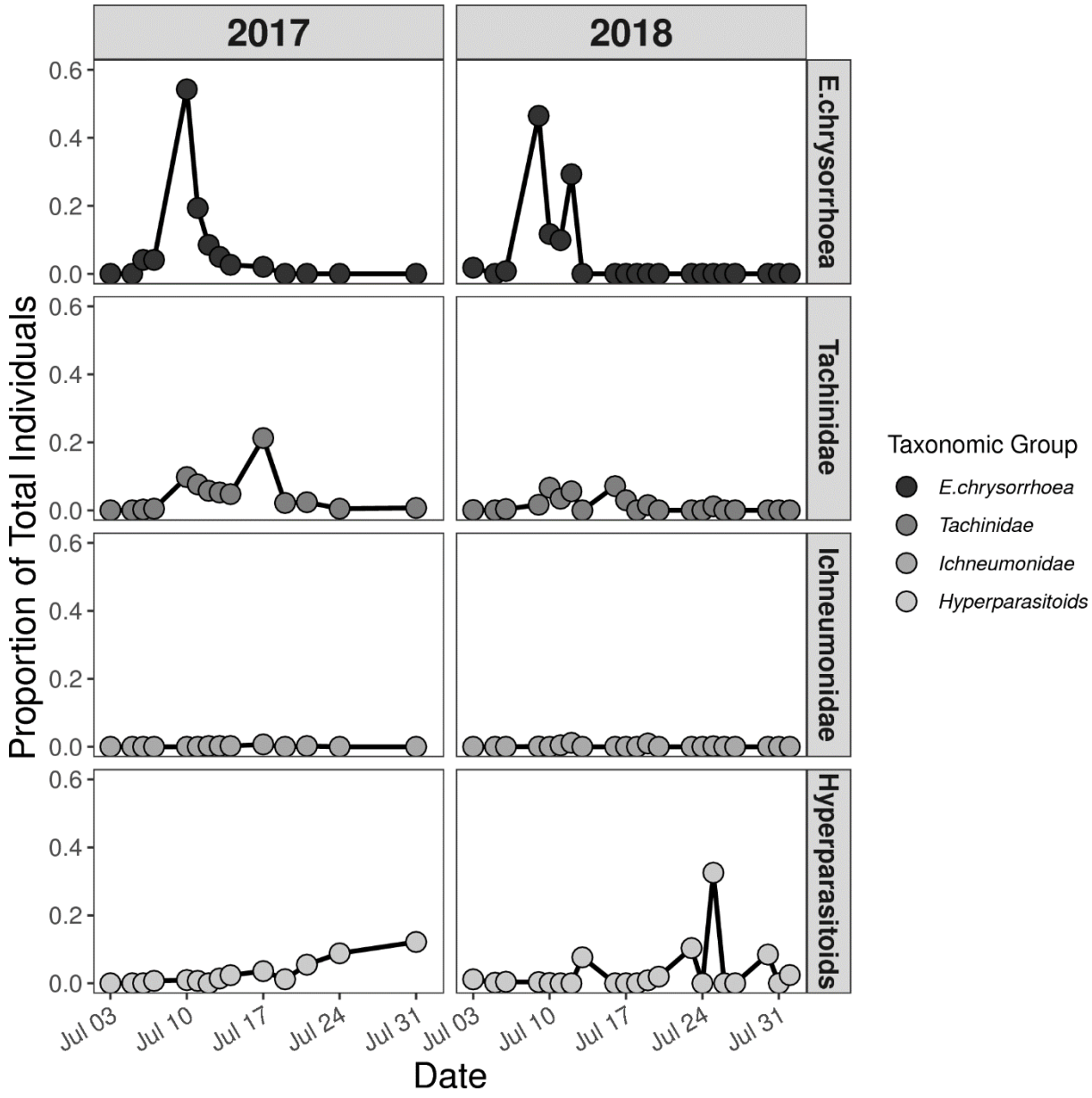

Figure 5. Emergence of *E. chrysorrhoea* and parasitoids from pupation nests in 2017 and 2018. Note: Years grouped because of similar rearing methods.

The percentage of browntail moth pupae attacked by parasitoids differed across years (Table 4). *Townsendiellomyia nidicola,* the only specialist parasitoid identified, had the highest parasitism across all years, and its impact increased considerably in 2018 over the previous two years. The hyperparasitoid, *M. aerus,* also had a sharp increase in mean percent parasitism

between 2017 and 2018 where it increased by 11.9 percent. All other parasitoids accounted for less than 3 percent parasitism. Both hyperparasitoids, *M. aerus* and *D. microgastri* represented a greater proportion of the total parasitoids recovered over the three years (Fig 6), while the proportions of Tachinid puparia and *C. concinnata* declined and Ichneumonidae species remained consistently low.

Table 4. Mean percent parasitism by each parasitoid species recovered from browntail moth pupal nests across all locations throughout the duration of the study. Hyperparasitoids were estimated to average three individuals per pupae for the percent parasitism calculations to account for superparasitism and polyembryony that occurs.

Species	**2016**	**2017**	**2018**
Tachinid puparia	42.11	4.03	6.42
Compsilura concinnata	1.58	0.22	0.42
Townsendiellmyia nidicola	14.89	13.86	24.33
Pimpla disparis Vier.	1.65	1.25	1.55
Itoplectis conquistador Say	0.44	0.07	0.49
Theronia atalantae Homg.	0.12	0.37	<0.01
Monodontomerus aerus Walk.*	5.28	4.72	16.6
Dibrachys microgastri Bouc.*	0.82	0.59	2.61
Brachymeria tibialis Walk*	<0.01	NA	NA
Total	66.89	25.1	52.42

* indicates hyperparasitoids acting at either the primary, secondary, or tertiary level

Abundance and Diversity of Parasitoids

The relative abundance of parasitoids attacking pupation nests was significantly different between years and within years (Table 5 and Fig 6). In 2016, the greatest number of parasitoids emerged from samples collected in Gardiner, ME, with *T. nidicola* occurring most frequently. In 2017 the highest number of parasitoids emerged from samples collected at a new inland infestation in Burnham, ME. In 2018 the highest number of parasitoids, which consisted mostly of hyperparasitoids, emerged from samples collected at another inland site in Waterville, ME. The highest density of parasitoids was in Gardiner in 2016 (n = 125), Yarmouth in 2017 (n = 14), and Waterville in 2018 (n = 50). Parasitoid relative abundance was highest at Bowdoinham in

2016 and Wiscasset in 2017 and 2018. Diversity was relatively low across all years with only seven species found across all years and did not differ significantly between years or sites (Table 5).

Table 5. Results from general linear models on relative abundance and diversity measures with site blocked for year.

Measure	**Variable**	**Sum Sq.**	**Mean Sq.**	***F***	***P***
Relative Abundance	Year	0.305	0.152	6.216	0.005
	Site	1.943	0.058	2.399	0.006
Shannon-Weiner Diversity Index	Year	0.001	<0.001	0.185	0.832
	Site	0.134	0.004	0.817	0.719

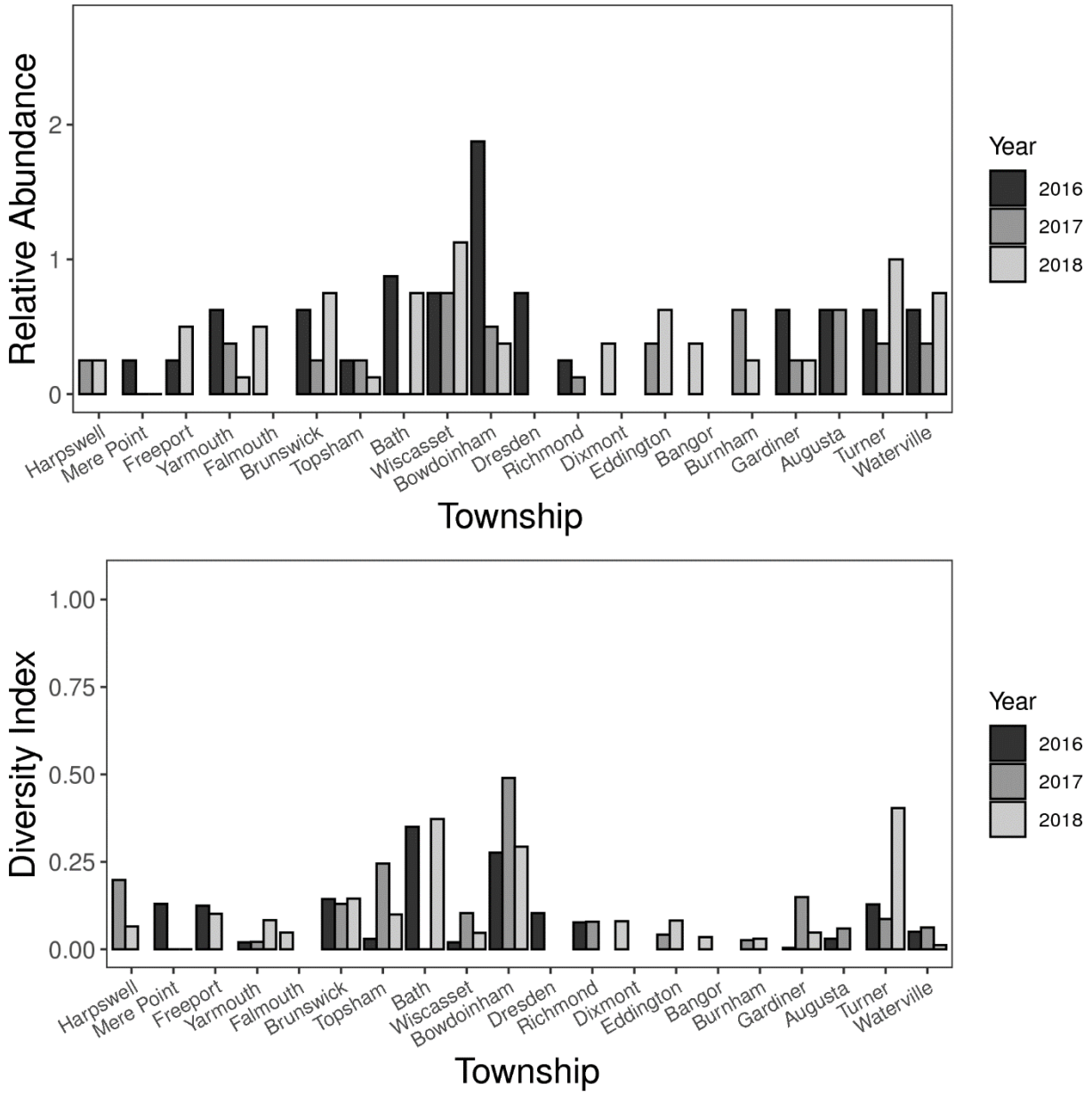

Figure 6. Relative abundance and diversity indices of pupal parasitoids collected from townships across Maine. Townships are ordered by their distance to nearest coastline (Harpswell = closest, Waterville = furthest).

Geographically Separated Parasitoid Assemblages

Tachinid puparia and *T. nidicola* were two groups that had the highest parasitoid abundance in most townships, accounting for greater than 50% of individuals observed in 2016 and 2017 (Fig 7). Hyperparasitoids *M. aereus* and *D. microgastri* overall increased in proportion

in 2018, usually at inland sites. Assemblages with greater than three species were found in Freeport in 2018, Yarmouth in 2016, Falmouth in 2018, Topsham in 2016, Wiscasset all years, Bowdoinham in 2016, Dresden in 2016, Eddington in 2018, Burnham in 2017, Augusta in 2016 and 2017, Turner in 2018, and Waterville in 2018. Tachinid puparia, *T. nidicola*, *M. aereus*, and *D. microgastri* were present at greater proportions than other species.

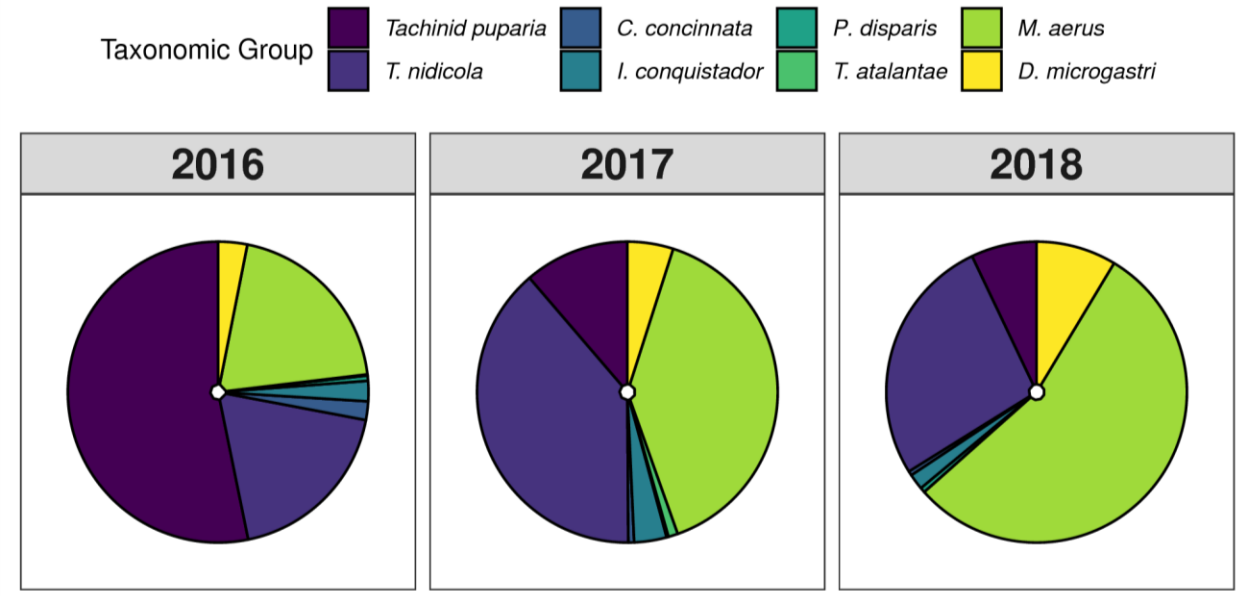

Figure 7. Each species proportion of the total parasitoids reared from browntail moth pupa across all sites sampled in Maine between 2016 and 2018.

Climate and Habitat Impact on Parasitoids

Negative binomial regressions revealed no significant relationships between the climate variables of average annual temperature and total annual precipitation and the abundance of all Tachinid puparia, *T. nidicola*, *C. concinnata*, *T. atalantae*, or *D. microgastri* (Table 6). There were also no significant relationships between these climate variables and the total abundance of all parasitoids, total Tachinidae, or total Ichneumonidae. The abundance of two species of Ichneumonidae, *P. disparis* and *I. conquistador*, decreased significantly as total precipitation increased. Of the habitat type variables, Ichneumonidae abundance was determined by the

amount of evergreen forests and high-intensity development. Whereas, the abundance of hyperparasitoids was negatively correlated with the amount of evergreen forest. The hyperparasitoid *M. aerus* had a similar trend, with a significant decrease in abundance as evergreen forest increased. Year was significant for all primary parasitoids ($Z_{(60, 53)} = -3.13$, $P = 0.001$, coefficient $\pm$ SE = -1.27 $\pm$ 0.404), Tachinidae ($Z_{(60, 53)} = -3.53$, $P = 0.001$, coefficient $\pm$ SE = -1.38 $\pm$ 0.404), and Tachinid puparia($Z_{(60, 53)} = -2.849$, $P = 0.001$, coefficient $\pm$ SE = -2.11 $\pm$ 0.537), in 2017. Tachinid puparia abundance was also significantly lower in 2018 ($Z_{(60, 53)} = -2.849$, $P = 0.004$, coefficient $\pm$ SE = -2.25 $\pm$ 0.79). In contrast, 2016 had significantly higher abundance in for *P. disparis* ($Z_{(60, 53)} = 2.24$, $P = 0.03$, coefficient $\pm$ SE = 31.833 $\pm$ 14.21).

Table 6. Significant negative binomial model results of taxonomic groups and individual species abundances tested with climate variables, habitat variables, and year.

Taxonomic group	Variable	Coefficient	Std. Error	Z^1	*P*
Ichneumonidae	Evergreen forest	17.45	3.87	4.50	<0.001
	Developed land	22.92	8.19	2.79	0.005
Hyperparasitoids	Evergreen forest	-9.09	2.68	-3.38	<0.001
	Total annual precipitation	-0.02	0.006	-3.39	<0.001
I. conquistador	Total annual precipitation	-0.01	0.005	-2.18	0.02
M. aerus	Evergreen forest	-15.57	3.54	-4.45	<0.001

Z^1 = Log-likelihood statistic

The second group of negative binomial models, which tested distance measures and year with parasitoid abundance, and resulted in significant predictors for all taxon except Ichneumonidae and hyperparasitoids (Table 7). Neither distance to coast, nor distance to Peak's Island (presumed origin of outbreak), were significant for any group or species. In 2016 abundance was significantly higher for all primary parasitoids, Tachinidae, Tachinid puparia and the two most abundant species, *T. nidicola* and *M. aerus*. The same year had significantly lower abundance for the two Ichneumonidae, *P. disparis* and *I. conquistador*. By contrast, both 2017

and 2018 had significantly lower abundance for all primary parasitoids, Tachinidae, and tachinid puparia.

Table 7. Significant negative binomial model results of taxonomic groups and individual species abundances tested with distance to coast, distance to Peak's Island, ME and year.

Taxonomic group	Variable	Coefficient	Std. Error	Z^1	*P*
All primary parasitoids	Year: 2016	3.39	0.28	11.89	<0.001
	Year: 2017	-1.18	0.33	-3.53	<0.001
	Year: 2018	-0.76	0.31	-2.41	0.01
Tachinidae	Year: 2016	3.35	0.27	12.2	<0.001
	Year: 2017	-1.36	0.32	-4.2	<0.001
	Year: 2018	-0.77	0.30	-2.54	0.01
Tachinid puparia	Year: 2016	2.90	0.38	7.57	<0.001
	Year: 2017	-1.86	0.45	-4.12	<0.001
	Year: 2018	-2.18	0.43	-5.02	<0.001
T. nidicola	Year: 2016	1.82	0.30	5.93	<0.001
P. disparis	Year: 2016	-2.10	0.66	-3.14	0.001
I. conquistador	Year: 2016	-1.72	0.73	-2.36	0.01
M. aerus	Year: 2016	1.68	0.63	2.67	0.007

Z^1 = Log-likelihood statistic

Analysis of proportion parasitism relative to independent estimates of host abundance (timed observations of pupal nests) did not reveal any significant density dependent relationships for any of the parasitoid groups (Fig 8).

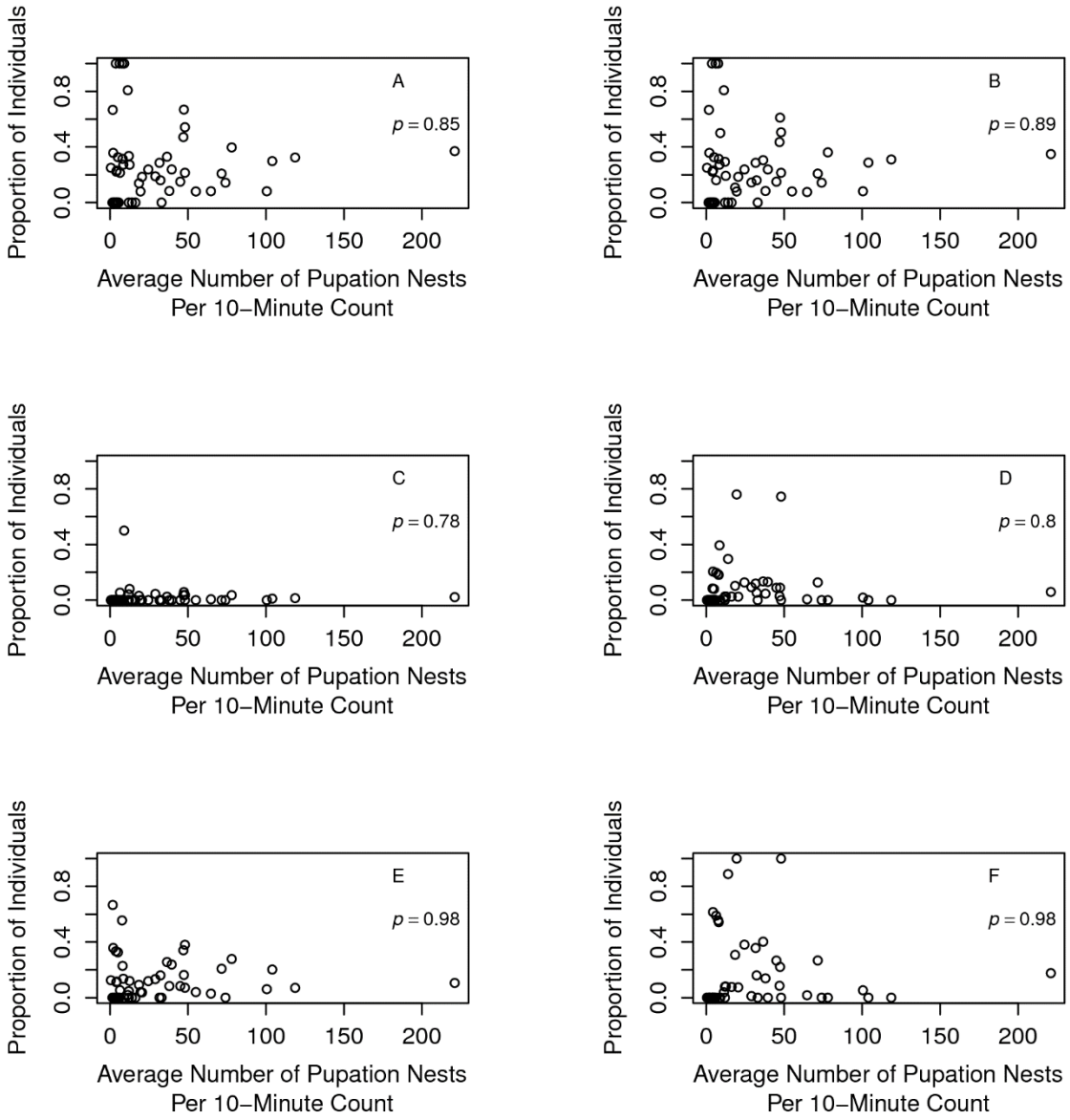

Figure 8. Proportion parasitism relative to host density (pupal counts) organized by taxonomic and trophic groups. A) Primary parasitoids, B) Tachinidae, C) Ichnuemonidae, D) Hyperparasitoids, E) *T. nidicola*, and F) *M. aerus*.

Discussion

The total parasitoid assemblages observed throughout the duration of this study consisted of eight parasitoids emerging from browntail moth pupae. As far as we know, this study represents the first record of *Dibrachys microgastri* attacking browntail moth in Maine.

According to Burgess and Crossman (1929) positively established larval and pupal parasitoids from the initial outbreak include *A. lacticolor, M. versicolor, T. nidicola* (*Sturmia nidicola*), *C. concinnata*, *Carcelia laxifrons* Ville., and *Trichomalopsis hemiptera* (*Euptermalus nidulans*) Walk. Although *E. nidulans* bares resemblance to the hyperparasitoid *D. microgastri* found in this study, it was likely a group of several similar species in Ptermalidae that have since been re-classified. Two Tachinidae species (Peters and Baur 2011). *Townsendiellomyia nidicola* and *C. concinnata* were positively identified in this study, with *C. laxifrons* and *Exorista larvarum* L. likely candidates for some of the unidentified tachinid puparia. *M. versicolor* was not found in browntail moth pupae but was discovered parasitizing browntail moth mid-stage larvae. Schaefer (1974) found 12 parasitoids during his study in a non-outbreak period, which included *T. nidicola, C. concinnata, C. laxifrons, Eusisyropa blanda* O.S., *Exorista sp., A. lacticolor, M. versicolor, I. conquistador, M. aereus, Hyposoter fugitivus* Say, *Brachymeria compsilurae* Cwfd., and *Trichogramma minutum* Riley. Of these, eight are pupal and prepupal parasitoids. Elkinton et al. (2006) described *T. nidicola, C. concinnata, C. laxifrons*, and several Ichneumonid wasps on Massachusetts populations.

Several factors are likely impacting parasitoid emergence from pupation nests, including trophic level at which the parasitoids attack hosts and competition with other parasitoids. Natural enemies can enhance or negate natural enemy species abundance and diversity through increased resource competition (Bonsall and Hassell 1999). We observed this throughout the present study as abundance of primary parasitoids decreased and abundance of hyperparasitoids increased (Table 4, Fig 7). The Tachinidae and Ichneumonidae parasitoid groups were similar in that they occurred synchronously with their hosts, likely a strategy that allows generalists to find other lepidopteran hosts and specialists to parasitize larval browntail moth two to three weeks after

they hatch in August (Meusebeck 1922). Hyperparasitoids were the most delayed in their emergence of the taxonomic groups observed (Fig 4 and Fig 5). This delay in emergence times could be a result of hyperparasitism occuring at a secondary and tertiary levels on Tachinidae, Ichneumonidae, and other Chalcidoidea (Schaefer 1974, Peters and Baur 2011). Tree host that browntail feed on could also impact parasitoid emergence, depending on nutrition values and their impact of browntail moth development.

Although there was not enough data to determine percent parasitism across several life stages, percent parasitism for the different species of parasitoids observed during the pupal stage varied by year (Table 4). *Townsendiellomyia nidicola*, the specialist tachinid, was the most abundant primary parasitoid across all years. Compared to Burgess and Crossman (1929) and Elkinton (2006), we recorded considerably higher *T. nidicola* parasitism in 2018, which increased from what we observed in 2016 and 2017 (Table 3), these earlier years being more consistent with previous studies. Levels of parasitism by this specialist tachinid ranges from 10 to 22 percent based on reared samples from field collected hosts during a non-outbreak assessment (Schaefer 1974). Other studies have shown an average of 13 percent parasitism of browntail moth from 1917 to 1929 (Burgess and Crossman 1929). This relatively low and fluctuating percent parasitism is likely due to the univoltine life cycle of this specialist that is often the target of hyperparasitoids and in competition with other primary parasitoids (Schaefer 1974). All other primary parasitoids had less than two percent parasitism for any given year. The hyperparasitoid *M. aerus* accounted for less than five percent parasitism in 2016 and 2017, then saw an increase to 16 .6 percent in 2018. Before averaging this hyperparasitoid to three individuals per pupa, estimates of mean percent parasitism (based upon a per nest estimate) were 15 percent in 2016, 14 percent in 2017, and 49 percent in 2018. Although we classify this

parasitoid as hyperparasitic, there is some variation in what trophic level it attacks browntail moth larvae at. In some cases, primary parasitism of browntail moth occurs between 30 and 45 percent of cases where *M. aereus* is present, with other studies finding primary parasitism as high as 48 percent (Museback 1931, Schaefer 1974). Of cases where hyperparasitism is occuring, it reportedly attacks 60 percent of *C. concinnata* when on browntail moth pupae (Proper 1934). Tertiary parasitism is reported on *A. lacticolor*, but this phenomenon is understudied and its possible this hyperparasitoid could attack any of the species present in this study at a secondary or tertiary level. If *M. aereus* was acting as a secondary or tertiary parasitoid, this could explain why average percent parasitism was low for other generalists found during this study.

Relative abundance of browntail moth parasitoids was significantly different across years when blocked by site (Table 5). Relative abundance was highest at Bowdoinham sites in 2016, and Wiscasset sites in 2017 and 2018 (Fig 6). These findings are consistent with the observation that Bowdoinham was one of the epicenters of the early outbreak in 2016 (DACF 2016). Diversity was low at most sites throughout all years of the study, due to the sample species assemblages of only eight parasitoid species found throughout the study. Diversity of parasitoids is higher in their native range, where 17 primary parasitoids and 10 hyperparasitoids have been found (Frago et al. 2012). Parasitoid diversity in the present study is similar to recent assessments by Scheafer (1974), where nine species were found attacking browntail moth populations. Bowdoinham, the site with the highest relative abundance of parasitoids in 2016, also had the highest diversity index in 2016. Abundance and diversity of parasitoid assemblages is understudied but could be influenced by fine-scale habitat characteristics and trophic levels of hosts (Fraser et al. 2007). All sites were ordered by their distance to the nearest coastline, but no trends were seen with this parameter.

The negative binomial regression results indicate that annual climate and habitat variables were not significant drivers for parasitoid abundance over the study period for most taxonomic groups and species (Table 6). In 2017, a major epizootic of *Entomophaga aulicae* Hum. was discovered across late-stage larval samples of browntail moth populations from the mid-coast sites, which may have influenced parasitoid abundance during this year. Abundance of all Ichneumonidae, *P. disparis*, and *I. conquistador* decreased with increasing annual precipitation. Precipitation and other climate factors can influence insect trophic structures, especially parasitoids that may not be directly correlated with the abundance of their hosts (Menedez et al 2007, Zhu 2014). This may be true for the parasitoids observed in the present study, as there was no density dependence observed for any group (Fig 8). The negative relationship observed with precipitation could be a result of delayed development because of unfavorable conditions or mortality within hosts because of disease. Annual average temperature was not a significant variable for any parasitoid group or species, but it is possible that annual measures of climate do not capture trends in specific months or times of the year when browntail moth parasitoids are active.

In our study Ichneumonidae abundance was greater in sites with higher amounts of evergreen forest habitat and high-intensity development, whereas hyperparasitoid abundance decreased with increased evergreen forests (Table 6). Landscape and local scale vegetation and land management can have a large impact on diversity and richness of Ichneumonidae (Gonzalez-Moreno et al 2018). Of the Ichneumonidae found in the present study, *P. disparis* has at least 72 hosts and *I. conquistador* has at least 82 hosts recorded, some of which are specialists on conifers (Arthur 1962, Choi et al 2015). *Dibrachys microgastri*, a Ptermalidae included in this category, is a generalist hyperparasitoid with a large host range and no known habitat

preferences and may be outcompeted by other Chalcidoidea species for hosts. (Peters and Abraham 2010, Peters and Baur 2011, Peters 2011). It is possible that the significant relationship resulting from our models is a result of browntail moth tree host preferences, which are deciduous trees in the US (Shaefer 1974). The hyperparasitoid with the highest abundance, *M. aerus* decreased in abundance as evergreen forests increased. This hyperparasitoid is understudied and its habitat preferences are largely unknown, but a similar relationship is likely as this is a generalist primary and hyperparasitoid that has a host range consisting of browntail moth hosts and most parasitoid species identified in the present study (Muesebeck 1931). It is also possible that some parasitoid abundances decreased in 2017 and 2018 because hyperparasitoid proportions increased during these years (fig 7).

It has been theorized that browntail moth parasitoid diversity, and particularly the abundance of the generalist parasitoid, *C. conncinata*, decreases with distance from the nearest marine coastline, and the subsequent lack of parasitoid pressure explains the persistence of localized relic populations of browntail moth observed for many years at coastal sites in Maine and Massachusetts (Elkinton et al. 2008). Elkinton et al. (2008) suggest that decreased host plant diversity in coastal habitats limits the diversity of suitable Lepidoptera host for these generalist parasitoids, which are more abundance in inland deciduous forests. In this study, distance to coast was not significant in explaining variation in abundance of any parasitoid taxa grouping or individual species. Parasitoid assemblages may differ between Maine and Massachusetts populations of browntail moth especially as Maine and Massachusetts have differences in coastal habitat (deciduous woodland vs. coastal scrub and sand dunes) with differences in some of the available host vegetation (Schaefer 1974, Elkinton et al 2006, Appendix C). Greater variation in

species composition and outbreak condition could mean that the variation in parasitoid counts is not explained by any previously studied factors and requires more investigation.

Similarly, distance to Peak's Island, Maine was also not a significant factor for any of the parasitoid taxa. Initially, we hypothesized that the age of a localized infestation would contribute to parasitoid abundance as parasitoids "follow" hosts to new areas of their range and that distance from Peak's Island would represent an index of age of an infestation. However, either distance is not a good representation of age or our findings do not support this theory that older infestations will have greater abundance and diversity of parasitoids.

Variation in abundance of parasitoid taxa between years was not explained by annual climate variables used in our models and may reflect variations in other factors not examined in this study, such as abundance of alternate hosts for these species, which are primarily generalists, specific climatic events that are not captured in the annual climate summary, or other competition or host factors impacting individual parasitoid species. In both models, relative abundance was always negatively significant for both 2017 and 2018 for some parasitoid groups and species. The epizootic outbreak of *Entomophaga aulicae* on host BTM in 2017 was likely the cause of the decline during that year, with abundances not fully rebounding to 2016 levels by 2018.

Parasitoid assemblages changed throughout the duration of the study (Fig 7). In 2016, Tachinidae puparia and *T. nidicola* accounted for the highest proportion of species throughout all regions. In 2017, those same two groups were still seen in high proportions, but across fewer sites, likely a result of the previously mentioned epizootic observed in the host population. Based on our findings, Tachinidae is the most important parasitoid group for the browntail moth. The specialist, *T. nidicola*, accounted for more primary parasitism than any other species. Although

this species is selective and caused upwards of 20 percent mortality, its slow univoltine lifecycle and high rates of hyperparasitism mean that it is not a solution to controlling BTM outbreaks like some other specialist tachinids attacking other hosts (Schaefer 1974, Roland and Embree 1995). The generalist tachinid, *C. concinnata*, was not highly prevalent in our study (Table 4 and Fig 7). The average percent parasitism for this species was less than two percent each year. Although *C. concinnata* was implicated as the parasitoid that may have caused the initial crash of the BTM population (Boettner et al 2000, Elkinton et al 2006), recent studies have shown that parasitism by this generalist has declined in recent years on native silk moths (Baranowski et al 2019). We were unable to positively confirm to species the nonadult tachinids collected in the current study based on puparia characteristics due to the variable condition of some puparia. Some of the candidates for these mentioned earlier include the specialist, *C. laxifrons,* and the generalist, *E. larvarum*, which were introduced to North America from the native range of BTM at the turn of the century (Burgess and Crossman 1929). There is evidence that suggests *C. laxifrons* requires alternate hosts when it persist in the absence of BTM, putting it in an oligophagous category (Elkinton et al. 2006). This parasitoid can cause considerable parasitism but if it was present, it may be competing for hosts with the other Tachinidae parasitoids found in this study (Table 3). Less is known about the multivoltine generalist *E. larvarum* other than that it has been a rare occurrence during the non-outbreak periods of BTM and may prefer gypsy moth (Burgess and Crossman 1929, Tallamy 1983).

In 2017, an increase in the proportion of hyperparasitoids, particularly *M. aerus,* likely led to a decrease in the population of the primary parasitoids observed, especially *T. nidicola* and *C. concinnata* (Fig 7). In 2018, this trend continued with a greater proportion of hyperparasitoids than primary parasitoids. In its native range, browntail moth population

dynamics are driven by moth density, and coastal outbreaks can decline due to intra-specific competition of hyperparasitoids (Frago et al 2012). Given the increase in *M. aereus* and *D. microgastri* in the present study, it is likely that a mixture of different trophic levels of parasitism are occurring, with hyperparasitoids potentially also increasing their function as a primary parasitoid. Overall, this proportional increase is seen as a detriment to browntail moth biological control, as it can decrease the most vital primary parasitoids by a considerable amount (Scheafer 1974, Frago et al 2012). In some cases, increases in hyperparasitism can cause outbreaks of forest pests by interfering with top-down regulation (Rosenheim 1998, Nenzen et al 2018). Although we can definitively say that parasitism is occurring at higher trophic levels, it is difficult to know what affect this has on population dynamics of browntail moth and if they are related to the origins of this recent outbreak.

M. aereus, which was classified as a hyperparasitoid during the present study, may act as a gregarious primary, secondary, or tertiary ectoparasitoid (Schaefer 1974). Although these parasitoids were assumed to develop and emerge as three adults per BTM pupa for analyses, *M. aereus* may have been over estimated as it may sometimes be parthenogenic and can lay multiple progeny at once. (Muesebeck and Dohanian 1927). Muesebeck (1931) found that in laboratory studies 37 percent of *C. concinnata* and 10 percent of *T. nidicola* were parasitized by *M. aereus.* In addition, *M. aereus* may also parasitize Braconidae hyperparasitoids. Observations during these studies also revealed that females may feed on and kill Tachinidae puparia without parasitizing them. Similarly, *D. microgastri*, the other hyperparasitoid observed at high densities in the present study, may also be difficult to pinpoint to a level of parasitism, although it acts primarily as a secondary parasitoid (Meusebeck and Dohanian 1927). As stated above, *D. microgastri* can be the inferior species when competing with other hyperparasitoids, but it may

also tolerate some superparasitism and unmated females are parthenogenic and will produce males (Muesbeck and Dohanian1927, Peters 2011). Levels of true hyperparasitism in the current study are unknown as the hazardous host material prevented the author from detailed dissections. The importance of such trophic interactions in browntail moth populations has not been highlighted before in America, but more research is needed to fully understand these dynamics.

Taxonomically, these Chalcidoidea species are complex and required professional identification in the present study. The author is under the suspicion that the Ptermalid, *Eupteromalus nidulans* (Valid name: *Trichomalopsis hemiptera*) originally reported to be released as a part of the biocontrol effort for gypsy moth and browntail moth, was a part of a complex of species from Ptermalidae, with *D. microgastri* as one of the potential candidates for correct identification. Evidence of this lies in the lack of material of *T. hemiptera* located on browntail moth in recent years. Also, Peck (1963) identified where the genus *Ptermalus*, *Eupteromalus*, and *Dibrachys* were often reported as misidentified during this period, a common occurrence in the family Pteromalidae as several changes have been made to this group rated the "most difficult" (Gibson et al 1997) of Chalcidoidea taxonomy. Although this correction may be unnecessary, it could be useful and bring clarity for future research elucidating the parasitoid trophic structure of browntail moth.

In conclusion, the parasitoid complex of browntail moth pupa in Maine's current outbreak is comprised of at least eight parasitoids, three of which are hyperparasitoids. Some of these parasitoid groups and species are influenced by abiotic and biotic factors. The mean percent parasitism of the two most abundance primary parasitoids, *T. nidicola* and *M. aerus* increased between 2016 and 2018. The overall proportion of hyperparasitoids also increased and surpassed that of the specialist tachinid, *T. nidicol*a. Based on our analyses, we can conclude that

some annual climate variables and habitat variables are significant predictors for browntail moth parasitoid relative abundance. Year was also a significant factor for the relative abundance of some parasitoid groups, whereas distance measures were not significant. None of the parasitoids displayed significant density-dependent relationships with hosts, likely a result of the majority of parasitoid species being generalists. From these results, we can conclude that parasitoids are not regulating in browntail moth populations, as new populations are establishing at more inland portions of Maine and older populations continue to persist. Overall, parasitoid assemblages in BTM populations are fluctuating and likely much larger than what the current study could observe. These findings provide an understanding of the pupal parasitoid natural enemies within the browntail moth population soon after an outbreak of a historical pest occurred, and what factors may influence these parasitoid populations.

CHAPTER 3:

THE INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON BROWNTAIL MOTH MORTALITY AT DIFFERENT LIFE STAGES ACROSS THREE YEARS IN MAINE

Abstract

The browntail moth (*Euproctis chrysorrhoea* L.) is an invasive forest pest that was accidentally introduced to Cambridge, MA in 1897 and caused widespread damage to forests in the early part of the 20th Century. During its peak range expansion in 1915 in the northeastern United States, this insect encompassed an area of 150,000 km^2. During this time, biological control efforts initiated by the United States Department of Agriculture introduced 47 natural species of natural enemies to combat the browntail moth and gypsy moth (*Lymantria dispar* L.) invasive outbreaks. A population decline of browntail moth soon followed until only small relic populations existed on Cape Cod, Massachusetts, and the Casco Bay Region in Maine, with small outbreaks typically < 4,000 ha^2. Recently, an outbreak of browntail moth originating from the Casco Bay Region and encompassing > 50,000 ha^2 in parts of mid-coast and inland Maine became cause for concern because of both public health and forest health problems associated with this insect. This student investigated the abundance and survival of browntail moth throughout infested areas of Maine from 2016 - 2018, and explores factors that may influence browntail moth population dynamics over this time. We sampled three life stages in the field: overwintering hibernacula, late-stage larvae, and pupal nests, and with these and subsequent rearing of collected samples estimated overwintering survival, larval, and pupal density, and healthy moth emergence. Statistical models were constructed to determine important factors for

predicting density and survival relative to year, distance to coast, age of infestation, habitat, and annual climate variables.

Results from the present study indicate that browntail moth abundance was significantly different across years for both pupae and adults. Emergence of adults was similar for both years that it was recorded. Mean survival of all life stages increased between 2017 and 2018 and was higher in 2016 for pupae. Negative binomial generalized linear models indicated that no singular factor was a significant predictor for abundance of all life stages. Late-stage larval abundance declined with average annual temperature, which may be due to some unaccounted density-dependent factor. Similarly, late-stage larvae and adult abundance decreased with increased number of emerged overwintered larvae, suggesting a density dependent mortality effect. Abundance of emerged overwintered larvae, though no other life stages, increased with distance to coast and decreased with the age of infestation. Overall, it seems that browntail moth populations may be determined by more fine-scale climate and habitat features, but there is not one factor that explained abundance. Decreases in survival and differences between browntail moth at different sites may be driven by epizootic outbreaks of *Entomophaga aulicae* Reich. while parasitoids play less of a role. More research is needed to understand exactly what determines these populations at different life stages, in addition to pinpointing cause of the population expansion over the past several years.

Introduction

Insect population dynamics are often strongly influenced by abiotic environmental conditions (Netherer and Schopf 2010). This includes temperature, which can influence rate of development, voltinism, population density, individual size, genetic composition, extent of host plant exploitation, and geographical distribution in insect populations (Bale et al 2002). Other

abiotic factors that influence insect population dynamics include precipitation, CO_2 concentration in the atmosphere, and shortwave ultraviolet irradiation (McCloud and Berenbam 1993, Stange 1997, Thacker et al 1997, Karuppaiah and Sujayanad 2012). The browntail moth, *Euproctis chrysorrhoea* (L.), is an invasive insect that has recently spread via outbreak throughout parts of its historic invasive range in Maine. Although there are some studies investigating winter temperatures and diapausing browntail moth larvae, there are only seven publications on other abiotic factors and how they influence other life stages of this insect (Sacharov 1930, Pantyukhov 1964, Skoptsov 1968, Sterling and Speight 1989, Elkinton et al 2006, Elkinton et al 2008, Frago et al 2010).

There is evidence that gypsy moth (*Lymantria dispar* L.), a closely related species to browntail moth, have population dynamics that are heavily influenced by abiotic factors, although disease and parasitoids are also important in population regulation. High temperatures affect larval and pupal development, allowing individuals to develop faster and escape some natural enemies, while precipitation can induce the spread of disease in the population (Leonard 1974). Minimum lethal temperature for gypsy moth ranges from -20°C to -29°C, but snow cover can increase the duration at which these temperatures are tolerated (Sullivan and Wallace 1972). It is difficult to directly compare gypsy moth overwintering to that of browntail moth as gypsy moth egg masses enter diapause close to the ground, whereas browntail moth early instar larvae enter diapause in winter hibernacula in the tree canopy.

The winter survival of browntail moth in hibernacula has been previously studied to determine the influence of winter temperatures. Elkinton et al (2008) explored the relationship between extreme minimum temperatures and larval survival in winter hibernacula over the period of expansion and collapse throughout North America. They found that there was no

correlation with survival and that the larvae are well adapted to New England and Southern Canadian minimum winter temperature regimes. Minimum lethal temperatures for browntail moth have not been accurately identified, with accounts ranging from -17.7°C (Schaefer 1974), to -29°C (Anon. 1940, see Schaefer 1974), to -31°C (Gilliatt 1920). Although there has been confusion on the supercooling point of browntail moth, duration of exposure to cold temperatures is equally as important as cold temperature alone (Schaefer 1974).

In its native range in Europe, Frago et al (2011) created life tables to examine factors influencing mortality of all browntail moth life stages at four sites in Spain. In this area of its native range, there is less of an impact on browntail moth populations from parasitoids, but there is an effect of distance from the coast where mortality is higher at costal sites vs inland sites. Several abiotic and biotic factors were tested, which included water deficits, degree-days, rainfall, temperature below 30°C, and temperature above 30°C, none of which were significant. Only the realized fecundity of females, which increased with increasing density explained the difference in abundance between populations. Although mortality is higher at coastal sites, fecundity of females is also higher, resulting in a density-dependent effect of mortality. The authors note that population dynamics in the native range of BTM are different than that of their introduced range. In Spain, this insect only feeds on the evergreen strawberry tree (*Arbutus unedo*) and enters diapause later in the autumn and for only two months, and the influence of parasitoids is a minimal contributor to mortality in (Frago et al 2009, Frago et al 2011). In North America, browntail moth feed on several deciduous hosts, enter diapause for six to seven months, and have a diverse community of insect natural enemies (Burgess and Crossman 1929, Schaefer 1974, see Chapter 2). These differences are also seen in the genetic composition of

populations, as the origin of U.S. populations of BTM have been traced to the U.K., which are significantly different from southern Europe genotypes (Marques et al 2014).

Similar to gypsy moth, browntail moth has two significant pathogens found to infect populations (Sterling and Speight 1989) in their native and introduced ranges, an entomopathogic fungus, *Entomophaga aulicae* Hum., and the nuclearpolyhedrosis virus (EcNPV). Both of these have been observed infrequently when relic populations were present in Maine and Massachusetts (Schaefer 1974) but have been observed in this most recent outbreak of browntail moth.

This study examines the survival of browntail moth larvae (diapausing and late-stage larvae) and pupae across different sites in Maine. We hypothesize that browntail moth at different locations in Maine will be similarly impacted by changing abiotic conditions as is *L. dispar,* a closely related invasive species that also causes outbreaks in Northeastern regions of the United States. These include positive effects of increased temperatures on populations due to enhanced growth, and negative effects of increased precipitation due to higher incidence of disease. Our objectives to address these hypotheses are as follows: 1) Examine the relative population densities of diapausing larva, late-stage larva, pupa, and emergence of adults at different sites across Maine, 2) Estimate survival of these life stages across geographically separated locations (inland or coastal), and 3) examine whether the relative density and survival of browntail at different sites are influenced by abiotic factors (temperatures, precipitation). If the survival and density of this insect is heavily determined by abiotic factors, it could provide a tool for predicting the extent of outbreaks for public and forest health managers.

Methods

This study was conducted across the mid coast (Cumberland, Sagadahoc, Lincoln) and parts of central Maine (Penobscot, Kennebec, Waldo) over three years, from 2016 through 2018. Initial sample sites were selected to encompass the geographic extent of the infestation in 2016 based on winter web density estimates provided by the Maine Forest Service, Department of Agriculture Conservation and Forestry (MFS). Twenty-one sites across 14 Maine townships were monitored throughout the entire study (Fig 2), and as browntail moth spread throughout the state, 25 additional sites were added in 2017 and 2018. Sites were selected to be evenly distributed across the geographic range of the outbreak, and that included low to moderate height (2 m - 10 m) trees that could be successfully observed from the ground and sampled with 6 m pole pruners.

Population Monitoring

Browntail moth densities were sampled at three different life stages over each year, winter hibernacula, late stage post diapausing larvae, and pupae. For diapausing larvae in winter hibernacula, winter web surveys were conducted in collaboration with the Maine Forest Service (Jan - Mar). Technicians from the Maine Forest Service conducted road surveys throughout the infestation areas and estimated the range of density of winter webs in the surrounding trees via visual inspection. Webs are observable in trees in the latter half of the winter after leaf abscission. Winter webs are located at the tips of tree branches, in the apical portion of the tree canopy. These webs vary in the amount of observable silk and leaf matter that is visible but are usually clusters of leaves with thick silk that is visible from the ground. Web densities were recorded as: 0 nests, 1 – 9 nests, 10 – 99 nests, 100 – 499 nests, 500 – 999 nests, 1,000 – 4,999, and >5,000 nests for groups of 10 or less trees at a given site. Sampling of late-stage larva and

pupation nests were conducted during June and July, respectively, via timed observations. Observers walked around and under the host plant trees at each site, using handheld counters to record all observed live and dead caterpillars for a 10-minute period. Feeding live caterpillars were readily visible along the edges and undersides of leaves and through leaves in the sun. Pupal nests consisted of a network of foliage loosely connected by silk with anywhere from 1 - 100 pupa contained within. One or more observers conducted observations per site carefully moving branches as necessary. In areas of high densities of browntail moth larvae or nests, multiple observers sampled separate areas within the site. These repeated counts were averaged across all observers. Browntail moth adults prefer to lay egg masses at the very top of trees. Egg mass density counts were attempted but unsuccessful due to our inability to accurately distinguish egg masses from leaf marks and damage when observing the undersides of leaves at the top of canopy from the ground.

Insect Rearing

Winter hibernacula were collected from late March through early April presumably after the majority of the winter mortality had occurred. One to five accessible webs were collected per site by clipping branches just below a web with hand pruners or pole pruners (maximum of 6m). Samples were then held in cold storage at 4°C until all collections were complete (2 - 3 weeks). One web per site was then removed from cold storage and placed in a Fabri-Kal® deli cup container (473 ml plastic cup or plastic freezer bag). These containers were then held at an ambient laboratory temperature ~21°C and monitored daily for emergence of larvae. This process was repeated two times over 2 weeks to provide emergence data for three individual nests per site.

Late-stage larvae were also collected if accessible by hand or pole pruners. These collections were made in early to mid-June before larvae began pupating. Larvae were transported to the laboratory in coolers and stored at 10°C for up to seven days until they were processed. Larvae were placed in groups of 10 - 12 in petri dishes lined with moist filter paper and fed fresh foliage. Dishes were sealed with parafilm and kept in an environmental chamber with a 12 hr daylight cycle at 20°C. Host foliage provided to rearing larvae was either oak (*Quercus rubus*) or apple (*Malus* spp.). Caterpillars were monitored daily for mortality and natural enemy emergence, with dead larvae removed daily. Up to 50 individuals were collected from each site, with one third of those individuals reared out as described above, and the others stored at -80°C in 70% ethanol for later studies.

Pupation nest collections were also made at each site after timed density samples were conducted in late-June to mid-July. Pupation nest collection and storage methods were the same as for diapausing and late stage larvae. Once collected, pupation nests were immediately set up in 473mL Fabri-Kal® clear plastic cups with Fabri-Kal® clear plastic dome lids that were covered with cloth or loose mesh to allow oxygen and humidity exchange. In 2016, individual pupae were removed from nests and reared in individual 60mL Fabri-Kal® clear plastic condiment containers with lids. In 2017 and 2018, entire nests were reared after they were examined for density of pupae. This method was adopted to reduce handling time of nests which had very high quantities of hazardous setae. Nests were kept on the laboratory bench at ambient temperature (~ 17.3 °C) in a room with open windows to ensure all samples were reared under the same conditions. All containers were checked daily for emergence of moths, parasitoids, and pathogenic fungi. After emergence was complete, nests were dissected to confirm moth sex ratios, survival of pupae, and incidence of fungi. Fungal cadavers were isolated and frozen at -80

°C until they could be identified. Fungi were identified using the appropriate taxonomic keys (Humber 2005).

Abiotic and Biotic Data

Abiotic climate data was obtained from the National Oceanic and Atmospheric Administration's Climate Data Online service (NOAA 2019). Sites were assigned to the climate stations nearest to them (no greater than 30 km^2) , with some climate stations representing multiple sites. Yearly climate variables were calculated and summarized based on browntail moth life cycle from $July_{t0}$ - $June_{t1}$. Annual (12 months averages) climate variables calculated included average annual temperature (TAVG) and total annual precipitation (TPRCP). Habitat vegetation data was obtained through the National Land Cover Dataset (MRLC 2016). Due to collinearity between habitat types, three habitat types hypothesized to be most relevant were used for analyses: high intensity developed land, deciduous forest, and evergreen forest. Vegetation types present at each site were determined by identifying a 1.5 km radius around the center of each site using ArcGIS (ESRI 2011). Total m^2 were calculated for each habitat type within each 1.5 km area to obtain the m^2 for each habitat type within a site. Separate models were also conducted for the distance to nearest ocean coastline and distance to Peaks Island, Portland, Maine. This island is the epicenter of where small relic populations existed during the non-outbreak period of browntail moth and is used as a measure of the age of the current outbreak. Year was also added to each model to capture any temporal variation. Estimates of the abundance of successfully overwintered post-diapausing larvae emerging from hibernacula at each site were calculated by multiplying the density estimate of hibernacula for the site from the Maine Forest Service survey, by the mean proportion of live larvae that emerged from the sampled hibernacula from the site (n = 3). The midpoint of the range of hibernacula number

corresponding to the rank density from the hibernacula survey was used for our calculations (i.e. site rank density = 2, hence, estimated midpoint = 50 hibernacula). This measure of estimated early spring emerged larval abundance was added as a covariate in models for predicting the abundance of late-stage larvae, pupation nests, and adults.

Data Analysis

Relationships between abiotic and biotic variables and four different browntail moth life stages were explored using negative binomial generalized linear regressions. The life stages included: 1) emerged overwintered post-diapausing larvae; (estimates described above), 2) late stage larvae, abundance estimated from timed counts, 3) pupal nests, abundance estimated from timed counts, and 4) adults, represented by the number successfully emerging from pupae collected at each site adjusted by sample size. Two separate models were conducted for each of the four life stages observed using variables described above (average temperature, annual total precipitation, habitat, distance, year). This resulted in a total of eight negative binomial models. An analysis of variance was also run for each life-stage to test the effect of year and site, with data log transformed + 0.1 to meet assumption of analysis of variance. Life-stages tested included mean density rank for winter hibernacula, survival of overwintered post-diapausing larvae reared, late-stage larval density counts, late-stage larval survival estimates from subsamples reared, pupation nest counts, and adult emergence. All analyses were run in RStudio (version 1.1.414) using the MASS and car packages.

Results

The Effect of Year and Site on Browntail Moth Life-Stages

The mean rank for winter hibernacula was significantly different between sites ($F_{(2, 32)} = 2.82$, $P = 0.01$) but did not differ between years (Fig 9). The mean rank estimate for number of webs per tree when converted to number was 1,908 ± 465 in 2016 which decreased to 455 ± 150 in 2017 and 435 ± 96 in 2018. Across sites, the mean proportion of emerging overwintered larvae surviving was moderate and averaged 0.65 in mid coast sites but was not different between sites or years (Fig 10). There was also no significant difference between sites and years for estimates of overwintered post-diapausing larvae. No fungi were observed on dead larvae in winter hibernacula.

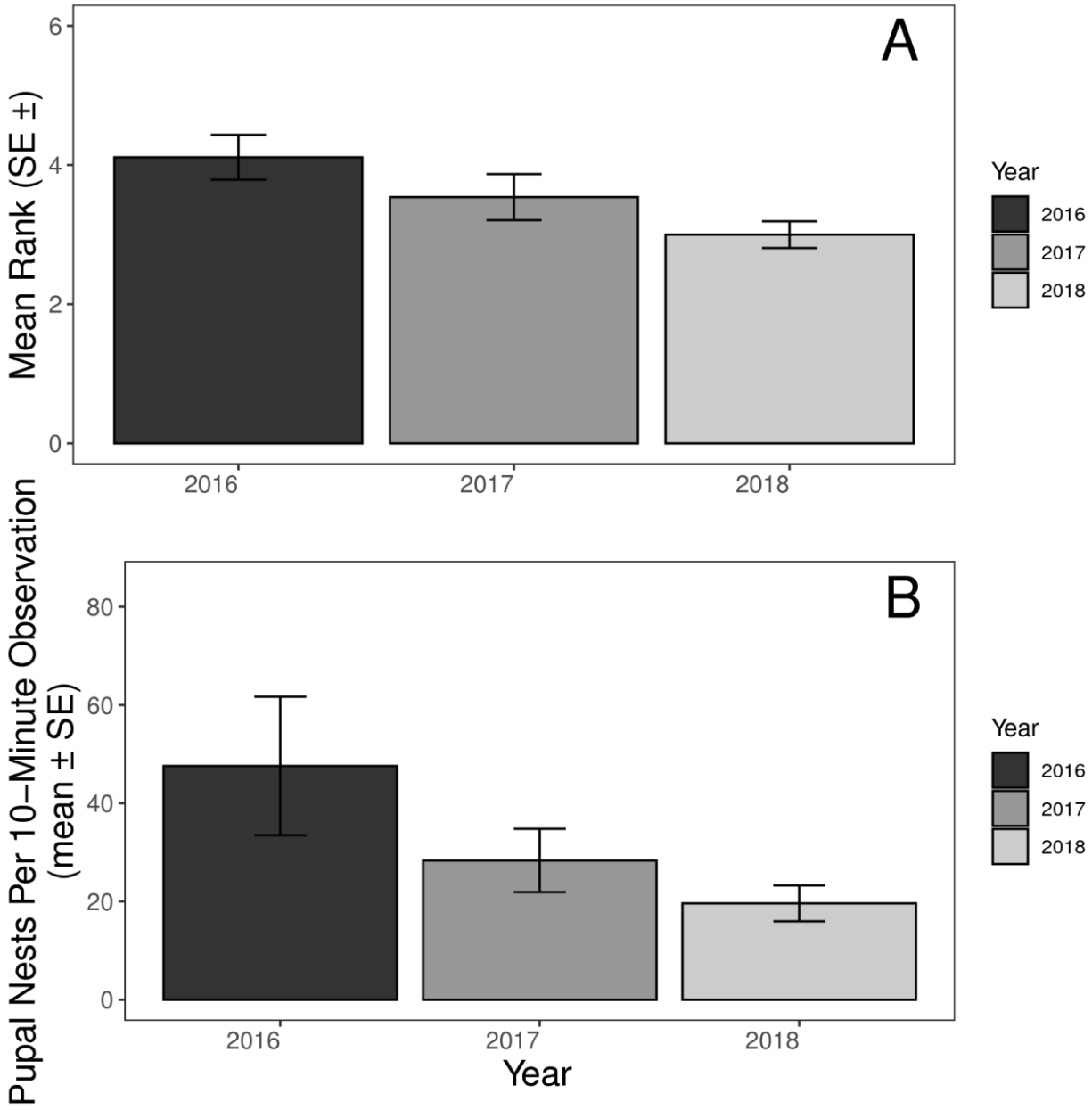

Figure 9. A) Mean rank density of winter hibernacula from the Maine Forest Service survey, across all sites for all years. B) Number of pupation nests counted per 10-minute timed density count averaged across all sites for all years. Note: Rank scales are as follows: 1 = 0 - 9 webs, 2 = 10 - 99 webs, 3 = 100 - 499 webs, 4 = 500 - 999 webs, 5 = 1,000 - 5,0000 webs, 6 = 5,000+ webs.

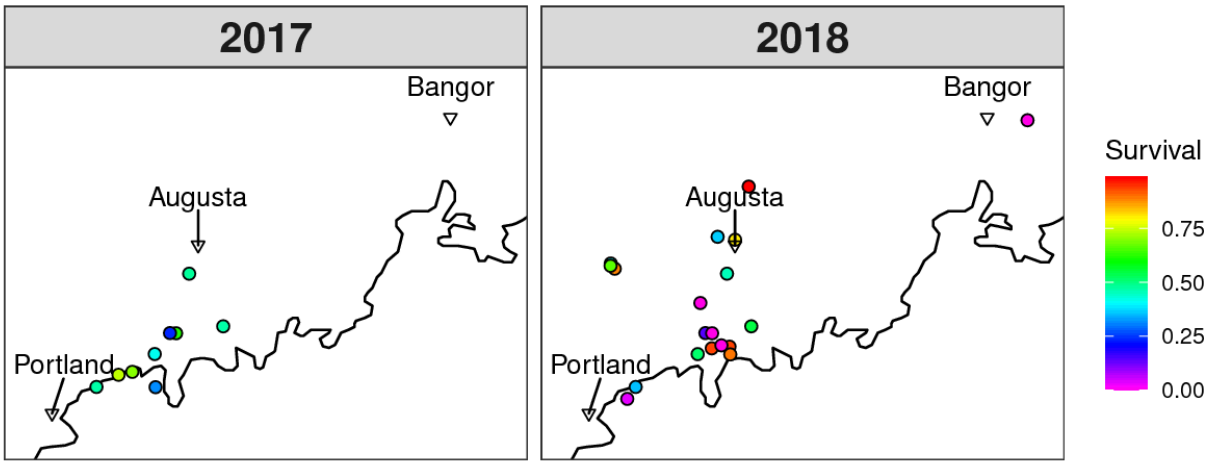

Figure 10. Mean proportion of overwintered post-diapausing *E. chrysorrhoea* that survived and emerged from winter hibernacula collected from sites across Maine between 2017 and 2018 (n=3 webs per site).

Late-stage larval counts were not significantly different between sites or years (Fig 10). The highest counts were in Waterville in 2017 with 322 larvae observed per 10-minute count and Wiscasset in 2018 with 58 larvae observed. Survival from subsamples of larvae collected in 2017 and 2018 indicated no significant differences between site and year. In 2017, 208 out of 1,050 reared larvae died of the fungus *E. aulicae*, and in 2018 this decreased with the decrease in larval samples, with only 10 out of 225 larvae sampled dying of the fungus.

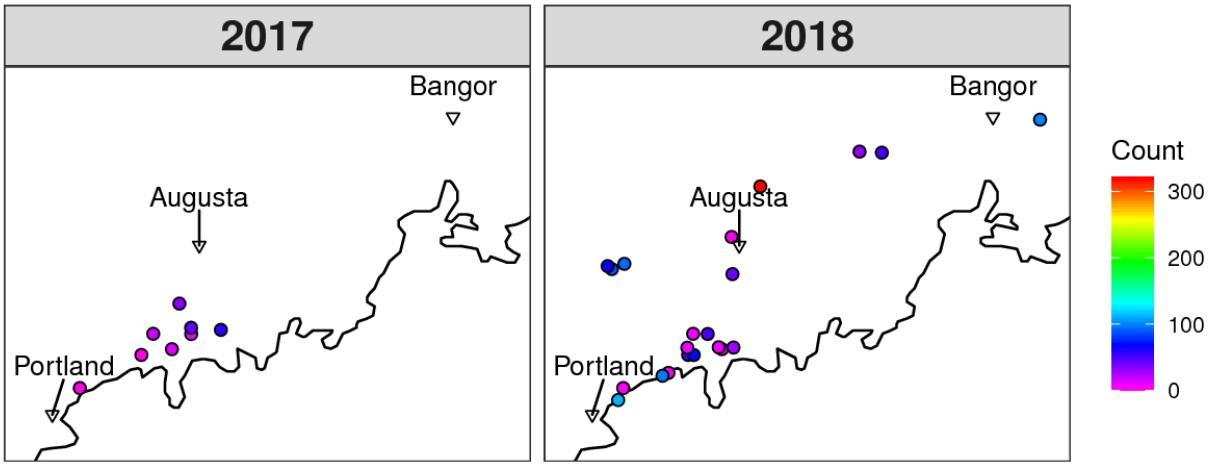

Figure 11. Mean number of *E. chrysorrhoea* larva counted over a ten-minute period across sites in Maine between 2017 and 2018.

Pupation nest abundance significantly varied between years ($F_{(2,42)} = 3.95$, $P = 0.03$) but not across sites between years (Fig 12). Nest abundance was higher in 2016 compared with the other two years, with a mean (± SE) of 50 ± 15 nests per site in 2016, 28 ± 6 in 2017, and 20 ± 4 in 2018. An outlier site in Burnham had a mean density count of 666 pupation nests per 10-minute density count in 2017, the largest across all years and sites, and was omitted from analyses. By comparison, the next highest pupation nest count was in Bowdoinham, Maine with a mean density count of 208 nests in 2016. In 2018 the highest mean count was in Wiscasset, Maine with 78 nests observed (Fig 12). In 2016, we collected and reared a total of 592 pupation nests containing 2,028 pupae from which 711 total adults emerged. In 2017, a total of 440 pupation nests were reared containing 1,364 pupae which resulted in 253 adults. In 2018, there were a total of 494 pupation nests reared which contained 1,418 pupae that resulted in a total of 419 adults. Of these, only 14 of the unermerged pupae in 2017, and 158 of the pupae in 2018 showed signs of a fungus. The presence of fungi in unemerged pupae was not recorded in 2016. In 2017, a mean of 11 percent of pupa reared emerged as adult females and a mean of 15 percent of pupa reared emerged as adult males.

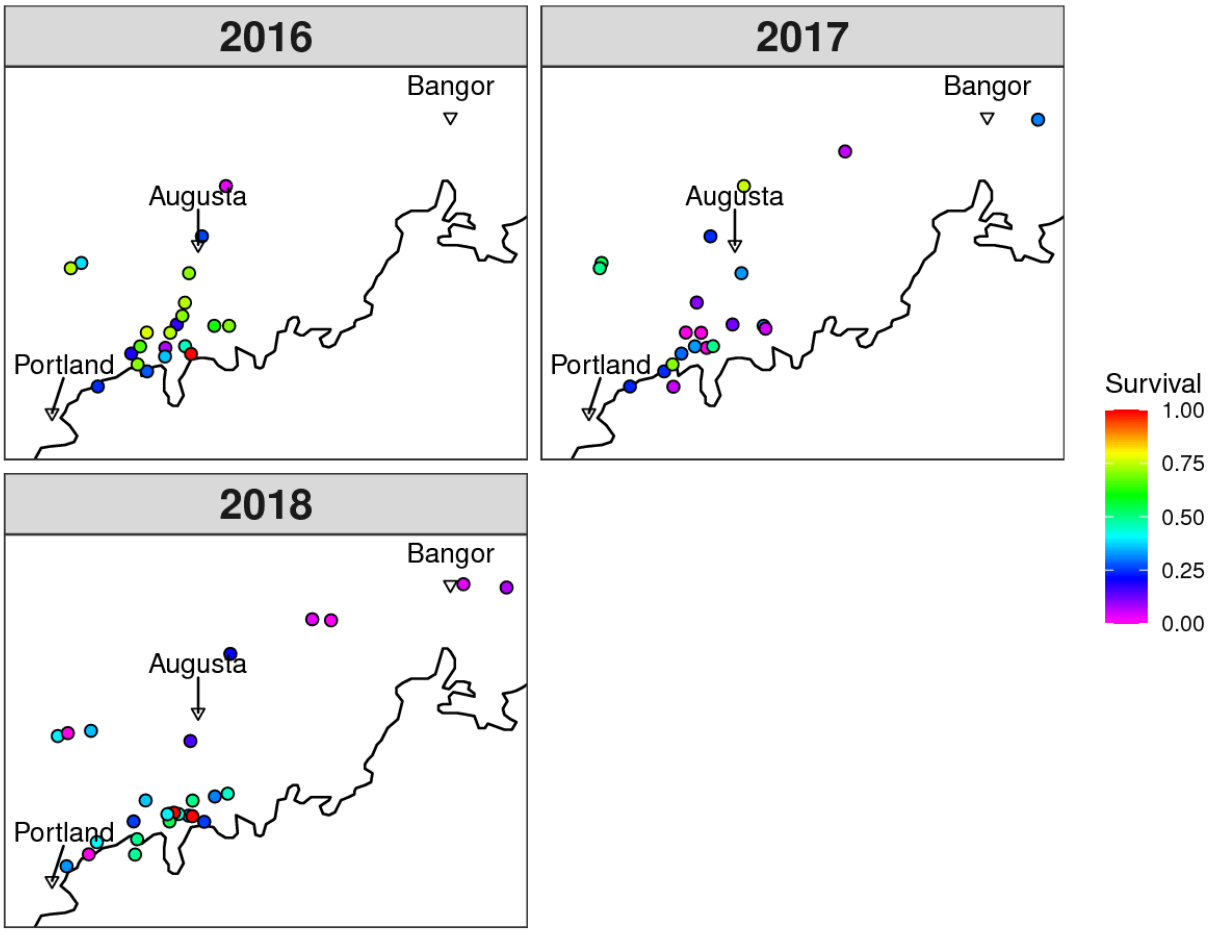

Figure 12. Mean number of *E. chrysorrhoea* pupation nests counted over a ten-minute period across sites in Maine between 2016 and 2018. Note: Burnham, Maine was divided in half to keep scale similar to other life stages (n = 666 / 10 m).

Adult emergence also changed across the study, with differences between years ($F_{(2,39)}$ = 5.42, P = 0.01) but not between sites between years (Fig 13). High emergence was concentrated across sites in the Mid-coast region in 2016 and 2018, while emergence was higher at peripheral sites like Burnham and Eddington in 2017. Emergence of male and female moths was recorded in 2017 and 2018 (Fig 14). In 2017, more males emerged than females (M:F = 1.16:1.00) while in 2018, the same number of males and females emerged.

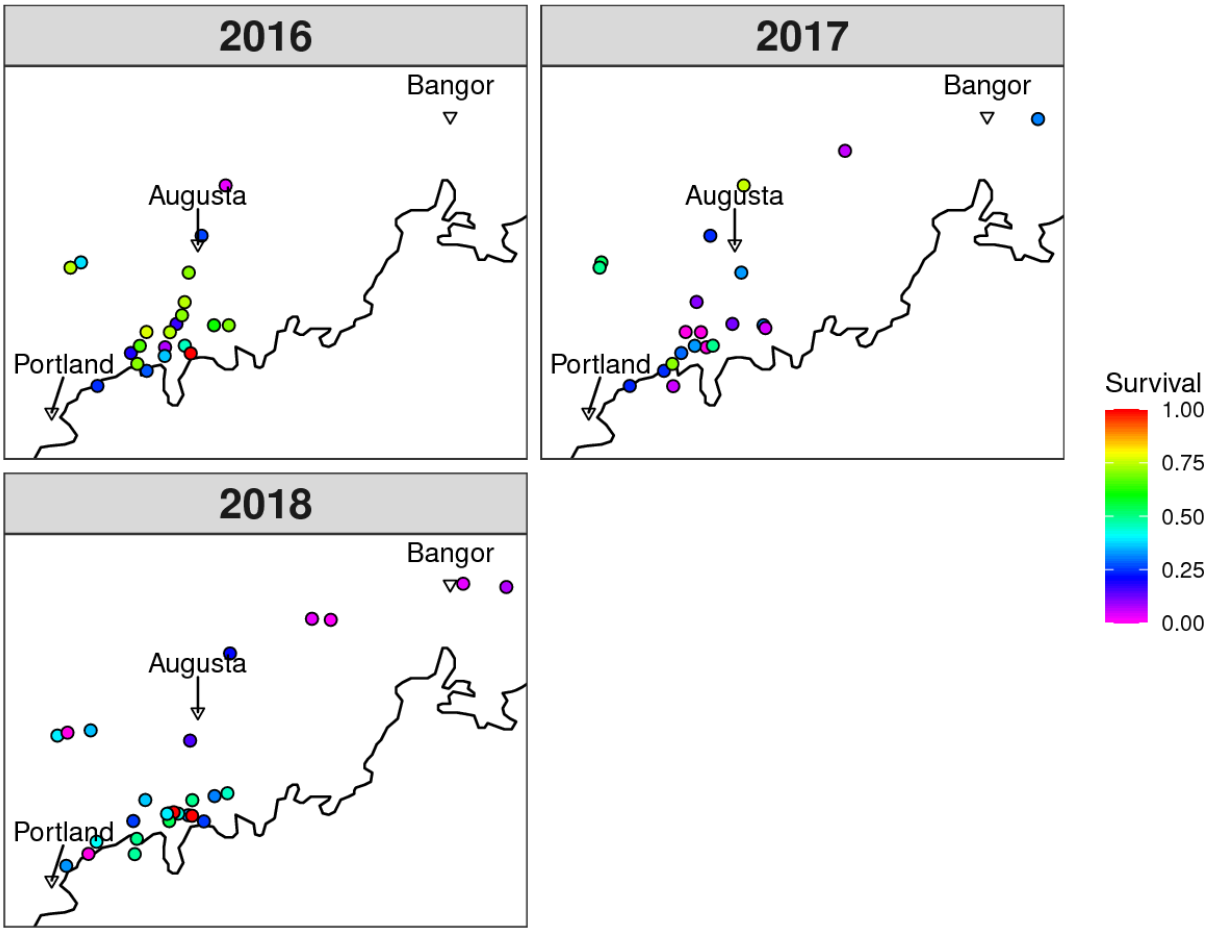

Figure 13. Proportion of adult *E. chrysorrhoea* emerged from pupation nests across Maine between 2016 and 2018.

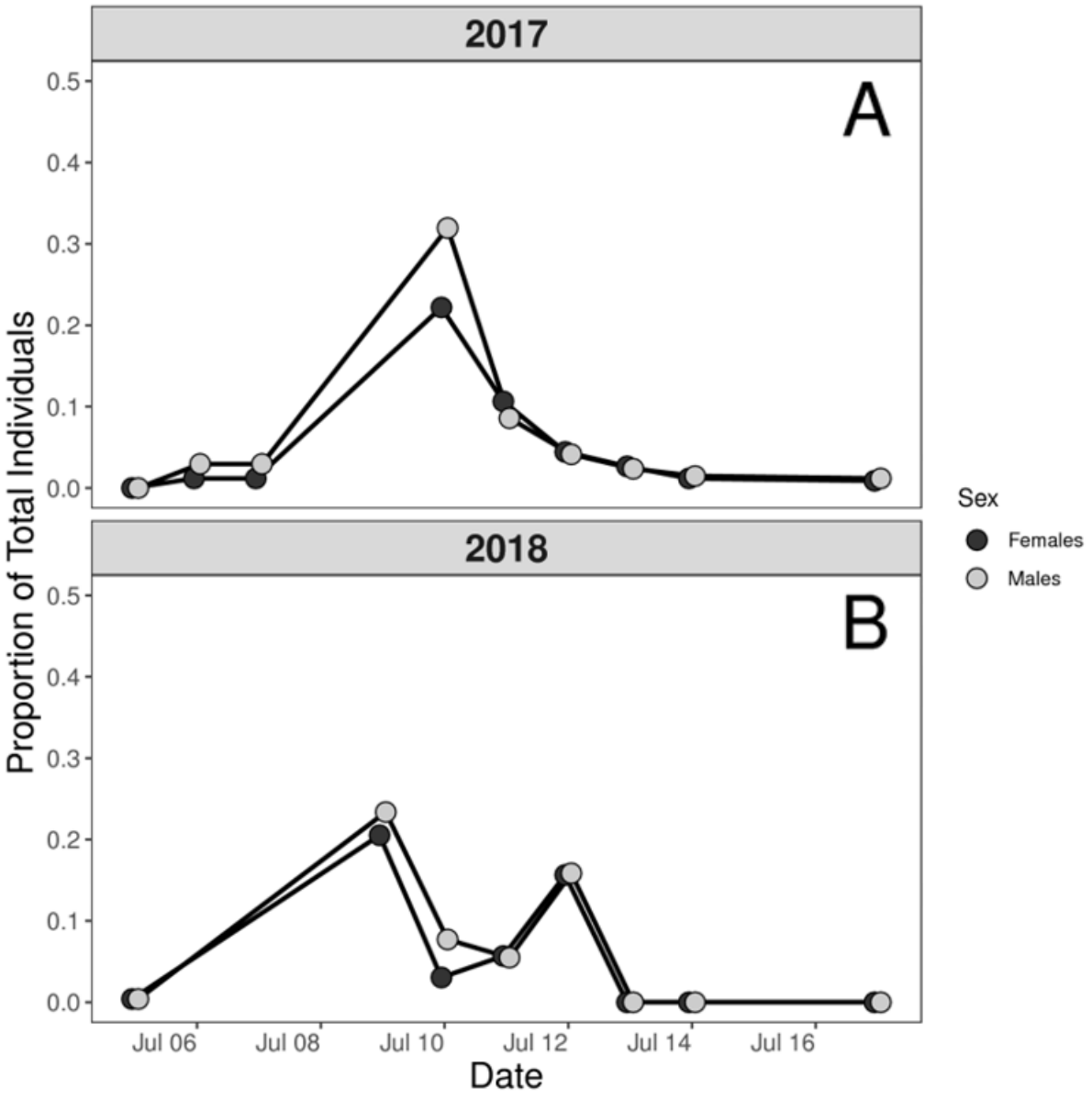

Figure 14. Proportions of adult male and female *E. chrysorrhoea* emergence from pupation nests in 2017 A) and 2018 B) collected across sites in Maine.

Climate and Habitat Impacts on Browntail Moth Life-stages

The negative binomial regression analyses for annual climate and proportion habitat indicated several significant relationships across browntail moth life stages (Table 8). The only climate variable that was a significant predictor of a browntail moth life stage was average annual temperature, which was negatively correlated with late-stage larval abundance. Habitat

variables were more consistent predictors of browntail moth abundance. Abundance of late-stage larvae and pupation nests increased with the amount of deciduous forest at a site and late-stage larvae decreased with the amount of evergreen forest. As the amount of high intensity developed land increased, the abundance of late-stage larvae decreased. Both the abundance of late-stage larvae and emergence of adult browntail moth were negatively correlated with the relative abundance of overwintered post-diapausing larval density. Pupation nest abundance was significantly lower in 2018 ($Z_{(21, 14)} = -2.53$, $P = 0.01$, coefficient $\pm$ SE = -1.72 $\pm$ 0.678).

Table 8. Negative binomial regression models of annual climate, vegetation, and year variables against counts across different life stages of browntail moth recorded across Maine from 2016 - 2018. Emerged OW larvae = overwintered larvae that survived and emerged from winter hibernacula.

Life-stage	Variables	Coefficient	Std. Error	Z^1	P
Emerged OW larvae	Evergreen forest	-10.487	4.41	-2.37	**0.01**
Late-stage larvae	Emerged OW larvae	-0.002	<0.001	-2.01	**0.04**
	Average annual temperature	-1.655	0.76	-2.16	**0.03**
	Deciduous forest	48.08	12.4	3.87	**0.001**
	Evergreen forest	-18.51	5.82	-3.17	**0.001**
	Developed land	-47.47	17.24	-2.75	**0.005**
Pupation nests	Deciduous forest	19.602	6.19	3.16	**0.001**
Adults	Emerged OW larvae	-0.002	0.6	-3.11	**0.001**

Z^1 = Log-likelihood statistic

Separate negative binomial regression models were also run for each browntail moth life stage for distance to nearest coastline and distance from Peak's Island, Maine, with year as a factor in the models. The relative abundance of overwintered post-diapausing larvae increased significantly with distance from the coast (z (24) = 2.314, $P = 0.02$, coefficient $\pm$ SE = 0.108 $\pm$ 0.046) and decreased significantly with distance from Peak's Island (z (24) = -3.159, $P = 0.001$, coefficient $\pm$ SE = -0.108 $\pm$ 0.034), but theses variables did not significantly predict the abundance of late-stage larvae or pupae, or the emergence of adults.

Survival of all life-stages increased between 2017 and 2018 (Table 9). In 2016, Pupae had the highest survival of all years, which subsequently decreased in 2017. Survival of overwintered larvae and late-stage larvae were not recorded in 2016.

Table 9. Mean proportion survival of overwintered larvae, late-stage larvae, and pupa from field collected and laboratory reared browntail moth during all study years. Note: Data missing in 2016 was before insect rearing began for the study. Value in parentheses is the total number of individuals (N) of the life stage per year.

	Mean survival		
Life Stage	2016	2017	2018
Overwintered Larvae	NA	0.45 (68)	0.73(115)
Late-stage Larvae	NA	0.8 (1050)	0.98 (225)
Pupa	0.52(2028)	0.29 (1364)	0.34(1418)

Discussion

Individual browntail moth life stages have not been previously examined to understand their fluctuating abundance during an outbreak period in North America. Characteristics of their winter hibernacula, including their height in host trees, weight, and volume, as well as the presence of parasitoids, can influence the survival of browntail moth larvae overwintering (Schaefer 1974). Additionally, Schaefer (1974) suggested that survival of overwintered larvae also varies with both the nutritional value of the tree leaves and insulation properties of the host leaves as well as parasitoid presence (Schaefer 1974). These were not measured in the present study but may have had an influence on our results. The cause of the decline in mean density rank estimates across the years of this study is difficult to speculate on. Winter webs are generally constructed in late summer by larvae feeding near their oviposition site. There is some evidence to support that females discriminate between and select for less damaged trees rather than those previously fed refoliated leaves produced the same year as severe spring defoliation (Shaefer 1974). The decrease in Maine Forest Service web estimates between 2017 and 2018 can

be explained by unfavorable conditions of high spring precipitation for larvae and pupae in the spring of 2017 that lead to lower populations of adults and subsequent eggs and later summer larvae. However, the decline in web abundance at sites between 2016 and 2017 may be due to another underlaying factor not examined in this study. There was considerable expansion of infestation area from 2016 to 2017. Adult browntail moth are known to be strong fliers (Schaefer 1974), and lower densities of winter webs at our sites in 2017 may represent dispersal of moths away from heavily damaged trees to new previously undamaged areas.

Emerged overwintered larvae, as previously described, are largely gregarious, and utilize winter hibernacula and newly constructed webs even after foliage has begun to leaf out. Overwintering survival and emergence of overwintered post-diapausing larvae changed across sites, with moderate survival across all sites in 2017 and variable survival as new sites were added in 2018 (Fig 10). Percent overwintering survival in our study (an average of 80%) was similar to that reported by Schaefer (1974) who found 85% survival in Maine. Winter survival of these larvae is typically not explained by low winter temperatures, as browntail moth can survive extreme lows of -17°C to -25°C (Gilliatt 1921, Schaefer 1974, Elkinton et al 2008). Temperature within webs may also influence survival, as webs with higher densities of larvae have higher temperatures than that of the atmosphere (Skoptsov 1968). Volume of organic matter incorporated into the web by larvae may also play a role in insulation of winter webs (Schaefer 1974). Under similar laboratory conditions, Schaefer (1974) described high humidity as factor that influences emergence of overwintered post-diapausing larvae, with larvae displaying less emergence under moist conditions. We found no relationship between total annual precipitation and overwintering survival of browntail moth larvae.

Based on field observations during the present study, browntail moth seem to display behavior similar to tent makers in Lasiocampidae, whereby pheromone trails are continuously laid with silk to provide "road maps" to where food and shelter is (Fitzgerald and Willer 1983). As larvae reach their latter instars, they become less gregarious and leave their communal webs altogether. Counts of late-stage larvae indicated that densities were not significantly different between sites and years (Fig 11). Survival of this life stage showed a trend of an increase between 2017 and 2018. An epizootic of the entomopathogen, *Entomophaga aulicae*, was widely distributed across the mid-coast browntail population during the very wet May and June of 2017 (mean 197 cm $\pm$ 40.63 cm) which likely caused a decrease in survival as this pathogen aggressively attacks larger larvae (Boyd and Groden, unpublished data). A drier, more favorable spring in 2018 resulted in a much lower incidence of disease (10 larvae) than in 2017 (208 larvae) and likely accounts for the higher late stage larval survival during this year.

Browntail moth larvae have an average of six instars before pupation, with a maximum of eight instars (Frago et al 2009). After solitary feeding, the late-stage larvae will often aggregate to create communal pupation nests. Throughout the duration of this study, this was observed with some large pupation nests containing upwards of 100+ pupae. At particularly high densities, larvae were observed spinning pupation webs in coniferous non-hosts. Counts of these pupation nests indicated that mean counts increased from 2016 to 2017, then declined in 2018. Sites with high counts were concentrated at mid-coast sites in 2016, inland sites in 2017, and remained lower across all sites in 2018 (Fig 12). By comparison, subsamples of larvae reared to emergence had the highest survival in 2016, which dropped to the lowest in 2017, and slightly increased in 2018 (Table 9). In developmental laboratory studies, Fargo et al (2009) found that the best predictors of pupation were induced diapause or "chilling", sex (males), number of instars, size

at maturity, and age (degree-days) at maturity. Partial life tables prepared by Schaefer (1974) and Frago et al (2011) indicate that parasitism had the highest impact on mortality of browntail moth during the larval and pupal stages. Many parasitoids, such as *Townsendiellomyia nidicola* Twns., are specialists that live within-host during the winter, through the spring feeding period, and emerge from pupae in the summer (see Ch. 2). Parasitism by *T. nidicola* was highest in 2018 (see Ch. 2) and hence did not account for the higher pupal survival in this year. Presence of *E. aulicae* in dead pupae was higher in 2018 than in 2017, but averages across sites were low 5.6 $\pm$ 7.9 in 2018 and 0.67 $\pm$ 1.32 in 2017. Earlier initiation of pupal development of larvae may have reduced mortality from disease during epizootic outbreaks in 2017, which is observed in *L. dispar* larvae during similar disease epizootics (Leonard 1974).

Adult males emerged at a slightly greater proportion than adult females (M:F = 1.16:1.00) in 2017 but were similar in the subsequent year (Fig 14). Survival of browntail moth from pupa to adult was highest throughout most mid-coast sites in 2016, inland sites in 2017, and again concentrated at mid-coast sites in 2018. Similar to the other life-stages observed throughout this study, mid-coast sites in 2017 were likely affected by a localized epizootic outbreak. Larvae that undergo more larval instars with higher pupal weights lead to higher realized fecundity of females (Frago et al 2009). This may also affect overall survival from pupa to adult, as heavier individuals likely have higher nutrient reserves for the process of pupation.

Negative binomial models revealed that average annual temperatures were negatively correlated with late-stage larval abundance, though not correlated with abundance of any other life stage (Table 8). It is not clear what is driving this relationship. The focus of climatic factors of browntail moth has previously been focused on minimum lethal temperature and winter temperature regimes on winter hibernacula (Schaefer 1974, Elkinton 2008, Frago et al 2011). In

L. dispar, temperature can influence development by increasing development rate at higher temperatures, allowing larvae and pupa to escape natural enemies (Leonard 1974, Alalouni 2012). Increases in average temperature could also increase female fecundity by allowing for larvae to develop quicker and into more instars, increasing female reproductive capacity (Frago et al 2009). Increases in average temperature had a negative impact during the present study. This could be a result of masked density-dependent mortality, where warmer temperatures promote faster growth and higher densities which then result in increased disease or decreased food quality due to defoliation causing density to decline. Total annual precipitation was not significantly correlated with abundance of any life-stage but could be important on a shorter timescale, especially during months and seasons where pathogens are active.

Both larval stages and pupation nest abundance was significantly influenced by at least one or all the forest habitat variables (Table 8). In each case, the amount of deciduous forest had a positive relationship with larval abundance while the amount of evergreen forest had a negative relationship with counts. Although browntail moth are polyphagus feeders, they do not survive on coniferous hosts in most populations, and highly prefer hosts in the *Quercus*, *Malus*, and *Prunus* genera (Schaefer 1974). Coniferous stands with fewer deciduous hosts could mean starvation when larvae cause complete defoliation and have no alternative hosts. High-intensity developed land was negatively correlated with late-stage larvae, likely a result of developed landscapes areas having shorter and more sparse hosts available when total defoliation occurs. It may also reflect increased pesticide use in more intensely managed landscapes.

Negative binomial regressions also revealed a significant positive relationship between abundance of overwintered larvae emerging from hibernacula and distance to nearest marine coastline. These relationships could be explained by this outbreak expanding into new areas,

allowing browntail moth to be released from some of their natural enemies (Jeffries and Lawton 1984, Schönrogge 1995, Davis et al 2018). In many areas, except for the mid-coast region of Maine, outbreaks have not been experienced on this scale for 75 - 100+ years. This current outbreak of browntail moth could be functioning as if it has been released from natural enemies or similar to a new introduced pest. Previous studies have shown that there are stark differences in survival at coastal (<1km) and inland (>1km) sites, likely a result of microclimate, host availability, and parasitoid prevalence (Shaefer 1974, Elkinton et al 2008). In the present study, we found different results, where distance to coast positively influenced abundance of emerged overwintered larvae. This is likely due to several factors described by differences in study methodologies and the outbreak state of the population (See Chapter 2).

Emerged overwintered larvae were the only life-stage that had a significant relationship with distance to Peaks' Island, Maine, the region where the recent outbreak was thought to originate. Abundance decreased with increasing distance. We used distance to Peaks Island, thought to represent the "history "of the outbreak, as a measure of the age of the infestation at our sites throughout Maine. Hence, these results suggest that the number of emerging overwintered larvae is higher in the older infestation sites than the newer sites, or distance from Peak's Island may not be a good proxy for the age of the infestation. In either case, this relationship and the influence of infestation age warrants further investigation.

The covariate of emerged overwintered larvae were significant for late-stage larvae and adults, where abundance decreased as emerged overwintered larvae emergence increased. This may also be due to an unaccounted-for density dependent factor, which may negatively influence abundance by increasing rates of starvation, disease transmission, and exposure to parasitoids.

In conclusion, populations of browntail moth seem to be influenced by both abiotic and biotic factors. Populations fluctuate differently closer to the coast than those of the inland populations but may synchronize given optimal conditions. Evidence of this is the increase in counts and survival across life-stages in 2018 after the high precipitation events of spring 2017 (See ch 2.). Based on our analyses, we can conclude there is not one factor that influences browntail moth abundances across different life-stages, a finding that does not reflect that of *L. dispar* larval populations that are heavily influenced by temperature and precipitation, but are influenced heavily by parasitoids similar to *L. dispar* (Leonard 1974). Average annual temperature was less important for browntail moth, but could be an important factor for larval development, as higher average annual temperatures could cause desiccation or increase rate of development for larvae. Landscape vegetation composition characteristics were significant for both immobile non-feeding life-stages and those that feed. Habitat types with more host trees were positively correlated with abundance, while those that had fewer hosts were negatively correlated with abundance. These vegetation features could be explored further, including host species and more fine-scale habitat characteristics. Overall, the current outbreak of browntail moth in Maine is a dynamic, changing system that requires further investigation into the drivers for expansion and fluctuation of populations. These findings give us a baseline knowledge of the survival and densities of populations at the beginning of the outbreak, the factors that may influence different life-stages of browntail moth, and how they change spatially and temporally.

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APPENDIX A: LIST OF SITES

Table 10. All sites, weather stations, their latitude and longitude, and the year sampled.

Site	Weather Station ID	Year	Latitude	Longitude
Augusta 1	USW00014605	2016	44.3527	-69.7850
Bath 1	USC00170409	2016	43.9269	-69.8587
Bath 2	USC00170409	2016	43.9332	-69.8244
Bath 3	USC00170409	2016	43.9036	-69.7975
Bowdoinham 1	USC00170409	2016	43.9852	-69.8914
Bowdoinham 2	USC00170409	2016	44.0160	-69.8320
Bowdoinham 3	USC00170409	2016	43.9845	-69.8320
Brunswick 1	USC00170409	2016	43.9050	-69.9660
Brunswick 2	USC00170409	2016	43.8938	-69.9059
Dresden 1	USC00173046	2016	44.0490	-69.7725
Dresden 2	USC00173046	2016	44.0571	-69.7731
Gardiner 1	USC00173046	2016	44.2118	-69.7844
Mere Point 1	USC00170409	2016	43.8370	-70.0105
Mere Point 2	USC00170409	2016	43.8636	-69.9801
Richmond 1	USC00173046	2016	44.0998	-69.8692
Richmond 2	USC00173046	2016	44.0991	-69.8658
Six River Farm	USC00170409	2016	44.0082	-69.8384
Topsham 1	USC00170409	2016	43.9324	-69.9399
Turner 1	USC00178817	2016	44.2508	-70.2354
Turner 2	USC00178817	2016	44.2308	-70.2436
Wiscasset 1	USW00094623	2016	44.0109	-69.6679
Wolfneck 1	USC00170409	2016	49.5090	-70.0456
Yarmouth 1	USW00014764	2016	43.7794	-70.1814
Augusta 1	USW00014605	2017	44.3527	-69.7850
Bath 1	USC00170409	2017	43.9269	-69.8587
Bath 2	USC00170409	2017	43.9332	-69.8244
Bath 3	USC00170409	2017	43.9036	-69.7975
Bowdoinham 1	USC00170409	2017	43.9852	-69.8905
Bowdoinham 2	USC00170409	2017	44.0160	-69.8320
Bowdoinham 3	USC00170409	2017	43.9845	-69.8320
Brunswick 1	USC00170409	2017	43.9050	-69.9660
Burnham 1	USC00179151	2017	44.6787	-69.3364
Cundy Harbor Cemetery 1	USC00170409	2017	43.8365	-69.8994
Dresden 1	USW00014605	2017	44.0572	-69.7727
Eddington	USW00094644	2017	44.7970	-68.5926
Gardiner 1	USW00014605	2017	44.2118	-69.7844

Table 10. Continued

Harpswell 1	USC00170409	2017	43.7785	-69.9364
Mere Point 1	USC00170409	2017	43.8370	-70.0105
Mere Point 2	USC00170409	2017	43.8636	-69.9801
Richmond 1	USC00170409	2017	44.0998	-69.8692
Six River Farm	USC00170409	2017	44.0082	-69.8384
Topsham 1	USC00170409	2017	43.9324	-69.9399
Turner 1	USC00178817	2017	44.2508	-70.2354
Turner 2	USC00178817	2017	44.2308	-70.2436
Waterville 1	USC00179151	2017	44.5444	-69.6651
Wiscasset 1	USW00094623	2017	44.0109	-69.6679
Wiscasset 2	USW00094623	2017	44.0001	-69.6450
Wolfneck 1	USC00170409	2017	43.8259	-70.0857
Yarmouth 1	USW00014764	2017	43.7794	-70.1814
Augusta 1	USW00014605	2018	44.3527	-69.7850
Augusta 2	USW00014605	2018	44.3413	-69.7971
Bangor 1	USW00014606	2018	44.8103	-68.7978
Bath 1	USC00170409	2018	43.9269	-69.8587
Bath 2	USC00170409	2018	43.9332	-69.8244
Bath 3	USC00170409	2018	43.9036	-69.7975
Bath Wiskeag	USC00170409	2018	43.9340	-69.8325
Bath Wooduck	USC00170409	2018	43.9382	-69.8467
Bath/Cemetery	USC00170409	2018	43.9251	-69.8285
Bowdoinham 1	USC00170409	2018	43.9852	-69.8905
Bowdoinham 3	USC00170409	2018	43.9845	-69.8320
Brunswick 1	USC00170409	2018	43.9050	-69.9660
Brunswick 2	USC00170409	2018	43.9048	-69.9150
Burnham 1	USC00179151	2018	44.6766	-69.3338
Dixmont 1	USC00179151	2018	44.6727	-69.1963
Eddington 1	USW00094644	2018	44.7979	-68.5913
Falmouth 1	USW00014764	2018	43.7339	-70.2104
Gardiner 1	USW00014605	2018	44.2118	-69.7844
Harpswell 1	USC00170409	2018	43.7785	-69.9364
Mere Point 1	USC00170409	2018	43.8370	-70.0105
Mere Point 2	USC00170409	2018	43.8636	-69.9801
Richmond 1	USW00014605	2018	44.0998	-69.8692
Topsham 1	USC00170409	2018	43.9324	-69.9399
Turner 1	USC00178817	2018	44.2508	-70.2354
Turner 2	USC00178817	2018	44.2308	-70.2436
Turner 3	USC00178817	2018	44.2421	-70.2390
Waterville 1	USC00179151	2018	44.5444	-69.6651

Table 10. Continued

Wiscasset 1	USW00094623	2018	44.0109	-69.6679
Wiscasset 2	USW00094623	2018	44.0001	-69.6450
Wolfneck 1	USW00014764	2018	43.8259	-70.0857
Yarmouth 1	USW00014764	2018	43.7794	-70.1814

BIOGRAPHY OF THE AUTHOR

Karla Stryker Boyd was born in Newburyport, MA. She was raised in Hilton Head Island, SC and graduated from Hilton Head Island High School in 2012. She attended the University of Maine in Orono, Maine and received a Bachelor of Science in Wildlife Ecology in 2016. During the 2015 spring semester, Karla took a Tropical Ecology course in Peru where she found her passion in Entomology. While in college, she worked on campus in several wildlife labs on various projects. After graduating, she started as a research technician at the University of Maine where she worked on several projects including European fire ant bioassays, winter moth bioassays, and browntail moth surveys. As the browntail moth population became a serious problem in the State of Maine, Karla entered the School of Biology and Ecology Master of Science program at the University of Maine in the summer of 2017. She is a member of the Entomological Society of America and the Maine Entomological Society. Her research focus has been on the natural enemies of the browntail moth (*Euproctis chrysorrhoea*). She is a candidate for the Master of Science degree in Entomology from the University of Maine in May 2020.