## Title

Ecology and Intraguild Relationships Among the Invasive Wasp Ophelimus maskelli and Two Associated Parasitoid Wasps Closterocerus chamaeleon and Selitrichodes neseri (Hymenoptera: Eulophidae) in California

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# Ecology and Intraguild Relationships Among the Invasive Wasp *Ophelimus maskelli* and Two Associated Parasitoid Wasps *Closterocerus chamaeleon* and *Selitrichodes neseri* (Hymenoptera: Eulophidae) in California

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of the requirements for the degree of

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in

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by

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# ABSTRACT OF THE THESIS

## Ecology and Intraguild Relationships Among the Invasive Wasp *Ophelimus maskelli* and Two Associated Parasitoid Wasps *Closterocerus chamaeleon* and *Selitrichodes neseri* (Hymenoptera: Eulophidae) in California

by

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Master of Science, Graduate program in Entomology
University of California, Riverside, September 2017
Professor Timothy D. Paine, Chairperson

The Eucalyptus gall wasp, *Ophelimus maskelli*, is a significant pest in areas throughout the world. Heavy galling significantly weakens trees as well as causing early defoliation leading to premature death. Eucalyptus species are grown all over the world for various uses including fuel and paper making. The plants are especially prized in plantations for their rapid growth and development. *O. maskelli* was recently discovered in southern California along with two associated parasitoid wasps. *Closterocerus chamaeleon* is a known parasitoid of *O. maskelli* and has been intentionally introduced in areas of Israel as well as throughout the Mediterranean for use as a biological control agent. The second parasitoid wasp, *Selitrichodes neseri*, has been intentionally introduced in South Africa as a biological control agent for another Eucalyptus gall wasp, *Leptocybe invasa*. Phenologies of each species in southern California are recorded and discussed using two different collection methods. Comparisons are made between species, inland and coastal populations of southern California, and populations in Israel. Morphological, molecular, and ecological data are combined in the description of a new strain of *S. neseri* in southern California. Our studies discuss ecological relationships between these three wasp species as well as provide a groundwork for further research involving the adjustment of invasive species to a new environment.

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## Chapter 1. General Introduction

Although native to Australia, eucalyptus species are now grown all over the world and have become a highly profitable investment. In fact, it is now the world's most widely planted hardwood species (Indufor Plantation Databank 2012). Eucalyptus is grown commercially in plantations on every continent, with the sole exception of Antarctica. The trees are favored for their rapid growth and excellence in both paper and energy production. Compared to other commercially grown timber, eucalyptus is ideal for its stable growth which occurs at a much faster pace than other wood species. In ideal conditions eucalyptus trees can yield rates of return of over 15% (Tree Plantation 2016). Of the total 54.3 million ha of industrial forest plantations across the world, 26% is made up of eucalyptus. The only species to surpass eucalyptus in total area is pine (Indufor Plantation Databank 2012). It has also been estimated that the growth of eucalyptus plantations will continue to rise over the next 30 years (Indufor Plantation Databank 2012).

Not surprisingly, eucalyptus trees are found all over California and are extremely valuable (Doughty 2000). Ninety different species of eucalyptus can be found throughout California. Eucalyptus trees are not only used for making paper and fuelwood, but essential oils are extracted from the foliage and trees are often planted as windbreaks. The trees are found in both landscapes and urban areas. One particular survey estimated the average value of individual eucalypt street trees at $5,978 in California (Paine et al. 2015).

Eucalyptus were first brought to North America in 1850 as seeds, to survive trans-Pacific crossing in the days of sailing ships and to avoid importing pests with them. The trees remained pest-free in North America for over a hundred years. In 1984, eucalyptus long horned borer

(*Phoracantha semipunctata)* was discovered in California and became a major pest, colonizing and killing many eucalyptus trees (Hanks et al. 1993). In June of 1998, *Glycaspis brimblecombei,* the red gum lerp psyllid, was discovered in Los Angeles County (Brennan et al. 1999). Within the next year, two more pests were discovered in California: the lemon gum lerp psyllid (*Cryptoneossa triangula*) and the eucalyptus tortoise beetle (*Trachymela sloanei*) (Garrison 1998). In the ten years following, eucalyptus snout beetle (*Gonipterus scutellatus)*, eucalyptus psyllid (*Blastopsylla occidentalis*), Tristania psyllid (*Ctenarytaina longicauda)*, and the lesser eucalyptus long horned borer (*Phoracantha recurva*) were all discovered in California (Paine et al. 2011). Over the last 25 years, a total of 16 new eucalyptus pest species have shown up in the state of California (Paine et al. 2011). The most recently discovered of these pests is *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) (Burks et al. 2015a).

The eucalyptus gall wasp, *Ophelimus maskelli* (Hymenoptera: Eulophidae), is a uniparental gall-inducing pest of various species of eucalyptus trees. The wasp originated in New South Wales, Australia and has invaded the Mediterranean Basin, Southeast Asia, Northern Africa, and most recently, California (Protasov et al. 2007a, Burks et al. 2015a). Along with the discovery of *O. maskelli* in California, two additional wasp species have been found emerging from the galls. The first, *Closterocerus chamaeleon* (Girault) (Hymenoptera: Eulophidae), is a known associated parasitoid of *O. maskelli* (Burks et al. 2015b). The remaining species has been identified as a California strain of *Selitrichodes neseri* (Hymenoptera: Eulophidae: Tetrastichinae).

All three of these wasps are members of the family Eulophidae. When dealing with invasive species, it is important to know the taxonomy of the insects. Other closely related insects may have similar biology or ecology, which may aid in determining more about a new species and developing appropriate control measures. Eulophidae contains 283 genera and over 4000 described species (Gauthier et al. 2000). Most of the individuals within this family are parasitoid wasps, with just a few species feeding on plant tissue as larvae. The group is quite abundant and species can be found in several niches across the world. *Ophelimus maskelli,* being a gall-former, is one of the few members of this group that is not a parasitoid. *Selitrichodes neseri* has previously been known to parasitize another eucalyptus gall wasp, *Leptocybe invasa* (Kelly and La Salle 2012). The genus *Selitrichodes* is relatively small including only 14 described species. Interestingly, as with the family Eulophidae, we continue to find both parasitoids and gall-formers within this small group. Recently, *Selitrichodes globulus* was found producing galls on blue gum eucalyptus in California (La Salle et al. 2009). However, *S. neseri* has been observed as a larval ectoparasitoid of *O. maskelli* in California and does not produce galls of any kind.

In the Mediterranean Basin, *Ophelimus maskelli* invaded and became an uncontrolled significant pest by 2004 (Protasov et al. 2007b). The wasp produces small round galls, about a millimeter in diameter on the leaf blades of eucalyptus. The galls may turn red or purple in color when exposed to sunlight for an extended period of time, making them very easily recognizable. Each gall contains one developing larva and a single female produces 109 galls on average. Wasps prefer to attack leaves of the lower canopy which are 30 to 45 days old (Protasov et al. 2007a). Heavy galling causes the tree to allocate nutrient resources to the forming of galls, resulting in increased amounts of stress and causes the tree to become more susceptible to pathogens and disease. Additionally, heavy galling causes early defoliation of the tree soon after wasp emergence. Major infestations can pose respiratory health problems to humans at peak emergence (Protasov et al. 2007a). *Ophelimus maskelli* is successful in colonizing several eucalypt host species including; *E. botryoides* (Smith), *E. bridgesiana* (R.Baker), *E. camaldulensis* (Dehnh), *E. cinerea* (F. Muell), *E. globulus* (Labill), *E. grandis* (W. Hill), *E. gunii* (J.D. Hook), *E. nicholii* (Maiden), *E. pulverulenta* (Sims), *E. robusta* (Smith), *E. rudis* (Endl), *E. saligna* (Smith), and *E. viminalis* (Labill) (Protasov et al., 2007a). However, particularly great success has been noted in Red River gum, *E. camaldulensis* (Protasov et al., 2007a).

In 2004 a classical biological control project was launched to control *O. maskelli* pest populations in Israel (Protasov et al., 2007b). Classical biological control consists of returning to the pest's native habitat to find a natural enemy with hopes of introducing the natural enemy alongside the pest and therefore naturally controlling pest populations. *Closterocerus chamaeleon* was identified from Australia as a suitable natural enemy and was released in several locations throughout Israel. It has since been shown to be successful as a biological control agent (Protasov et al. 2007b). *Closterocerus chamaeleon* is also a uniparental species and a larval ectoparasitoid of *O. maskelli* attacking late second and third instar larvae (Protasov et al. 2007a). Uniquely, *C. chamaeleon* is active during the winter months when the development of *O. maskelli* is essentially arrested, contributing to its success as a biological control agent.

As a newly invasive species to California, nothing is known about *O. maskelli* relative to this new environment. It is important to determine whether or not emergence patterns are similar to those exhibited in the Mediterranean Basin. Management techniques can be tailored with such information, particularly when attempting to use chemical controls on insects protected within a gall. Along with phenology of *O. maskelli*, it is important to monitor emergence and population size of the associated parasitoids, *C. chamaeleon* and *S. neseri*. This information can give insight into the success of both parasitoids in California. Monitoring both a coastal and an inland site gives us valuable information regarding the relationship between these wasps and their environment. This relationship could be used to predict adult emergence as well as provide insight into wasp development and temperature-dependent survivability. Sampling with sticky traps can additionally help to monitor for other eucalyptus gall wasps that may be introduced at any time.

In its native habitat of New South Wales, Australia, *O. maskelli* is considered a minor pest (Inghilesi et al. 2013). This is most likely due to the presence of natural enemies. Although *C. chamaeleon* has proven to be a successful biological control agent in Israel and the surrounding areas (Protasov et al., 2007b), there are heavy infestations of *O. maskelli* in California where *C. chamaeleon* is also present. This provides a unique opportunity to answer both species-specific and general questions that lend helpful information to areas of ecology, pest management, and insect behavior. Because the California strain of *S. neseri* parasitizes a different host, it is important to learn more about the biology for this species, especially in this area. Monitoring and sampling the population of *S. neseri* can determine the phenology and emergence periods relative to the other two wasps. This system affords the unique opportunity to examine the intricate interactions between this new strain and two additional invasive species. Understanding the relationships among these wasps is crucial in determining how we can control *O. maskelli* populations in California.

The competitive exclusion principle states that two individual species cannot occupy the same niche within an environment. There must be some environmental, ecological, behavioral, or morphological factor that separates the two species' niches. Over-lapping of this niche space results in competition for resources. *Selitrichodes neseri* and *C. chamaeleon* are host-specific parasitoid wasps which compete for hosts. Competition among parasitoids, in general, is intentionally avoided by the mother when choosing where to lay her eggs (Salt 1961). Many other insects lay as many eggs as possible, regardless of the potential competition among their progeny. This offers a unique ecological study system with regards to competition, and particularly competition within a gall. Many parasitoid wasps are capable of detecting whether or not a host has previously been parasitized and thereby significantly reduces the amount of interspecific competition (Godfray 1994; Hanks et al. 2001; Taylor 1988). However, in a system where more than one parasitoid species shares a particular host, the mother is no longer strictly avoiding intraspecific competition, but interspecific competition as well. Under circumstances where available hosts are scarce, we are likely to see an increase in both interspecific and intraspecific competition (Salt 1961; Taylor 1988). These types of situations result in phenomena such as superparasitism, multiparasitism, hyperparasitism, and facultative hyperparasitism.

For this particular system, the presence of another parasitoid wasp, *S. neseri,* may play some type of interference role with regards to pest management. It is known that *C. chamaeleon* attacks late second and third instar larvae *of O. maskelli* (Protasov et al. 2007b). However, the host preference of *S. neseri* with regards to larval instar remains a mystery. It is likely that *S. neseri* may operate in a facultative hyperparasitoid capacity due to constraints placed on it by a strong presence of *C. chamaeleon* and the reproductive advantages of this uniparental species. This hypothesis is further supported by our knowledge of differing host species parasitized by the South African strain of *S. neseri*. Elucidating some of this intraguild competition in the future may have important resulting effects on ecology and pest management.

This unique ecology involving competition among parasitoids can give insight into the use of parasitoids as biological control agents. Specifically, intraguild competition has been a topic of extreme interest as it pertains to the effectiveness of multiple biological control agents (Denoth et al. 2002; Kakehashi et al. 1984; Pedersen and Mills 2004; Snyder and Ives 2001). Intraguild competition is not only affected by prey abundance, but can have an impact on prey abundance itself. Interactions among competing parasitoids may result in a greater risk of parasitism through additive mortality or conversely result in parasitism interference lessening the effects of a potential biological control agent (Brodeur and Rosenheim 2000; Müller and Brodeur 2002; Rosenheim et al. 1995). Biological control agents play an extremely significant role in the control of both *O. maskelli* and the many other pests we find on eucalyptus. The primary control method for most eucalyptus pests utilizes a natural enemy (Hanks et al. 2001; Hodkinson 1999; Kay 1990; ). When developing an efficient integrated pest management protocol for eucalyptus pests the protection of all natural enemies is vital to its success. Therefore, understanding the role of competition and interactions among various parasitoids is a crucial part of developing these control methods.

The system in Southern California involving *O. maskelli* and its associated parasitoids, *C. chamaeleon* and *S. neseri* poses several interesting questions regarding both parasitoid competition and the effects of intraguild competition within a biological control setting. *O.*

*maskelli* occurs naturally in New South Wales, Australia with several different parasitoids including *C. chamaeleon.* However, the pest population in Southern California does not appear to be controlled which may be caused by some type of interference effect resulting from competition.

Furthermore, these types of studies not only examine the relationships among these particular wasps, but can lend further understanding of relationships among invasive pests worldwide that invade along with a natural enemy. This particular situation may have important implications for biological control of invasive species in the future. These studies give helpful insight into the ecological transition of invasive insects and how that may change their life history and success within a new niche.

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## **Chapter 2**: "Seasonal phenology of two parasitoid wasps (*Closterocerus chamaeleon, Selitrichodes neseri*) and their gall-forming host (*Ophelimus maskelli*) at two sites in Southern California"

### Abstract

A eucalyptus gall wasp, *Ophelimus maskelli* (Ashmead) (Hymenoptera; Eulophidae), and two associated parasitoid wasps, *Closterocerus chamaeleon* (Girault) (Hymenoptera; Eulophidae) and *Selitrichodes neseri* (Hymenoptera; Eulophidae), have been found in Southern California. Two infested areas were monitored and sampled for a 12-month period, comparing inland and coastal phenologies. Adult *O. maskelli* emerge from their galls once per year inland and twice per year near the coast. Sampling was done using both green sticky traps and foliage collection. Greater numbers of *O. maskelli* were collected from green sticky traps as compared to foliage samples. Conversely, greater numbers of both parasitoids were collected from foliage samples over green sticky traps. Data support a correlation between leaf availability and *O. maskelli* emergence. We also observe a positive correlation between temperature and *O. maskelli* emergence.

### Introduction

The gall-forming pest, *Ophelimus maskelli,* was first discovered in California in March of 2014 (Burks et al., 2015a). *Closterocerus chamaeleon* and *Selitrichodes neseri* were reared from *O. maskelli* galls and determined to have been introduced to California along with this particular population of *O. maskelli* (Burks et al., 2015b). To determine emergence periods and the relationships between these three coinciding populations, it is important to sample their seasonal phenology. Timing of emergence not only provides insight into the developmental biology of these wasps, but can also assist in the development of a cohesive integrated pest management approach in controlling the *O. maskelli* population.

California shares a similar Mediterranean climate to Israel, another location where *O. maskelli* has invaded; however, the emergence periods appear to differ in California. While there are three major emergences a year in Israel (Protasov et al., 2007a), there are at most two in California (KW Pers. Obs.). Emergence periods of adult pests like *O. maskelli* are crucial to the understanding and timing of control methods. Most chemical controls are inadequate due to the physical protection provided by the gall (Protasov et al., 2007b). This leaves the emerged adult stage as the most vulnerable, as well as the most problematic.

Monitoring pest populations is extremely important, as is careful monitoring of natural enemies. Studying parasitoid populations, particularly during adult emergence, give us insight into the feasibility and effectiveness of biological control. In southern California, we also have the unique opportunity to learn more about the biology of the California strain of *S. neseri*. Although the South African strain of *S. neseri* parasitizes *Leptocybe invasa*, the California strain has been observed to be a larval ectoparasitoid of *O. maskelli* (KW Pers. Obs.). This parasitoid species may play a very important role in the biological control of *O. maskelli*. What exactly that role might be remains to be seen; however, monitoring the phenology of these wasps can help elucidate their potential role.

Phenology describes the recurring, seasonal events or life cycle stages in plants and animals. It involves studying the timing of such events and determining seasonal patterns and annual cycles (Ascerno, 1991). A very important phenophase, with regards to gall wasps, would be the emergence of adults. Because gall wasps live the majority of their lives as larvae within the galls, the adult stage includes various important events in a very short period of time, including oviposition and any major movement from one location to another. Determining these important phenophases is critical to developing effective and efficient pest management strategies.

It is also important to compare the population sizes and activity under different environmental conditions to understand how these populations vary within important parameters. A key aspect of studying phenology is looking at relationships with insect timing and weather. A coastal area for example, where temperatures show limited fluctuations, may be more or less suitable to insect development than an inland area where temperatures widely fluctuate. Temperature, as well as rainfall and available moisture, can have indirect effects on insects that rely on particular plants for reproduction, oviposition, and development. Both population density and emergence periods may be significantly affected by the local environmental conditions.

Population census data can be highly influenced by collection method. Two different methods were used in this study in an attempt to gain a more accurate representation of the population sizes. Developing an accurate method for monitoring is crucial for developing and maintaining effective control.

Successful control strategies are predicated on solid data and understanding of the system. With any invasive species, it is imperative that we determine the timing of certain crucial biological processes. Particularly with gall-forming insects, where most life stages are protected, understanding the timing of adult emergence is paramount to implementing effective pest control measures. Natural enemies are the most useful tool in controlling such protected pests and should also be carefully monitored and studied to be successful in their management.

## Materials and Methods

### *Description of study sites*

Sampling of all three wasp species was conducted at an inland study site located in the Agricultural Experiment Station property of University of California, Riverside CA. A second coastal study site was located in the Laguna Niguel Regional Park in Orange County CA. At each site, 10 infested eucalyptus trees (*E. camaldulensis* and *E. rudis*) were randomly selected to be monitored. Selected trees were clearly infested with *O. maskelli,* showing heavy galling of several leaves. Average daily temperature data were obtained through The Weather Channel (https://weather.com/weather/monthly/l/92501:4:US). Available leaves suitable for oviposition were estimated by the percentage of new foliage relative to the total foliage in the tree canopy.

### *Wasp Sampling*

The sampling occurred between June 2015 and June 2016. Two different methods were used for the sampling of wasps. The first method utilized one green sticky trap hung about 5 feet from the ground in each selected tree. Sticky traps follow methods described in Eatough Jones et al. (2011). Transparent 10-cm plastic lids (polypropylene deli container lid) were spray painted with Neon Green (Rust-oleum [Vernon Hills, IL]) and attached to a wire hanger. The wire hanger was wrapped around a eucalyptus tree branch to suspend the plastic green lid. Another clear plastic deli lid was then coated in a thin layer of motor oil additive (STP® Oil Treatment) (Armored AutoGroup Inc., Danbury, CT, USA) and placed over the green lid background. The lids were attached to each other by two clips. The bright green color has been proven to be most attractive to both *O. maskelli* and *C. chamaeleon* (Protasov et al., 2007a). One trap was set out on each of the 10 infested trees at each location. Traps were collected and refreshed every two weeks over the entire year-long sampling period. Collected traps were taken back to the lab where the wasps were identified and counted using a microscope. Sample data reflects the number of wasps counted in a randomly selected 5x5cm sub-sample area of the sticky lid.

The second method used to monitor wasp populations required a collection of foliage samples. One galled leaf from each infested tree was randomly selected and sealed in a plastic sandwich bag with a small damp paper towel to prevent desiccation. Leaves were collected at the same time new traps were set up to standardize emergence periods. Emerging wasps were then collected from each plastic bag after the 2-week period to be counted and identified.

### Statistical Analysis

Negative binomial regression model in R (R Core Team, 2016) was used to test for differences between sampling sites, methods, and parasitoid populations. The numbers of wasps collected per trap were checked for normality using the Shapiro test. Data fit to a Poisson regression using packages 'arm' (Gelman and Su, 2016) and 'AER' (Kleiber and Zeileis, 2008) showed overdispersion, so a negative binomial regression model was used to analyze wasp numbers per trap where Julian date was included as a factor for each comparison within a general linear model using package 'MASS' (Venables and Ripley, 2002). To compare locations, the count data for each wasp species were compared separately for each collection method. To compare methods, the count data for each wasp species were compared separately for each location. To compare the mean number of parasitoids observed, both location and method were compared separately.

To determine correlation between wasp species and leaf availability, as well as wasp species and temperature, we conducted Spearman's correlation. Each sampling location and each sampling method was tested separately for each species. When data failed to meet Pearson's correlation assumptions, the non-parametric Spearman's correlation coefficient (rho) was calculated and used to determine correlation significance. All analyses were conducted using R Studio version 3.1.2.

## Results

### *Comparing Wasp Abundance Between Locations*

Significantly more *O. maskelli* were collected from sticky traps in Laguna Niguel than from sticky traps in Riverside (z= -10.434, df= 499, P < 0.005; Figure 2.1). The number of *O. maskelli* observed were significantly affected by date as well (z= 4.357, df= 499, P < 0.005; Figure 2.1). However, there were no significant differences between sites in the number of *O. maskelli* collected from leaf emergence (Figure 2.2).

Significantly more *C. chamaeleon* were collected from sticky traps in Laguna Niguel than from traps in Riverside (z= -3.561, df= 499, P < 0.005; Figure 2.3), while date was not significant. Similar to what was observed in the number of O. maskelli, there were no significant differences in the number of *C. chamaeleon* collected from leaf emergence in Riverside or Laguna Niguel (Figure 2.4). However, differences between dates are marginally significant (z= 1.162, df= 499, P = 0.04; Figure 2.4).

As was observed for the other two wasp species, significantly more of the *S. neseri* were collected from sticky traps in Laguna Niguel than from traps in Riverside (z= -12.125, df= 499, P < 0.005; Figure 2.6). Differences in the number of *S. neseri* observed were significantly different among multiple dates as well (z= -2.239, df= 499, P < 0.005; Figure 2.6). Similarly, more wasps were collected from leaf emergence in Laguna Niguel than Riverside (z= -4.654, df= 499, P < 0.005; Figure 2.5). Differences in the number of *S. neseri* observed were significantly different between multiple dates as well (z= 5.747, df= 499, P < 0.05; Figure 2.5).

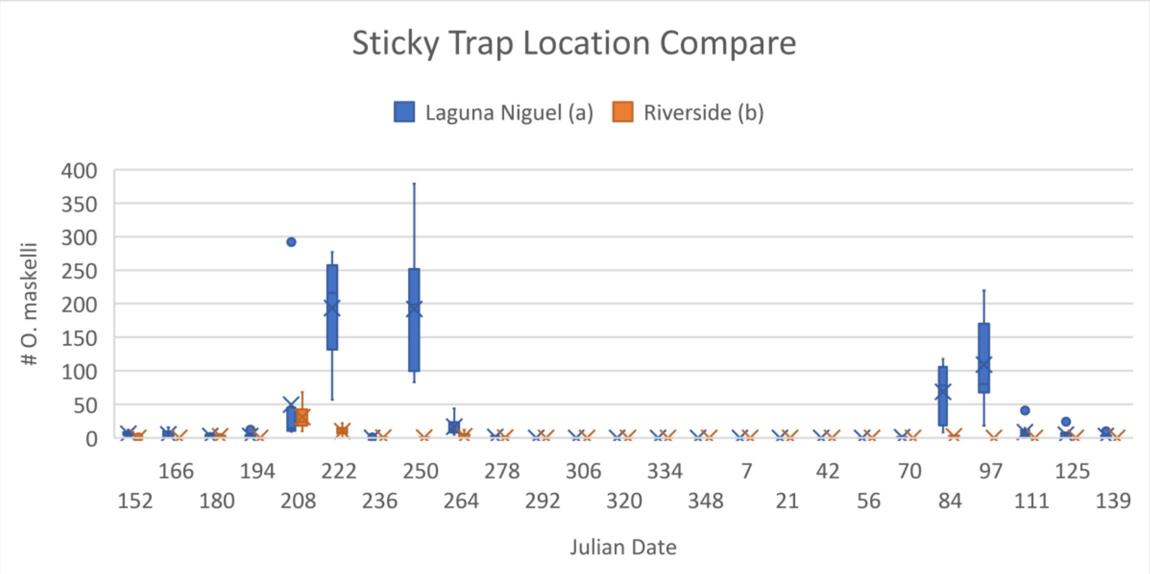

**Figure 2.1. Comparison of *O. maskelli* between locations for the sticky trap method showing standard deviation. Site locations are Laguna Niguel, CA and Riverside, CA. Locations with different letters are significantly different (Negative Binomial Regression Model, P < 0.005). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, P < 0.005).**

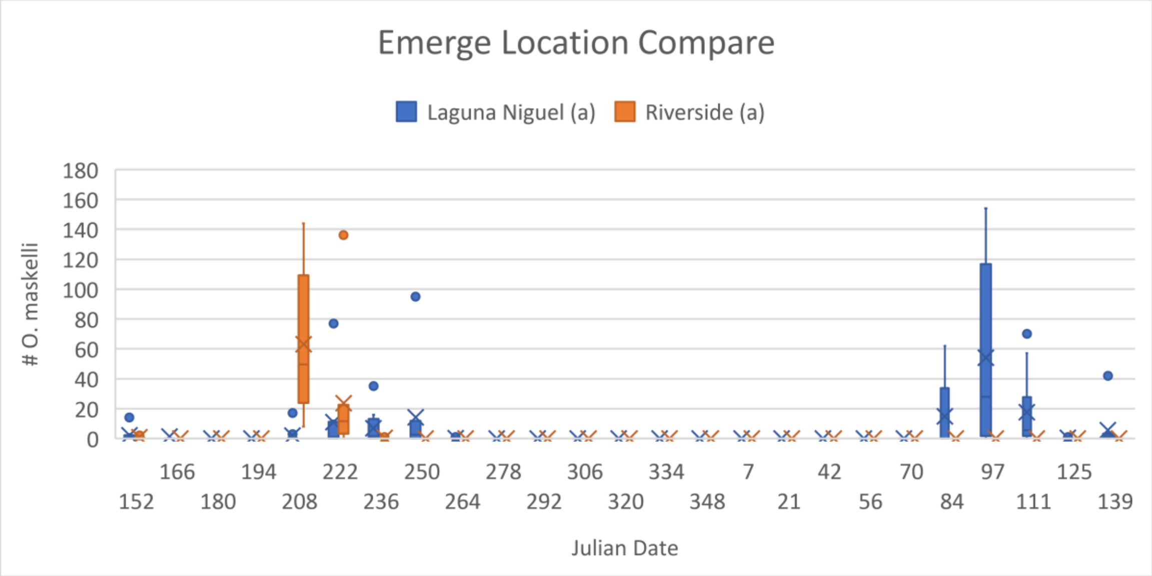

**Figure 2.2. Comparison of *O. maskelli* between locations for the leaf emergence method showing standard deviation. Site locations are Laguna Niguel, CA and Riverside, CA. Locations were not significantly different (Negative Binomial Regression Model, P $\geq$ 0.05).**

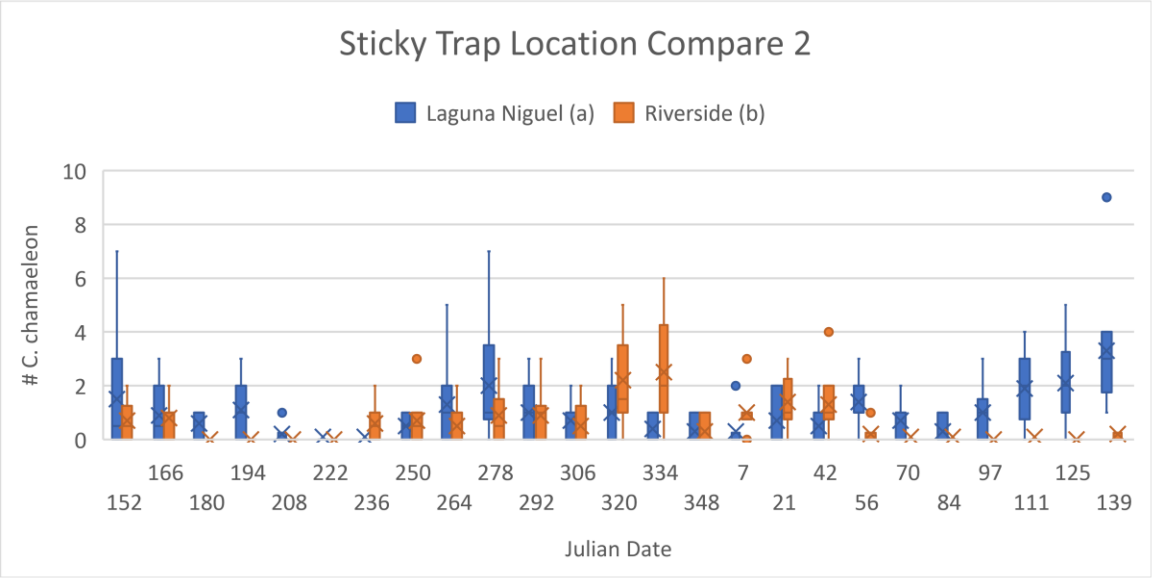

**Figure 2.3. Comparison of *C. chamaeleon* between locations for the sticky trap method showing standard deviation. Site locations are Laguna Niguel, CA and Riverside, CA. Locations with different letters are significantly different (Negative Binomial Regression Model, $P \leq 0.005$).**

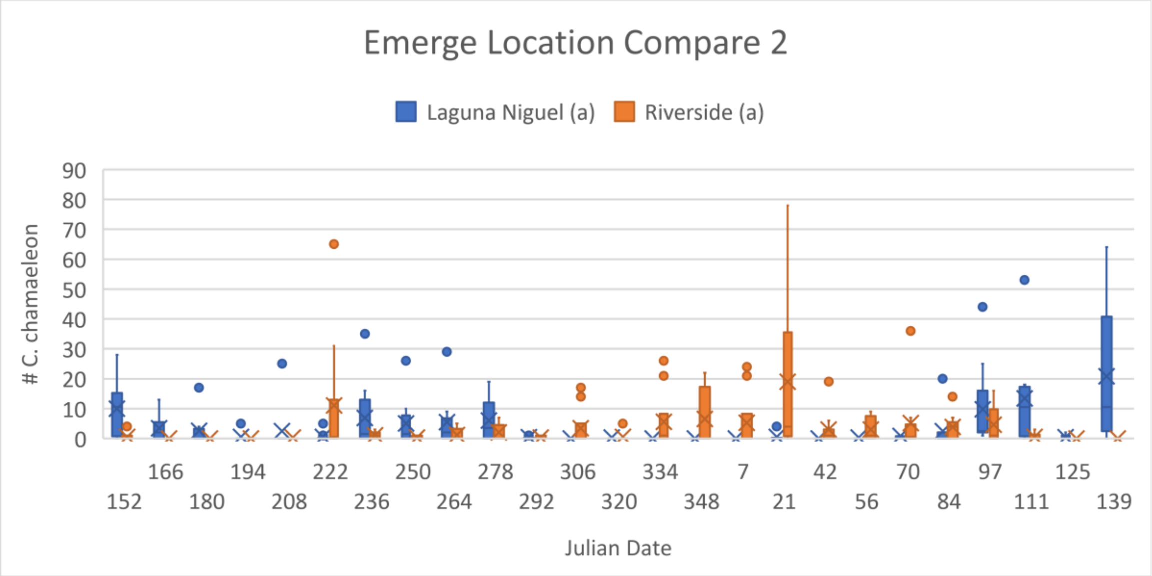

**Figure 2.4. Comparison of *C. chamaeleon* between locations for the leaf emergence method showing standard deviation. Site locations are Laguna Niguel, CA and Riverside, CA. Locations were not significantly different (Negative Binomial Regression Model, P $\geq$ 0.005). An asterisk denotes the date to be a marginally significant factor (Negative Binomial Regression Model, P = 0.04).**

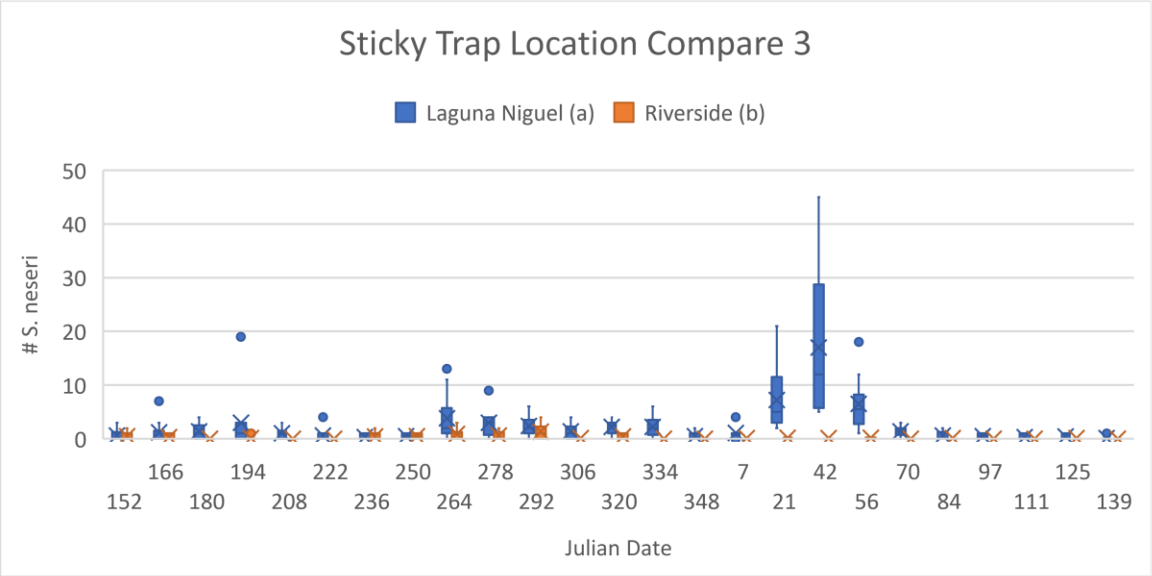

**Figure 2.5. Comparison of *Selitrichodes neseri* between locations for the sticky trap method showing standard deviation. Site locations are Laguna Niguel, CA and Riverside, CA. Locations with different letters are significantly different (Negative Binomial Regression Model, P ≤ 0.0005). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, P ≤ 0.05).**

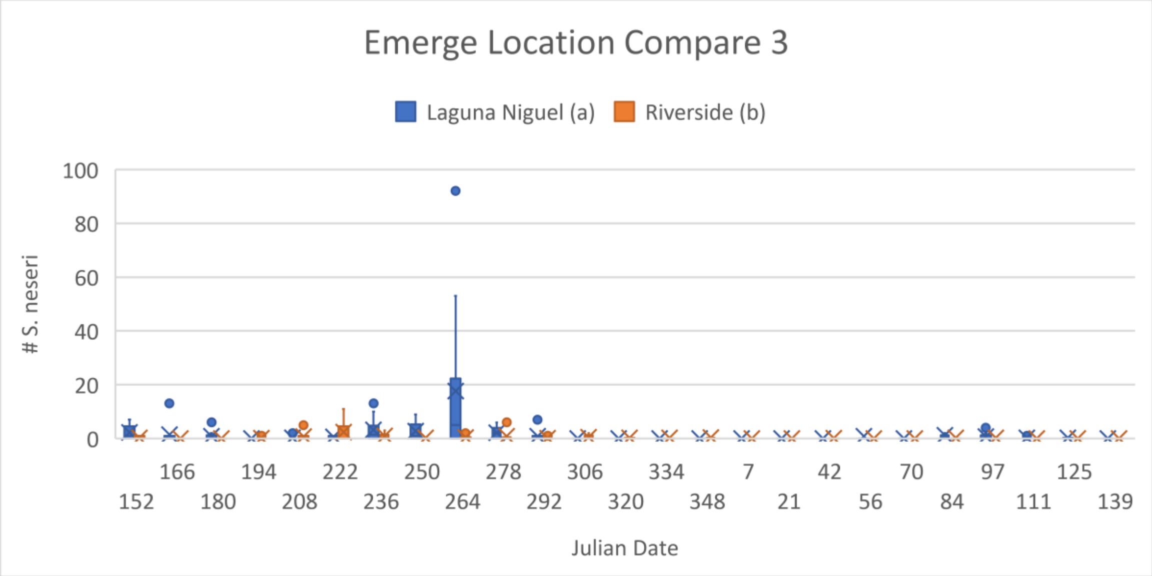

**Figure 2.6. Comparison of *Selitrichodes neseri* between locations for the leaf emergence method showing standard deviation. Site locations are Laguna Niguel, CA and Riverside, CA. Locations with different letters are significantly different (Negative Binomial Regression Model, P $\leq$ 0.005). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, P $\leq$ 0.005).**

***Comparing Sampling Methods***

When comparing the number of *O. maskelli* in Laguna Niguel, we observed a significant difference between methods of capture. Sticky traps showed significantly more *O. maskelli* than the leaf emergence method ($P < 0.005$; Figure 2.7). The number of *O. maskelli* observed significantly differed by date as well ($P < 0.05$; Figure 2.7). However, we did not observe a significant difference between methods of capture in Riverside (Figure 2.8), although date had a significant effect on the number of *O. maskelli* observed ($P < 0.05$; Figure 2.8).

Similarly, there were significant differences between the two collection methods for the parasitoid *C. chamaeleon* collected in Laguna Niguel. The leaf emergence method collected significantly more wasps than the sticky trap method ($P < 0.05$; Figure 2.9) and there was a significant effect by date ($P < 0.05$; Figure 2.9). In Riverside, we also observed a difference in *C. chamaeleon* ($P < 0.005$; Figure 2.10), between collection methods. However, the factor date was not statistically significant (Figure 2.10).

When comparing the number of the parasitoid *S. neseri* collected in Laguna Niguel, we observed a significant difference between the two collection methods ($P < 0.05$; Figure 2.11). However, the factor date was not statistically significant (Figure 2.11). In Riverside, there were no differences between capture methods for this wasp (Figure 2.12), although the factor date was statistically significant ($P < 0.005$; Figure 2.12).

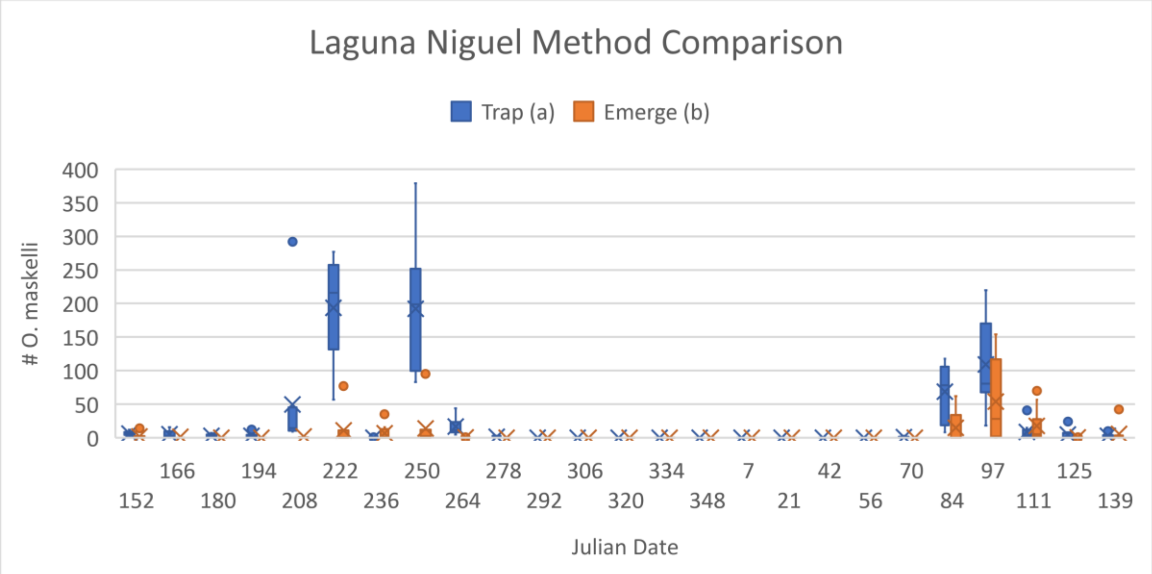

**Figure 2.7. Comparison of *O. maskelli* between methods in Laguna Niguel showing standard deviation. Methods are the green sticky trap method and the leaf emergence method. Methods with different letters are significantly different (Negative Binomial Regression Model, $P \leq 0.005$). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, $P \leq 0.005$).**

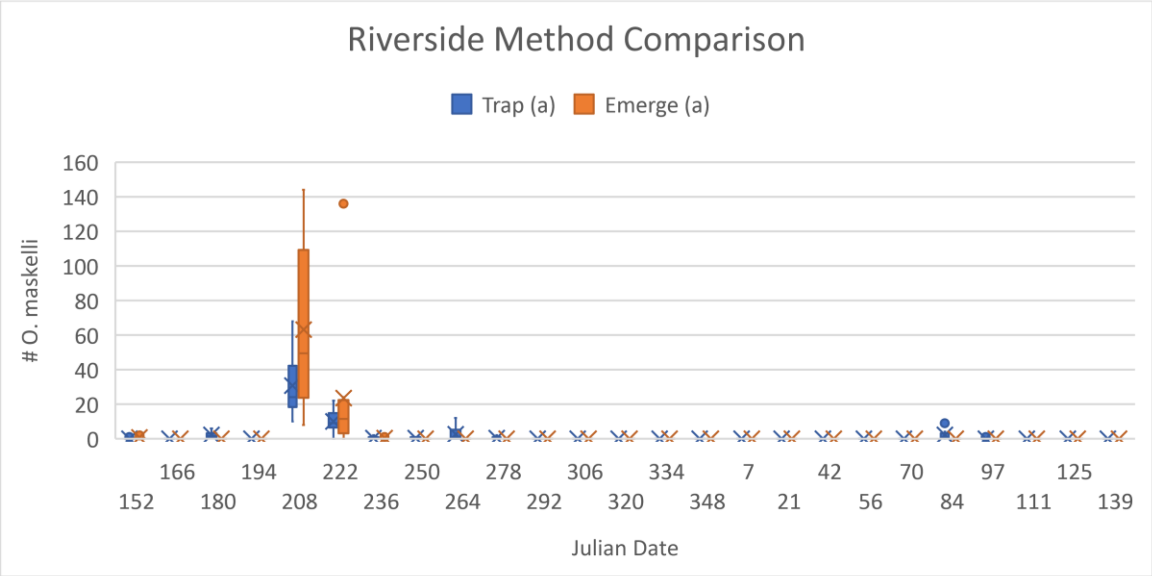

**Figure 2.8. Comparison of *O. maskelli* between methods in Riverside showing standard deviation. Methods are the green sticky trap method and the leaf emergence method. Methods are not significantly different (Negative Binomial Regression Model, $P > 0.05$). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, $P \leq 0.05$).**

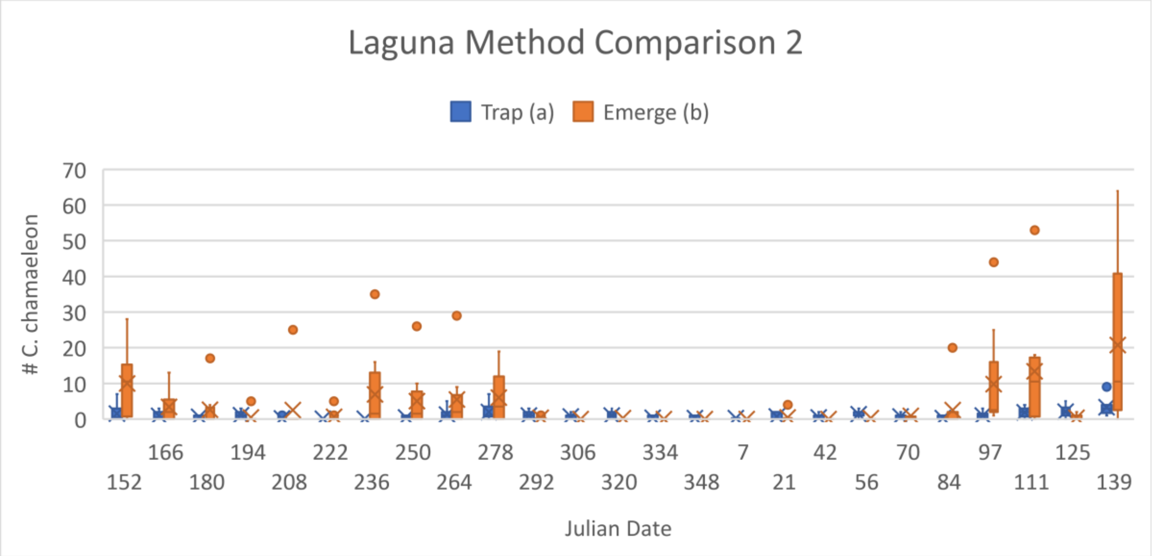

**Figure 2.9. Comparison of *C. chamaeleon* between methods in Laguna Niguel showing standard deviation. Methods are the green sticky trap method and the leaf emergence method. Methods with different letters are significantly different (Negative Binomial Regression Model, P $\leq$ 0.005). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, P $\leq$ 0.005).**

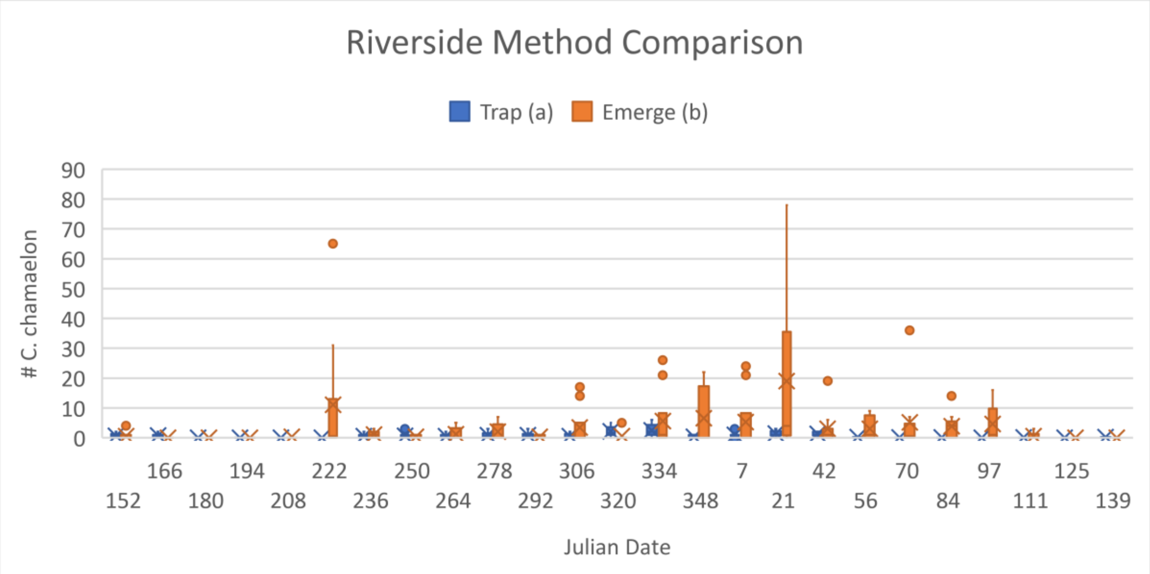

**Figure 2.10. Comparison of *C. chamaeleon* between methods in Riverside showing standard deviation. Methods are the green sticky trap method and the leaf emergence method. Methods with different letters are significantly different (Negative Binomial Regression Model, P $\leq$ 0.005).**

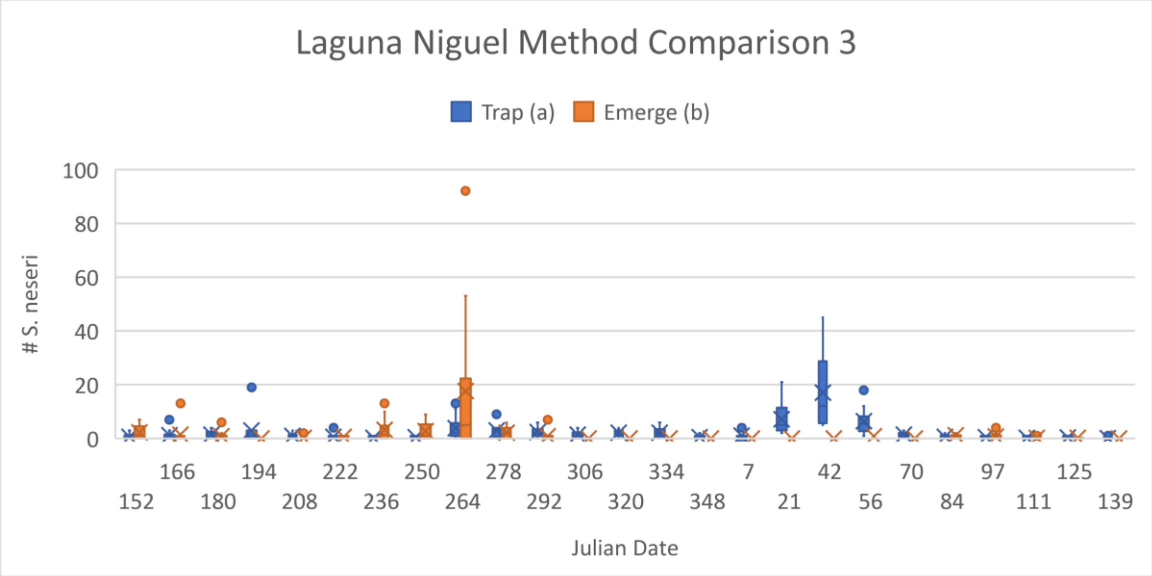

**Figure 2.11. Comparison of *Selitrichodes neseri* between methods in Laguna Niguel showing standard deviation. Methods are the green sticky trap method and the leaf emergence method. Methods with different letters are significantly different (Negative Binomial Regression Model, P $\leq$ 0.005).**

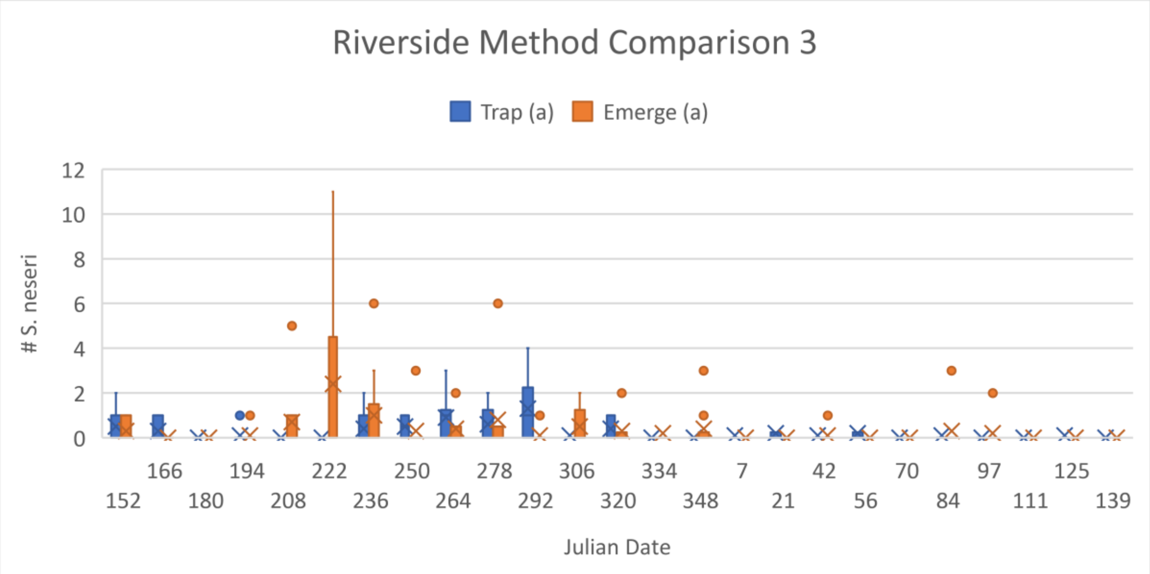

**Figure 2.12. Comparison of *Selitrichodes neseri* between methods in Riverside showing standard deviation. Methods are the green sticky trap method and the leaf emergence method. Methods are not significantly different (Negative Binomial Regression Model, P $\geq$ 0.05). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, P $\leq$ 0.005).**

### ***Comparing Parasitoid Species***

Significantly more *S. neseri* than *C. chamaeleon* were collected in Laguna Niguel on sticky traps (z= 6.261, df= 499, P < 0.005). There was a significant effect of date with peak abundance occurring in February (z= -4.337, df= 499, P < 0.005; Figure 2.13). However, significantly more *C. chamaeleon* were collected from leaf emergence samples than *S. neseri* in Laguna Niguel (z= -4.388, df= 499, P < 0.005). The factor date is also significant (z= 2.904, df= 499, P < 0.005; Figure 2.14).

This pattern was slightly different in the Riverside site, where significantly more *C. chamaeleon* were collected on sticky traps than *S. neseri* (z= -5.184, df= 499, P < 0.005) and leaf emergence samples (z= -7.942, df= 499, P < 0.005). The factor date is significant for sticky traps but not leaf emergence samples (z= 3.712, df= 499, P < 0.005; Figure 2.15).

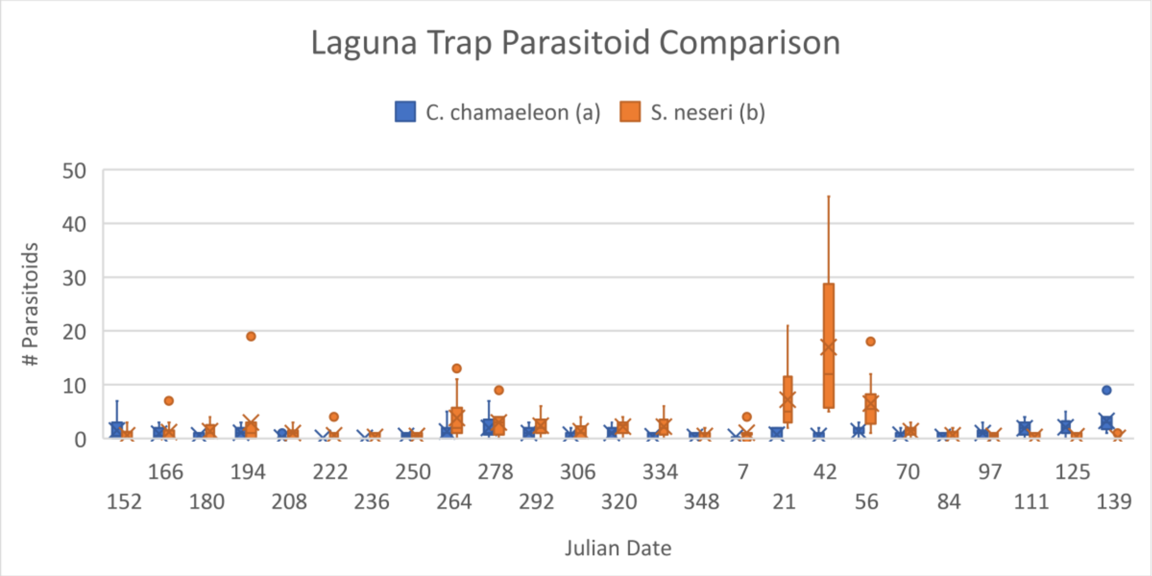

**Figure 2.13. Comparison of parasitoids collected by green sticky trap in Laguna Niguel showing standard deviation. The parasitoid species compared are *Closterocerus chamaeleon* and *Selitrichodes neseri*. The number of parasitoids collected is significantly different, as marked by the different letters in parentheses (Negative Binomial Regression Model, $P \geq 0.005$). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, $P \leq 0.005$).**

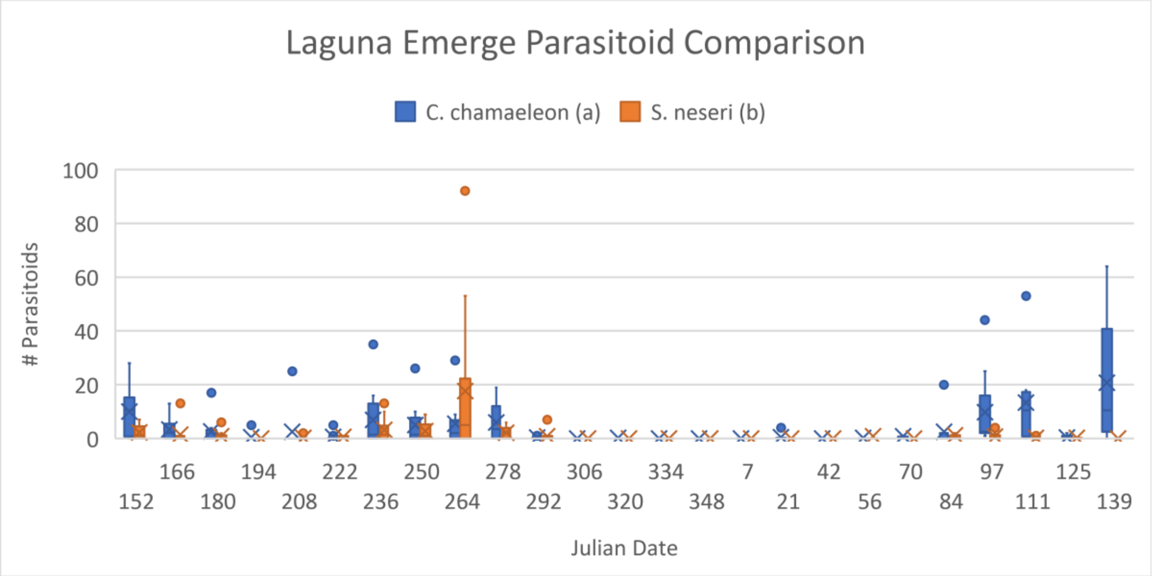

**Figure 2.14. Comparison of parasitoids collected by leaf emergence in Laguna Niguel showing standard deviation. The parasitoid species compared are *Closterocerus chamaeleon* and *Selitrichodes neseri*. The number of parasitoids collected is significantly different, as marked by the different letters in parentheses (Negative Binomial Regression Model, P $\geq$ 0.005). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, P $\leq$ 0.005).**

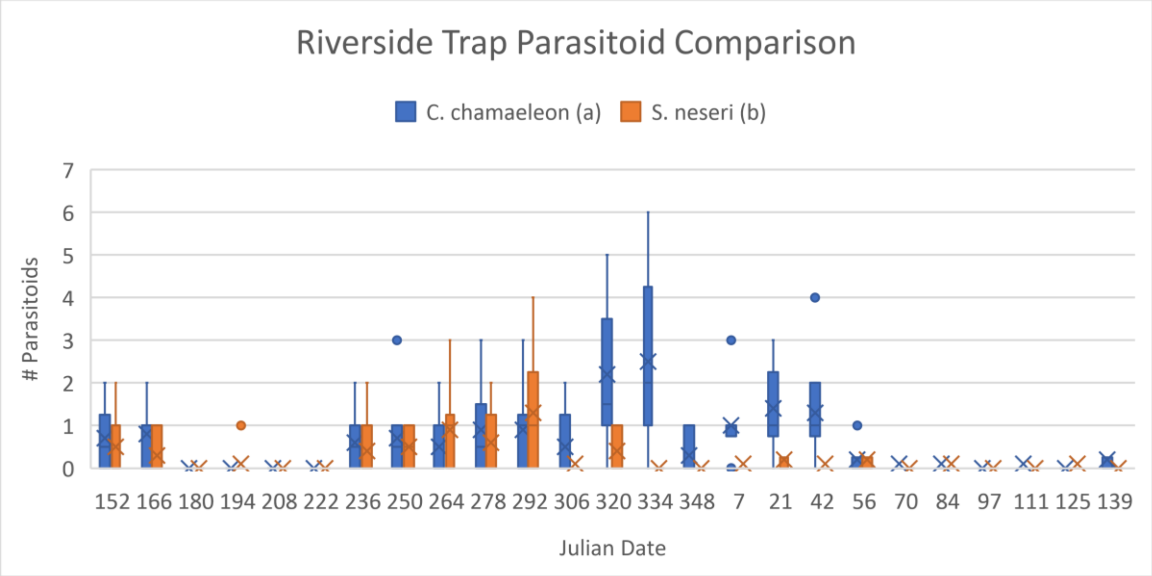

**Figure 2.15. Comparison of parasitoids collected by green sticky trap in Riverside showing standard deviation. The parasitoid species compared are *Closterocerus chamaeleon* and *Selitrichodes neseri*. The number of parasitoids collected is significantly different, as marked by the different letters in parentheses (Negative Binomial Regression Model, $P \geq 0.005$). An asterisk denotes the date to be a significant factor (Negative Binomial Regression Model, $P \leq 0.005$).**

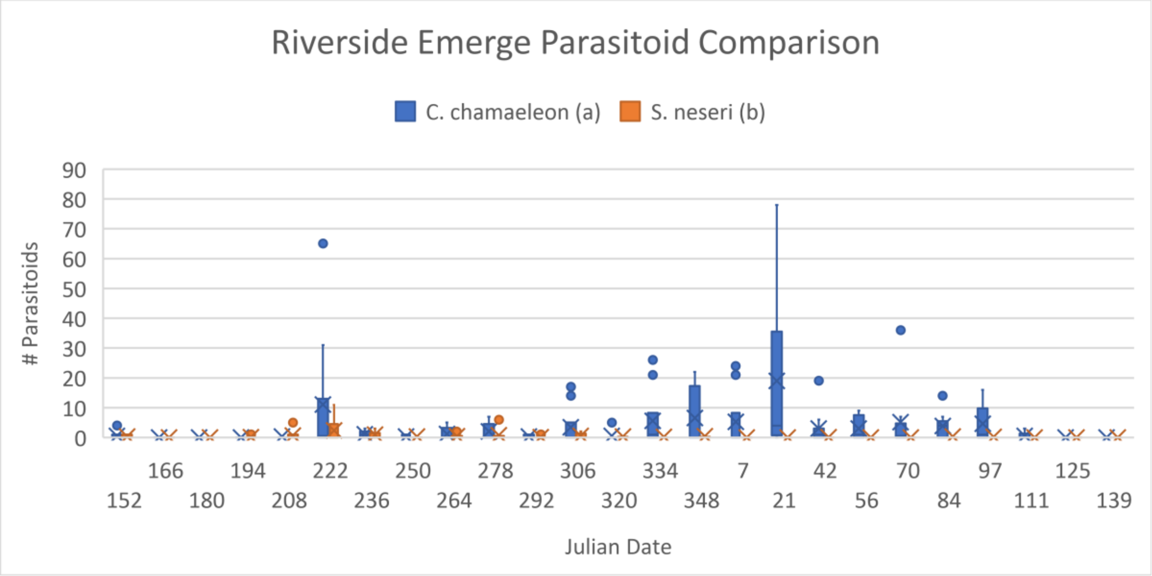

**Figure 2.16. Comparison of parasitoids collected by leaf emergence in Riverside showing standard deviation. The parasitoid species compared are *Closterocerus chamaeleon* and *Selitrichodes neseri*. The number of parasitoids collected is significantly different, as marked by the different letters in parentheses (Negative Binomial Regression Model, P $\geq$ 0.005).**

### ***Leaf Availability***

When assessing the relationship between the average number of *O. maskelli* collected and the percentage of leaves suitable for oviposition, neither emergence samples at Laguna Niguel nor sticky traps at Riverside show strong $R^2$ values (Table 2.1; Figures A1- A2). However, although data collected from Laguna Niguel via the sticky trap method showed significant positive correlation with leaf availability ($R^2 = 0.032$, $\rho = 0.412$, df= 23, $P < 0.05$; Figure 2.17), it was weak. Similarly, data collected from Riverside via the leaf emergence method showed a weak significant negative correlation with the amount of leaves available for oviposition ($R^2 = 0.0627$, $\rho = -0.560$, df= 23, $P < 0.005$; Figure 2.18). There were no significant correlations ($P > 0.05$) for either site in the average number of *C. chamaeleon* collected as a function of the percentage of leaves suitable for oviposition ($P > 0.05$) (Table2.2; Figures A3-A6). Similarly, there were no significant correlations for either site in the average number of *S. neseri* collected by the percentage of leaves suitable for oviposition ($P > 0.05$) (Table2.3; Figures A7-A10).

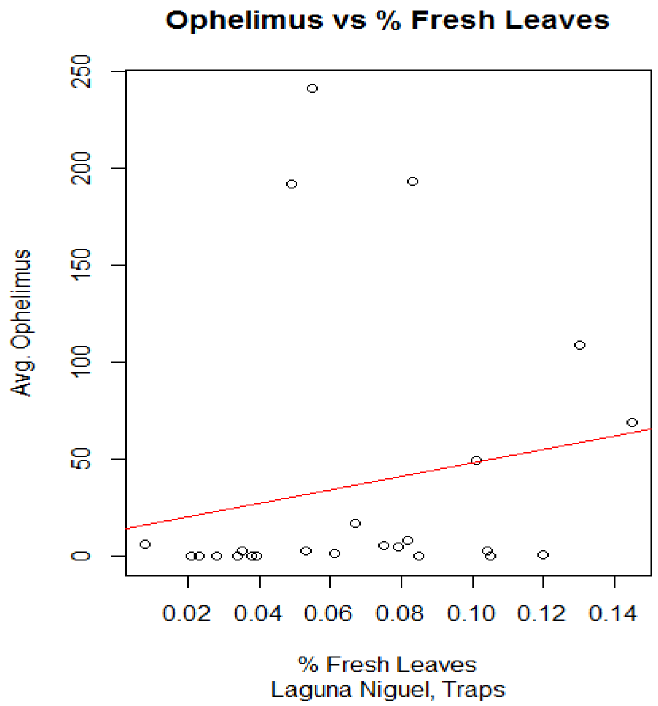

**Figure 2.17. The number of *Ophelimus maskelli* collected from green sticky traps in Laguna Niguel correlated with the percentage of fresh leaves available (Spearman's correlation; $R^2 = 0.032$, $P < 0.05$).**

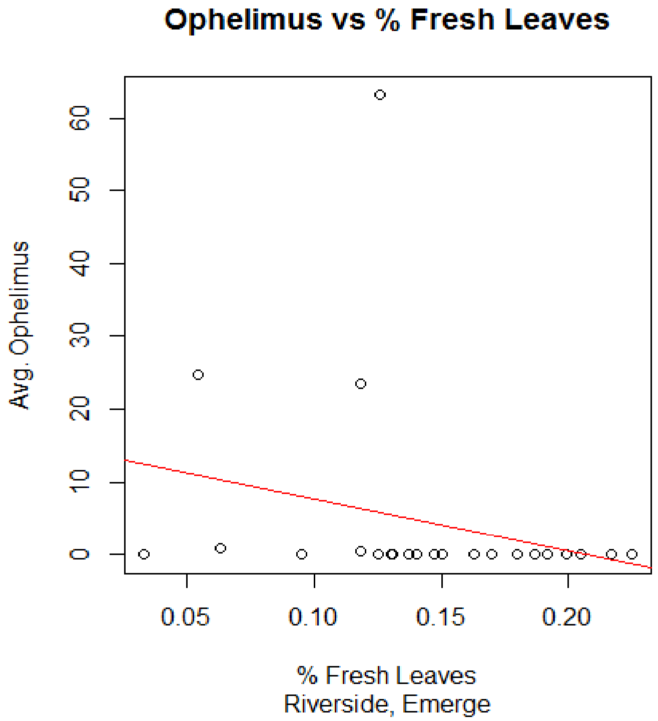

**Figure 2.18. The number of *Ophelimus maskelli* collected from leaf emergence in Riverside correlated with the percentage of fresh leaves available (Spearman's correlation; $R^2 = 0.0627$, $P < 0.005$).**

| Location | Method | Equation | $R^2$ Value | P-Value |
|---|---|---|---|---|
| Laguna Niguel | Trap | y = 9E-05x + 0.0629 | 0.032 | 0.0405* |
| Laguna Niguel | Emerge | y = 0.0013x + 0.0589 | 0.176 | 0.158 |
| Riverside | Trap | y = -0.002x + 0.1499 | 0.0921 | 0.127 |
| Riverside | Emerge | y = -0.0009x + 0.1482 | 0.0627 | 0.004* |

**Table 2.1. The number of *Ophelimus maskelli* collected correlated with the percentage of fresh leaves available. An asterisk denotes a significant P-value (Spearman's Correlation).**

| Location | Method | Equation | $R^2$ Value | P-Value |
|---|---|---|---|---|
| Laguna Niguel | Trap | y = -0.0038x + 0.0699 | 0.0064 | 0.932 |
| Laguna Niguel | Emerge | y = -0.0004x + 0.0678 | 0.0031 | 0.447 |
| Riverside | Trap | y = -0.0063x + 0.1484 | 0.0101 | 0.887 |
| Riverside | Emerge | y = 0.0005x + 0.1426 | 0.0017 | 0.742 |

**Table 2.2. The number of *Closterocerus chamaeleon* collected correlated with the percentage of fresh leaves available. There were no significant correlations (Spearman's Correlation).**

| Location | Method | Equation | $R^2$ Value | P-Value |
|---|---|---|---|---|
| Laguna Niguel | Trap | y = 0.0007x + 0.0648 | 0.0041 | 0.987 |
| Laguna Niguel | Emerge | y = -0.0025x + 0.0701 | 0.0961 | 0.437 |
| Riverside | Trap | y = 0.0078x + 0.1428 | 0.0015 | 0.888 |
| Riverside | Emerge | y = -0.0049x + 0.1458 | 0.0026 | 0.638 |

**Table 2.3. The number of *Selitrichodes neseri* collected correlated with the percentage of fresh leaves available. There were no significant correlations (Spearman's Correlation).**

### ***Temperature***

When assessing the effect of ambient temperature on the average number of *O. maskelli* collected emergence samples from Laguna Niguel show weak $R^2$ values (Table 2.4; Figure A11). However, data collected from Laguna Niguel via the sticky trap method showed significant correlation ($R^2 = 0.2599$, $\rho = 0.466$, df= 23, $P < 0.05$; Figure 2.19). Data collected from Riverside via both the sticky trap ($R^2 = 0.1563$, $\rho = 0.739$, df= 23, $P < 0.005$; Figure 2.20) and leaf emergence ($R^2 = 0.1302$, $\rho = 0.453$, df= 23, $P < 0.05$; Figure 2.21) methods also showed weak significant association between ambient temperature and *O. maskelli* abundance. There was no significant relationship between the average number of *C. chamaeleon* collected and the average temperature observed at either location or by either method (Table 2.5; Figures A12-A15). The average number of *S. neseri* collected was significantly correlated to the average temperature observed only for wasps collected via the leaf emergence method in Laguna Niguel ($R^2 = 0.0019$, $\rho = 0.486$, df= 23, $P < 0.05$; Figure 2.22) (Table 2.6; Figures A16-A18).

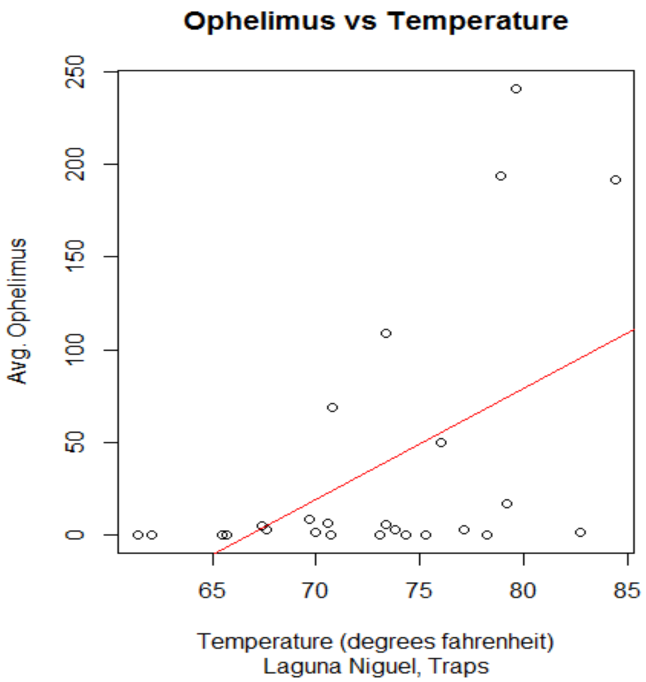

**Figure 2.19. The number of *Ophelimus maskelli* collected from green sticky traps in Laguna Niguel correlated with the temperature (Spearman's correlation; $R^2$ = 0.2599, $P < 0.05$).**

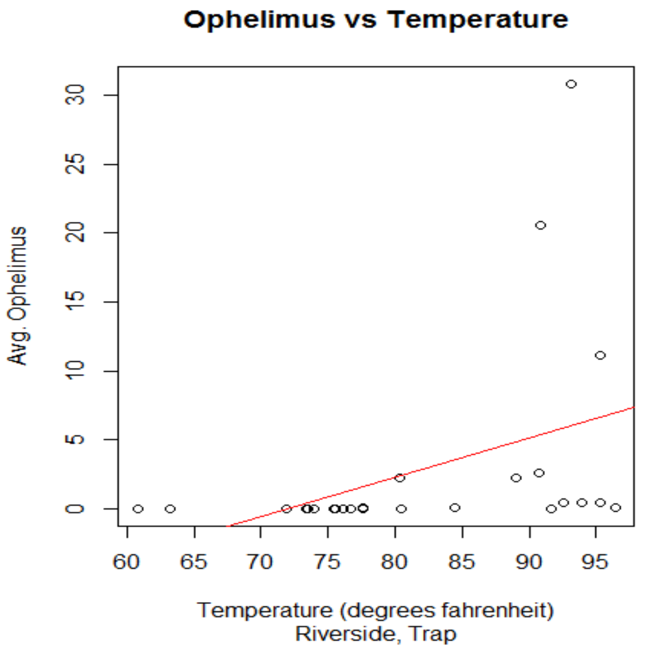

**Figure 2.20. The number of *Ophelimus maskelli* collected from green sticky traps in Riverside correlated with the temperature (Spearman's correlation; $R^2$ = 0.1563, $P < 0.005$).**

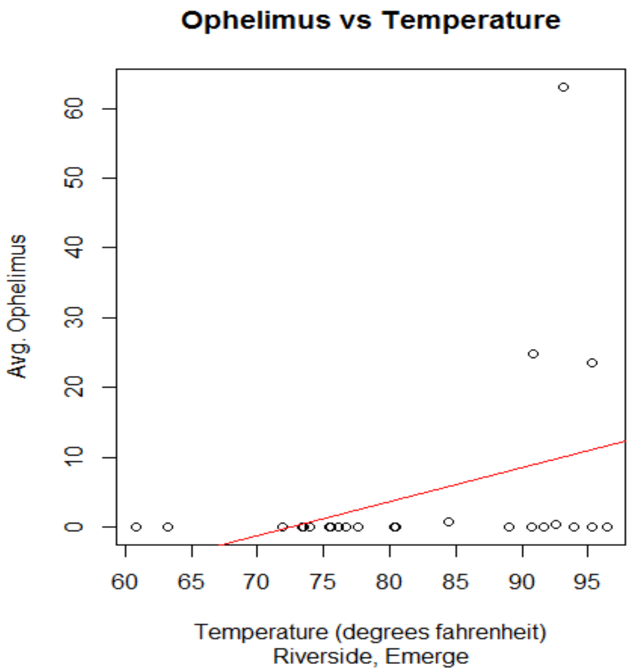

**Figure 2.21.** The number of ***Ophelimus maskelli*** collected from leaf emergence in Riverside correlated with the temperature (Spearman's correlation; $R^2 = 0.1302$, $P < 0.05$).

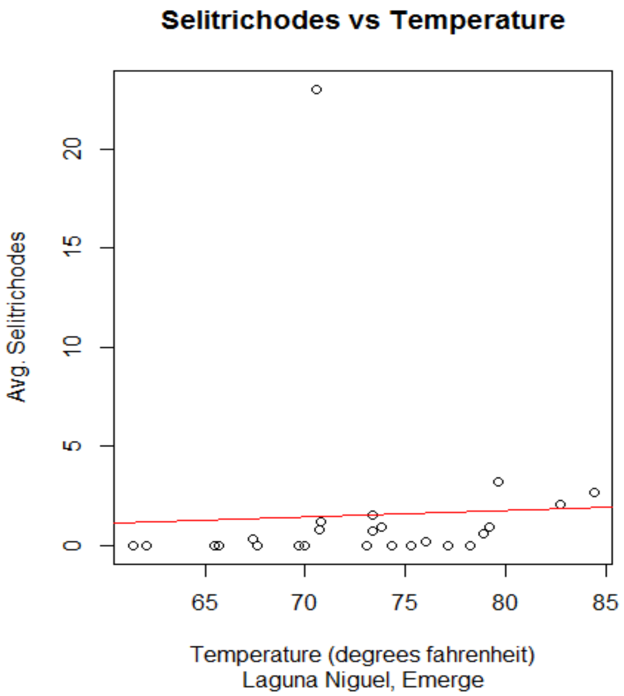

**Figure 2.22.** The number of ***Selitrichodes neseri*** collected from leaf emergence in Laguna Niguel correlated with the temperature (Spearman's correlation; $R^2 = 0.1776$, $P < 0.05$).

| Location | Method | Equation | $R^2$ Value | P-Value |
|---|---|---|---|---|
| Laguna Niguel | Trap | y = 0.0434x + 71.264 | 0.2599 | 0.01886* |
| Laguna Niguel | Emerge | y = 0.0827x + 72.361 | 0.027 | 0.3188 |
| Riverside | Trap | y = 0.5505x + 80.42 | 0.1563 | 2.432e-05** |
| Riverside | Emerge | y = 0.267x + 80.78 | 0.1302 | 0.02303* |

**Table 2.4. The number of *Ophelimus maskelli* collected correlated with temperature. An asterisk denotes a significant P-value, a double asterisk denotes a highly significant P-value (Spearman's Correlation).**

| Location | Method | Equation | $R^2$ Value | P-Value |
|---|---|---|---|---|
| Laguna Niguel | Trap | y = -0.9329x + 73.705 | 0.0143 | 0.4691 |
| Laguna Niguel | Emerge | y = 0.0518x + 72.645 | 0.002 | 0.2215 |
| Riverside | Trap | y = -1.4238x + 82.929 | 0.0115 | 0.4092 |
| Riverside | Emerge | y = -0.7555x + 84.486 | 0.0996 | 0.05303 |

**Table 2.5. The number of *Closterocerus chamaeleon* collected correlated with temperature. There were no significant correlations (Spearman's Correlation).**

| Location | Method | Equation | $R^2$ Value | P-Value |
|---|---|---|---|---|
| Laguna Niguel | Trap | y = -0.4687x + 73.942 | 0.0773 | 0.8032 |
| Laguna Niguel | Emerge | y = 0.057x + 72.749 | 0.0019 | 0.01383* |
| Riverside | Trap | y = 16.157x + 79.011 | 0.141 | 0.2433 |
| Riverside | Emerge | y = 8.4877x + 79.2 | 0.1776 | 0.06652 |

**Table 2.6. The number of *Selitrichodes neseri* collected correlated with temperature. (Spearman's Correlation). An asterisk denotes a significant P-value (Spearman's Correlation).**

## Discussion and Conclusion

### *Comparing Locations & Methods*

Using the green sticky trap method, we consistently saw a difference between sites in the number of individuals caught for all three species. The number of *O. maskelli* caught in green sticky traps in Laguna Niguel was significantly higher than the number of individuals caught in green sticky traps in Riverside. Over the year-long period of observation, we see two clear peak emergence periods in Laguna Niguel of *O. maskelli*: the first emergence began in the end of March and ended in the middle of April, while the second emergence began in the end of July and ended in the middle of September (Figure A.19). However, in Riverside, we only observed one emergence period near the end of July and only lasting about 2 weeks. We also observed higher population numbers in Riverside via the leaf emergence method, rather than the green sticky traps. Both of these observations differ from the emergence periods documented in Israel (Protasov et al. 2007a), which occur near the middle of March, the beginning of July, and the middle of October (Figure A.19). Two of these periods are very similar to what we observed in Laguna Niguel, differing only by a week or two. The emergence with highest population numbers in Israel occurs in March, similar to observations made from the leaf emergence method in California, however, this major emergence was observed in August on green sticky traps (Protasov et al. 2007). The climate in Bet Dagan, Israel is similar to the climate in Riverside, California with average temperatures ranging from 50°F to 90°F and differing slightly from an average of 23 inches' annual precipitation to about 10 inches in Riverside (World Weather Online). Temperatures in Laguna Niguel fluctuate less, but with slightly greater average annual precipitation. Differences between emergence at a coastal site versus an inland site suggest climate may play a role in the timing of this emergence. However, with such similar climates and yet such varying emergence cycles, it is reasonable to suggest the timing of adult emergence is dependent upon factors other than temperature.

The number of *C. chamaeleon* caught in green sticky traps in Laguna Niguel was generally observed to be more than those caught in Riverside. This significant difference is consistent with the prediction that population density and parasitoid survivability are affected by climate. As observed, we would also expect to find a greater number of this parasitoid in the area with a more abundant host species. However, we do not find the date factor to be significant for sticky traps, suggesting there are not particular emergence periods for the adult parasitoids. It is possible that the numbers observed were too small to signify any significant differences among dates.

The leaf emergence method tells a slightly different story from the green sticky traps concerning *C. chamaeleon*. We observed much higher numbers of individuals from both locations. In general, population numbers were higher in Laguna Niguel, with just a few exceptions. The date factor was statistically significant, suggesting that there are particular emergence periods annually. Interestingly, timing of emergence differs throughout the year for each location. Very few dates overlap with emergence in both locations, suggesting location plays a significant role in the timing of these events. Despite the singular emergence period of the host in Riverside, we observe *C. chamaeleon* emerging three times over the course of the year. The development time of the parasitoid is much shorter than that of the host, allowing for multiple generations of the parasitoid to be produced within the time required for one generation of the host to develop (Protasov et al. 2007). This significant biological difference also allows *C. chamaeleon* to build up population numbers much more rapidly than its host, making it an extremely prolific natural enemy.

The number of *S. neseri* caught in green sticky traps in Laguna Niguel was significantly greater than those caught in Riverside, although very small numbers were observed in Riverside from either collection method. This significant difference suggests that climate may influence parasitoid density and survival. We would also expect to see this trend following the population density of the host. Interestingly, the factor date was significant suggesting three particular emergence periods, rather than scattered emergence throughout the year. Two of these parasitoid emergence periods consistently ensue host emergence periods, suggesting a strong link between parasitoid and host emergence. This is particularly important concerning *S. neseri* as very little is known about the biology or phenology in California.

Overall, we observe higher population numbers from green sticky traps in Laguna Niguel. Two yearly *O. maskelli* emergences suggest two generations per year which would significantly aid in the building up of populations numbers, when compared to the population in Riverside which only displays one emergence period. The parasitoid *C. chamaeleon* appears to be in much greater numbers than *S. neseri* in general. This may be due to differences in development time or reproduction. Further observation over multiple years would significantly aid in the accuracy of these conclusions and the predictability of future emergence patterns, as well as aiding in pest management techniques in Southern California.

### ***Comparing Parasitoids***

We would expect to see some variation among the prevalence of these two species due to the similarities in host preference and reproductive needs. Although both species require the same host to reproduce in California, there must be a conducive differentiation between niches for both species to survive. All locations and methods showed significant differences between the two species of parasitoids collected. Coastal emergence in Laguna Niguel showed more *S. neseri* collected from sticky traps. Leaf emergence showed more *C. chamaeleon* collected, except for two outliers in September (Fig. 2.14). Both methods in Riverside showed more *C. chamaeleon* collected as well. It is not surprising to observe much greater numbers of *C. chamaeleon* when considering the differing biology between the two parasitoid species. *Closterocerus chamaeleon* is a uniparental species which does not require males in its reproduction, allowing it to reproduce much more effortlessly and rapidly than *S. neseri* which requires males for mating and reproduction. There were also notable differences in the timing of emergence for both parasitoids. It appears that *C. chamaeleon* emerges three times a year in Riverside, and twice per year in Laguna Niguel. On the other hand, *S. neseri* emergence does not exhibit consistent patterns among the different locations and methods. However, this may be due to the small numbers of *S. neseri* that were collected. These differences in the timing of emergence may be a key contributor to the success of these two species sharing the same host in the same geographical area.

### ***Leaf Availability***

In Israel, the large spring emergence of *O. maskelli* coincides with the high availability of leaves suitable for oviposition (Protasov et al. 2007b). Despite having the greatest availability of leaves suitable for oviposition occurring in the spring in southern California, we do not observe the same high wasp emergence numbers at this time. However, we do see a significant correlation between the leaves available for oviposition and the numbers of *O. maskelli* retrieved from sticky traps in Laguna Niguel. The weak $R^2$ value suggests that leaf availability is only one among other factors that contribute to the timing of emergence. Curiously, the only significant correlation found in Riverside was a negative one, which resulted from data gathered by the emergence method. Notably, this data was mainly made up of zero counts. One possible explanation for this result may be the nature of the areas used in this study. The infested trees used in both locations are cared for year-round, meaning regular watering and consequently fresh growth. With fresh leaves being produced constantly, we did not see a strong pattern. Further observations in a more natural setting may help to clarify this relationship.

### ***Temperature***

Sample data show a clear offset in periods of *O. maskelli* emergence between the inland and coastal sites. From this we would suspect that host density correlates with average temperature. We do indeed observe a significant correlation between the observed average temperature and the number of *O. maskelli* individuals observed for both locations ($P < 0.05$). However, the $R^2$ value was fairly weak (0.2599 and 0.1563), suggesting temperature is merely one factor involved. We would expect temperature and climate to have somewhat of a bearing on host density, as temperature plays a significant role in insect development (Ratte 1984; Protasov et al. 2007a). Insects within galls are very much protected during their developmental stage and are mostly susceptible to outside influences during the exposed adult emergence. Interestingly, *O. maskelli* emerges in greatest numbers during the warmest part of the year in California, differing from Israel where the largest population numbers emerge after the population has built up over the winter months and *O. maskelli* development is essentially arrested (Protasov et al. 2007a).

We did not detect any significant correlation between average temperature and either of the parasitoid collections, aside from the *S. neseri* leaf emergence which may be due to very low collection numbers (Table 2.6; Figure 2.22). These parasitoid wasps are much more likely to depend on host density and availability rather than climate. Parasitoids must rely upon the host for opportunities to oviposit and produce offspring, therefore highly influencing population numbers. Temperature likely has much more of a bearing on development, indirectly influencing emergence patterns via development time (Protasov et al., 2007b).

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**Chapter 3:** "New host record: *Selitrichodes neseri* Kelly found parasitizing the eucalyptus gall wasp *Ophelimus maskelli* Girault (Hymenoptera: Eulophidae)"

## Abstract

A strain of *Selitrichodes neseri* (Hymenoptera: Eulophidae: Tetrastichinae) found in southern California is described as a parasitoid of the invasive eucalyptus gall wasp *Ophelimus maskelli* Ashmead (Hymenoptera: Eulophidae). Specimens of this strain were originally collected in California in 2015, reared from *O. maskelli* galls alongside another eulophid parasitoid, *Closterocerus chamaeleon*. Prior to this observation, *S. neseri* has only been known to parasitize another eucalyptus gall wasp, *Leptocybe invasa*. The California strain was identified as *S. neseri* because it is identical with *S. neseri* reared from *L. invasa* morphologically and in 28S D2 rDNA. It differs from South African populations of *S. neseri* in the COI mtDNA barcoding region by 2%, but this value is not normally high enough to suggest a species difference.

## Introduction

The eucalyptus gall wasp, *Ophelimus maskelli* Ashmead (Hymenoptera: Eulophidae) is an invasive pest of *Eucalyptus*. *Ophelimus maskelli* forms small pimple-like galls on the leaves of several different Eucalypt species causing early defoliation. Populations can build up to extremely high numbers, causing tremendous damage to host trees and presenting a serious nuisance to humans at peak emergence (Protasov et al., 2007a).

*Ophelimus maskelli* is native to New South Wales, Australia and has invaded the Mediterranean Basin, Southeast Asia, Northern Africa, and most recently, California (Protasov et al. 2007a, Burks et al. 2015a). In 2004, *O. maskelli* became an uncontrolled significant pest throughout the Mediterranean Basin and required immediate control measures (Protasov et al. 2007b). Due to the protective nature of the gall, chemical control measures are far less effective than biological control. *Closterocerus chamaeleon* Girault (Hymenoptera: Eulophidae) was demonstrated to be a successful biological control agent in Israel (Protasov et al. 2007b). Two other parasitoids were also identified as natural enemies of *O. maskelli*: *Stethynium ophelimi* and *Stethynium breviovipositor* Huber (Hymenoptera: Mymaridae) (Huber et al. 2006). However, *S. neseri* was not found among these other potential biological control agents.

Along with the discovery of *O. maskelli* in California in 2015, both *C. chamaeleon* and *S. neseri* were also reared from *O. maskelli* galls (Burks et al. 2015a, 2015b). Over the course of 18 months and throughout more than 300 leaf samples, no other wasp species besides these have been observed emerging from *O. maskelli* galls in southern California. While *S. neseri* is known to be a parasitoid of *L. invasa* Fisher & LaSalle, no *L. invasa* specimens have been obtained in southern California to date.

## Materials & Methods

*Parasitoid collection site.* Specimens of *S. neseri* were collected from two different sites in southern California. Throughout the summer of 2015, both females and males were reared from galls on leaves collected from several eucalyptus species including *E. rudis*, *E. camaldulensis*, and *E. globulus*, at Laguna Niguel Regional Park (33°32’45N, 117°42’22W). Both females and males were also reared from galls on leaves collected from *E. rudis* at the Agricultural Experiment Station property of University of California, Riverside (33°58’19N, 117°20’24W).

*Molecular methods.* Fresh or dried specimens were used for DNA extraction. Non-destructive sampling was done using chelex + proteinase-K for extraction. Thus, the wasp could then be used as a voucher specimen. All voucher specimens of the California strain were reared from *O. maskelli* and stored at the University of California, Riverside Entomology Research Museum (UCRC). Table 3.1 lists the specimens used, their sex, locality, voucher numbers, and GenBank accession numbers of all sequences. Specimens of the South African strain were provided by the University of Pretoria, and were reared from *L. invasa*. To compare the California strain of S. neseri to the South African strain, we amplified two gene regions were sequenced: the 28S D2 nuclear ribosomal expansion region using forward primer 28S D2 F and reverse primer 28S D2 R, and the mitochondrial cytochrome oxidase I (COI) region, using forward primer LCO-1490 and reverse primer HCO-2198 (Folmer et al. 1994). We followed the PCR protocol and conditions of Burks et al. (2011).

**Table 3.1**

| Specimen (*S. neseri*) | Sex | Locality | Voucher # | GenBank |
|---|---|---|---|---|
| 1 | Male | Riverside | UCRC_ENT00434583 | TBA |
| 2 | Female | Riverside | UCRC_ENT00412685 | TBA |
| 3 | Female | Riverside | UCRC_ENT00412687 | TBA |
| 4 | Female | Riverside | UCRC_ENT00412688 | TBA |
| 5 | Female | South Africa | UCRC_ENT00496091 | TBA |
| 6 | Male | South Africa | UCRC_ENT00496092 | TBA |
| 7 | Female | South Africa | UCRC_ENT00496093 | TBA |

## Results

**Morphology** (Figs 3.1-3.3).

The California strain of *S. neseri* is almost identical with other populations of its species in morphology. Some features are discussed here to report observed variation, and to clarify some details. Terminology used follows Gibson (1997).

***Coloration of both sexes*** Head and mesosoma light brown to dark brown, occasionally with some pale areas flanking sulci and along midline of mesoscutum. Antenna brown to pale brown. Mesosoma light brown to dark brown, occasionally with some pale areas flanking sulci and along midline of mesoscutum; gaster dark to pale brown. Coxae brown (may be pale apically); trochanters dark brown; femora brown with pale apex; tibiae light brown with pale tips and another pale area near midlength.

***Female*** (Figs 3.1-3.2). Length 0.82-1.14 mm.

*Antenna* with 2 anelli, 3 funiculars and 3 clavomeres. Funiculars about as long as wide. Clava 2-2.5 times as long as wide, wider than funicle, with terminal spine.

*Forewing* usually, but not always, with brownish area posterior to marginal vein.

***Male*** Length 0.54-.93 mm. Antenna with unsegmented clava (Fig. 3.3, this is true for all *S. neseri*).

**Molecular Data**

Both strains were identical for 28S D2. However, the sequences for the COI barcoding region differed for 13 out of 645 comparable bases between the strains. This 2% sequence divergence does not provide strong evidence that the strains are separate species according to conventional criteria (Ratnasingham and Hebert 2007). Further investigation could test this value, using several populations reared from each host, but such data were not available for populations reared from *L. invasa*. This variation suggests that the populations of *S. neseri* could be distinct or a recent divergence, but do not conclusively indicate this. Therefore, we refer to them as two different strains.

## Notes on Biology

Several specimens of *Selitrichodes neseri* were exposed to *Eucalyptus globulus* potted saplings to test whether this California strain might produce galls. However, it was determined that they do not form galls.

Unlike specimens collected in South Africa (Kelly and La Salle 2012), the California strain of *S. neseri* was reared only from galls formed by *O. maskelli*. Leaf samples were collected from two locations in southern California. The leaves were placed in a sealed plastic sandwich bag with a damp paper towel to keep the leaves from desiccation. Although *S. neseri* has been observed as a larval ectoparasitoid of *O. maskelli* (K.W. Pers. Obs.), it is unclear whether or not *S. neseri* functions as a primary parasitoid, hyperparasitoid, or facultative hyperparasitoid. *Ophelimus maskelli* was discovered in California along with an associated parasitoid, C. chamaeleon. A suitable technique has not yet been developed to determine the exact contents of the galls prior to emergence, therefore it is possible that *S. neseri* also attacks *C. chamaeleon*.

The source populations for these three wasp species in California remains unclear. This is the first record of *S. neseri* found as an invasive species. Populations of *S. neseri* in South Africa were introduced from Australia as part of a biological control program to combat the negative effects of *L. invasa* (Kelly and La Salle 2012). It is unlikely these wasp populations originated from Europe, as *S. neseri* has not been introduced nor observed in that area. All three species are currently found in South Africa, in addition to their native habitat of Australia (Bush et al. 2016; Kelly and La Salle 2012).

It may also be important to note that the relative abundance of *S. neseri* is much lower than that of *C. chamaeleon* and *O. maskelli* in both California locations.

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## Figures

**Fig. 3.1** *Selitrichodes neseri* female, lateral view

**Fig. 3.2** *Selitrichodes neseri* female, dorsal view

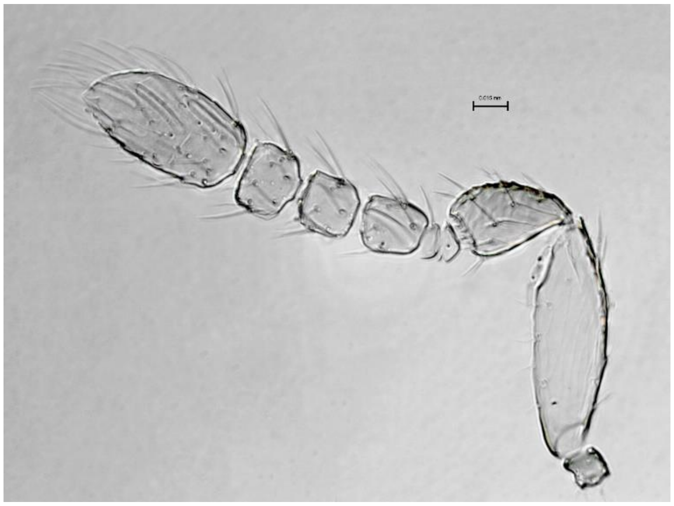

**Fig. 3.3** *Selitrichodes neseri* male antenna

## **Chapter 4:** Summary of studies on ecology and intraguild relationships among the invasive wasp *Ophelimus maskelli* and two associated parasitoid wasps *Closterocerus chamaeleon* and *Selitrichodes neseri* (Hymenoptera: Eulophidae) in California

### Conclusions

The eucalyptus gall wasp, *O. maskelli*, is an understudied, yet damaging pest of eucalyptus. Studies such as those described here provide key insights into the biology and natural history of the pest which information is valuable to future biological control endeavors. Eucalyptus is an important plant in several countries throughout the world; useful in various ways and valued for its efficient growth. *Ophelimus maskelli* poses an international threat to eucalyptus, especially within plantations where pests spread both easily and rapidly.

Understanding the phenology of *O. maskelli* in California and comparing that to what we know of the species in other countries can help elucidate how invasive species adapt to different environments. Not only do we see differences in phenologies between Israel and California, but also between inland and coastal sites within California. These differences include: the timing of adult emergence, the number of emergences per year, correlation between emergence and temperature, and a correlation between emergence and leaf availability.

Despite having very similar climates, we observe major differences in adult emergence between southern California and Israel. There are clearly three periods throughout the year in Israel when adult *O. maskelli* emerge from their galls (Protasov et al. 2007). However, we observe only two clear emergence periods in Laguna Niguel and just one in Riverside. This difference in emergence periods suggests a variance in the developmental period for *O. maskelli*. Yet, we observe similar timing of emergence for all three locations, indicating a significant role of climate in the wasp development accompanied by some other contributing factors. The significant positive correlation between temperature and the observed number of *O. maskelli* individuals provides further evidence in support of this conclusion.

In addition to the sampling of both inland and coastal sites in southern California, we used two different methods of sampling: passive trapping with green sticky traps and leaf emergence. The green sticky trap method has been used successfully for monitoring both *O. maskelli* and *C. chamaeleon* in Israel (Protasov et al. 2007b). This method provides information about the population of active adults in the area, as well as capturing any other wasp species that we may not yet be aware. The second method, capturing adults as they emerge from a specific leaf, guarantees that the captured specimens originated from that particular leaf/tree/area. It also can provide the ratio of hosts to parasitoids on a single leaf, which indicates the effectiveness of the parasitoid as a means for biological control.

The phenology of *C. chamaeleon* also differs between the inland and coastal sites of southern California. Although we do not have any phenological data for *C. chamaeleon* in Israel, we can still compare our data for two sites in California. As expected, we observed greater numbers of *C. chamaeleon* in Laguna Niguel where we observed greater numbers of the host. The timing of adult wasp emergence varied so greatly between the two locations that very few dates overlap. However, the significance of the date factor suggests that there are particular emergence patterns annually. These patterns are most likely influenced by temperature and climate. Despite its success in Israel, the green sticky trap caught fewer *C. chamaeleon* than the leaf emergence method. This difference may indicate that *C. chamaeleon* can rapidly control small areas, such as a leaf, but may not necessarily be as successful in controlling the entire population. This is especially interesting because adult *C. chamaeleon* only produce about seven offspring on average (Protasov et al. 2007b). However, the development time for *C. chamaeleon* is much less than that of *O. maskelli*, allowing for the parasitoid population to build much more rapidly than that of the host, making it an excellent natural enemy.

The presence of this second parasitoid, *S. neseri*, is particularly interesting for many reasons. The only record of this species anywhere outside of its native habitat of Australia is in South Africa where it has been utilized as a biological control agent for another eucalyptus gall wasp, *L. invasa* (Kelly and La Salle 2012). Therefore, we do not know very much about the phenology of this species in any area of the world. In southern California, we continue to observe these phenological differences between inland and coastal sites. As with *C. chamaeleon*, much greater numbers of *S. neseri* were found in the coastal area. We would expect to see this species correlation with population density of the host. It is also possible all three species were introduced to the coast of southern California and have since spread to more inland areas. Unlike *C. chamaeleon*, two emergence periods of *S. neseri* consistently parallel host emergence periods, suggesting a strong link between parasitoid and host emergence. As the first record of *S. neseri* phenology in the world, this information is valuable not only to further our knowledge of this species, but its capabilities as a biological control agent.

This the first record of *S. neseri* in California, in addition to the parasitizing of a different species of eucalyptus gall wasp than previously reported, *O. maskelli*. To determine relatedness between the *S. neseri* specimens found in southern California and those reared in South Africa, both morphological and molecular data were analyzed.  Morphologically, the two strains are almost identical aside from generally longer body length in the California strain. Molecularly, both strains were found to be identical for ribosomal DNA. However, there was a 2% sequence difference for the mitochondrial barcoding region. Without much more molecular data from several different populations we are unable to further test this variation between these two strains. Noting these biological, ecological, and molecular differences, it is possible that *S. neseri* may be currently undergoing a speciation event.

Due to the unintentional introduction of these species into California, the origins of these populations remain uncertain. *Ophelimus maskelli* has spread across various countries throughout the world unaccompanied by parasitoids (Branco et al. 2009; Burks et al. 2015; Doğanlar and Mendel 2007). In response to this invasion, scientists from Israel intentionally introduced populations of *C. chamaeleon*, as well as *Stethynium ophelimi* and *Stethynium brevioviipositor* from Australia, but did not include *S. neseri* (Huber et al. 2006; Protasov et al. 2007b). In South Africa, *S. neseri* was introduced to control a different pest species, *Leptocybe invasa* (Kelly and La Salle 2012). The situation in California was unique, where there was the unintended introduction of not only the pest species, but two associated parasitoid species.

All three wasp species present such curious dynamics within the realms of pest control, intraguild relationships, species variation, and phenology. The eucalyptus gall wasp, *O. maskelli*, affords us many opportunities to study several different important aspects of pest control including competition among parasitoids; an influential part of natural enemy biology and behavior. Our studies provide a starting point for future investigations and the implications of this information reach much further than pest control in southern California. As time passes and more information is gathered, we are able to illuminate not only these specific relationships, but the implications these relationships have for invasive species and biological control of pests around the world.

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## Appendix

**Figure A.1. Effect of leaf availability on *O. maskelli* abundance from leaf emergence samples in Laguna Niguel**

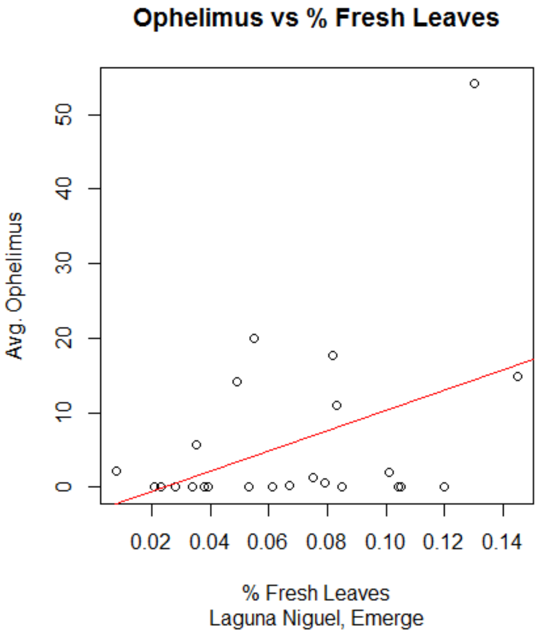

**Figure A.2. Effect of leaf availability on *O. maskelli* abundance from sticky trap samples in Riverside**

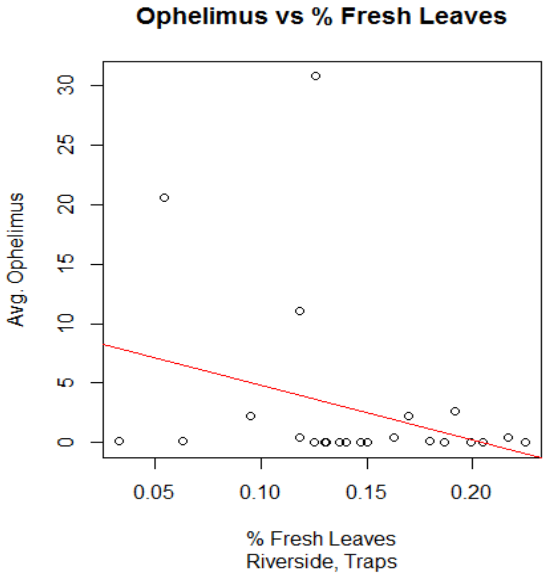

**Figure A.3. Effect of leaf availability on *C. chamaeleon* abundance from sticky trap samples in Laguna Niguel**

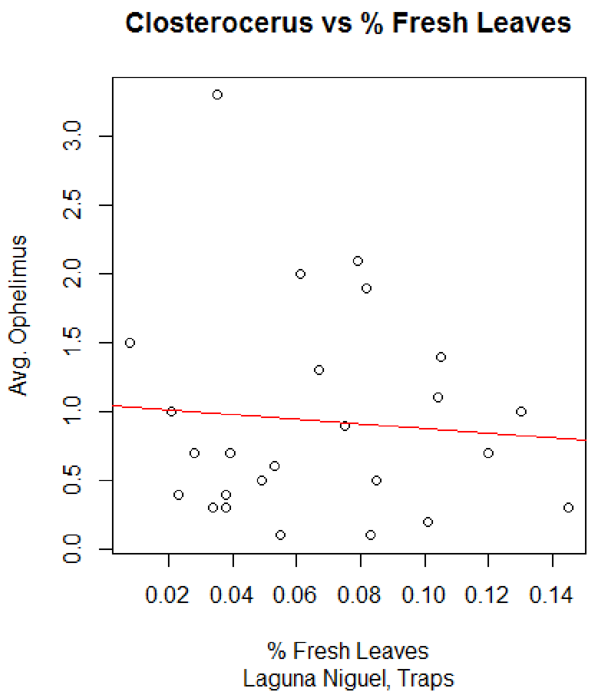

**Figure A.4. Effect of leaf availability on *C. chamaeleon* abundance from leaf emergence samples in Laguna Niguel**

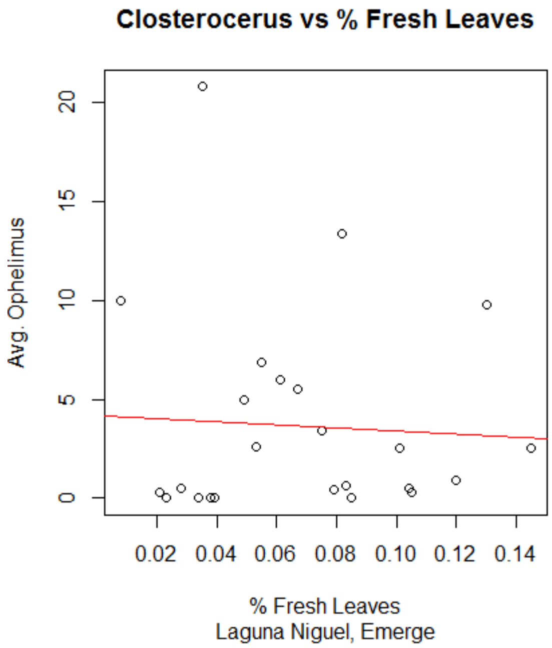

**Figure A.5. Effect of leaf availability on *C. chamaeleon* abundance from sticky trap samples in Riverside**

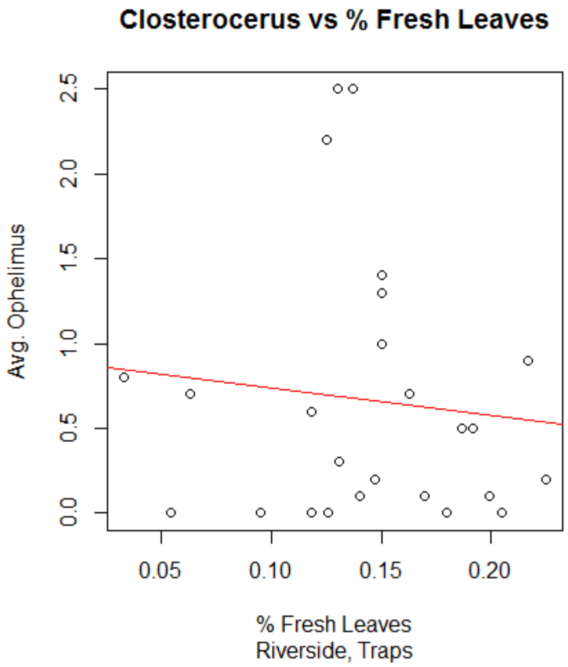

**Figure A.6. Effect of leaf availability on *C. chamaeleon* abundance from leaf emergence samples in Riverside**

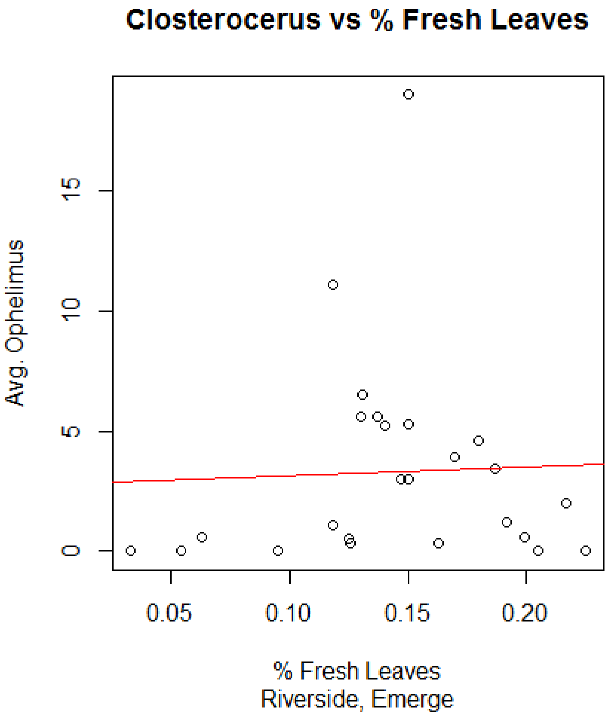

**Figure A.7. Effect of leaf availability on *S. neseri* abundance from sticky trap samples in Laguna Niguel**

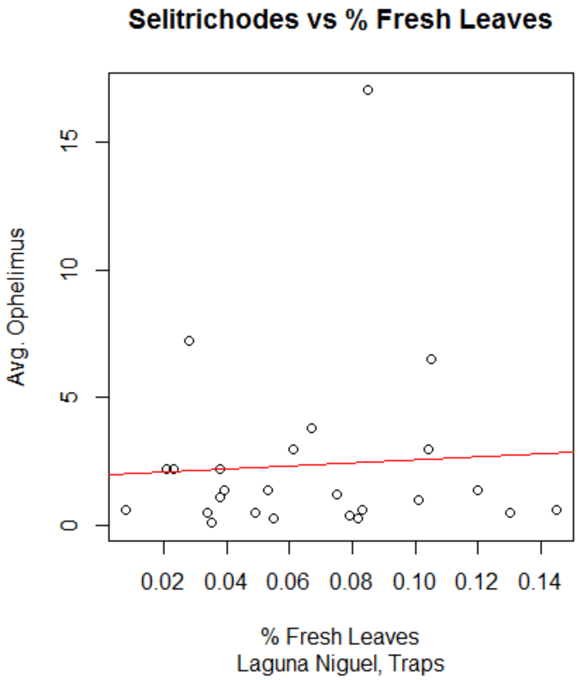

**Figure A.8. Effect of leaf availability on *S. neseri* abundance from leaf emergence samples in Laguna Niguel**

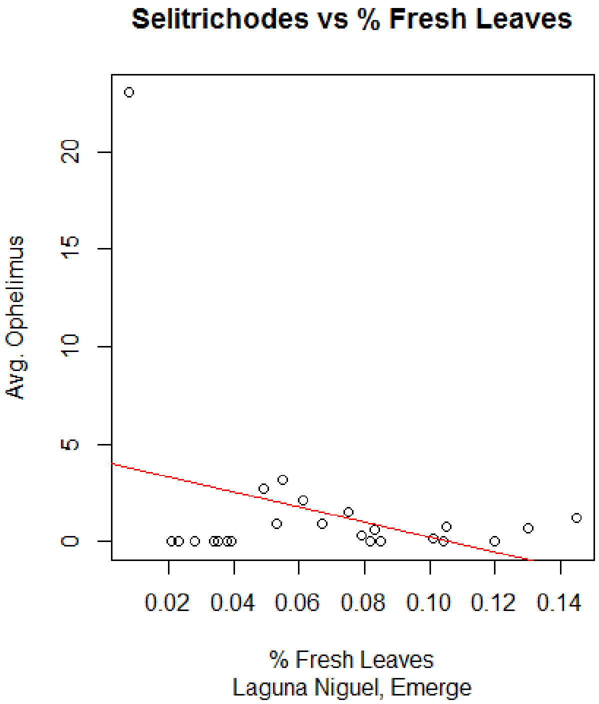

**Figure A.9. Effect of leaf availability on *S. neseri* abundance from sticky trap samples in Riverside**

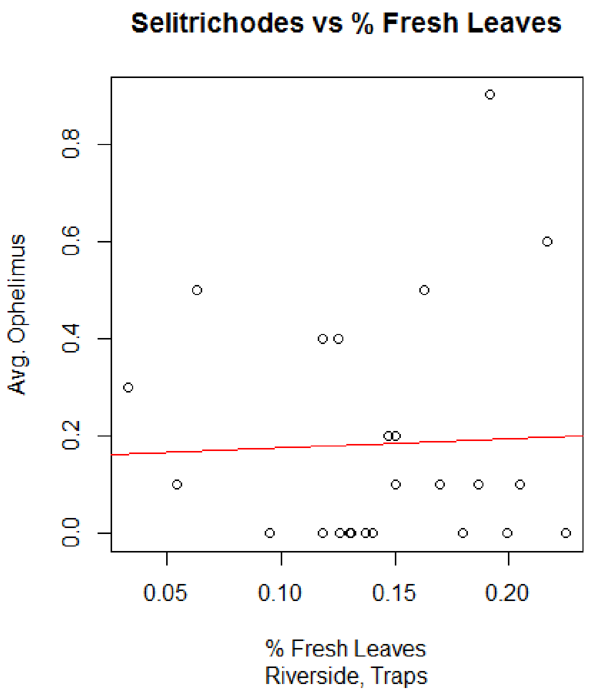

**Figure A.10. Effect of leaf availability on *S. neseri* abundance from leaf emergence samples in Riverside**

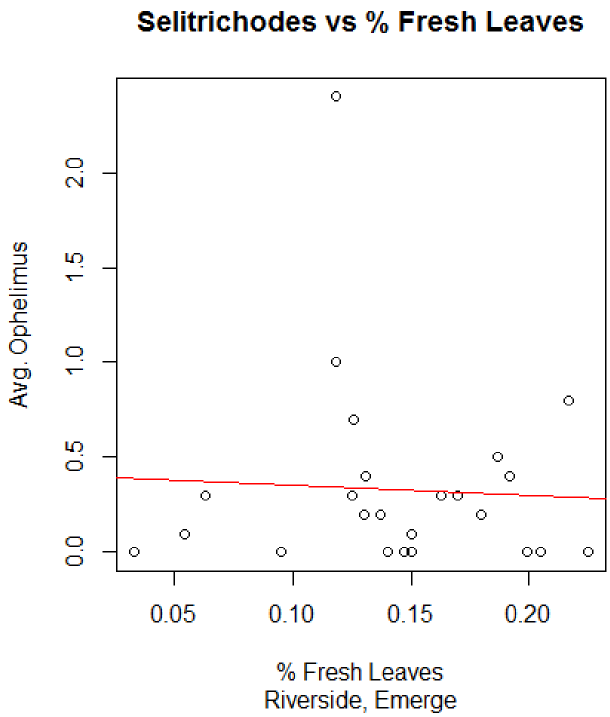

**Figure A.11. Effect of temperature on *O. maskelli* abundance from leaf emergence samples in Laguna Niguel**

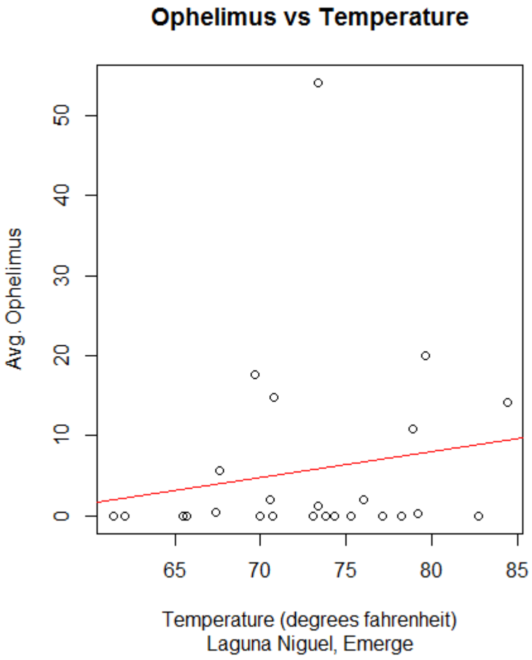

**Figure A.12. Effect of temperature on *C. chamaeleon* abundance from sticky trap samples in Laguna Niguel**

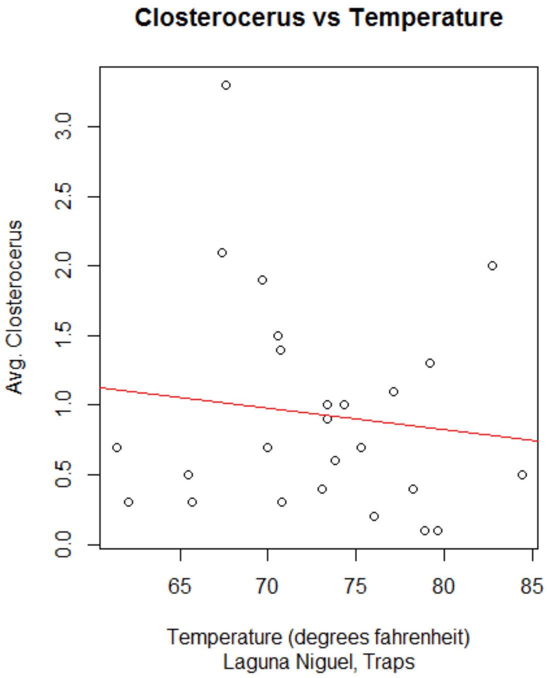

**Figure A.13. Effect of temperature on *C. chamaeleon* abundance from leaf emergence samples in Laguna Niguel**

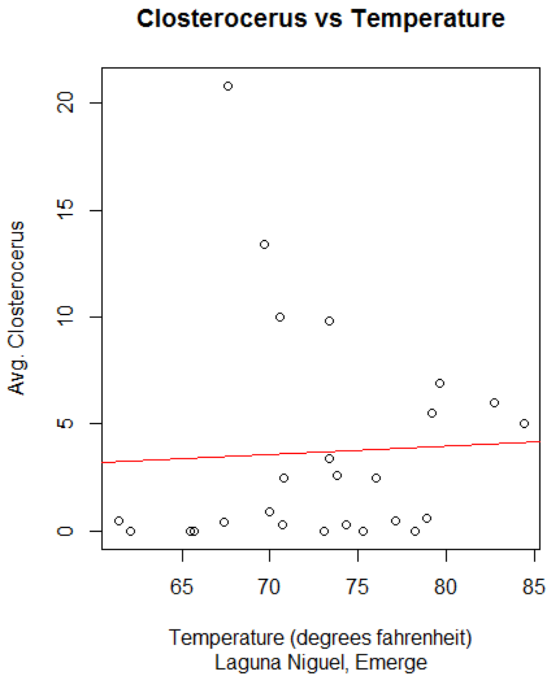

**Figure A.14. Effect of temperature on *C. chamaeleon* abundance from sticky trap samples in Riverside**

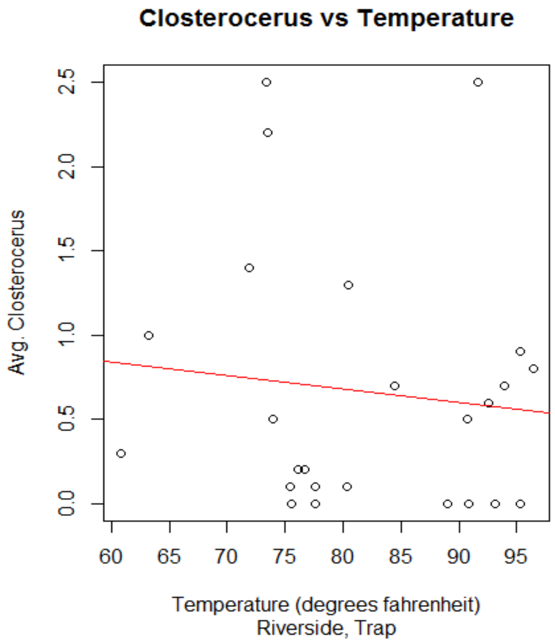

**Figure A.15. Effect of temperature on *C. chamaeleon* abundance from leaf emergence samples in Riverside**

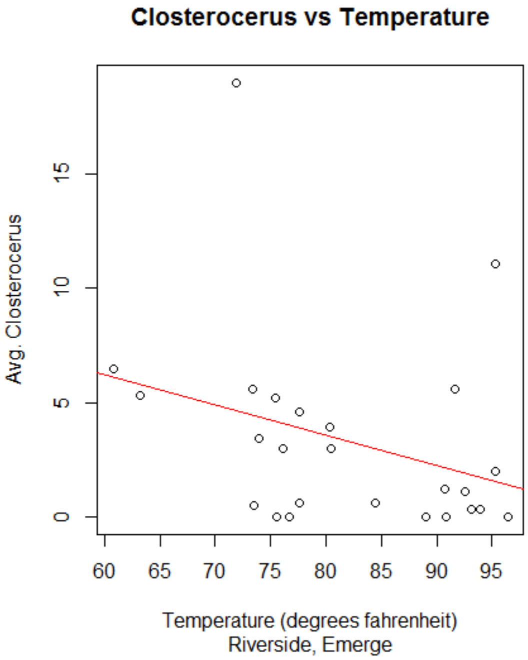

**Figure A.16. Effect of temperature on *S. neseri* abundance from sticky trap samples in Laguna Niguel**

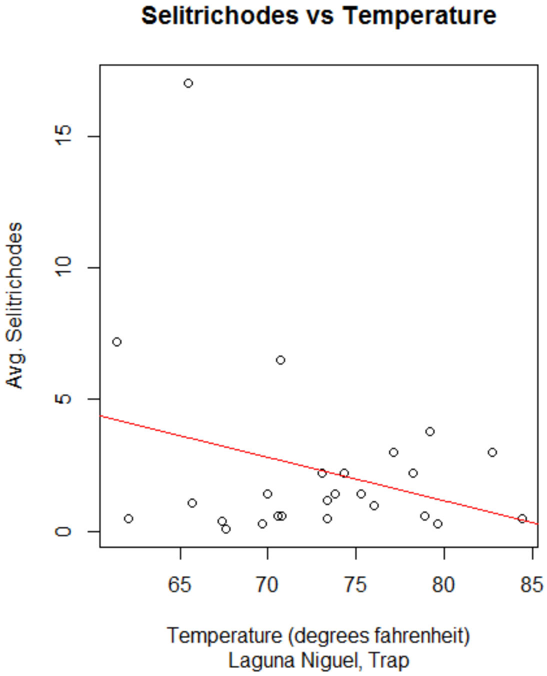

**Figure A.17. Effect of temperature on *S. neseri* abundance from sticky trap samples in Riverside**

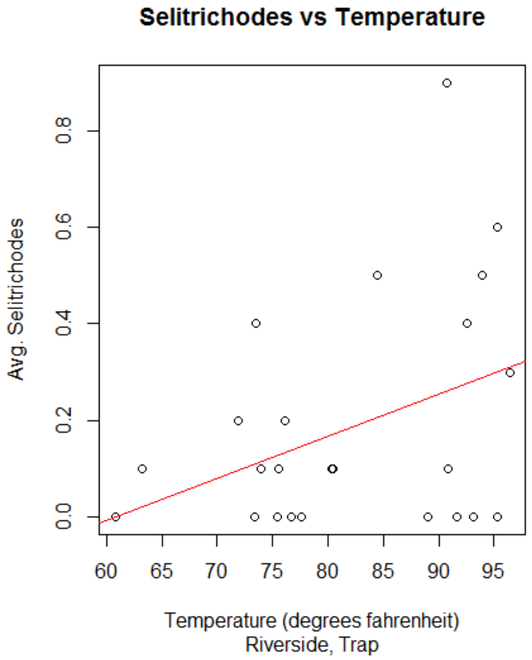

**Figure A.18. Effect of temperature on *S. neseri* abundance from leaf emergence samples in Riverside**

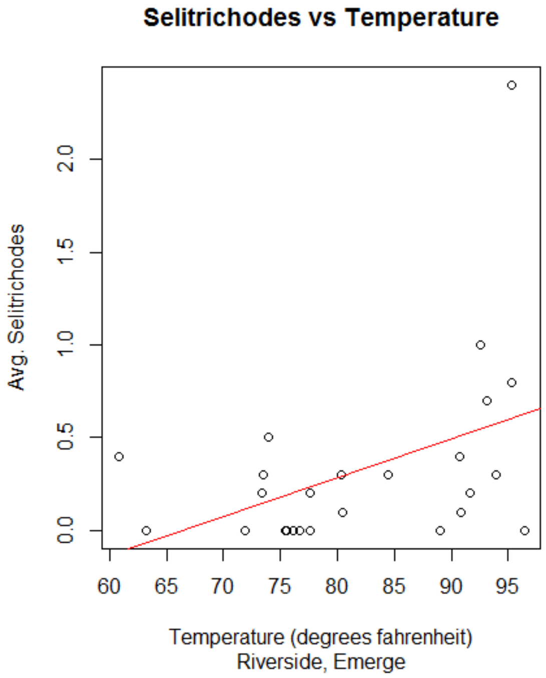

**Figure A.19. Showing the emergence periods for *Ophelimus maskelli* in all three locations.**