Oviposition Behavior of the Female Coconut Rhinoceros Beetle, *Oryctes rhinoceros*

(Coleoptera: Scarabaeidae)

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DEDICATION

To my mother, Marilyn Noble Manley, for sacrificing so much to raise me and my sisters and for showing us what a strong woman is. Thank you for never giving up on me and helping me to grow into a woman I know Lolo would be proud of.

I love you Mom.

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ABSTRACT

The coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* (L.) (Coleoptera: Scarbaeidae), has become one of the most important coconut and oil palm pests to date. CRB recently emerged in December 2013 as a new invasive pest of coconut palms on Oʻahu, Hawaiʻi. Since its detection, efforts have been expended performing delimiting and monitoring surveys, detecting and sanitizing breeding sites, and developing methods for eradication and control. To aid these efforts, information on the factors influencing female adult oviposition behavior and identifying these behaviors is needed. In this study, data and observations were collected from a series of substrate choice tests and depth observations to assess if both lab-reared and wild-caught CRB have a preference for oviposition site or egg-laying pattern. Small or large particle sizes, as well as high (8dS/m) or low (2 dS/m) salinity substrates, were tested and oviposition depths were observed using an observational chamber. Female CRB were found to prefer small particle size substrate and lay their eggs in an aggregated dispersion pattern at various depths up to 119 cm. No preference for substrate salinity was found. Additionally, female CRB were found to differ significantly in their oviposition behavior between lab-reared and wild-caught beetle types. These results can help to identify potential breeding sites and can also be implemented into management programs for future eradication and prevention efforts.

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CHAPTER 1.

INTRODUCTION AND LITERATURE REVIEW

Native to south and southeast Asia (Bedford, 1980), the coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* (L.)(Coleoptera: Scarabaeidae), has been unintentionally introduced throughout the South Pacific to countries and territories such as Papua New Guinea, Samoa, American Samoa, Tonga, Fiji, Wallis Island, Micronesia, the Cocos Islands, Saipan, and Guam. The spread of CRB throughout the Pacific is believed to have occurred more rapidly during World War II with the increase in aircraft and shipping activity in the region (Bedford, 1980). However, CRB was not found reproducing in Hawai‘i until December 2013 (USDA APHIS, 2015) although it had been intercepted previously five times (Molet, 2013). In Hawai‘i, as in other areas where it has been introduced, the beetle has become a major problem because adult feeding causes moderate to severe damage to palms, and there are few natural enemies that control populations. Without control, the beetle can decimate coconut palm populations and is now considered one of the most important coconut and oil palm pests worldwide (CABI, 2015). In Hawai‘i, palms are important in natural ecosystems, for the commercial nursery industry, are part of the iconic scenery that draws tourists, as well as culturally significant to many Pacific Islanders (USDA APHIS, 2015). The multiagency response to CRB incursion in Hawai‘i relies on detection of adults through deployment of traps equipped with pheromone lures and solar powered LED/UV lights to attract adult beetles (USDA APHIS, 2015), surveys of damaged palms, and location and sanitation of breeding sites.

One of the major challenges in mitigating the spread and achieving eradication of CRB in Hawai‘i is identifying active and possible oviposition sites. It is unclear what factors

stimulate a beetle to choose a particular site in which to oviposit or even how deep a female will dig to lay her eggs. To further CRB prevention and management programs, an understanding of egg laying behaviors is needed.

Impact of CRB

The coconut palm (*Cocos nucifera*) is the most extensively grown and used nut in the world (Broschat and Crane, 2014). It is an important commercial crop in many countries of the South Pacific, which contributes significantly to their economies in different ways. Copra, dried coconut kernel, is the main product of coconut palms being a source for coconut oil, and in many countries the fruit of the palm is consumed locally for food or exported around the world. Alongside the coconut palm as a host plant, are the oil (*Elaeis guineensis*) and date (*Phoenix dactylifera*) palms, which can also be severely damaged by CRB (Bedford, 1980). The oil palm produces bunches of fruits that yield both palm oil and kernel oil, while the date palm produces edible sweet fruits as well as fruit clusters that can be used for brooms, and seeds that can be ground up to use for animal feed (Bedford, 1980).

The adult stage of the coconut rhinoceros beetle are nocturnal, gregarious and burrow through the palm crown to the growing point to feed on tissues and plant liquids (Hinckley, 1973). Multiple beetles can attack a single palm at once, with taller prominent palms being attacked more frequently, and one attack increasing the probability of future attacks (Bedford, 1980). Adult beetles can burrow about 10 - 50 cm down to the center of the spear cluster most often damaging the midrib of the leaves (Giblin-Davis 2001, Lever 1979). By doing so, they damage young plant tissue and can reduce coconut production as

well as result in complete tree death, with younger palms, between 1 - 3 years old, being more susceptible than mature palms (Giblin-Davis, 2001). Adult beetles are the only life stage that can cause damage, while larvae cause no damaging economic impact. Damage can be caused to the inflorescence, which can also delay fruit development. CRB attacks may also provide entry points for secondary attacks by other insect pests or pathogens.

Signs of CRB infestation of palms include boreholes, typically on the stems of fronds, which are created and can be seen visibly after the adult beetle has burrowed through the frond leaving behind a fibrous frass that has been pushed from the burrow (Molet, 2013). Hinckley (1973) found that adult beetles can make burrows towards the crown ranging from 2 to 50 cm (average 21 cm), and when beetles were placed in artificial holes 4 cm deep, they showed an average duration inside the holes of 6 days and a burrowing rate of 5 cm per day for 3 days, followed by 3 days of less activity. V-shaped damage to the palm fronds, which result from adult beetles boring through developing fronds, can be seen after the frond grows out and expands. Burrowing lower will damage more fronds, and the closer CRB will get to the inflorescence and growing point (Bedford, 1980), which was also observed by Young (1975) who found that most attacks begin in the axils of opened fronds closer to the growing point.

Landscapes in Hawai‘i and Guam where the beetle incursion is still fairly new, are also at high risk of economic loss as the beetles have shown to cause severe economic and ecological damage in non-native lands. Economic losses such as reduced yields, cost of control measures and eradication efforts, replanting to replace damaged or dead trees, removal of trees, and quarantine procedures are all costs that should be taken into consideration when thinking of the economic impact of CRB. For instance, in Palau 50% of

their coconut palms were destroyed by CRB within 10 years of its introduction in 1942 (Gressit, 1953). In 1968, CRB caused $US 1,100,000 in damage to the South Pacific alone (Bedford, 1980). In India, CRB is responsible for 10% of all coconut yield loss, and in the atoll of Diego Garcia a third of all seedlings planted are killed due to CRB damage (Catley, 1969).

Although CRB prefer to feed on coconut palm (*Cocos nucifera*), oil palm (*Elaeis guineensis*), and date palm (*Phoenix dactylifera*), they have also been found to feed on banana (*Musa*), sugarcane (*Saccharum officinarum*), papaya (*Carica papaya*), sisal (*Agave sisalana)*, and pineapple (*Ananas comosus*) as well as ornamentals like the royal palm (*Roystonea regia*), the latanier palm (*Latania loddigesii*), the talipot palm (*Corypha umbraculifera*), and the raphia palm (*Raphia*) (CABI, 2015). These are less preferred targets, but if attacked can result in potentially severe economic loss of crop and ornamental industries. The incursion of CRB in Hawai‘i is also of proximate concern to the date palm industry in California. Estimated at $US 68 million in 2015 (USDA NASS, 2017), this industry is at high risk for CRB damage should this beetle establish in California.

Life History

The coconut rhinoceros beetle life cycle usually lasts around 4-9 months, with more than one generation possible to occur per year, and is comprised of four stages: egg, larva, pupa, and imago or adult stage (Manjeri et al. 2014, Giblin-Davis 2001). Life stages are variable depending on climatic conditions and nutrition in the location that development occurred (Kamarudin et al., 2004). Unfavorable climatic or nutritional conditions for CRB can delay larval development, extending it to as long as 14 months (Bedford, 1980). Through life cycle observations in Western Samoa, Hinckley (1973) found that CRB larvae

with a prolonged development produce smaller than average and short-lived adult beetles. A dry climate and low nutritional conditions commonly delay the development of CRB and result in smaller adults (Catley, 1969).

Egg

CRB eggs are a whitish brown color about 3 - 4 mm long and are initially soft and oval shaped, but will swell into a circle about 4 - 5 days after oviposition (Giblin-Davis 2001, Hinckley 1973). Fertilized eggs can hatch between 8-12 days (Molet, 2013).

Larva

Complete development of the larval stage takes between 82 - 207 days. The first instar lasts for about 10 - 21 days, the second lasts for about 12 - 21 days, and third instar lasts for 60 - 165 days (CABI 2015, Bedford 1980). All instars of larvae are a whitish color with a brown head capsule and legs, and a grey posterior end of the abdomen. Instar stages can be differentiated according to the size of the head capsule (Giblin-Davis, 2001). First instar larvae are ~7.5 mm long (Lever, 1979), while the third instar larvae are C-shaped and about 60 to 105 mm long (Giblin-Davis 2001, Gressitt 1953). CRB spend their entire larval stage inside the breeding site, and prefer to feed on dead or decaying plant material. Larvae have shown to prefer temperatures of 27 - 29°C, 77% moisture content, and a soil pH lower than 4.2 to increase population density (Kamarudin et al. 2004, Manjeri et al. 2014, Costa 1967). Larvae also showed preference for higher humidity and showed behavior that was dominated by light factors, which could help select for areas promoting survival or development (Costa, 1967). These beetles can develop in many types of media including decaying organic matter, many types of soil, mulch, compost heaps, and rotting palm material including dead standing trees. In countries such as the Philippines, Malaysia,

Palau Islands, Tonga, Samoa, Fiji, New Ireland, Mauritius, India, Burma, and Papua New Guinea, breeding sites have been found in rubber trees stumps and other types of decaying wood, compost, cocoa pod shells, cow dung and sawdust heaps, decaying *Pandanus* trunks, and rotting paddy straw (Bedford, 1980).

Pupa

Following the larval stage, CRB then enter the non-feeding prepupal stage that lasts for about 8 - 13 days and continues on to the pupal stage, lasting for about 17 - 30 days (Schmaedick 2005, Bedford 1980). The larvae will build an oval pupal casing around themselves, usually measuring about 55 x 35 x 33mm and enclose it with frass (Gressitt, 1953). Pupation can occur in the soil or breeding site and the casing can be constructed of materials like plant tissue, sawdust, or frass (Giblin-Davis 2001, Vargo 1995). Pupae are a yellowish brown color and can range between 39.5 and 51.5mm in length, 19 to 23.6mm in width, and 16 to 19.4mm in depth (Gressitt, 1953). After pupation is complete, adult beetles can remain in the pupal enclosure for about 11 - 20 days to allow the exoskeleton to darken and harden (Giblin-Davis 2001, Hinckley 1973). Adult beetles will then emerge and leave the developmental site around 20 - 30 days after ecdysis to feed (Bedford, 1980).

Adult

Adult beetles are large; usually about 30 to 57 mm long, with a shiny black or brown exoskeleton, a reddish hue on the ventral surface, and a backward curved horn on its head giving it the iconic name, rhinoceros beetle (Giblin-Davis, 2001). Adults are gregarious insects and are attracted to host kairomones while also employing either an aggregation or sex pheromone, or both (Bedford, 1980). Bedford (1976) found that male beetles live on average 6.4 months as an adult, while female beetles live on average 9.1 months. CRB have

sexual dimorphism and can be sexed according to the tufts of orange hairs that grow on the abdomen, the size of the horn, and if the abdomen is pointed or rounded. Female beetles typically have a smaller horn with a more pointed abdomen covered in small orange hairs, while male beetles typically have large horns with a rounded abdomen, usually having very few small orange hairs, or no hairs at all. Adult horn and body size can vary greatly between the two sexes, and is correlated with the larval environment (Gressitt, 1953).

According to Vander Meer (1986), CRB adults reach a maximum mass when they emerge from their pupal casing and go through three behavioral phases correlated with body mass. The first phase is ~30-day period of non-feeding and weight loss until adult beetle weight is down to about 65% of its emergent weight. The beetle then locates a food source and increases its weight to about 80% of its emergence weight. The second phase begins when the beetle feeds, lasting about 120 days of flying and feeding. Beetle weight will fluctuate between about 60 - 80% of its emergent mass until its third phase where the beetle stops feeding completely and decreases in weight continually until death occurs, usually at about 40% of its emergent mass. CRB showed to take flight, usually between dusk and dawn, lasting between 2 - 3 hours, and distances traveled were up to 4 km (Catley 1969, Hinckley 1973).

Reproduction

After copulation, female coconut rhinoceros beetles make serpentine burrows compressing breeding site material behind her, and laying eggs in clutches. According to a study by Hinckley (1973), CRB were found to have a clutch size ranging from 11 - 62 eggs and averaged 27, with beetles being able to lay between 3 - 4 clutches of eggs in a lifetime. Similarly, Bedford (1976) found the number of eggs oviposited per female ranged from 24 -

65 (average 51), with between 70 - 100 eggs laid in a lifetime. Higher egg numbers were seen with larger female beetles rather than smaller ones (Hinckley, 1973) and were deposited at a decreasing rate throughout the lifespan (Zelazny, 1973). Female beetles that do not feed are still able to develop eggs, but without fertilization, these eggs are unable to hatch (Hinckley, 1973).

Bedford's (1980) laboratory study showed that oviposition could occur before beetles emerge from the sites in which they developed. However, mating and oviposition usually take place after the young beetles have left the pupation site and after the first feeding (Bedford 1980, Manjeri et al. 2014). Adult female CRB can utilize spermatozoa that retain their vitality up to six months in the female's spermatheca so that multiple matings are not necessary but can occur; enabling a female beetle to continue laying fertilized eggs for several months (Bedford 1980, Hinckley 1973). More than one generation can occur per year with different broods at different developmental stages being able to occur simultaneously (Bedford, 1976).

Pest Management

There has been limited success in managing coconut rhinoceros beetle populations not only in Hawai'i, but worldwide. Effective control has been achieved in some locations through the use of a virus, *Oryctes rhinoceros nudivirus* (OrNV). Others have used cultural management or chemical management, or an integrated pest management program incorporating many of the various approaches.

Monitoring and Trapping

Pheromone trapping is currently the most common procedure to detect and reduce infestations of CRB (Manjeri et al., 2013). This is done through use of a male produced

aggregation pheromone and a female produced sex pheromone. However, the species-specific aggregation pheromone, ethyl 4-methyloctanoate, showed to work best for mass trapping and monitoring (Hallett, 1995). Hallett et al. (1995) discovered this pheromone in Indonesia, which aided in mate selection, finding breeding sites, and searching for food (Manjeri 2005, Alfiler 1999). Kamarudin et al. (2004), found that 91.9% of female beetles caught in pheromone traps were gravid, suggesting that these traps can reduce the chance of breeding by capturing the gravid females and preventing them from finding a mate or breeding site. However, Manjeri et al. (2013) found that not all populations of *O. rhinoceros* in the field were observed to be attracted to the aggregation pheromone.

Cultural Management

The only documented example of CRB eradication was achieved on Niuatoputapu Island. Eradication was accomplished by destroying the beetle's breeding sites, which spanned over a seven-year period ending in 1930 (Bedford, 1980). Shredding and burning of felled trunks and organic matter were common practices to reduce CRB populations, and although it was an effective method, it was very expensive and was banned in different countries to reduce air pollution in the region (Hallet et al., 1995). Instead, other cultural management methods were practiced to attempt to decrease CRB populations. Three pulverization techniques, the Enviro Mulcher Method, The Mountain Goat Method and The Beaver Method, were used in Malaysia to reduce the decomposition period of felled palms that could potentially be used as CRB breeding sites (Manjeri et al. 2014). Kamarudin et al. (2004) also suggested reducing breeding sites before a rainy period since beetles have shown to be more active during this time. Cover crops with a height above 70 cm tall were found to be an adequate cultural control method by acting as a barrier to breeding sites and

influencing the field population of CRB to decrease (Kamarudin et al., 2004). It was suggested by Owen (1961) that vegetative barriers could interfere with the beetle's ability to locate palms for feeding as well as be used to conceal breeding sites, and provide a physical barrier to flight. Other suggestions for mitigation include chopping and burning decaying logs, cutting stumps as close to the soil surface as possible, and growing vines or ground covers that can be planted to grow over logs or stumps that cannot be destroyed (Schmaedick, 2005).

Chemical Management

Various methods of chemical control have been attempted. Leaf axils of palms were fitted with sawdust containing pesticides to prevent CRB from spreading in the Fiji Islands (Huger, 2005) and naphthalene balls were placed at the base of palm fronds in various countries (Gressitt, 1953). However, these attempts proved futile.

Overall, chemical control methods have been found to be mainly ineffective in controlling CRB populations below the economic threshold due to its lack of direct contact with the insect, which spends a majority of its life within a breeding site or feeding gallery avoiding direct exposure to chemical applications. Chemical control may also cause additional health and environmental hazards, and its cost makes it a less appealing method for control and eradication efforts, particularly in developing nations.

Biological Control

In 1965, biological control via the release of natural enemies into the Pacific Islands was endeavored with the initiation of the UNDP/SPC Project for Research on the Control of the Coconut Rhinoceros Beetle (Young 1986, Jackson 2009). From observations made over time, CRB were seen being eaten by rodents, larvae of the elaterid beetle (*Lunelater*

fuscipes), centipedes (*Scolopendra nzorsitmzs*), or parasitized by the scoliid wasp (*Scolin ruficornzis*) (Hinckley, 1973). Other predators and parasitoids of CRB include insects like Coleoptera (Elateridae, Histeridae and Carabidae), Hemiptera (Reduviidae), hymenopteran (Scoliidae) and dipteran (Tachinidae) parasitoids, along with ants, and vertebrates (lizards, rats, chickens, mice, shrews, squirrels, lemurs, monkeys, mongoose, heron, barn owls and pigs) (Hinckley, 1967). However, none of which could reduce CRB populations significantly or be used as a biocontrol agent.

One biological control agent found and used to control *Oryctes* species, is the fungus *Metarhizium majus* (formerly *M. anisopliae* var. *majus*), which has been implemented since 1913 (Bedford, 1980). *M*. *majus*, a green Muscardine entomopathogenic fungus, can attack CRB larval stages (Manjeri et al., 2014). The fungus can be spread through spores that can germinate on or penetrate the cuticle of CRB entering the insect, as well as through dead larvae that contain local pockets of spores (Hochberg and Waage, 1991). Infected adults can spread the fungus only after they have died, therefore making the fungus dependent on adult movement after infection (Hochberg and Waage, 1991). *M. majus* can also be applied as a microbial pesticide, as seen in Malaysia, to the surface of breeding sites with the spores remaining viable for at least 24 months, and larvae killed three months after surface application (Bedford, 1980). Ramle's (1999) study similarly sprayed a broadcasted spore solution and solid substrate of spores onto breeding sites in field trials, and found that they significantly reduced larvae and the overall CRB population with up to 100% of third instars affected and killed between 12 - 14 days after treatment.

Another biological control agent used to control populations of CRB, considered a classical biological control success, is OrNV. OrNV is a non-occluded dsDNA virus and was

previously named *Rhabdionvirus oryctes*, and later *Oryctes virus* (Bedford 1986, Huger 2005). OrNV is a peroral infection able to attack the larval and adult stages of CRB (Zelazny 1973, Bedford 1986). The virus was introduced into Western Samoa in 1967 and became established in the CRB population within a year, reducing the population greatly (Marschall, 1970). In 1970, the virus was also introduced to Wallis Island via artificial log heaps reducing the adult beetle population by 60 - 80% and palm damage by 82% within one year (Hammes, 1974). OrNV has also established at sites in Fiji, Tonga, Tokelaus, and Papua New Guinea with a decline in CRB populations (Bedford, 1986). Symptoms of larval OrNV infection include the abdomen becoming turgid and glassy, the fat body disintegrating, an extroverted rectum, and increased amounts of hemolymph (Bedford 1986). Adult infection leads to decreased fecundity, decreased longevity, and a discontinuance of feeding reducing palm damage (Jackson, 2009). OrNV can be spread by ingestion of the pathogen, or when larvae come in contact with other larvae or adults, while adults are usually infected through mating or feeding (Zelazny, 1976). Ramle et al. (2005) found that there was more infection among mature beetles than neonates or larvae suggesting the virus is horizontally transmitted between adults. Infected adult beetles can shed the virus about 3 - 9 days after infection and can live for weeks post-virus (Mohan et al., 1986). Bedford (1986) stated that the release of the virus can be most economical and effective by releasing infected adults. However, a new haplotype of CRB has been recognized as CRB-G, which is resistant to OrNV and has become a major issue in the Pacific (Marshall et al., 2017).

Monitoring and Eradication in Hawai‘i

Once a beetle infestation has been detected, either by evidence of damage to palms, catches in pest survey traps, or discovery of a breeding site, a number of management tactics may be implemented. Bird or fish netting to trap adults may be placed on mulch piles that are identified as a CRB contaminated breeding site, or at the base of palm fronds near the spear to deter burrowing through the crown. Mulch piles may also be incinerated or heat-treated to kill different beetle life stages. Palms can be sprayed or injected with insecticide, or removed completely. Outreach and extension to local farms, companies, and the public has also been conducted. These stakeholders have been advised to keep mulch piles to a minimum and reduce potential for breeding site activity, although this cannot technically be enforced, only suggested. Similarly, Hinckley (1973) emphasized identification, destruction, or removal of existing or potential breeding sites as being crucial for CRB eradication efforts.

In association with the above, a primary approach to reduction of CRB spread on O‘ahu, Hawai‘i to date includes sanitation by eliminating plant and mulch debris that could potentially be used for breeding sites. However, this approach often relies on assumptions of where a breeding site could be without confirmation that the plant or mulch material is being used, or could be used by CRB for oviposition. Populations of CRB have shown to exist in areas where no known breeding sites can be found and population numbers have remained the same with occasional increases depending on the time of year. This indicates that without the ability to detect and find the actual breeding site, mitigation efforts are futile and CRB will continue to reproduce and the population will persist. In other cases, detection of breeding sites due to mulching surveys have been found only after CRB have

already established multiple stages of their life cycle in that particular site. This does not create a form of prevention, but rather requires response efforts instead, expending resources to reduce potential future generations, adult emergence and damage created. The challenges in detecting breeding sites, even in known infested areas, is due to the ability of the CRB to establish themselves in many types of substrates, even some that may not have been considered before, or breeding sites that may be hidden.

In spite of the efforts to eradicate the CRB infestation on Oʻahu, the beetle continues to persist. Female choice for oviposition site is a primary driver for this persistence. It is known from Hinckley (1973), that CRB lay about 27 eggs on average, and lay them in clutches. However, little is known about oviposition behaviors of CRB in regards to oviposition site preference and what factors may influence them to choose where to oviposit. If active or potential sites are able to be identified, then management can be effected. Understanding female preferences and behaviors in relation to oviposition can give insight as to where these breeding sites may be found.

To gain an understanding of female preference for oviposition site, factors that could potentially influence oviposition behaviors are investigated, such as substrate salinity and particle size. Salinity within a substrate can influence oviposition behavior of beetles. In a study investigating a tiger beetle species, *C. circumpicta*, females selected sites for oviposition with soil salinities of 4.0 ppt or higher (Hoback et al., 2000).

Likewise, substrate particle size can also influence oviposition behavior. From previous work with the CRB colony testing different substrates for rearing purposes, it seemed that CRB life stages survived in certain particle size substrates better than others (personal observation). A study done by Cornelisse and Hafernik (2009) on two species of

tiger beetles found that grain size was a factor influencing oviposition behavior, with the *C. hirticollis* species preferring finer sand and *C. oregona* species preferring coarser sand.

Lastly, the various depths at which *O. rhinoceros* can oviposit needs investigation. No studies have been done examining if there is an egg laying pattern and the depths at which a female will lay her eggs. Knowledge of oviposition depth can aid in both detection and management efforts. By understanding CRB burrowing behavior and where eggs are likely to be laid, researchers or field technicians can develop a better sampling plan and target management tactics that can benefit from information about the depths a particular chemical, biological, or culture management method will have to reach to exert control over a breeding site.

RESEARCH OBJECTIVES

The primary aim of this research is to understand coconut rhinoceros beetle oviposition behavior in order to more effectively detect and treat breeding sites. The specific objectives are:

1. To evaluate the preference of female CRB for substrates (Chapter 2) in relation to
    i. salinity and,
    ii. particle size
2. To determine the range of depths and average depth female CRB will burrow and oviposit (Chapter 3)

It is expected that coconut rhinoceros beetle oviposition behavior will be influenced by substrate particle size and salinity, and eggs will be laid at various depths in an aggregated pattern primarily limited by the depth of suitable breeding material.

CHAPTER 2.

PREFERENCES OF LAB-REARED AND WILD-CAUGHT COCONUT RHINOCEROS BEETLE (*ORYCTES RHINOCEROS,* COLEOPTERA: SCARABAEIDAE) FOR OVIPOSITION SITES IN RELATION TO SUBSTRATE PARTICLE SIZE AND SALINITY

INTRODUCTION

Most insects carefully select oviposition sites to provide the best environment for their offspring and promote their fitness (Resetarits 1996, Hoback et al. 2000). In order to differentiate and choose the best site for oviposition, some beetles may use cues like plant volatiles, pH, moisture, and temperature (Szendrei and Isaacs 2005, Sowig 1995, Cherry 1990, Brandhorst-Hubbard et al. 2001). Oviposition site preference among soil-nesting Scarabaeidae has been demonstrated in the green June beetle (*Cotinis nitida*). Brandhorst-Hubbard et al. (2001) showed that they prefer to oviposit in sandy-loam soil treated with cow manure, broiler litter and hay, rather than milorganite or being left untreated, and suggests that this may be due to the soil nitrogen content and ammonia gas. Other Coleoptera also show oviposition site selectivity, for example, tiger beetle species which used shade as a cue, possibly due to temperature reduction compared to sites that were in direct sunlight (Hoback et al., 2000). Previous researchers have made, observations regarding fecundity and clutch size in coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) (CRB) (Bedford 1976, Hinckley 1973). However, no studies have been done on preference for oviposition sites that differ in physical or chemical characteristics.

The coconut rhinoceros beetle has recently become one of the most prominent invasive insect pests in Hawai‘i since its detection on the island of O‘ahu in December 2013. The beetle has spread from South to West O‘ahu, and has caused visible damage to numerous coconut palms, resulting in more than 100 being cut down and removed from various landscapes in the infestation zone. Currently, a program is in place attempting to eradicate the CRB population. However, new locations where CRB is reproducing continue to be detected. Without control, the beetle may continue to spread throughout O‘ahu and to other Hawaiian islands, exponentially increasing the rate of damage to not only coconut palms, but potentially other palm species as well.

A main challenge in management and eradication of CRB is identifying active and potential breeding sites. It is uncertain what factors motivate a female beetle to choose a particular site in which to oviposit. CRB larvae have been observed to occur most frequently in sites with a pH less than 4.2, prefer temperatures between 27-29°C and moisture level of 77% or higher (Kamarudin et al. 2004, Manjeri et al. 2014, Costa 1967, King et al. 1981), suggesting that adult females may select sites for their offspring using environmental conditions and soil characteristics as cues to locate a preferred breeding site in which the larvae will develop. However, no experimental studies have been done on adult female CRB preference for oviposition site conditions.

To improve CRB management programs, an understanding of the female egg laying behaviors is needed. This study seeks to determine if CRB are selective in their oviposition behavior and if they have a preference for media in relation to particle size or salinity. Through a series of choice tests, both lab-reared and wild-caught individual CRB were evaluated for oviposition site preference of either a small or large particle size substrate, as

well as a high or low salinity substrate. Particle size was of interest as literature had stated that CRB pack their eggs with oviposition site material, and pupae create a pupal casing using materials "soft enough for burrowing yet firm enough to provide compacted frass" (Hinckley, 1973). This led me to hypothesize that an oviposition site of smaller material may be preferred as this would make egg clutches and pupal chambers easier to produce. The size of the substrate was of interest as particle size could potentially be used as a cultural management method to reduce suitable CRB oviposition sites. Salinity was another factor of interest as the same study mentioned earlier from Hoback et al. (2000) also found tiger beetles discern between oviposition sites of different salinities. This was thought to be primarily through chemoreception for sites with soil salinities of 4.0 ppt or higher. In this study, it is hypothesized that female CRB will exhibit substrate preferences and be able to discern between the different factors being tested: salinity and particle size.

This study also examines if lab-reared and wild-caught beetles exhibit similar behavior. Previous studies on various insects found that oftentimes these two groups demonstrated different behaviors and life histories. One study by Nagarkatti and Nagaraja (1978) on *Trichogramma* wasps showed that although laboratory reared wasps lived longer than wild-caught wasps, they also had a higher rate of sterility and produced fewer progeny. In another study, Simoes et al. (2006) found that laboratory reared fruit flies had increased fecundity with domestication, however they also had reduced sensitivity to their environment. In this study, it is expected that lab-reared beetles will exhibit oviposition behavior different than that of wild-caught beetles. The results of this study will inform management plans and aid in identifying potential CRB breeding sites.

MATERIALS AND METHODS

Insect Colony

Adult *O. rhinoceros* for the study were collected in the field and maintained in a laboratory colony, or reared in the lab from field caught eggs, larvae, and pupae. The colony used was initiated by the Hawai'i Department of Agriculture in 2015 and is now established in a containment laboratory at the University of Hawai'i at Manoa campus. Adult beetles were collected from panel and barrel traps (USDA APHIS, 2015), and larvae and pupae collected from mulch sites. Lab-reared adults developed in entirety from wild-caught eggs to adult stage in lab conditions. Two groups of *O. rhinoceros* were used in this study: wild-caught and lab-reared. Both groups of beetles were selected from the colony. After trial completion, beetles were returned back to the colony or disposed of if found dead.

In the colony, adult beetles were placed in plastic breeding boxes (41.9cm L x 33.0cm W x 16.8cm H and 43.2cm L x 28.3cm W x 6.5cm H) (Sterilite Corporation, MA) with a medium of 50:50 commercial mulch (Menehune Magic Mulch, Hawaiian Earth Products, HI) and coconut coir (Pacific Growers Supplies, Inc., HI). Starting in 2017, eggs and larvae were transferred to plastic containers (41.0cm L x 28.6cm W x 27.6cm H) (Sterilite Corporation, MA) containing mulch acquired from the University of Hawai'i at Manoa Landscaping Department. This mulch is comprised of trimmings and organic matter collected by the landscaping department from across the UH Manoa campus. Immature stages were reared to the adult stage. All adult beetles were fed an artificial diet made of vanilla-flavored whey protein, sugar, agar and water that was melted, mixed together and set as a soft gelatinous cube using ice-cube trays

Choice Tests

To determine whether female oviposition is influenced by substrate salinity or particle size, a series of choice tests were conducted under laboratory conditions. A single gravid beetle was placed in an arena and given a choice between two mulches that varied in size or salinity as described below, or a sand substrate in which to lay her eggs. Washed sand was used as a negative control as CRB have been observed to actively avoid and rarely burrow directly into sand (personal observation). The female beetle was held in the arena for one week and allowed to dig freely throughout the arena. The site and number of eggs laid in each of three available substrates was recorded at the end of one week. Each beetle was scored as laying all eggs in either substrate (high or low salinity, or, large or small particle size, or sand), some eggs in multiple substrates within the same trial, or no eggs in any substrate.

The arena for the choice tests consisted of a wooden chamber (50.8cm L x 30.5cm W x 33.0cm H) with three inner compartments, one containing sand (25.4cm L x 17.3cm W x 25.7cm H) and two containing different oviposition choices (25.4cm L x 13.3cm W x 25.7cm H), and a lockable lid. Plastic containers (25.4cm L x 12.7cm W x 27.3cm H) (InterDesign, OH) were placed inside the oviposition choice compartments for better ease of removing the substrate for digging out eggs. The compartments were filled with either sand (The QUIKRETE Companies, Inc., OH), as a negative control, or one of two mulch substrate options. Basic mulch used for the experiments was gathered from the University of Hawai'i at Manoa Landscaping Department and manipulated to produce one of the levels of either particle size or salinity. Each choice arena containing a single female was kept with the lid closed within a biological incubation chamber at a setting of 29°C with a RH of 69% (Model

I-41LLVL, Percival Scientific, Inc., IA). Three replicates, of one female beetle per arena, were placed in the incubation chamber at the same time for one week. At the end of the period, the beetle and substrate were removed and eggs counted. The containers were refilled with new substrate prior to the introduction of a new female beetle.

A total of 24 wild-caught female beetles and 24 lab-reared female beetles were tested in each particle size and salinity comparison. Lab-reared beetles were on average 42 days post-eclosion and had been placed in a mating arena with a male for 3 days prior to placement in the arena. The mating arena consisted of a (19.4cm L x 16.5cm W x 11.4cm H) plastic container (Sterilite Corporation, MA) containing the 50:50 mulch and coconut coir mixture. The age of wild-caught beetles was unknown as they were collected as adults from the field. Wild-caught beetles used in these experiments were taken from colony boxes where they had been previously cohabited with males with a male:female ratio of 1:2, and left to mate freely.

Particle Size

Beetles were given a choice of substrates with two particle sizes: large and small. To produce substrates of different particle size, mulch was oven dried at 120°C for a minimum of 24 hours and mixed thoroughly, then sifted through one of two sizes of steel screen. Steel screen size 1.6 mm was used to produce a substrate with small particle size (Phifer Incorporated, AL), and size 12.0 mm was used to produce a substrate with large particle size (Everbilt Hardware Company). Substrate with the larger particle size was placed in one compartment of the choice arena, in another, small particle-sized substrate, and in the third, sand. The surface of the arena was level.

Salinity

To produce mulch of different salinities, mulch was oven dried at 120°C for a minimum of 24 hours and mixed thoroughly, and then sifted through 12.0 mm steel screen to produce a standard particle size. The mulch was then saturated with a salt solution of either high or low salinity until 25% moisture was attained. Moisture was measured using a soil moisture meter (Extech Instruments, NH). To produce levels of the desired salinity (measured as electrical conductivity), the soil was saturated with a deionized water and table salt (NaCl) solution that had an EC of either 2 dS/m or 8 dS/m, measured with an electrical conductivity meter (Soil Test Direct Soil EC Tester, Hanna Instruments, RI). The sand used as a negative control had an EC of 0 dS/m. Mulch with high salinity was placed in one compartment, low salinity in the other, and sand in the third. The surface of the arena was level.

Biometrics

All 48 wild-caught beetles were measured for what was defined in this study as body length (mm), width (mm), depth (mm), and mass (g). The same biometric data were collected from thirty six lab-reared beetles. Using a caliper, beetle body length was measured from the anterior tip to the posterior tip, width was measured on the widest part of the abdomen, depth was measured from the area of the body with the greatest girth, and mass was measured using a digital balance (A&D Weighing).

Data Analysis

For each particle size and salinity level experiment, for both lab-reared and wild-caught females, the number of eggs laid per beetle in each substrate was calculated, and compared using a nonparametric Wilcoxon Ranked-Sum Test (JMP Pro 13). If no eggs were found in a substrate, this category was not included in the analysis. The average total number of eggs laid per beetle per choice test was also calculated and statistical comparisons conducted.

The oviposition pattern for each beetle was scored and analyzed using categorical data analysis techniques. A single female beetle was scored as either laying her eggs in one mulch substrate or the other, sand, multiple substrates in the same trial, or none. The null hypothesis that oviposition would be equally distributed across substrates was tested using a Chi-Squared Goodness of Fit Test (JMP Pro 13) for wild-caught and lab reared beetles separately. If the chi-square test showed there was not an equal distribution between the choices of none, both, or either of the three mulch substrate options, then a subdivided chi-square goodness of fit test (Zar, 2010) was conducted to determine if there was an equal distribution between the option of ovipositing in both mulch substrates within the same trial, or just in one of the two mulch substrate options. The sand category was not included in any subsequent analysis as no beetles were ever found to oviposit in that substrate.

Additionally, a Chi-Squared Test of Independence with a contingency analysis (JMP Pro 13) was used to compare wild-caught vs. lab-reared beetle preference in each of the parameter choice experiments. A One-Way ANOVA (JMP Pro 13) was used to analyze the total number of eggs laid per wild-caught beetle compared to lab-reared beetles. Female

beetle biometric measurements compared to the number of eggs laid for both wild-caught and lab-reared beetles were analyzed using a full factorial backwards-elimination stepwise multiple regression (JMP Pro 13) to assess if length, width, depth, or mass could be used as a predictor of fecundity. Lastly, wild-caught biometric measurements were compared to lab-reared biometrics using a One-Way ANOVA (JMP Pro 13).

RESULTS

Particle size choice

Wild caught beetles laid on average (± SEM) 43 ± 5 eggs (range: 4 – 104) when given the choice of small or large particle size substrate. Significantly more eggs were laid in the small particle size mulch (range: 0-89) rather than large particle size (range: 0-28) (Table 2.1). No beetles laid eggs in sand.

Table 2.1 Mean (±SEM) number of eggs laid and mean (±SEM) percent of eggs laid by wild-caught CRB when given the choice of either sand, small, or large particle size mulch.

Treatment	**Eggs**	**Percent of Eggs**
Small Particle	41 ± 6	91.0 ± 0.1
Large Particle	3 ± 2	9.0 ± 0.1
Sand	0	0
t = 6.18, d.f. = 23,		
p < 0.0001		

Of the 24 wild-caught females given a choice between small and large particle size substrate, all of them made an oviposition choice, however, unequally (Figure 2.1; χ^2 = 61.00, df = 4, p < 0 .0001). Only two beetles laid eggs in both mulch options in the same trial (χ^2= 27.00, df = 2, p < 0.0001), with 20 of the remaining beetles ovipositing in small particle size mulch and 2 in the large particle size (χ^2 = 14.73, df = 1, p = 0 .0001). This suggests that wild-caught CRB prefer to oviposit their eggs in mulch comprising of small particle size rather than large, as defined in this study.

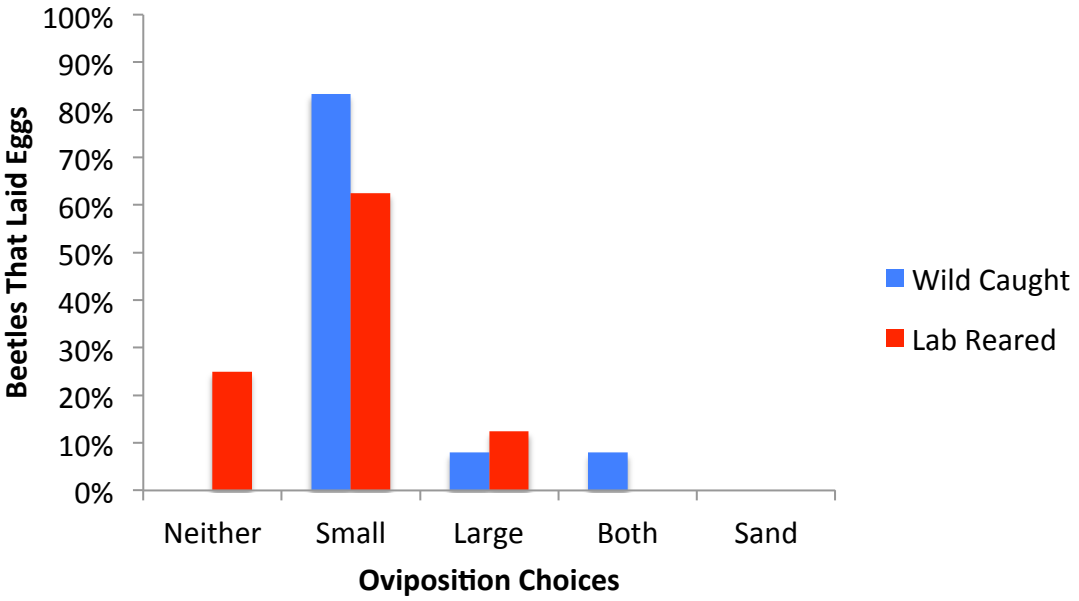

Figure 2.1 Percent of wild-caught and lab-reared beetles choosing to oviposit in substrates of either sand, small or large particle size mulch, lay no eggs at all or lay eggs in both mulch substrates within the same experiment.

On average, lab-reared females laid (± SEM) 8 ± 2 eggs (range: 0 – 28) when given the choice of small or large particle size substrate. Significantly more eggs on average were

laid in small particle size mulch (range: 0 – 28) compared to large particle size (range: 0 – 10) (Table 2.2). No beetles laid eggs in sand.

Table 2.2 Mean (±SEM) number of eggs laid and mean (±SEM) percent of total eggs laid by lab-reared CRB when given the choice of either sand, small, or large particle size mulch.

Treatment	**Eggs**	**Percent of Eggs**
Small Particle	8 ± 2	62.5 ± 0.1
Large Particle	1 ± 0	12.5 ± 0.1
Sand	0	0
	t = 3.27, d.f.=23,	
	p=0.0014	

Of the 24 lab-reared beetles given a choice between small and large particle size substrate, 6 beetles did not make an oviposition choice with the remaining choosing one of the two mulch substrates (Figure 2.1; $\chi^2 = 32.25$, df = 4, $p < 0.0001$). The majority (n=15) laid all their eggs in the small particle size mulch and 3 laid in the large particle size ($\chi^2 = 8.00$, df = 1, p = 0.0047).

Salinity choice

Wild-caught beetles also laid on average (± SEM) 33 eggs ± 4 (range: 1- 73) when presented with the choice of low salinity or high salinity substrate. Slightly more eggs were

laid in low salinity mulch (range: 0 – 59) versus high salinity mulch (range: 0 – 61), however, the difference in choices was not statistically different suggesting there was no preference of female beetles for either salinity level choice (Table 2.3). No beetles laid eggs in sand.

Table 2.3 Mean (±SEM) number of eggs laid and mean (±SEM) percent of total eggs laid by wild-caught CRB when given the choice of either sand, low, or high salinity mulch.

Treatment	**Eggs**	**Percent of Eggs**
Low Salinity	17 ± 4	54.7 ± 0.1
High Salinity	16 ± 4	45.3 ± 0.1
Sand	0	0
	t = 0.2128, d.f. = 23,	
	p=0.7606	

All of the 24 wild-caught beetles given a choice between high and low salinity substrate made an oviposition site choice (Figure 2.2; χ^2= 18.92, df = 4, p = 0.0008). Five of the 24 beetles, oviposited in both high and low salinity mulch within the same trial, and the remaining 19 chose either low (n=10) or high salinity (n=9) mulch. Eggs laid in either high, low, or both salinity mulch substrates within the same trial were equally distributed (χ^2 =

1.75, df = 2, p = 0.4169) and there was no significant preference for low or high salinity mulch (χ^2 = 0.05, df = 1, p = 0.8185).

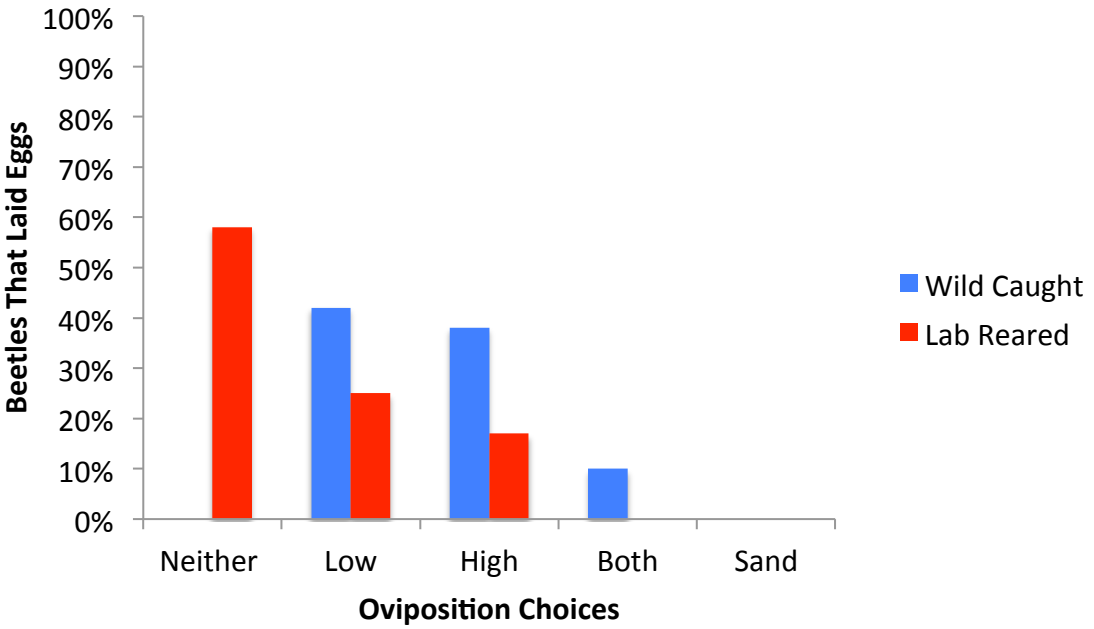

Figure 2.2 Percent of wild-caught and lab-reared beetles choosing to oviposit in substrates of either sand, low or high salinity mulch, lay no eggs at all or lay eggs in both mulch substrates within the same experiment.

Lab-reared females also laid on average (± SEM) 3 ± 1 eggs (range: 0 – 21) when given the choice of low salinity or high salinity substrate. More eggs on average were laid in low salinity mulch (range: 0 – 21) than in high salinity mulch (range: 0 – 12), however, there was no significant difference in CRB for oviposition preference between substrate salinities tested (Table 2.4). No beetles laid eggs in sand.

Table 2.4 Mean (±SEM) number of eggs laid and mean (±SEM) percent of total eggs laid by lab-reared CRB when given the choice of either sand, low, or high salinity mulch.

Treatment	Eggs	Percent of Eggs
Low Salinity	2 ± 1	25.0 ± 0.1
High Salinity	1 ± 1	16.7 ± 0.1
Sand	0	0
t = 0.71, d.f=23, p=0.5427		

Of the 24 lab-reared beetles given a choice between high and low salinity, 14 beetles did not oviposit ($\chi^2 = 27.67$, df = 4, $p < 0.0001$). These beetles were excluded from further analysis. The remaining beetles selected the low salinity (n= 6) and high salinity (n=4) mulch as an oviposition site almost equally (Figure 2.2; $\chi^2 = 0.40$, df = 1, $p = 0.5271$).

Wild-caught vs. lab-reared

Across both choice experiments, wild-caught beetles laid significantly more eggs on average (± SEM), 38 ± 3 eggs (range: 1 – 104), than lab-reared beetles when presented with either the choice of low or high salinity mulch, or, small or large particle size mulch ($F = 83.11$, df = 1, $p < 0.0001$). Lab-reared beetles laid on average 6 ± 1 eggs (range: 0 – 28). Of the 48 lab-reared beetles used in this study, 42% did not lay eggs.

Lab-reared and wild-caught beetles exhibited different oviposition patterns when given a choice between large and small particle size mulches (Figure 2.1; χ^2= 8.91, df = 3, p = 0.0305). The proportion of females ovipositing in both mulch substrate choices within the same trial was only seen in wild-caught beetles, whereas the proportion of beetles laying no eggs in either mulch substrate was only seen in lab-reared beetles.

Wild-caught and lab-reared beetles also had different patterns of oviposition when offered a choice between low or high salinity mulch (Figure 2.2; χ^2= 21.92, df = 3, p < 0.0001). A higher proportion of lab-reared females laid no eggs in either mulch substrate than wild-caught females. There did not appear to be a preference for either low or high salinity for either group.

Body depth and mass, as well as the multiplicative effects of length*mass, length*width*mass, depth*mass, and width*depth*mass were predictors in a model of the number of eggs a wild-caught beetle will lay after one week (Table 2.5; F = $5.14_{6;41}$, df = 6;41, p = 0.0005). However, for lab-reared beetles, no suitable model using biometric parameters could be generated to predict fecundity (F = $2.56_{2;33}$, df = 2;33], p = 0.0925).

Table 2.5 Wild-caught beetle estimates, standard error, degrees of freedom, F-ratio, and P-value for each biometric predictor of fecundity.

Measurement	Estimate	SE	df	F	p
Depth	-99.15	35.77	1	7.68	0.0083
Mass	17.08	4.15	1	16.97	0.0002
Depth*Mass	-315.43	144.98	1	4.73	0.0354
Length*Mass	-89.22	29.74	1	8.99	0.0046
Width*Depth*Mass	700.33	297.72	1	5.53	0.0235
Length*Width*Mass	200.53	61.31	1	9.72	0.0033

Wild-caught beetles were found to be larger in terms of depth (Figure 2.3, $F = 5.9$, $df = 1$, $p = 0.0173$), mass (Figure 2.5, $F = 45.1$, $df = 1$, $p < 0.0001$) and length (Figure 2.4, $F = 13.7$, $df = 1$, $p = 0.0004$) than lab-reared beetles (Table 2.6). The two groups were not different with regard to width ($F = 0.2$, $df = 1$, $p = 0.6579$). Larger wild-caught beetles also laid more eggs than smaller beetles (Figures 2.3, 2.4, 2.5).

Table 2.6 Average (±SEM) body length, width, depth, and mass for wild-caught and lab-reared beetles.

Measurement	Wild Caught	Lab Reared
Length (mm)	42.3 ± 0.5	39.8 ± 0.5
Width (mm)	19.4 ± 0.2	19.3 ± 0.2
Depth (mm)	15.2 ± 0.3	14.4 ± 0.2
Mass (g)	5.2 ± 0.2	3.5 ± 0.1

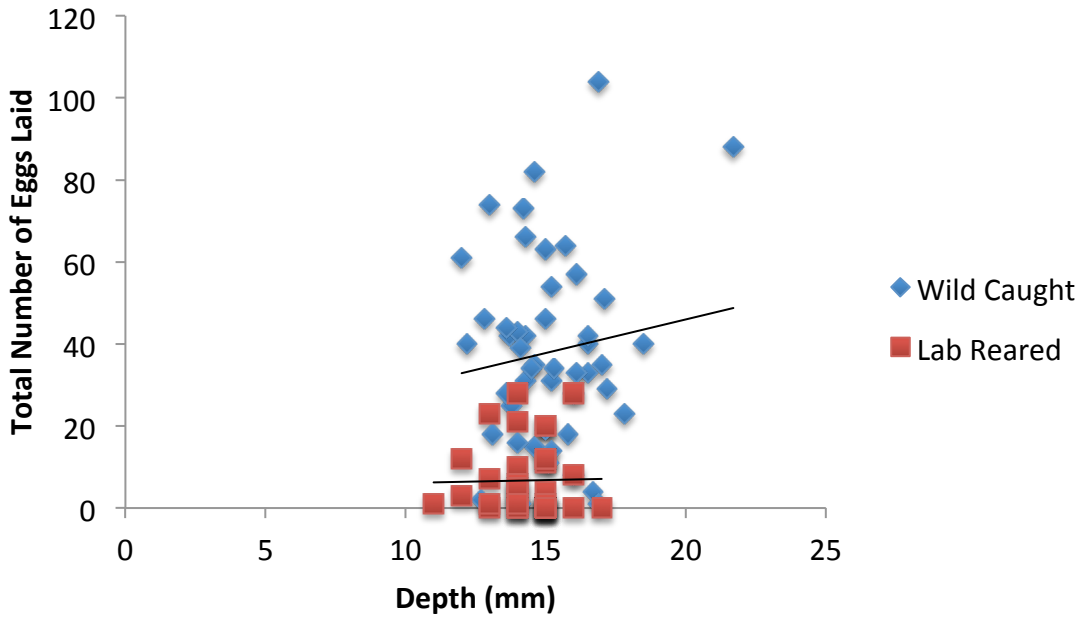

Figure 2.3 Total number of eggs laid per wild-caught (blue) and lab-reared (red) beetle compared to body depth.

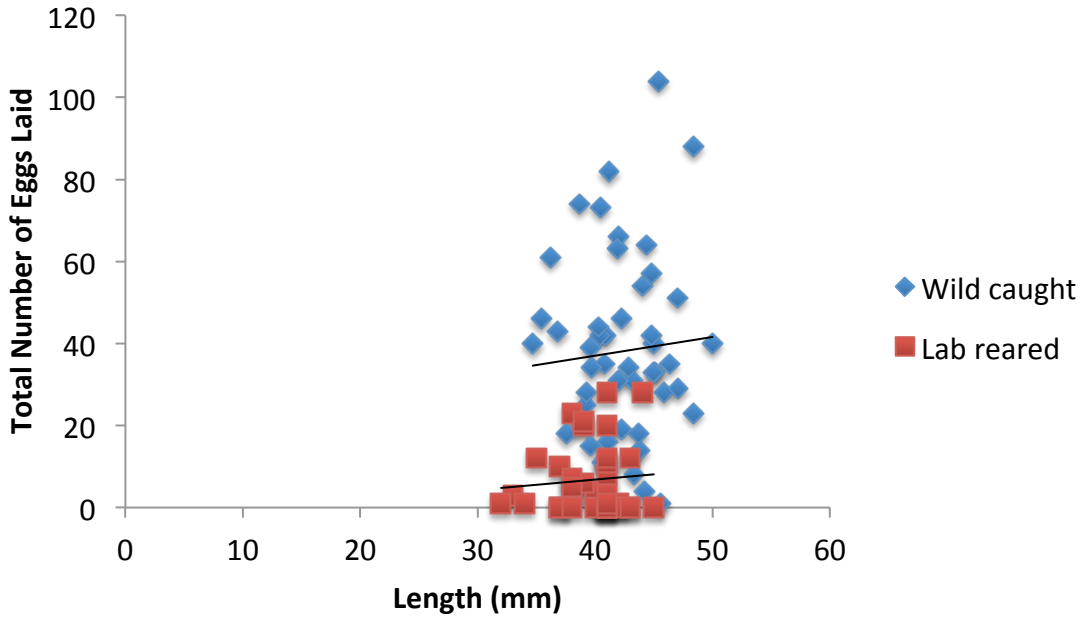

Figure 2.4 Total number of eggs laid per wild-caught (blue) and lab-reared (red) beetle compared to body length.

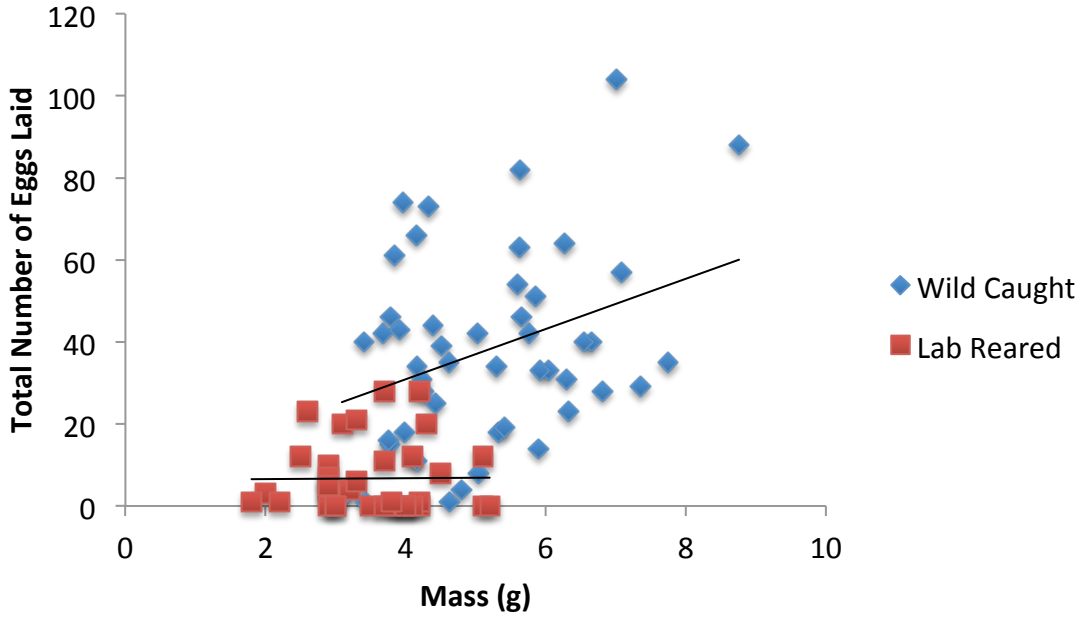

Figure 2.5 Total number of eggs laid per wild-caught (blue) and lab-reared (red) beetle compared to body mass.

DISCUSSION

Both wild-caught and lab-reared female CRB appeared to demonstrate the ability to discern where to lay their eggs and exhibited a preference for smaller particle substrate over large particle for oviposition. This preference for small particle substrate over large particle in both wild-caught and lab-reared females was evidenced both in terms of the average number of eggs laid and the proportion of beetles that laid in that substrate. In terms of potential breeding site management, these results suggest that keeping mulch or compost piles made up of organic material chipped to a larger size could help deter females from choosing them as a breeding site and ovipositing. However, there is a likelihood that CRB will be able to adapt to this changed resource, so a suite of management techniques should be incorporated together to reduce strong selective pressure. Small particle mulch could also be used as a potential trap-site to attract beetles to burrow inside, which could then be treated with a control method targeting CRB life stages.

Neither wild-caught nor lab-reared beetles appeared to have a preference for oviposition sites with different salinity levels. This finding may have been due to the methods used or the levels of salinity tested. In this study, table salt and DI water were mixed to create different salt solutions for a desired EC, and then added to the mulch until saturated. In a similar study, Vowell et al. (unpublished), created different salinities according to molarity and measured the EC of the soil instead of the solution. Their approach to manipulating substrate salinity led to a difference in the type and amount of salt used, and different results. Further testing with a range of salinities and different types of salts might demonstrate whether CRB females can or care to discriminate oviposition sites on the basis of soil salinity. From the salinity choice tests, given the salinity levels

used, the results indicate that altering salinity in a mulch or compost pile would not be an appropriate management tactic for deterring CRB from choosing that site to oviposit. However, salinity may be a useful characteristic as a treatment for managed breeding sites, as research by Vowell et al. (unpublished) found certain salts to hinder immature development.

This study also highlighted the difference in wild-caught and lab-reared CRB oviposition behaviors. Given their developmental background being different, this is not surprising. It is not uncommon for insects in a laboratory colony to become physically different or even exhibit different behavior (Simoes et al. 2006, Nagarkatti and Nagaraja 1978). In the present case, the choice experiments indicated that these factors might lead to meaningful differences in reproductive behavior in terms of both fecundity and oviposition patterns. In this study, wild-caught beetles laid on average more eggs than lab-reared beetles overall, as well as in each separate experiment. The proportion of beetles that oviposited in the small particle size substrate was higher for wild-caught beetles than lab-reared. The proportion of beetles that oviposited in both the low and high-level substrates was also greater for wild-caught beetles than lab-reared. This could be due to lab-reared beetles being kept in captivity where they do not receive the same nutrition or conditions as wild-caught beetles and are also not likely to be exposed to the same environmental stressors that CRB in the natural environment encounter. These lab-reared beetles may also not have laid eggs due to potentially being too young, not being mated with enough male beetles, or not being mated long enough. Additionally, lab-reared beetles used at the beginning of the experiments were unhealthy and had a shorter longevity than beetles used later in the experiments, as rearing practices were still being fine-tuned and

beetles with a greater longevity were produced later. Overall, these findings indicate that CRB produced in the colony may not be appropriate indicators for CRB oviposition behavior in the field, and any studies conducted with lab-reared beetles should be interpreted with caution.

Oviposition behavior was also investigated in relation to CRB biometrics measuring length, width, depth, and mass. Parameters that predicted beetle fecundity were only found for wild-caught beetles. Wild-caught beetles were also found to be on average larger in length, depth, and mass compared to lab-reared beetles. However, the widths of the two groups were similar. Larger wild-caught beetles, in regards to mass and depth, were found to lay a larger number of eggs than smaller beetles. This agrees with a study by Hinckley (1973) that also found larger CRB to lay a greater number of eggs than smaller beetles. However, this model cannot be applied to lab-reared beetles as results showed that none of the biometric measurements were sufficient predictors for the number of eggs laid.

This is the first study to investigate the individual oviposition preference of CRB. This study demonstrated that female CRB do discriminate on the basis of some environmental parameters in terms of oviposition site, e.g. substrate particle size, but may not rely on others such as substrate salinity. It is also the first direct comparison of lab-reared and wild-caught CRB behavior and demonstrates that there is a difference in physical size and behavior in the two groups. This study has produced important information with regards to CRB oviposition site preference and lab-reared versus wild-caught oviposition behavior. There is potential for future research furthering comparisons of lab-reared to wild-caught CRB behaviors, as well as oviposition site preference using different salts and salinity levels. It is still unknown what prompts a female *O. rhinoceros* to

oviposit her eggs in a particular site, and more research can be done to investigate this behavior.

CHAPTER 3.

OBSERVATIONS ON COCONUT RHINOCEROS BEETLE (*ORYCTES RHINOCEROS,* COLEOPTERA: SCARABAEIDAE) OVIPOSITION DEPTH IN A CONTROLLED ENVIRONMENT

INTRODUCTION

Various types of beetles, including numerous species of scarabs, can burrow to different depths in soil or organic matter. This may be done to lay eggs, create brood chambers, diapause, or even mate (Hinckley 1973, Bedford 1976, Sowig 1995, Facundo et al. 1998, Costanzo 1997). As seen in the white grub beetle, *Dasylepida ishigakiensis (Coleoptera: Scarabaeidae*), burrows were made from depths of 2 to 10 cm (Harano et al., 2010). One type of tiger beetle, *Cicindela formosa* (Coleoptera: Carabidae), was found to make a vertical burrow from 30 to 50 cm deep (Shelford, 1908). In another study done on the dung beetle *Typhaeus typhoeus*, burrow depths of up to 100 cm were observed (Brussaard, 1987). Fossorial behavior is an essential component in the reproductive behavior of many beetles, including a number of pest species. Elucidating nesting behavior has the potential to contribute to developing methods aimed at disrupting pest reproduction, and thus pest management. Despite its pest status, little is known about the burrowing behavior of the coconut rhinoceros beetle (CRB) (*Oryctes rhinoceros*, Coleoptera: Scarabaeidae). Adult CRB burrowing behavior has only been recorded in relation to the feeding behavior of adults as they burrow through the crowns of palms (Hinckley, 1973). No literature has been found on the depth a CRB will burrow within a site to oviposit.

In Hawai‘i where CRB has become one of the most prominent invasive insect pests, issues with eradication and control of the beetle have surfaced. A main challenge in management and eradication of CRB is finding active breeding sites, and choosing appropriate management methods to treat these sites. An obstacle preventing researchers or field technicians from discovering breeding sites is the lack of knowledge about the depth one would have to dig into the substrate to find any CRB life stages. By understanding CRB burrowing behavior and where eggs can be laid, egg targeting management methods can benefit from information about the depths a particular chemical, biological, or culture management method will have to reach to exert control over a breeding site.

Another primary aim of this study was to determine the range of depths a female coconut rhinoceros beetle can burrow for oviposition, and to evaluate the distribution of egg deposition across the range of burrowing distances. Using a mulch-filled observational chamber, adult female CRB egg-laying patterns were observed.

MATERIALS AND METHODS

Insect Colony

Adult *O. rhinoceros* for the ovipositional study were collected in the field and maintained in a laboratory colony, or reared in the lab from field caught eggs, larvae, and pupae. The colony used was initiated by the Hawai‘i Department of Agriculture in 2015 and is now established in a containment laboratory at the University of Hawai‘i at Manoa campus. Adult beetles were collected from panel and barrel traps (USDA APHIS, 2015), and

larvae and pupae collected from mulch sites. After trial completion, beetles were returned back to the colony or disposed of if found dead.

In the colony, adult beetles were placed in plastic breeding boxes (41.9cm L x 33.0cm W x 16.8cm H) and (43.2cm L x 28.3cm W x 6.5cm H) (Sterilite Corporation, MA) with a medium of 50:50 commercial mulch (Menehune Magic Mulch, Hawaiian Earth Products, HI) and coconut coir (Pacific Growers Supplies, Inc., HI). Female beetles had been previously cohabited with males with a male:female ratio of 1:2, and left to mate freely. All adult beetles were fed an artificial diet made of vanilla-flavored whey protein, sugar, agar and water that was melted, mixed together and set as a soft gelatinous cube using ice-cube trays.

Observation chamber

A large vertically oriented observation chamber (121.9cm H x 45.7cm W x 4.5cm D) modeled after Thomas (2001) was constructed by a commercial company (Min Plastics & Supply Inc., Honolulu, HI). The observation chamber consisted completely of clear plastic (Plexiglas), 4.8 mm thick, sealed together at all edges. The chamber was divided into three equal compartments (119.4cm H x 15.2cm W x 4.1cm D) using Plexiglas dividers. One side of the chamber was attached to the main frame using hinges to allow access to the interior contents. Round rubber plugs, 38 mm in diameter, were inserted at the top of each of the three chambers to be used for ventilation as well as an opening through which to introduce gravid female CRB into the chamber.

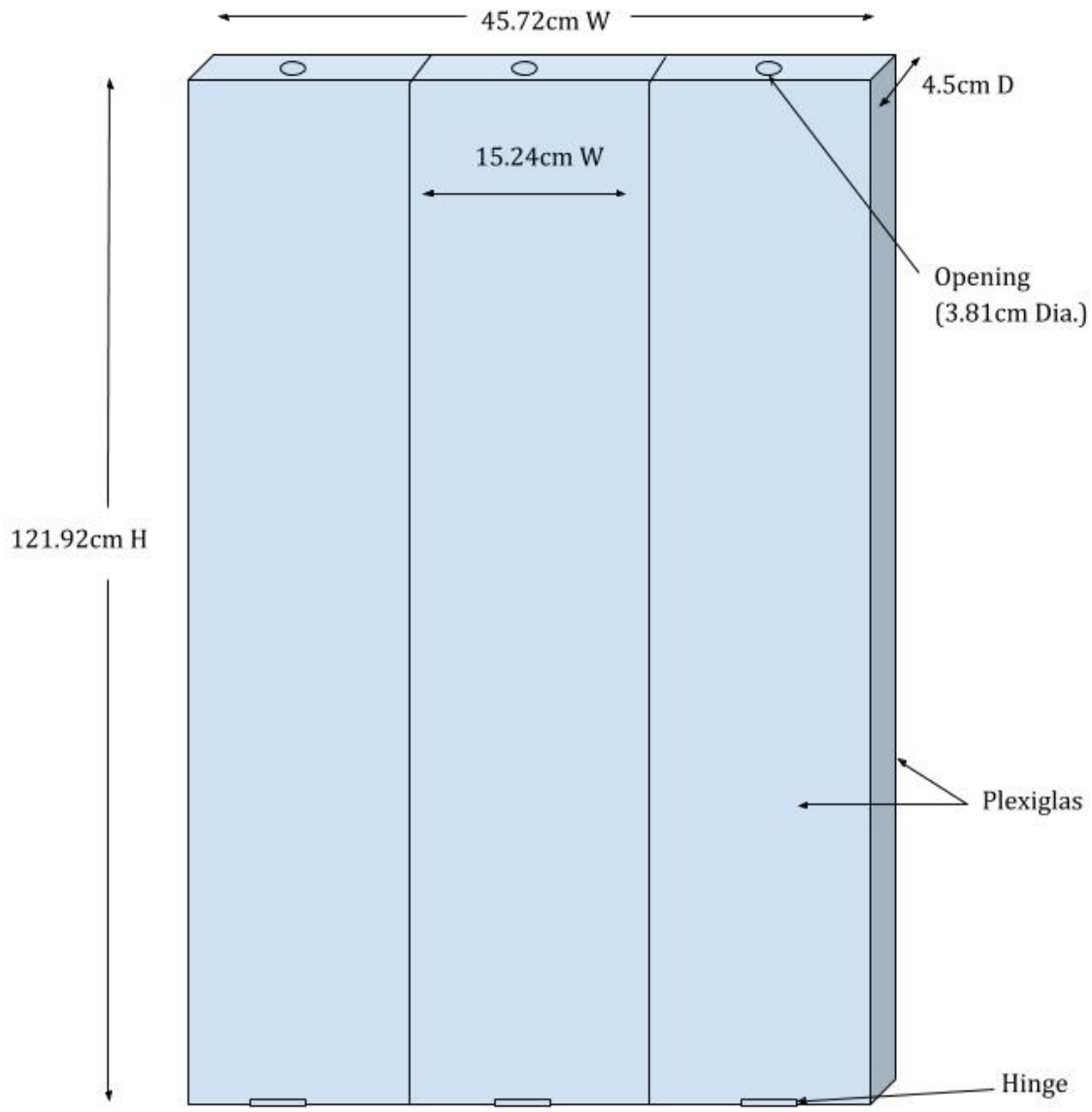

Figure 3.1 Observation chamber drawing and measurements.

Oviposition Depth

Each compartment was filled with mulch collected from the University of Hawai‘i at Manoa Landscaping Department. Mulch was initially oven dried for a minimum of 24 hours at 120°C, sifted through 12.0 mm steel screen, and rehydrated with water until 25% moisture was reached. Moisture was measured using a direct soil moisture meter (Extech Instruments).

A single gravid CRB was placed in each of the three compartments and allowed to burrow freely and oviposit for one week. At the end of the week, the distance burrowed by the beetle was recorded. Eggs were located, and the number of eggs laid per 2.5 cm in depth was quantified, enabling the calculation of the range of depths at which eggs were laid. Depth was measured from the top of the surface to the bottom of the observation chamber using a tape measure. All mulch was removed and replaced before a new female was placed within the observation chamber. There were 24 replicates. The chamber was held in a containment lab at the University of Hawai'i at Manoa that had an average temperature of 25 – 29°C.

Biometrics

All 24 beetles were measured for what was defined in this study as body length (mm), width (mm), depth (mm), and mass (g). Using a caliper, beetle length was measured from the anterior tip to the posterior tip, width was measured on the widest part of the abdomen, depth was measured from the area of the body with the greatest girth, and mass was measured using a digital balance (A&D Weighing).

Data Analysis

In this study, the mean and variance of the number of eggs laid per 2.5 cm in depth of substrate were calculated for each individual female beetle. Taylor's Power Law was used to analyze the dispersion of egg laying. Taylor's Power Law coefficients were estimated using standard least squares regression (JMP Pro 13) to calculate if the relationship between the mean and variance of eggs oviposited per inch was random,

uniform, or aggregated. Data were log-transformed for linear regression analysis. The average number of eggs laid per female, average number of eggs laid per inch, average depth female beetles were found, and the average depth range eggs were laid was calculated.

Beetle biometric measurements compared to the number of eggs laid were analyzed using a backwards elimination stepwise multiple regression (JMP Pro 13) to assess if either beetle length, width, depth, or mass can be used as a predictor for CRB fecundity.

RESULTS

Beetle Depth and Egg Dispersion

On average (± SEM), female beetles laid 21 ± 4 eggs (range: 0 – 74). The average depth (± SEM) females were found after one week was 42 cm ± 6 (range: 8 – 114 cm). The average (± SEM) minimum depth eggs were laid at was 41 cm ± 7, and the average (± SEM) maximum depth eggs were laid was 78 cm ± 9 (range: 5 – 119 cm). The greatest proportion of beetles laid eggs at 53 cm (42%), while only a few eggs were laid within the first 3 cm of substrate (Figure 3.2).

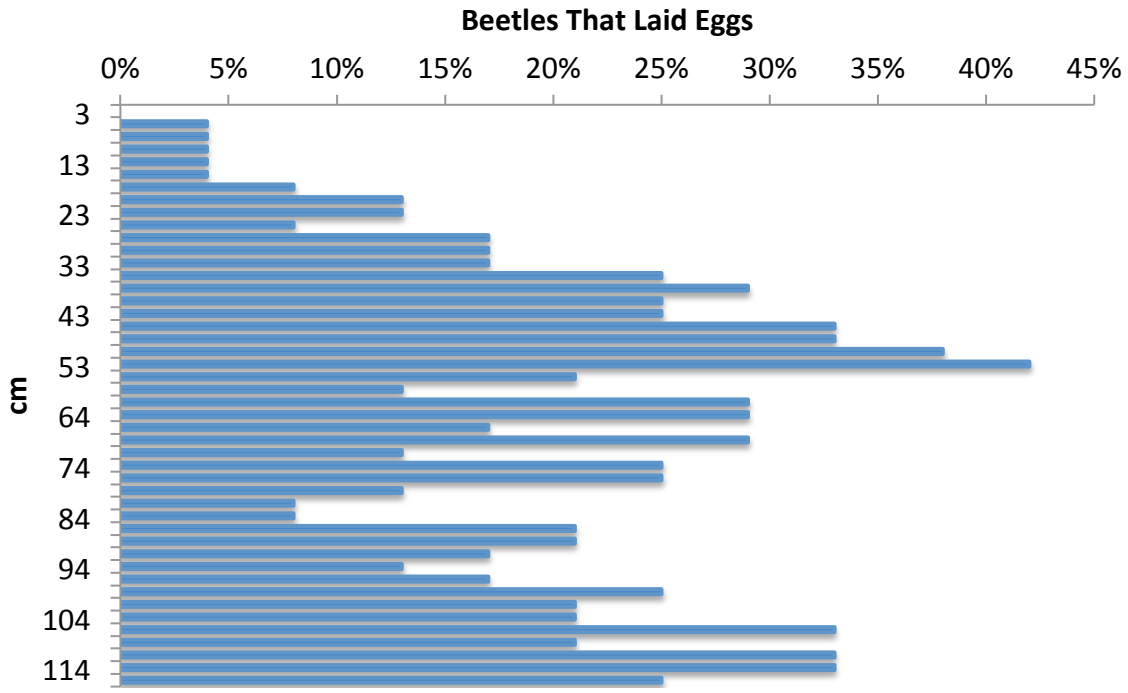

Figure 3.2 Proportion of beetles that laid eggs every 2.5 cm.

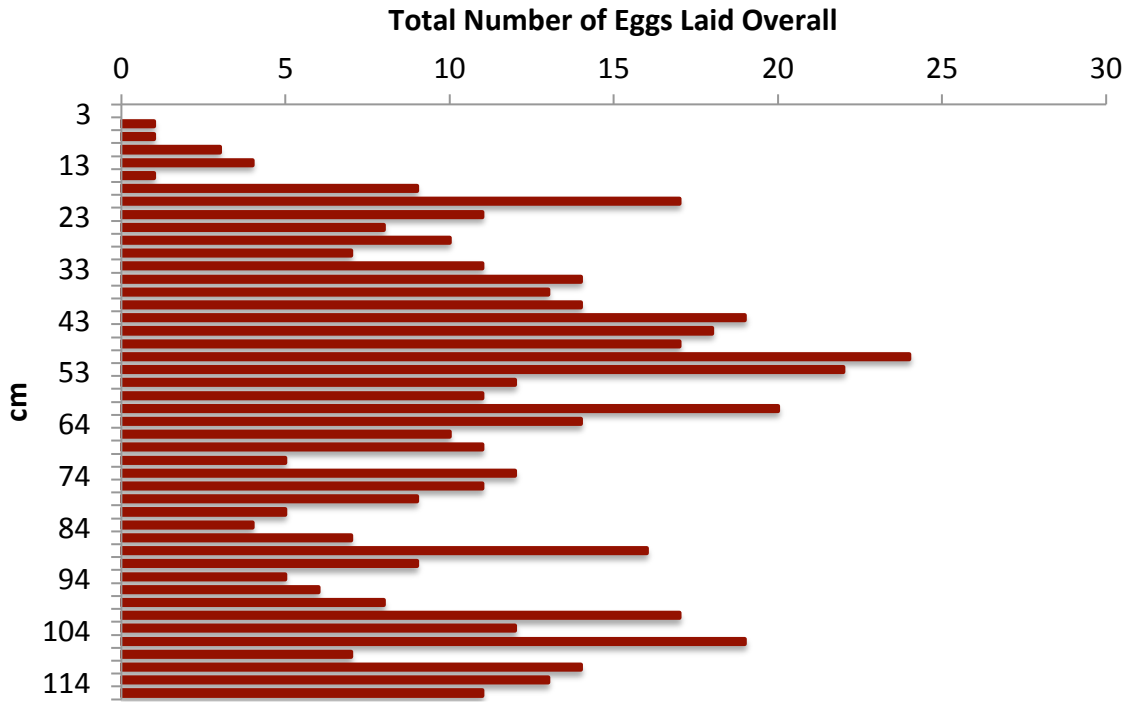

Figure 3.3 Total number of eggs laid, for all 24 beetles, every 2.5 cm in depth.

The highest mean number of eggs laid was between 47 – 61 cm, while the lowest number of eggs oviposited was within the first 15 cm (Table 3.1). Only 2% of eggs were laid within the first 15 cm. The total number of eggs laid per inch ranges from 0 – 24, while the average number of eggs laid per inch ranges from 0 – 1 (Figure 3.3).

Table 3.1 The total number of eggs laid for all 24 beetles, mean (± SEM) number of eggs laid, and percentage of total eggs laid per 15 cm in depth.

Depth (cm)	Total	Average	%
1 - 15	10	2 ± 1	2
16 - 30	62	10 ± 2	12
31 - 45	89	15 ± 1	18
46 - 60	106	18 ± 2	21
61 - 75	63	11 ± 1	13
76 - 90	50	8 ± 2	10
91 - 105	67	11 ± 2	13
106 - 120	76	13 ± 2	15

Taylor's Power Law analysis indicated an aggregated dispersion of eggs with the regression slope (b = 1.28), significantly greater than 1 (t = 3.27; df = 1; p = 0.002). The variance and mean of total eggs laid per 2.5 cm were log-transformed (y = 1.28x + 0.51, R^2 = 0.83, p < .0001) (Figure 3.4).

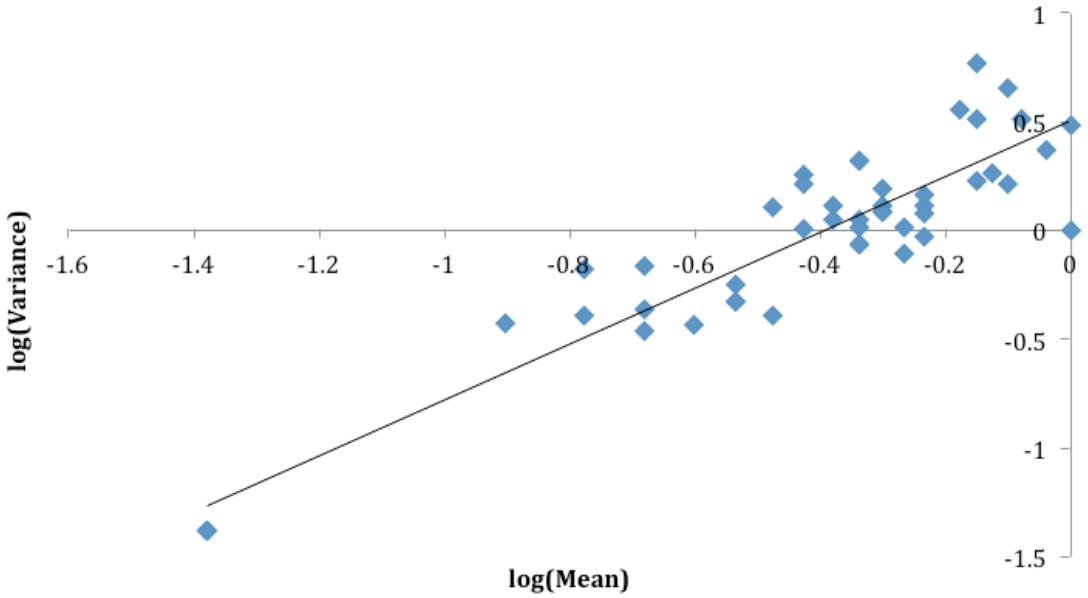

Figure 3.4 Log variance to mean relationship for total eggs laid per 2.5 cm.

Biometrics

The average measurements for female beetles used in the choice experiments were 41mm L x 19mm W x 15mm D and a mass of 4.8 grams. The body length of the beetle as well as the multiplicative effects of length*width, length*depth, width*depth, length*mass, width*depth*mass, and length*width*depth*mass showed to be predictors of the number of eggs a wild caught female beetle will lay (Table 3.2; $F = 4.99_{7;16}$, df = 7;16, p =0 .0037). Smaller beetles laid a greater number of eggs than larger beetles (Figure 3.5).

Table 3.2 CRB estimates, standard error, degrees of freedom, F-ratio, and P-value for each biometric predictor for fecundity.

Measurement	Estimate	SE	df	F	p
Length	-75.33	17.23	1	19.10	0.0033
Length*Mass	-68.77	32.08	1	4.60	0.0478
Length*Width	437.28	171.89	1	6.47	0.0217
Length*Depth	838.67	269.76	1	9.67	0.0068
Width*Depth	-2033.6	458.83	1	19.65	0.0004
Width*Depth*Mass	1003.93	219.67	1	20.89	0.0003
Length*Width*Depth*Mass	1489.59	517.46	1	8.29	0.0109

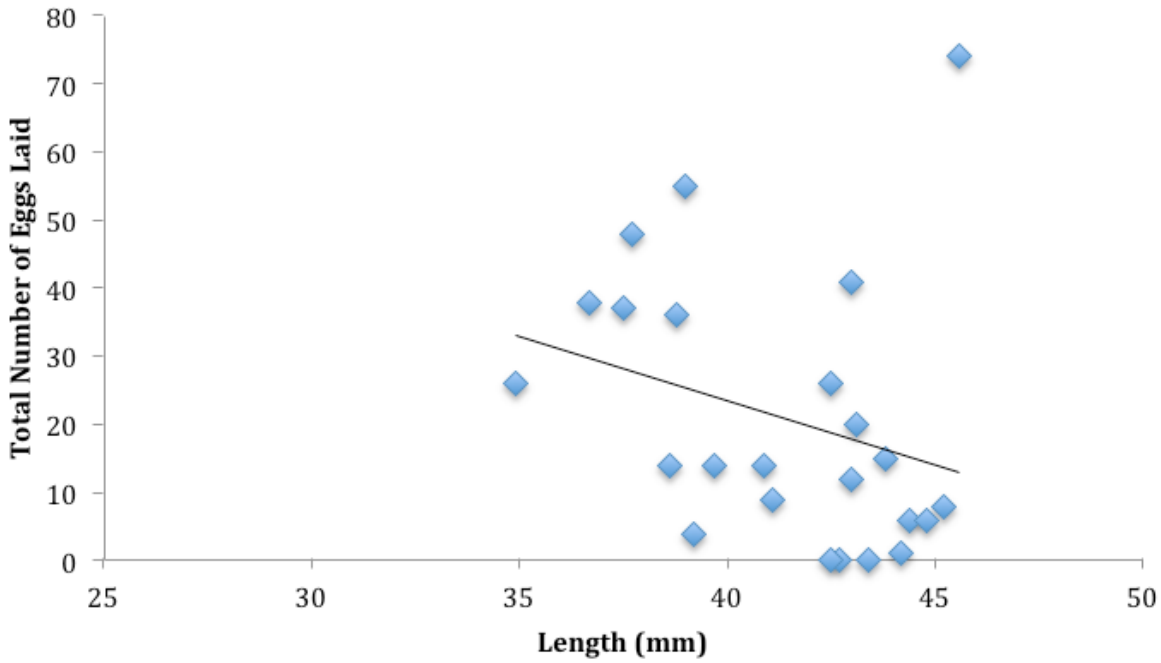

Figure 3.5 Total number of eggs laid per beetle compared to body length.

DISCUSSION

One of the main challenges associated with CRB management is the detection of oviposition sites. This study demonstrated that when given the opportunity to lay eggs at different depths in the observation chamber, CRB laid them ranging from 5 – 119 cm deep showing the vast range over which a female CRB may burrow and oviposit. Eggs were never laid within the shallowest 3 cm, and the fewest number of eggs laid was in the first 15 cm, suggesting that this may be the minimum depth needed by CRB to oviposit within a site. The average depth females were found after one week was 42 cm. The greatest proportion of beetles (42%) laid eggs at 53 cm while the highest average number of eggs laid (24 eggs) was at 51 cm. Therefore, future attempts to identify oviposition sites for CRB should at the very minimum excavate at least 51 cm or more if possible. If detection

methods do not dig to an appropriate depth, certain oviposition sites may be misdiagnosed as CRB free, allowing the population to proliferate and spread. To help prevent this from occurring, information from this study could help to develop a sampling plan providing an optimized procedure for surveying mulch piles for oviposition sites. In terms of breeding site management, these results also suggest that keeping potential breeding-site material spread thinly (i.e. below a maximum depth) may help to reduce the potential for CRB to select that site and oviposit. Future research further investigating the minimum depth needed by a CRB to oviposit any eggs could help to develop a management plan that incorporates spreading breeding-site material below a maximum depth. Additionally, information about the depths CRB can oviposit is also useful for management targeting the egg life stage. This can help management plans to incorporate the various depths treatment would have to reach to effectively kill a certain percentage of the population.

CRB used in this study laid about 21 eggs on average across a range of depths. Fewer eggs were laid than what was observed during the choice experiments (Chapter 2). Additionally, instead of laying eggs randomly or uniformly across the gradient, coconut rhinoceros beetles laid their eggs in aggregations, consistent with Hinckley's (1973) observation that female beetles lay eggs in clutches compacted with oviposition site material. Beetles used in this study may have laid fewer eggs than wild-caught beetles from the choice experiments (Chapter 2) due to the difference in shape of the arena they were in. These beetles had limited horizontal movement but greater vertical movement than those in the choice experiment. This suggests that breeding sites may be dependent on both horizontal and vertical size, but further research is needed.

This is the first study to investigate the depths at which individual CRB prefer to lay their eggs. However, studies of other soil ovipositing scarabs have been conducted. Karam et al. (2000) conducted a study on the life cycle of the rose chafer in Egypt, *Tropinota squalida* (Coleoptera: Scarabaeidae), and found that eggs were oviposited at an average depth of 50 cm. The results from this study indicate that in a given breeding site, CRB may be able to burrow as far down as 120 cm to lay eggs and will oviposit in an aggregated pattern. The maximum depth at which CRB can lay eggs has not been determined, even in this study oviposition depth was constrained by the apparatus.

Lastly, the results for female beetle biometrics as predictors for fecundity after one week agrees with results found from the choice experiments (Chapter 2) using wild-caught beetles; fecundity can be modeled on female CRB biometric measurements. From this study, body length was found to serve as a reliable predictor for fecundity. However, smaller beetles seemed to lay more eggs than larger beetles, contrary to the findings of Hinckley (1973) and Kajita and Evans (2010). We also see that different parameters, such as depth and mass, were used to predict wild-caught beetle oviposition in this study than in the choice experiments (chapter 2), such as length. Again, this may be due to the size of the arena.

This study has yielded important information with regard to the depths at which CRB will oviposit, in spite of the constraints of the apparatus. There is potential for future research of oviposition behaviors within an arena mimicking more closely breeding sites found in the field that allow extensive horizontal and vertical movement for oviposition. It is still unknown what prompts a female CRB to burrow to various depths, or even the maximum depth CRB can burrow, and more research can be done to investigate this.

CHAPTER 4.

GENERAL DISCUSSION

The oviposition behavior of the coconut rhinoceros beetle (CRB) (*Oryctes rhinoceros*, Coleoptera: Scarabaeidae) and the factors that influence this behavior is understudied. This study examined coconut rhinoceros beetle oviposition behaviors to evaluate if physical factors such as substrate particle size or salinity, influence CRB preference for oviposition site, and the range of depths at which they can lay eggs. In addition, this study also compared lab-reared CRB to wild-caught CRB in terms of oviposition behavior and physical parameters. Understanding CRB oviposition behaviors is essential as it could aid in developing appropriate detection and management methods for the prevention and control of oviposition sites and the continuing reproduction and spread of CRB populations in Hawai'i.

In this study, CRB were found to be selective about where they lay eggs. In assessing what factors could potentially influence oviposition, a relatively small and large particle size, and low and high salinity mulch were tested for site preference. The choice tests revealed that CRB will discern between media when given different particle sizes, however, they do not appear to discriminate between media of different salinity levels. However, lab-reared beetles demonstrated oviposition behaviors that differed significantly from that of wild-caught beetles, and analyzed biometric measurements were predictors of fecundity in wild-caught beetles only.

Coconut rhinoceros beetle discriminated among oviposition sites when given the choice of either small or large particle mulch. Both wild-caught and lab-reared beetles in this experiment preferred to oviposit in small particle rather than large. This indicated that

female beetles were able to differentiate between the two media options and were selective in both oviposition site and number of eggs laid. Female beetles could prefer smaller particle size to provide a suitable environment for larval development and pupation, as well as the smaller material providing a more easily handled material to pack eggs with, as reported by Hinckley (1973). This suggests that managing particle size within mulch piles should be considered a factor for improved coconut rhinoceros beetle management, for example, milling plant material to consistently large particle size may reduce reproductive success of the beetles. Other management methods can also be integrated with the information found in this study, such as making sure that small particle size substrate that can't be disposed of is covered with netting to prevent CRB from entering, or by destroying it. Small particle substrate can also be used for controlled breeding sites as a trap-site. This could attract beetles to burrow into the substrate and then the site can be treated with an effective management treatment. However, further research conducting field trials and examining a more narrow range and combination of particle sizes should be assessed.

Although female CRB discriminated between different particle sizes, both lab-reared and wild-caught CRB were not seen to be able to discern between mulch of high or low salinity levels. In this study, the method of providing females with mulch mixed with either a high or low EC salt solution was evaluated. Female beetles laid close to equal amounts of eggs in both mulch choices, therefore it seems that CRB have no preference for oviposition at the trial salinity levels. A possible reason why no preference was seen could be due to the low concentration of salt used. In this study, vastly smaller amounts of table salt (NaCl) were used in comparison to a recent unpublished study done by Vowell et al. that used almost 40 times that amount of salt and found it influenced CRB to prefer one media over

the other. However, Epsom salt ($MgSO_4$) rather than table salt (NaCl) was used. This suggests that perhaps the amount of salt or type of salt used for my study may not be beneficial for management efforts in regards to deterring CRB oviposition, however, further studies examining higher concentrations of salt and using different types of salts may provide different results. Potentially, these different types of salts may also be used in management as a treatment for breeding sites to hinder immature life stages, as this effect was observed in a study conducted by Vowell et al. (unpublished).

Although factors influencing oviposition site preference of CRB are of interest for management efforts, depth of burrowing for oviposition is also important for oviposition site detection and management. In this study, a 122 cm observation chamber was used to evaluate the range of depths at which a female CRB can burrow and lay eggs. Female beetles were seen to burrow vertically and demonstrate oviposition in an aggregated dispersion pattern rather than uniform or random across the gradient offered in the trials. Female beetles laid a majority of eggs between 31 and 61 cm (39%) below the surface, with the greatest average number of eggs laid at 51 cm, and laid the fewest eggs within the first 15 cm (2%) below the surface of the observation chamber. For detection efforts, this suggests that digging deeper than 51 cm may be needed before reliably locating any eggs in an oviposition site. However, the females were also able to burrow and oviposit up to depths of 119 cm. This suggests that if management efforts are targeting a known or potential oviposition site, the various control methods used would be most successful if it could reach the full depth range over which CRB are known to oviposit eggs. Future studies could include researching female burrowing behaviors in an arena that allows for different dimensions of horizontal and vertical movement as well as examining this behavior in the

field. Questions to consider are: is there a maximum depth female beetles will burrow? Will female beetles not burrow as deep if there is more room to move horizontally? What is the minimum depth female beetles will burrow to lay any eggs? Answering these questions could help to more accurately assess a potential oviposition site and apply treatment accordingly.

Additionally, this study underscored that biological differences are often significant between lab-reared and wild-caught insects, and was the first make this comparison with coconut rhinoceros beetle. In this study, lab-reared beetles were found to lay on average fewer eggs compared to wild-caught beetles. These two groups also displayed some differences in their egg laying patterns in different oviposition sites. Jandt et al. (2015) examined the effects of laboratory rearing on factors like behavior and colony dynamics of paper wasps, *Polistes fuscatus*, and stated that lab and field environment differences could possibly skew the biological significance of results in experiments. This was observed even within the first generation of lab reared CRB. Wild-caught beetles laid more eggs in all mulch treatments, laying 38 eggs on average per week, while lab-reared beetles laid 6 eggs on average per week. Wild-caught females were more fecund than lab-reared beetles overall, suggesting that lab-reared beetles should not be used as indicators of wild-caught beetle oviposition behaviors in the natural environment. A potential reason why different patterns of egg laying were observed between these two groups could be due to the developmental environment. Lab-reared beetles do not have the same diet, space, environmental factors, microbiome, predators, and natural photoperiod as wild-caught beetles. They do not need to locate or search for food, whereas wild-caught beetles may be food limited, or may have to actively search for breeding and oviposition sites. These

factors could contribute greatly to the differences affecting oviposition behaviors and possibly other behaviors not yet studied. Future studies looking at behaviors of lab-reared and wild-caught CRB could include lengthier comparison studies following the life cycle and oviposition behaviors of these two groups.

Lastly, biometric parameters (body length, width, depth, and mass) were measured and analyzed as predictors for the number of eggs CRB will lay within a week, and to compare beetle biometrics in relation to origin of the beetle. Wild-caught beetles were the only group that produced adequately consistent egg laying to permit the development of models predicting the number of eggs a beetle will lay. The wild-caught beetles were also found to be on average larger than lab-reared beetles. Substrate choice tests showed larger beetles laid more eggs than smaller beetles, however, oviposition depth experiments showed that smaller beetles laid more eggs than larger beetles. The difference could be due to the type of arena used in the different tests (substrate choice vs. burrowing depth). The choice test arenas had a greater horizontal area for movement, while the arena used for oviposition depth studies had a greater area vertically but were very limited horizontally. It is possible that smaller beetles could move throughout the oviposition depth chamber with greater ease, therefore they could burrow and oviposit a higher number of eggs than larger beetles. This suggests that different dimensions of an oviposition site may produce different results for the number of eggs laid in relation to beetle size, and leads to the conclusion that further studies using a site of different horizontal and vertical measurements should be conducted.

In conclusion, CRB do exhibit preference for oviposition sites as well as burrow vertically to a variety of depths, and oviposit in an aggregated pattern up to 119 cm deep in

substrate. This study is the first to evaluate the oviposition preference and depths of the coconut rhinoceros beetles not only in wild-caught beetles, but in lab-reared beetles as well. Additionally, this study also examines the effects of laboratory rearing on oviposition behaviors compared to wild-caught beetles and takes into consideration biometric parameters. Substrate particle size and depth of an oviposition site should be considered for CRB management and can be implemented to improve management methods. However, further research should be done on different levels of substrate salinity, oviposition preference, and depth under field conditions.

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