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Size and Population Dynamics of Native Ghost Crabs, *Ocypode* spp., in Response to an Invasive Ant Population in a Native Wildlife Refuge, O‘ahu, Hawai‘i



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Biology 400 (Marine Option Program Skill Project) (OPIHI Internship)
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*Invasive species are harmful to ocean environments especially fragile ecosystems, like intertidal island environments. Ant populations are among the most aggressive invaders. Ants have been known to cause individual harm to endangered birds, plants and other arthropods in Hawai‘i. The yellow crazy ant (YCA), *Anoplolepis gracilipes*, is one of the worst invasive ants as it can form supercolonies and spew out formic acid. This study investigates the effect of YCA on the abundance, sizes and distribution of ghost crabs, *Ocypode* spp., at James Campbell National Wildlife Refuge (JCNWR), a native wildlife restoration area. Ghost crab’s burrows were counted and measured (a proxy for crab size), in order to see if there is a relationship between ghost crab size/number to the density of ants (both invasive YCA and others) present. Ghost crabs are vital to sandy intertidal regions as they are the link to land and water ecosystems and are opportunistic. This study is important because it tell us how the ants are affecting the ghost crab populations and the ecosystem in general. This study can be used in future research to determine conservation techniques in order to control invasive ant populations, like the YCA.*

Introduction

Intertidal zones are areas between the ocean and land ecosystems that encompasses a more sensitive to change area compared to other terrestrial environments (Ansari et al. 2014). The challenges of the intertidal zone come from its many hardships including, temperature extremes, wave intensity, foot

traffic from humans and risk of desiccation (Przeslawski 2004; Nordlund et al. 2014; Pombo et al. 2017). As temperature increases or decreases it can have an effect on the embryonic development which may reflect how a species in the intertidal zone is distributed (Przeslawski 2004). Organisms are affected by both abiotic and biotic factors, some of which affect species in the intertidal zone more so than others (Brancho et al. 2010). Sandy intertidal zones are characterized by variations in



This has been an amazing opportunity to show how an invasive species changes the ecology of an ecosystem. I learned more about research in the field and learn more about crabs and ants. I also learned how to do better data analysis and come up with my own research question, hypothesis and other scientific method processes. It was a great way to meet new people and develop a network of friends in the community. Thank you to OPIHI (Our Project in Hawaii’s Intertidal) and NSF (National Science Foundation).

sand grain size, sun exposure time, differences in the swash zone width, temperature variations and inter/external species interactions (Zardi et al. 2007; Przeslowski 2004). In the sandy intertidal zone species like the ghost crab (*Ocypode* spp.) are affected by wind and human traffic as well as the other factors mentioned, which may reflect fragility in their species which could allow for better management tactics as well (Chan et al. 2006; Brancho et al. 2010).

Included in the biotic factors, is organismal competition which may dictate the spatial distribution of the intertidal zone (Connell and Gillanders 2007; Nordlund et al. 2014). For the genus, *Ocypode*, some spatial distributions are based on gender differences, where the males are found in the upper intertidal/dry areas and females/juveniles are found in the vegetation zone (Fellows 1975), though some indicate that juveniles are found more towards the wet swash zone due to desiccation awareness, which may be an indication of differences in burrow diameters as adult males tend to leave behind larger burrows (Chan et al. 2006). It may also indicate gender-based pyramidal formation, an important sign of colony formation where males build burrows with pyramid-like sand pellet behind them, in order to attract females (Trott 1998; Fellows 1975). Lighter (1977) describes how *Ocypode* used all three kinds of spatial dispersal patterns, randomness, aggregation and uniformity based on sex and crowding distances. He used a similar method to this study to characterize the sizes of crabs, by measuring their hole diameter (Lighter 1977; Pombo et al. 2007). Species distribution and spatial variation can change based on different biotic and abiotic factors and can be based on species interactions as well (Brancho et al. 2010).

Species interactions can be beneficial, harmful, or neither (Connell and Gillanders 2007). Sometimes species that are more dominant 'take-over' or invade an area. If invaders are unmanageable, they can compromise the spatial distribution and/or densities of *Ocypode*, interfering with their natural processes (Bergstrom et al. 2009; Dejean et al. 2010). Due to the fragility of island-like environments, allowing many generations for species to evolve and co-exist together, invasive species are a detriment to these environments, like in Hawai'i (Reimer 2004; Bergstrom et al. 2009). This phenomenon threatens Hawaiian ecosystems as there are 30% endangered and rare species and more than 1000 native Hawaiian species already extinct (Allen 2000). Therefore, it is vital that conservation efforts go into place in order to protect the remaining species present.

Many ant species including *Solenopsis invicta* (Red Fire Ant), *Linepithema humile* (Argentina Ant), *Pheidole megacephala* (Big Headed Ant), *Wasmannia auropunctata* (Electric Ant) and *Anoplolepis gracilipes* (Yellow Crazy Ant), which have been known to cause detrimental ecological effects around the world (Wetterer 2005; Invasive ants...c2018). A successful invader has low intraspecific aggression (unicolonial nests), high interspecific aggression and mutualistic relationships (Kirschenbaum 2007; Dejean et al. 2010). Ants are invasive due to their ability to form high

densities or supercolonies causing 'invasional meltdowns' of species in the area (Abbott 2005). There are about 45 different ant species in Hawai'i all of which are non-native and invasive with about half found in urban, agricultural and natural environments (Reimer 2004; Kirschenbaum 2007). The invasive ant species, *Anoplolepis gracilipes* or the yellow crazy ant, originated from West Africa, India or China. They are found mostly in tropical, moist lowlands environments but not found in areas above 1200m or in arid environments. *A. gracilipes* has been known to have affects on nesting birds and native invertebrates in Seychelles as well as endemic crabs found on Christmas Island, Australia (Wetterer 2005). *A. gracilipes* forms a mosaic of high-density supercolonies that can last for more than 24 hours a day, year-round as well as their ability to spit out formic acid into the eyes or weak areas of birds and crabs (Abbott 2005; Gül 2017). On Christmas Island they have had the ability to kill the native red land crabs within 24 hours (Abbott 2005), which makes YCA a substantial threat to native Hawaiian crabs.

Ocypode spp. is an ecologically important part of the sandy intertidal food webs. They are highly mobile and feed on a variety of foods which making them an important balance in the food web ecosystem. *Ocypode* spp. are also a key link between inland ecosystems and the marine environment as they are the predator/prey item for higher level consumers like birds, as well as some of their food is obtained through terrestrial resources (Rae et al. 2019). Ghost crabs are also an important part of bioindication of oil spills and other such chemical contamination (Ghost Crabs... unknown copy-right date). Hawai'i has two ghost crab species, *Ocypode ceratophthalmus* (horn-eyed ghost crab) and *Ocypode laevis* or *Ocypode pallidula* (pallid ghost crab), The horn-eyed ghost crab is found all over the world whereas the pallid ghost crab is found on different islands throughout the pacific (Lighter 1977).

Invasive species have become one of the causes for the 91 (54%) extinctions of the 680 extinct species around the world of which 34 (20%) of the extinctions, invasive species were the only cause of extinction (Clavero and Garcia-Berthou 2005). Many species have become introduced, since the first arrival of humans over 1,000 years ago and many have also become extinct including the important land-dwelling crab, *Geoprapus severnsi*, once common on the Hawaiian islands. The loss of the land-dwelling crab allowed for there to be an ecological imbalance, that was once harmonious, in an ecosystem (Gonzaga 2011). This emphasizes the importance of understanding invasive species ecology as ecosystems do not want to allow more species to go extinct as well as disrupt the normal harmony of the ecosystem present. The effect of invasive species could influence the environment and species around them in the refuge.

Therefore, this study was focused on James Campbell National Wildlife Refuge (JCNWR) where a restoration of four native water birds has taken place (Hunt and Eric 2000). The focus is to examine how the invasive ant population of *A. gracilipes* and other ants affects the native crab population, *Ocypode* spp. and the vegetation zone at JCNWR.

The main objectives of this study are first to investigate population changes over time (comparing 2019 and 2020 data sampling seasons), second, to compare crab densities in different zones of the beach (sand vs. vegetation), and third, to determine whether or not invasive ant species are affecting crab populations as well as the vegetation within JCNWR. Understanding the ant populations mutualistic relationship will allow us to better monitor them and how they influence the ecosystem. We may also be able to even eradicate them from the reserve to help the species in that area and hopefully limit the number of extinctions or catch extinctions before they occur.

Materials and Methods

SITE MAPPING

Our site is located on the North Shore of O'ahu from Kahuku to James Campbell National Wildlife Refuge (JCNWR) to Turtle Bay (Fig. 1). The site is on a sandy and rocky intertidal zone in-front of a protected reef which allows there to be smaller waves on the beach front. Tides were looked up using computer data bases (<https://www.surf-forecast.com/breaks/Turtle-Bay/tides/latest>) and start time was set at 0830–0900.

In order to map out the different transect locations or sample areas we walked along the beach from Kahuku to Turtle Bay and used the Global Position System (GPS) to pinpoint the locations. We walked along the beach and found 12 sites that were large enough to have two transects in the sandy areas ($2 \times 12 = 24$) and plotted them on Google Earth with the GPS coordinates (Fig. 1). Lines mark the area of the reserve boundary.



Figure 1 Location (GPS points 1-24) of James Campbell National Wildlife Refuge (JCNWR) on the island of O'ahu, Hawai'i. Each site was found by walking along the 6.4 Kilometers beach finding 12 sandy zones and dividing the zones into 2 for a total of 24 sites. Rocky intertidal areas in the ocean were not surveyed. Only 22 of the 24 GPS points were actually surveyed due to COVID-19.

We wanted each area to have a patch of sand in the water where the mole crabs survey could be conducted as well as enough sand for the ghost crab surveys. Only 22 of the 24 surveys were conducted due to the COVID-19 pandemic.

ANTS SURVEYS

For the ants at each transect point (marked by GPS) we placed a container with a smear of peanut butter and honey on the sides of each container and SPAM at the bottom in order to attract the ants (Fraiola K., *pers. comm.*). We placed the container in a shady area at each transect point with a marker for the location away from the wind so that minimal sand went into the container. Location (GPS point), date and time in/out were put onto the container. We left the ants out for 30 minutes to 2 hours and upon collection, capped the bottles. After the surveying day was done the bottles were placed in a freezer and kept frozen until counting. After freezing, we counted how many yellow crazy ants were present using a dissecting microscope; we also counted the number of other ants present but did not classify them to species (Fig. 2). Due to the global SARS-CoV-2 pandemic, ants were not able to be placed under a microscope towards the end of the survey. Ants were visual identified with the naked eye, so our ant identification may not be as accurate therefore classification of the ant species was called other or the total number of ants in the container was used.

GHOST CRAB

Ghost crabs are in the subphylum Crustacea and the genus *Ocypode*. They are characterized by their white almost ghost like appearance in addition to their burrowing behavior



Figure 2 Containers to collect the ants were placed in shaded areas away from the wind for 30 minutes to 2 hours. Each container was capped and placed in a freezer until time for counting. Samples were placed under a dissection microscope to identify if they were yellow crazy ants on the left or other ant on the right (Pharaoh). Due to global SARS-CoV-2 pandemic, microscopes were not available towards the end of the surveying and samples were visually identified with the naked eye.

(Pearse and Vicki 1987). Lighter (1977) used a similar method to this study to characterize the sizes of crabs, by measuring their hole's diameter (also Pombo et al. 2007). *Ocypode* make large burrows in order to defend the space surrounding their burrows (Fellows 1975). At each GPS point starting from the swash zone, where the tidal line ends, we counted the number of ghost crabs. When we counted the number of ghost crabs we also looked at the sizes of their holes. Hole sizes were measured by measuring the diameter of the shaft of each burrow. If we could not see into the hole or there was no darkness the hole was not counted (had to be a fresh hole). At the swash zone three people lined up side by side with arms spread out, we walked up the beach counted and measured the hole diameter using a ruler until we reached the vegetation zone, noticing what type of vegetation and continued till the last hole was observed (Fig. 3). In the vegetation zone some of the holes could have been made by the vegetation and wind. Measurement of the distance from the swash zone to the last hole were taken using a transect (measured in meters to the nearest decimal point). Observations of wind, surf and tides were taken into account along with vegetation type.

DATA ANALYSIS

We determined the relative density of yellow crazy ants to other ants over an area using a correlation analysis, density of ants was measured in ants per second. For the average number of crabs we used a T-test. For the ghost crabs we used density of ghost crabs as the number of crabs per meter squared. We compared the yellow crazy ants to ghost crab hole diameter, number and density using correlation tests. For comparing vegetation and abundance/hole diameter of the crabs to the presence/absence of ants we used the program R (GLM) (Data Analysis credit Patrick Nichols).



Figure 3 On the bottom right shows the holes that we measured using a ruler and on the left shows a live ghost crab. The hole diameter corresponds to the size of the ghost crabs (Lighter 1977; Pombo et al. 2007).

Results

The average number of crabs in 2019 ($n=1065$; 39.444 ± 20.363) was not significantly different from the total number of crabs in 2020 ($n=554$; 30.7778 ± 16.491) Therefore we accepted the null hypothesis (T-test; $df=41$, $p=0.12406$, Fig. 4).

The average number of crabs in the sand in 2019 ($n=557$; 20.6292963 ± 14.6633) was not significantly different from the average number of crabs in the sand in 2020 ($n=404$; 22.4444 ± 16.8531). Therefore we accepted the null hypothesis (F-test, $df(2019)=26$ and $df(2020)=17$; $p=0.254710648$; T-test; $df=33$, $p=0.711939178$, Fig. 5).

The average number of crabs in the vegetation zone from 2019 ($n=532$; 19.7037 ± 17.0064) was significantly higher than the average number of crabs in the vegetation zone from 2020 ($n=150$; 8.3333 ± 7.1702) therefore we rejected the null

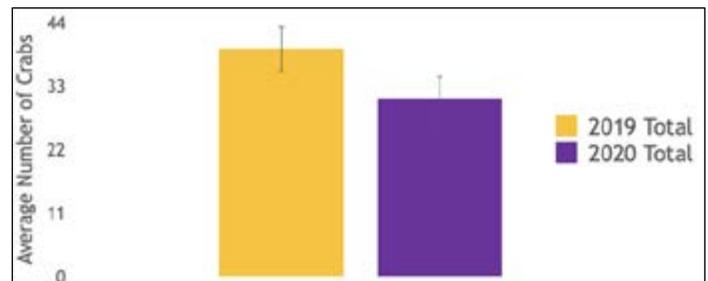


Figure 4 Average total number of ghost crabs in the vegetation and sand zones combined from 2019 ($n=1065$ crabs; 39.444 ± 20.363 crabs, orange) to 2020 ($n=554$; 30.7778 ± 16.491 crabs, purple). The numbers of crabs total from 2019 to 2020 were not significantly different therefore the null hypothesis was not rejected (F-test, $df(2019)=26$ and $df(2020)=17$; $p=0.184777246$; T-test; $df=41$, $p=0.12406$).

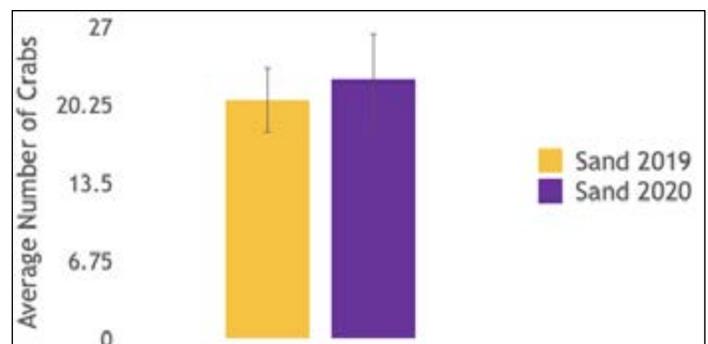


Figure 5 Average number of crabs in the sand from 2019 ($n=557$; 20.6296293 ± 14.6633 , orange) and the average number of crabs in the sand from 2020 ($n=404$; 22.4444 ± 16.3782 , purple). The average numbers of crabs total from 2019 to 2020 were not significantly different therefore the null hypothesis was accepted (F-test, $df(2019)=26$ and $df(2020)=17$; $p=0.254710648$; T-test; $df=33$, $p=0.711939178$).

hypothesis (F-test, $df(2019)=26$ and $df(2020)=17$; $p=0.00027$; T-test; $df=38$, $p=0.003766$, Fig. 6).

Data from 2020 season comparing the sand zone ($n=404$; 24.4444 ± 16.3782) and the vegetation zone ($n=150$; 8.33333 ± 7.17019977) showed that there was a significant difference (F-test; $df=17$, $p=0.0005$; T-test; $df=23$; $p=0.003373899$, Fig. 7) between the two zones, therefore we rejected the null hypothesis.

There was not a significant difference between the number of crabs for the 2019 season in the vegetation zone ($n=532$; 19.703704 ± 17.006367) compared to the sand ($n=557$; 20.6296 ± 14.6633), therefore we accepted the null hypothesis (F-test; $df=26$, $p=0.227438875$; T-test; $df=27$, $p=0.831197$, Fig. 8).

The density of ghost crabs also did not change in response to the total amount of ants being present including the other ant populations (correlation; $df=17$, $r=-0.211$, $p=0.385$, Fig. 9). Fig. 9

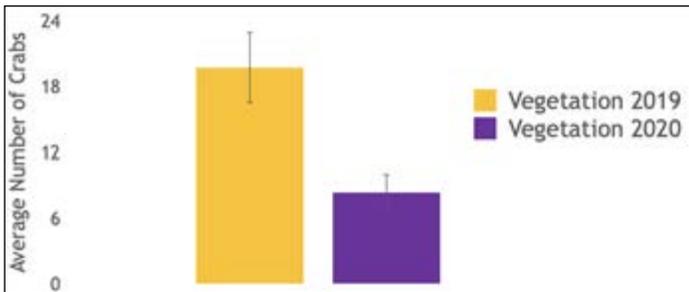


Figure 6 Average number of crabs in the vegetation from 2019 ($n=532$; 19.7037 ± 17.0064 , orange) and the average number of crabs in the vegetation from 2020 ($n=150$; 8.3333 ± 7.1702 , purple). The average numbers of crabs total from 2019 to 2020 were significantly different therefore the null hypothesis was rejected (F-test, $df(2019)=26$ and $df(2020)=17$; $p=0.00027$; T-test; $df=38$, $p=0.003766$).

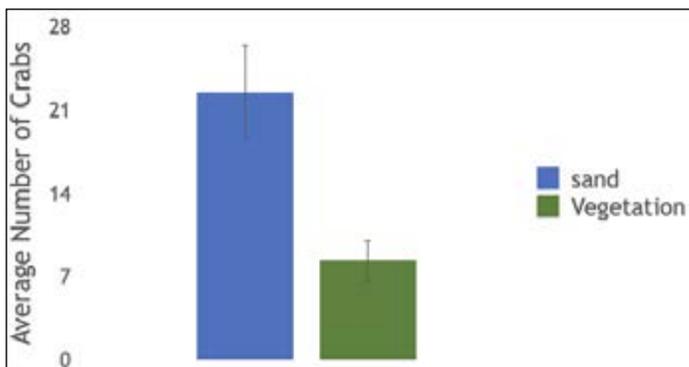


Figure 7 Comparison between average number of crabs in the sand for only 2020 data ($n=404$; 24.4444 ± 16.3782 , blue) and the average number of crabs in the vegetation ($n=150$; 8.3333 ± 7.1702 , green). The average number of crabs in the vegetation zone was significantly smaller the the average number of crabs in the sand, therefore reject the null (F-test, $df=17$; $p=0.00027$; T-test; $df=23$, $p=0.003766$).

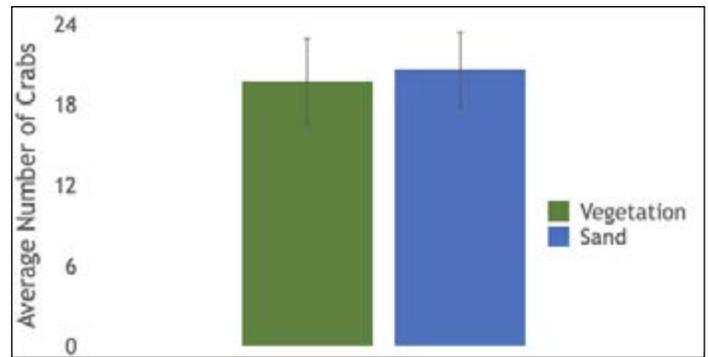


Figure 8 Average number of ghost crabs in the vegetation zone ($n=532$; 19.703704 ± 17.006367 , green) compared to the average number of crabs in the sand ($n=557$; 20.6296 ± 14.6633), therefore we accepted the null hypothesis (F-test; $df=26$, $p=0.227438875$; T-test; $df=27$, $p=0.831197$) for the 2019 year.

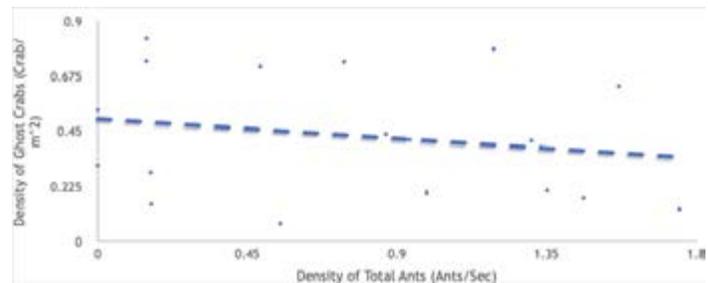


Figure 9 Density of total ants present (ants/sec) compared to the density of ghost crabs (crabs/m²). These two values were negatively not significant correlated (correlation; $df=17$, $r=-0.211$, $p=0.385$).

Density of total ants present (ants/sec) compared to the density of ghost crabs (crabs/m²). These two values were not significantly correlated (correlation; $df=17$, $r=-0.211$, $p=0.385$).

There was a negative, non-statistically significant relationship between the total density of crabs and the density of yellow crazy ants (Correlation; $df=17$, $r=-0.319$, $p=0.183$, Fig. 10). There was also a negative, non-statistically significant relationship between the density of crabs in the sand and the density of yellow crazy ants (Correlation; $df=17$, $r=-0.369$, $p=0.120$, Fig. 10). Though positive, there was no statistically significant relationship between the density of crabs in the vegetation and the density of yellow crazy ants (Correlation; $df=17$, $r=0.107$, $p=0.662$, Fig. 10).

There was a negative not statically significant correlation between the total average sizes of ghost crabs and the total ant density (correlation; $df=16$, $r=-0.233$, $p=0.2411$, Fig. 11).

There were no significant effects of vegetation type (GLM; $F=13.4$, $df=11$, $p=0.27$, Fig. 12) or yellow crazy ants presence/absence (GLM; $F=1.54$, $df=2$, $p=0.46$, Fig. 12) on ghost crab abundances. More diversity was present with the absence of yellow crazy ants. There was only Akiaki and Akuikuli present with

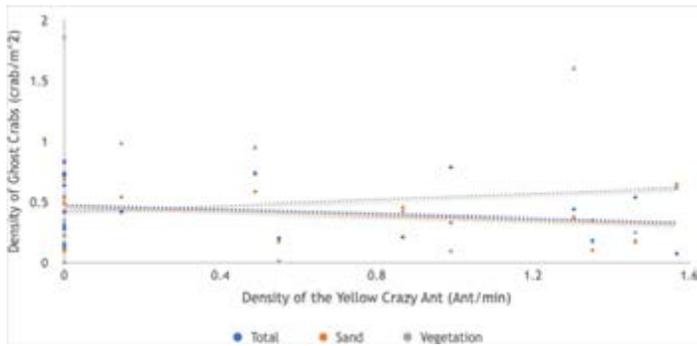


Figure 10 Density of the yellow crazy ant (2020) in ants per unit time (minutes) was affecting the density of ghost crabs, in crab per unit area (m^2). There was a non-statistically significant relationship between the total density of crabs and the density of yellow crazy ants (correlation; $df=17$, $r=-0.319$, $p=0.183$), a negative, non-statistically significant relationship between the density of crabs in the sand and the density of yellow crazy ants (correlation; $df=17$, $r=-0.369$, $p=0.120$) and positive, non-statistically significant relationship between the density of crabs in the vegetation and the density of yellow crazy ants (correlation; $df=17$, $r=0.107$, $p=0.662$).

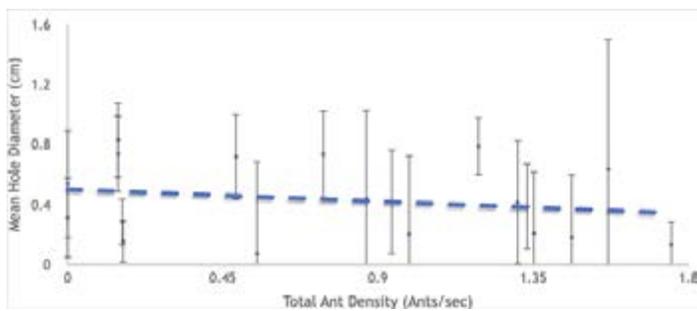


Figure 11 Density of the total ant population (ants/sec) versus the average hole diameter (cm). The correlation showed that the two were negatively non-significantly correlated relationship for the average mean hole diameter between the crabs and the total ant density (correlation; $df=16$, $r=-0.233$, $p=0.2411$).

the presence of yellow crazy ants. However, there was a significant effect of other ant presence/absence on ghost crab hole diameter (GLM; $F=19.5$, $df=2$, $p<0.001$, **Fig. 13**). There was also a significant interaction of vegetation type and other Ant presence/absence on ghost crab hole diameter (GLM; $F=4.73$, $df=1$, $p=0.03$, **Fig. 13**). Meaning that both vegetation and whether or not other ants were present had combined effects to significantly impact the diameter of burrows. Finally, there was a marginally significant (meaning almost at $p=0.05$) effect of the interaction between vegetation type and yellow crazy ant presence/absence on ghost crab hole diameter (GLM; $F=4.74$, $df=2$, $p=0.06$, **Fig. 13**).

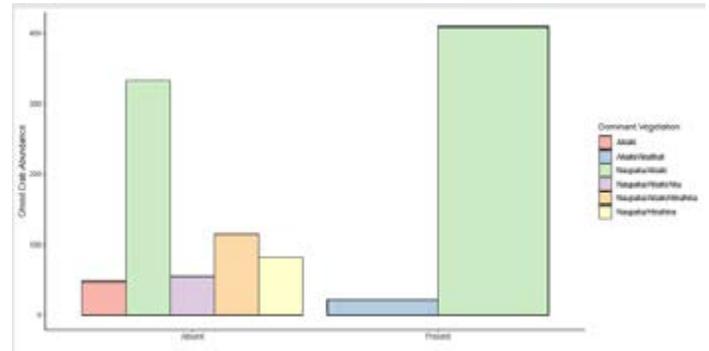


Figure 12 Comparing the ghost crab abundance, the presence and absence of yellow crazy ants and the vegetation type (Akiaki-pink, Akiaki/Akulukuli-blue, Naupaka/Akiaki-green, Naupaka/Akiaki/Aku-purple, Naupaka/Akiaki/Hinahina-orange, Naupaka/Hinahina-yellow). There were no significant effects of vegetation type (GLM; $F=13.4$, $df=11$, $p=0.27$) or YCA presence/absence (GLM; $F=1.54$, $df=2$, $p=0.46$) on ghost crab abundances. More diversity was present with the absent of yellow crazy ants. Data analysis done by Patrick Nichols.

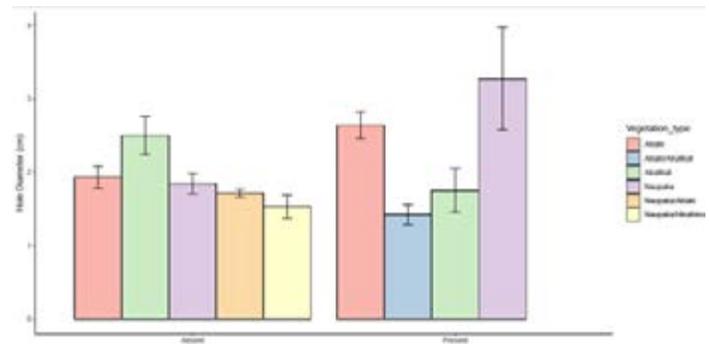


Figure 13 Comparing the ghost crab hole diameter in centimeters (corresponds to the size of the crab, Pombo et al. 2007) with the presence (right)/absence (left) of ants and the vegetation type (Akiaki-pink, Akiaki/Akulukui-blue, Naupaka/Akiaki-green, Naupaka/Akiaki/Aku-purple, Naupaka/Akiaki/Hinahina-orange, Naupaka/Hinahina-yellow). There was a significant effect of other ant presence/absence on ghost crab hole diameter (GLM; $F=19.5$, $df=2$, $p<0.001$). There was also a significant interaction of vegetation type and other ant presence/absence on ghost crab hole diameter (GLM; $F=4.73$, $df=1$, $p=0.03$). There was a marginally significant effect of the interaction between vegetation type and yellow crazy ant presence/absence on ghost crab hole diameter (GLM; $F=4.74$, $df=2$, $p=0.06$). Data analysis done by Patrick Nichols.

Discussion

GHOST CRABS FROM 2019 TO 2020

This study is an on-going study from Spring 2019 to Spring 2020; comparing the data showing if there were any trends for

number of crabs in the vegetation and sand zones and seeing if there are any differences in where the crabs and ants are located along the beach and if this affects the crab's size. The average number of crabs from 2019 to 2020 in the sand and total did not change (Fig. 4 and 5).

The number of crabs found in vegetation decreased from 2019 to 2020 indicating that something was occurring in the vegetation zone in regards to physical, chemical or the social structures (Fig. 6, $p < 0.05$).

Also in the 2019 season there were no significance between the number of crabs in the vegetation zone and the sand zone but in the 2020 season there was a significantly smaller number of crabs in the vegetation zone compared to the sand zone (Fig. 7 and 8).

There was also the possibility of error due to our inability to count the ants under the dissection microscope because of the SARS-CoV-2 pandemic, therefore we combined all ants instead of grouping them into yellow crazy ants and other ants (Fig. 9).

GHOST CRABS

Two main species of the genus *Ocypode* were found at James Campbell National Wildlife Refuge (JCNWR), *Ocypode ceratophthalmus* (horn-eyed ghost crab) and *Ocypode laevis* or *Ocypode pallidula* (pallid ghost crab) (Fellows 1975). Ghost crabs are scavengers and predators which may dictate where they are spaced as well (Rae et al. 2019). Size differences in crabs may reflect differences in sexual and reproductive cycles (Jonah et al. 2015). The sizes of burrows were found not to be related to the total ant density (Fig. 11). Small differences in sizes may be due to their gender as well as locational patterns (Fellows 1975; Lighter 1977). Smaller sized crabs tend to live lower on the beach and larger crabs live more in the upper littoral zone, because juveniles may desiccate if out of their burrows for too long (Branco et al. 2010).

Average burrow size as measured by hole diameter was not correlated with the total density of ants (Fig. 11). The presence of YCA appeared to limit ghost crab distribution to Naupaka/Akiaki vegetation (Fig. 12) and vegetation type had a significant impact on ghost crab burrow sizes (Fig. 13).

COMPARING ANTS TO OTHER ANTS

Dr. Sheldon Plentovich indicated that some of the ant species that looked like yellow crazy ants in our study were actually of the species *Monomorium pharoanis* (a Pharaoh ant) or possibly a cardiocondyla. *M. pharoanis* are smaller compared to the *A. gracilipes* but otherwise look similar. The yellow crazy ants were only positively identified in the cemetery (reserve edge). Many species of ants are successful at invading due to their ability to have low intraspecific aggressions, high interspecific aggression and mutualistic relationships (Kirschenbaum

2007). Along with *Anoplolepis gracilipes*/*Monomorium pharoanis* there were a variety of ant species in the containers we collected, some of which may be more aggressive than others in regards to aggregation. *A. gracilipes*/*M. pharoanis* was not found in similar locations as the other species of ants, perhaps due to their inability to aggregate with other ant species (Kirschenbaum 2007; *Monomorium pharoanis*...c1996–2020). James Campbell National Wildlife Refuge (JCNWR) provides an excellent location for *A. gracilipes* to congregate as they require large rocks or rock-lined areas for nesting grounds (Fluker and Beardsley 1970). The presence of yellow crazy/pharaoh ants seems to have an effect on the diversity of the different vegetation types, as in there were only two types of vegetation when yellow crazy/pharaoh ant were present compared to five vegetation types in the absence of yellow crazy/pharaoh ants (Fig. 12). The vegetation could have changed based on certain ant species being present, as certain ant populations alter the soil composition (Fig. 12 and 13; Majer 1985).

IMPORTANCE

Ghost crabs were not found too far into each vegetation zone, where most of the ant populations resides. Ghost crabs are a good indication of a healthy ecosystem as they are top omnivores of the sand dune community (Brancho et al. 2010; Jonah et al. 2015). Differences in vegetation and the presence of ants have an effect on the distribution of crab burrows (Fig. 13). Spatial distribution of the different colonies of ants may play a role in species competition and how the ants are affecting the ecosystem as some ants are more aggressive than others therefore affecting the ecosystem in different ways (Fluker and Beardsley 1970; Majer 1985; Green et al. 2011). Understanding the distribution and densities of certain ants (ie. yellow crazy/pharaoh ant) could allow us to successfully locate the ants in order to be able to eradicate them. It is better to understand the ant population now before they cause massive destruction to the ecosystem, as has been the case on Christmas Island with the yellow crazy ant as well as in other ecosystems with other ant species (Wetterer 2005; Green et al. 2011). Although the pharaoh ants are not as costly to the ecosystem as the yellow crazy ants, they still cause damage and may be affecting the vegetation types or vegetation zone in general (Fig. 12 and 13). Finding yellow crazy ants in the reserve allows us to monitor them before they increase enough to have an impact on nesting bird populations in the reserve (Fluker and Beardsley 1970; Boland et al. 2011).

Yellow crazy ants (similarly with the pharaoh ant) prefer low wetland areas, JCNWR is a key place for these species to reside and flourish. The yellow crazy ant look-alike, the pharaoh ant, also forms polydymonus nests, which are made up of multiple colonies with more than one queen. Humans can easily spread the pharaoh ants through scattering of rubbish.

It is possible that there were some burrows that we're too

small to see or blown-out by the wind to be found through visual observations. Differences in grain size and temperature may also reflect the density distribution of the ghost crabs which tend to hide in the burrows during the day to resist predation or desiccation (Jonah et al. 2015; Pombo et al. 2017). Some of the beaches had bigger grains of sand, while others had finer sand which would affect their burrow sizes as well as their spatial distribution as beaches with smaller grain sizes tend to have more species (Fellows 1975; Pombo et al. 2017). Areas with smaller grain sizes tended to have higher density and mean size of crabs. Although grain size was not measured, this could explain why different vegetation had a range of burrow sizes as different vegetation types could promote smaller or larger grain sizes (Fig. 13; Wang et al. 1998; Pombo et al. 2017). The slope or gradient of the beach may also have an effect on spatial distribution (Pombo et al. 2017).

Future studies could examine whether ants prefer certain kinds of food bait in traps, as the pharaoh ants have been shown to prefer honey (Kirschenbaum 2007; *Monomorium pharaonis*... 1996–2020). This would enable us to specifically isolate the species of interest and hone in on their effects on the environment. It would also be interesting to add ant traps up the beach in different orientations and see if there are more yellow crazy ant hatchlings near the rocky areas in order to find out more about their reproductive success as well as their hierarchical structure (Kirschenbaum 2007). Some successful eradication procedures have been done on Christmas Island using a fipronil bait and allowing for the workers to bring the poisoned food back to the queen (Boland et al. 2011).

We know very little about the behavior and burrowing techniques of crabs or their larval life stages which may play into where the crabs prefer to reside (Chan et al. 2006; Brancho et al. 2010; Jonah et al. 2015). Most work on ants has mostly been done on just a single species affecting an ecosystem, but the ant community as a whole can affect or reflect the dynamics of the ecosystem and how they are changing it (Green et al. 2011). As more species of ants invade we are going to most likely see changes in community structure (Reimer 2004).

Changes in community composition may reflect an ecosystem imbalance which can be addressed by improving management. Humans can reduce threats to ecosystems by being more mindful of the environment and preventing the spread of invasive species (Clavero and García-Berthou 2005). Ecosystems in Hawai'i are particularly vulnerable because many species have arrived or evolved here in the absence of predators (Reimer 2004). Sandy intertidal zones are vital to humans as they provide resources, shelter from storms, and are of economic importance (Jonah et al. 2015). Understanding this fragile strand ecosystem is important as it is the link between land and sea and a connection between humans and the ocean.

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