Biology Honors Thesis

Can an Invasive Species of Crayfish Help Save a Population of a Threatened Species of Bird, the King Rail?

Weston Lee Beamon

May 2018

| APPROVED BY: | |
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| Director of Thesis: | SB Malue |
| | Name |
| Faculty assessor: Raul | Att |
| | Name |
| Director of Undergraduate Stu | dies: |
| · · | Jean-Luc Scemama, PhD |
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Weston L. Beamon

A thesis submitted to the Department of Biology, East Carolina University, in partial fulfillment of the requirements for Biology Honors Thesis

Advisor: Susan B. McRae, Ph.D East Carolina University Department of Biology

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I hereby declare I am the sole author of this thesis. Part of this work, the collection of King Rail call data using Autonomous Recording Units and analysis using Kaleidoscope software, was the result of a collaboration with Katie M. Schroeder. This work has not been submitted elsewhere as coursework for this or another degree.

Signed:_

Weston L. Beamon

Date: 65/03/18

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Weston L. Beamon

Department of Biology, East Carolina University, Greenville N.C. 27858-4353, U.S.A.

ABSTRACT - Invasive species are frequently harmful to native species and to ecosystem stability. Yet, in a few cases, alien species have been found to benefit native residents. I investigated the relationship between a threatened species of marsh bird and an invasive species of crayfish on which it feeds. The Red Swamp Crayfish, *Procambarus clarkii*, was deliberately introduced into an impoundment at Mackay Island National Wildlife Refuge (NWR) 25 years ago. The refuge hosts one of the largest breeding populations of King Rails *Rallus elegans* on the east coast of the United States. This secretive marsh bird is globally Near Threatened (Birdlife International). The King Rail is mainly carnivorous and feeds in the shallows on fish, crustaceans, and other aquatic animals, with crayfish being a preferred food. The invasive crayfish is fast-growing and a prolific breeder. I investigated whether the crayfish could be providing these rare birds a resource that allows them to prosper here relative to other sites.

Data were collected over the summer breeding season on both crayfish and rail abundance. I determined the distribution of crayfish among ten predetermined sites that were being surveyed for rail breeding density as part of an ongoing study. Crayfish were caught using food-baited traps. Carapace remains from consumed crayfish were collected as a representative sample of the segment of the population that fell prey. The rail population was surveyed via passive recording of calls using autonomous recording units (Wildlife Acoustics).

Both species preferred areas of natural marsh compared to impoundments. I investigated whether peak numbers of the largest size class of the invasive species, *P. clarkii*, coincided with

the brood rearing period, when King Rails would likely be most nutritionally stressed. The temporal data revealed that King Rail hatching dates peaked when the largest crayfish size classes were most abundant. That King Rails timed their breeding so that hatching coincided with larger sizes of the invasive crayfish suggests that *P. clarkii* may have a positive effect on King Rail population growth at this site. Relative rail abundance based on calling rates among sites was then compared to crayfish abundance at the same sites based on trap data to see if these were correlated. The spatial results of the study showed that when comparing *P. clarkii* abundance and King Rail relative density at ten locations, there was no significant relationship between higher numbers of crayfish and where rails chose to nest.

Dietary constraints may have contributed to the decline of King Rail populations across its range, and its extirpation from marshes where vegetation, water depth and other habitat variables appear suitable. Further research will be needed to reveal what proportion of the diet of Mackay Island King Rails the crayfish represent, and whether the invasive species, *P. clarkii*, is a contributing substantially to their reproductive success.

Acknowledgements

I would sincerely like to extend my thanks to the individuals and organizations that made this study possible: the staff at Mackay Island NWR, the U.S. Fish and Wildlife Services, the McRae Lab, the Department of Biology, and East Carolina University. I would especially like to extend a special thanks to my mentor Dr. Susan B. McRae for her knowledge and guidance, along with Katie Schroeder. Funding was received from an East Carolina University Undergraduate Research and Creativity Award.

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Introduction

Slight changes in the food web of an ecosystem can cause major changes in the energy flow of an ecosystem (Chapin et al. 2000). The introduction of prolific invasive species into new environments can have catastrophic consequences on that ecosystem. Approximately 50,000 non-native species have been introduced to the United States (Pimentel et al. 2004), and the effect that each invasive species has had on the ecosystem is difficult to measure. Studies on individual populations introduced can be very labor and time intensive but are necessary to determine the extent to which a new species affects an existing environment.

There have been instances of invasive species modifying ecosystems with benefits to specific native species. The Florida everglades is home to an invasive species of apple snail, the Island Apple Snail *Pomacea maculata*, that has higher feeding and growth rates than the native species *P. paludosa* and has previously harmed wetlands in Southeast Asia (Morrison & Hay 2011). Because of the higher growth rate and efficiency of the invasive species, it also accelerates the consumption of some native plants in the food web (Morrison & Hay 2011). The decline of the endangered Snail Kite *Rostrhamus sociabilis*, which is a long-time native species of the area, is associated with the decrease of their native primary food source *P. paludosa* due to a decline in wetland area in Florida (Bennetts & Kitchens 1997). The Snail Kite has expanded its diet to prey on this new, larger, species in its environment (Cattau et al. 2018). Recent surveys show the beak size of the Snail Kite has increased over the period of the apple snail's invasion. Cattau et al. (2018) believe that phenotypic plasticity acted as a precursor to rapid evolutionary change in feeding behavior, and they expect the bill size of the raptor to continue to adapt to its most effective range. If so, this invasive species could be helping an endangered species by

replacing its previous primary resource with a more abundant, larger, and rapid reproducing species.

In another example of an invasive prey species introduction, a study of the effects of the Red Swamp Crayfish *Procambarus clarkii* on predatory species was carried out in Spain (Tablado et al. 2010). The crayfish escaped from nearby aquaculture farms Guadalquivir marshes in the early 1970's. The study focused on the effects crayfish had on various species of birds and a few other vertebrates, by isolating predators and non-predators based on a threshold of 10% or more of diet, which was determined by collection and dissection of bird pellets, nest remains or chick regurgitations. The species that preyed on crayfish showed an increase in breeding abundance, and crayfish were found to be a significantly higher percent of their diets. Species that did not prey upon the crayfish showed no real effect during the same period of time. This supports their claim that predator species are benefitting from the alien species. These data also suggested that there was a ten year period in which these predator species had to adjust to the new invader crayfish in order to incorporate them into their diet.

The King Rail *Rallus elegans* is a species of secretive marsh bird whose populations are declining in numbers and in range due to drainage and reduction of wetland habitat in the eastern United States. The decline of the inland migratory populations of rails has been more severe leaving the majority of breeding populations occupying coastal estuaries (Glisson et al. 2015). Secretive by nature and rarely seen, King Rails spend their lives in freshwater marshes where they feed and nest in the tall marsh grasses (Meanley 1969). Their diet consists of crustaceans, insects and other prey found in shallow bodies of water that the birds are able to wade in (Meanley 1969). In describing their habitat requirements, many studies have focused on the vegetation structure and species composition (Pickens 2012, 2013; Kolts 2014). Few have

addressed the dietary needs of this mostly carnivorous bird.

The Red Swamp Crayfish (hereafter *P. clarkii*) is semi-aquatic species of crustacean typically raised commercially for food and bait (Loureiro et al. 2015). They are able to endure travel over long distances over land, which allows them to escape from aquaculture ponds and establish in natural wetlands (Aquiloni et al. 2010). It is a very quick developing species, reaching sexual maturity around three months of age, growing up to 15 cm, relatively large for crayfish species (Dörr et al. 2006; Loureiro et al. 2015). Notably, *P. clarkii* breeds prolifically and is able to do so in a large range of temperatures (Loureiro et al. 2015). The fact that these crayfish are behaviorally aggressive, and also serve as vectors for crayfish plague, has allowed this species to outcompete and drive out native species in the area for habitat and resources (Dick et al. 1955; Loureiro et al. 2015). The female exhibits maternal behavior after laying eggs, protecting the brood by carrying them under her abdomen (Huner and Barr 1991; Huner 1994; Loureiro et al. 2015). These traits make *P. clarkii* well suited to invade a variety of environments outside of their natural range in the southern parts of the United States and Mexico (Hobbs 1988).

Located in the northeastern most corner of coastal North Carolina, Mackay Island National Wildlife Refuge (NWR), hereafter 'the refuge', is comprised of over 3000 h of natural marsh managed by the U.S. Fish and Wildlife Service (US Fish and Wildlife Service 2010). Controlled burns are performed on the refuge in order to maintain the marshes in an early successional state (Rogers et al. 2013; Kolts 2014). The refuge is home to a resident breeding population of the globally Near Threatened King Rail *Rallus elegans* (Rogers et al. 2013).

The invasive *P. clarkii* was intentionally introduced into an impoundment at the refuge in 1992, 25 years before this study. It has since spread throughout the refuge from the isolated

introduction point. Crayfish are a major component of the King Rail's diet (Meanley 1969). Across the North American range of King Rails, crayfish were found in more King Rail stomach contents than any other prey (Meanley 1969). Tablado et al. (2009) found that predators of *P. clarkii* showed a statistically significant increase in abundance of breeding individuals compared to non-predator species.

While the King Rail has declined across most of its range, a relatively robust breeding population can be found at Mackay Island NWR. Conservation efforts have been invested in identifying habitat requirements for the species, but these have focused mainly on the structure and diversity of vegetation communities. Little attention has been paid to food availability of this mainly carnivorous bird with a penchant for crustaceans. I therefore proposed that the high density of breeding King Rails at the refuge could be related to the presence of *P. clarkii*.

Specifically, the purpose of this study was to determine if there is a temporal or spatial correlation between numbers of crayfish and numbers of King Rails across the refuge. First, I predicted that the ontogeny and population dynamics of *P. clarkii* are timed appropriately for breeding King Rails. I sought to determine if peak availability of preferred size classes coincides with King Rail hatch dates, since the brood rearing period is assumed to be the time of greatest nutritional need for King Rails due to the need to feed chicks. My second prediction was that variation in King Rail density should be related to the spatial distribution of crayfish. Finding of a positive relationship could mean that King Rails are using the non-native crayfish as a significant food source during their breeding period when nutritional demands are highest. Dietary constraints may have contributed to the decline of King Rail populations across its range, and this could in part explain its extirpation from marshes where vegetation, water depth and

other habitat variables appear suitable.

A preliminary investigation in 2015 revealed *P. clarkii* to be the only crayfish species on the island. In that year, the spatial distribution of these crayfish was not found to be related to the breeding King Rail distribution (Susan McRae, Brittney Graham, and Amanda Clauser, unpublished data). However, this study suffered from the lack of a reliable and standardized method for assessing King Rail breeding density in locations where nesting data were incomplete. Here, callback surveys were restricted to mornings only. Further, it has been noted that King Rails do not always respond to callback, especially when they are incubating (Kolts 2014). In order to improve upon these shortcomings I implemented, in collaboration with graduate student, Katie Schroeder, a novel means for surveying the rails using autonomous recording systems (Wildlife Acoustics). These recording systems were deployed for forty-eight hours of passive recording, providing a significant improvement over past methods that only used listening periods of six minutes in order to determine breeding density.

Methods

The study took place at Mackay Island NWR (36.5310° N, 75.9521° W), from 19 March to 20 July, 2017, where a study of King Rail reproductive ecology has been underway since 2011. Description of the characteristics of the study site can be found in (Clauser and McRae 2017; Kolts and McRae 2017).

In order to determine the distribution of crayfish, they were trapped using baited minnow funnel traps, previously shown to proficiently trap crayfish. Every two weeks, traps were set at each of the ten locations selected where we conducted rotational deployments of the two autonomous recording units (ARU's). Traps were deployed around mid-day and retrieved around the same time the next day, no sooner than 24 hours. Traps were baited with two different types of food. A tablespoon (~15 mL) of canned cat food (Purina, Friskies) was placed in a perforated tupperware box secured within each trap that provided a broad scent radius in the water. A cup (~200 mL) of dry dog food (Purina or Ol' Roy) was also provided in each trap where the captured crayfish could eat it, and this amount was sufficient to last each 24-hour trap deployment. Environmental data were measured upon collection of traps. These data included water depth (measured with meter stick ± 0.1 cm), water temperature (measured with electronic thermometer ± 0.1 °C), and air temperature (measured by local station ± 1 °C). Data collected from individuals caught included weight (measured with a Pesola ± 0.5 g), sex, and total carapace length (TCL, measured with dial calipers, \pm 1mm) (Figure 1). Later in the season, supplemental data were collected opportunistically from the depredated remains of *P. clarkii* found in and in close proximity to the ten locations used. The TCL of the remains were similarly measured.

King Rail breeding densities were estimated from nest densities and auditory surveys in

the same ten sites during the previous season. ARUs were rotated among sites for 48-hr deployments. The ARU's were set to record continuously from one hour before to three hours after sunrise and two hours before to one hour after sunset. They were set to record ten minutes on, ten minutes off during the remainder of the day. The auditory data were saved as .wav files and later downloaded and visualized as a spectrograph that can be analyzed using new software produced by Wildlife Acoustics.

In order to process hundreds of hours (1 TB) worth of data, Kaleidoscope software (Wildlife Acoustics) was used. Partially automated analysis of audio files was achieved using Kaleidoscope signal detection software. This program is capable of automating the analysis of a large number of .wav files to quickly and accurately select and categorize only sound types specified by the user. Based on operator-entered parameters, a recognizer was developed from training files of known King Rail and non-King Rail calls. The software applies this to new sound files in order to recognize signature wavelengths and intensities in a sound, and clusters them with similar sounds. Clusters are then user-validated. The machine-learning program can prioritize and cluster data by user-controlled parameters. It does this by using a clustering technique that allows it to identify sounds of syllable length and wavelength ranges specified by the user (Schroeder 2018). Validation and quality control was then implemented: each file identified by the program as a King Rail vocalization was confirmed by human ear.

The acoustic data output included the total number of King Rail calls and the number of files (five minute segments of recording) per deployment at each location from which a call rate could be calculated. In order to compare these data with the crayfish location data, relative densities by location needed to be established. Total King Rail calls per files was to be used, but due to an unequal sample size, some locations had more or less files. To normalize the data and

create ratios with a common denominator (number of files) that could be used for comparison between locations, the number of files was set to 1000 and the total call number was adjusted accordingly. The average of each deployment was then used to determine an average call number per 1000 files at each location (Figure 2). The relative density of King Rails was then based on percent of total calls at each site, and this was then compared to the relative crayfish abundance based on percent of total crayfish caught at those sites using a χ^2 comparison. JMP Pro 13 was used for all statistical analyses.

Results

A consistent, repeatable measure for body size in crayfish is the total carapace length (TCL; Figure 1). Use of this measure also allowed me to compare directly the sizes of live-captured crayfish and of crayfish remains. Weight was also measured for each live individual caught, and TCL was plotted against weight and found to be significantly positively correlated $(R^2 = 0.816, P < 0.0001; Figure 2)$.



Figure 1: Total carapace length measurement on a captured crayfish *P. clarkii*. Brackets show length used in study (distance from the tip of the rostrum to the cervical groove).

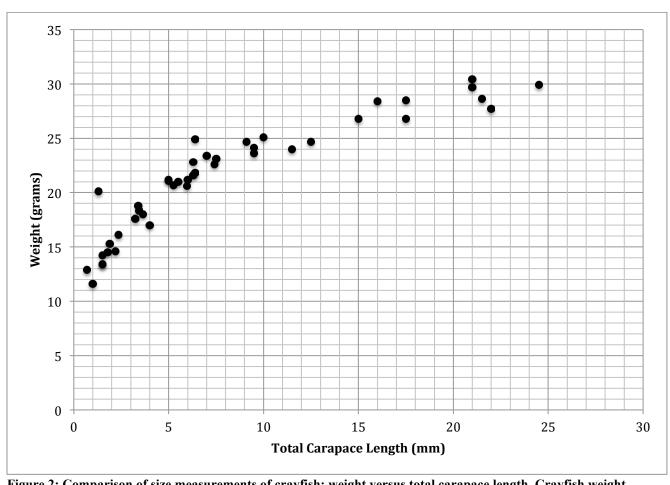


Figure 2: Comparison of size measurements of crayfish: weight versus total carapace length. Crayfish weight was significantly positively correlated with total carapace length (standard least squares fit model $R^2 = 0.816$, P < 0.0001).

To determine if there was a significant change in abundance of crayfish from the beginning to the end of the season, and particularly relative to King Rail mean hatch dates, I compared the numbers of crayfish caught in traps on each date. Date and total crayfish caught per trap were placed into a ZIP model because of the high quantity of zero count data in the trap data set. There was no significant correlation when comparing number of crayfish caught per trap with the date of capture (P = 0.73).

Based on my captured sample, mean crayfish size increased significantly during the course of the summer. A linear regression of total carapace length versus date revealed a significant positive correlation ($R^2 = 0.402$, P < 0.01). The largest individuals (TCL up to 34 mm) only occurred in my samples in the later dates of the King Rail's breeding season.

The sample sizes of trapped crayfish were quite small, and included many zeroes. These numbers declined further later in the season, so the decision was made to supplement the live sample with depredated remains of P. clarkii collected opportunistically. This supplemented the total crayfish sampled, but also provided a comparison between the available size classes of crayfish (live trap sample) and the sample of crayfish eaten by birds. The sizes of crayfish eaten were comparable to the sizes of crayfish caught during the same date ranges (2-week intervals), with statistical analyses showing no significant difference between live or depredated carapace lengths (MANOVA, $F_{3.15} = 0.62$, P = 0.61).

King Rail hatch dates were either objectively recorded (N = 13) or were estimated using the average incubation period of 21 days (Clauser and McRae 2017), for nests that had begun to incubate, but failed before hatching (N = 16). Based on the fact that one egg is laid per day, the mean clutch size (ten) first egg was also taken into account for estimating the hatch dates for the

first egg of each clutch that laid but did not start incubating before failing due to factors such as depredation or flooding. A box plot for the real or estimated hatch dates of 29 nests King Rail nests is overlaid onto the scatter plot of crayfish TCL versus date revealing that the peak in rail hatching coincided with the larger crayfish prey (Figure 3). An independent samples t-test was performed to compare carapace lengths of crayfish before and after the first King Rail hatch date. There was a significant difference in the mean scores for TCL measured before and after the King Rail mean hatch date (Mean_{before} = 18.99, SD = 4.24; Mean_{after} = 26.41, SD = 3.47; t $_{34.7}$ = 1.98, P < 0.0001).

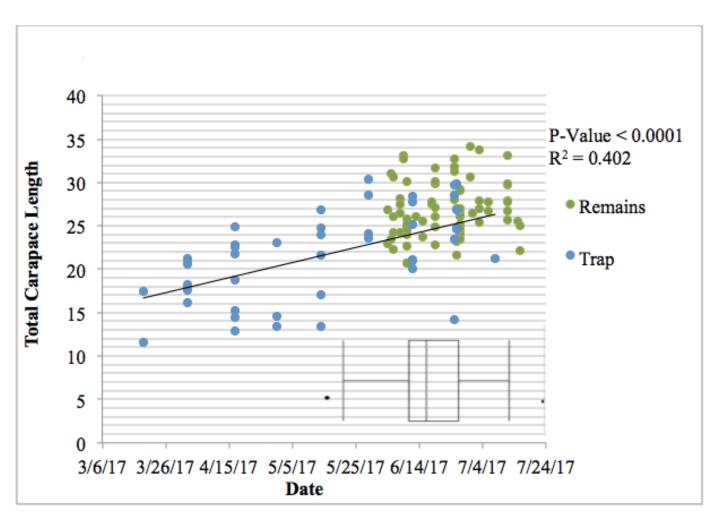


Figure 3: Crayfish size class and King Rail hatch dates in relation to season. Depicts total carapace length of both trapped individuals and collected remains over the King Rail breeding season. The superimposed box plot (showing one standard deviation around the mean, median, 5^{th} and 95^{th} percentile error bars, and outlier points) represents the distribution of real or estimated hatch dates of King Rail clutches (N = 29 nests).

Crayfish densities were measured using trapped individuals from the ten sampling locations. A Zero Inflated Poisson (ZIP) model was used to determine the relationship between the trap data and parameters in each location (Figure 4). The ZIP model was used due to the high numbers of traps that caught no crayfish and exclusion of these count data would not accurately represent the sample. The model attempts to include these zero counts by using binary distribution that give the zeros structure and then runs it through a Poisson model. Two parameters were found to notably affect total crayfish caught by the traps: water depth and whether the trap was set in an impoundment or natural marsh. Originally, other factors such as water and air temperatures were included in the analysis, but these were dropped out due to a lack of significance.

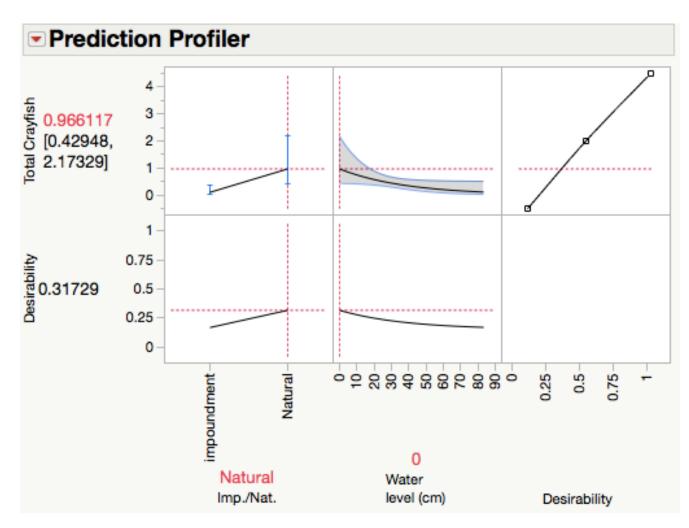


Figure 4: Maximum desirability plot: effect of habitat and water depth on catch rate. Prediction model completed by ZIP analysis comparing water level and habitat (natural vs. impoundment) to total crayfish caught.

| Term | Estimate | Std Error | Wald ChiSquare | Prob > ChiSquare |
|---------------------|----------|-----------|----------------|------------------|
| Intercept | 0.91 | 0.37 | 6.04 | 0.014 |
| Impoundment/Natural | -2.28 | 0.57 | 16.24 | < 0.0001 |
| Water Level | -0.03 | 0.01 | 3.83 | 0.050 |
| Zero Inflation | 0.61 | 0.08 | 55.20 | < 0.0001 |

Table 1: Parameter estimates: water level and impoundment/natural. Results from ZIP analysis of the effect of water depth and habitat on total crayfish caught.

The effect of water level on number of crayfish caught was marginally significant (P < 0.05; Table 1). The ZIP model revealed that as water level approached zero, there was a greater likelihood a crayfish would be caught (Figure 4). As an example, location 5 had an average depth of 31.3 cm and caught 29 crayfish over the course of the season (63.2% of all crayfish caught). Location 1 had an average depth of 44.0 cm and caught 2 crayfish, 4.3% of the total (Figure 4).

Placing the trap in natural marsh or an impoundment had a significant effect on crayfish caught (P < 0.0001; Table 1). The model predicted that traps placed in natural marsh would have a higher catch rate than those in the impoundments (Figure 4). I observed that natural marshes also contained the highest number of crayfish caught in one trap (4 individuals). This occurred two times, both in traps set in natural marsh.

Total crayfish caught through trapping was divided into location in which each individual crayfish was captured, shown as a percentage of the total, and placed in ascending order. When looking at effect of a single location on total capture, there are only four locations (2, 3, 8, and 5) that have significant relationships with total crayfish caught when compared in a ZIP analysis. It is clear to see that location 5 had a positive relationship, catching the majority of the total crayfish captured (63.2% shown in Figure 5) (P < 0.0001). Location 5 is an area of both natural marsh and shallow water depth. Other areas such as 2, 3, and 8 caught no crayfish and were areas of impoundment and deeper water depth. These areas had a negative relationship with total crayfish caught (P < 0.0001).

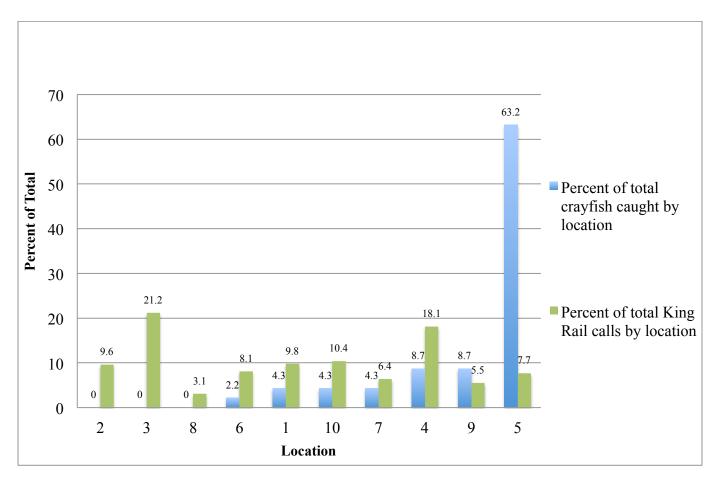


Figure 5: Relative densities of King Rails and crayfish by location. Locations are ordered by ascending crayfish proportional abundance. Crayfish density is shown as a percent of the total crayfish caught by location caught. King Rail density is measured by the percent of total calls by location per 1000 files (Breakdown of process is shown in Table 2). Numbers above bars are actual percent values.

| Location | Deployment | No. of Calls | No. of Files (5 min. sections) | Ratios | Normalized Ratios (per 1000 files) | Average of Location |
|----------|------------|--------------|--------------------------------|---------|------------------------------------|---------------------------|
| 3 | 1 | 78 | 288 | 78/288 | 270.8/1000 | |
| 3 | 2 | 74 | 376 | 74/376 | 196.8/1000 | |
| 3 | 3 | 123 | 368 | 123/368 | 334.2/1000 | ≈258 calls per 1000 files |
| 3 | 4 | 130 | 373 | 130/373 | 348.5/1000 | |
| 3 | 5 | 52 | 369 | 52/369 | 140.9/1000 | |

Table 2: Explanation of King Rail density estimation. Actual data here illustrate how King Rail relative densities were determined by location. Call numbers from each deployment was set to a common denominator of 1000 files and were averaged together to create a call rate at each location.

After determining the distribution among sites of crayfish, I compared these to the distribution of breeding King Rails based on the passive recordings. When percentages of the total crayfish abundance and percent of total King Rail calls per 1000 files were compared by location, there was no significant relationship ($R^2 = 0.019$, P = 0.71; Figure 5).

Discussion

My temporal results showed an increase in crayfish size over the sampling period with the largest size class of individuals appearing at later dates. Crayfish are known to burrow to avoid environmental stressors and females will burrow with their clutches (Huner & Barr 1991). The increase in temperature during these months may have caused crayfish, especially mature females to burrow in order to protect themselves and increase fitness. In this study, however, there were no significant effects of date or of water temperature on crayfish trap rate even with two discoveries towards the end of the season of females with clutches of eggs under their abdomens. A larger sample size may be required to determine if there is a seasonal change in population size due to strategies to avoid environmental stress or increase fitness.

Despite no significant difference in abundance of crayfish caught over the season, there was a significant increase in size. This could be due to multiple factors. Larger crayfish may survive longer due to competitive advantages over smaller individuals. Another possibility is that crayfish mature quickly in the warm climate provided by eastern North Carolina; *P. clarkii* requires approximately three months to reach sexual maturation (Dörr et al 2006), though this can vary according to environmental conditions, warmer temperatures are usually associated with higher feeding rates and therefore quicker growth rates (Croll & Watts 2007, Sommer, 1984). The sampling period for this study was slightly over three months, and represented a significant growth period of *P. clarkii*. This annual cycle is conducive to allowing the King Rail to temporally align its breeding with the emergence of larger size classes. The timing of appearance of larger crayfish coincided with my estimate of the peak hatching dates of King Rail nests. This could be an indication that King Rails are timing their breeding so that hatching occurs during peak crayfish biomass availability. Due to the lack of breeding data on King Rails

in years previous to this ongoing study, we do not know when the peak in hatching occurred prior to the introduction of *P. clarkii*. To determine if there is a significant temporal difference in King Rail breeding periods only at Mackay, timing of breeding should be analyzed from locations in close proximity, similar habitat and climate, but without the introduced species of crayfish. If there is a significant difference in peak hatching, this could be strong evidence supporting timing of breeding of the King Rails at Mackay are being influenced by this invasive species.

Significant factors affecting crayfish capture were habitat and water depth. In a comparison of habitat, more crayfish were caught in areas of natural marsh as compared to impoundments. In a comparison of water depth, crayfish were more likely to be captured in shallower water depths. Shallow water depth is also a characteristic of natural marshes. Crayfish may be more likely to be caught in these areas due to a couple of factors. For example, crayfish may be able to escape more easily from traps in deeper water by swimming out. Looking at water depth, shallow water presents a smaller total volume and things such as scent from the baited traps and less area for larger predators to reside may be influencing factors. Shallower bodies of water heat up faster, and warmer water temperatures are associated with higher feeding rate in crayfish (Croll & Watts 2007). Locations of natural marsh may contain more biodiversity and therefore more foraging opportunities for the crayfish than impoundments. An extensive survey would be needed to determine the difference in biodiversity of both habitats, as well as how biodiversity may impact crayfish movements and foraging.

My results on the spatial distributions showed no significant relationship between crayfish abundance and where King Rails chose to nest. However, a study of radio-tagged King Rails revealed that breeders at this site will move their broods an average of ~300 meters away

from the nest to forage (Kolts 2014; Kolts & McRae 2017). Rails will also spend a significant amount of time foraging in areas away from their nest while still in the incubation phase.

Another possibility is that the King Rails could be exerting high consumer pressure onto crayfish at the locations with less crayfish abundance. Testing this would entail something such as tethering a number of crayfish in each locations and looking at predation rates by the King Rails. If rails are foraging in areas away from their nests often, this may give a more accurate representation of the effect of crayfish abundance in an area by using feeding rates instead of rail density. These factors were not taken account of when hypothesizing the spatial component.

In Spain, a ten-year period of low rate of incorporation of *P. clarkii* into predatory birds' diet was observed (Tablado et al 2010). It is possible that the same invasive species is benefitting this population of rails. The deliberate introduction occurred twenty-five years ago, and the King Rail population size at the refuge is already substantially larger than at other regional sites. A similar study to that of Tablado et al. (2010), comparing growth over time of the King Rail population and of that of other bird species that do not prey on *P. clarkii*, is warranted. The temporal part of this study produced promising results showing the possibility that King Rails at Mackay Island NWR time their breeding so that hatching coincides with larger crayfish biomass, though we lack pre-introduction data on reproductive timing to test this theory. If such an essential part of their fitness as a population is being affected by this invasive species, continued investigation could reveal *P. clarkii* as a major contribution to the success of this population of King Rails at Mackay, just as it was for predatory bird species in Spain (Tablado et al. 2010), and of Snail Kites in Florida (Bennetts & Kitchens 1997).

More research is required to determine the effect the invasive *P. clarkii* is having on this population of King Rails at Mackay Island NWR. This study has shown a correlational

relationship of the temporal component of King Rail breeding and crayfish biomass. While it would be difficult to estimate the population density of the rail using other means than by call rate due to its elusiveness, the food-baited traps caught fewer crayfish than expected. Allowing for more or longer deployments may help to increase these numbers. A larger sample size would enable us to understand better the dynamics of the crayfish population, and to determine whether *P. clarkii* is benefitting King Rails and other predatory bird species at Mackay as was demonstrated in Spain.

Literature cited

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