Population Dynamics of the King Rail, *Rallus elegans*

A Final Report

Susan B. McRae, PhD
Department of Biology &
North Carolina Center for Biodiversity
East Carolina University
Greenville, NC
27858-4353
mcraes@ecu.edu
Tel: (252) 328-6515

Submitted to:

Michael J. Hoff
U.S. Fish and Wildlife Service
Refuge Manager
MacKay Island and Currituck NWR's
P.O. Box 39 (Shipping: 316 Marsh Causeway)
Knott's Island, NC 27950-0039

August 31st, 2012

Photo: S. McRae

Contents

Introduction

This report describes the findings of research conducted by a team of biologists from East Carolina University. The project 'Population Dynamics of the King Rail, *Rallus elegans*', was supported during the period of May 2011 through August 2012, by a CESU Cooperative and Joint Venture Agreement between the USFWS Southeastern Region refuges (MacKay Island NWR, Mike Hoff, Refuge Manager), and East Carolina University (Dr. Susan McRae, Department of Biology). This report summarizes the findings to date from this research.

The North American Conservation Action Plan lists the king rail as a species of high concern based on recent declines in population estimates across its range (Cooper 2008). Recent studies of king rail have focused on the effectiveness of identifying nesting habitat using call back surveys in multiple regions (Pierluissi and King 2008; Darrah and Krementz 2009; Collazo and Drew 2011). However, distribution patterns alone are inadequate to predict population dynamics. Building on the valuable contributions of these studies, we should now focus our efforts on increasing knowledge of the population dynamics and reproductive ecology of the species.

Coastal development in the Carolinas has left few natural wetlands suitable as breeding grounds for rails. The NC Wildlife Action Plan specifically identifies a need for long-term demographic studies of birds to better understand their life histories and their responses to habitat manipulations (NC Wildlife Resources Commission 2008).

Positioned on the border of North Carolina and Virginia, MacKay Island NWR remains one of the only coastal areas in the region managed for overwintering waterfowl that also includes non-impounded natural wetlands with a burn management regime. Since 2009, call back surveys have been used to assess habitat occupancy by king rails during the breeding season, and a small number of nests were monitored intensively on the refuge (Rogers, 2011). While continuing to survey the area for occupancy rates, we have expanded the study of king rail reproductive ecology. We are trying to understand the behavior and population ecology of this species by focal study of marked individuals. We also describe here the development of genetic tools with which to assess regional population structure, and compare king rails on the East coast with populations elsewhere in their range.

Faculty and students at East Carolina University (ECU) are well placed to do research on regional coastal species. We have a record of strength in ecological studies of coastal systems through the Institute for Coastal Studies Program. Faculty allied through the newly formed 'North Carolina Center for Biodiversity', a research center within ECU, are further committed to research on biodiversity at all levels, from genetic diversity through organisms, populations, and communities, throughout North Carolina, but particularly to organisms inhabiting the coastal plain.

Scope of work (as proposed in the agreement)

1. Search for king rails at Mackay Island NWR. Use appropriate Service-approved or appropriate State search techniques. Set up traps and ensure they are checked at appropriate intervals.

2. Monitor all nests for success estimations.

3. Capture, mark, and release rails using approved NWR and animal handling protocols.

4. Provide report to refuge by August 31, 2012, and seek publishing in peer-reviewed journals.

5. Make markers developed during this study available for use to further national population structure identification efforts.

Field Methods

Finding and monitoring king rail nests

Starting as early as mid-March, general areas occupied by king rails were identified via call-back surveys using the *kek-kek-kek* and *grunt* calls, and listening for responses. The locations of positive responses were only used to guide general search areas for nests, as we quickly learned that birds with nests often remained quiet. Therefore, we generally found nests by intensively searching areas of marsh with suitable vegetation types and densities (using both systematic and random approaches). Nest searching in this habitat is extremely laborious and involves long hours of wading under inhospitable conditions. Having a fit and experienced field crew (see section on *Training young professionals*) available to do this most days during the peak of the breeding season greatly enhanced our ability to find nests, and many were found early (during the laying period).

Nests found during the laying period were monitored once daily. Each egg was measured (weight, width, length), and marked individually with a Sharpie on the day it was laid. This enabled us to determine if there were any irregularities in the laying sequence, for example due to conspecific brood parasitism, or to detect if eggs went missing.

Complete king rail clutch 2011 laid out in a standardized photo, MacKay Island NWR (Photo: C. Brackett)

Full clutches were photographed for later analysis of female eggshell pattern distinctiveness. If females exhibit distinctive 'signature' eggs, this could provide a method of recognizing whether certain females continue to breed in the same locations on the refuge in subsequent years. We reduced the frequency of nest visits to every three days during the incubation period, to reduce both disturbance to the parents and risk of attracting predators.

Hatch dates of nests found after clutch completion could be estimated using the technique of floating the eggs (Rush *et al.* 2007). As eggs develop, the air sac increases in volume causing the eggs to float more readily, enabling us to predict the hatch date, and often also the hatching order. Nests were visited on the predicted day of hatching initiation so that chicks could be caught and sampled soon after hatching. Each chick was caught, measured (weight, tarsus length), and a small blood sample was drawn. After they left the nest, it became much harder to catch them. Unfortunately, our attempt to use single adult colored leg bands adjusted with draught excluder (previously used on moorhen chicks – McRae 1995) was unsuccessful. This marking method proved poorly suited to the habitat: the chicks got their leg bands caught between the reeds, and some simply slipped off. We abandoned the method, and have so far been unable to find an alternative humane way of permanently marking day-old chicks. Instead, chicks were marked impermanently with a dab of white paint on the back of the head to help us focus on catching unsampled chicks on repeat visits to the nest during the hatching interval.

Catching adult king rails

The majority of successful catches were accomplished at attended nests during the day. All 17 of the adult king rails caught during the summer were captured while on incubation duty during the last week of the 21-day incubation period. Our capture method works on the premise that late in the nesting cycle, king rail parents sit very tight on the nest (i.e. flush late). We invoked stealth in surrounding the nest with 4 overlapping mistnets no more than 5 m from the nest. These were fully extended downward into the mud. We left open a gap of 6-10 feet through which the researchers slowly walked. A sudden clap of the hands served to gently flush the incubating bird into the mistnets, where it could quickly be caught by hand by pinning it against the net. Out of 22 birds targeted for capture using this method over two breeding seasons, 17 (77%) captures were successful.

A variety of other methods of capture were attempted, but none proved successful during the breeding period. These alternative methods included V-shaped lines of mistnets with callback, and spotlighting with callback at night.

Havahart 2-door Large Easyset® live traps were placed in channels where king rails were observed (checked at intervals of 2 hours). On a dozen occasions, between April 21 and June 16, we used a combination of Havahart traps placed at gaps along zigzag lines of drift nets mounted on poles. The traps were opened shortly after sunrise, and king rail calls were played intermittently from an iPod and speaker system from the vicinity of the traps until they were closed at noon. This method was also attempted twice in the evenings, also unsuccessfully.

Non-breeding season capture attempts occurred between November 12th, 2011 and March 2012. About once monthly, we attempted night captures by spotlighting from an airboat driven by Mike Hoff, refuge manager. These captures were attempted between 6:30-10:00pm, in order to minimize disturbance to residents of Knott's Island with late night boat noise.

Carol Brackett holds a night-captured king rail (Photo: S. McRae

As we traveled through the marsh, we used a three million candlepower spotlight to locate king rails. Once a rail was dazzled with the light, personnel pursued it on foot and the bird was caught by hand or with a dip net. Out of approximately 15 hours of attempted capture over five days, we caught 2 adult king rails using this method. The captures occurred on March 10th and 12th, respectively. Though the sample size was low this first winter, we gained better knowledge of how weather variables and water levels varied seasonally, and how these in turn affect the distribution and accessibility of rails on the refuge. We will use this knowledge to guide our future attempts.

We observed Virginia rails and soras in the marshes during nighttime airboat outings in late winter, and obtained permissions to capture, blood-sample and band a small number of these birds per Federal Bird Banding permit #22047 (Master bander: Michael Hoff), ECU IACUC AUP#253 (PI: Susan McRae). The samples will be used to test our genetic markers for cross-species amplification.

Standard morphometrics (weight, flattened wing chord, tarsus length, tarsus and middle toe length) were obtained for all adult rails captured. In addition, we measured bill (length, width, depth) on all king rail adults. A 50uL blood sample was drawn by brachial venipuncture, and stored in 100% ethanol for genetic analysis. Adult king rails were fitted with a USGS permanent band, and an individually distinctive combination of 3 color bands per Federal Bird Banding permit #23728 (Master bander: Susan McRae), ECU IACUC AUP#253 (PI: Susan McRae).

Morphometric measures will be used to determine whether adults in this population are sufficiently sexually dimorphic in size to be sexed in the field. Discriminant function analysis will be used in conjunction with molecular sex determination based on a diagnostic PCR test (Griffiths *et al.* 2000) that we have modified to sex king rails (unpublished data).

Laboratory methods

Development of new microsatellite markers for king rails

In collaboration with James M. Maley and Dr. Robb T. Brumfield of Louisiana State University (LSU), we have developed a panel of variable microsatellite markers to analyze population genetic structure at local and regional scales. The work at LSU was independently funded by NSF. The sequencing formed part of Maley's PhD thesis research. For this project, we conducted additional analyses to screen his sequence data for microsatellite loci.

Louisiana State University:

Genomic DNA was isolated from pectoral muscle tissue of 10 unrelated king rails (*Rallus elegans*) and 10 unrelated clapper rails (*Rallus longirostris*) collected in southern Louisiana between April and July 2009. Next-generation sequencing was performed as part of James Maley's dissertation research in which he identified Single Nucleotide Polymorphisms to distinguish Louisiana King and Clapper rails along a hybrid zone. Amplified Fragment Length Polymorphisms (AFLPz) cut enzymes and primers were used to enrich the DNA and barcode all individuals for segments adjacent to restriction fragment cut sites. Each sample of genomic DNA was initially digested with *Eco*RI and *Mse*I cutters, then ligated with a primer sequence. Restriction digests were subjected to agarose gel electrophoresis and DNA fragments in the size range of 350 to 450 bp were selected and cut from the gel. These were isolated and used in two rounds of PCR extending the primer by one base the first round, size selecting again, then adding two more bases and size selecting again. These steps removed the majority of the "junk" DNA, by selecting specific identical sequences for all 20 individuals and barcoded each individual to ease downstream analyses.

The AFLPs were then sequenced on a Roche 454 pyrosequencer using standard protocols. For the initial assembly, individuals were separated by barcode and all low-quality sequences were removed. Settings required an overlap of 95% once all of the erroneous single base insertions are removed, those with more than two possible bases and obvious sequencing errors (see McCormack *et al.* 2012). A computational pipeline called PRGmatic was used to clean up the data, align everything, and provide allele calls per individual (Hird *et al.* 2011). A program called MSAT commander (Faircloth 2008) was used to screen the concatenated sequence data for microsatellite repeats. The selection process produced 1419 DNA sequence fragments between 48-477 base pairs long containing di-, tetra- and pentamer repeat microsatellite sequences.

East Carolina University:

We received the 1419 sequences from James Maley as FASTA sequence files. We sorted the sequences, assembled all those containing identical microsatellite motifs, and manually aligned them in Se-Al v. 2.0 (Rambaut 2002). This produced alignments of 4 to 47 sequences (Mean = 15, some with multiple copies of the same alleles, likely multiple copies from the same individuals), and revealed variability in microsatellite repeat numbers indicating up to 12 alleles per locus for both species combined. We then used a consensus of the unique flanking sequences around the microsatellite targets to design primers using the program Primer3 (Rozen & Skaletsky 2000). We did alignments for a total of 20 different, variable microsatellite loci.

Products were successfully amplified from ten of the 20 loci. Though all of the loci for which primers were designed were variable, primer design was challenging in some cases because of limited flanking sequence. In 6 cases, we were only able to design one good primer. Since all of the loci have high allelic variability among king and clapper rails, we are in a position to go back to these and resequence them, should more loci be needed. In 4 other cases, we were unable to find conditions in which the locus would amplify reliably. Results of variability tests using samples from our population are shown in Table 1.

Results

King rail nesting ecology

In the summer of 2011, we initiated a study at MacKay Island NWR on breeding king rails. We developed methods of catching breeding adults, and we use unique combinations of color bands, in addition to the numbered permanent USGS bands to mark them. Sightings of these individually identifiable birds around the refuge by members of the field crew enable us to better understand the home ranges of individuals. During the breeding period, video of nests of color-banded birds allowed us to determine which parent was in attendance. Though few sightings of banded adults were made during the first year of the study, we were able to establish in a couple of cases breeding success of banded adults based on observations of breeders from known nests accompanying their broods (see below).

Funding from the Southeastern (Region 4) Refuge Competition significantly enhanced our ability to find more nests in 2012 than in the previous year (Table 2, Figure 1). This injection of support provided experienced personnel and essential equipment. We were able to hire 3 exceptionally talented young graduate field assistants, each unconstrained by academic calendars and able to start before the peak of nesting in April. Each brought with them experience relevant to the project (see below, *Training young professionals*). The purchase of two kayaks and other field gear such as waders for the entire crew enabled us to cover more thoroughly parts of the great marsh we were unable to access in 2011.

A selection of king rail nests monitored at MacKay Island NWR (photos S. McRae and C. Brackett)

Over the last two years, we collected vital rate data for the king rail on the refuge. Clutch size ranged widely both within and between years. The mean clutch size was 9 in 2011, but decreased to 7 in 2012. This difference between years was significant (two-tailed $t = 3.6$, 39 d.f., $p = 0.0008$). This year, king rails suffered a higher rate of nest predation (Table 2). Circumstantial evidence and video footage suggest nest predation was mostly by raccoons (C. Brackett, unpublished obs.). Smaller average clutch sizes may reflect that the sample included many renests after predation.

The mean daily temperature in 2012 was warmer in March but cooler in April, by comparison with 2011. The mild Spring likely also contributed to earlier nest initiation dates in 2012. Our data suggest that king rails do not nest synchronously (C. Brackett, unpublished), though we have not yet analyzed the data for evidence of local temporal patterns of nest initiation dates.

Brood survival

In 2011, brood survival data were limited to two nests: in one nest, 3 chicks out of 5 hatched were known to have survived to 4 weeks, and in another, at least 2 chicks out of 11 were known to have survived 2 weeks. In 2012, estimates for the average number of chicks surviving to 2 weeks was 3.6 chicks (N=8 nests), to 4 weeks, 2.8 chicks (N=4 nests), and to 6 weeks, 2 chicks (N=5 nests). This assumes that broods were not divided between the parents, since both parents were not always seen during brood observations. Three of these sightings were of broods attended by a color-banded parent. We expect to improve brood monitoring in 2013 by radio-tracking selected parents during the brood rearing period.

Catching and monitoring adults

In 2011, seven breeding adults were captured at five different nests, sampled and banded. In 2012, a total of ten breeders were banded, all at different nests. This includes four that were fitted with radio-transmitters late in the season, three of which are still currently being tracked.

Carol Brackett releasing a banded king rail (Photo S. McRae)

We were able to supplement our inventory of genetic samples of adult king rails from road kills found on the causeway. These supplements provided 13 more summer samples (including both members of two probable breeding pairs), and one additional winter sample.

With the preliminary data we were able to collect based on this grant, and the support from our regional partners, we successfully competed for a USFWS grant from the Webless Migratory Game Bird Program at the national level. This grant will fund another graduate student's fieldwork on king rail, and enable our research to continue at MacKay Island NWR through 2013. We have budgeted for 20 radio-transmitters and receivers in the new grant. Thus, we have begun to make use of the reliable capture methods we have developed, and will be able to track the movements of adult birds around the refuge for up to 6 months at a time. This new dimension to the research will allow us to better understand habitat use, and help us to determine whether breeders overwinter on the refuge.

Genetic results

The grant funded laboratory consumables and equipment, such as a benchtop microcentrifuge and small footprint PCR machine, essential for the development and testing of hypervariable microsatellite markers. These markers will enable us for the first time to conduct population genetic analyses of king rails across the species range, and once published, they will be remain available for future analyses of population structure, dispersal, and admixture in king rails.

Moreover, we have screened additional microsatellite loci characterized from other rail species for variability in king rails. Of 20 markers screened that were developed from black rail (*Laterallus jamaicensis coturniculus*; Girard *et al.* 2010), three were found to be variable: BlRa A116 (7 alleles, 11 individual king rails typed), BlRa B106 (4 alleles, 15 individuals) and BlRa D11 (11 alleles, 14 individuals). Of ten markers developed in Tasmanian native hen (*Tribonix mortieri*) (Buchan 2000), three are variable: *Tm*18 (8 alleles, 13 individuals), *Tm*31B (8 alleles, 11 individuals) and *Tm*38 (5 alleles, 21 individuals). These have been added to our panel of markers bringing the total number of informative loci identified so far to 16.

Tests reveal that a subset of the markers amplify and are variable in both the Virginia rail and soras. Preliminary tests on samples collected from MacKay Island NWR, indicate that at least five loci have variable alleles in Virginia rail (KiRa 7, KiRa 10, KiRa 16, KiRa 17 and BlRaD11), and three in soras (KiRa 7, KiRa 10 and KiRa 16). These are so far based on small sample sizes, comprising six presumed unrelated Virginia rails and two soras. Thus, these genetic markers will be a long-lasting tool for measuring genetic diversity in king rail populations as well as related species.

We will complete the typing of all of the 2011-2012 samples from 33 king rail adults and 68 chicks this Fall. We have additional samples taken from eggs abandoned before hatching, and from shell membranes collected opportunistically from leftover eggshells of both hatched and depredated eggs. In cases where we have a blood sample from only one (or neither) parent but several chick or eggshell samples, we can reconstruct parental genotypes to use in these analyses. This relies on the assumption of genetic monogamy, but we will be able to detect if this assumption is violated. Samples from breeding adults will be used to quantify genetic diversity in the population in comparison to samples from Louisiana. We will determine whether the birds caught in the winter have alleles not found among breeders, which would suggest migration to the island from other breeding locations.

Broader impacts

Training of young professionals

Eight students of ornithology, conservation and ecology have so far received training during the course of this research. **Carol Brackett**, now approaching the writing stages of her **MS in Biology at ECU**, has led the field team throughout the 2011-2012 seasons at MacKay Island NWR. A graduate of University of Massachusetts (Boston), Carol came to ECU with field experience, notably working as a field assistant on breeding soras and Virginia rails in Maine in 2010. She conducted radio-telemetry studies of of whip-poor-wills on Cape Cod and of three-toed sloths in Costa Rica before that. Carol found the majority of the nests, mapped the nests using ArcGIS, developed methods of capturing adult king rails at the nest, helped refine the design of a harness for securing radio-transmitters and initiated the telemetry study of the rails. While at ECU, Carol contributed to the development of microsatellite markers for king rail, and will complete the typing of all of the 2011-2012 samples this Fall. The population genetic analyses will be a major part of her thesis. She has learned molecular ecological techniques while studying at ECU, and plans to apply these new skills in pursuing a PhD in zoology or wildlife ecology.

Our team in the summer of 2011 consisted of three enthusiastic **volunteer** assistants. Each was enrolled in a wildlife or environmental sciences program: **Chelsea Weithman** (Clemson University), **Michelle Badolato** (State University of New York College, Syracuse) and **Nathan Ingbretsen** (University of Maine at Machias, ME). Their participation enabled Carol to increase survey areas, facilitated the erection of mistnets, and greatly increased the coverage of nest-searching in the pilot year of the study. However, as enrolled undergraduates, academic terms meant they were unable to arrive before mid-May, well into the breeding season. Their experience working on the king rail project helped them to secure other field positions. Chelsea has since worked as an assistant on a woodduck study through the University of Alabama, and since graduating, secured a position at the River Beaver Wildlife Management Area where she studies movements of bobwhite quail. Since obtaining her BS, Michelle has been working as a technician for USDA APHIS Wildlife Service. Nathan has since worked as a husbandry assistant for Sylvan Heights Bird Park, in Scotland Neck, NC, and has subsequently worked with a graduate student from University of Illinois on grassland birds inhabiting fragmented habitat in Chicago.

The USFWS refuge competition grant for the 2011-12 year, enabled us to advertise **paid** positions for 3 highly experienced **graduates** for the 2012 breeding season. We selected them from among 64 candidates nationwide that responded to our advertisement on the Texas A&M University conservation job board.

Chelsey Faller (BS in Environmental Conservation, Univ. of New Hampshire 2009) stood out because of her extensive field experiences with birds and mammals all over the country, most notably work as a field assistant with Brad Pickens (PhD, LSU 2012) on king rail habitat use in Louisiana. Chelsey contributed valuable experience with radio-transmitter harness attachment based on her previous experience with radio-telemetry of king rails. While a member of our team, she learned and became very competent at nest-finding, catching and banding chicks, and navigating around the muskrat mounds with which the king rail nests are often associated. She will begin her own MS research in Wildlife Ecology at the University of Florida this Fall studying muskrat ecology.

Elizabeth Lesley (BS in Biological Sciences, Clemson University 2008) came to us after 2 years in a position in Loxahatchee National Wildlife Refuge in Florida. This was her third USFWS position after graduation, and she had already gained relevant experience conducting water quality surveys in wetlands, call surveys of anurans and birds including secretive marsh birds, and handling and transporting shorebirds. She expressed a desire to gain additional experience in population ecology. While working on the king rail project, she honed her skills catching and banding rails, and finding nests. Her specific contributions included technical skills with equipment learned while in the FWS.

She provided valuable continuity in the radiotelemetry data collection at the refuge late in the season when Carol was presenting our research at the North American Ornithological Congress in Vancouver. Elizabeth will be moving this Fall with her fiance to Gatlinsburg, Tennessee, and plans to pursue a MS in environmental biology or wildlife ecology at a nearby university.

Alexandra Mankofsky (BS Environmental Sciences, Richard Stockton College, NJ) arrived with abundant field experience locating shorebird nests (piping plovers, least terns and American oystercatchers on the New Jersey coast), GPS mapping, mist-netting and banding birds, conducting shorebird surveys, identifying bird species by sound for secretive marsh bird surveys as well as conducting vegetation surveys. She was able to start at the beginning of April and monitored crucial early nests when Carol still had teaching obligations at ECU. Alex began applying her newly acquired rail nest-finding skills immediately after leaving us; she began working for a PhD student at the Univ. of Maryland studying clapper rail nesting ecology in New Jersey salt marshes. She intends to enroll in a MS program in wildlife ecology or environmental biology.

Jaan Kolts is a new **MS candidate in Biology at East Carolina University**, enrolled Fall 2012. A graduate of Virginia Tech, Jaan also comes to the project with 8 post-graduate years of ornithological and wetland experience. Jaan brings to the table expertise in quantifying habitat characteristics of coastal marshes, and catching, banding and observing rare and secretive birds, including most recently the Maui parrotbill. Jaan assisted Ellen Robertson (University of Maine, Orono) with her research on Virginia rails and soras in Maine. He gained experience with fire-managed ecosystems while working on red-cockaded woodpeckers at Virginia Tech, and as a fire-CREW member for The Nature Conservancy. We were delighted to welcome Jaan to MacKay Island in July, well in advance of his graduate degree start date, to become acquainted with the king rail system and the refuge. He received training in radiotelemetry of king rails, and helped to process and band the last adults caught this summer. He will take over as field team leader during the coming year.

Engaging the public in our research

We engage the public in our research in several ways. We distribute pamphlets at the headquarters and at information kiosks at the refuge entrance sites and public wildlife viewing areas. The pamphlets provide information about our king rail research, and solicit sightings of color-banded rails from visitors to the refuge. We also present information and pictures of our research through posters displayed in the Department of Biology at ECU, as well as through the North Carolina Center for Biodiversity website. Moreover, we frequently engage refuge visitors in informal conversation about our research, and some island residents and regular visitors report their observations of king rails to us on a routine basis. On 10 March, 2012, we met with members of the MacKay Island NWR Friends group, and participated in their annual causeway clean-up, as a means of introducing them to our personnel and research.

Management implications of this research

Our observations of king rail behavior and ecology on the refuge will benefit the refuge staff's efforts to facilitate its conservation. In particular, nest location and spatial distribution data based on callback monitoring have been shared with colleagues from North Carolina State University to be incorporated into existing distribution models (Collazo and Drew 2011). These models are being used to help guide the Refuge Management Plan (USFWS 2008).

In 2012, unusually mild spring weather was presumed to be the reason for early nesting by some resident bird species. Based on back-dating the initiation dates of the earliest king rail nests in 2012, we estimated the first king rail egg was laid March 15st or possibly earlier. Early nests may have better fledging success on average than later ones since that year predation rates increased over the course of the season (C. Brackett, unpublished data). Further data are still needed, but a trend for early nesting may lead us to recommend modifying the latest date for conducting controlled burns on areas of the refuge where king rails are now known to nest.

Adaptive habitat models produced by Rogers (2011) predict that king rails remain in open marsh areas, and avoid wooded areas whether flooded or not. During our study, king rails have been observed or detected (by calls and/or radiotelemetry) in habitat not previously known to support this species. The movement of rails around the refuge during different seasons will be a major focus of the next year's work. For example, wooded areas of the refuge are not covered under the current management plan. Thus, adjustments may be implemented in accordance with our findings.

Anecdotal discussions with refuge staff have led to innovation in strategies to protect and enhance king rail breeding success. For example, in response to observations of broods of king rail chicks being unable to follow their parents from water channels up onto the steep braced levees built for the refuge roads, Mike Hoff has proposed adding purpose built ramps at sites where king rail broods have been observed.

Conclusions

A recent study (Behrman and Brown 2012) focused on the effect of imminent sea level rise on the persistence of marshes in MacKay Island NWR, with particular attention to breeding king rails, based on nests found in 2008-2009 (Rogers 2011). Sea-Level Affecting Marshes Models (SLAMM) presented in this paper suggest a likely reduction in suitable nesting habitat for king rails under several different plausible scenarios of sea level rise in the next 100 years. However, the authors acknowledge the need for better breeding data, and additional data on foraging areas and movements of king rails around the refuge. During the course of the last year, with funding from the CESU agreement, we have developed methodology to conduct intensive study of king rail breeding success. Thanks to supplemental funding from the USFWS Webless Migratory Game Bird Program, this research will continue through 2013.

We have added to the conservation genetic toolbox, microsatellite markers useful for studying rails. Microsatellites are neutral genomic markers that will be used to genotype individual king rails. Several levels of analysis are possible ranging from the family (parentage, inbreeding), to the local population (structure, recruitment), to regional and species-range scales (assessment of dispersal and migratory status, inter-population comparisons). We have completed preliminary variability testing, and are currently genotyping samples collected at the refuge.

Products

Presentations at scientific meetings:

Brackett, C.L. and S.B. McRae 2011. Reproductive ecology and population genetics of the king rail, *Rallus elegans*, at MacKay Island NWR, NC (poster). Southeastern Population Ecology and Evolutionary Genetics Meeting, Reidsville, NC (hosted by UNC – Chapel Hill), Oct. 21-23.

Brackett, C.L. and S.B. McRae 2012. Reproductive ecology and population genetics of the king rail, *Rallus elegans*, at MacKay Island NWR, NC (poster). Partners in Flight Southeastern Working Group Annual Meeting 'Conserving Birds in a Changing Environment', NC Museum of Natural Sciences, Raleigh, NC, Feb. 7-9.

Brackett, C.L. and S.B. McRae 2012. Reproductive ecology and population genetics of the king rail (oral). East Carolina University Research and Creative Activity Week (hosted by the Office of Research and Graduate Studies), Greeville, NC, Mar. 20-25

Brackett, C.L. and S.B. McRae 2012. Reproductive ecology of the king rail, *Rallus elegans*, with implications for east coast population dynamics (poster). North American Ornithological Congress, Vancouver, BC, Canada (hosted by University of British Columbia), August 14-18.

McRae, S.B. and Brackett, C.L. 2012. Reproductive Ecology of the King rail, *Rallus elegans*, at MacKay Island National Wildlife Refuge, NC. Invited talk for Greenville Bird Club, River Park North, Greenville, NC, May 7.

Planned publication in scholarly journals **(CESU agreement Objective 4.)**

Brackett, C.L., Maley, J. Brumfield, R. and S.B. McRae (in prep) Characterization of microsatellite markers for the king rail *Rallus elegans* derived from new-generation sequence data. (*to be submitted to Conservation Genetic Resources*)

Brackett, C.L. and S.B. McRae (in prep) Behavior and ecology of the king rail in coastal North Carolina

Brackett, C.L. and S.B. McRae (in prep) Genetic structure of an island population of king rail in a hyposaline coastal sound

Popular articles stemming from this research:

Beamon, C. 6-16-12 'Hail the King Rail: rare fowl studied on MacKay Island' The Daily Advance, Elizabeth City, NC

Gray, L. 2012 ECU Press Release: 'Rare birds:ECU research team studies King Rails on Mackay Island' (publicized across the university including the Harriot College of Arts and Sciences and main University website home pages), June 20.

Gray, L. 2012. Rare peeks at king rails. Edge: ECU alumni magazine (Fall edition)

Grant application:

McRae, S.B. 2011. Population dynamics of the king rail on the Atlantic coast: reproductive ecology, population genetics and dispersal. Webless Migratory Game Bird Research Program, US Fish and Wildlife Service (funded, $65K)

King rail with 3 chicks, MacKay Island NWR Photo: S. McRae

Table 1. Variable microsatellite loci characterized for the king rail

Locus	Primer sequence 5'-3'	Motif	Motif Repeats*	Allele size range (bp)	No. of alleles†	*N*
Kira 1a	AAGTGCTGGAGTGTGTCC ACTGTACCTCATCAACACAGAG	CACAT	12-18	209-261	10	14
Kira 5	TGCTGCACTGAGACAACATCT TGATCATGAGTAGAAGGAATAACCA	TC	10-11	284-322	10	20
Kira 6	CCTGCTGGAGGTACAAGGAG ACAACGCAGGAGAAGGTGTT	TC	10-11	227-247	3	16
Kira 7	TACTCGTATGCCAGTGTTG CAGAGATTATGTTCTCAATGACT	TAGA	7-12	165-194	12	22
Kira 8a	GGCTGTGCAGAGAGGAAG GTGACACTGATACAGTGTGCCT	ATGG	8-18	264-334	14	13
Kira 9	TGATCTGGGCAGGCTTCTAC GTCGAATAATGGCAGCAATG	GT	9-23	171-203	9	11
Kira 10	CCAAGTACCATCTGCGAAGC AACCCGAACGAGAGATGTGA	CA	9-16	126-139	7	21
Kira 16	CCAGGTGGAAACTCTGCATT ACAGTTGTGATGTGGCTGGA	TG	10-15	267-297	11	13
Kira17a	TTACCAGCAGCCAACTGTGA AGTAGTGGTATCCTGGTGAGAGG	CA	14-21	234-264	13	22
Kira 20	GAATCCAGCGCATCCTTACC CGGCTGAGCAGCAGCAG	AC	10-16	169-199	7	12

* Number of repeats detected among sequenced Louisiana king and clapper rails
† Number of alleles detected in a sample of *N* king rails from MacKay Island NWR

Table 2. Nest success of king rails at MacKay Island NWR over two breeding seasons. The total nest number includes all nests found that contained king rail eggs. A small proportion of these were discovered after they were no longer active, and so whether or not some of their eggs hatched is not known. These nests are not included in clutch size calculations, nor in estimates of hatching success or predation rates.

Breeding parameter	2011	2012
Total # of nests	25	51
Mean clutch size (complete clutches)	9.1 (*N*=13)	7.0 (*N*=28)
Clutch size range	5 - 11	4 - 10
Estimated first egg date	April 28, 2011	March 15, 2012
Hatching success (percent nests hatched)	37% (*N*=14)	25% (*N*=42)
Predation Rate (percent depredated nests)	53% (*N*=15)	70% (*N*=40)
Percent eggs hatched (hatched nests only)	80% (*N*=6)	87% (*N*=12)

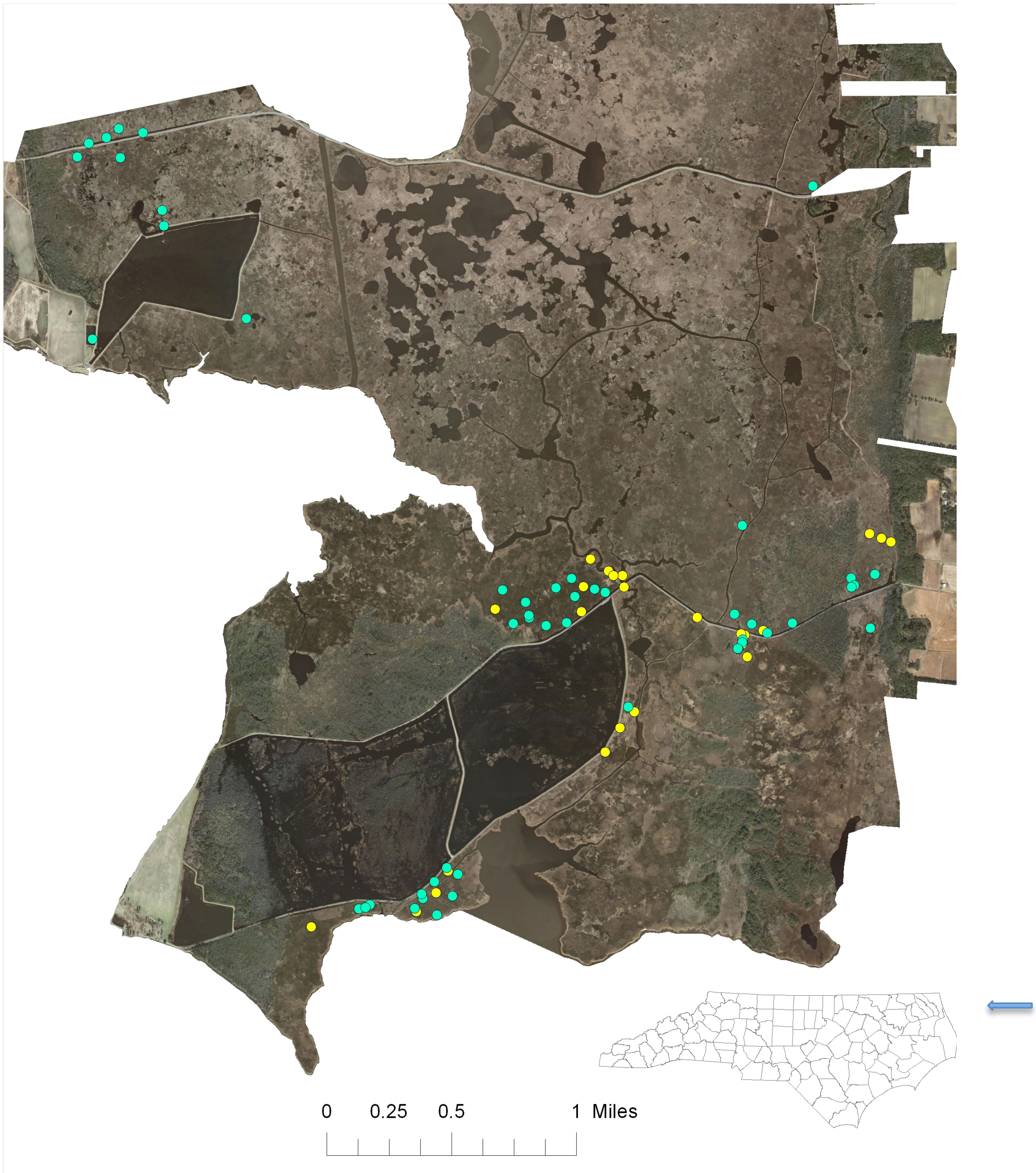
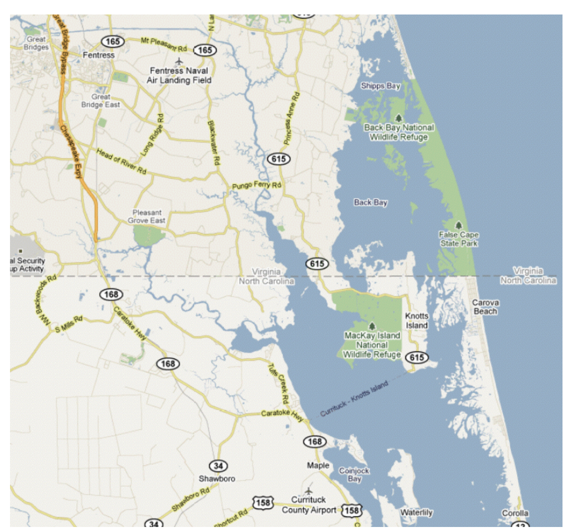


Figure 1. Aerial photograph of MacKay Island NWR indicating locations of monitored king rail nests based on GIS mapping in 2011 (yellow) and 2012 (green). Pale lines are refuge roads. Dark areas indicate open water. A county map for the State of North Carolina is illustrated as a tracing below. The arrow indicates the level of MacKay Island, on the Virginia border. See inset (Google maps) for location of the refuge (green) in relation to Currituck Sound and Back Bay NWR in Virginia.



Literature cited:

Buchan, J.C. 1999. *Behavioral and genetic aspects of mate-sharing in the Tasmanian native hen (Gallinula mortierii) and dusky moorhen (Gallinula tenebrosa).* PhD Thesis. Department of Zoology and Entomology, The University of Queensland, Brisbane St. Lucia.

Cooper, T. R. (Plan Coordinator). 2008. King Rail Conservation Plan, Version 1. U.S. Fish and Wildlife Service, Fort Snelling, Minnesota. 121pp.

Darrah, A.J. and Krementz, D.G. 2009. Distribution and habitat use of king rails in the Illinois and Upper Mississippi River Valleys. *Journal of Wildlife Management* 8: 1380-1386.

Collazo, J.A. and Drew, C.A. 2011. Linking Site Occupancy, Reproductive Success, and Management Outcomes for Strategic Habitat Conservation of King Rail (*Rallus elegans*). WMGBP Final Report http://www.fws.gov/migratorybirds/NewReportsPublications/Research/WMGBMR/WMGBMR.html

Faircloth, B.C. 2008. MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Molecular Ecology Resources 8:92-94. doi:10.1111/j.1471-8286.2007.01884.x.

Girard, P., J.Y. Takekawa, and S.R. Beissinger. 2010. Uncloaking a cryptic, threatened rail with molecular markers: origins, connectivity and demography of a recently-discovered population. *Conservation Genetics* 11:2409-2418.

Griffiths, R., Double, M.C., Orr, K., and Dawson, R.J.G. 1998. A DNA test to sex most birds. *Molecular Ecology* 7: 1071-1075.

Maley, J.M. 2012. *Ecological speciation of king rails (Rallus elegans) and clapper rails (Rallus longirostris).* PhD Thesis, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA.

McRae, S.B. 1995. Temporal variation in responses to intraspecific brood parasitism in the moorhen. *Animal Behaviour* 49: 1073-1088.

North Carolina Wildlife Resources Commission 2008. Protected Wildlife Species of North Carolina.

http://www.ncwildlife.org/Portals/0/Conserving/documents/protected_species.pdf

Pierluissi, S. and King, S.L. 2008. Relative nest density, nest success and site occupancy of king rails in Southwestern Louisiana rice fields. *Waterbirds* 31: 530-540.

Rambaut, A. 2002. Se-Al sequence alignment editor. V2.0a11. University of Oxford, Oxford, UK.

Rogers, S. 2011. King Rail (*Rallus elegans*) *Occupancy, Reproductive Activity and Success in Fire Managed Coastal Marshes of North Carolina and Virginia*. Master's thesis, North Carolina State University, Raleigh, NC

Rozen S. and Skaletsky H.J. 2000. Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S). Humana Press, Totowa, NJ.

Rush, S.A., Cooper, R.J. and Woodrey, M.S. 2007. A nondestructive method of estimating the age of clapper rail eggs. *J. Field Ornith.* 78:407-410.

U.S. Department of Fish and Wildlife. 2008. Mackay Island National Wildlife Refuge Comprehensive Conservation Plan. U.S. Department of Interior.