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Is the LCP Superfund Site an Ecological Trap for Clapper Rails?

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Abstract - *Rallus longirostris* (Clapper Rail) is considered a good indicator species for toxicants because of its strong site fidelity and predictable diet of benthic organisms. High levels of the rare PCB Aroclor 1268 have been found in Clapper Rail adults, chicks, and eggs from the marshes associated with the Linden Chemical Plant (LCP) in Brunswick, GA. Recently, sampling and testing feathers has successfully been used as a non-lethal tool to trace exposure and assimilation of Aroclor 1268 in rails from the LCP site. This approach allows us to infer how and when these birds are exposed to contaminants in the marsh due to predictable molting patterns coupled with the fact that a feather will accumulate the toxicant only at the time it is grown. This study is the start of a process to investigate the possibility that this ecosystem contamination is the foundation of an ecological trap for Clapper Rails at the LCP site. Aroclor 1268 was isolated in Clapper Rail feathers from all birds collected from the contaminated marsh. This finding suggests that these birds either hatched or resided in this system the previous year and thus were not new recruits, which would be expected if fitness was being compromised from the contamination. Based on samples taken during the same fall and winter from the LCP site and control areas, the ratios of hatch-year (HY) birds to adults were similar. However, based on samples taken during the spring and summer, the ratios of HY birds to adults from LCP were lower than reported previously in studies in the southeast. This result suggests that the population age structure may have been affected by the contamination. Future studies specifically focused on reproductive success and fitness are needed at LCP and throughout the Brunswick estuary.

Introduction

Most examples of ecological traps in nature are linked to habitat selection miscues resulting from modern agricultural activities (Best 1986, Bollinger et al. 1990), urbanization (Kolbe and Janzen 2002), and forestry practices (Angelstam 1986, Flaspohler et al. 2001). An ecological trap must (1) somehow increase the habitat's attractiveness, (2) decrease habitat suitability, or (3) do both concurrently (Robertson and Hutto 2006). Ecotoxicological studies have traditionally focused on genetic or other physiological stressor endpoints to quantify fitness, but have not focused on the life-history cues that may endanger the organism. Only recently has the discipline of evolutionary toxicology emerged, acknowledging the need to include these underlying mechanisms to better understand the broader impact to ecosystem health (Bickham and Smolen 1994, Fox 1995).

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This study aims to investigate if an extreme ecosystem contamination event has lead to an ecological trap for a *Rallus longirostris* Boddaert (Clapper Rail) population. Initial responses to large contamination events around the world are designed to address the impacts of toxicant exposure on environmental health, which in turn focuses on acute toxicity. Clean-up plans often consider action levels to minimize chronic toxic effects, but fail to regard the broader impacts of long-term exposure to populations. The Clapper Rail population in this study has been used as an indicator population for acute effects (Cumbee et al. 2008; Novak et al. 2006; Rodriguez-Navarro et al. 2002, 2006). However, the long-term consequences of this perturbation have not been examined. Specifically, the purpose of this study was to determine if Clapper Rails that either bred or hatched from a Superfund site in Brunswick GA were coming back to nest the following season.

The Linden Chemical Plant (LCP), located in the center of the Golden Isles region of coastal Georgia (Fig. 1), was convicted of dumping 150 tons of mercury (Hg) into Purvis Creek, a tributary of the surrounding tidal marshes, between the mid-1980s and its closure in 1994 (Gardner 1978, USEPA 2002). In addition, LCP purchased a polychlorinated biphenyl (PCB) mixture known as Aroclor 1268, from the sole manufacturer, Monsanto Corporation. Only a limited amount was produced (Kannan et al. 1997), and no other chemical company purchased it within the eastern United States, making it a direct fingerprint to the point source of the pollution. This PCB mixture has been found at very high levels in the marshes directly adjacent to LCP, and contamination has spread via tidal action through the entire Brunswick estuary (Kannan et al. 1997, Novak et al. 2006). The LCP facility was declared a Superfund site after it closed in 1994 (USEPA 2002, USFWS 1999). On 17 June 1996, the site was officially placed on the Superfund National Priorities List (NPL) by the US Environmental Protection Agency (USEPA). The USEPA considers the direct contamination threats mitigated by physical clean-up work at small (<10 ha) specific locales with high concentrations. All work summarized below has been performed post-mitigation in areas that were never physically disturbed by clean-up efforts. Studies performed post-mitigation have found that the Aroclor 1268 mixture is still present in the sediment throughout the marshes associated with LCP, has gone through little physical or chemical weathering, and is highly bioavailable (Cumbee et al. 2008; Novak et al. 2006; Rodriguez-Navarro et al. 2002, 2006).

Gaines et al. (1999, 2000) assisted the US Fish and Wildlife Service (USFWS) to document the extent of the post-mitigation damage to wildlife resources, using Clapper Rails as an indicator of ecosystem health. Specifically, this research reported that birds had elevated levels of DNA strand-breakage, egg-shells had been weakened, and chicks had neurological damage and bone-structural problems (Cumbee et al. 2008; Novak et al. 2006; Rodriguez-Navarro et al. 2002, 2006). Although this research helped the USFWS quantify damage to the ecosystem, it left many unanswered questions. One hypothesis was that the LCP marsh had become an ecological trap. Previous studies reported that the LCP marsh has excellent habitat for Clapper Rail courtship and nesting (Cumbee et al. 2008, Gaines et al. 2003). However, the high levels of contaminants may be reducing or possibly eliminating

successful nests (i.e., chick survival to recruitment). Information regarding age structure is important to gather at varying times of year because it can lend insight as to whether the site has become a metapopulation sink (Kristan 2003).

Clapper Rails only molt once per year at the end of the nesting season, and Aroclor 1268 mixture has only been used at the LCP Superfund site. Therefore, presence of the contaminant in feathers of individuals at the site will indicate that they either nested or hatched from that location the previous year. In the southeast, birds should have completed this molt by September (Eddleman and Conway 1998). Hatch year (HY) birds will also have completed their first prebasic molt and acquired their first basic plumage by that time (Eddleman and Conway 1998). Although PCBs are lipophilic, recent studies have shown that feathers can be used as a nondestructive biomonitor for persistent organic pollutants (Dauwe et al. 2005, Summers et al. 2010). Also, Aroclor 1268 concentrations in tissues from birds used in this study for other toxicological investigations (Cumbee et al. 2008; Gaines et al. 2003; Novak et al. 2006; Rodriguez-Navarro et al. 2002, 2006) demonstrated that there is limited movement of the PCB hydrologically and there is no evidence that birds from the nearby reference sites forage in the LCP marsh. Therefore, to explore the broader impacts of the contamination event, our specific objectives were to: (1) determine if birds nesting in the LCP marsh nested or hatched the previous year from this polluted site based on the presence or absence of the PCB mixture Aroclor 1268 in the birds' feathers, and (2) document and contrast the age structure of the Clapper Rail populations from this study to previous reports of populations inhabiting uncontaminated ecosystems.

Field Site Description

This study was conducted in an estuarine marsh system near Brunswick, GA (Glynn County, 31°10'N, 81°30'W; Figs. 1, 2). The study sites included an impacted area situated near Linden Chemical Plant (LCP). Three control sites (Troupe Creek, Frederica Island, and Blythe Island) were used for comparative purposes. The vegetation and habitat structure of these locations were consistent with each other as well as with most other southeastern salt marshes of the United States, comprised mainly of *Spartina* spp. interspersed with small patches of *Juncus* spp. and intersected by tidal creeks (Gaines et al. 2003). The Brunswick estuary has an average tidal amplitude of 1.12 m.

Methods

Ten full size, flighted Clapper Rails were collected using a shotgun during and outside the hunting season from the LCP estuary, and 23 were collected from nearby control sites Frederica and Troupe Creek during the months of November and December 1999 and January 2000 (Fig. 2). Additional Clapper Rails were collected from the LCP area in August/September 2006 ($n = 35$) and in May/June 2007 ($n = 21$). All birds were collected via airboat during high tide when marshes were flooded and birds had no cover to hide in. Additionally, birds were collected throughout these sites to prevent locational bias. Birds were stored at -20 °C until

dissection. During necropsies, sex was determined by gonadal examination for 61 birds from LCP marshes and 20 birds from control sites. Presence of a bursa, plumage, and bill and gape coloration were used to distinguish between adults and full-grown HY birds (Bellamy and Mohammed 1982, Eddleman and Conway 1998). Since Clapper Rails have only a post-nuptial molt in the late fall and fledglings also grow their adult feathers at that time (Meanley 1985), collection months should have no influence on feather PCB load.

PCB extraction methods for Clapper Rail feathers were adapted from Covaci et al. (2002). The specific PCB extractions for the feathers used in this study are described in detail by Summers et al. (2010). In brief, the primary PCB congeners that compose Aroclor 1268 were quantified in primary flight feathers. Aroclor 1268 standards (Supelco, Inc.) were used as spikes to determine procedural recovery rates, as well as to determine analytical accuracy and precision. Using these congeners and their respective ratios allows accurate identification of Aroclor 1268 in biological media. An experiment using chicken feathers incubated in contaminated sediment was performed to determine if PCBs could be washed

Figure 1. Relative position of the Linden Chemical Plant (LCP Superfund Site) located near Brunswick, GA in the southeastern coast of the United States.

off the outside of the feather completely (Summers et al. 2010). No PCBs were detected in these chicken feathers regardless of washing methods.

Gas chromatography (GC) analyses were performed on an Agilent (Atlanta, GA) 6890 gas chromatograph equipped with a DB-5 capillary column (30 m $\times$ 0.25-mm I.D., 0.25-mm film thickness; J & W Scientific, Folsom, CA), electronic pressure control (EPC), and an electron capture detector (ECD). Samples were introduced via auto-injection at 250 °C, splitless mode. Chromatographic conditions were: 54 °C, held isothermal for one minute, 54–160 °C at 20 °C min⁻¹, isothermal for 5 minutes, then 160–270 °C at 3 °C min⁻¹, held isothermal for 3 minutes, with constant pressure at 11 psi. Samples were quantified using a six-point calibration curve derived from dilutions of certified congener (Ultra Scientific) and Aroclor 1268 (Sigma-Aldrich) standards. All congeners were identified based on retention time and elution order relative to the standards. Previous analyses performed on GC-MS confirmed GC-ECD identification (Cumbee et al. 2008). Six major congeners were selected from the Aroclor mixtures. All selected congener peaks were at least 25% of the highest Aroclor component. Fifteen percent of the feather samples were replicated for quality assurance purposes including chicken feathers spiked with a certified Supelco Aroclor 1268 standard (50 ng.g⁻¹) to determine recovery rates.

A *t*-test (SAS ver. 9) was used to test the hypotheses that Aroclor 1268 concentrations found in Clapper Rail feathers did not differ between sexes or between

Figure 2. The Linden Chemical Plant (LCP) study site and the three control sites (Troupe Creek, Frederica, and Blythe Island) that were used to collect Clapper Rail samples, located near Brunswick, GA.

age classes. Previous studies indicated that collection year had no effect on Aroclor 1268 concentrations (Cumbee et al. 2008; Gaines et al. 2003; Novak et al. 2006; Rodriguez-Navarro et al. 2002, 2006). Therefore, data were combined for these hypotheses tests. A Fisher's exact test (SAS ver. 9) was used to compare sex ratio between seasons (November 1999–Jan. 2000 to August 2006 as well as to May 2007) for birds collected from the LCP site.

Results

Of the 61 LCP birds that could be examined for the presence of a bursa and coloration (some birds were too damaged), 23 (38%) were identified as HY, while 38 (62%) individuals were categorized as adults. Of the birds classified as adults, 20 were female (56%), while 16 were male (44%) (Table 1). Of those classified as HY, 6 were female (19%) and 15 male (81%) (Table 1).

All feathers collected from birds at the LCP site had measureable levels of Aroclor 1268, and the congener profiles were the same as other media quantified from the site as well as the Aroclor 1268 standard (Fig. 3). Feathers collected from birds inhabiting the control sites had no Aroclor 1268 above the detection limits of the analysis. The Aroclor 1268 toxicant load in feathers from male and female rails from the LCP marsh site did not differ significantly ($df = 60$,

Table 1. Ratios of hatch-year (HY) to adult Clapper Rails collected from marshes contaminated by the LCP chemical plant and nearby control sites in Brunswick, GA compared to other published works.

			Female	Male	Unknown	Total	Ratio
This study							
November 1999–January 2000 (Control)							
	HY		10	6		17	5.67
	Adult		0	3		3	
	Total					20	
November 1999–January 2000 (LCP)							
	HY		1	7		8	4.00
	Adult		0	2		2	
	Total					10	
August 2006 (LCP)							
	HY		4	6	2	12	0.55
	Adult		14	6	2	22	
	Total					34	
May 2007 (LCP)							
	HY		1	2		3	0.21
	Adult		6	8		14	
	Total					17	
Other studies							
Season	# Individuals	HYs	Adults	Ratio	Study		
Summer	212	176	36	4.9:1	Adams and Quay 1958		
Fall	263	139	24	5.8:1	Adams and Quay 1958		
Fall–Winter	42	35	7	5.0:1	Adams and Quay 1958		
Fall	80	60	20	3.0:1	Meanley 1985		

t [two-tailed] = 2.00, P = 0.43). Similarly, the 95% confidence limits of toxicant feather loads overlapped based on age (df = 59, t [two-tailed] = 2.00, P = 0.09). Feather toxicant load ranged from 119–4828 ng.g⁻¹ for adults and 89–3858 ng.g⁻¹ for HYs. Female feather toxicant load ranged from 130–4828 ng.g⁻¹, while males ranged from 2.7–3858 ng.g⁻¹.

The ratio of HY birds to adults in Clapper Rails collected from marshes contaminated by the LCP chemical plant ranged from 0.21–4.0:1, while ratios from the control site as well as other studies ranged from 3.0–5.8:1 (Table 1). Though it was not possible to compare statistically among sites, comparison of the age ratios of samples taken at the LCP site revealed significant differences among sampling intervals (November 1999–January 2000 versus August 2006, Fisher's exact P = 0.02; November 1999–January 2000 versus May 2007, Fisher's exact P = 0.002).

Discussion

If Clapper Rail fitness has been compromised from events linked to anthropogenic activities associated with LCP, it is crucial to consider the components that lead to that diminishment. Previous studies reported that birds from the control sites had no measureable levels of Aroclor 1268 (Cumbee et al. 2008, Novak et al. 2006). In this study, all HYs and adults from LCP had measurable levels of Aroclor 1268 in their feathers, which indicates that those individuals hatched from LCP the previous year or, less likely, moved into LCP in July/August just prior to growing their plumage. This conclusion is supported by the fact that there

Figure 3. Percent composition of Aroclor 1268 (6 major congeners–Ballschmitter-Zell (BZ) numbering system) in feather samples collected from the LCP superfund site in Brunswick, GA. Samples were quantified using gas chromatography. Percent compositional breakdown for Aroclor 1268 (standard) is shown as a reference and shows little to no chemical weathering (e.g., chemical breakdown).

were no differences in feather PCB load between sexes or between age classes. Differences would not be expected if these birds spent equal amounts of time in the marsh so that PCB blood levels would be in equilibrium at the time the feather was grown. In contrast, if HYs had no Aroclor 1268 in their feathers, then it could be assumed that they hatched elsewhere. Similarly, if an adult had no Aroclor 1268 in its feathers, then it could be assumed to be a new recruit that had not spent significant time in the LCP marsh the previous year. If this were the case, the data would suggest an ecological trap based on ecotoxicological pressure, as animals would not survive to breed the following year. The data do not support this conclusion. Although numerous ecotoxicological endpoints have demonstrated that Clapper Rails from LCP have experienced biological damage (e.g., DNA strand-breakage, eggshell integrity, hatching success, chick toxification; Cumbee et al. 2008; Novak et al. 2006; Rodriguez-Navarro et al. 2002, 2006), at this time, the ecotoxicological pressure does not seem sufficient to prevent their breeding. However, more data are needed to determine hatching success in the field, and this will be the focus of future work planned for this population.

The ratio of HY to adults from the nearby control sites were consistent with other studies. Moreover, age ratios, at least in fall populations, have been shown to be variable. For example, Sharp (1976) reported HY to adult ratios of 2 in Louisiana. Age ratios of Clapper Rails in North Carolina ranged between 4.9–5.8:1 HYs to adults with varying seasons and sample sizes (Table 1; Adams and Quay 1958). Reports from the summer were likely to include younger pre-flight birds, whereas the fall–winter ratios are comparable to the HY birds collected in the LCP study. Our study showed variable age ratios of 0.21 in May to 4.0 in the winter at LCP. Although sex ratios are not necessarily expected to stay the same throughout the year, one explanation for such a low ratio could be that hatchability and brood survival may be diminished at LCP. However, more data are needed to explore this possibility. It is not surprising to find a low proportion of HY birds in May, as many birds may have lost their bursa and developed more adult coloration. However, in Brunswick, GA, one would expect to have the opportunity to collect many HY birds since nesting starts as early as February. No studies have reported sex ratios of Clapper Rails in Brunswick, so data from this study provide insight into populations from this region that are affected by anthropogenic activities. Previous studies from LCP (Gaines and Stephens 1999; Gaines et al. 2000, 2003; Novak et al. 2006) indicated that Clapper Rail chicks often were too weak to stand after hatching as compared to birds from control sites, most likely from mercury toxification. Mercury is a problem not only throughout the Brunswick region from industrial processes, but throughout the Clapper Rail's range. Both mercury and Aroclor 1268 have long ecological half-lives. Higher chlorinated PCBs like Aroclor 1268 tend to be more persistent in sediments, with half-lives up to 38 years (Sinkkonen and Paasivirta 2000). Similarly, elemental mercury will not break down over time, and its bioavailability is mostly based on the methylation rate within the system, which tends to be steady in estuarine environments. Sediments measured for mercury that were collected from LCP during the time of this study (Cumbee et al. 2008) were similar to those reported in the late 1970s (Gardner et al. 1978).

At this point, there is insufficient evidence to suggest that the Clapper Rail population at LCP is being drawn into an ecological trap. Specifically, more information regarding the HY to adult ratio and chick survivability are required to understand better the pressures that may be influencing fitness. To our knowledge, this is the first study that has used the techniques of extracting a unique PCB from bird feathers to explore an evolutionary ecotoxicological problem. Further, data and techniques from this study can lend insight into the conservation issues associated with endangered Clapper Rail populations. This study is relevant also to the San Francisco Bay area, which has similar toxicological issues. The key element that differs between these locales is habitat conservation. The Georgia coast has been left relatively untouched, which gives these birds the chance to respond or adapt, even in the presence of anthropogenic selective pressures.

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