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Clapper rails as indicators of mercury and PCB bioavailability in a Georgia saltmarsh system

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Abstract Clapper rails (*Rallus longirostris*) were used as an indicator species of estuarine marsh habitat quality because of their strong site fidelity and predictable diet consisting of mostly benthic organisms. Mercury (Hg) and the polychlorinated biphenyl (PCB) Aroclor 1268 concentrations were determined for sediments, crabs, as well as clapper rail adults and chicks collected from salt marshes associated with the LCP Superfund site in Brunswick, Georgia. Home ranges were established for adult rails, and sediment and crab samples were taken from each individual's range. The study was designed to minimize the spatial variability associated with trophic transfer studies by choosing an endpoint species with a potentially small home range and specifically sampling its foraging range. The mean home range for clapper rails was 1.2 ha with a median of 0.28 ha. Concentrations of Hg and Aroclor 1268 were shown to increase with each trophic level. Transfer factors between media followed the same pattern for both contaminants with the highest between fiddler crabs and clapper rail liver. Hg and PCB transfer factors were similar between sediment to fiddler crab and fiddler crab to muscle, however the PCB transfer factor from fiddler crabs to liver was over twice as large as for Hg. PCB congener profiles did not significantly differ between media types.

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Introduction

Salt marsh habitats along the Atlantic and Pacific coasts are valuable natural resources both biologically and economically. However, the tidal dynamics and geochemical properties of these systems can spread contamination from a local level (hectares), to the landscape level (square kilometers). To approach the ecotoxicological impact contaminant mixtures have at multiple scales, the proper species must be utilized to identify deleterious effects. Since it is not practical to monitor every potential response a species may have to environmental impacts, investigators must also choose appropriate endpoints. The clapper rail (*Rallus longirostris*) is a secretive marsh bird found throughout coastal salt marshes from the Gulf of Mexico to Rhode Island (where population numbers are high and are hunted) to and along the California's Pacific coastline (where populations are endangered). Throughout their range, this species is an integral part of the salt marsh ecosystem in which they reside. The rails' strong fidelity to their breeding grounds (Zembal et al. 1989) and predictable diet feeding relatively high on the food chain (Eddleman and Conway 1998), makes it an ideal organism to study the movement and fate of contaminants in disturbed ecosystems. Therefore, using rails as an indicator species addresses both ecosystem and human health risks over the long-term.

Over the past 70 years an oil refinery, paint manufacturing company, power plant, and chlor-alkali plant have all operated at the Linden Chemical Plant (LCP) Superfund site in Brunswick Georgia, USA. The facility was declared a Superfund site when it closed in 1994 (EPA 2002). Over this

time frame, Aroclor 1268 was purchased from the sole manufacturer, Monsanto Corporation, which produced only a limited amount (Kannan et al. 1997). LCP was the only user of this congener mixture in the southeast (Maruya and Lee 1998). This PCB has been found at very high levels in the marshes directly adjacent to LCP (EPA 2002; Kannan et al. 1997), thus making it a direct fingerprint to the point source of the pollution. Further, previous studies have shown that LCP marsh has excellent habitat for clapper rail courtship and nesting (Gaines et al. 2003), thus luring birds in to nest. However, the high levels of contaminants may be reducing or possibly eliminating the number of successful nests (i.e. chick survival to the next generation to breed). This would imply that these types of contamination events may be constructing ecological and/or evolutionary traps at the population level. Therefore quantifying the level of contamination in these birds and their immediate habitat, as well as their food source is essential. The purpose of this study is to use clapper rails as an indicator species to characterize trophic transfer of Hg and the PCB Aroclor 1268, using spatially explicit sampling methods in the disturbed estuary located adjacent to LCP. Laboratory studies on fish (*Fundulus heteroclitus*) from the estuary have suggested that both contaminants have direct and transgenerational effects (Baker Matta et al. 2001). Numerous studies have described the distribution and characterization of both Hg and Aroclor 1268 in sediments and soil from this system, as well as report contaminant concentrations from biota opportunistically collected from the estuary (Kannan et al. 1997, 1998a, 1999; Maruya and Lee 1998). However, no study to date has characterized the contaminant burden of a population residing at known spatial locations within LCP and the contaminant loads of their offspring. Information gleaned from this study by using animals with such limited home ranges during nesting will build a better understanding of the transfer rate of these contaminants at the local level.

Specifically, the main objective of this study was to quantify the trophic transfer of contaminants from the sediment into preferred prey items (fiddler crabs, *Uca* spp.) to the clapper rail by determining: (a) home ranges of individual clapper rails and (b) the contaminant contents of the sediment, fiddler crabs, clapper rail adults and chicks within this species home range. Based on this objective, we wanted to determine if contaminant loads from nearby reference locations were significantly distinguishable from LCP to further ensure the clapper rail as an appropriate indicator species for site specific contamination events. Since clapper rails feed in both tidal creek and tidal pool habitats within their home range and detritivorous fiddler crabs are their preferred food items, we tested the following hypotheses to determine if contaminant distributions differed between these two microhabitats: (1) there is no difference between tidal creek and tidal pool contaminant loads; (2) there is no difference between fiddler crab contaminant load from those inhabiting tidal creek versus tidal pool habitats; (3) there is no difference between carbon content of tidal creek sediment and tidal pool sediment. Moreover, since this study was designed to control for some of the spatial variation associated with field contaminant studies, we tested to see if there were correlations between contaminant loads and the different media samples within a clapper rail's home range. Concurrently, we tested the hypothesis that the major congener profiles (relative percent) did not differ between media types. Finally, we tested to see if there were differences in contaminant loads (muscle and liver) between male and female clapper rails since depuration to eggs/chicks is a well known elimination pathway for both Hg and PCB's in birds (Bargar et al. 2001; Lewis and Furness 1993).

Materials and methods

Study sites

The LCP Superfund Site is located on the Turtle River in Brunswick, Georgia (31.1 N, 81.5 W; Fig. 1). Most of the 223-ha site consists of 195-ha of tidal marsh that is considered navigable waters and therefore is owned by the federal government. Purvis Creek, a tidal tributary of the Turtle River, flows through the LCP marsh. An east-west causeway, running from Purvis Creek to the edge of the marsh, divides it into northern and southern sections (Fig. 2). Primarily, Hg and Aroclor 1268 contaminate the marsh sediments, groundwater, and biota although other metals (especially Pb and Cr) have been found in elevated levels. Most of this contamination was the result of the

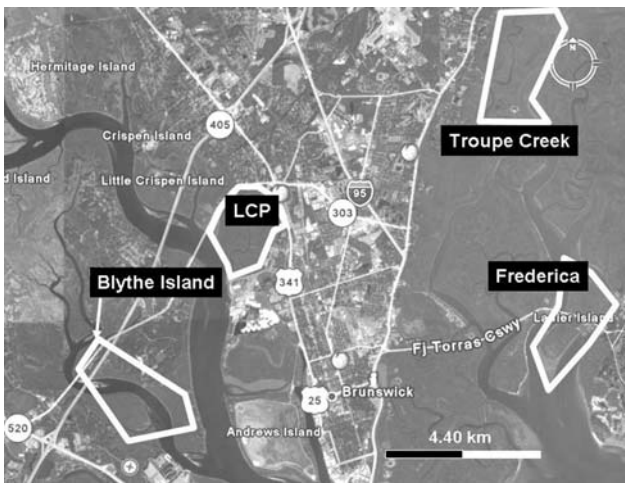

Fig. 1 Aerial photo of Glynn county in southeastern Georgia. This figure shows the relationship of the LCP study site to the city of Brunswick and all control sites used in this study. All control sites were similar to LCP in habitat type and structure

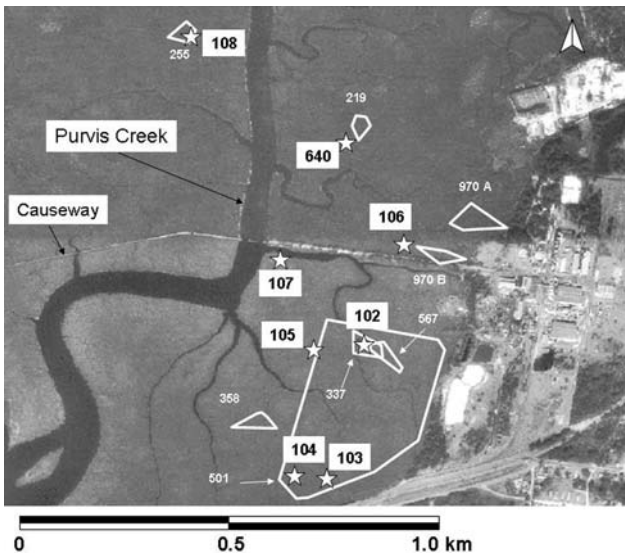

Fig. 2 Known and estimated home ranges for clapper rails ($n = 15$) collected during the 2002 breeding season adjacent to Purvis Creek at the LCP study site. Outlined ranges with white labels represent clapper rails ($n = 7$) that had sufficient telemetry data ($n > 15$ locations) to establish their core home range using the Minimum Convex Polygon (MCP) method. Stars with black labels represent clapper rails ($n = 8$) taken from known active nesting locations. These home ranges were estimated by calculating the median (0.28 ha) of the known home ranges ($n = 16$) and applying a buffer of that area around the nest

chemical manufacturing process undertaken by Allied Signal and LCP between 1955 and 1979 (Odom 1975). The EPA estimates that more than 173,000 kilograms of Hg were released into the estuary during this period. Hg has been detected in fish from a 1-mile portion of the Turtle River and in all of Purvis Creek at concentrations exceeding the EPA's limit of 0.5 ppm, requiring a ban on commercial fishing as well as a seafood consumption advisory in these areas (EPA 2002).

Three separate marsh areas around the Brunswick vicinity were chosen for use as reference sites (Fig. 1). Troupe Creek, Frederica Island and Blythe Island were all similar to the LCP estuary in habitat type and structure. We specifically chose reference sites within and near Brunswick to determine if clapper rails have the appropriate foraging range and feeding habits to distinguish LCP from nearby populations. These marshes consisted primarily of smooth cordgrass (*Spartina alterniflora*), hereafter spartina, interspersed with patches of needle rush (*Juncus roemerianus*) and were inundated with tidal brackish water twice a day.

Capture of rails

Clapper rails ($n = 52$) were captured from the LCP marsh between January 29, 2002 and March 27, 2002 (after the fall—winter hunting season) by hand or with a dip net from an airboat during the highest tides of the month. A subset of rails ($n = 25$) were fitted with 7.9-g backpack radio transmitters (Holohil Systems Ltd., Ontario, Canada; model RI-2CM). These transmitters did not exceed approximately 3–5% of the rails body weight. Transmitters were attached to the rails in a backpack fashion by tying an elastic string over the hips and looping it around the legs. This allowed the bird to fly normally while keeping the transmitter in place for the duration of the study. All birds were released within one hour and 500 m of the point of capture. All clapper rails captured in this study were handled according to the guidelines set forth by the State of Georgia scientific collecting permit (29-9WMB-03-96). All rails were handled according to the guidelines set forth by the University of Georgia IACUC (#A2003-10017-0).

Home range

Clapper rails ($n = 15$) fitted with backpack transmitters were located 213 times February to June 2002. In order to provide useful home range analyses, a telemetered rail had to have at least 15 locations evenly distributed among all hours of the day and tide levels. Furthermore, the bird had to be recaptured after radio monitoring, so that contaminant concentrations in its tissues could be compared to those of sediment and crab samples taken from within its home range. Birds were located both day and night, and during high and low tides. Clapper rail locations were recorded using a handheld Global Positioning System (Garmin Corporation, Olathe, Kansas, USA). To minimize spatial and temporal autocorrelation of animal locations (Swihart and Slade 1985), only one location was taken per bird per high or low tide and a minimum of 12 h was allowed between consecutive locations for any given rail. This allowed a tidal event to occur which influences the birds movement, thus assuring locational independence of consecutive points. Each individual was located at least twice a week until transmitter failure or it was recaptured and sacrificed once sufficient data had been collected ($n \geq 15$ locations). Home ranges were estimated using the Minimum Convex Polygon Method (MCP) which represents 100% of the animals observed home range (Kie et al. 1996).

Individuals that met the home range criteria to be used in contaminant analyses were recaptured and sacrificed. Adult birds ($n = 20$) were also collected throughout the control sites for comparison. The rail was then dissected to determine sex and collect tissue samples. The whole liver (3–6 g fresh weight) was taken and divided equally for metal and PCB analyses. Muscle samples (5–10 g fresh weight) were excised from the right breast muscle using a surgical scalpel. Each sample was placed into a 30 ml vial, labeled and kept in an upright commercial freezer (−10–0°C) for later analysis of PCBs and metals.

Collection of sediment and prey items

Sediment and fiddler crabs were collected from within each of the radio telemetry determined or estimated home ranges. Within each home range, feeding areas were divided into two categories: tidal creeks and pools. Tidal creek areas were those areas where a small steep-banked tidal drain passed through the home range. Tidal pools were those areas that commonly flooded at high tide, but consisted of an exposed mud flat with short (<20 cm) exposed vegetation at low tide (Gaines et al. 2003). Twenty fiddler crabs (ten creek; ten pool) and ten sediment samples (five creek; five pool) were collected from each type of feeding area within a home range. Sediment samples were collected by hand (± 500 g fresh weight) from the top ten centimeters of sediment and mixed together (by hand) in one bag for each location. The same procedure was repeated for control samples near nests from where clapper rail eggs were collected. Percent carbon for sediment was determined using a Perkin Elmer 2400 analyzer by the Chemical Analysis Laboratory at the University of Georgia, Athens GA. Fiddler crabs were collected by hand from the surface of the exposed tidal pools and creeks at low tide. All ten crabs from each type of feeding area were mixed and ground together to comprise one composite sample from each type of feeding area from every home range.

Collection of eggs and chicks

During the breeding season of 2000, nest searches were conducted at LCP and Blythe Island to collect eggs for eggshell integrity and chick studies (Rodriguez-Navarro et al. 2002, 2006). The eggs collected directly correspond to the home ranges used in this 2002 study, as this study was designed to spatially match/overlap the locations where eggs were collected from the 2000 study. If a nest was found with 4 (minimum clutch size) or more eggs, the eggs were removed from the nest and brought back to the Savannah River Ecology Laboratory (SREL) within 4 h. The eggs were incubated (99°F and 87°F temperature set for the relative humidity) and rotated automatically every 12 h. After a chick successfully hatched and remained out of its shell for at least 12 h, it was weighed (g), euthanized, and placed in a freezer for trace metal and PCB analyses.

Metal analysis

Liver, muscle, whole chick, crab and sediment samples were freeze-dried prior to microwave digestion. Wet weights (g) were taken to account for percent moisture for each sample before it was placed in a LABCONCO freeze dry system for approximately 7 days and reweighed upon removal from the freeze-drier. After the samples were dried they were homogenized using a SPEX CertiPrep© 6750 Freezer/Mill (all were calculated on a dry weight basis). For metal analysis approximately 100 mg of homogenized, freeze-dried, sample was weighed and digested separately, using 2.5 ml of trace metal grade HNO_3 (Fisher Scientific), and placed into a Teflon microwave digestion vessel. The vessel was then capped and digested in a CEM MDS-2000 microwave using a variable-powered program with increasing microwave power applied over a 45-min program. After cooling, the vessels were uncapped and 1 ml of certified ACS 30% H_2O_2 (Fisher Scientific) was added. The vessels were then recapped and placed into the microwave for a repeat of the previous procedure. Once the samples were cooled, each digestion was brought up to a final volume of 25 ml with double deionized water using volumetric flasks. Within each digestion set, there was a duplicate set of samples, a blank sample, and a certified reference material standard (SRM: Dorm-2, Dolt-2, Tort-2, or Mess-2; National Research Council Canada, Ottawa, Canada). Total mercury (THg) analyses were performed using a Perkin-Elmer Sciex Elan DRC Plus inductively coupled plasma mass spectrometer (ICP-MS, Norwalk, CT) in standard operating mode following the methodology outlined in EPA method 6020. Quality control procedures were based on EPA method SW-846. Calibration standards covering a range of 1–500 µg/l were prepared daily by serial dilution of NIST traceable primary standards. Certified reference material recovery ranged from 86% to 136% with an average of 109% ($n = 11$). Mean difference between digestion replicates were 3% ($n = 14$). Mean difference between dilution replicates were 2% ($n = 8$). Mean analytical spike recovery at approximately 2-times the un-spiked sample concentration ranged from 82% to 97% ($n = 3$). Mean THg concentrations within analytical blanks were 0.194 µg/kg ($n = 8$). Mean method detection limit (MDL) was calculated as 0.544 µg/g on a dry weight basis.

PCB analysis

PCB's were extracted from the tissue using ultrasonic extraction (EPA Method 3550B). Tissues were freeze dried and macerated prior to extraction. For chicks, the whole chick was ground since they were too small to dissect individual tissues. Dibromooctofluorobiphenyl and tetrachlorometazylene were added as internal surrogate standards. The extractions were performed by sonicating the tissues in 150 ml of acetone: hexane (1:1 v/v) using a Tekmar Sonic Disruptor operated at 100% power in the pulsed mode with a 50% duty cycle for 3 min. The mixture was filtered and the extraction repeated twice with fresh solvent. The combined solvent extracts were dried with Na_2SO_4, solvent exchanged, and concentrated. Lipids were removed by treatment with 1:1 sulfuric acid solution and the solution back-extracted

into hexane. The aqueous phase was discarded and the procedure repeated until a clear hexane extract was obtained. The hexane extracts were concentrated to about 1 ml and then charged onto a precleaned silica gel column to isolate the PCB's from other organic contaminants. The column was sequentially eluted with a series of organic solvents and the PCB fraction collected. The isolated fraction was then concentrated and analyzed using gas chromatography (GC) and gas chromatography—mass spectrometry (GC-MS). PCB analyses were performed on a Hewlett Packard (Atlanta, GA) 6890 gas chromatograph equipped with an electron capture detector (ECD), splitless injection, electronic pressure control (EPC), and autoinjector. Separation of PCB congeners was achieved using a capillary chromatographic column (30 m DB-5, 0.025 mm I.D., 0.25 um film thickness; J &W Scientific, Folsom, CA). Samples were quantified as Aroclor 1268 using a five-point calibration curve derived from dilutions of certified standards. Six characteristic peaks were selected from the Aroclor mixtures. All selected congener peaks were at least 25% of the highest Aroclor component. A Hewlett Packard 5890 Series II gas chromatograph with splitless injection, EPC, and a 5972 mass spectrometer (GC-MS) was used to confirm GC-ECD identifications. All samples were analyzed by GC-MS using the selected ion monitoring (SIM) acquisition mode. Selected samples were also analyzed using full scan acquisition in a separate sample injection/analysis. All of the 12 congeners in the Aroclor 1268 mixture were determined in the GC-MS analysis. Selected ions in the SIM mode for different retention time windows were determined from the analysis of an Aroclor 1268 standard. Analyses of spectra obtained in the full scan mode (mass 50–550) were performed by comparing the mass spectra with Aroclor 1268 standards as well as the NIST reference library.

Transfer factors

Transfer factors for Hg and PCB concentrations were calculated between trophic levels. To calculate the transfer factors from the sediment to fiddler crabs the concentrations found in fiddler crabs were divided by the concentrations found in the corresponding sediments. Then the concentrations found in the adult rails' muscle or liver samples were divided by the amount found in the corresponding fiddler crabs. Percent carbon values for sediment are referenced but not adjusted for either sediment or fiddler crab, since the focus of this study is to determine what the clapper rail ingested.

Statistical analysis

We first examined Hg and PCB distributions using Shapiro-Wilk statistics (PROC UNIVARIATE, version 8.1; SAS Institute©). This tested the hypothesis that these data were random samples from normal distributions, which was rejected at $\alpha = 0.05$. Stem-and-leaf plots suggested a log-transformation, which successfully transformed the data to a normal distribution in all cases. To examine the relationship between Hg and PCB concentrations in the different sampling locations (creek or pool) and media types (sediment, crabs or adults) and to test for differences in congener profiles between media types, we used analysis of variance models (ANOVA; PROC GLM; SAS Institute©). For all tested models, Type III (partial) sums of squares and associated F-statistics were interpreted and least-squares means procedures were used to provide estimates of dependent variables that were adjusted for all effects in the models and to provide mean separation tests. To examine the relationship between Hg and PCB concentrations as well as percent carbon in the different sampling locations (creek or pool), paired t-tests were used where the clapper rail's home range site was used for pairing. All statistical tests were considered significant at $P < 0.05$. Means and standard errors were presented as geometric means (i.e., back-transformed values of log least-squares means estimates).

Results

Home range

Transmitters were placed on 25 of the 52 birds that were captured between January 29, 2002 and March 27, 2002. Due to predation and loss of transmitters, only seven (females = 4, males = 3) of the 25 radio-tagged clapper rails had 15 or more locations and were successfully recaptured. Eight birds with >15 locations could not be recaptured. To insure an acceptable sample size ($n = 15$), eight additional non-telemetered rails from active nests with known locations were shot. A home range was estimated for each of these eight birds using the median home range area (0.28 ha) data from the remaining telemetered birds ($n = 16$; $\bar{x} = 1.2 \pm 0.8$ SE ha, range: 0.06–13.27 ha). Sediment and fiddler crabs were collected within a hypothetical circular home range boundary around the nest with a radius of 30 m, which gave a total contained area of 0.28 ha.

Contaminant concentrations (sediment, crabs, adult rails and chicks)

All samples analyzed for Hg and PCBs from LCP and the control sites were above the minimum detection limits (Hg: 0.004 μg g^{-1}, PCB: 0.04 μg g^{-1}; Table 1). Hg concentrations in the sediments were found to be significantly higher at LCP than at the control sites ($F = 65.31$, $P \leq 0.0001$, Fig. 4). Sediment PCB concentrations were

Table 1 Sex, number of telemetry locations (Loc.), home range estimates (ha) of adult clapper rails and concentration ($\bar{x}$(SD)) of Hg and Aroclor 1268 (Hg/PCB) found in all sample types (μg g^{-1} dry weight) from the LCP estuary in Brunswick, Georgia during the 2002 breeding season

Bird ID/Sex	Loc.	Home range (ha)	Creek sediment[a]	Pool sediment[b]	Crab[c]	Muscle[d]	Liver[e]
102/F	19	0.19	4.7/18	1.9/3.8	0.6/3.4	4.7/13	20/32
103/F	18	0.21	3.0/1.6	1.6/1.0	0.3/0.8	3.5/9.4	NA
104/F	25	0.33	2.8/1.8	1.1/0.7	0.3/0.8	1.7/1.7	5.3/8.3
105/M	15	0.27	1.6/1.3	1.6/1.6	0.3/1.2	7.3/8.9	14/108
106/M	25	13.3	1.8/1.0	1.7/1.0	0.3/0.6	4.6/5.0	21/20
107/M	23	0.28	2.2/1.1	1.1/1.6	0.6/1.3	3.4/18	11/56
108/F	16	0.77	2.7/3.0	1.3/1.1	0.3/0.7	3.6/49	18/499
219/F	1	0.28	2.4/4.1	0.6/1.1	0.4/1.2	3.7/5.8	12/38.7
255/M	1	0.28	2.0/3.2	0.9/1.6	0.4/1.0	8.8/25	24/164
337/F	1	0.28	1.0/3.5	0.9/3.7	0.5/4.0	11/71	31/568
358/F	1	0.28	1.2/20	0.5/0.8	0.2/0.9	9.7/19	35/157
501/M	1	0.28	0.1/1.6	0.7/0.5	0.9/3.2	8.9/27	36/167
567/F	1	0.28	3.0/3.1	1.2/2.1	0.8/3.5	9.0/18	41/126
640/F	1	0.28	1.3/2.5	0.8/1.2	0.2/0.6	1.7/4.6	4.9/54
970/M	1	0.28	2.7/2.5	3.5/2.9	0.5/1.6	6.5/22	19.5/84
$\bar{x}$(SD)/$\bar{x}$(SD)			2.4(1.2)/4.6(6.1)	1.3(0.7)/1.7(1.0)	0.4(0.2)/1.6(1.2)	5.9(3.1)/ 21.9(18.5)	20.9(11.3)/155.9(172.2)

Any values labeled "NA" were samples that could not be collected

[a] $\bar{x} \pm$ SD% moisture content: 70% ± 3%, $n = 30$; $\bar{x} \pm$ SD% carbon content: 6.7% ± 2.5%, $n = 15$

[b] $\bar{x} \pm$ SD% carbon content: 8.4% ± 3.8%, $n = 15$

[c] $\bar{x} \pm$ SD% moisture content: 75% ± 7%, $n = 10$

[d] $\bar{x} \pm$ SD% moisture content: 73% ± 2%, $n = 46$

[e] $\bar{x} \pm$ SD% moisture content: 74% ± 5%, $n = 22$

also significantly higher at LCP ($F = 92.74$, $P \leq 0.0001$). Concentrations of Hg and PCBs of crabs were also found to be significantly higher at LCP than at the control sites ($F_{Hg} = 86.13$, $P \leq 0.0001$; $F_{PCBs} = 65.36$, $P \leq 0.0001$, Figs. 3, 4). Hg concentrations in the sediments from LCP were significantly higher than those in the crabs ($P \leq 0.0001$). However, PCB concentrations did not differ between sediment and crabs ($P = 0.7365$). Muscle and liver samples from adult birds were significantly higher in both Hg and PCBs than were either the sediment or crab samples from the LCP site ($P \leq 0.0001$). Hg concentrations in chicks from LCP were also significantly higher than in the chicks from the control site ($P \leq 0.0001$), and were also significantly higher than those found in the crabs and sediments from the same site ($P \leq 0.0001$). PCB concentrations were significantly higher in chicks from LCP than in those from the control marsh ($P \leq 0.0001$). PCB levels in chicks from LCP were significantly higher than in adult muscle but not liver ($P_{muscle} \leq 0.0001$; $P_{liver} = 1.000$). Male and female clapper rails did not significantly differ in liver or muscle burden for either Hg or PCB. Since sample sizes were low from this study (6 male, 9 female), we pooled data from a previous study

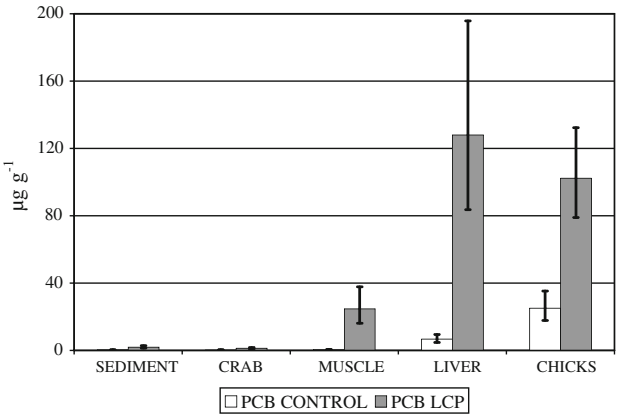

Fig. 3 Geometric means and associated 95% confidence levels of Aroclor 1268 concentrations found in all sample types from control and LCP sites collected during the breeding seasons of 2000 and 2002. Values are in μg g^{-1} dry weight. $\bar{x} \pm$ SD percent moisture content for chicks was 74% ± 3%, $n = 10$

(Novak et al. 2006) to add 9 additional males and 1 additional female from LCP and still no differences were found. PCB congener profiles (6 major) did not significantly differ between media (Fig. 5).

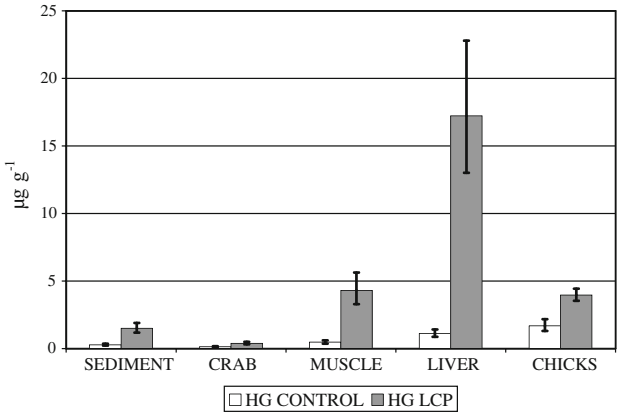

Fig. 4 Geometric means and associated 95% confidence levels of Hg concentrations found in all sample types from control and LCP sites collected during the breeding seasons of 2000 and 2002. Values are in μg g^{-1} dry weight. $\bar{x} \pm$ SD percent moisture content for chicks was 74% ± 3%, $n = 10$

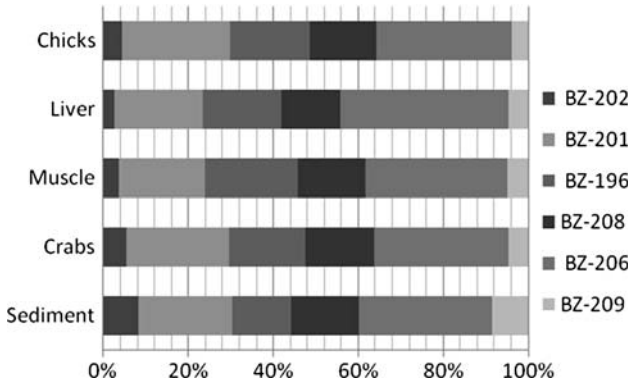

Fig. 5 Composition (%) of the 6 major PCB congeners (Ballschmiter-Zell (BZ) numbering system[1]) for Aroclor 1268 in the biota and sediments collected from the LCP super fund site in Brunswick, Georgia during the 2000 and 2002 breeding season. [1]To adhere to IUPAC rules, changes have been made to the BZ structural nomenclature; specifically BZ-201 was formally identified as BZ-199 (Mills et al. 2007)

For Hg, creek and pool crab samples, crab pool and muscle, crab pool and liver, and muscle and liver all correlated well with r-values ≥ 0.50 (Table 2). PCB concentrations correlated well between creek and pool crab samples ($r = 0.75$; Table 2).

Transfer factors

Percent carbon content was higher ($P = 0.0557$) for pool samples ($\bar{x} = 6.66$, SD = 2.4) than for creek samples ($\bar{x} = 8.43$, SD = 3.78). In LCP, Hg and PCBs concentrations in the sediment samples were significantly higher in the tidal creek than the tidal pool feeding areas ($P_{Hg} = 0.0432$, $P_{PCB} = 0.0157$) and normalizing for carbon made no difference for this result. However, crab samples from the two types of feeding areas were not significantly different ($P_{PCB} = 0.3711$, $P_{Hg} = .9903$). Because the contaminant concentrations of crabs from creek vs. pool locations were not significantly different, transfer factors (Table 3) for the creek and pool samples were combined. The average transfer factor for Hg from the sediment to the crabs was 0.4, from crabs to muscle was 14.8 and from crabs to liver was 50.1. The average transfer factor for PCB from the sediment to the crabs was 0.8, from crabs to muscle was 15 and from crabs to liver was 119.

Discussion

Home range

Although clapper rails will expand their home range to the square kilometer level during the non-breeding season (Eddleman and Conway 1998), birds will most likely be accumulating most of their body burden (especially for Aroclor 1268) during the pair-bonding and breeding months (February–September) when their home ranges reflect those

Table 2 Correlation coefficients (r-values) for media sampled within 15 clapper rail home ranges from the LCP estuary in Brunswick, Georgia during the 2000 and 2002 breeding season

	Creek sediment	Pool sediment	Crab creek	Crab pool	Muscle	Liver
Creek sediment		0.22	−0.13	−0.13	−0.16	0.01
Pool sediment	0.28		0.03	0.05	−0.12	−0.13
Crab creek	0.09	**0.54**		**0.79**	0.15	0.32
Crab pool	0.00	0.35	**0.75**		**0.50**	**0.66**
Muscle	−0.01	0.43	0.41	0.29		**0.87**
Liver	−0.11	−0.15	−0.21	−0.33	0.38	

Values on the upper right side of the correlation matrix are for Hg and values on the lower left side of the matrix are for Aroclor 1268

Correlations where $P \leq 0.05$ are in bold

Table 3 Transfer factors for all sample types for Hg/Aroclor 1268 from samples collected from the LCP estuary in Brunswick, Georgia during the 2002 breeding season

Bird ID	Sediment to crab	Crab to muscle	Crab to liver
102	0.2/0.3	7.7/3.9	33.0/9.5
103	0.1/0.6	12.3 /12.3	NA/NA
104	0.1/0.7	6.3/2.0	19.5/9.9
105	0.2/0.9	27.0/7.1	52.7/87.3
106	0.2/0.6	15.6/8.9	70.2/34.6
107	0.4/0.9	5.6/14.4	18.2/43.5
108	0.2/0.3	10.9 /73.4	53.5/744
219	0.3/0.4	8.5/5.0	28.3/33.5
255	0.2/0.4	25.1/24.6	67.2/162
337	0.5/1.1	22.3/17.9	63.6/144
358	0.1/0.1	39.5/21.5	141.0/178
501	2.1/3.0	10.4/8.5	42.0/52.7
567	0.4/1.3	11.8 /5.2	53.7/36.3
640	0.2/0.3	6.9/7.3	20.3/85.3
970	0.2/0.6	12.5/13.5	37.6/51.1
$\bar{x}$(SD)	0.4(0.5)/ 0.8(0.7)	14.8(9.6)/ 15.0(17.4)	50.1(31.6)/ 119.4(187.8)

Transfer factors for crabs were calculated as a function of the concentration of contaminant found in the sediment while muscle and liver transfer factors were calculated as a function of the concentration of contaminant found in the crabs

quantified by this study. Establishing the life history home range characteristics of clapper rails in the Brunswick marsh system was extremely important, since previous studies investigating nest preferences in this population definitively showed that clapper rails choose nest sites differently than their counterparts in other coastal areas due to vegetation structural and tidal amplitude differences (Gaines et al. 2003). Specifically, clapper rails will nest near tidal pool locations just as much as tidal creek areas in the Brunswick vicinity. This is markedly different from Virginia populations that only nest adjacent to tidal creeks, which is why both tidal creek and tidal pool sediment/crabs were sampled in each home range and compared. The average clapper rail home range of 1.2 ± 0.8 SE ha found in this study, however, was consistent within the range found for previous studies, which generally varied from 0.4 to 1.8 ha (Eddleman and Conway 1998; Roth et al. 1972; Zembal et al. 1989). In this study, most rails had small home ranges and tended to stay within 1 ha of the nest. However, some rails did tend to have larger movement patterns with one female (# 970; Table 1) appearing to shift her home range midway through the study and another male (#501) appearing not to have successfully mated (e.g. never found at or near a nest; Fig. 2). Such shifts in home ranges are likely due to renesting attempts (Blandin 1963). This variability in home range may help explain possible variation in contaminant burdens. For example, male 501 with the largest home range also had the highest transfer factor from sediment to crab for both Hg and PCBs possibly since the bird potentially foraged (and samples were collected) over a larger area, thus not providing an accurate picture of the true contaminant ratio between sediment and crabs. These findings reinforce the notion the area of contamination to home range ratio is extremely important to understand contaminant mobility (Gaines et al. 2004). However, even the sediment sampled in the smallest home ranges (e.g. <1.0 ha) most likely still do not represent the true contaminant load of the sediments since contaminant deposition is so patchily distributed in almost any terrestrial or aquatic system.

Contaminant concentrations (sediment, crabs, adult rails and chicks)

Since inorganic Hg does not tend to bioaccumulate because it is less efficiently absorbed and more readily eliminated from the body than methylmercury (MeHg) (EPA 1997), Hg was not speciated. Further, MeHg was found to be negligible in LCP tidal creek (0.32–1.49%) and tidal pool (0.006–1.04%) sediment samples (NOAA 1998). Studies in estuaries from this region show that Hg methylation rates are correlated with sulfur reduction rates in the anoxic slurry, which as such is a better predictor of potential bioaccumulation into the food chain than quantifying sediment MeHg (King et al. 1999, 2001). The (total) Hg concentrations in the sediments of the control sites used in this study were found to be within normal background levels of <1 μg g^{-1} (Eisler 2000a). Hg found in sediments associated with chlor-alkali plants around the world have been reported to exceed 300 ppm (Eisler 2000a). However, our study support previous studies conducted on the Turtle River estuary in Brunswick that report sediment levels to be approximately 1.5 μg g^{-1} (Gardner et al. 1978; Windom et al. 1976). This pattern was also found for Aroclor 1268 with elevated sediment concentrations (0.43–20 μg g^{-1}) at LCP and lower concentrations of Aroclor 1268 at the control sites (<0.5 μg g^{-1}). Our results showing that congener (6 major) profiles did not differ significantly between media contrast those of Kannan et al. (1998a) who collected sediment and biota from LCP which showed large variation between fauna and organs within the same species. This is most likely due to the low sample size (n = 1–5) of individuals of each species collected for that study as well as their life history traits (home range size and foraging preferences).

Since fiddler crabs are the major food source for clapper rails (Heard 1982), sampling them helped control for diet variability when establishing transfer factors. Since this study focused on the bioavailability of Hg and Aroclor 1268, the contents of the crab gut were not purged, thus the

Hg measured is a potential mixture of what was acquired within the body of the crab as well as media that would be excreted, which would most likely be a mixture of both inorganic and methylated forms. The average percent MeHg in fiddler crabs from the exact same sampling locations at LCP where the sediment was purged from the gut was found to be 72% with a 3.31% mean dry wt. lipid content (NOAA 1998).

The hypotheses that were tested to determine if contaminant distributions differed between tidal creek and tidal pool microhabitats indicated that there were differences in contaminant loads with Hg and PCBs concentrations in the sediment samples both higher in the tidal creek than the tidal pool feeding areas regardless of carbon content normalization. This was an interesting result since carbon levels were slightly higher in tidal pools. The fact that carbon content was not dramatically different between the two microhabitats was not too surprising since the entire marsh will flood and drain with each tide making the depositional hydraulics similar. The normalization was performed since studies have shown predictable associations of lipophilic contaminants as well as MeHg with detrital carbon (Castro and Vale 1995; King et al. 2001; Nhan et al. 2001). However, other studies from the southeast have shown the relationship between Hg and carbon content to be complex (Kannan et al. 1998b), most likely because carbon is only one component that can influence methylation processes.

The population of clapper rails nesting in the Brunswick estuary is likely one of year-round residents. Moreover, toxicants may accumulate in the adult clapper rails in high concentrations due to this species' relatively long life expectancy (5–10 years) (Eddleman and Conway 1998). This coupled with the fact that PCBs and MeHg tend to deposit in fatty tissues and do not depurate easily will allow them to accumulate to high levels over extended periods. Since clapper rails for this study were also used in a human health risk study for those who may consume these birds, PCBs were not adjusted for lipid content. However, clapper rails collected from our study area have been documented to have a wet weight fat content of 2.5% for muscle and 4.1% for liver (Kannan et al. 1999).

Whole body concentrations of Hg and Aroclor 1268 in clapper rail chicks indicated that both contaminants were actively passed to the chicks. These data suggest that egg-laying may be a significant pathway of elimination of both toxicants from adult females. However, interestingly, there was no difference in muscle or liver burden between males and females collected in this study for either analyte. This could be due to the fact that females are actively eating such highly contaminated food items that any burden depurated was quickly reestablished. Although sampling for chicks predated the home range study, egg collections (hatched to chick in laboratory) were performed in the exact area where the home range study took place. In fact, the experimental design for this study was engineered specifically to make sure the sampling locations would overlap the egg/chick study. Elevated levels of Hg in eggs have been reported to cause low reproductive success and behavioral abnormalities in several species of waterfowl, fish-eating predators and songbirds (Heard 1982; Heinz 1975; Odom 1975). Likewise, many studies have shown that an increased concentration of organochlorines in bird eggs has been related to lower reproductive success and interference with developmental processes (Eisler 2000b). Rail chicks hatched from the eggs from the LCP marsh in this study have been shown to have both behavioral and physical abnormalities (Novak et al. 2006), as well as bone structural problems (Rodriguez-Navarro et al. 2006). Moreover, eggs that did not hatch or were archived (not allowed to hatch) also demonstrated egg shell integrity problems (Rodriguez-Navarro et al. 2002). These endpoints were linked to the toxicant mixtures that were passed in ovo.

Contaminant transfer

The chemical characteristics of the contaminant, environmental conditions, behavior and physiology all play important roles in regulating the transfer of pollutants through food chains. Collecting prey and sediment samples from the delineated home range of each individual rail lent insight into the variability in and among home ranges that is often overlooked in trophic mobility studies. By using clapper rails as indicator species, we were able to control for large movements and assumed limited diet variability, improving our ability to quantify the transfer of the target contaminants. This was supported by the differences found in contaminant loads between LCP and nearby control sites. In 1999, over 5 ha of the most contaminated portions of the LCP marsh were remediated and in 2006, the EPA considered this site remediated. However, this study showed that both Hg and Aroclor 1268 are still bioavailable and occur at levels that may be harmful to both clapper rails and their young. Although this study shows that there is a large amount of spatial and individual variability in clapper rails from the LCP marsh, rails from the LCP site have been shown to have elevated frequency of double-stranded DNA breaks and there was a relationship between those breaks and contaminant concentrations (mostly Hg) within the rails (Novak et al. 2006). Understanding the movement, feeding, breeding and social characteristics of the target species will lead to more accurate assessment of exposure tailored to specific species or populations.

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