American Journal of Environmental Protection, 2019, Vol. 7, No. 1, 13-16
Available online at http://pubs.sciepub.com/env/7/1/3
Published by Science and Education Publishing
DOI:10.12691/env-7-1-3

Environmental Health Assessment Using Sentinel Species near Placencia Lagoon, Belize

Sheila A. Scoville[1,*], J. Peter Doherty[2], Marisa Tellez[3,4], Sierra McLinn[4]

[1]Department of Pathology and Anatomy, Eastern Virginia Medical School, Norfolk, VA USA 23547
[2]214 84th St, Virginia Beach, VA 23451
[3]Crocodile Research Coalition
[4]Ecology and Evolutionary Marine Biology, University of California Santa Barbara, Santa Barbara, CA USA 93106
*Corresponding author: scovilsa@evms.edu

Received February 07, 2019; Revised March 21, 2019; Accepted March 28, 2019

Abstract Unexplained bird deaths discovered around Placencia Lagoon, Belize over a 2-year period sparked an investigation into the environmental health of the lagoon and two of its major tributaries. In this study, conducted from January 23 to February 28, 2018, we assessed avian health within two commercial aquaculture facilities, each with a substantial outflow into the lagoon; a shrimp farm and a tilapia farm. Birds were chosen as our study subjects not only because of previous bird deaths, but also as sentinel species to assess overall possible contamination. Both facilities had previously been affected by die-offs of their cultivated product. The variety of sampled bird species, both year-round and winter residents, fed on small fish, airborne insects, mud-dwelling invertebrates and/or plant material, i.e. seeds and fruits, thereby sampling air, soil and water as possible bioaccumulation vectors. We trapped birds in, or nearby outflow containment ponds which emptied into the lagoon. Additionally, we assessed avian health at two reference locations on Placencia Penninsula, not known for avian health problems. Birds from multiple families representing different ecological niches were captured using two net types. We conducted standard ornithological assessments, measured hematocrit, made an evaluation of ectoparasite load and types, and for shorebirds, examined fecal swabs for parasites. Measured values were compared to published data and a previously established database from data collected elsewhere in the Caribbean. For each of the major sites, an extensive period of evaluation was later followed with a shorter, second evaluation at each location. Except for a single, second-year, Brown Pelican *(Pelicanus occidentalis*) evaluated one day at the close of the study, 257 birds were found to be healthy on all parameters including hematocrit and parasite load. We observed that the pelican had not been present previously and inferred that its health problems were acquired at another site. This conclusion was corroborated by the concurrent discovery of dead or dying pelicans along the Belize coast near the end of this investigation.

Keywords: *sentinel species, avian hematocrit, Belize, Brown Pelican, ectoparasite, neotropical migratory birds*

Cite This Article: Sheila A. Scoville, J. Peter Doherty, Marisa Tellez, and Sierra McLinn, "Environmental Health Assessment Using Sentinel Species near Placencia Lagoon, Belize." *American Journal of Environmental Protection*, vol. 7, no. 1 (2019): 13-16. doi: 10.12691/env-7-1-3.

1. Introduction

The use of sentinel species to establish threats to environmental health is a well-recognized strategy [1]. Three general types of studies can be implemented. One of these is the laboratory study in which animals are dosed with suspected environmental contaminants. A second is an *in situ* study in which subject animals are placed in a possibly contaminated environment and monitored for changes. This is the scenario of the canary in the coal mine which has become the metaphor for sentinel species. A third type is the observational study in which inhabitants of a specified environment are monitored for signs of physiological, anatomical or behavioral problems. This is the form of the current study. The research objective was to assess the health of wintering and year-round resident birds near Placencia Lagoon, Belize, using well-defined ornithological standards to determine possible environmental contamination.

An investigation was conducted from January 23 to February 28, 2018 in response to unexplained episodic bird deaths discovered at locations around Placencia Lagoon, Belize. Dead, disabled and apparently ill birds including Roseate Spoonbills *Ajaia ajaja*, Snowy Egrets *Egretta thula*) and Cattle Egrets (*Bubulcus ibis*) were discovered by team members of the Crocodile Research Coalition (CRC) during routine diurnal biodiversity surveys or nocturnal crocodile population surveys around the Placencia Lagoon, as well as by community members of the Placencia Peninsula. Observations of episodic mortalities began in mid-2016, with a cycle of every 3-6 months. Among the possibilities for the source of an affecting pathogen or toxin were containment ponds that held the outflow from two aquaculture facilities. While

waterbirds were among those previously reported, a broad evaluation was undertaken of neotropical and resident birds including waders, shorebirds, passerines and near-passerines present in, or bordering the outflow containment ponds. Birds were captured using mist and drop nets [2] at two commercial aquaculture facilities and at two additional reference locations on the Placencia Peninsula. In Belize, sporadic bacterial infections have been widespread, affecting the shrimp production industry including the two operations in this investigation. It is unknown whether bird deaths in adjacent locations were related or were caused by a different pathogen or toxin. In natural systems, causation is often multifactorial [1].

The two commercial facilities were Belize Aquaculture Ltd. (BAL) and Texmar in the Stann Creek District, Belize. BAL is a large actively managed shrimp aquaculture facility in the northern area of Placencia Lagoon. The site chosen for investigation was a final waste water impoundment before its release into Placencia Lagoon via a drainage creek. During our investigation, that impoundment was mostly empty of water, apparently due to the failure of a berm at one end of the containment pond. The remaining water was a narrow stream flowing through the impoundment. Recent continuing rains provided the water source for two, small wet sites on the floor of the containment pond. The habitat surrounding the pond consisted of a grassy area including ferns, small shrubs, and pine trees. The consistency of the floor of the impoundment was highly variable with solid footing on some surfaces and soft muck 12-16 inches (or more) deep in areas of standing water or adjoining the streams. Foraging shorebirds, predominantly sandpipers and plovers, fed on the wet areas of the containment pond floor in two locations, (N 16° 39.406" W 088°23.054" and N 16°39.294" W 088°22.971"). Drop nets, actively triggered capture devices, were erected at these sites where it was determined that birds could be safely captured and retrieved for evaluation. Mist nets were erected along the corridor between the grassy road, spanning the flowage, with the scrub-edged savannah on the opposite side. This area was primarily used by a variety of passerines and other forest edge birds.

Texmar, formerly a shrimp farm, now a managed tilapia farm, was arranged in dozens of rectangular impoundments in a block-type formation separated from one another by grassy and sandy berms. Water levels were variable throughout our investigation. Waste water was channeled into parallel ditches and flowed to containment ponds before emptying into the nearby creek, which also flowed into Placencia Lagoon. One of the containment ponds featured a sandbar where wading birds, waterfowl, and shorebirds routinely roosted and fished nearby. Waders such as herons, stilts and egrets were of special interest at this location. A drop net was erected on the sandbar and was triggered from a position on the grassy road embankment. To the outside of that embankment was a small scrub border and extensive savannah. Mist nets were erected in the savannah (N 16°33.459" W 088° 24.756").

2. Methods

A total of 257 birds were evaluated between January 23 and February 28, 2018. Birds were captured and extracted from the nets and placed in lightweight cotton bags prior to processing. The investigators complied with all standards of ornithological health assessment which are established through the North American Banding Council. These standards consist of visual evaluation of fat and muscle tissue and are compared to well-defined guidelines, while weight and size are directly measured. Additionally, ornithological ageing and sexing guidelines were followed according to detailed species accounts used for scientific reports by Pyle [6,12]. In addition to "in hand" evaluation of health, including assessments of weakness or incoordination, hematocrit was chosen as screening tool. Both hematocrit and closely related blood marker hemoglobin [4] are standard evaluation tools. A small blood sample (less than 1% of body weight) was taken by venipuncture from the brachial vein and collected into microcapillary tubes treated with ammonium heparin. Tubes were held in an insulated container until they were processed. Hematocrit was read within 6 hours. Micro-hematocrit tubes were centrifuged at 12,000g for a minimum of 5 minutes [5]. Additionally, birds were examined for ectoparasites and for waders and several species of shorebirds, fecal (cloacal) swabs were taken and analyzed for parasites. Birds present at two additional reference locations were similarly evaluated. No species listed as endangered or threatened in the United States or Belize was captured or sampled. All wildlife safety protocols were approved by the Forest Department of Belize.

3. Results and Discussion

In order to determine possible niche contamination, birds were evaluated by group: passerines, shorebirds and waders. Both resident and migratory passerine (songbird) species were captured at all locations. Among the neotropicals, Gray Catbird (*Dunetella carolinensis*) was the species with the largest capture (26 individuals), while warblers (Paradae) were the largest neotropical songbird family that we examined (53 individuals). At BAL and TexMar, all were within appropriate parameters for size [6]. Hematocrit, and other standard measurements were compared to published data (Table 1) as well as from the authors' additional data collected in The Bahamas and Hawaii [7,8,9,10]. For HCT, all individuals were within the previously established reference intervals or were within normal seasonal values for their respective families, including Thraupidae (tanager), and Vireonidae, (vireo). For all migrants, HCT averaged in the mid-40s, with a slight pre-migratory elevation (especially in males) towards the end of February, a phenomenon established elsewhere in the Caribbean [7]. All resident passerine species at both BAL and TexMar were in good health as measured by the above parameters. These birds consisted of families including Columbidae, Vireonidae, Tyrannidae, Thraupidae and Troglodytidae. Many of these birds were in breeding plumage or showed evidence of breeding activity such as a brood patch. Again, hematocrit followed that of the breeding residents in The Bahamas with HCT measurements averaging around 49%, higher than for migrants [7]. Increased testosterone in breeding males is known to upregulate hematocrit levels [11].

Table 1. Hematocrit values from neotropical migrants or resident birds are compared to best available normal values. [a]Scoville & Doherty 2017, [b]Owen & Moore 2000, [c]Scoville & Doherty database, [d]Celdran, et al. 1993, [e]Zaias et al. 2000

	Species or Family	N	HCT range	normal HCT RI, mean or range
Neotropicals				
Gray catbird	*Dunetella carolinensis*	25	39-47.5	39.55-48.89 RI [a]
Wood warblers	Paridae, 8 species	52	39-52	38.17-54.34 RI [a]
Shorebirds	Charadriidae, *Calidridris*, 3 species	19	39-52	39.9-51.8 RI [a]
Wood thrush	*Hylochicha mustelina*	5	43-49	$\overline{X} = 47$ [b]
Breeding Residents or Partial Migrants				
Common Ground Dove	*Columbina passerina*	2	46.5-56	$\overline{X} = 51.6$[c]
White-collared seedeater	*Sporophila morelleti*	31	43-56	unknown
Other songbirds	Passeriformes, 23 species	85	41-61	unknown
Waders	Ardeidae, 2 species	3	44-49	42.9-50[d]
Black-necked stilt	*Himantopus mexicanus*	3	48-53	45.5-55.5 [c]
Green Heron	*Butorides virescens*	1	36	42.9-50[d]
Brown Pelican*	*Pelicanus occidentalis*	1	24.5	45.5 [e]

One additional parameter was examined; the proportion of resident (non-migratory) birds at different ages and was compared to previously collected data from resident birds of The Bahamas. Here, the possible presence of a disproportionate number of young birds (second-year) would indicate a biological sink. In that situation, older breeding birds are present in reduced proportions due to poor breeding habitat. While exact age proportions are unknown that would indicate good breeding habitat, a higher proportion of older resident birds was present at both aquaculture facilities than was present in The Bahamas.

For migratory birds, the addition of fat stores in late winter is considered essential for migration. For warblers, fat in January at BAL averaged a rating of .77, while in February the average increased to 1.3 at BAL and 1.8 at TexMar, surveyed more extensively towards the end of February. Substantial increases in fat stores throughout the winter at both locations indicated good availability of food items.

Among shorebirds, the most frequently captured was the Least Sandpiper (*Calidris minutilla*) (19). Others included Spotted Sandpiper (*Actitus macularia*) and Semipalmated Plover (*Charadrius semipalmatus*). This group of birds was considered of special importance since their main diet consisted of invertebrates found on the containment pond floor and would likely have had more exposure to bioaccumulated contaminants. All shorebirds were found to be within normal parameters of size [12]. All birds were within the range of values previously collected for muscle and fat. Hematocrit ranged from 42 to 54, within the range previously gathered from other shorebirds [7]. Small numbers (< 5) of ectoparasites were found on these birds but did not otherwise appear to affect the birds' general health. The presence of ectoparasites on all birds in our study is presented in Table 2.

Waders were also considered likely targets for bioaccumulation of possible toxins or pathogens. For these birds, small fish and amphibians such as frogs are the usual prey items. Captured wader species were Little Blue Heron, *Egretta caerulea*, Tricolored Heron, *Egretta tricolor*, and Black-necked Stilt, *Himantopus mexicanus*. For Black-necked Stilts, the range of HCT values was within the range of a Hawaiian subspecies *Himantopus mexicanus* knudseni (45-53), with males ranging higher than females. For neotropical migrants, all health parameters, including hematocrit were within the range of health standards determined in previous investigations [10].

Fewer birds were captured at TexMar than at BAL. Waders were difficult to capture safely except in small numbers. During the first capture period, all birds were found to be healthy. On the return visit all passerines, shorebirds and waders were found to be healthy. A single, second-year, Brown Pelican *Pelicanus occidentalis* was captured with a heavy ectoparasite load, estimated to be several thousand lice of an undetermined species. Hematocrit measurement found it to be anemic (24.5) compared to a mean of 45.5 HCT [9]. Upon release, the bird showed reduced activity, consistent with the reduced blood oxygen carrying capacity of its anemia and possibly indicating other diseases. During our investigation, Brown Pelicans had not been seen previously at this location, and it is our conclusion that it arrived after it acquired its current affliction.

At reference locations birds were captured and evaluated at two sites on the Placencia Peninsula. One was in a residential section of Maya Beach, Stann Creek Distrrict, Belize with sparse housing on a vacant wooded lot within a block of Placencia Lagoon, a similar distance from the lagoon as the aquaculture facilities (N 16°39.920" W 088°21.262"). All birds were found to be healthy. Perhaps the most surprising observation was the very high fat levels measured at this location in migratory species. Fat is necessary for energy during migration so that high fat levels are a positive indicator. We observed large numbers of birds feeding continuously in daylight hours at this location. It is our supposition that substantial rain had forced invertebrates to the soil surface where the birds were feeding.

Table 2. Shorebirds were sampled for ectoparasites and for the presence of parasites in fecal swabs. Two resident bird species (not systematically sampled) are included indicating the parasites found in the shorebirds are not unique to those species. The Brown Pelican was found to be anemic and the Green Heron was found to have low HCT

Species/Genus/Family	Trematode *Paramaratremopsis*	nematode *Capillaria*	Trematode and nematode *Capillaria*	Cestodes tapeworm	Ectoparasite *Actornthopolus umbrinus*
Least Sandpiper n=6	3		1		n=3 <5 individuals
Semipalmated Plover n=2		1		1	
Dusky-capped Flycatcher n=1	1				
Yellowthroated euphonia n=1				1	
Brown Pelican n=1					unidentified lice >1000
Green Heron n=1					< 5 unidentified lice

The second reference site was a commercial property along the southeast shore of the Placencia Lagoon known as the Turtle Inn (N 16° 31.883" W 088° 21.741"). Here, 4 of 5 sites had birds entirely within healthy parameters and were present in impressive numbers and diversity. A single Green Heron captured at the southern edge of the property bordering an area being actively dredged had low hematocrit values (36 HCT) when compared to published values of herons and egrets [10] and had a light ectoparasite load. Implications of this apparently low HCT are unknown, but may implicate possible contamination in the dredged material.

4. Conclusions

The current study made use of sentinel species, resident and migratory birds, to evaluate the environmental health of two aquaculture facilities bordering the Placencia Lagoon, Belize. Birds present at these facilities were found to be healthy when indicators of health were compared to a published database, leading us to conclude that the two aquaculture facilities were not currently contributing to problems in the Placencia Lagoon. Hence, we were unable to explain the previously reported bird deaths. The implications of our findings are that other factors such as bacterial or viral diseases may have been responsible for the Placencia Lagoon bird deaths. At the aquaculture facilities, only a single Brown Pelican that initially appeared during the last 2 days of our study was found to be anemic and have an ectoparasite infestation. Contemporaneous reports of dead or dying pelicans throughout the Belizean coastline at that time, (reported to the CRC's Tellez) suggests to these authors a possible offshore source, perhaps a brevetoxin exposure similar to one reported off the United States coastline in 2005 [13].

Acknowledgements

We thank the Belize Forest Department and the Belize Bird Rescue for supporting the project, as well as Melvin Arevalo from Turtle Inn, and Miriam Boucher from the Crocodile Research Coalition for their assistance in the field. We are also grateful to Belize Aquaculture Limited, TexMar, and Turtle Inn for granting us permission to conduct research on their property. Additionally, we thank E. Paxon, U.S. Geological Survey Pacific Island Ecosystems Research Center for use of the Hawaiian Stilt data.

References

[1] National Research Council (U.S.). Committee on Animals as Monitors of Environmental Hazards, "Animals as Sentinels of Environmental Health Hazards: Committee on Animals as Monitors of Environmental Hazards," National Academy Press: 1991, ISBN 0309040469.

[2] Doherty, J.P. (2009) A modern, portable drop net can safely capture a suite of shorebirds. Waterbirds Vol. 32, No.3 pp. 472-475.

[3] The North American Bander's Study Guide. The North American Banding Council, Publications Committee, 2001.

[4] Minias, P. (2015). The use of haemoglobin concentrations to assess physiological condition in birds: a review. Conserv Physiol 3(1):cov007.

[5] Campbell, T.W. (1994) Cytology. In: Ritchie, B.W., Harrison, G.J., Harrison, L.R. (Eds) Avian Medicine: Principles and Applications. Winger, Lake Worth: pp. 199-221.

[6] Pyle, P. (1997). Identification Guide to North American Birds. Part 1. Slate Creek Press.

[7] Scoville, S.A., J.P. Doherty (2017). Baseline hematocrit values in birds: an important tool for monitoring exposure to polycyclic aromatic hydrocarbons. Environmental Pollution and Protection, Vol. 2, No. 3, pp. 145-152.

[8] Owen, J. C., F. R. Moore (2006) Seasonal differences in immunological condition of three species of thrushes. Condor 108 389-398.

[9] Zaias, J.A., WP Fox, C Cray, NH Altman, (2000). Hematologic plasma protein, and biochemical profiles of brown pelicans (*Pelicanus occidentalis*) Am J Vet Res 61, 771-774.

[10] Celdran, J., FJ Polo, VI Peinado, G Viscor, and J. Palomeque. (1994). Haemotology of captive herons, egrets, spoonbill, ibis and gallinule. Comp. Biochem. Physiol. 107A 337-341.

[11] Murphy, W.G., (2014). The sex difference in haemoglobin levels in adults -mechanisms, causes, and consequences. Blood Reviews 28 41-47.

[12] Pyle, P. (2008). Identification Guide to North American Birds. Part 2. Slate Creek Press.

[13] Fauquier DA, LJ Flewelling, JM Maucher, M Keller, MJ Kinsel, CK Johnson, M Henry, JG Gannon, JS Ramsdell. (2013). Brevetoxicosis in seabirds naturally exposed to *Karenia brevis* blooms along the west coast of Florida J Wildlife Diseases. Vol. 49, No. 2 (April 2013) pp. 246-260.