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Flow Cytometric Analysis of Hematocytes from Brown Pelicans (*Pelecanus occidentalis*) Exposed to Planar Halogenated Hydrocarbons and Heavy Metals

J. K. Wickliffe,[1] J. W. Bickham[2]

[1] 640-7 Gaines School Road, Athens, GA 30605, USA
[2] Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77643, USA

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The Brown Pelican (*Pelecanus occidentalis*) has endured a tremendous decline and recovery over the past fifty years (Schreiber 1980, Anderson and Gress 1983, Guravich 1983, Johnsgard 1993). The precipitous decline, primarily affecting populations inhabiting the U.S. Atlantic, Gulf, and Pacific coasts, coincided with the heavy use of organochlorine pesticides (e.g. DDT, Endrin Dieldrin). The decline has implicated reproductive failure associated with DDT dispersal and accumulation (i.e. eggshell thinning), acute mortality, ecological disruption (i.e. prey base decline), and natural causes (i.e. meteorological, climatic, disease) (Blus 1977, Schreiber 1980, Anderson and Gress 1983, Guravich 1983, Johnsgard 1993). Listed as an endangered species in 1970, the Brown Pelican has since made a remarkable recovery and demonstrated the goal of the Endangered Species Act of 1973. However, the sensitivity exhibited by this species to organochlorine compounds and ecological disruption clearly defines its role as a past, present, and future sentinel of environmental degradation.

Currently, a number of natural resource managers are concerned that Brown Pelicans nesting on spoil islands adjacent the South Carolina coast may be exposed and/or exposing their chicks to planar halogenated hydrocarbons (PHHs) and heavy metals present in forage fish from the surrounding waters. PHH compounds include polychlorinated dibenzodioxins (PCDD's) and polychlorinated dibenzodifurans (PCDF's) and derive from a number of sources associated with chlorine chemistry including pulp mill effluent (i.e. bleached paper), polyvinyl chloride (PVC) plastics production, organochlorine solvents and pesticides, and chlorine intermediates (Zook and Rappe 1994). These compounds are very stable and lipophilic which results in their environmental persistence and trophic biomagnification. Biological effects including liver hyperplasia, microcephaly, mixed-function oxidase (MFO) enzyme activation (e.g. CYP450, CYP1A1, EROD, PROD), depleted Vitamin A and retinoid reserves, wasting syndrome, biomagnification and developmental abnormalities have been associated with PHH exposure (Mineau et al. 1984, Gallo et al. 1991, Yamashita 1993, Giesy et al. 1994, Jones et al. 1994, Schecter 1994, Van den Burg et al. 1994, White and Hoffman 1995, Williams et al. 1995, Custer et al. 1997). Furthermore, Brown Pelicans, which are exclusively marine, feed entirely on fish (Johnsgard 1993). As specialized predators, they, in particular, run a high risk of

Correspondence to: J. K. Wickliffe

accumulating potentially deleterious, persistent compounds. In an effort to determine exposure levels of PHHs and elemental contaminants and if this exposure was genotoxic, blood samples were collected from chicks reared on spoil islands adjacent to the coast of North Carolina. Eggs were analyzed for the presence of PHHs (TCDD-EQs; H4IIE bioassay) and elemental contaminants (arsenic, selenium, and mercury) (see Tysklind et al. 1994 for an explanation of the H4IIE bioassay). The flow cytometric method (FCM) was then used to estimate the coefficient of variation in DNA content within individuals. These measures are used to estimate the extent of somatic chromosomal damage within an individual (see Bickham 1990 for a review). The FCM has been previously used to document the clastogenecity of such agents as polycyclic aromatic hydrocarbons (PAHs), radionuclides, and pesticides (McBee and Bickham 1988, McBee and Bickham 1990, Bickham et al 1992, Bickham et al. 1994, Custer et al. 1994, Lamb et al. 1995, Lowcock et al. 1997). While PHHs have been implicated as mutagens, their possible clastogenic properties have not been indicated, therefore the goal of this study was to further investigate the possible clastogenecity of these compounds and heavy metals in Brown Pelicans. In addition, physical abnormalities were reported in Royal Terns (*Sterna maxima*) from areas inhabited by Brown Pelicans in this study and provided further impetus for this investigation.

MATERIALS AND METHODS

Blood samples were collected in June 1995 from Brown Pelicans reared on two spoil islands along the North Carolina coast (Augspurger et al. unpubl.). Twenty-four samples were taken from Wainwright island (34°59'N, 22°51'W) located midway between Cape Hatteras and Cape Lookout. The remaining twenty-four samples were taken from Ferry Slip island (33°59'N, 77°57'W) which is located to the south of Wainwright island near Cape Fear. Both pelican colonies are large with several different species of nesting waterbirds. Samples were shipped frozen to Texas A&M University and immediately placed in an ultracold freezer (-80°C). Egg samples were taken from two colonies, Wainwright island and South Pelican island (33°56'N, 77°51'W, adjacent to Ferry Slip island), in 1994 (Augspurger et al. unpubl.). Samples from South Pelican island were used in contaminant residue analyses, and pelicans inhabiting both Ferry Slip and South Pelican island are believed to be exposed to similar levels of pollutants.

Whole blood cell suspensions were prepared according to Vindelov and Christensen (1994) and analyzed on a Coulter Epics Elite Flow Cytometer. DNACheck™ microfluorospheres (Coulter Corp., Hiahleah, FL.) and a biological standard, isogenic *Gallus domesticus* blood cells, were prepared and analyzed to minimize mechanical error and ensure laboratory procedures. Ten thousand cells/individual were then assayed on the flow cytometer to estimate mean DNA content and the half-peak coefficient of variation (HPCV) in DNA content.

A two-tailed Student's t-Test (SAS ver. 6.08, Cary, NC) was used to test for significant differences between localities based on mean HPCV. A critical value

of P<0.05 was used as the limit for significance.

RESULTS AND DISCUSSION

Pelican egg samples from South Pelican island had significantly higher concentrations of arsenic, selenium, TCDD-EQs, and a higher concentration of mercury than those from Wainwright island (Table 1). TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is considered to be the most toxic PHH congener and is typically used as a benchmark of toxicity in PHH analyses (Safe 1990).

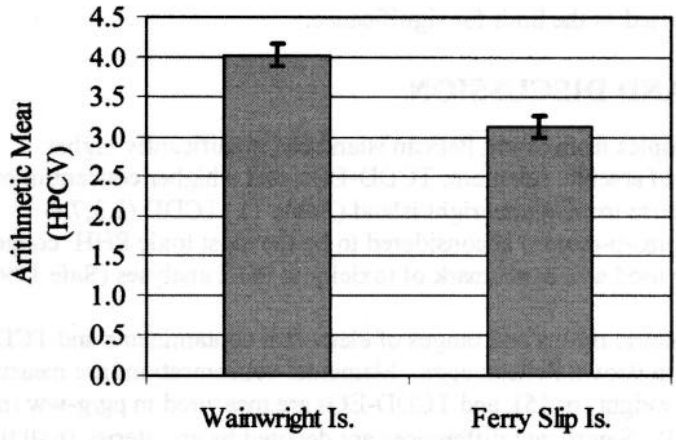
Table 1. Geometric means and ranges of elemental contaminants and TCDD-EQs measured in Brown Pelican eggs. Elemental concentrations are measured in ug/g-fresh wet weight (n=15), and TCDD-EQs are measured in pg/g-ww (n=5 composites of 3). Significant differences are denoted by an asterisk ($p \leq 0.05$). Data from Augspurger et al. (unpubl.)

Location	Arsenic*	Selenium*	Mercury	TCDD-EQs*
South Pelican island	0.03 (0.01-0.06)	0.38 (0.32-0.48)	0.34 (0.12-0.91)	(0.55-7.99)
Wainwright island	0.01 (0.01-0.02)	0.34 (0.29-0.40)	0.26 (0.14-0.52)	<1 (<1-6.05)

It should be noted that TCDD-EQs (PHHs) levels correlating strongly with adverse effects in species such as the Double-crested Cormorant (*Phalacrocorax auritus*), Herring *Gull* (*Larus argentatus*), Caspian Tern (*Sterna caspia*), Bald Eagle (*Haliaetus leucocephalus*) and Wood Duck (*Aix sponsa*) are 4-50x those observed in this study (Giesy et al. 1994, White and Hoffman 1995). Contaminant levels (heavy metals and PHHs) measured in this study are below those typically constituting the lower limit of the accepted avian-effect threshold. Furthermore, no physical abnormalities or differences in eggshell thickness were noted in the Brown Pelicans inhabiting these sites in contrast to Royal Terns from nearby colonies. However, the lack of thorough investigation of this biomarker (HPCV in DNA content) in birds exposed to PHH compounds, and the myriad effects seen across avian taxa exposed to a range of TCDD-EQs (PHHs) render this baseline information valuable (Mineau et al. 1984, Giesy et al. 1994, White and Hoffman 1995).

In any case, statistical analysis of the flow cytometric data indicated control blood samples from Wainwright island had a significantly higher mean HPCV when compared to those samples from Ferry Slip island (Fig. 1). These results were unexpected in light of the chemical residue analyses both in terms of significant differences bewteen sites and the low levels of contamination at both sites. The lack of positive association between elemental contaminants and TCDD-EQs and mean values of HPCV suggest exposure at these levels does not induce chromosome breaks in a dose-response fashion.

The molecular mechanisms by which PHH compounds effect transcription of



*p<0.0001

Figure 1. Mean HPCV in DNA content in Brown Pelican hematocytes from Wainwright island (n=24) and Ferry Slip island (n=24) colonies. Vertical bars represent ±l standard error.

MFO systems is well understood (Okino and Whitlock 1995, Wu and Whitlock 1992). In conjuction with transcriptional enhancement, alteration of local chromatin structure has been observed however, DNA strand breaks have not been associated with this molecular phenomena. In fact, the clastogenecity of dioxins has only been reported from a single source (Green et al. 1977). Meyne et al. (1985) found no evidence for chromosomal damage when resistant and susceptible mouse strains were exposed in vivo to dioxins (50-150ug/kg body weight) using standard metaphase spreads, the micronucleus assay, and sister-chromatid exchange. There is the possibility the function of dioxins used in their study were not fully representative, and thus there might remain congeners or PHH compounds which are clastogenic, but there is no foundation for this argument. Subsequent studies have not encountered chromosomal anomalies in association with toxic levels of TCDD-EQs. In addition, Custer et al. (1997) found that Great Blue Heron chicks exposed to TCDD-EQs levels twice those in this study did not present a clastogenic response. This evidence is further supported here as the control colony on Wainwright island had a significantly higher mean HPCV than the colony on Ferry Slip island. Recall the present study assumes Wainwright island represents a “relative control”. An additional assumption is that PHHs and/or elemental contaminants, if clastogenic, manifest their effects in a classical dose-response relationship. In light of this, four possible explanations are given for these unexpected results.

It is possible that those birds inhabiting the Wainwright colony have been exposed to an undetected clastogen either not present at the Ferry Slip colony or present in non-genotoxic concentrations. This case assumes that the contaminant screen from South Pelican island represents the distribution and concentration of contaminants one would observe at Ferry Slip island. For example, if an

undetected clastogen were found at Wainwright island, it would not be found at Ferry Slip island if it were not detected at South Pelican island. It is also plausible differing mixtures between the two sites are in some manner exhibiting an effect (possibly synergistic) at Wainwright island and relatively no effect (possibly antagonistic) at Ferry Slip island. In these situations, we would expect a response in the birds inhabiting Wainwright island relative to those on Ferry Slip island.

Another possibility is that South Pelican island is not synonymous with Ferry Slip island in terms of contamination and concentration A highly, non-random pattern of contaminant distribution would thus be expected. Though not incomprehensible we would expect, in conjunction with this pattern adults from each island to feed in different areas with very little overlap. Therefore, those birds from Wainwright island would be exposed to clastogenic levels of the contaminants observed while those from Ferry Slip island would not. At this point however, there is no evidence to support this reasoning.

It might be possible that the combination of compounds at Ferry Slip island, assuming synonymity with South Pelican island, has served to enhance strand break repair beyond background (Wainwright island). In other words, there has been an adaptive response to this suite of contaminants. This phenomena has been observed in human lymphocytes following exposure to low doses of ionizing radiation (Wolff 1996). Wolff found there was an initial increase in DNA double-strand breaks followed by a subsequent decrease in double-strand breaks relative to control cells following chronic exposure to low levels of ionizing radiation. This might explain the apparent negative relationship observed between mean HPCV and contaminant concentration. Again there is no evidence to support this explanation over the previous two.

A final possibilty that could explain the observed differences results from historical demographic differences. A relatively recent population or colony-wide bottleneck resulting from exposure (e.g. acute mortality, reproductive impairment, developmental anomalies reducing recruitment) at Ferry Slip island could reduce the natural DNA content variation below that observed in the Wainwright colony. A molecular genetic survey might serve to elucidate the validity of this hypothesis.

This study serves to highlight the need for a more intensive survey and monitoring of potential contaminants present at both sites. In light of the existing evidence, previous research rejecting the clastogenecity of low levels of these elemental and PHH contaminants as presently measured is supported.

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