

# Great Lakes Colonial Waterbirds as Sentinels for Continuing Reproductive and Health Impairments at Contaminated Sites in Michigan during 2010-17

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## Introduction

Significant concentrations of persistent organic pollutants including polychlorinated biphenyls (PCBs), chlorinated dioxins and furans, and organochlorine pesticides have contaminated the Great Lakes since their introduction during the 1940s-60s. Studies from the late 1960s onward have shown that fish-eating birds of the Great Lakes are excellent sentinel species for assessing and monitoring effects of contaminants including reproductive problems, deformities, and immune suppression (Grasman et al. 1996, 1998). PCBs and dioxins have been associated with Great Lakes Embryo Mortality, Edema, and Deformities Syndrome (GLEMEDS; Grasman et al. 1998). GLEMEDS in wild birds parallels chick edema disease in chickens, which is caused by these same contaminants (Gilbertson et al. 1991) and produces similar symptoms such as crossed bills, edema, gastoschisis, and embryonic mortality. Suppression of T cell-mediated immunity has been associated previously with organochlorines in young herring gulls and Caspian terns at contaminated Great Lakes sites (Grasman et al. 1996).

The Great Lakes Water Quality Agreement recognizes 14 Beneficial Use Impairments (BUIs) caused by contaminants at specially designated Areas of Concern (AOCs) and in lake-wide regions. Two wildlife-related BUIs are directly relevant to the background discussed above and to the present study: 1) bird or animal deformities or reproductive problems and 2) degraded fish and wildlife populations. The present study is a reassessment of these BUIs in the Saginaw Bay and River Raisin AOCs as part of the US Fish and Wildlife Service's broader efforts under the Great Lakes Restoration Initiative (GLRI) Funding for AOCs and Remediation and Restoration of Contaminated Sediments. In 2014, Grand Traverse Bay was added as a new site. Herring gull eggs from Bellow Island in the Grand Traverse Bay have elevated concentrations of dioxin toxic equivalents (TEQs) despite medium PCB concentrations, and also elevated DDE concentrations (Bowerman et al. 2011). This unique mixture of contaminants may be a result of the herbicides and insecticides used on orchards surrounding the Grand Traverse Bay.

The specific purpose of this study was to reassess the reproduction, growth, and immunological health of colonial waterbirds in the Saginaw Bay and River Raisin AOCs and Grand Traverse Bay. Endpoints used in this assessment included embryonic mortality, deformities, overall reproductive success, chick growth, and T cell and antibody-mediated immune responses in chicks. Low immune responses, including the phytohemagglutinin (PHA) skin response (a measure of T cell function), are associated with reduced survival and dispersal in wild song birds (Moeller and Saino 2004, Moeller and Cassey 2004). Herring gulls from the reference colonies chosen for this study (Fig. 1) have low concentrations of organochlorines compared to those collected from the Saginaw Bay and River Raisin AOCs and Grand Traverse Bay (Bowerman et al. 2011).

## Materials and Methods

### Embryonic Nonviability

- Herring gull nests containing one to two eggs were marked during laying. Embryonic viability was assessed during mid incubation (19-21 days) using an Avitronics Digital Egg Monitor (Cornwall England), which measures the embryo's heart rate and movement. Nonviable eggs were opened and inspected to identify infertility, stage of development, and deformities.

### Growth and Reproductive Success

- Body mass was measured at 3 and 4 weeks to evaluate the growth rate of chicks.
- Chick productivity was calculated as the number of 3 week chicks per nest in enclosures.

### Immune Function

- T cell-mediated immune response:** The initial wing web thickness of three week gulls and terns and two week herons was measured with pressure sensitive calipers. The chicks were then injected with a irritant, phytohemagglutinin (PHA) in phosphate buffered saline (PBS), in one wing web and a placebo of PBS in the other. 24 hours post injection, the wing web thickness was re-measured to quantify the T cell-mediated response. The PHA stimulation index was calculated as the difference between the change in the PHA wing web and the change in the PBS (placebo) wing web thickness (Grasman et al. 1996).
- Antibody response:** Sheep red blood cells (SRBCs) were injected into three week chicks to stimulate antibody production. Antibody titers were measured in plasma of 4 week old chicks by serial dilution of plasma and incubation with SRBCs. The highest dilution in which agglutination formed between the antigen and antibody constituted the titer value for total antibody response. IgG antibody response was assessed by adding 2-mercaptoethanol to denature IgM antibodies prior to addition of SRBCs (Grasman et al. 1996).

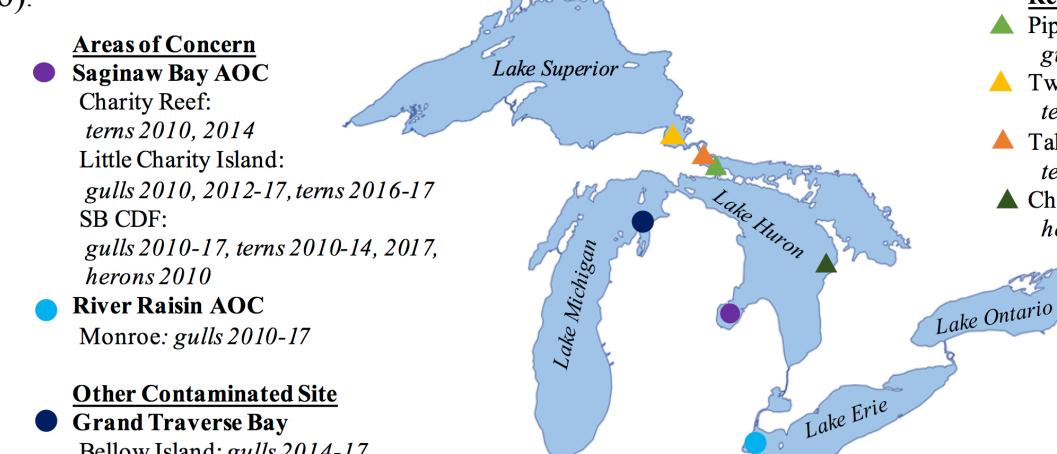


Figure 1. Colonial waterbird colonies studied in Great Lakes AOCs, Grand Traverse Bay, and reference sites for this assessment of health and reproduction in fish-eating birds during 2010-17.

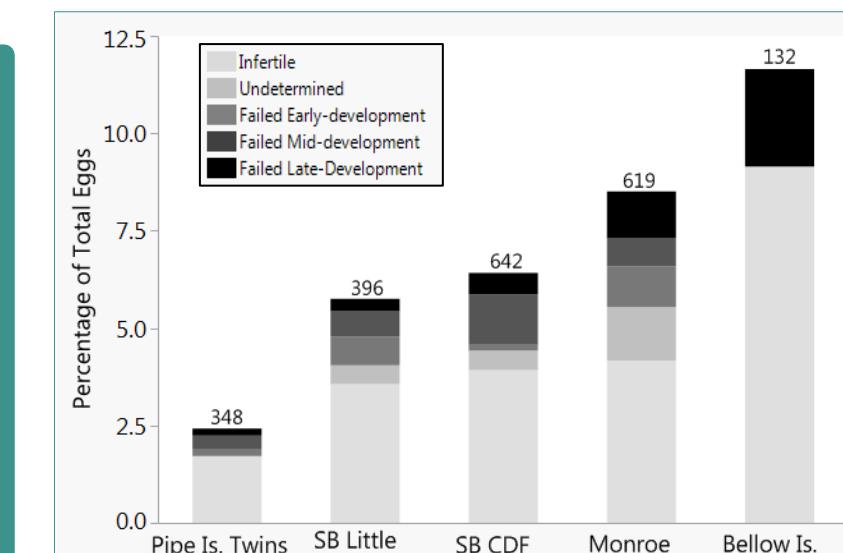


Figure 2. Embryonic nonviability in herring gulls at the Reference site, Saginaw Bay and River Raisin AOCs and Grand Traverse Bay during 2010, 2012-17. Numbers on bars are egg totals. "Undetermined" describes infertile or failed eggs that could not be distinguished due to addling.

Table 1. Relative risk ratios for incidence rates of embryonic nonviability, fertility, and failed development in herring gulls in the Saginaw Bay and River Raisin AOCs and Grand Traverse Bay compared to the reference site (Pipe Island Twins) during 2010-17.

Location	Relative Risk Ratio (one way exact p-value)		
	Overall Nonviability	Infertile <sup>a</sup>	Failed Development <sup>a</sup>
All contaminated sites combined	2.48 (0.0006)	2.47 (0.0053)	2.82 (0.012)
Saginaw Bay AOC Both islands combined	2.13 (0.0062)	2.25 (0.016)	2.26 (0.055)
SB CDF	2.12 (0.010)	2.09 (0.036)	2.44 (0.047)
Little Charity Island	2.16 (0.014)	2.51 (0.015)	1.98 (0.13)
River Raisin AOC	2.71 (0.0005)	2.41 (0.013)	3.67 (0.0034)
Grand Traverse Bay (Bellow Island)	4.07 (0.0001)	4.52 (0.0007)	3.30 (0.044)

<sup>a</sup> includes undetermined eggs that were either infertile or early failed



Figure 3. Cross-billed colonial waterbird chicks and embryos observed at AOCs during this study: herring gulls at Monroe in 2012 (A), 2013 (B), and 2016 (C); a Caspian tern on L. Charity Is. in 2016 (D); herring gull embryos on the SB CDF in 2016 (E) and L. Charity Is. in 2017 (F); and a comorant on L. Charity Is. in 2017 (G).

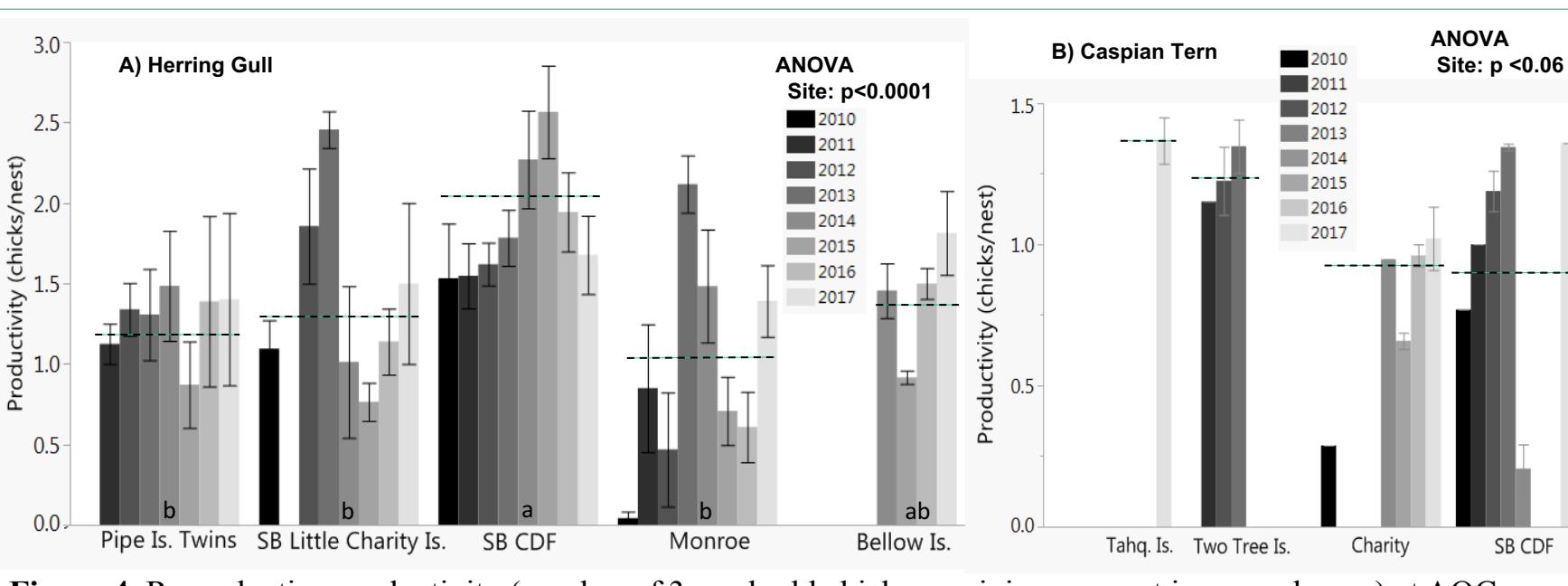


Figure 4. Reproductive productivity (number of 3 week old chicks surviving per nest in an enclosure) at AOCs, Grand Traverse Bay, and reference sites in 2010-17. For herring gulls, productivity of 0.75 chicks/nest or greater is necessary to maintain a stable population (Kadlec and Drury 1968). Dotted lines indicate site means. Sites with the same letter(s) were not statistically different by Tukey's test ( $p < 0.05$ ).

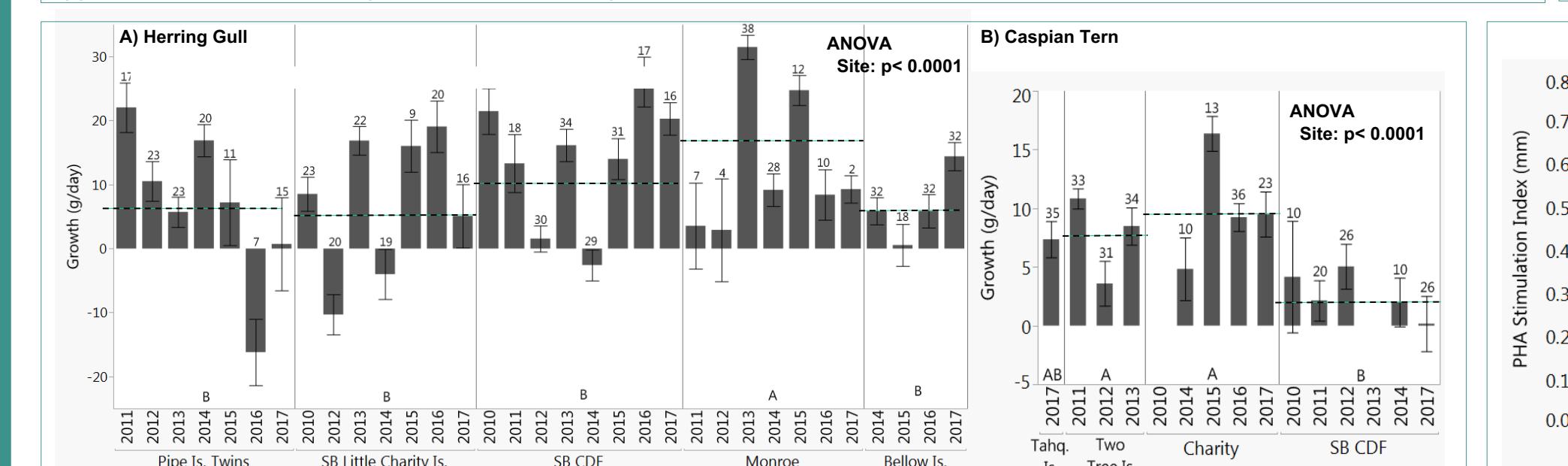


Figure 5. Mean change in body mass between 3 and 4 weeks of age in pre-fledgling fish-eating birds at Great Lakes AOCs, Grand Traverse Bay, and reference sites in 2010-17. Numbers on bars indicate sample sizes, and error bars indicate standard errors. Dotted lines indicate site means. Sites with the same letter(s) were not statistically different by Tukey's test ( $p < 0.05$ ).

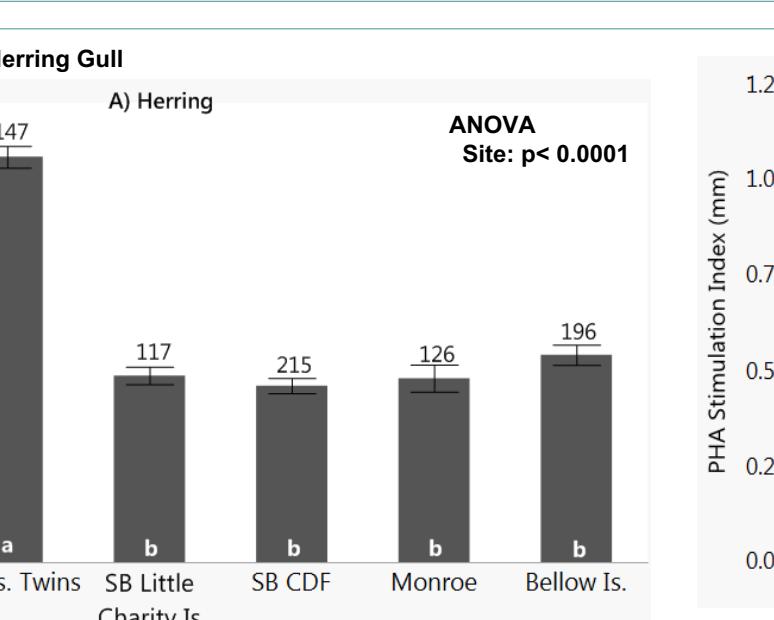


Figure 6. Mean phytohemagglutinin (PHA) stimulation index, a measure of T cell-mediated immunity, in pre-fledgling fish-eating birds at AOCs, Grand Traverse Bay, and reference sites in 2010-17. Numbers on bars indicate sample sizes, and error bars indicate standard errors. Dotted lines indicate site means. Sites with the same letter(s) were not statistically different by Tukey's test ( $p < 0.05$ ).

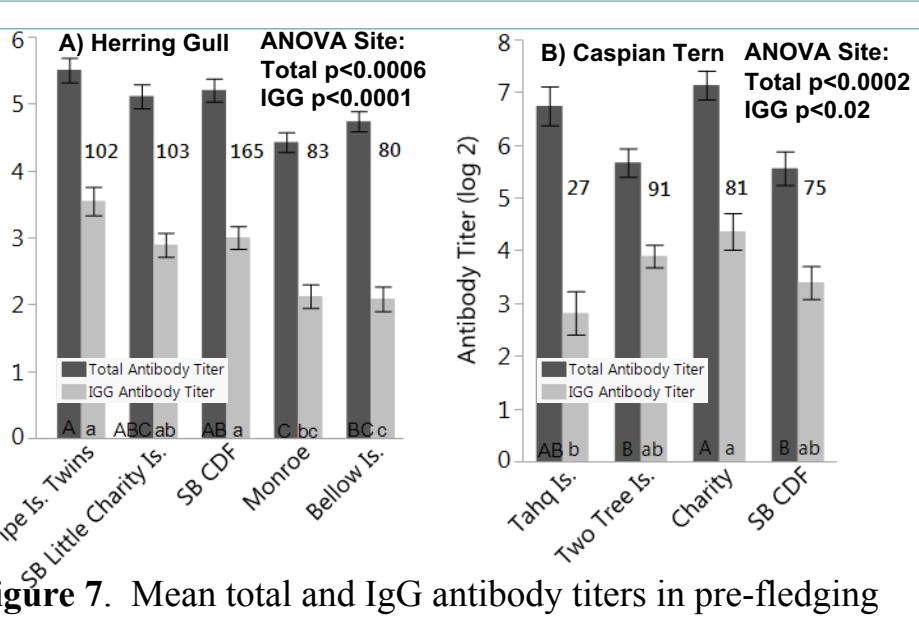


Figure 7. Mean total and IgG antibody titers in pre-fledgling herring gulls at AOCs, Grand Traverse Bay, and reference sites in 2010-17. Sites sharing letter(s) were not significantly different by Tukey's test ( $p < 0.05$ ). Numbers indicate sample sizes. Error bars indicate the standard error.

## Results and Discussion

### Embryonic Nonviability and Deformities

Embryonic nonviability rates in late incubation herring gull eggs were elevated in the River Raisin AOC (8.6%), both islands in the Saginaw Bay AOC (6.7-6.8%), and in Grand Traverse Bay (12.9%) compared to the Pipe Is. Twins reference site (3.2%; Fig. 2). Infertility was the primary cause of nonviability at the reference site and was further elevated at all AOC colonies and Grand Traverse Bay. Embryonic death (failed development) also accounted for a sizable portion of total nonviability at all contaminated colonies including both AOCs and Grand Traverse Bay.

Nonviability data were analyzed statistically by calculating relative risk (RR) ratios based on the incidence rates of abnormalities at the contaminated sites relative to the reference site. RR ratios of 1 indicate no difference in incidence rates between groups. There was strong statistical evidence that RR ratios for overall nonviability ( $p$  values from  $<0.014$  to  $<0.0001$ ) and infertility ( $p$  values from  $<0.036$  to  $<0.0007$ ) were significantly elevated two to four fold at contaminated sites (Table 1). This conclusion held for individual islands, grouping islands within AOC for Saginaw Bay, and grouping all contaminated sites together. Failed development was also significantly elevated for both AOCs, Grand Traverse Bay, and all contaminated sites together ( $p$  values from  $<0.055$  to  $<0.0034$ ).

During the course of this study a number of embryos and chicks have been observed with deformities such as crossed bills (Fig. 3) and gastoschisis, but only at AOC colonies. Embryonic nonviability and deformities such as crossed bills and gastoschisis are characteristic of GLEMEDS and are associated with exposure to PCBs and dioxins (Gilbertson et al. 1991, Grasman et al. 1998).

**Reproductive Success**  
While productivity was consistently good at reference colonies, herring gulls in the River Raisin AOC and Caspian terns in the Saginaw Bay AOC had the lowest mean productivity and experienced complete (or nearly complete) colony-level reproductive failure in several years (Fig. 4). Mean productivity of 3 week herring gull chicks differed significantly among sites (ANOVA  $p=0.0001$ ; Fig. 4A), with highest mean productivity (2 chicks/nest) on the SB CDF and the lowest productivity (1 chick/nest) in the River Raisin AOC. All other site means were above the level necessary to maintain a stable population (0.8 chicks/nest, Kadlec and Drury 1968). However, productivity at Monroe was below 0.8 chicks/nest in 4 of 8 years, including complete reproductive failure in one year. Mean productivity of 3 week Caspian tern chicks was approximately 40% lower in Saginaw Bay compared to reference sites (ANOVA  $p=0.0001$ ; Fig. 4B). When the four sites were ordered by PCB contamination, there was strong statistical evidence for decreasing productivity with increasing PCBs (Jonkheere  $P<0.011$ ). When the reference sites were pooled into one group and the two Saginaw Bay colonies into an AOC group, the difference in productivity was highly significant (one-tailed  $T$ -test  $p<0.0001$ ). Overall these data indicate a continuing and consistent reproductive impairment in Caspian terns, a state-threatened species, in the Saginaw Bay AOC.

### Growth

Growth (weight gain) in pre-fledgling gulls differed significantly among sites (ANOVA  $p<0.0001$ ), with Monroe in the Raisin River AOC significantly higher than all other colonies (Fig. 5A). Only Monroe had a mean multiyear growth rate in the expected range for herring gull chicks of 14-20 g/day established by a previous study (Grasman et al. 1996). However, the high mean at Monroe was driven by extremely high growth in two years, with poor growth in the other five of seven years of study.

Chicks at both AOCs and Bellow Island had poor rates of growth in almost half of the years studied. Mean growth at Bellow fell below the normal range in three of four years in the study. Mean weight loss or wasting occasionally was observed in the Saginaw Bay AOC (two years on Little Charity Island and one year on the SB CDF) and during one year on the Pipe Is. Twins reference site. Growth in tern chicks was significantly lower on the SB CDF compared to all other colonies (ANOVA  $p<0.0001$ ; Fig. 5B) and was below the the expected range of 4.0-18.0 g/day for Caspian terns (Grasman et al. 1996). The low growth rates at the herring gull reference colony are most likely due to low food supplies. However, food is abundant in the highly productive Saginaw Bay and River Raisin AOCs, so food shortages are not likely to be responsible for poor growth in gulls and terns in these areas.

### T Cell-Mediated Immunity

Herring gulls and Caspian tern chicks had lower T cell-mediated immune responses at all AOC sites as well as Grand Traverse Bay compared to the reference sites (ANOVA  $p<0.0001$ ; Fig. 6). The mean PHA skin response of gulls was 52-55% lower at AOCs than at the Pipe Island Twins reference site. Gulls in Grand Traverse Bay had a 46% lower mean PHA skin response than the reference site. Caspian terns at AOCs had a 46% lower response as compared to Two Tree Island. Black-crowned night herons on the SB CDF showed a 39% lower T cell response compared to herons at the Chantry Island reference site ( $T$ -test  $p=0.05$ ) (Fig. 6C). The magnitude of the suppression in the PHA response at contaminated sites was biologically significant and comparable to the complete removal of the thymus gland in chickens, which causes a reduction in the stimulation index by approximately 50-60% (Grasman et al. 1996). Furthermore, lower PHA responses have been associated with reduced survival and dispersal in wild birds (Moeller and Saino 2004, Moeller and Cassey 2004).

### Antibody Response

Pre-fledgling herring gulls at the River Raisin AOC and in Grand Traverse Bay had significantly two to three fold lower total antibody and IgG responses than herring gulls at the Pipe Island Twins reference site (ANOVA  $p=0.0006$  for total  $p=0.0001$  for IgG; Fig. 7A). Total and IgG responses in gulls in the Saginaw Bay AOC were similar to those at the reference site. Total antibody responses in Caspian terns were significantly higher at Charity than Two Tree Is. and the SB CDF (ANOVA  $p=0.0002$ ; Fig. 7B). IgG responses were significantly lower at Tahquamenon Is. than at Charity (ANOVA  $p=0.023$ ).

## Conclusions

Herring gulls, Caspian terns, and black-crowned night herons in the Saginaw Bay AOC showed impairments in immune responses and (or) reproduction consistent with past studies, which were consistent with past studies

- Embryonic nonviability, primarily infertility but also failed development, was elevated in gulls
- Terns had lower overall productivity in the AOC when compared to reference sites
- Growth of tern chicks was significantly lower on the SB CDF than the reference site
- Suppressed T cell-mediated immune was demonstrated by herring gulls, Caspian terns, and black-crowned night herons

### Herring gulls at the River Raisin AOC showed impairments in immune responses and reproduction, consistent with past studies

- Embryonic nonviability, including both infertility and failed development, was elevated in gulls in the River Raisin AOC
- Complete reproductive failure in one year, and low chick productivity in three other years
- Low growth rates in gull chicks in five out of seven years
- Suppressed T cell-mediated immune response
- Suppressed total antibody and IgG responses

### Herring gulls at Grand Traverse Bay, a site with high concentrations of TEQs and DDE, showed impairments in immune responses and reproduction

- Elevated embryonic nonviability, including both infertility and failed development, in gulls
- Low growth rates between three and four weeks of age
- Suppressed T cell-mediated immune response
- Suppressed total antibody and IgG responses

## Acknowledgements

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## References

- Bowerman,