Immunological and Reproductive Health Assessment in Herring Gulls and Black-Crowned Night Herons in the Hudson Raritan Estuary

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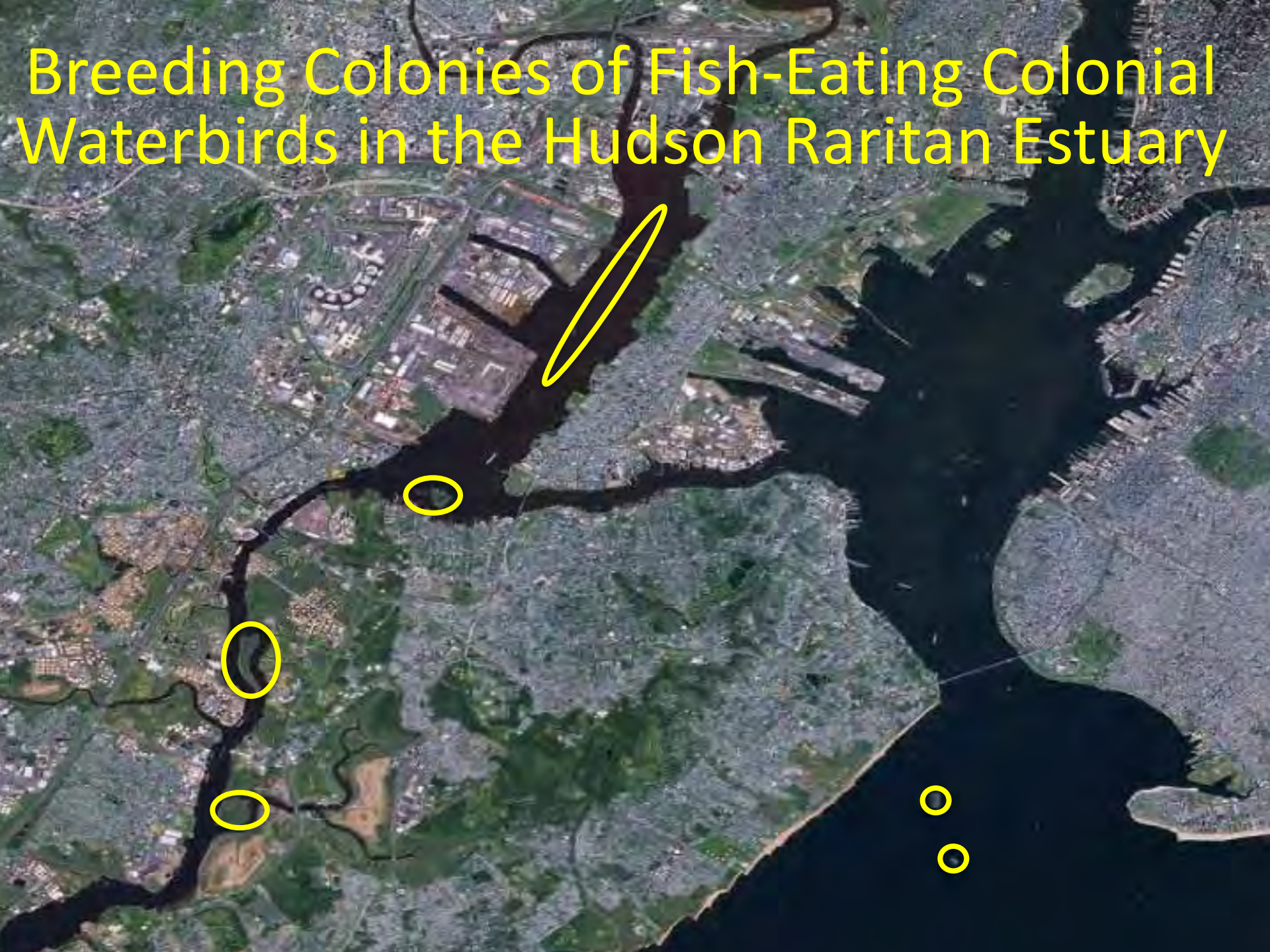
Breeding Colonies of Fish-Eating Colonial Waterbirds in the Hudson Raritan Estuary



Fish-Eating Birds Colonial Waterbirds

- Excellent sentinel species for contaminants
  - Persistent organic pollutants that biomagnify
  - Both exposure and effects

- Few previous studies in Hudson Raritan
  - Population declines and colony disappearances
  - Significant concentrations of dioxin-like compounds (DLCs) (PCDDs, PCDFs, and planar PCBs) in cormorant eggs

Objectives of the 2003 Study

1) To investigate the reproductive success of herring gulls in lower New York Harbor

2) To assess the immunological health of herring gulls and black-crowned night herons

3) To explore potential associations between environmental contaminants and impaired immune function or reproduction

Immunotoxicity of DLCs

- Many mechanisms of immunotoxicity
  - Thymic atrophy and suppressed T cell function
    - Acute, chronic, and developmental exposure
  - Altered antibody responses (often acute exposure)
  - Decreased/increased lymphocyte proliferation *in vitro*
  - The developing immune system is particularly sensitive

- Associated with increased infections:
  - Mallard ducklings challenged with duck hepatitis virus
  - Marine mammals--cetaceans and pinnipeds
  - Norwegian glaucous gulls--intestinal nematodes
  - Inuit children in northern Quebec--ear infections

Liver OCs & metals

Growth b/w 3-4 wk
Survival to 3 & 4 wk

PHA skin response
(T cell function)

Lymphocyte
mitogenesis

Lymphocyte
mitogenesis

# of lymphoid
cells in thymus
and bursa of
Fabricius

Swinburne Island

Herring gulls

PHA skin response
(T cell function)

Hoffman Island

Black-crowned night herons

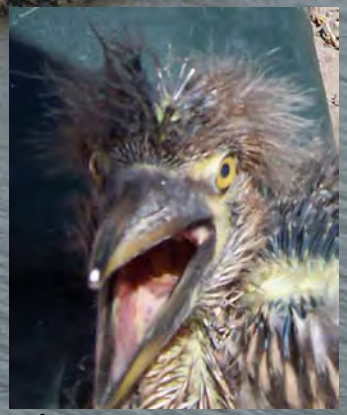
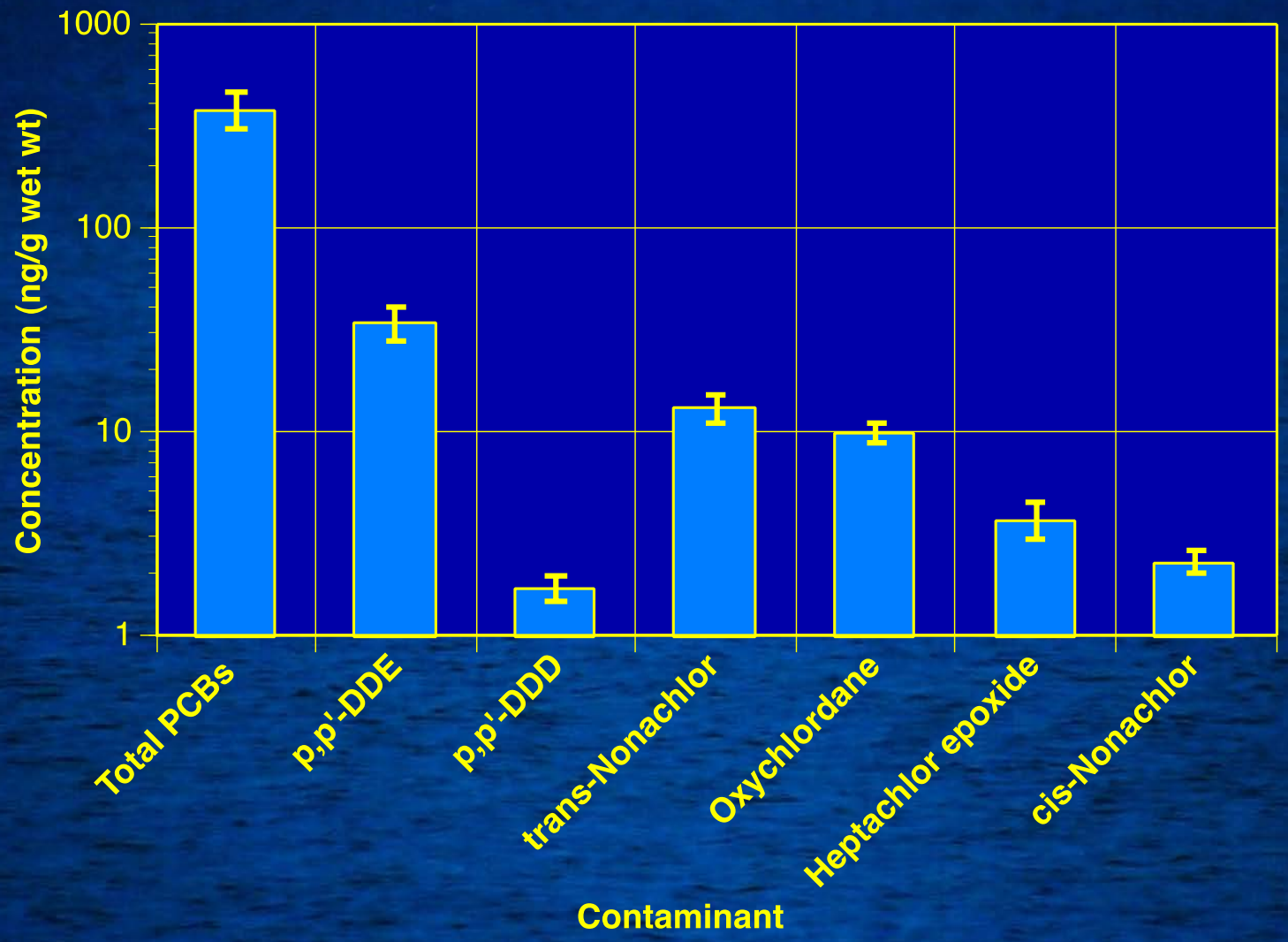


Photo Credit: USFWS-NJFO

Organochlorines in Livers of Herring Gull Chicks

4 Weeks Old

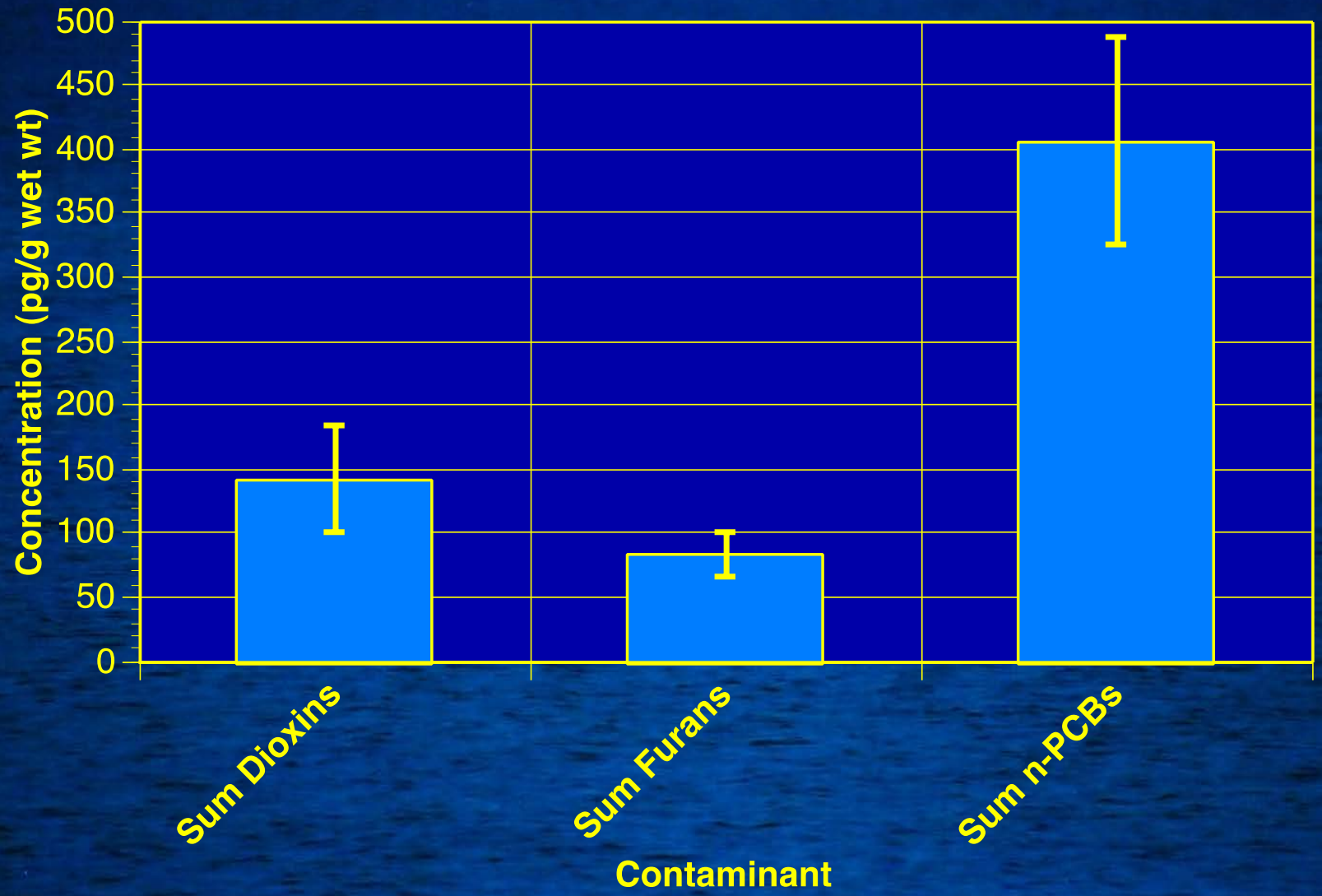
Lower New York Harbor



DLCs in Livers of Herring Gull Chicks

4 Weeks Old

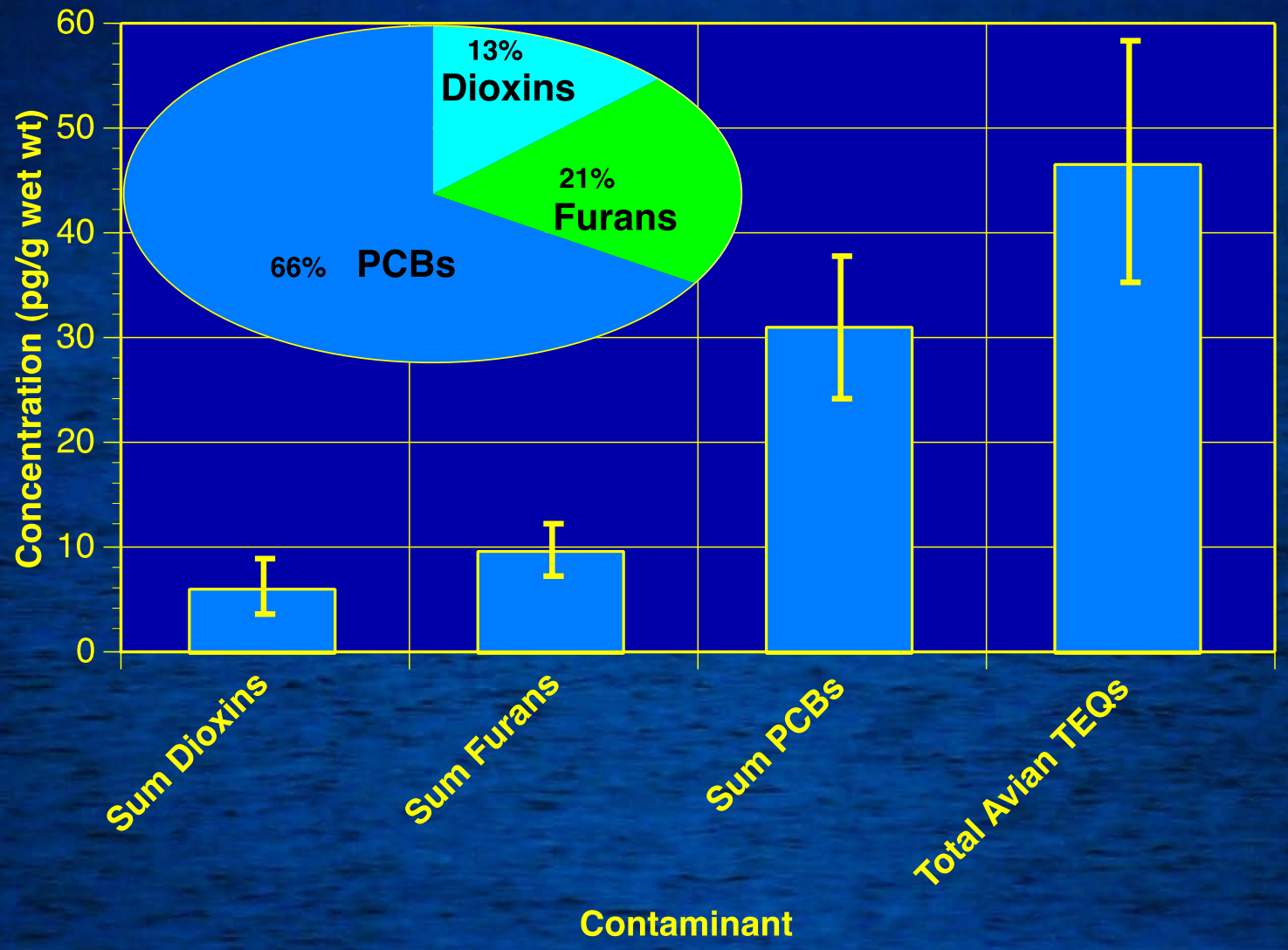
Lower New York Harbor



TEQs in Livers of Herring Gull Chicks

4 Weeks Old

Lower New York Harbor



Reproductive Success in Herring Gulls

- Usually lay 3 eggs per nest
- Population models show that an average of 0.8 young/nest at 3 weeks will maintain a stable population, although higher values are frequently observed

- In lower NY Harbor, chick productivity was only 0.68 young per nest at 3 weeks
- Decreased further to 0.5 young per nest 4 weeks

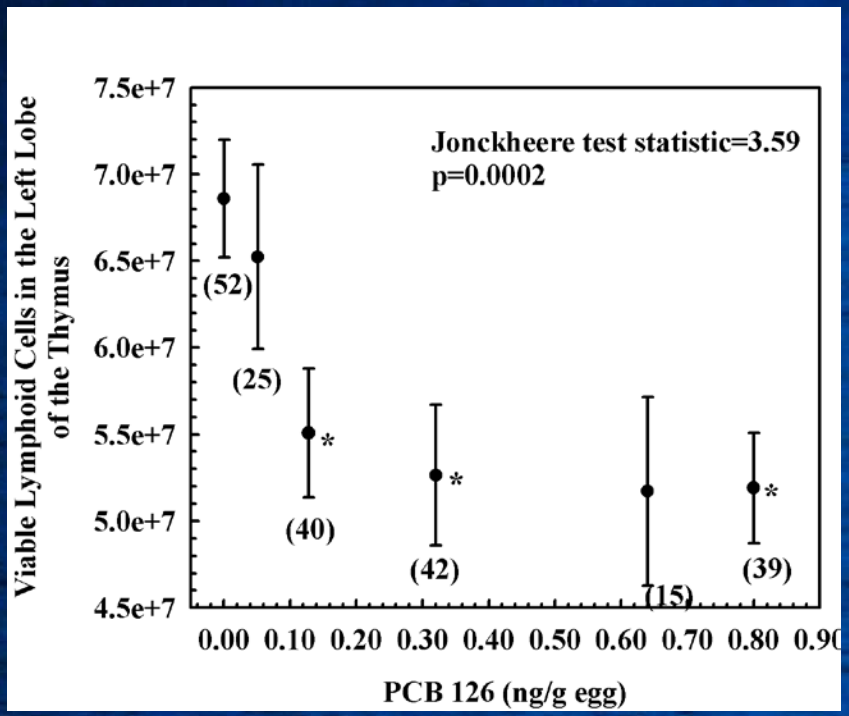
Growth in Herring Gull Chicks

- Herring gull chicks often grow at a rate of 15-20 g/day between 3 and 4 weeks

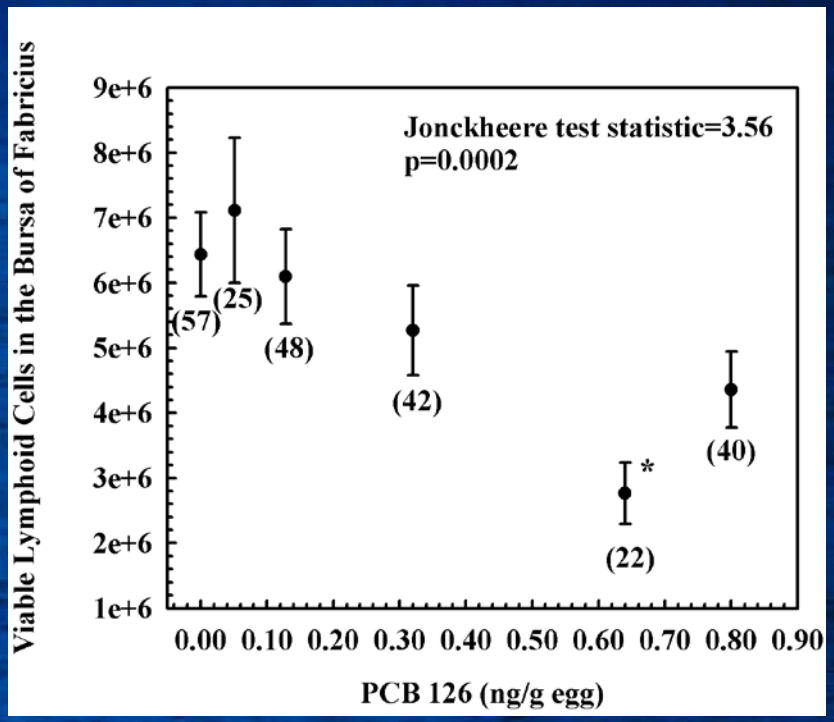
- In lower NY Harbor, the mean growth rate was only 4.7 g/day
- Only 50% of the chicks gained body mass during this time period, with 40% losing weight and 10% gaining none

Developmental Immunotoxicity of DLCs in Chicken Embryos

Thymus: T cells



Bursa of Fabricius: B cells

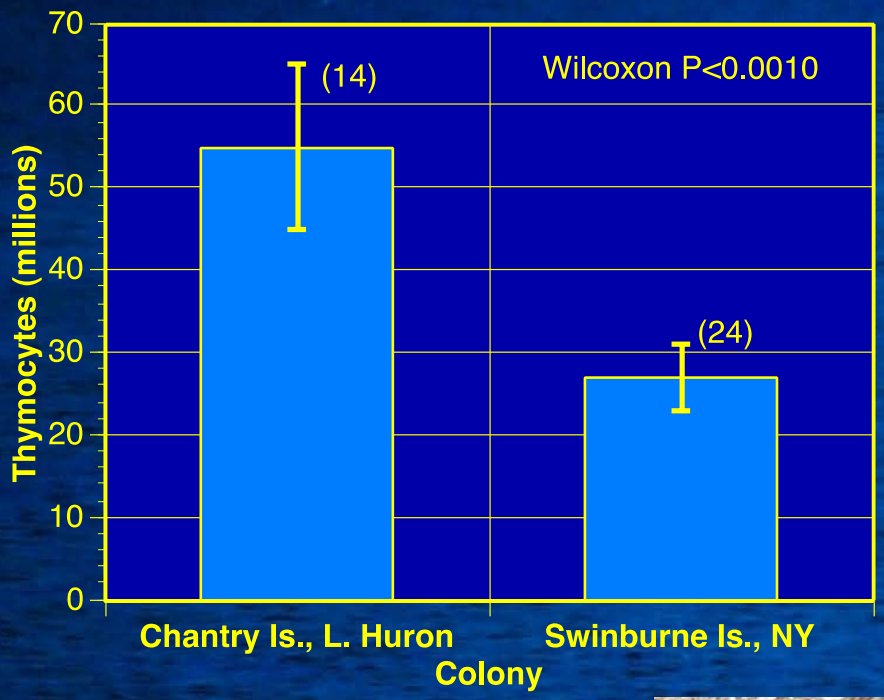


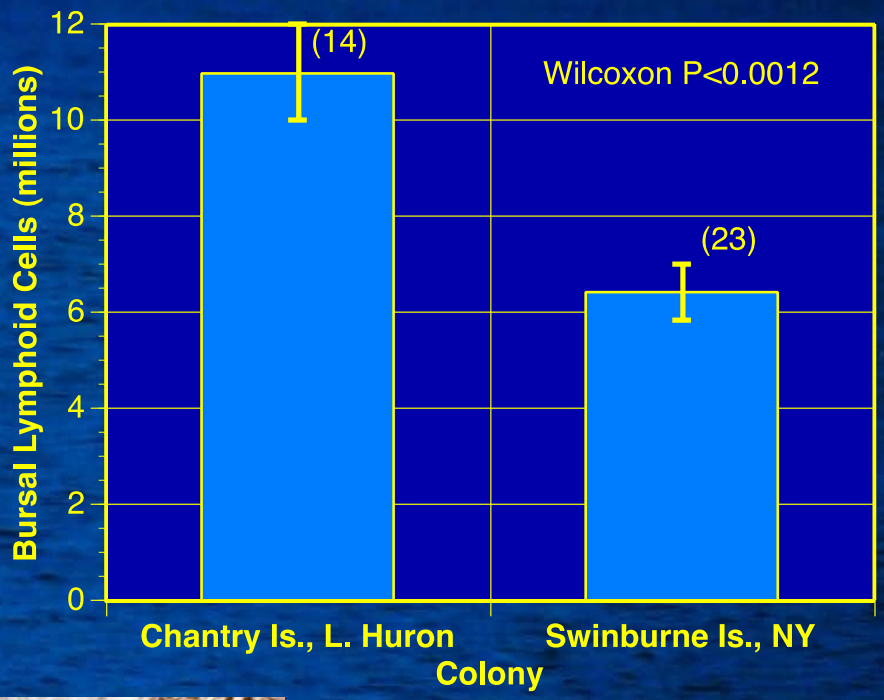
PCB 77 and Aroclor 1254 cause similar immunotoxicity

Environmentally relevant doses after accounting for sensitivity

Lymphoid Cells in Thymus and Bursa

Developmental Immunotoxicity in Pipping Herring Gull Embryos





Lymphocyte Isolation & Cryopreservation

Heparinized blood sample

↓

Slow spin centrifugation & remove lymphocytes & plasma

↓

Pellet & re-suspend lymphocytes in media & DMSO

↓

Slow cooling and freezing on dry ice & then LN_2

Proliferation Assay

Thaw, dilute DMSO & incubate

↓

Count, assess viability, plate into 96 well plates

↓

Culture with and without T and B cell mitogens

↓

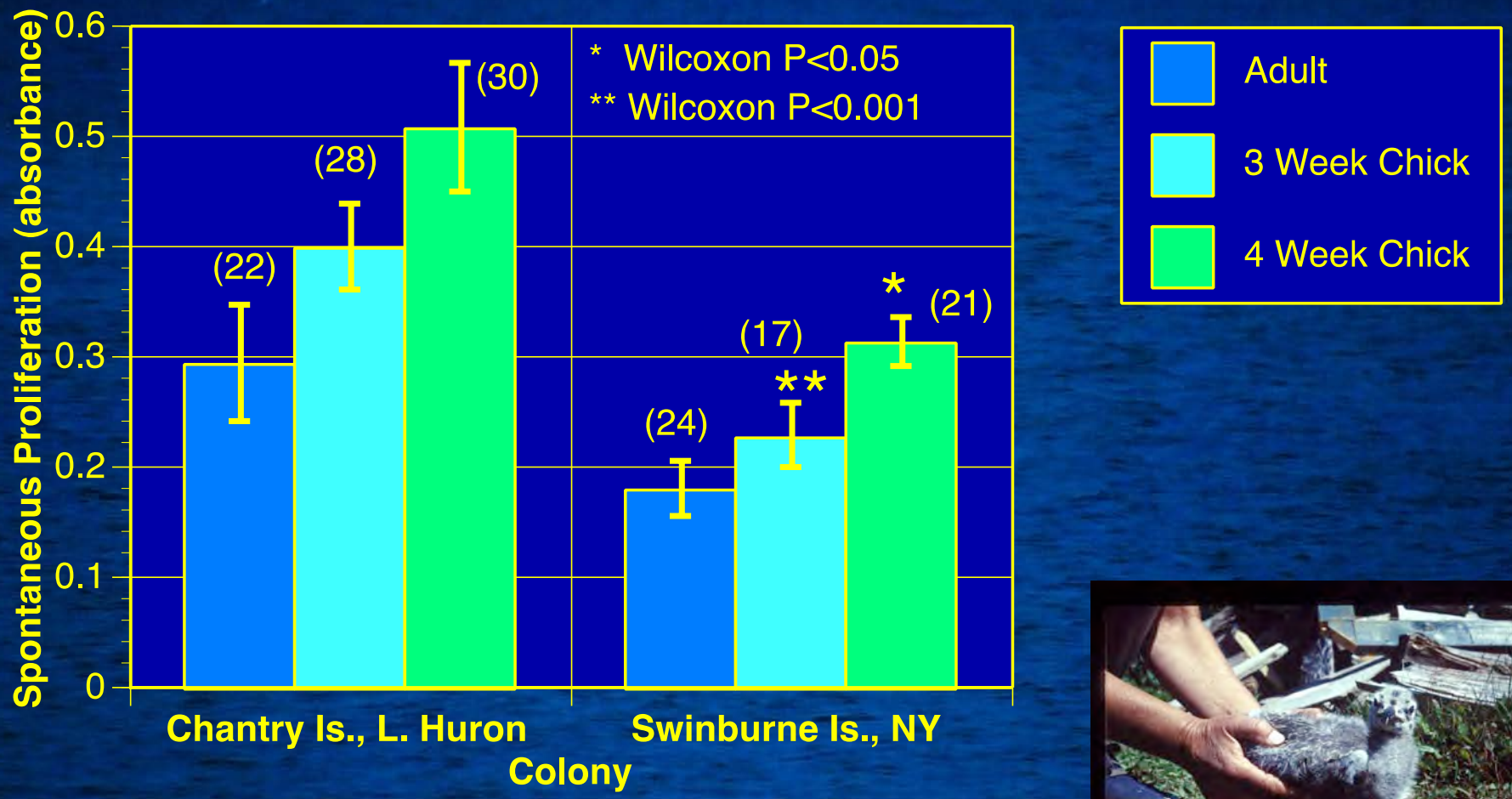
Add Brdu: incorporated into new DNA

↓

Brdu ELISA provides a measure of cell proliferation

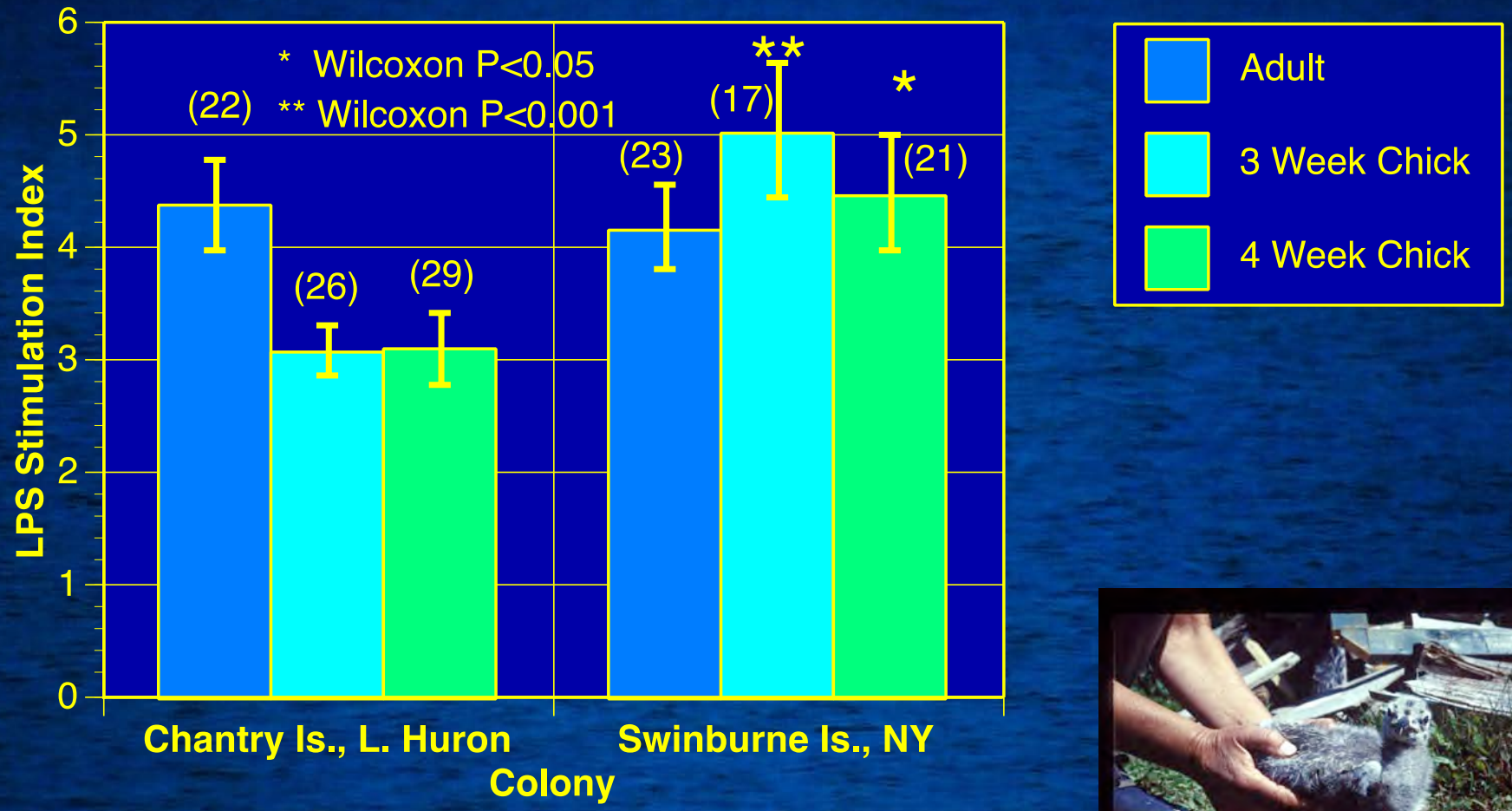
Lymphocyte Proliferation

Spontaneous Cell Division



B Lymphocyte Proliferation

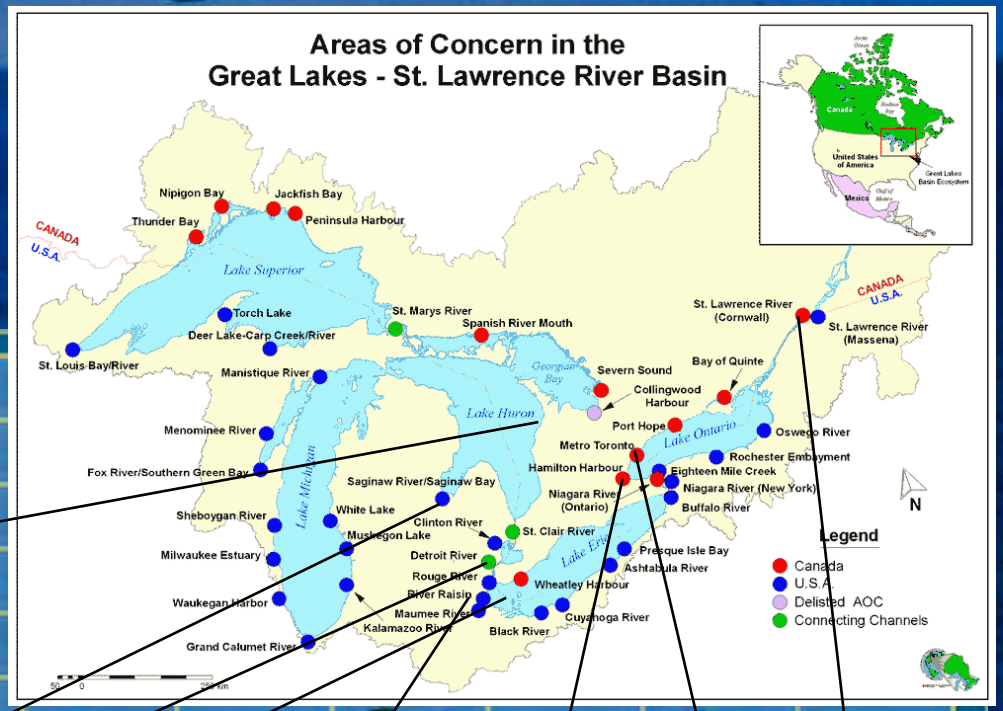
Division of Antibody-Producing Cells

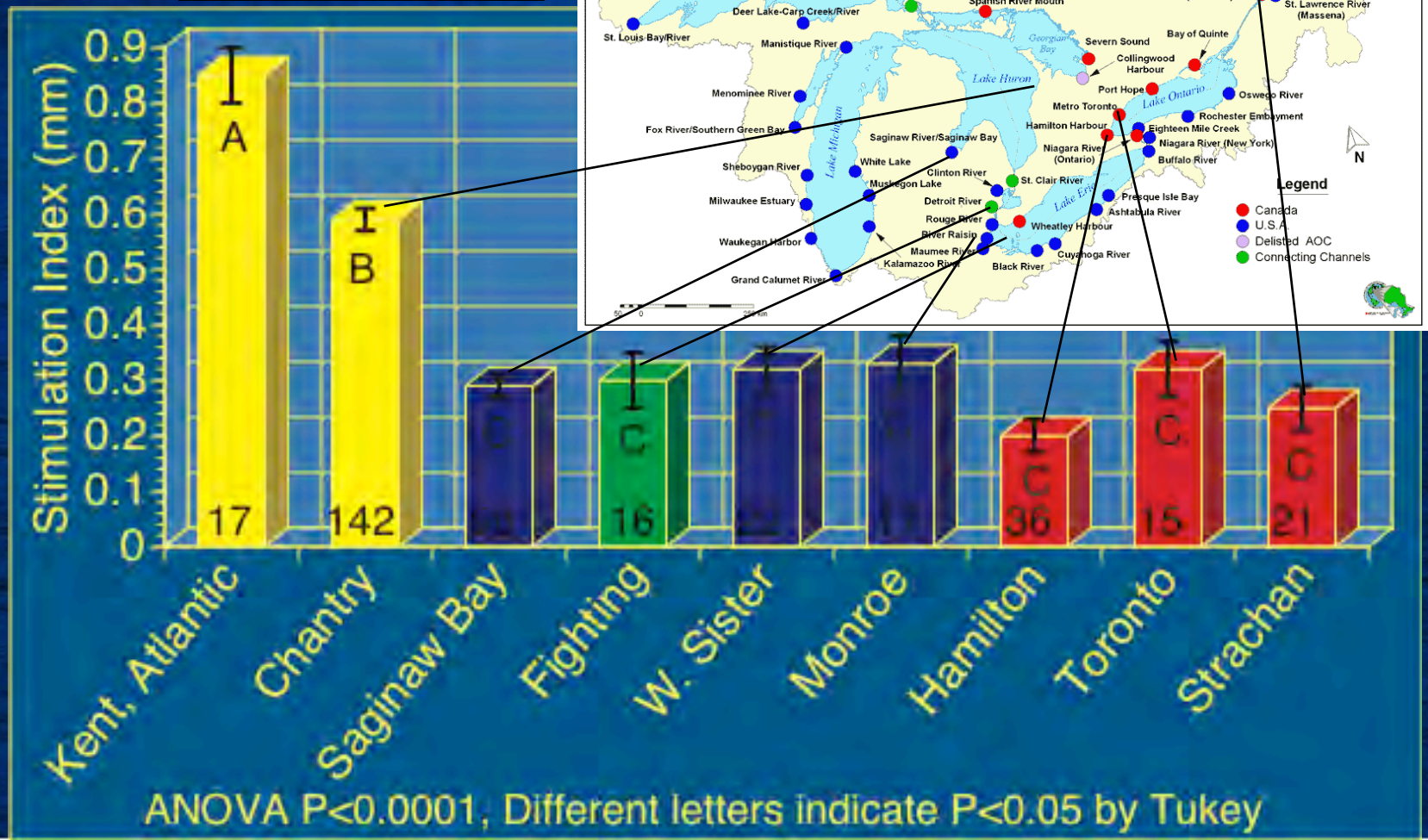


Phytohemagglutinin Skin Response

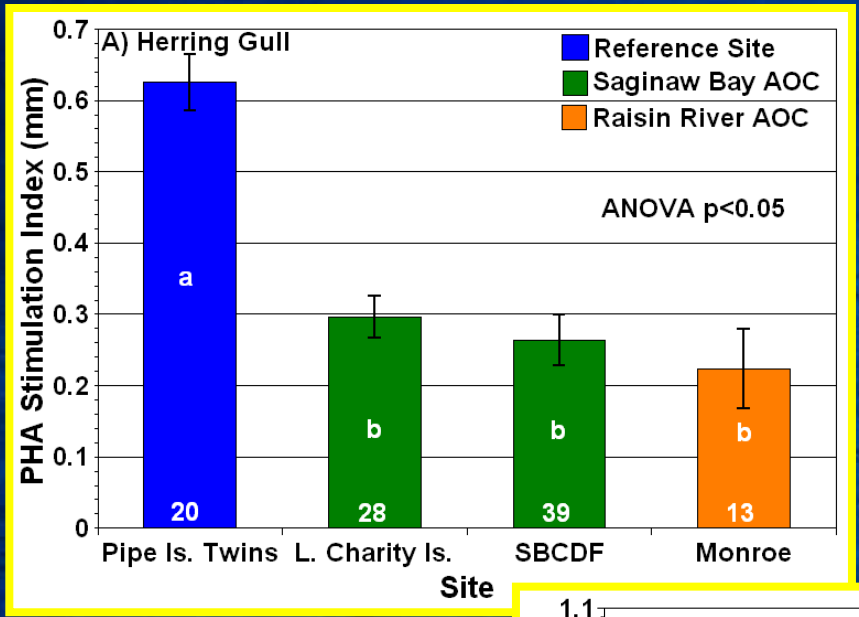
- Intradermal PHA causes a T cell-dependent inflammation in 12-48 h
- Integrates multiple T cell functions:
  - Proliferation, differentiation, cytokine, WBC infiltration
- Elimination of T cells with drugs or irradiation reduces the response by 50-60% in captive birds
- Similar to human tuberculin skin test
- One of the most common immune assays in avian immunotoxicology and immuno-ecology
  - Low response = low survival in wild birds

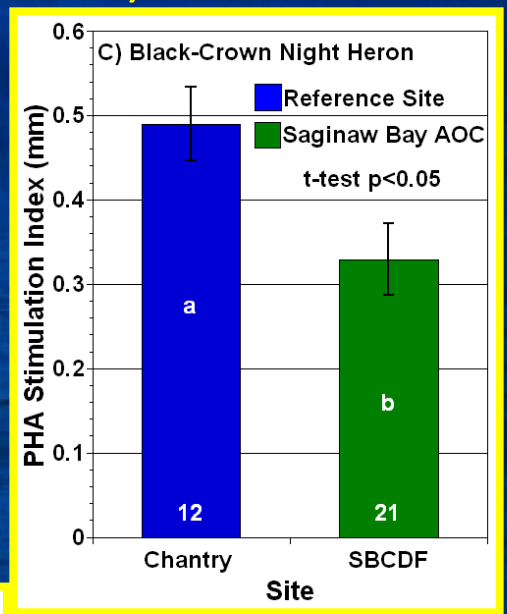
T-Cell Function in Young Herring Gulls, 2001-04

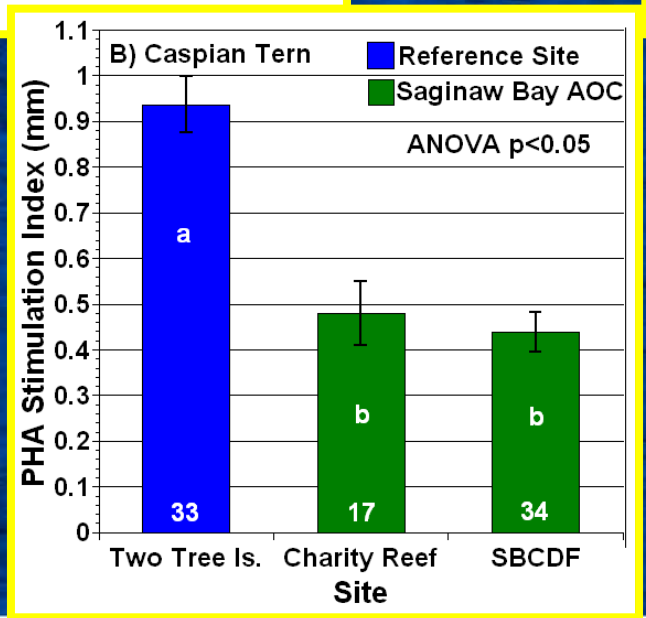




PHA Skin Response at Michigan's Areas of Concern (poster WP113)

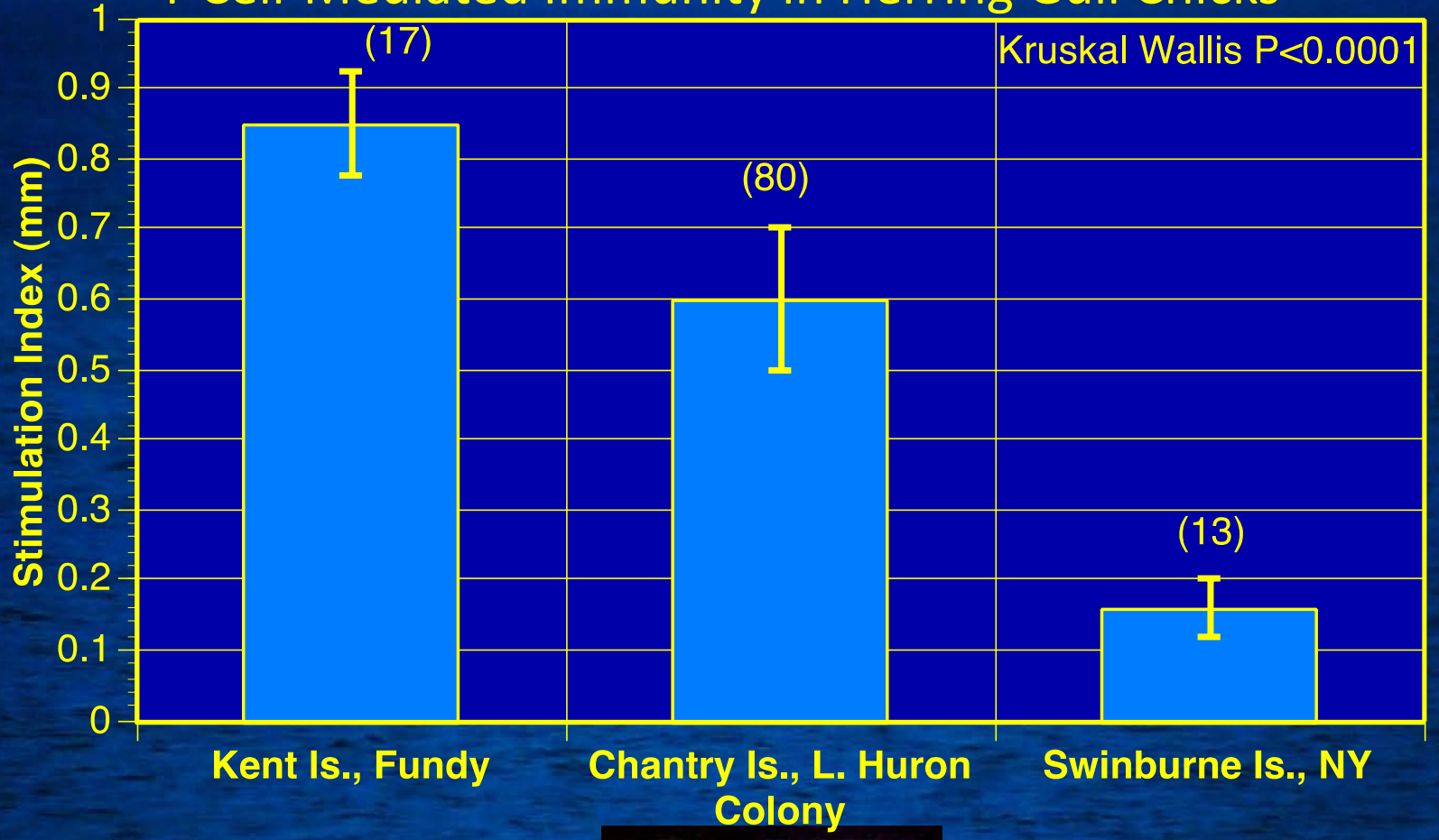






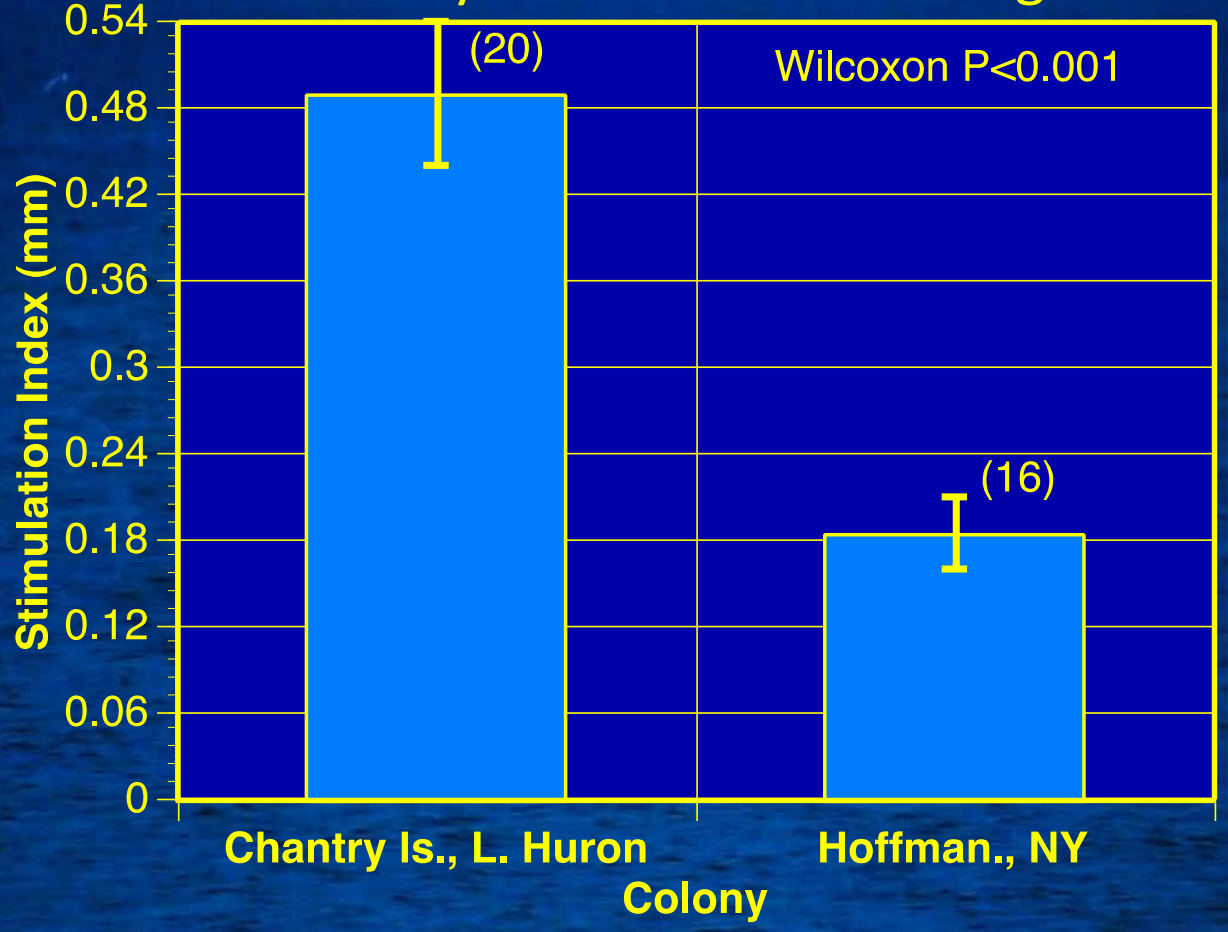
PHA Skin Response

T Cell-Mediated Immunity in Herring Gull Chicks



PHA Skin Response

T Cell-Mediated Immunity in Black-Crowned Night Heron Chicks



Ecological Significance of a Suppressed PHA Skin Response in Wild Birds

- 12 studies on immune response and subsequent survivorship (9 PHA)
  - "The relationship between immune response and survival accounted for 18.4% of the variance, while three other studies of potential predictors of survival (secondary sex characteristics and symmetry) only accounted for 1.4, 1.5 and 6% … Thus, immune response is by far the best predictor identified so far."

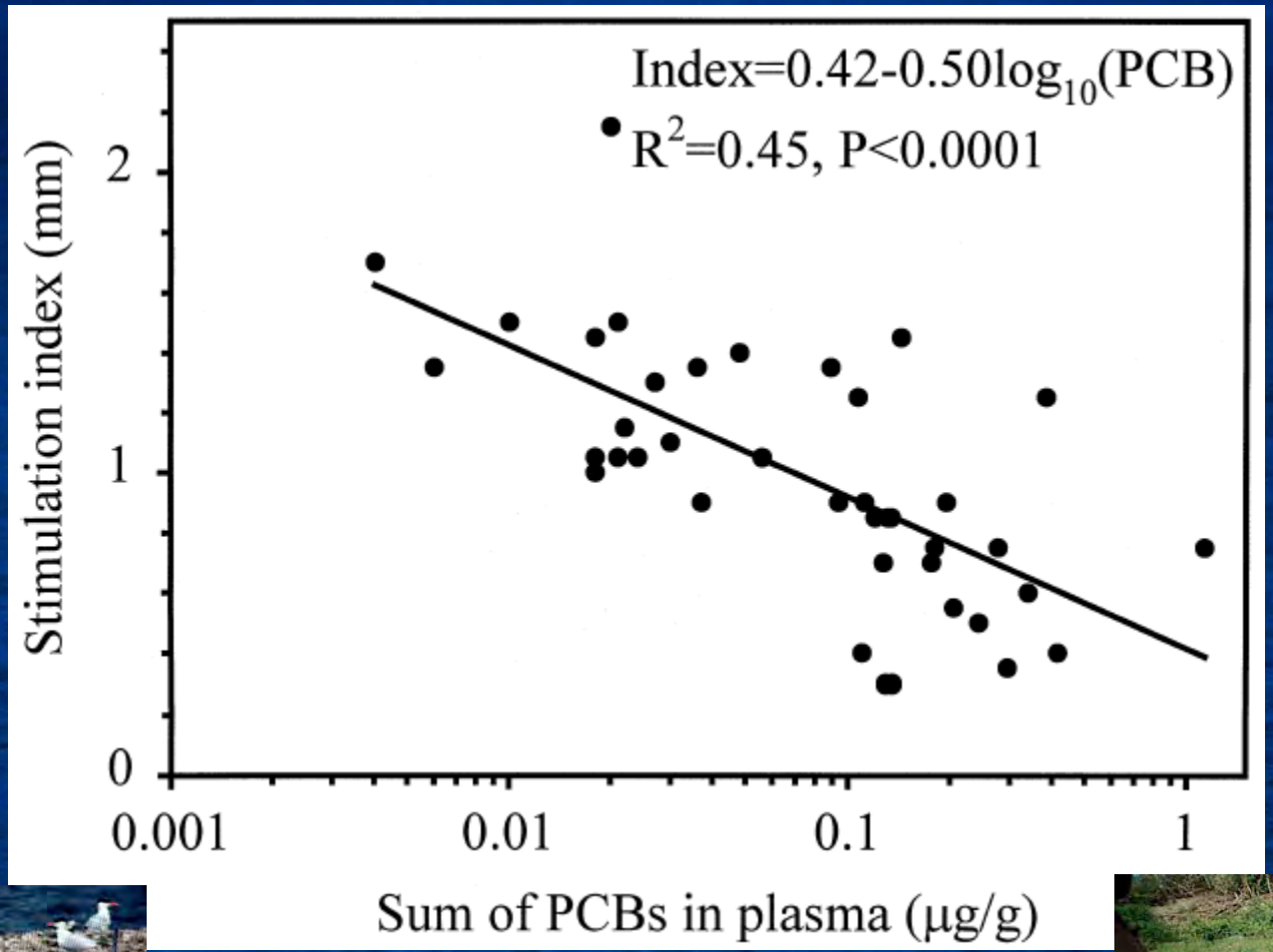
    (Moeller & Saino, 2004, Oikos 104:299-344)

- Another meta-analysis concluded that higher PHA responses in nestlings increased the probability of establishing a new local population, presumably because a strong immune system helped fight novel diseases (Moeller & Cassey, 2004, J Animal Ecol 73:1035-42)

Fixed effect	Parameter estimate	Standard error	*t* statistic
Life history			
Log_{10}(Body mass)	0·40	0·69	0·59
Log_{10}(Annual fecundity)	1·55	1·77	0·88
Ecology			
Habitat generalism	1·11	0·25	4·39**
Migratory habit	−0·40	0·33	−1·21
Sexual monochromatism	0·30	0·59	0·51
Introduction event			
Log_{10}(no. of propagules)	0·73	0·18	4·15**
Immune response			
Nestling T-cell response	0·75	0·40	1·88*
Adult T-cell response	2·96	4·35	0·68

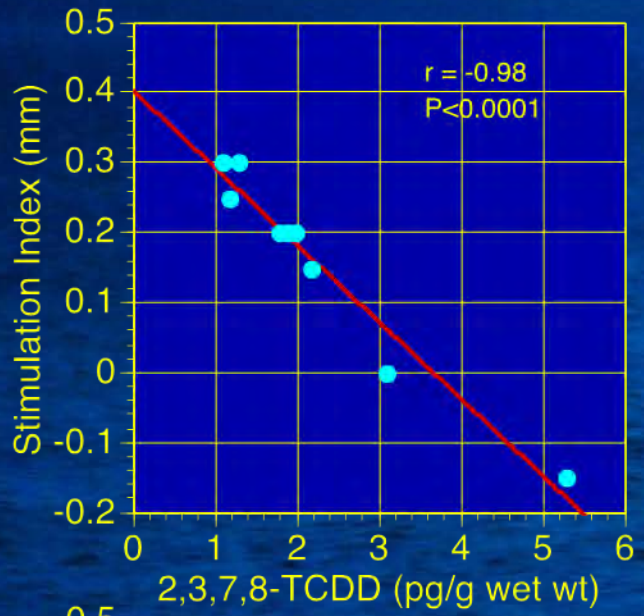
*$P < 0·05$, **$P < 0·01$.

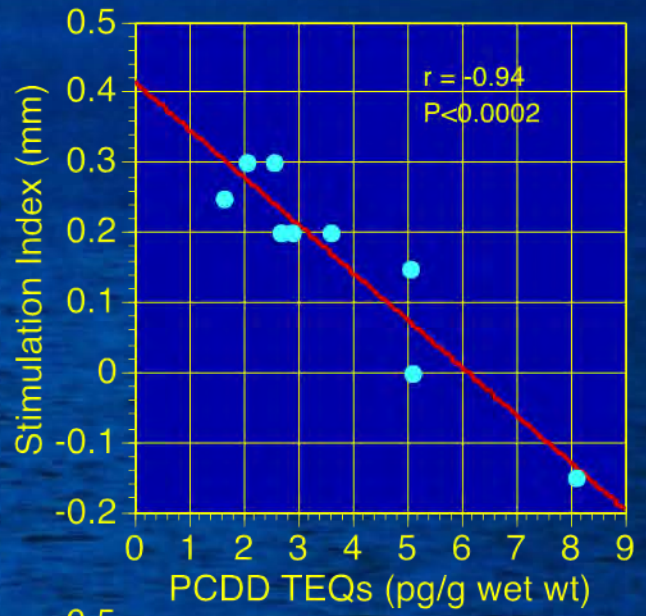
T-Lymphocyte Function in Great Lakes Caspian Terns

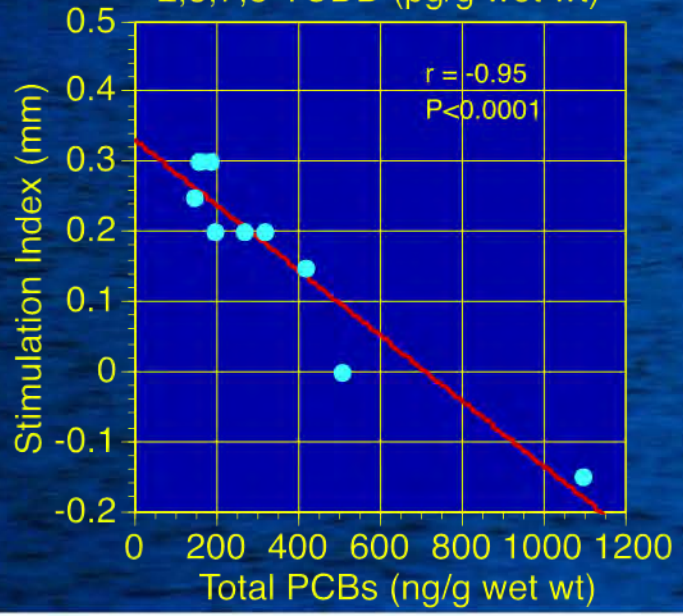


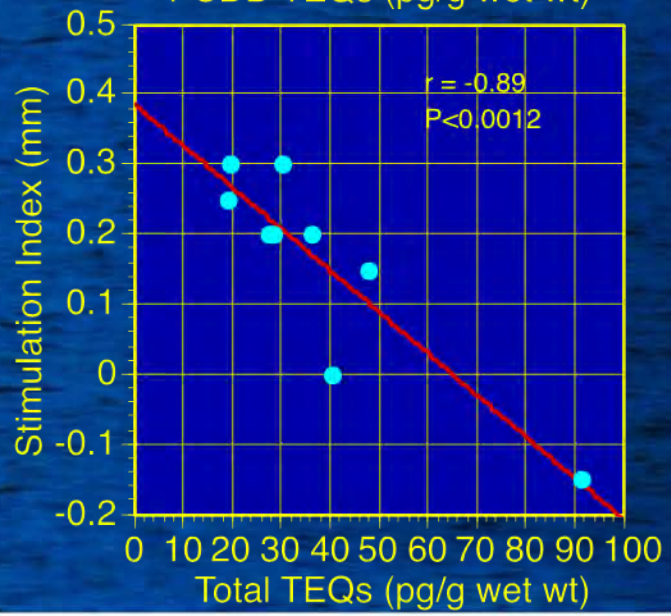
Grasman and Fox Ecotoxicology, 10, 101–114, 2001

Associations between PHA Response & DLCs









Conclusions

- Significant concentrations of PCDDs, PCDFs, and PCBs in livers of herring gull chicks in lower New York Harbor
  - Other OCs and metals were comparably low
  - DLCs were lower than those at highly contaminated Great Lakes sites
- Herring gulls had poor chick productivity and growth
- Numerous measures of altered immune status
  - Reduced developing lymphoid cells in gull embryos
  - Altered lymphocyte proliferation in gull chicks
  - Suppressed PHA response for T cell function in gulls and herons
- Immune effects were consistent with other studies of DLCs in wild and captive birds
  - Strong negative associations between the PHA response and dioxins and PCBs in gull livers suggested that these chemicals contribute significantly to immunosuppression in New York Harbor waterbirds

Acknowledgments

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  - Tim Kubiak of USFWS, project officer

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