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Fall 2005

# ES/RP 531
# Fundamentals of Environmental Toxicology

Lecture 11

Reproductive Toxicity

Case Studies:

Frogs & Atrazine; Eggshell Thinning

## Reproductive Physiology: General Processes

- Timing and type of breeding behavior
- Successful mating and fertilization
- Fecundity (how many eggs produced per female
- Fertility (how many eggs successfully hatch or number of live young produced)
- Embryonic development
- Organizational vs. activational effects
- Gender determination
- Hatchling or offspring growth
- Parental care

## Overview of Reproductive Physiology in Selected Animal Groups

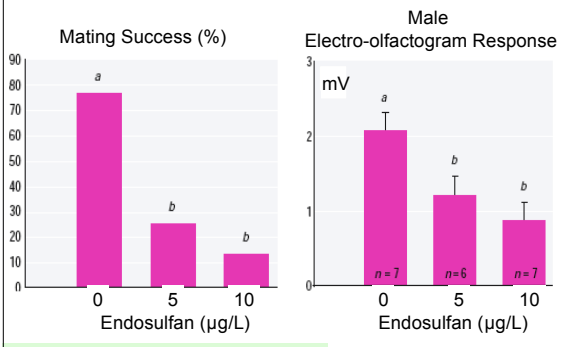
(See Lecture Outline Notes)

- Daphnia (representing Crustacea)
- Fish
- Amphibia
- Birds

## Reproductive Toxicity: General Principles

- Effects include:
  - Reduced survivorship of breeding adults
  - Reduced ability to successfully fertilize eggs
  - Reduced egg viability and hatchability
  - Unsuccessful development of embryo
  - Adverse effect on mating displays and male/female attraction

Hypothesis: Endosulfan disrupts successful mating of red-spotted newts through effects on sex pheromone release in females




Park et al. (2001) Environ. Hlth. Perspect. 109:669

## Reproductive Toxicity of Contaminants

- In vivo laboratory studies
  - Measure/observe physiological parameters of reproductive physiology, including any aspect of endocrine system function that is tied to embryonic development, neonatal development, and adult reproduction
  - Tissue histology
  - In invertebrates, such as *Daphnia* spp., will examine fecundity/fertility
- Field studies
  - Biomarkers of endocrine system function
  - Residues of contaminants in tissues
  - Hatching success; no. of fledglings (birds)

## EPA Requirements for Mammalian Developmental Reproductive Toxicity Testing

**EPA** **Health Effects Test Guidelines**
OPPTS 870.3550
Reproduction/ Developmental Toxicity Screening Test

**EPA** **Health Effects Test Guidelines**
OPPTS 870.3650
Combined Repeated Dose Toxicity Study With the Reproduction/ Developmental Toxicity Screening Test

**EPA** Health Effects Test Guidelines
OPPTS 870.3700
Prenatal Developmental Toxicity Study

**EPA** Health Effects Test Guidelines
OPPTS 870.3800
Reproduction and Fertility Effects

## EPA Requirements for Developmental & Reproductive Toxicity Testing of Invertebrates, Fish, & Birds

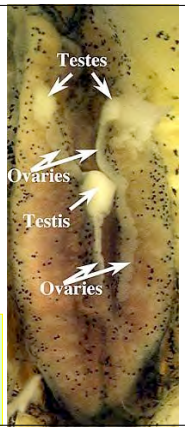
**EPA** **Ecological Effects Test Guidelines**
OPPTS 850.1500
Fish life cycle toxicity

**EPA** **Ecological Effects Test Guidelines**
OPPTS 850.2300
Avian Reproduction Test

**EPA** **Ecological Effects Test Guidelines**
OPPTS 850.1400
Fish Early-Life Stage Toxicity Test

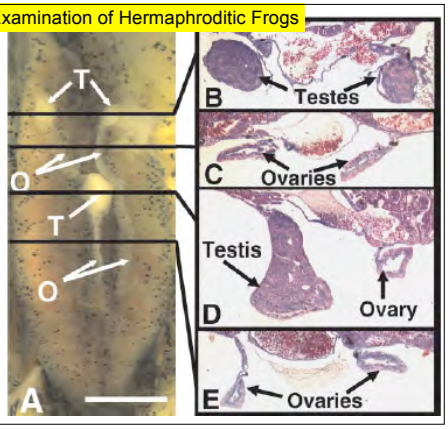
**EPA** **Ecological Effects Test Guidelines**
OPPTS 850.1300
Daphnid Chronic Toxicity Test

## 'Alarming' Atrazine Studies

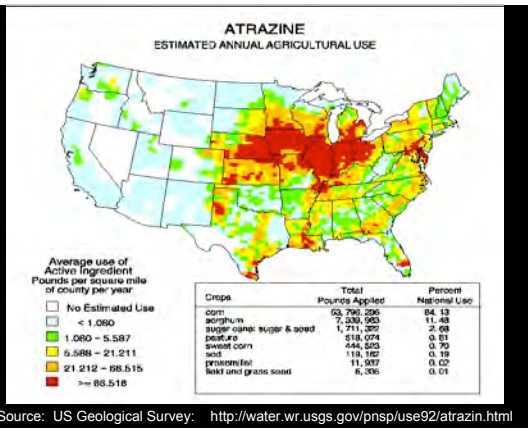



**African clawed frog tadpoles exposed to 0.1 ppb atrazine develop into hermaphrodites. (Hayes et al., 2002, PNAS)**

## Histological Examination of Hermaphroditic Frogs

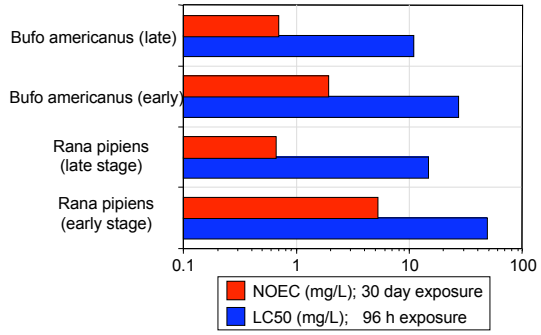



Hayes et al. (2002)



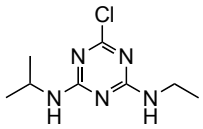

Source: US Geological Survey: http://water.wr.usgs.gov/pnsp/use92/atrazin.html

## Atrazine Acute & Chronic Toxicity (Lethality) Is Low Compared to High End Environmental Concentrations



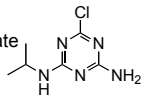

Howe et al. (1998) Environ. Toxic. Chem. 17:519

## About Atrazine

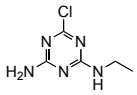



atrazine

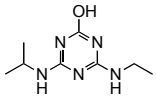
- Whole body half-life ~31 hours (rodents)
  - Does not bioaccumulate (not stored in fat)
- 95% eliminated after 7 days (rodents)
  - 75% in urine
  - 25% in feces
- Metabolites
  - Dechlorinated
  - Dealkylated




desethyl atrazine

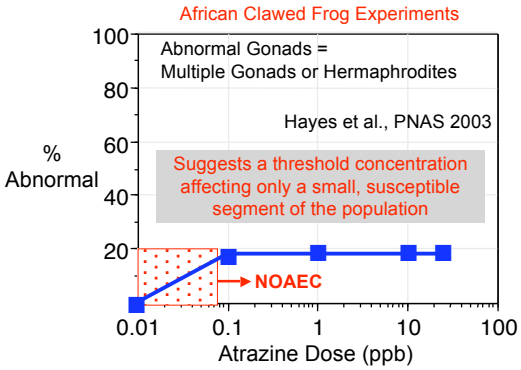



desisopropyl atrazine

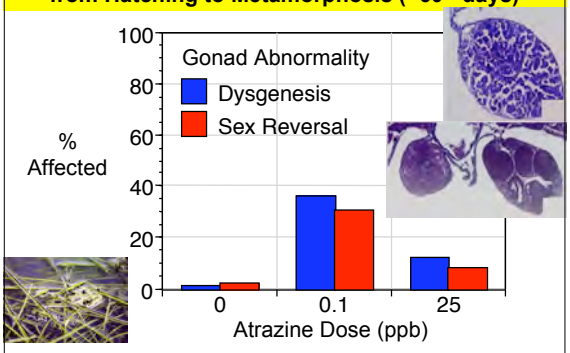



hyrdroxy atrazine

## No Dose-Response Trend for Abnormal Gonads!!

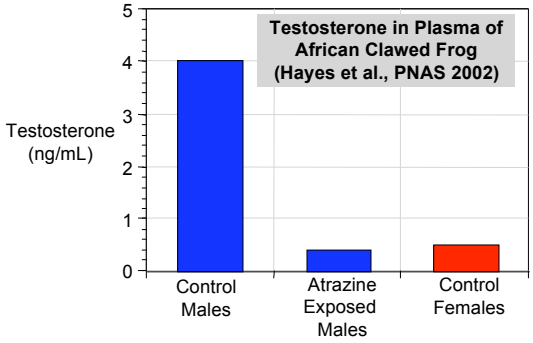


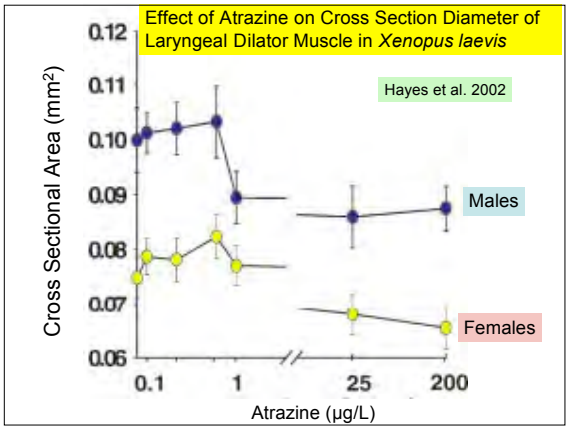

## Exposure of Leopard Frogs to Atrazine from Hatching to Metamorphosis (~60+ days)




Hayes et al., EHP 2003

## Exposure of males to 25 ppb atrazine for 46 days reduced testosterone levels

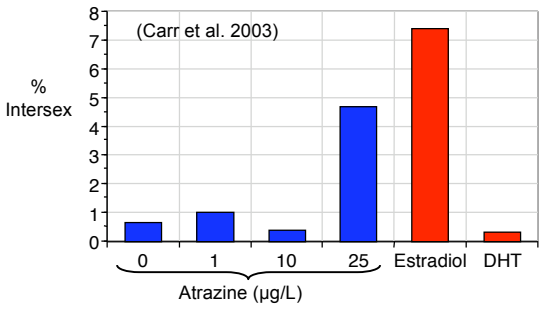


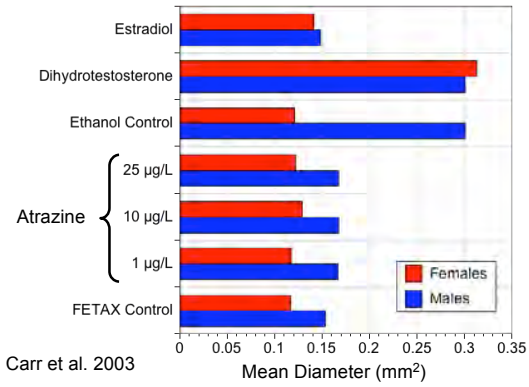

## Plausible Hypothesis?

- Atrazine induces aromatase, thus speeding up the oxidation of testosterone to estradiol
  - Evidence seems supported by rodent studies (although at high ppm doses of atrazine in cell cultures)

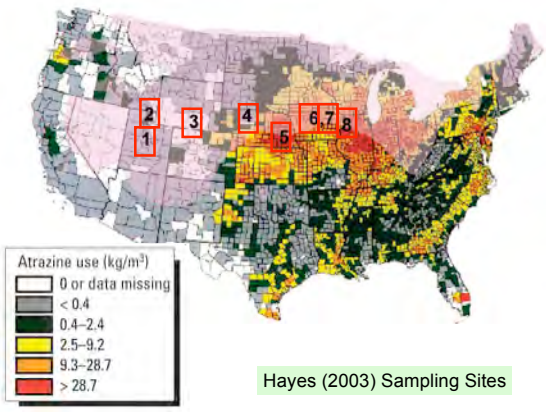
Effect of Atrazine, Estradiol, & Dihydrotestosterone (DHT) On Incidence of Intersex Frogs



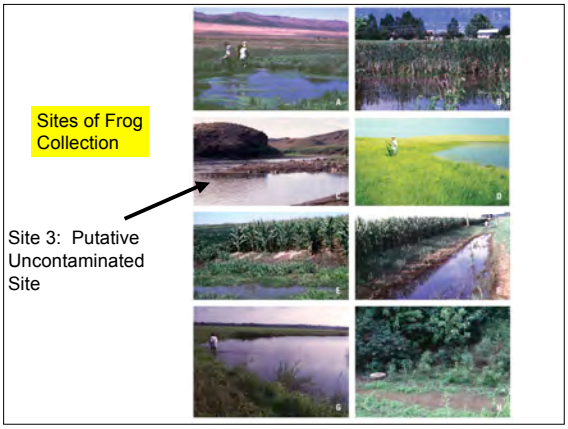

Effect of Atrazine & Steroid Hormones on Xenopus Larynx Muscle



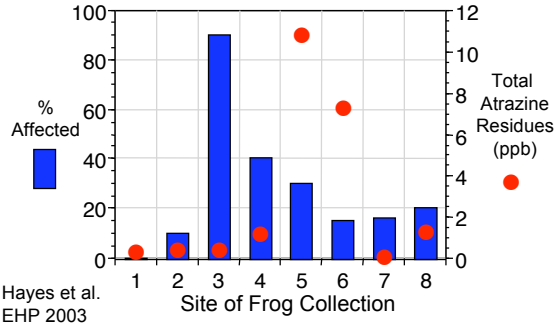

Carr et al. 2003




Hayes (2003) Sampling Sites

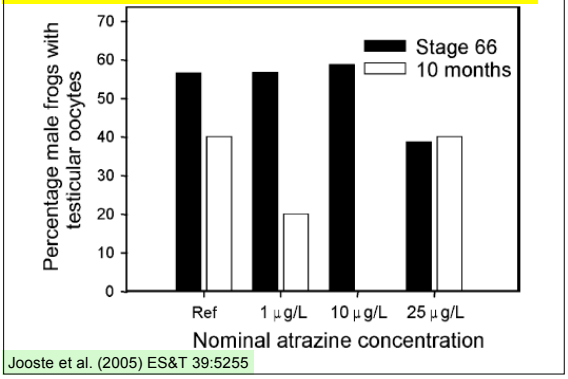



**Reality Bites from the Field: % Frogs Affected (Hermaphrodites) & Atrazine + Metabolite Residues (ppb)**



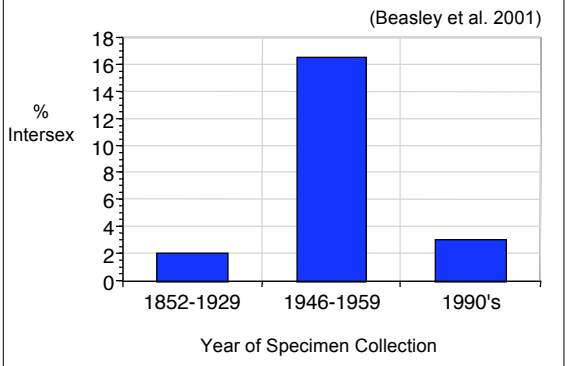

Hayes et al. EHP 2003

Effect of Atrazine on Anamolous Testis Development in Xenopus Living in Outdoor Mesocosms

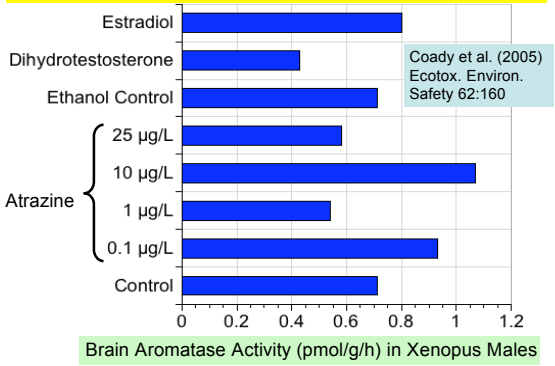



Jooste et al. (2005) ES&T 39:5255

## Incidence of Intersex Cricket Frogs in Museum Collections




## What Happened to the Aromatase Hypothesis?



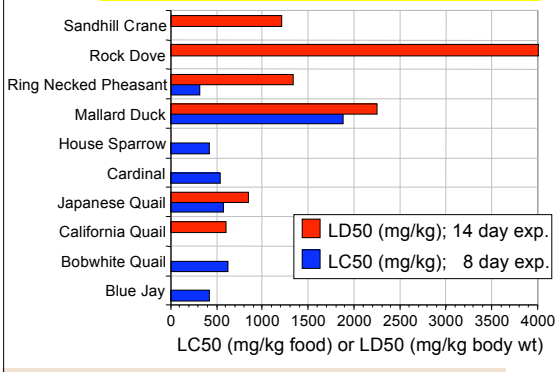

## Do Atrazine Residues Cause Sexual Confusion??

- Magnitude of atrazine concentration does not seem correlated with field observations of frog 'sex' effects
- Furthermore, in the field study showing high numbers of intersex individuals, frogs were very abundant
- Experiments have not been conducted to determine if frogs with abnormal gonads can reproduce successfully

## Hypothesis: Eggshell Thinning Caused Bird Population Declines

- **Proposed several yrs after *Silent Spring***
- **Declines in raptorial bird populations**
- **Inverse correlations between DDE in eggs and eggshell thickness**
- **"i.e., the crushing by incubating birds of eggshells thinned by DDE caused reproductive failure and population decreases in many species of birds"**
  **(Keith 1996)**

## DDT Chronic Oral & Dietary Toxicity to Birds is Low




Data compiled by University of Guelph, Center for Toxicology, Ontario, Canada

## Rationale for Hypothesis

- DDT of only moderate toxicity to birds
  - very high levels (>>10 ppm) in brains of birds found dead or symptomatic
- Moore & Ratcliffe (1962) noted DDE in eggs of birds
- Ratcliffe (1967) noted weights of raptor eggs lower than "historical" values
- Hickey & Anderson (1968) first to correlate DDE levels & shell thickness

## Link to Xenoestrogens?

- Ratcliffe (1967, Nature *215*:208) first proposed the link
  - **attributed decrease in eggshell weights (as surrogate for thickness) to changes in calcium carbonate fraction**
  - **noted calcium metabolism under endocrine control**
  - **cited earlier paper (Jeffries 1967) where DDT delayed ovulation in finches**

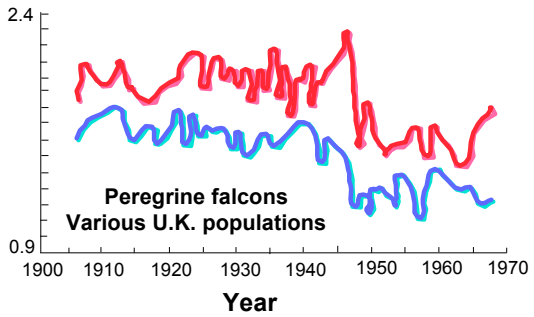
## Supporting Evidence

- **Eggshell thickness measurements benchmarked to museum specimens pre-1947**
  - **Compared to field-collected eggs**
  - **Disparate geographic populations**
- **Laboratory feeding studies**
- **Measurements of reproductive success**

## Skeptical Questions

- How much variation in shell thickness is explained by DDE residues?
- Is shell thickness related to breeding success?
- How important is egg survivability in comparison to other natural mortality factors?
- Can alternative hypotheses explain population declines?

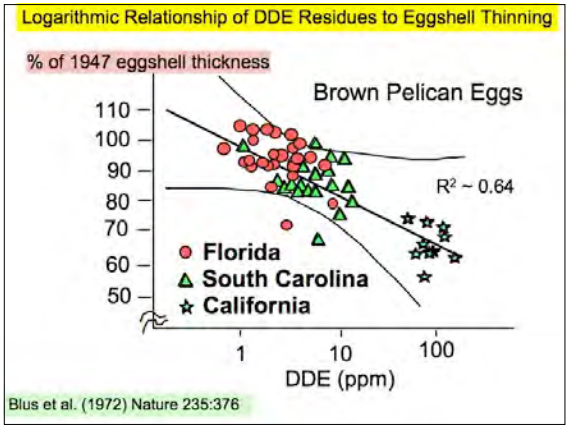
**Upper & Lower Bounds for Thickness Index (adapted from Ratcliffe 1967)**




## A Curious Conclusion

- "Recovery of locally depleted populations of peregrine began in 1946 and continued for several years after eggshells became lighter and egg eating frequent..."
- "Population "crashes" in peregrine and sparrowhawk after 1955 probably involved greatly increased adult mortality..."

Ratcliffe 1967




Linear Regression

**Slope**

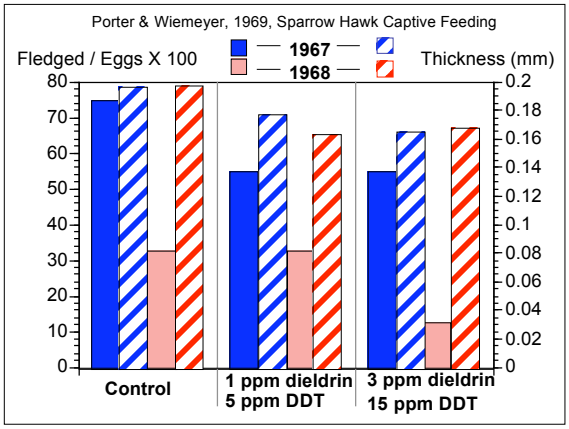
**Tells if and how fast the dependent variable changes relative to the independent variable**

**Correlation Coefficient**

**Tells if a linear relationship actually exists**

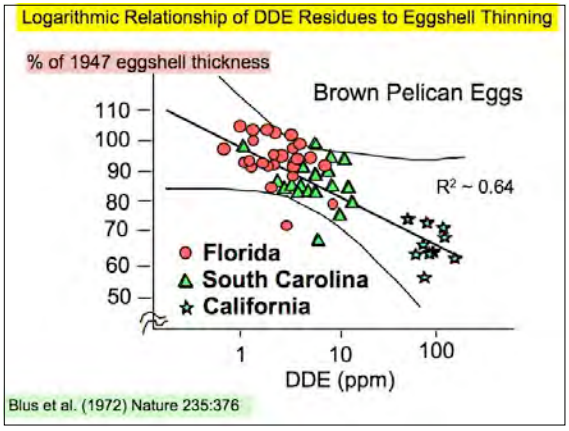
**Regression Coefficient**

**Tells the proportion or percentage of variance in the dependent variable that is accounted for by the independent variable**




## Plausible Hypotheses for the Mechanism?

- Induction of hepatic enzymes that metabolize steroid hormones necessary for calcification (i.e., estrogen)
  - Some studies did show that chlorinated hydrocarbon insecticides could induce cyt P450 dependent microsomal oxidases
- Inhibition of medullary bone deposition, the major calcium source during eggshell formation
- Inhibition of thyroid or parathyroid function
- Inhibition of intestinal calcium absorption
- Stimulation of nervous system resulting in premature egg laying

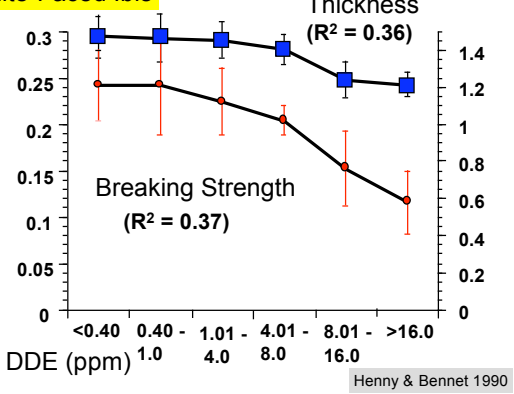



## Alternative Hypothesis

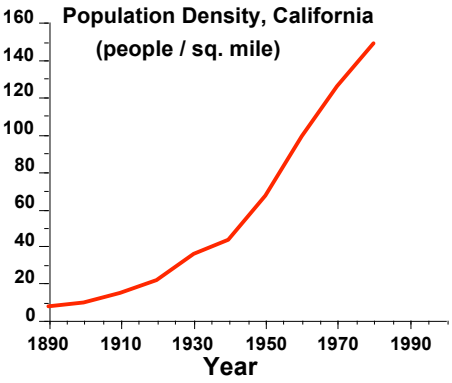
- **Polymorphic characters**
  - **Occurrence of several different discontinuous phenotypes within a single interbreeding population**
- **Polygenic characters**
  - **Continuous variation in phenotype under the control of several genes**

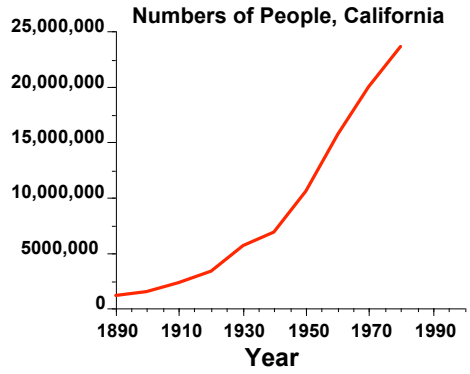
Mayr 1971
"Population, Species, & Evolution"

White-Faced Ibis




Henny & Bennet 1990

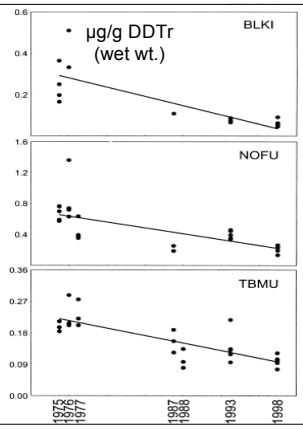



Is the Eggshell Thinning Hypothesis Now a Mute Question?

"Contaminant residues in seabird eggs from the Canadian Arctic" Temporal Trends 1975-1998

Braune et al. (2001) Environ. Pollut. 114:39




## Lessons Learned

- Correlation is not causation
- Pay attention to the regression coefficient
- Examine alternative hypotheses
- Use the weight of the evidence and don't ignore work that disagrees with your own
- Use positive hormone controls
- Do experiments under field conditions (if feasible)
- Hazard is NOT equivalent to risk
- Questioning dogma leads to new knowledge but is distinct from risk management decisions