Sublethal Effects to Individuals

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Sublethal Effects

**Sublethal effects**: changes in physiological processes, growth, reproduction behavior, development, etc.

- common theme: adverse effect on individual’s fitness

**Ecological context and concepts**:

- sublethal effects may have lethal consequences
- individuals must compete for food, avoid predation, mate, etc.
- *ecological mortality v. somatic mortality*
  - toxicant-related diminution of fitness may equal somatic mortality (effects expressed in the next generation)
General Adaptation Syndrome

**Selyean stress** - response- specific suite of responses to generalized stress

**General Adaptation Syndrome**

1) Alarm reaction - short-term
   - catecholamine release; epinephrine, norepinephrine)
   - glucocorticosteroid release; e.g. cortisol, corticosterone
     - increased blood pressure
     - increased blood sugar
     - immune suppression

2) Adaptation or resistance – mid/long term
   - tissue-level response
   - hypertrophy, atrophy

3) Exhaustion - long term
   - depletion of reserves
   - failure to compensate
   - eventually leads to death
**Sublethal Effects - Growth**

**Growth** - common *response variable* for sublethal effects
- easy to measure
- integrates a suite of biochemical and physiological effects
- often related to individual fitness

**Toxicant-influenced growth** – potential biomarker
- often shows dose-response effect of toxicants
- caution – be aware of threshold effects; i.e. no effect at low dose

**Nomenclature:**

| Lethal – L | 50% mortality – LD$_{50}$, LC$_{50}$ |
| Sublethal Effect – E | 50% response – EC$_{50}$, ED$_{50}$ |
| Dose – D | 50% reduction in normal response – IC$_{50}$, EC$_{50}$ |
| Concentration – C | by definition, 50% mortality (response) = **median dose** |
| Inhibitory - I | test duration: e.g. 48, 96 h |
| | - 48 hour LC$_{50}$, 96 h EC$_{50}$ |
Sublethal Effects - Growth

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**Hormesis** may also be evident
- biphasic response
- usually not toxicant-specific
- may reflect regulatory overcompensation; e.g.
  - detoxification enzymes
  - immune response
  - heat stress proteins
  - metallothioneins
  - antioxidants
Developmental Toxicity

Developmental stages are generally more sensitive to toxicants. (Why?)

**Effects** (e.g.)
- developmental mortality
- malformations (teratogenesis)
- functional deficiencies
- slow growth
- behavior

**Toxicant exposure:**
- egg stage
  - many vulnerabilities
  - contaminants deposited in yolk
- embryonic/larval stage
- some contaminants cross placenta

**Critical periods** or windows for developmental toxicity; e.g.
- Hg effects (Minamata disease)
- thalidomide
- endocrine disruptors
Teratogenesis Assays

**FETAX assay** (frog embryo teratogenesis assay – *Xenopus*)

- 96 h exposure to contaminants
- mortality and abnormal development scored
- compare concentrations producing 50% death (LC$_{50}$) and 50% abnormalities (TC$_{50}$)
- teratogenic index (TI) = LC$_{50}$/TC$_{50}$
  - reflects developmental hazard of a contaminant
Developmental Toxicity

**Behavioral teratology** – behavioral abnormalities arising after embryonic exposure to toxicant; e.g.
- mummichogs exposed to Hg as embryos had decreased ability to capture prey
- green frogs exposed to pesticides as embryos had decreased predator avoidance response

**Developmental stability** – capacity of an organism to develop into a consistent phenotype
- correlated to fitness
- deviations measured from norm; e.g.
  - bilaterality: fluctuating asymmetry (FA)
  - FA thought to reflect perturbations in normal developmental processes

polymelia (*Rana pipiens*)
Sexual Characteristics

**Endocrine Disrupting Chemicals (EDCs)**

- xenobiotic estrogens (xenoestrogens) mimic estrogens
- regulate activity of estrogen-responsive genes by binding to estrogen receptor (ER)
- disrupt hormonal signaling
- affect sex organ development, brain development, behavior, fertility, physiology; e.g.
  - male vitellogenesis (fish)
  - male gulls ignore nesting colonies
  - female gulls pair, lay infertile eggs (DDT/DDE)
  - imposex (mollusks)
  - intersex (fish, amphibians)
  - sex reversals (fish)
  - abnormal sex ratios
  
  testicular oocytes (leopard frog)
Reproduction

**Effect:** lowered fitness of individuals due to reproductive impairment; e.g.

- DDT/DDE induced eggshell thinning (inhibition of Ca-dependent ATPase in eggshell gland)
- imposex in mollusks
- various fish:
  - reduced egg size
  - reduced egg and larval survival
  - reproductive failure
  - lower fry survival
  - lowered fertilization rates
  - lower embryo success
- lab experiments; e.g.
  - *Daphnia* – reduced number of young
  - *Gambusia* – low number of embryos
Sublethal Effects - Physiology

Physiological biomarkers: deviations from homeostatic state
- may reflect threshold effects
- may be used to infer mode of action
  - e.g. AChE inhibitors may affect feeding, respiration, etc.
- imply lowered fitness

Physiological biomarkers include:
- impaired performance; swimming speed, stamina
- changes in respiration
- excretion
- ion regulation
- osmoregulation
- bioenergetics (i.e. food conversion efficiency)
- immunological capabilities/disease resistance
- etc.
Physiological Biomarkers

**Respiratory activity:**
- e.g. fish “coughs” (gill purges)

**Adenylate energy charge (AEC)**
- reflects balance between anabolic and catabolic activities

\[
AEC = \frac{ATP + \frac{1}{2} ADP}{ATP + ADP + AMP}
\]

**Respiration (oxidative phosphorylation)**
- \(O_2\) consumption under minimal and maximal activity levels
- compare with N excretion rate (protein degradation) to determine CHO v. protein catabolism
Behavior

Contaminants/toxicants cause a wide variety of behavioral changes.
- often measured in lab settings; occasionally in the field
- difficult to objectively score
- considerable “normal” variability
- difficult to extrapolate accurately from structured lab experiments to field situations

Behavioral biomarkers include:
- preference/avoidance
  - e.g. light, temp, salinity
  - movement towards or away from stimulant
- activity levels; e.g. fatigue, hyperactivity
- feeding; e.g.
  - diminished or cessation (lab)
  - deviations from predictions from optimal foraging theory (field)
Behavioral Biomarkers

Behavioral biomarkers, cont.

- performance
  - swimming against current
  - maintaining proper orientation
  - critical swimming speed
- learning
  - memory impairment (humans)
  - memory loss (humans)
- predation
  - avoidance
  - sub-optimal foraging behavior
- reproductive behavior
- social interactions
Toxicant Delivery and Exposure

Terrestrial organisms are typically administered a **Dose** of toxicant; i.e. toxicant is injected into or fed to the test organism.

Aquatic organisms are typically exposed to a **Concentration** of toxicant; i.e. toxicant is in solution with the water.

**Nominal Exposures** – Toxicant is weighed/diluted/mixed as necessary to theoretically match the target exposures.

**Measured Exposures** – Identical to nominal exposure, except that actual exposures are quantified by analytical chemistry methods.

**Nomenclature:**

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- 50% mortality – **LD**\(_{50}\), **LC**\(_{50}\)
- 50% response – **EC**\(_{50}\), **ED**\(_{50}\)
- 50% reduction in normal response – **IC**\(_{50}\), **EC**\(_{50}\)

by definition, 50% mortality (response) = median dose

- test duration: e.g. 48, 96 h
- 48 hour **LC**\(_{50}\)
Detecting Toxic Effects

Effects detected and quantified in tests involving different exposures, replicates, time series (intervals or single time points)

**Experimental Designs**

**Sublethal** – no death to the test organism; however, effects to its behavior and/or biochemical & physiological processes occur.

**Acute Lethal** – death to the test organism following a brief, and often intense, exposure to a toxic substance.

**Chronic Lethal** – death to the test organism following a prolonged low-intensity exposure to a toxic substance.
Toxicity Tests

Proportion of Life Span and System Complexity

Aquatic Toxicity Study Methods

Acute

Chronic

Field studies

Microcosms

Mesocosms

Pre-reproductive

Reproduction

Interspecific interactions

Community interactions/
abiotic factors

Ecosystem interactions

Successional states
Toxicity Tests

**Acute (lethal)** - short period of life span
- fish, daphnids, rats, birds
- 24, 48 h

**Chronic (lethal)** - significant portion of lifespan
- must include gestational period (female)
- must include portion of spermatogenesis (male)
Test Systems

Acute - Aquatic single species tests

Static: one-time addition of toxicant (test compound), water, food, etc.

+ simple, cost effective
+ small amount of chemical, waste

- $O_2$ content and toxicant decrease over time
  - high COD or BOD can be dangerous
- metabolic waste products increase
- least sensitive; chemical degradation may reduce apparent sensitivity

Static renewal: periodic addition/removal of toxicant, water, food, etc.; or, organisms are periodically placed in new solutions

+ toxicant concentrations and $O_2$ returned to original values
+ animals fed; waste products removed
+ small amount of waste products

- more handling of test vessels and organisms
  - increased chance of accidents
- increased stress on animals
- less sensitive than flow-through; chemical degradation, absorption
Test Systems

Acute - Aquatic single species tests

Flow-through (recirculating, intermittent flow, continuous flow): continual replacement of toxicant, O₂, etc. removal of waste

- provides more representative evaluation of acute toxicity; sample can be administered directly from source
- higher loading factor (biomass) may be used
- maintains water quality
- constant toxicant exposure; loss due to volatilization, absorption, degradation, uptake, reduced

+ large volumes of sample and dilution water required
+ increased complexity; space, equipment
- uncertainty about effect of water treatment on toxicant concentration
- opportunities for mechanical failure
- large increase in waste water and toxicant

Acute - Solid Phase (terrestrial, sediment) – cages, pens, soil (flats or plots), sediments, controlled-environment chambers.
- use contaminated substrates
- use spiked substrates
Test Organisms

- a few of many examples:

**Aquatic** – freshwater & marine fish, invertebrates and algae

**Semi-aquatic** – amphibians and mallard duck

**Terrestrial** – earth worms, quail, honey bees, and plants
Test Considerations and Parameters

Test substance properties – e.g. solubility, volatility and stability

Dilution water properties – e.g. D.O., hardness, alkalinity, pH, conductivity, salinity

Test organism considerations
- source, age, condition, handling, acclimation or acclimatization

Acclimatization – same processes applied to natural or field settings

Acclimation – (controlled environments or laboratory settings)
Physiological adjustment to a change in environmental conditions;
maintains or minimizes deviations from homeostasis

Test organisms must be subjected to an acclimation period where
all key environmental parameters match the actual test conditions
in the absence of toxicant.
Test Considerations and Parameters

**Loading** – minimize biomass per unit volume of test solution

**Environmental variables** – temperature and photoperiod

**Feeding** – generally avoided, unless organisms are cannibalistic or test duration significantly overlaps organism’s life span
Concentration or Dose Units

ppm – part per million
ppb – part per billion
ppt – part per trillion

Aquatic

mg / L = ppm
μg / L = ppb
ng / L = ppt

Terrestrial

mg / kg = ppm
μg / kg = ppb
ng / kg = ppt
Experimental Design

exposure environment

experimental units

various concentrations = dose/response

replicates
Design Considerations

Randomization schemes; e.g.
- Latin square
- modified block

Physical Design
Results and Analysis

Independent variable – concentration

Dependent variables – response (e.g. growth, reproduction, behavior, development, etc.)

- how potent is the toxicant?
- what is a “safe” concentration
- at what concentration can we expect lethal effects?
Analysis

**Hypothesis Testing**: Which of the treatment groups is significantly different from the others? Includes:

1. ANOVA (analysis of variance)
   - ANOVA compares variance within treatments (i.e. replicates) to variance among treatments
     - variance within treatments assumed to reflect sampling
     - or error variance
     - variance among treatments includes error variance plus any additional variance associated with the treatment
   - Null hypothesis states that there should be no difference between, within, and among variance
   - F statistic tests the null hypothesis of equal means among treatments
Analysis

Hypothesis Testing: continued

- ANOVA assumptions:
  1) equal variances among treatments
  2) normally distributed data (Shapiro-Wilk’s test)

NOTE – data can be transformed (e.g. arcsine, log, square root)
  to achieve normality/linearity

- Also, observations must be independent
  - depends on good experimental design
  - random assignment of treatments
Post-ANOVA Tests

ANOVA - differences in variances indicate different means
- null hypothesis accepted (no difference in variances) - no further analysis
- null hypothesis rejected (differences in variances) - post-ANOVA tests show which treatments differ significantly from each other
  e.g. Dunnett’s test, students t-test, Williams’s test
- assign a significance to the response means for different treatments
- level of significance (α) set; most often 0.05 (i.e. 0.95)

- various biological effects can be predicted

However: statistical hypothesis testing only demonstrates that something that is present in the data set differs significantly from the null hypothesis
Non-Parametric Post-ANOVA Tests

Some data sets will not qualify for ANOVA
- ANOVA assumptions:
  1) equal variances among treatments
  2) normally distributed data
- various tests can be used to establish significant differences
  - Steel’s many-one rank test
  - Wilcoxon rank sum test
Sublethal Effects Concepts/Terminology

**NOEC (NOEL)** - no-observed-effects concentration:
- highest test concentration for which there was no statistically detectable difference of the control response

**NOAEC** - no observed *adverse* effects concentrations

**LOEC (LOEL)** - lowest-observed-effects concentration:
- lowest concentration in a test with a statistically significant difference from the control response

**LOAEC** - lowest observed *adverse* effects level

**MATC** – maximum acceptable toxicant concentration:
- undetermined concentration within the interval bounded by the NOEC and LOEC that is presumed safe

\[ \text{NOEC} < \text{MATC} < \text{LOEC} \]
Sublethal Effects Concepts/Terminology

Note:
- NOEC and LOEC totally dependent on test concentrations
- can be considered artifacts of experimental design
- process can produce higher than optimal NOEC and LOEC with suboptimal experimental design
- therefore, poor technique can be “rewarded” with high NOEC and LOEC

also:
- “safe” MATC is dependent on species, exposure duration, etc
Regression Methods

Data fit to a concentration-effect model by various regression methods; e.g.
- least-squares
- maximum-likelihood

- concentrations and their associated confidence intervals having biologically
  significant effects can be calculated by interpolation
  - e.g. IC$_{10}$, LC$_{5}$, etc.