December 10, 2003

Lecture 25: Ecotoxicological Issues—Measuring Effects at Higher Levels of Organization

I. The Current State of Environmental Toxicology

A. Thus far in this course, we have largely focused on the individual and its physiological systems.

B. When we talked about measuring toxicity, we necessarily had to work with a defined group of organisms that were dosed at increasing levels of toxicant.

1. We could measure a population response that we estimated as an LD50 or LC50.
2. However, our application of this toxicological endpoint was always to the individual.
   a. In other words, if exposure was greater than the equivalent of the LD50 or LC50, than the probability that the exposed individual would die was great.
   b. But knowing how many individuals will die does not tell us anything about hazards to higher levels of organization such as the whole population or the population within the context of its relationships in the community. (Figure 1)
      1. Attributes such as diversity and functionality become crucial at ecosystem level scales.
         a. Effects on individuals or populations can have unforeseen effects on biological scales larger than the population.

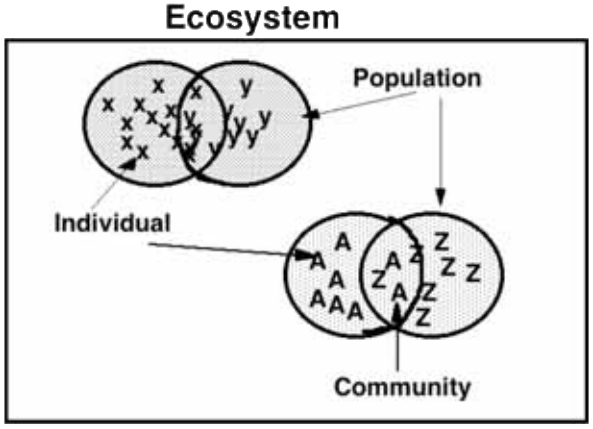

Figure 1. The relationship of individuals in a population and the population to higher levels of ecological organization.

3. While ecological risk characterization by the EPA is deterministic, and thus largely individual-organism centric, the use of certain sensitive endpoints may be surrogates for indirect, and therefore community level effects.
   a. For example, EPA has focused on the most sensitive endpoint for atrazine as affects on primary productivity as applied to aquatic phytoplankton (you will recall that the endpoint chosen was a NOAEC of ~2.3 ppb).

b. Given that phytoplankton forms the base of aquatic food chains, any "massive" disruption could affect the population of many other organisms.

C. To redress the problem of extrapolating the effects of contaminants to higher levels of ecological organization, some researchers have turned their attention to demographic (or population based) toxicology, mesocosm studies, and ecoepidemiology.
   1. Of these three focal areas for research, demographic toxicology and mesocosm studies can be carried out under controlled laboratory, greenhouse, or field conditions.
      a. However the "systems" studied still suffer from doubts about relevance to the complexity of the field.
      b. The studies do generate usable hypotheses about how the "real" world may be working upon exposure to high concentrations of contaminants.
         1. A high concentration in this case would be one that is approaching known lethal levels (perhaps somewhere between the LC5 and the LC50)
   2. Ecoepidemiology will depend on field observations, and thus requires a lot of sampling to make conclusions about the relationship between a population as a whole and the "smoking" gun causing or influencing adverse effects.
   3.

II. Population Level Effects (aka Demographic Toxicology)

A. It is assumed that if enough individuals in a population are killed or otherwise prevented from reproducing that the population of one species will suffer.
   1. Owing to the dependency of one species population on another species, harm at the population level could translate to harm at the community level.
      a. For example, if the prey of a species with predatory characteristics is affected, the population of the predatory species will also be affected.
   2. However, it is not a foregone conclusion that acute toxicity and the health of the population are one and the same.

B. Experiments with fast reproducing insects, namely aphids, by Stark et al. show that the intrinsic rate of population increase (denoted r_m) is about the same in aphids exposed to doses of imidacloprid at the LC50 for neonates as in the unexposed neonates (note that there were numerical differences but not statistically significant).
   1. Stark first exposed adult aphids on treated bean foliage to different concentrations of the neonicotinoid insecticide imidacloprid
      a. Walthall, W. K. and J. D. Stark. 1997. A comparison of acute mortality and population growth rate as endpoints of toxicological effect. Ecotoxiocology & Environmental Safety 37:45-52.
   2. First note that the LC50 of imidacloprid to adults (0.468 mg/L) and neonate aphids (0.225 mg/L) differ by a factor of about 2 (Figure 2 below).

C. Second, differences in the absolute value of the intrinsic rate of increase between imidacloprid exposed and unexposed aphids could be accounted for solely by the magnitude of mortality as doses of imidacloprid increased. (Figure B below)

D. Third, as a result of the reservoir of neonates unaffected by the concentrations of imidacloprid, the intrinsic rate of increase over time plateaued at about the same

time frame, and as long as aphids weren't killed outright (i.e., there were survivors), the intrinsic rate of increase was similar to rate exhibited by unexposed aphids. (Figure 4 below)

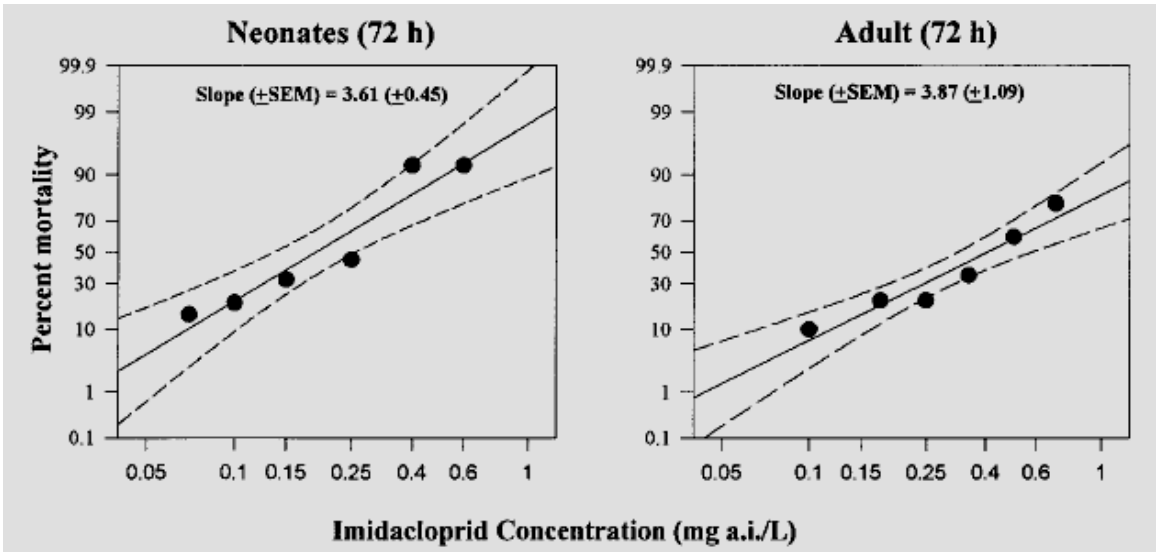

Figure 2: Dose-response curves for imidacloprid exposed aphids caged on bean leaves (Walthall & Stark 1997)

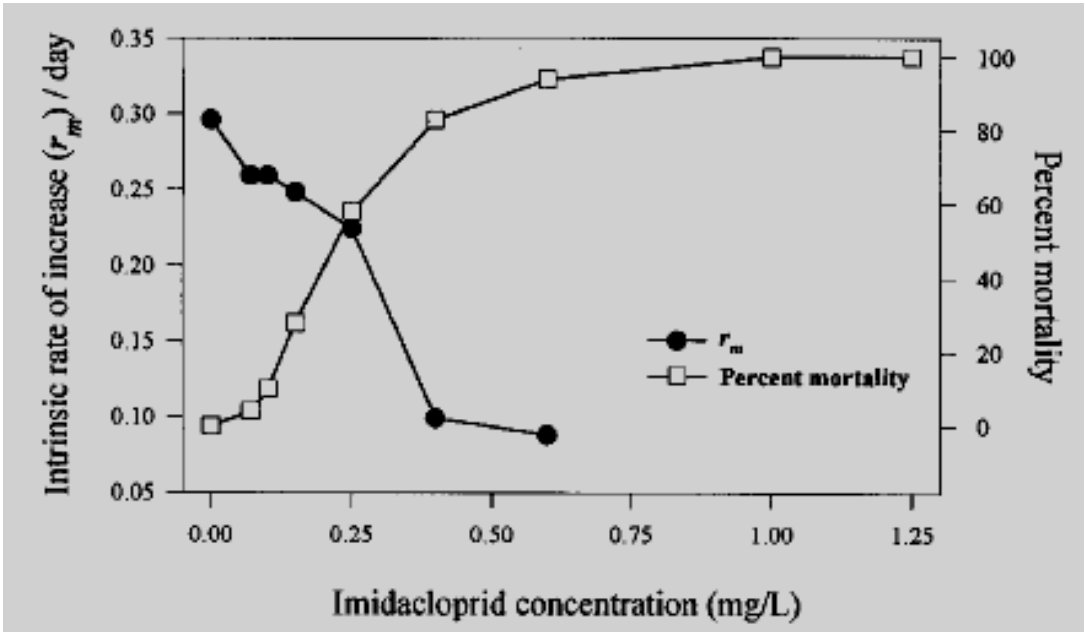

Figure 3: Relationship between intrinsic rate of increase and percentage mortality of aphids at different concentrations of imidacloprid on bean leaves. Note that intrinsic rate only significantly decreases when the LC50 is substantially exceeded. (Walthall and Stark 1997)

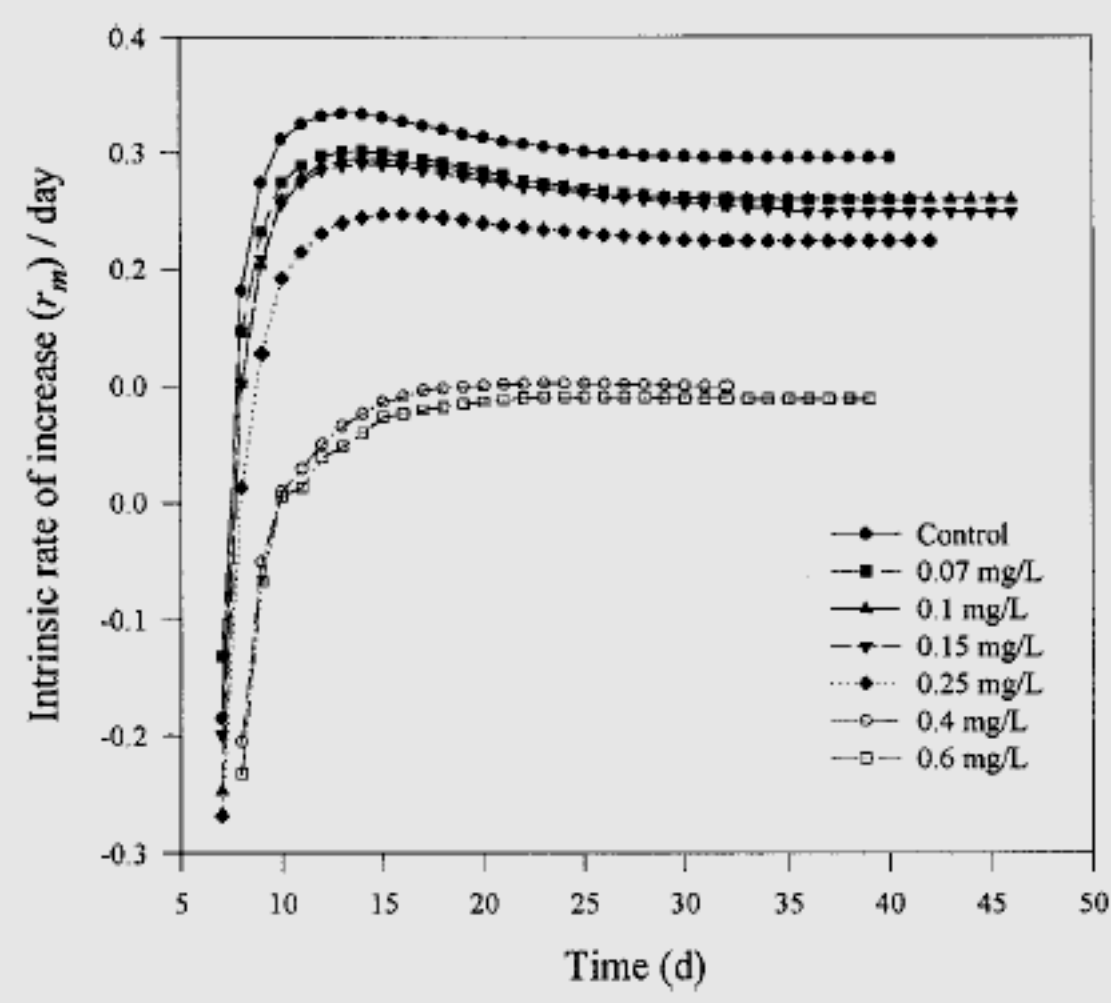

Figure 4: Intrinsic rate of increase of aphid cohorts on bean plants over time; exposure was continuous. (Walthall and Stark 1997)

III. Measuring Effects at Higher Levels of Organization (System Level Effects)

A. Microcosms and mesocosms
   1. Experimental systems that contain miniature representations of very simple ecosystems
   2. Contain a representation of diversity and functionality
      a. Examples: Metcalf Model Ecosystem (1971) [Metcalf, R. L., G. K. Sangha, and I. P. Kapoor. 1971. Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. Environmental Science and Technology 5(8):709-713.] (Figure 5)
      b. Metcalf developed the first lab model of food chain assimilation of pesticide residues
         1. Radiolabelled (14C) pesticides were sprayed on the sorghum; then after specific time intervals the various components were harvested and extracted

2. A mass balance of the parent compound and metabolites could be established
   a. One pitfall of the model ecosystem is that it could not measure volatilization of residues
3. The substrate was a quartz sand, and therefore not very realistic;

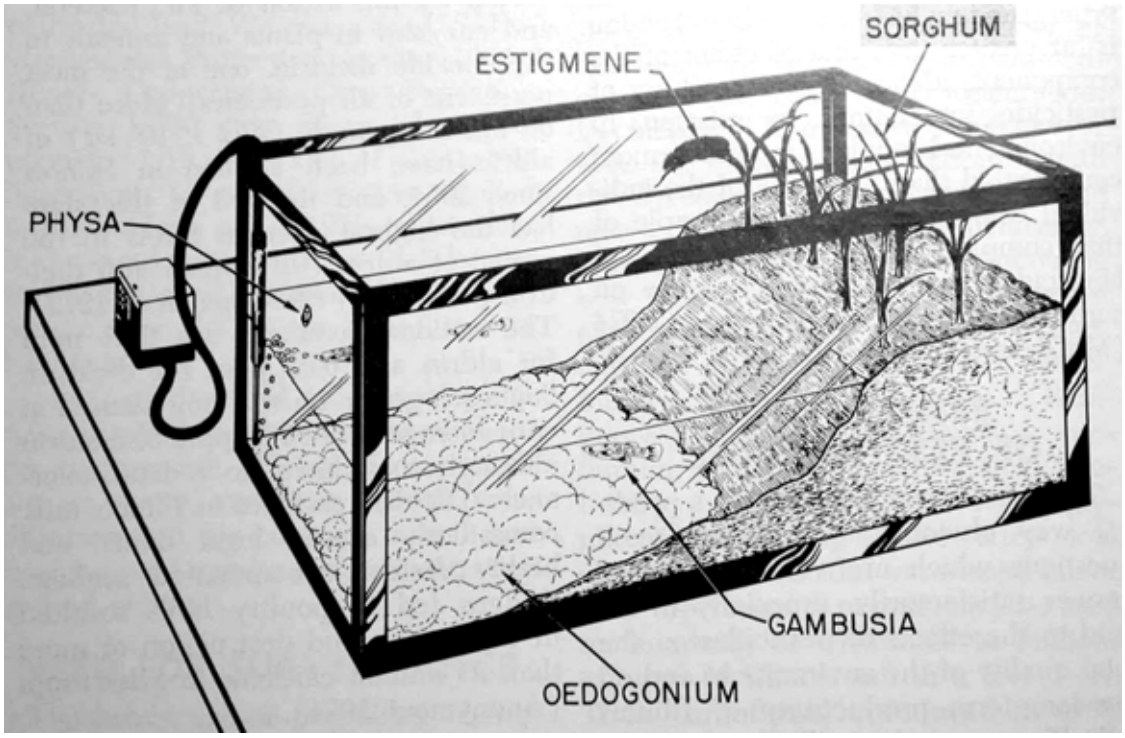

Figure 5. Schematic drawing of the Metcalf model ecosystem, first published in Environmental Science & Technology during 1971.

4. However, the real purpose of the model ecosystem was to estimate bioaccumulation factors (or BCF) in simple food chains
5. The food chain established in the Metcalf model ecosystem was
   a. sorghum---->saltmarsh caterpillar (*Estigmene* sp.)
   b. saltmarsh caterpillar excreta----->algae (*Oedogonium* sp.)
   c. algae------->snail (*Physa* sp.)
   d. caterpillar excreta----->diatoms
   e. diatoms----->plankton
   f. plankton------->mosquito larva
   g. mosquito larva-------->fish (*Gambusia* sp.)
6. Appropriate uses of microcosms/mesocosms in ecotoxicology
   a. Effects of perturbants on biogeochemical cycles
      1. Decomposition or alterations of organic substrates
      2. Phosphatase activity, ammonification, nitrification, denitrification, nitrogen fixation
      3. Nutrient assimilation and immobilization by plants and microbes

b. Effects of perturbants on small organisms (small relative to the size of the microcosm)
    1. Tests on sensitive and/or critical species (limited to species that can be removed from the system)
    2. Alteration of seasonal succession patterns (for ex., due to change in reproductive rates, competitive ability, predation, etc.)
    3. Changes in primary or secondary productivity of whole systems and of selected components
    4. Dose-response curves for organisms
c. Fate and pathway studies
    1. Toxicity-enhancement or toxicity-reduction processes (for ex., methylation of mercury)
    2. Trans-media movement of toxins (for ex., sedimentation of pollutants; metal release from soil or sediment as a result of acidification)
    3. Use of microcosms as a conditioning medium for pollutant with subsequent use of water as a perturbant in a bioassay
    4. Budget pathways (i.e., mass balance), speciation, degradation, and fate of perturbants
d. Fundamental studies on mechanisms of toxicity
e. Effects that are not directly researchable in the lab because of time- and space-scale problems, but that could be studied by appropriate interfacing of microcosm and field studies
    1. Effects on fish production, as indicated by damage at lower trophic levels
    2. Damage to agricultural or public water supplies resulting from toxic metal mobilization by acid precipitation (or other pH changes)
    3. Long-term damage of pollutants
    4. Stimulation of nuisance organism
f. Validation of other test procedures
    1. Mathematical models
    2. Bioassays

B. Effects on Diversity (Structure) and Functionality

1. Structural indices (numbers and kinds)of species
2. Functionality of primary producers, for ex., reduction in chlorophyll *a* content.
3. Case Study of Morton et al. (1985) as described by Duke and Mount (1991) (Toxic effects on individuals, populations and aquatic ecosystems and indicators of exposures to chemicals. Methods for Assessing Exposure of Human and Non-Human Biota (John Wiley & Sons Ltd.):393-404.)
    a. Tested the effects of drilling fluids and/or associated clays on an experimental seagrass community; developed treated and untreated microcosms; microcosms exposed for 6 weeks; took another three weeks to harvest the communities and make the necessary measurements.

1. Drilling fluids are a complex mixture of chemicals, clay and water. The fluids usually consist of bentonite and other clays, barium sulfate as a weighting agent, lignosulfonates as thinners, and other chemicals like diesel for lubrication. Recycled until efficiency declines and then discharged.

b. Studied the macroinvertebrate assemblage, growth, chlorophyll content of grass and associated epiphytes, and rates of decomposition of grass leaves

4. Results:
   a. When treated with both drilling fluid and clay, microcosms contained approximately one-half the number of individuals as control microcosms (this is a structural effect on the system)
   b. The effect was attributed to physical layering of drilling fluid and clay on bottom of microcosm, altering the particle size of the sediment substrate
   c. Found a significant reduction in chlorophyll *a* per dry weight of leaf tissue after adding drilling fluid plus clay (this is a functional effect)
   d. Significant decrease in rate of decomposition of seagrass with drilling fluid only treatments, suggesting a toxic impact (drilling fluid has diesel in it)
5. Conclusions: Physical changes in substrate as well as toxic effects of drilling fluids alone disrupted community structure and function

C. In summary, contaminant effects can be direct on individuals, but whether there are population level effects depends on whether potential intrinsic rate of increase is also affected. However, effects can be indirect, such as shown for the example above that examined drilling fluids.
   1. Arguments have been made for examination of a daily activity and resource budget of individual organisms (i.e., resource allocation-based life history analysis) to conduct ecological analyses of polluted systems (Congdon et al., ETAC 20:1698-1703).
   2. The resource allocation approach hypothesizes an operative environment of individuals (i.e., environmental factors influencing birth, death, or migration). Contaminants can have a strong influence on operative environments by modifying resource allocation strategies that reflect changes in energy assimilation and demands.
   3. As shown in the figure below, contaminants can have direct effects on individuals (and thus the population) or indirect effects by modifying the operative environment).
      a. In the figure 4 below, the letters in the resource allocation circle represent respectively, growth (G), storage (S), maintenance & activity metabolism (M), and reproduction (R).

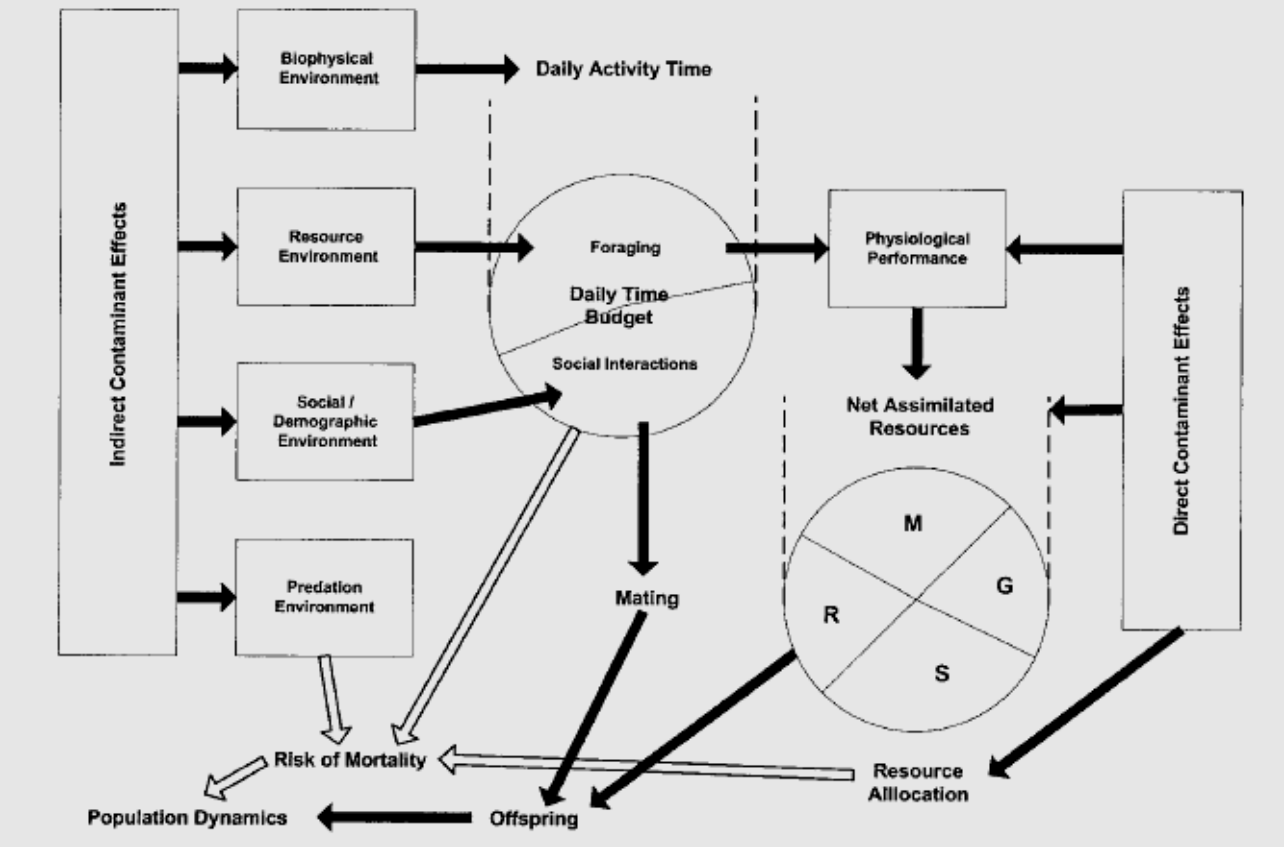

Figure 6. The resource allocation model for ecological risk assessment of a population within an ecosystem.

D. Mesocosm Case Study for Measuring Effects at Higher Levels of Organization
   1. The use of mesocosms allows a more community based perspective on risk. Such studies have been done for a few pesticides, but the data are often difficult to interpret given the natural fluctuations in populations.
   2. One example of a mesocosm study with fairly well-explained outcomes was useful for testing the predictive value of the LC_{50} and adequacy of ambient water quality guidelines (Giddings, J. M., R. C. Biever, M. F. Annunziato, and A. J. Hosmer. 1996. Effects of diazinon on large outdoor pond microcosms. Environmental Toxicology and Chemistry 15:618-629)
   3. Giddings et al. set up 8 model ecosystems (a.k.a. mesocosms because of their size, 8 m^2) (Figure 7)
      a. Each mesocosm was filled with sediment and pond water, and the fauna allowed to develop
      b. Pre-dosing measurements were made of each mesocosm to determine ecological parameters
      c. Then each mesocosm was dosed with an increasing concentration of diazinon (except for the zero level control)
      d. The systems were maintained for over two months
      e. The diazinon concentration was monitored periodically to establish its concentration

1. A time-weighted average concentration of diazinon could be calculated

4. The concentration parameter would be useful to calculated the lowest observed effect concentration (LOEC)
   a. The LOEC could be compared to the known LC50 (96 h) for a species of the same taxa involved in the mesocosm study

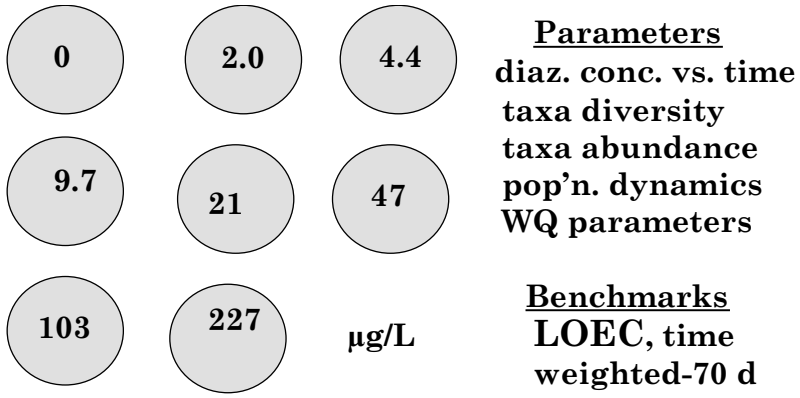

Figure 7. Experimental Setup of the Mesocosm Study reported by Giddings et al. 1996.

5. Results
   a. The various taxa studied with the observed LOEC and literature LC50 are compared below in Figure 8.
      1. When the LOEC (gray) bar is longer than the LC50 (striped) bar, then the LC50 is more conservative than the actual "field" toxicity
      2. When the LOEC bar is shorter than the LC50 bar, than the LC50 is under predictive of any effects in the field
      3. Note the aquatic guideline for diazinon is 9 ppt (0.009 ppb)
      4. In no case did the LOEC exceed the guideline;
         a. Indeed, the most sensitive taxa, Cladocera, responded with an LOEC and LC50 that was at least 30-40 times greater than the guideline, suggesting the guideline is incredibly conservative
6. Summary of the effects (LOECs for numbers and species richness)
   a. Zooplankton
      1. Total numbers: 4.3 μg/L
      2. Taxonomic richness: 2.4 μg/L
   b. Insects
      1. Total numbers: 9.2 μg/L
      2. Taxonomic richness: 9.2 μg/L
   c. Fish
      1. Survival: 54 μg/L
      2. Biomass: 22 μg/L

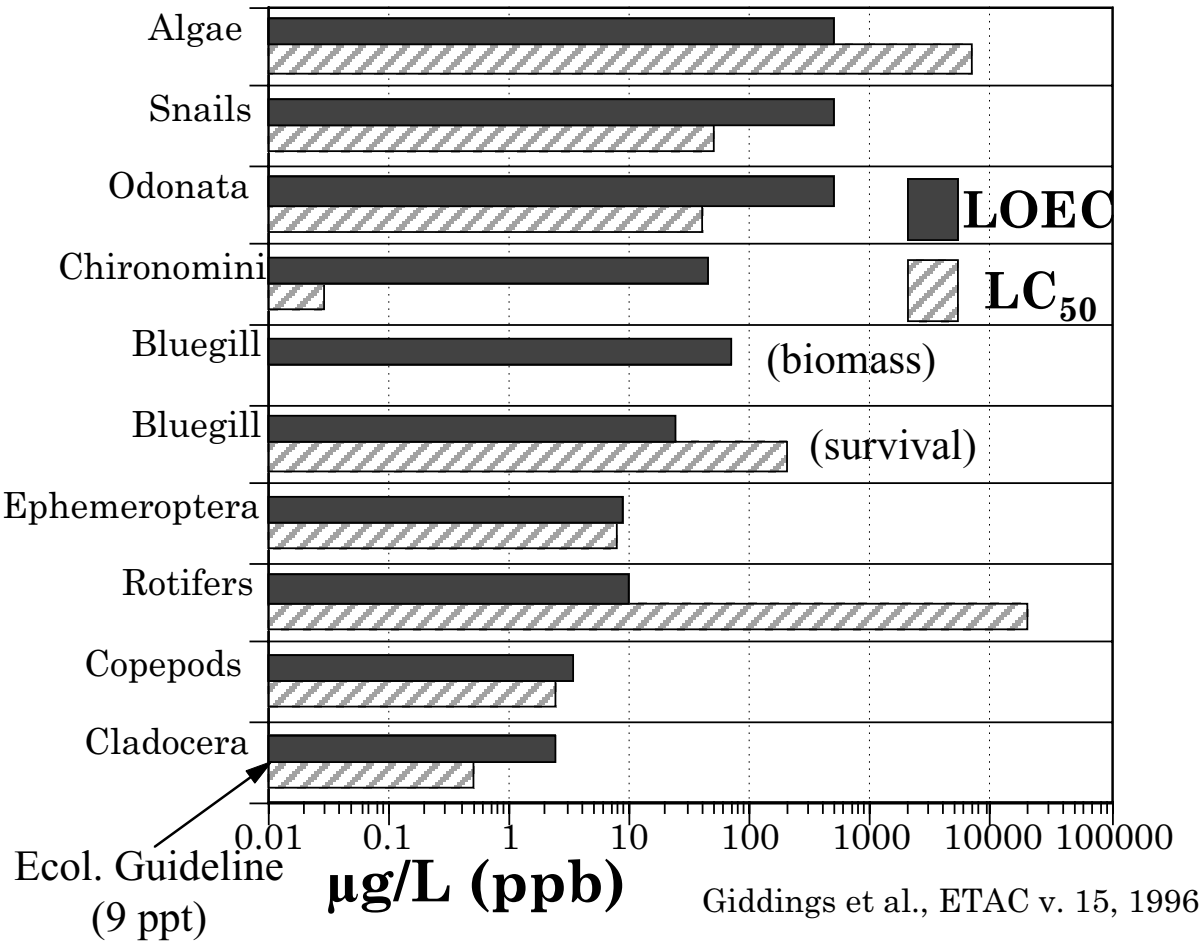

Figure 8. The relationship between the observed LOEC for various taxonomic groups in the Giddings et al. (1996) study and the LC50 derived from laboratory assays that were published in the literature.

7. Aggregate LOEC and NOEC (over all species for duration of the study—70 days)
   a. LOEC: 9.2 μg/L
   b. NOEC: 4.3 μg/L
8. Although the Giddings et al. (1996) study shows that the aquatic criteria of 9 ng/L (i.e., 0.009 μg/L) is extremely well protective of many taxonomic groups, the assumption remains that the interactions of the various organisms at a community level is not impacted beyond 70 days.

IV. Ecoepidemiology

A. Ecoepidemiology asks similar questions about wildlife and aquatic biota population in natural ecosystems as epidemiologists would about human populations.

1. What is the incidence of a disease (or incidence of some effect) and what factors correlate with this incidence
2. As opposed to human epidemiology as applied to chemicals, wildlife can be directly tested for exposure by examining contaminant residues in the body of captured animals, and many different types of biomarkers can be used to assess overall health.
3. However, ecoepidemiology still has to overcome interpretation of many confounding factors.

B. Case Study: Hypothesis that the herbicides 2,4-D and 2,4,5-T affected a population of tortoises near Olympia in southern Greece (Willemsen, R. E. and A. Hailey. 2001. Effects of spraying the herbicides 2,4-D and 2,4,5-T on a population of the tortoise Testudo hermanni in southern Greece. Environmental Pollution 113:71-78).
   1. The tortoise population had been studied for a number of years in a region where herbicides (specifically paraquat and atrazine) were used to manage unwanted vegetation. However, starting in 1980, one part of the region was treated with a combination of 2,4-D and 2,4,5-T. (Figure 9)
   2. Thereafter, the investigators noted a population crash. (Table 1)

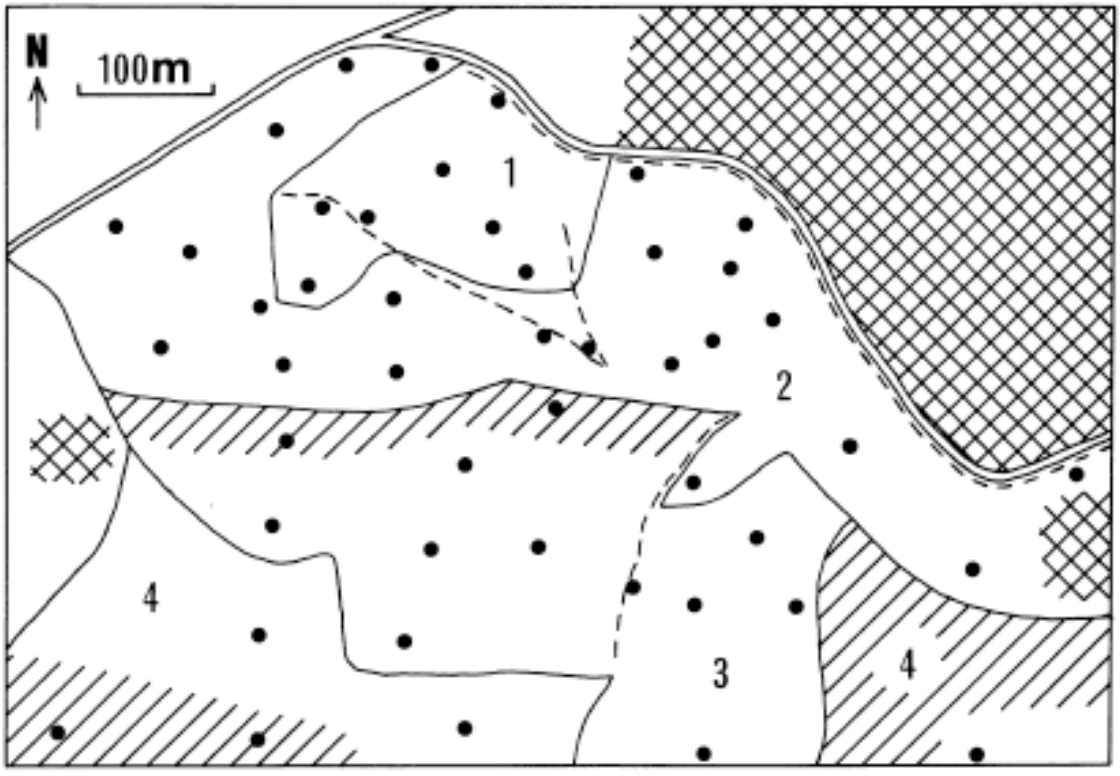

Figure 9. Map of area near Olympia, Greece showing 2,4-D sprayed area (1), and non-sprayed areas (2, 3, 4). The cross-hatched area represents urbanized areas, and the two parallel lines represent a road. The single hatched area represents a plant-covered slope and the dashed line represents a ditch. Black circles are turtle collection sites.

Table 1. Survival (based on capture-mark-release-capture studies) and population size during the time when 2,4-D and 2,4,5-T were used (Willemsen and Hailey 2001)

Year	Site 1 Tortoises		Site 2-4 Tortoises	
	Survival	Population Size	Survival	Population Size
1980	0.90	68	0.87	300
1981	0.45	59	0.92	329
1982	0.50	29	1.06	442
1983		18		784

3. Turtles exhibited symptoms of acute toxicity after spraying characterized by the symptoms of death (i.e., dead tortoises were found after spraying), swollen eyes, fluid discharge from the nose, and immobility).
   a. No data were presented regarding the LC50 or NOEC concentrations or dietary concentrations for the herbicides.
   b. However, loss of population in site 1 was not attributed to emigration nor to loss of vegetative structure.
      1. Furthermore, chronic toxicity due to stress was not evident because the condition and body weights of the captured tortoises did not differ among the sites (Figure 10).
4. Willemsen et al. concluded that the "crash" of the population was due to acute toxicity (lethality) leading to a lack or recruitment of young. (Table 2)
   a. They further hypothesized that the 2,4,5-T may have been contaminated with the highly toxic dioxin congener, TCDD (2,3,7,8 tetrachloro-para-dibenzodioxin.

Table 2. Population structure made up by juveniles in different areas over several years (Willemsen and Hailey 2001)

Year	Site 1 Tortoises		Site 2-4 Tortoises	
	% Juveniles	Sample Size	% Juveniles	Sample Size
1980	8.0	87	6.2	208
1981	3.4	59	15.6	211
1982	4.2	24	6.5	153
1983	0	20	8.4	415
1984		0	12.9	193

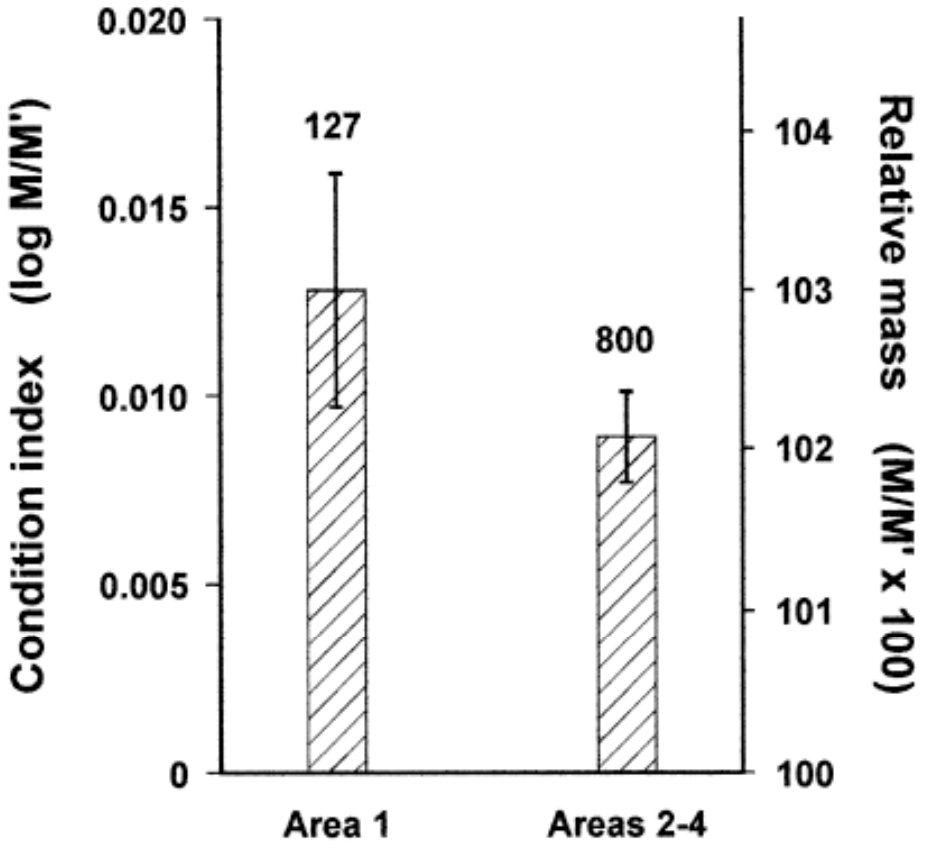

Figure 10. Condition index (relative measure of mass to mass expected based on body length) and relative mass did not vary between sprayed area 1 and areas 2, 3, 4, suggesting that chronic illness was not a factor in turtle decline.