Ecotoxicology & ERA

Toxicity measures

Biomarkers

General theory of stress

Effect of toxic chemicals on populations

Ryszard Laskowski
Institute of Environmental Sciences, JU
Gronostajowa 7, Kraków
Room 2.1.2

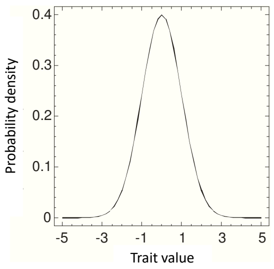
https://home.cyf-kr.edu.pl//~uxlaskow

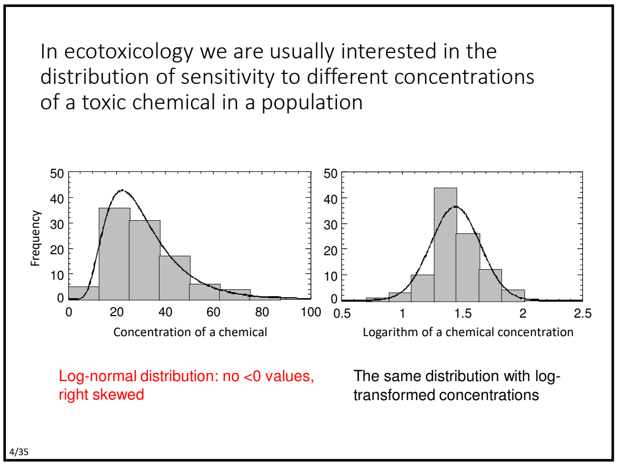
Toxicology *vs.* ecotoxicology

- **Toxicology**:
  - well established methodology (observing individual organisms or cells)
  - clear toxicity measures (Lethal Dose → LD_{50}, Lethal Concentration → LC_{50}, etc.)
- **Ecotoxicology** :
  - can we use the same methods and measurs in ecotoxicology?

<u>Remember</u>: „...higher organization levels than an individual...” – population is not an organism!

- Individuals differ in their tolerance to environmental factors → the distribution of any trait in a population usually follows normal distribution (*Gaussian curve*)

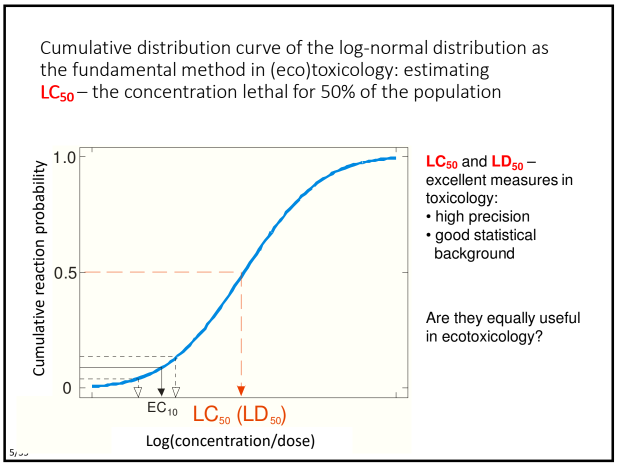




In ecotoxicology we are usually interested in the distribution of sensitivity to different concentrations of a toxic chemical in a population

Log-normal distribution: no <0 values, right skewed

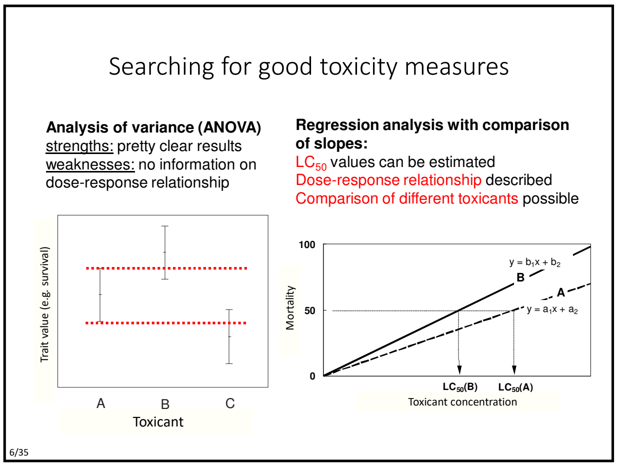
The same distribution with log-transformed concentrations



Cumulative distribution curve of the log-normal distribution as the fundamental method in (eco)toxicology: estimating **LC_{50}** – the concentration lethal for 50% of the population

LC_{50} and **LD_{50}** – excellent measures in toxicology:
- high precision
- good statistical background

Are they equally useful in ecotoxicology?



Searching for good toxicity measures

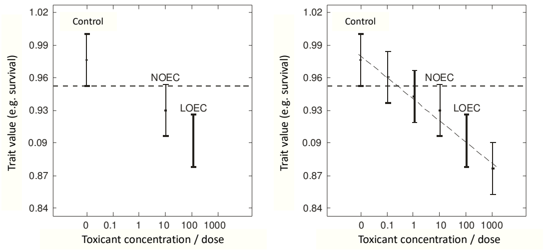
Analysis of variance (ANOVA)
strengths: pretty clear results
weaknesses: no information on dose-response relationship

Regression analysis with comparison of slopes:
LC_{50} values can be estimated
Dose-response relationship described
Comparison of different toxicants possible

ANOVA approach in ecotoxicology...

- **NOEC – "No Observed Effect Concentration"**
- **LOEC – "Lowest Observed Effect Concentration"**

...makes no sense!

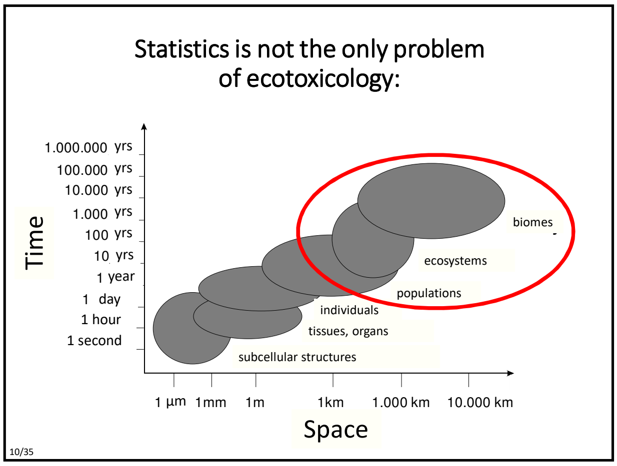


Results of six different *post hoc* tests, used to compare means of ten random samples from two populations: X with the mean **μX=90** and Y with the mean **μY=100** and equal standard deviations *s*X=*s*Y=10; n=10, α=0.05, β=0.11. The same letter in a column means no significant difference at probability 95%.

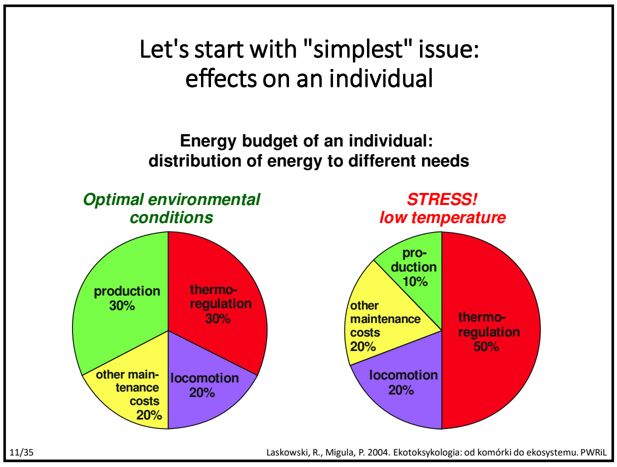
Sample	Mean	Method (*post hoc* test)					
		Tukey	LSD	Scheffé	Bonferroni	Newman-Keuls	Duncan
X1	86.5	A	A	A	A	A	A
X2	89.4	A B	A	A	A B	A B	A B
X3	89.5	A B	A	A	A B	A B	A B
X4	91.9	A B	A B	A	A B	A B	A B C
X5	94.5	A B	A B C	A	A B	A B	A B C D
Y1	97.7	A B	B C	A	A B	A B	B C D
Y2	99.6	A B	B C	A	A B	B	C D
Y3	99.6	A B	B C	A	A B	B	C D
Y4	101.7	B	C	A	B	B	D
Y5	102.1	B	C	A	B	B	D

Examples showing the non-adequacy of NOEC i LOEC

Species	Parameter (toxicant)	LOEC [mg/kg]	EC_{50} [mg/kg]
Cognettia sphagnetorum	Body mass increase (Cu)	**100**	**8**
Folsomia candida	Reproduction (Cu)	**800**	**658**
Folsomia candida	Reproduction (LAS)	**400**	**91**
Platynothrus peltifer	Reproduction (LAS)	**1000**	**467**
Eisenia fetida	Cocoon number (LAS)	**800**	**558**
Eisenia fetida	Cocoon number (DMT)	**10**	**5.3**
Eisenia fetida	Offspring number (DMT)	**10**	**7.1**



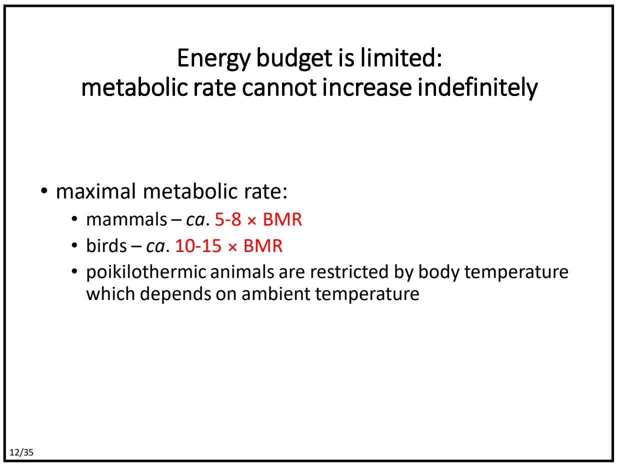
Statistics is not the only problem of ecotoxicology:



Let's start with "simplest" issue: effects on an individual

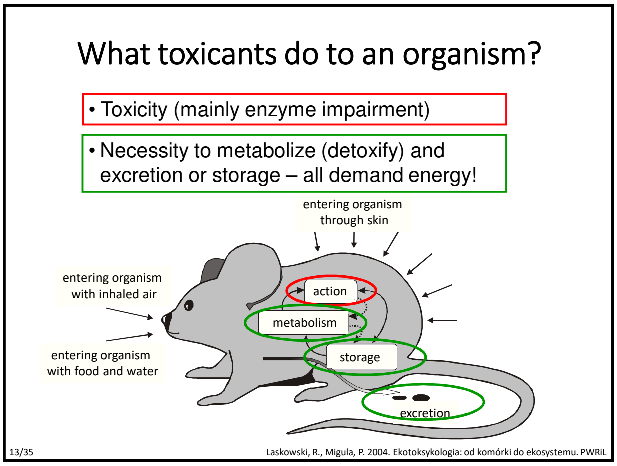
Energy budget of an individual: distribution of energy to different needs

Laskowski, R., Migula, P. 2004. Ekotoksykologia: od komórki do ekosystemu. PWRiL



Energy budget is limited: metabolic rate cannot increase indefinitely

- maximal metabolic rate:
  - mammals – *ca*. 5-8 × BMR
  - birds – *ca*. 10-15 × BMR
  - poikilothermic animals are restricted by body temperature which depends on ambient temperature



What toxicants do to an organism?

- Toxicity (mainly enzyme impairment)
- Necessity to metabolize (detoxify) and excretion or storage – all demand energy!

entering organism through skin

entering organism with inhaled air

entering organism with food and water

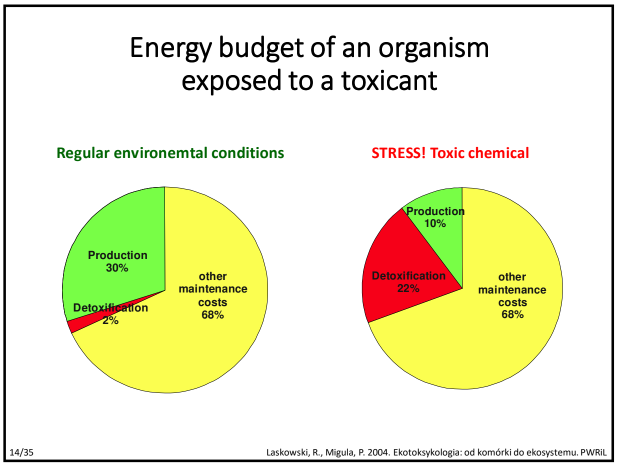
action

metabolism

storage

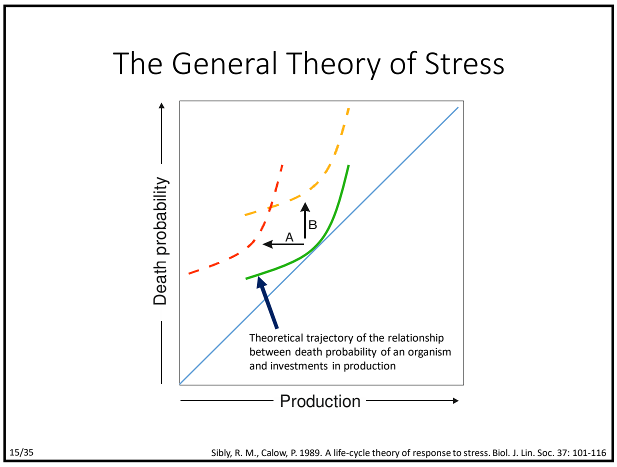
excretion

Laskowski, R., Migula, P. 2004. Ekotoksykologia: od komórki do ekosystemu. PWRiL



Energy budget of an organism exposed to a toxicant

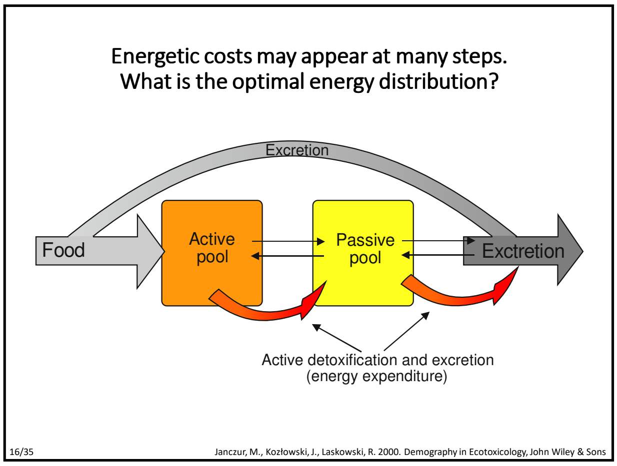
Laskowski, R., Migula, P. 2004. Ekotoksykologia: od komórki do ekosystemu. PWRiL



The General Theory of Stress

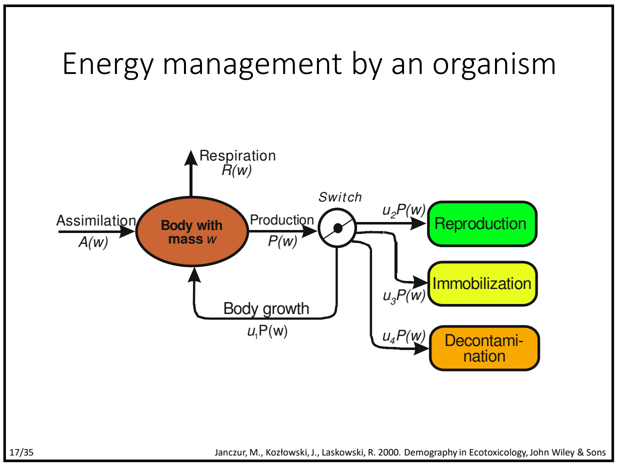
Theoretical trajectory of the relationship between death probability of an organism and investments in production

Sibly, R. M., Calow, P. 1989. A life-cycle theory of response to stress. Biol. J. Lin. Soc. 37: 101-116



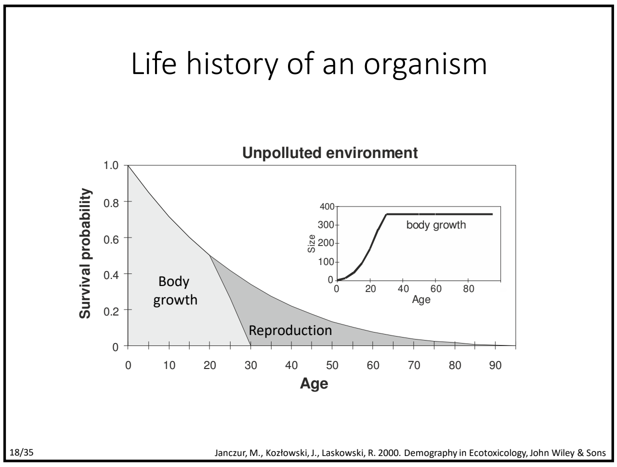
Energetic costs may appear at many steps. What is the optimal energy distribution?

Janczur, M., Kozłowski, J., Laskowski, R. 2000. Demography in Ecotoxicology, John Wiley & Sons



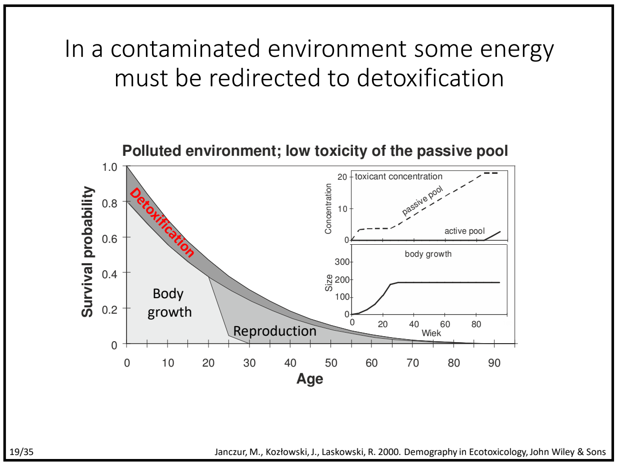
Energy management by an organism

Janczur, M., Kozłowski, J., Laskowski, R. 2000. Demography in Ecotoxicology, John Wiley & Sons



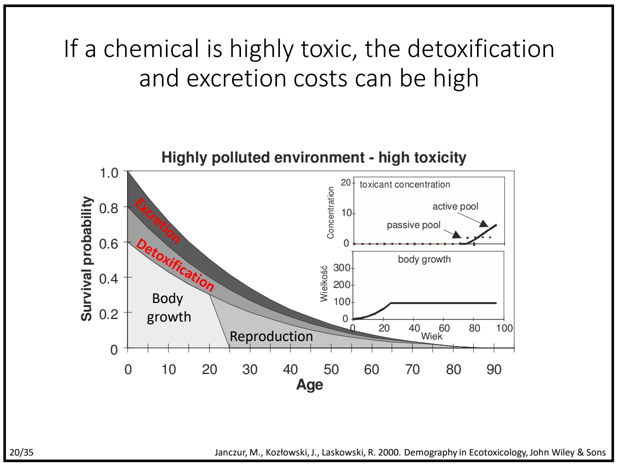
Life history of an organism

Janczur, M., Kozłowski, J., Laskowski, R. 2000. Demography in Ecotoxicology, John Wiley & Sons



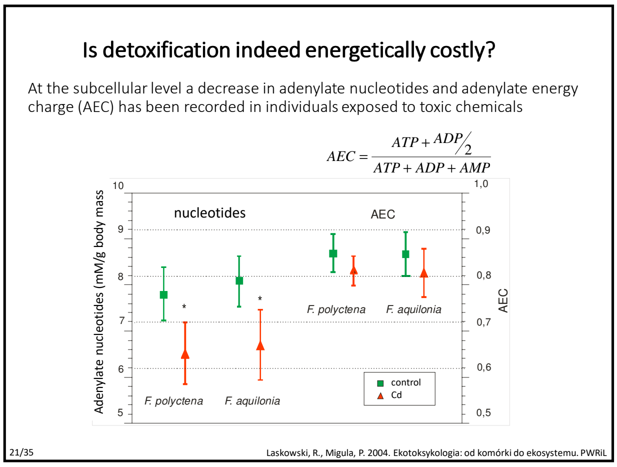
In a contaminated environment some energy must be redirected to detoxification

Janczur, M., Kozłowski, J., Laskowski, R. 2000. Demography in Ecotoxicology, John Wiley & Sons



If a chemical is highly toxic, the detoxification and excretion costs can be high

Janczur, M., Kozłowski, J., Laskowski, R. 2000. Demography in Ecotoxicology, John Wiley & Sons

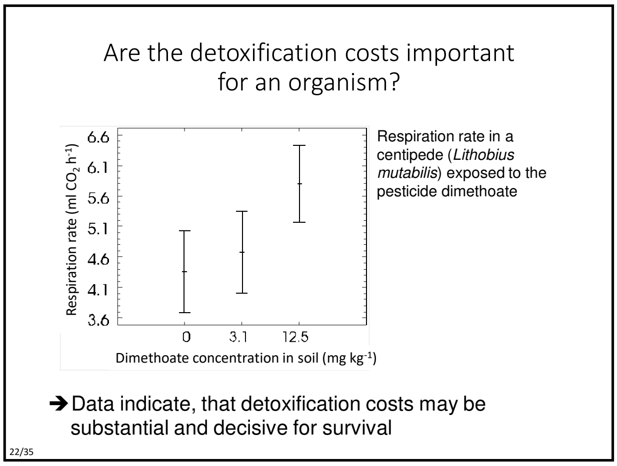


Is detoxification indeed energetically costly?

At the subcellular level a decrease in adenylate nucleotides and adenylate energy charge (AEC) has been recorded in individuals exposed to toxic chemicals

$$AEC = \frac{ATP + {}^{ADP}\!/_{2}}{ATP + ADP + AMP}$$

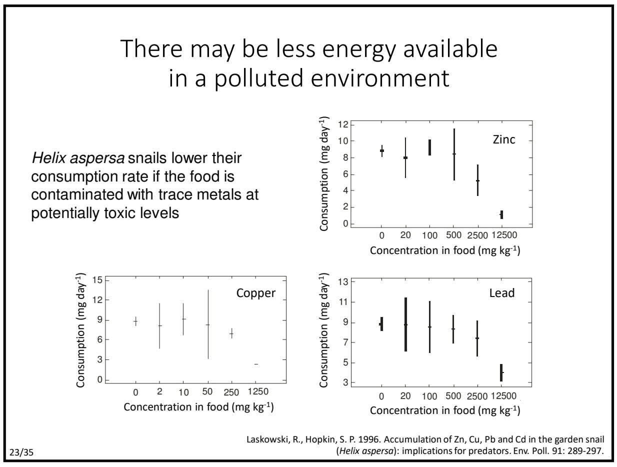
Laskowski, R., Migula, P. 2004. Ekotoksykologia: od komórki do ekosystemu. PWRiL



Are the detoxification costs important for an organism?

Respiration rate in a centipede (*Lithobius mutabilis*) exposed to the pesticide dimethoate

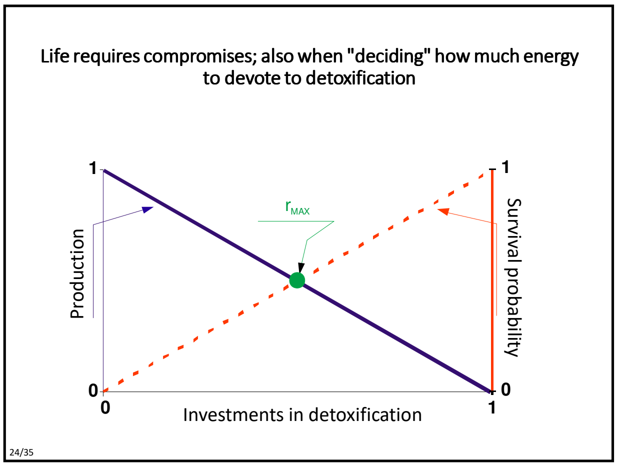
➔ Data indicate, that detoxification costs may be substantial and decisive for survival



There may be less energy available in a polluted environment

Helix aspersa snails lower their consumption rate if the food is contaminated with trace metals at potentially toxic levels

Laskowski, R., Hopkin, S. P. 1996. Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): implications for predators. Env. Poll. 91: 289-297.



Life requires compromises; also when "deciding" how much energy to devote to detoxification

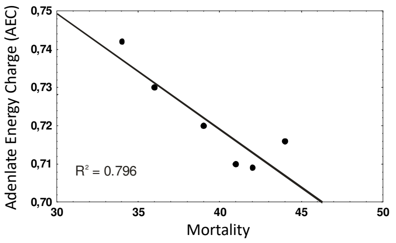
Biomarkers of toxicity

- Biomarker: "Any biological response to the presence of a toxic substance in the environment, at the individual level or below, showing a deviation from the norm"
- Types of biomarkers:
  - biochemical
  - physiological
  - histological
  - morphological
  - behavioral

Examples of biomarkers

Organization level	Biomarkers
Biochemistry	inhibition of AChE; induction of mono-oxygenases; induction of metallothioneins; hsp induction; decrease in AEC / nucleotides
Physiology	egg shell thinning; masculinization ("imposex"); feminization of embryos
Tissues	pathological changes in tissues (kidneys, liver, etc.)
Individual	Morphology: fluctuating asymmetry; b0dy growth rate Behavior: consumption decline; loss of orientation; increase or decrease in locomotor activity

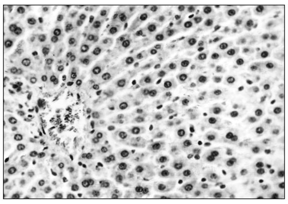
Links between biomarkers and life history



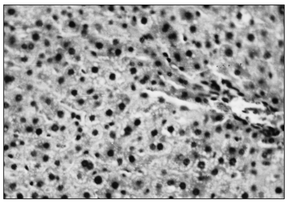
AEC is significantly negatively correlated with larvae mortality of the housefly (*Musca domestica*)

Laskowski, R., Migula, P. 2004. Ekotoksykologia: od komórki do ekosystemu. PWRiL

Histopathological changes in the liver of a rat exposed to elevated Cd concentration



Control rat liver (x300)



Liver of Cd-exposed rat (x300); spongy cytoplasm, filled with vacuoles, more compact nuclear chromatin, necrosis of individual hepatocytes

Brzóska et al. 2003. Liver and kidney functions and histology in rats exposed to cadmium and ethanol. Alcohol and Alcoholism, 38: 2-10.

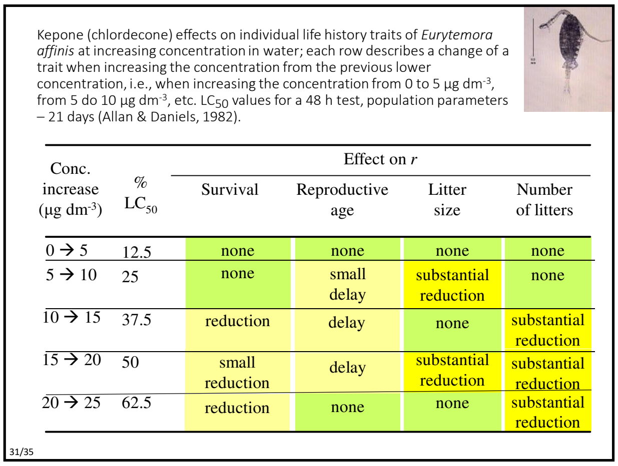
The effect of toxicants on what trait should we then measure?

- It depends on the question:
  - if we want to detect the effects "at any cost" → most sensitive life story traits or biomarkers
  - if we need a precise impact assessment at the population level → population dynamics measures (*r*, *λ*, *K*)
  - if the goal is to protect a population from extinction → probability of extinction and predicted time to extinction

Which life history traits or biomarkers are most sensitive to toxic substances?

Species	LC_{50} (14 days)	EC_{50} (cocoon production)	EC_{50} (NRR)
	mg Zn kg^{-1}		
Eisenia fetida	3172	1898	>2000
Lumbricus terrestris	2378	1029	542
Lumbricus rubellus	1734	599	168
Aporrectodea caliginosa	1695	442	252

Spurgeon et al. 2000. Relative sensitivity of life-cycle and biomarker responses in four earthworm species exposed to zinc. Environmental Toxicology and Chemistry, 19: 1800-1808.



Kepone (chlordecone) effects on individual life history traits of *Eurytemora affinis* at increasing concentration in water; each row describes a change of a trait when increasing the concentration from the previous lower concentration, i.e., when increasing the concentration from 0 to 5 μg dm^{-3}, from 5 do 10 μg dm^{-3}, etc. LC$_{50}$ values for a 48 h test, population parameters – 21 days (Allan & Daniels, 1982).

Conc. increase (μg dm^{-3})	% LC$_{50}$	Effect on *r* Survival	Reproductive age	Litter size	Number of litters
0 → 5	12.5	none	none	none	none
5 → 10	25	none	small delay	substantial reduction	none
10 → 15	37.5	reduction	delay	none	substantial reduction
15 → 20	50	small reduction	delay	substantial reduction	substantial reduction
20 → 25	62.5	reduction	none	none	substantial reduction

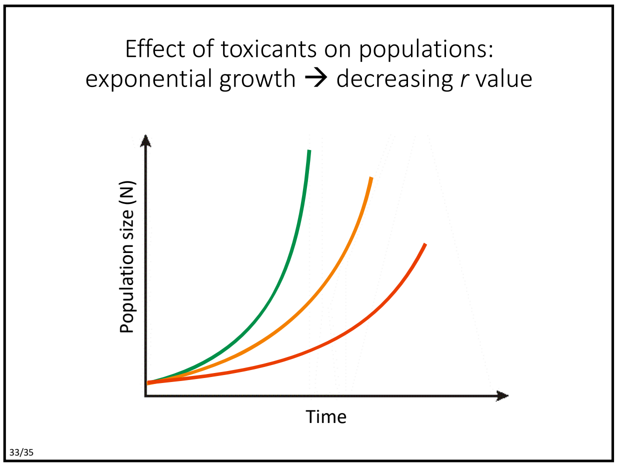
When we need to estimate effects of a toxicant on a population, it's better to use population dynamics measures

$$R_0 = \sum_{x=0}^{n} l_x m_x \qquad 1 = \sum e^{-rx} l_x m_x$$

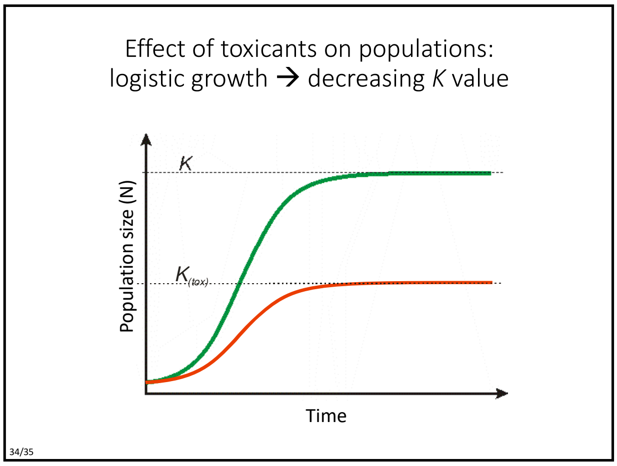
$$r_i = \frac{\ln \frac{N_t}{N_0}}{\Delta t}$$

λ - the dominant eigenvalue of the Leslie matrix

K – environmental capacity



Effect of toxicants on populations: exponential growth → decreasing *r* value



Effect of toxicants on populations: logistic growth → decreasing K value

Take-home messages

- **LD$_{50}$** – *Lethal Dose for 50% individuals*
- **LC$_{50}$** – *Lethal Concentration for 50% individuals*
- **EC$_{50}$** – *50% Effect Concentration* (e.g., 50% decrease in fecundity or life span, etc.)
- **NOEC** – *No Observed Effect Concentration* = the highest concentration that does not cause significant negative effects
- **LOEC** – *Lowest Observed Effect Concentration* = the lowest concentration at which significant negative effects appear
- Statistical method of choice does matter!
- The General Theory of Stress tells that detoxification and decontamination may be energetically costly → effects!
- Biomarkers can help in early detection of intoxication
- Useful measures of population-level effect of toxicants: r, λ, K