Dynamic Energy Budget models in Ecotoxicology

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Special thanks to Tin Klanjscek, Dina Lika, Erik Muller, Cheryl Murphy
Louise Stevenson and all members of NIMBioS working group
“Molecules to individuals”

Lecture 1: "Dynamic Energy Budget theory in ecotoxicology"

- Ecotoxicology: effects of toxic substances on living organisms at multiple levels of ecological organization
- Why develop general theory? Too many chemicals, organisms, environments
- Toxicokinetics (TK) and toxicodynamics (TD)
- Modeling triad: DEB/TK/TD
- DEB-based modeling of lethal effects: damage and survival (GUTS)
- DEB-based modeling of sublethal effects: physiological modes of action (pMoA)
- Practical challenges

Reference:

Jager T (2019). *Making Sense of Chemical Stress. Application of Dynamic Energy Budget Theory in Ecotoxicology and Stress Ecology*. Leanpub: https://leanpub.com/debtox_book.

T. Jager. Making sense of chemical stress. https://leanpub.com/debtox_book

Ecotoxicology and Ecological risk assessment (ERA)

Ecotoxicology: effects of toxic substances on living organisms at multiple levels of ecological organization

ERA*: *the process for evaluating how likely it is that the environment may be impacted as a result of exposure to one or more environmental stressors.*

ERA involves predicting effects of exposure on populations, communities and ecosystems – including "ecosystem production functions" such as nutrient cycling and "ecosystem services".

One approach uses process-based, dynamic models of exposure and response to exposure to predict "step-by-step" up and down levels of organization.

* http://www.epa.gov/risk_assessment/ecological-risk.htm

Why predictive toxicology is hard

Need general theory:

- Too many chemicals, organisms, environments

Feedbacks

- *Physiology*: e.g. regulatory processes within and among cells and organs
- *Physico-chemical environment*: e.g. excretion products may impact toxicity
- *Ecological interactions:* e.g. resource limitation, mutualism
- *Plasticity, acclimation and adaptation:* e.g. evolutionary rescue

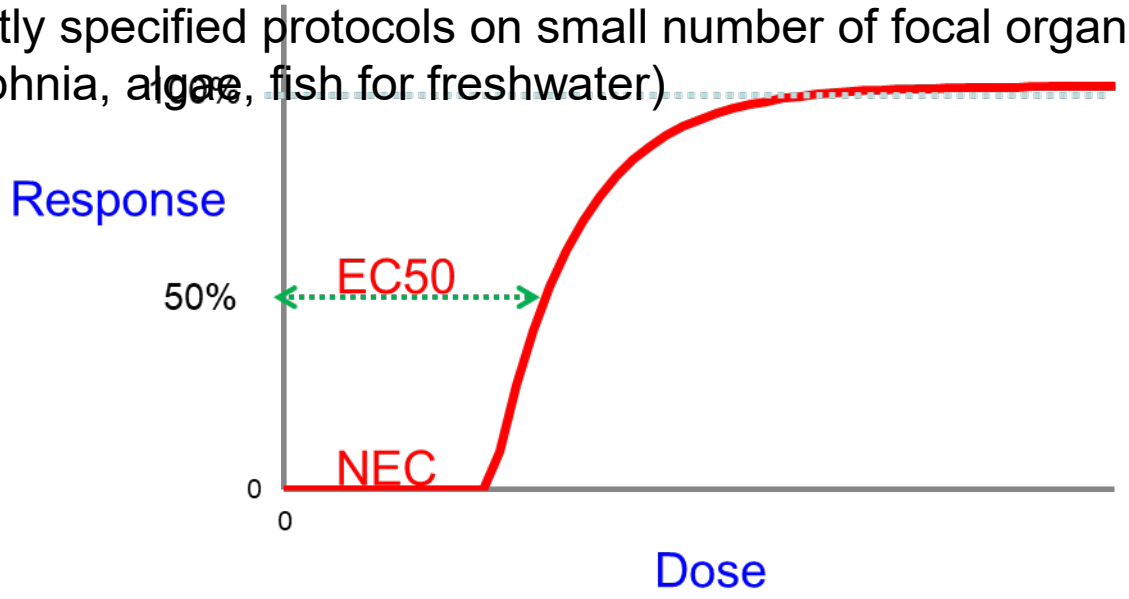
Emergent properties

- Found at every link between levels of organization, e.g. tipping points

Rohr, Salice, Nisbet, *Critical Reviews in Toxicology,* 2016.

Standardized toxicity tests

- Primary aim is to guide regulation of chemicals by identifying “safe” levels in the environment
- Tests for both acute (lethal) and chronic (non-lethal) toxicity
- Use strictly specified protocols on small number of focal organisms (e.g. Daphnia, algae, fish for freshwater)



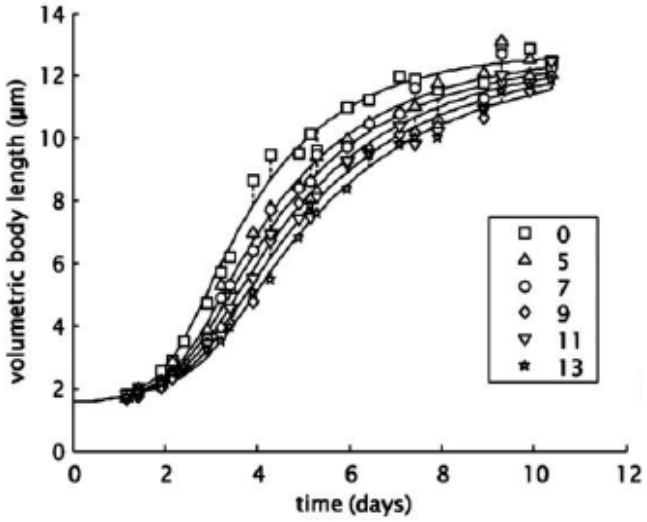
- EC50 (LC50) = dose at which response (mortality) is 50% of maximum
- NEC = dose below which there is no “harm” to organism
- Permitted level some fraction of NEC or EC50

EC_x depends on duration of experiment

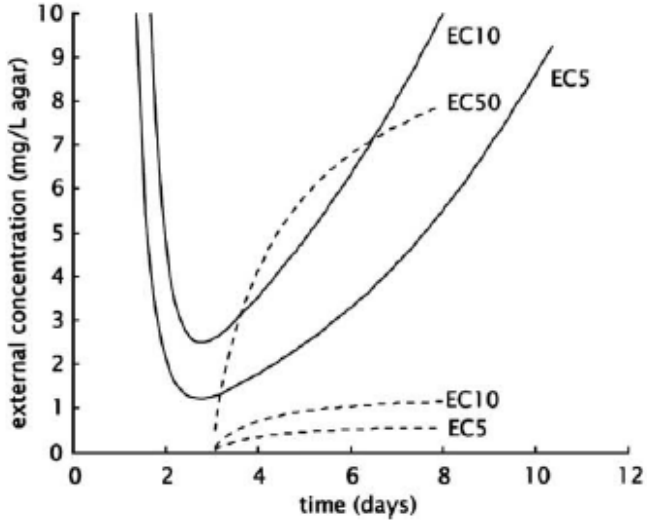
Definition: EC_x is concentration of a compound where x% of its maximal effect is observed.

Problem: value depends on duration of study

Example: *C. elegans* growth exposed to pentachlorobenzene

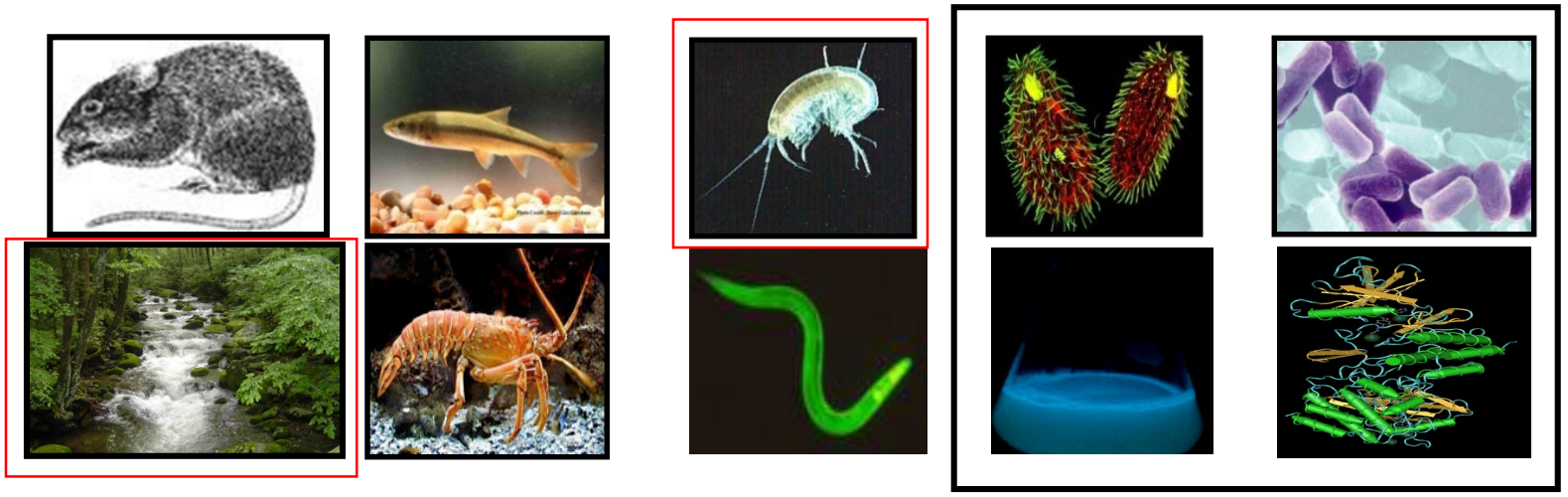


Length versus time for different toxicant levels



EC_x related to duration of exposure (cont. lines – length; broken lines – reproduction)

Other sources of data



few/year | 100's/year | 1000's/year | 10,000's/day | 100,000's/day

High Throughput Bacterial, Cellular, Yeast, Embryo or Molecular Screening

Expensive *in vivo* testing and ecological experiments

Challenge for theorists: to use information from organismal and suborganismal studies to prioritize, guide design, and interpret ecological studies and inform ERA, i.e. progress towards predictive ERA

Remarkably little ecotox research by ecologists

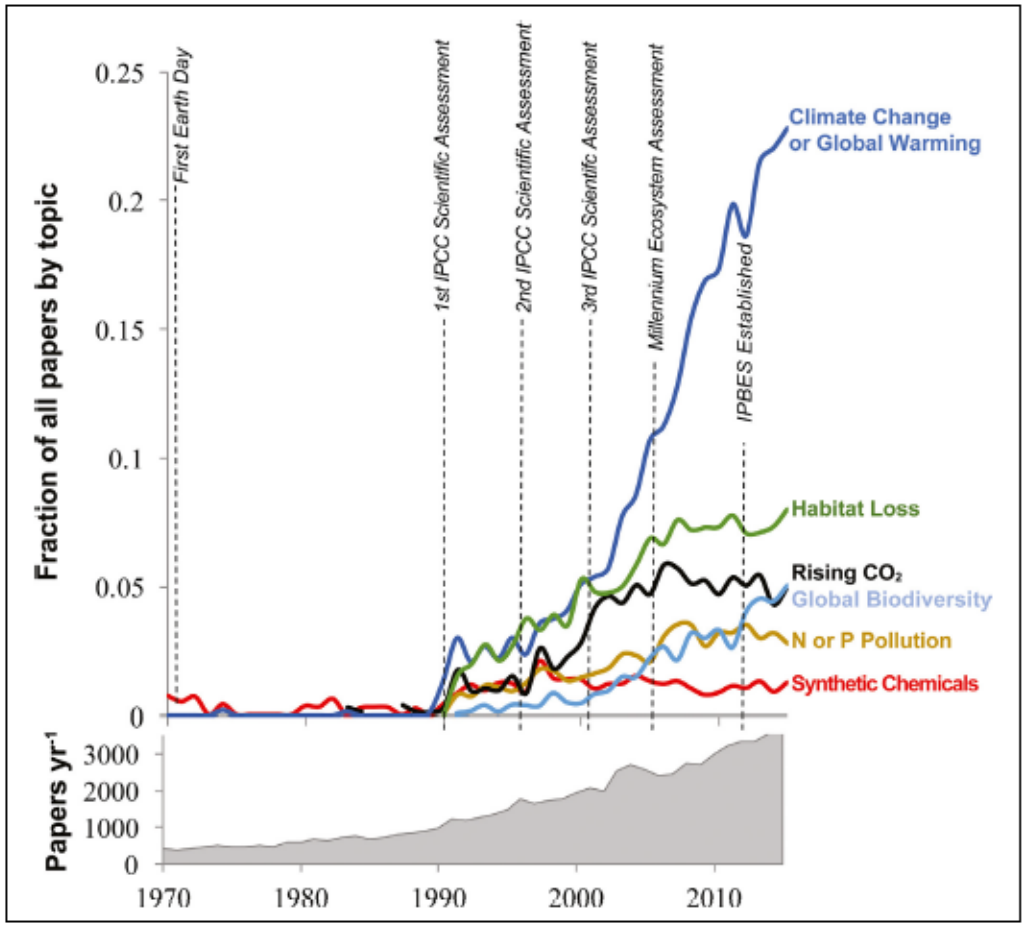


Figure 2. Total publications and the proportion of published papers including global-change driver terms in the top 20 ecology journals (Table 1) according to the highest total citations reported in the ecology section of the ISI Web of Science for the period 1970–2015.

E. Bernhardt et al. *Front Ecol Environ* 2017;

Routes to general theory

Option I: follow the chemical

- Absorbed by organism
- Distributed within organism
- Chemically transformed
- Excreted

} **TOXICOKINETICS (TK)**

Routes to general theory

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- Distributed within organism
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TOXICOKINETICS (TK)

- Interacts with tissue
- “Damages” tissue
- Impacts survival(hazard)
- Sublethal physiological impacts

TOXICODYNAMICS (TD)

Routes to general theory

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- Absorbed by organism
- Distributed within organism
- Chemically transformed
- Excreted

TOXICOKINETICS (TK)

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- Sublethal physiological impacts

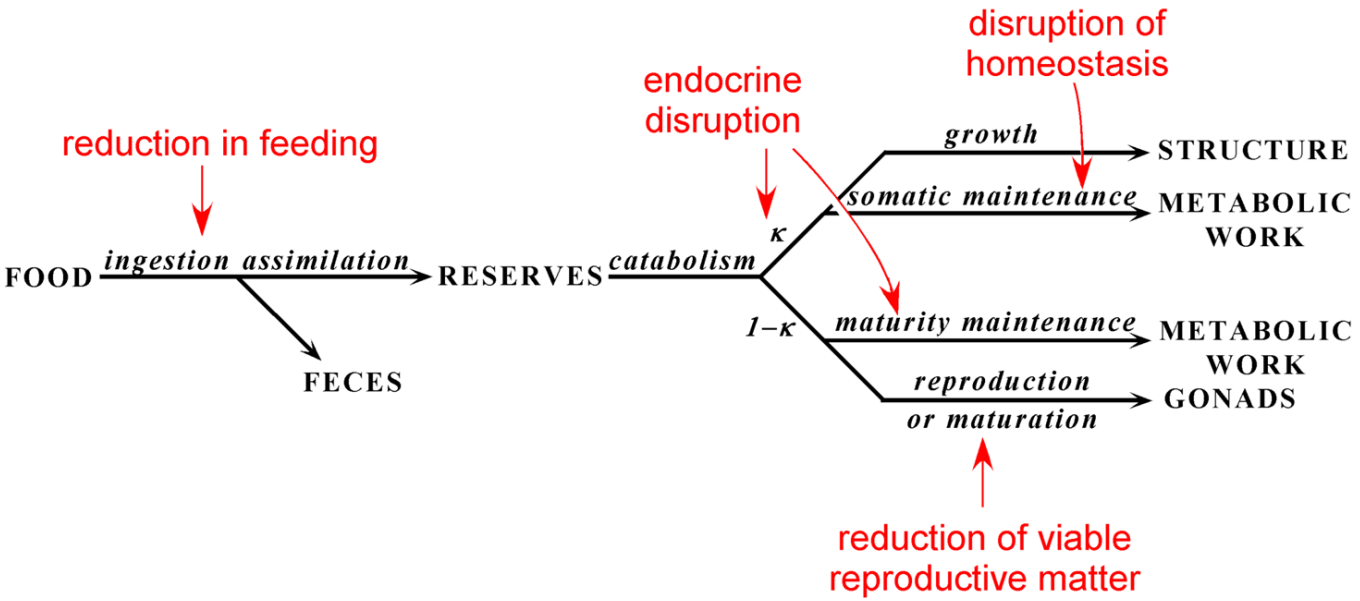
TOXICODYNAMICS (TD)

TOXICODYNAMICS CAN BE COMPLEX

Routes to general theory

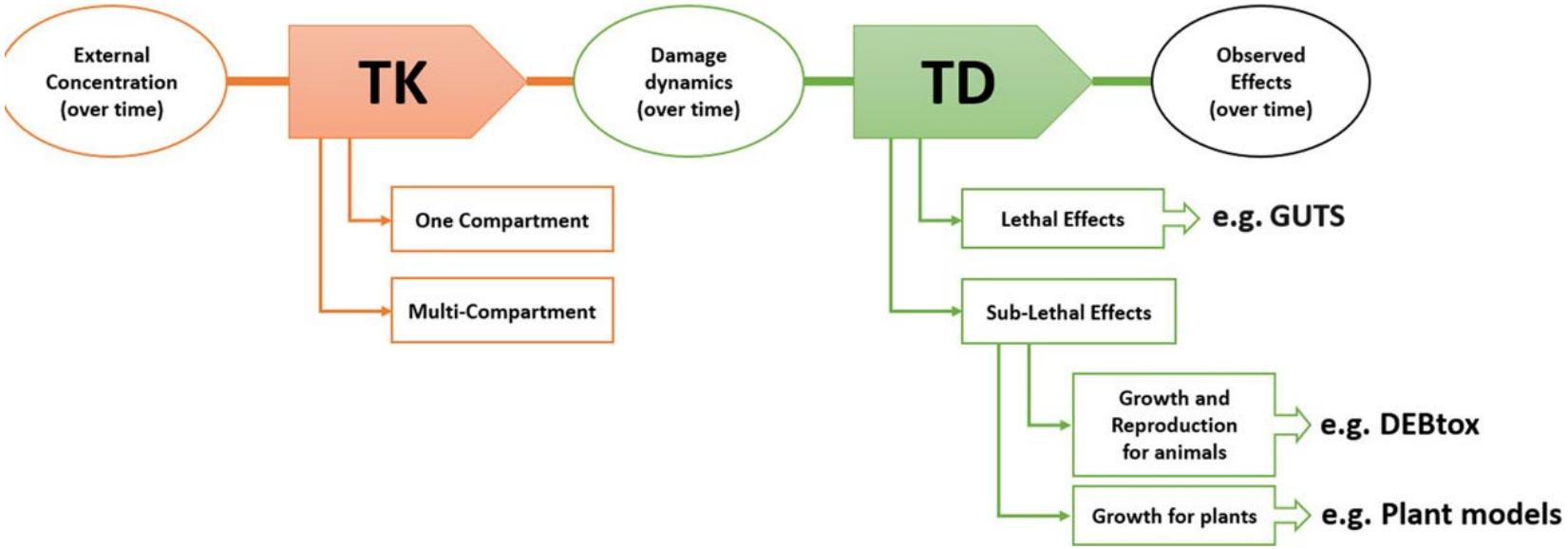
Option II: follow the complete organism

- Use DEB!!
- Each flow could in principle be affected by chemical stress – different Physiological Models of Action (pMoA)
- Identify some measure of stress and assume that different DEB (primary) parameters are functions of stress
- Fit data on growth, reproduction, or mortality assuming different pMoA(s).
- Identify “winner” statistically



SYNTHESIS: The TK-TD-DEB triad

First Step: *Scientific Opinion on the state of the art of Toxicokinetic/ Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms*



Ockleford et al (2008): doi: 10.2903/j.efsa.2018.5377

The TK-TD-DEB triad

- Dynamic energy budget (DEB) model describes the assimilation and utilization of energy and elemental matter by living organisms
- Toxicants may enter organism directly from environment or via food – represented by toxicokinetic (TK) model.
- Toxicants impact one or more energy and material flows ("mode-of-action"-MoA) – represented by toxicodynamic (TD) model

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MISSSING LINK: DEB TO TOXICODYNAMICS

USE NEW DEB VARIABLE REPRESENTING "DAMAGE"

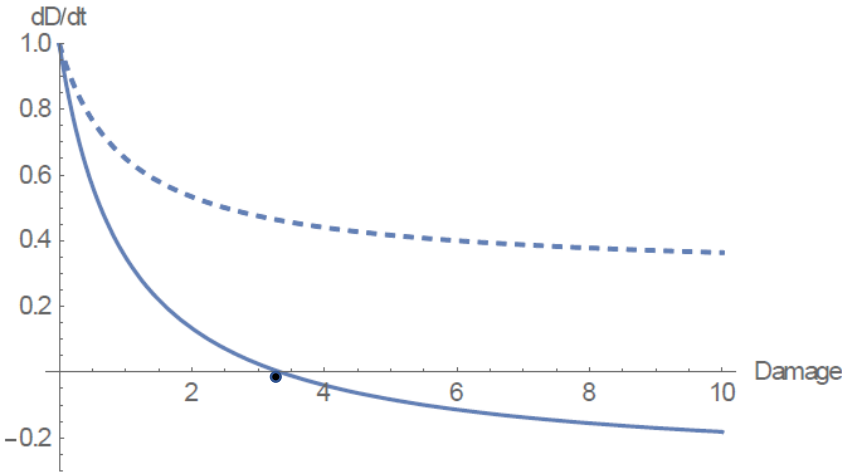
Damage variable for lethal and sublethal effects

$$\frac{dD}{dt} = P - R \quad \text{with } P = \text{ damage production rate}$$

$$R = \text{ damage repair rate}$$

Assume P is constant but R is a saturating function of D

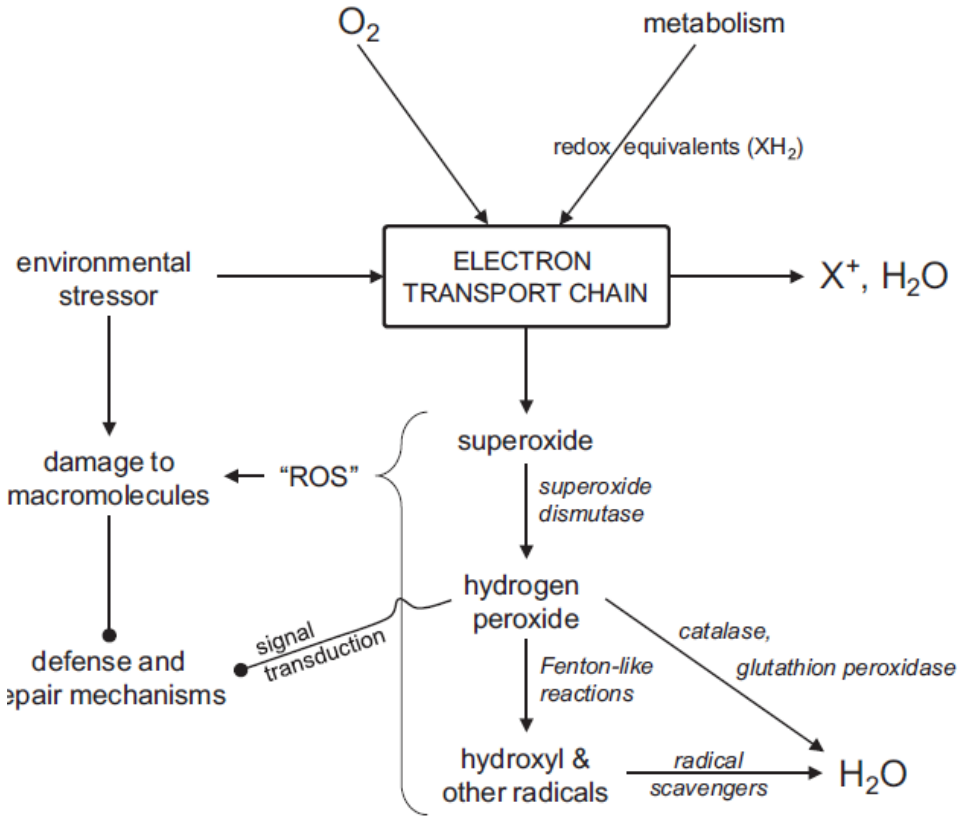
Then there are two possibilities:



No equilibrium; unbounded growth of damage - LETHAL

Stable equilibrium; damage controlled - SUBLETHAL

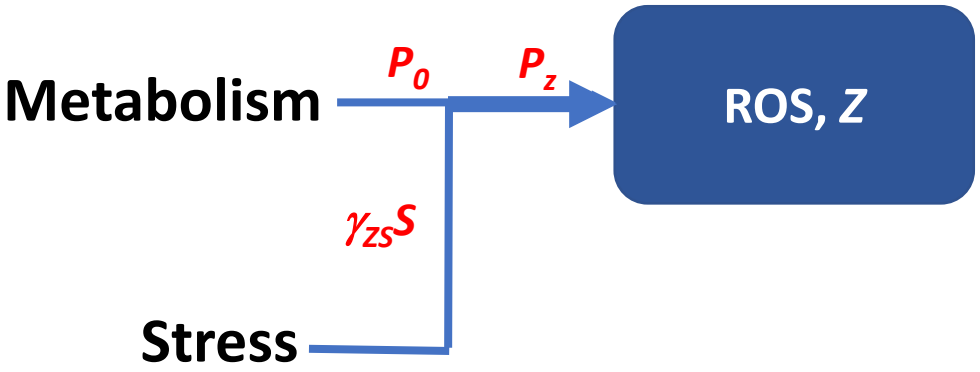
Detailed example – oxidative stress



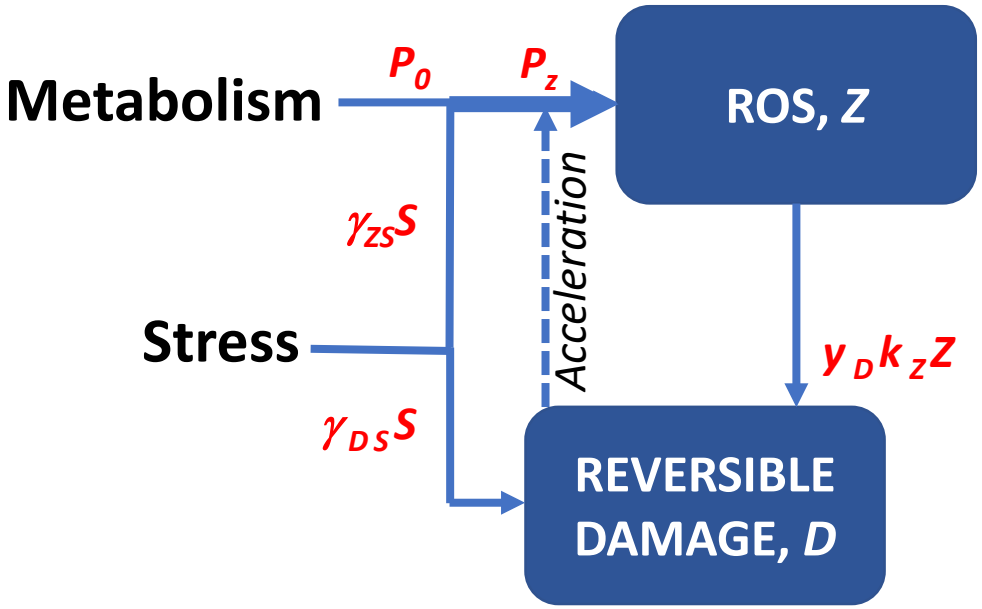
Real world toxicodynamics – greatly simplfied

Klanjscek et al, J Theor Biol 2016

Oxidative Stress – Damage Model



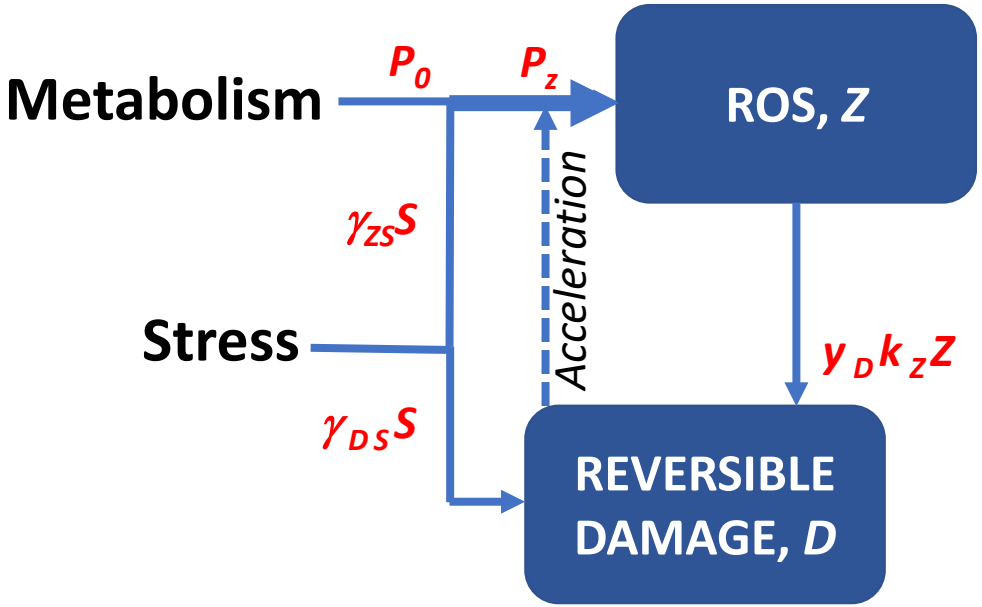
Oxidative Stress – Damage Model



Oxidative Stress – Damage Model

ROS production rate, P_Z, with acceleration being

- additive feedback of damage: $P_Z = P_0 + \gamma_{ZS} S + \gamma_{ZD} D$
- multiplicative feedback of damage: $P_Z = (P_0 + \gamma_{ZS} S)(1 + \gamma_{ZD} D)$

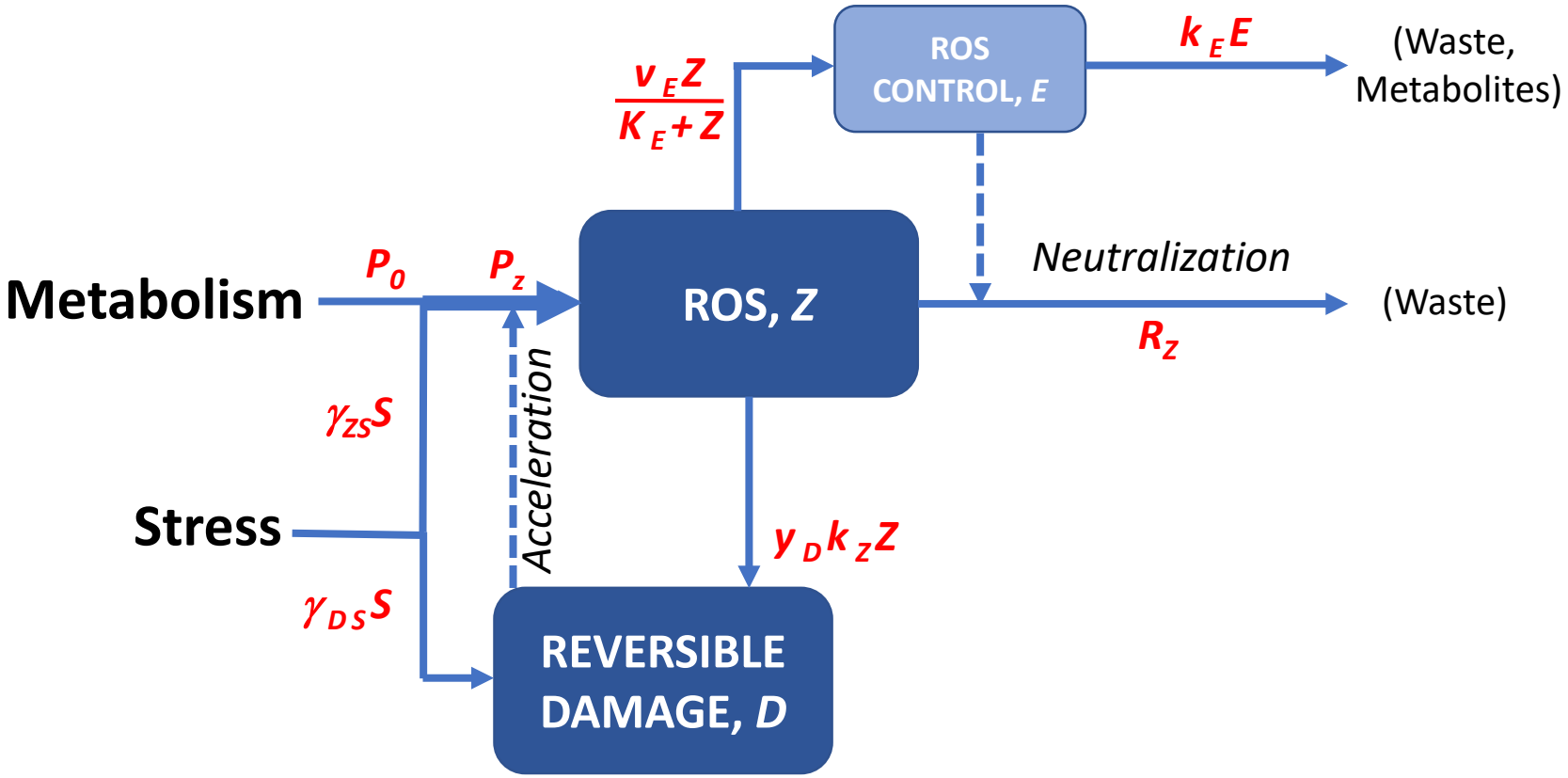


*

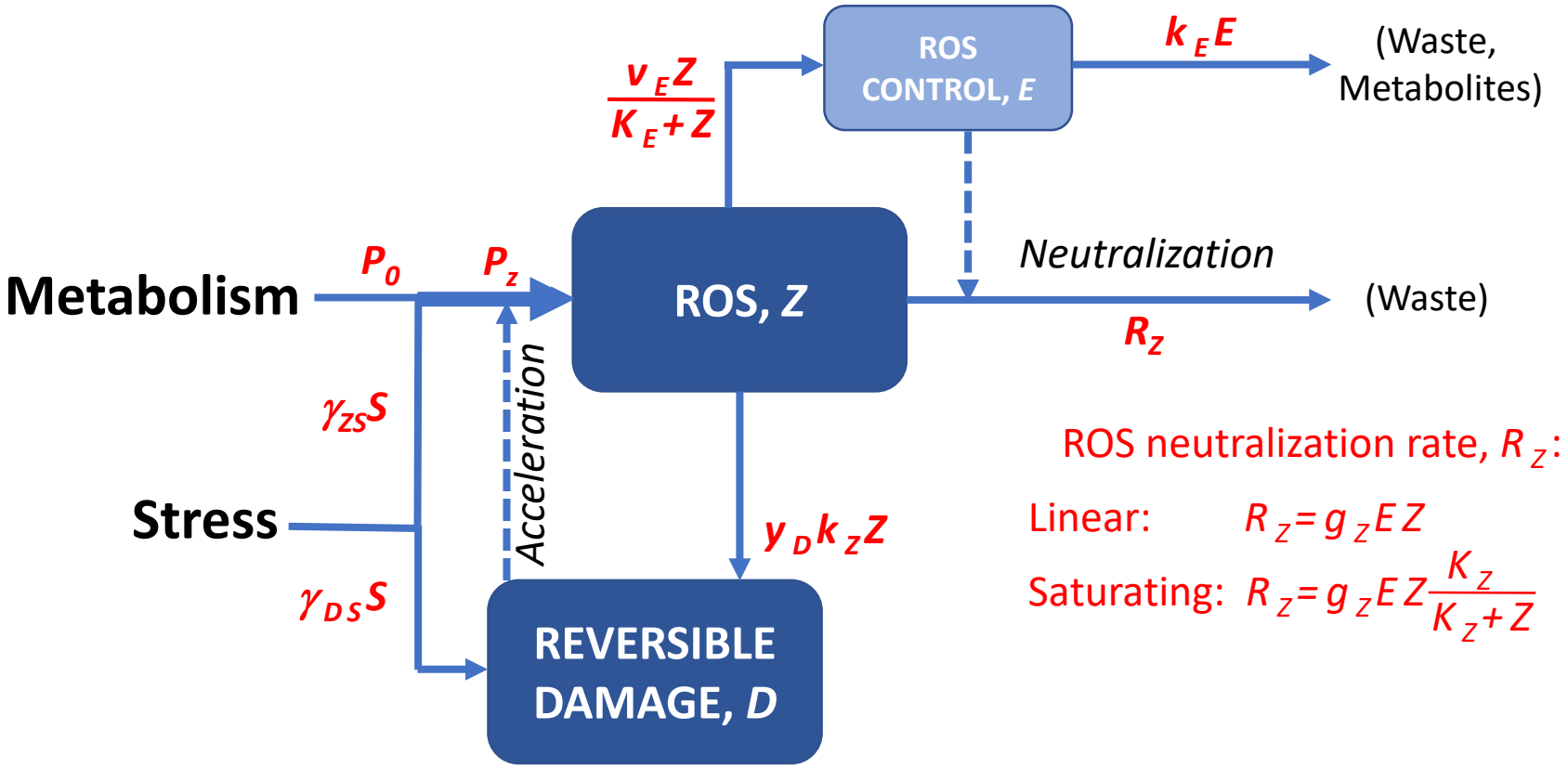
Damage production rate, P_D:

$P_D = \gamma_{DS} S + y_D k_Z Z$

Oxidative Stress – Damage Model



Oxidative Stress – Damage Model

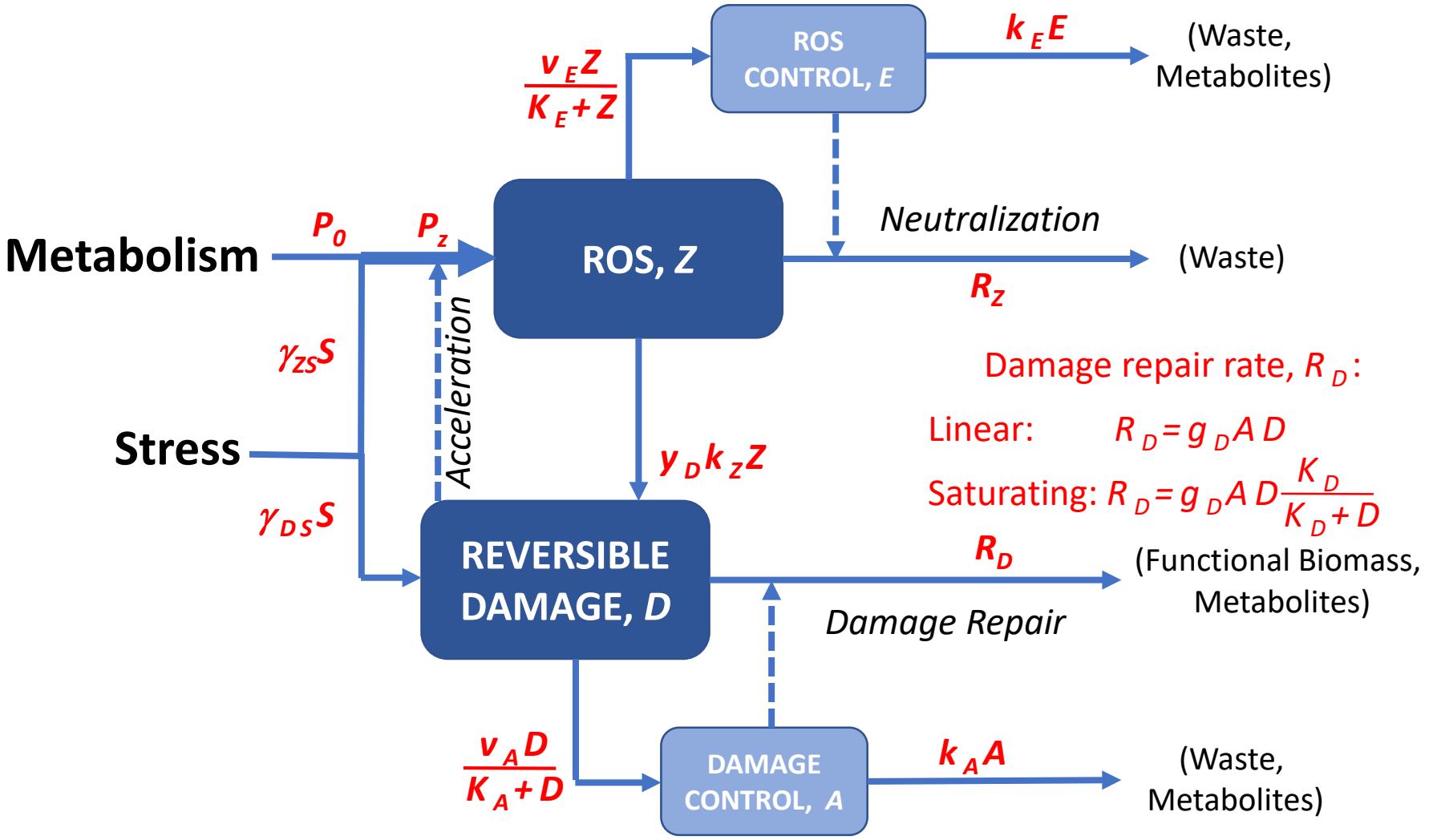


ROS neutralization rate, R_Z:

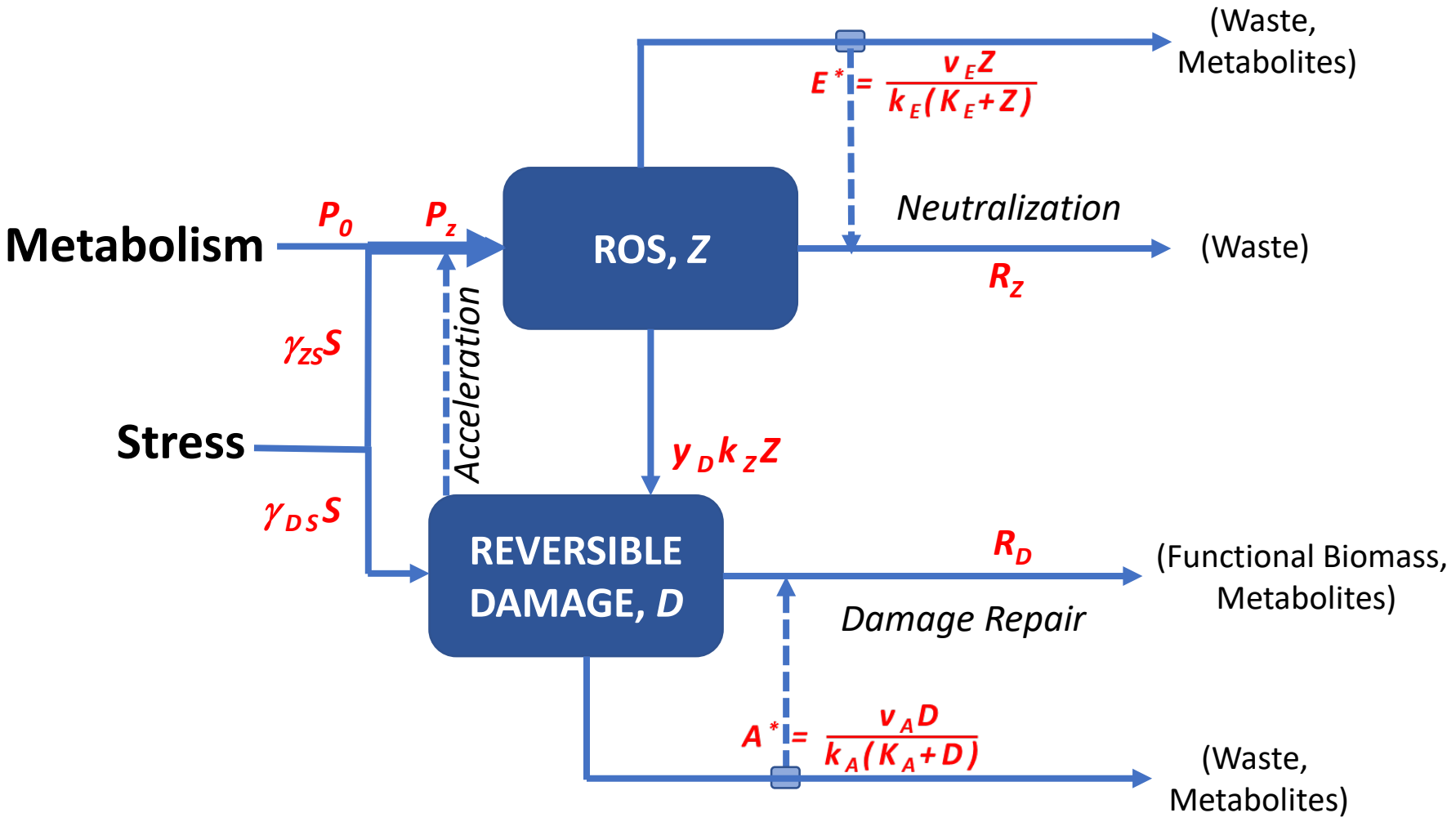
Linear: $R_Z = g_Z E Z$

Saturating: $R_Z = g_Z E Z \frac{K_Z}{K_Z + Z}$

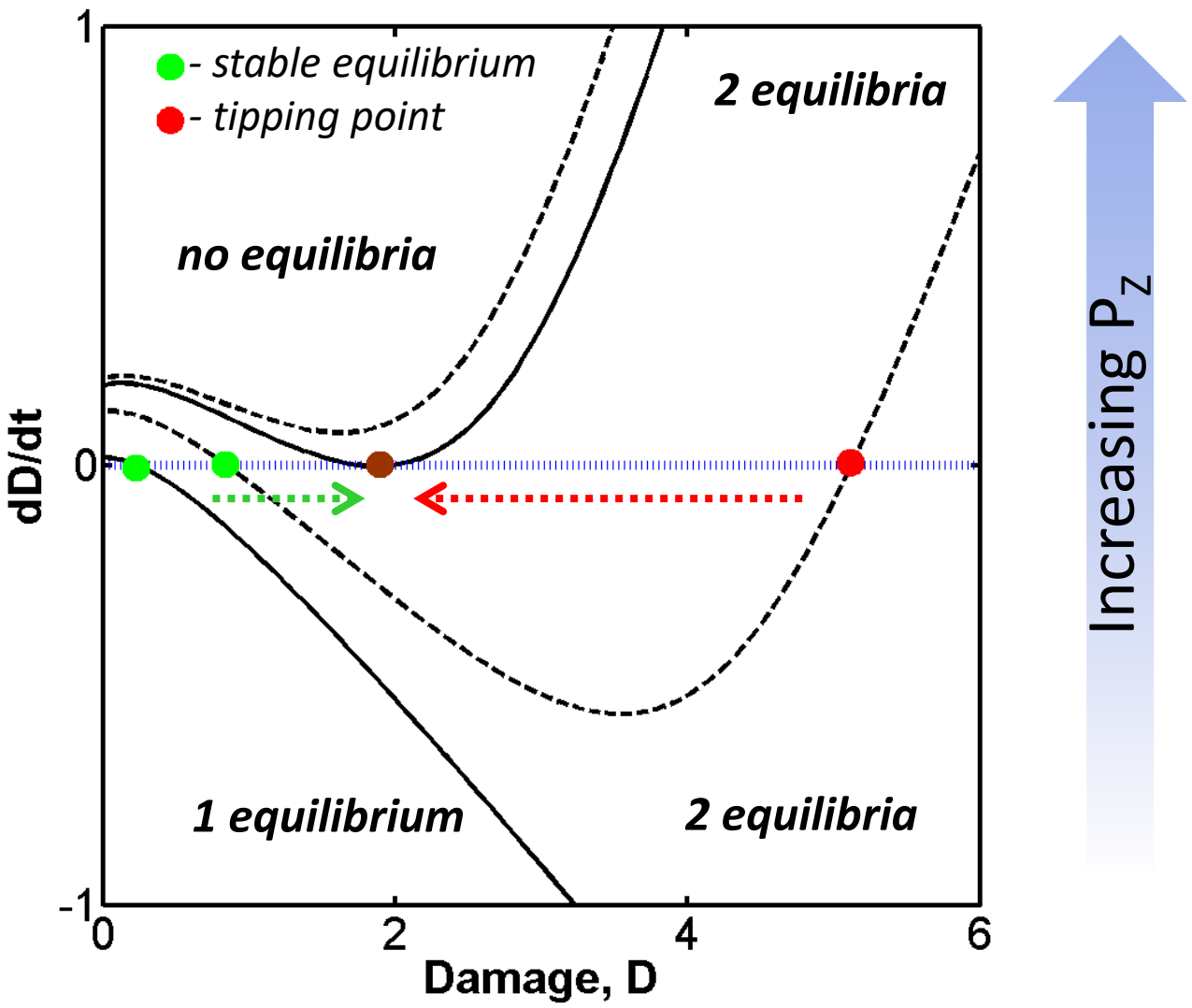
Oxidative Stress – Damage Model



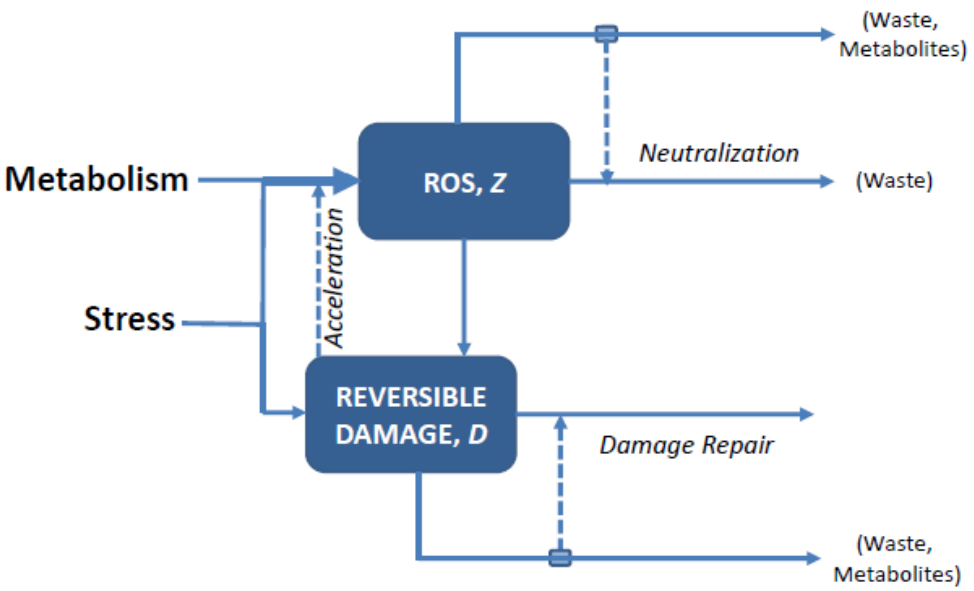
Relatively Fast Dynamics of Controllers
for Steady State Analysis



Damage Equilibria with Increasing ROS production, P_Z



Oxidative stress model properties



- Predicts co-variation of ROS and damage in response to NP exposure
- Takes account of exposure history
- Predicts "tipping points" caused by break-down of regulation (previous slide)
- Provides mechanistic basis for no-effect concentrations
- **Testing requires time-series data – consideration for future HTS studies**

Added value to data by using DEB models?

Could careful extrapolation from toxicity testing achieve similar results?

Design of toxicity tests involves many **choices**:

- **Species of organism** → DEB has recipe for interspecies comparisons
- **Exposure mode** → DEB allows natural coupling to TK and TD models
- **"Endpoint"** (measured response) → DEB model output, related to basic biology
- **Duration** → DEB model is dynamic, so output is time-dependent
- **Environmental conditions** → DEB can handle multiple environmental stressors

Lecture 2: "Lessons learned in ecotoxicology crossing scales of organization"

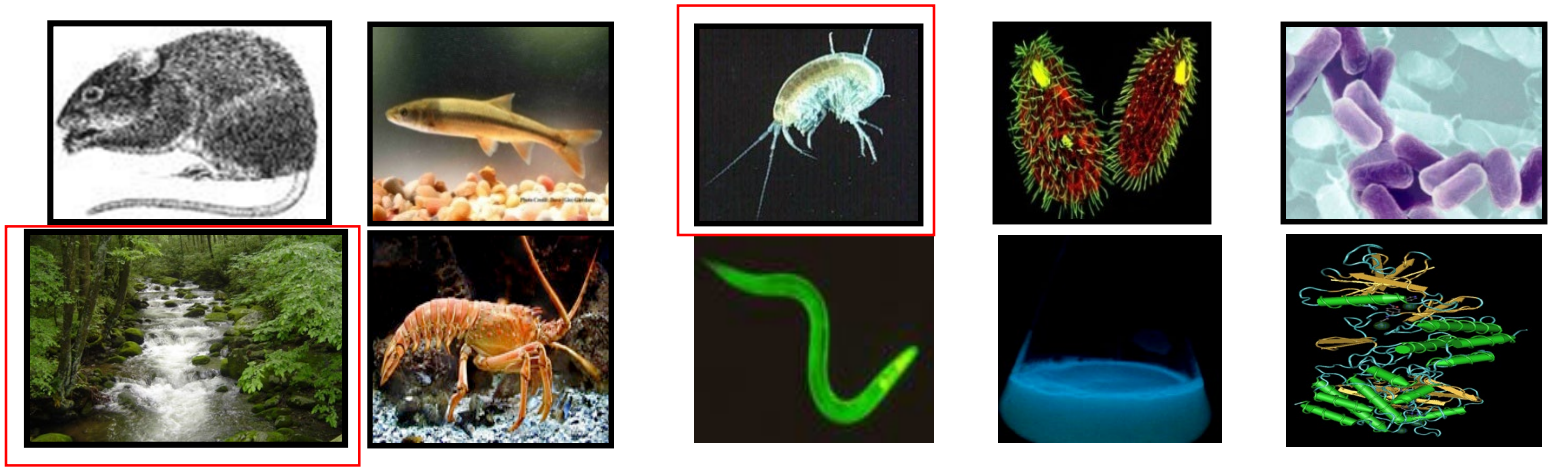
- DEB as "pivot" linking sub-organismal biology to higher levels of ecological organization
- Individual-to-population: DEB-IBM (connects with DEB-in-Practice: "Importance of toxicants' Mode of Actions to predict population outcomes")
- Adverse Outcome Pathways (AOP)
- AOP-to-DEB: challenges in linking AOP to pMoA in DEB theory
- So much more needed!

References:

B. Martin et al. (2014). *Limitations of extrapolating toxic effects on reproduction to the population level.* Ecological Applications, 24, pp. 1972–1983.

C.A. Murphy et al. (2018). *Incorporating Suborganismal Processes into Dynamic Energy Budget Models for Ecological Risk Assessment*, Integrated Environmental Assessment and Management, DOI: 10.1002/ieam.4063

Toxicant hazard at different levels of biological organization



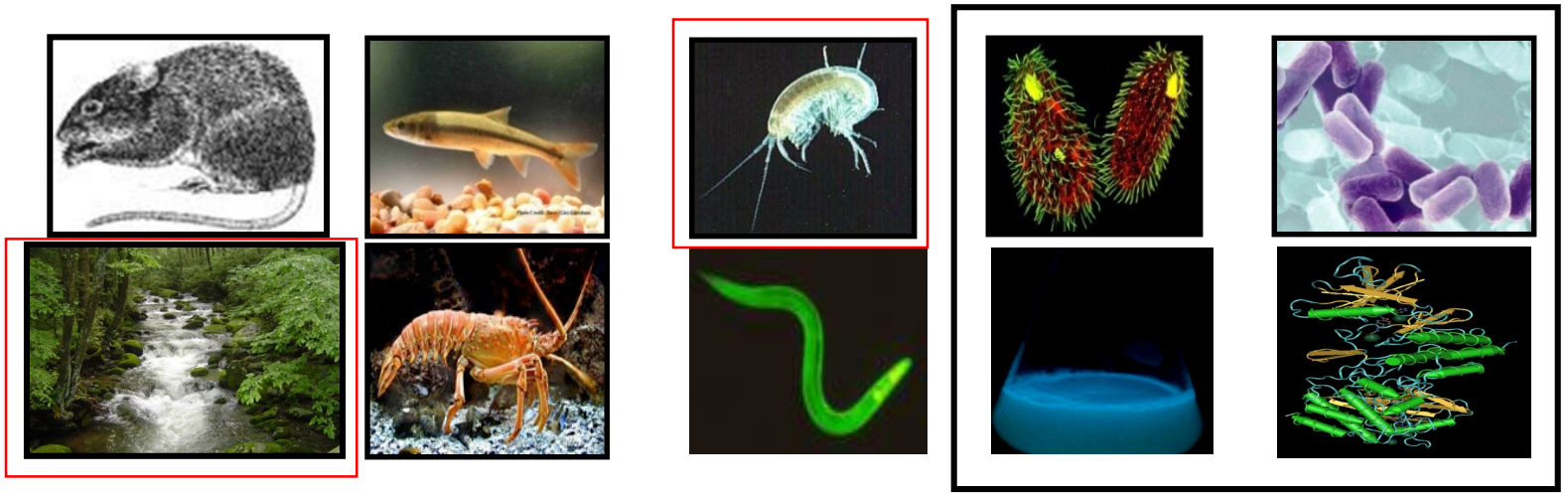
few/year | 100's/year | 1000's/year | 10,000's/day | 100,000's/day

High Throughput Bacterial, Cellular, Yeast, Embryo or Molecular Screening

Expensive *in vivo* testing and ecological experiments

Challenge for theorists: to use information from organismal and suborganismal studies to prioritize, guide design, and interpret ecological studies and inform ERA, i.e. progress towards predictive ERA

Toxicant hazard at different levels of biological organization



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Why predictive toxicology across levels of organization is hard

Feedbacks

- *Physiology*: e.g. regulatory processes within and among cells and organs
- *Physico-chemical environment*: e.g. excretion products may impact toxicity
- *Ecological interactions:* e.g. resource limitation, mutualism
- *Adaptation:* e.g. evolutionary rescue

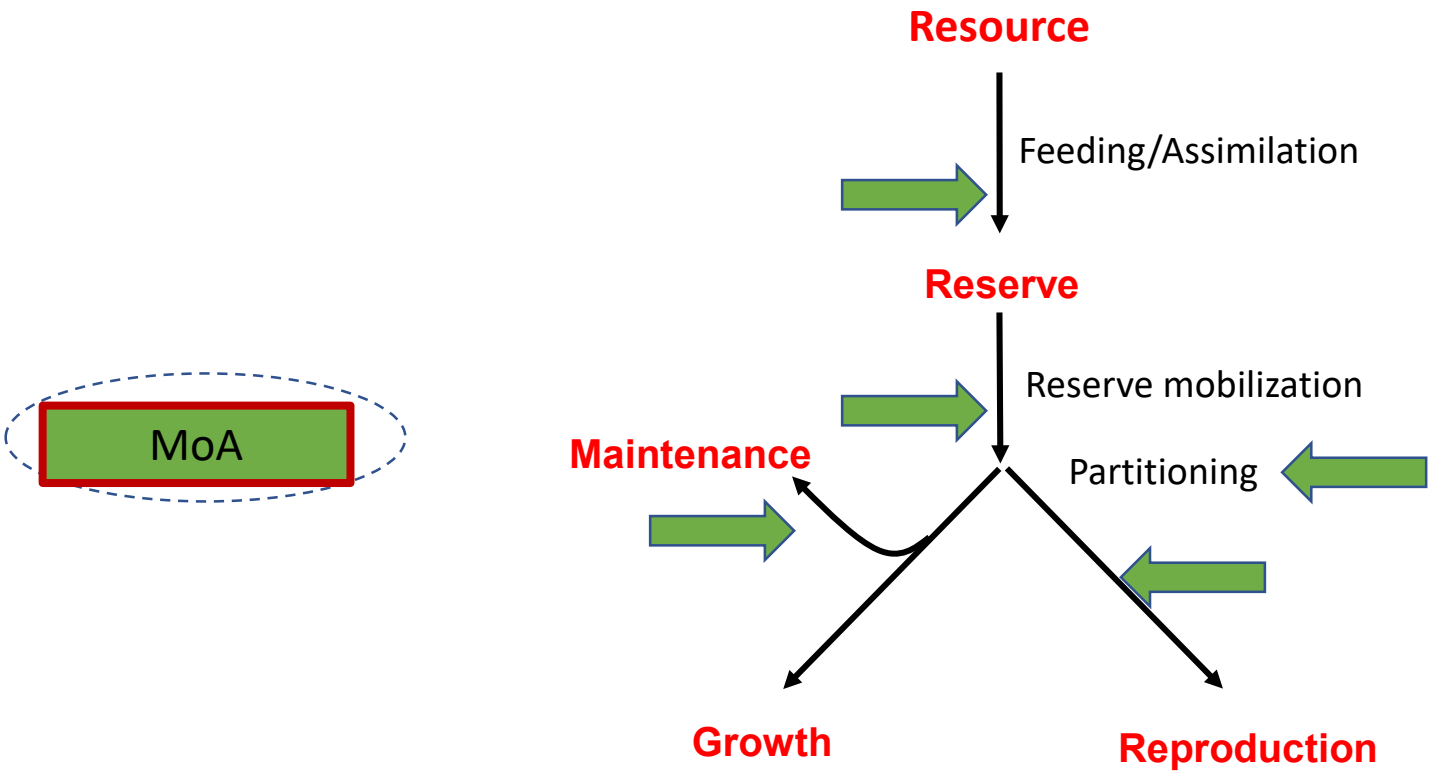
Emergent properties

- Found at every link between levels of organization, e.g. tipping points

NEED NON-LINEAR MATHEMATICAL/COMPUATIONAL MODELS

Rohr, Salice, Nisbet, *Critical Reviews in Toxicology,* 2016.

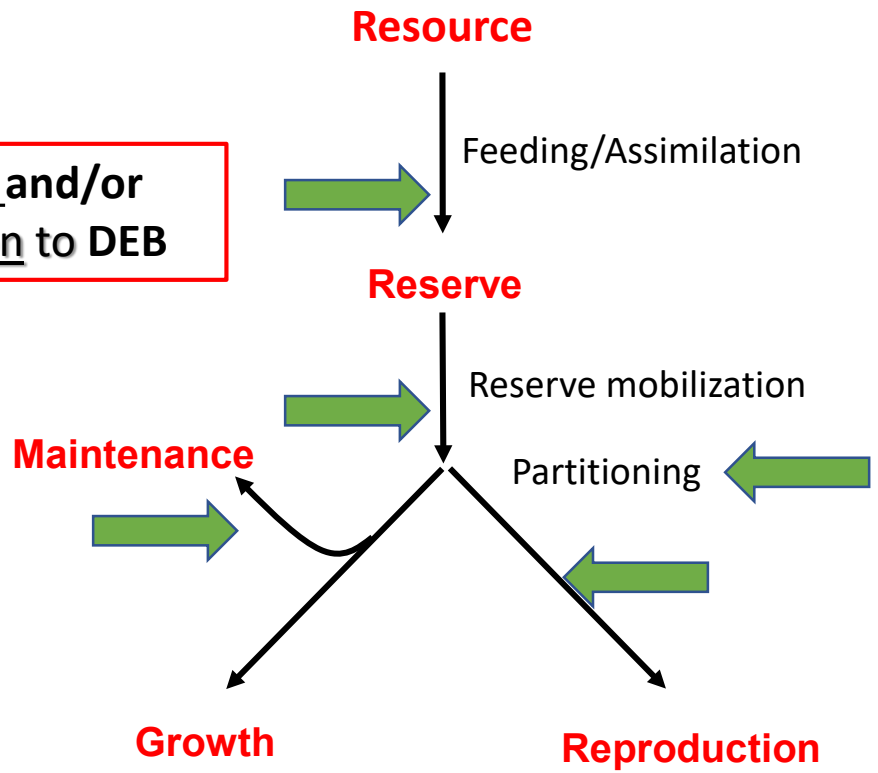
Conceptual model linking AOP and DEB approaches



DEB model

Conceptual model linking AOP and DEB approaches

Connect Key Event Relations and/or Physiological Modes of Action to DEB

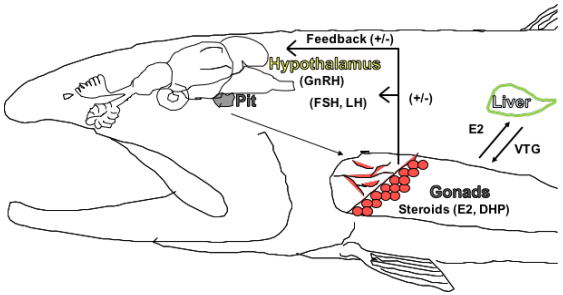


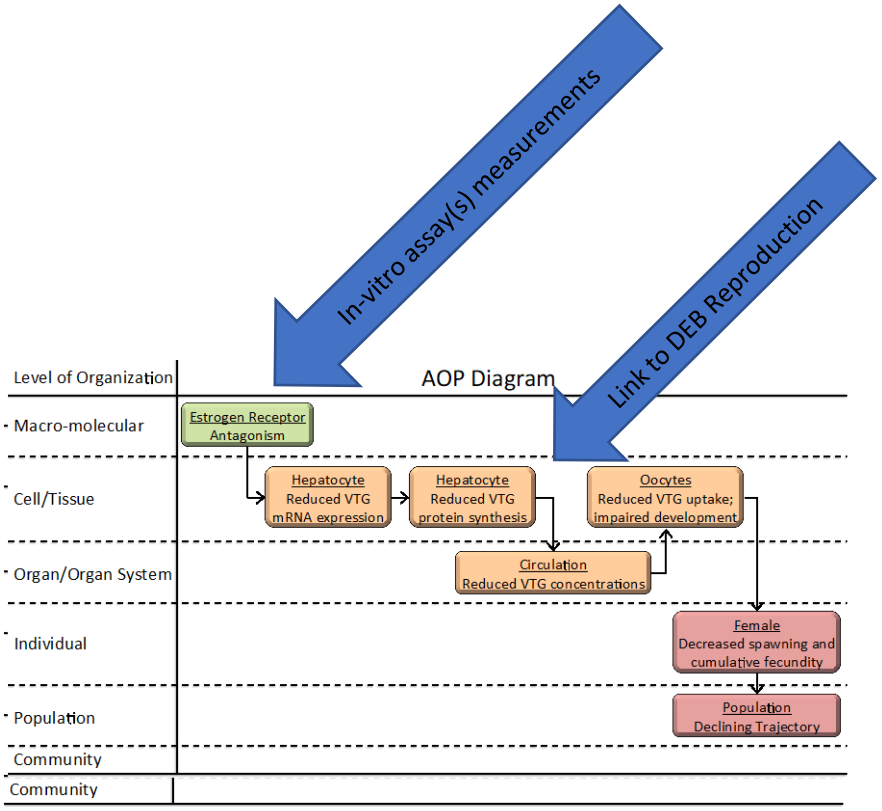
Link to AOP

DEB model

C. A. Murphy + NIMBioS group, *Integrated Environmental Assessment and Management*, in review

Example: AOP for Estrogen Receptor Antagonism in trout

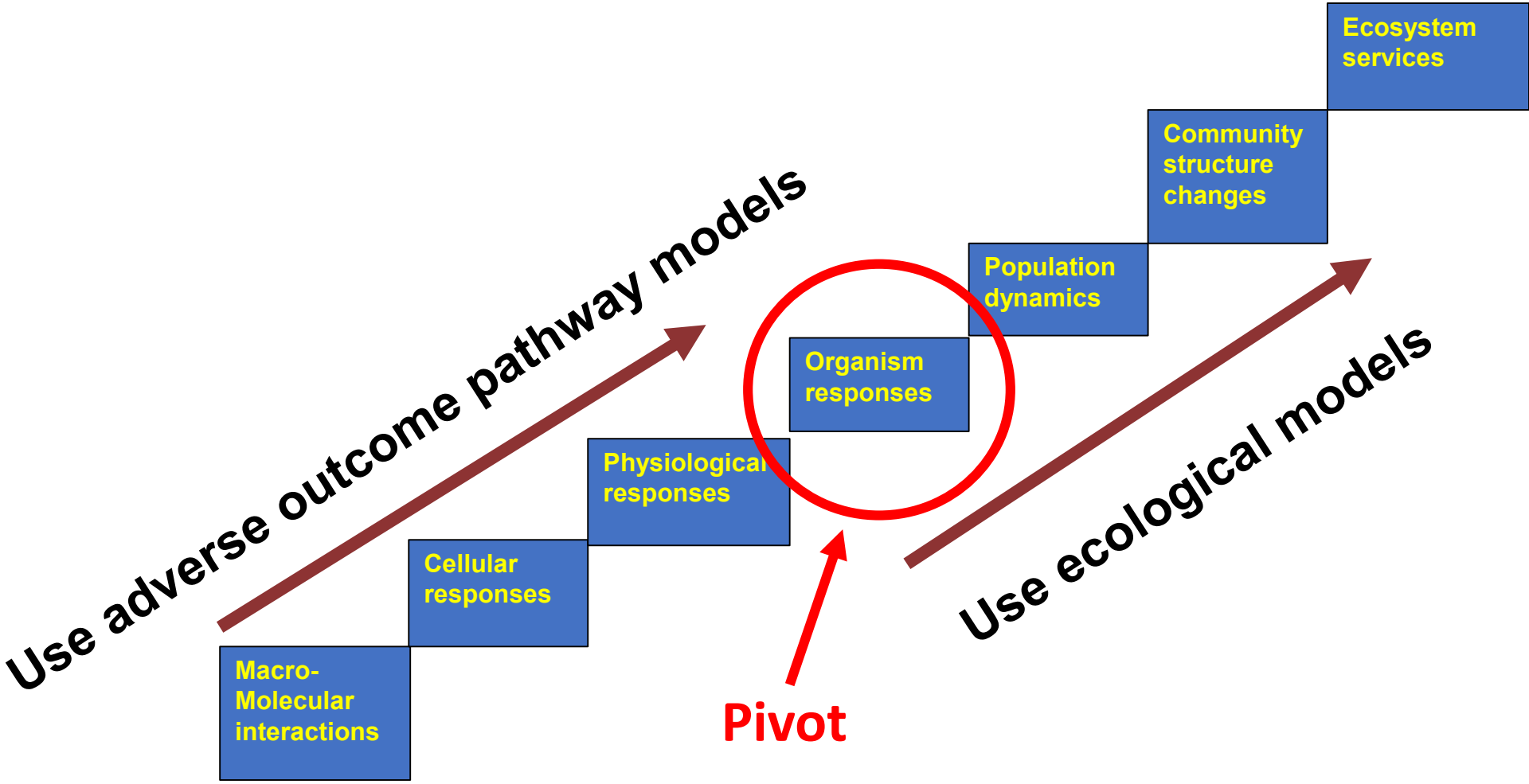




Slide from Karen Watanabe

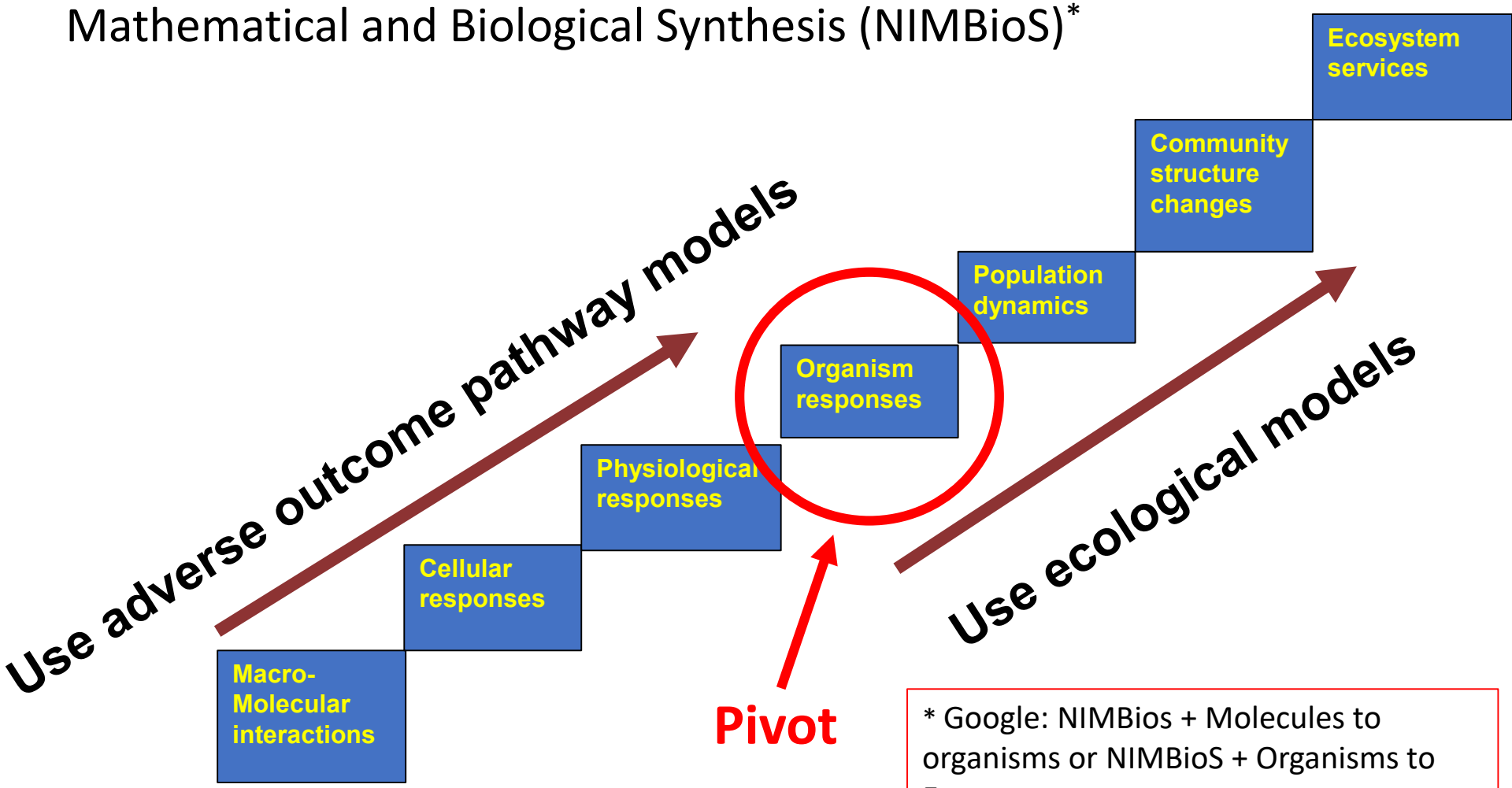
Watanabe, K. H. and Schultz, I. R. (2015). Development of quantitative adverse outcome pathways for ecological risk assessment. In *SETAC North America 36th Annual Meeting Abstract Book*: p. 347.

DEB models as “pivot point” linking suborganismal to ecological processes?



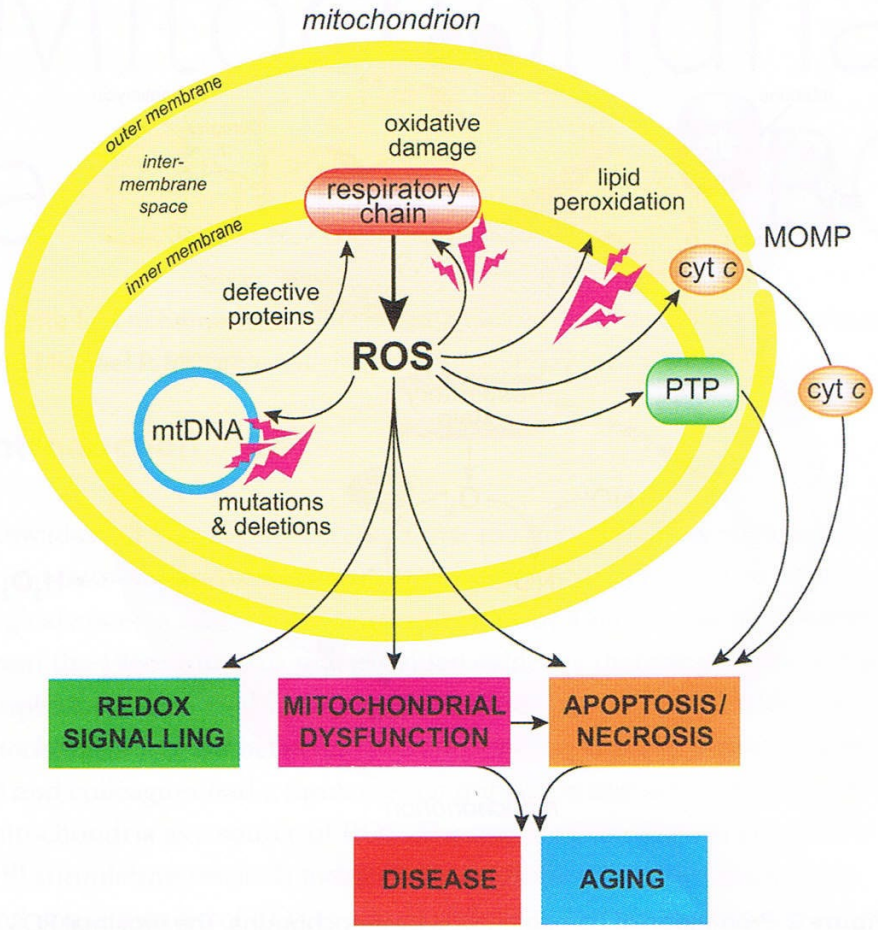
DEB models as "pivot point" linking suborganismal to ecological processes?

Goal of two working groups at National Center for Mathematical and Biological Synthesis (NIMBioS)*



* Google: NIMBios + Molecules to organisms or NIMBioS + Organisms to Ecosystems

Kooijman's chacterization of damage inducing compounds, damage, and mortality*



Reactive oxygen species (ROS) and immediate products

↓

Damaged proteins, membranes, DNA

↓

increased risk of mortality

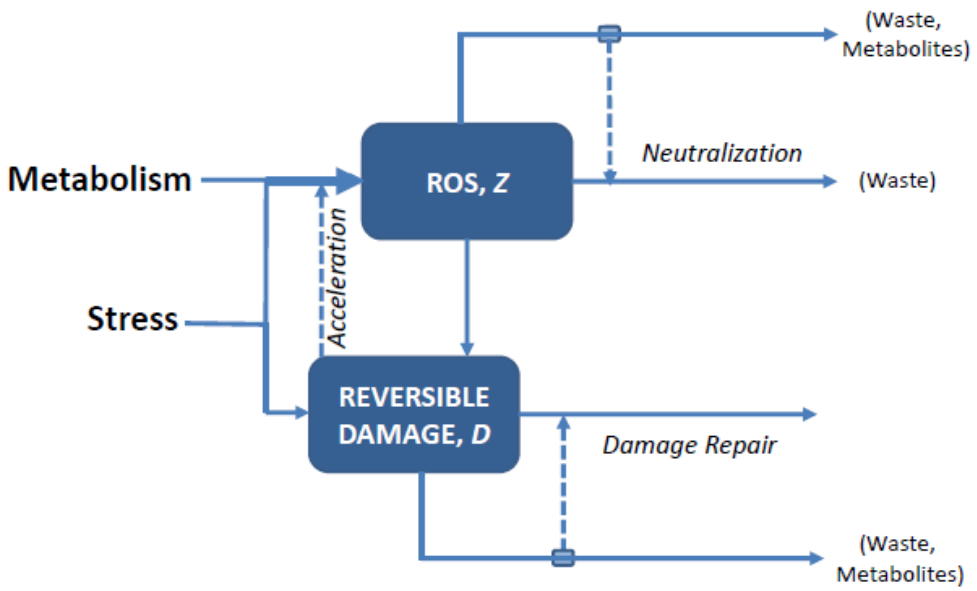
* Figure from Kooijman's "Comments" at http://www.bio.vu.nl/thb/deb/

Dynamic energy budget (DEB) modeling of oxidative stress, cellular damage and mortality

- Many studies report changes of ROS level in response to environmental stress (e.g. many toxicants)
- Many studies report metrics characterizing "damage" to cells or organs (e.g. lipid peroxidation, coral bleaching
- ROS has important function in cells (e.g. signaling) and is regulated.
- Much damage is repairable
- Generic systems model[1] explores the implications of the feedbacks
- Model is generalizable; Z need not represent ROS

1. T. Klanjscek, E.B. Muller and R.M. Nisbet. *J Theor Biol. (2016).* https://doi.org/10.1016/j.jtbi.2016.05.034

Oxidative stress and damage model



- Predicts co-variation of ROS and damage in response to toxicant exposure
- Takes account of history of exposure to stree
- Predicts "tipping points" caused by break-down of regulation (previous slide)
- Tipping points can be interpreted as transition point from sublethal to lethal responses to stress
- Provides mechanistic basis for no-effect concentrations (Klanjscek talk)

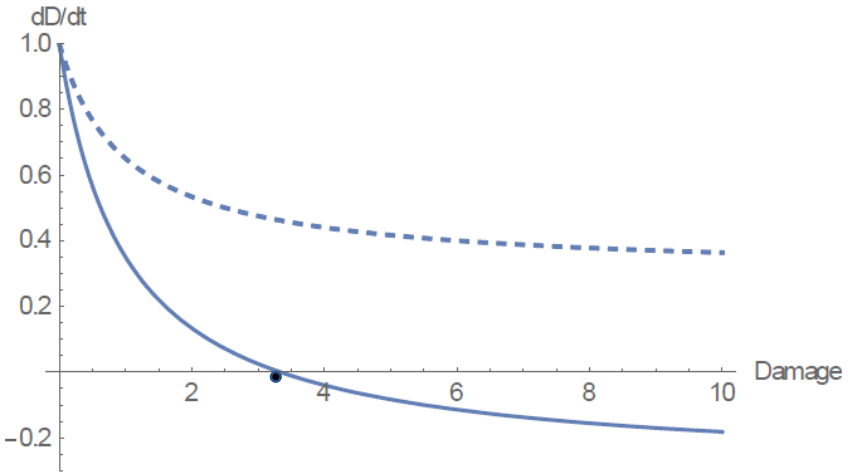
Lethal and Sublethal effects of stress

$$\frac{dD}{dt} = P - R \quad \text{with } P = \text{ damage production rate}$$

$$R = \text{ damage repair rate}$$

Assume P is constant but R is a saturating function of D

Then there are two possibilities:

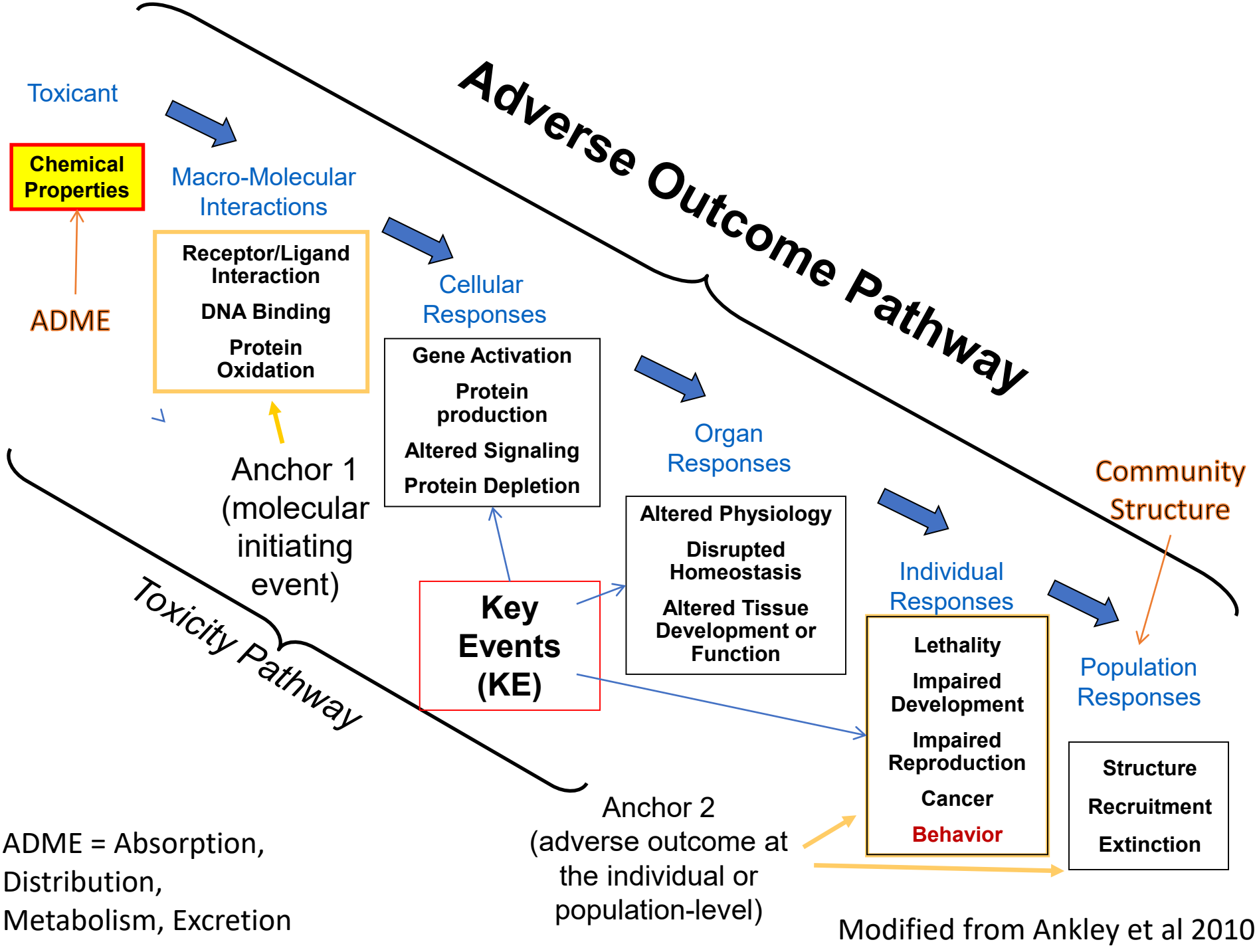


No equilibrium; unbounded growth of damage - LETHAL

Stable equilibrium; damage controlled - SUBLETHAL

Sub-organismal Characterizations of Damage: Adverse Outcome Pathways (AOP)

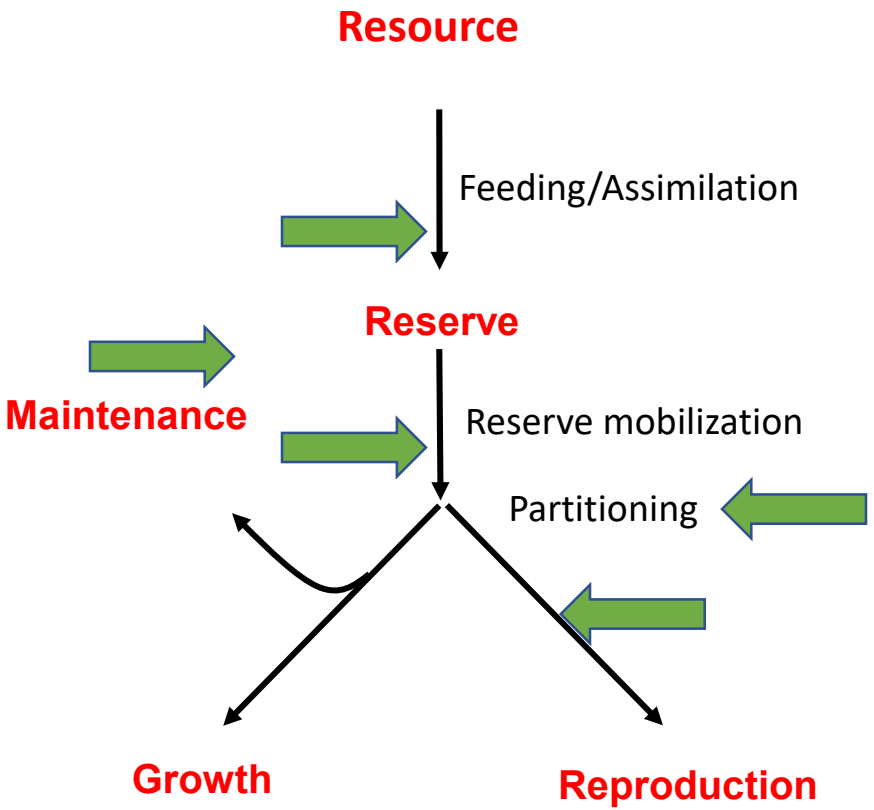
- "AOPs are conceptual representations of key events, spanning multiple levels of biological organization that link molecular initiating events … to adverse outcomes…." (Villeneuve and Garcia-Reyero, 2009)
- Concept increasingly used in ecotoxicology


Adverse Outcome Pathway
Toxicant
Chemical Properties
ADME
Macro-Molecular Interactions
Receptor/Ligand Interaction
DNA Binding
Protein Oxidation
Anchor 1 (molecular initiating event)
Cellular Responses
Gene Activation
Protein production
Altered Signaling
Protein Depletion
Organ Responses
Altered Physiology
Disrupted Homeostasis
Altered Tissue Development or Function
Individual Responses
Lethality
Impaired Development
Impaired Reproduction
Cancer
Behavior
Population Responses
Structure
Recruitment
Extinction
Community Structure
Toxicity Pathway
Key Events (KE)
Anchor 2 (adverse outcome at the individual or population-level)
ADME = Absorption, Distribution, Metabolism, Excretion
Modified from Ankley et al 2010

Conceptual model linking AOP and DEB approaches

Connect key events in AOP with "damage" variable(s) that impacts one or more flows in DEB model

Use data from transciptomics (or other 'omics')



C.A. Murphy et al. (2018), Integrated Environmental Assessment and Management, https://doi.org/10.1002/ieam.4063 - PUBLCATION OF NIMBioS WWORKING GROUP

Applications of "damage" concept from DEB

- Many examples for modeling survival (e.g. GUTS framework – Jager et al 2011)
- Several "DEBtox" studies *implicitly* utilize the concept in models of sublethal effects (e.g. Klansjcek et al 2013; bacteria exposed to CdSe quantum dots (nanoparticles)
- Few *explicit* DEB applications for modeling sublethal effects – example is study of response of snails parasitized with schistosomes (Civitello et al 2018)
- Work in progress using molecular data to help identify toxicant modes of action:
  - killifish embryos exposed to dioxin-like compound (transcriptomics)
  - freshwater microalgae exposed to dissolved Cu and Cu nanoparticles (metabolomics)
  - Daphnia exposed to coal ash (transcriptomics)

Applications of "damage" concept from DEB

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Killifish modeling overview

Step I: Choose version of DEB model to use

Standard DEB – gives access to databases
DEBkiss (simplified DEB model due to Jager (2013)
DEBlipid (variant of *DEBkiss* for lipid dynamics in fish)

Step II: Use molecular data to inform toxicokinetics and toxicodynamics

Step III: Hypothesize equations for damage variable(s)

Step IV: Use information from step II to make hypothesis about MoA

Step V: Test predictions!

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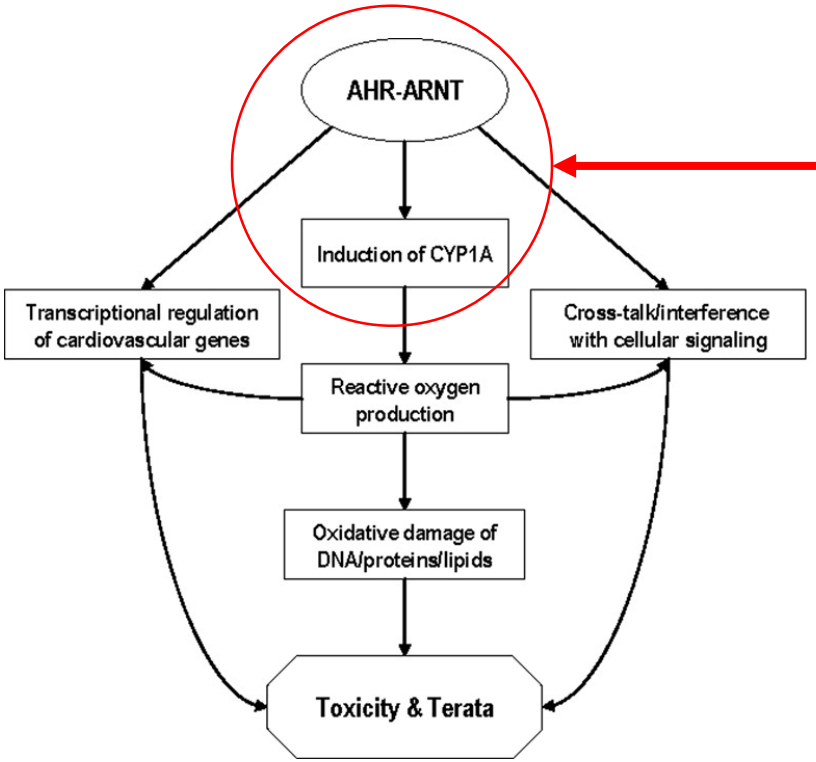
Step V: Fit, then Test predictions on new data!

Steps 2 and 4 – using molecular information

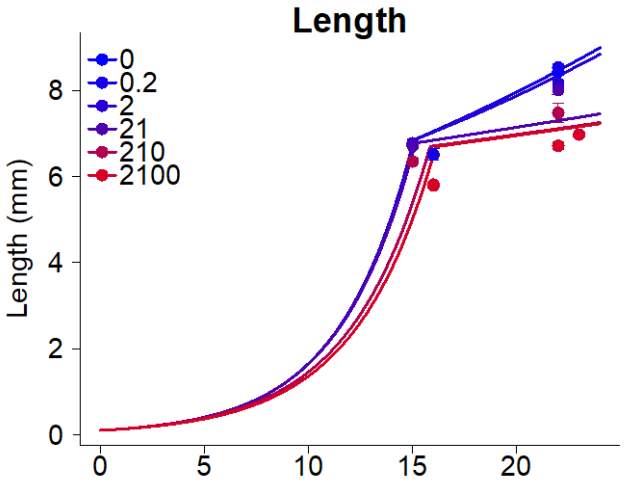
- Established AOP for dioxin-like compounds DLC – AHR (Aryl Hydrocarbon Receptor) pathway activation
- Transcriptomics indicate: (i) increase in detoxification response and (ii) less energy toward translational management (protein synthesis)

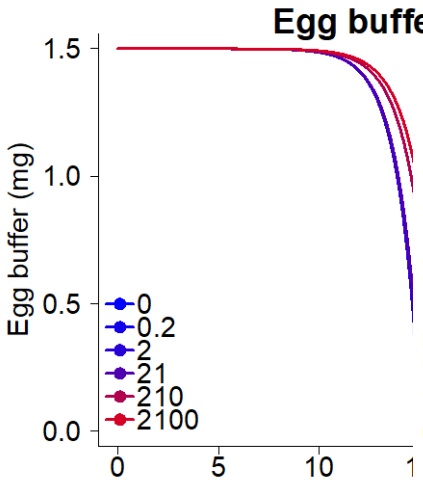
→ Hypothesis: <u>maintenance costs increasing with DLC exposure</u>

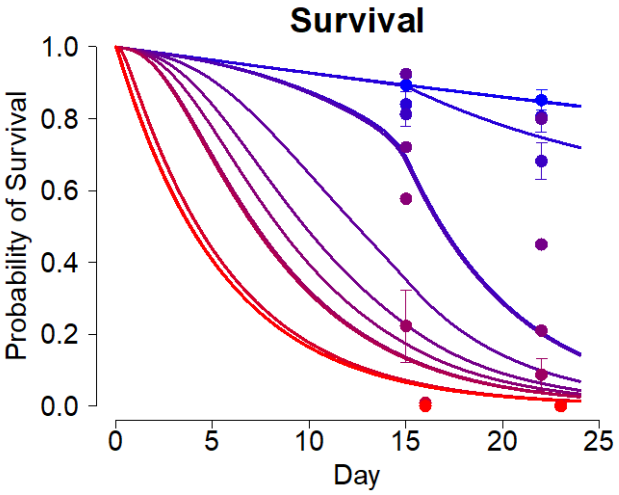


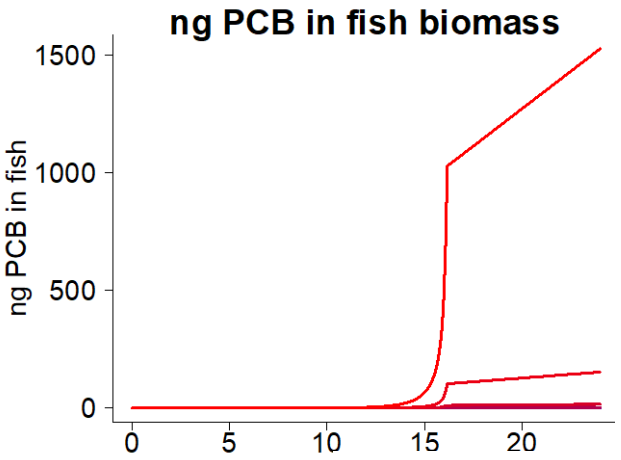
Used to parameterize TK

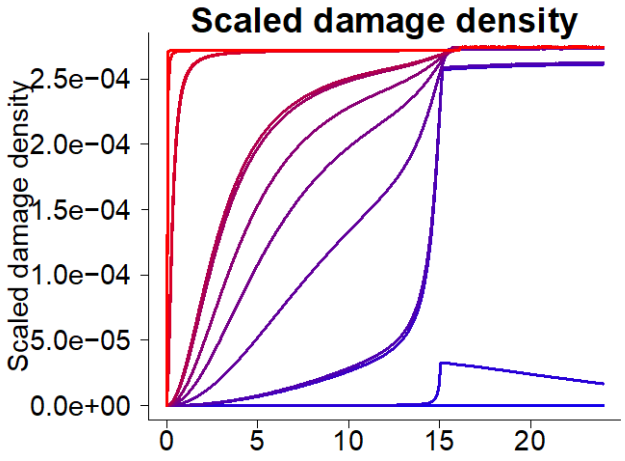
Model Fits – fish from "sensitive" population

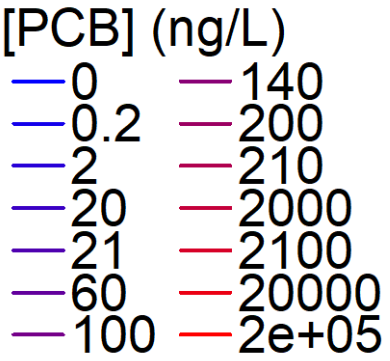












Day (days past fertilization)

Model captures both sublethal and lethal effects

Transcriptomic response to sublethal exposure to DLC

- Clusters common among concentr

Huang et al. 2008 *Nature protocols* 4:1.

- Up regulated
  - Endoplasmic reticulum
  - Cytochrome P450
  - Protein synthesis
  - Transmembrane proteins
- Down regulated
  - Ribosome
  - Oxidoreductase
  - Methyltranseferase
  - mRNA degradation
  - Filament proteins

OVERALL:

- Increase in detoxification response
- Less energy toward translational management

→ Hypothesis: maintenance costs increasing with DLC exposure

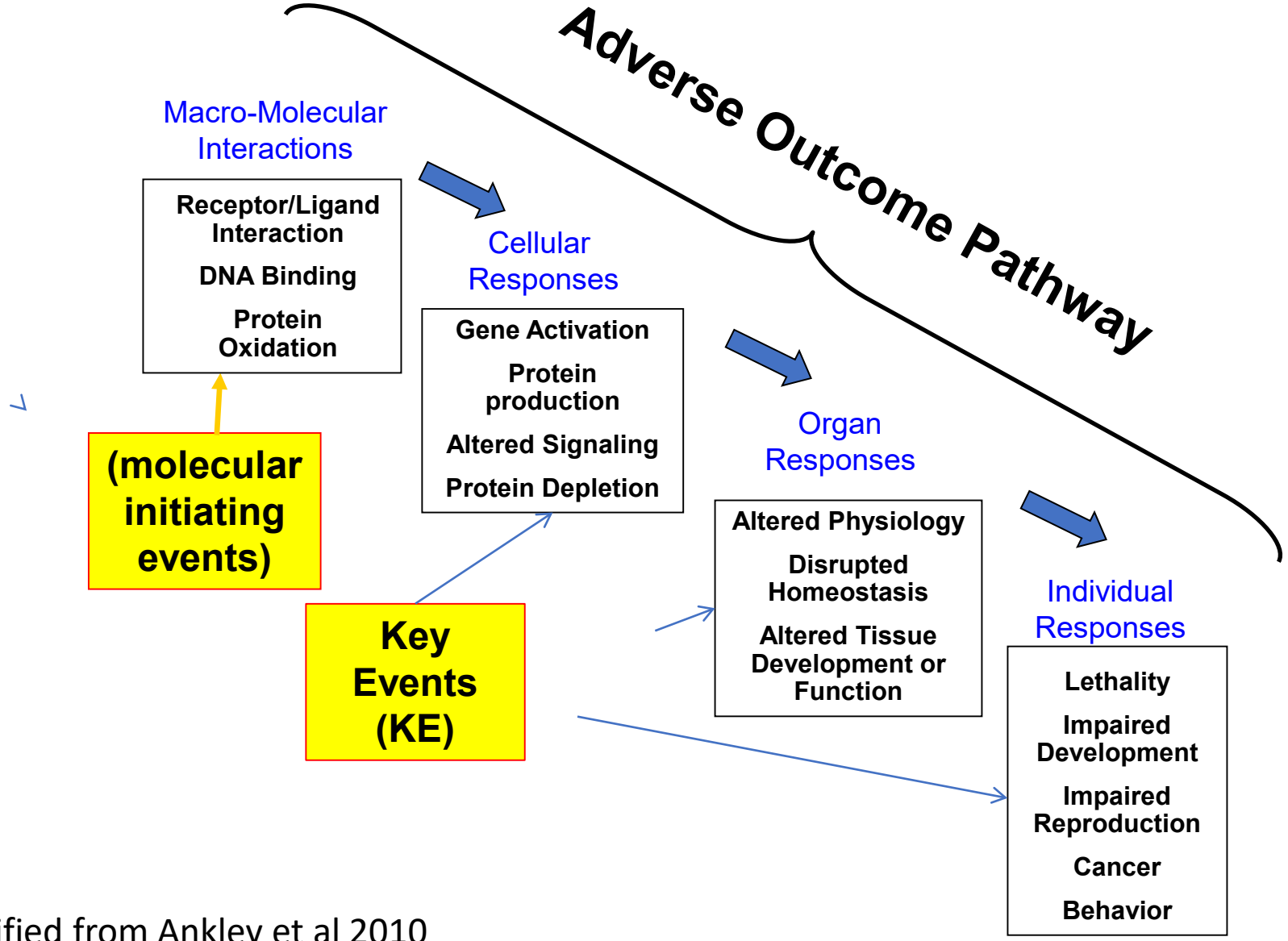
Brown et al. 2017 *GBE* 9.9: 2251-2264

The Great Divide

WHAT WE OFTEN MEASURE

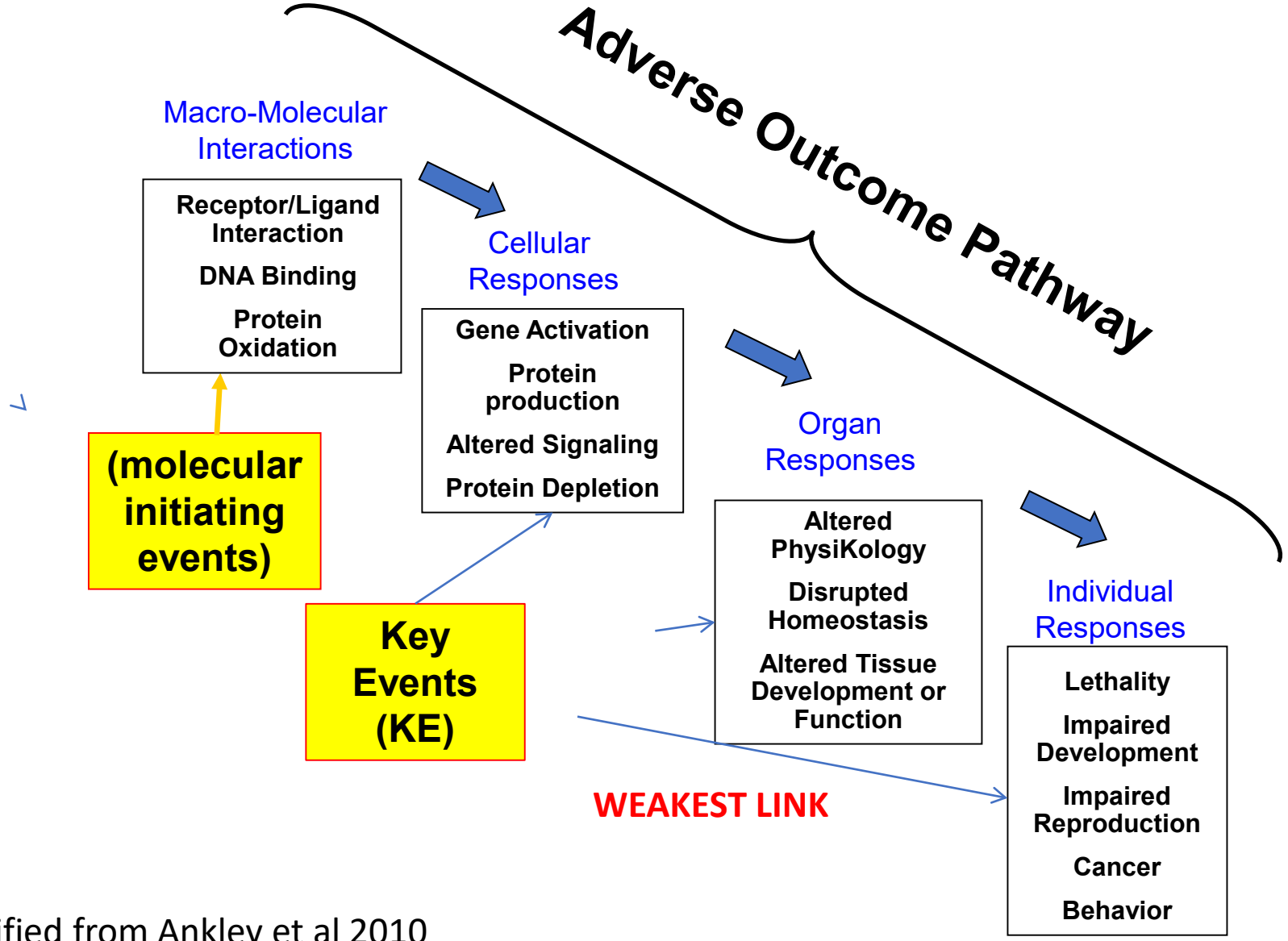
WHAT WE CARE ABOUT

Adverse outcome pathways



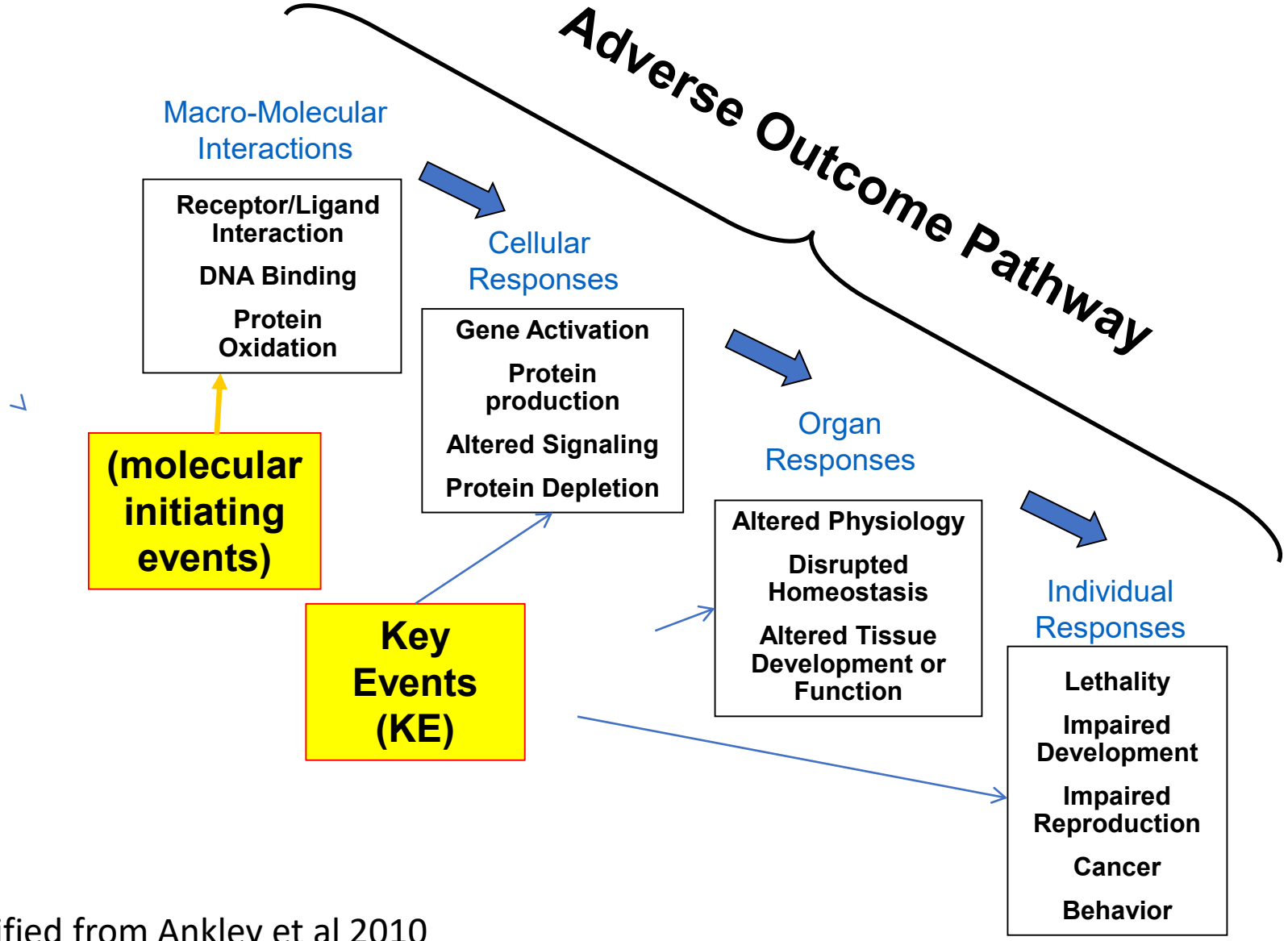
Modified from Ankley et al 2010

Adverse outcome pathways



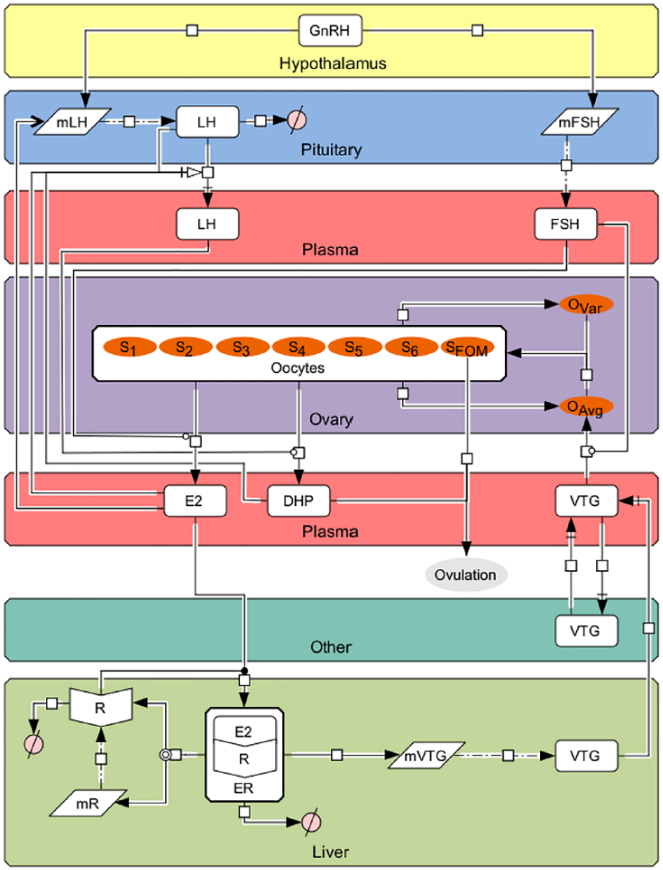
Modified from Ankley et al 2010

WHAT ARE AOPs?

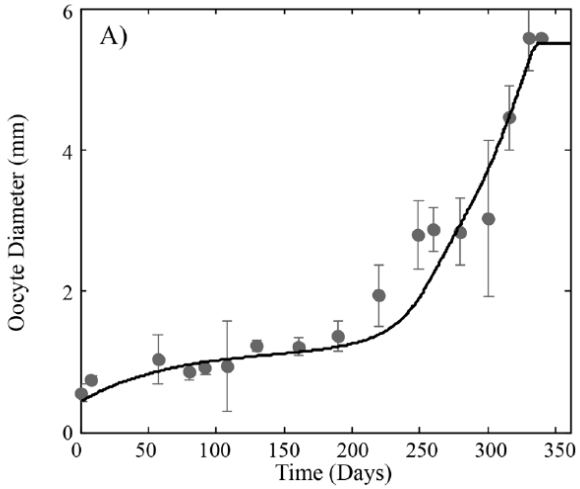


Modified from Ankley et al 2010

Quantitative AOPs: More complex physiological Model*



- Many feedbacks – positive and negative
- Link to DEB is prediction of rate of oocyte growth (primarily driven by Vitellogenin ("VTG" in diagram) supply.
- NEED TO DEVELOP VARIANT OF "STANDARD" DEB WITH TIME RESOLVED REPRESENTATION OF REPRODUCTION



*Gillies K, Krone SM, Nagler JJ, **Schultz IR** (2016). PLoS Comput Biol 12(4): e1004874. doi:10.1371/journal.pcbi.1004874

Why do we need DEB models?

Could careful exptrapolation from toxicity testing achieve similar results?

Design of toxicity tests involves many **choices**:

- **Species of organism** → DEB has recipe for interspecies comparisons
- **Exposure mode** → DEB allows natural coupling to TK models
- **"Endpoint"** (measured response) → DEB model output, related tobasic biology
- **Duration** → DEB model is dynamic, so output is time-dependent
- **Environmental conditions** → DEB can handle multiple environmental stressors

Dynamic energy budget (DEB) modeling of oxidative stress, cellular damage and mortality[1]

- Many CEIN studies report changes of ROS level in response to NP exposure (e.g. Cd-Se quantum dots and bacteria)
- Many CEIN studies report metrics characterizing "damage" to cells or organs
- ROS has important function in cells (e.g. signaling) and is regulated.
- Much damage is repairable
- Current DEB models (e.g. CEIN modeling of Cd-Se quantum dots) do not include feedback mechanisms associated with ROS regulation or damage repair
- New generic systems model explores the implications of the feedbacks

1. T. Klanjscek, E.B. Muller and R.M. Nisbet, *in prep*. Feedbacks and tipping points in organismal response to oxidative stress.
2. T. Klanscek