Feeding Measurements

Methods used to Estimate Grazing Impacts

- Inferences from Natural Populations
  - no incubations
  - use natural properties to infer feeding rates
- Tracer Techniques
  - short incubations
  - uptake of labeled prey
  - relatively minor disruption of natural community
- Community Manipulations
  - long incubations (to measure significant changes in population abundances)
  - substantial disruption of natural community

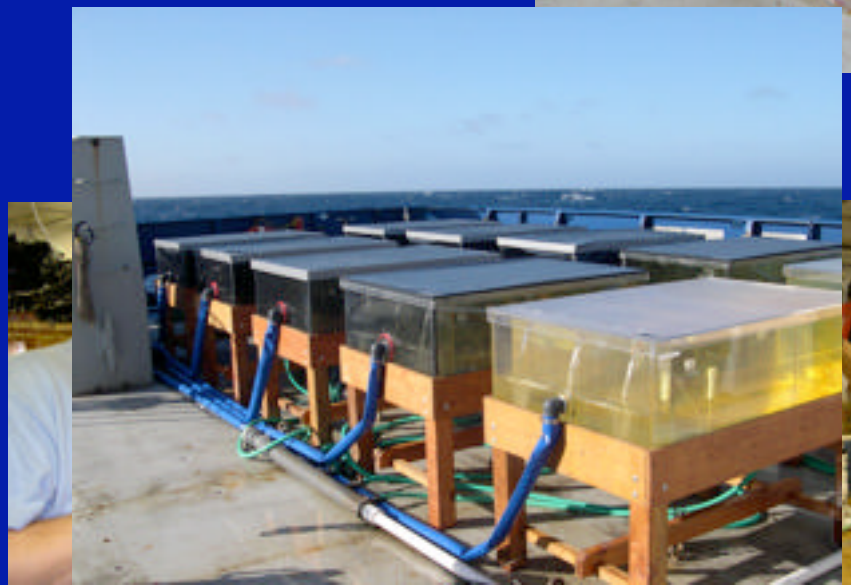
How do we sample zooplankton?

1) get a ship/boat

2) appropriate collection gear

3) treat the sample gently

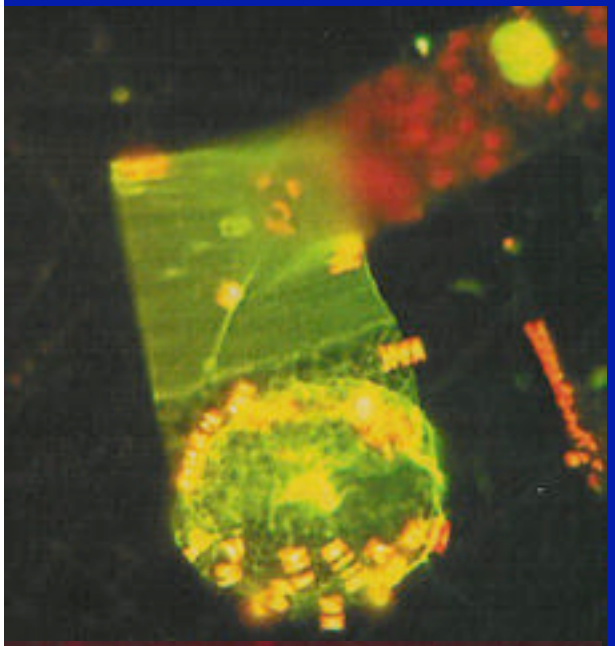
- manipulate and then incubate the sample
- The end? Usually filter the sample for later analyses on shore...

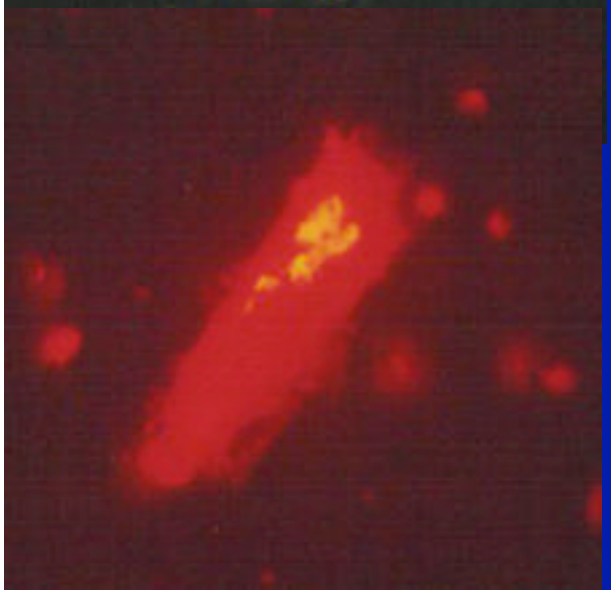


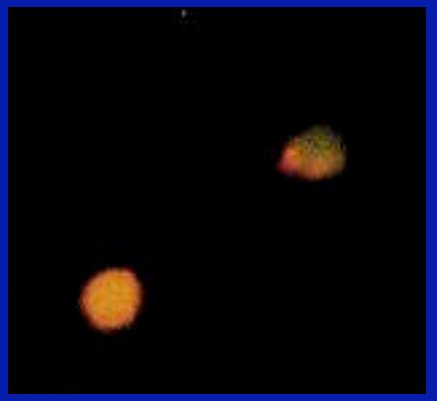
Inferences from Natural Populations

- Digestive Enzyme Assay
  - activity of acid lysozyme in grazer cell lysates
  - bacterivores only (peptidoglycan-bearing cells)
  - problem: substrate specificity
  - only really useful in eutrophic environments (e.g., coastal)
  - analogous method in metazoans stymied by their ability to store digestive enzymes in their guts, so the activity doesn't represent current food conditions
- Food Vacuole (protist) or Gut Content (metazoan)
  - assess average number of prey/predator
  - rate of prey digestion (vacuole turnover rate or gut clearance rate)
  - laborious method

Vacuole Contents







What is it?

Prey recognition

How long has it been there?

Digestion times

Best use: to reveal significant trophic pathways among dominant predators and prey

Gut Fluorescence

1) Net tow to get organisms

2) Anesthesize, extract or freeze immediately to prevent evacuation of guts (*critical step*)

3) Measure amount of pigment (chlorophyll) in gut (fluorometer)

4) Calculate grazing rate =

Gut pigment content * gut evacuation rate

-- *this will give you the grazing impact on phytoplankton (but not on non-pigmented cells)*

Detection of Prey DNA in Predator

- New method applied to copepods (Nejstgaard et al., 2008): not extensively tested yet
- Design primers to specific prey groups (18S rRNA)
- Extract DNA from predator and its gut
- Perform Q-PCR on material to get number of prey cells in sample (prey cells/predator)

Potential Issues:

Number of copies of target molecule in prey cells: varies with prey physiological state

Must design primers to targets that are known already

Digestion of DNA in gut may limit what is detected

Tracer Techniques

- Radio-isotope labeled prey
  - Effective separation of prey and predator (not so hard with metazoans)
  - Very sensitive (easy to measure low amounts of radioactivity)
  - isotope cycling causing interpretation problems
    - *example:* ^{14}C-labeled algae release ^{14}C-DOC, bacteria take up DOC, and then are eaten by heterotrophic flagellates -- ^{14}C in predator fraction but not from herbivory

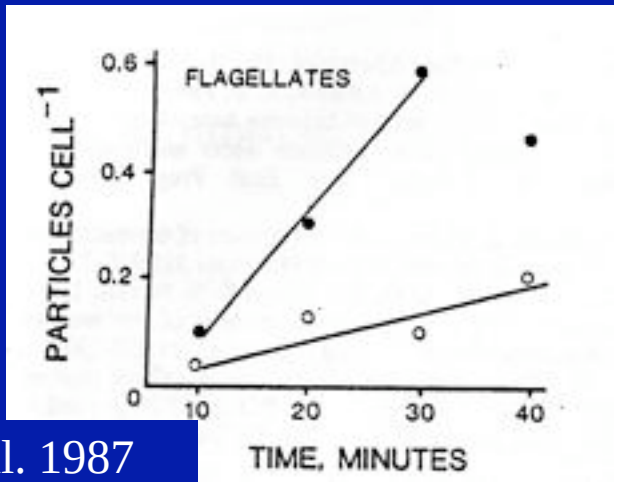
Tracer Techniques, continued:

Fluorescently-labeled Prey (FLB: bacteria, FLA: algae)

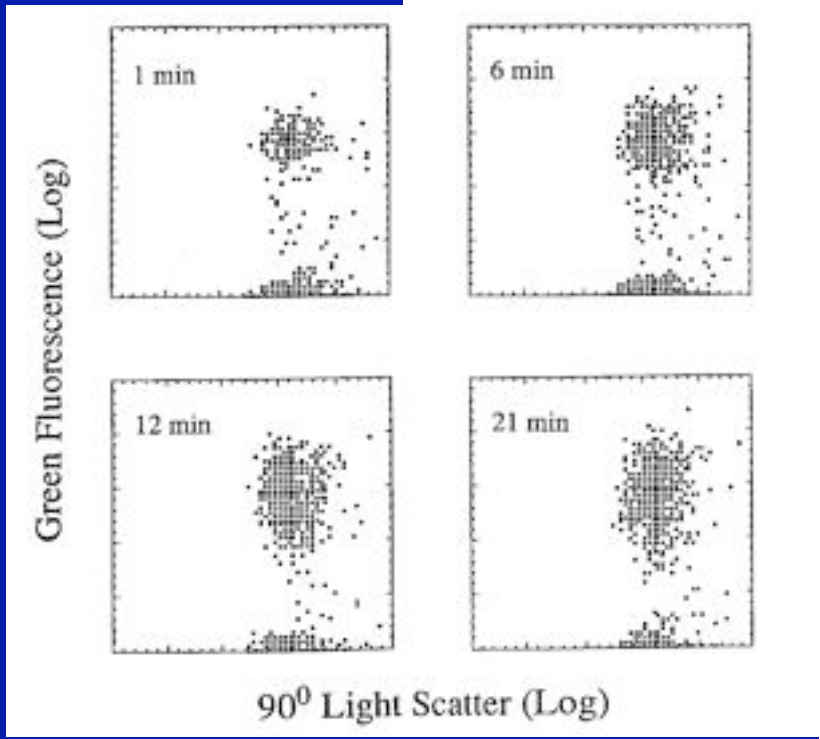
- Identify a prey culture that is the correct size/type for grazer of interest
- Grow up thick batch cultures, stain with a dye, determine concentration: FLB or FLA**
- Add tracer quantities to experimental flask (usually 1/10 - 1/100 of total prey concentrations), have a parallel control with fsw and FLP
- Take aliquots at frequent time intervals: examine them microscopically or with the flow cytometer to determine how many prey/grazer.
- Prey/grazer/time = ingestion rate (I)

***can also use genetically modified prey that express GFP or RFP*

closed circles: FLB
open circles: microspheres



Sherr et al. 1987



Monger & Landry 1992

Fluorescently-labeled Prey

Advantages:

-- FLP will be digested, so become colorless: short-term uptake or long-term disappearance

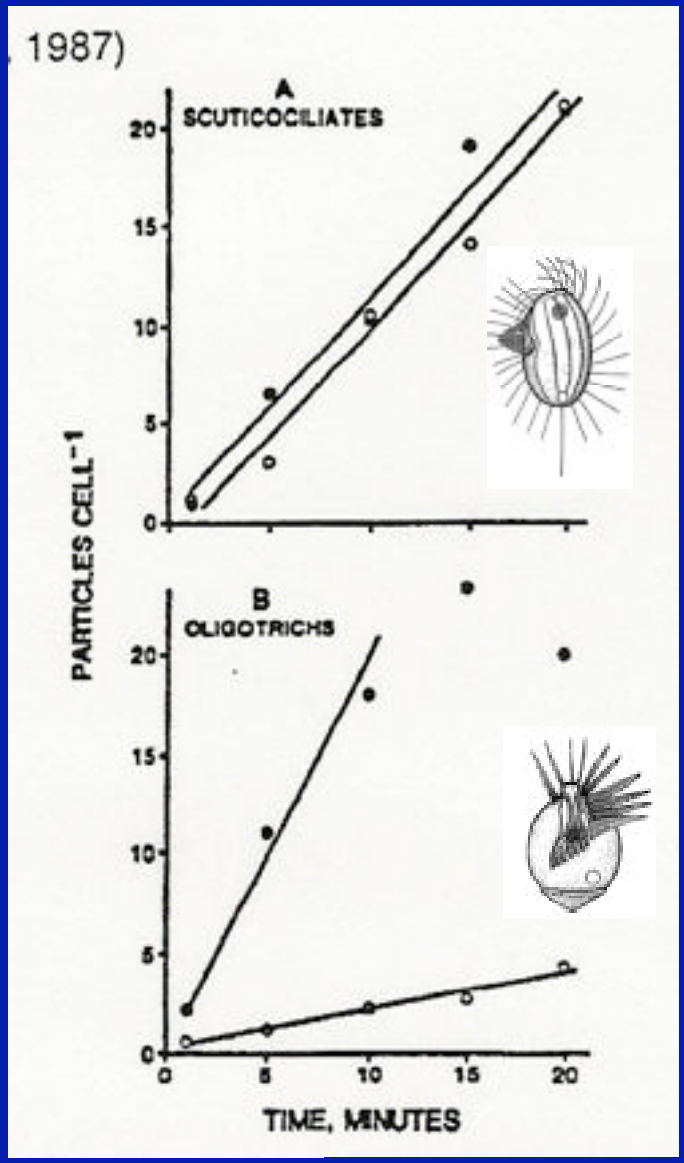
-- If use microscopic examination, can see what fraction of grazing community “ate” the surrogate

Caveats/Cares:

-- tracer concentrations?

-- discrimination - rejection?

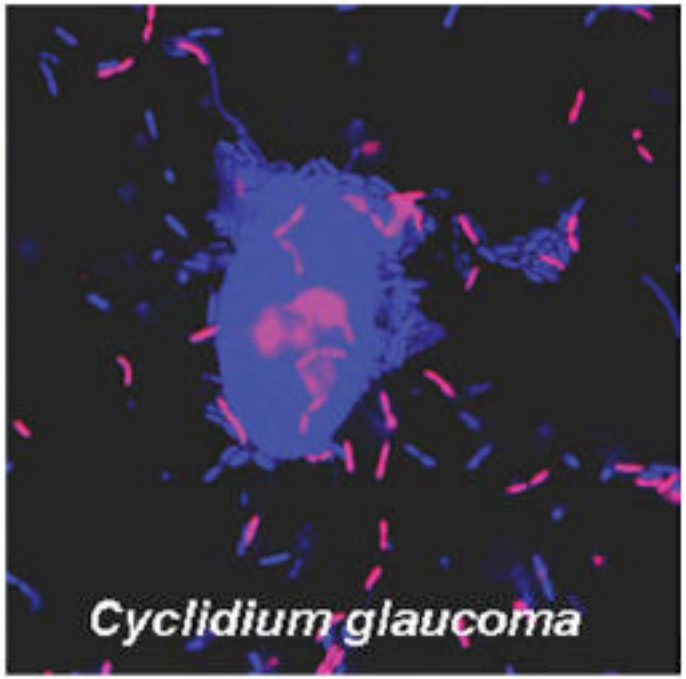
-- egestion - preservation

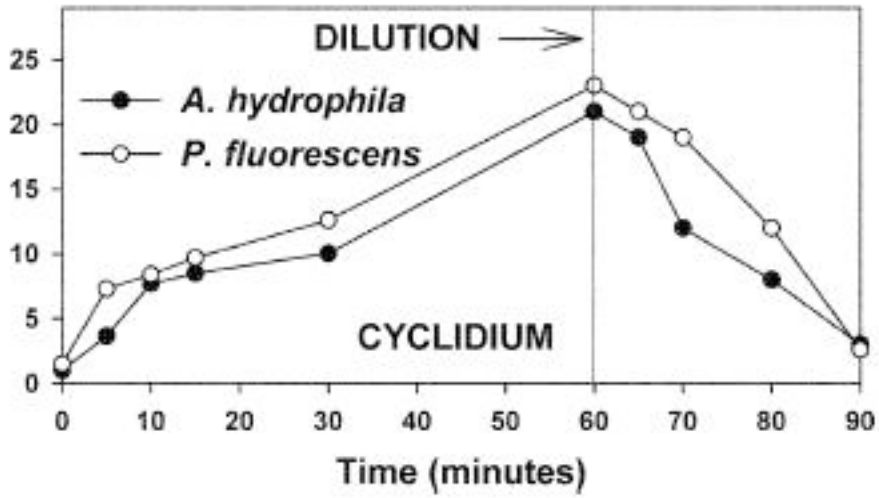


Sherr et al. 1987

Newer tracer method for Protist Grazing

- FISH (*F*luorescence *I*n *S*itu *H*ybridization) method to probe specific DNA in protist food vacuole





Jezbera et al. 2005

Community Manipulations

- Size Fractionation (separate predator & prey)
  1) Pick a filter pore size that separates predator and prey (or manually remove predators)
  2) Incubate sample after filtration and get net growth rates in both filtered (no predator, μ) and unfiltered sample (with predator, k)
  3) Calculate grazing impact (μ - k)

Main problem: effective separation of predator and prey

Other concerns:

organic enrichments from cell breakage?

loss of “nurturing” relationship

Size Fractionation: Grazer Removal

Location: Station ALOHA

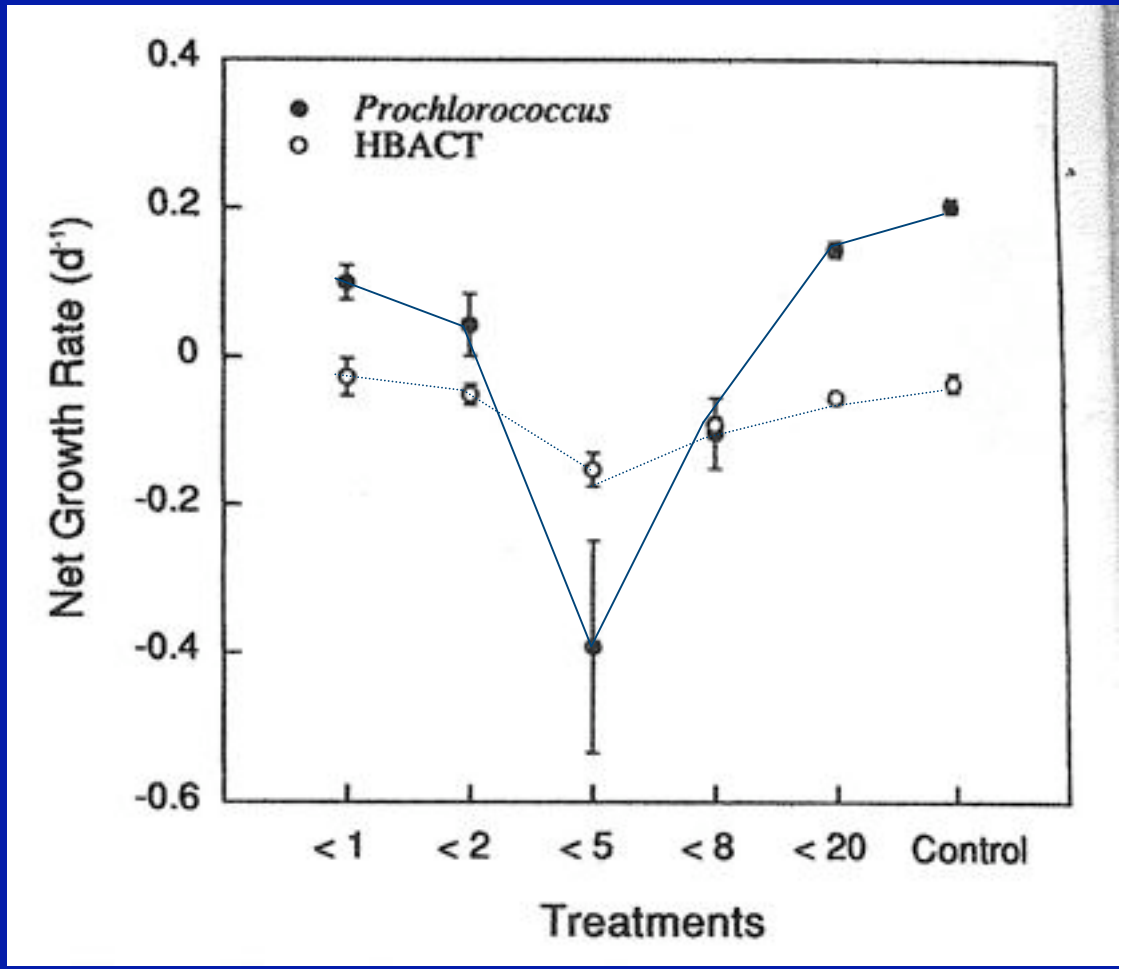
Manipulation:
Bacterial net growth in a size-truncated food web

Implied Grazer Chain:

5 - 20 µm HF

2 - 5 µm HF

Bacteria



Calbet & Landry 1999

Community Manipulations, continued: Seawater Dilution Method

– main advantage: results in an estimate of μ and m for phytoplankton community in a single experiment

- Keep phytoplankton “happy” by keeping light, nutrients and temperature the same
- Reduce grazing impact by diluting the grazers (progressive dilution with grazer-free seawater)
- Depends upon the assumptions that

  1) grazers were consuming at their maximum rate prior to dilution and

  2) dilution does not reduce prey density below a “threshold” level, eliciting reduced grazing effort

How do you do it?

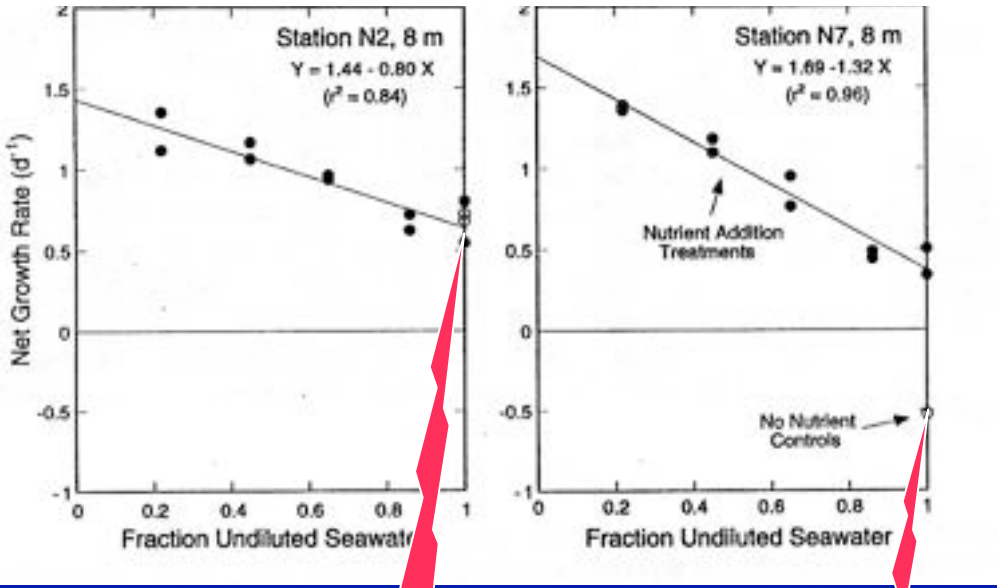
1) get water and store in clean bottle (WSW)
2) filter some of it (0.2 µm) to get rid of organisms, but keep dissolved nutrients (FSW)
3) Progressively dilute the whole seawater with the filtered seawater (e.g., 100%WSW, 75%WSW:25%FSW, 50%WSW:50%FSW, etc.)
4) measure abundance/biomass in WSW bottle (e.g., cell numbers, Chl, other pigments, POC, etc.)
5) incubate all bottles under “in situ” conditions for 24 hours
6) measure abundance/biomass again in all bottles
7) Plot net growth rates of all bottles against the dilution factor

Microzooplankton Grazing by Dilution

Principle: Reduce grazer-prey encounter rate

Arabian Sea, Dec. 1995 (Landry et al. 1998)

μ_n = 1.4 d^{-1}, m = 0.8 d^{-1} μ_n = 1.7 d^{-1}, m = 1.3 d^{-1}

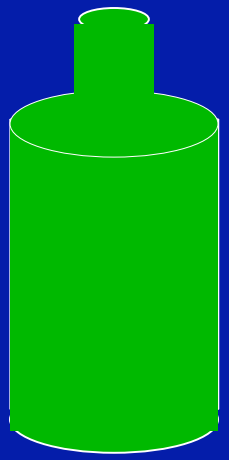


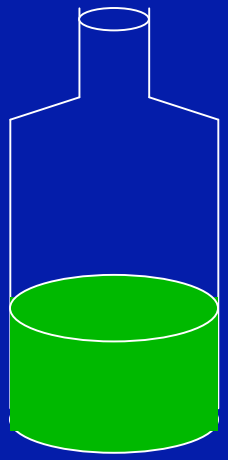
no nutrient effect

nutrient effect

$k_j = \mu_n - (m \times D_j)$;
$k_0 = \mu_0 - m$

2-Bottle Dilution Experiments: can use this method if regular dilution response is shown to be linear





D = 0.33

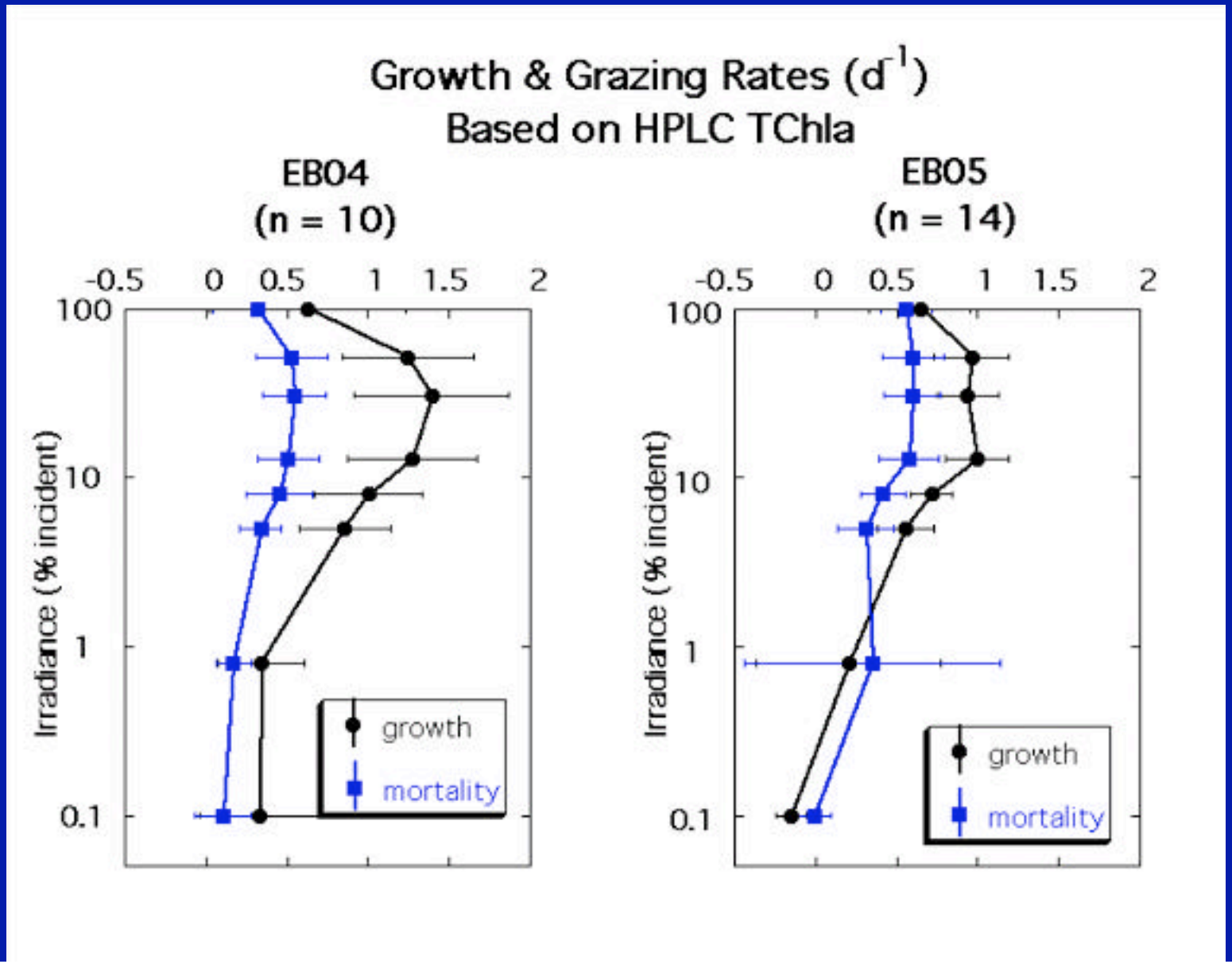
$$k = 1/t \ln (P_t/P_o)$$

$$k = \mu - m \qquad k' = \mu - D \bullet m$$

$$m = (k' - k)/(1-D)$$

$$\mu = k + m$$

2-point-Dilution Experiments



References

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