Microbial Grazers Lab

Objective: Measure the rate at which bacteria are consumed by predators.

Overview

- Size based food webs
- Microbial loop concepts
- Bacterial predators
- Methods to assess microbial grazing rates

Readings (see class web site)

1. Caron,D.A. et al. (2012) Marine Protistan Diversity, *Annu. Rev. Mar. Sci*. **4**:467–93.
2. Azam et al. (1983) *Marine Ecology Progress Series*, **10**: 257-263

Size Classification (Revisited)

Unlike terrestrial systems, primary production in aquatic systems is dominated by microorganisms with sizes typically less than 200 μm.

Femtoplankton: 0.02 - 0.2 μm
- Mostly viruses

Picoplankton: 0.2 - 2 μm
- Bacteria, cyanobacteria

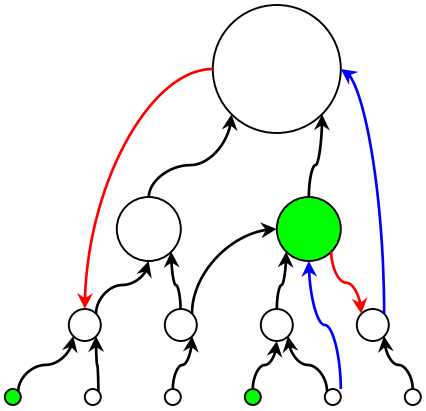
Nanoplankton: 2 - 20 μm
- **Flagellates**, dinoflagellates

Microplankton 20 - 200 μm
- Diatoms, **ciliates**.

Mesoplankton > 200 μm
- Zooplankton (copepods)

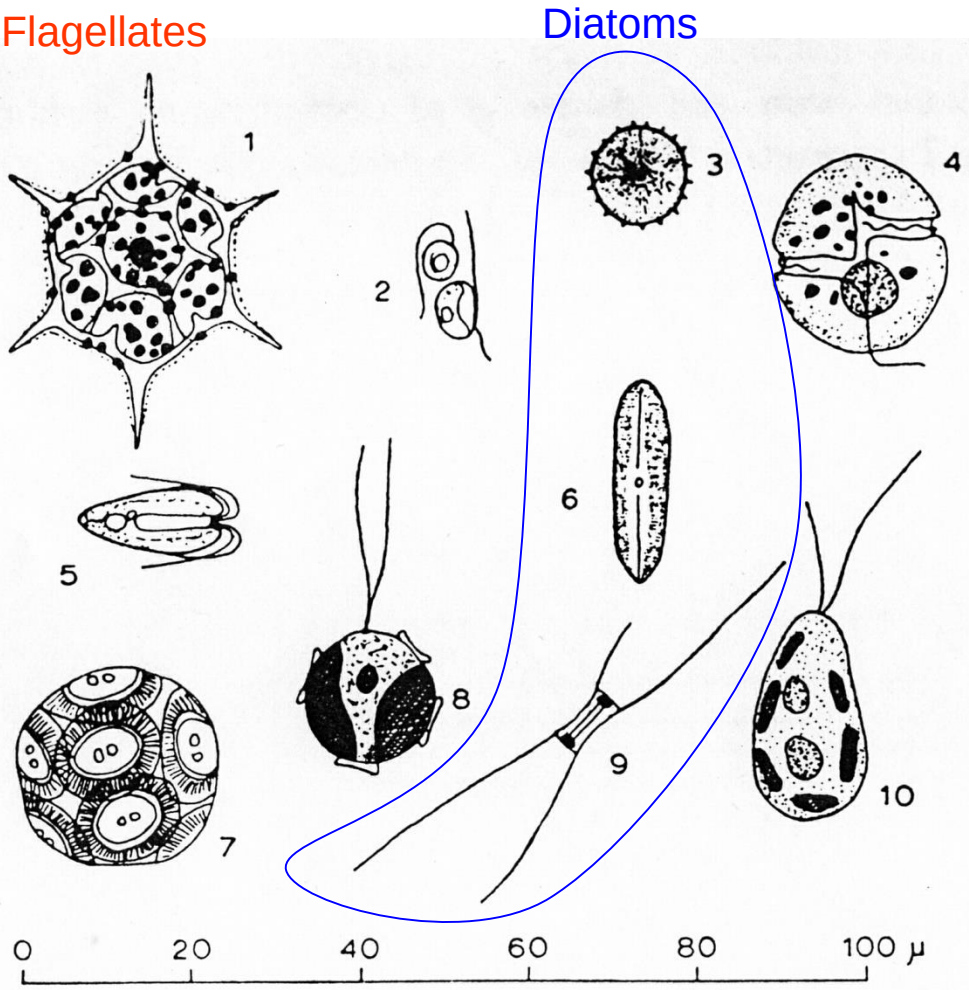
Bacteria: 0.2 μm - 1000 μm (1 mm)
- Typically 1 - 2 μm culture, or < 1 μm natural environments.

Size classifications are used because:
- Functional definition (filter cutoffs)
- Feeding approximately based on relative sizes
- Identification not always helpful



Schematic of size based feeding. Some organisms might be mixotrophs (both auto- and heterotrophy, shown in green), and some may feed across trophic levels (blue lines), or feed on organisms larger than themselves (red lines).

Nanoplankton Examples (2 - 20 μm)



G. 5A. Examples of nanoplankton flagellates [*Distephanus* (1), *Thalassomonas* (2), *Gymnodinium* (4), *Tetraselmis* (5), *Coccolithus*
), *Pontosphaera* (8), *Cryptochrysis* (10)], diatoms [centrate (3), pennate (6), *Chaetoceros* (9)] (redrawn from Wailes, 1939, Cupp,
1943, Fritsch, 1956 and Newell and Newell, 1963).

Microplankton Examples (20 - 200 μm)

Zooxanthellae, coral symbionts, are dinoflagellates. Their expulsion from coral animal is what is called coral bleaching

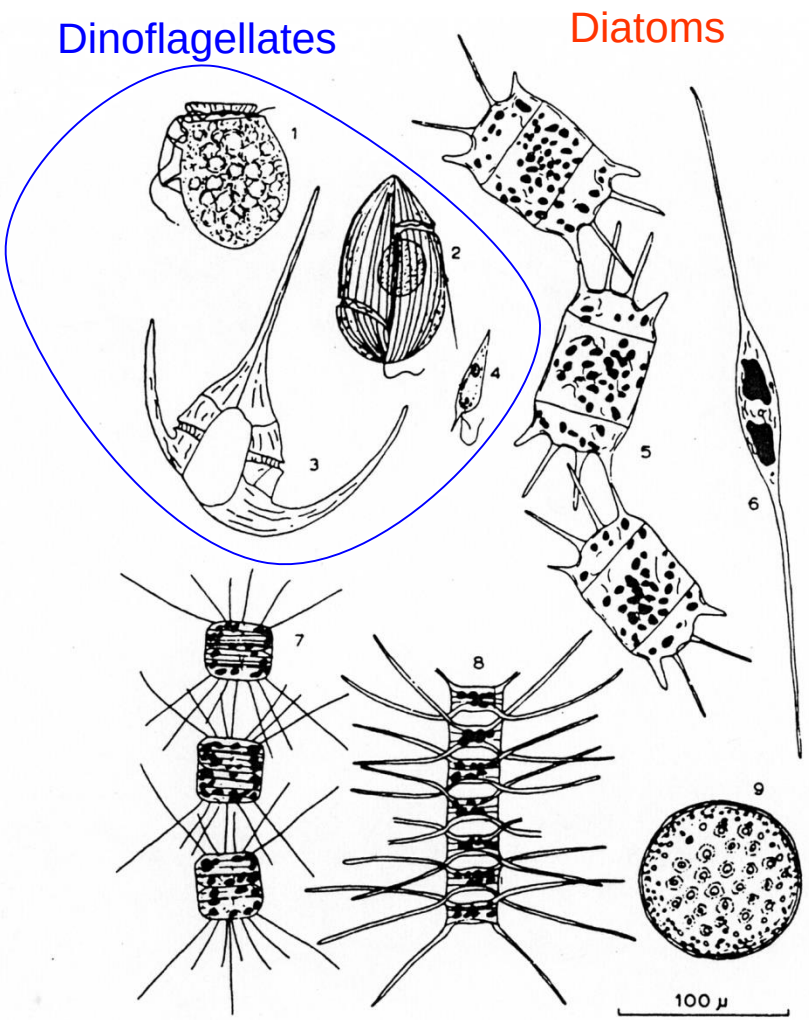


FIG. 5B. Examples of microphytoplankton: dinoflagellates [*Dinophysis* (1), *Gyrodinium* (2), *Ceratium* (3), *Prorocentrum* (4)], diatoms [*Biddulphia* (5), *Nitzschia* (6), *Thalassiosira* (7), *Chaetoceros* (8), *Coscinodiscus* (9)] (redrawn from Wailes, 1939, and Cupp, 1943).

Mesoplankton Examples (> 200 μm)

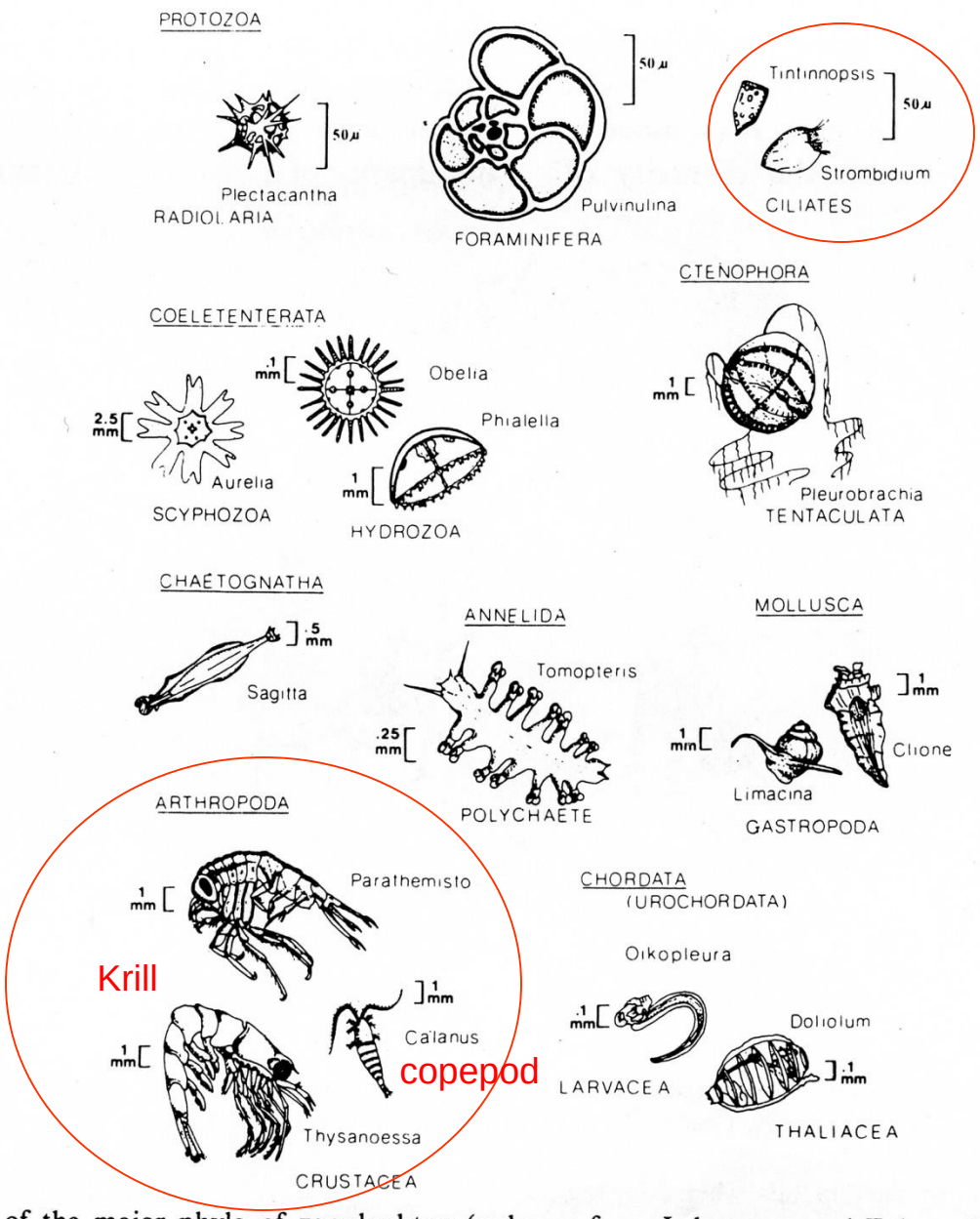
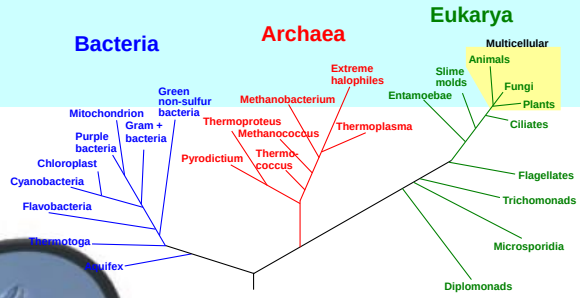
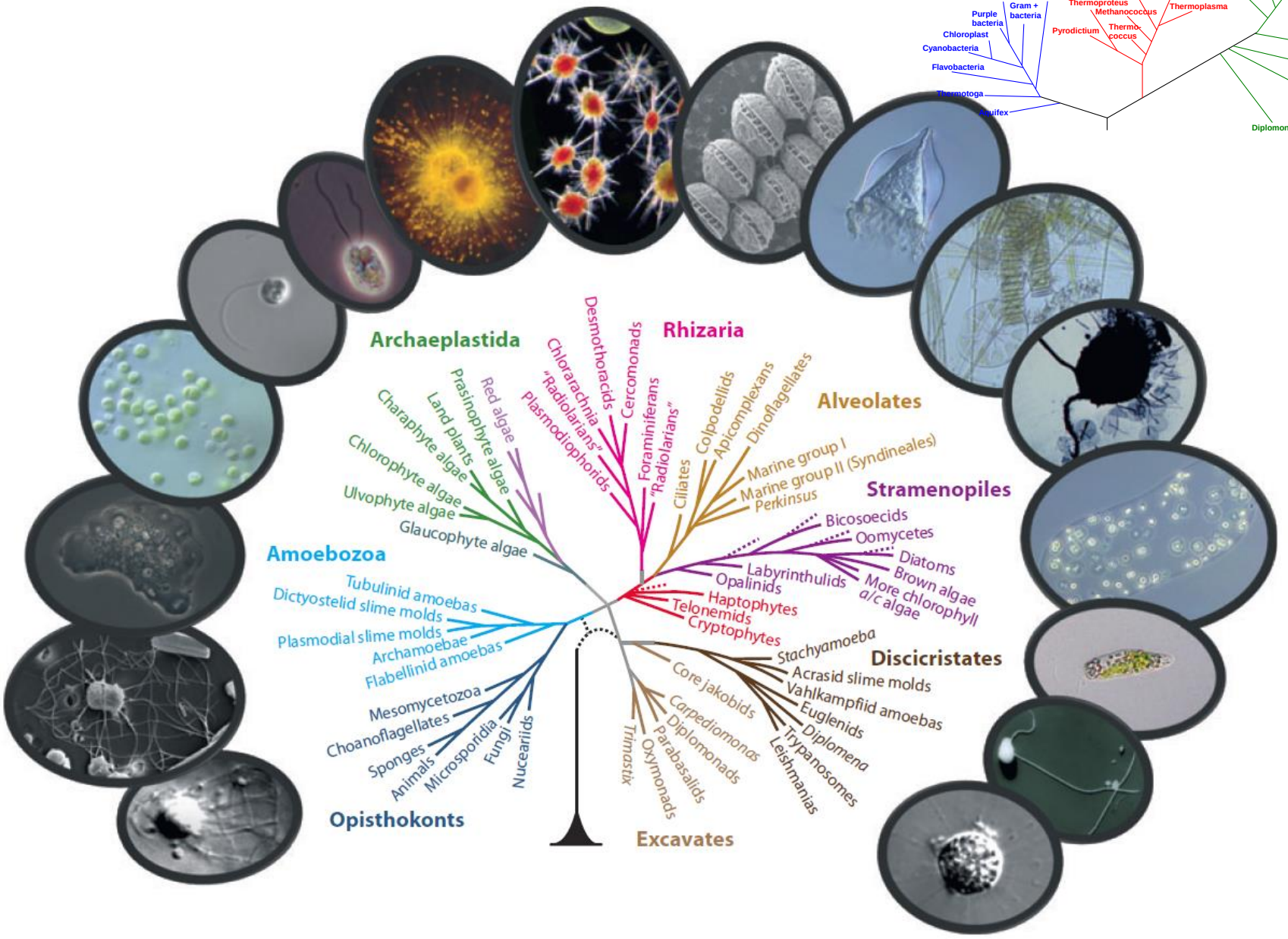


FIG. 5C. Illustrations of the major phyla of zooplankton (redrawn from Lebrasseur and Fulton, 1967; Wailes, 1937 and 1943; Cushman 1931).

Phylogenetic Marine Protistan Diversity

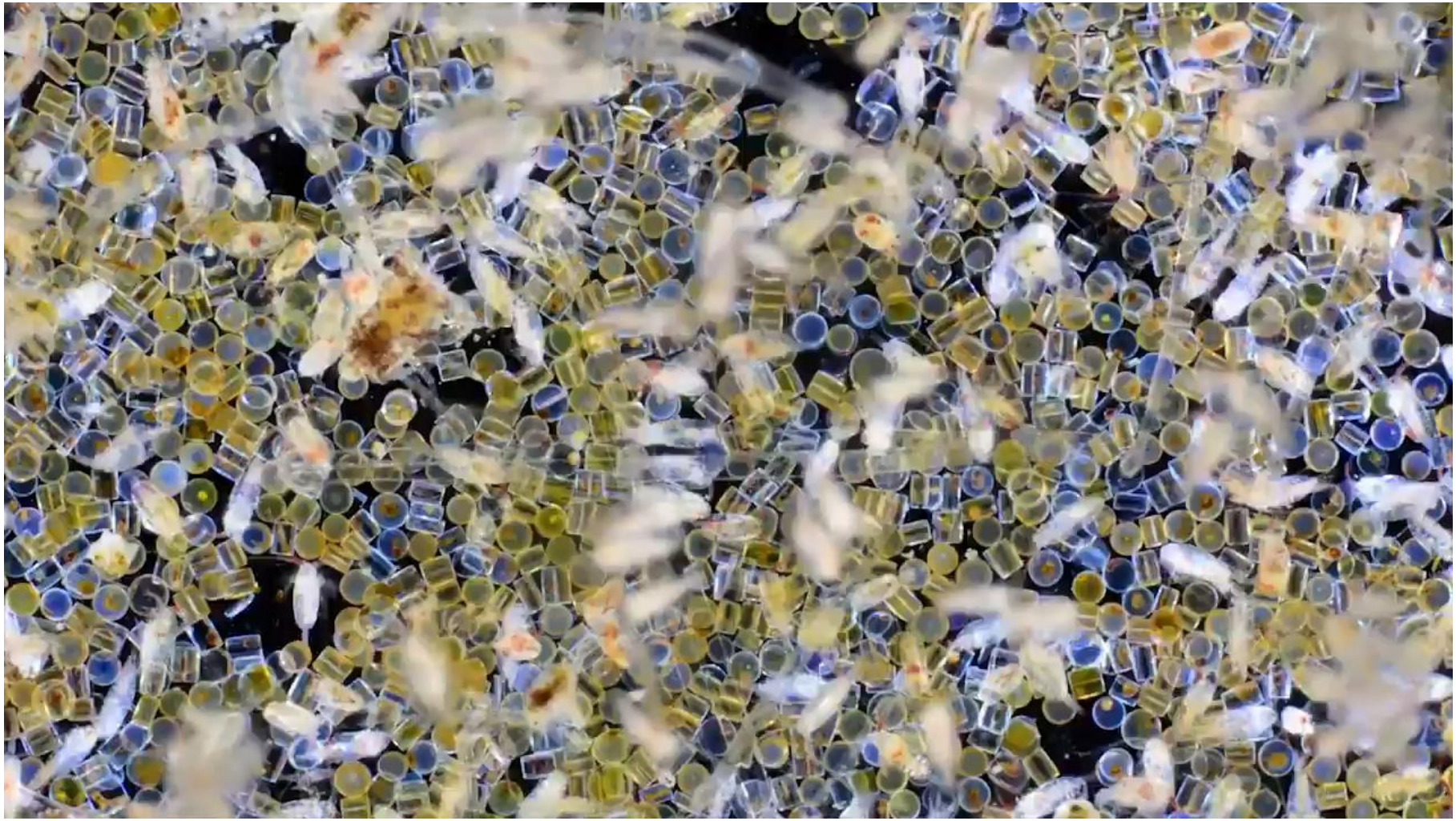




From Caron et al. (2012)

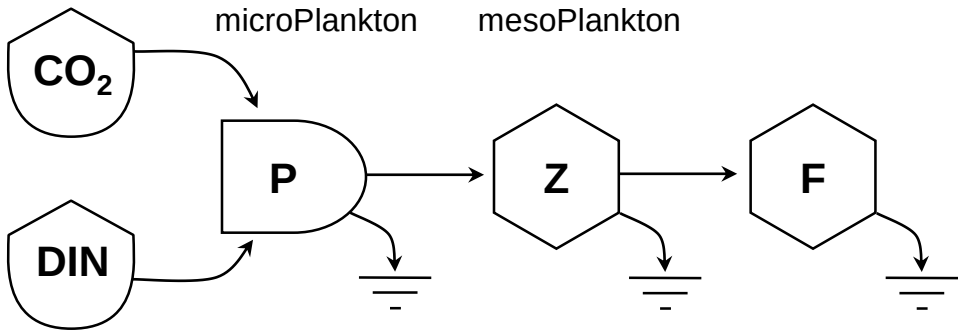
Phylogenetic Marine Protistan Diversity

Example of results from a plankton tow
~1.5 kt, 10 min, 48 cm dia net, 180 µm mesh
From Richard Kirby, (https://twitter.com/PlanktonPundit/status/918549329471787008?s=03)



Classic Food Chain

- The classic view of aquatic food webs was the linear food chain from phytoplankton to fish.
- Although bacteria were know to exist, they were not thought to be significant consumers of carbon or energy.



P: Phytoplankton (e.g., Diatoms)
Z: Zooplankton (e.g., Copepods)
F: Fish (both planktivors and piscivors)

Bacterial vs Phytoplankton Productivity

- Development of epi-fluorescence reveals large number of bacteria (10^6 mL^{-1})
- Development of bacterial productivity assay shows large fraction of NPP is processed by bacteria (50%?).

From: Cole et al. MEPS (1988).

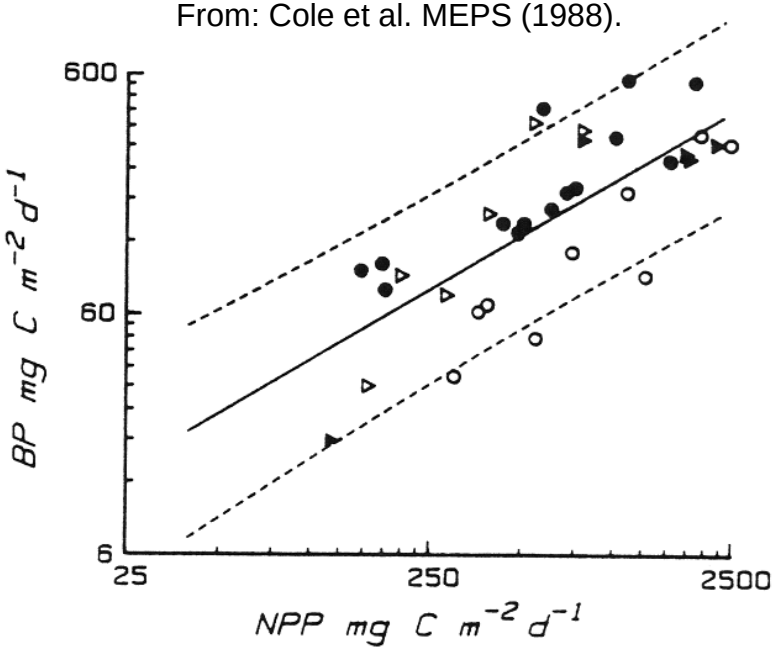
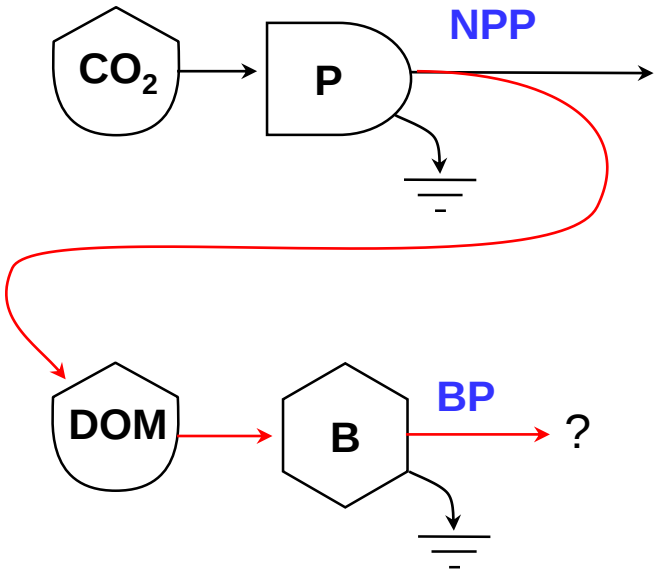


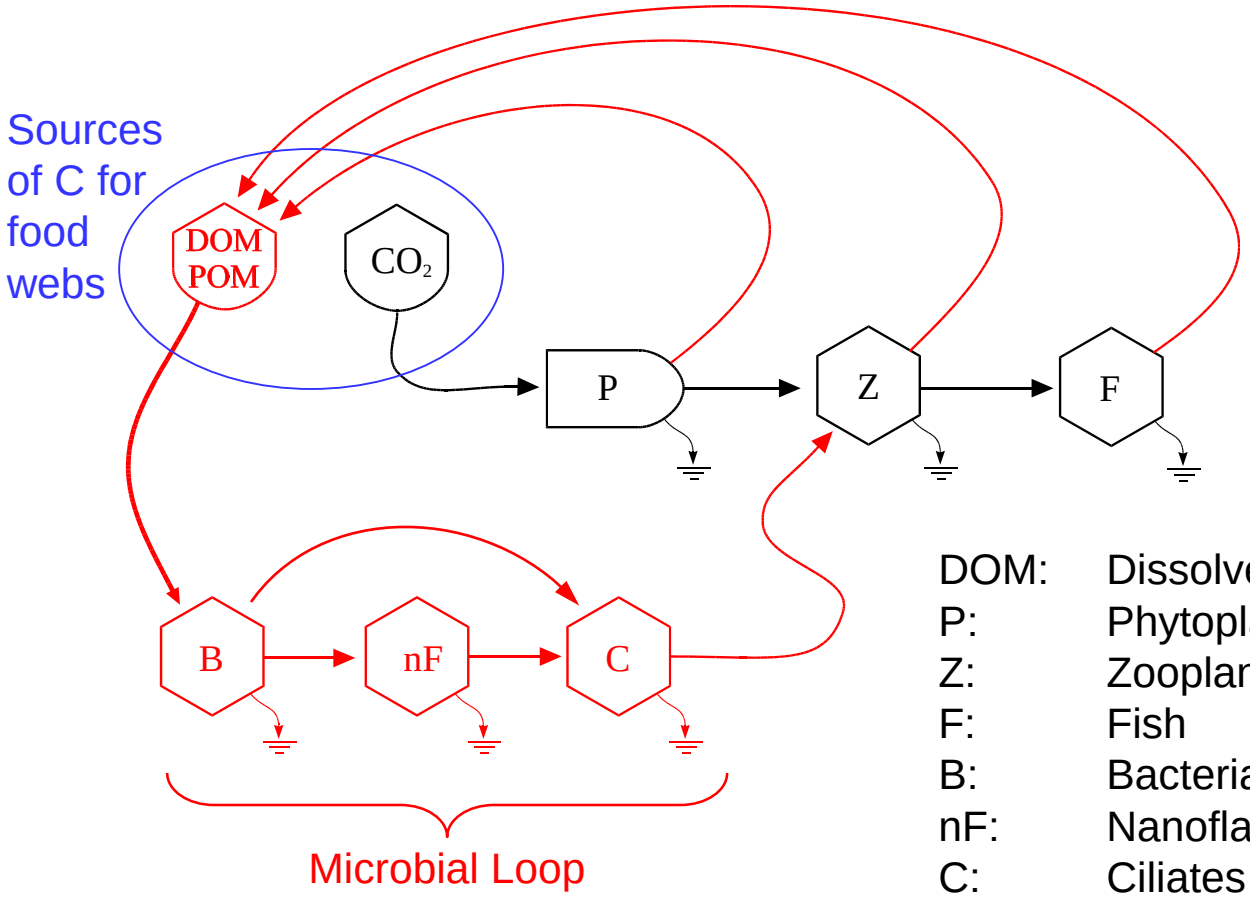
Fig. 3. Areal relation between primary production (NPP; X-axis) and bacterial production (BP; Y-axis) expressed per unit area for the entire water column. Symbols are as in Fig. 1. Regression line (Log Y = 0.75Log X + 0.093) is shown with 90 % confidence limits for the individual predictions of BP



Copepods cannot eat bacteria though

Microbial Loop

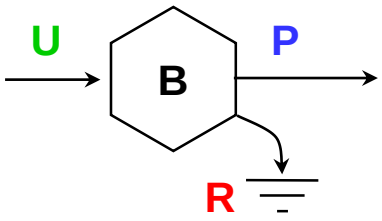
The microbial loop is a conceptualization by which DOM can be routed into the classic food chain via bacteria and their grazers. (Pomeroy 1974, Azam et al. 1983)



DOM:	Dissolved organic matter
P:	Phytoplankton
Z:	Zooplankton
F:	Fish
B:	Bacteria
nF:	Nanoflagellates
C:	Ciliates

Is the Microbial Loop a Link or Sink for Organic Carbon?

Efficiency

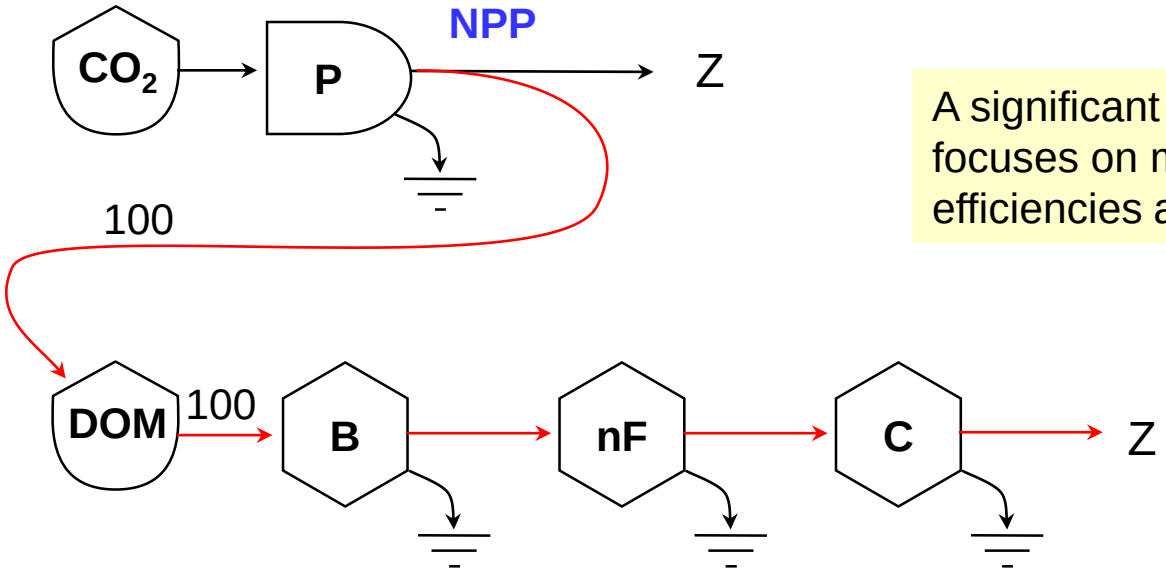


$U = P + R$

$$\varepsilon = \frac{P}{U}$$

Typical Efficiencies:
$0.1 \leq \varepsilon \leq 0.6$

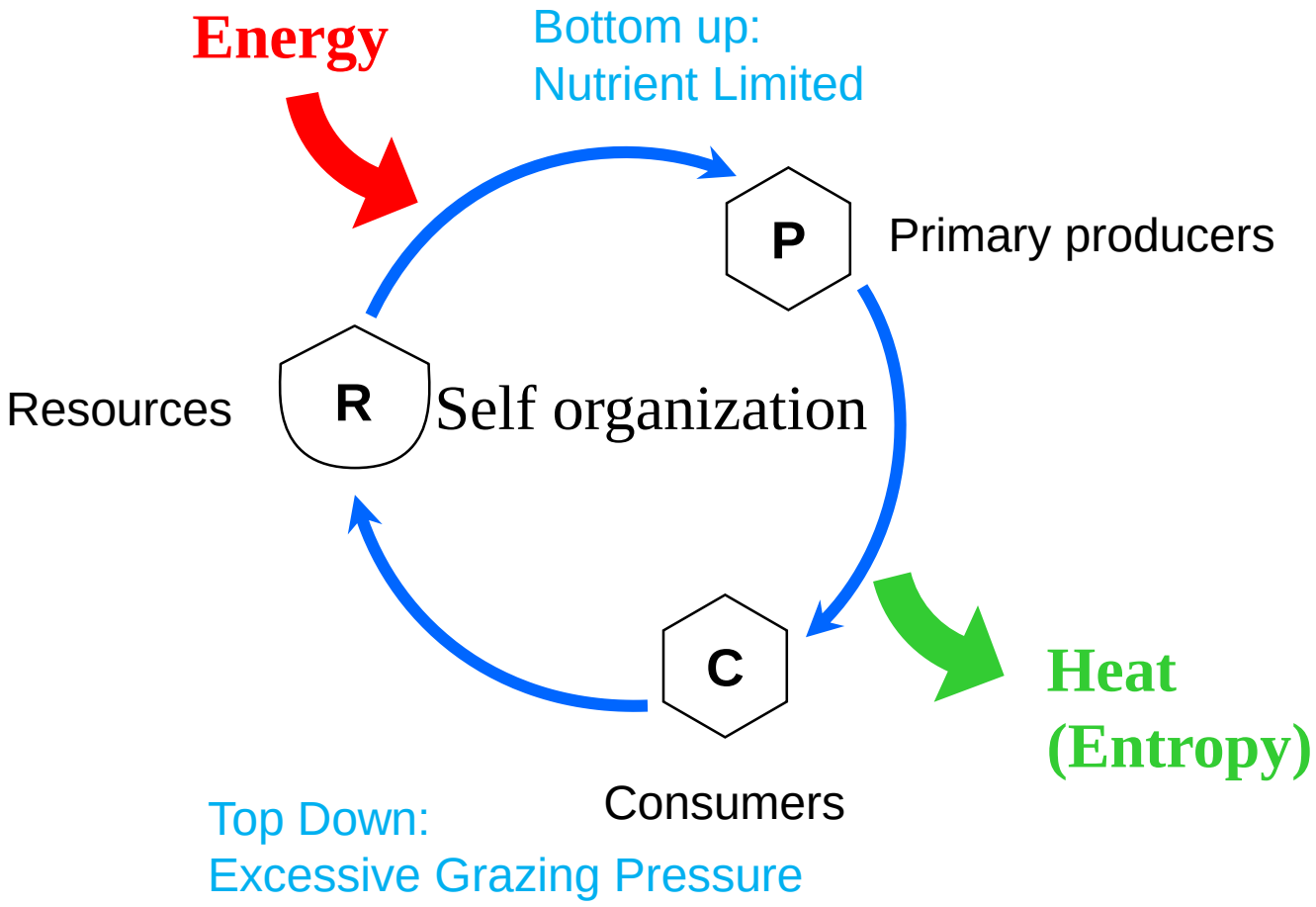
How much bacterial C makes it to zooplankton via the microbial loop?



A significant amount of research focuses on measuring growth efficiencies and feeding rates

ε	B→nF	nF→C	C→Z
10%	10	1	0.1
50%	50	25	12.5

Top Down or Bottom Up Limitation?



- Both views are myopic, in that they are transient assessments and likely to change over time, but the time scale may be long (decades or more).
- Likewise, the microbial loop, as a link, may be important over short periods when food resources are scarce.

Protozoa

Single-celled, eukaryotic, heterotrophs ranging in size from 2 μm to 1 mm or more.
Feed mostly by phagocytosis (engulfment).
Three basic types:

Flagellates

Use one or two (sometimes more) flagella (little whips) for motility.
Size: 2-100 μm
Representative taxa: Dinoflagellates, Chrysomonads, Bicosoecids, Choanoflagellates, Kinetoplastids

Ciliates

Range from uniformly covered with cilia (hair-like tubules) to mostly naked with tufts of cilia.
Size: 10-200 μm
Representative taxa: (planktonic) Oligotrichs, Tintinnids, Scuticociliates, (benthic) Hypotrichs, Peritrichs, Heterotrichs

Sarcodines

Amoeba-like species without flagella or cilia. Many possess skeletal structures or “shells”
Size: 5 μm to > 1 mm
Representative taxa: Gymnamoebae, Testacea, Foraminifera, Radiolaria, Acantharia, Heliozoa

Feeding and Motility

Phagocytosis

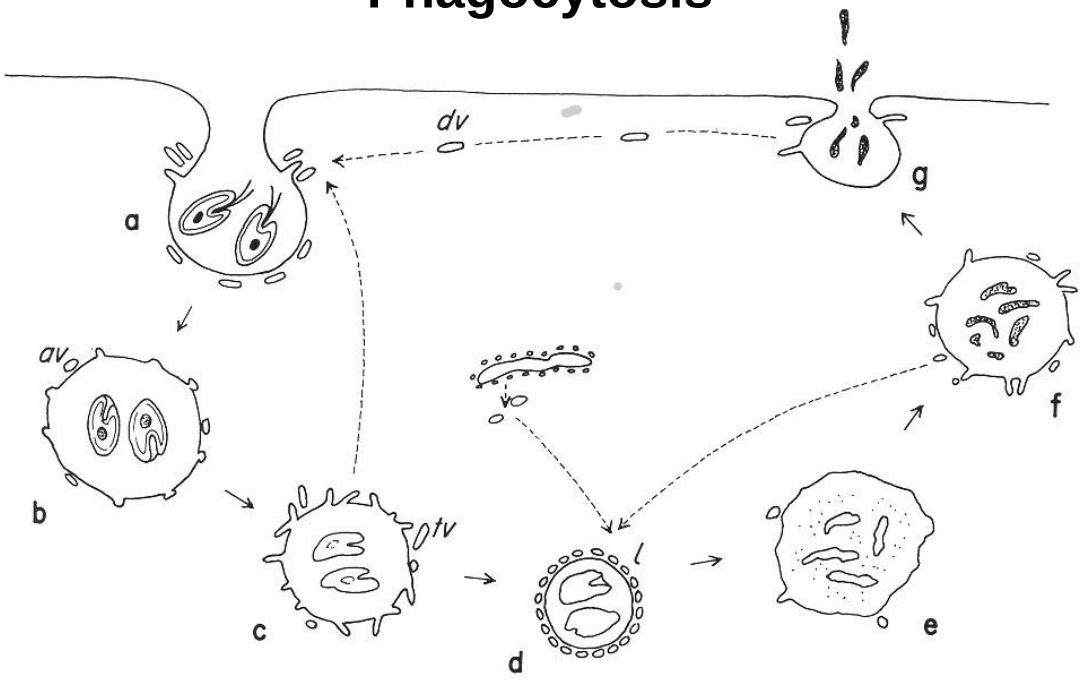
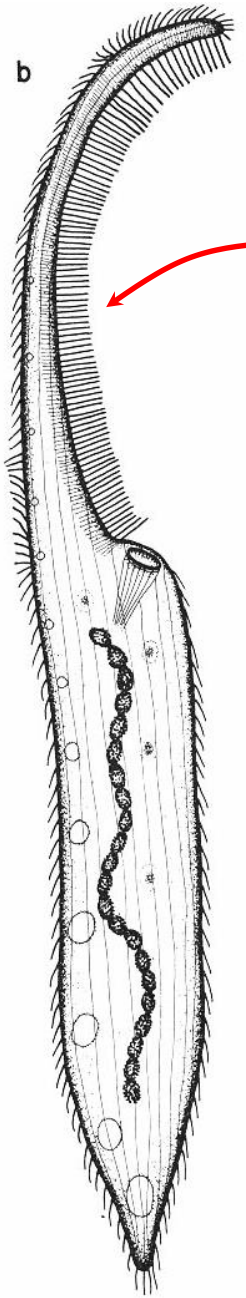
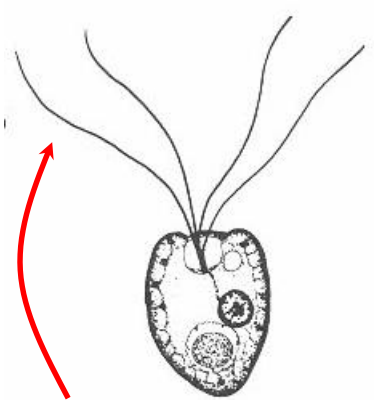


Fig. 2.6 The food vacuole cycle of a ciliate like ***Paramecium***. The food vacuole **(a)** receives captured food particles at the cytostome, where it also receives membrane from discoidal vesicles *(dv)*; the food vacuole is pinched off from the surface membrane, and as it moves away (**b**) it fuses with acidosome vesicles (*av*); fluid is removed from the vacuole (**c**) into tubular vesicles (*tv*), which may recycle to the cytostome; the shrunken vacuole (**d**) receives enzymes in lysosomes (*l*), that are either newly produced from ER or recycled from old food vacuoles; following digestion (**e**) micropinocytosis occurs around the vacuole and later (**f**) some lysosomal materials may be retrieved and recycled before the spent vacuole reaches the cytoproct (**g**), where undigested materials may be released and membrane retrieved to be returned to the cytostome as discoidal vesicles. (Information from Nilsson, 1979 and Allen, 1984).



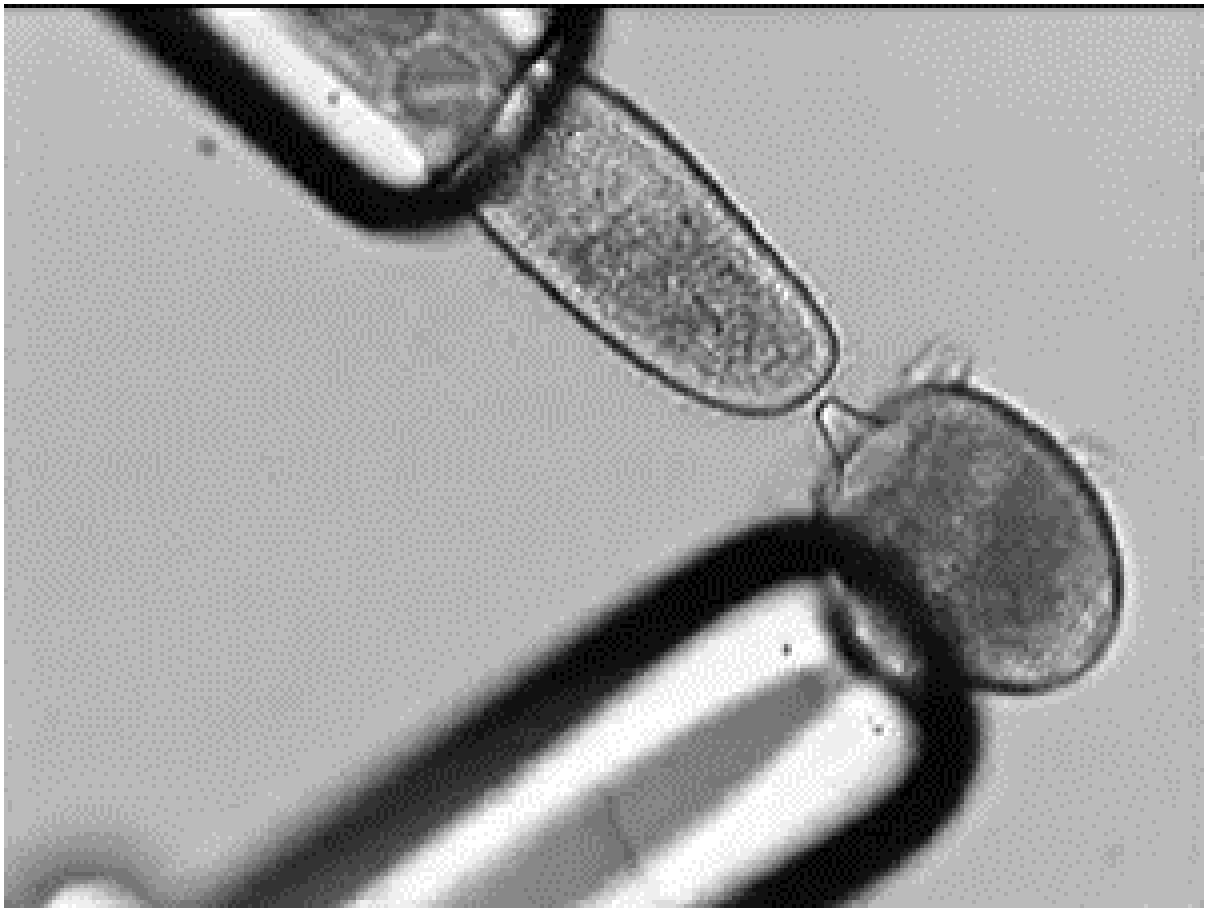
Cilia used from motility and particle feeding



Flagella used for motility

Extreme Examples

Didinium Eats Paramecium

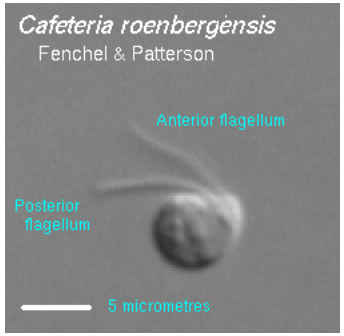


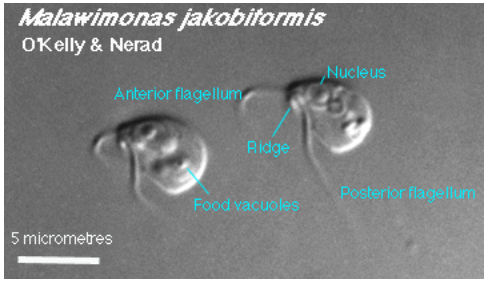
(Schiegeli Acanthopagrus;
(https://www.youtube.com/watch?v=arLutw0b-AY)

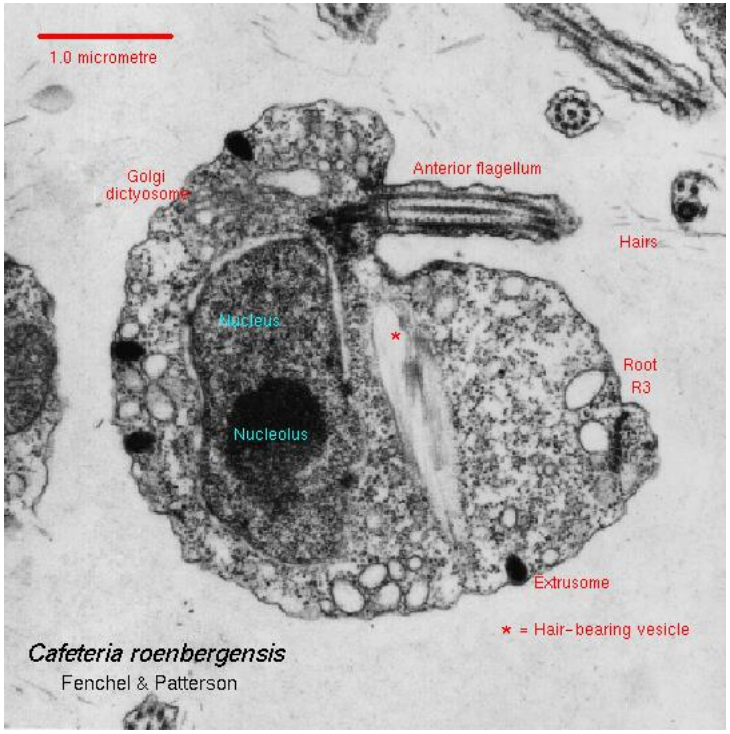
Heterotrophic Nanoflagellates (HNF)

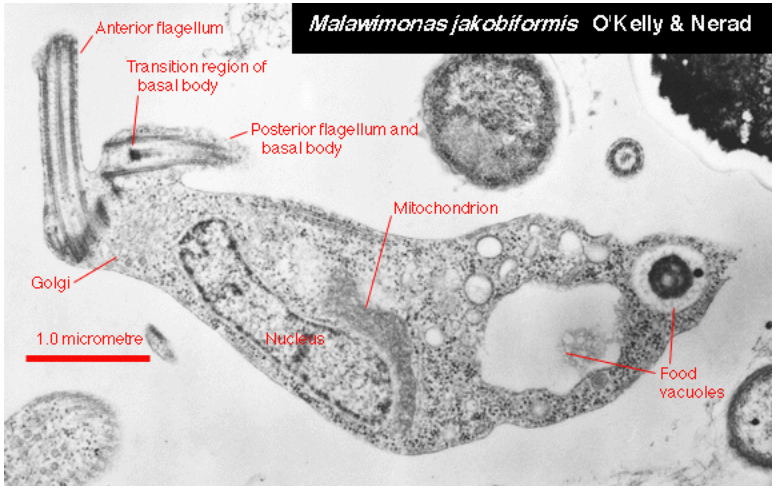
These are some of the smallest eukaryotes (2-3 μm)

Typical HNF densities around 10^2 - 10^3 ml^{-1}







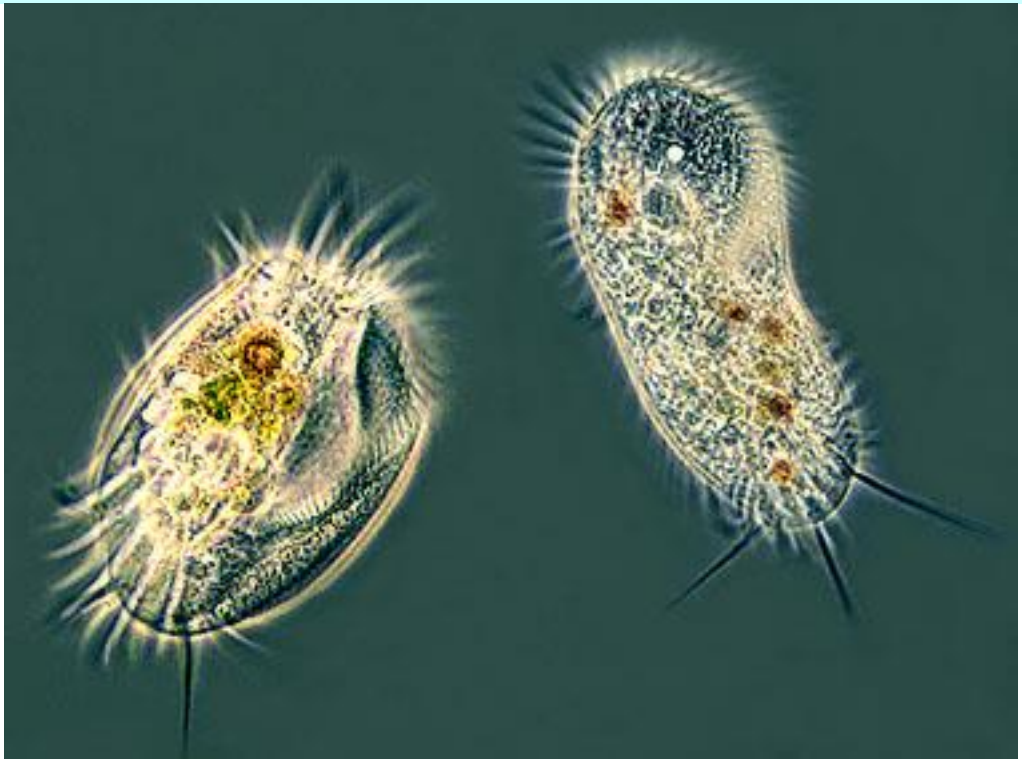


Pictures from http://megasun.bch.umontreal.ca/protists/protists.html

Ciliates

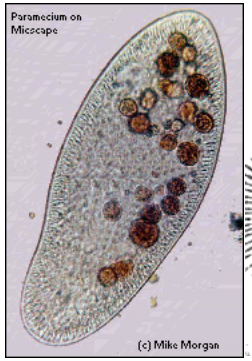
Ciliates are protozoans (single cell) that can be identified by the cilia that surrounds most of the body. Classic example is the *Paramecium sp*. Also see: http://www.microscopy-uk.org.uk/index.html

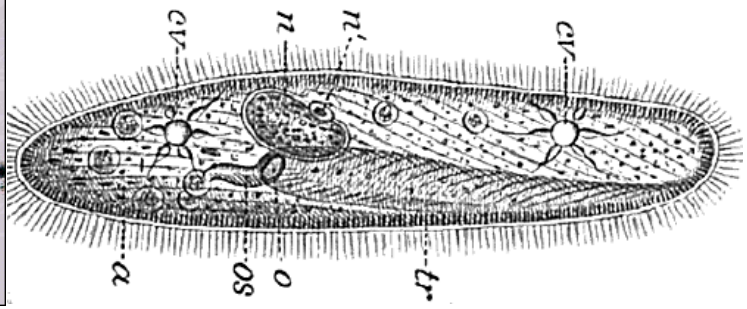
Densities around 1-100 ml^{-1}



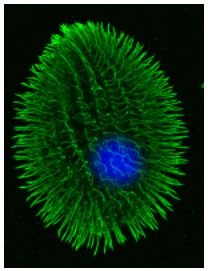
Two hypotrich ciliates: *Euplotes* (left) and *Stylonychia* (right)

Colony of *Carchesium*



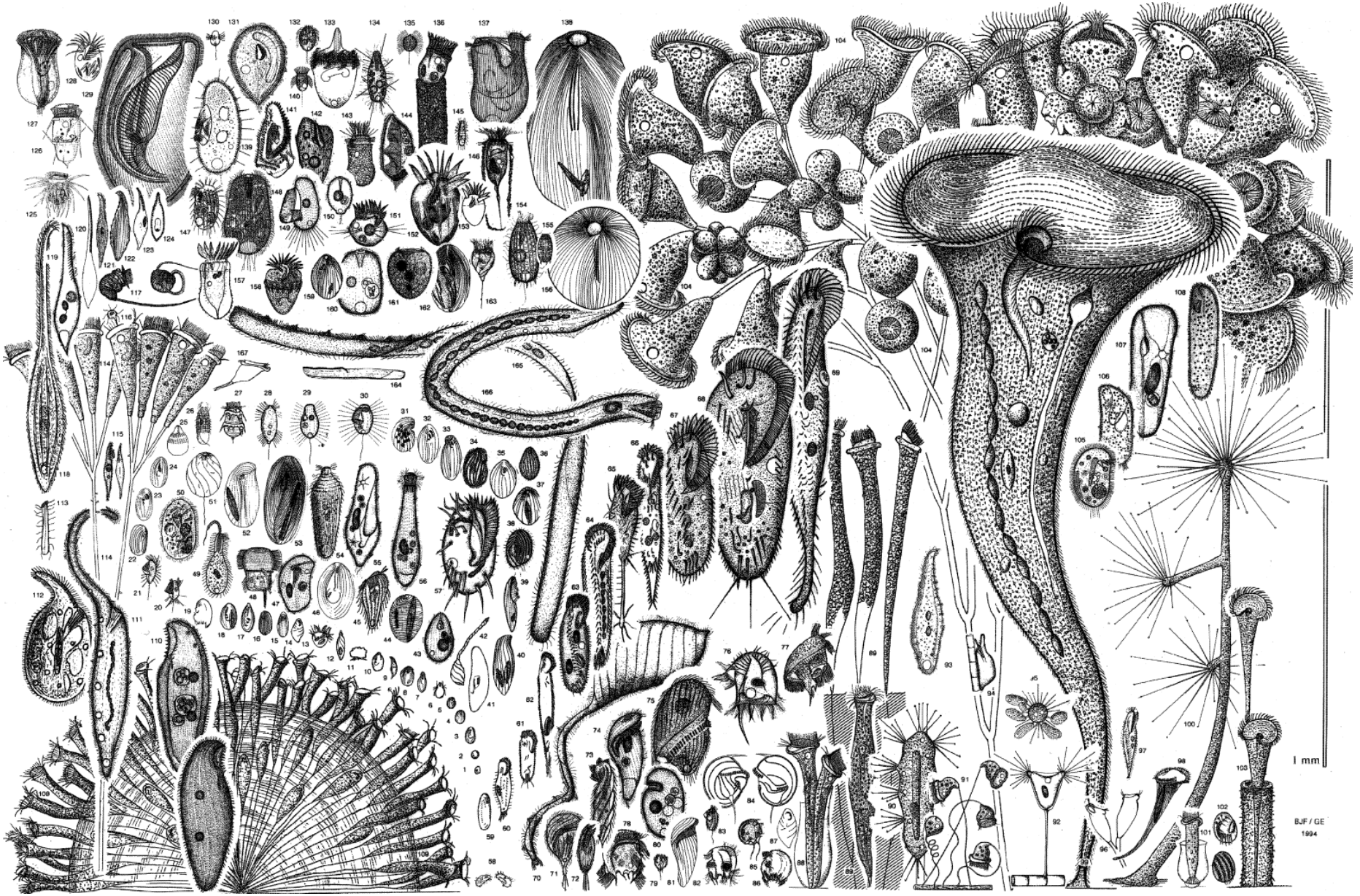


Paramecium



Tetrahymena

Ciliate Diversity (lakes and rivers)

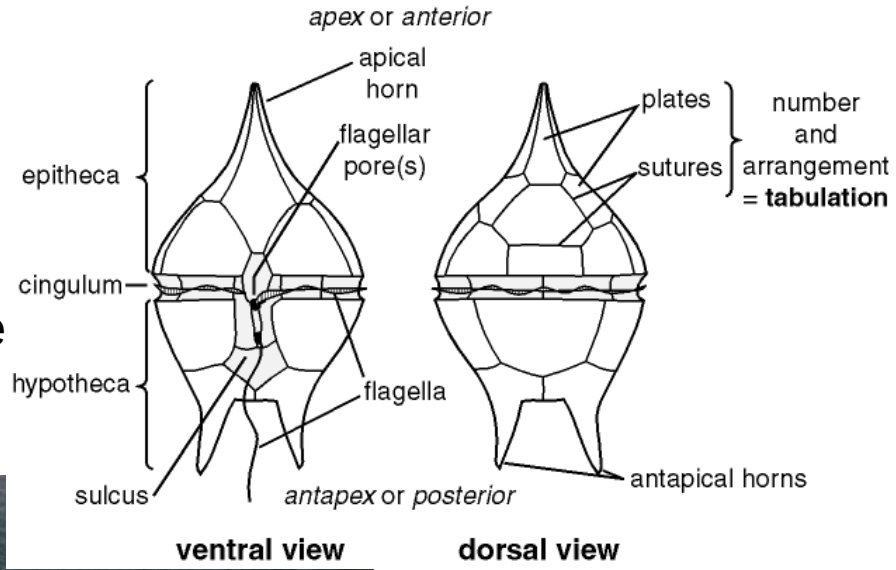


B.J. Finlay and G.F. Esteban (see http://members.aon.at/peigner/Ciliate%20Diversity.htm)

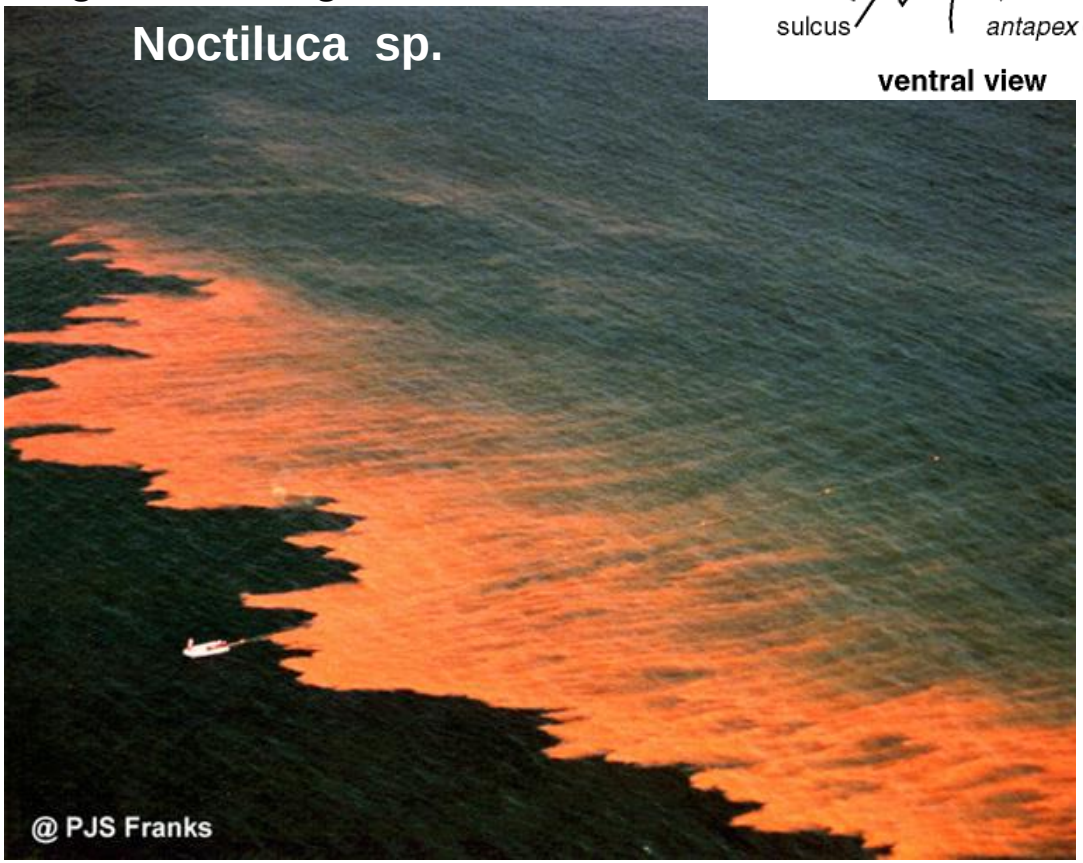
Dinoflagellates and Toxic blooms

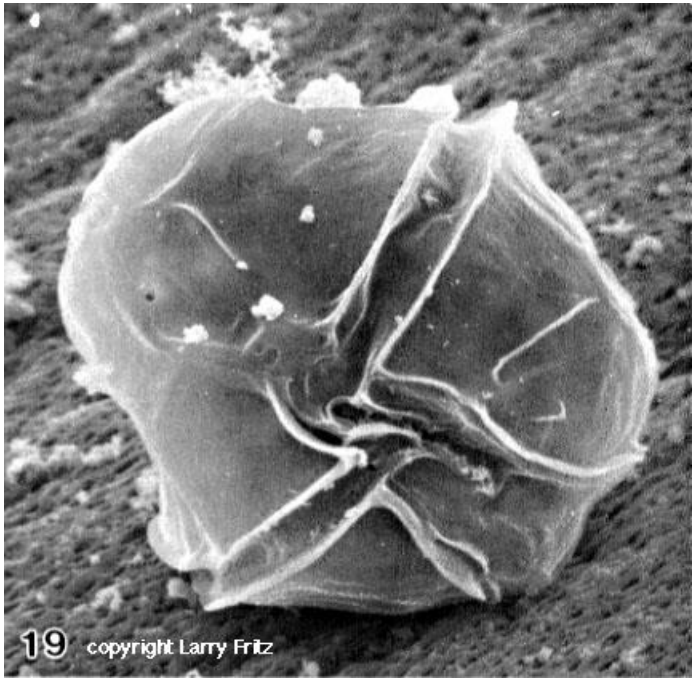
Dinoflagellates are the cause of “red tides”. Production of neurotoxins lead to fish kills and paralytic shellfish poisoning. See http://www.whoi.edu/redtide/

Harmful algal blooms appear to be on the rise, due to eutrophication and global change?



Cyanobacteria

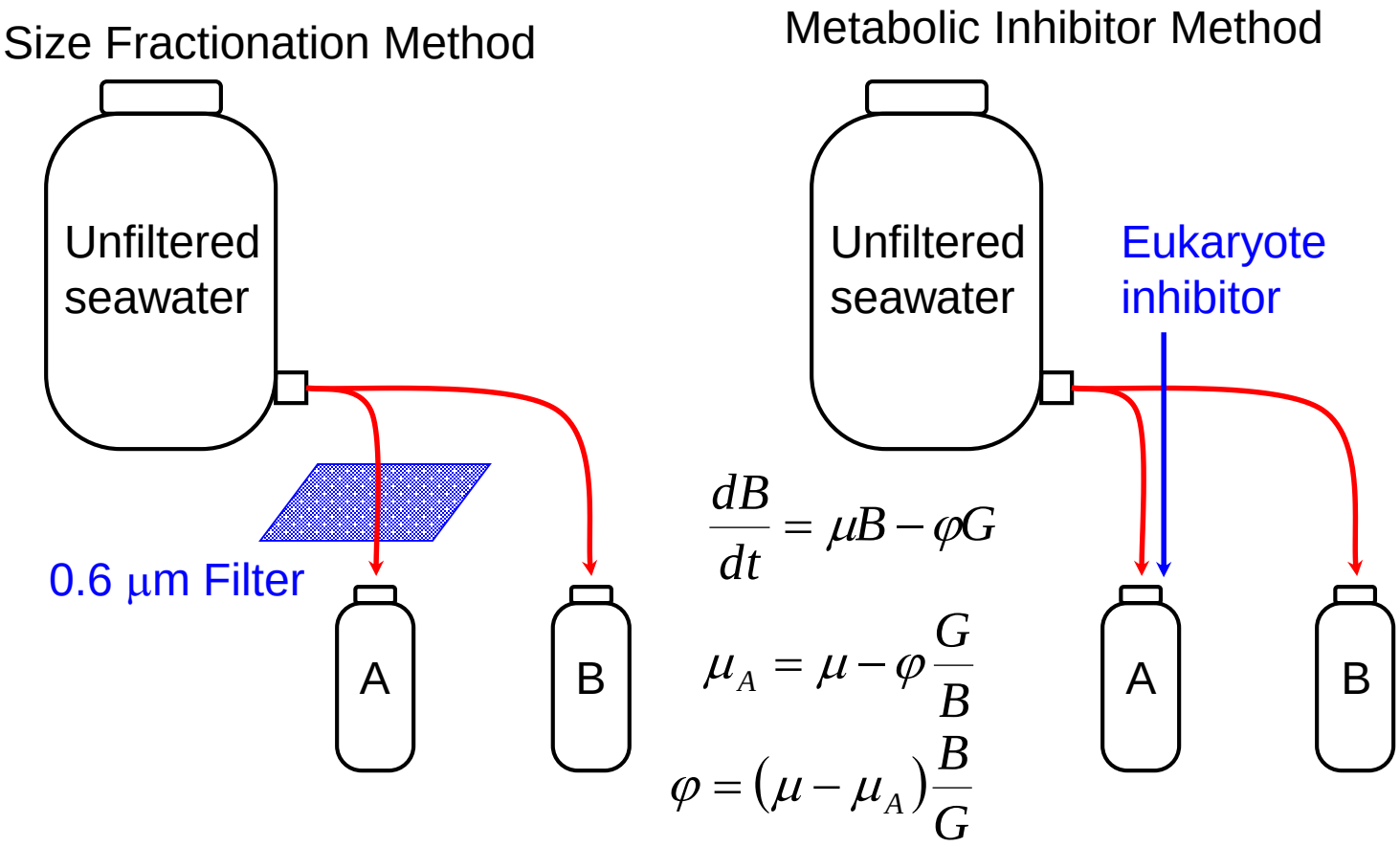




Techniques for measuring feeding rates

Metabolic inhibitors	Use an inhibitor (i.e., antibiotic) specific for eukaryotes. Measure increase in bacterial numbers in the presence and absence of inhibitor.
Size fractionation	Filter predator out of sample, and measure bacterial growth.
Dilution method	Measure bacterial growth rates at several sample dilutions
Radiolabeled bacteria	Feed predators radiolabeled bacterial
Fluorescently label particles	Feed predators fluorescently labeled particles or bacteria.

Metabolic inhibitors and size fractionation



$$\frac{dB}{dt} = \mu B - \varphi G$$

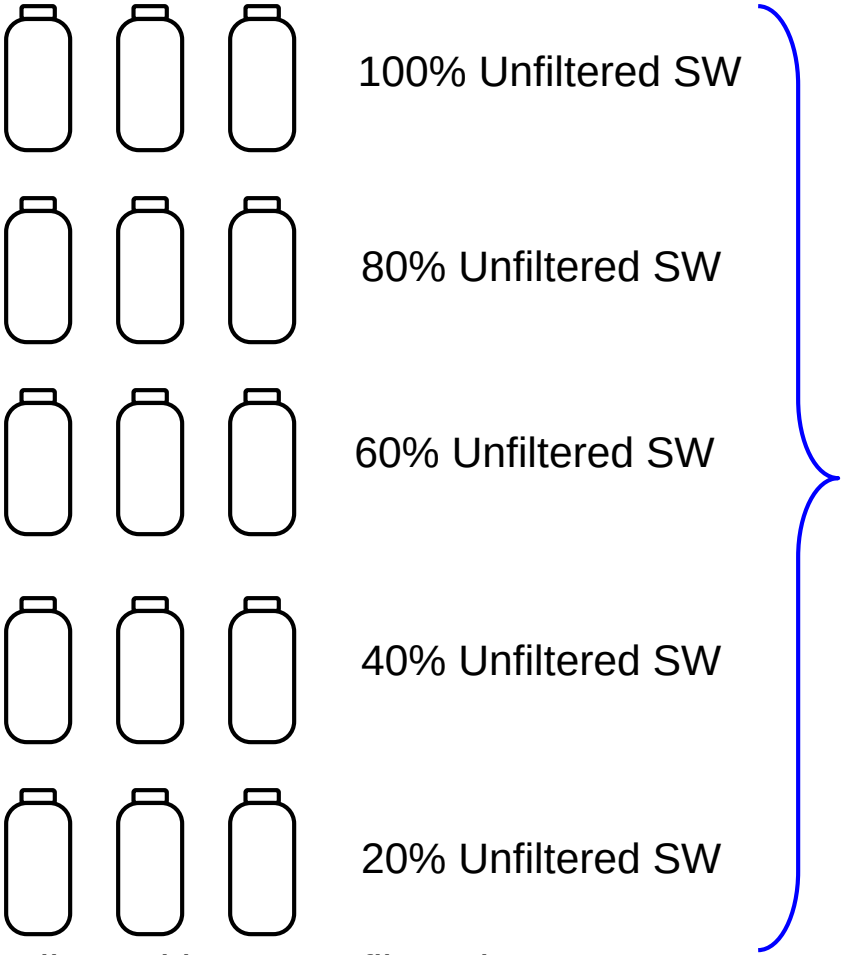
$$\mu_A = \mu - \varphi \frac{G}{B}$$

$$\varphi = (\mu - \mu_A)\frac{B}{G}$$

Measure bacterial number increases in treatment A's compared to treatment B's.

Problems: Filtration can cause cell lysis.
Inhibitors may not be perfectly selective, and my be consumed by bacteria.
Cannot look at species-level grazing.
Incubation time is long.

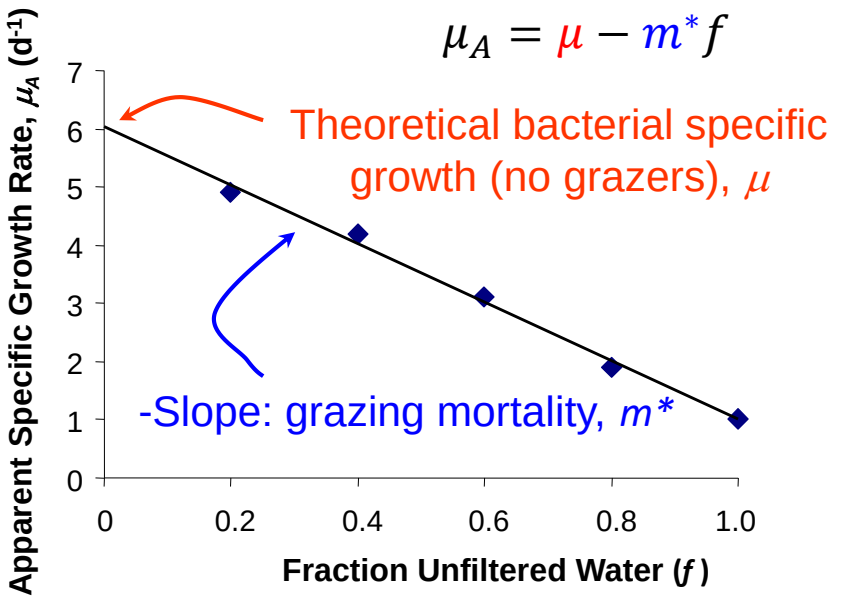
Dilution Method



Measure bacterial numbers at t-zero, and again at a later time (one or more days).

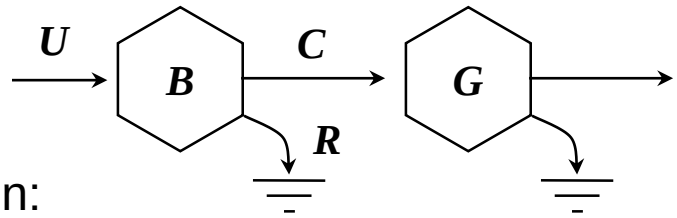
Calculate apparent bacterial specific growth rate for each: $\mu_A = \ln(x(t)/x(0))/t$

Plot μ versus fraction unfiltered water.



Problems: Dilution alters system.
Cannot look at species-level grazing.
Incubation time is long.

Grazing Rate from Dilution Cultures



Line from dilution plot:

$$\mu_A = \mu - m^* f$$

What is *m?**

Bacteria production:

$U - R = \mu B$ True growth rate

Grazer uptake:

$$C = \varphi G \qquad \varphi = \frac{\varphi^M B}{B + K_B} \approx \frac{\varphi^M}{K_B} B \quad if \quad B << K_B$$

Assumption behind dilution technique

Mass balance around bacteria: ***B***

$$\frac{dB}{dt} = U - R - C = \mu B - \varphi G = \mu B - \frac{\varphi^M}{K_B} BG = \left(\mu - \frac{\varphi^M}{K_B} G \right) B = \mu_A B$$

where,

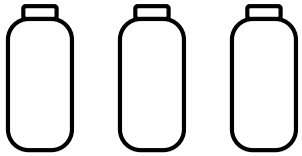
$$\mu_A = \mu - \frac{\varphi^M}{K_B} G \qquad G = fG^* \qquad \mu_A = \mu - \frac{\varphi^M}{K_B} G^* f$$

$$m^* \equiv \frac{\varphi^M}{K_B} G^* \qquad \varphi^* = \frac{m^*}{G^*} B^*$$

*m**	Mortality (1/d)
f	Fraction unfiltered
*	Denotes conditions in original sample

Fluorescently or Radiolabeled bacteria or particles

Water samples



Added FLP or RLP at <20-50% of natural bacterial abundance

- At specific times, take sample and preserver.
- Filter sample on 0.8 μm filter to remove unconsumed particles.
- Either microscopically count abundance of ingested particles per specific group of protozoa, or measure radioactivity.
- Accounting for bacterial abundance relative to added particles, calculate total number of bacteria consumed per protozoan per unit time. Can also calculate total bacterial removal rate.

Advantages: Can obtain species specific grazing rates
Short incubation times.

Problems: Predators may discriminate against particles.