Ecology and energetics of free-living pelagic protists

Karen Selph
Marine Microplankton Ecology
Ocean 626, Fall 2007
Office: MSB 608
ph. 956-7941
selph@hawaii.edu
Basic Microbial Food Web

hv

Phytoplankton

Inorganics (nutrients/energy)

REMINERALIZERS

DOM

PHAGOTROPHIC PROTISTS

LINKS TO HIGHER TROPHIC LEVELS

Bacteria

GRAZERS

GRAZERS
Lecture Topics

#1: What are protists?
• What are Protists?
• Protistan diversity, as exemplified by feeding modes

#2: How do we measure protist feeding, in the context of protist physiology?
• Functional Responses and how to estimate feeding impacts
• Materials Balance Approach to Protist Metabolism, Growth, Reproduction

#3: Role of protist remineralization, in the context of pelagic food webs
• Trophic transfer efficiencies: consideration of remineralization
• Structure of pelagic food webs
Today’s Lecture

• What is a protist?
• Protist abundance and size ranges
• Protist Feeding Modes
• Factors Affecting Feeding Selectivity
What are Protists?

Example: *Ochromonas* (flagellate)

- **Single celled:**
  All cells equal, no division of labor or specialized cells unlike metazoans

- **Eukaryotes:**
  Have organelles (nucleus, mitochondria, endoplasmic reticulum, etc.)
Common Groups

Sarcodina (pseudopodia): Amoeba, Foraminifera, Actinopoda

- Amoeba
  - Patterson 1992
  - www.markweber.org

- Foraminifera (CaCO$_3$)
  - www.ucmp.berkeley.edu
  - www.whoi.edu

- Radiolarian (Si)
  - www.markweber.org

- Acantharian (SrSO$_4$)
  - www.whoi.edu

- Heliozoan (Si)
  - www.whoi.edu
  - Patterson 1992

Flagellates

- www.bigelow.org
5-Kingdom Concept of Whittaker

organizational complexity

trophic mode

Kingdom Protista

FIG. 7-1 Five-kingdom system of classification, showing postulated evolutionary relationships. (From Whittaker, R. H. 1969. Science 163: 150-160.)
Size Range of Organisms

Protist Size ~ 1/2 the size range for all life forms.

Potential for great diversity in their functional roles within ecosystems.
Limits to Cell Size

- **smallest**: accommodate organelles (at least one mitochondrion, a nucleus, phagocytic vacuoles...)
  - smallest free-living eukaryote: *Ostreococcus tauri*
- **largest**: limited by gas diffusion rate, theoretical single cell max (spherical) ~800 µm

- Adaptations for large cells
  - elongated, flattened shape
  - high vacuole content
  - reduced activity and O$_2$ consumption/mass
  - O$_2$ producing symbionts

*background foram by O. Roger Anderson*
Protist Abundance

world/ocean wide

2-20 µm: ~1000/ml

20-200 µm: ~10-100/ml

<table>
<thead>
<tr>
<th>Determination</th>
<th>Cells/ml</th>
<th>C (mg/m³)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Nanoplankton, 2-20 µm</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>World ocean</td>
<td></td>
<td></td>
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<tr>
<td>Oligotrophic waters</td>
<td>$0.4 \times 10^3$–$3.0 \times 10^3$</td>
<td>1.3–8</td>
</tr>
<tr>
<td>Mesotrophic waters</td>
<td>$4.0 \times 10^3$–$9.5 \times 10^3$</td>
<td>16–35</td>
</tr>
<tr>
<td>Eutrophic waters</td>
<td>$13 \times 10^3$–$23 \times 10^3$</td>
<td>58–120</td>
</tr>
<tr>
<td>Atlantic Ocean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open ocean</td>
<td>$0.1 \times 10^3$–$2.8 \times 10^3$</td>
<td>(0.4–11)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Continental shelf</td>
<td>$0.9 \times 10^3$–$5.1 \times 10^3$</td>
<td>(3.6–20)</td>
</tr>
<tr>
<td>Estuaries</td>
<td>$0.9 \times 10^3$–$37 \times 10^3$</td>
<td>(3.6–149)</td>
</tr>
<tr>
<td>Atlantic Ocean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer shelf</td>
<td>$0.2 \times 10^3$–$0.8 \times 10^3$</td>
<td>0.6–8</td>
</tr>
<tr>
<td>Nearshore shelf</td>
<td>$0.3 \times 10^3$–$2.3 \times 10^3$</td>
<td>1.0–14</td>
</tr>
<tr>
<td>Estuary</td>
<td>$1.6 \times 10^3$–$3.3 \times 10^3$</td>
<td>5.4–15</td>
</tr>
<tr>
<td>Chesapeake Bay</td>
<td>$0.5 \times 10^3$–$15 \times 10^3$</td>
<td>(2.0–60)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Limfjord, Denmark</td>
<td>&lt;0.2 \times 10^3$–$3.0 \times 10^3$</td>
<td>(&lt;0.8–12)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>B. Microplankton, 20–200 µm</strong></td>
<td></td>
<td></td>
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<tr>
<td>Ciliates, world ocean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligotrophic waters</td>
<td>0.04–9</td>
<td>0.6–15</td>
</tr>
<tr>
<td>Mesotrophic waters</td>
<td>17–30</td>
<td>34–65</td>
</tr>
<tr>
<td>Eutrophic waters</td>
<td>40–145</td>
<td>67–144</td>
</tr>
<tr>
<td>Ciliates, Southampton Estuary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tintinnida</td>
<td>—</td>
<td>0.4–2.3</td>
</tr>
<tr>
<td>Naked ciliates</td>
<td>—</td>
<td>0.07–2.1</td>
</tr>
<tr>
<td>Protozoa, California coast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tintinnida</td>
<td>0.08–5.6</td>
<td>0.04–1.69</td>
</tr>
<tr>
<td>Other ciliates</td>
<td>0–5.0</td>
<td>0–5.26</td>
</tr>
<tr>
<td>Foraminifera</td>
<td>0–0.02</td>
<td>0–0.08</td>
</tr>
<tr>
<td>Radiolaria</td>
<td>0–0.09</td>
<td>&lt;0.02–0.29</td>
</tr>
<tr>
<td>Protozoa, Central Pacific Gyre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(92% ciliates)</td>
<td>0.7–2.0</td>
<td>2.0–4.4</td>
</tr>
</tbody>
</table>

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Protist Reproduction

Asexual

Binary fission produces two identical daughter cells by mitotic division.

Multiple fission involves repeated division of nucleus to produce several to many daughter cells. Occurs in a wide range of protists.

Paramecium: binary fission

Sexual

Widespread, but absent among some amoebae and some flagellate groups.

Ciliates are diploid and conjugative genetic exchange occurs in many forms.

Alternating haploid and diploid generations appear to be the rule among forams.

Paramecium: conjugation

www.ebiomedia.com

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Food Vacuoles

Intracellular vacuoles digest the food (no gut as in metazoans)

Example of a food vacuole cycle in a protist:

a: food vacuole “pinching off” from outer membrane, enclosing food items (phagocytosis)

b: it fuses with acid-containing vesicles (av)

c & d: it shrinks as liquid is removed, then merges with lytic (lysosomal) enzymes (digestion)

e & f: after food digestion, digested vacuolar contents are pinched off into cytoplasm, as are the enzymes for re-use

g: undigested material may be released to the “outside” environment (remineralization) and the membrane retrieved to be re-used for another cycle

Sleigh 1989
Protist Feeding Modes

- Sarcodines: Diffusion feeding (except amoeba)
- Flagellates: Direct interception
- Ciliates, some flagellates: Filter feeding
- Dinoflagellates: peduncle and pallium feeding
Diffusion Feeding

*Actinophrys sol* (heliozoan) capturing the ciliate *Colpidium*.

a) adhesion  
b) pseudopod extension  
c/d) pseudopod wrapping around prey  
e/f) prey completely enclosed  

scale bar = 50 µm

Hausmann & Patterson 1982  
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Movie of Actinophyrs capturing food

(movie in class, not pictured here)
Direct Interception Feeding

- Performed by the smallest protists (flagellates)
- Results in “bacterivory”
- Ubiquitous in aquatic ecosystems

Images courtesy of Bay Paul Center
http://starcentral.mbl.edu/microscope/portal.php

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On the use of video-microscopy for the analysis of protist feeding behavior

J. Boenigk, Institute for Limnology, Austrian Academy of Sciences, Mondsee, Austria

The mechanisms of food selection were the focus of the study. Food selectivity of heterotrophic nanoflagellates can be subdivided into: (1) passive food selection (contact probability and morphological properties of the feeding structures are responsible for a particle-specific response); and (2) active food selection (flagellates may actively select food during food uptake). These experiments revealed a high variability between species, and also high intraspecific variability. Advantages and disadvantages of the technique will be discussed. These include photochemical effects, experimental artifacts and the general suitability of the method for investigating behavioral patterns in microbial populations.


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Flagella Diversity

fat arrows: protist body movement

thin arrows: water movement

Sleigh 1989

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Protist Motility: pseudopods

- Sarcodines: e.g., amoeba

http://www.isengrim.com/lasaterd43.html
Filter feeding - choanoflagellates

Acanthocorbis unguiculata (dry prep)

Parvicoribcula quadricostata (Southern Ocean)

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Protist Motility: cilia & flagella

Structure: 9 microtubule doublets surround a core of 2 (total diameter 0.2 μm); interconnected by fibers of elastic proteins (dynein & nexin). Proteins activated by ATP; microtubules slide relative to one another.

Mechanics: cilia - short paddles (10-20+ μm); "powerstroke" & recovery stroke; coordinated action - "metachronal wave", hydrodynamic coupling due to water viscosity
flagella - long structures (20 - 50+ μm); propagating wave; "pseudopodium" (smooth) vs "tractellum" (hairy - "mastigonemes")

More filter feeding: ciliates

scale bar = 10 µm

Ciliate (oligotrich, *Haltheria*): particles intercepted on inside of membranelle zone.

Position of a latex bead at 20 µs intervals, showing acceleration of water into the membrane.

Ciliate (scuticociliate, *Cyclidium*): particles intercepted on a paroral membrane of parallel immovable cilia.

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Tintinnid Feeding with Cilia

Video by Harvey Marchant

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Flagellates and Ciliates have different swimming velocities

Swimming speed is a function of mode of propulsion

Cilia are shorter, but can beat at a higher velocity than flagella

Swimming velocities of 15 species of flagellates and 21 species of ciliates (cell lengths per sec) as a function of cell length. Slopes of -1 indicate constant, absolute swimming velocities independent of cell length (From Sleigh and Blake, 1977).
Pallium Feeding by Dinoflagellates

figures from Jacobsen & Anderson 1986
Peduncle Feeding by Dinoflagellates (Myzocytosis)

e.g., *Pfiesteria spp.*: fish killed by dinoflagellate predation

A larval sheepshead minnow (*Cyprinodon*) being fed upon by *Pfiesteria*

fish skin lesion w/dinos attached

Vogelbein et al. 2002
Mixotrophy

- Mixed mode of nutrition, definition broad enough to include symbiotic relationships
- Widespread amongst protistian groups
- Diverse: manifests in many ways
Advantages & Functions of Mixotrophy

- Gas exchange--oxygenation of large cells
- Additional sources of nutrition
- Different perspective, source of nutrients/organics for basically autotrophic cell in an oligotrophic environment
- Protection, advantage to symbionts

- For corals, symbionts supply the added nutrition required for secretion of CaCO$_3$ skeletons

Three General Types of Mixotrophy

1. Endosymbiotic relationships: true symbiotic relationships, like algae in corals
   - mostly associated with Sarcodines (e.g., foraminifera) -- symbionts are often dinoflagellates, monads, diatoms, red algae
   - usually only one symbiont type per host species
   - enclosed in vacuolar membrane, “respectful distance”

Globigerinoides ruber with dinoflagellate symbiont

2 mm
image courtesy of D. Lea, UCSB
2. Borrowed chloroplasts

Example: Laboea strobila, a tintinnid ciliate, borrows chloroplasts, it may eat some at night -- gets mainly polysaccharide sugars and LMW molecules from chloroplasts.

• Implication: since “prey” may continue to produce organics for predator after ingestion, efficiency of growth might be higher than predicted simply from ingestion of prey biomass.

http://www.liv.ac.uk/ciliate
3. Inherent part of Organism’s Structure (genome)

*Example:* Mixotrophic flagellate (chrysophyte) *P. malhamensis*, phagotrophy dominates if bacteria are present, phototrophy only when bacterial abundance becomes limiting.

- Other organisms may show the opposite preference for trophic mode (e.g., many red-tide dinoflagellates are phagotrophic).
Implications of Mixotrophy

- How to distinguish autotrophic and heterotrophic organisms?
- Energy flows; newly fixed carbon can come in at various places
- Growth: increased “apparent” gross growth efficiency (growth/ingestion)
Summary of Feeding Modes

• Feeding mechanisms: 4 main types
  – Filter feeding
  – Diffusion feeding
  – Pallium and peduncle feeding
  – Direct interception (raptorial) feeding

• Mixotrophy: 3 main types
  - Endosymbiosis (true partnership)
  - Kleptochloroplasts ("borrowed" chloroplasts)
  - Genetically capable of switching ("autonomous" mixotrophs)
Factors affecting Feeding: result in selective feeding

- Prey Size
- Prey Motility
- Prey surface chemistry
- Predator chemosensory behavior
- Physics: turbulence
Heterotrophic flagellate feeding on bacteria

Size Selection

Dinoflagellate feeding on algae

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Andersson et al. 1986

Weisse & Kirchhoff 1997

Fig. 8. Size-selective grazing of *Peridinopsis berolinense* (dark shaded area) versus the initial algal size distribution (light shaded area) measured by EPCS in the first experiment. The y-axis denotes count rate, i.e. number of particles per each of the 1024 channels of the equivalent spherical diameter (ESD, x-axis), the latter is a measure of cell size. The dark area thus indicates the size range where feeding was effective and the number of algae in each channel that have been grazed during the experiment. Cursors mark the abundance peak of *Rhodomonas minuta* (left) and *Cryptomonas* sp. (right), respectively. The corresponding ESD are given in the top right corner (CL: cursor left, R. minute, CR: cursor right, Cryptomonas sp.)
Selection based on Prey Size

Monger & Landry 1992

Graph showing the clearance rate (nl HNAN-l-h-1) of prey based on their diameter (µm). The graph includes data points for live prey (circles) and heat-killed prey (dots).
Motility of Prey

Uptake of fluorescently-labelled, living and heat-killed cultures of a highly motile marine bacteria (Kaneohe Bay isolate) by the flagellate HNAN.

Monger & Landry 1992
Prey Hydrophobicity

Monger et al. 1999

Clearance Rate (nl h^-1 flag^-1)

HIC Index

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Hydrophobicity of *Prochlorococcus*
Chemosensory Behavior?

closed circles: FLB
open circles: latex beads

data reprinted in Sherr & Sherr 1987

closed circles: FLB
open circles: microspheres

ciliate w/fixed filter apparatus

ciliate w/membranelle filter

Sherr et al. 1987

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Landry et al. 1991
Pallium Feeding

- Preferred diatoms over dinoflagellate prey
- Appeared to respond to chemosensory cues
- Had greater capture success with non-motile prey

Higher growth rate on diatoms (0.7 d\(^{-1}\)) vs. dinoflagellates (0.4 d\(^{-1}\))

Table 2. Observed feeding interactions between *Protoperidinium pellucidum* and 4 potential food types: *Ditylum brightwellii*, *Thalassiosira* sp. 1, *Gonyaulax polyedra* and *Prorocentrum micans*. If *P. pellucidum* formed a pallium around its food cell, it was scored as a successful capture. If the cell was lost after the tow thread was attached, it was scored as an escape. If *P. pellucidum* circled the cell in a stereotypical feeding behavior, but failed to attach a tow thread, it was scored as a lost contact. n = no. of observations.

<table>
<thead>
<tr>
<th>Prey</th>
<th>Prey speed (mm s(^{-1}))</th>
<th>Successful capture</th>
<th>Escape</th>
<th>Lost contact</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ditylum brightwellii</em></td>
<td>0</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>115</td>
</tr>
<tr>
<td><em>Thalassiosira</em> sp. 1</td>
<td>0</td>
<td>98.3%</td>
<td>1.7%</td>
<td>0</td>
<td>116</td>
</tr>
<tr>
<td><em>Gonyaulax polyedra</em></td>
<td>0.4</td>
<td>61.9%</td>
<td>21.2%</td>
<td>16.9%</td>
<td>118</td>
</tr>
<tr>
<td><em>Prorocentrum micans</em></td>
<td>0.1</td>
<td>46%</td>
<td>43%</td>
<td>11%</td>
<td>100</td>
</tr>
</tbody>
</table>

Buskey 1997

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Turbulence as a factor?

Shimeta et al. 1995

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Shimeta et al. 1995
Summary of Selective Feeding
All predators are selective:
different parameters determine selective impacts

- diffusion feeding:
  - prey swimming speed and size
- filter feeding:
  - filter spacing relative to prey radius
- pallium feeding:
  - not by prey size* - probably chemoreception/motility of prey
- direct interception:
  - prey size and surface chemistry