ter and Lewis (1990). Phytoplankton cells are thought to have a "phycosphere" about them, a boundary layer in which cell products are diffusing away from them at the slow rates of molecular diffusion. Price and Paffenhöfer (1984) showed an intriguing aspect of this detection mechanism problem. They took repeated films of *E. pileatus* after various durations of experience with a given phytoplankton food. Inexperienced feeders responded to plants at a mean distance of 273 μm away from the maxilla, while those that had had the food available for 24 or more hours responded to them at 345 μm. The experienced also responded to 31% of cells appearing somewhere in the vicinity (not defined) of the maxilla, as opposed to only 12% for the inexperienced. Price and Paffenhöfer took this to be evidence that olfaction is indeed the sense by which phytoplankton are detected. At least it shows that experience tells the animal that weaker signals of the effective kind, whatever that is, are associated with food. Bundy *et al.* (1998) have shown that copepods will also move to and capture polystyrene beads, not likely to have much odor. They speculate that the presence of the bead distorts the streamlines of the feeding current as the flow approaches the animal's boundary layer, and that sensing organs (innervated setae) on the limb surfaces, particularly the antennule, detect the disturbations.

While the mechanism described by Koehl and Strickler is definitely filter feeding, it is not that of strainer cleaning plants out of a thin soup to get either a thick porridge for swallowing. Rather, the plant or protozoan is found, then moved to, separated from the surrounding water, ingested, and eaten or rejected individually.

We know from large differences in feeding rates that not all filter-feeding copepods collect particles by this exact mechanism. Old studies of forms like *Centropages* and *Acartia* suggest that they pull the long setae on the second maxilla forward through the water in spread position, collecting particles against the setae, then folding them to move the particles forward to the mouth. The filtering current as such seems to be involved. Movies of *Eucalanus* are all made with the animal held before the lens by a hair glued to its back. The *Acartia* mechanism cannot be studied in that way. Strickler has suggested that flow is along the midline close to the body, but reverses along the spines and moves toward the tips. Capture occurs when particles catch on the setules.

One variant mechanism, that of *Diaptomus sicilis*, a freshwater copepod, is described by Vanderploeg and Paffenhöfer (1985), but again based on films of tethered individuals. In *D. sicilis*, the limbs generate a flow from anterior to posterior. Particles coming quite close to the maxillar setae will invoke a fling and clap response comparable to that of *Eucalanus*. However, part of the flow is deflected along the inner surface of the maxillar setules which seem to funnel it. Some particles appear to be ingested from this directed flow without any fling and clap, a passive capture mode. However, Vanderploeg and Paffenhöfer insist that the setal web does not act as a filter. According to observations by H. Price, *Eucalanus* is also capable of passive captures. It is unfortunate that we have no proper film study of feeding in *Calanus*, which in other respects is the best-studied genus of copepod.

Feeding rates and factors affecting them

From a trophic-dynamic viewpoint, the interesting aspect of zooplankton feeding is not how it is done, but the rate of consumption. Feeding rates are determined in several ways presently. The before-and-after method is to bottle some plankters with a known concentration of plants for a period of several hours or a day, then re-measure the plant concentration. The measures can be done by direct microscopic counting on hemocytometer slides or by electronic particle counter (Box 7.3).

The results are usually expressed as a filtering or clearance rate, F, with units of volume per time per animal. Since filtering as such is not necessarily how an animal feeds, clearance rate is the better term. Filter feeding is not like you straining peas from a pan. You would pour the water and peas through a sieve, each lot of water removed from the pan being discarded and separated from the peas. The rate of change of peas, N, remaining in the pot would be:

$$dN/dt = -FN/V$$

Box 7.3

Electronic particle counters made experimental analysis of filter feeding very simple, probably deceptively simple. These devices, deriving from an original design by Wallace Coulter, count microscopic particles moving with a stream of electrolyte (blood or seawater) through the space between two electrodes embedded in the wall of a glass tube. Counts are electronically cumulated of the changes that the particles cause in the resistance to electric current flow between the electrodes. It is possible to gather changes in resistance in separate counts according to their magnitude, partly a function of particle size, and thus to obtain particle counts in a number of size-related "channels". Resistance change is roughly proportional to particle volume, so feeding experiments are often characterized in terms of total particle volume in a feeding chamber before and after an animal has fed there for some known time.

where F is the volume filtered per time and V is the original volume of cooking water. A plot of N against time would be a straight line with negative slope, intercepting the time axis ($N = 0$) when $Ft = V$. In filter-feeding the water filtered is not removed from the container (or ocean) after the animal removes particles from it; it returns to the suspending volume, diluting the remaining particles. Therefore, reduction in concentration follows an exponential decay law. That is, the animals must filter progressively more water for each unit of food obtained. This is expressed by:

$$dN/dt = (-FC/V)N$$

where N is the number of plants, V is the container volume, C is the number of animals, and F is the volume cleared by each animal per unit time at an assumed 100% filtering efficiency. Harvey (1937) apparently first applied this equation to filtering rate determinations. Note that the model here is a strainer, not an encounter predator. However, since the animal is very small relative to the scale of the water it must "search" by the Koehl–Strickler or other mechanism, the model is still the right one. The per capita clearance rate is an appropriate measure of the search effort the animal applies in feeding. Separating variables and integrating, we have:

$$\ln(N_t/N_0) - FCt/V$$

or

$$N_t = N_0 \exp(-FCt/V)$$

where N_0 and N_t are the cell concentrations at the beginning and end of the experimental interval t.

If the plants are growing significantly, a control container without the grazers must be used to evaluate that rate, μ, and the associated equations must take account of plant growth:

$$\ln(N_t/N_0) = (\mu - FC/V)t.$$

This, or a mathematical equivalent, is often termed the Frost equation (Frost 1972). Frost also showed that the average concentration, N_{mean}/V, of cells over the interval of grazing in the experiment is calculated as follows: let $FC/V = \gamma$, then:

$$N_{mean}/V = N_0[e^{(\mu-\gamma)t} - 1]/Vt(\mu - \gamma).$$

This number can be used in calculating the average individual ingestion rate: $I = FN_{mean}/V$, or from the units: (mL cleared h^{-1}) $\times$ N_{mean} mL^{-1} = cells ingested h^{-1}.

Copepod studies

The effects on F and I of a variety of factors have been studied. These include: food density, container volume, particle size of food, animal size, life cycle stage and individual size, mixtures of several types of food, previous feeding history, and temperature. More work remains in each area. Frost's 1972 paper gives the basic result for copepod studies of the effect of food density, the so-called "functional response". Frost used an electronic counter, plunger-jar stirrers to keep the food evenly suspended, and uniformly shaped centric

diatoms as food. The experimental vessel was 3.5 liters, well above the threshold of any described volume effects (very small containers reduce feeding rates, a very early result). Each vessel contained 10 to 30 adult female *Calanus pacificus* which had not been starved prior to the experiment but were feeding steadily. The functional response (Fig. 7.4) has the following basic features.

1 Filtering rates are variable, but do not change significantly with food concentration at low concentrations.
2 When a sufficient food density is reached, filtering rates begin to decrease so as to keep the ingestion rate constant. Exactly this sort of functional response represents zooplankton grazing in practically all pelagic ecosystem models.
3 Copepods do not (at least in this data set) eat superfluously despite high food availability.
4 The maximum ration is obtained more rapidly on large cells than on small ones.

Calanus pacificus females are about 170 μg dry weight, which at 40% carbon includes 68 μgC. Thus the ingestion of 1.1 μgC copepod^{-1} h^{-1} (26.4 μgC day^{-1}) is 39% of the body carbon per day.

The maximum filtering rate exercised by the animals at low food density is about 8 mL copepod^{-1} h^{-1}, or 192 mL day^{-1}. This turns out to be less than it can do when previously starved. Runge (1980) has shown F values for this species up to 49 mL h^{-1} (over 1 L day^{-1}) feeding on large cells, applying prior starvation, and collecting in particular seasons. Debate about the adequacy of these rates to provide enough food at natural phytoplankton concentrations appears to be endless. Many copepods are found where less than 1 μg of particulate carbon is available in 200 mL; they should be on short rations. Older (e.g. Mullin & Brooks 1976; Derenbach *et al.* 1979) and newer (Cowles *et al.* 1998) observations of thin horizontal layers with phytoplankton concentrations two to five times above background suggest that maybe copepods solve the problem by sojourns in such layers. As this is written, the association has not been proved. The fact that hungry copepods can suddenly filter very fast when finally given a meal implies that they are equipped to take advantage of happening into such strata.

Perhaps a hundred worthy papers could be cited here to illustrate various refinements and extensions of these observations on copepod feeding. We will consider only a few of the important results.

1 The functional response curve is not very fixed. Ingestion measured quickly at a range of food concentrations will tend to become asymptotic at close to the field concentration of phytoplankton at the time copepods were collected (Mayzaud & Poulet 1978). More food will not stimulate more eating immediately. However, copepods collected from high food situations will have higher asymptotes than those from more dilute situations. Thus the functional response is flexible over some interval longer than the term of the usual feeding experiment. This is not true of all species, but it has been found for a number of smaller, coastal forms (Fig. 7.5; Donaghay 1980)
2 There probably are subtle feeding thresholds. At low food concentrations filtration rates are reduced (Frost 1975). Thresholds are potentially important because they give the phytoplankton a refuge from annihilation, although that is not likely to be the reason that grazers have thresholds. These thresholds are difficult to observe, because they only occur when N/V is small, making changes in N/V small and hard to measure accurately. When observable at all, thresholds are at very low food concentrations.
3 There are a variety of selection processes. Copepods can eat different sorts or sizes of particles at different rates. Many factors affect which available foods will be eaten fastest. Particle size is not the primary determinant of this. A nice experiment by Richman *et al.* (1977) demonstrated selection by *Acartia tonsa* from Chesapeake Bay feeding on natural particle assemblages. They found that any peak in the particle size spectrum could be consumed at significant rates, while particles just larger and just smaller were ignored completely (Fig. 7.6). It has been confirmed (Donaghay & Small 1979) with mixtures of foods and plastic beads that copepods can eat particles of two distinct sizes

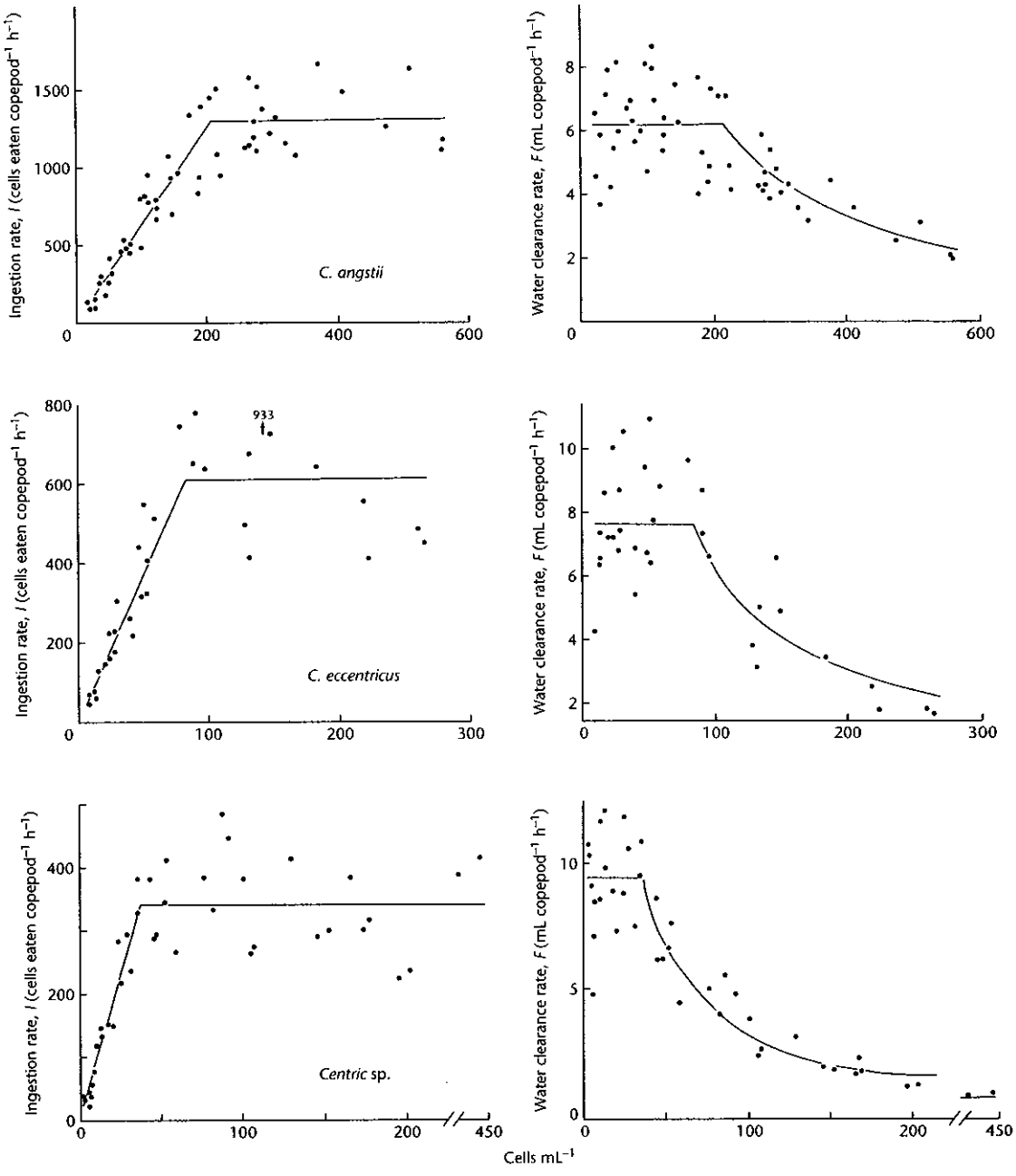


Fig. 7.4 Effect of cell concentration on ingestion rate (I, cells eaten copepod^{-1}h^{-1}, left) and water clearance rate (F, mL copepod^{-1}h^{-1}, right) in *Calanus pacificus* females feeding on three small-, medium-, and large-sized diatoms. (After Frost 1972.)

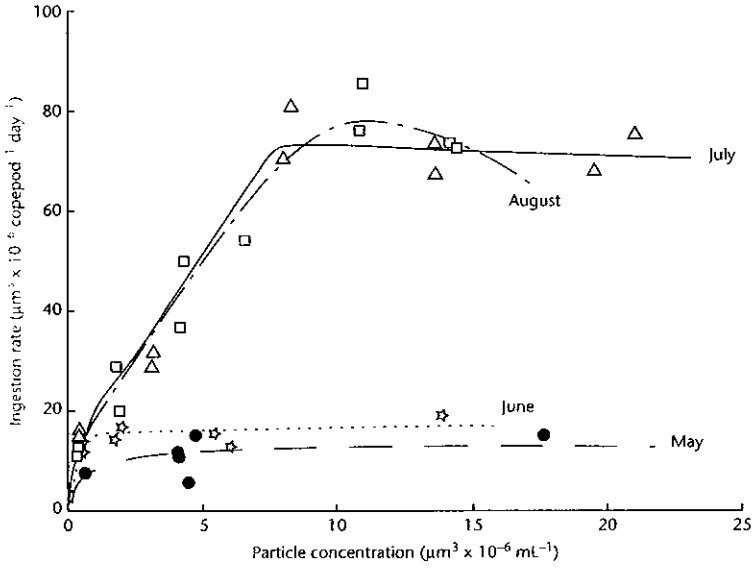


Fig. 7.5 Shift in the asymptote of the ingestion rate curve in *Acartia hudsonica* from Yaquina Bay, Oregon between May and June (lower curves) and July and August (upper curves). When food is more abundant in the field (summer in this estuary), more food is consumed than when it is sparser (spring). (After Donaghay 1980.)

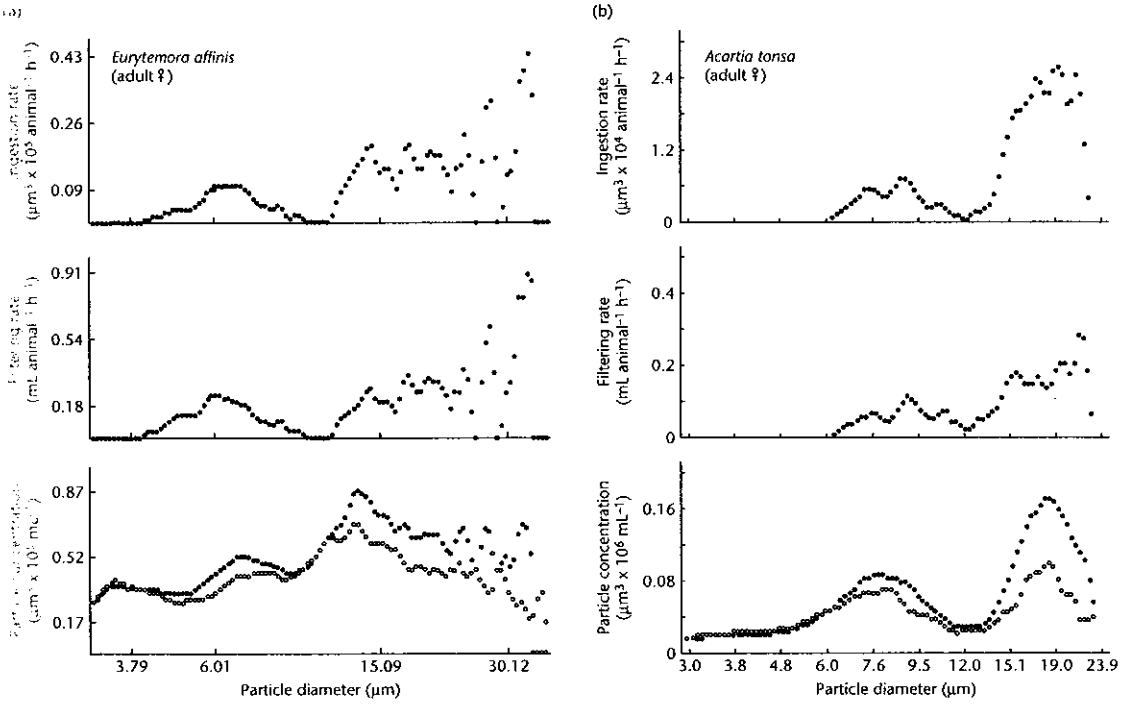


Fig. 7.6 Two examples of selection by particle-feeding copepods from Chesapeake Bay. (a) The particle spectra before (dark circles) and after (open circles) a day of feeding by female *Eurytemora affinis*. No particles of about 10 μm equivalent spherical diameter were removed and the filtering rate and ingestion rate spectra reflect that. (b) A similar demonstration for *Acartia tonsa* females, which avoided eating particles near 12 μm diameter. (After Richman *et al.* 1977.)

and manage not to eat particles of an exactly intermediate size. This cannot be done with a feeding mechanism that is essentially a flour sifter, unless particles can be identified and chucked back out after sifting. The feeding mechanism proposed by Koehl and Strickler allows selection, and particle rejections are obvious in the high-speed films.

4 Animal size affects feeding as you would expect: bigger individuals filter faster than smaller ones. Paffenhöfer (1971, 1984) has provided some good comparisons among developmental stages of *Calanus pacificus* (Fig. 7.7) and *Paracalanus parvus*.

5 The importance of olfactory cues from the phytoplankton is suggested by a variety of experiments, although none of them is very critical. Poulet (e.g. Poulet & Oullet 1983: "Copepods are French, they prefer foods that taste good.") has shown that Sephadex® beads with attached plant flavor are ingested more readily by copepods than those without it. Buskey (1984) has done a peculiar test of the role of odor. He compared the speed of individual swimming bouts in filtered seawater with those of feeding animals. They slow down while feeding. Adding phytoplankton "odor" to the water made no difference in the swimming speed, but adding plastic spheres slowed them and a combination of plastic spheres and food odor slowed them as much as phytoplankton food.

6 In the 1990s experimenters pursued a suggestion of Rothschild and Osborn (1988) that small scale turbulence in the sea should enhance encounter rates between grazers and prey, enhancing ingestion rates. The basic idea is that a grazer and the array of prey around it will have their relative velocities increased by small-scale shear, thus passing more prey within the detection radius of the grazer. Peters and Marrasé (2000) reviewed the entire literature and concluded that results are inconclusive. However, in at least some cases there is a dome-shaped relation with some increase of ingestion rate at intermediate turbulence levels. For example, Caparroy *et al.* (1998) found a level of turbulent energy dissipation, $\varepsilon \sim 0.3\,\mathrm{cm^2\ s^{-3}}$, that enhanced feeding capability compared to calmer conditions in experiments with *Centropages typicus*. That level of turbulence enhanced encounters (or capture efficiency, it is impossible to say which) such that ingestion was readily saturated, and clearance rate fell off rapidly with increasing prey density. Higher turbulence seemed to interfere with prey capture. Intense or recurring shear perhaps eroded the feeding current or disrupted transfer of prey location information. It has proved very difficult to duplicate convincingly in laboratory containers the dissipation of ocean turbulence at the size scales of plankton animals. For example, in the Caparroy experiments, a grid is moved up and down through the feeding chamber. Mixing is high just as the grid passes any point, then decays. Different mean energy dissipation (estimated ε) is achieved by different speeds (and frequency) of grid pulsing. Thus the effects can be due to the frequency of extreme disturbance rather than the general level of turbulence. Nevertheless, turbulence in some sense is shown to matter.

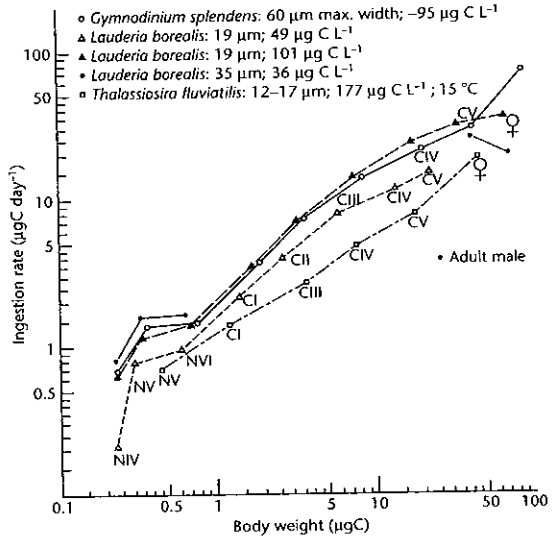


Fig. 7.7 Ingestion rate of *Calanus pacificus* as a function of body weight. Filtering rates increase similarly. Naupliar and copepodite stages, indicated by Roman numerals, varied somewhat in weights attained and ingestion rates among four species of food, represented by the separate lines. Ingestion increases roughly as weight to the 0.87 power. (After Paffenhöfer 1971.)

It can be argued that most turbulence occurs at length scales (basically eddy diameters) large relative to body sizes of mesozooplankton. This argument comes from estimates of the Kolmogorov length scale, the length at which viscosity effectively damps transfer of momentum into progressively smaller eddies. However, a plankter of whatever length within an eddy will be turned over by it, and many of them, particularly copepods, have preferred orientations but small righting moment (vertical stability). Thus, turbulence will require recurring righting moves regardless of scale. There is more to be learned about the interaction of turbulence with planktonic activities.

Other mesozooplankton groups

Filtering rate determinations for euphausiids, also important in the planktonic economy, are fewer. One by McClatchie (1985) shows a different approach to the measurement. He used a spherical 250 L flow-through chamber with a magnetic stirring bar at the bottom. Diatom culture was added to the inflow in pulses, mixed through the chamber quickly, then diluted away at a rate measured from chlorophyll fluorescence. The increase in apparent rate of dilution with 25 *Thysanoessa raschii* present was the basis for determining their ingestion rate, and thus water clearance rate, F. The euphausiid ingestion rate closely tracked the diatom concentration through 4-fold pulses (Fig. 7.8a), which implied a constant $F = 15.4$ mL krill^{-1} h^{-1}. The functional response curve (Fig. 7.8b), then, increases linearly over the range of food concentration tested. While there must be an upper limit to the ingestion rate for this, or any, animal, it wasn't reached at the very high food levels represented by 7 µg chlorophyll L^{-1}. Results from clearance rate measures for other euphausiids vary with animal size. McClatchie's *T. raschii* were 17 mg dry weight. The very large antarctic krill, *Euphausia superba* at approximately 250 mg dry weight, filters diatom suspension at 25 to 300 mL krill^{-1} h^{-1} (Antezana *et al.* 1982). The lower rates occur at less than 3 µg chlorophyll L^{-1}. At 4 to 12 µgL^{-1} they average 210 mL krill^{-1} h^{-1}. This appears to be a threshold effect, although some feeding occurs even at 0.6 µg L^{-1}.

Some clearance rate estimates and functional response curves are available for other particle-feeding plankton. Values determined for appendicularian grazing allow illustration here of a different approach to grazing rates (see Box 7.4). Bochdansky *et al.* (1998) give clearance rates (Fig. 7.9) for a large appendicularian, *Oikopleura vanhoeffeni*, from Newfoundland waters, derived

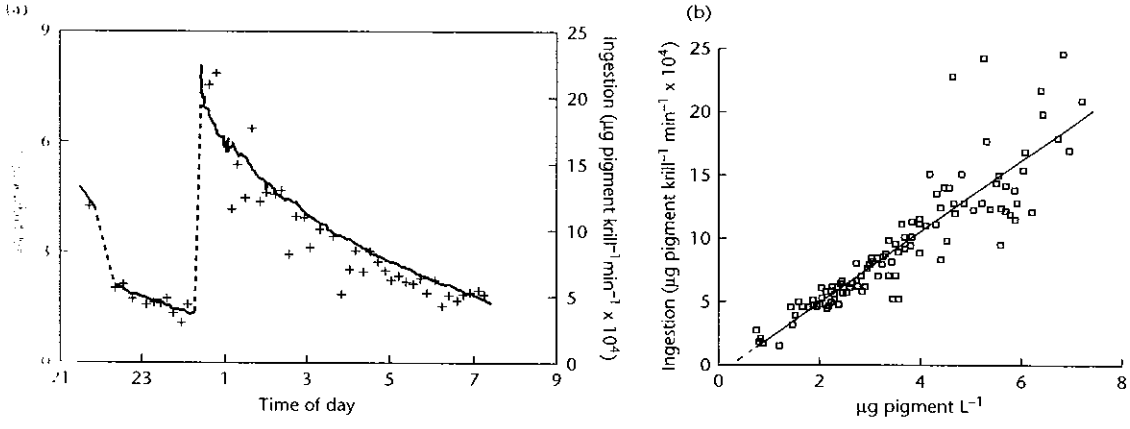
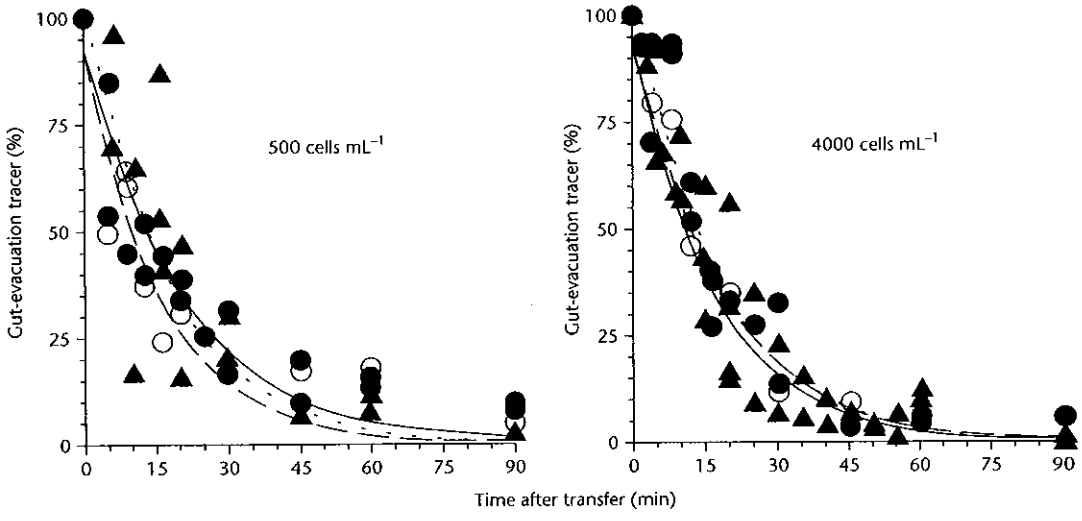


Fig. 7.8 (a) Time course of pigment in a feeding chamber containing 25 specimens of *Thysanoessa raschii*, an euphausiid. A pulse of phytoplanton culture (5.4-fold increase) was provided, then traced as it was both washed out of the flow-through chamber and eaten by the animals. Ingestion rate is calculated from the implied filtration rate and concentration. (b) Ingestion rate varies directly with food concentration over the range provided. (After McClatchie 1985.)

Box 7.4 Clearance rates from pigments in gut contents

Mackas and Bohrer (1976) developed an ingestion rate measurement technique that has mostly been evaluated for use with copepods. An animal eating plants containing chlorophyll will have some in its gut. If that food is suddenly taken away, the gut chlorophyll, or (chlorophyll + breakdown pigments) will decline at a rate equal to the sum of internal pigment degradation and pigment defecation rates. Typically this decline with time is a steep exponential curve, but the pattern depends upon the type of animal, and whether individual gut clearance or reduction of total gut content of a group is examined. In an animal feeding steadily, the input rate might equal this output rate, sustaining its gut pigment content close to constant. Gut chlorophyll or total fluorescent pigments are readily measured by fluorometry. A captured animal is immediately(!) ground in acetone, extracting the pigments, which have a calibratable fluorescence signal. Standard laboratory instruments give strong signals from the amounts of pigments in one zooplankter, and the fluorescence blanks of animals with empty guts are very small. Considerable averaging among animals should be done, since at any moment a fraction will not be feeding. Sets of animals are also immediately sorted live into filtered seawater, then sacrificed every few minutes for an hour or more to determine the rate (say, min^{-1} or h^{-1}) of decrease of contained pigment (see figure below). Emphasis on sets of animals comes from the fairly wild individual variability (Mobley 1987). The time course of decrease is generally well fitted by one model curve or another. Then it is assumed that pigment must be removed from the habitat at the same rate to maintain the initial value, and the clearance rate is calculated as the volume of water at the ambient chlorophyll concentration that has to be cleared per time to replace the internal pool at this rate.

A great deal of discussion has been occasioned by the fact that some, or often most, of the pigment is actually digested to non-fluorescing breakdown products, with components perhaps assimilated. Supposedly this makes the estimates biased. It would matter if the pigment input were evaluated by fecal pigment output. But that is not the measurement. Both egestion and destruction are included in the rate based on time series of whole body measures. Pigment destruction would make the measurement useless if it were instantaneous, that is it would make the gut content zero with no measurable rate of decrease when input was stopped. It only works if there is a substantial quantity present internally to allow measurement of its decline. The temptation is to multiply the rate by the



Box Fig. 7.4.1 Time course of decline in gut pigment content (triangles) in *Calanus marshallae* originally feeding on two different cell concentrations. Circles are decline in gut content of germanium-68 labeled food in filtered seawater (open circles) and in unlabeled food suspension (filled circles). (After Ellis & Small 1989.)

ratio of chlorophyll (or pigment) ingested to that defecated, thus supposedly accounting for the chlorophyll "lost" internally, which it appears necessary to replace along with that defecated. Following this temptation can also be correct, as in the appendicularian case considered in the main text.

There are assumptions to test. Food is possibly retained longer in filtered water, than while food is available. If so, the reliability of the pigment loss rate depends upon how fast the animal recognizes that food has been withdrawn. One test (Ellis & Small 1989) showed that the change to longer retention is slow enough not to matter. Rates of decline were checked by feeding diatoms labeled with radioactive germanium, then switching to unlabeled food just before the loss rate determination. This allowed a comparison based on defecation of germanium-labeled frustules. Pigment loss rates were greater than label loss rates, which is not a problem. Rates of label evacuation were the same for fed animals as for those switched to filtered seawater, which verifies the assumption that withdrawal of food doesn't change behavior instantly. This result is not consistent; Penry and Frost (1991) found the shift to filtered water accelerated defecation, and others have seen that also. Clearly, caution is required.

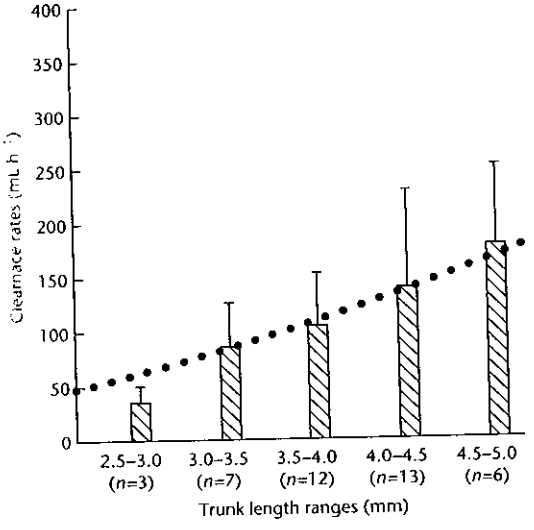


Fig. 7.9 Water clearance rates of *Oikopleura vanhoeffeni* determined from estimates of gut pigment content and approximate pigment destruction rates (bars) compared to a measure based on uptake by animals of ^{14}C-labeled diatoms (dots). Bigger individuals filter faster. (From Bochdansky *et al.* 1998.)

from a modified pigment replacement method. In this animal the defecation rate is essentially constant, one pellet about every 13 minutes regardless of either small temperature changes or food level. It was observed that the digestive tract consistently contained three fecal pellet volumes of food, and that the pigment in the gut was only 21% of that required to replace it (shown by ratio comparisons to an unassimilated tracer, ^{68}Ge, in shells of diatoms used as experimental food). Thus, the turnover of gut content occurs every 39 minutes and replacing the pigment requires 4.76 (= 1.0/0.21) times that content for each turnover. The clearance rate in field-captured *O. vanhoeffeni* (mL h^{-1}) was determined as the volume of water containing that scaled-up content each 0.65 h (39 min). Results (Fig. 7.9) were close to those obtained by Knoechel and Steel-Flynn (1989) by enclosing animals in the field and feeding them ^{14}C-labeled food to determine their uptake rate. Rates depend upon body size, measured as trunk length.

These are higher rates than those of copepods, and the maximum rates for this appendicularian are even higher. Bochdansky and Deibel (1999) show successive estimates of cell abundance in 500 mL bottles, each with one feeding *O. vanhoeffeni* (Fig. 7.10). Because of the logarithmic scale, the slopes of the lines are proportional to the filtering rates. Food levels were allowed to drop, the time course of which was well fitted by an exponential clearance rate relation (straight line on the semi-log plot), then replenished. At low food levels filtering is faster (order 250 mL h^{-1} for one animal, up to 500 for two others) than at medium or high levels (60–70 mL h^{-1}). The time series for one animal feeding at low food concentrations showed progressive slowing as the filters of its house clogged and deteriorated. Upon

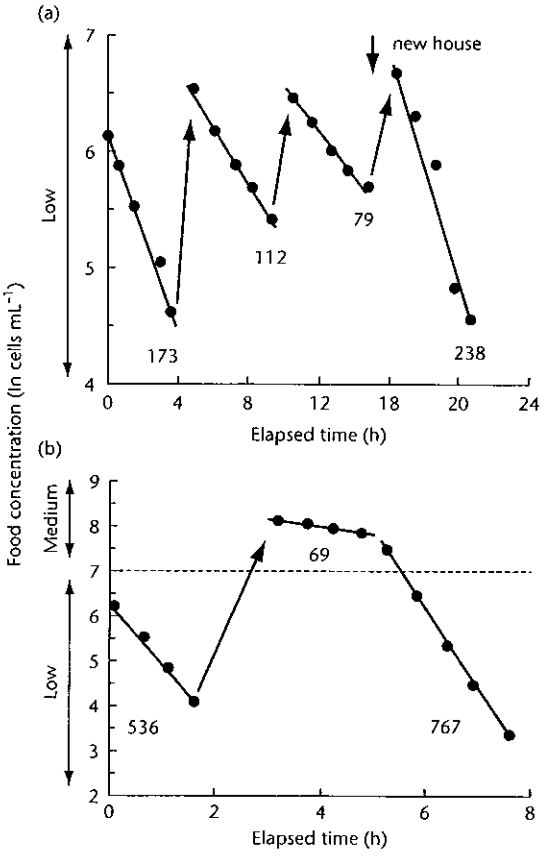


Fig. 7.10 Time course of cell abundance in containers with feeding *Oikopleura vanhoeffeni*. Clearance rates in mL h^{-1} are given beneath each down slope. Arrows represent additions of concentrated food particles. (a) Rates decline as feeding progresses; houses eventually become clogged and are changed, restoring higher rates. (b) Rates are high at low food concentrations (< 1100 cells mL^{-1}), low at higher concentrations; ingestion becomes saturated. (From Bochdansky & Deibel 1999.)

replacing its house, a matter of a few minutes, it returned to high rates. Apparently a house can deteriorate substantially before replacing it is worth the cost. The results overall show that appendicularians have a saturable functional response. In this case it saturated at high levels of offered diatom concentration, greater than 10^7 cells mL^{-1}. Actual rates in these experiments varied greatly between individuals, more than was explainable by size as in Fig. 7.9.

Protozoan feeding rates

Since about 1985, surprisingly late, we have been aware of the importance of heterotrophic protozoans in marine habitats, particularly the pelagic. They have now been shown to consume a majority of pelagic primary production, so they provide a large pathway in the marine food web. The delay was occasioned by (i) the intense interest generated by larger invertebrate grazers, and (ii) the extreme fragility and near perfect transparency of high seas protists. The most abundant planktonic microheterotrophs are small flagellates. The swimming behavior and feeding mechanisms of these animals are dominated by the properties of viscous flow and by boundary layer processes. Flagellates move toward their food, attracted and directed by gradients in scent. They grapple with it by several mechanisms. Some are capable of phagocytosis at specific sites on the cell surface. Some, like the colorless cryptophyte *Katablepharis* (Lee *et al.* 1991) have modestly elaborate "mouths" which can manipulate and engulf food using microtubular organelles that extend into the cell as a digestive tract. Choanoflagellates have feeding collars coated with sticky material to which nanoparticulate food adheres, followed by eventual ingestion at a localized site. Ciliates move to particulate food items, ingesting them with mouth-like organelles specialized for phagocytosis. Various mechanisms are reviewed in Hausmann *et al.* (1996).

Two techniques have been applied to determine the feeding rates of marine protists, often referred to as "microheterotrophs". The **FLB** technique ("fluorescently labeled bacteria"; Sherr *et al.* 1987) involves gently mixing a tracer meal of bacteria from a culture, usually *Escherichia coli*, into a sample of seawater. The tracer cells are stained with a fluorescent dye. Preparation for staining kills the tracer bacteria, but a wide range of protists eats them anyway. After incubating a few tens of minutes, the sample is passed through a black membrane filter, which is then mounted on a microscope slide and examined by epifluoresence microscopy. Eaten FLB are visible, glowing with fluorescence, in food vacuoles inside the protists. The rate of water clearance, F, by a cell is given by

F = [count of FLB in a cell]/
([FLB concentration] × [incubation duration]).

Given suitable averaging and a density estimate from the same slide for protists, an overall rate (volume cleared time^{-1}) of microheterotrophy can be obtained. There are biases, of course, because normal marine bacteria will be encountered at different rates and selected for, or against, differently from FLB. But the measurement is a basis for comparisons among sites, depths or regions.

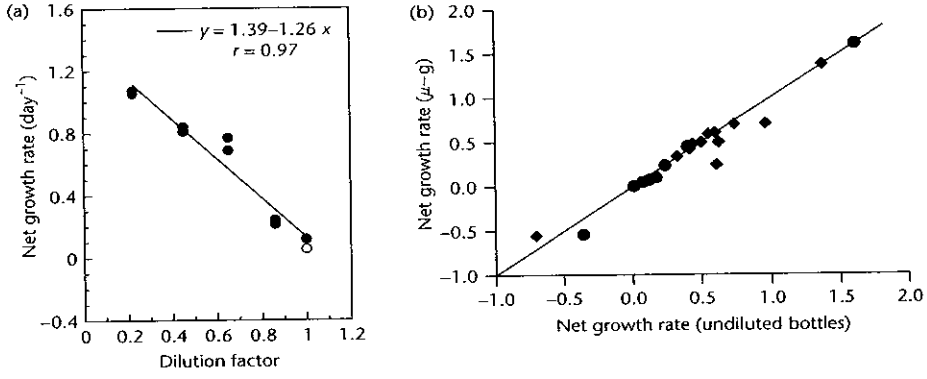
The **dilution series technique** (Landry & Hassett 1982; Box 7.5) has been more widely applied, perhaps because some versions do not require extensive microscopy.

Box 7.5 Microzooplankton grazing rates by dilution series (Landry & Hassett 1982)

Dilution series produce bulk estimates of the rate of microheterotroph ingestion, not feeding rates of individual protists. Collect seawater from a station and depth of interest using a clean and gentle sampling bottle. Filter a suitable quantity (F) with membrane filters (0.45 or 0.2 μm pores) to remove all microheterotrophs. Remove mesozooplankton from another portion (–M) of original sample. Establish a series of incubations of –M diluted in different proportions with F, e.g. 1.0, 0.75, 0.5, 0.35, 0.2 and 0.1 of –M. Determine the per capita rates of increase during an incubation period, say 24 hours, for phytoplankton (usually, but heterotrophic bacteria can also be evaluated) by increase of cell counts, chlorophyll content, or ^{14}C-uptake. The concept is that dilution will not change the per capita growth rates, but it will decrease per capita mortality rates from grazing.

These rates are then regressed (see figure below) against fraction –M; the expectation is a negative slope, with higher net (growth – grazing) rates at greater dilutions. The regression intercept is the "true" phytoplankton growth rate (μ, day^{-1}); the slope is the bulk fractional grazing rate (g, day^{-1}). Problem: derive that relationship.

As the figure from Strom *et al.* shows, this often works. There are, however, frequent cases of excess scatter or positive slope (apparent "negative grazing"). The usual practice is to discard such results as bad runs, even if no reason for the problem is evident. Most such outcomes are never seen in public. Debate continues about the reliability of the apparently good runs.



Box Fig. 7.5.1 (a) A dilution series regression for an experiment from the eastern tropical Pacific. Net growth was determined from chlorophyll concentrations before and after 24 h in shipboard incubators. This is an extreme, so very clear, example, in which net growth in undiluted seawater (dilution factor = 1.0) was nearly zero, and net growth at dilution factor = 0.0 was very large, 1.4 day^{-1}. (After Landry *et al.* 2000.) (b) Comparison for dilution runs in coastal waters (Puget Sound and northern Gulf of Alaska) of net growth in undiluted incubations with the estimated difference between dilution experiment intercepts (μ) and slopes (g). The line is the 1:1 line, showing excellent agreement. (After Strom *et al.* 2001.)

Strom *et al.* (2001) used dilution experiments to show that very large fractions of phytoplankton production are consumed by protists, even in coastal waters and even for relatively large phytoplankton including diatoms. Over a large series of experiments, the ratio $g:\mu$ was 80% for cells smaller than 8 μm and 42% for cells larger than 8 μm, with an overall average of 64%. They found that large ciliates and heterotrophic dinoflagellates tend to increase as stocks of large phytoplankton increase, consuming larger relative fractions of production at high phytoplankton standing stocks. This is possible in part because many protists, particularly heterotrophic dinoflagellates, can digest phytoplankton externally, inserting a feeding tube into prey cells or surrounding them with a pallial web.

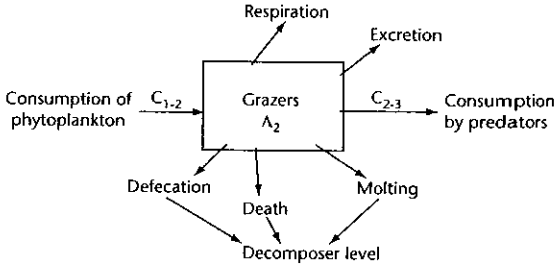
Another aspect of microheterotrophy is that protists are capable of growth rates similar to those of phytoplankton, thus the overall rate of grazing can often keep pace with phytoplankton stocks. Recent estimates for the ciliates *Strombilidium* and *Strombidium* (Montagnes 1996) were mostly in the range $0.6-1.0\ \text{day}^{-1}$ (recall the comparison that one doubling each day equals an exponential rate of $0.69\ \text{day}^{-1}$), about the same as typical phytoplankton growth rates, and reached $2.2\ \text{day}^{-1}$ with saturating food at the highest habitat temperatures. Strom and Morello (1998) found coastal ciliates were growing at 0.77 to $1.01\ \text{day}^{-1}$. Heterotrophic dinoflagellates increased more slowly, at 0.41 to $0.48\ \text{day}^{-1}$, which is still fast enough to make them significant contributors to grazing. These are maximum rates from laboratory batch cultures, not net rates after predation.

Biological oceanographers are still assimilating the importance of protistan grazing. It is clear that well over half of the metabolic processing of pelagic primary production occurs in the microbial food web. That is true both in oligotrophic regimes far out to sea and in richer coastal zones. Mesozooplankton like appendicularians, copepods, pteropods, and euphausiids take a large fraction of their nutrition from the microbial food web as giant carnivores, and planktivorous fish have stepped out a full trophic level compared to the place they were believed to have 30 years ago. Recall the experiment by Calbet and Landry, described in Chapter 5, showing a trophic cascade of three or perhaps four steps among organisms less that 20 μm in cell diameter. None of this is yet fully accounted for in pelagic ecosystem models.

Evaluation of secondary production

Next, zooplankton must be fitted into the scheme of production analysis we started on with phytoplankton. Zooplankton are "secondary" producers, so we want to determine the rates of secondary production, the amount of new zooplankton tissue elaborated each day or year. However, mesozooplankton are not finely dispersed particles like phytoplankton, and they do not take up a well-defined and easily labeled substrate (CO_2) such that they can simply be filtered and their productivity equated to substrate incorporated. The problem is very hard, nearly uncrackable, and we have no complete estimates. However, as we work on the problem, we come up with insights about zooplankton themselves. If we knew the rates of secondary production in pelagic ecosystems, we could estimate the ecological efficiency of the lower trophic levels and begin to develop an expectation for amounts of higher level production (fish, squid, shrimp, ...) that can be harvested.

The place of herbivores in the trophic-dynamic scheme can be diagramed as follows:



Secondary production is the rate of change in the biomass of herbivores ($\Delta A_2/\Delta t$), plus the portion of increase of biomass that is balanced by predation, $C_{2\rightarrow 3}$. This instantaneous rate is appropriate for a system in steady state, or it is a suitable