Chapter 9. Methods for the Study of Feeding, Grazing and Assimilation by Zooplankton

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1 Introduction

In terms of energy or mass flow, ingestion represents the greatest of all interactions between an animal and its environment (Spomer 1973). Consequently, many aquatic ecologists have attempted to describe and measure the feeding process of zooplankton. They have evolved a formidable number of methods.

Any investigation must be tailored to the hypotheses to be tested; it is, therefore, important to consider briefly the major questions to which zooplankton ingestion and assimilation are relevant. At a practical level, there are two: we wish to know the future amount and composition of the algal community and we wish to predict fish stocks. The former is determined, in part, by the pressure of zooplanktonic grazing on the algae and the latter, in part, by the amount of zooplankton production available to the fish. Of course, accurate descriptions of the feeding process of zooplankton alone will not permit predictions about fish stocks or algal composition: such descriptions can only be used as components in larger models of the aquatic ecosystem which will yield such predictions. At a more academic level, zooplankton lend themselves to the testing of ecological theory because of the animals' small size, ease of culture, aquatic habitat and the seemingly reduced heterogeneity of their environment. Determinations of ingestion and grazing rates have been used in studies dealing with energy flow (Slobodkin and Richman 1956; Richman 1958), mineral cycling (Johannes 1968; Peters & Rigler 1973), competition (Dodson 1970; Hall *et al.* 1976; Lynch 1977), optimal foraging (Lam & Frost 1976; Lehman 1976), and predator-prey interactions (Holling 1959; Wilson 1973) among others. Beyond these general theories lie an unlimited number of specific hypotheses regarding the fine structure of aquatic communities and the behavior of their components. This review primarily presents the basic methods which have been used to measure feeding, grazing and assimilation rates. I hope these methods will not be adopted uncritically by other workers but will be adapted to suit their own needs and their questions.

2 Basic Concepts

2.1 Definition of terms

The same term or concept may apply to values which necessarily differ because they were determined in different ways and under different assumptions. For example, the term 'grazing rate' is applied both to values determined using Gauld's (1951) formula (which implies an exponential decline in food concentration) and to values from short-term radiotracer experiments (which assume a linear reduction in food concentration). These two estimates can differ considerably (Rigler 1971a) despite their conceptual identity. Because the three terms in the title (feeding, grazing, and assimilation) are used widely and variously in the literature, one must clarify their usage each time they are presented by giving their method of calculation. Consequently, only idealized definitions are given in this section. The appropriate method of calculation for each technique will be provided later.

2.2 Feeding and grazing

Feeding rate (f), or ingestion rate, is a measure of mass or energy flow into the animal. It is usually expressed in cells ingested individual^{-1} time^{-1}. However, differences in cell size and animal weight render such a formulation difficult to interpret for most quantitative purposes. A preferable, though far less common formulation, would express ingestion rate in terms of ingested biomass, which can be presented as cell volume, dry weight, elemental composition, energy content, etc. No standard expression has been accepted in the literature, but perhaps dry weight or volume might be suggested as a compromise, for these are easily determined and can approximate other units through relatively simple conversions.

Grazing rate (G) is the volume of food suspension from which a zooplankter would have to remove all cells in a unit of time to provide its measured ingestion. Synonyms in the literature include searching rate, filtering rate, filtration rate, clearance rate, and volume of water cleared per unit time. Each of these terms involves a mechanistic image of the food collection process which may be false. Suspension-feeding organisms may not actively search and zooplankton do not always (and may never) force the suspension through a sieve-like device and so may never actually filter-feed. Similarly animals do not remove cells from suspension with an efficiency of 100 % and so it is misleading to speak of clearance. For these reasons the more neutral term, grazing rate, has been preferred in this account; however, even this may imply a false homology with terrestrial grass-eaters. It is unlikely that such a suggestion for terminological change will find widespread acceptance

because all terms are entrenched in the literature. Consequently, we should be careful not to over interpret the intentions behind such synonyms when they are encountered. Perhaps it is easier to change our interpretations of these phrases than to change the phrases themselves.

Feeding rate, which is equal to the product of grazing rate and food concentration, is an approximation of the sustenance which an animal draws from its environment. It is most appropriate in considering problems of zooplankton production for fish stocks, while grazing rate is more appropriate in considering the effect of zooplankton on algae, for it is equivalent to the mortality rate of algae per zooplankter. Both feeding and grazing rates vary with the environmental conditions of the animals.

2.3 The feeding process

Before discussing assimilation or techniques for the measurement of feeding and grazing, I will review the current descriptions of feeding and grazing by zooplankton, in part to review terms and in part to outline our current conception of the process. It is this imagery which often determines our approach to the subject. For example, it was once thought that grazing was an automatic behavior and that each species had a typical clearance rate independent of external conditions. If this were true, grazing rate could be determined in very artificial laboratory conditions and the results could then be applied readily to nature. We now know the process to be far more sensitive and our prerequisites for laboratory measurement and field application of grazing rates are far more demanding.

Our conception of the mechanism of suspension feeding is currently undergoing a revolution. 'Pre-revolutionary' conceptions are still widely held and continue to influence experimental design. Consequently this account begins with a traditional picture of filter-feeding and then provides a brief sketch of a contemporary alternative.

The processes involved in food collection by zooplankton were studied in detail by many authors (Naumann 1921; Cannon 1928, 1933; Storch 1928; Eriksson 1934; Lowndes 1935; Fryer 1957a; Gauld 1959, 1966). Their accounts sometimes differ in detail, but a traditional picture of feeding by the major groups of zooplankton may be had without doing great violence to any particular researcher.

The most studied suspension-feeding zooplankton are calanoid copepods and cladocerans. Traditionally, both animals were said to force a current of water through a fine mesh of setae and setules which sieve food particles from the water. These particles should then pass to the mouth where they are swallowed, with or without mastication. The other major group of freshwater, planktonic crustaceans, the cyclopoid copepods were thought not to depend

on filtering currents but were considered raptorial feeders. Protozoans and rotifers were similarly not thought to filter-feed because neither group is obviously equipped with sieve-like appendages, although rotifers certainly create feeding currents and Fenchel (1980a) believes that ciliates use their cilia as screens. Most research effort has concentrated on cladocerans and calanoids which will be given disproportionate coverage here. This account ignores suspension feeding by the zoobenthos, which has been less studied. Cummins & Klug (1979) and Wallace & Merritt (1980) provide points of entry to this literature.

Among calanoids, the feeding current is traditionally thought to consist of secondary eddies created by the locomotory activity of the second antennae and by movement of the mouth parts. This current would draw water and suspended food forward into a filtering chamber bounded dorsally by the body, laterally by the filtering setae of the maxillae and ventrally by the forward-directed abdominal appendages. Water flows through the setae of the maxillae which strain out suspended matter; this then collects between the appendages (Fig. 9.1). Actions of the mouth parts then propel the food forward to the mandibles which push the food between the labrum and the body to the mouth (Lowndes 1935; Gauld 1966). Conover (1966a) found that marine calanoids, and presumably their freshwater relatives, are capable of two other modes of food collection when confronted by larger particles. The maxillipeds may be extended below the filter basket to seine for food or they may be extended, praying mantis-like, to grasp larger prey. The mandibles serve to masticate large prey before swallowing. Rosenberg (1980) found that the marine calanoid, *Acartia*, will use its thoracic legs to supplement the seining action of the mouth parts.

Cladocerans are classically thought to create a filtering current by the active, rhythmic pumping of their thoracic legs. This action would draw water and suspended food into the anterior part of the filter chamber formed dorsally by the ventral body wall, posteriorly by the post-abdomen, and laterally and ventrally by the moving filtering appendages. Water in the filtering chamber would then pass through the setae of these appendages, towards the valves of the carapace and then away from the animal, leaving the food to collect in the food groove between the filtering appendages along the ventral body wall whence it passes, propelled by water currents, to the mandibles and the mouth (Naumann 1921; Cannon 1933). The post-abdomen is used to clean the food groove and the filtering appendages of excess or undesirable material (Cannon 1933; McMahon & Rigler 1963; Burns 1968a).

For both groups, the significant aspects of the process are the rate of movement of the appendages creating the current, the volume of flow, the efficiency of retention of the filtering setae, the efficiency of transfer of the collected food to the mouth, and the amount of nutrient extracted in the gut.

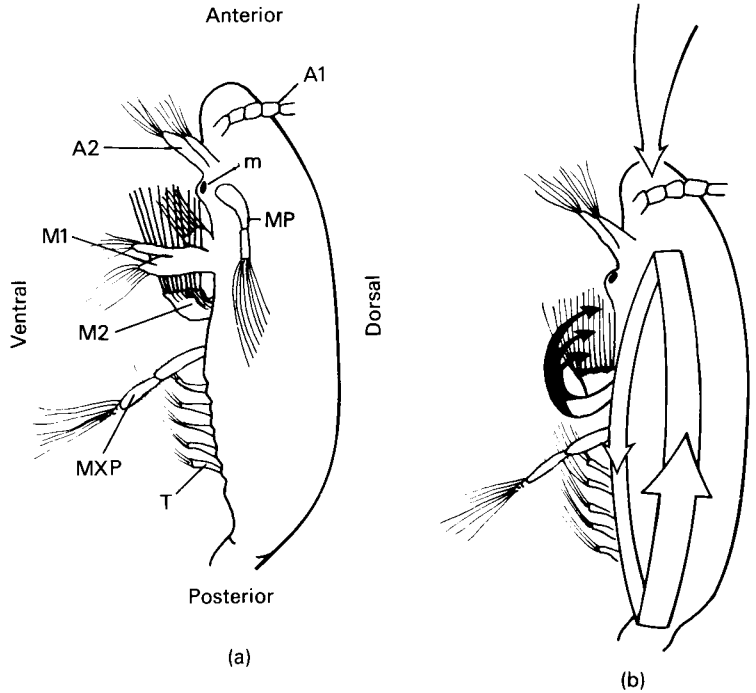

Fig. 9.1 The feeding appendages (a) and the traditional conception of filter-feeding (b). Only the left appendage of each pair is shown in schematic form. Movement of the appendages draws water from in front of the animal into a large posterio-lateral whorl. A portion of this swirling water is sucked anterio-medially by the outward swing of the maxillipeds. The inward swing then forces water through the fine meshes of the maxillipeds' setae which strains food from the water. Arrows with a large head and narrow shaft indicate movement out of the plane of the page and towards the reader. A thick shaft and narrow head indicate medial movement away from the reader. A1: first antenna; A2: second antenna; m: mouth; MP: mandibular palp; M1: first maxilla; M2: second maxilla; MXP: maxilliped; T: Thoracic or swimming legs (modified from Koehl & Strickler 1981).

The image of suspension feeding as a seiving of particles larger than the filter's mesh from a stream of flowing water may not be accurate. Rubenstein and Koehl (1977) point out that such filters may collect particles by direct interception, inertial impaction, gravitational deposition, motile particle deposition, or by electrostatic impaction. Gerritsen and Porter (1982) found that charge associated with the food particle may affect filtering rate.

Recently, Strickler has suggested that the conception of zooplankton as sieves or filters is grounded in misconception. He believes that, given the viscosity of water and the rapid rates of movement of the appendages, it is

impossible that water should flow through the tiny apertures between the setae. Instead the appendages must act like paddles pushing food particles out of the feeding current to the mouth (Fig. 9.2) and the concept of filtration by suspension-feeders must be abandoned. Instead, all food collection is essentially a raptorial selection of individual particles. An increasing amount of information appears to support this interpretation of 'filter'-feeding (Alcaraz *et al.* 1980; Friedman 1980; Zaret 1980; Koehl & Strickler 1981; Gerritsen & Porter 1982; Paffenhöfer *et al.* 1982). These arguments may eventually have far reaching effects on experimental studies of suspension-feeders: for example, studies of the intersetule distance (i.e. filter mesh size) are rendered largely irrelevant under this conception of suspension feeding (Porter *et al.* in press).

It is less easy for this writer to imagine how experimental determinations of feeding or grazing rates themselves would distinguish between these hypotheses. The value of any alternative scientific hypothesis rests in the critical experiments they suggest to distinguish between competing theories. For example, B.Marcotte (personal communication) pointed out that Strickler's conception allows a greater role for prey selection by grazers. This

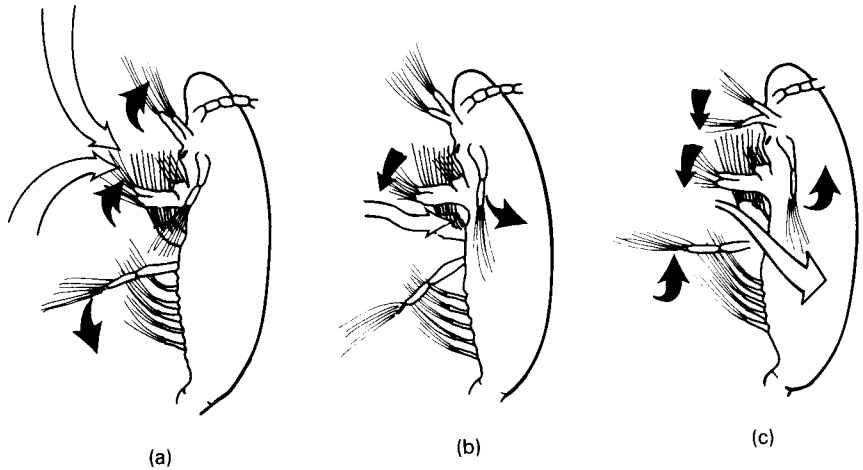

Fig. 9.2 A more contemporary view of copepod feeding currents and appendage movements showing how outward movement of the second antennae and maxillipeds sucks water towards the maxillae (a), postero-medial movement of the first maxillae and dorso-lateral movement of mandibular palps sucks water laterally (b), inward movements of second antennae and maxillipods coupled with dorso-lateral movement of mandibular palps shoves water postero-laterally. The second maxillae collect (and reject) food particles carried in the feeding current (c). Appendage movements are indicated by closed arrows and water currents as open arrows. Movement out of the plane of the page is indicated by varying widths of the arrows as noted in Fig. 9.1 (modified from Koehl & Strickler 1981).

may provide a basis for critical tests. In any case, our earlier conceptions and misconceptions about suspension feeding have been extremely fruitful. Their simplicity has allowed development of theories and methods for feeding behavior which are second to none.

2.4 Assimilation

Assimilation rate is usually defined as the amount of material or energy which passes from the lumen of the gut into the body of the animal, per unit time. It must be expressed in terms of a particular element (P, C, N, etc.) or compound (glucose, vitamin B_{12}, etc.) or energy (Joules), for different materials are assimilated to different degrees. Assimilation (A) is often expressed as assimilation efficiency (A.E.), the percentage of the ingested food (I) which is assimilated.

$$\text{A.E.} = (\text{A}/\text{I}) \times 100 \qquad (9.1)$$

where A and I are in the same units. In the physiological literature, assimilation often implies the difference between ingestion and the sum of excretion and defecation. Johannes & Satomi (1967) suggest that 'retention' be used for this concept.

This section has described current, widely held conceptions of food collection by zooplankton. Ideally, the quantities measured by our methods would correspond closely to these concepts. However, since alternative techniques with the same conceptual goal yield different values, it seems that this ideal is not always achieved. This lack of correspondence between measured value and concept argues that the researcher take as many approaches as possible. Although this is a truism for good research, it provides a strong rationale for a short examination of the advantages and disadvantages of at least the most popular methods used to measure the feeding behavior of zooplankton.

3 Techniques for Measurement of Feeding and Grazing Rates

3.1 Morphology and microscopy

Although careful examination of the feeding appendages has yielded a great deal of information, this approach has been less extensively employed in recent years. Most basic descriptions were completed in the nineteenth century by Daday, Leydig, Sars and others, even if questions of detail remain. These early authors did not usually deal with function; this was investigated most intensively in the early part of this century (e.g. Naumann 1921; Cannon 1928; Lowndes 1935). The ecological significance of the morphology of the feeding apparatus is still an open question.

In general, plumose setation and flat, grinding mandibles are associated with herbivorous species whereas carnivores are armed with spiny, widely spaced setae and carinate mandibles (Naumann 1921; Anraku & Omori 1963). The mandibles of herbivorous copepods also carry long sharp projections which may imply a cracking rather than grinding function. (Sullivan *et al.* 1975). There is, however, no sharp division in diet between these types and opportunistic feeding is to be expected (Marshall 1924, 1973; Fryer 1975b). The inter-setular distance has been measured (Marshall 1973; Nival & Nival 1976; Geller & Müller 1981) as an indicator of minimum food size, for the filtering apparatus may function as a 'leaky sieve' (Boyd 1976) allowing small particles to pass (Peterson *et al.* 1978). Gliwicz (1977, 1980; Gliwicz & Siedlar 1980) has analyzed the width of the opening between the valves of cladocerans since this could set a maximum size to the edible particle, as could the so-called guard setae which protect the entry to the filter basket of calanoid copepods (Gauld 1966). Egloff & Palmer (1971) measured the filtering area of the thoracic appendages of two *Daphnia* species in an attempt to test apparently conflicting hypotheses (McMahon & Rigler 1963; Burns & Rigler 1967; Burns 1969a) regarding the relationship of grazing rate (and, by their reasoning, filter area) and animal size. Strickler and his coworkers (Strickler & Bal 1973; Friedman & Strickler 1975; Strickler 1975; Friedman 1980) have used electron microscopy to identify putative chemoreceptors and thigmoreceptors on the appendages of copepods. Such receptors would be required if these animals are capable of selective feeding on the basis of taste or of response to moving prey at some distance, respectively. Gut and fecal analyses have been used more widely (Fryer 1957b; Porter 1973, 1975; Infante 1973, 1981; Pourriot 1977; among others). Despite these initiatives, microscopic examinations are used too rarely in the modern testing of ideas and the following comments are written in the hope that future workers will employ them more frequently.

Animals may be studied from whole mounts or sections of animals and exuvia (Frey 1973; Fryer 1968) or from dissections of the limbs. Since isolated parts may misrepresent the function of the whole, it is good practice to compare dissections with whole mounts and living animals. Care should be taken in preserving material since killing agents, such as formalin, may distort the animals. Haney and Hall (1973) recommend that animals be anaesthetized with carbonated water before killing with a solution of 4% formalin and sucrose. Prepas (1978) reported that chilling the formalin and sugar solution reduces distortion still more. Manipulations of the limbs or animals is simplified if performed in a viscous medium like glycerol or polyvinyl lactophenol (Frey 1973; Fryer 1968).

It is often not necessary to stain the animals. However, Egloff and Palmer (1971) used a chitin stain, crystal violet, to examine the setae of *Daphnia* and Eriksson (1934) found that methyl violet B was useful in observing hairs and

bristles'. Fryer (1968) recommends Mallory's triple stain for most uses. Polarized light and a camera lucida or other drawing system (Frey 1973) have also been employed. Researchers hoping to use such techniques can refer to Pantin (1964), a microscopical handbook or, in the case of drawing aids, to the manufacturer's directions. Electron microscopy is finding increasing application in the analysis of the fine structures associated with feeding (Sullivan *et al.* 1975; Geller & Müller 1981; Gerritsen & Porter 1982).

Gut contents of zooplankton have long been a focus of study: the presence or absence of food in the gut may identify diel rhythms of feeding behaviour (Fuller 1937; Gauld 1953; Kajak & Ranke-Rybickova 1970; Singh 1972) and the identification of food types in the gut may reflect selective feeding and food preferences (Lebour 1922; Lowndes 1935; Fryer 1957b; Gliwicz 1969; Rapport *et al.* 1972; Infante 1973; Porter 1973, 1975, and others). However, such analyses should be treated critically. Handling and fixing of the animals may cause regurgitation or defecation of the gut contents (Gauld 1953; Swift & Fedorenko 1973; Hayward & Gallup 1976). This should be avoided by narcotizing the animals: Gannon & Gannon (1975) compared 20 different narcotizing and killing agents and finally recommended that the animals be anaesthetized by adding carbonated water to their container (1:20 by volume). Many animals will not completely empty their gut even after prolonged starvation (Cannon 1928; Lowndes 1935; Lemcke & Lampert 1975), thus empty guts may not indicate fasting and food in the gut may not indicate recent feeding.

The interpretation of analyses of gut contents also requires considerable caution. A number of food organisms, especially naked flagellates, disintegrate in the gut and analysis may show only a green mush. Gliwicz (1969) found identifiable remains in less than 10% of the 150 animals he examined and Infante (1973) obtained results from only one third of the animals she dissected. Marshall (1924) found that only 52% of the *Calanus* she studied contained recognizable remains. Moreover, a long series of observations have shown that some cells, although ingested by zooplankton, may pass through the gut apparently unharmed (Lefèvre 1942; Fryer 1957b; Grygierek 1971; Porter 1973, 1975, 1976; Pourriot 1977). Identification of prey in the gut of predators is made difficult because the animals frequently ingest only a part of their prey (Confer 1971; Ambler & Frost 1974; Dagg 1974; Brandl & Fernando 1975); in addition, the food of the prey may appear in the predator's gut (Fryer 1957b). Murtaugh (1981) found no evidence for differential digestion of animal prey in the guts of *Neomysis*. The implications of these problems are very serious. Because only a small, highly resistant portion of the gut contents is identifiable, analyses invariably deal with an unrepresentative sample. Because food must be destroyed in the gut, whereas indigestible items are unaffected, the presence of a particular item in the gut does not indicate that it is food and its absence is no proof that the animal does not eat that item. Nevertheless, the presence of a particle in the gut proves ingestion, while the presence of cell fragments or empty tests shows mortality of the cell and probably indicates utilization of the cells by the zooplankton.

The examination of the gut contents is usually performed by gently crushing the dissected gut (Marshall 1924; Fryer 1957b) or the entire animal (Porter 1973; Pourriot 1977) between slide and coverslip. This extrudes the gut contents. Fryer (1957b) suggests that the extruded material be further macerated and claims that many difficulties in identification of the gut contents could be reduced by this step. It may be advantageous to examine the gut contents in living animals, for the gut peristalsis allows the researcher to see the food from different angles (Gliwicz 1969). The discrete fecal pellet produced by copepods allows their gut contents to be identified by examining the pellets rather than the gut (Marshall & Orr 1955).

Chemical analyses can offer a less time-consuming alternative to microscopic examination of the gut. Gut enzymes may provide important clues to food type and digestibility (Hasler 1937; Mayzaud 1980; Mayzaud & Mayzaud 1981). Fluorescence analysis for chlorophyll derivatives in extracts from whole zooplankton (Mackas & Bohrer 1976; Dagg & Grill 1980) has been used to study diel variation in feeding activity (Boyd *et al.* 1980).

In summary, gut analyses and morphological studies can yield useful and rare information. However, the techniques are time-consuming and require great patience; they are open to misinterpretation and the results should be compared to the behavior of living animals.

3.2 *Behavioral studies*

Close examination of the actions of individual, living animals complements both static, morphological observations and feeding or grazing rate determinations, which tend to be 'black-box' experiments. Behavioral studies have provided the descriptions of the feeding process outlined above and have given strong evidence that zooplankton are not automatic filtering machines, but are capable of a considerable range of feeding behaviors (Porter *et al.* 1982). McMahon (1968; McMahon & Rigler 1963) discovered that the reduced grazing rate of *Daphnia magna* in high concentrations of food is achieved by a reduction in the rate of movement of the thoracic appendages and by rejection of excess food in the food groove by the post-abdomen. Burns (1968a) showed that this same movement can be used to discard undesirable foods, such as blue-green algae. This behavior is widespread in cladocerans (Hayward & Gallup 1976; Webster & Peters 1978; Porter & Orcutt 1980), although gut analyses of both laboratory and field specimens have shown that not all blue-green algae are rejected (Bogatova 1965; Arnold 1971; Infante

1973, 1981; Geller 1975; Nadin-Hurley & Duncan 1976; and others) and some rotifers (Starkweather 1981) and cladocerans are capable of assimilation (Lampert 1977a), growth and reproduction on a diet containing blue-green algae (Hrbáčkova-Esslova 1963; de Bernardi *et al.* 1981). Studies of the behavior of calanoids have added greatly to our picture of their feeding process. *Calanus* may feed discontinuously (Conover 1966a; Paffenhöfer 1971; Rosenberg 1980), a habit which could cause considerable variation in short-term measurements of feeding rate. Conover (1966a) also noted that these animals may shred larger food particles and lose some fragments. This observation involves modification of the cell size spectrum, which has implications for the interpretation of feeding or grazing rates determined from the size spectra produced by electronic size analyzers like the Coulter counter and may result in overestimation of ingestion rates based on microscopic determinations of the food cell mortality through cell counts. Cells which are collected in the filter basket are not always successfully ingested (Conover 1966a; Alcaraz *et al.* 1980); moreover *Calanus* uses different behaviors to deal with foods of different size (Conover 1966a; Gauld 1966) and may persist in one mode despite changes in food type. Others (Poulet & Marsot 1980) argue for more opportunistic switching among foods. These observations suggest that the animals feed less effectively on certain foods at particular times. Active selection of foods has also been observed in cyclopoids (Williamson 1980), rotifers (Gilbert & Starkweather 1977; Gilbert & Bogdan 1981), and ciliates (Rapport *et al.* 1972). Obviously, most of these studies require observation through a microscope, but useful information can be gained by simply watching zooplankton in an aquarium. For example, both *Diaptomus* (Lowndes 1935) and *Daphnia* (McMahon & Rigler 1963; Burns 1969b; Horton *et al.* 1979) were observed to scavenge or browse on container surfaces when the suspended food concentration is low. This implies that suspended food concentration may not reflect available food for zooplankton in shallow lakes or in the littoral zone.

Microscopic examination of the feeding process requires that the animal be enclosed in a small volume of water, and this frequently means that it is fixed in position. Observation chambers may be as large as a watch glass or syracuse dish (Gauld 1966; Conover 1966a; Williamson 1980) but small animals may be observed in a depression slide (Fryer 1968, 1974) or in a drop of water suspended from a coverslip (Edmondson 1965; Brandl and Fernando 1975). Closer observation requires that the animals be held in position. Cladocerans may be constrained by enclosing the animal in a very small drop of water and then pressing the dorsal surface of the carapace into a small dab of petroleum jelly or stopcock grease, care should be taken to allow the swimming antennae and thoracic appendages free movement. The animal is then given more water and mounted under a microscope with the ventral

surface towards the microscope's objective lens (McMahon & Rigler 1963; Webster & Peters 1978). This gives the animal an abnormal position with respect to gravity, although a side mounted microscope partially circumvents this problem (Burns 1968a). Ringelberg (1969) has positioned *Daphnia magna* by driving a fine pin through the brood pouch and using a side mounted microscope with dorsally directed illumination. This should permit the animal free rotation to assume a more natural position. The same effect may be had with less damage to the animals if they are fixed to the end of a fine wire (Fox *et al.* 1951) or a glass rod (Porter *et al.* 1979) with petroleum jelly or glued onto a hair (Alcaraz *et al.* 1980). These are probably the only measures suitable for copepods (Alcaraz *et al.* 1980). All manipulations are greatly facilitated by the use of fine Erwin loops (Sargent-Welch Co.) which minimize damage to the animals. Webster & Peters (1978) felt that restrained small cladocerans may reject food more frequently than free-swimming animals, but McMahon & Rigler (1963) found no such effect with *Daphnia magna*. The effect of such restraints must be investigated further.

It is difficult to believe that the behavior of planktonic animals is unaffected by these conditions of observation. Paffenhöfer *et al.* (1982) feel that tethering copepods may reduce the range of behaviors of free-living animals. Fryer (1968) argues that no benthic cladoceran behaves normally on a clean, brightly lighted, slide. Certainly, light should be as dim as possible. The spectral composition of light may influence animal behavior (Baylor & Smith 1954; Hairston 1976): red illumination is sometimes used to reduce any light effect but the insensitivity of zooplanktonic crustaceans to red light is by no means a certainty (Lumer 1932; Viaud 1951; Smith & Baylor 1953; Stearns 1975). *Daphnia* do not respond to infra-red light (Baylor 1959; Young 1974) and Strickler (1970, 1977) used infra-red light with the appropriate film to follow the behavior of cyclopoid copepods. Since microscope lights may inadvertently heat the animal's water, one should take care to control the water temperature by the use of water jackets, flow-through chambers or simply by using a relatively large volume of water and a short observation period. Small chambers may also distort laminar flows generated by calanoid copepods when feeding, thus inhibiting food particle perception and capture (B.Marcotte, personal communication).

Observation chambers have been used to describe the feeding currents produced by the animals. Food cells, inorganic particles, or dye are introduced to such systems and the patterns of flow observed. Among the substances used have been methylene blue, coloured starch particles (Cannon 1928), charcoal, carmine red (Naumann 1921) and India ink (Eriksson 1934; Koehl & Strickler 1981).

More quantitative studies have determined the rate of movement of the feeding apparatus. The appendages move at a greater speed than one can

normally count by eye, although larger cladocerans may move slowly enough for manual methods (McMahon & Rigler 1963, McMahon 1968) and for some purposes zooplankton may be slowed down by the use of an anaesthetic like MS222 (Conover 1966a) or chloral hydrate (Eriksson 1934), by starvation (Rigler 1961) or by thickening the water with methyl cellulose (Fryer 1968). However, such techniques are intentionally unnatural and should be compared with normal behavior whenever possible. A number of mechanical aids have been used to resolve the problem of rapid movement. For example, if the movement is continuous and rhythmic, one can 'freeze' the actions of the appendages stroboscopically. An animal is fixed under a microscope and the light directed to the eye is pulsed. When the rate of pulsing is equivalent to the rate of movement of the appendages (or to any integral multiple of that rate) the appendages appear to stand still; slightly higher or slower pulse rates slow the motion of the appendages. This 'freezing' can be achieved by pulsing the light source but rapidly fluctuating light may upset the animals unnecessarily. A more effective device is the stroboscope (Cannon 1928; Gray 1930; Tonolli 1947; Schröder 1961): a dark dish, slit at regular intervals around its perimeter, is rotated between the objective and the animal, so that the animal is only visible through the slits. The speed of rotation is increased until appendage motion appears to cease. At that point, the speed of the appendages (in cycles per unit time) equals the product of the number of slits and the rate of revolution of the disk. For effective stroboscopic measurements, the animal must move its legs at a fixed speed for some time. Lowndes (1935) concluded that the stroboscope was ineffective for the study of calonoid copepods but Schröder (1961) obtained stroboscopic estimates which were comparable to those obtained cinemagraphically by Lowndes (1800–3600 versus 600–2400 beats per minute, respectively). In any case, the stroboscope cannot reveal inter-flash or irregular behaviors, which may also play a significant role in food collection (B.Marcotte, personal communication), McMahon (1968) and Burns (1968a) have circumvented the problem of discontinuous and arrhythmic movement by continuous and automatic recording. A photocell replaced one of the oculars of a stereoscopic microscope which was focused on the moving appendages. These movements produced changes in light intensity, and, therefore, in the current through the photocell, which were automatically recorded on a strip chart recorder. Porter *et al.* (1982) have used a similar device. High speed cinematography has provided an alternative means of observation for many years (Erikkson 1934; Lowndes 1935). Strickler (1975) filmed zooplankton at 250 frames/sec and subsequently (Alcaraz *et al.* 1980; Koehl & Strickler 1981; Paffenhöfer *et al.* 1982) at 500 frames/sec; such films can then be viewed in slow motion and a wealth of information extracted. Videotape can provide a useful alternative to film and is preferable in that the tape can be viewed immediately. However, the

resolution provided by the television screen is less than that of film and the range of filming speeds is reduced. Technological advance may soon permit the application of equipment with much higher time resolution which is now prohibitively expensive. (J.A.Downing, personal communication).

To date, much of the literature has been confined to verbal descriptions of processes without attempts to quantify or score such behavior. Altmann (1974) suggests that such studies are best seen as initial reconnaissance preparatory to further quantitative investigation. She maintains that the danger of biased description based on selective observations is too great to be ignored; such studies must be followed with careful testing of selected hypotheses based on objective systems of scoring behavior. One danger of quantification by the development of unambiguous criteria for scoring is that one may study events which are easily scored but of little or no ecological relevance. Marler & Hamilton (1967, Chapter 20) point out that compromise may be necessary between the significance of the behavior to the researcher's ultimate objectives and the ease with which it may be measured. Such trade-offs are commonplace in ecological research.

Behavioral studies can provide independent tests of mechanistic models of zooplankton feeding and are a rich source of ideas and inspiration for hypotheses to be tested with other techniques. They also permit the biologist to treat zooplankton as complex and interesting organisms rather than simple pump-and-filter automata. However, many ethological studies of zooplankton feeding have limited themselves to qualitative descriptions and often do not provide objective tests of hypotheses.

3.3 Laboratory determinations of feeding and grazing rate

Most studies of zooplankton feeding have concentrated on the estimation of G and f in laboratory vessels. The animals are introduced to a suspension of food and the rate of accumulation of food by the animals or its rate of loss from suspension is measured. The former method usually involves labeling the food with a radioactive tracer and the latter involves sequential estimates of food biomass (cell number, cell volume, chlorophyll, carbon, etc.). Both approaches are quantitative, relatively rapid and conceptually simple, but uncritical use can confuse the interpretation of results.

3.3.1 Cell counts

The oldest technique for estimating grazing and ingestion rates is based on the observed change in the number of suspended cells counted before and after a suitable period of exposure to feeding animals. The change in food concentration is then considered a measure of the amount of food eaten. The

grazing rate (G, in ml animal^{-1} unit time^{-1}) is calculated as:

$$G = V(\ln C_0 - \ln C_t)/(tN) \quad (9.2)$$

where V is the volume of the container in ml, t is the length of time the animals were allowed to feed, N is the number of animals in the container, C_0 is the initial cell concentration and C_t is the final cell concentration. This, of course, simply assumes that cell number is reduced by a constant fraction per unit time (i.e. that grazing rate is unaffected by changes in cell concentration) and is thus a simple negative exponential relationship. This formula is popularly referred to as Gauld's (1951) equation. Since subsequent studies (Rigler 1961; Mullin 1963; Richman 1966; Burns & Rigler 1967; Frost 1972; Corner *et al.* 1976; and many others) have shown that above some critical concentration (Fig. 9.3), termed the 'incipient limiting level' (Rigler 1961), grazing rate is a negative function of food concentration, the assumptions of Gauld's equation do not apply universally. In his seminal paper, Gauld (1951) was careful to ensure that the cell concentration did indeed decline exponentially, but this precaution has often been ignored by other workers. Rigler (1971a) has calculated that this oversight may result in as much as a 100% overestimate of feeding rate. Above the incipient limiting level, feeding rate (f) is constant (Burns & Rigler 1967; Frost 1972; Mullin *et al.* 1975; Corner *et al.* 1976; Harris & Paffenhöfer 1976a) or nearly so (McMahon 1965; Mullin 1963; Parsons and LeBrasseur 1970; Geller 1975; Horton *et al.* 1979) and f (in cells animal^{-1} unit time^{-1}) can be calculated as:

$$f = V(C_0 - C_t)/(Nt) \quad (9.3)$$

(The reader should be aware that precise identification of the incipient limiting level may not be possible and that it depends on the model used to describe the effect of food concentration on feeding behavior—see Section 3.6). Although grazing rate is not constant above the incipient limiting level and feeding rate is not constant below, it may sometimes be necessary to calculate these values, regardless. At low concentrations of food, an average value for the food concentration (C) can be estimated as:

$$C = VC_0 \exp(-GNt/V) - 1/tGN \quad (9.4)$$

(modified from Frost 1972) and an average feeding rate (f) as:

$$f = CG \quad (9.5)$$

Above the incipient limiting level, an average G can be calculated as:

$$G = (C_0 - C_t)2/t(C_0 + C_t) \quad (9.6)$$

and C can be taken as the arithmetic mean of the initial and final cell concentrations ($[C_0 + C_t]/2$). Both calculations are only approximations of

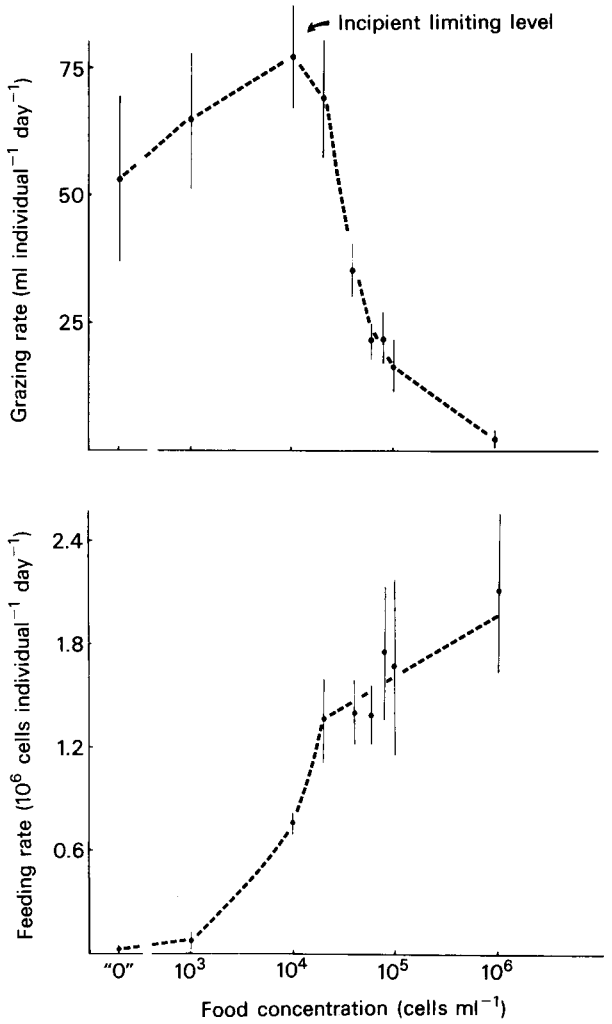

Fig. 9.3 The effect of food concentration on filtering and feeding rates of *Daphnia pulex* (Horton *et al.* 1979) showing the decline in filtering rate and near plateau of feeding rate above the 'incipient limit concentration'.

the animals' changing behavior over the period of the experiment. The errors involved are increased as the reduction in food concentration during the experiment (Rigler 1971a) becomes greater.

At the incipient limiting food concentration, animals pass from a concentration at which feeding rate is constant to one in which grazing rate is constant. A survey of the literature (Fig. 9.4) suggests that this change occurs between 1 and 10 p.p.m. (vol./vol.) of food, and experiments conducted in this

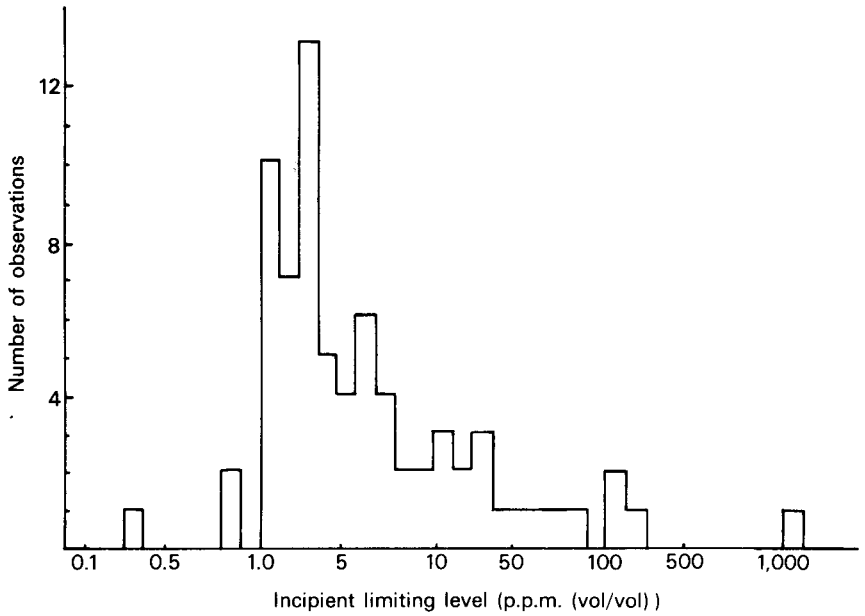

Fig. 9.4 Frequency distribution of reported incipient limiting food concentrations (p.p.m., wet weight/volume) for calanoid copepods (Richman 1966, Kibby 1969, McAllister 1970, Corner *et al.* 1972, Esaias & Curl 1972, Frost 1972, Gaudy 1974, Poulet 1977) Cladocera (Ryther 1954, Rigler 1961, McMahon 1965, McMahon & Rigler 1965, Burns & Rigler 1967, Crowley 1973, Infante 1973, Geller 1975, Hayward & Gallup 1976, Kersting and van der Leeuw 1976, Kring & O'Brien 1976) and rotifers (Gilbert & Starkweather 1977a). When necessary, wet weight of cells was calculated from volumes published elsewhere for the same species or by conversion from other indices of biomass such as carbon content or dry weight. In general, the incipient limiting level lies between 1 and 10 p.p.m.

range should include assurance that change in cell concentration is approximated either by a linear (i.e. f constant) or by a negative exponential (i.e. G constant) function of time. Otherwise, the researcher should use radiotracer or electronic cell counting techniques, which minimize the change in cell concentration, or simply avoid working over this range of concentrations.

A number of problems have arisen in using cell counts. Because relatively long exposures (2–24 h) are required, algal sedimentation may produce variations in cell concentration. This problem may be alleviated by using motile algae like *Chlamydomonas* (Marshall & Orr 1955), but most researchers now routinely stir or rotate the experimental vessels. Anraku (1964) and Nauwerck (1959) both report an increase in observed feeding rates during stirring but neither Mullin (1963) nor Schindler (1968) found a

significant effect. Ryther (1954) found that agitation of the experimental container stopped feeding. Long exposures also raise the possibility that algal growth may seriously influence the change in cell numbers (Nauwerck 1963; Sheldon *et al.* 1973; Nival & Nival 1976) even with little or no light (Anraku 1964; Roman & Rublee 1980). Most workers try to reduce algal growth by keeping their experimental flasks in dim light and a number have corrected their estimates of grazing rate by following the increase in cell numbers in control flasks without zooplankton. Coughlan (1969) reviews the equations for such a correction which reduce to the consideration of the food growth rate in the control flask assuming an exponential increase. The initial (CC_0) and final (CC_t) concentrations in the control are determined and a growth rate constant (b) is calculated as:

$$b = [\ln(CC_t) - \ln(CC_0)]/t \tag{9.7}$$

A similar calculation for changes (C_t and C_0) in the experimental chamber yields 'a' (a negative value which is equal to Gauld's G [equation 9.2] times N/V):

$$a = (\ln C_t - \ln C_0)/t \tag{9.8}$$

The grazing rate can then be calculated as:

$$G = V(b - a)/N \tag{9.9}$$

Since a is usually negative this estimate of G is larger than that estimated by equation 9.2. The average cell concentration is calculated by substituting (b – a) for GNt/V in equation 9.4 (Frost 1972).

Strictly interpreted, these corrections can only be applied when both the algal growth rate in the controls and its rate of decline in the experimental vessels are shown to be exponential. Such determinations are rarely made because the number of cell counts required is considered prohibitive unless electronic counting systems are used (Sheldon 1979). The possibility exists that any external control is inappropriate because the growth rate of algae in the experimental flask may be enhanced by the excretions of zooplankton (Gliwicz 1975; Porter 1976; Roman & Rublee 1980). If this is so, feeding and grazing rates will be underestimated, but strong evidence for zooplanktonic enhancement of algal growth is scarce (Frost 1972). The conditions for algal growth must vary greatly with experimental conditions and no set rule about the magnitude of error introduced can be established. A maximum value of b ($= r_{max}$: day^{-1}) for algal growth can be established on the basis of the allometric equation of Blueweiss *et al.* (1978):

$$r_{max} = 33M^{-0.26} \tag{9.10}$$

where M is the wet weight of the food cell in pg. This would usually

overestimate algal growth under the conditions provided by grazing rate experiments, since such conditions are rarely ideal for algal growth.

When microscope counts are used to determine cell concentrations, care should be taken to obtain representative counts. This is rarely a problem with pure cultures, but in mixed cultures of food cells or natural waters, one may be tempted to push the method beyond its limits and to count cells for which statistically meaningful counts are unobtainable. Hobero & Willén (1977) recommend:

(1) The use of KI_2 as a preservative.
(2) The use of an Utermöhl inverted microscope as a counting system.
(3) The counting of at least 100 cells per species.
(4) The counting of only the most abundant species.

Theoretically, cells should settle randomly in an Utermöhl chamber and the variance around each count should be equal to the mean; hence the standard deviation for a count of 100 cells is 10 (see Chapter 7). However, since the cells frequently settle unevenly, a Poisson distribution cannot be assumed and the variance in counts should be determined directly (Nauwerck 1963). Because grazing rate is calculated as the ratio of the means of C_0 and C_t (i.e. $\ln C_t - \ln C_0 = \ln C_t/C_0$) the standard deviation of the filtering rate is increased over that of the estimates of C_0 and C_t:

$$\text{standard deviation of } C_t/C_0 = (\text{variance of } C_t)/C_0^2 + (\text{variance of } C_t)/C_t^2 \qquad (9.11)$$

(Mood *et al.* 1974). Because G is calculated from the logarithm of this ratio, the probability distribution of G is negatively skewed and extremely low values of G will be more frequent than extremely high values. Most publications of grazing rates have ignored statistical treatments of the individual estimates, apparently more by tradition than by design. An exception to this rule is the work of Downing (1979, 1981; Downing & Peters 1980).

A frequent ploy in cell count experiments is to confine a large number of animals in a small volume of water so that large changes in cell concentration will be achieved in a relatively short time. This sould be avoided since crowding has been shown to influence metabolic rates (Zeiss 1963; Hargrave & Geen 1968; Santomi & Pomeroy 1968) and to depress feeding rates of zooplankton (Anraku 1964; Hargrave & Geen 1970). Marine calanoids appear to increase their grazing rates with increases in volume up to at least 7 liters (Paffenhöfer 1971, 1976; Harris & Paffenhöfer 1976b; Paffenhöfer & Harris 1976; Paffenhöfer & Knowles 1978). However, these workers consistently use equation 9.9, which gives higher values of G than the calculations used by others. Hayward & Gallup (1976) have found that the grazing rate of

Daphnia pulex is inhibited when the animals are held at concentrations of >1 animal per 20 ml. Apparently, this is the only investigation of the effects of crowding on feeding by freshwater animals.

A number of authors have suggested that zooplankton feed on detritus (Nauwerck 1962; Tappa 1965; Paffenhöfer & Strickland 1970; Saunders 1972; Corner *et al.* 1974; Buscemi & Puffer 1975; Nadin-Hurley & Duncan 1976; Lenz 1977). Detrital feeding cannot be effectively measured with visual counts; detrital particles are practically impossible to count with a microscope and because the particles vary so greatly in size and shape that the size of each must be measured individually. This is best left to automatic particle counters.

Despite their disadvantages, direct counts can provide more information than any other estimate of feeding rate, and they are particularly useful in determining differential rates of algal mortality in suspensions of mixed species and in natural waters (Gliwicz 1969; McQueen 1970; Gaudy 1974). Because the method necessarily deals with large numbers of animals and longer periods of exposure, it circumvents the variations introduced by diel patterns in feeding activity (Nauwerck 1963; Duval & Geen 1975; Haney & Hall 1975; Starkweather 1978), discontinuous activity, and individual variations (Conover 1966a; Paffenhöfer 1971). Cell count techniques avoid the dangers of radiotracers (Section 3.3.5) and the ambiguities of electronic particle counters (Section 3.3.2). They also require equipment which is less expensive and widely available. It is unfortunate, if understandable, that this time-consuming approach has not been used more often.

3.3.2 Electronic particle analysis

Coulter counters®, and their analogues (e.g. Coulter's 'Cell Sorter'® system, or image analyzers like Bausch and Lomb's Omnicon® and Quantimet's 720®) represent a significant advance in the techniques of cell counting. These systems alleviate many of the problems and most of the tedium involved in microscopical cell counts and are rapidly finding acceptance among marine workers. Only the Coulter counter has been widely used and this discussion is limited to that machine. Again, while the device is extremely powerful, uncritical use can be misleading (Sheldon & Parsons 1967a, b: Allen 1975; Deason 1980a; Harbison & McAlister 1980).

The basis of the Coulter counter is very simple (Sheldon & Parsons 1967a). The particles to be counted are suspended in an electrolyte and passed through a small aperture containing an electric field. As they pass through this field, the cells change the suspension's resistance (R) and, because the change in resistance is very nearly proportional to the cell volume, both the volume (V_i) of cells and their number can be determined from the relationship:

$$R = q \cdot V_i(r_p - r_e)/r_p \qquad (9.12)$$

where q is a constant of proportionality, V_i is the cell volume, r_p is the resistivity of the particle and r_e is the resistivity of the electrolyte. Usually, r_p is assumed to be so large that the term $(r_p - r_e)/r_p$ is practically unity. The value of q is determined by calibration of the instrument with different particles of known size, usually pollen grains (Sheldon & Parsons 1967a; Allen 1975). For smaller particles, the electric current must be increased, but since very high currents cause the electrolyte to boil there is a minimum particle size of about 0·8 μm (Allen 1975). This excludes the use of the machine for very small cells such as populations of natural bacteria (Peterson *et al.* 1978). The size of the aperture sets a maximum limit to diameter (= 0·4 × aperture diameter), but utilization of a series of three apertures permits the sizing of phytoplankton up to 800 μm in diameter (Sheldon & Parsons 1967a) which is sufficient for most uses. Analysis of the output from the counter is greatly facilitated by the use of a Coulter Channelyzer which determines and prints a copy of the particle distribution in the suspension.

Coulter counters determine the total cell volume in each of a series of up to 100 channels of size classes for each aperture used. Each class corresponds to a range of cell volumes (say V_1 to V_2) and results are expressed as total volume or number of cells each of which cause a change in resistance equivalent to that produced by a cell with a volume between V_1 and V_2. The scales are frequently converted to 'equivalent spherical diameters' calculated as $(6 . V_1/\pi)^{0·333}$ to $(6 . V_2/\pi)^{0·333}$.

The Coulter counter readily analyzes the size spectrum of particles in natural waters. The range is usually so great that the size classes are plotted on a logarithmic scale, usually to the base 2 (Sheldon & Parsons 1967b) so that the size range doubles over each class. This requires some mental flexibility in interpretation, since a doubling of diameter implies an 8-fold increase in volume whereas a doubling in volume only entails a 26 % increase in diameter. The range of diameters or volumes in each class, expressed in μm or μm^3, increases exponentially with increasing logarithmic size class (i.e. 1–2, 2–4, 4–8, . . ., 1024–2048, etc.). The counter measures the size of all particles and so the recent trend to refer to measured volumes as 'biovolumes' appears to introduce a misnomer; the volumes need not be biotic at all. The approach is sometimes frustrating to the biologist in that our traditional taxonomic categories and distinctions between living and non-living particles are necessarily ignored. However, these difficulties are perhaps more apparent than real and diminish with familiarity.

The great advantage of these systems is that they can count and measure thousands of particles in a matter of seconds. Researchers can, therefore, determine statistically significant effects of feeding from small changes in cell concentration and without crowding their animals or using long exposure times. In practice, however, both animal concentration and exposure times have often been similar to those employed for the cell count methods.

The Coulter counter has a number of sources of error. The number of small cells may be underestimated and dense cultures can cause an underestimate of cell number if two or more cells pass the aperture together (Allen 1975). The volume of large particles may be underestimated (Paffenhöfer & Knowles 1978; Vanderploeg 1981) and large particles may block the aperture (Sheldon & Parsons 1967a). The equality of cell volumes determined microscopically and electronically has rarely (Harbison & McAlister 1980) been investigated over a range of cell sizes and types. Harbison & McAlister (1980) list 6 sources of variation in Coulter counts:

(1) Variations in particle shape.
(2) Changes in the geometry of the orifice.
(3) Differences in particle path.
(4) Variations in applied current.
(6) Variations in the conductivities of the medium.
(6) Variations in the conductivities of the particles.

They cite C.Boyd as holding that the first three items can produce an error of 20 %. Allen (1975) warns that electronic failure may go undetected and result in erroneous counts. He suggests that, notwithstanding the simplicity of operation, users should be 'experienced'. Presumably, frequent controls and calibrations would eliminate the dangers of equipment failure. It is possible that osmotic stress created by the electrolyte may distort or destroy small freshwater cells, such as naked flagellates. Perhaps the greatest problem with Coulter counters is the cost: in 1980, the price of a Coulter TA-2 counting system in Canada was $32 000·00.

Once the counts in each channel are obtained in both control and experimental vessels (or before and after feeding), grazing rates and feeding rates are calculated from the same formulae used in the cell count method. The ease and rapidity of the method allows the experimenter to work with very small changes in cell concentration and one can easily determine the relationships of cell growth and mortality over time required for accurate assessment of feeding and grazing rate. Occasionally, grazing rates, calculated from Coulter counts, are presented as ml individual^{-1} litre^{-1} unit time^{-1} (Berman & Richman 1974; Duval & Geen 1975). These units are converted to the more usual ml individual^{-1} unit time^{-1} on multiplication by the volume of water in the experimental vessel.

The Coulter counter has been used extensively to investigate the selection of foods of different size by zooplankton (Richman & Rogers 1969; Poulet 1973, 1974; Berman & Richman 1974; Allan *et al.* 1977; Richman *et al.* 1977). These studies purport to show that marine calanoids, and perhaps even freshwater cladocerans, demonstrate a remarkable capacity to 'track' or feed selectively upon the most abundant size class of food particles, to the exclusion of less abundant particles from the diet. When the food size spectrum is more

evenly distributed, the animals feed non-selectively. This would be an extremely exciting discovery, for it would imply that zooplankton impose a density dependent control on their food and greatly influence the course of algal succession. Unfortunately, two sources of error could, under certain assumptions, produce the same results even though the animals feed non-selectively at all times (Poulet & Chanut 1975; Poulet 1976; Vanderploeg 1981).

Peter Starkweather (personal communication) points out that the variance associated with grazing rate estimates decreases as the number of particles counted increases (equation 9.11). Thus, if the animals removed a constant proportion of all size classes, statistically significant estimates of G would first be obtained in the most abundant class; with time, and hence with further reduction in the number of particles in this most abundant class, significant reductions in cell numbers would be observed in other classes. A naive interpreter might think that the animals fed selectively when once class was very abundant but increased the range of particle size ingested as the number of cells in the most abundant class fell. The solution is obviously to give the confidence limits for grazing rate in each channel; these should be determined by repetitive counts rather than by assuming a Poisson distribution.

A second source of error arises because the animals may modify the size spectrum of suspended particles in ways other than by simply consuming cells. For example, defecated or masticated cell fragments may be released into the medium and be counted as food particles. Since most food particles are likely to be derived from the most abundant size class, a disproportionate number of defecated aggregations or cell fragments would appear in other channels, reducing the observed changes in these channels. The results could then be falsely interpreted to indicate selective feeding on the most abundant size classes (Nival & Nival 1976; Poulet 1974; Frost 1977).

Many observations suggest that particle modification could occur. Both calanoids and cladocerans lose masticated fragments of larger cells (Conover 1966a; Lampert 1978) and the animals break apart chains of algal cells (Martin 1970; O'Connors *et al.* 1976; Alcaraz *et al.* 1980; Deason 1980b); both processes result in the production of smaller particles. Even gentle agitation may disrupt some cells (Harbison & McAlister 1980) and the feces of cladocerans quickly disintegrate once released into the water (Rigler 1971b). The feces of copepods are frequently contained by the peritrophic membrane of the fecal pellet and may be less likely to influence the counts generated by electronic analysis. Although some fecal pellets are very resistant (Ferrante & Parker 1977), Marshall & Orr (1955) observed that others disintegrate immediately after defecation. Lautenschlager *et al.* (1978) observed that the peritrophic membrane of *Gammarus* disappears in 7–24 h at 10 °C and Turner (1977) believes that the fecal pellets of some marine calanoids lack peritrophic membranes altogether. Conover (1966a) has observed that calanoids may tear open and then discard fecal pellets. Kersting & Holterman (1973) suggest that the Coulter counter may record electrolyte-filled tests from the feces as cell fragments because of their changed resistance. All of these effects must increase cell counts in at least some channels and so reduce grazing rates for those size classes.

Kersting & Holterman (1973) showed that defecation gives the impression of selective feeding although none occurs. They used a Coulter counter to follow the decline in cell concentration induced by previously starved *Daphnia magna*. The animals appeared to feed selectively only after the onset of defecation, when released cell fragments appeared in some channels. Harbison & McAlister (1980) warn that sieves sense particle size by largest linear dimension while sensing zone counters sort by volume: this difference in sorting may give the appearance of selectivity where none exists. They show that a metal screen can select cells of intermediate volume (but greater length) over volumetrically larger but shorter cells. Runge & Oman (1982) showed that cells do not necessarily orient along one axis when passing through a sieve.

Although it has long been recognized that Coulter counters cannot distinguish between particle modification and selective feeding (Sheldon & Parsons 1967a), many workers continue to interpret all net reductions in cell number as the result of selective feeding alone. Typically, only channels which show a net gain in cells (and therefore negative feeding and grazing rates) are thought to show particle modification; the grazing and feeding rates in these channels are arbitrarily set to zero. Channels in which the net change is reduced (but not reversed) are treated quite differently, for in them the role of particle modification is ignored. Current models of cladoceran feeding do not permit much selection; because Coulter counters seem to show remarkable powers of selection in *Daphnia* (Berman & Richman 1974), one is lead to doubt the evidence for selection in copepods, although their richer behavioral repertoire should permit more selective feeding. Deason (1980a) used a computer simulation to show that Coulter counters are effective only in estimation of total ingestion rate, not of ingestion rate on any one size class.

Recently, the phrase 'particle modification' has become increasingly applied to the results of electronic particle counters. Unfortunately, the change seems cosmetic not conceptual: the phrase 'particle modification' is becoming a synonym for 'feeding'. In summary, alternative approaches and methodologies are required to test the models based on particle counter techniques and to resolve the doubts they have engendered. Nevertheless the discriminatory power and ease of operation of particle counters hold great promise for increasing our knowledge of suspension feeding.

3.3.3 Other estimates of biomass

Although cell numbers are the most frequently used representation of biomass in expression of feeding rate, a number of other estimators have been used which are probably preferable for comparisons among studies. The Coulter counter provides one appropriate alternative, cell volume, which has found wide acceptance. Energy or carbon content are also suitable, but dry weight seems the most easily obtained of the various measures. Chlorophyll concentration has been used to estimate algal biomass but the ratio of chlorophyll to cell volume is quite variable (Krey 1958; Nicholls & Dillon 1978). In each case, the initial and final concentrations of biomass replace C_0 and C_t in equations 9.2 through 9.9. Again, defecation and cell growth can lead to underestimates of ingestion and grazing rates.

It will frequently be necessary to convert among the different indices. Cell volume is approximately three times cell dry weight (Parsons *et al.* 1961; Nalewajko 1966). Cell carbon (C_0 in pg) is 40–50 % of dry weight or may be estimated from cell volume (V_i in μm^3) as:

$$C_c = 0{\cdot}40V_i^{0{\cdot}712} \tag{9.13}$$

(Strathmann 1967). The ratio of chlorophyll to cell volume varies from 0·001 to 0·097 pg/μm^3 (Nicholls & Dillon 1978) with a median of about 0·008.

3.3.4 Beads and inorganic particles

Many characteristics of the food cells influence grazing and ingestion rates, thus comparisons among experiments with different foods often entail simultaneous changes in more than one variable. Since strong conclusions are difficult to draw from such multivariate experiments, several attempts have been made to standardize the particles. For example, Frost (1972) investigated the effect of cell size on the feeding rate of *Calanus* by using different sized clones of the same alga. Other workers have used various non-food particles to achieve the same standardization: Burns (1968b) and Gliwicz (1977) used plastic spheres, Wilson (1973) used glass beads, and Gliwicz (1969) used sand and natural diatomite. Poulet and Marsot (1978, 1980) have manufactured membrane bound particles which permitted the flavouring of these artificial cells with different materials. Burky & Benjamin (1979) describe a rapid spectrophotometric analysis for filtering rate of latex beads. All such beads provide the animals with a standard 'food' which is not destroyed by digestion.

These experiments are much the same as those which use natural cells. Animals are allowed to feed in a bead suspension. After a short time, they are killed and rates of feeding and grazing are estimated from the number of particles removed from the water or present in the gut (Gliwicz 1969a, 1977).

The calculations required are analogous to those used in the cell count method, and the problems encountered are identical. Since these particles may be resuspended after gut passage, experimental exposures should be less than the gut passage time. An alternative to shortened feeding times is to determine gut passage time (G_p) then to expose the animals for a longer period of time, count the number of beads (B) in the gut and calculate feeding rate (f) in beads per unit time per individual as:

$$f = B/G_p \tag{9.14}$$

Grazing rate could be calculated from the concentration of beads in suspension (S):

$$G = B/(SG_p) \tag{9.15}$$

For such use, gut passage time should be determined for the experimental conditions. This is probably an inappropriate measure for ingestion by some zooplankton, since *Bosmina* and *Daphnia longiremis* apparently select against all beads (Burns 1968b) as do *Acartia clausi* (Donaghay & Small 1979; Donaghay 1980). In practice, beads are not normally used to provide absolute estimates of feeding rates. Instead, they provide an index of selective feeding among particles which are similar in all respects but size (Burns 1968b, 1969b, Gliwicz 1969) or taste (Poulet & Marsot 1978). Grazing rates obtained from beads in the guts are frequently (Wilson 1973; Frost 1977; Gliwicz 1977), but not always (Gliwicz 1969), lower than those determined with other techniques. Rigler (unpublished data) compared the number and size distribution of both natural and artificial particles in the guts and the environment of *Limnocalanus macrurus*. He found that, although these copepods grazed beads at a lower rate than natural foods, the size distribution of both particles was similar. This suggests that beads are best used to determine feeding selectivity, not feeding rate. Gerritsen & Porter (1982) found that the surface charge of the beads influences grazing rate determinations. This may be a widespread phenomenon and may indicate the inadequacy of size alone in determining selection behavior.

Because the volume of a zooplankton gut is small and analyses are tedious, the number of beads counted is frequently low. Moreover, the most interesting regions in the size frequency distributions (usually the larger size categories) are usually represented by the smallest number of particles because size distributions of offered particles are skewed. Small sample size imposes wider statistical limits on any counts of these beads and may lead to misinterpretations. Such estimates must include a statement of properly calculated statistical variation (equation 9.11) and researchers should ignore size classes in which only a few beads are counted.

One should also recognize that hard, spherical beads are only imperfect

models of natural foods. Since natural particles may be more easily ingested (Nadin-Hurley & Duncan 1976) or may be broken in ingestion (Infante 1973), natural particles eaten by cladocerans may be larger than the maximum sizes suggested by experiments with glass beads (Burns 1968b).

3.3.5 Radiotracer techniques

The prime alternatives to cell counts for the determination of feeding and grazing rates are obtained through estimates of the amount of radioactivity that animals accumulate from a suspension of radioactively labeled cells. If the activity of N animals is A_a after t minutes of exposure in a suspension of particles with a radioactivity as A_s (ml^{-1}), then grazing rate on the tracer particles (G, in ml individual^{-1} h^{-1}) is:

$$G = A_a 60/A_s Nt \tag{9.16}$$

Feeding rate (f) in units of biomass per individual per unit time can be calculated as:

$$f = GS \tag{9.17}$$

where S is the food concentration ml^{-1} expressed in the appropriate units (cell number, volume, dry weight, carbon, etc.). Equation 9.17 is simply a more general statement of equation 9.5. In most modern studies, t is less than the time required for food to pass through the gut. The great precision of this technique makes it extremely useful in dealing with short exposure times, single animals and dilute food concentrations. Since exposure times are short there is no need to correct for changes in cell concentration as in cell count methods. However, determination of selection among a variety of foods or of feeding and grazing over extended periods requires considerably more effort than would estimates from Coulter counting.

The older literature contains several variations on this basic experiment. Marshall & Orr (1955) measured the loss of tracer from suspension and Sorokin (1966) advocated monitoring the gain in incorporated tracer plus fecal radioactivity over periods of up to several hours. Both approaches require the quantitative recovery of all tracer in the body, eggs and feces and both assume that no radioactive material will be respired or excreted during the experiment. Early work suggested that these assumptions may hold (Marshall & Orr 1955; Sorokin 1966; Schindler 1968), but more recent studies (Sorokin 1968; Conover & Francis 1973; Brandl & Fernando 1975; Peters 1975a; Lampert 1977a) suggest that a considerable proportion of assimilated tracer is excreted in only an hour. Lampert (1977a) suggests that previous checks for respiration losses of ^{14}C-CO_2 were insufficiently sensitive to detect rapid dilution of a small labile pool of ^{14}C which mediates all tracer exchange: as the animals feed upon labeled food this labile compartment excretes

significant amounts of ingested tracer but such excretion ceases soon after the labeled food is removed. The similarity of various models for carbon (Brandl & Fernando 1975; Lampert 1975) and phosphorus (Conover 1961; Peters & Rigler 1973) metabolism for both copepods (Conover 1961; Brandl & Fernando 1975) and cladocerans (Peters & Rigler 1973; Lambert 1975) suggests that tracer excretion is probably a general phenomenon. Since it is improbable that defecated or excreted tracer can be completely recovered, it is unlikely that long-term tracer experiments will yield accurate data. In consequence, only short-term radiotracer experiments will be considered here. Those interested in longer term experiments may refer to Sorokin (1966).

Selection of an appropriate radioisotope is obviously a first step. A number of radionuclides have been used in feeding experiments: ^{14}C (Nauwerck 1959; Geller 1975; Lampert 1981), ^{59}Fe (McMahon 1970), ^{3}H (Gophen *et al.* 1974; Lampert 1974; Hollibaugh *et al.* 1980; DeMott 1982), ^{32}P (Rigler 1961; Webster & Peters 1978) and many others are possibilities. Primarily, one seeks a label which is easily incorporated by the food cell (usually algae, bacteria or yeast), inexpensive, and which emits low energy particles for safety's sake. For Geiger–Müller counting, the most practical isotope is ^{32}P. Although this is a hard beta emitter and is potentially more dangerous than ^{14}C or ^{3}H, a higher energy particle is necessary to avoid self-absorption, i.e. the absorption of the emitted particle by the body of the animal. Less penetrating isotopes can be used with these counting systems if self-absorption corrections are applied (Sorokin 1966, 1968; Rigler 1971a). Correction is necessary because the calculation of grazing rate (equation 9.16) compares the number of disintegrations per minute in the animals' bodies to that in a sample of dried food suspension which has low self-absorption. Generally, zooplankton absorb 15–40 % of the low energy beta particles emitted by ^{14}C in their guts (Richman 1966; Bell & Ward 1968; Kibby 1969) although the exact amount depends greatly on animal size (Sorokin 1966, 1968). Even using ^{32}P, the counting efficiency of the Geiger–Müller system (i.e. the number of counts per 100 disintegrations) is less than that of liquid scintillation counters. Despite these disadvantages, Geiger–Müller counting is simpler and cheaper than liquid scintillation and does not involve the use of dangerous fluors and organic solvents. Moreover, liquid scintillation systems are in continuous use in many institutes where Geiger–Müller systems are left unused. Nevertheless, liquid scintillation counting is preferred for most work because of the greater range of possible isotopes, higher counting efficiencies, the capacity to count two or more isotopes simultaneously and because these systems can be serviced more readily. In either case, the isotope should be chosen in relation to available counting systems. The most economical isotopically-labeled compounds are ^{14}C-bicarbonate and ^{32}P-phosphoric acid.

Labeling of an appropriate food is quite simple. If ^{32}P is used, the cells may simply be grown in or exposed to a medium containing ^{32}P-PO_4 and low levels of ^{31}P-PO_4. Such a system will label both heterotrophic and autotrophic cells (Rigler 1961; Burns & Rigler 1967; Haney 1973; Gilbert & Starkweather 1977; Downing & Peters 1980). Primary producers are easily labeled with ^{14}C-bicarbonate, but labeled organic materials are required to label heterotrophes with ^{14}C or ^{3}H (Sorokin 1966, 1968; Gophen *et al.* 1974; Lampert 1974; Hollibaugh *et al.* 1980; De Mott 1982). Since both C and H will be lost from the culture as gases, special precautions should be taken to render the vessel air-tight if long experiments are anticipated (Sorokin 1966; Conover & Francis 1973; Copping & Lorenzen 1980). Many workers have chosen to label cultures for short periods of 6–24 h. This treatment may result in incomplete labeling of the cells—a point of minor interest in feeding rate estimates if pure cell cultures are used but a crucial problem in measurements of feeding rates in multispecific cultures or assimilation (Conover & Francis 1973). Radiotracer estimates of assimilation must derive from cells which were grown in an environment of constant specific activity.

In some cases, ^{14}C (Nauwerck 1959, 1963; Bogdan & McNaught 1975; Griffiths & Caperon 1979), ^{3}H (Hollibaugh *et al.* 1980) and ^{32}P (Webster & Peters 1978) have been used to label natural sestonic assemblages which were subsequently used to estimate grazing rates. It is improbable that the label is spread evenly or consistently among all potential food particles (Knoechel & Kalff 1978); hence the nature of the labeled food must change from day to day and lake to lake. This method is appropriate only for simultaneous comparisons within a single study, and not for quantitative comparisons among different studies or dates, unless uniform labeling can be shown.

Radiotracer techniques usually involve pure cultures. The amount of radioactivity introduced to the culture cannot be specified because variations in counting efficiency, exposure time, and growth conditions will strongly affect the amount of tracer collected by each cell. Ideally, each estimate of A_a in equation 9.16 should be based on a total of 10 000 counts. If small numbers or small animals are to be used, this level may be difficult, though not impossible, to attain. To increase the counts in the animals, workers try to keep the specific activity of the food cell medium as high as possible, usually by reducing the amount of 'carrier' (non-radioactive isotope) to a minimum. Downing & Peters (1980) obtained very radioactive food particles by first introducing a small inoculum of log phase yeast cells to a ^{32}P-labeled carrier-free medium for 1 day and then promoting cell growth by the addition of small amounts of P-rich yeast extract. Smith & Wiebe (1977) found that algae could be made highly radioactive with ^{14}C by incubating the cultures in water which had been purged of dissolved inorganic carbon. The concentration of food cells achieved in a nutrient medium is frequently much greater than that in nature and also, therefore, greater than that which should be used in feeding experiments. This means that levels of radioactivity in the cell cultures must be elevated in order to allow for subsequent dilution of the cell suspension.

After exposure of the food to the tracer for a period ranging from several hours to several days, the cells are usually centrifuged, the supernatant drained off, and the cells resuspended in unlabeled medium. A second or third rinse is a wise precaution. However, excretion by cells may be so rapid (Johannes 1964; Brock & Brock 1969; Hollibaugh *et al.* 1980) that such washes are effective only immediately before the experiment. The actual level of particulate activity should be determined during the experiment by filtering at least 5 ml of feeding solution through a 25 mm, $\leq 0{\cdot}45\,\mu m$ porosity membrane filter. Absorption of dissolved tracer by the filters produces significant overestimates of particulate tracer if larger filters or smaller volumes are used (Arthur & Rigler 1967). The radioactivity of the material retained on the filter can then be used to determine A_s in equation 9.16.

Unless the researcher wishes to investigate the effects of sudden change on rates of food collection (Runge 1980), the animals should be acclimatized to the experimental concentration of food. The length of this 'pre-feeding' period is normally determined as the time the animals require to feed on unlabeled cells in order to yield consistent feeding rates. Typically, animals are allowed to feed on unlabeled cells for a short time and then grazing and feeding rates are determined. These decline to a more or less constant value after a finite period of feeding, which then determines the minimum pre-feeding time (McMahon and Rigler 1963, 1965; Geller 1975). The appropriate length of the pre-feeding period appears to be 30–60 min for cladocerans (McMahon & Rigler 1963, 1965; Geller 1975) but may last several days in the case of marine calanoids (Mullin 1963; Rigler 1971a; Frost 1972). For those animals, one can only wonder if rates measured early or late in acclimatization approximate normal values.

Soon after the tracer cells are added, the animals must be removed from the suspension, using filtration (Burns & Rigler 1967) before (Haney 1971; Geller 1975) or after (Hayward & Gallup 1976; Horton *et al.* 1979) narcotization with carbonated water or after gentle heating (Saunders, in Rigler 1971a). A number of methods have been proposed to stop feeding. Burns and Rigler (1967) allowed their animals a 2 min 'post-feeding' period in unlabeled cells to allow ingestion or rejection of any radioactive cells in the food groove, while Rigler (1971a) recommends that animals be rinsed to remove any adhering particles. However, any manipulation may cause the animals to lose tracer (Peters & Lean 1973) and it is not possible to recommend any one method. Until careful comparative studies are performed, one can only caution that the method of removal from the feeding suspension should be as gentle as possible.

Exposure time must be less than gut passage time; this should be determined in separate experiments, and two methods are commonly used. The microscopic method involves feeding the animals in a suspension of one colour and then transferring the animals to a suspension of contrasting colour. Suspensions of chalk dust, India ink, green algae and yeast have all been used (Naumann 1921; Bond 1933; Pacaud 1939; Burns & Rigler 1967; Peters 1972). This produces a sharp transition which moves down the gut with time. By sampling a number of such animals over time (or following the movement of the edge in one animal) one can determine the time required for food to pass through the gut. The same parameter may be estimated isotopically (Nauwerck 1959; Rigler 1961; Burns & Rigler 1967; Haney 1971), with the amount of tracer accumulated by different animals feeding on identical radioactive food for various lengths of time being measured. As Fig. 9.5 shows, tracer is accumulated at a constant rate, presumably as the gut fills, until a break in the uptake curve appears. This is presumed to be the point at which defecation begins. Isotopic and microscopic estimates of gut passage time concur (Peters 1972; Starkweather & Gilbert 1977b). Gut passage time increases with body size (Geller 1975), with decreasing temperatures, and with decreasing food concentrations (Peters 1972). Copepods may have longer gut passage times than cladocerans (Table 9.1).

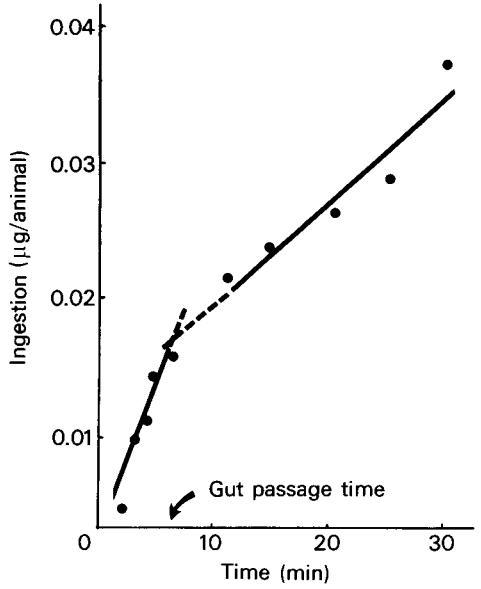

Fig. 9.5 The accumulation of radioactive phosphorus by *Daphnia rosea* feeding on radioactively labeled yeast showing the decline of tracer uptake rate when defecation begins (Peters 1972).

Table 9.1 The gut passage time (*GPT*, in min) for zooplankton of different types and weights (W, in μg dry weight) feeding upon different foods. The food concentrations (C, in mg dry weight per litre) are also listed.

Animal	W	Food	C	GPT	Ref.
Calliopus laeviusculus	6000	*Calanus*	—	210	1
Calanus hyperboreus	3100	Large Cells		40	2
		Small Zooplankton			
Rhincalanus nasutus	475	*Prorocentrum*	2	37	3
Pleuromamma xiphias	450	*Streptotheca*	3	28	3
Daphnia pulex	410	Phytoplankton	—	60	4
D. magna	290	*Chlorella*	5	50	5
D. magna	250	*Saccaromyces*	5	45	6
D. magna	140	—	—	55	7
D. magna	140	*Chlorella*	—	25	8
D. pulex	50	—	—	25	7
D. pulex	50	*Scenedesmus*	0·4	59	9
D. pulex		*Asterionella*	1·1	28	9
D. pulex		*Nitzschia*	1·7	48	9
Sida crystallina	30	—	—	60	10
S. crystallina	30	Phytoplankton	—	60	11
Centropages	25	*Gymnodinium*	2	125	3
D. schodleri	20	*Ankistrodesmus*	0·4	135	12
Diaptomus sicilis	11	Phytoplankton		200	13
Daphnia rosea	10	Phytoplankton	—	6	14
D. rosea	10	*Rhodotorula*	5	5	15
D. longispina	10	—	—	25	10
D. longispina	10	—	—	15	7
Diaptomus	8	—	—	30	7
Eudiaptomus gracilis	8	Phytoplankton	—	60	16
Holopedium	7	—	—	15	10
Acartia tonsa	7	*Prorocentrum*	1	55	3
		Prorocentrum	2	60	3
		Prorocentrum	10	37	3
A. tonsa	7	Neutral red	—	25	17
Ceriodaphnia	6	—	—	15	10
Ceriodaphnia	6	Phytoplankton	—	4	14
Daphnia galeata	5	Phytoplankton	—	5	14
D. galeata	5		—	4	7
Bosmina	5	Nannoplankton	—	15	10
Scapholeberis	4	—	—	15	10
Chydorus sphaericus	1	*Rhodotorula*	—	40	11
Brachionus calyciflorus	0·2	*Euglena*	70	20	18
Tintinnid	0·0001	Starch grains		20	19

References: (1) Dagg 1974; (2) Conover 1966a; (3) Arashkevich 1975; (4) Bell & Ward 1970; (5) McMahon 1970; (6) Rigler 1961; (7) Bond 1933; (8) Schindler 1968; (9) Geller 1975; (10) Naumann 1921; (11) Downing 1979; (12) Hayward & Gallup 1976; (13) Kibby 1969; (14) Haney 1971; (15) Burns & Rigler 1967; (16) Nauwerck 1963; (17) Hargis 1977; (18) Starkweather & Gilbert 1977b; (19) Heinbokel 1978b.

Since animals are exposed to labeled cells for a short time, the disturbance involved in adding labeled cells or removing animals may result in anomalous feeding rates. Experiments such as that in Fig. 9.5, should reveal such effects as a discontinuity in tracer uptake after very short (< 5 min) exposures. If such a plot extrapolates through zero one may assume that ingestion rates were constant over the course of the experiment.

The animals should be removed quickly from the sieve or rinse water and placed on planchets or in liquid scintillation vials (Downing & Peters 1980). Delays at this point may result in leaching of tracer to the rinse water by autolysis (Golterman 1964; Krause 1964), by loss of the haemolymph (Rigler 1971a; Ikeda *et al.* 1982) or by defecation of the gut contents. Such losses may reduce grazing rate estimates by up to 70 % (Downing & Peters 1980; Holtby & Knoechel 1981) depending on post-experimental treatment of the animals. Holtby & Knoechel (1981) suggest that losses from ^{32}P-labeled yeast and ^{14}C-labeled algae are minimized if ethanol or Lugol's solution, respectively, serve as killing agents or if chemical preservatives are avoided entirely. They recommend killing in near boiling water and then drying. Downing & Peters (1980) chose only to minimize the lag between collection and plating of the animals.

If Geiger–Müller counting systems are used, the animals should be placed in a single stratum close to the centre of the planchet to increase counting efficiency. If a weakly penetrating isotope is used, they should be dismembered. The animals may be covered with Parafilm® discs to prevent loss of material and to ease subsequent handling (Rigler 1971a).

If liquid scintillation is anticipated, two methods of counting may be employed, depending on the isotope. Hard beta emitters, like ^{32}P, produce a light called Cerenkov radiation, which may be effectively counted by a liquid scintillation system though the glass liquid scintillation vial contains only water (Haberer 1966; Fox 1976; J.Haney, personal communication). Weaker emitters are normally digested overnight in tissue solubilizer, an organic base like NCS®, Protosol®, or Soluene® (Ward *et al.* 1970; Lampert 1977a) before adding the scintillation 'cocktail'. Addition of tissue solubilizers to scintillation cocktails can alter their pH, causing high background counts due to chemoluminescence and unpredictable shifts in apparent energy spectra. It is a wise precaution to acidify the sample with a small amount of glacial acetic acid—Hall (1978) added 0·3 ml of acid per litre of scintillation fluid, Downing (1979) suggests 30 μl or 'two tiny drops' and DeMott (1982) 50 μl of glacial acetic acid per 10 ml of cocktail. Combustion of animals and food in an oxygen atmosphere either manually (Bell & Ward 1968) or automatically (using a system like Intertechnique's Oximat®) for ^{14}C and ^{3}H provide alternatives to digestion in an organic base. In any liquid scintillation system, there is a possibility that emissions will be 'quenched' to different degrees, because contaminants in the sample absorb some of the scintillations. Quench corrections must be routinely applied to all work. Researchers who intend to use any radioisotopic technique should acquaint themselves with the basic of these methods by reference to standard works (e.g. Wang & Willis 1965; Fox 1976).

Liquid scintillation can distinguish between isotopes which emit particles of different energy (e.g. ^{3}H and ^{14}C; ^{14}C and ^{32}P; ^{32}P and ^{33}P). This allows simultaneous estimation of grazing on two different particles (Lampert 1974; Starkweather & Gilbert 1978; Downing 1981a). In such experiments, the activity of the low energy emitter is counted over one, low, range or 'window' of emitted energies and the activity of the high energy emitter in three. Standard curves permit an estimation of 'crosstalk', the proportion of all counts in the lowest window which are derived from the high energy particle, from the ratio of counts in the two upper windows. Although this method is much more limited than Coulter counters, it can be used to compare grazing rates on similar sized particles and could be used to check Coulter counter estimates. Dual isotope experiments are also possible with Geiger–Müller counters. If one selects two isotopes with different decay characteristics, levels of activity of each isotope may be determined.

(1) By curve splitting (Riggs 1970) using plots of total activity against time.
(2) By waiting until one isotope disappears and then determining initial levels of the decayed isotope by difference.
(3) By using isotopes that penetrate different materials to different degrees.

In the latter case, the counts are determined before and after the weak emitter is blocked by some material such as aluminum foil, and the counts of each isotope are determined by difference; self-absorption corrections will be necessary. Although these possibilities have existed for some time they have rarely been exploited.

There are several limitations to radioactive estimates of feeding rate. Because the experiments must be short, discontinuous feeding (Conover 1966a; Rosenberg 1980) or diel patterns in feeding behavior (Haney & Hall 1975; Starkweather 1975) can result in considerable variations. The necessary disturbance of the animals caused by adding the labeled cells and removing the zooplankton may create unnatural rates. If natural foods or mixtures of species are used, it is impractical to have identical labeled and unlabeled cell suspensions for pre-feeding and experimental food. Instead, highly radioactive cells of one species, usually yeast (Burns & Rigler 1967), small algae (Chisholm *et al.* 1975), or bacteria (Haney 1973) are introduced into the food suspension at a very low concentration (< 1000 cells ml^{-1}). Such experiments yield grazing rates on this radioactive particle, but rates on the unlabeled cells are often assumed to be the same. The validity of this assumption has not been

rigorously tested (Haney 1973). Loss of tracer by excretion or mastication usually results in underestimates of only a few percent (Rigler 1971a; Peters 1975a) but mastication loss may rise to 10–15 % if large cells are used, at least for *Daphnia* (Lampert 1978).

3.4 *Field estimates of feeding and grazing*

If our laboratory experiments are to be of ecological significance they must be relevant to the field. A number of workers have tried to extend laboratory estimates of ingestion to lakes by multiplication of laboratory feeding or grazing rates and population densities (Heinle 1974; Nauwerck 1963). The usual goal of such calculations is to show whether zooplankton ingestion can significantly affect phytoplankton populations. Thus Jassby & Goldman (1974) were able to show that, in Castle Lake, zooplankton populations were far too low (apparently $< 1\,l^{-1}$) to explain algal population dynamics. Coveney *et al.* (1977) reached the same conclusions for a small eutrophic lake with much higher zooplankton densities. Lampert & Schober (1978) applied laboratory feeding rates to field population densities and showed that zooplankton may control summer phytoplankton density in Lake Constance. Gliwicz & Hilbricht-Ilkowska (1975; Gliwicz 1970) considered both grazing rate and food selection by zooplankton and found that, in eutrophic lakes, phytoplankton are less affected by zooplankton grazing than are bacteria. Nauwerck (1963) and Gulati (1975) calculated that observed primary production was insufficient to meet the demands of zooplankton; they suggested that bacteria and detritus must be utilized by the animals.

These calculations are extremely interesting, but unless field rates can be shown to correspond to laboratory values, one should treat such extrapolations with suspicion. Unfortunately, few field measurements have been made and still fewer have been made in a manner which would allow comparisons with laboratory data.

The simplest approximation to field measurements is achieved by placing animals in a beaker of natural lake water. Grazing rate can then be estimated by either microscopic (McQueen 1970) or electronic (Allan *et al.* 1977; Poulet 1977; Richman *et al.* 1977) cell counts or by changes in suspended carbon or chlorophyll (Hargis 1977; Taguchi & Ishi 1972). Radiotracer estimates are possible, using either radioactively labeled phytoplankton (Nauwerck 1959, 1963; Zankai & Ponyi 1974a, 1974b, 1976; Bogdan & McNaught 1975; Gulati 1975) or by introducing a low concentration of radioactively labeled cells of one species (Burns & Rigler 1967) into the lake water. Such experiments require handling, enclosure, and changes in a number of other factors; it is, therefore, questionable how accurately these measurements reflect field rates.

The measurement of grazing rates on natural bacteria presents a special problem. Such cells are so small (Peterson *et al.* 1978), that they lie below the lower limit of the Coulter counter, and they are not obvious under normal microscopy: These can be isotopically labeled but there is a risk of uneven labeling of freshly collected natural populations, and cultured bacteria cannot be used because they are much larger in size. The only practicable alternative appears to be cell counts after staining the bacteria with a fluorescing stain such as 4′6-diamidino-2-phenyl indole (DAPI; Coleman 1980; Porter & Feig 1980) or fluorescein isothiocyanate (FITC: Fliermans & Schmidt 1976).

Very few workers have attempted to measure feeding rates *in situ*, and most of the experiments have been performed using some modification of Gliwicz's (1968) experimental chamber (Fig. 9.6). This is a clear plastic

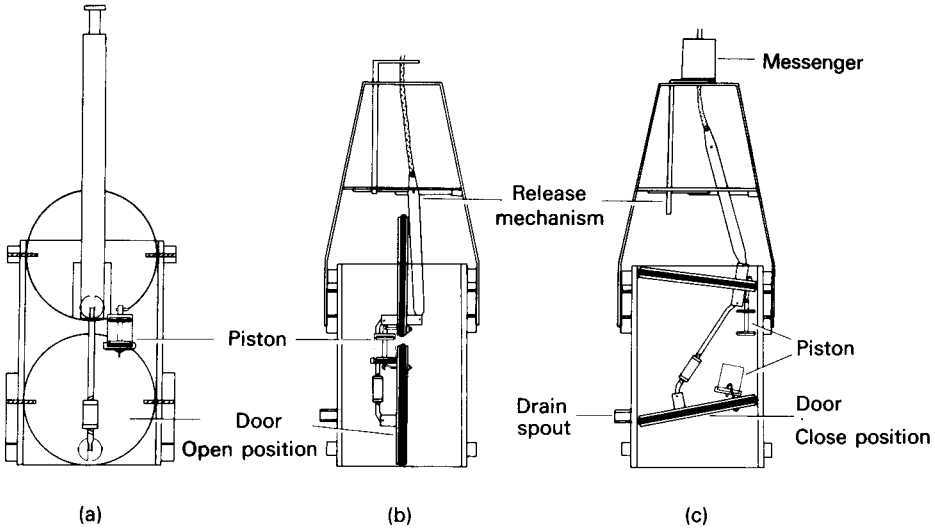

Fig. 9.6 Haney's (1971) modification of Gliwicz' (1968) *in situ* grazing chamber for the measurement of filtering rate by planktonic filter feeders (modified from Haney 1971).

plankton trap or water sampler which, when lowered into a lake and closed, encloses a volume of water and simultaneously opens a small piston inside the chamber. Gliwicz (1968) used two such samplers—one simply trapped and held zooplankton in lake water at a particular depth; the other released a narcotic (physostigmium salicyclum) from the piston into the water. This substance stopped zooplankton feeding in 5–30 min. Both chambers were then held at the collection depth and inverted every 20 min to prevent settling of the seston. After 4 h, subsamples from each chamber were preserved for later microscopic determination of the number of particles in each chamber, from which the feeding rates of the zooplankton community were calculated.

The obvious advantage of this system is the reduction of any change in the physical, chemical, or biological environment of the animals.

Haney (1971) used a similar device for *in situ* radiotracer experiments: the piston was filled with ^{32}P-labeled cells (yeast, bacteria or algae) and the closure of the chamber released and mixed these particles into the water. After 5 min, the trap was withdrawn from the lake and drained through a sieve. The animals were then anaesthetized with carbonated water and killed with formalin. Haney (1971, 1973; Haney & Hall 1975) then either measured the radioactivity in the entire sample for total grazing rates of the zooplankton community or picked animals from the sieve for individual grazing rates. This technique has most of the advantages of Gliwicz's approach but is much more rapid and permits estimates of both spatial and temporal variation in grazing rates. Using this approach, Haney (1973) identified a new parameter for lake ecosystems, community grazing rate. (G_c, day^{-1}) which is calculated as:

$$G_c = A_a 60 \times 24/VA_s t \qquad (9.18)$$

where A_a is the radioactivity of all animals in the feeding chamber (c.p.m.) at the end of the experiment, A_s is the radioactivity of the suspended food (c.p.m./ml), V is the volume of the chamber (ml) and t is time the animals were allowed to feed (min). Community grazing rate represents the mortality that the entire animal plankton imposed on all algae which are as suitable as the tracer particle as food. Haney found that the predation pressure on the nannoplankton was far more intense in eutrophic than in oligotrophic or dystrophic lakes. This might lead to increased sedimentation in the latter systems, affecting both patterns of nutrient flow and the balance between benthic and planktonic secondary production in lakes (Rigler, personal communication). Regrettably, few researchers have adopted Haney's technique and those who have used it (Kibby & Rigler 1973; Peters 1975b; Waite 1976) have usually measured individual rather than community rates.

These *in situ* techniques are not without problems. Only one type of radioactive particle is used at a time, so that measured grazing rates refer only to this particle type. Sieving of the animals at the end of the feeding period may be a harsh treatment which results in loss of radioactivity. The method measures the animal's behavior only over a small segment of time, and repetitive sampling is necessary to determine daily rates (Haney & Hall 1975). However, the method requires less effort than laboratory radiotracer measurements and certainly yields data which are closer to nature.

Haney's method has been modified to measure the feeding rate of littoral animals associated with macrophytes (Downing & Peters 1980). A 6-litre plexiglass box was placed around a macrophyte (Fig. 9.7), trapping animals associated with the plant. A suspension of radioactively labeled yeast cells was injected from a syringe into the chamber. After 10 min, the chamber was

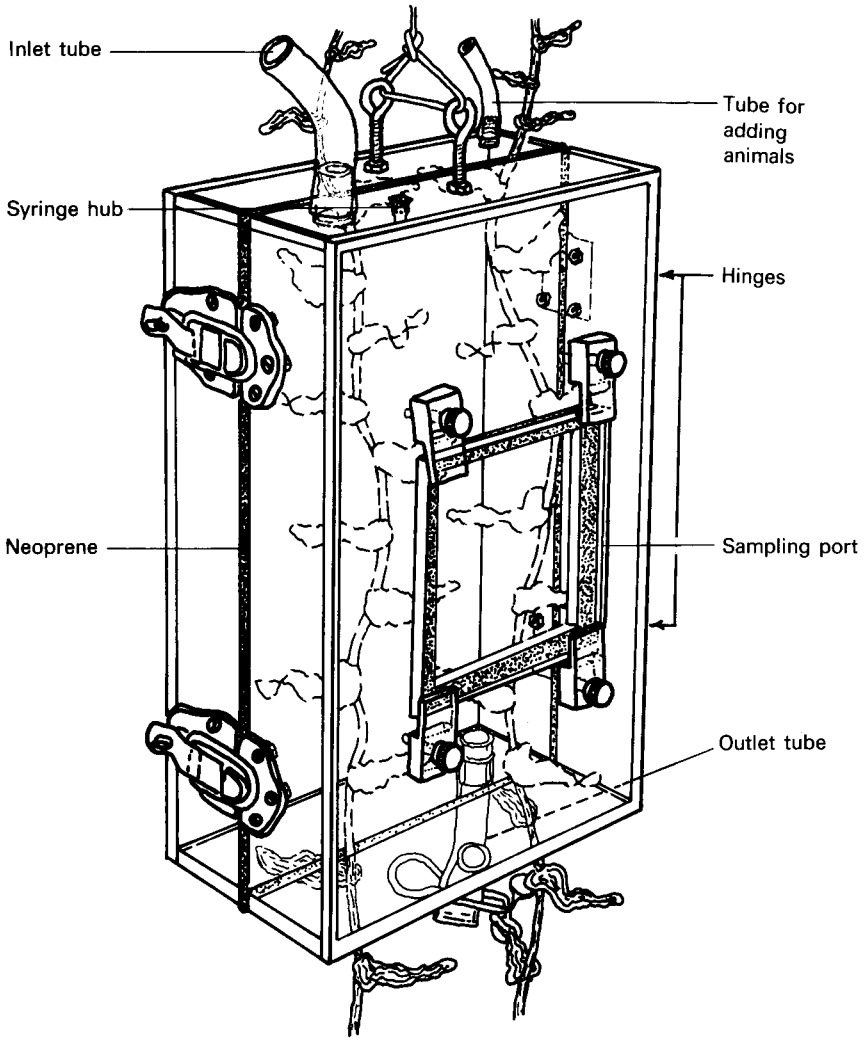

Fig. 9.7 Downing's (1981) *in situ* chamber for the measurement of feeding and filtering by littoral microcrustaceans.

drained through a Nitex® screen. Animals so collected were anaesthetized with carbonated water and then preserved in sucrose-formalin solution (Haney & Hall 1973) until they could be separated into individual liquid scintillation vials. Grazing rates were calculated from equation 9.16 and feeding rates were approximated as the product of grazing rate and the concentration of seston which passed a 35 μm mesh screen.

Downing (1981) further modified this technique to estimate the rates of

ingestion of both suspended and periphytic foods. After the plant was enclosed, he injected 0·1–0·5 mCi of $^{32}PO_4$ solution into the chamber. After 1–3 days, the tracer was distributed proportionately among different size fractions. Suspended ^{32}P was then reduced to a minimum by pumping unlabeled lake water through the chamber. Animals already in the chamber were unsuitable for feeding experiments because they were, of course, labeled with ^{32}P. Instead, animals were collected from nearby plants and introduced into the chamber with a wide bore syringe. Immediately afterwards, a suspension of tritium-labeled yeast cells was also injected into the chamber. Samples of macrophyte were then withdrawn to determine the amount of $^{32}P(A_p)$ in 'loose' periphytic material (Cattaneo & Kalff 1978) per unit weight of macrophyte. Samples of suspension were taken for later estimates of the amounts of tritium (A_{st}) and $^{32}P(A_{sp})$ in suspension. After 10 min the animals were collected as above and the amounts of tritium (A_{at}) and $^{32}P(A_{ap})$ in each animal was measured. Grazing rates (G, ml day^{-1}) were determined from the tritium counts using equation 9.16. Rates of ingestion of periphytic material (I_p, in μg animal^{-1} day^{-1}) were calculated as:

$$I_p = 24(A_{ap} - G.A_{.sp}.t/24)/A_p t \qquad (9.19)$$

where t is the length of the experiment in hours.

An approximation of *in situ* feeding rates may be made from the contents of animals' guts and the turnover time of these contents or gut passage time (Mackas & Bohrer 1976; Boyd *et al.* 1980; Dagg & Grill 1980). First, one estimates fullness of the gut at a series of sample times. Gut passage time is then estimated in separate experiments. The sampling interval divided by the gut passage time yields an estimate of the number of times that the gut has filled over the interval. Multiplication by gut contents gives ingestion over the interval and division by interval yields the feeding rate. Grazing rate is calculated by division by food concentration in the medium. This is a very rough estimate because gut passage time is variable (Table 9.1) and 'food concentration' of natural waters is difficult to assess.

3.5 *Feeding rates of other organisms*

Estimates of predation rate (number of prey killed individual^{-1} unit time^{-1}) and ingestion rate of predaceous zooplankton such as cyclopoids (Anderson 1970; Smyly 1970; Confer 1971; Brandl & Fernando 1975), calanoids (Anraku & Omori 1963; Ambler & Frost 1974; Dodson 1974) raptorial cladocerans like *Leptodora* (Hillbricht-Ilkowska & Karabin 1970) and dipterous larvae like *Chaoborus* (Kajak & Ranke-Rybickova 1970; Swift & Fedorenko 1973; Lewis 1977) are becoming more frequent in the literature. The basic techniques and problems are identical to those used in the cell count

method except that small animals, nauplii, copepodites, rotifers, or cladocerans, replace algal or bacterial cells. The prey are counted at intervals and their mortality rate is calculated, generally by assuming an exponential decline in prey numbers (equation 9·2). Since many predators only partly ingest their prey (Ambler & Frost 1974; Dagg 1974; Brandl & Fernando 1975), measurement of mortality rate of the prey will give an overestimate of the amount of food ingested and researchers should, therefore, be careful to collect discarded animal parts if ingestion rates are important to their study. Lawton (1970) points out that prey animals may lose a considerable proportion of their weight if not fed during experiments (Lemcke & Lampert 1975; Threlkeld 1976), thus ingestion rate would be over-estimated if initial prey weights were used in calculation. Since animal prey are more motile than algae they can aggregate in parts of the experimental vessel, producing heterogeneous prey distribution. This might result in apparent food selectivity due to differential availability of prey and will certainly affect estimates of prey density. Finally, some prey can escape from certain predators more easily than others (Confer 1971; Dodson 1974; Fedorenko 1975; Kerfoot 1977; Lewis 1977), so that the type of prey offered may influence the results of experiments. Ambler & Frost (1974) say that the widely used *Artemia* nauplius is slow compared to other tiny zooplankton and estimates based on these prey items may overestimate both predation and ingestion rates.

Less effort has been placed on the measurement of feeding and grazing rates by rotifers or ciliates than on crustaceans. Studies of rotifers (Hirayama & Ogawa 1972; Doohan 1973; Gilbert & Starkweather 1977; Pilarska 1977; Pourriot 1977; Starkweather & Gilbert 1977a; Gilbert & Bogdan 1981; Starkweather 1981) have usually employed variations of the cell count or radioisotopic methods. These can also be applied to ciliates (Laybourne 1975; Laybourn & Stewart 1975). Fenchel (1975) has employed an interesting technique, similar to that of Mackas & Bohrer (1976), to measure the ingestion rate of protozoans. After a feeding period on algae or bacteria, he stained the animals with acridine orange and determined the rate constant of decline in the number of food cells in vacuoles. He then assumed that the number of vacuolar food items was maintained in the field by an ingestion rate which balanced these laboratory loss rates and calculated ingestion rate as the product of the number of food particles in field specimens and the rate constant of loss. Subsequently, Fenchel (1980b) has determined the grazing rate of ciliates in a suspension of latex beads by counting the number of beads in the food vacuoles. Rassoulzadegan & Etienne (1981) have successfully used a Coulter counter technique to estimate feeding rates of a tintinnid ciliate. Heinbokel (1978a, 1978b) has also estimated the feeding rates of tintinnids using cell counts in laboratory cultures and by counting starch grains ingested by natural protozoan populations.

Gliwicz's chamber has also been used to measure rates of predation. Hillbricht-Ilkowska & Karabin (1970) placed several *Leptodora kindtii* in the piston and measured the reduction in zooplankton populations after closure of the chamber relative to the lake. Kajak & Ranke-Rybickova (1970) used a similar technique with *Chaoborus*, but used a second sampler without *Chaoborus* as a control. Lane *et al.* (1975) put prey organisms, marked with acridine orange, in the piston and counted the decline of these organisms. Both approaches require handling of either prey or predators but again seem preferable to experiments performed completely in the laboratory. It should be noted that some brands of acridine orange are toxic to cladocerans (Downing 1980); this could have contributed to the very high rates of zooplankton mortality reported by Lane *et al.* (1975). In addition, stain may be lost from potential prey in the course of longer experiments. Lasenby (1979) believes that realistic predation rates may be estimated using this basic technique even if a Haney trap is unavailable. He marked *Limnocalanus* with Rhodamine b and added a known number to carboys containing *Mysis* and uncounted, unmarked copepods. He then estimated mortality rate from the loss of marked animals and the final concentration of unmarked copepods. Lasenby (1979) warns that prolonged staining (>3 min) increased mortality and that neither cyclopoids nor cladocerans stain well. Other stains might circumvent some of these problems.

3.6 *Factors influencing grazing and feeding rates*

It is not my purpose to review the published literature exhaustively (see Jørgensen 1966; Marshall 1973; Porter 1977; Pourriot 1977; Conover 1978). However, the researcher who intends to work with suspension feeding should be aware of the range of factors which have been shown to be important, if only so that more effective controls may be devised.

Although there may be disagreement as to the magnitude of effect, physical and chemical factors which influence metabolic rates of any animal may be assumed to influence grazing and feeding by zooplankton. There is typically a thermal optimum (Anraku 1964; Zankai & Ponyi 1974; Geller 1975; Kersting & van der Leeuw 1976), which is influenced by acclimatization (Kibby 1971). Low oxygen levels (20–40 % saturation) reduce grazing rates of *Daphnia* (Fox *et al.* 1951; Heisey & Porter 1977; Hoshi & Kobayashi 1971; Kring & O'Brien 1976a). The intensity and quality of light have not been shown, unequivocally, to influence feeding rate (McMahon 1965; Buikema 1973, 1975), although they obviously influence behavior (e.g. Smith & Baylor 1953; Hairston 1976; Horton *et al.* 1979). Haney & Hall (1975) argue that diel changes in light intensity at dawn and dusk induce a strong increase in the grazing rate of cladocerans, but not of copepods. Similar observations have subsequently been made in the laboratory (Starkweather 1975, 1978). The effect of pH also seems highly variable (Ivanova 1969; Ivanova & Klebowski 1972) perhaps reflecting, in part, physiological plasticity (Kring & O'Brien 1976b).

The physiological status of the zooplankter may influence rates of food collection: male calanoids graze more slowly than females (Conover 1956; Nauwerck 1959; Harris & Paffenhöfer 1976b) but the reverse may be true for cladocerans (Hayward & Gallup 1976). Egg-bearing *Diaptomus*, which are presumably healthy animals, graze faster than non-ovigerous females (Nauwerck 1959; Zankai and Ponyi 1976) and ephippiate *Daphnia* graze at slower rates than parthenogenetic females (Haney & Hall 1975). There is some evidence that the grazing rate of juvenile animals may be less affected by experimental conditions than that of adults. Haney & Hall (1975) report that diel variations are more pronounced among larger daphnids. Chisholm *et al.* (1975) found that large *Daphnia* are more sensitive to temperature than small animals. Both feeding and grazing rates increase with animal body size (McMahon & Rigler 1963; Suschenya 1967, 1970; Burns 1969a; Kibby & Rigler 1973; Allan *et al.* 1977; Paffenhöfer & Knowles 1978; and many others).

As one would expect, zooplankton have some maximum and minimum sizes for foods which they consume and, within this range, some food sizes are more effectively handled than others. While Gliwicz (1977) offers some evidence for this, most studies on the effect of food size depend upon gut analyses without effective statistical treatment (Burns 1968b; Wilson 1973; Nadin-Hurley & Duncan 1976), or on Coulter counter experiments which confuse ingestion and particle modification. As a result, there is little good evidence for size selection. Figure 9.8 shows that experimenters believe that cladocerans prefer smaller cells than copepods, and these frequency distributions may summarize our intuitions regarding food sizes eaten by members of the two groups. Feeding experiments suggest that calanoids may feed less effectively upon small particles (McQueen 1970; Kibby & Rigler 1973; Paffenhöfer & Knowles 1978) but their behavior is quite variable (Bogdan & McNaught 1975; Gliwicz 1977). It is also possible that freshwater copepods prefer smaller particles than their marine relatives. Cladocerans, and especially small cladocerans, may prefer smaller particles (Nadin-Hurley & Duncan 1976; Gliwicz 1977) but the size range over which effective collection is possible ranges from $0{\cdot}1\ \mu m^3$ to $10^6\ \mu m^3$ (McMahon & Rigler 1963; Peterson *et al.* 1978; Porter *et al.* 1979).

A number of authors have found that the quality of the food organism influences feeding rates. In particular, blue-green algae are frequently rejected as food (Burns 1968a; Webster & Peters 1978; Porter & Orcutt 1980) or are grazed at a lower rate (Tezuka 1971; Crowley 1973; Infante 1973; Geller 1975; Lampert 1981). Some interactions probably occur among food types. For

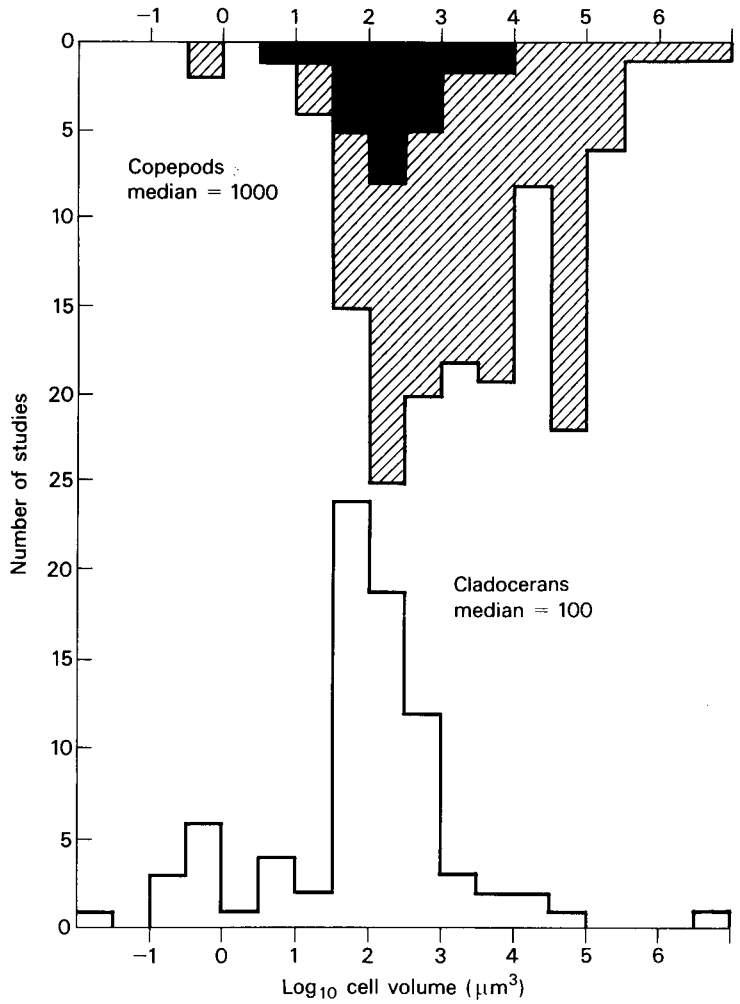

Fig. 9.8 Frequency distribution of food sizes used in the measurement of feeding as filtering rates for calanoids and cladocerans. The contribution of freshwater copepods is indicated by the solid histogram. This figure suggests that most researchers believe that cladocerans prefer smaller particles than do calanoid copepods.

example, rejection of a food bolus containing blue-green algae must reduce feeding rates on all cells. The presence of larger algal cells appears to increase feeding rates on smaller bacterial foods (DeMott 1982). Not all strains of blue-green algae have this effect (O'Brien & DeNoyelles 1974; Porter & Orcutt 1980) and some animals ingest and grow on a diet of blue-greens (Arnold 1972; De Bernardi *et al.* 1981). Esaias & Curl (1972) found that

bioluminescent dinoflagellates were eaten less readily than non-luminescent cultures.

Finally, food concentration has a marked influence on both feeding and grazing rates. A number of mathematical formulae have been applied to curves such as those in Fig. 9.3 (Rigler 1971a) which relate feeding rate (f) to food concentration (S). These are usually called:

(1) The rectilinear model, which implies a constant grazing rate below the incipient limiting food concentration.
(2) The curvilinear model, which implies a decelerating grazing rate as food concentration increases.
(3) The Ivlev model, which permits a reduction in grazing rate at very low food concentrations (Fig. 9.9).

Porter *et al.* (1982) found that a power curve ($f = aS^b$), a logarithmic curve ($f = a + b \ln S$), and a hyperbolic curve ($f = a + b/S$) also fit such data. However, attempts to distinguish which of these various models is best (Frost 1972; Mullin *et al.* 1975; Corner *et al.* 1976; Harris & Paffenhöfer 1976a; Heinbokel 1978a) have not been able to identify any one as better than the others. The rectilinear model typically has higher correlation coefficients. However, since the same data (i.e. food concentrations) are frequently incorporated in both axes, these plots involve a degree of autocorrelation and least squares regression may be biased and inappropriate. If low enough food levels are used, plots of grazing rate against food concentration can distinguish the rectilinear model from others (Fig. 9.9). By plotting grazing, rather than feeding, rate against food concentration, Frost (1975) has clearly shown that *Calanus* will reduce its grazing rate at very low concentrations.

The foregoing factors may be expected to have an influence in nature and we should obviously control for their effects in laboratory experiments.

Factors which are clearly artefacts of our experimental techniques also influence our determinations of feeding and grazing rates. Crowding reduces the grazing rate of both copepods (Lucas 1936; Hargrave & Geen 1970) and cladocerans (Hayward & Gallup 1976). Starvation may lead to rapid initial rate of grazing and feeding (Lucas 1936; McMahon & Rigler 1963; McAllister 1970; Frost 1972; Hirayama & Ogawa 1972; Geller 1975). A number of workers have found that the grazing rate of copepods is reduced in very small vessels (Marshall & Orr 1955; Cushing 1958; Anraku 1964; Corner *et al.* 1972), but most of this earlier work assumed that maximum and natural rates were achieved in the largest container used (about 500 ml). Paffenhöfer (1971, 1976; Harris & Paffenhöfer 1976a, 1976b; Paffenhöfer & Harris 1976; Paffenhöfer & Knowles 1978) have since shown that the rates can increase another order of magnitude if vessels of several litres are used. This raises the possibility that most published values for calanoids are underestimates.

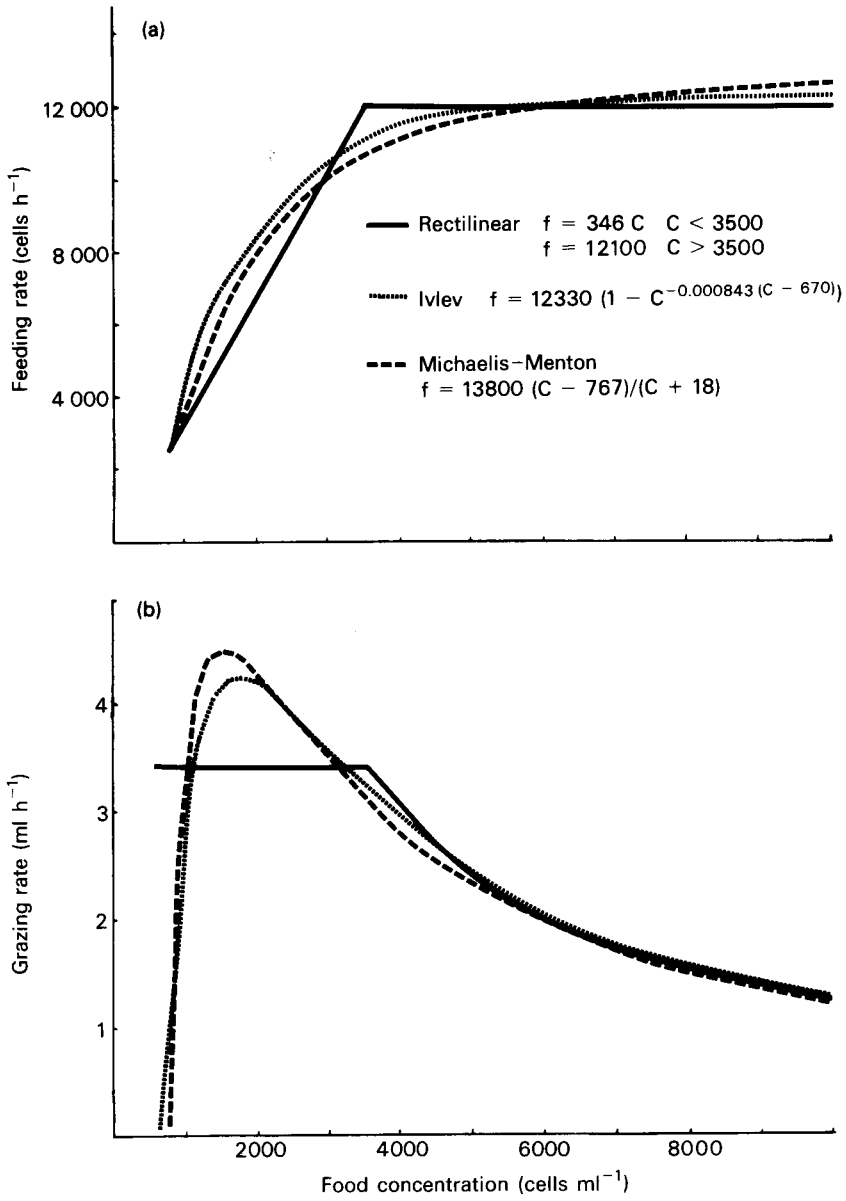

Fig. 9.9 Three models which describe feeding rate as a function of food concentrations (a) and their solution to provide grazing rates (b). Each model is an equally good description of the data (Frost 1972) on which the models are based. Panel (a) is modified from Mullin *et al.* (1975). The rectilinear model provides quite different descriptions of filtering rate at low food concentrations and this may permit one to distinguish the appropriate model (Frost 1975).

Finally, the age of the cell culture used in feeding experiments has been shown to influence feeding; senescent cultures are eaten less readily than those in exponential growth (Ryther 1954; McMahon & Rigler 1963; Mullin 1963; Marshall & Orr 1966).

The effects of these sources of variation on estimates of feeding or grazing rates are difficult to judge. Certainly, they reduce the absolute value; however, it is also possible any qualitative trends observed in such experiments—such as the effects of food size or animal body weight—are also unnatural. Rigler (1971a) pleaded for greater care in experimentation and further study of these artefacts. Although most of us are not interested in the behavior of starving animals feeding on unpalatable and monotonous foods, crowded into small beakers etc., these effects can bias our results. We should put more effort into the evaluation of such effects.

3.7 *Average experimental conditions*

The number of factors which can influence determinations of grazing and feeding rates may daunt a researcher seeking to enter this field. Ideally, one would examine the effect of all aspects of the experimental treatment on the animals and the results. However, few of us have the time to study all potential sources of error, and we must turn instead to the literature for some direction in planning our experiments.

Such searches are often haphazard. To provide a more quantitative summary of some aspects of experimental design, the literature surveyed in preparing this review is summarized in Table 9.2. This shows the mean, median, mode and range of values reported for seven variables which are likely to affect grazing rate estimation. One cannot assume that any of these values are good, in the sense that observations made under these conditions are unbiased. At best, one can only hope that such values are no worse than others in the literature.

For comparison, it may be instructive to consider the range which such variables may take in nature. Straškraba (1980) compiled the average annual surface temperature of moderate-sized lakes. He found a range between 1 and 20 °C and a range of 0–30 °C is probably sufficient to represent the range in lakes. Ponds and puddles may be somewhat warmer. The concentration of naturally occurring phytoplankton varies between 0·3 and 100 p.p.m. by volume (McCauley & Kalff 1981). Nannoplankton concentrations, which may better reflect available food, range from 0·1 to 10 p.p.m. (Watson & Kalff 1981). Planktonic crustacean concentrations range from 0·06 to 70 p.p.m. (by volume). This corresponds to about 1–700 animals, each weighing 10 μg dry weight, per litre. The volume of water per animal is therefore between 1·4 and 1000 ml.

Table 9.2 Summary of typical conditions and independent variables used in the estimation of filtering and feeding rates of cladocerans and calanoid copepods. The values listed in this table summarize those used in the literature and do not represent recommended values. W = animal dry weight, μg; S = food concentration, p.p.m. by volume; V_i = cell volume, μm^3; T = temperature, °C; H = volume per animal, ml; M = experimental duration, min; L = total volume of experimental vessel, litres.

	Median	Mode	Mean	Range	n
Cladocerans					
W	20	10	26	0·5–300	191
S	4·5	0·8	4·5	0·38–180	160
V_i	50	50	68	0·1–6 150 000	190
T	20	20	18·6	5–35	179
H	6	8	15·8	1–80	86
M	30	30	198	2–1440	158
L	0·125	0·125	0·441	0·004–10	190
Calanoids					
W	11	195	35	0·4–74 000	429
S	2·8	0·5	2·9	0·02–510	411
V_i	2500	2500	5370	0·5–6 900 000	303
T	15	15	12·4	2–35	340
H	20	3	88·7	1–400	245
M	1440	1440	1219	5–13 300	364
L	0·500	0·700	0·187	0·010–10	352

3.8 Comparison of methods

Surprisingly, few scientists have made direct comparisons of available methods for the estimation of feeding by zooplankton. To augment these comparisons, I have plotted weight specific rates of grazing for calanoid copepods and cladocerans measured with four basic techniques. Figure 9.10 shows first that workers with cladocerans have favoured short-term tracer experiments while workers studying calanoids prefer to use Coulter counters.

Grazing rates generated by the various techniques are quite variable and hence there is considerable overlap among methods. Long-term tracer experiments may yield values slightly lower than other techniques. Those grazing rates obtained using Coulter counters or cell counts are the most variable. However, the variability of grazing rates (5 orders of magnitude) makes it difficult to separate the distributions for different techniques.

When frequency diagrams of the weight specific grazing rates of the two groups are prepared, ignoring the methods used, the histograms in Fig. 9.11

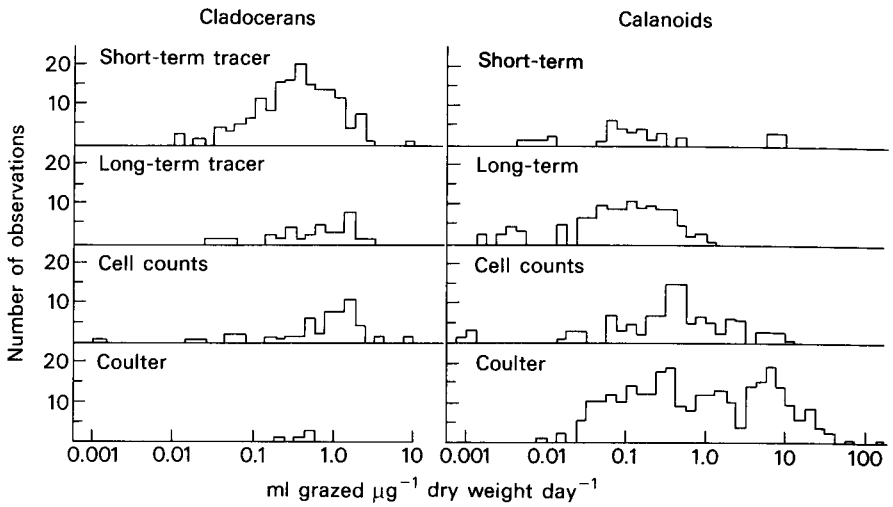

Fig. 9.10 Frequency distribution of weight specific grazing rates for cladocerans and calanoid copepods measured with four different techniques. Data were collected from an intensive literature survey, but only one point for each combination of food type and zooplankton species was taken from each publication. The variance in the literature does not seem to result from methodological differences.

are obtained. These show that, although calanoid copepods may filter at higher rates than cladocerans and show more variation, the median values for the two groups are identical. The higher variation in copepods probably represents their greater range of feeding behavior and their greater sensitivity to experimental conditions. The high values almost always reflect the use of large (i.e. $\geq$2·5 l) experimental vessels. Figures 9.12 and 9.13 are similar plots for feeding rate.

The similarity of the median values is in contrast to the results of several experiments which compared cladoceran and calanoid grazing rates (Nauwerck 1963; Haney 1973; Bogdan & McNaught 1975). These suggest that copepods graze at lower rates than cladocerans. However, most of these experiments used methods (small volumes, small cells) which are more appropriate for cladocerans and may underestimate the potential of copepods.

The comparisons in Figs 9.10 and 9.12 are necessarily coarse and they must be interpreted in the light of direct experimental confrontation obtained by various methods. Conover & Francis (1973) measured grazing rate with cell

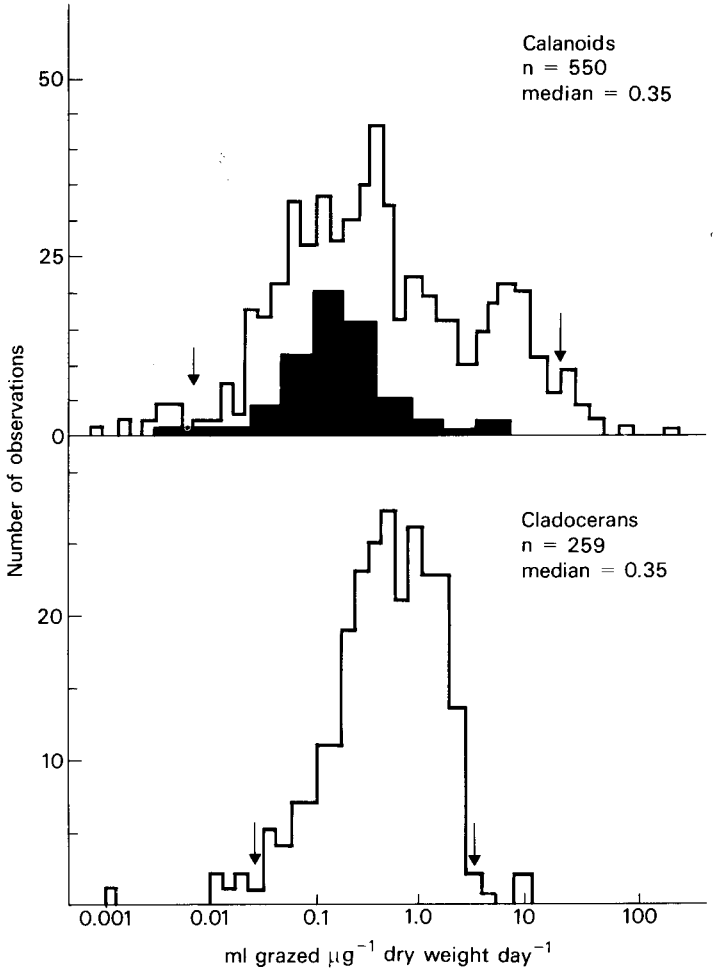

Fig. 9.11 Frequency distribution of weight specific grazing rates for cladocerans and calanoid copepods, regardless of method used. 95% of all points lie between the arrows. Although calanoids have shown a greater range in measured rates the median values are similar. Freshwater calanoids are represented by the solid histogram, which uses a larger interval.

counts and long-term tracer experiments. They concluded that the latter underestimate G and so confirmed the reservations of Sorokin (1968). Nauwerck (1959, 1963) employed both cell counts and short-term tracer experiments and found that the cell count method gave somewhat higher values (5 versus 3 ml individual^{-1} day^{-1}). However, his experiments were not paired and he preferred the tracer method because of its greater rapidity and

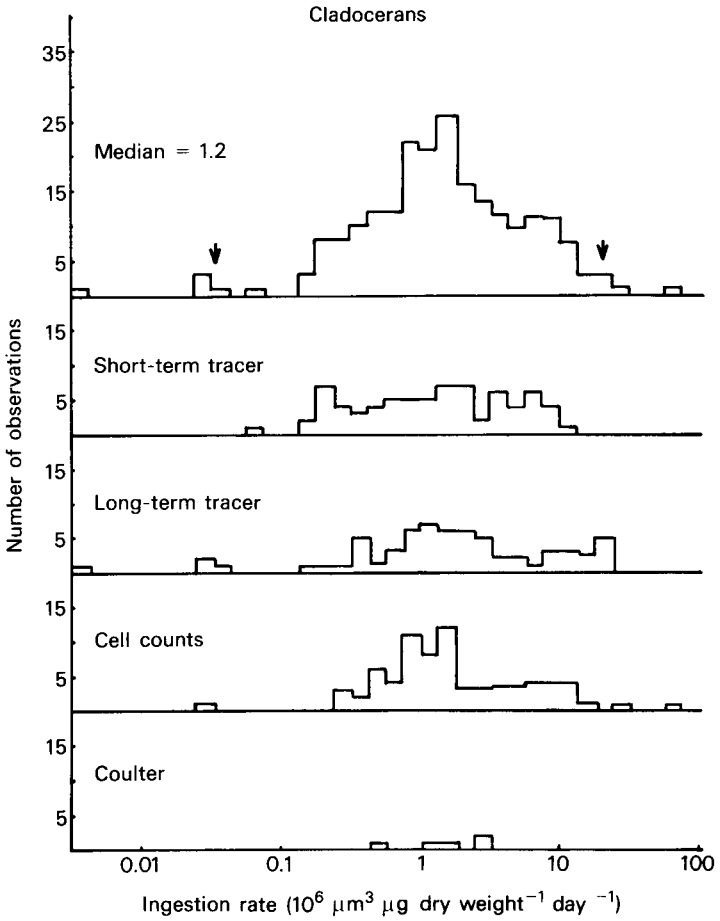

Fig. 9.12 Frequency distributions of weight specific ingestion rates for cladocerans measured with four different techniques. The upper panel shows a cumulative distribution ignoring methodology.

precision. Richman & Rogers (1969) measured the feeding rate of *Calanus* on single and paired cells using a Coulter counter and a radiotracer technique. They reported that Coulter counts showed higher grazing rates on double than on single cells, but the short-term radiotracer estimate for animals feeding on a labeled mixture of both types was as high as that measured for paired cells using a Coulter counter. This suggests that breakage of the paired cells resulted in underestimates of the grazing rates on single cells as measured by the Coulter counter. If both methods were equally effective the tracer estimate would have been lower than observed. Taguchi & Fukuchi (1975) and Taguchi & Ishi (1972) compared the grazing rate of *Calanus* measured from changes in

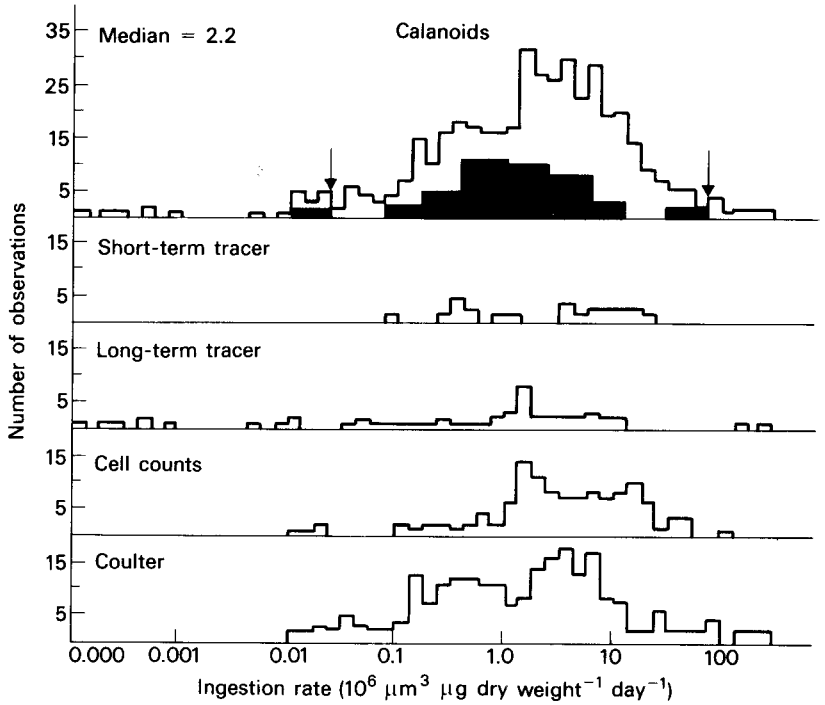

Fig. 9.13 Frequency distributions of weight specific ingestion rates for calanoids measured with four different techniques. The upper panel shows a cumulative distribution ignoring methodology. Freshwater calanoids are represented by the solid histogram which uses a larger interval.

cell number, chlorophyll concentration, sestonic carbon concentration, and (in smaller vessels) long-term incorporation of ^{14}C from labeled cells. They found that chlorophyll and ^{14}C gave similar estimates but were lower than grazing rates estimated from changes in carbon concentration or cell counts. Hargis (1977) compared the grazing rates of *Acartia* on small natural phytoplankton using Coulter counts, change in chlorophyll and short term uptake of ^{14}C from labeled cells. His results were quite variable and no statistical difference existed among the methods. Haney's (1973) *in situ* estimates of the grazing rate of *Daphnia* can be compared to those obtained by adding radioactive cells to a sample of lake water in a beaker (Burns & Rigler 1967). Although these experiments were conducted in different years, both studies used the same species of zooplankton from the same lake, and measured grazing rate using short-term tracer experiments in which ^{32}P-labeled *Rhodotorula* was the tracer particle. Both experiments gave grazing rates which were $\frac{1}{2}$ to $\frac{1}{6}$ of those predicted on the basis of measured grazing rates in single species suspensions of food.

These comparisons suggest that long-term tracer experiments may

underestimate grazing rates and that the other techniques are roughly comparable. However, no set of comparisons has been conducted under a range of conditions and available comparisons often differ in more than the method of evaluating changes in cell concentration. More intensive study is required before any one method can be unhesitatingly recommended.

3.9 Calculated grazing and feeding rates

One often wishes to estimate the feeding or grazing rates of zooplankton but is unable to invest the time and effort required to make adequate measurements. To meet this need, Peters & Downing (in preparation) performed multiple regression analyses of the data used to construct Figs. 9.10–9.13. These analyses predict grazing rate (in ml animal^{-1} day^{-1}) or feeding rate (in μg wet weight of food animal^{-1} day^{-1}) from animal dry weight (W, in μg), food concentration (S, in p.p.m. volume/volume), individual food cell size (V_i, in μm^3), temperature (T, in degrees centigrade), volume of water per animal (H, in ml), experiment duration (M, in minutes), and container volume (L, in liters). Of these factors only those which had a significant ($p < 5\%$) effect were retained in their regression equations. For cladocerans, the equations are:

$$\log_{10} G = 0{\cdot}173 + 0{\cdot}75 \log_{10} W - 0{\cdot}43 \log_{10} S - 0{\cdot}33L + 0{\cdot}014H \quad (n = 70;\ R^2 = 0{\cdot}68) \qquad (9.20)$$

$$\log_{10} f = -1{\cdot}34 + 0{\cdot}49 \log_{10} S + 0{\cdot}59 \log_{10} W + 0{\cdot}014H - 0{\cdot}16(\log_{10} V_i)^2 + 0{\cdot}89 \log_{10} V_i + 0{\cdot}027T \quad (n = 81;\ R^2 = 0{\cdot}66) \qquad (9.21)$$

For marine calanoids, they found:

$$\log_{10} G = -1{\cdot}25 + 0{\cdot}53 \log_{10} W + 0{\cdot}13L + 0{\cdot}68 \log_{10} V_i - 0{\cdot}000\,18M - 0{\cdot}067(\log_{10} V_i)^2 \quad (n = 272;\ R^2 = 0{\cdot}69) \qquad (9.22)$$

$$\log_{10} f = -0{\cdot}039 + 0{\cdot}37 \log_{10} W - 0{\cdot}000\,30M + 0{\cdot}48 \log_{10} S + 0{\cdot}59 \log_{10} V_i - 0{\cdot}060(\log_{10} V_i)^2 \quad (n = 246;\ R^2 = 0{\cdot}52) \qquad (9.23)$$

For all data including cladocerans, calanoids plus rotifers, euphausiids etc.:

$$\log_{10} G = 0{\cdot}11 + 0{\cdot}55 \log_{10} W - 0{\cdot}26 \log_{10} S + 0{\cdot}099L + 0{\cdot}12 \log_{10} V_i - 0{\cdot}000\,20M \quad (n = 350;\ R^2 = 0{\cdot}66) \qquad (9.24)$$

$$\log_{10} f = -0{\cdot}353 + 0{\cdot}54 \log_{10} W + 0{\cdot}58 \log_{10} S - 0{\cdot}000\,22M + 0{\cdot}47 \log_{10} V_i + 0{\cdot}060L - 0{\cdot}045(\log_{10} V_i)^2 \quad (n = 355;\ R^2 = 0{\cdot}63) \qquad (9.25)$$

The order of entry of the independent variables in the above equations reflects their partial significance in the multiple regression analysis. The most significant factor is listed first. The equations use all data available to the authors and so must include erroneously high and erroneously low values. Residual analysis of these regressions suggests that rotifers and fresh water calanoids feed and graze more slowly than other animals. Radioisotopic techniques give lower values than average, while cell and Coulter counts give higher values. The average equations represent the most complete composite value which can be derived from the literature and this cannot be assumed to be more correct than carefully measured rates. However, these equations are more representative than a casual selection of values from the literature.

3.10 The expression of selectivity

The past decade has seen increased interest in the food preferences of zooplankton and other animals. Increased grazing mortality on any one food class and the possibility that animals may switch their preferences with food availability have powerful implications for patterns of phytoplankton succession and competition. Since zooplankton are no longer thought to have a similar effect on all potential food items, grazing rates must be adjusted to account for differences in catchability (Gliwicz 1970) if they are applied to real systems. Measurement of these capacities requires a quantitative index of food preference.

Many indices of selectivity or electivity have been suggested and several reviews of these proposals have recently been published (Jacobs 1974; Chesson 1978; Cock 1978; Paloheimo 1979; Vanderploeg & Scavia 1979). All indices seek to compare the proportion of each food type in the environment with the proportion in the diet—high values of whatever index is used should indicate preference and low values should indicate avoidance. Lechowicz (1982) reviews the desirable characteristics of electivity indices and compares the adequacy of the existing alternatives.

For zooplankton, the amount of available food of each type in the environment is determined from plankton samples; the amount of each food type in the diet is determined from feeding experiments or by gut analyses. Both estimates are only operational approximations. Zooplankton can, and do, browse on surfaces and in sediments (Lowndes 1935; Nadin-Hurley & Duncan 1976; Horton *et al.* 1979) so plankton samples may not reflect available food resources, at least in shallow waters. Since particle modification may affect estimates of feeding rate, and differential digestion obviously affects gut analyses, the actual diets may be quite different from those determined. These pitfalls in the experimental methods cannot be circumvented by the choice of index.

The most common indices used are the forage ratio (F.R.) and Ivlev's index (I) (Cock 1978). For both, the experimenter must determine the proportion of food type i which is eaten (r_i), from the number of this type in the ration or gut (N_{r_i}):

$$r_i = N_{r_i}/\Sigma N_{r_i} \tag{9.26}$$

and p_i, the proportion of food type i in the environment is determined from counts (N_{p_i}) of each type in the environment:

$$p_i = N_{p_i}/\Sigma N_{p_i} \tag{9.27}$$

The forage ratio is then calculated as:

$$FR = r_i/p_i \tag{9.28}$$

and Ivlev's index as:

$$I = (p_i - r_i)/(r_i + p_i) \tag{9.29}$$

Both indices have serious disadvantages.

(1) Both change as the ambient proportion of various foods change through grazing (Jacobs 1974), even though the animals maintain the same behavior.
(2) The value of the index for any one food type is not independent of the other values; thus, positive selection on one class imposes negative selection on the others (Boyd 1976) and an error in counts in one class imposes an error on all other classes.
(3) Because both indices are ratios of ratios, the values obtained will have very broad statistical limits (equation 9.11) and are subject to bias (Chapter 8).

Several alternatives to these indices have been presented (Jacobs 1974; Chesson 1978; Cock 1978; Vanderploeg & Scavia 1979), however those of us who work with suspension-feeders need not consider these alternatives in detail since they amount to comparisons of mortality rate in each class which, of course, is the same as a comparison of grazing rates on each class. Grazing rate is a simple, effective and practical index of selectivity among a series of food classes: the class on which grazing rate is highest is that most selected. In theory, the grazing rate on each class is independent of the other classes and this value is calculated as a simple ratio rather than as a ratio of ratios. More complex or obscure formulations give no more information.

Larger animals will have higher grazing rates than smaller ones, although they may be no more selective in the sense that their predation draws a greater proportion from some food class. In comparison of the impact of animals of different size of algal succession, this is quite appropriate because larger animals will have more effect. However, if one desires to compare the selective

behavior of two such animals, the data may be standardized by plotting the results on graphs of identical size (i.e. in cm) (Richman *et al.* 1977; Allan *et al.* 1977). The same effect is achieved by dividing the measured grazing rate on each class by the sum of all grazing rates (Vanderploeg & Scavia 1979). Because food selection is a complex behavior which depends at least upon the relative and total densities of predator and prey, it should not be compressed into a single, one-dimensional measure or index. The only exception to this is the case in which only two foods are available or in which pair-wise comparisons are made. In this case, the ratio of the grazing rates on the two foods would suffice.

Theoretically, grazing rate in ml cleared individual^{-1} day^{-1} is equal to the mortality rate imposed on algae in 1 ml of water by a single zooplankter. Multiplication of G by the concentration of animals per ml yields a value which is the mortality rate of the algae or food particles, the dimensions of which are day^{-1}. This should be a useful index of electivity in other systems as well.

4 Assimilation

Viable gut passage (Porter 1973, 1975) and differential digestability (Lefèvre 1942; Arnold 1971; Lampert 1977a) of food cells underline the difference between the ingestion of food by zooplankton and its use in secondary production or respiration. The absorption of material from the gut, or its ecological assimilation, is in part measured in an attempt to differentiate between these processes. Although assimilation rate is not measured as frequently as grazing and feeding rates, a number of methods have been developed.

4.1 Estimates based on defecation rates

The simplest approach, conceptually, to the determination of assimilation rate is based on the measurements of rates of ingestion and defecation:

$$\text{Assimilation rate} = \text{Ingestion rate} - \text{defecation rate} \qquad (9.30)$$

in which all rates are expressed in the same units. Because techniques for the estimation of ingestion rates have been reviewed above only the measurement of defecation need be considered in this section.

Defecation rates are measured from quantitative collection of feces produced over a known time interval. These feces are then weighed (if gravimetric analyses are intended), burned (if calorimetric estimates of assimilation are necessary), or analyzed chemically (if the assimilation rates of some element or compound are sought). The amount of material defecated is then divided by the length of the collection period to yield a defecation rate.

The conceptual simplicity of this method should not obscure its practical complexity. Since the estimate will be subtracted from the ingestion rate it is essential that the conditions of the experiment should be identical to those used in the accompanying ingestion experiments. The animals should be kept in a steady state (i.e. defecation rate should be constant over the experiment).

The method is restricted to species which always produce discrete and coherent fecal pellets—this certainly excludes cladocerans and may not include all copepods or any copepod at certain times (see Section 3.3.2). Moreover, once released from the body, fecal pellets may undergo rapid changes in composition both through bacterial colonization and loss of soluble fecal material (Johannes and Satomi 1966, 1967). Some elements, like phosphorus, quickly leach from dead plankton (Golterman 1964; Krause 1964). Coprophagy must be avoided (Frankenberg *et al.* 1967). In the absence of information to the contrary, it is prudent to assume that any fecal material will alter rapidly and so must be removed as soon as possible after release from the animal. This may prove difficult to do without disturbing the animal and thereby altering the rate of ingestion or defecation.

Fecal pellets contain not only unabsorbed food but also products of the animals themselves: the chitinous peritrophic membrane, cells sloughed from the gut, mucus, intestinal bacteria and so forth. The amount of material in the feces can, therefore, only approximate the unutilized and unabsorbed portion of the food. However, such an approximation is probably sufficient for most ecological applications.

4.2 Estimates based on fecal analysis

For some experiments, it may be inconvenient to measure the rate of feces production but quite feasible to analyze a non-quantitative sample of the feces. Conover (1966b, 1966c) has found that assimilation rate may be estimated from ingestion rate if some component of the food is completely unassimilated. If an animal has an ingestion rate (I), and the food contains an unassimilable fraction (U), such that:

$$\text{U} = \text{mass of unassimilable material/food mass} \qquad (9.31)$$

The feces will contain a higher proportion of the unassimilable fraction (U′), since a unit of this material is unaffected by gut passage but the total amount of food will have been reduced by the amount of the food which was assimilated. The assimilation rate (A) can then be calculated as:

$$\text{A} = \text{I} \times \text{U}/\text{U}' \qquad (9.32)$$

The numerator and denominator used in the calculation of U′ (equation 9.31) and U must be expressed in appropriate terms (grams, Joules, μg N, etc.).

The advantage of this technique is that it eliminates the need for quantitative collection of the defecated material. The method assumes:

(1) That the unassimilable material is indeed unassimilated.
(2) That the animal does not select against this indigestible substance.
(3) That this material moves through the gut at the same rate as the digestible fraction.
(4) That this substance is not lost from the feces once defecated (Wightman 1975).

Conover (1966b) suggested that the ratio of ash-free dry weight to ash in the food and feces be used to provide U and U′. He showed that these ratios can give estimates of assimilation which are comparable to those measured gravimetrically (Conover 1966c). However, assimilation rates of some components of the ash, such as phosphorus, have repeatedly been shown to be significant (Peters & Rigler 1973). Lasenby & Langford (1973) estimate that 70 % of the ash in the food of *Mysis relicta* is assimilated. This would lead to overestimates of assimilation rate, since U′ would be decreased. Conover (1966b) supposed that U could be measured fron the ratio of ash-free dry weight to ash in plankton samples, but growing evidence for selective feeding suggests that total plankton may not represent zooplankton food.

Even if the original formulation of the method is not correct in detail, a number of modifications are possible. Calow & Fletcher (1972) proposed that an unassimilated radioactive tracer (e.g. ^{51}Cr) could be used in place of ash. Unfortunately, it is difficult to label a food cell with a material which cannot be assimilated by the cell's predator. In practice, a compromise is reached and corrections for the assimilation of this material are applied (Wightman 1975). In principle, other materials could substitute—for example, one could use the number of chitinous remains of prey animals or the tests of food cells in the feces (Rigler 1971b) as the numerator in equation 9.31. These methods would require extensive examination before use. For example, B.Marcotte (personal communication) suggests that some copepods may ingest only the contents of diatom cells, not their tests. It might be possible to label cells which are ingested but not assimilated with one isotope and other cells with a second. In any case, the tracer material must not be selected for or against, it must pass through the gut with the food but must not be assimilated, and it must not leach from the feces before collection.

4.3 Direct measurement of assimilation

Isotopic estimates of assimilation are possible only for elements or non-degradable compounds. Extrapolations to assimilation in terms of total mass,

energy or other materials are only possible through conversion factors and should not be considered more than rough approximations.

The methods are essentially the same as those used for isotopic measurement of ingestion rate. Basically, the uptake of tracer by the animals is measured over a time series, such as that shown in Fig. 9.5. Assimilation rate is approximately proportional to the slope of the uptake curve after the inflection point which indicates the initiation of egestion of radioactive feces. If the radioactivity per animal at t_2 minutes, and later at t_3 minutes is A_2 and A_3 respectively, then assimilation rate (A) can be calculated as:

$$A = (A_3 - A_2)/(t_3 - t_2)s \tag{9.33}$$

where s is the specific activity (radioactivity/unit mass) of the substance for which assimilation is to be determined. Lampert (1977a) has reviewed the methodological difficulties with this approach.

One of the major problems results from the excretion of assimilated tracer. This reduces the uptake rate calculated in equation 9.33. The error becomes more severe as the experiment becomes longer. Lampert (1977a) points out that the amount of excreted tracer declines rapidly after removal of the animal from its labeled food. Thus, the size of the underestimate due to tracer excretion can only be estimated by determining the reduction in tracer excretion with time after removal from the radioactive food and extrapolation to zero time (Peters & Rigler 1973; Peters 1975b) or by measuring the rate of appearance of soluble tracer simultaneous with measurement of the rate of assimilation (Lampert 1977a). The former is the only method advisable if rates of re-uptake of excreted tracer by the animals or by the remaining food cells are significant (Peters & Lean 1973; Ganf & Blazka 1974). This is certainly the case with phosphorus, but probably not so with carbon. Tracer excretion can result in underestimates of assimilation of 10 % in experiments which last only 30 minutes (Peters 1975a).

The measurement of assimilation by isotopic accumulation requires that any tracer uptake between t_2 and t_3 represents only uptake by the body of the animal. The radioactivity of the gut remains unchanged because all non-radioactive food in the gut was, in principle, flushed out before t_2. This may not be so. Smirnov (1974), Schultz & Kennedy (1976) and Marcotte (1977) report that antiperistaltic movement may lead to refluxing of the gut contents. This would lead to overestimates of assimilation rate in short-term experiments as some of the tracer accumulated after defecation begins would represent further filling of the gut. However, microscopic estimates of time to defecation suggest that the amount of mixing of the gut contents is rather small.

A more serious problem in the measurement of tracer assimilation is

incomplete labeling of the food cells. This leads to low values of s in equation 9.33 and so causes overestimation of assimilation rate. Since most work has used algal cultures which were labeled for only a day (Bell & Ward 1970; Arnold 1971; Lampert 1977a) it is unlikely that all compartments within the food are fully labeled. For example, it is probable that structural components, such as cell walls, become labeled with ^{14}C-bicarbonate more slowly than metabolically active sugars. This problem may be avoided if the cell cultures are grown in a medium of constant specific activity (Conover & Francis 1973; Peters & Rigler 1973).

Lampert (1977a) checked for the influence of incomplete labeling by comparing assimilation with the sum of growth and respiration. The close correspondence probably indicates that most of the carbon in his cells was labeled. Since it is improbable that the cell walls were labeled (Lampert reported little growth in population during labeling), one must speculate that, under the culture conditions used, the cells fixed and stored a large quantity of uniformly labeled carbohydrate (Morgan 1976). A more effective check for complete labeling of the food cells may be made by dividing the cells into two or more arbitrary fractions. Cells may be sonified and filtered and the specific activity of material in the filtrate and particulate fractions determined or one may measure the specific activity of acid extractable and residual components (Lampert 1977a). In fully labeled cultures, all fractions should have identical specific activities. Since the fractions are arbitrary, it is conceivable that two such different fractions could have similar specific activities before complete labeling was achieved. If possible, two or more distinctive fractionations should be used.

In any measurement of assimilation, the researcher should be aware that absorption of the material does not indicate that the assimilated substance is used in production by the zooplankton. High assimilation rates do not necessarily correlate with high growth rates for animals feeding on that food (Arnold 1971; Lampert 1977b). This may reflect antibiotic effects of the food, some nutrient deficiency in the algae (Taub & Dollar 1968) or an error due to incomplete labeling of the cells. Researchers should test for the effect of easily assimilated foods in zooplankton growth experiments.

Finally, a comment on the presentation of assimilation rates is in order. Most estimates are presented as assimilation efficiencies (equation 9.1). In the case of the ash-free dry weight method and its analogues, assimilation efficiency must be calculated to determine assimilation rate from ingestion rate. Because assimilation efficiency is a ratio of two experimental values, the variance around the mean is magnified. Partly as a result of this, published values for assimilation are notoriously variable (i.e. 8–100%—Conover 1964; Peters 1972). Assimilation should be presented as a rate, not a ratio, whenever possible.

5 Conclusions

Every experimental technique is open to abuse and the data obtained from any method must be interpreted in light of the capabilities of the techniques employed. Careful researchers should approach their hypotheses from as many experimental directions as possible and hypotheses resting solely on one technique should be treated with a healthy scientific skepticism until tested with alternative techniques. It is not enough to state the assumptions of the method chosen; the validity of these assumptions should be tested explicitly. Techniques for the measurement of feeding and grazing by zooplankton are among the most developed, and, when used properly, give us some of the best estimates available for feeding of any group. I hope this review will increase our already high level of achievement.

The research community involved in measurements of feeding and grazing is now so large that it risks defining a 'normal science' of Zooplanktophagology, one which creates and tests theories which are interesting only to members of that community. We must continually examine and re-affirm the ecological relevance of our studies. While the minute aspects of zooplankton feeding may be of interest to us, we must also consider their utility and relevance. The techniques for the measurement of feeding, grazing, and assimilation rates are powerful; it is for us to find a place for these measurements in the broad scheme of material and energy flow both in lakes and ecosystems in general.

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Chapter 10. The Measurement of Respiration

WINFRIED LAMPERT

1 Introduction

The aim of estimating secondary production cannot only be to obtain a number for the productivity of a certain lake or river. In order to understand the functioning of a system, one must know which factors limit secondary production and how production changes with changing environmental conditions. The production of different species may respond differently to the change of one factor. Production is the visible result of many physiological processes; it is, therefore, worthwhile to examine closely the processes which contribute to it and the effect of environmental changes. Losses of energy or matter during metabolism are one of the processes important to production.

When production is considered as the sum of accumulated matter in somatic growth and reproduction, it can be determined in long-term experiments without measuring any metabolic losses. However, when the energy or material budget is the center of interest, exact knowledge of the losses and the way in which they vary is essential. It should be mentioned that there is no coupling between the 'metabolic activity' of an animal and its production, *per se* even though animals that exhibit a high metabolic turnover also have a high potential rate of production under optimal conditions, this production rate may not always be realized. Large-scale interspecific comparisons resulted in linear correlations between log R and log P (McNeill & Lawton 1970, Humphreys 1979, Lavigne 1982). Different regression lines could be calculated when metabolically similar species were grouped. For a group of short-lived poikilotherms including several freshwater animals, McNeill & Lawton (1970) established the regression line:

$$\log P = 0{\cdot}8262 \log R - 0{\cdot}0948 \qquad (10.1)$$

(both expressed as kcal $m^{-2} yr^{-1}$). If reliable measurements of respiration during the season are available, accumulated production can be estimated in this way, even if this can only be a rough approximation due to the considerable scatter of the points obtained. Predictions for individual species under certain environmental conditions should not be made (Lavigne 1982).