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Chapter IX

Physiolgical Diversity in Widely Distributed Microzooplankton: Digestion in the Ciliate *Euplotes vannus*

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Abstract

Many planktonic microbes exhibit nearly global distributions. Apparent adaptability to wide ranges of environmental conditions in cosmopolitan species could involve physiological diversity among individuals. However, variability between individuals among microorganisms is rarely considered. We examined individual variability in the common marine ciliate *Euplotes vannus*. Digestion was followed in individual cells subjected to different levels of ambient food concentration. Average digestion times were not significantly different because, in the absence of food or in the presence of super-saturating food concentrations, there was a large variability between individuals. Some ciliates completed food vacuole processing under 1 h while others took as long as 17 h. The range of digestion times, which increased under extreme conditions, may correspond to range of growth rates or growth potentials among individuals.

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Introduction

Limits of Averages

Variation between individuals is a component of biological diversity which has received very little attention; commonly attempts are made in to minimize, rather than characterize, individual variability (Spicer & Gaston 1999). Establishing and comparing "averages" is the standard means of describing populations or species. This is true despite the fact that employing averages has familiar pitfalls such as masking distinct differences within a group and thus representing a descriptor of little value. Obviously, averages are acknowledged to be mathematical representations, not literal descriptions, but the possibility that averages may be unrealistic representations can be easy to neglect. Consider for example, estimating population reproductive rates with averages and a hypothetical human population with an "average" birth rate of 2.5 children per female. Let the number 2.5 represents the average of two sets of individuals (equal in abundance and mortality rates) but with different fertilities, say one with 1 child and the other with 4 children per female. Not only does t the "average" female does exist in this population but more importantly, if any factors differentially influence the two distinct sets of individuals, it would be impossible to predict the net effect using a single arithmetic average value of fertility. Thus, consideration of average rates may be not only unrealistic in the sense the "average does not exist", but can lead to erroneous conclusions. It is then of some significant interest to consider physiological diversity within a population. This has long been recognized and most recently underlined by Spicer & Gaston (1999). For any particular species, its capability to colonize new habitats, or react to shifts in climatic conditions, or adapt to changes in resources are the types of topics difficult if not impossible to address without some knowledge of the physiological diversity found within the species in terms of diversity among individuals.

With regard to planktonic organisms, physiological diversity between individuals is very rarely assessed. Firstly, as most planktonic organisms are microscopic observations of individual microorganisms are techniquely difficult. Secondly, they live in time and space scales very distinct from those with which we are familiar. Perhaps though the most important reason is that individual variability among microbes are simply not considered to be of much importance. Microbes and most planktonic organisms are characterized by very large population sizes, and as large sample or population size is often equated with 'normality' of distribution, average values are thought to be good descriptors.

Here we report on physiological diversity in the planktonic protist ciliate *Euplotes vannus*. Data on the variability of digestion with feeding conditions, ambient food levels, was gathered. The experiments were not designed originally to establish variability or ranges of rates, but rather to produce averages. We were able to examine physiological diversity only because we followed individual ciliates. However, we show the inadequacy of using averages to describe a physiological rate in the ciliate. Below, we place planktonic ciliates in their ecological rôle and briefly describe the nature of previous studies on *Euplotes vannus*, on which a great many studies have been conducted but very few of which documented differences between individuals.

Ecological Role of Plankton Ciliates and Euplotes Vannus

In the plankton, primary production is carried out by organisms occupying 3 different size-classes: 1) picoplankton-size organisms (0.2 - 2.0 μm in size) autotrophic prokaryotes such as *Synechococcus* and *Prochlorococcus* and small eukaryotic flagellates, 2) nanoplankton-sized organisms (2.0 - 20 μm) mainly eukaryotic flagellates and small diatoms, and 3) microplankton-size organisms (20 -200 μm in size) primarily large dinoflagellates and diatoms. Microzooplankton, composed of planktonic ciliates and heterotrophic dinoflagellates between the sizes of 20 and 200 microns, are the consumers of much of this primary production. Calbet & Landry (2004) estimated that a surprisingly constant 70 % of total planktonic primary production is consumed by ciliates and dinoflagellates across marine systems. This consistency and magnitude of microzooplankton grazing has been challenged (Dolan & McKeon 2005) but there is little doubt that microzooplankton are important in plankton food webs. Microzooplankton, are the link between nanoplankton, and to a lesser extent pico-plankton, and the higher trophic levels which prey on microzooplankton in the form of copepods, larval fish, etc. Among the ciliate component of the microzooplankton, distinct trophic guilds (groups of ciliate species whose diets appeared specialized to some degree) can de distinguished (Dolan 1991). Ciliates whose prey are mainly pico-plankton sized prey are microphagous, those feeding on larger prey are macrophagous and ciliates capable of feeding on other ciliates are predatory. *Euplotes vannus* feeds ciliates as well as other prey types and so is considered a predatory ciliate.

Euplotes Vannus

We isolated *Euplotes vannus* from the Chesapeake Bay. However, the ciliate is very widely distributed, as are many species of the microzooplankton. Euplotes are described as "marine cosmopolitan" and indeed *Euplotes vannus* is found in a wide range of ecosystems from the Chesapeake Bay, the origin of the cultures used in the present work (Dolan & Coats 1991a), to the coastal waters of Denmark (Fenchel 2004), Japan, Italy and California (e.g., Dini & Nyberg 1999). In common with most species of *Euplotes*, while often found in the plankton, it is ordinarily found among the benthic ciliates. In size it is about 100 microns in length. *Euplotes vannus* appears to feed upon algae, other ciliates as well as bacteria. It can be among the ciliates which colonize marine snow particles (Artolozaga et al. 1997).

Euplotes vannus has been the subject of many investigations. The species was used in some of the first studies on nutrient regeneration by protists (Johannes 1965; Gast & Hörstman 1983). The relationships between food type and food concentration and population growth have been investigated (Capriuolo et al. 1988; Dini & Nyberg 1999; Dolan & Coats 1991a). Selective feeding has been examined (Premke & Arndt 2000; Lewitus et al. 2006; Scott et al. 2003). Behavioral experiments have been conducted with *Euplotes vannus* (Tomaru et al. 2001; Fenchel 2004). Taken as a typical coastal ciliate, it has been subjected to several toxicology studies (Stebbing et al. 1990; Coppellotti 1988; Ricci et al. 1997; Xu et al. 2004; Mori et al. 2003). Many membrane physiology investigations have focused on *Euplotes vannus* (Stock et al. 1977; Kruppel & Wissing 1996; Kruppel et al. 1995; Lueken et

al. 1996). Molecular and phylogenetic data have become available in recent years (Petroni et al. 2003; Shang et al. 2002; Cheng & Song 2002; Petroni et al. 2002). While diversity, including physiological, between strains or mating types has been considered (Walton et al. 1995; Jones & Gates 1994; Caprette & Gates 1994) physiological diversity between individuals, to our knowledge, has not been addressed.

Use of Digestion Data

The interest in examining digestion in ciliates may appear obscure but it is potentially very useful in the estimation of feeding in natural populations. Estimating feeding rates in field populations is generally problematic with regard to planktonic organisms. One manner, possible in principle, is using 'gut contents' combined with digestion rate data. The ingestion rate, assuming steady-state conditions, equals food contents multiplied by digestion rate. The interest in the approach lies in the possibility of combining experimentally derived digestion rate data with estimates of average food contents from *in situ* or natural populations. Food content data can often be obtained relatively easily by analyzing freshly caught animals with little manipulation. This approach has been applied to benthic ciliates (Goulder 1972, 1973; Fenchel 1975), planktonic ciliates (Kopylov & Tumantseva 1987; Dolan & Coats 1991b) and heterotrophic nanoflagellates feeding on Synechococcus (Dolan & Simek 1999). Thus digestion rates, and their variability with ambient food levels and food type have been studied in ciliates fed algae and other ciliates (Capriuolo & Degan 1991; Dolan & Coats 1991a, Dolan & Simek 1997) and have concluded that digestion rate is insensitive to ambient food concentrations and food type. However, these studies sampled populations with time and did not attempt to asses individual variability.

Significance of Physiological Diversity

We report the results of a set of experiments designed to determine the effects of ambient food levels on digestion in the ciliate *Euplotes vannus*. The experimental design of following individual cells is a design rarely employed with regard to microbes and allowed us to assess physiological diversity in *Euplotes vannus*. Our results show that a population of *Euplotes vannus* contains individuals with distinct reactions to a given set of conditions. We followed digestion of labeled prey by monitoring individual ciliates digesting prey under different conditions of ambient food levels. Comparison of average values suggested no significant effect of ambient food levels on digestion rates. However, examination of the distribution of digestion times among individual ciliates showed that when food was abundant, individual *Euplotes vannus* cells reacted in different fashions- including notably rapid or prolonged digestion. The different durations of digestions among individuals likely correspond with different growth rates. Thus, *Euplotes vannus* populations are composed of individuals which will probably exhibit distinct growth responses when exposed to high prey concentrations. This hypothesis would not have been supported based on the evidence of no differences in digestion with ambient food level, a conclusion based on population averages.

Methods and Materials

Isolation, cultivation and generation of growth curves of *Euplotes vannus* and its food organism, the bactivorous scuticociliate *Metanophrys* sp. are given in detail in Dolan & Coats (1991a). For each of the 4 experiments, log-phase cultures of *Euplotes vannus* and *Metanoprys* were used. Prey ciliates (*Metanophrys* sp.) were labeled with fluorescent microspheres and fed to *Euplotes vannus* as described in Dolan & Coats (1991a). *Euplotes vannus* were washed free of labeled *Metanophrys* and individual cells were micro-pipetted into, and isolated in, 1 ml well plates. Ciliates in the well plates were examined using an inverted microscope equipped with epifluorescence permiting visual verification of ciliate cell contents. For each experiment, a set of 30 individual *Euplotes vannus* containing *Metanophrys* labeled with fluorescent microspheres (Figure 1) assembled. Elapsed time from separating *Euplotes vannus* from labeled *Metanophyrys* to composing a set of 30 Euplotes, each isolated in an individual well of a well plate, was about 20 minutes.

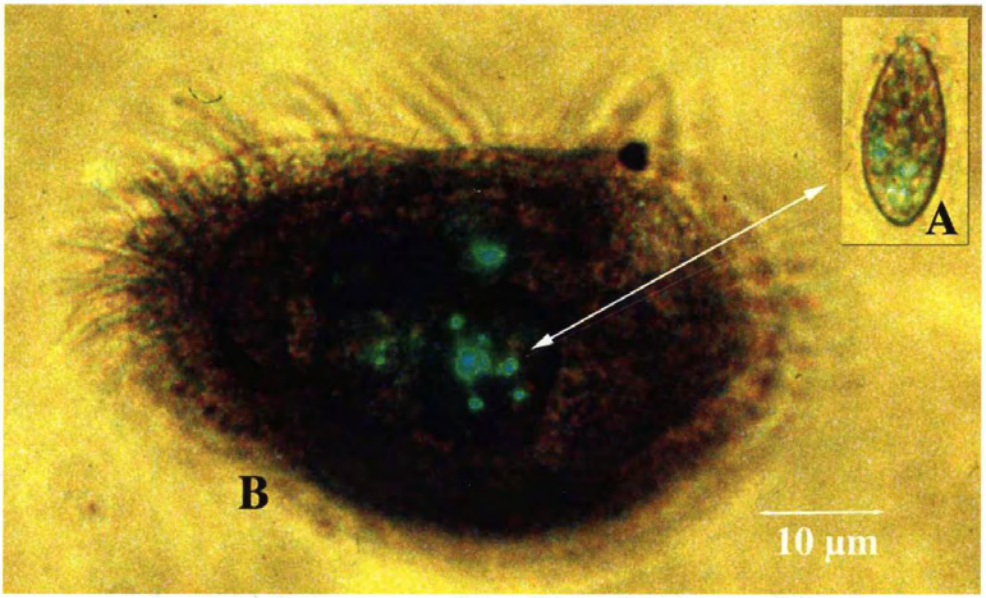

Figure 1. Photomicrographs of a *Metanophrys* (A) labeled with fluorescent microspheres and a *Euplotes vannus* containing several labeled Metanophyrys. The photomicrographs were obtained by using transmitted phase-contrast and epifluorescence illumination.

For each experiment, individual *Euplotes vannus* were examined at 30 minute intervals until the fluorescent microspheres contained within the labeled prey were expelled, marking the end of prey digestion. We thus obtained a digestion time for each *Euplotes* cell. All cultures, labeling and observations were conducted at 20° C in a temperature-controlled chamber.

In the first experiment *Euplotes vannus* were monitored in wells containing culture medium filtered free of food organisms. Thus the ciliates were digesting prey while being starved. In the second, third and fourth experiments, the solutions in the well plates contained, respectively, 100,1000 and 10,000 *Metanophyrys* ml^{-1}. In the latter 3 experiments,

Euplotes vannus cells were transferred to fresh prey solutions approximately every 2 hours. It should be noted that feeding rates reach a maximum in *Euplotes vannus* preying on *Metanophrys* saturates at a food concentration of about 100 *Metanophrys* ml^{-1} (Dolan & Coats 1991). Thus, the 1000 and 10000 prey concentrations represented super-saturated food concentrations for *Euplotes vannus*.

Results

Digestion time, in terms of averages, while appearing to increase with ambient food level, were not significantly different using a t-test comparing results of the 4 experiments due to the relatively large standard deviations (Figure 2). At 20° C, the 'average' Euplotes digesting *Metanophrys* while held in prey-free water and therefore not feeding, digested its food vacuole contents and expelled the inert fluorescent microsphere which labeled its food item, in about 5 hours. The 'average' value for Euplotes digesting in the presence of 100 prey per ml was also about 5 hours. Population averages for ciliates held digesting in the presence of high concentrations of food increased to about 10 h but with a considerable amount of variability. Considering the averages alone and their associated standard deviations, no effect of ambient food level on the digestion time of *Euplotes vannus* can be shown. Thus, digestion time appeared fixed and not responsive to the availability of food.

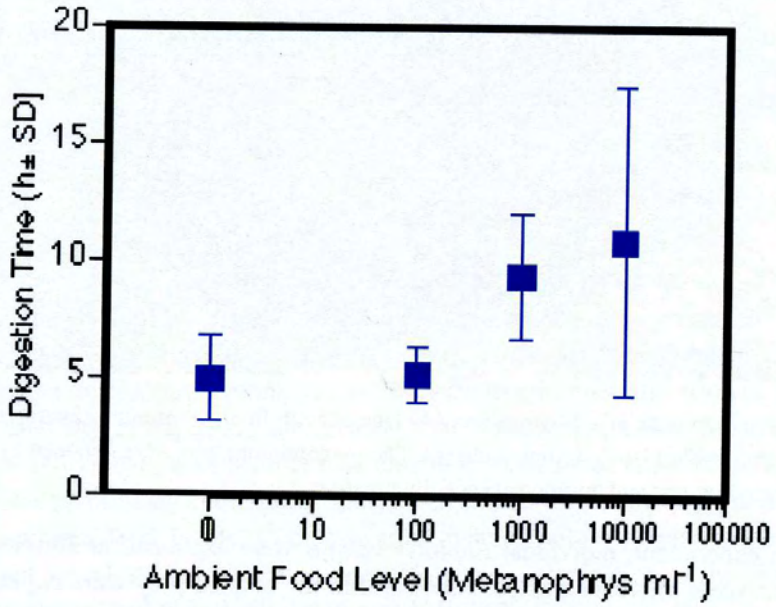

Figure 2. Average digestion times (±standard deviations) in Euplotes vannus digesting Metanophrys as a function of ambient concentrations of Metanophrys. Note that wityh the relatively large standard deviations, no significant differences in digestion times based on a comparison means could be shown.

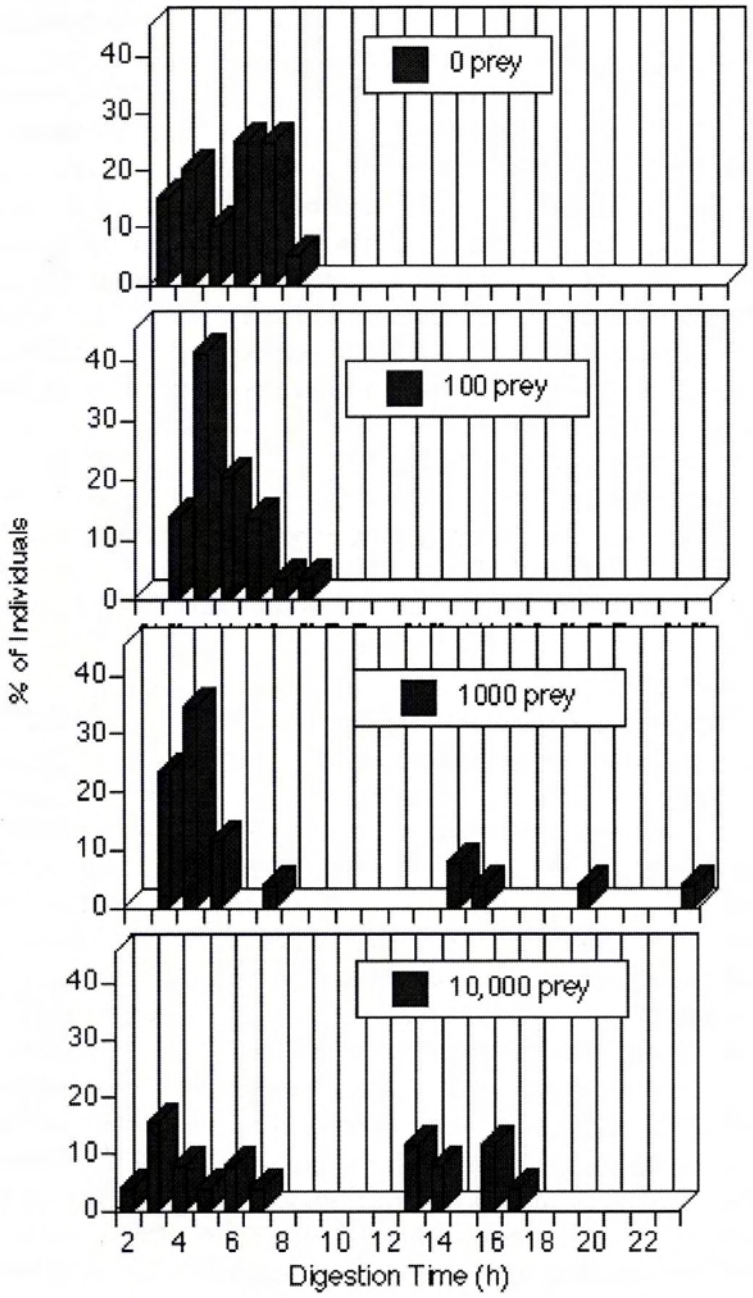

Figure 3. Distribution of digestion times in the 4 experiments. Note that rapid digestion times (1 - 2 h) were detected only in experiments with *Euplotes* digesting while being starved (top panel) or while digesting in the presence of super-abundant prey. Long digestion times (> 9 h) were observed in presence of high food concentrations.

However, distributions of digestion times, in contrast to average values, showed distinct differences with ambient food levels (Figure 3). In non-feeding cells, digestion times appeared to show a bi-modal distribution. Approximately equal and relatively large proportions (about 30 %)of individuals ciliates were rapid digesters (≤ 2 h) and others slow

digesters (≥ 6h). The small numbers of cells used precludes a rigorous test of distribution. In *Euplotes vannus* held in prey concentrations of 100 prey per ml, the distribution of digestion times approached normality with a modal digestion time of about 3 h accounting for about 45 % of the individuals. Despite the differences in distributions between digestion in starving and ciliates feeding in 100 prey ml-1, average digestion times for the entire populations were very similar (Figure 3). With high prey concentrations of 1000 or 10,000 prey ml^{-1}, some *Euplotes vannus* showed digestion times similar to ciliates held in lower food concentrations of 2 or 3 h, while others showed very long digestion times, approaching 24 h. At 1000 prey ml-1, there was a relatively high proportion, about 60% of 'fast digesters' , showing digestion times ≤ 2 h). Thus, the existence of two distinct populations, or individuals showing distinct digestion durations, were apparent in *Euplotes vannus* when subjected to super-abundant prey levels and perhaps starved (held in food-free water).

Discussion

Physiological Diversity

The manner in which we gathered data on digestion in *Euplotes vannus*, following individual ciliates, allowed us to see the physiological diversity among individual ciliates. In experiments sampling with time an entire population (e.g., Capriuolo & Degnan 1991; Dolan & Coats 1991a,b) this information can not be extracted. To return to our example in the "Introduction" concerning rates birth rates in a human population, - net rates of change can be estimated by sampling a population over time and simply noting shifts in abundances, but important demographic details will be masked. Our experimental data showed that the ciliate population was not homogenous but rather it appears that ciliates can react in 2 distinct fashions to the presence of super-saturating food concentrations and perhaps a lack of food.

Euplotes vannus digesting prey in a solution free of food showed a dispersed population, relative to those processing food in the presence of 100 prey ml^{-1}, a concentration supporting exponential population growth (Dolan & Coats 1991a). Under very high food concentrations, 1000 or 10,000 *Metanophrys* ml^{-1}, the tendency was towards a dispersal of digestion times within a population was exaggerated. Thus, under perhaps unusual circumstances of no food or very abundant food, the some individual ciliates tended to either process food rapidly or very slowly. Presumably the distinct digestion times recorded would correspond with different digestion efficiencies, biomass production and different rates of cell division. Before considering if there are indeed different patterns employed by different individuals, or rather in some individuals the digestion process simply malfunctions when challenged, it is worth briefly reviewing food vacuole formation in ciliates. The following account is based on studies of food vacuole formation and membrane processing in *Paramecium* (Allen & Fok 1993; Fok & Allen 1990,1993; Fok & Schockley 1985; Fok et al. 1982), Kaneshiro et al. (1992), Plattner & Kissmehl 2003; Ramoino (1996).

One of the characteristics which distinguish most ciliates from members of many other protist groups is the existence of a differentiated mouth region where food vacuole formation begins (e.g., Hausmann & Radek 1993). Thus, in contrast to amboeba, in ciliates the food

vacuole membrane is formed from disk-shaped vesicles in the mouth region, rather than simply from the plasma membrane. In addition to discodial vesicles, acidosomes, at about 1 μm in dia considerably larger than discodial vesicles, are also found at the mouth region. As the food vacuole forms from the discoidal vesicles, the acidosomes 'dock' but do not fuse with the forming vacuole. Acidosomes, as the name implies are low pH vesicles. When the food vacuole reaches a certain size, or yet some other unknown cellular trigger is reached, the vacuole is pinched off from the mouth region and is transported to the interior of the cell. The acidosomes then fuse with the vacuole and the original vacuole membrane is retrieved with an end result of the nearly entire replacement of the membrane with that derived from the acidosomes. The food vacuole, once formed of acidosome membrane is then associated with docking of lysosomes. These membrane bound sacs contain acidic hydrolyses. It is possible that a second membrane replacement occurs with the fusion of lysosomes. The lysomes appear to simultaneously fuse with the vacuole and actual digestion begins. After a short time (in *Paramecium* 20 minutes) another change in membrane characteristics occurs. Lysosome membrane appears to be removed from the vacuole. At this point, the food vacuole may be 'defecation competent' or another change in membrane properties may be necessary before binding at the cytoproct region is possible. The vacuole is transported to the cytoproct region and at this point is acid phosphatase negative. The cytoproct opens and the undigested matter is expelled but the vacuole membrane remains inside the cell. Membrane from the food vacuole is tubularized and the tubules released into the cell. From this scenario it is clear that membrane supply and flow is primordial in the formation and processing of food vacuoles. Most if not all membranes appear to be recycled. Thus, the original food vacuole membrane formed from the discodial vesicles are themselves derived, at least in part, from re-cycled food vacuole membrane transported back to the mouth region after indigested matter is expelled from the cell. Likewise, acidosomes, based on antigen results, seem to have a heterogeneous origin but recycling of membrane is suspected to occur.

The complex sequence of events based on studies of *Paramecium* may or may not characterize most ciliates. Food vacuole processing has been examined in few species in any detail and there is at least one report of a different pattern. *Pseudomicrothorax dubius* feeds on filamentous blue-green algae and in this ciliate, lysosomes fuse with food vacuole as it is formed in the mouth region (Peck & Hausmann 1980). The processing of food vacuoles in *Euplotes vannus*, if strictly sequential and following that known from *Paramecium*, can be short-circuited as we observed a few instances in which prey-containing food vacuoles merged. Indeed, a breakdown in the sequence is an explanation for long digestion times.

In the scenario based on *Paramecium*, a food vacuole will remain in the cell if it is not first fused with acidosomes and the membrane replaced and/or not subsequently fused with lysosomes. The longest digestion times in *Euplotes vannus* were observed in cells held in solutions of 1,000 prey ml^{-1} (Figure 3). In circumstances of over-abundant prey one may hypothesize an imbalance between the formation of early stage food vacuoles and the supply of acidosomes and lysosomes. For example, some of the membrane generally used for the fabrication of lysosomes or acidosomes was perhaps monopolized in the form of early stage food vacuoles. It is difficult to explain the retention of ingested matter in the presence of abundant food as a useful strategy. Therefore, it seems most likely that in some individuals there was a breakdown in coordination among the distinct components of the digestion

process. What is noteworthy is the fact that long digestion times characterized only a portion of the ciliates subjected to high food concentrations while digesting.

Our data show that a treatment effect of ambient food level can be discerned on a portion of the ciliates, a portion hidden in the averages, a comparison of which suggested no significant differences among treatments. We conclude then perhaps re-stating the obvious (but often ignored or under-appreciated): individuals vary. In the ciliate *Euplotes vannus*, we found considerable variability in terms of patterns of digestion found in even the small populations examined. Such an apparent physiological diversity may in part explain its wide distribution.

Using Food Contents and Digestion Rates to Estimate Ingestion Rates

Our results indicate that while average population rates of digestion appeared insensitive to ambient food levels, parts of the ciliate populations displayed long digestion times. The portion of the ciliate population thus effected by food level could be substantial, with nearly 40% of individuals showing long (> 12h digestion rates (Figure 3) but the phenomenon masked by individual to individual variability. If long digestion times are associated with inefficient exploitation of food, a population feeding under conditions of high prey availability will possibly have a lower average growth rate. However, as we noted, a substantial portion of the populations showed very rapid digestion times perhaps with corresponding generation times.

Conclusion

Previous studies have underlined the differences between strains of the same ciliate species or clones derived from a single strain, largely in terms of growth rates or temperature responses (e.g., Wiesse & Montagnes 1998; Weisse & Rammer 2006). Differences between individuals, while suspected to exist, have received very little, if any, attention even with regard to basic activities of ecological interest such as feeding rates (Weisse 2002). However, differences between individual cells can exist, as we have shown. Some *Euplotes vannus* showed digestion times in the presence of over-abundant prey similar to those found in simply optimal prey concentrations, others showed faster digestion or slower digestion rates. In the absence of food, some individuals slowed while other increased digestion times. Such physiological diversity among individuals is likely in part an explanation for the wide geographic distribution of *Euplotes vannus*.

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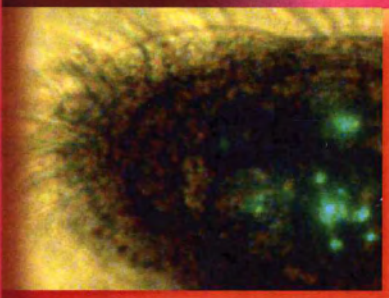

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