EFFECTS OF SMALL-SCALE TURBULENCE ON MICROZOOPLANKTON PREDATOR-PREY INTERACTIONS

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by

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A thesis submitted to the School of Graduate Studies

in partial fulfillment of the

requirements for the degree of

Master of Science

Department of Biology

Ocean Sciences Centre

Memorial University of Newfoundland

April 1998

St. John's Newfoundland

ABSTRACT

The biological and chemical processes which affect bacterioplankton growth and mortality have been well studied. Little is known about physical controls on microbial systems. Turbulent mixing can influence the rates of prey encounter and grazing characteristics of small metazoan grazers, however models suggest that small-scale turbulence (Kolmogorov length scale) should not have an effect on microorganisms less than 10 micrometers. Recent studies suggest that, despite theory, turbulent mixing can influence microflagellates. This study has examined the interactions between temperature (0°,5°,10°,15°C) and turbulent mixing on the growth and trophodynamics between the heterotrophic microflagellate *Paraphysomonas imperforata* and its bacterial prey, *Vibrio splendidus*. It was found that growth rates of *P. imperforata* at 5 to 15°C were 1.5 to 2 fold higher under turbulent compared to static conditions. However, as the temperature decreased from 15 to 0°C, ingestion and clearance rates increased 10 fold, but no significant difference was found between the turbulent and static conditions. It is believed that the increased growth rates in the flagellates at the warmer temperatures in the turbulent condition were due to increased encounter rates between the microflagellate and bacteria. The higher ingestion and clearance rates at the colder temperatures are believed to be due to the increase in the viscosity of seawater, allowing flagellates to move a greater volume of water across its boundary layer. These results suggest that growth and ingestion rates determined during static incubations of *in situ* samples under 15°C from previously published studies may be over or underestimated since turbulence is continuous in nature.

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μ	Dynamic viscosity, Pa s *or* specific growth rate, divisions h^{-1}
ρ	Density of seawater (15°C, 32‰), 1.024 g cm^{-3}
ν	Kinematic viscosity, m^2s^{-1}
η	Kolmogorov length microscale of turbulence, mm
ε	Turbulent kinetic energy dissipation rate, W kg^{-1}
S_s	Smallest turbulent eddy size, mm
D	Diffusion coefficient, cm^2s^{-1}
A	Predator cell radius, µm
K_B	Boltzmann's constant, 1.38×10^{-23} J K^{-1}
T_K	Temperature in degrees Kelvin (273.15 + 15°C = K)
R_0	Prey cell radius, µm
α	Wavenumber $\mathbf{k} = 2\pi$
$\sigma^2_{x,y,z}$	Standard deviations of particle velocities
Ω^2	Sum of σ^2_x, σ^2_y, and σ^2_z
T	Period of one oscillation, 1 sec

Acknowledgments

I would like to thank an enormous number of people for helping me through this project these last couple of years. First and foremost, to my supervisor, Dr. Richard Rivkin, who not only put his confidence in me and invited me to his lab over two and half years ago, but has provided me with a wealth of knowledge in the microbial world. Sincere thanks to Paul Matthews, for without his help on a day to day basis I would have truly been lost, and most likely have taken another year to finish this project. Thanks to Diane Mooney and Heather Bussey for loaning some of their valuable time to assist me in bacterial and flagellate counts. Much thanks to Zanna Chase, a student of Dr. Neil Price at McGill University, for supplying me with that cute little flagellate, *Paraphysomona imperforata*. My gratitude to Steve Sooley for the actual construction of my experimental apparatus; he took my drawings and made them a reality. Thanks to Jim Millan of the Institute for Marine Dynamics for the use of that fantastic machine called a laser Doppler velocitimeter--the chance to control a 300mW argon-ion laser was quite the rush. Thanks to Dr. Charles Shirkey for taking that huge amount of data obtained from that fantastic laser and helping me process it for human consumption. Again, add another year to this project if he had not come and rescue me from the perils of signal processing. A big thank you to Dr. John Gow who not only provided editorial advice on this somewhat massive document, but also for supplying me with the initial cultures of *Vibrio splendidus* so my poor little flagellates would not starve. Thanks to Dr. Don Deibel for his guidance, patience and editorial advice on this thesis. A small thank you to Hong Chen who

provided me with some very valuable statistical advice. Allow me to extend my gratitude to my wonderful and dear family and friends who have cheered me on these last couple of years. Many blessed thank you's to my mother for not only allowing her little girl to move to another country away from her, but also for continually providing so much emotional support each and every day. And last but hardly ever least, I would like to show an immense amount of appreciation to my father, my phantom committee member, who contributed a great deal of input on the physics of my research. Most importantly, I am eternally grateful because he has put so much faith in me, not only as his daughter, but as a true scientist.

CHAPTER 1

1.1. Background

The marine microbial trophic levels process a considerable portion of dissolved and particulate carbon in the world's ocean. The marine bacteria are responsible for the remineralization of dissolved organic material emanating from phytoplankton and heterotrophic grazers (Davis and Sieburth 1984, Jumars *et al.* 1989 and ref. cited within). Bacterial production alone, has been reported to constitute 20 to 60% of primary production in the ocean (White *et al.* 1991, Ducklow and Carlson 1992). However, bacteria-controlled carbon fluxes in the ocean can be highly variable and range from zero to more than 100% of local primary production (Pomeroy *et al.* 1991, Hoch and Kirchman 1993, Azam *et al.* 1994). Even though photosynthesis limits the amount of atmospheric CO_2 absorbed into the ocean's surface layer, it is the function of the marine bacteria and microbial food web to export the carbon from the surface layer to the mixed layer of the ocean. Nonetheless, the energy consumed by the marine bacteria must be transferred to the next trophic level by means of predation by protozoans and other microzooplankton (e.g. pelagic tunicates; King *et al.* 1982, Urban *et al.* 1992, Deibel and Lee 1993).

The concept of the microbial food web was recently modified by Legendre and Rassoulzadegan (1995) to introduce the idea of a multivorous food web where mesozooplankton omnivory or herbivory, and microzooplankton herbivory or bactiverory co-exist. For example, heterotrophic microflagellates are major bactiverous grazers which play a substantial role in the cycling of biogenic carbon (Fenchel 1982a, b, c and d,

Sherr and Sherr 1983). Flagellates can repackage bacterial biomass into particles accessible to mesozooplankton, and hence recycle carbon back to the microbial loop (Caron *et al.* 1985 and ref. cited within). To fully understand the factors that regulate the dynamics and flow of the biogenic carbon in the marine microbial food web, it is necessary to determine how physical factors, such as small scale turbulence and low seawater temperatures, influence the interactions between these trophic levels. Turbulent motion that has been dissipated to a micro-scale can increase the relative velocity of a microzooplankton and its bacterial prey, and possibly increase their encounter rates. Increased encounter rates usually result in increased grazing rates, and hence the cycling of carbon in the microbial food web could occur at a faster rate.

Microflagellates experience life at low Reynold's numbers where, at least in theory, inertia has little effect. The Reynold's number is a dimensionless index which relates the drag of a solid through a viscous fluid; the higher the viscosity, the lower the Reynold's number (Reynolds 1883). The forces exerted on the microorganism to assist in its movement are forces acting on its body at that moment and that moment alone (Purcell 1977). A microflagellate swimming through seawater is analogous to a human attempting to swim through a pool of molasses. Hence, when the microflagellate stops its propulsion through the fluid, the animal's movement immediately ceases. With such low Reynold's numbers to consider, small-scale turbulence can possibly benefit a microflagellate because the increased water velocities created by the turbulent eddies can increase the animal's relative velocity through the fluid.

Small-scale turbulence is the result of the dissipation of larger turbulent eddies created by strong disruptions to the water's surface, such as high winds creating waves. Any small scale oceanic motion (smaller dissipated eddies) relies only on the rate at which it is provided with energy by a larger scale motion (large turbulent eddies) and on the kinematic viscosity of the seawater (Tennekes 1972). The smallest turbulent scale is determined by the Kolmogorov length scale. This theoretical micro-scale exists where viscous drag begins to dominate and viscosity starts to smooth out turbulent water fluctuations (Tennekes 1972, Vogel 1987, Mann and Lazier 1989). Therefore, the equation for the Kolmogorov length scale (η) is governed by the kinematic viscosity (ν) or the molecular diffusivity of momentum, and the kinetic energy dissipation rate (ε) which can range in the ocean from 10^{-3} to 10^{-10} W kg^{-1} (Osborn 1978, Oakey and Elliot 1982, Yamazaki and Osborn 1988):

(1) $$\eta = \left(\frac{\nu^3}{\varepsilon} \right)^{1/4}$$

To further emphasize that small scale motion is dominated by viscous forces, the Reynold's number will equal "1" when η and υ (fluid velocity) are combined:

(2) $$\frac{\eta \upsilon}{\nu} = 1$$

emphasizing that the dissipation of the viscosity can modify itself to the amount of energy supplied by the turbulent eddies (Tennekes 1972). At scales smaller than the Kolmogorov scale, flow is classified as laminar (or "smooth") and microorganisms will only experience the laminar shear created by the dissipated eddies (Shimeta *et al.* 1995).

Turbulent motion is a complicated concept that has been investigated for years by physicists and physical oceanographers (see Nelkin 1992). Quantifying turbulence at any scale is difficult since it must be determined through a set of theoretical equations. Modern technology permits the measurement of turbulence at the larger scales, but small scale turbulence is impossible to measure directly. Furthermore, in most cases turbulence and plankton have been studied independently in natural systems, particularly due to the difficulties in controlling the physical parameters (Alcaraz *et al.* 1988, Sanford 1997). Therefore, a number of theoretical equations and standards exist for micro-scale fluid motion which all originate from Kolmogorov's Universal Equilibrium Theory (1941). This physical effect on a biological system has been investigated conventionally by a number of researchers. Purcell (1978) was the first to derive a model characterizing the effect of stirring on a predator's (< 50 μm diameter) absorption of prey particles based on a theory developed by Smoluchowski (1916). Purcell had determined that particles (or organisms) less than a few microns in size would not be affected by vigorous stirring, but those swimming microorganisms greater than 5 μm in size could enhance the absorption of prey particles. Lazier and Mann (1989) further explored Purcell's ideas, focusing their theory on the diffusive boundary layer around a microorganism. Their model suggests that turbulence does not affect spherical organisms less than 100 μm in diameter. Lazier and Mann (1989) believe the difference from their results and others published results arose from the misuse of the Kolmogorov length scale, underestimating the length of the smallest turbulent eddy by a factor of ten. Rothschild and Osborn (1988) created a

turbulence induced predator-prey encounter model which suggests that zooplankton feeding rates, as well as other plankton rates such as the optimal foraging theory and nutrient exchange in oligotrophic systems, may be underestimated by failure to consider turbulent motion. Kiørboe and Saiz (1995) further expanded Rothschild and Osborn's model because they believed the results did not apply to larger predators, such as copepods. They enhanced the equations by adding behavioral components to include characteristics such as random walk patterns and ambush predation.

The general argument in the field of small scale turbulence is that only meso-sized organisms (> 50 μm) can benefit from turbulence, and even though there has been a considerable amount of research on the effects of turbulence on copepods (Saiz *et al.* 1992, Marrasé *et al.* 1990, Costello *et al.* 1990) and fish larvae (MacKenzie and Leggett 1991, Muelbert *et al.* 1994, Sundby and Fossum 1990) studies of smaller organisms, such as ciliates and microflagellates, have been neglected. Kiørboe and Saiz (1995) had reported that turbulence is to be considered "unimportant for very large predators and for most very small predators", but possibly effective to meso-size predators which function around the Kolmogorov length scale. Hill *et al.* (1992) concluded their study with the same hypothesis in which "the encounter rate of particles similar in size to the Kolmogorov scale is controlled by turbulent eddying motions." However, this is only theory, and all theory must be tested and applied. A survey of the literature has presented only four articles on the effects of turbulent motions on microorganisms smaller than 10 μm. Peters and Gross (1994) reported the grazing rates of *Paraphysomonas imperforata* feeding on marine bacteria in the Gulf of Mexico during stagnant and turbulent water

conditions. They found no difference in the ingestion rates between static and turbulence conditions, but results showed a change in flagellate abundance and cell size. A higher flagellate abundance predominated under the turbulent condition, however, cell size appeared smaller than those found under the static condition. Shimeta *et al.* (1995) subjected planktivorous suspension feeders to laminar shear fields just below the Kolmogorov length scale. They found that the shear created by small scale turbulence had no significant effect on the flagellate and ciliate species (*Paraphysomonas* sp., 2 chrysomonads, *Diaphanoeca grandis*, *Favella* sp, and an unidentified heterotrich), except for the choanoflagellate *Monosiga* sp. This led to a hypothesis that turbulence effects may be species-specific, having a stronger influence on nonmotile organisms or weak swimmers such as foraminiferans and radiolarians. Peters *et al.* (1996) investigated the effect of turbulent mixing on *P. imperforata*'s ingestion rates. Microflagellates were exposed to 12 to 24 hour turbulent periods over a range of turbulent intensities. They found no significant difference ($p < 0.05$) in the ingestion rates among turbulent intensities and suggested that turbulence may not have an effect on the organisms unless they are exposed to turbulence for more than 12 h. The effect of turbulence on bacterial production was reported by Moseneder and Herndl (1995). They found that turbulence increased bacterial production when phytoplankton was present, but not when phytoplankton was absent. Since individual bacterial cells are much smaller than the Kolmogorov scale (Logan and Kirchman 1991), turbulence should, in theory, not influence the rates of production unless they are in larger aggregates, such as attached to clumps of phytoplankton cells. Therefore, Moeseneder and Herndl (1995) suggest that

turbulence may only alter a bacterioplankton community structure and not affect its actual rates of production.

For the research proposed herein, the heterotrophic microflagellate *Paraphysomonas imperforata* was the chosen animal because it is a ubiquitous microflagellate, easy to culture and grows over a wide range of temperatures. It is spherical in shape, covered with siliceous spines, with two flagella, one short and one long, which assist in locomotion (Figure 1.1) (Fenchel 1982a). The longer of the two flagella has a bilateral array of heterokont tubular hairs, whereas the shorter is smooth (Eccleston-Parry and Leadbeater 1994). It is a colorless microflagellate, and for it to feed, a bacterium or algal cell must come in direct contact with the flagellate's ventral furrow where the prey is then phagocytized (Fenchel 1982a). Caron *et al.* (1986) did an extensive study on the effect of increased temperatures on the physiological properties of growth and ingestion rates and cycling of carbon and nitrogen by *P. imperforata*. They found that increasing temperature from 14 to 26°C resulted in increased growth and grazing rates of the microflagellate. However, there was no relationship between increased temperature and gross growth efficiency. Choi and Peters (1992) measured the feeding rates of two cold ocean strains of *P. imperforata* at a temperature range of -1.8 to 20°C. Their results agreed with those of Caron *et al.* (1986) in which there was an increase in feeding rates with the increase in temperatures. However, the results of Choi and Peters (1992) also showed an increase in the gross growth efficiency of *P. imperforata*. Most research concerning grazing and growth rates of microflagellates have been conducted at higher temperatures (~20°C), leaving a great need for more

information on how lower seawater temperatures can affect the feeding and growth of *P. imperforata*.

1.2. Thesis Objectives

No two experimental results or models absolutely agree upon how small scale turbulence affects microorganisms and their encounter probabilities. In addition, no study has yet combined the effects of turbulence with decreasing seawater temperatures to consider the consequences of the increased viscosity of the fluid on microzooplankton in a turbulent environment. Nonetheless, many studies do have one underlying idea, and that is, if turbulence does prove to have an effect on the physiological rates of microorganisms, then all previously published rates assessed under stagnant incubations may be under or overestimated. Hence, the purpose of this research is to determine whether turbulence influences the growth and grazing rate of *Paraphysomonas imperforata* at seawater temperatures below 15°C. It is hypothesized that small scale turbulence will increase the growth and grazing of *P. imperforata* at all temperatures by augmenting the encounter rates between predator and prey.

Within this thesis, Chapters 2 and 3 accomplish the following objectives: Chapter 2 discusses theory developed to discern whether turbulence impacts the heterotrophic microflagellate, *P. imperforata* and its contact with its bacterial prey by modifying the equations of Purcell (1977). The theory is then applied to determine growth, ingestion and clearance rates of *P. imperforata*, in a turbulent environment. Chapter 2 also quantifies the turbulence or calculates the energy dissipated in the experimental environment. Chapter 3 investigates and reports the combined effects of small-scale

turbulence and seawater temperatures below 15°C on the growth, ingestion and clearance rates of *P. imperforata*. Chapter 4 simply concludes this thesis with a variety of future directives aimed at this field of research.

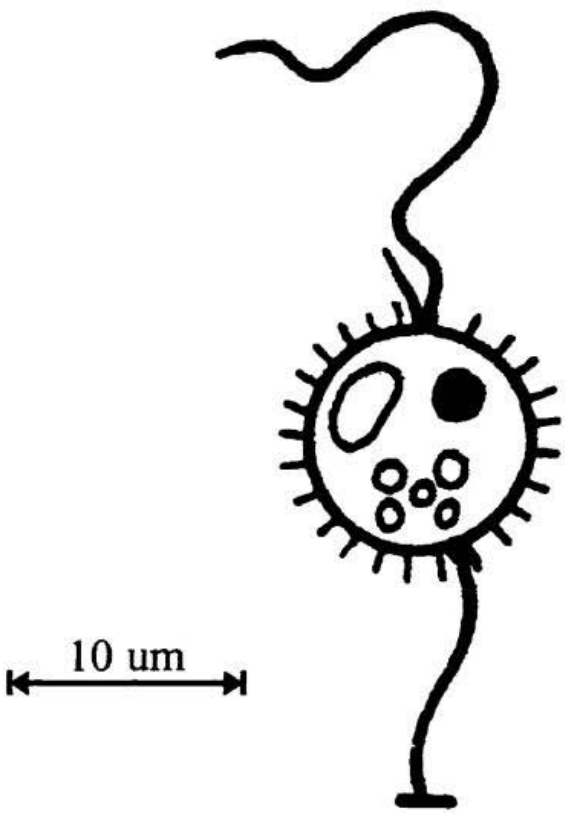


Figure 1.1: Schematic drawing of *Paraphysomona imperforata* from Eccelston-Parry and Leadbeater (1994).

CHAPTER 2

The Effects of Turbulence on Protistan Grazers Smaller than the Kolmogorov Length Scale, in Theory and Applied.

2.1.1. Introduction

It is widely believed that the microbial food web is a dominant pathway for energy flow (Azam *et al.* 1983). Heterotrophic microflagellates are known to be primary predators of bacteria (Sherr and Sherr 1984, Fenchel 1986) and are considered to be a critical trophic link in the microbial food web (Azam *et al.* 1983, Choi and Peters 1992, Fuhrman 1992). *Paraphysomonas imperforata* is a ubiquitous heterotrophic microflagellate. It can rapidly increase its population when conditions are optimal and consume a variety of sizes (0.5 μm^3 to 200 μm^3) and types of prey (bacteria and algal cells) (Fenchel 1982a, Choi and Peters 1992, Eccleston-Parry and Leadbeater 1994). Until recently, all reported growth, ingestion and clearance rates of this flagellate, and other microbial groups, have been determined in non-turbulent conditions. In many cases, microorganisms that were collected from the field were obtained from turbulent waters, and growth and grazing rates were ascertained during static incubation conditions.

The dissipation of turbulent kinetic energy from the large to small size scales is an inherent characteristic of the ocean. Turbulence has been known to contribute to the formation of marine aggregates (Kiørboe 1993) and dispersion of plankton populations (Lasker 1975, Hary *et al.* 1990). Rothschild and Osborn (1988) suggested that small scale turbulence could also significantly influence predator-prey interactions and the flow

of energy within and between marine food webs. Several studies on the effects of turbulence on copepods and fish larvae proposed that contact and feeding rates may be seriously biased when excluding the contribution of small scale turbulence to these rates (Marassé *et al.* 1990, Costello *et al.* 1990, MacKenzie and Leggett 1991, Sundby and Fossum 1990). In consideration of these studies, a question arises whether turbulence could have the same effect on smaller organisms (10 μm or less) and consequently, what impact it could have on the marine microbial food web.

Only recently have the effects of turbulence on a flagellate's ingestion rates been examined (Peters and Gross 1994, Peters *et al.* 1996). The first of these two papers investigated ingestion rates of fluorescently labeled bacteria by *Paraphysomonas imperforata*. Peters and Gross (1994) found that there was an increase in the abundance of *P. imperforata* under a turbulent environment, but cell size had decreased over time. Their results also showed ingestion rates were slightly higher under turbulence, but the rates were not significantly different than those of the static condition. These authors proposed that the turbulence causes a change in behavioral response which resulted in increased grazing, further suggesting that this response is similar to that reported in experiments using calanoid copepods (Saiz and Alcaraz 1992a, Saiz *et al.* 1992). Peters *et al.* (1996) expanded on this in studies of the ingestion rates of *P. imperforata*, but this time concentrated on the effect of different turbulent intensities (0.05, 0.15, 15 cm^2s^{-1}). Their results showed that the flagellates influenced only by "high turbulent levels" (15 cm^2s^{-1}) for twelve hours were more abundant than the non-turbulent condition.

Shimeta (1993) postulated that microorganisms would encounter prey at a higher rate because of laminar shear created by the smallest turbulent eddies which are just below the Kolmogorov length scale. They tested their hypothesis in order to determine if shear rate affected clearance rates of a variety of planktivorous suspension feeders, such as flagellates and ciliates (Shimeta *et al.* 1995). Their results suggested that the effects of shear seemed to be species-specific. For example, *Paraphysomonas* sp. and the choanoflagellate *Monosiga* sp. were both fed FLB of similar concentrations (10^6 ml^{-1}). The clearance rates determined from the *Paraphysomonas* sp. exposed to the laminar shear fields were not significantly different from the clearance rates of those in the static condition. In contrast, *Monosiga* sp., which is similar in size to *Paraphysomonas* sp., did show a significant difference with a much greater clearance rate when exposed to the laminar shear field.

2.1.1. Objectives

The objective of this study is to quantify the effects of small scale turbulence on the growth and ingestion rates of the microflagellate *Paraphysomonas imperforata* feeding on bacterial prey. Equations of Purcell (1978) are first discussed and then applied to micro-sized (5 to 6 μm) grazers. Then to test Purcell's theory, experiments were done to evaluate the effects of turbulence upon feeding on the psychrotrophic marine bacteria, *Vibrio splendidus*, by *P. imperforata*. These experiments test the hypothesis that microflagellate growth and its ingestion of bacteria will be greater under turbulent than stagnant conditions. If turbulence enhances the rate of growth of microheterotrophs and their grazing of marine bacteria then it is likely that published

rates of growth and ingestion determined under static incubation conditions may be under or overestimated.

2.2. THEORY

The size of the smallest turbulent eddies is typically referenced to the Kolmogorov length scale, η. This is a scale which is determined by the kinematic viscosity of a fluid and the velocity shear (or otherwise known as the kinetic energy dissipation rate). Kinematic viscosity differs from dynamic viscosity (μ) in that the latter is the friction of a fluid. It is a measure of a fluid's resistance to shear when a fluid is in motion and can be defined as:

(1) $$\mu = \frac{F * z}{U * S}$$

where F (Newtons) is the force acting on one fluid layer or body moving across the other, z (meters) is the distance between the two bodies, U (ms^{-1}) is the velocity resulting from the force acting upon the layer, and S (m^2) is the area of the fluid velocity or body (Vogel 1994). The dimension of dynamic viscosity is a Pascal second (Pa s) in SI units. Kinematic viscosity (ν) is then simply a ratio of the dynamic viscosity and the fluid's density (ρ):

(2) $$\nu = \frac{\mu}{\rho}$$

The SI unit dimension for kinematic viscosity is m^2s^{-1}. This ratio is defined as the measurement of the "ability of molecular transport to eliminate the non-uniformities of fluid velocity" (Batchelor 1967, Vogel 1994). Table 2.1 lists the dynamic and kinematic viscosities calculated for seawater characterized for Logy Bay, Newfoundland at four experimental temperatures.

The Kolmogorov length scale relates the kinematic viscosity to the energy dissipation rate (ε) of a turbulent eddy. It is defined as:

(3) $$\eta = \left(\frac{\nu^3}{\varepsilon}\right)^{1/4}$$

where ε can range from 10^{-3} to 10^{-10} W kg^{-1} or 10 to 10^{-6} cm^2 sec^{-3} (Osborn 1978, Oakey and Elliot 1982, Yamazaki and Osborn 1988) (1 W kg^{-1} = 10^4 $cm^2 s^{-3}$). By using this range of ε and assuming ν is approximately 10^{-6} m^2 s^{-1}, then η can vary between 1 to 6 mm.

According to Lazier and Mann (1989), the size of the smallest turbulent eddy (S_s, mm) has been routinely underestimated in the past by some factor α, and therefore:

(4) $$S_S = \alpha\,\eta = \alpha\left(\frac{\nu^3}{\varepsilon}\right)^{1/4}$$

The value of 2π has been opted for α by Lazier and Mann (1989) because 2π is often used in the definition of the wavenumber **k** for mathematical convenience. It is relatively unimportant what the absolute value is for α, just as long as it is clear what is the percent of shear energy that is used by the smallest turbulent eddy. When α=2π, the Kolmogorov length scale would range from 6 to 37 mm and the smallest turbulent eddy would contain approximately 3% of the maximum shear energy of the original turbulent flow. Consequently, any turbulent motion which spans less than a few millimeters will diminish to linear shear.

For suspended particles (e.g. predators and prey) to encounter one another, they must move at different relative velocities. This can occur by either swimming or sinking

at different velocities, generation of a feeding current, or fluid motion bringing particles into contact. A question arises as to how a prey particle is absorbed by a predator. Does the predator experience direct interception or "diffusional or motile particle deposition" (Vogel 1994)? For direct interception, a prey particle must pass within the radius of the predator's cell and be streamlined directly to the predator, a technique typical for a motile predator and non-motile prey. For diffusional deposition, a predator captures a prey particle as a result of the prey's Brownian motion, or otherwise stated as the prey's random motion (Figure 2.1). For the purpose of this study, we will assume the latter situation since *Vibrio splendidus* is a motile bacterium. Two other assumptions which must be made for mathematical convenience are: (1) all the particles in question are perfect spherical bodies and (2) turbulence is isotropic at the small scale.

If we are to assume that turbulent motion increases the contact of predator and prey particles, then the next question to ask is how much "stirring" or turbulent energy is required to double the contact rate of prey particles. Purcell (1978) derived an equation which involves the diffusion coefficient (D, cm^2s^{-1}) of a prey particle, the dynamic viscosity (μ, $W\ s^2cm^{-3}$) of a fluid and the predator's cell radius (A, μm) to determine the "stirring-power density for doubling the rate of absorption" ($<S>_{min}$, $W\ cm^{-3}$):

(5) $$<S>_{min} = \frac{500\mu D^2}{A^4}$$

The diffusion coefficient was ascertained with the Einstein-Smoluchovski equation:

(6) $$D = \frac{k_B T_K}{6\pi\mu^2 R_o}$$

where k_B is Boltzmann's constant (1.38×10^{-23} J/ degree K), T_K is the temperature in Kelvin (15°C = 288K), μ is the dynamic viscosity of seawater (at 15°C and 32‰, μ=1.1 x 10^{-9} W s^2cm^{-3}), and R_0 is the radius of the prey particle (in our case, 0.5 μm = the radius of a 1 μm bacterium).

Purcell tested the equation by first using a predator radius of 1 μm which is absorbing a particle with a D of 10^{-5} cm^2s^{-1}. This results in a stirring power of 0.5 W cm^{-3}, a very powerful and impractical value for a predator of this size. He then applied the same situation to a predator of 10 μm radius which results in a stirring power of 5×10^{-7} W cm^{-3} which is much more realistic and obtainable. Let us now examine how much energy it would take to double the absorption rate of a predator with the radius of 2.5 to 3.0 μm (similar in size to the microflagellate *P. imperforata*), a D of 3.8 x 10^{-9} cm^2s^{-1} and μ of 1.1 x 10^{-9} W s^2cm^{-3} (for 15°C, 32‰ salinity) :

$$<S>_{min} = \frac{500(1.1 \times 10^{-9}\, W s^2 cm^3)(3.8 \times 10^{-9}\, cm^2 s^{-1})^2}{(2.5\ to\ 3.0 \times 10^{-4}\, cm)^4}$$

(7)
$$<S>_{min} = \frac{(7.9 \times 10^{-24}\, W s cm^{-1})}{3.9\ to\ 8.1 \times 10^{-15}\, cm^4}$$

$$<S>_{min} = 2 \times 10^{-9}\, W cm^{-3}\ to\ 9 \times 10^{-10}\, W cm^{-3}$$

This range is similar to Purcell's estimation of a 10 μm predator and therefore a reasonable value to attain. This, in fact, means that the turbulent energy created at 2 x 10^{-9} W cm^{-3} to 9 x 10^{-10} W cm^{-3} can be effective and increase the rate of diffusional deposition of a bacterium to a microflagellate of 5 to 6 μm in size.

2.3. MATERIALS AND METHODS

2.3.1. Experimental apparatus

The experimental apparatus consisted of three insulated plexiglass containers (46 cm x 20 cm) containing two smaller plexiglass incubation containers (11 cm x 11 cm x 14 cm height). The three tanks were connected to a temperature controlled recirculating water bath (Neslab RTE-210). Of the six smaller incubation chambers, three are the control (i.e. non-turbulent) condition and three chambers are the turbulent condition (Figure 2.2).

The turbulence was created by vertically oscillating plungers consisting of a plastic wand and a perforated polyvinyl chloride (PVC) plate (plate size was 9.5 cm^2, perforation diameter was 2 mm, ~93% solidity). The plates were centered in each chamber with 2.5 mm clearance between the inside walls of the tank and the edge of the plates. The vertical amplitude of the plunger motion was 3 cm, travelling in the upper portion of the water column, and oscillating at a rate of 1 Hz $\pm$ 0.3 Hz. (Figure 2.3)

2.3.2. Quantification of turbulence

Turbulence was measured with a 2-axis 300 mW argon-ion laser Doppler velocitimeter (Dantec Electronics) and processed with a Flow Velocity Anemometer (Dantec Electronics). Vertical and horizontal components of water velocities were measured at different positions within the water column using a computer controlled traverse mechanism. Latex fluorescent beads (1 μm), similar in size to the bacterial prey, were used as tracer particles in the water column. Each velocity component was measured at a sampling rate of ~120 Hz with a bandwidth of 0.12 MHz and ~2 mm fringe

spacing (n=35). Data were collected at random locations within the tank for an accumulation of 10,000 sample velocities or a maximum of 10 minutes per site. The standard deviations were calculated for each U_x (velocity component in the x-direction) and V_y (velocity component in the y-direction). A third standard deviation for the velocities in the z-direction was assumed to be similar to U_x and V_y. The energy per volume, or the kinetic energy dissipation rate, ε (W kg^{-1}), was determined first by finding the sum of the standard deviations of the three velocity components:

(8) $$\Omega^2 = \sigma_x^2 + \sigma_y^2 + \sigma_z^2$$

This sum was applied to an energy formula to determine the energy per volume (m^{-3}):

(9) $$\varepsilon = \left(\frac{1}{2}\right)\rho\,\Omega^2$$

where ρ is the density of seawater at 15°C (1.0236 g cm^{-3}). Then to equate Watts kg^{-1} into the formula, (9) is divided by ρ and T, the period of one oscillation of the plunger (1 sec; Tritton 1988):

(10) $$\varepsilon = \left(\frac{1}{2}\right)\frac{\Omega^2}{T}.$$

2.3.3. Maintenance of Cultures

Cultures of the heterotrophic microflagellate *Paraphysomonas imperforata* were maintained in 70 ml glass culture tubes at 15°C. Stock cultures were transferred into 50 ml of fresh medium every 3 to 4 weeks . The medium used was sterile, 0.2 μm filtered seawater enriched with 1 ml of 10% yeast extract (Difco) and 1 ml of 0.2% proteose peptone (Difco). A sterile rice grain was added to each culture tube. *Vibrio splendidus*

cultures were maintained on agar plates for up to one month. Two steps were required for the preparation of a stock culture to be used for an experiment. First, 25 ml of deionized water (DI) was enriched with 0.935 g of prepared nutrient mix (Marine Broth 2216, Difco) and autoclaved in 100 ml Erlenmyer flasks. A swab off a marine broth agar plate was transferred to the aqueous medium. The flask was then placed on a reciprocating shaker ca. 12 h at room temperature. This time was sufficient for *V. splendidus* to reach exponential growth. The next day, 1 ml of the culture was diluted to 800 ml of sterile, 0.2 μm filtered seawater, and replaced on the shaker for 6 to 12 hours. This working stock culture was then divided among the six incubation chambers (~ 130 ml each), diluted to 1.3 L with sterile 0.2 μm filtered seawater, and allowed to acclimate to the experimental temperatures before the addition of *P. imperforata*.

2.3.4. Flagellate Growth and Ingestion of Bacteria

Four of the six incubation tanks (two static and two turbulent) containing the diluted bacterial cultures were inoculated with 30 ml of the flagellate culture and allowed an unstirred acclimation period of ca. 12 h before the initial (t=0) sampling. The two remaining incubation tanks (one static and one turbulent) were grazer-free controls to assess the effect of turbulence on bacterial growth. Samples were taken at 6 to 12 h intervals for 72 h intervals. All samples (5 ml) for enumerating bacteria and flagellate abundances were immediately preserved with 1 ml of gluteraldehyde (5% final concentration), stored at room temperature in glass 20 ml liquid scintillation vials, and processed within 3 to 5 days of their collection.

2.3.5. Sample Analysis

Bacterial abundance was determined with the acridine orange direct count method (Hobbie *et al.* 1977). Due to high bacterial abundances, samples were diluted up to 20 fold with 0.2 μm filtered seawater. The diluted subsamples were collected onto a pre-stained black 0.2 μm Poretics polycarbonate filter and post-stained with acridine orange. Two filters per slide were placed on smeared drops of Cargille Type A immersion oil on a glass microscope slide. Each filter was topped with another drop of immersion oil and the coverslip was gently pressed on so that the oil was evenly dispersed over the filter. Bacteria were counted using a BH2 - RFC Olympus epifluorescence microscope under a magnification of 1000x. A mercury lamp (100 W) was used to emit blue excitation (BP440, DM455, AFC+Y475). At least 300 cells on each filter were counted by randomly selecting fields.

Flagellate abundances were determined by diluting 2-ml aliquots to 5 ml (with 0.2 μm filtered seawater) and filtering the entire 5 ml onto 1.0 μm Poretics pre-stained black polycarbonate filters. Each filter was post-stained with 0.2 ml of acridine orange. Filters were prepared as described above. Flagellate abundances were enumerated by counting 10 random fields on each filter using epifluorescence microscopy.

2.3.6. Calculations

Each bacterial and flagellate abundance from the microscopic counts were calculated by using the following equation:

(11)
$$N * \left[\frac{A^2}{100/X} \right] * \frac{1000}{V}$$

where N is the average number of cells counted per filter, A^2 is the area of filter occupied by the stained sample (mm^2), X is the number of squares counted in the ocular grid (100 squares, $1 mm^2$), and V is the volume of the filtered undiluted sample.

For each experimental tank, cell division rates (μ, h^{-1}) of flagellates and bacteria were determined from the linear regression of the natural logarithm (ln) of abundances plotted against time. For each grazed tank, each plot of bacterial abundance was divided into three intervals, representing lag, growth, and depleted cells (negative slope), in which linear regression was applied individually. The net grazing mortality, or apparent growth rate (AGR, h^{-1}), of bacteria in the grazing chambers was also determined with linear regression of the plotted data. Lines of best fit for each interval were considered to be those that maximized the r^2 and minimized the standard error of each division rate. The growth (μ) and AGR were then applied to Frost's equations (1972) to equate mean bacterial concentration ([C], cells ml^{-1}), flagellate clearance (CR, nl $flagellate^{-1}h^{-1}$) and ingestion (IR, cells $flagellate^{-1}h^{-1}$):

(12) $$[C] = C_1\left(\frac{e^{(\mu - AGR)(t_2 - t_1)} - 1}{(\mu - AGR)(t_2 - t_1)}\right)$$

(13) $$CR = \frac{AGR}{N}$$

(14) $$IR = CR * [C]$$

where C_1 is the concentration of bacteria in the non-grazer tanks at the beginning (t_1) of the time interval, t_2 is the end of the time interval, and N is the concentration of flagellates ml^{-1} at that specified time interval. Ingestion rates were normalized by

dividing IR with the mean flagellate population for a given time interval. Student paired t-tests or two-way ANOVA with integration were used for statistical analyses of each experiment. Each rate (growth, grazing and ingestion) was tested for significant differences between experiment replications and for the static and turbulent conditions.

2.4. RESULTS

Three grazing experiments were analyzed for the results presented in Tables 2.2 and 2.3. In all experiments, the specific growth rate of *P. imperforata* was significantly higher ($p=0.0001$, ANOVA) in the turbulent than the static condition. Flagellate populations reached a maximum between 30 to 48 hours (ca. 5×10^5 cells ml^{-1}) in the turbulent incubation tank whereas the populations in the static incubation tanks reached maximum abundance at ~60 hours (ca. 3×10^5 cells ml^{-1}; Figures 2.4, 2.5, 2.6). The mean growth rate of *P. imperforata* was 2.35 ± 0.67 d^{-1} and 1.01 ± 0.4 d^{-1} in the three turbulent and static tanks, respectively (Table 2.2).

Bacterial growth in the grazer-free tanks was not significantly different ($p>0.05$, Student t-test) between the turbulent and static conditions. The mean growth rate of *V. splendidus* was 0.89 ± 0.54 d^{-1} and 0.65 ± 0.77 d^{-1}in the turbulent tanks and static tanks, respectively. It should be noted that although growth in the two conditions was not significantly different, the final bacterial abundances after 60 hours tended to be approximately 1.5 fold higher in the turbulent condition (Figure 2.7).

The community grazing of the bacteria by *P. imperforata* was significantly ($p=0.001$, ANOVA) greater in the turbulent than static tanks (Table 2.3). For the three experiments, bacterial growth exceeded grazing mortality in the first 24 to 30 h for both conditions. After 30 h, bacterial abundances declined in the turbulent tanks. However, bacterial abundances of the static tanks showed no obvious grazing mortality for the next 6 to 12 h, then continued for the remainder of the incubation with a low grazing mortality

(Figures 2.4, 2.5, 2.6). Final bacterial abundances were always greater in the static than the turbulent conditions. Experiment A showed a decrease in bacterial numbers after 30 h in the turbulent condition, whereas in the static tank, a noticeable decrease did not occur until after 48 hours (Figure 2.4). In experiments B and C, bacterial abundances were diminished to zero soon after 36 hours (Figures 2.5 and 2.6); this corresponded with the peak abundances of *P. imperforata*.

Ingestion and clearance rates varied among the experiments and are summarized in Table 2.3. In spite of the variability, the mean ingestion rates between the two conditions are almost equal, where the static tanks showed 4.2 cells flagellate^{-1} h^{-1} and the turbulent tanks showed 4.1 cells flagellate^{-1} h^{-1}. The mean clearance rates were slightly higher in the static tanks (1.04 x 10^{-6} ml flagellate^{-1} h^{-1}) than the turbulent tanks (7.28 x 10^{-7} ml flagellate^{-1} h^{-1}).

Cell volumes were not routinely measured throughout the experiments. However, there was a noticeable decrease in the cell volume of the flagellates in the turbulent conditions. Average flagellate cell volumes during the first 24 to 30 hours were ca. 20 μm^3, with some cells $\geq$ 50 μm^3 (~1 out of 20 cells counted had a cell radius 4 to 5 μm). Towards the end of the experiment, though, mean cell volumes were ca. 7 μm^3.

2.5. DISCUSSION

2.5.1. Theoretical versus measured kinetic energy

The energy dissipation rate estimated for three random locations in the stirred tank was approximately 1.35×10^{-5} W kg^{-1}. The kinetic energy dissipation rates measured in the open ocean range from 10^{-3} to 10^{-10} W kg^{-1} (Osborn 1978, Oakey and Elliot 1982, Yamazaki and Osborn 1988), so the measured rate observed in this study is similar to that found in nature. In regards to the amount of energy required to "double the absorption rate" (Purcell 1978) of a 5 to 6 μm predator, 10^{-5} W kg^{-1} is more than enough energy to enhance the capture of prey particles.

In most cases, experiments that are based on theory tend to produce results more ideal than what would be found in nature. This experimental set-up may have generated dissipation rates similar to those found in the open ocean, but it provided constant, steady stirring. Environmental turbulence can be characterized as intermittent and unpredictable. Furthermore, the equations used to determine the energy needed to double the absorption rate of a predator assumed the prey particle and predator to be spherical. *V. splendidus* is a rod shaped bacterium, and even though *P. imperforata* is spherical, it is covered in siliceous spines (Fenchel 1986a). The implications of the equation are slightly questionable. The spherical shape was assumed only to make the mathematics easier. Hence, it would be a mathematical challenge to determine how a rod-shaped particle, or any non-spherical body, is considered into a similar equation for the diffusion coefficient.

2.5.2. Biotic parameters

Purcell's (1978) first example of $\langle S \rangle_{min}$, which was explored earlier, showed that a bacterium would require an enormous amount of energy to increase the rate of nutrient uptake. If it is assumed that nutrient uptake controls bacterial production, then the results of this study are consistent with those of Moseneder and Herndl (1994). The bacterial production (BP) was measured in seawater samples after agitation for 24 hours and in non-agitated controls. In the samples which contained only bacteria, BP was similar in stagnant and turbulent conditions. This is consistent with the results presented in this study. There was no significant difference in the bacterial growth between the turbulent and static grazer-free conditions. Since a single bacterium is much smaller than the Kolmogorov scale, it experiences only laminar shear and must depend upon its own motility to encounter and absorb its nutrients. The higher bacterial abundance at the end of the experiment in the turbulent condition would then be a result of a better homogeneous mix of nutrients within the tank, already an assumed characteristic of turbulence (see Section 2.2).

It is evident that turbulence did have a clear effect on the growth and grazing of the microflagellate. Growth rates were ≥ 2 fold higher in the turbulent (2.3 d^{-1}) than static conditions (1.1 d^{-1}; Table 2.3). Consequently, with higher flagellate abundances, the bacteria were depleted from the turbulent system more rapidly than those of the static. These results suggest that flagellate grazing does have control of the bacterial population in the presence of turbulence.

The ingestion rates of the microflagellate were variable under the two conditions. Ingestion rates were higher in the first 6 to 24 h of all three turbulent experiments, however, there was a massive increase in flagellate numbers and the number of prey particles ingested per flagellate decreased to the mean rates listed in Table 2.3. The opposite occurred for the static condition. For the first 6 to 24 h flagellate grazing and ingestion had very little control on the bacterial abundance. After 36 hours, when grazing mortality surpassed bacterial growth, flagellates of the static conditions still ingested the same number of cells h^{-1} as those flagellates of the turbulent conditions. These results are similar to the results of Peters and Gross (1994) and Peters *et al.* (1996). Peters and Gross (1994) found that turbulence effectively increased the grazing of *P. imperforata*, but the ingestion rates in the two experimental conditions were not significantly different. Peters *et al.* (1996) found that only at very high levels of turbulence (15 cm^2s^{-1}) did there appear to be differences in the flagellate abundances, feeding rates on fluorescently labeled bacteria and gross growth efficiency. They attributed the lower ingestion rates of the turbulent condition to the decrease in cell size as the flagellate population increased. According to Fenchel (1982b), microflagellates will continue to divide at low food concentrations, but there will be little increase in the total biomass, suggesting that these protozoa may resort to a survival technique when confronted with little or no food available, or periods of starvation. Cell division without growth may have occurred in this experiment because of the flagellate abundance outnumbering the bacterial abundance in the turbulent conditions at the end of the experiments. In addition, there

was a noticeable size difference in the flagellate cells at the end of the turbulent incubations.

Shimeta *et al.* (1995) also reported no prominent effect of applied laminar shear on the clearance rates of *P. imperforata*. They used Couette tanks to produce laminar shear fields. Couette tanks use a rotating platter and spindle to gently spin the water within the tank. As previously discussed, laminar shear is characteristically found just below the Kolmogorov turbulent length where viscosity is dramatically smoothing the smallest turbulent eddies. Shimeta *et al.* (1995) did, however, observe a significant increase in the clearance rates of *Monosiga* sp., and suggested that the effect of turbulence is species specific. *Monosiga* sp. is a choanoflagellate and is typically the same size (5 to 6 μm diameter), or smaller than *P. imperforata*. How turbulence effects the feeding rate of one microflagellate, but not another, may reflect the difference in the mode of feeding and how prey particles are captured. Fenchel (1982a) describes *Monosiga* as having a collar with pseudopodia arising from the base of the collar. Prey particles only need to come into contact with the pseudopodia to then be carried down to the posterior end of the organism. *P. imperforata*, on the other hand, must have a prey particle come into direct contact with its ventral furrow in order for it to be phagocytized. The pseudopodia of *Monosiga* may provide a larger surface area than the ventral furrow of *P. imperforata*. A larger surface area could be translated to better chances at encounter, and therefore a higher ingestion or clearance rate.

Higher encounter rates caused by the fluid motion surrounding an organism does not necessarily result in higher ingestion rates, as was previously reported in Table 2.3.

The question still remains: Why do increased water movements created by rapid stirring or turbulence influence the grazing of microorganisms? Many microflagellates, including *P. imperforata*, have been observed to have a random walk pattern (Berg 1982, Peters 1994). Hence, it could be argued that increased water fluctuations would only reinforce the random patterns of the swimming organism. As it was discussed in Section 2.2, equations (5) through (7) showed that weak stirring would not enhance encounter rates with prey particles anymore than simple diffusion. However, higher turbulent energies will allow smaller grazers to "outrun" diffusion by moving faster and covering larger distances (Purcell 1977). Purcell had also suggested that perhaps turbulence is only leading microorganisms to "greener pastures" where higher food concentrations exist. It has been reported that small-scale turbulence may actually promote plankton patchiness (Kiørboe 1993) or particle concentration rather than uniform distribution (Squires and Yamazaki 1995). Hence, small-scale turbulence may allow small grazers a better chance for survival by transporting them to nutrient rich areas within the water column.

2.5.3. Conclusions

This research shows that turbulence can augment the community growth and grazing of the microflagellate species, *P. imperforata*. This further suggests that growth and ingestion rates of some protistan grazers that have been determined under static incubations, may be underestimated compared with the rates which occur in nature, where turbulent conditions exist. Results from various other researchers do not yet agree as to why small-scale turbulence effects microorganisms and by how much. Therefore

more research in this field is needed for a better understanding of this phenomenon and how it impacts upon the trophic interactions of the microbial food web.

A B

Figure 2.1: a) Prey particle encountering predator through Brownian motion and b) prey particle encountering predator by direct interception. (Vogel 1994) Arrows represent the streamline of the fluid's flow.

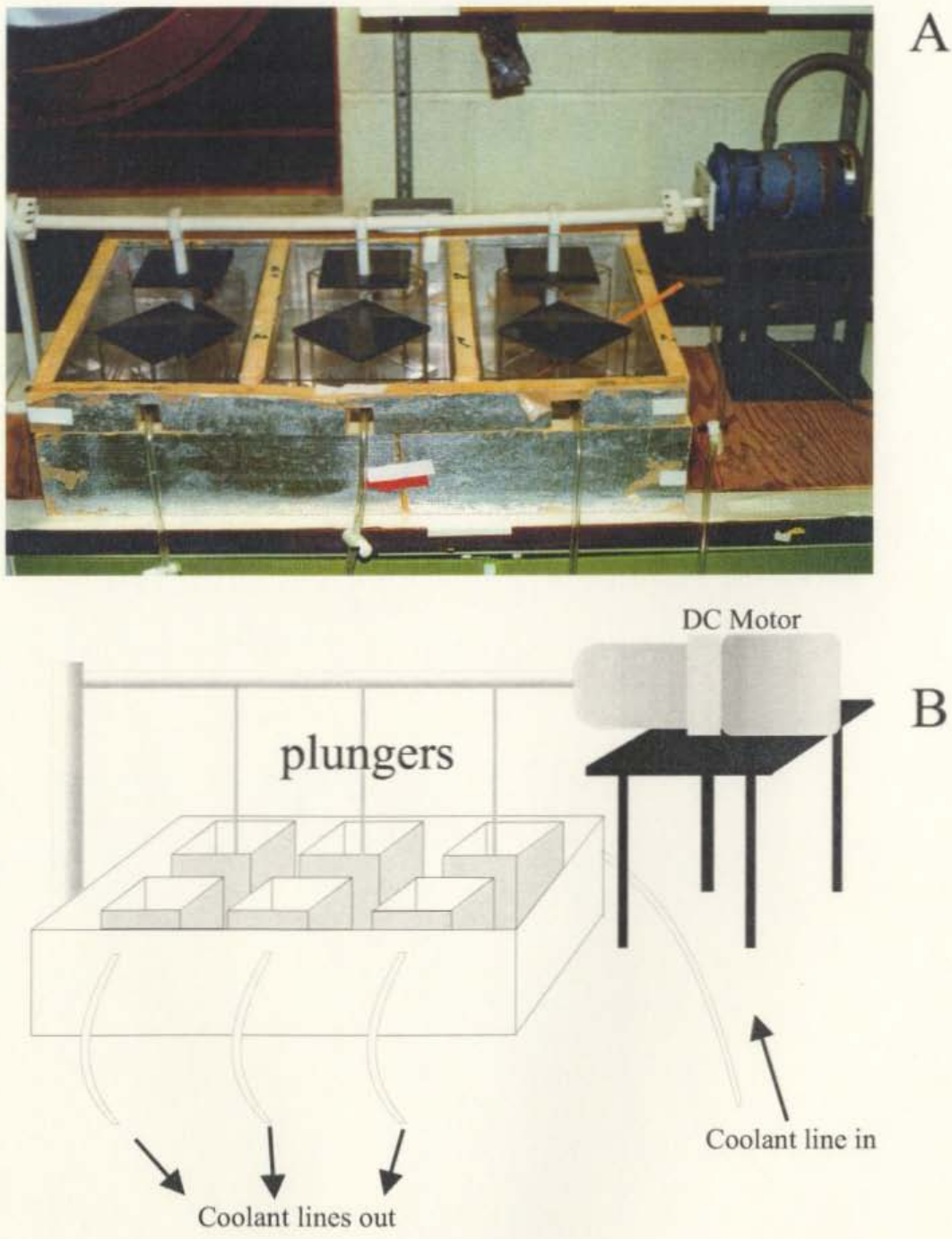
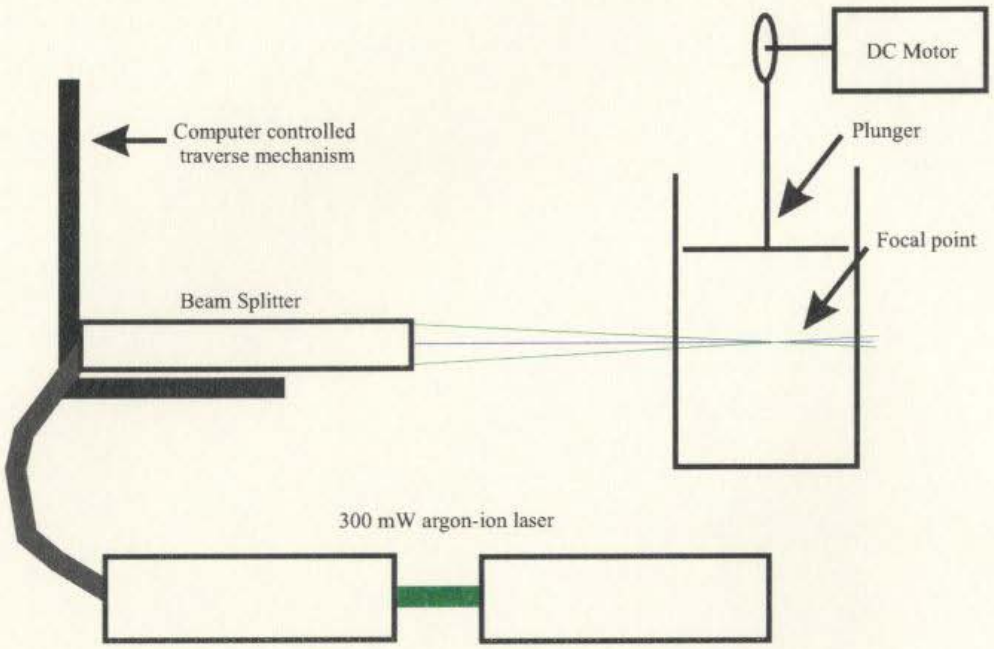


Figure 2.2 Experimental apparatus consisting of 3 static and 3 stirred (turbulent) incubation tanks. Turbulence created by vertically oscillating plungers consisting of a plastic wand and a perforated PVC plate. A) Still-photo of experimental system. B) Schematic drawing of system.

A



B

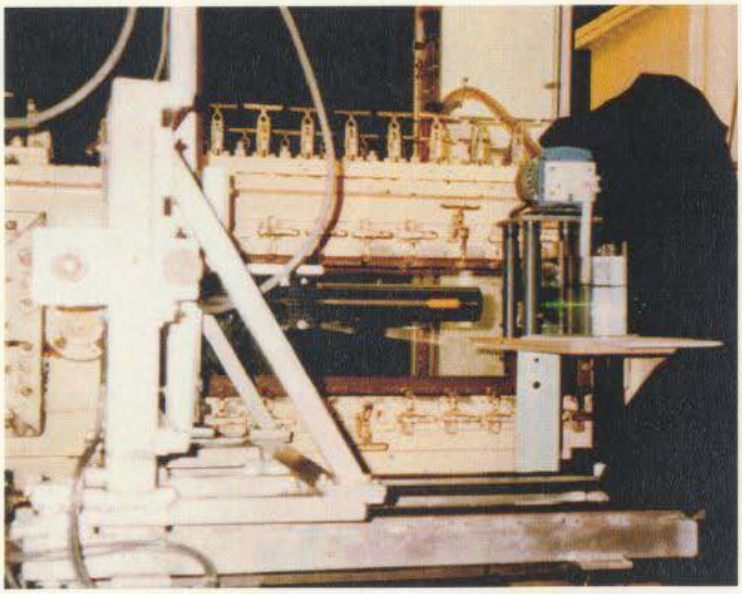


Fig 2.3 Laboratory set-up of the laser Doppler velocitimeter. Laser beam is split into 4 beams and positioned just below the PVC plunger. A) Schematic drawing of experimental set-up. B) Still-photo of set-up.

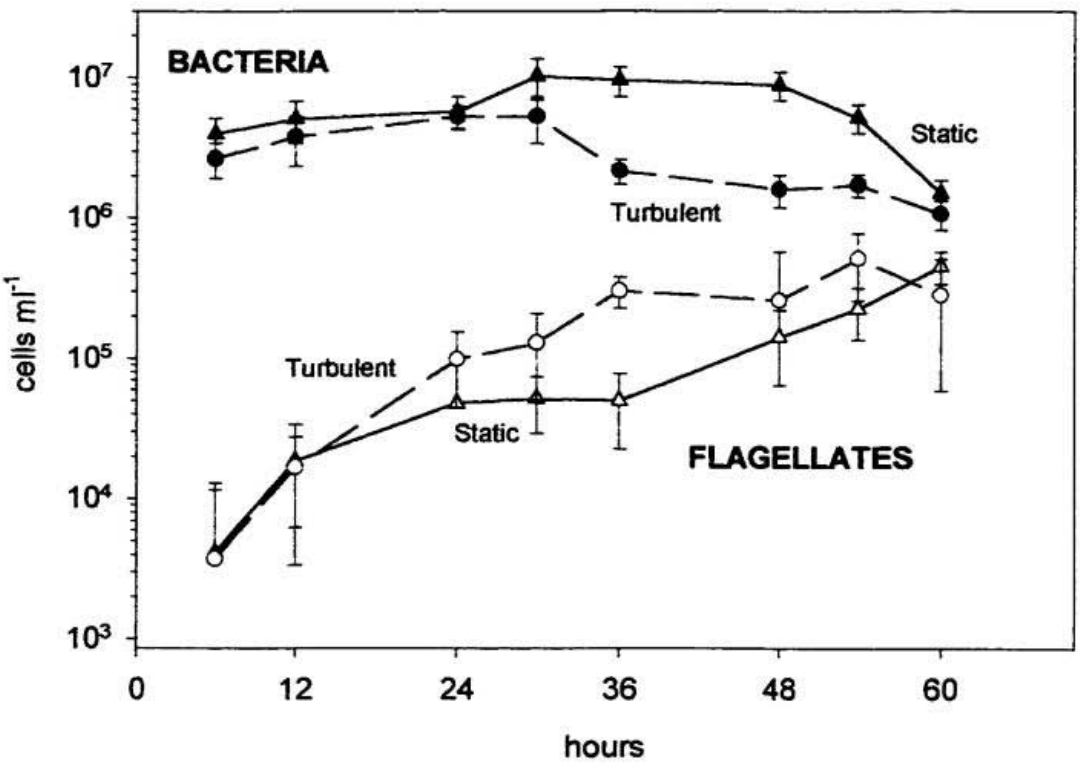


Figure 2.4: The change in abundance (± SE) of the heterotrophic flagellate *Paraphysomonas imperforata* (open symbols) and bacteria *Vibrio splendidus* (filled symbols) of Experiment A at 15°C in turbulent (circles) and static (triangles) conditions.

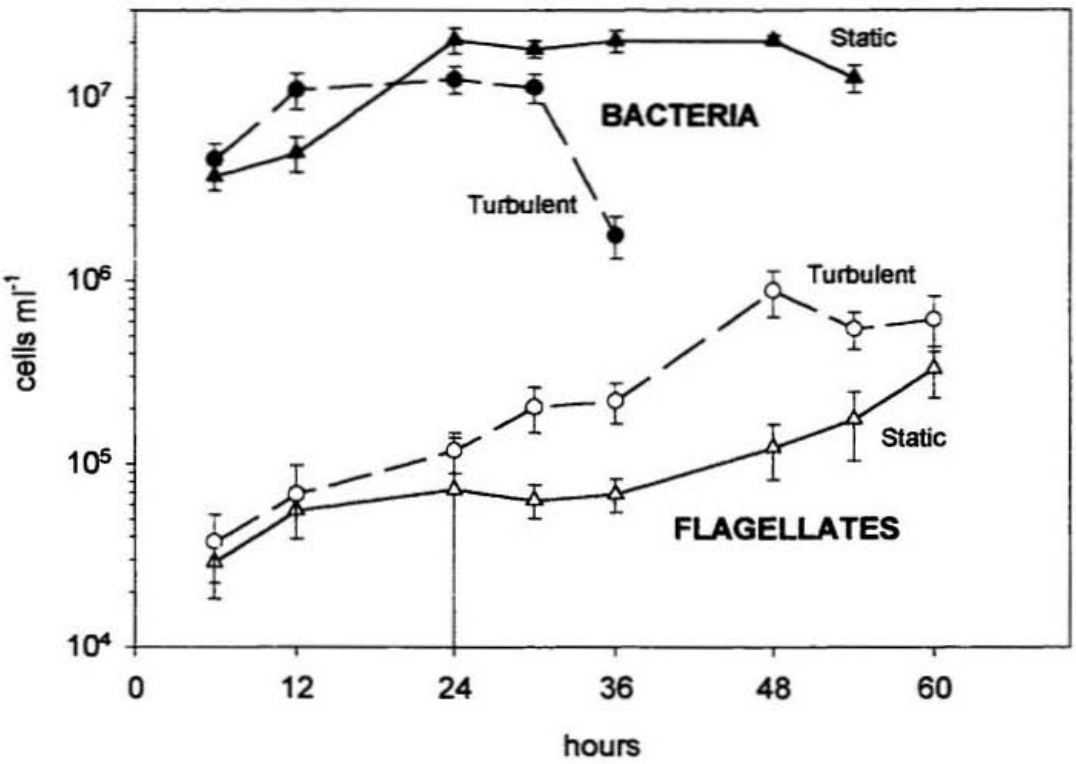


Figure 2.5: The change in abundance (± SE) of the heterotrophic flagellate *Paraphysomonas imperforata* (open symbols) and bacteria *Vibrio splendidus* (filled symbols) of Experiment B at 15°C in turbulent (circles) and static (triangles) conditions.

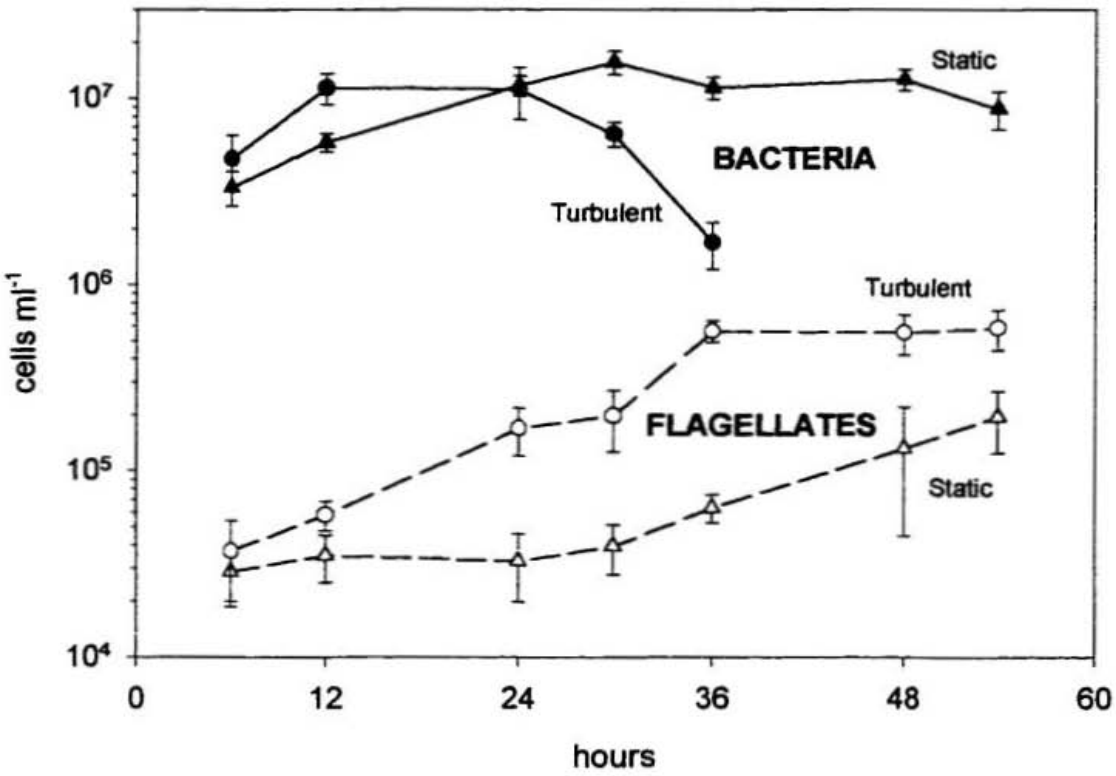


Figure 2.6: The change in abundance (± SE) of the heterotrophic flagellate *Paraphysomonas imperforata* (open symbols) and the bacteria *Vibrio splendidus* (filled symbols) of Experiment C at 15°C in turbulent (circles) and static (triangles) conditions.

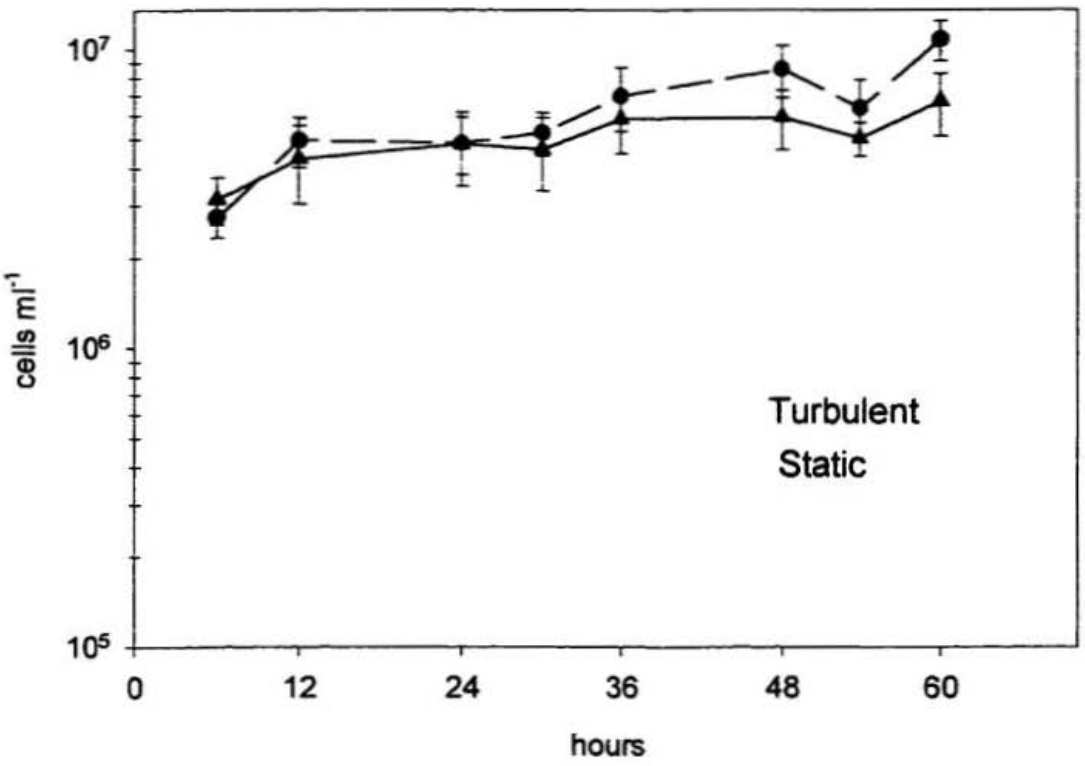


Figure 2.7: Change in bacterial abundance (±SE) in a grazer-free environment of Experiment A at a temperature of 15°C in the turbulent (triangles) and static (circles) conditions.

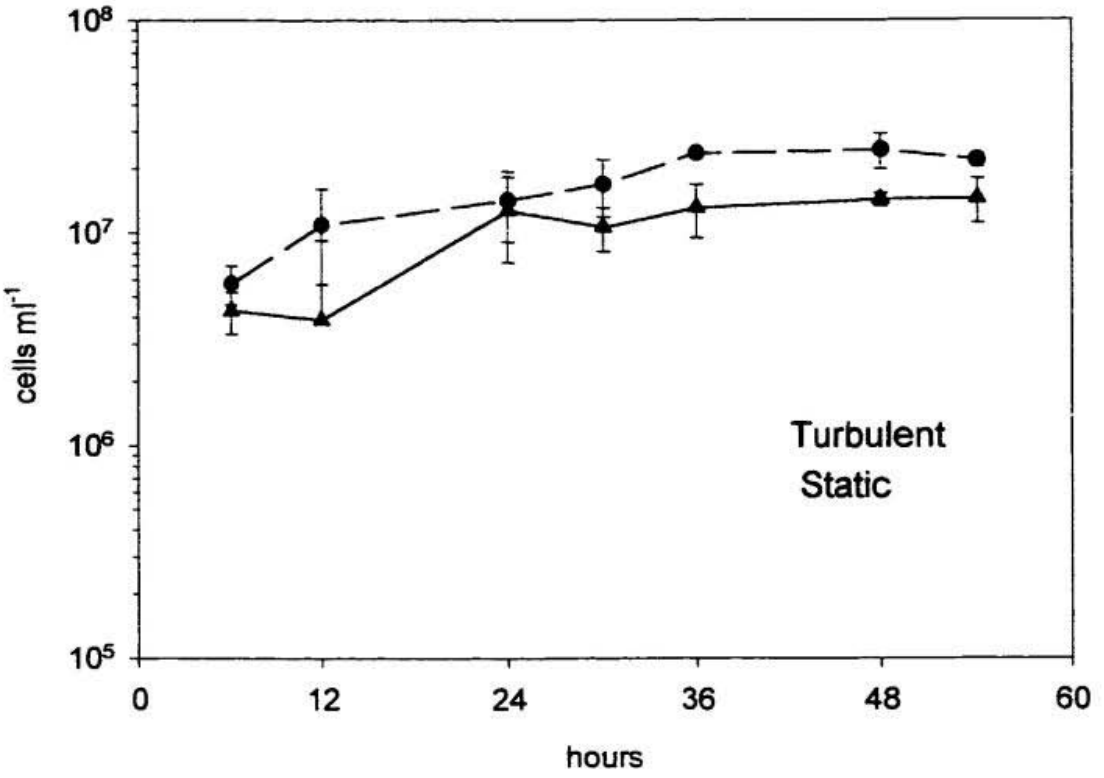


Figure 2.8: Change in bacterial abundance (±SE) in a grazer-free environment of Experiment B at a temperature of 15°C in the turbulent (triangles) and static (circles) conditions.

Table 2.1: Viscosities of seawater collected from Logy Bay, Newfoundland (32‰), calculated from a set of equations listed in Appendix A from Jumars *et al.* (1993).

Temperature (°C)	Dynamic viscosity (Pa s)	Kinematic viscosity ($m^2 s^{-1}$)
0	1.83×10^{-3}	1.78×10^{-6}
5	1.56×10^{-3}	1.53×10^{-6}
10	1.33×10^{-3}	1.30×10^{-6}
15	1.11×10^{-3}	1.09×10^{-6}

Table 2.2: The growth rates of the heterotrophic flagellate *Paraphysomonas imperforata* and the bacteria *Vibrio splendidus* (± 90%) for each experiment at 15°C.

Experiment	Bacterial growth (divisions d^{-1})	Flagellate growth (divisions d^{-1})
Experiment A		
Static	0.24±0.12	1.10±0.43
Turbulent	0.77±0.50	3.55±1.30*
Experiment B		
Static	1.06±0.60	0.86±0.24
Turbulent	0.98±0.34	1.67±0.31*
Experiment C		
Static	1.06±0.60	1.03±0.26
Turbulent	0.98±0.34	2.04±0.46*
Grand Mean		
Static	0.74±0.39	1.01±0.31
Turbulent	0.77±0.29	2.35±0.52

* denotes a significant difference ($p < 0.05$, ANOVA) between the static and turbulent conditions.

Table 2.3: The ingestion and clearance rates of the heterotrophic flagellate *Paraphysomonas imperforata* for each experiment at 15°C. Mean bacterial abundance was 10^6 to 10^7 cells ml^{-1}.

Experiment	Ingestion rate (bacteria flagellate^{-1} h^{-1})	Flagellate clearance rate (nl flagellate h^{-1})	Total bacteria consumed (ml^{-1} h^{-1})
Experiment A			
Static	1.12	0.26	1.09×10^5
Turbulent	1.37	0.43	2.83×10^5
Experiment B			
Static	7.03	1.8	8.72×10^5
Turbulent	7.52	1.2	9.71×10^5
Experiment C			
Static	4.34	0.46	5.10×10^5
Turbulent	3.46	0.54	1.27×10^6

CHAPTER 3

The Effects of Small-Scale Turbulence and Low Seawater Temperatures on the Growth and Grazing of the Heterotrophic Microflagellate, *Paraphysomonas imperforata*

3.1.1. Introduction

It is widely accepted that heterotrophic bacteria are the foundation of the marine microbial food web (Pomeroy 1974, Azam *et al.* 1983). Bacteria take up and recycle dissolved organic matter and other nutrients in the ocean which are produced by protistan and metozoan grazers. Moreover, bacteria are too small to be efficiently ingested and utilized by most metazoan grazers (Fortier *et al.* 1994). Hence, it is the bacteriverous protists, such as flagellates and ciliates, which incorporate the energy of that organic matter into the marine food web by repackaging the small cells into particles which can be efficiently ingested by larger zooplankton (Goldman *et al.* 1985, Goldman *et al.* 1987). Although marine bacteria have the potential for rapid growth, the abundances remain relatively constant through time within a range of 0.5 to 3 x 10^6 cells ml^{-1} (Andersen and Fenchel 1985, Ducklow and Carlson 1992). It is believed that heterotrophic microflagellates are the major grazers which control bacterial abundance (Sherr and Sherr 1984, Porter *et al.* 1985, Fenchel 1987). Sherr *et al.* (1986) suggested that microflagellates are capable of consuming 60% or more of the bacterial biomass, and Fenchel (1982d) calculated that 10 to 70% of the water column is cleared of bacteria each day by microflagellates. With such a large grazing potential, it is not surprising that both

phototrophic and heterotrophic protozoa dominate the plankton community in the euphotic zone (Goldman and Caron 1985).

Many models for the marine microbial food web are qualitative models which require that biological and physical processes be parameterized and constrained to the grazing impact on bacteria. This is essential to understand the functionality of the microbial system (Wright 1988a). Many physical processes influence the conditions that control important biological processes, such as growth, grazing, and particle distribution. Clearly, biological processes cannot be considered in isolation of the physical and chemical environment (Mann and Lazier 1990). Physical processes, such as turbulence and seawater temperatures, can potentially impact the pathways of transfer of microbial production to larger consumers (e.g. mesozooplankton). In the past decade, several studies suggested that small-scale turbulence could influence the encounter rates of a plankton grazer and its prey (Rothschild and Osborn 1988, MacKenzie and Leggett 1991, Kiørboe and Saiz 1995). This theory has been examined numerous times with predators such as copepods (Marrasé *et al.* 1990) and fish larvae (Dower *et al.* 1996), but very few studies have investigated the impact of turbulence on heterotrophic microflagellates. Moreover, to date, studies examining the effect of small-scale turbulence on microorganisms, including microflagellates, have been carried out at seawater temperatures above 15°C. It seems that studies on temperature dependent bacterial and heterotrophic processes have been under-represented from cold or polar environments (Rivkin *et al.* 1996). Considering that more than 70% of the ocean is always below 5°C, and 90% of the ocean is seasonally below 5°C (Baross and Morita 1978, Levitus 1982,

Rivkin *et al.* 1996), it is necessary to assess the effects of small-scale turbulence and cold seawater temperatures on the grazing of bacteria by heterotrophic microflagellates.

3.1.2. Objectives

This chapter examines the effect of turbulence on the temperature dependent growth of the heterotrophic microflagellate, *Paraphysomonas imperforata*, and the grazing on its bacterial prey, *Vibrio splendidus*, at 15°C, 10°C, 5°C and 0°C. The experiments were designed to encompass the seasonal temperature variability of Logy Bay, Newfoundland, eastern Canada. It is hypothesized that microflagellate growth and its ingestion of bacteria will be higher in the turbulent condition at all four experimental temperatures.

3.2. MATERIALS AND METHODS

3.2.1. Experimental apparatus

The experimental apparatus consisted of three insulated plexiglass containers (46 cm x 20 cm) containing two smaller plexiglass incubation containers (11 cm x 11 cm x 14 cm height). The three tanks were connected to a temperature controlled recirculating water bath (Neslab RTE-210). Of the six smaller incubation chambers, three are the control (i.e. non-turbulent) condition and three chambers are the turbulent condition (Figure 2.2).

The turbulence was created by vertically oscillating plungers consisting of a plastic wand and a perforated polyvinyl chloride (PVC) plate (plate size was 9.5 cm^2, perforation diameter was 2 mm, ~93% solidity). The plates were centered in each chamber with 2.5 mm clearance between the inside walls of the tank and the edge of the plates. The vertical amplitude of the plunger motion was 3 cm, travelling in the upper portion of the water column, and oscillating at a rate of 1 Hz $\pm$ 0.3 Hz. (see Figure 2.3)

3.2.2. Quantification of turbulence

Turbulence was measured with a 2-axis 300 mW argon-ion laser Doppler velocitimeter (Dantec Electronics) and processed with a Flow Velocity Anemometer (Dantec Electronics). Vertical and horizontal components of water velocities were measured at different positions within the water column using a computer controlled traverse mechanism. Latex fluorescent beads (1 μm), similar in size to the bacterial prey, were used as tracer particles in the water column. Each velocity component was measured at a sampling rate of ~120 Hz with a bandwidth of 0.12 MHz and ~2 mm fringe

spacing (n=35). Data were collected at random locations within the tank for an accumulation of 10,000 sample velocities or a maximum of 10 minutes per site. See Section **2.3.2** for complete details on the data analysis of the laser doppler anemometer.

3.2.3. Maintenance of Cultures

Cultures of *Paraphysomonas imperforata* were maintained in 70 ml glass culture tubes at all four temperatures. Stock cultures were transferred into 50 ml of fresh medium every 3 to 4 weeks. The medium used was sterile, 0.2 μm filtered seawater enriched with 1 ml of 10% yeast extract (Difco) and 1 ml of 0.2% proteose peptone (Difco). A sterile rice grain was added to each culture tube. *Vibrio splendidus* cultures were maintained on agar plates for up to one month. Two steps were required for the preparation of a stock culture to be used for an experiment. First, 25 ml of deionized water (DI) was enriched with 0.935 g of prepared nutrient mix (Marine Broth 2216, Difco) and autoclaved in 100 ml Erlenmyer flasks. A swab off a marine broth agar plate was transferred to the aqueous medium. The flask was then placed on a reciprocating shaker ca. 12 h at room temperature. This was sufficient time for *V. splendidus* to reach exponential growth. The next day, 1 ml of the culture was diluted to 800 ml of sterile, 0.2 μm filtered seawater, and replaced on the shaker for 6 to 12 hours. This working stock culture was then divided among the six incubation chambers (~ 130 ml each), diluted to 1.3 L with sterile, 0.2 μm filtered seawater, and allowed to acclimate to the experimental temperatures before the addition of *P. imperforata*.

3.2.4. Flagellate Growth and Ingestion of Bacteria

Four of the six incubation tanks (two static and two turbulent) containing the diluted bacterial cultures were inoculated with 30 ml of the flagellate culture and were maintained in static condition for ca. 12 h before the initial (t=0) sampling to allow for acclimation. The two remaining incubation tanks (one static and one turbulent) were grazer-free controls to assess the effect of turbulence on bacterial growth. The duration of the flagellate growth experiments was dependent upon each temperature. For the 15°C and 10°C experiments, samples were taken at 6 to 12 h intervals for three days. For the 5°C and 0°C experiments, samples were collected every 24 h for the first 72 h, and at every 12 h for the next 72 h (with the exception of one 5°C replication where sampling was similar to those of the higher temperatures). All samples (5 ml) for enumerating bacteria and flagellate abundances were immediately preserved with 1 ml of gluteraldehyde (5% final concentration), stored at room temperature in glass 20 ml liquid scintillation vials, and processed within 3 to 5 days of their collection.

3.2.5. Sample Analysis

Bacterial abundance was determined with the acridine orange direct count method (Hobbie *et al.* 1977). Due to high bacterial abundances, samples were diluted up to ~20 fold with 0.2 µm filtered seawater. The diluted subsamples were collected onto a pre-stained black 0.2 µm Poretics polycarbonate filter and post-stained with acridine orange. Two filters per slide were placed on smeared drops of Cargille Type A immersion oil on a glass microscope slide. Each filter was topped with another drop of immersion oil and the coverslip was gently pressed on so that the oil was evenly dispersed over the filter.

Bacteria were counted using a BH2 - RFC Olympus epifluorescence microscope under a magnification of 1000x. A mercury lamp (100 W) was used to emit blue excitation (BP440, DM455, AFC+Y475). At least 300 cells on each filter were counted by randomly selecting fields.

Flagellate abundances were determined by diluting 2-ml aliquots to 5 ml (with 0.2 um filtered seawater) and filtering the entire 5 ml onto 1.0 μm Poretics pre-stained black polycarbonate filters. Each filter was post stained with 0.2 ml of acridine orange. Filters were prepared as described above. Flagellate abundances were enumerated by counting 10 random fields on each filter using epifluorescence microscopy.

3.2.6. Calculations

Each bacterial and flagellate abundance from the microscopic counts were calculated by using the following equation:

(1) $$N * \left[\frac{A^2}{100/X} \right] * \frac{1000}{V}$$

where N is the average number of cells counted per filter, A^2 is the area of filter occupied by the stained sample (mm^2), X is the number of squares counted in the ocular grid (100 squares, $1 mm^2$), and V is the volume of the filtered undiluted sample.

For each experimental tank, cell division rates (μ, h^{-1}) of flagellates and bacteria were determined from the linear regression of the natural logarithm (ln) of abundances plotted against time. For each grazed tank, each plot of bacterial abundance was divided into three intervals, representing lag, growth, and depleted cells (negative slope). The net grazing mortality, or apparent growth rate (AGR, h^{-1}), of bacteria in the grazing chambers

was also determined with linear regression of the plotted data. The best lines were considered to be those that maximized the r^2 and minimized the standard error of each division rate. The growth (μ) and AGR were then applied to Frost's equations (1972) to equate mean bacterial concentration ([C], cells ml^{-1}), flagellate clearance (CR, nl $flagellate^{-1}h^{-1}$) and ingestion (IR, cells $flagellate^{-1}h^{-1}$):

(2) $$[C] = C_1\left(\frac{e^{(\mu - AGR)(t_2 - t_1)} - 1}{(\mu - AGR)(t_2 - t_1)}\right)$$

(3) $$CR = \frac{AGR}{N}$$

(4) $$IR = CR * [C]$$

where C_1 is the concentration of bacteria in the non-grazer tanks at the beginning (t_1) of the time interval, t_2 is the end of the time interval, and N is the concentration of flagellates ml^{-1} at that specified time interval. Ingestion rates were normalized by dividing IR with the mean flagellate population for a given time interval. Student paired t-tests or two-way ANOVA with integration were used for statistical analyses of each experiment. Each rate (growth, grazing and ingestion) was tested for significant differences between experiment replications and for the static and turbulent conditions.

3.3 RESULTS

At each of the four temperatures, there was no difference in the mean bacterial growth rate between the static and turbulent treatments ($p>0.05$, ANOVA). The bacterial growth rate ranged from 0.22 to 0.68 divisions d^{-1}, except for one experiment at 15°C where the bacterial growth rate was ~1 d^{-1} for both the turbulent and static conditions (Table 3.1).

Flagellate abundance was significantly higher at 15°C ($p=0.0001$, ANOVA) and 10°C ($p=0.002$, ANOVA) under turbulent than the static conditions (Table 3.1). Flagellate growth at 5°C was almost twice as high in the turbulent compared with the static condition, but the difference was not significant ($p=0.08$, ANOVA). Similarly, flagellate growth was slightly higher in the turbulent condition at 0°C, but the variance in the growth rate was higher in the turbulent than the static condition. Flagellate growth also declined with the decreasing temperature. Growth decreased 3 fold in the static condition and decreased approximately 5 fold in the turbulent condition (Fig 3.2).

At 15°C and 10°C, bacteria were grazed from the seawater faster under turbulent than static conditions. At 15°C, there was a noticeable decrease in bacterial abundance by 24 to 30 h, whereas under the static conditions, bacterial abundance did not decrease until 36 to 48 h. Indeed, in the experiments at 15°C, flagellate grazing depleted the bacterial population by 48 h in the turbulent conditions (see Figures 2.5 and 2.6). Hence, the mean total bacteria grazed by the flagellate community at 15°C was significantly higher in the turbulent than static conditions ($p=0.0001$, ANOVA, Table 3.2). Bacterial grazing at 10°C was slower than at 15°C, and differences between the static and turbulent

conditions were significant ($p=0.0011$, ANOVA, Table 3.2). In three of the four experiments at 10°C, bacterial abundances decreased after 36 h in the turbulent condition, whereas in the static condition, bacterial abundances decreased after 48 h. In one experiment at 10°C, the bacterial population ml^{-1} remained relatively constant (no noticeable decrease) throughout the duration of the experiment (Figure 3.3). For the experiments at 5°C and 0°C, there was no difference ($p>0.05$) in the grazed bacterial population between the static and turbulent conditions, although the 5°C experiment showed a slightly higher grazing mortality (7.2 to 8.9 x 10^5 bacterial cells $ml^{-1}h^{-1}$) than the 0°C experiment (3.1 to 6.6 x 10^5 bacterial cells $ml^{-1}h^{-1}$; Table 3.2).

The apparent growth rates of the bacteria (AGR) were calculated from the decreasing slope of the bacterial abundance for each experiment, from which ingestion rates (bacteria ingested $flagellate^{-1}$ h^{-1}) and clearance rates (nl $flagellate^{-1}$ h^{-1}) of *P. imperforata* were calculated. The flagellate ingestion and clearance rates were similar in the turbulent and static conditions. Moreover, the ingestion and clearance rates were higher at cold (0 and 5°C) than warm temperatures (10 and 15°C). For example, 4.1 to 4.2 bacterial cells were consumed $flagellate^{-1}$ h^{-1} at 15°C whereas 34.7 to 48.9 cells were consumed $flagellate^{-1}$ h^{-1} at 0°C. A similar pattern occurred in the clearance rates. There was no difference between the static and turbulent conditions, but higher clearance rates were estimated in the colder temperatures (Table 3.2). For example, only 0.73 to 0.90 nl $flagellate^{-1}$ h^{-1} were cleared at 15°C, but the clearance rate had increased to 3.2 to 3.3 nl $flagellate^{-1}$ h^{-1} at 0°C.

Q_{10} values were calculated for temperature-dependent growth, ingestion and clearance rates for each 5 and 10°C temperature increment between 0 and 15°C (Table 3.3). The Q_{10} value for growth over the entire 15°C range was 2.2 in the static condition and 2.9 in the turbulent condition, with the highest Q_{10} value (3.8) for growth occurring for the temperature interval from 10 to 15°C. The Q_{10} for ingestion over the 15°C range was 0.19 in the static condition and 0.24 in the turbulent condition. The Q_{10} for clearance was greater than the ingestion rate, but still smaller than growth with 0.43 in the static condition and 0.36 in the turbulent condition. The Q_{10} value for bacteria growth over the entire 15°C range was 1.1 for the static condition and 1.6 for the turbulent condition, with the highest value of 5.3 for bacterial growth occurring during the 10 to 15°C increment (Table 3.4).

3.4. DISCUSSION

3.4.1. Biotic parameters, static versus turbulent conditions

As was discussed in Chapter 2, a bacterium would require an unnatural amount of turbulent, or stirring, energy to increase its rate of absorption of nutrients, and hence its growth and production. The results of this study showed no significant difference in the bacterial growth in the static and turbulent conditions (Table 3.1). These results are consistent with those of Moseneder and Herndl (1994), who investigated effects of turbulence on bacterial production (BP). The BP was measured in seawater samples after agitation for 24 hours and in non-agitated samples. In the samples which contained only bacteria, BP was similar in stagnant and turbulent conditions. Logan and Kirchman (1991) showed that the uptake of leucine in attached bacteria increased by 8 fold when fluid flow increased from 20 to 70 m d^{-1}. However, Logan and Kirchman (1991) also reported that increases in fluid motion did not increase nutrient uptake in free-living (unattached) bacteria. Therefore, it appears that a single bacterium must rely on its own motility rather than the fluid motion created by turbulence to encounter and absorb its nutrients since it is much smaller than the Kolmogorov scale.

The growth rates of *P. imperforata* determined during these experiments were consistently higher than their bacterial prey. These rates (0.32 to 2.35 d^{-1}) are comparable to those rates determined in other studies (Table 3.5). Choi and Peters (1992) examined the growth rates of "Arctic" and "Newfoundland" isolates of *P. imperforata* at temperatures ranging from -1.5 to 15°C. They reported growth rates of 0.53 to 2.37 d^{-1} when the flagellate grazed at a saturated food concentration of a marine bacterium.

These authors (Choi and Peters 1992) suggested that their growth rates of *P. imperforata* were relatively high and that *in situ* rates may be lower due to a smaller bacterial size and lower bacterial abundances typically found in nature. Eccleston-Parry and Leadbeater (1994) reported a maximum growth rate of ~5.04 d^{-1} for *P. imperforata* incubated at 20°C when fed a marine bacterium and Caron *et al.* (1986) reported a growth rate of 1.37 d^{-1} at a temperature of 14°C when *P. imperforata* was feeding on the diatom *Phaecodactulum tricornutum*. In all these studies, though, the growth of *P. imperforata* was measured under static incubations and the length of incubation varied in each study.

The ingestion rates by *P. imperforata* estimated during this study (4 to 49 bacterial cells flagellate^{-1} h^{-1}) were low relative to other studies (Table 3.5). However, the abundance of flagellates in this study was relatively high in the system, especially at the peak growth rates in the turbulent conditions of the warmer (10 and 15°C) temperatures. High flagellate abundances can result in lower ingestion rates per flagellate due to the number of prey available, hence the increased competition for a food resource (Peters 1994). For example, during the 15°C experiments, at t=0 h, the ratio of bacteria : flagellates was ~750, but by 60 h, the ratio decreased to ~10. Within the 15°C experiments, by the time maximal grazing affected the bacterial population, there were not enough bacteria available for each flagellate to ingest at a high rate and the bacteria could not divide quickly enough to recover its population. Moreover, flagellate growth at 15°C was 3 fold greater than bacterial growth (Table 3.1).

In this study, ingestion rates were computed from clearance rates, which in turn, were computed from the Frost (1972) equations. Since ingestion was not measured

directly in this study, it is difficult to assess whether turbulence truly influenced the number of bacteria encountered and ingested by the flagellates. In contrast, several previously published studies measured ingestion rates of *P. imperforata* using fluorescently labeled bacteria (FLB) or non-growing bacteria (either heat killed or starved of nutrients; i.e. Fenchel 1982b, Sherr *et al.* 1988, Choi and Peters 1992, Choi 1994). Certainly, the results reported here suggest that there is no difference in the ingestion rates between the static and turbulent conditions (Table 3.2) which agrees with the results of Peters and Gross (1994) who measured ingestion rates of 2.11 to 2.70 FLB flagellate^{-1} h^{-1} from direct observation of ingested FLB into the food vacuoles of *P. imperforata*. Sherr *et al.* (1988) also reported ingestion rates of a mixed group of bactiverous flagellates by directly measuring the ingestion of FLB. They reported that flagellates ingested 5.2 to 27.4 FLB flagellate^{-1} h^{-1}. The results of Peters and Gross (1994) and Sherr *et al.* (1988) could suggest one of two things: a) the calculated ingestion rates of this study are not that different than rates directly measured, or b) the flagellates showed selectivity in prey ingestion and perhaps consumed less due to the bacteria being fluorescently labeled. After examining data from numerous studies, Vaqué *et al.* (1994) found that grazing rates measured through dilution, inhibition, filtration or uptake of genetically marked bacteria were higher than those grazing rates measured by the uptake of FLB. However, they reported no significant difference among 372 cases that measured grazing rates by the FLB observation method, and equated an average ingestion rate of 9.7 bacterial cells flagellate^{-1} h^{-1} among those studies.

By multiplying the ingestion rates with the number of flagellates in the system, the total number of bacterial cells grazed ml^{-1} by the community shows a substantial difference between the static and turbulent conditions at the two warmer temperatures. At 15°C, the average total bacteria consumed $ml^{-1}h^{-1}$ in the static condition was 5.93 x 10^5 whereas the flagellates in the turbulent tanks grazed about twice this amount (9.32 x 10^5 bacteria $ml^{-1}h^{-1}$). Similarly at 10°C, the flagellates of the static tanks grazed approximately the same amount as the flagellates at 15°C, 5.53 x 10^5 bacteria $ml^{-1}h^{-1}$, but those flagellates in the turbulent tanks consumed more than twice that (1.62 x 10^6 bacterial cells $ml^{-1}h^{-1}$). The amount of bacteria consumed from the water column of this study was approximately 10 to 100 times greater than *in situ* grazing rates reported from other studies (Table 3.6). This is likely because the only prey available to the *P.imperforata* of this study was the bacteria, whereas in the ocean, other food sources are available (i.e. algal cells) for the flagellates to prey upon (Fenchel 1982a, Choi and Peters 1992, Eccleston-Parry and Leadbeater 1994).

3.4.2. Biotic Parameters, temperature dependence

The growth of *Vibrio splendidus* was variable within the 0 to 15°C temperature range. The growth rates steadily declined between 15°C (0.77 and 0.74 divisions d^{-1}) and 5°C (0.22 and 0.32 divisions d^{-1}). However, the rate of growth increases by 2 fold in the static and turbulent conditions at 0°C (Figure 3.2). Bacteria can have a higher growth efficiency in colder water than those growth and production rates of warmer waters found in lower latitudes (Christian and Weibe 1974, Bjørnsen 1986, Legendre *et al.* 1996). This increased rate of ingestion reported in this study may have resulted in the rapid recycling

of organic nutrients, leading to the compensation of high nutrient effects on low temperature growth (Pomeroy *et al.* 1991). Q_{10} values for the bacterial growth were less than those values for flagellate growth. Moreover, it was suggested by Jumars *et al.* (1993) that an organism with a larger Q_{10} could be limited as the temperature decreases, but the one with the smaller Q_{10} would be limited by increasing temperatures. In the case of this study, bacterial growth for the entire 15°C range and the 0 to 10°C increment had a smaller Q_{10} than the flagellate growth for the same intervals. Therefore, it is possible that as the temperature decreases, it could actually benefit bacterial growth where it would hamper flagellate growth.

The growth, ingestion and clearance rates of *P. imperforata* at the four temperature regimes follow a pattern with the decreasing water temperatures (Figures 3.2 and 3.4). Growth rates tend to decrease and ingestion rates increase with the decreasing temperature. It is generally believed that microorganisms will have higher rates of metabolism and growth at warmer than at colder temperatures, but that cold temperatures do not necessarily hamper the ability of the microorganism to grow at high rates. Thus far only Choi and Peters (1992) have examined some physiological rates of *P. imperforata* below 15°C. *P. imperforata* isolated from Arctic and Newfoundland waters incubated under static conditions grew at similar rates to those reported in this study (Table 3.5), with higher growth rates (~2.54 divisions d^{-1}) at 15°C, and lower growth at -1.5°C (~0.65 d^{-1}). However, Choi and Peters (1992) reported a decrease in the flagellate ingestion rates as the temperature decreased and the clearance rates remained the same between 15 and -1.5°C (Table 3.5).

Dependent upon the temperature interval, Q_{10} values for the growth of *P. imperforata* are near the theoretical value of 2 (Table 3.3), and are in good agreement with other studies. Caron *et al.* (1986) had reported a Q_{10} of 2.5 between 14 and 26°C for *P. imperforata*, and Sherr *et al.* (1988) reported a Q_{10} of 2.5 between 3 and 18°C for a *Monas* sp. The Q_{10} for ingestion for the entire temperature range of this study was much smaller than reported in other studies. A Q_{10} of 0.19 and 0.24 (0 to 15°C) is reported in this study for the static and turbulent conditions versus a Q_{10} of 3.73 for *P. imperforata* between 14 and 26°C (Caron *et al.* 1986) and 3.12 for a mixed group of flagellates between 3 and 18°C (Sherr *et al.* 1988).

The flagellate-specific clearance and ingestion rates of this study are inversely related to temperature (Fig. 3.4). The ingestion and clearance rates at 0°C were 10 fold and 3 to 5 fold, respectively, greater than those rates at 15°C. The increase in ingestion rates was partly due to the number of bacteria available per flagellates at each of the four experimental temperatures because peak flagellate abundances were greater at the warmer temperatures (10^5 ml^{-1}) than those abundances in the colder temperatures (10^4 ml^{-1}). Nevertheless, the results cannot be ignored, especially since it has been suggested that bactiverous flagellates should have relatively high clearance rates at low seawater temperatures typical of polar regions (Legendre *et al.* 1996) and that microflagellates do have the potential to exert a significant grazing pressure on bacteria in colder temperatures (Choi and Peters 1992).

3.4.3. Conclusion

The lower growth rates could possibly be due to a lower prey encounter in the colder temperatures. This, in turn, could be a function of the higher dynamic viscosity of seawater with decreasing temperatures. Higher viscosity makes an animal's mobility more difficult because of decreasing Reynold's numbers and as a result, more energy is required for a predator to swim to its prey. The colder temperatures can also limit the transport of nutrients across a microorganism's boundary layer that could, in turn, reduce the animal's growth (Jumars *et al.* 1993, Legendre *et al.* 1996).

The increased viscosity will also affect the size range of small-scale turbulence. If the Kolmogorov length scale is classified as the length of the smallest turbulent eddy before it is smoothed into laminar shear by viscosity, then the length of the scale would change with the changing temperature. The kinematic viscosity of the seawater of this study, at 15°C, was 1.0875×10^{-6} m^2 s^{-1} while at 0°C it was 1.7841×10^{-6} m^2 s^{-1}. The 1.5 fold increase in viscosity between 0°C and 15°C will have a profound effect at such a small scale. Therefore, the lower the temperature the greater the viscosity, and the turbulent eddies have to be larger to dominate the viscous forces. If the smallest turbulent eddies have increased in size, then the probability of small-scale turbulence affecting organisms 5 to 10 μm in size is extremely low. Small-scale turbulence had a significant effect on the flagellate growth and grazing in the 15 and 10°C experiments. A noticeable difference, but not significant, occurred during the 5°C experiments, but no difference was seen between the two conditions at 0°C.

The growth and ingestion rates obtained in this research may be higher than the rates which exist in nature due to a higher abundance of bacteria available to the flagellates and nutrients added to the seawater cultures. However, the distinct effects on these rates between turbulent and static conditions at all the temperatures cannot be ignored. The results of this study show that turbulence has a clear effect on the growth of *P. imperforata* (above 5°C) and also implies that cultures which require incubation periods longer than 12 to 24 hours, to determine growth rates, should be stirred. This is because major differences between the static and turbulent cultures of this study appeared after 24 hours. It should also be noted that if studies are to continue on the physical-biological interactions of the marine microbial food web, then turbulence (or stirring) should be incorporated as a standard in laboratory experiments. Turbulence is just as important a physical process on a biological system as light, temperature, salinity and nutrient concentrations (Sanford 1997). Furthermore, growth responses in the colder temperatures should be examined more closely, especially to discern what type of control viscosity really has on the microbial community at such low seawater temperatures.

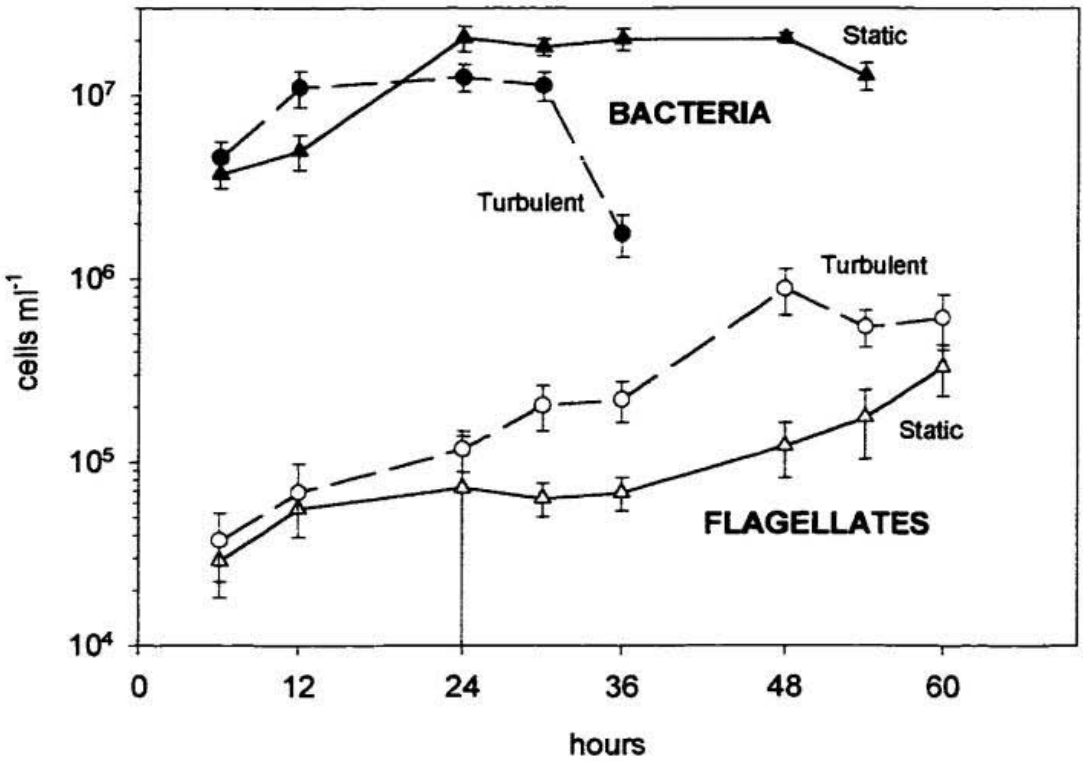


Figure 3.1: The change in abundance (± SE) of the heterotrophic flagellate *Paraphysomonas imperforata* (open symbols) and bacteria *Vibrio splendidus* (filled symbols) of an experiment at 15°C in turbulent (circles) and static (triangles) conditions.

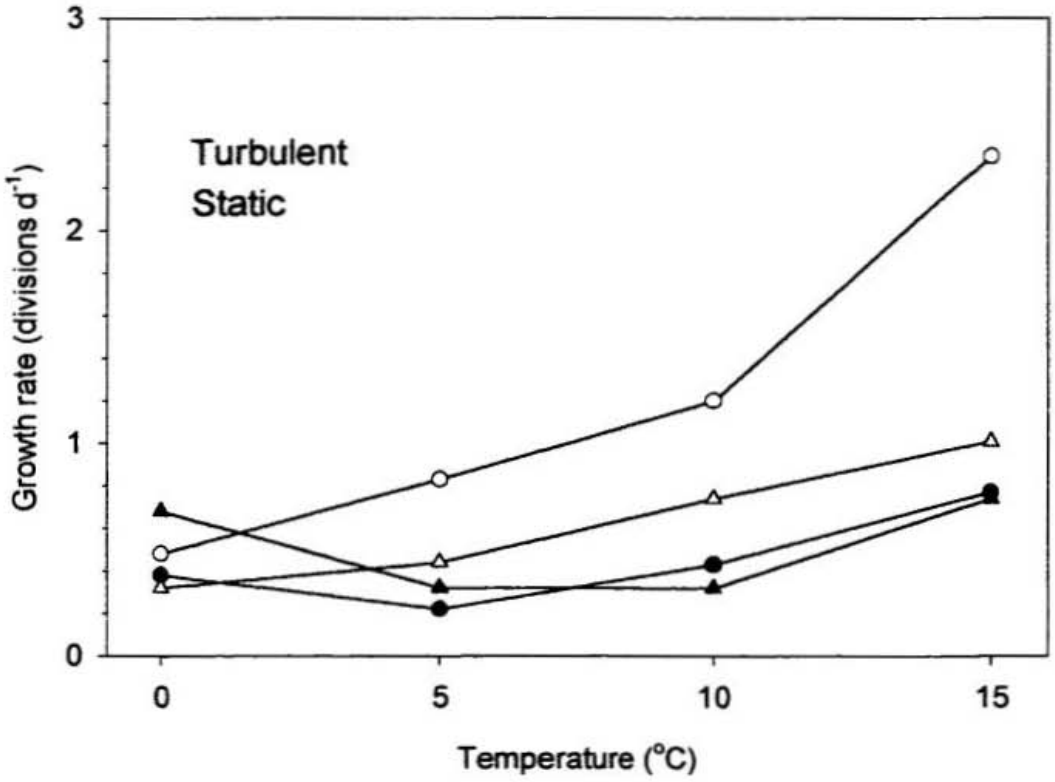


Figure 3.2: The effect of temperature on the mean growth rates of *Paraphysomonas imperforata* (open symbols) and the marine bacterium *Vibrio splendidus* (filled symbols) in turbulent (circles) and static (triangles) conditions.

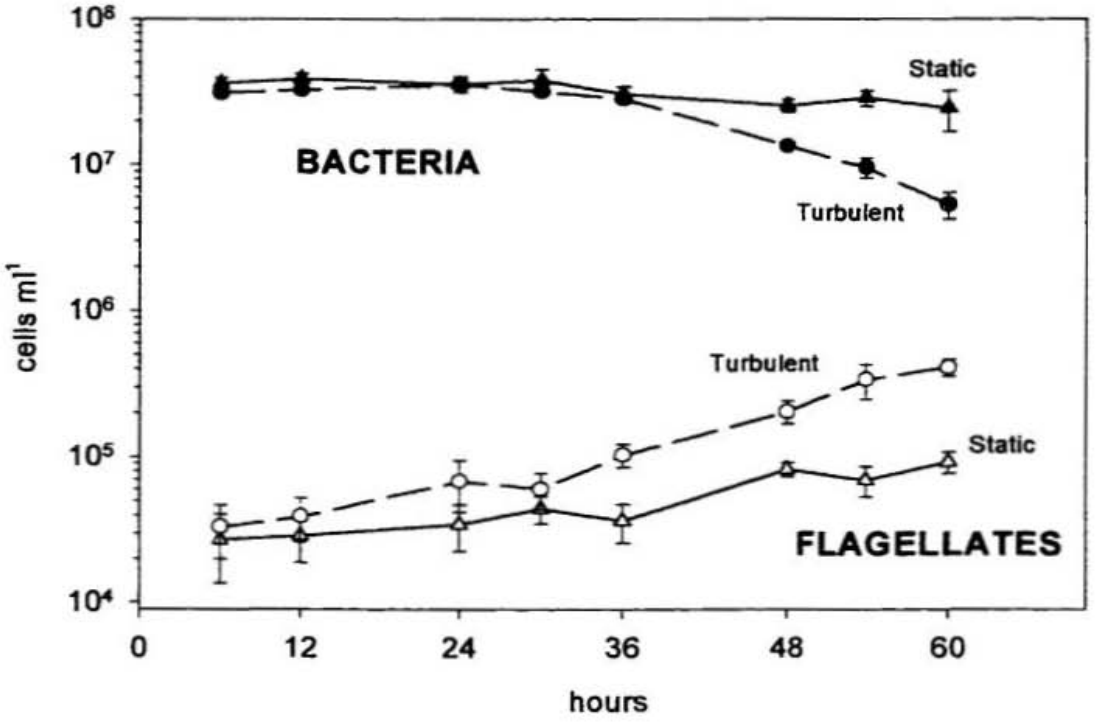


Figure 3.3: The change in abundance (±SE) of *Paraphysomonas imperforata* (open symbols) and bacteria (filled symbols) of an experiment at 10°C in turbulent (circles) and static (triangles) conditions.

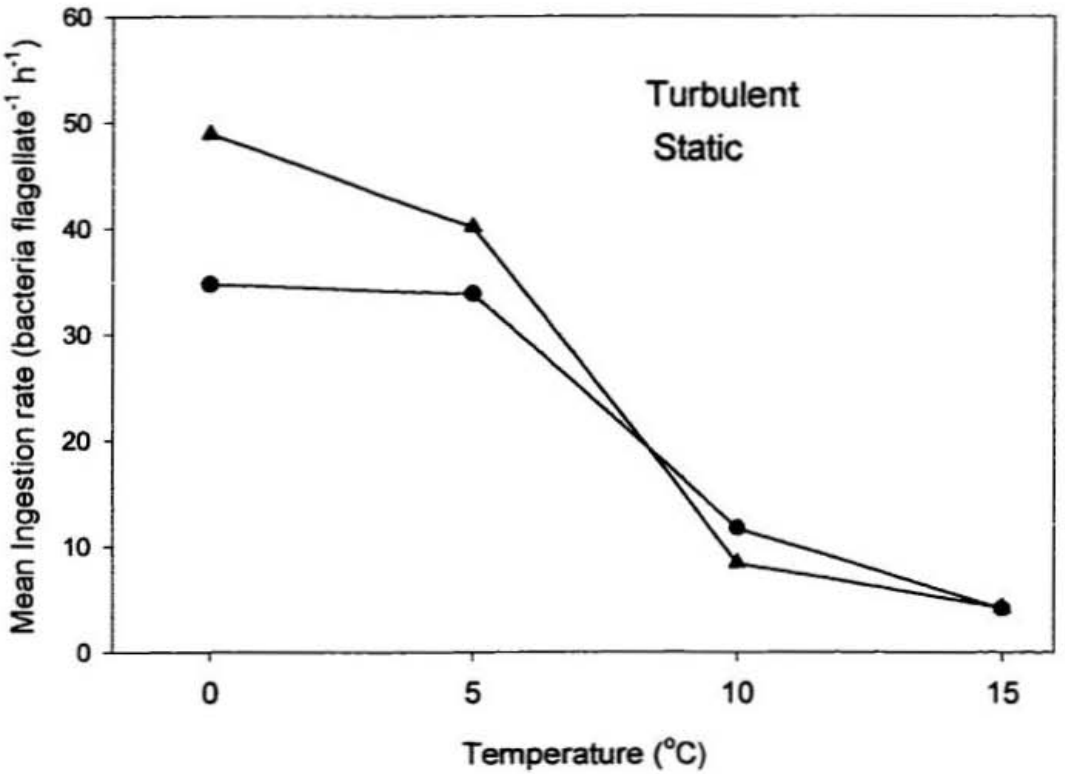


Figure 3.4: The effect of temperature on the mean ingestion rates of the heterotrophic microflagellate *Paraphysomonas imperforata* under turbulent (circles) and static (triangles) conditions.

Table 3.1: The mean growth rates (± 90%) of the heterotrophic flagellate *Paraphysomonas imperforata* and the bacteria *Vibrio splendidus* for each experiment.

Experiment		Bacterial growth (divisions d^{-1})	Flagellate growth (divisions d^{-1})
15°C			
Static	n=3	0.74 ± 0.39	1.01 ± 0.31
Turbulent	n=3	0.77 ±0.29	2.35 ± 0.52*
10°C			
Static	n=3	0.32 ± 0.29	0.74 ± 0.29
Turbulent	n=3	0.43 ± 0.42	1.20 ± 0.43*
5°C			
Static	n=2	0.32 ± 0.29	0.44 ± 0.18
Turbulent	n=2	0.22 ± 0.14	0.83 ± 0.34
0°C			
Static	n=1	0.68 ± 0.43	0.32 ± 0.05
Turbulent	n=1	0.38 ± 0.17	0.48 ± 0.12

* denotes a significant difference ($p < 0.05$, ANOVA) between the static and turbulent conditions.

Table 3.2: The clearance, ingestion, and community grazing rates of all four experimental temperatures. Mean bacterial abundances available were 10^6 to 10^7 cells ml^{-1}. Each rate is a mean of 1 to 4 experiments.

Experiment	Flagellate clearance rate (nl h^{-1})	Ingestion rate (bacteria flagellate^{-1} h^{-1})	Total bacteria consumed (ml^{-1} h^{-1})
15°C			
Static	0.90	4.2	5.93×10^5
Turbulent	0.73	4.1	9.32×10^5*
10°C			
Static	0.42	8.4	5.53×10^5
Turbulent	0.53	11.7	1.62×10^6*
5°C			
Static	1.35	40.1	8.85×10^5
Turbulent	1.33	33.8	7.20×10^5
0°C			
Static	3.20	48.9	6.56×10^5
Turbulent	3.34	34.7	3.14×10^5

* denotes a significant difference ($p<0.05$, ANOVA) between the static and turbulent conditions.

Table 3.3: Q_{10} values* for the growth of the heterotrophic microflagellate *P. imperforata* fed on the marine bacterium *V. splendidus* over the temperature range of 0 to 15°C.

Temperature Range	Growth Rate	Ingestion Rate	Clearance Rate
0 – 5°C			
Static	1.9	0.67	0.17
Turbulent	3.0	0.95	0.16
5 – 10°C			
Static	2.8	0.04	0.10
Turbulent	2.1	0.12	0.16
10 – 15°C			
Static	1.9	0.25	0.22
Turbulent	3.8	0.12	0.53
0 – 10°C			
Static	2.3	0.17	0.13
Turbulent	2.5	0.34	0.16
5 – 15°C			
Static	2.3	0.10	0.67
Turbulent	2.8	0.12	0.55
0 – 15°C			
Static	2.2	0.19	0.43
Turbulent	2.9	0.24	0.36

* $Q_{10} = (k_2 / k_1)^{10/(T_2 - T_1)}$ where k_1 and k_2 are the rates at temperatures T_1 and T_2 respectively.

Table 3.4: Q_{10} values* for the growth of the marine bacterium *V. splendidus* over the temperature range of 0 to 15°C.

Temperature Range	Growth Rate
0 – 5°C	
Static	0.2
Turbulent	0.3
5 – 10°C	
Static	1.0
Turbulent	3.8
10 – 15°C	
Static	5.3
Turbulent	3.2
0 – 10°C	
Static	0.5
Turbulent	1.1
5 – 15°C	
Static	2.3
Turbulent	3.5
0 – 15°C	
Static	1.1
Turbulent	1.6

* $Q_{10} = (k_2 / k_1)^{10/(T_2 - T_1)}$ where k_1 and k_2 are the rates at temperatures T_1 and T_2 respectively.

Table 3.5: Comparison of growth, ingestion and clearance rates of *P. imperforata* from other published results. Results of this study are in bold.

Temperature	Prey	Growth Rate (d^{-1})	Ingestion Rate (bacteria flagellate^{-1} h^{-1})	Clearance Rate (nl flagellate^{-1} h^{-1})	Citation
≥ 20°C	*Vibrio* sp.	----	180	18 – 145	Davis & Sieburth (1984)
≥ 20°C	*Vibrio* sp.	5.04 to 5.28	249 to 278	19 – 21	Edwards (1989)
20°C	B1 (bacteria)	5.04	63	58	Eccelston-Parry & Leadbeater (1994)
15°C	T1 (bacteria)	2.34 to 2.37	62 to 99	0.10 to 0.32	Choi & Peters (1992)
-1.5 to 6°C	T1 (bacteria)	0.53 to 1.53	14 to 55	0.10 to 0.20	Choi & Peters (1992)
14 to 26°C	*P. tricornutum*	1.37 to 4.04	43 to 209	18 to 40.5	Caron *et al.* (1986)
0 to 15°C	***Vibrio splendius***	**0.32 to 2.35**	**4.1 to 48.9**	**0.42 to 3.34**	*** this study**

Table 3.6: Comparison of mean grazing rates on bacteria by heterotrophic microflagellates in the ocean from other published results. Results of this study are in bold.

Site	Mean Grazing Rate (bacteria ml^{-1} h^{-1})	Citation
Sargasso Sea	2.5×10^4	Davis and Seiburth (1984)
Off Hawaii	1.5 to 3.4×10^4	Landry *et al.* (1984)
Mediterranean Sea	2.6×10^4	Wikner *et al.* (1986), Hagström *et al.* (1988)
Red Sea	2.9×10^4	Weiss (1989)
***this study**	**3.1×10^5 to 1.6×10^6**	----------

CHAPTER 4

4.1. Summary

This research has shown convincing evidence that small-scale turbulence can have some effects on the interactions between a 5 to 6 μm microzooplankton and its bacterial prey. These results have shown that increased water movements can alter the boundary layers around a microorganism as well as influence the transport of nutrients and the encounter of its prey particles (Lazier and Mann 1989). The theory developed in Chapter 2 showed that a minimum dissipation rate of 10^{-5} W kg^{-1} is required to double the encounter rate between a predator (5 to 6 μm in size) and its prey (1 μm). The dissipation rates of the ocean are more than adequate to accomplish this.

In Chapter 3 it was found that flagellate growth rates in three of four temperature regimes were higher in the turbulent condition than the static condition. Grazing rates at the warmer temperatures were noticeably higher in the turbulent conditions . Nevertheless, it has been generally believed that grazers which are similar in size to the Kolmogorov length (mm to cm) are those organisms which will be influenced by the turbulence. The results of Hill *et al.* (1992) had supported their hypothesis that turbulent motion did control the encounter rate of particles similar in size to the Kolmogorov scale. Kiørboe and Saiz (1995) also concluded that turbulence was insignificant to extremely large or extremely small predators, but potentially significant to the meso-sized predators. The results the study reported here disputes these empirical studies and theoretical arguments and suggest that more experimental work is needed to fully elucidate the effect of turbulence on the smaller microzooplankton. Browman (1996)

suggested that this area of small-scale turbulence is theory rich but lacking substantially in true reported data from laboratory or field experiments. Much more research is a requisite before we, as a scientific community, begin to link small-scale turbulence to large-scale population dynamics (Dower 1997).

4.2. Future Research

If *Paraphysomona imperforata* is a good representative of the natural microzooplankton community, then the grazing on marine bacteria in the microbial food web should be reexamined. The recycling of carbon due to microzooplankton grazing should be investigated not only at the different seasons and latitudes, but under the different oceanic conditions as well. The first question to arise is whether turbulence is always present at the small scale. At the large scale, turbulence is intermittent. Hence, how long will it take for all the smallest size eddies to manifest into laminar flow before another large-scale turbulent eddy is created? If turbulence is consistently present, it becomes problematic whether the physiological rates of protozoa isolated from the field are actually an accurate representation of what occurs in nature when the animals are incubated in static conditions.

Can microzooplankton survive in lower food concentrations when in the presence of turbulence? This is a question that is of vital importance. Examining this would in fact be a representative of whether increased encounter rates could translate to higher ingestion rates. This would be one experiment that must be carried out in a laboratory-controlled environment. As it was seen in the 15°C experiments, the flagellates under turbulence practically consumed all the bacteria of the culture, therefore an experiment

must be carefully designed so to administer small prey concentrations every so often to keep the animals from starvation (i.e. controlled continuous culture).

Another area to be explored is whether turbulence really does promote marine aggregates, especially with the marine bacteria. Even though this research only investigated the growth of bacteria and found no significant change in abundances between the two conditions, the appearance of bacterial clumps did not go unnoticed. Does turbulence actually increase the number of bacterial clumps or does it break the aggregates apart? It has been argued by van Leussen (1988, 1997) that small-scale turbulence sets an upper limit on aggregate size as well as promote or hinder the aggregation of particles. Perhaps the flagellate growth which showed positive influence under the turbulence was due to the number of cells encountering a bacterial aggregate which allowed the flagellates to attach themselves to it and feast.

As suggested by Shimeta *et al.* (1995), the effect of small-scale turbulence could, in fact, be species-specific. Mackas *et al.* (1993) had concluded that the distribution of copepods and fish larvae may be modified by variations in turbulent intensities and that different species will respond to turbulence differently. Small-scale shear has also been responsible for the inhibition of growth of some dinoflagellate species (Thomas and Gibson 1992, Gibson and Thompson 1995). Therefore, future research should be expanded to include other similar species in the same size range as *P. imperforata*. They should be examined as a community and not as individuals isolated in individual tanks. How, as a whole, are the growth and grazing rates influenced by small-scale turbulence?

It is evident that many more studies are needed in the field. The research that has been published so far is only a beginning. We still need to discover if there is a similar general response among the microzooplankton community to turbulence so it can be defined in the workings of the marine microbial food web.

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