INFLUENCES OF TEMPERATURE ON ANTARCTIC FRESHWATER DIATOM GROWTH RATES IN CULTURE

by

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ABSTRACT

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Influences of Temperature on Antarctic Freshwater Diatom Growth Rates in Culture
Thesis directed by Professor Diane McKnight

The McMurdo Dry Valleys, Antarctica represent one of the coldest and driest deserts on the planet. Stream microbial life, however, flourishes within glacial meltwater streams during the austral summer. It has been previously estimated that 60% of the diatom species in the dry valley streams are endemic to Antarctica. Beyond hydrologic controls on diatom community structure, there may be species-level adaptations that help explain taxa distribution and abundance in Dry Valley streams. This research sought to quantify intrinsic growth rates at different temperature regimes for multiple Dry Valley taxa.

It was hypothesized that Antarctic species would have faster growth rates at cold temperatures, illustrating psychrophilic behavior. Furthermore, it was thought that intrinsic growth rate would vary by temperature, providing insight into current known species distributions in the Dry Valleys. Using a fluorometer to track growth and microscopy to visually count cells, growth curves were developed for 7.6 and 10 degrees Celsius for 4 species. It was found that temperature impacted 3 of the 4 benthic taxa growth rates. *Hantzschia amphioxys f. muelleri*, had the highest overall intrinsic growth rate at both temperatures and grew fastest at 10°C. The intrinsic growth rate of *Psammothidium papilio* did not change with temperature.

Hantzschia abundans and *Hantzschia amphioxys* both exhibited stimulated higher growth rates at 7.6°C as compared to 10°C.

Previous research has shown that there are hydrologic controls on these mat communities, but perhaps there are temperature controls at the species level. These results present the idea that inter-genus growth rate relationships may provide a better understanding of taxa habitat preferences and variation in abundance by stream.

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To my parents who have always been my number one fans and provided unrelenting moral support throughout my academic career. To Kit Davidson, for his boundless encouragement and for sharing his curiosity about the world.

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CHAPTER 1: BACKGROUND

1.1 Phototrophic Organisms: Diatoms

Diatoms are single-celled, eukaryotic microorganisms that are ubiquitous to nearly all aquatic environments. A diatom is a photosynthetic autotroph meaning that it acquires energy via sunlight to produce organic compounds, though there are some species that can live heterotrophically (Round et al. 1990). Diatoms have cell walls composed mainly of silica dioxide and are often preserved in sediments which allows them to be used as bioindicators (Sec. 1.3) (Smol and Stoermer 2010). Like many other single-celled organisms, asexual mitotic divisions dominate diatom reproduction, although sexual reproduction is an important aspect of their life cycle (Julius and Theriot 2010).

Diatoms play integral roles as primary producers at the base of the food web, accounting for more than 40% of net primary production in oceans (Nelson et al. 1995). A sought after food source for many aquatic heterotrophs, diatoms produce and store high-energy lipids. These lipids then become the basis for essential fatty acids within higher trophic levels. Furthermore, the high lipid content of diatoms makes them attractive in algal biofuel research (Hu et al. 2008). Diatoms have also been credited with the mediation of silica in oceans (Conley 2002). Existing as either planktonic or benthic organisms, diatoms can grow alone or in colonies. Some zooplankton have adapted to feeding on planktonic diatoms while grazers target benthic diatoms. The abundance and diversity of diatom grazers reflects on the abundance and diversity of the diatom species themselves.

There are an estimated 200,000 diatom species on the planet with a rate of new species descriptions occurring at 180 each year (Julius and Theriot 2010). There are greater than 24,000 diatom species that have scientific names, but there is little ecological, genetic, or physiological study that has occurred with these species (Julius and Theriot 2010). This situation leaves the scientific community with lots of room for research. Using diatoms for historical climate reconstruction in sediment records would also benefit from thorough research on ecological controls at the species and community level (Sec. 1.3).

Diatoms can be viewed under the microscope in either valve or girdle view. If examining a diatom in plan view, the observer will be looking at valve view.

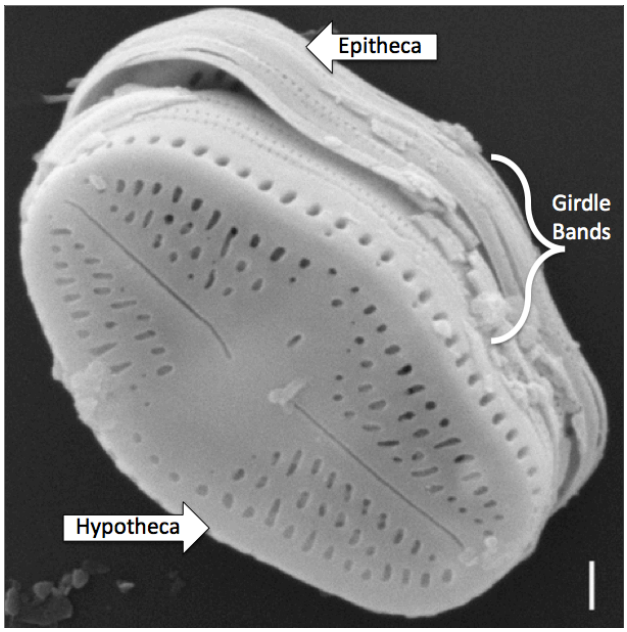


Figure 1: Basic diatom anatomy. Scanning electron microscope image of *L. muticopsis f. reducta* adapted from huey.colorado.edu/diatoms

Valves have various striations and profiles and can be used to identify diatom species (Smol and Stoermer 2010). The girdle view occurs when the observer sees the siliceous belts designated as girdle bands in side view. Together the 2 valves,

termed epitheca and hypotheca, and girdle bands comprise the cell wall known as the frustule illustrated in Figure 1 (Round et al. 1990).

Most diatoms can be classified as either pennate or centric, names derived from their valve symmetry. Centric diatoms, generally found in marine environments, illustrate radial symmetry in valve view while pennate diatoms have bilateral symmetry (Round et al. 1990). Many freshwater diatoms are pennate diatoms. All of the diatoms cultured and analyzed in this thesis work are raphid pennate diatoms.

1.2 McMurdo Dry Valleys Climate and Limnology

The diatoms examined in this work were collected from the McMurdo Dry Valleys (MDV) of Antarctica. Located within the McMurdo Dry Valleys Long-Term Ecological Research (MCMLTER) site, research has been conducted in the MDV since 1986. Located directly south of New Zealand, the Dry Valleys represent the largest ice-free area of Antarctica between the Polar Plateau and the Ross Sea (Figure 2).

Figure 2: McMurdo Dry Valleys, Antarctica

Arguably comparable to the Martian environment, the Dry Valleys represent the coldest and driest desert on earth (Priscu 1998). The Transantarctic Mountains prevent the advancement of the polar ice sheet and air temperature generally ranges from -45°C to 5°C (Alger et al. 1997). Intermittent glacial meltwater streams flow during the Antarctic austral summer dependent on temperature and solar irradiance. These ephemeral streams can be up to 10 °C warmer than air

temperature (Cozzetto et al. 2006). Stream temperatures can range from 0.1 °C to 15 °C and experience daily swings of 6 °C to 9 °C (Cozzetto et al. 2006). The Antarctic continent has been experiencing a net cooling trend since 1986 with periodic high flow (warmer) years in recent past that are expected to increase in frequency with changes in circulation and climate (Doran et al. 2002).

Although there is no terrestrial plant life living in the extreme conditions of the MDV, the intermittent streams support abundant, perennial microbial communities including benthic algal mats that achieve high levels of biomass (McKnight and Tate 1997). The microbial communities that thrive in the dry valley stream conditions have to be tolerant to extreme cold, unsteady flow, periodic desiccation, and intense solar radiation. The microbial mats containing diatoms exist in a freeze-dried state during the winter and are then reactivated once flow begins in the austral summer (McKnight et al. 2007).

Antarctica is known for endemic biota (Rogers 2007). Low species diversity, but high levels of endemism within the diatom community has been found in Dry Valley streams (Alger et al. 1999; Esposito et al. 2006). As illustrated on the Antarctic Freshwater Diatoms website, 41 species have been documented from these seasonal streams, of which 17 are endemic to the continent of Antarctica (Esposito et al. 2008). The cosmopolitan, or widespread, diatom species that can survive in these ephemeral streams have to be tolerant to the desiccation periods and varying electrolyte concentrations (Konfirst et al. 2011).

The characteristics of Antarctic algal mats and their distribution have been examined in detail (Davey and Clarke 1992; Alger et al. 1997), but studies of the abiotic controls on mat composition have just gained momentum in recent years (Esposito et al. 2006; Stanish et al. 2012).

1.3 Diatoms As Indicators For Environmental Change

Diatoms have long been used as bioindicator organisms due their rapid generation times and sensitivity to abiotic and biotic fluctuations (Smol and Stoermer 2010). Inorganic cell walls composed of silica dioxide (SiO_2) are a distinct hallmark of diatoms. These silica frustules can be preserved in sediments for millennia and can provide evidence for climate reconstruction including pH, salinity and nutrient history (Battarbee and Charles 1986; Smol and Stoermer 2010). Studies have shown changes in diatom growth rates at various temperatures and furthermore, temperature has been shown to be an important factor in explaining the variation in diatom community composition (Battarbee et al. 2001)

Because of their vast geographical coverage and species diversity, diatoms can play an important role in understanding global climate patterns (Julius and Theriot 2010). Diatoms have been used as proxies to understand past, present, and future ecosystem trends and water body health (Harding et al. 2005; Wang et al. 2005; Juettner et al. 2012). While diatoms hold great potential to serve as environmental indicators, bioindicator use is dependent on accurate taxonomy and autecology. To correctly use diatoms to estimate paleoenvironmental conditions, correlations need

to be established to connect limnological and abiotic controls to species assemblages. The permanently ice-covered lakes in the dry valleys emphasize the importance of the relationship between dry valley streams and lakes. For example, in the MDV a diatom record was recently created using sediment cores taken from Lake Fryxell by Konfirst et al. (2011) and cores have been previously examined from Lake Hoare (Spaulding et al. 1997). It was noted that the "precise environmental interpretation of the cores are hampered by limited knowledge of environmental constraints on the diatom taxa present in the core" (Konfirst et al. 2011).

As Madigan et al. (2009) state, temperature can arguably be the most important environmental factor in determining growth rates and survival of microorganisms. This argument is especially relevant in the MDV where light, temperature and flow are prominent abiotic controls in a desert with little trophic interaction. Studying microbes from an environment with little trophic interaction makes laboratory studies on abiotic controls much more applicable to reality as compared to temperate ecosystems.

1.4 McMurdo Dry Valley Context for Intrinsic Growth Rate Experiments

Greater than 70% of the earth exists as cold ecosystems with temperatures that hover at or below the freezing point of water and the ecosystems are often dominated by microorganisms (Morgan-Kiss et al. 2006). The MDV are certainly a cold, and geographically isolated, ecosystem where the streams support abundant algal communities and nematodes represent the largest predator thriving in the

valleys. The ephemeral streams act as nutrient and biological highways linking the glaciers and lakes in the valleys for up to twelve weeks each year.

A critical adaptation for Antarctic diatoms as phototrophic organisms involves the coordination of the temperature-independent process of light absorption with the temperature-dependent production of organic energy products like ATP and NADPH (Morgan-Kiss et al. 2006). Because of the MDV climate, these diatoms can potentially experience a consistent state of energy imbalance due to high irradiance and low temperatures. Antarctic endemic diatoms, those taxa that evolved in Antarctica, have previously illustrated a higher abundance within extreme stream conditions than cosmopolitan species (Esposito et al. 2006).

The Antarctic Diatom Index (ADI), developed by Esposito et al. (2006), represents the relative abundance of Antarctic endemic diatoms in a sample. A stream harshness index, first developed by Fritz and Dodds (2005), was adapted to MDV streams by Esposito et al. (2006):

> Stream harshness in the Dry Valleys is calculated using mean annual flow for each year, annual maximum flow for each year, days without flow during the summer, mean annual flow for historical record, mean annual days without flow for historical record and flood events per year for historical record.

Historically, the ADI-harshness relationship has illustrated a trend linking increased ADI values in streams of increasing harshness (Esposito et al. 2006). Additionally, previous analysis of diatom mat communities in the MDV have illustrated increased ADI values in Canada Stream during cold periods (Kimball et al. unpublished data). These previous results indicate that the endemic diatom

taxa may be more efficient at adapting, or have a higher tolerance, to harsher stream flows induced by decreased solar radiation and lower temperatures. It should be noted that these historical ADI values were in part driven by taxa that now have new distribution ranges. For example, *Psammothidium papilio* was formally known as *Psammothidium chlidanos* and thought to be a cosmopolitan taxa and the distribution has now been updated to include just the Antarctic continent (Kopalova et al. 2012).

Even though there are many taxa defined as cosmopolitan in the Dry Valleys, research has shown that distinct Antarctic lineages within some cosmopolitan species (diatoms, cyanobacteria, and green algae) may have evolved due to long periods of isolation on the Antarctic continent (De Wever et al. 2009; Souffreau et al. 2013; Taton et al. 2003). These distinct lineages may mean that the taxa defined as cosmopolitan could actually be a complex of cryptic or pseudo-cryptic species. Cryptic means that they are a morphologically identical, but genetically different species (Lundholm et al. 2012).

Understanding the response of specific diatom taxa to changes in temperature can contribute to explaining why diatom assemblages change under cold or low flow conditions. A key portion of the fundamental niche for diatoms in the Dry Valleys include the cold, varying flow conditions experienced by all streams during austral summer periods. It can be hypothesized that habitat selection filters out less well-adapted species that are unable to survive long periods of desiccation in the Dry Valley streams.

The science in the MDV has illustrated knowledge about the patterns and distributions of diatoms. Recent research suggests that diatom size may impact distributions in Dry Valley streams and streams with higher discharge have been shown to contain higher abundances of smaller taxa (Stanish et al. 2011). This research works to understand the fundamental species level characteristic of intrinsic growth rate as it pertains to diatom distributions. This knowledge will help in interpreting aquatic ecosystems present in the MDV, along with other dry deserts, and will contribute to expanding the Antarctic Freshwater Diatoms website with intrinsic growth rate information.

The daily temperature swings, along with harshness induced community changes, highlight the importance of understanding the impacts of temperature at the species level. Stream hydrology and temperature in the geographically isolated MDV exert a strong control over algal mat community structure. This thesis will test the following hypotheses:

1. Antarctic diatoms will exhibit psychrophilic behavior with higher growth rates at lower temperatures.
2. Intrinsic growth rate will vary by temperature, providing insight into current known Dry Valley distribution.

1.5 Structure of Thesis

This research works to understand temperature effects on the fundamental species level characteristic of intrinsic growth rate.

Chapter 2 will outline the methods developed for this thesis work. It will describe the stock cultures and selection of the taxa analyzed. The chapter will end by outlining intrinsic growth rates and how they are determined in this work.

Chapter 3 will focus on temperature and intrinsic growth rate experiments. One main method was primarily used and a second, more time-efficient, method was explored for future use and is outlined in the Appendix.

Chapter 4 will integrate the results of the growth rate experiments into a discussion of broader impacts and conclusions. The chapter concludes with some recommendations for future work.

CHAPTER 2: MATERIALS AND METHODS

2.1 Stock Cultures

The Antarctic diatom culture collection at INSTAAR was started by Lee Stanish and other collaborators. The cultures were started to promote the exploration of the genetic and physiological mechanisms governing the responses of diatoms to their environment.

The diatoms are cultured in a DY-V medium from the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA) located in Maine, USA (Culture protocols and DY-V recipe in Appendix). Each culture is kept in 50mL glass test tubes in DY-V medium. DY-V medium is replenished periodically and tubes are passaged to support healthy cell populations. Passaging represents the transferring of cells to a tube with fresh medium on a regular basis. The cultures are consistently stored in a cold (7 – 8 °C) environmental chamber under low light conditions on a 12-hour light/dark cycle. Photosynthetically Active Radiation (PAR) is measured periodically with a LICOR light meter and reads between 14-20 μE. Photosynthetic organisms in the Antarctic experience extreme changes in irradiance from 24:0 to 0:24 hours light/24 hour day, thus there is inherent difficulty in choosing a consistent light regime for Antarctic cultures.

The INSTAAR diatom cultures are unialgal, but non-axenic due to the potential interactions between diatoms and bacteria. Round et al. (1990) state that some diatoms may not persist in axenic culture, especially if they utilize substances produced by bacterial metabolism. Correlations have recently been found in the

MDV between diatoms and bacterial taxa suggesting ecologically relevant interactions (Stanish et al. 2013).

To date, 9 taxa have been successfully maintained in unialgal culture at INSTAAR. These taxa include: *Diadesmis perpusilla*, *Hantzschia abundans*[*], *Hantzschia sp. # 5*, *Hantzschia amphioxys*, *Hantzschia amphioxys f. muelleri*, *Luticola muticopsis f. capitata*, *Luticola muticopsis f. evoluta*, *Psammothidium papilio*, *Stauroneis latistauros*. The stock cultures represent diatoms from a range of currently known geographic distributions (Table 1) including widespread (W), southern hemisphere endemic (SHE), Antarctic endemic (AE) and South Victorialand endemic (SVE) as historically compiled on the Antarctic Freshwater Diatoms website (Spaulding et al. 2010).

[*] Molecular 16S genetic information suggests that the *H. abundans* in culture at INSTAAR may be a strain of *H. amphioxys*. A full genetic work up will be conducted by the J. Craig Venter Institute (JCVI) during the summer of 2013. INSTAAR morphological analysis suggests that the species are distinct.

2.2 Antarctic Taxa Analyzed

Of the 9 taxa in culture, 8 taxa were analyzed in growth rate experiments beginning in 2010 (Figure 3). These taxa were chosen based upon their success in culture as well as their range of distribution and morphological characteristics (Figure 4, Table 1). Because the growth rate experiments took place over 3 years and culture tubes were passaged to maintain clean, healthy tube densities. Futhermore, care was taken to ensure that the taxa strains used in each growth rate experiment originated from the same parent tube.

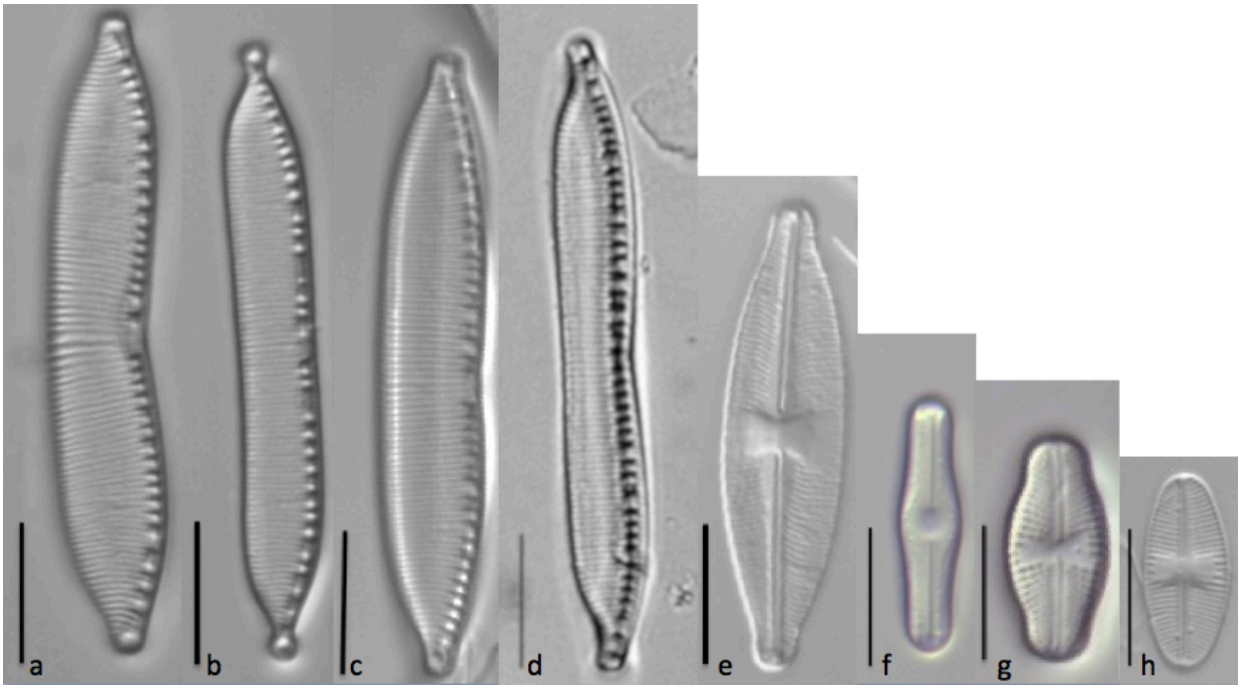


Figure 3: Light microscope images of taxa in culture at INSTAAR. ***(a) H. abundans (b) H. amphioxys f. muelleri (c) H. amphioxys (d) H. sp. # 5 (huey.colorado.edu/diatoms) (e) S. latistauros (f) D. perpusilla (f) L. muticopsis fo. capitata (g) P. papilio***

Table 1: Taxa analyzed and key morphological characteristics and distribution (L=length, W=width, n= number of tubes proceeding through logistic growth)

Taxa	Morphology	Current Known Distribution
Hantzschia abundans (n=8)	Nitzschioid L: 49-94 µm W: 7-9.5 µm	Widespread
Hantzschia amphioxys (n=8)	Nitzschioid L: 27-61 µm W: 7-10 µm	Widespread
Hantzschia amphioxys f. muelleri (n=16)	Nitzschioid L: 33-44 µm W: 5-7 µm	Antarctica and Patagonia
Hantzschia sp. #5	Nitzschioid L: 38.5-54.5 µm W: 4-6 µm	South Victorialand endemic
Luticola muticopsis f. capitata	Naviculoid L: 15-31 µm W: 8-11 µm	Antarctic endemic
Psammothidium papilio (n=26)	Monoraphid L: 10-17 um W: 5-6 um	Antarctic endemic
Stauroneis latistauros	Naviculoid L: 25-46 um W: 6-8.5	Southern hemisphere

Figure 4: Diatom distribution map (Source: huey.colorado.edu/diatoms, Perry-Casteñeda Library Map Collection)

2.2.1 General Taxa Descriptions

Hantzschia, described by Grunow (1877), is a small genus with fifty species recorded globally that can persist in freshwater, marine, intertidal, and terrestrial habitats (Zidarova et al. 2010; Round et al. 1990). *Hantzschia amphioxys* (Ehrenberg) Grunow, mostly found in semi-wet soils, is the most common species in the genus (Zidarova et al. 2010). *Hantzschia abundans,* another common species, is morphologically similar to *H. amphioxys* and was first differentiated from *H. amphioxys* by Lange-Bertalot (1993). *Hantzschia amphioxys* (Ehrenberg) Grunow *f. muelleri* Ko-Bayashi (1965) is similar to both of the previous species and is known to be present in Antarctica and Patagonia.

Psammothidium papilio, an Antarctic endemic, was first identified as *Navicula papilio* by Kellogg et al. (1980). This taxon was renamed to *P. papilio* by Kopalova et al. (2012), but was formally known as *Psammothidium chlidanos* (Esposito et al. 2008; Spaulding et al. 1997) and thought to be a cosmopolitan taxon. Kopalova et al. (2012) recognized that due to confusion with other species, it is likely that *P. papilio* has a larger than currently recognized geographic distribution.

The genus *Luticola* was first described by D.G. Mann (Round et al. 1990) and most species within the genus are found in soils, terrestrial mosses, seepage areas, and moist stones (Van De Vijver and Mataloni 2008). *Stauroneis* Ehernberg 1843 is a widespread genus found in freshwater, marine and terrestrial habitats (Van de Vijver et al. 2005). The *Stauroneis latistauros* taxon found in the MDV does not entirely conform to the described species (Esposito et al. 2008). It is hypothesized

that both *Luticola muticopsis f. capitata (L. muticopsis f. capitata)* and *Stauroneis latistauros (S. latistauros)* have not been sexually reproducing in culture as evidenced by their small size and deviation from accepted morphological descriptions noted on the Antarctic Diatoms website (huey.colorado.edu/diatoms) and in field sample observations.

2.3 Temperatures Analyzed

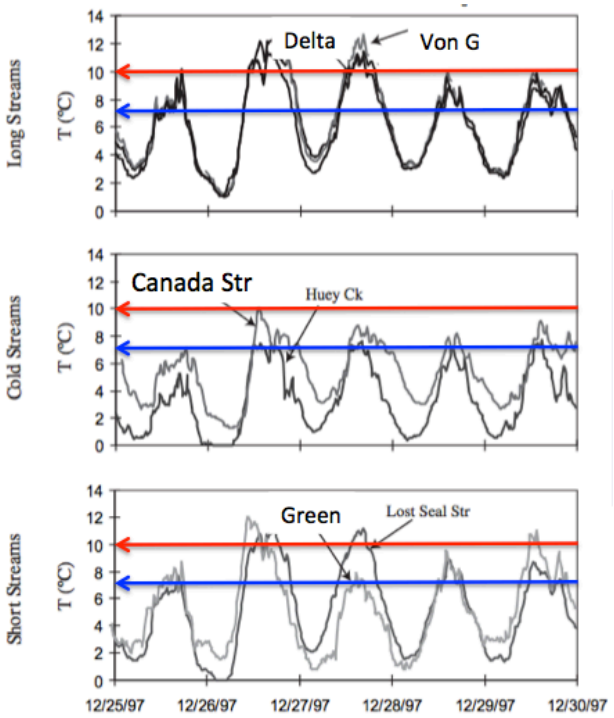


Figure 5: Range of stream temperatures in the Fryxell Basin over a 5-day period in mid-summer. Temperatures analyzed are shown in blue (7.6C) and red (10C). Graph adapted from Cozzetto et al. (2006)

As previously described in Section 1.2, Dry Valley stream temperatures can range from 0.1 °C to 15 °C and can experience daily swings of 6 °C to 9 °C (Cozzetto et al. 2006). Figure 5 above shows the diel temperature change each day for a 5-day period in mid-summer with stream temperature maxima occurring between 8°C to 15°C. Because phototrophic organisms living in cold ecosystems have to couple the temperature independent and dependent components of photosynthesis (Sec. 1.4), it is reasonable to expect that these diatoms will be most active during the warmest portion of the day. High temperature and high sunlight are expected to represent the most optimal growing conditions for the diatom taxa and two temperatures were tested with this in mind: 7.6°C and 10°C. Although the temperatures in this study

varied by only 2.4°C, they are representative of the daily maximum temperatures encountered in mid-summer.

2.4 Growth Rate Tubes and Taxon Isolation

Taxa were aseptically isolated from the stock cultures for the growth rate experiments. Strains selected for the growth rate experiments were recorded to ensure that all future growth rate experiments were conducted on the same taxon strain. Live cells were aseptically isolated and transferred from the stock tubes to the experiment tubes using a pasteur pipette and microscopy (Nikon TS100). Between 15 – 30 single, live cells were transferred into 10mL glass, growth rate test tubes at the beginning of each experiment. Every growth rate analyzed in this study began with the same inoculation population regardless of taxon for consistency and each taxon had 4 – 5 replicate tubes during each experiment. The only exception to this starting population occurred in 2013 and is outlined in section 3.2. During the growth rate experiments, the volume of DY-V was maintained at 7mL and periodically replenished following *in vivo* fluorescence readings of cell concentration.

2.5 *In Vivo* Fluorescence Method

Contrary to *E. coli* or other bacteria that have doubling times on the minute or hour scale, diatoms generally have doubling times on the scale of days. Rivkin (1986) noted that in optimal conditions some diatoms can divide once per day, however, Antarctic diatoms grow much more slowly than this even in our culture

conditions. Due to the slow growing nature of Antarctic diatoms, an *in vivo* chlorophyll-a fluorescence method was developed to monitor growth in a non-damaging manner similar to the approach used by McKay et al. (1997). Since Serôdio et al. (1997) first explored the uses of chlorophyll-a fluorescence to quantify biomass, it is now often used as a proxy to estimate algal biomass. Chlorophyll-a was measured on a Turner Designs field fluorometer between 2 – 4pm, 2 – 3 times each week. Unlike planktonic diatoms, benthic diatoms attach to solid substrates, thus the cells in the growth rate tubes needed to become suspended in the medium for reliable fluorescence measurement. Tubes were disturbed to cause cell suspension prior to measuring fluorescence either by using a pasteur pipette or quickly inverting the tube.

Fluorescence intensity and time were recorded and plotted to track logistic growth. Exponential phase growth was considered finished once the tube was exhibiting stationary phase behavior (see section 2.6). When the tubes had reached stationary phase, cell counts were conducted using a hemocytometer and microscopy (e.g. Guillard 1980) to correlate tube cell concentration with fluorescence intensity. In order to obtain fluorescence values that span the range encountered over the growth rate experiment, serial dilutions were performed on the growth rate tubes and cell counts repeated. An assumption was made here that assumed the physiological state of the cells in stationary phase was representative of their state within exponential phase. The tubes were counted and serially diluted quickly after stationary phase in the tube was identified.

To test for statistically significant differences in the regressions correlating the growth rate tube cell densities and fluorescence values at 7.6°C and 10°C a two-tailed t-test was performed for all years. A ratio of cell count to fluorescence value was used in the t-test to determine if there was a significant difference in cell concentration:fluorescence value among temperatures. Some species showed a divergence in the regressions at the two temperatures (*H. f. muelleri and H. abundans*) and some did not (*P. papilio and H. amphioxys*) as described in Chapter 3.

The sensitivity of the Turner Designs fluorometer was determined for each taxa and thus, only intensities greater than the species detection limit were included in the resulting regression. The relationship between cell concentration and fluorescence was expected to be linear and was used to convert recorded fluorescence values to cell concentrations. The points located within exponential phase were then used to calculate intrinsic growth rate and doubling time (Sec. 2.6) as described by Guillard (1980).

Records were kept throughout the duration of the growth rate experiments to monitor any distinct visual changes in morphology, cell size, or intracellular contraction or expansion of chloroplasts. While some taxa identified in this thesis are undergoing morphological changes and size reduction (*H. sp#5*, *L. capitata and S. latistauros*), none of the 4 analyzed taxa (*H. f. muelleri, H. abundans, H. amphioxys, P. papilio*) demonstrate such variation as described in Chapter 3.

2.6 Intrinsic Growth Rates

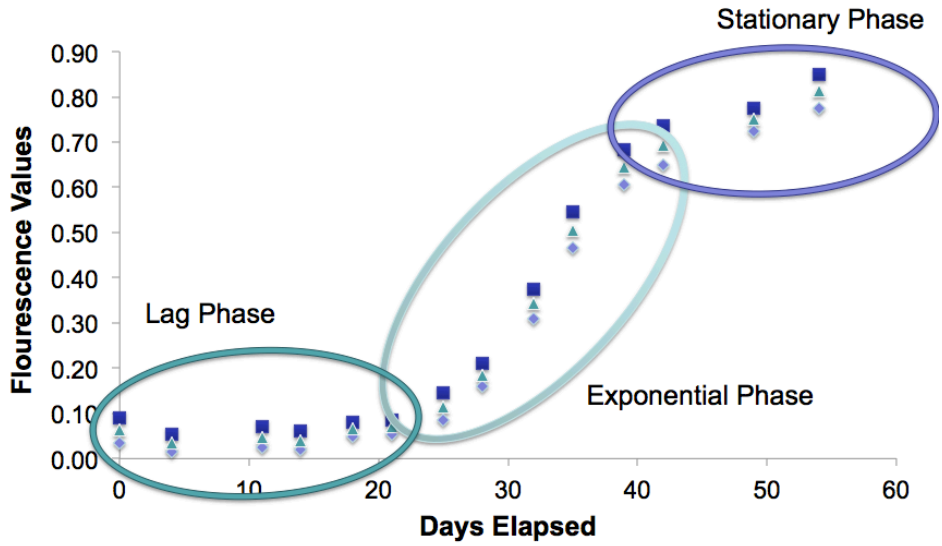


Figure 6: Logistic growth rate stages illustrated by *Psammothidium papilio* 7.6°C

With abundant resources populations can grow exponentially (Molles 2010). The environment fostered by the tubes in which these growth rate experiments took place was replete with nutrients and irradiance that encouraged the exponential growth of each taxon. Exponential growth eventually slowed due to some constraint such as space or nutrient limitation or perhaps a build up of metabolic by-products within the tube. The slowing of exponential growth shifted the overall trend to illustrate logarithmic growth.

The sigmoidal logistic curve can be separated into three distinct stages: lag phase, exponential phase, and stationary phase (Maier, Pepper, and Gerba 2009a). The lag phase and stationary phase represent periods of time where the growth rate is zero and the exponential phase is where the logarithm of the population increases

linearly with time (Figure 6). Stationary phase occurs at the carrying capacity that represents the population size that can thrive in the given space with the available resources. If cell death surpasses growth during the stationary phase it becomes known as the death phase.

Intrinsic growth was calculated as described in equations 1 to 4. Each growth rate tube had its own plotted logistic growth curve as shown in Figure 7. The intrinsic growth rates (k) for each tube in the experiments were calculated and included in statistics and averages presented in Chapter 3. Figure 7 illustrates that even with varying lag phase durations, intrinsic growth within exponential phase is comparable among different tubes.

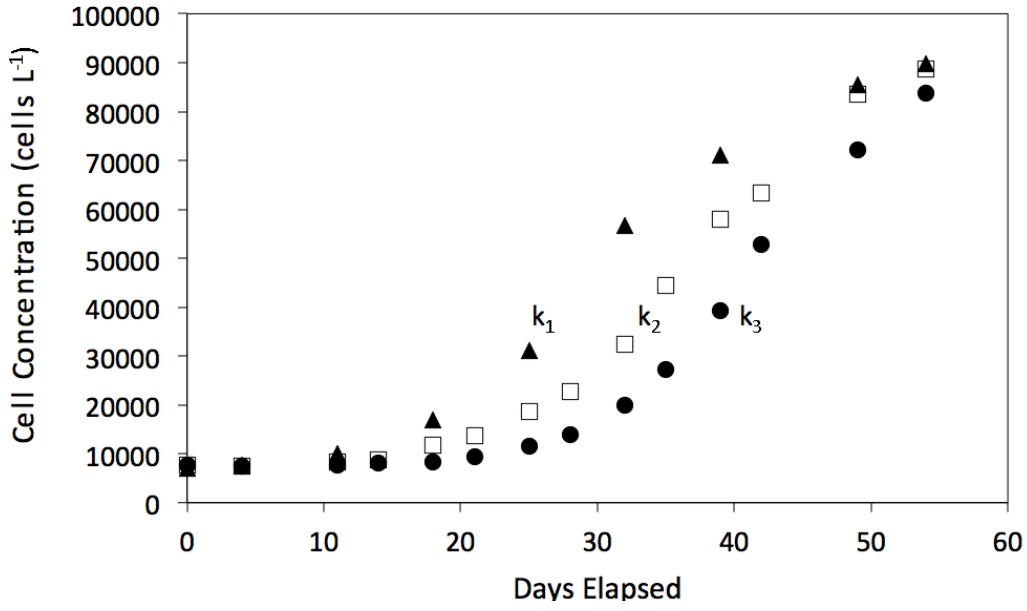


Figure 7: Logistic growth curves for three *P. papilio* tubes at 7.6°C, where k_1, k_2, k_3 represent intrinsic growth rate values for each log phase

I used the logistic model of growth as defined by P. F. Verhulst (1838):

$$\frac{dN}{dt} = rN\left(\frac{K-N}{K}\right)$$

Equation 1: Verhulst logistic Growth Model

Where $\frac{dN}{dt}$ represents the rate of change of growth over time, r is the intrinsic growth rate (cells/day), N is population at a given time, and K is carrying capacity. Population development during exponential phase only divides in a characteristic time and follow the equation:

$$\frac{dN}{dt} = K_e N$$

Equation 2: Guillard exponential phase population development model

Where N is the concentration of cells in the culture and K_e is the growth constant of 'logarithm-to-base-e' units of increase per day. K_e is obtained as the slope of the exponential phase points on a semi-log plot. To convert to k, cell divisions per day:

$$k = \frac{K_e}{.6931}$$

Equation 3: Guillard conversion to cell divisions per day

To calculate the population doubling time, T_d:

$$T_d = \frac{1}{k}$$

Equation 4: Guillard doubling time calculation

CHAPTER 3: INFLUENCE OF TEMPERATURE ON DIATOM GROWTH RATES

3.1 Results

The results are presented by taxa in this chapter. The chapter will proceed from the Antarctic endemic taxa to the widespread species. The chapter will open by presenting the results for the most analyzed taxa in the growth rate experiments first, *Hantzschia amphioxys f. muelleri* and *Psammothidium papilio*. Results for *Hantzschia abundans* and *Hantzschia amphioxys* will be presented next, followed by the taxa that yielded inconclusive results. *Hantzschia sp. #5* is discussed first followed by the mucilage producing *Luticola muticopsis f. capitata* and *Stauroneis latistauros*. The data are presented in this manner to help clarify the results as they pertain to the species distribution and species characteristics.

3.1.1 *Hantzschia amphioxys f. muelleri*

Hantzschia amphioxys f. muelleri was included in all growth rate experiments conducted between 2009 and 2013 making it one of the most analyzed species. Two temperatures were tested: 7.6°C in 2009, 2010 and 2013 and 10°C in 2010 and 2013. As seen in Table 2, a high correlation was found when testing the cell concentration:fluorescence value ratios across the years of the repeated 7.6°C experiments, as well as in the repeated 10°C experiments. However, when the ratios between the two temperature experiments were tested, a statistically significant p-value of 0.005 was found.

Table 2: T-test results for cell counts:fluorescence value ratios by year and temperature experiment for *Hantzschia amphioxys f. muelleri,* p< 0.05 in bold.

Temperature	7.6°C	7.6°C	7.6°C	10°C	7.6°C vs. 10°C
Comparison Years	2009, 2010	2009,2013	2010,2013	2010, 2013	2009 – 2013
p-value	0.83	0.94	0.85	0.27	**0.005**

The p-value found when comparing the ratios between the 7.6°C and 10°C experiments illustrates that there is a significant difference in the cell concentrations at specific fluorescence values. When comparing the same fluorescence value in the 10°C experiment with the 7.6°C experiment, more cells are found in the growth rate tube. Separate regressions correlating cell concentration to fluorescence value were generated for both the 7.6°C and 10°C experiments (Figure 8). The 7.6°C regression yields an equation with a R^2 value of .85. The 10°C regression has a R^2 value of .93. It is likely that the higher R^2 value of the 10°C regression is due to the detection levels of the fluorometer and the higher

concentrations found within the 10°C tubes. The slopes of the 7.6°C and 10°C regressions are significantly different by temperature with a resultant p-value of 0.001. As observed in Figure 8c, the extended regression view shows the deviation of cell concentration with increasing fluorescence values. *Hanztschia amphioxys f. muelleri* calculated regressions used to translate the recorded fluorescence values to cell concentrations:

$$\mathbf{7.6°}\boldsymbol{C\ Cell\ Concentration} = 7630.9 \times Fluorescence\ Value + 907.19$$
$$\mathbf{10°}\boldsymbol{C\ Cell\ Concentration} = 9841.5 \times Fluorescence\ Value + 241.3$$

Equation 5: *Hantzschia amphioxys f. muelleri* regressions

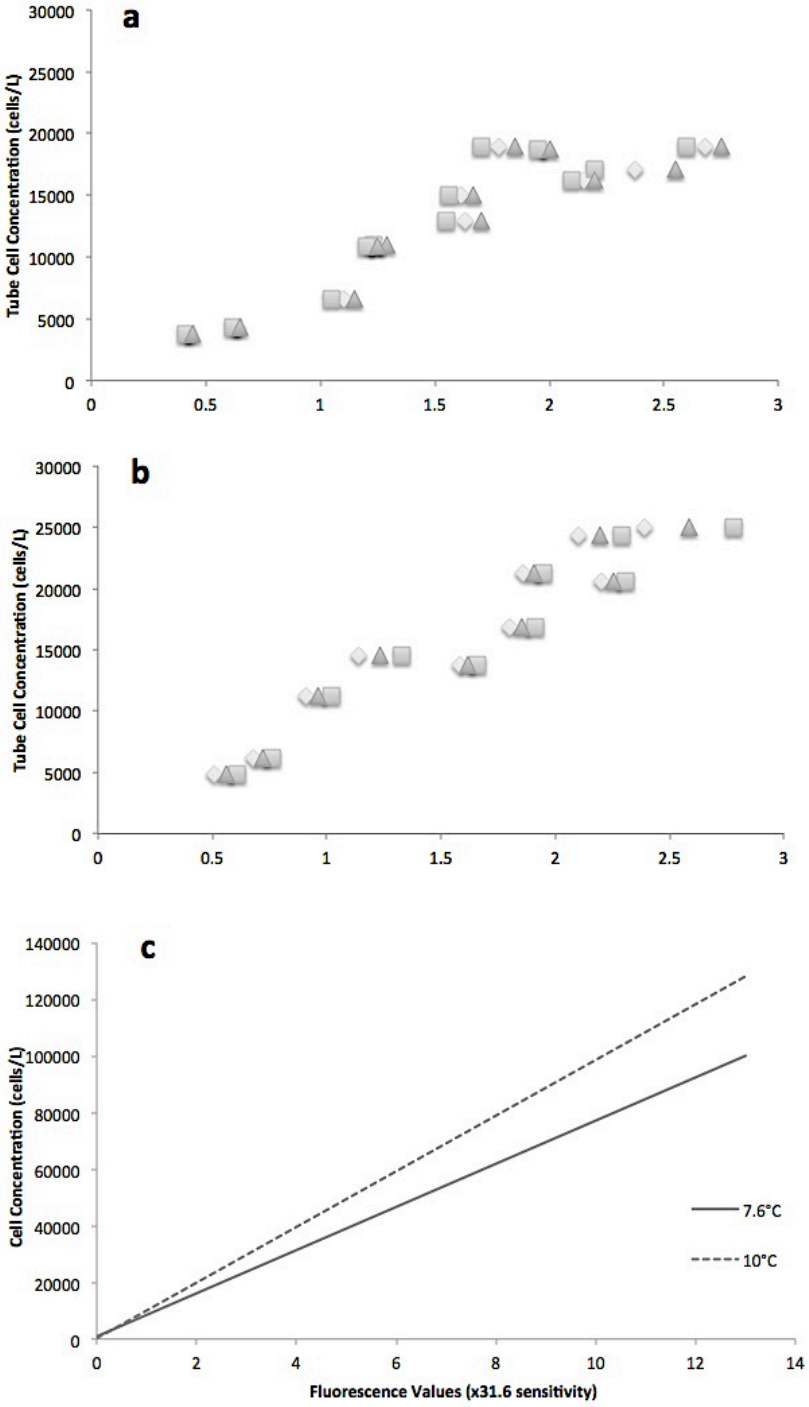


Figure 8: Correlation of *Hantzschia amphioxys f. muelleri* cell counts and fluorescence values at (a) 7.6°C, (b) 10°C, and (c) the extended regression comparison of both temperatures.

Table 3 summarizes the key growth parameters found for each temperature experiment. It was found that *Hantzschia amphioxys f. muelleri* grows significantly faster at 10°C than at 7.6°C with a doubling time nearly 2 days shorter when compared to 7.6°C.

Table 3: Calculated growth rate and doubling time for *Hantzschia amphioxys f. muelleri*, σ=standard deviation among tubes, n=number of tubes included in average

Experiment	Average Growth Rate (cells/day)	Average Doubling Time (days)
7.6°C	0.13 (σ=0.01, n= 9)	7.5 (σ=0.92, n=9)
10°C	0.18 (σ=0.03, n=7)	5.8 (σ=0.98, n=7)
p-value	**0.0026**	**0.0027**

The lag time, log phase duration, and carrying capacity were monitored for each tube. The lag time was not significantly different between temperature experiments, but with p-values <0.05 it was significantly different by experiment year. It was found that with each increasing year in culture, the lag times lengthened (Table 4). The log phase duration seemed to increase in 2010, but then dropped again in 2013 with no significant trends.

Table 4: Lag time and log phase duration by experiment year for *Hantzschia amphioxys f. muelleri*

Year of Exp	2009	2010	2013
Lag Time (days)	26.6	43.75	65.5
Log Phase (days)	17.5	22.75	18.5

Cell concentration at stationary phase varied some by experiment year, but was significantly different between temperature experiments with a p-value of 0.002. The average carrying capacity for 7.6°C was 15,000 cells/L and for 10°C was 20,000 cells/L. An example of the logistic growth curves for each temperature

experiment are shown below with their respective semi-log plots for the determination of intrinsic growth rate (Figure 9).

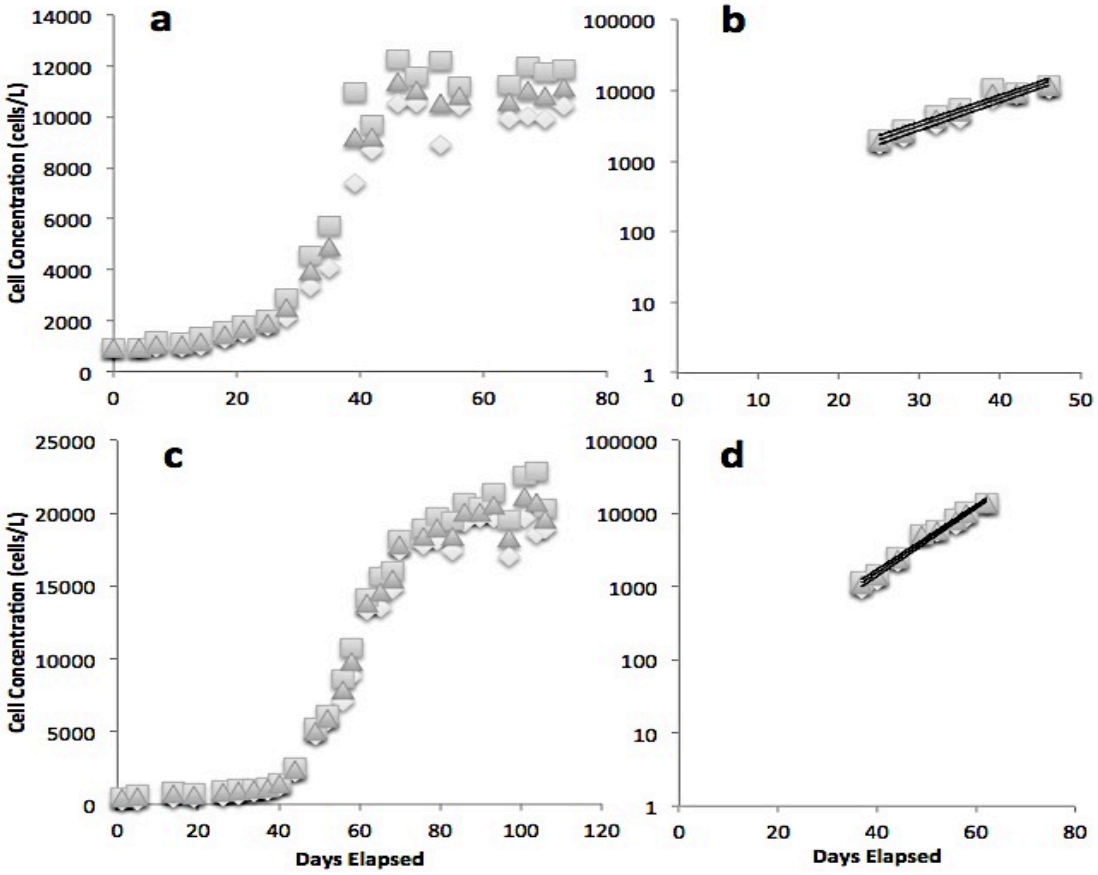


Figure 9: ***Hantzschia amphioxys f. muelleri*** **logistic growth curves at (a) 7.6°C 2009 and (c) 10°C 2010, semi-log plot to obtain exponential growth phase slope of (b)7.6°C and (d) 10°C**

3.1.2 *Psammothidium papilio*

Psammothidium papilio is the other taxon that was studied throughout all of the growth rate experiments. The growth responses for 7.6°C were tested in 2009 and 2010 and the growth at 10°C was analyzed in 2010 and 2013. The t-test of the cell count to fluorescence ratios in each year and temperature experiment yielded no statistically significant values. This indicates that at both temperatures tested, there was no significant difference in tube cell concentration and their associated fluorescence values. As seen in Table 5, a high correlation was found across both experimental year and temperature.

Table 5: Ratio t-test results by year and temperature for *Psammothidium papilio*

Temperature	7.6°C	10°C	7.6°C, 10°C
Years	2009, 2013	2010, 2013	2009 – 2013
p-value	0.94	0.95	0.67

One regression ($R^2 = .94$) was generated to convert fluorescence values to cell concentrations for both temperature experiments (Figure 10).

$$\boldsymbol{Cell\ Concentration}\left(\frac{\boldsymbol{cells}}{\boldsymbol{L}}\right) = 20901 \times Fluorescence\ Value + 6812.6$$

Equation 6: *Psammothidium papilio* regression

Table 6 summarizes the key growth parameters found for each temperature experiment. It was found that the growth rate of *Psammothidium papilio* does not significantly change between 10°C and 7.6°C with doubling times around 10 days. However, as will be discussed later in this chapter (3.3), an experiment was

conducted to assess the effects of inoculation concentration on lag time and growth rate for the 2013 experiments. The growth (Table 6) and lag time (Table 7) results of the 10°C 2013 experiment are significantly different from both the 10°C 2010 experiment as well as the 7.6°C experiment. Due to the differing inoculation concentration and significantly different growth rate and lag time, the 10°C 2013 results were not included in the 10°C 2010 averages, but are discussed in section 3.3.

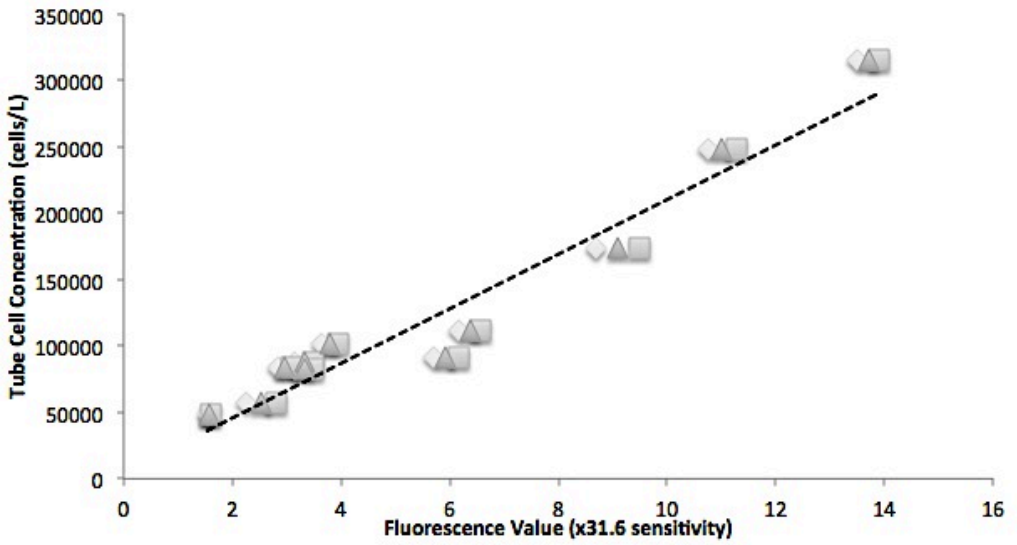


Figure 10: *Psammothidium papilio* fluorescence and cell count correlation. The hatched regression line is overlayed.

Table 6: Calculated growth rate and doubling time for *Psammothidium papilio*. σ=standard deviation, n= number of tubes included in average

Experiment	Average Growth Rate (cells/day)	Average Doubling Time (days)
7.6°C	0.10 (σ=0.02, n= 19)	10.1 (σ=2.04, n=19)
10°C 2010	0.10 (σ=0.007, n=4)	9.7 (σ=0.72, n=4)
p-value (7.6°C, 10°C 2010)	0.993	0.632
10°C 2013	0.34 (σ=0.01, n=3)	2.9 (σ=0.08, n=3)
p-value (10°C 2010,10°C 2013)	**3.4E -7**	**1.8E-5**

The lag time and log phase durations are recorded in Table 7. Between 2009 and 2010 the lag time lengthened substantially (p-value <<0.005) for the 7.6°C experiments and the 10°C 2010 experiment had a lengthy lag time as well. The period of exponential growth, however, did not significantly change between 2009 and 2010. The lengthened lag time led to the interest in examining the effects of an increased inoculation concentration on lag time with the hope of reduction as described in section 3.3. The higher inoculation concentration reduced the 7.6°C lag time and log phase to around 2009 levels, but significantly reduced both the lag time and log phase duration (p-values <0.05) for the 10°C 2013 experiment.

Table 7: Changes in lag time and log phase duration by year for *Psammothidium papilio* *=higher inoculation concentration, ----- = no experiment conducted that year

Year of Exp	2009	2010	2013*
7.6°C Lag Time (days)	23.5	67.5	26.3
10°C Lag Time (days)	-----	40.3	15
7.6°C Log Phase (days)	23	25	18.3
10°C Log Phase (days)	-----	22.8	9

The cell concentration at stationary phase for all growth rate tubes during the growth rate experiments were variable, but not significantly different by temperature or year. Correlation by both year (p-values> 0.5) and temperature (p-value = 0.63) was found. The maximum tube cell concentrations varied between 23,000 cells/L and 125,000 cells/L with no clear trend or relationship to tube growth rate or doubling time. Figure 11 shows the logistic growth of the tubes with the respective minimum and maximum cell concentration at stationary phase.

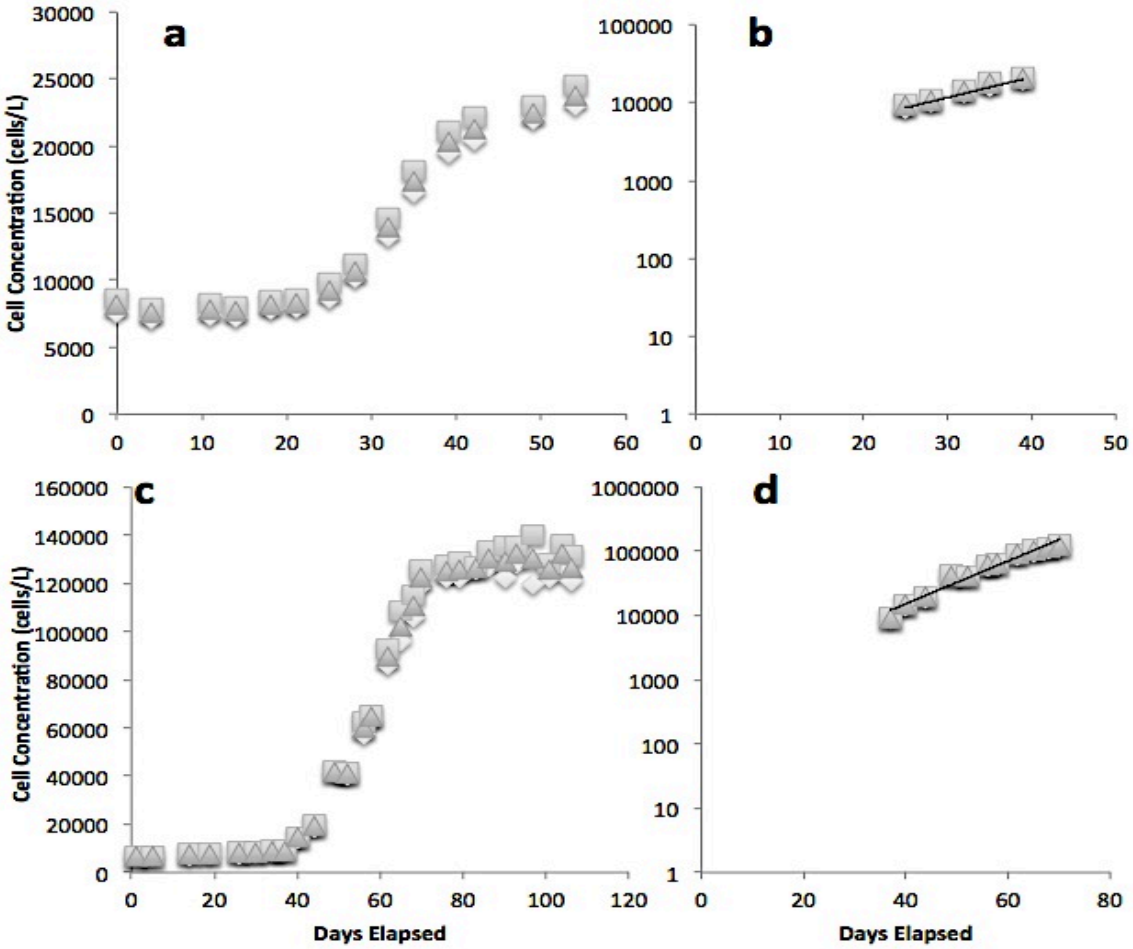


Figure 11: ***Psammothidium papilio*** **logistic growth curves at (a) 7.6°C 2009, (c) 10°C 2010 and their respective semi-log plots at right (b), (d)**

3.1.3 *Hantzschia abundans*

Hantzschia abundans was a relatively slow growing taxon analyzed at 7.6°C in 2009 and 2010 and at 10°C in 2010. When testing the ratios of cell count to fluorescence value, 7.6°C ratios were significantly different from 10°C values (p-value=0.011). Two regressions were generated to correlate cell concentration to fluorescence value for each temperature regime (Equation 7; Figure 12). The 7.6°C regression equation has an R^2 value of 0.92 and the 10°C equation has an R^2 of 0.79.

$$\mathbf{7.6°C\ Cell\ Concentration} = 6754 \times Fluorescence\ Value + 586.52$$
$$\mathbf{10°C\ Cell\ Concentration} = 4503 \times Fluorescence\ Value + 724.76$$

Equation 7: *Hantzschia abundans* generated regressions

These regressions indicate that at the same fluorescence value there are fewer cells present in the 10°C experiment than the 7.6°C experiment. Table 8 summarizes the growth parameters found for *Hantzschia abundans* at the two temperatures. Consistent with the regressions, a significantly lower growth rate was found for the 10°C temperature experiment with a doubling time 3 days longer than at 7.6°C.

Table 8: Calculated growth parameters for *Hantzschia abundans* by temperature.

Experiment	Average Growth Rate (cells/day)	Average Doubling Time (days)
7.6°C	0.088 (σ=0.011, n= 6)	11.4 (σ=1.5, n=6)
10°C	0.071 (σ=0.013, n=4)	14.4 (σ=2.5, n=4)
p-value	**0.033**	**0.031**

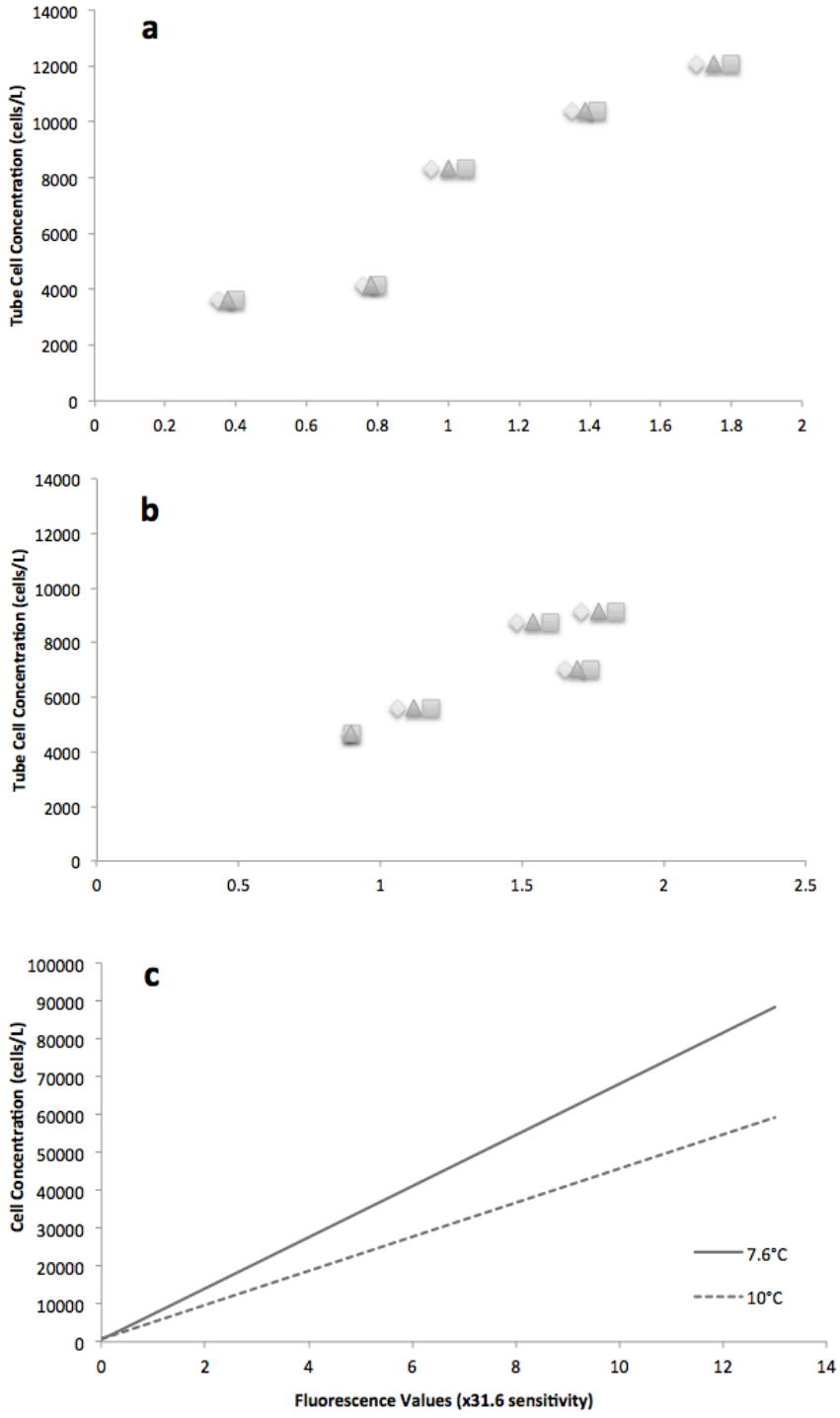


Figure 12: Correlation of *Hantzschia abundans* cell counts and fluorescence values at (a) 7.6°C 2010, (b) 10°C 2010, and (c) the extended regression comparison of both temperatures

The log phase was fairly consistent across temperature experiments (p-value=0.73) and years (p-value=0.21) with no significant differences. However, the lag time of the experiments increased by year and by temperature. The 2010 experiments had longer lag times, with the most extensive lag times found within the 10°C experiment Table 9. It is important to note, however, that the 10°C experiment occurred earlier in 2010 than the 7.6°C experiment. This means that a younger *Hantzschia abundans* stock culture illustrated a longer lag time at a higher temperature. While the 7.6°C lag time dropped later in 2010, it still did not drop to 2009 levels (p-value=0.05). The lag time variation among temperature experiments was significantly different (p-value<<0.005).

Table 9: Variation in lag time and log phase duration by year for *Hantzschia abundans*

Year of Exp	7.6°C 2009	7.6°C 2010	10°C 2010
Lag Time (days)	39	53	87
Log Phase (days)	27	25	32

The cell concentration at stationary phase was consistent for the 7.6°C experiments around 5000 cells/L. At 10°C however, maximum cell concentration significantly increased to an average of 7800 cells/L (p-value=0.02). Figure 13 shows the logistic growth curves for *Hantzschia abundans* and illustrates the differences in lag time and maximum cell concentration between 7.6°C and 10°C.

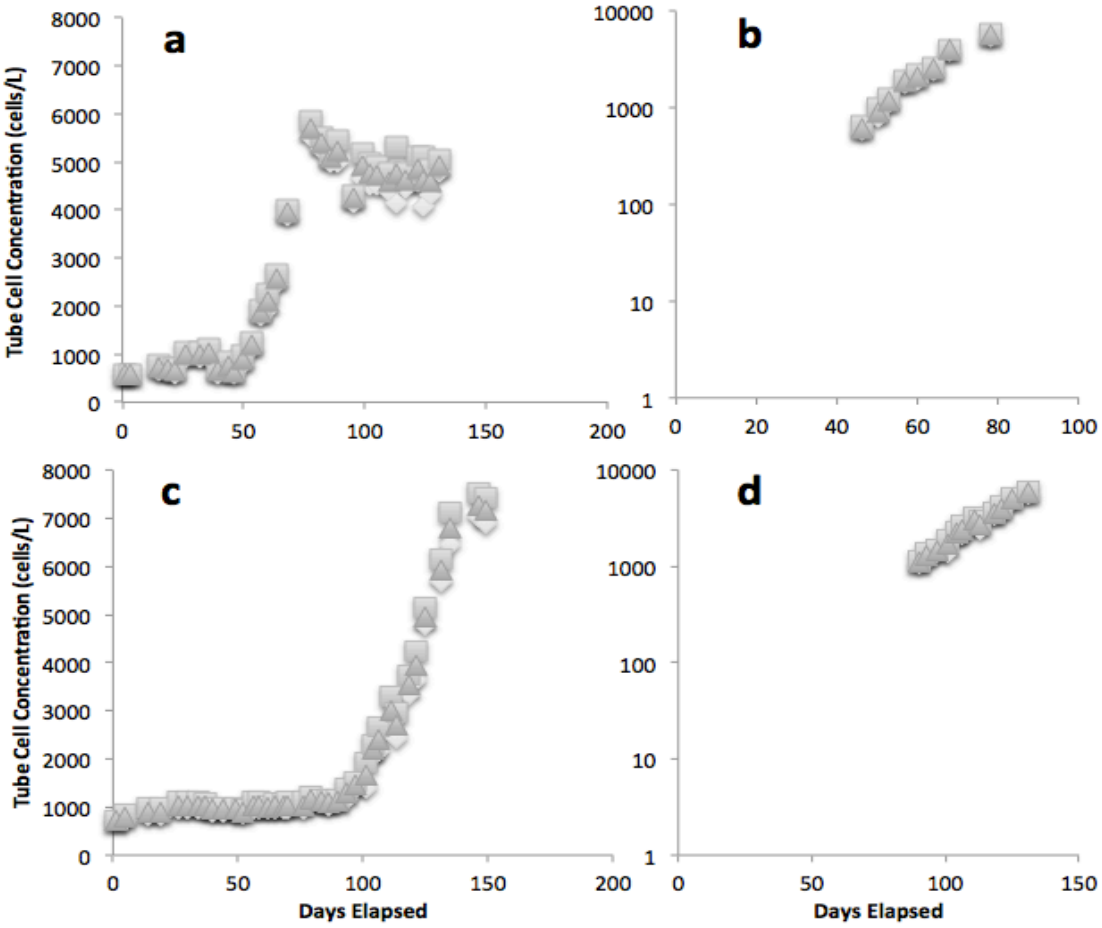


Figure 13: Logistic growth curves for *Hantzschia abundans* at (a) 7.6° C 2010, (c) 10° C 2010 and the respective semi-log plots at right

3.1.4 *Hantzschia amphioxys*

Hantzschia amphioxys was included in one 7.6°C and one 10°C experiment in 2010. *Hantzschia amphioxys* is a slow growing taxa demonstrating a long lag time and thus, was only tested twice. The cell count to fluorescence ratios found for 7.6°C and 10°C were not significantly different (p-value=0.83) and one combined regression was developed. Figure 14a shows the original regression trend, but the y-intercept was near -20,000 and translated fluorescence values into cell concentrations inaccurately. This error is again, likely due to the detection limits of the Turner Designs fluorometer at low fluorescence values. Figure 14b shows the adjusted regression trend crossing near the origin. While the R^2=0.58 of regression 14b is low, the overall trend is similar to the other *Hantzschia* taxa analyzed and thus may be a reasonable representation of tube cell concentration. It is recognized that this underestimates the cell concentration. Additional regression trends were examined, but it is hypothesized that a non-linear trend may imply that something is changing internally in the cell. The calculated regression equation for *Hantzschia amphioxys* at both temperatures is:

$$\boldsymbol{Cell\ Concentration}\ (\frac{\boldsymbol{cells}}{\boldsymbol{L}}) = 11510 \times Fluorescence\ Value + 762.02$$

Equation 8: *Hantzschia amphioxys* calculated regression trend

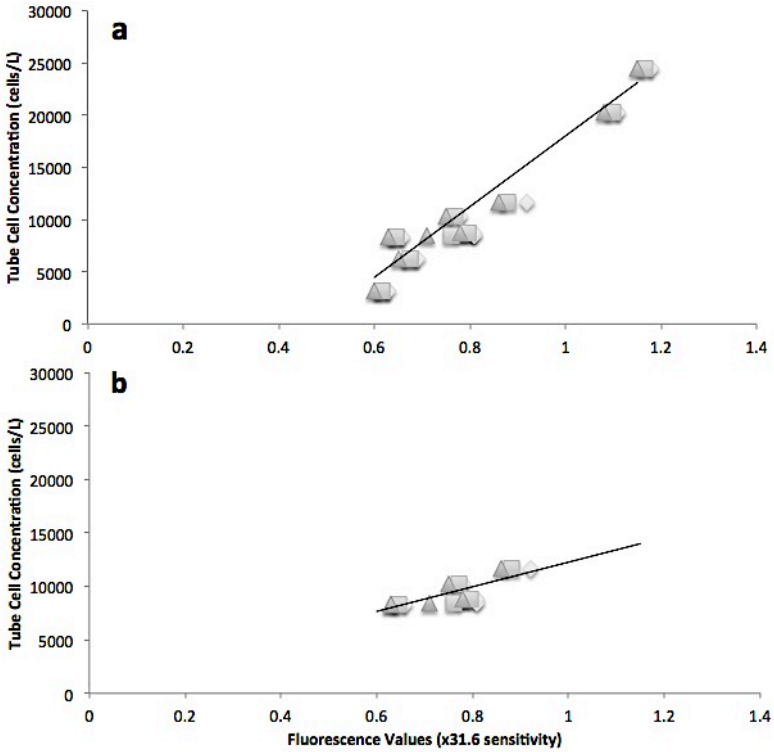


Figure 14: Calculated (a) and adjusted (b) regression for *Hantzschia amphioxys*

The 10°C experiment occurred before the 7.6°C experiment and thus represents a younger stock culture of *Hantzschia amphioxys*. The younger culture could explain why the 7.6°C lag time is longer than the 10°C lag time though not significantly. Table 10 shows the 7.6°C growth rate and doubling time to occur at a significantly faster rate than at 10°C even though the 7.6°C experiment occurred later in the year. Additionally, the cell concentration at stationary phase was significantly lower at 7.6°C than at 10°C.

Table 10: Key average growth parameters for *Hantzschia amphioxys*

Experiment	Growth Rate (cells/day)	Doubling Time (days)	Lag Time (days)	Log Phase Duration (days)	Carrying Capacity (cells/L)
7.6°C (n=4)	0.072 (σ=0.008)	14.0 (σ=1.6)	64	35.5	8700
10°C (n=4)	0.050 (σ=0.003)	19.9 (σ=1.4)	52.5	42.75	15200
p-value	**0.003**	**0.002**	0.20	0.19	**7.1E-5**

Figure 15 clearly shows the differences in the growth behavior of *Hantzschia amphioxys* at both temperatures.

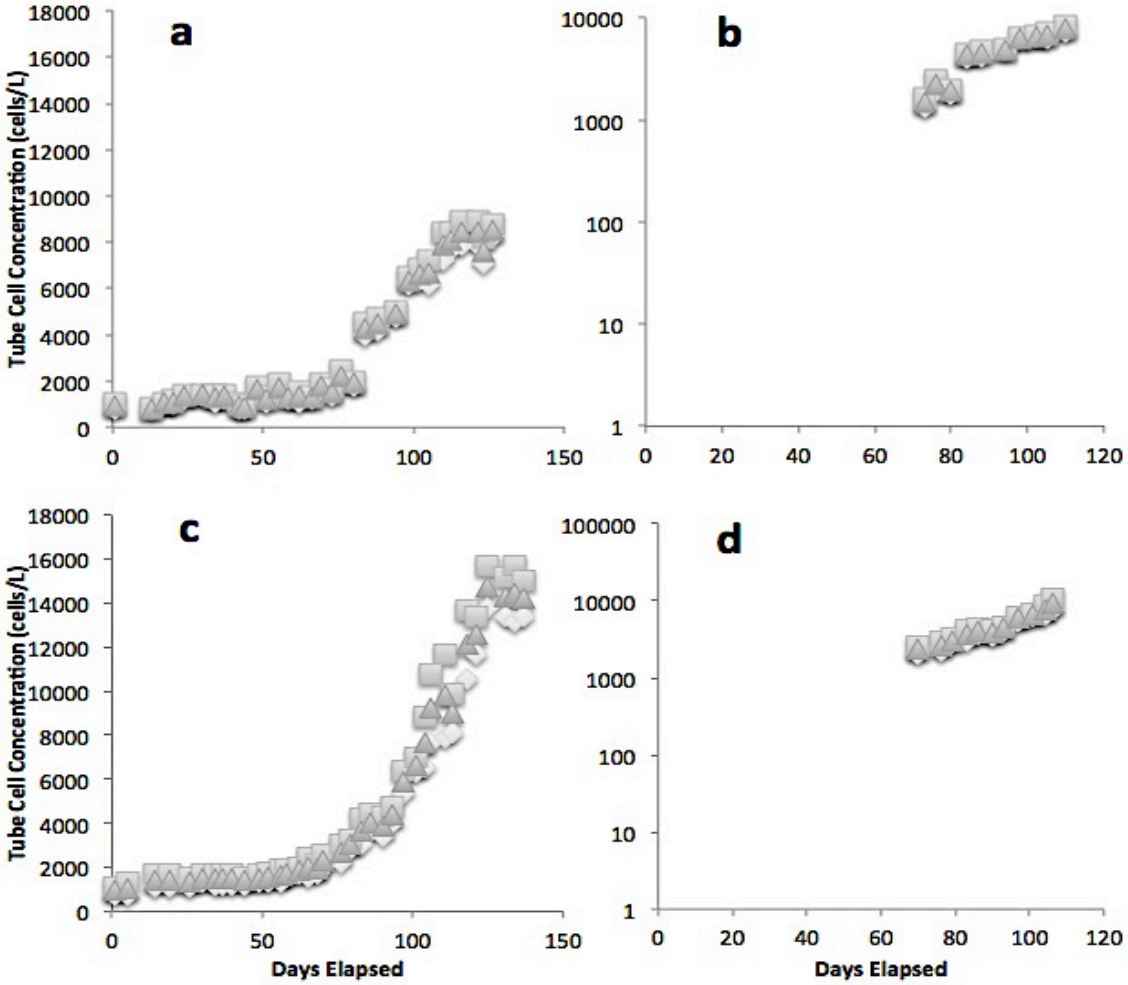


Figure 15: Logistic growth curve for *Hantzschia amphioxys* at (a) 7.6° C 2010, (c) 10° C 2010 and the respective semi-log plots at right

3.1.5 *Hantzschia sp. #5*

Hantzschia sp. #5 was only included in one growth rate experiment at 10°C in 2010. *Hantzschia Sp. #5* was included in a growth rate experiment to replace two taxa, *Luticola muticopsis f. capitata* and *Stauroneis latistauros,* that had issues with non-homogeneous tube mixing and mucilage production. During the growth rate experiment, however, it was found that the *Hantzschia sp. #5* stock culture and growth rate tubes were in decline. The cell morphology had changed and there was a significant reduction in cell length. When cultures reproduce asexually for extended periods of time, cell shrinkage can occur (Round, Crawford, and Mann 1990). Figure 16 shows a typical cell image compared to a cell in culture with a 50% reduction in length.

Figure 16: Typical *Hantzschia sp. #5* cell at left (Antarctic Freshwater Diatoms Website) and cell found in culture at right. Scale bars are 10µm.

The growth curves illustrated extremely long lag times of 80 – 110 days, likely as a result of the culture decline and size reduction. Lag times of this length

would prevent any taxa from growing during the 84 day (12 week) Antarctic austral summer. While the growth curves show logistic growth (Figure 17), the results are from a cell culture that is in a physiological state that is not comparable to other strains. The *Hantzschia sp. #5* growth results are not presented in this thesis.

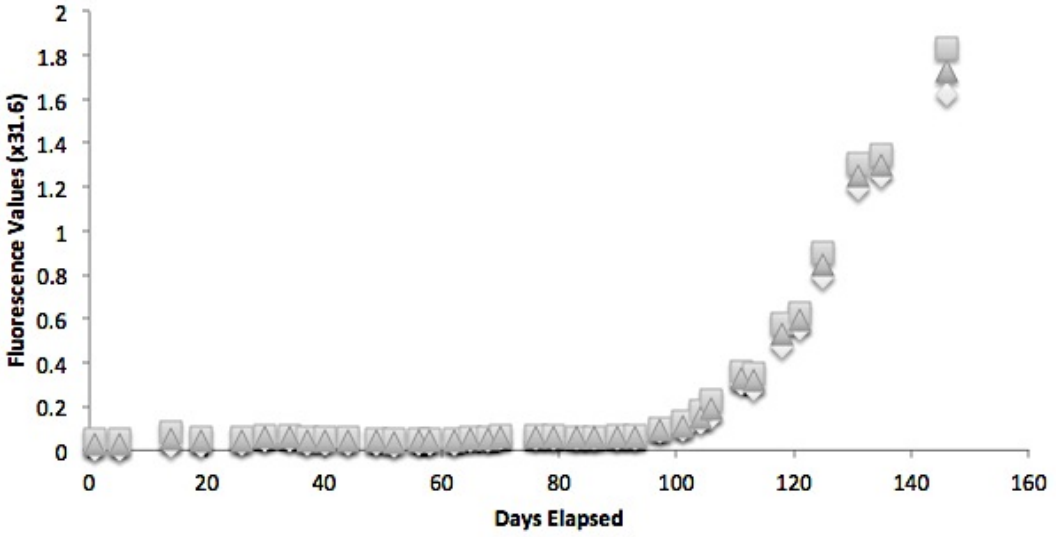


Figure 17: *Hantzschia sp. #5* logistic growth and 100 day lag time for the 2010 experiment.

3.1.6 *Luticola muticopsis f. capitata*

Luticola muticopsis f. capitata was only included in the first 7.6°C experiment conducted in 2009 due to difficulty in releasing cells from visible mucilage clumps. When visible growth was apparent after 35 days, the tubes were still reading rather low fluorescence values. To remedy the situation, prior to fluorescence readings the cells were vigorously resuspended in the tubes using pastuer pipettes. This method caused a slight increase in fluorescence values, but large deviations between high and low fluorescence readings persisted (Figure 18). It was noted that there were large mucilage clumps in the tube that did not break apart even after shaking. The *in vivo* fluorescence works well for taxa that are able to suspend homogeneously into the tube and that do not excrete large amounts of extracellular polymeric substances (EPS). *L. muticopsis f. capitata* is difficult to homogenize and thus *in vivo* tube fluorescence is not an accurate method for calculating growth rate. Another method of *in vivo* fluorescence was tested for use with species of high EPS production and is described in the Appendix.

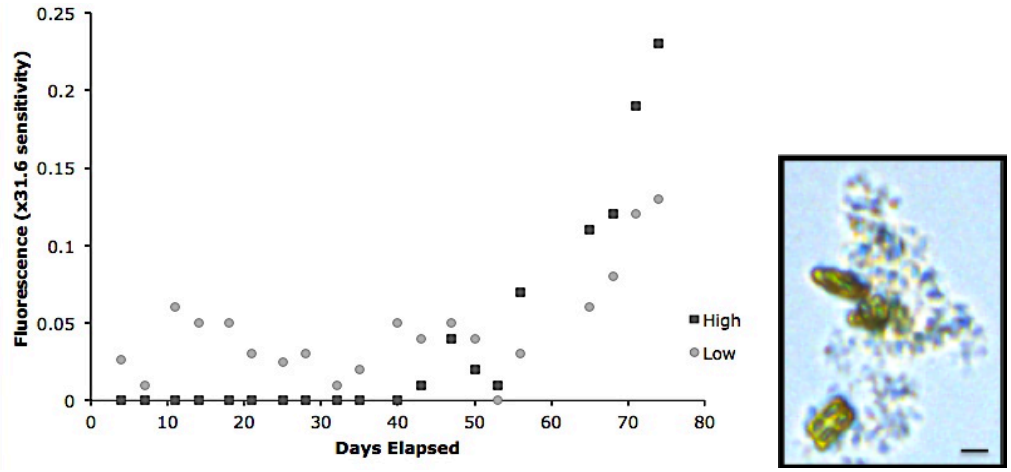


Figure 18: *Luticola f. capitata* fluorescence by time and a FlowCAM®* image of live cells in mucilage.

3.1.7 *Stauroneis latistauros*

Similar to *L. muticopsis f. capitata*, *Stauroneis latistauros* was only included in the 2009 7.6°C temperature experiment due to clumping and resuspension issues. When running the *Stauroneis latistauros* strain through a FlowCAM®*, dense mucilage surrounding the cells was observed (Figure 19).

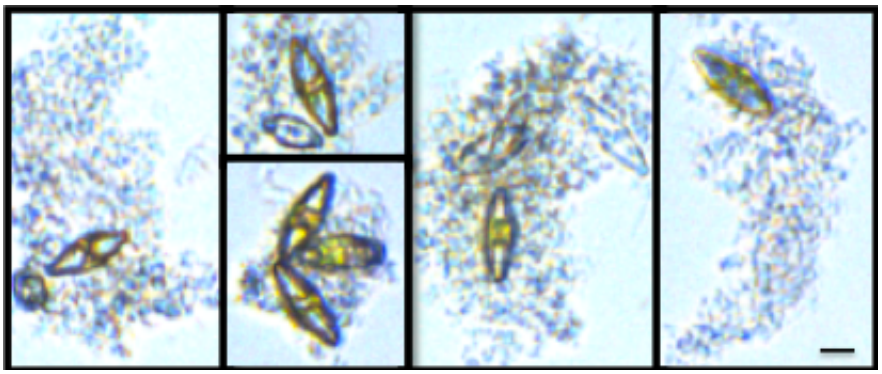


Figure 19: FlowCAM®* images of live *Stauroneis latistauros* with mucilage matrix. Scale bar = 10µm.

The growth curves show logistic growth, but no distinct correlation between cell concentration and fluorescence was found (Figure 20). The increase in fluorescence may not be entirely attributable to cell growth due to the extracellular mucilage in the tube. The growth rate of *Stauroneis latistauros* is not presented in this thesis.

* The FlowCAM® is an instrument developed by Fluid Imaging Technologies that is a microscope with a camera and a flow-through cell attached. Images are automatically captured of particles flowing through the flow-cell. These images were captured with a 10x objective and collimator with a 100µm flowcell.

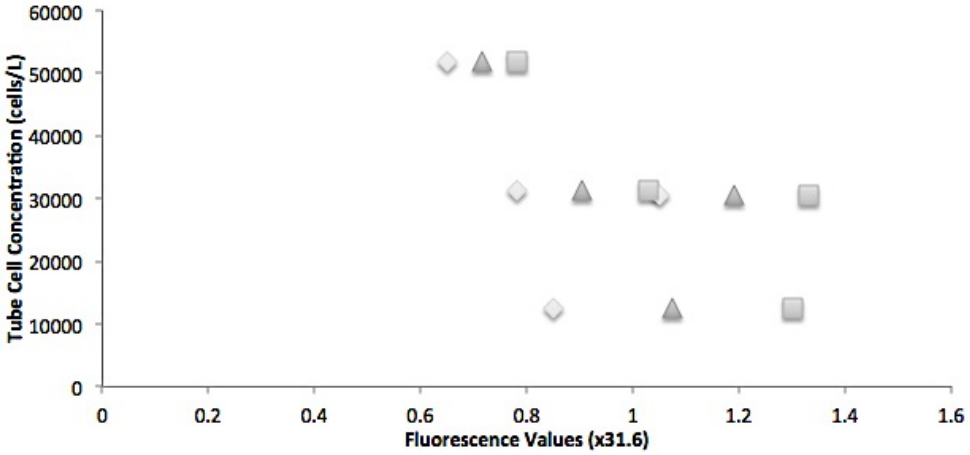


Figure 20: *Stauroneis latistauros* cell count and fluorescence relationship

3.2 Effect of inoculation concentration on lag time and growth rate

According to Segota et al. (2012) the lag phase is the most poorly understood of growth phases where the cells can increase in mass, but not in population. The lag phase is a period of time in which cells are adjusting to their new environment. In bacterial populations, this adjustment has been shown to happen by induction of mRNA and protein synthesis or the increase of exoenzymes (Maier, Pepper, and Gerba 2009a). The lag-log transition is not well understood in eukaryotic organisms, but a recent study has shown that the allee effect due to endrocrine signaling in a unicellular organism can incite this transition (Segota et al. 2012).

The final 10°C experiment conducted on *Psammothidium papilio* in 2013 was designed to test the effect of the inoculation concentration on the lag time. The lag time decreased by more than half, but the growth rate also significantly increased. The cell concentration at which the culture went into exponential phase remained the same, however, indicating that there may be some required cell concentration before physical signaling will stimulate the transition. Although these results are interesting, this growth rate experiment is not included in the discussion or taxon analysis in this thesis. All of the growth rate experiments, with the exception of the 10°C 2013 *Psammothidium papilio* were inoculated with the same population and followed consistent methods.

CHAPTER 4: DISCUSSION OF GROWTH RATE EXPERIMENT RESULTS

4.1 Regression Trends

An overall comparison of the calculated regression trends is illustrated in Figure 21. The *Psammothidium papilio* regression has a much higher slope than any of the *Hantzschia* taxa. *Psammothidium papilio* shows a higher cell concentration at the same fluorescence value when compared to any of the *Hantzschia* taxa. This difference is an expected result due to the size differences between the *Hantzschia* taxa and the smaller *Psammothidium papilio* cells. Cells were viewed under a Nikon TS100 before and after growth rate experiments. The regressions for *Hantzschia abundans (Hant abund)* and *Hantzschia amphioxys f. muelleri (Hant f muell)* were differentiated by temperature due to their significant separation by cell concentration:fluorescence value ratios. *Hant abund* 7.6°C and *Hant f muell* 10°C reveal higher cell concentrations at the same fluorescence intensity than at the alternative temperature regression. A higher cell concentration at the same fluorescence value could indicate that there is less chlorophyll per cell or that the cell concentration is high enough that a "masking" effect is taking place. The masking effect means that at some critical cell concentration in the tube, the cells begin to hide other cells (mask), preventing the fluorometer from picking up the fluorescence from those masked cells. The regression slopes agree with their demonstrated intrinsic growth rates (Figure 22).

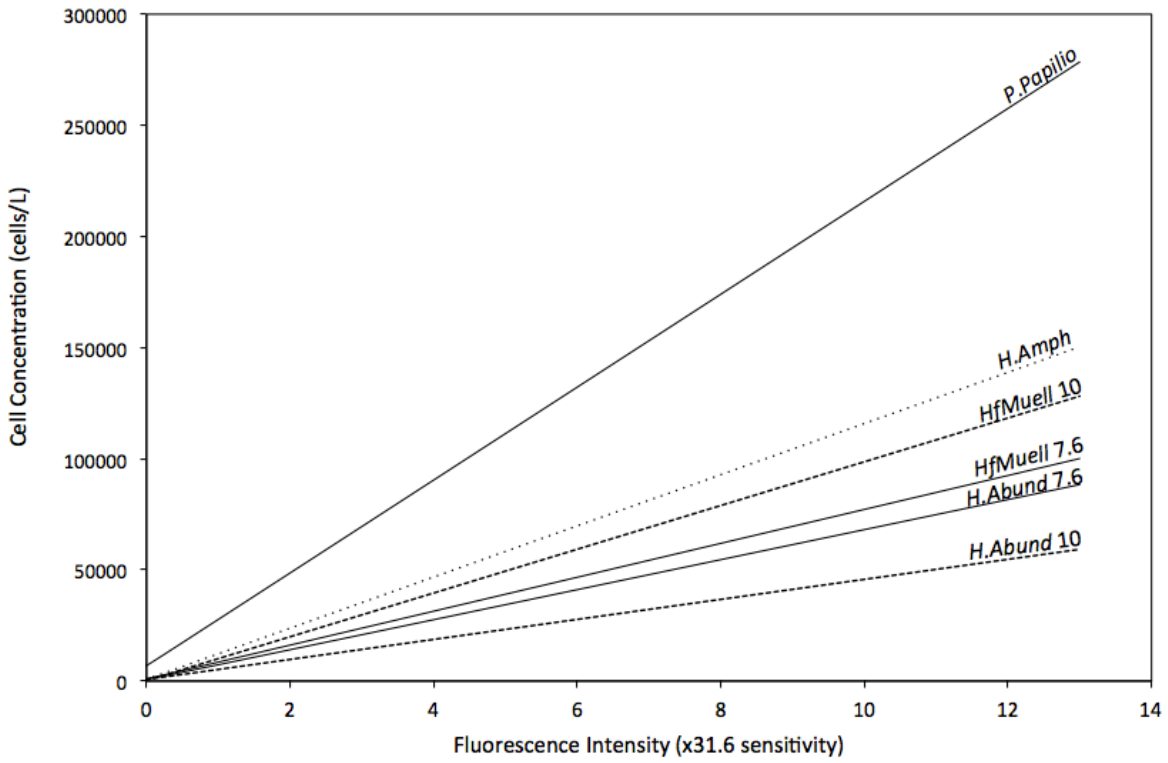


Figure 21: Cell concentration to fluorescence intensity regression comparison among taxa included in growth rate experiments.

4.2 Intrinsic Growth Rates

Diatoms thriving in the Dry Valleys are likely to be classified as psychrophiles, microorganisms that have optimal growth below 15°C (Madigan et al. 2009). Cold-adapted organisms synthesize enzymes that have evolved to provide structural flexibility for the enzyme to function efficiently at low temperatures (Maier, Pepper, and Gerba 2009b). It was expected that the Antarctic species would illustrate growth optima at lower temperatures in this experiment.

While *Hantzschia amphioxys f. muelleri* illustrates faster growth at 10°C, it has the fastest growth rate of all taxa at 7.6°C. *Hantzschia amphioxys f. muelleri* is the only South Victorialand endemic included in the 4 analyzed taxa. Evolving in the Dry Valleys and being entrained in the circadian cycle perhaps explains why *Hantzschia amphioxys f. muelleri* has a doubling time at 7.6°C that is 3 – 6 days faster than the other 3 taxa. Average intrinsic growth rates with standard deviation are summarized in Figure 22.

Hantzschia abundans and *Hantzschia amphioxys*, both considered cosmopolitan taxa, show enhanced growth at lower temperatures. These two taxa demonstrate optimal growth at 7.6°C. Although widespread in distribution, the higher growth rate at 7.6°C provides evidence that both of these taxa may be cold-adapted. This cold adaptation is likely a result of the geographic isolation in the Dry Valleys. The cells that initially made it to the Dry Valleys likely were extreme individuals within the species to be able to survive well in transit to Antarctica. These cells also had to thrive given the species radiation and competition filters

once in the Dry Valleys so it quite possible these species are unique in their cold adaptations within the species.

Psammothidium papilio, an Antarctic endemic, showed no significant growth preference between the two tested temperatures. This result may indicate that *PsamPap* is well acclimated to the daily fluctuation of stream temperatures found in the MDV.

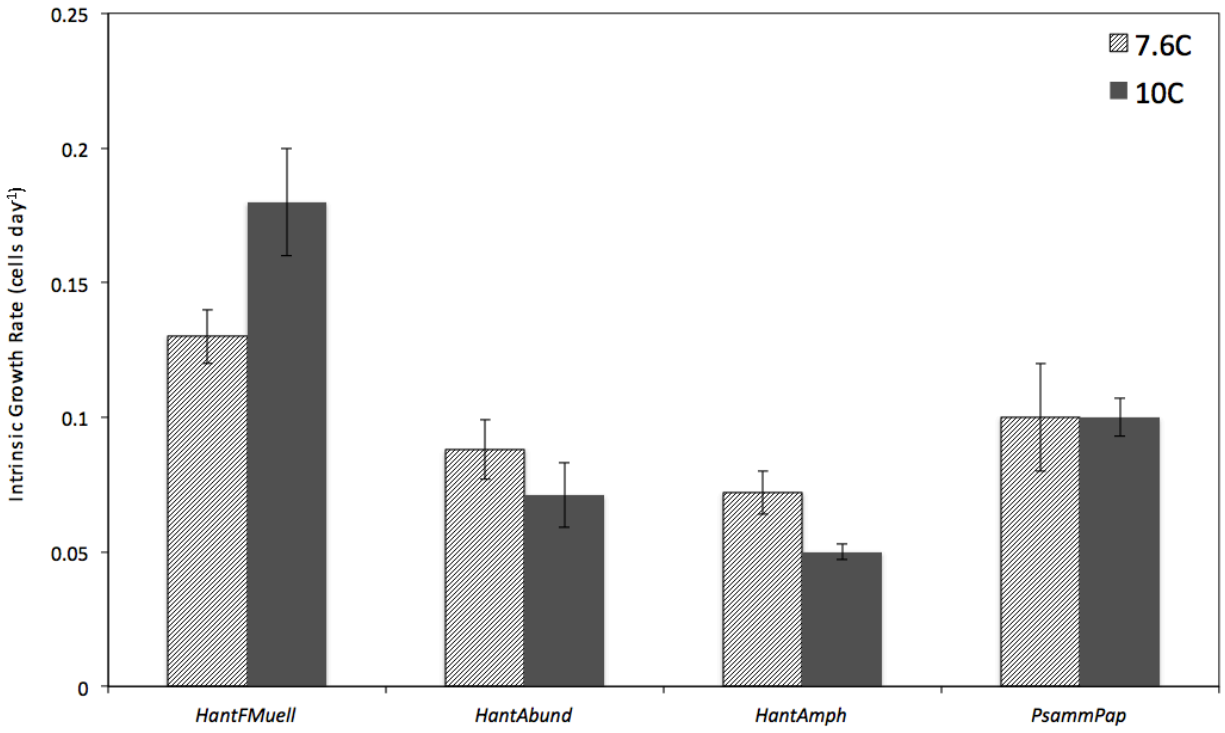


Figure 22: Average intrinsic growth rate of all taxa with standard deviation shown.

Two-tailed t-tests were performed across all taxa and at both temperature regimes to determine statistical significance of growth rates and are summarized in

Table 11 and

Table 12. With the exception of the correlation of *PsamPap* and *HantAbund* at 7.6°C, all of the intrinsic growth rates showed significance at p-values < 0.05 and most showed significance at the 0.005 level.

Table 11: p-values for intrinsic growth rate comparison across taxa at 7.6°C

Taxa, 7.6C	PsamPap	HantFMuell	HantAbund	HantAmph
PsamPap		6.71E-7	0.204	0.022
HantFMuell			5.23-5	1.88E-5
HantAbund				0.038

Table 12: p-values for intrinsic growth rate comparison across taxa at 10°C.

Taxa, 10C	PsamPap	HantFMuell	HantAbund	HantAmph
PsamPap		0.001	0.005	1.58E-5
HantFMuell			9.16E-5	7.05E-7
HantAbund				0.02

4.3 Doubling Times

Doubling time, Td, represents the amount of time it takes a population to double. The average doubling times for each taxon are shown in Figure 23.

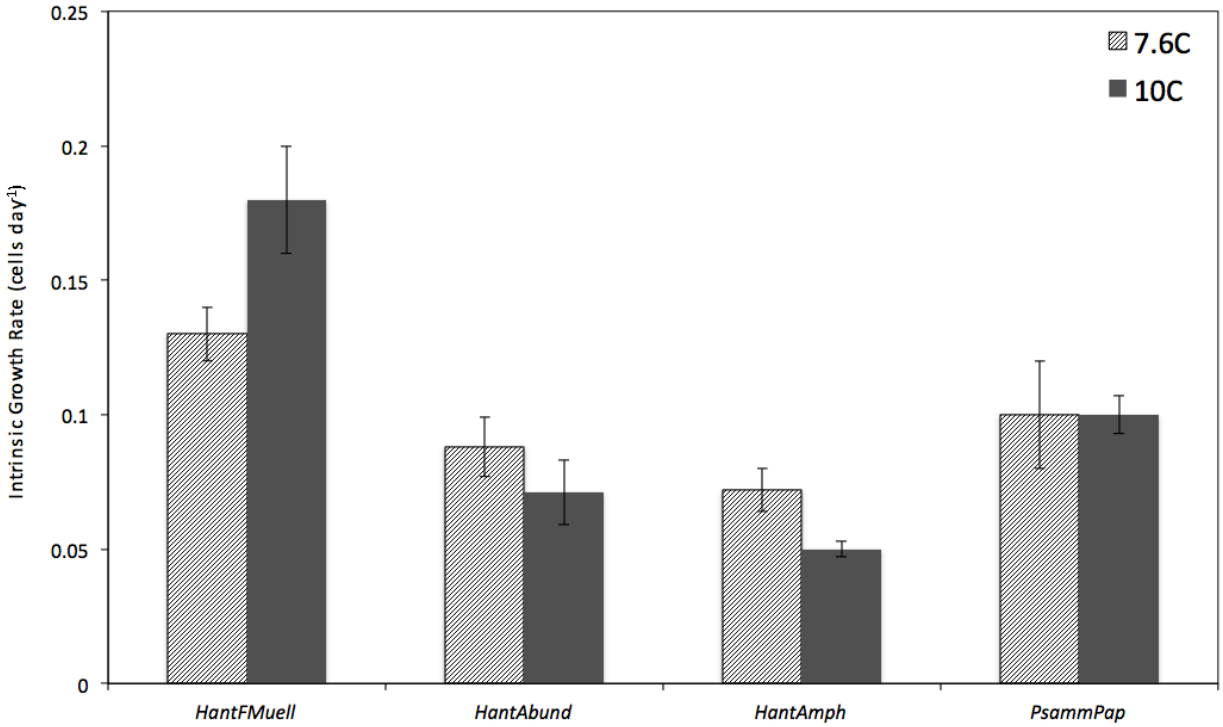


Figure 23: Average doubling times, T_d with standard deviation shown.

Thinking about doubling times in the context of the potential growing season during the Antarctic austral summer provides a theoretical estimation of how many times a population could double during the season. Assuming a long growing season of 12 weeks (84 days) and an average doubling time for each taxon, the number of population doublings that can occur at 7.6°C and 10°C are summarized in Table 13.

Table 13: Theoretical calculated number of population doublings per growing season.

Taxon	*Hant. f. muelleri*	*H. abundans*	*H. amphioxys*	*P. papilio*
Doublings per season (#)	11 – 14	6 – 7	4 – 6	8

4.4 Abundance and Distribution in Dry Valley Streams

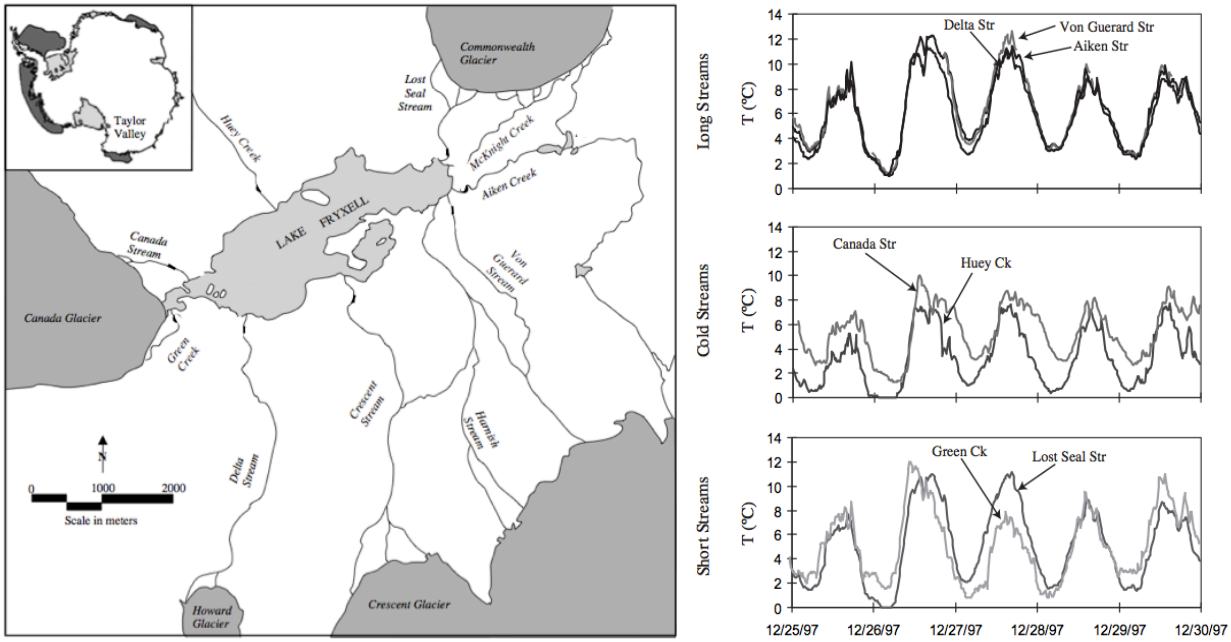


Figure 24: Fryxell Basin streams (Map adapted from Gooseff et al. 2003). Daily temperature range for a 5 day period in 7 Fryxell basin streams (Cozzetto et al. 2006).

The diatoms included in this study were all initially collected within the Fryxell basin. Research has shown that streams within the Fryxell basin exhibit similar diel temperature trends (Cozzetto et al. 2006). Temperatures in the streams peak at approximately the same time on a daily basis and evaporative cooling prevents temperatures from increasing above 15°C (Cozzetto et al. 2006). Though the two temperatures tested in this work vary by only 2.4°C, they are representative of the daily maximum temperatures (8 – 15°C) experienced within Fryxell streams during mid-summer. Furthermore, what may be considered minute temperature variation in temperate zones may have a great impact on the hydrologic regime in the Dry Valleys (Fountain et al. 1999).

Diatom communities vary by stream. Using previously known taxa distributions, Esposito et al. (2006) found increasing levels of Antarctic endemic species with increasing stream harshness. Harshness is impacted by climate variability and diatom populations have the potential to be altered in the future depending on climate trends. Understanding species level adaptations and growth with temperature variation will help identify which changes in communities or distribution might result in the future. Current species level abundance by stream for all taxa in culture are shown in **Error! Reference source not found.**.

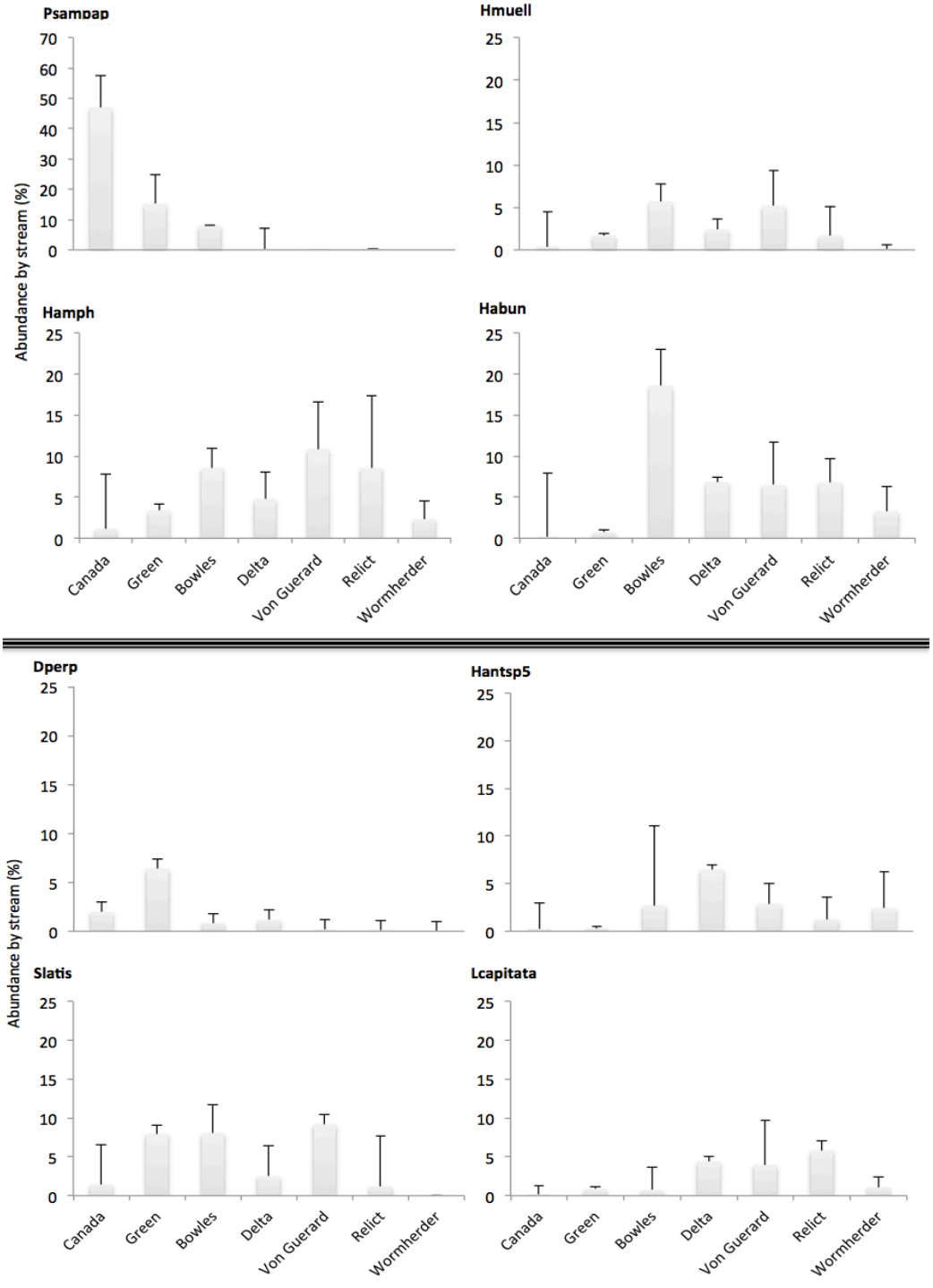


Figure 25: Taxa abundance by stream. Taxa from growth rate experiments (top) and taxa in culture (bottom). Streams arranged from least to most harsh as defined by Stanish et al. 2012.

In addition to focusing on harshness trends, current taxa abundance for the species tested is illustrated in

Figure 26. This figure shows abundance after rearranging the streams by water temperature.

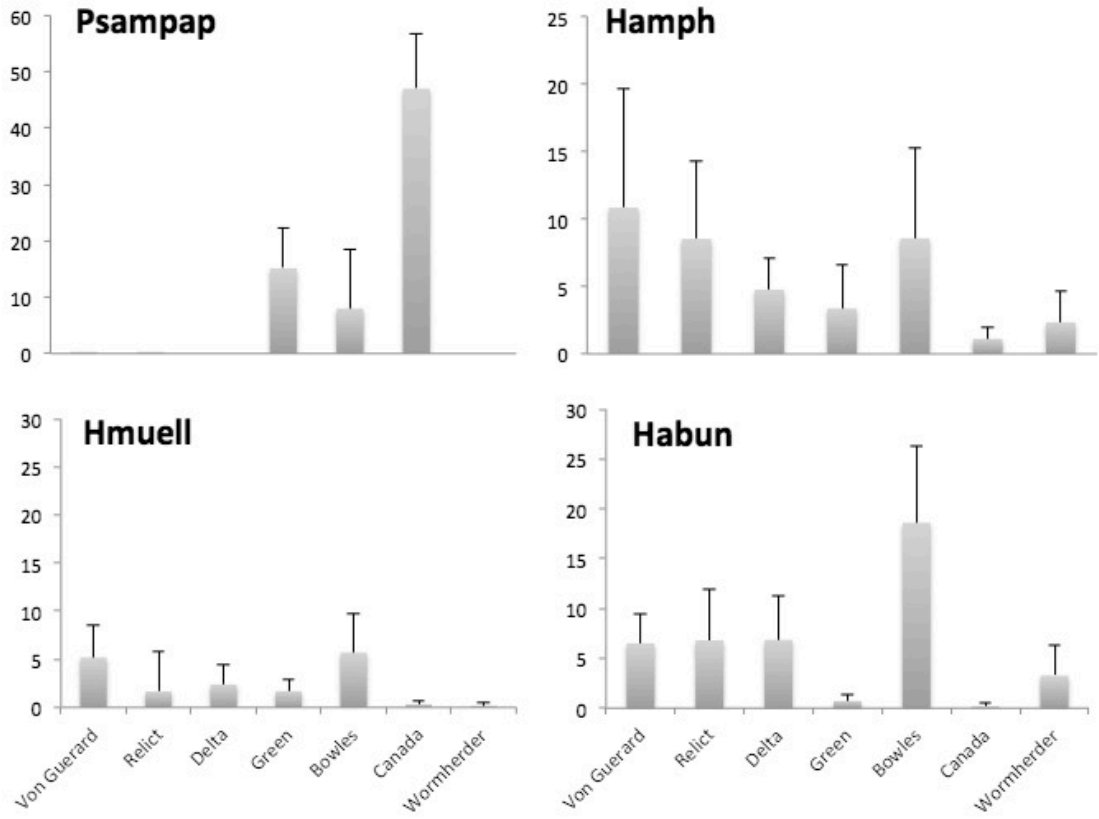


Figure 26: Taxa abundance by stream with streams arranged from warmer water temperatures on the left to colder water temperatures on the right.

It is noticed that distribution trends change when the stream arrangement changes and this will be noted in the individual species distribution summaries. Another way to visualize taxa distribution is by looking at Figure 27. This figure is a dot blot plot showing the abundance of most known diatom species in the MDV by stream. The taxa overlap in interesting ways. The species within the *Hantzschia* genus have different distribution trends and different calculated growth rates. However, *H. amphioxys* and *H. abundans* show similar distributions and illustrated similar

growth rate characteristics. Figure 27 also emphasizes the *P. papilio* dominance in Canada Stream and virtually non-existent abundance in all other MDV streams. More specific taxon level analysis of these trends is presented in the next section.

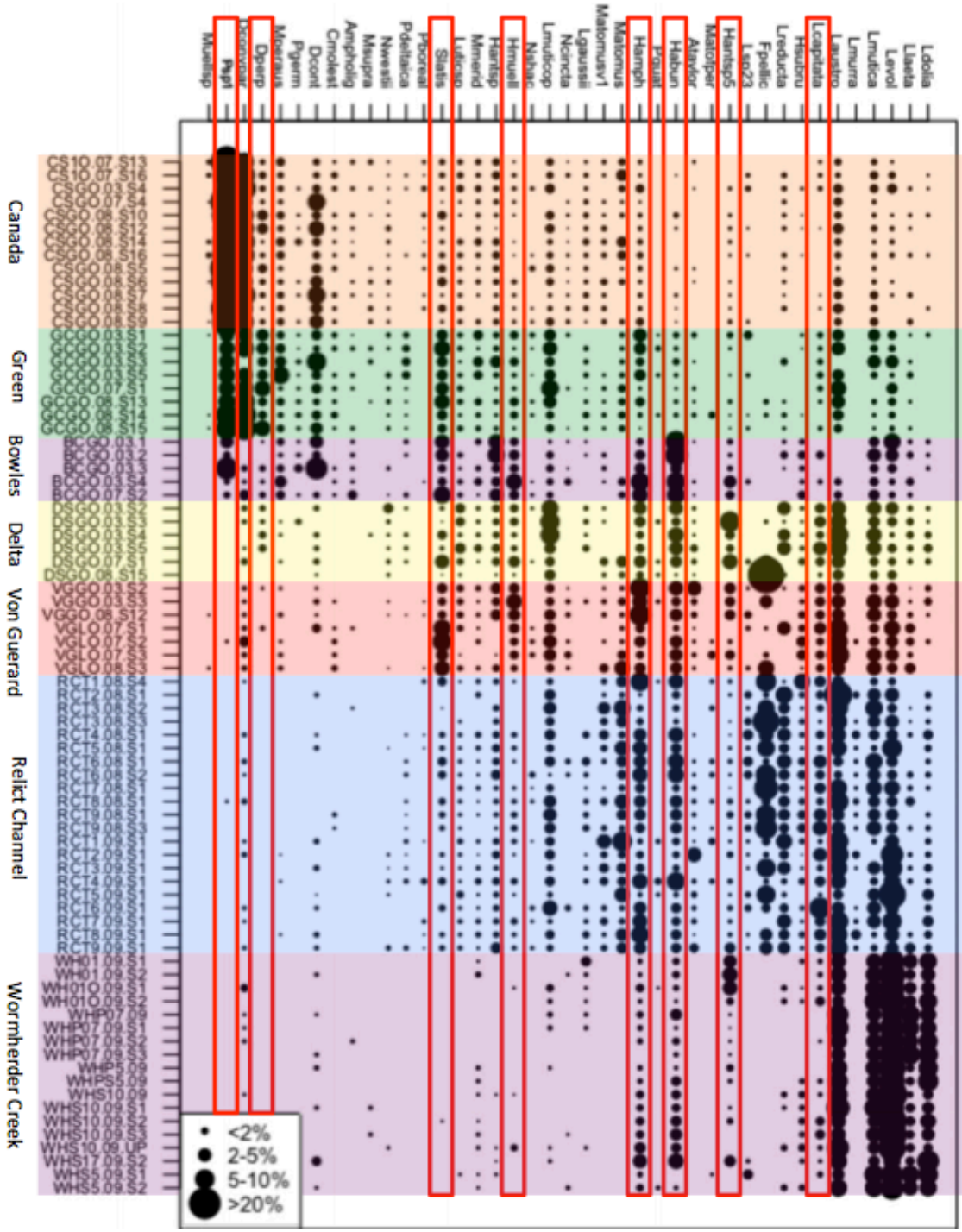


Figure 27: Dot blot plot showing abundance of all culture taxa (in red) by stream. Plot adapted from Stanish et. al 2012.

4.4.1 Dry Valley Stream Characteristics

Table 14 summarizes key Dry Valley stream information.

Table 14: Stream Fast Fact. Arranges least to most harsh (left to right) (a) Stanish et. al 2011 (b) Cozzetto et. al 2005 (c) Minimal data points, but Bowles is assumed to behave similarly to Green. Q = discharge, Qvarhist= deviation from the historical annual mean

Stream	Canada	Green	Bowles	Delta	Von Guerard
Season Length (days)[a]	**71**	**57**	**57**	**55**	**44**
Mean Total Q (m3)[a]	**2.06E5**	**1.42E5**	**2.62E4**	**1.05E5**	**5.97E4**
Qvarhist[a]	**18.2**	**12.5**	**12.5**	**531.3**	**383.6**
Temperature max[b]	**7-10**	**9-12**	**10-12[c]**	**10-12**	**10-12**
Temperature range[b]	**3-8**	**8 – 11**	**---[c]**	**7-11**	**6-11**

Canada Stream originates from Canada Glacier and is usually the first stream to begin flowing (Conovitz et al. 1998), thus providing the the longest growing season for microbial mats of all Fryxell basin streams. Canada Stream carries a high, relatively constant discharge and while it is not the warmest stream, it has a small range in temperature. Research has shown that Canada Stream has a relatively low species evenness due to the dominance of *Psammothidium papilio*.

The source for both Green Creek and Bowles Creek is a meltwater pond at the base of Canada Glacier that carries high thermal inertia. Both creeks have a long growing season, but Green Creek has a wider channel and carries more discharge than Bowles Creek. Green and Bowles have similar species diversity and evenness values.

Delta Stream is the longest stream and drains Howard Glacier in the Kukri Hills. Considered by Cozzetto et al. (2006) as one of the warmest streams during

mid-summer, along with Von Guerard Stream and Green Creek, Delta Stream has a growing season comparable to Canada, Green and Bowles Creek. The discharge in Delta Stream is lower than that found in Canada or Green. The diatoms found in Delta stream are not significantly different from those in Von Guerard or Bowles and Delta Stream illustrates high species evenness. Species within the genus *Hantzschia* are very abundant in the lower flow Bowles, Delta, and Von Guerard streams.

Von Guerard Stream, similar to Delta Stream, has high interannual variability in flow. Von Guerard is the second longest stream and drains an alpine glacier in the Kukri Hills. It has the steepest gradient of all dry valley streams (Conovitz et al. 1998). Although it has a shorter growing season, Von Guerard boasts the highest diatom diversity and evenness values of the streams. The diatom community response after flooding shows an increase in *Hantzschia abundans* and *Hantzschia amphioxys* (Stanish et al. 2012).

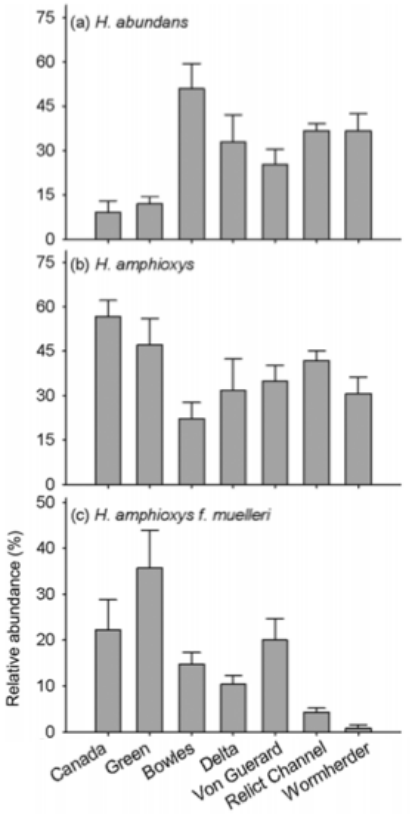
The Relict Channel and Wormherder Creek, Lake Bonney Basin, are both highly intermittent, harsh streams. (Stanish et al. 2011).

4.5 Individual Taxon Distribution Summaries

4.5.1 *Hantzschia amphioxys f. muelleri*

Hantzschia amphioxys f. muelleri is primarily found in Bowles Creek and Von Guerard Stream, but only at relative overall abundances between 5 – 10 percent (**Error! Reference source not found.**). *Hantzschia abundans* is found primarily in Bowles Creek (17%) and *Hantzschia amphioxys* is found within Bowles, Von Guerard, and the Relict Channel. While it is helpful to look at the overall abundances of these *Hantzschia* species, another visual tool involves comparing relative abundances scaled to within a genus as seen in Figure 28.

Figure 28: Relative abundance by stream within the genus Hantzschia. Adapted from Stanish et. al (2012). Error bars show standard error.



When scaled by genus, an entirely different distribution and abundance is observed. It is clear that *Hantzschia abundans* favors most streams with the exception of the high, constant flow streams of Canada and Green. *Hantzschia amphioxys* illustrates a very broad distribution with low abundances seen only in Bowles Creek. *Hantzschia amphioxys f. muelleri* shows a trend opposite that of *Hantzschia abundans* with preference of all streams except the Relict Channel and Wormherder Creek. Although *Hantzschia amphioxys f. muelleri* is classified as a South Victorialand endemic, it illustrates stimulated growth at higher temperatures. This growth rate information may help explain its habitat preference and higher abundance in the warmer waters of Von Guerard Stream and Green Creek. With a faster growth rate overall, it is possible that *Hantzschia amphioxys f. muelleri* has a competitive advantage in these warmer waters and is able to outcompete other taxa within the genus. Furthermore, *Hantzschia amphioxys f. muelleri*, while showing a lower growth rate at 7.6°C, is still growing more rapidly than *Hantzschia abundans* and *Hantzschia amphioxys* and this may indicate an increased tolerance to low temperatures. This tolerance may allow *Hantzschia amphioxys f. muelleri* to maintain its competitive advantage during the diel temperature variation. Furthermore, the fast growing nature of *Hantzschia amphioxys f. muelleri* may allow it to quickly populate streams like Von Guerard where growing seasons are short and flow is intermittent.

4.5.2 *Psammothidium papilio*

Psammothidium papilio did not illustrate a particular temperature preference in the growth rate experiments. It is most abundant, and dominant, in Canada Stream. Though Canada Stream may exhibit a smaller diel temperature range, *P. papilio* may be able to maintain a somewhat consistent growth rate throughout the austral summer. Furthermore, if temperature does not significantly impact the growth rate of *P. papilio*, this taxon may be more efficient in the production of organic energy products during photosynthesis. According to Passy (2008), smaller diatoms, like *P. papilio*, can maintain larger populations due to their ability for efficient resource uptake. I hypothesize that due to *P. papilio*'s indifference to temperature for growth, along with their small size, this taxa is able to successfully proliferate in the low nutrient conditions of Canada Stream (Stanish, Nemergut, and McKnight 2011).

4.5.3 *Hantzschia abundans* and *Hantzschia amphioxys*

Both widespread, this study suggests that *Hantzschia abundans* and *Hantzschia amphioxys* illustrate cold adaptations and grow faster lower temperatures. *Hantzschia amphioxys* shows the highest mean abundance across all streams with a preference for Canada Stream and Green Creek. Although a lower growth rate is exhibited by *Hantzschia amphioxys* as compared to *Hantzschia amphioxys f. muelleri*, the preference for growth at lower temperature may give *Hantzschia amphioxys* the competitive advantage during the diel cycle when the

temperature drops and during the initial periods of stream flow. *Hantzschia abundans* exhibits high relative abundances for all streams except Canada Stream and Green Creek. Though the growth rates for *Hantzschia abundans* and *Hantzschia amphioxys* are similar, *Hantzschia abundans* has a doubling time that is 3 – 5 days faster than *Hantzschia amphioxys*. This may allow *Hantzschia abundans* to occupy and proliferate a mat community faster than *Hantzschia amphioxys*. As neither taxa show preference via growth rates for the higher temperature tested, I hypothesize that this may explain why *Hantzschia abundans* illustrates low abundance values for Canada Stream and Greek Creek. *Hantzschia abundans* shows an apparent ecological preference for cooler temperatures in the Dry Valleys and due to its higher growth rate it may more quickly colonize colder water streams.

4.5.4 *Luticola muticopsis f. capitata and Stauroneis latistauros*

Although intrinsic growth rates were not obtained for *Stauroneis latistauros* or *Luticola muticopsis f. capitata* due to their high production of mucopolysaccharides, this is a significant observation of taxon behavior in culture. Stanish et. al 2012 report that the genus *Luticola* is most abundant in highly variable, low flow streams. *Luticola* is the most diverse genus found in the Dry Valleys, but *L. muticopsis f. capitata* seems to follow this trend and illustrates highest overall abundance in the Relict Channel (**Error! Reference source not found.**). When caling the relative abundances to be based only on the genus, *L. muticopsis f.*

capitata shows abundance in Delta Stream, Von Guerard Stream and the Relict Channel (Figure 29). Research has shown that stress can cause increases in EPS production (Abdullahi, Underwood, and Gretz 2006). Hoagland et al. (1993) mention the avoidance of desiccation via EPS production, but also point out the many other benefits of mucilage that can include cell attachment and sediment biostabilization among others. These streams are highly intermittent and I hypothesize that the mucilage production by *L. muticopsis f. capitata*, an Antarctic endemic, is an adaptation that has evolved as a protection from desiccation in these harsh streams. It should also be noted that the diatoms exist in a microbial mat that contains mucilage from the cyanobacterial matrix and are not solely dependent on their own mucilage production. This may also be the case for *S. latistauros,* a taxon that is also found in the Relict Channel and Von Guerard Stream.

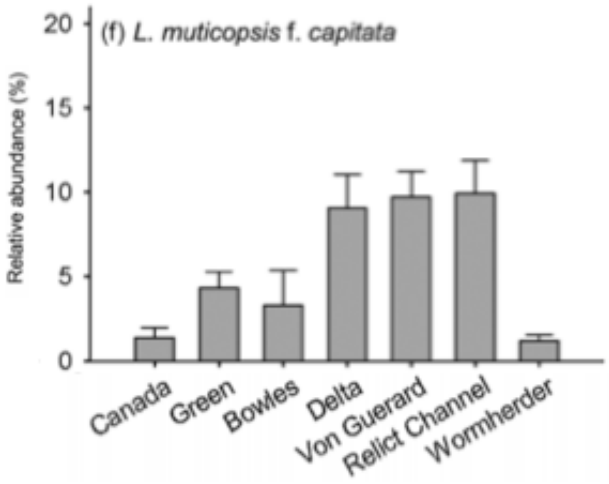


Figure 29: Luticola muticopsis f. capitata genus scaled abundance. Adapted from Stanish et. al (2012)

4.6 Summary

Hantzschia amphioxys f. muelleri, had the highest overall intrinsic growth rate at both temperatures and grew fastest at 10°C. The intrinsic growth rate of *Psammothidium papilio* did not change with temperature. *Hantzschia abundans* and *Hantzschia amphioxys* both exhibited stimulated higher growth rates at 7.6°C as compared to 10°C. A relationship was explored between biovolume and growth rate at both temperatures, but no clear trend was found (Appendix A5). These results present the idea that inter-genus growth rate relationships may provide a better understanding of taxa habitat preferences and variation in abundance within MDV streams.

4.7 Future Work

Much more work can be done to further characterize the autecology of the Antarctic freshwater diatoms that are in culture at INSTAAR. Although the hydrologic controls on algal communities have been documented, much work remains to understand the species level adaptations and responses to environmental conditions. More temperatures should be tested to identify the optimal growth temperature for each taxon. I would suggest growth rate experiments be conducted at 5°C as well as 15°C. There is an opportunity to test the photochemical efficiency of the diatoms in culture. This test would serve to better characterize the effects of temperature at the taxa level similar to Butler and Kitajima (1975).

Additionally, analyzing morphological changes in response to temperature, nutrient variation, freezing or desiccation stress would help understand the physiological adaptations at the species level. To be able to utilize diatoms to their full potential as bioindicators morphological variation should be examined similar to Trobajo, Cox, and Quintana (2004).

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Appendix

A1 Cell Viability After Desiccation and Freezing Events

Dry valley ecosystems are dominated by the presence and absence of water. In the autumn, and even at times during the spring and austral summer, there are freeze-thaw and desiccation-rehydration cycles (Davey and Clarke 1992). During the winter periods, temperature reaches far below freezing and algal mats are completely desiccated (Davey and Clarke 1992). Antarctic algae undergo winter freezing and this event may represent a control or reduction of annual taxa (Hawes 1990). Cell viability for 3 of the 4 taxa that had calculated growth rates was tested after being subjected to 4-hour treatments. The taxa experienced a 4-hour freezing event to -20°C and a 4-hour desiccation event. Additional freezing and drying experiments are being conducted in the summer of 2013 within the REU program at the University of Colorado Boulder.

A1.1 Methods

One hundred cells per taxa were isolated and placed in 24-well plates with a similar setup to Souffreau et al. (2010). The taxa were tested in triplicate and held for 24hours in the dark within the environmental growth chamber to avoid photo-oxidative stress. The entire experiment was conducted in the dark. This replicates conditions similar to what might be experienced in the polar regions during freezing or desiccation events. The freezing was manually conducted in a programmable freezer and reduced the temperature at a rate of 0.2 to 0.5°C per minute and then held at -20°C for 4 hours. The desiccation experiment was also conducted in the

dark. 2 of the 2.5mL of DY-V medium in each well were physically removed and the remaining medium was allowed to naturally evaporate. The wells were then left dry for 4 hours.

Cell viability was determined based on live dead counts using neutral red dye to aid in live/dead determination. All cells in the well were included in the count.

A1.2 Results

The average cell viability is shown in the figure below. The endemic taxon, *Hantzschia amphioxys f. muelleri* exhibited the greatest cell viability after both events. *Psammothidium papilio* exhibited lower, but consistent response to both treatments. *Hantzschia abundans* showed increased viability after desiccation as compared to freezing. More replicates and longer periods of time are needed to determine if these results are consistent.

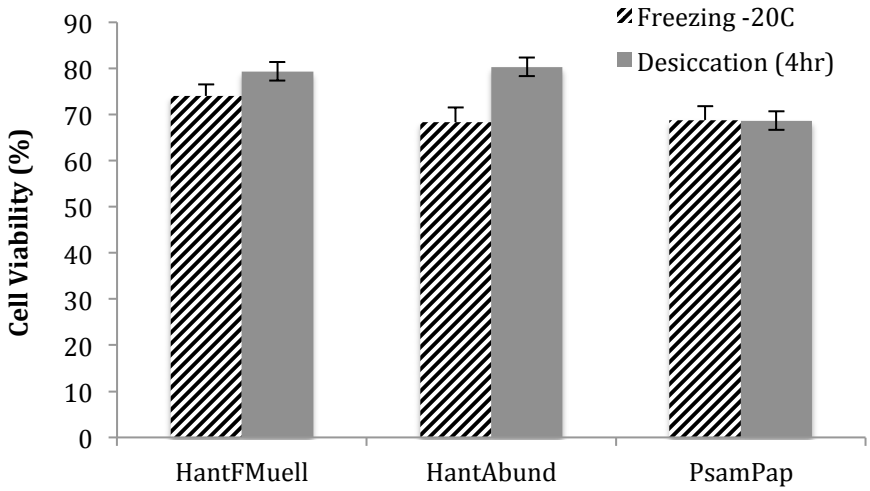

Figure 30: Average cell viability after freezing and desiccation events. Error bars show standard deviation.

Table 15: p-values across taxa for cell viability after freezing

p-values, Freezing	HantfMuell	HantAbund	PsamPap
HantfMuell		**0.0223**	**0.0084**
HantAbund			0.821

Table 16: p-values across taxa for cell viability after desiccation

p-values, Desiccation	HantfMuell	HantAbund	PsamPap
HantfMuell		0.6932	**0.0085**
HantAbund			**0.0132**

A2 Biotek Plate Reader Method: Growth Rates of Mucilage Producing Taxa

A2.1 Methods

This method was created to monitor *in vivo* algal fluorescence for the taxa that proved difficult to homogenize in suspension, *Stauroneis latistauros* and *Luticola muticopsis f. capitata*. This method was inspired by an application note written by P. Held of BioTek Instruments. Held (2011) conducted chlorophyll-based fluorescence of Chlorella vulgaris and Microcystis aeruginosa using a BioTek plate reader.

Because our cultures are benthic diatoms, instead of culturing them in an agitated beaker and removing aliquots to sample, we wanted to try and grow them in 96 well plates. An experiment was conducted to analyze the feasibility of growing the cultures in the plates for use in our Biotek Synergy HT plate reader with Gen5 v 2.0 software. The Gen5 software has an amazing capability to generate a "heat map" of each well showing the distribution of cells.

Isolating the same number of cells as in the Turner Designs tubes, each well began with 20 cells and 200uL of DY-V medium. The Synergy HT plate reader and Gen5 software limit well plate sizes to 96 or higher. Although it had been previously shown that our taxa in culture can grow in 24 well plates, this option was not available for this experiment.

A2.2 Results

These experiments were conducted 4 times with 2 dependable growth rate taxa, *Hantzschia amphioxys f. muelleri* and *Psammothidium papilio*. In each

experiment, the fluorescence values and visually inspected numbers of live cells declined over time. The 96 well plates simply did not offer enough well volume for an ample amount of medium to support healthy culture growth. The first experiment was conducted with the plate lid in place and we thought that perhaps this may have limited air exchange. The follow-up experiment was conducted with a clear, plastic Tupperware inverted to surround the plate and provide 3 inches of headspace above each well. That experiment also showed signs of declining fluorescence and live cells. The third experiment was conducted with routine DY-V medium aspiration and exchange to provide fresh nutrients to the cultures. It was believed that the cultures were depleting the nutrients too fast to support growth. This experiment too, showed signs of declining growth. The fourth experiment was conducted with all changes in place, and included suspending cells in the wells for a more consistent coverage throughout the well. This too, lead to an unsatisfying end. Additional problems with growing the cultures in the wells include a build up of salts due to evaporation and medium replacement or vitamin and/or iron deficiency due to chlorophyll a production.

Although the cultures are benthic diatoms, a 5th experiment was started to test the feasibility of growing a taxa in a well-stirred beaker and removing homogenous aliquots bi-weekly and monitoring growth via the plate reader. This experiment is on-going and shows promising results for benthic organism growth rate analysis using a high throughput method. The implications of forcing the cultures to stay in suspension by stirring are still under consideration. While this

method may work for *in vivo* fluorescence, it may not provide an accurate representation of the benthic growth rates.

A3 Diatom Culture Protocols

A3.1 Methods

*Only take caps off of culture tubes if underneath the prepared hood. Never expose tubes to ambient air or they will get contaminated.

PCR Hood Preparation:

1. Use ethanol to cleanse hands
2. Turn on fan in pressure hood and wait 10secs for it to push air through
3. Fill beaker on hot plate 1/3 full with DI water and turn dial to 550 (want to heat/bubble water, but don't run it at a boil)
4. Remove dirty slides if needed and take to wash at sink
5. Close doors and switch "UV" lamp on (will run for about 10-15mins)
6. When UV turns off, if water is bubbling, turn regular lamp on and begin
7. NEVER put food/phones/personal items in the hood-this is a sterile environment
8. After you have finished using the hood, make sure microscope is turned off, hot plate is turned off, all culture tubes are back in the incubator, doors are closed, then turn off fan and turn ON UV lamp.
9. Make sure any slides or pipettes you just used are getting directly contacted by the UV light (don't want them shaded by microscope tray etc). The UV lamp will turn off by itself after 10-15mins.

Incubator Preparation:

1. Use ethanol to cleanse hands
2. Check temperature of incubator on outside of unit. If it isn't right, let Deena know
3. Check top incubator tray for water spillage and discard any water in the dish down the sink
4. If spill over has occurred: Dump dish and use sponges (near sink) to sop up water
5. Check amount of DY-V (medium) in tubes and replenish if needed
6. Avoid keeping door open for long periods of time

Data Preparation:

1. Turn on computer
2. Open "Diatoms" file on desktop
3. Open "Culture Tubes Species List" and "Grand Passage Worksheet" excel files.
4. "Culture Tubes Species List": A listing of tubes CURRENTLY in the incubator, by species.
5. "Grand Passage Worksheet": A complete list of all previous and current tubes. Strikethrough font indicates the species tube has been discarded/died/had DNA extracted/etc.- and is no longer in the incubator. The tabs at the bottom

of the worksheet divide the pages up by "passage" number and the tubes are organized by date after that.

6. When updating spreadsheets with new observations/tubes/etc.- make sure to UPDATE both appropriately: For example:
    a. You just looked at Hant Muell species 018, passage (**P2-5**) and it looks healthy. You need to find this tube on the current "Culture Tubes Species List" and write your observations in the next empty cell for that tube's row. Save. Then open "Grand Passage Worksheet" and click on the tab for "second passage" at the bottom of the screen. Look up the tube by date and write your observations in the next empty cell for that tube's row. Save.
    b. You just looked at Hant Muell species 018, passage (**P2-1**) and it looks unhealthy, you are going to discard it. You need to find this tube on the "Grand Passage Worksheet". Click on the tab for "second passage" at the bottom of the screen. Look up the tube by date and write your observations in the next empty cell for that tube's row. Indicate that you are now discarding that tube. Highlight the entire row for that tube and change the font to ~~strikethrough~~ to indicate that tube is no longer in the incubator and why. Save. Then find this tube on the current "Culture Tubes Species List" and highlight the row and delete it, because it is no longer a tube that is currently in the incubator. Save.

Recording Culture Data/Observations:

1. Every time a culture is observed/passaged/discarded/isolated it needs to be recorded as described above.
2. Each entry in the excel sheets should include:
    a. *Date Observed* (not date observation is entered on spreadsheet)
    b. What culture looks like with *naked eye* in the tube before disturbing it
    c. What culture looks like *under the scope*
        i. Healthy/Unhealthy? Live/Dead cells?
        ii. Length of cells (live)
        iii. Bacteria or Debri present?
    d. *Recommendations*? Should this tube be passaged? Discarded? Cleaned?

Culture Maintenance/Observation-Microscope

1. Follow protocols for Hood/Incubator/Data preparation-Record observations AS YOU GO.
2. Turn scope on using switch on left side, turn knob to adjust light intensity (the lower the light the better, for your eyes and the organisms)
3. If using depression slides, make sure it is right side up on the scope tray
4. Using the appropriate pipette for the species you are going to look at, immerse the tip in the hot water beaker and suck up hot water 2-3 times.

Dispel water back into beaker and then let the tip cool (~20secs) before putting it in a culture (will burn diatoms if you don't wait)

5. Once tip is cool, place it in the culture tube and eject air onto the cells concentrated at the bottom of the tube. Take pipette out of tube medium and then again push air in tube to suspend cells.
6. Once cells are suspended, remove just enough medium/cells from the tube to observe on the slide and place in a depression
7. Let cells settle on slide (~1min) before trying to focus
8. Put culture tube back in incubator (to prevent heating up)
9. Adjust magnification to 20x by rotating them below the slide tray (it is much easier to focus and find cells initially on 20x)
10. To focus, bring the field of view over the edge of the depression
11. Using the fine focus (smaller knob), define the edge of the depression into a crisp line
12. As you move the field of view across the depression, you will need to adjust the fine focus because the slide "dips" in the middle. Remember to adjust down as you go to the center and up and you go towards the edges of the depression
13. If you need more magnification, once cells are found, rotate tray to 40x and slightly adjust fine focus
14. If looking at flat-bottomed slides or trays, once the cells are in focus, there shouldn't be much adjustment needed

15. SWITCH PIPETTES WHEN SWITCHING SPECIES! Otherwise cross contamination may happen.

Dirty Dishes/Slides/Tubes

1. If there are dishes/slides/tubes to be washed (don't watch pipettes), remove them from the hood and take them to the sink basin
2. Rinse 3x with ethanol into dish on counter before putting into soapy wash basin
3. Discard ethanol/culture waste into the Amber glass container on the counter labeled "Diatom Waste" (don't want to release these Antarctic organisms to the Boulder environment)
4. Let dishes soak (~5-10mins) in soapy basin
5. Using toothbrush/tools on the counter thoroughly wash
6. Rinse 3 times with tap water
7. Rinse 3 times with DI water
8. Let air dry on counter
9. Autoclave glass utensils once you have enough to warrant the use of the autoclave. Ask for help when autoclaving. Generally run ~20mins.

Adding DY-V to Culture Tubes/Dishes

1. Make sure dishes in incubator are always ½ full of DY-V. Make sure culture tubes don't dry out-Ask Deena how much should be in them

2. When adding DY-V make sure you are doing so under the prepared hood
3. Use the pipettes in the right-hand drawer underneath the hood and the DY-V in the hood
4. Never touch the tip of the pipettes to the tubes or dishes - it can cause contamination
5. Minimize the amount of time the pipette is exposed to the air and the amount of time the lid is off of the DY-V beaker.

A4 DY-V Medium Recipe

A4.1 Methods

Medium Recipe and Instructions from ncma.bigelow.org

This artificial freshwater medium was developed for chromophytes, especially chrysophytes and synurophytes. It is derived from DY-III medium developed by Lehman (1976). The medium was first modified as DY-IV by adding more trace metals (Keller and Andersen, in Andersen et al. 1997) and further modified by increasing the nitrogen and phosphorus concentrations. Its primary disadvantage is that fungi grow very well in the medium, probably due to the combination of glycerophosphate and ammonium, and therefore careful sterile technique is required. The MES buffer is pH adjusted to outside its buffering range, but removal of the MES hinders growth.

To prepare, begin with 950 mL of dH_20, add the following components and bring the final volume to 1 liter using dH_2O. Adjust pH to 6.8 with NaOH. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
MES	---	200 mg	1.02×10^{-3} M
$MgSO_4 \cdot 7H_2O$	50 g/L dH_2O	1 mL	2.03×10^{-4} M
KCl	3 g/L dH_2O	1 mL	4.02×10^{-5} M
NH_4Cl	2.68 g/L dH_2O	1 mL	5.01×10^{-5} M
$NaNO_3$	20 g/L dH_2O	1 mL	2.35×10^{-4} M
Na_2 b-glycerophosphate (see table below for DY-Vm substitution)	2.16 g/L dH_2O	1 mL	1.00×10^{-5} M
H_3BO_3	0.8 g/L dH_2O	1 mL	1.29×10^{-5} M
$Na_2SiO_3 \cdot 9\ H_2O$	14 g/L dH_2O	1 mL	4.93×10^{-5} M
$CaCl_2 \cdot 2\ H_2O$	75 g/L dH_2O	1 mL	6.76×10^{-4} M
trace element solution	(see recipe)	1 mL	---
f/2 vitamin solution	(see recipe)	0.5 mL	---

A5 Biovolume and Growth Rate

The relationship between biovolume and growth rate was explored as inspired by previous findings related to diatom biovolume in (Stanish, Nemergut, and McKnight 2011). Using the shape guidance set forth in Hillebrand et al. (1999), biovolumes were calculated for *H. amphioxys, H. amphioxys f. muelleri, H. abundans,* and *P. papilio.* The following figures show the resultant relationship between biovolume and growth rate at both temperatures.

Table 17: Biovolume and growth rate for each taxon

Taxon	Intrinsic Growth Rate at 7.6°C	Intrinsic Growth Rate at 10°C	Biovolume (μm^3)
H. abundans	.088	.071	1179
H. amphioxys	.072	.05	748
H. amphioxys f. muelleri	.13	.18	462
P. papilio	.10	.10	116

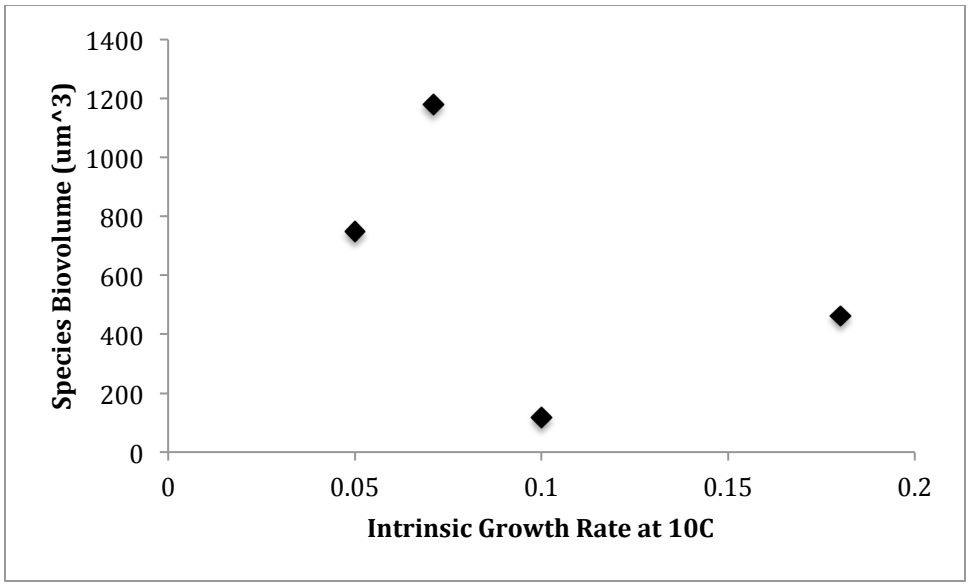

Figure 31: Intrinsic growth rate vs biovolume at 10C

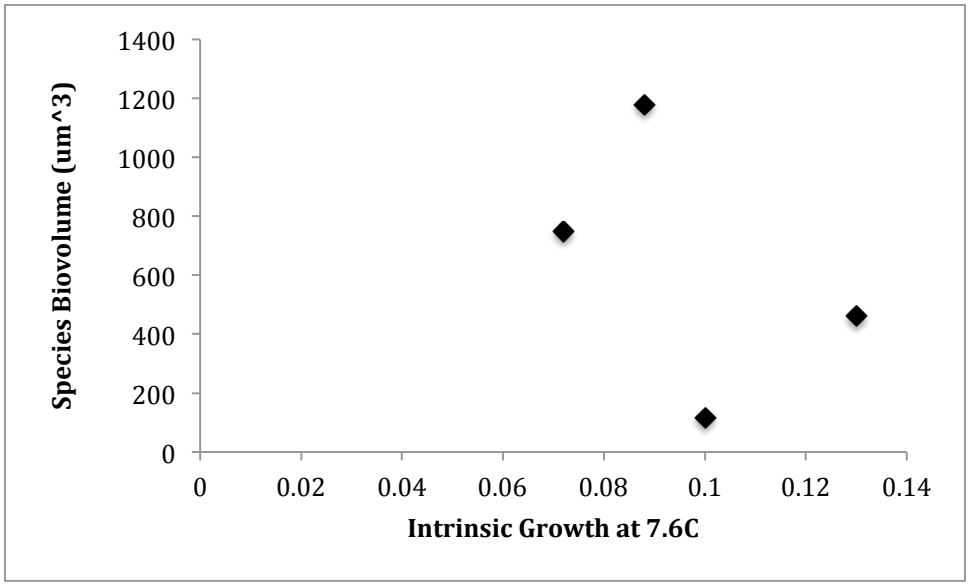

Figure 32: Intrinsic growth rate vs biovolume at 7.6C