TEMPERATURE-DEPENDENT GROWTH RATES AND RELATIVE ACTIVITY OF EXTRACELLULAR HYDROLYTIC ENZYMES IN A TROPICAL FUNGAL STRAIN OF *Fusarium equiseti* AND POLAR STRAINS OF *Pseudogymnoascus* spp.

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

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Field of Study: Marine Science

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TEMPERATURE-DEPENDENT GROWTH RATES AND RELATIVE ACTIVITY OF EXTRACELLULAR HYDROLYTIC ENZYMES IN A TROPICAL FUNGAL STRAIN OF *Fusarium equiseti* AND POLAR STRAINS OF *Pseudogymnoascus* spp.

ABSTRACT

The relative growth rates and relative activity (*RA*) of extracellular hydrolytic enzymes (EHEs) in a marine-derived tropical strain of *Fusarium equiseti* and polar (Arctic and Antarctica) strains of *Pseudogymnoascus* spp. under different solid-state nutrient assays would differ across culture temperatures between 5°C and 40°C. Relative growth rates and *RA* indices of protease, amylase and cellulase in these strains were screened in seawater solid-state nutrient assay plates augmented with skim milk, soluble starch, or carboxylmethylcellulose with trypan blue, respectively. Fungal colony and clear zone diameters were measured in the mid-log phase of growth to calculate relative growth rates and *RA* indices of these EHEs in selected strains. Relative growth rate values were fitted into third-degree polynomial and Brière-2 temperature-dependent models to estimate optimum temperatures for growth (T_{opt}) and maximum growth rates (R_{max}) of the selected strains under different nutrient assays. Estimates of growth rate values from the Brière-2 model were used to calculate the temperature coefficient (Q_{10}) and activation energy (E_a) for growth in all three fungal strains under different nutrient sources across the experimental culture temperature range. Similarly, specific growth rates in the three selected strains grown for 10 d under a liquid culture system comprised of seawater Mueller-Hinton Broth would differ across the same experimental culture temperature range. Values of T_{opt}, R_{max}, Q_{10}, and E_a were calculated using specific growth rate values obtained from the experiment. Changes in pH over time in the culture medium were also recorded. The findings indicated that *Fusarium equiseti* is better adapted to utilising

higher levels of thermal energy for growth than *Pseudogymnoascus* spp., consistent with general definitions that classify the former as a mesophile and the latter psychrophiles. EHE activity of protease, amylase, and cellulase in the three strains corresponded to the composition and abundance of particulate organic matter in the marine environment in tropical and polar latitudes, in which all three strains showed protease and amylase activities. Only *Pseudogymnoascus* spp. showed no cellulase activity during growth. This may suggest *Pseudogymnoascus* spp. might have achieved adaptation through loss of function under environments that typically have limited cellulosic materials. Increment of pH was observed during the growth (increased biomass) of *Fusarium equiseti* and *Pseudogymnoascus* spp. in the liquid culture system, which implies utilisation of substrates through EHE activity of mainly protease and amylase were facilitated by more alkaline conditions.

Keywords: filamentous fungi; thermal adaptation; extracellular hydrolytic enzymes; growth thermodynamics; temperature.

KADAR PERTUMBUHAN DAN AKTIVITI RELATIF ENZIM HIDROLITIK EKSTRASELULAR BERSANDARKAN SUHU DI DALAM KULAT PEROLEHAN MARIN STRAIN TROPIKA *Fusarium equiseti* DAN STRAIN KUTUB *Pseudogymnoascus spp.*

ABSTRAK

Kadar pertumbuhan relatif dan aktiviti relatif (*RA*) enzim hidrolitik ekstraselular (EHE) di dalam kulat strain tropika perolehan marin *Fusarium equiseti* dan strain kutub (Artik dan Antartika) *Pseudogymnoascus* spp. adalah berbeza pada julat suhu kultur di antara 5°C dan 40°C. Kadar pertumbuhan relatif dan aktiviti relatif protease, amilase, dan selulase di dalam strain-strain ini disaring di dalam esei bernutrien air laut berkeadaan pepejal yang dibekalkan susu skim, kanji boleh-larut atau karbosilmetilselulosa bersama tripan biru. Diameter koloni kulat dan zon jernih diukur pada pertengahan fasa log pertumbuhan untuk menghitung kadar pertumbuhan relatif dan indeks *RA* bagi EHE dari strain-strain yang dipilih. Nilai kadar pertumbuhan relatif disuaikan kepada model bersandarkan suhu polinomial darjah ketiga dan Brière-2 bagi menganggar suhu optimum pertumbuhan (T_{opt}) dan kadar pertumbuhan maksimum (R_{max}) strain-strain yang dipilih di dalam esei nutrien berbeza. Anggaran kadar pertumbuhan daripada model Brière-2 diguna untuk mengira pekali suhu (Q_{10}) dan tenaga pengaktifan (E_a) pertumbuhan ketiga-tiga strain di dalam setiap esei nutrien disepanjang julat suhu kultur eksperimen tersebut. Selepas itu, kami membuat hipotesis bahawa kadar pertumbuhan spesifik untuk ketiga-tiga strain kulat yang dipilih dan dikulturkan selama 10 hari di dalam kaldu Mueller-Hinton berair laut adalah berbeza bagi julat suhu kultur eksperimen yang sama. Nilai-nilai T_{opt}, R_{max}, Q_{10} dan E_a dihitung dengan menggunakan nilai-nilai kadar pertumbuhan spesifik yang diperolehi dari eksperimen tersebut. Perubahan aras pH juga dicatatkan. Hasil kajian kami menunjukkan bahawa *Fusarium equiseti* teradaptasi untuk

menggunakan tenaga termal yang lebih tinggi untuk pertumbuhan berbanding dengan *Pseudogymnoascus* spp., dan ini adalah konsisten dengan definisi umum yang mengelaskan *F. equiseti* sebagai mesofil manakala *Pseudogymnoascus* spp. adalah psikrofil. Aktiviti EHE protease, amilase dan selulase bagi ketiga-tiga strain kulat adalah sepadan dengan komposisi dan limpahan bahan organik zarahan di dalam ekosistem marin di latitud-latitud tropika dan kutub, di mana kesemua strain menunjukkan aktiviti protease dan amilase. Hanya *Pseudogymnoascus* spp. yang tidak menunjukkan aktiviti selulase ketika berlakunya pertumbuhan. Ini mencadangkan bahawa *Pseudogymnoascus* spp. mungkin mencapai adaptasi melalui kehilangan fungsi di persekitaran yang secara kebiasaanya mempunai limpahan bahan-bahan selulos terhad. Peningkatan aras pH diperhatikan ketika pertumbuhan (peningkatan biojisim) *Fusarium equiseti* dan *Pseudogymnoascus* spp. di dalam sistem kultur cecair. Ini memberi implikasi bahawa keadaan yang beralkali akan membolehkan penggunaan substrat-substrat dalam aktiviti EHE terutamanya bagi protease dan amilase.

Kata kunci: kulat berfilamen; adaptasi termal; enzim hidrolitik ekstraselular; termodinamik pertumbuhan; suhu.

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Cheers to all broken filter funnels,

Natasha Tajuddin
October 20th, 2017

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LIST OF SYMBOLS AND ABBREVIATIONS

EC	:	Enzyme Commission
EHE	:	Extracellular hydrolytic enzyme(s)
DOM	:	Dissolved organic matter
POM	:	Particulate organic matter
CO_2	:	Carbon dioxide
O_2	:	Oxygen
HCO_3	:	Carbonic acid
HCO_3^-	:	Bicarbonate ion
K^+	:	Potassium ion
Na^+	:	Sodium ion
H^+	:	Hydrogen ion
OH^-	:	Hydroxyl ion
NH_4^+	:	Ammonium ion
NUV	:	Near-ultraviolet
UV-B	:	Ultraviolet-B
T_{opt}	:	Optimum temperature
T_{min}	:	Minimum thermal limit
T_{max}	:	Maximum thermal limit/upper lethal temperature
R_{max}	:	Maximum growth rate
R_r	:	Relative growth rate
R_s	:	Specific growth rate
E_a	:	Activation energy (for growth)

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Research background

Fungi are a heterotrophic eukaryotic group of organisms which typically grow through apical extensions of hyphae. Repeated branching of hyphae behind their tips gives rise to a network called mycelium. Some species, however, are dimorphic – alternating between yeast and hyphal phases under specific environmental triggers. Fungi typically exhibit haploidy in asexual life stages, but many budding yeasts are diploid in sexual life stages. Fungi produce spores of various shapes, sizes, and properties as they evolve and adapt to specific environments. Cell walls of fungal cells are generally composed of chitin and glucan. Storage compounds in fungal cells are most often sugar alcohols (e.g. mannitol and trehalose), and glycogen. These compounds are similar to those in arthropods, but are different from plants (Deacon, 2006).

The uniqueness of fungi is reflected in the fact that they have a status of a kingdom equivalent to Plantae and Animalia, hence representing one of the three major evolutionary branches of multicellular organisms. Fungi can be found across all of Earth's ecosystems, having significant ecological impacts as pathogens to economically-important crops, and animals including humans, and as decomposers of complex materials found in both natural and artificial environments. These significant ecological roles stem from their nutritional mode. As non-diazotrophic heterotophs, fungi do not photosynthesise to convert light energy into chemical energy for utilisation in their physiological processes. Instead, fungi secrete a suite of functional proteins called extracellular hydrolytic enzymes (EHEs) into their surroundings to acquire nutrient sources for growth and survival. These enzymes then 'capture' and 'digest' complex organic and inorganic matter for subsequent transportation across fungal cell walls and

absorption into their cells, before these simpler molecules are further utilised for growth in fungal cells (Feller, 2013).

Enzymes are biocatalysts that help accelerate chemical and biological reaction rates that otherwise would be relatively slow when driven by abiotic factors alone. Extracellular enzymes can either be proteins that are embedded on the phospholipid layers of cell membranes, or are directly secreted outside the cells (true extracellular enzymes) (Ramaley, 1979). Most extracellular enzymes of microbial origins are hydrolases (Figure 1.1). Thermal energy, conventionally measured in terms of temperature, is the primary energy form driving these reactions in biological systems (Davenport, 1997; Klok & Chown, 1998; Angilletta *et al.*, 2006; McCullough *et al.*, 2009). All organisms, including fungi, are adapted to function at various ranges of temperature. In natural environments, there is a complex interaction between a multitude of abiotic factors (temperature, pH, salinity, etc.) that ultimately influence adaptation and evolutionary processes in fungi. Singling out and studying thermal effects on fungal growth under experimental conditions could provide fundamental insights into their adaptation to specific environments.

Meta-analysis on fungal enzyme data obtained from *BRENDA* (**BR**aunschweig **EN**zyme **DA**tabase) shows that there are 696 unique enzymes that have been characterised in an economically-important fungi *Fusarium*, of which a majority of these enzymes are hydrolases. A majority of extracellular hydrolytic enzymes that have been characterised in *Fusarium* fungi are peptidases (91), esterases (65), and glycosylases (48). Aminases and amidases, acid anhydridases, ketonases, and halidases comprise the remaining 51 hydrolases in the EC 3 class (Figure 1.1).

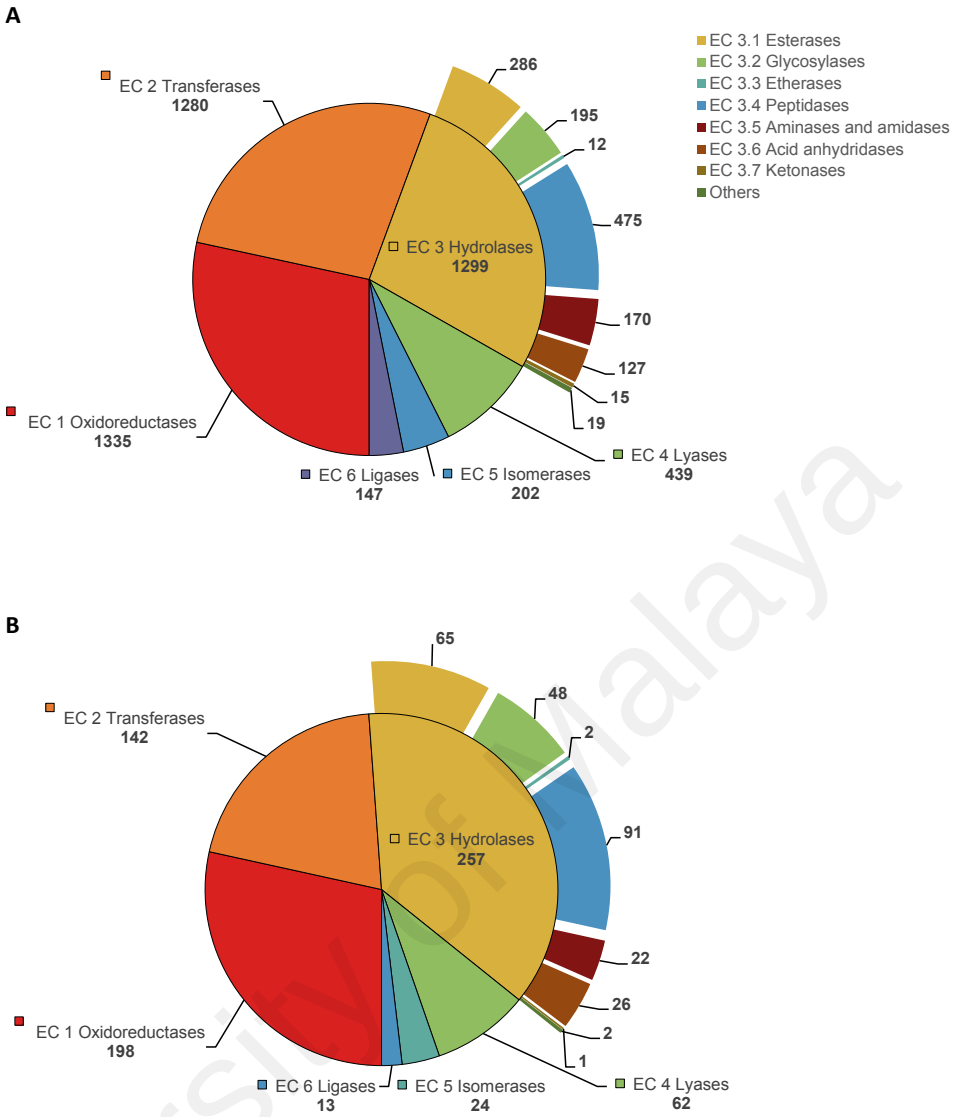

Figure 1.1: Classes of enzymes and subclasses of hydrolytic enzymes that have been characterised in (A) fungi (4702 unique enzymes in total) and (B) *Fusarium* fungi (696 unique enzymes in total). Data were screened on and obtained from *BRENDA* (**BR**aunschweig **EN**zyme **DA**tabase), with organism synonyms included. Enzyme Commission (EC) numbers are based on the official nomenclature developed by the International Union of Biochemistry and Molecular Biology (IUBMB). Characterised fungal hydrolases were isolated from various environments and substrates. Source: https://www.brenda-enzymes.org.

The marine environment is vast, with the ocean covering approximately 70% of the Earth's surface. Yet, our knowledge on how organisms survive and thrive in this dynamic environment is relatively scant. The majority of experimental research is medically- and industrially-driven, and involves the use of terrestrial/freshwater fungal strains. Additionally, these strains are often tested or studied under freshwater conditions (Arnosti

et al., 2014). Therefore, the physiology of marine-derived and true-marine fungal strains is not fully understood. Fungi respond differentially to environmental variability in a variety of ways, which (interestingly) could even be readily observed in their phenotypic features. With this background, this study set out to tackle questions on thermal adaptation in marine-derived fungi originating from different bioclimatic regions when grown under various nutrient sources and culture conditions.

1.2 Research questions

Following the premise above, our research questions for this study were:

a) How do marine-derived fungi from different biogeographical regions adapt to varying ambient temperatures when grown under seawater conditions?;
b) What is the preferred substrate for growth in marine-derived fungi under seawater conditions?; and
c) What is the growth performance of marine-derived fungi grown under solid- and liquid-state culture systems?

The hypothesis of the current study is marine-derived fungi from specific biogeographical regions would have been pre-adapted to the climatic conditions of their respective habitats, and would exhibit differences in growth rates and relative enzyme activities under different nutrient sources in response to temperature variation.

1.3 Research objectives

a) To profile growth rates and relative activity of selected extracellular hydrolytic enzymes (EHEs) across a range of experimental temperatures in selected

marine-derived fungal strains originating from different biogeographical regions;

b) To determine the relationship of fungal growth rates and enzyme activity with temperature; and

c) To determine energetic requirements for growth in these strains across the experimental temperature range under different culture conditions.

1.4 Significance of research

Studies on region-specific biological indicators from different latitudes can give an important contribution to understanding the impacts of climate change on ecosystems and their biological communities. The ubiquity of fungi across different ecosystems, including in coastal ecosystems (coastlines, mangroves, salt marshes, and coastal oceans), make them good candidates for studies elucidating the roles of the heterotrophic microbial ecosystem component in coastal ecological processes across latitudes. Also, enzyme activities are a direct measurement of microbial perceptions of their environment. The presence and activity of extracellular enzymes can link environmental nutrient availability to microbial nutrient demand.

1.5 Research flow

The diagram below illustrates the flow of the study:

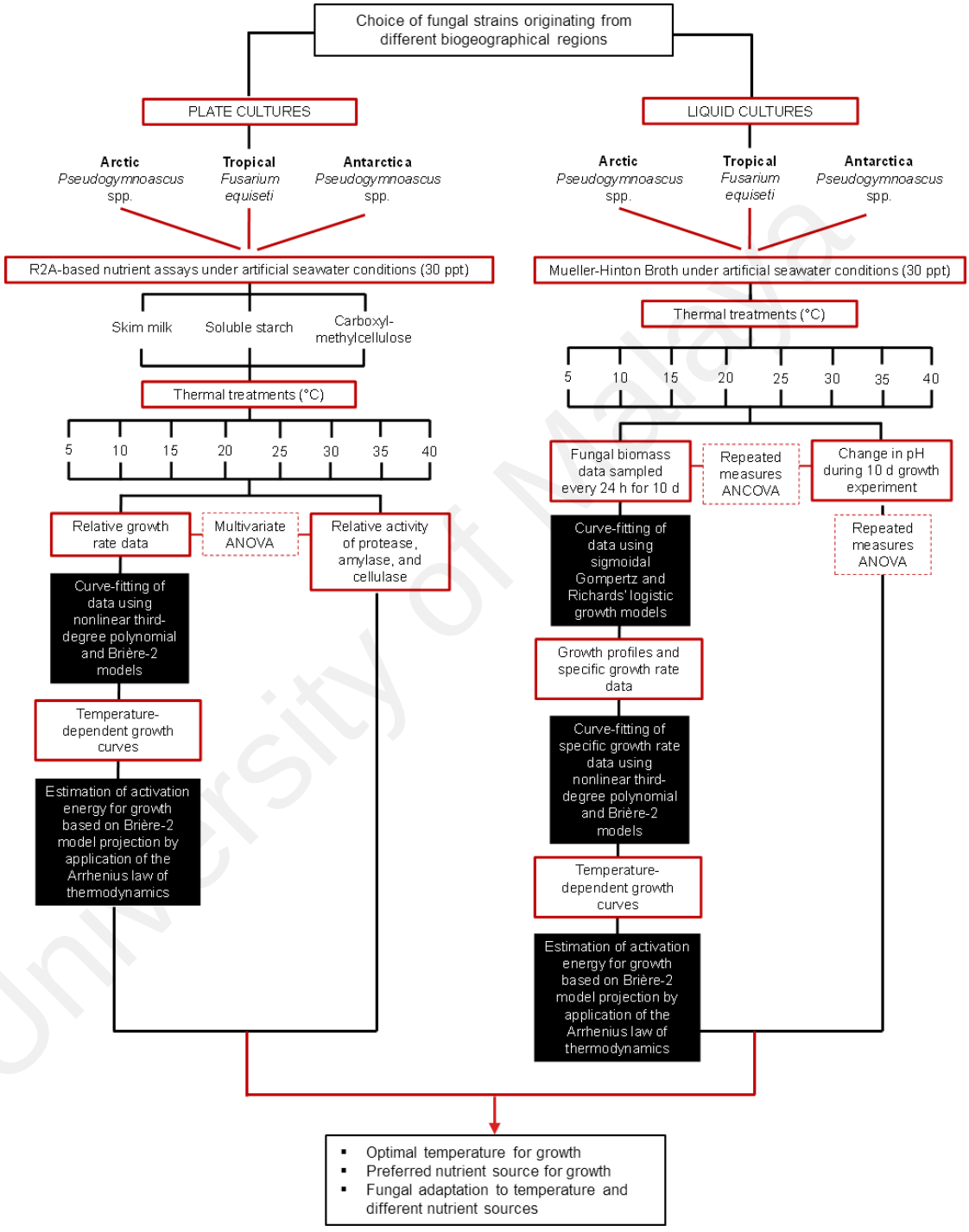

Figure 1.2: Workflow of the study.

CHAPTER 2: LITERATURE REVIEW

2.1 Fungi in the marine environment

Members of the fungal kingdom have been found in all environments, and are dispersed globally. Fungi are also most often isolated from terrestrial and freshwater substrates, as demonstrated in countless studies outlined in papers investigating their morphology, physiology, and pathogenicity in plant hosts, and mycosis in infected animal and human tissues. Fungal spores can be dispersed over continental distances through airborne drift. The ubiquity of fungi across different environments makes them a relevant biological analog in the attempt to elucidate the role of heterotrophic microbial components in biogeochemical processes including in coastal ecosystems. The spread of fungi in soil, although slower than through airborne drift, may be aided by flow of water, dust storms, spread of host plants and associated debris, and human movements. When there is an anthropogenic component factoring into the distribution of fungi, at least some species can move very rapidly across large distances (Summerell *et al.*, 2010). Prior distribution studies on fungi point to at least two major fungal associations – host- and climate-associated patterns. Fungal diversity also decreases with declining vegetation coverage from lower to higher latitudes (Tedersoo & Nara, 2010; Wardle & Lindahl, 2014).

Most of Kingdom Fungi is composed of true fungi (Eumycota). True fungi are assigned into five different phyla: Chytridiomycota, Zygomycota, Ascomycota, Basidiomycota, and the recently erected (2001) Glomeromycota (Schüβler *et al.*, 2001). Within the kingdom, the Ascomycota and the Basidiomycota share many common features, clearly pointing to a common ancestry. Around 75% of all the fungi that have been described to date are ascomycetes. All members of the phylum are characterised by the presence of septae between cells, and the ascus when in their sexual stage. In the more advanced members of the group, many asci are produced within a fruiting body, termed

an ascocarp. The sexual stage (teleomorphic) in fungi is absent, rare, or unknown. One fifth of all described fungal species found in both natural and artificial environments are mitosporic (also known as anamorphic) because fungi most often reproduce through asexual and/or parasexual (mitotic combination) mechanisms. Parasexuality is a reproductive mechanism which fungi employ to preserve genetic integrity while maintaining limited genetic diversity that promotes adaptation to less rapidly changing host and environmental niches. This clearly contributes to the natural and common occurrence of mitosporic fungi. However, fungi are highly successful group due to diverse reproductive strategies and are a speciose kingdom (Taylor, 1999; Pringle *et al.*, 2002; Kohn, 2005).

Fungi may experience fluctuations in environmental factors across and within their habitats. Temperature, salinity, nutrients and other abiotic parameters can vary dramatically both spatially and temporally in these habitats because of the interaction of various abiotic factors governed by regional climates. To survive these fluctuations, fungi adjust phenotypic features to accommodate their physiological processes to specific energetic needs. Fungi have been subjected to various treatments in a wide range of medically-driven research, usually testing hypotheses on the relationship between fungal pathogenicity and temperature in an increasingly warming world (Paterson and Lima, 2010; Fisher *et al.*, 2012). The influence of temperature on fungal adaptation to their ecological function in carbon cycling, however, has received relatively little research attention to date.

Most existing studies have examined growth responses of fungi to different temperature levels under terrestrial/freshwater conditions, not under marine/seawater conditions (Newsham *et al.*, 2016). Some fungi are known to alter their growth forms under different conditions through several mechanisms, including growing in pellets

when grown in liquid cultures, producing dormant cells called resting spores in unfavourable environmental conditions, and exhibiting dimorphism as seen in a model fungus *Histoplasma capsulatum* Darling which can grow as a yeast from mould (thus becoming pathogenic) at 37°C in human bodies, and *vice versa* in *Candida albicans* (C.P. Robin) Berkhout (reverse morphogenesis) (Lambowitz *et al.*, 1983; Maresca and Kobayashi, 1989; Ignatov and Keath, 2002; Casas López *et al.*, 2005; Nemecek *et al.*, 2006; Klein & Tebbets, 2007; Liu *et al.*, 2008). Evidently, fungi respond to environmental variability differentially, and much is left to be discovered in understanding how these members of a successful kingdom adapt to various environmental niches and change.

2.1.1 Defining 'marine-derived fungi'

Definitions of marine-derived fungi have been frequently revisited over time. Marine fungi are generally designated to two groups based on their association with marine habitats. Obligate marine fungi are fungal species that are exclusively found in the marine environment. Facultative marine fungi are species that are found in terrestrial environments, but are also able to sporulate and thrive in marine habitats (Kohlmeyer & Kohlmeyer, 1979). A large number of fungal species may be facultative marine fungi, particularly when they are established in the coastal regions, known as the 'border' between terrestrial and marine ecosystems. Overy *et al.* (2014) have described marine-derived fungi as fungal species that are established from substrates which are found anywhere in marine-impacted areas regardless of their habitats of origin. Moreover, Pang *et al.* (2016) state that fungal strains subjected to experimentation can be considered 'marine-derived' only if they are treated under seawater/saline conditions.

Sachs (1874) proposed the Floridean hypothesis – members of Ascomycota evolved from the parasitic red algal-like ancestors of the family Florideae (phylum Rhodophyta)

(Kohlmeyer, 1975). Phylogenetic analyses of the genes that code for the small subunit ribosomal DNA (SSU rDNA) among eukaryotes do not support the Floridean hypothesis, even though similarities in life histories, nutritional modes, and morphology of reproductive structures between the Ascomycota and Rhodophyta are present. Conversely, researchers turn to the extension of the Floridean hyphothesis which states that secondary (facultative) marine fungal species represent the reintroduction of fungi into the marine environment and share more recent common ancestry with terrestrial lineages as a conceptual basis in further investigating the evolutionary relationships between marine-derived and true marine fungi (Jones & Pang, 2012). Despite the many efforts directed into molecular phylogenetics of marine-derived and true marine fungi, the polarity of terrestrial-marine transitions within the Ascomycota is still unresolved due to contradicting results in phylogeny (Bhattacharya *et al.*, 1990; Cavalier-Smith *et al.*, 1994).

2.1.2 Biogeographical and ecological classifications of fungi

True marine fungi have been classified into three groups according to their biogeographical distribution: (a) tropical to subtropical, (b) temperate to polar, and (c) cosmopolitan (Jones, 2000). There is often a mixture of temperate and tropical fungi where intermediate sea temperatures occur between the tropics and subtropics. Marine fungal communities are more similar within a single ocean basin than between different basins although, in the tropics, mangrove-associated fungal species have wide longitudinal distributions (Jones, 1993, 2000; Jones & Pang, 2012). Fungi (inclusive of marine-derived and true marine fungi) are also assigned to various ecological groups based on the substrates they colonise and organisms they form symbiotic relationships with (Table 2.1).

Table 2.1: Ecological groups of fungi based on their ecological functions as either saprotrophs, mutualistic partner to various organisms, or pathogens.

Group	Colonised substrate/ symbiotic partner	References
Arenicolous	Soils, sand, and other types of sediments	Kirk Jr. (1983)
Endolithic	On surfaces and within interstitial spaces of rocks	Golubic *et al.* (2005)
Endophytic	Internal tissues of vascular plants	Petrini (1991)
Entomopathogenic	Preying upon insects	Zimmermann (1986)
Lichenicolous	Mutualism with algae in lichens	Hawksworth (1975)
Lignicolous	Driftwood	Booth & Kenkel (1986)
Manglicolous	Mangrove flora	Jones & Tan (1987)
Mycorrhizal	Associated with roots of vascular plants	van der Heijden *et al.* (1998)
Nematophagous	Preying upon nematodes	Jansson & Nordbring-Hertz (1979)
Phytopathogenic	Causing parasitism in plants	Kombrink & Hahlbrock (1986)
Pyrophilous	Growing on burnt or fired substrata	Seaver (1909)

2.1.3 Physico-chemical characteristics of the marine environment across latitudes

The marine environment is characterised by its constant exposure to seawater. There are generally three ecosystems included in considerations of the marine environment: (a) coastal/sublittoral (intertidal zones, mangroves, estuaries, salt marshes, seagrass meadows), (b) coral reef, and (c) deep-sea ecosystems. Seawater is slightly alkaline (approximately pH 8.1), and is a complex mixture of approximately 96.5% pure water, 3.5% salt, dissolved organic matter, dust particles, and minute amounts of organisms (Millero *et al.*, 1973). Seawater has a specific heat capacity of 3985 J kg^{-1} $°C^{-1}$ in comparison to Earth's crustal materials (830 J kg^{-1} $°C^{-1}$ for sand, 2512 J kg^{-1} $°C^{-1}$ for wet clay). Hence, terrestrial sediments (soils, etc.) only need smaller amounts of thermal energy to increase as much as 1°C in temperature compared to seawater. The implication of this is that oceans serve as Earth's thermal buffer. Introduction of disturbances to the planet's energy equilibrium could impact long-term regional and global climatic processes (http://climate.nasa.gov).

Coastal ecosystems are the most productive in the marine environment. Coastal habitats are exposed to a wide range of environmental variability that is a result of exposure to seawater, tidal activities, wind-induced circulations, riverine influxes, land-atmospheric interaction, and anthropogenic activities. A large percentage of organic matter (approximately 80%) that is present in the marine ecosystem is preserved in deltaic and coastal marine deposits. Sediments in coastal regions are also predominantly comprised of clastic materials (materials that are made up of smaller fragments of rocks) introduced from terrestrial environments by rivers (Hedges, 1992). In recent decades, anthropogenic activities have greatly influenced seaward fluxes of macronutrients, and the riverine flux of dissolved silicate into the coastal oceans, ultimately affecting phytoplankton communities (largely comprised of diatoms and dinoflagellates) and productivity in the coastal regions (Friedl & Wüest, 2002; Zhang, 2014). Hence, fungal communities benefit from this terrigenous deposition of organic matter and high productivity that are present in coastal environments.

2.1.4 Organic matter as a nutrient source in the marine environment

Much of the organic matter in the marine environment is derived from various sources, both living and non-living. Phytoplankton is generally the most important source of organic matter, with a carbon production of 50 – 150 g per m^2 annually in major ocean basins. However, benthic algae and rooted aquatic vegetation of the intertidal zone are more productive than phytoplankton, and riverine fluxes can be important locally. Transformation of organic matter can occur rapidly but this cannot counter pre-existing and ongoing accumulation rates of dissolved and particulate organic matter (DOM and POM), leaving large amounts of organic matter preserved in the marine environment (Riley, 1970).

Productivity is not only a function of nutrients and availability of light energy, but also temperature. It is important to understand that temperature influences fluid dynamics of seawater, affecting the formation of thermoclines and consecutively dissolution and mixing of gases and organic matter throughout the water column. Dissolution of particulates decreases with increasing ambient temperature. Therefore, productivity across world's oceans does not follow the patterns seen on land (Behrenfeld *et al.*, 2006; Holmgren *et al.*, 2012). High levels of productivity can be seen in estuarine water, and upwelling areas nearing the equator. Productivity levels in polar oceans are comparatively lower all-year round. However, spikes of productivity surpassing those of low-latitude marine waters may be observed due to the presence of phytoplankton blooms during austral summers in the Arctic and boreal summers in the Antarctic regions, respectively (Fabiano *et al.*, 1993; Fabiano & Danovaro, 1998; Fabiano & Pusceddu, 1998).

Dissolved organic matter (DOM) includes compounds that contain reduced carbon often bound to heteroatoms such as oxygen, nitrogen, phosphorus, and sulphur. Marine DOM is largely produced through three mechanisms: (a) abiotic rearrangement, (b) modification by extracellular enzymes, and (c) release of chemically simple organic molecules by organisms. DOM may also be released into the environment when cells die through viral lysis, predation by bacteria and protozoa, and senescence. Overall DOM status of an environment is influenced by the relative contribution of primary production and mortality-related processes. Productive regions have significant DOM production directly from photosynthesis by phytoplankton and photosynthetic bacteria, whereas oligotrophic regions have significant DOM production from grazing processes by protozoa (Kujawinski, 2011).

Just as primary productivity affects DOM levels, the predominance of compounds derived from phytoplankton, and to some extent zooplankton, influences the composition

of particulate organic matter (POM) distributed in the marine environment. Large amounts of POM generally sink to the sea floor from surface waters, undergoing several physico-chemical alterations during the descent. When POM reaches the sea floor, it may be preserved as sedimentary organic matter. Some may also remain suspended in the marine water column. Levels of POM generally decrease with depth in the marine water column. Particles exported from surface waters consist of, in decreasing concentrations, proteins, carbohydrates, and lipids. Phytoplankton dominate composition of POM in surface waters, but heterotrophic consumers play a vital role in selectively degrading these substances here, consequentially influencing further transformations of POM (Huston & Deming, 2002).

2.1.5 Marine environment in the face of climate change

Environmental parameters have been monitored over several decades through the technology of remote sensing to quantify environmental anomalies on the Earth's surface. Mean surface temperatures in the polar regions can be as low as -20°C, but increase to short-term maxima of 25°C or more, while shaded surface temperatures in the tropics narrowly range between 28 and 32°C (Nagelkerken, 2009; Convey, 2012; Peck, 2015). Vegetation coverage generally decreases from low to high latitudes, while productivity may vary due to seasonal shifts between subtropical and polar latitudes in the Southern and Northern Hemispheres (Figure 2.1).

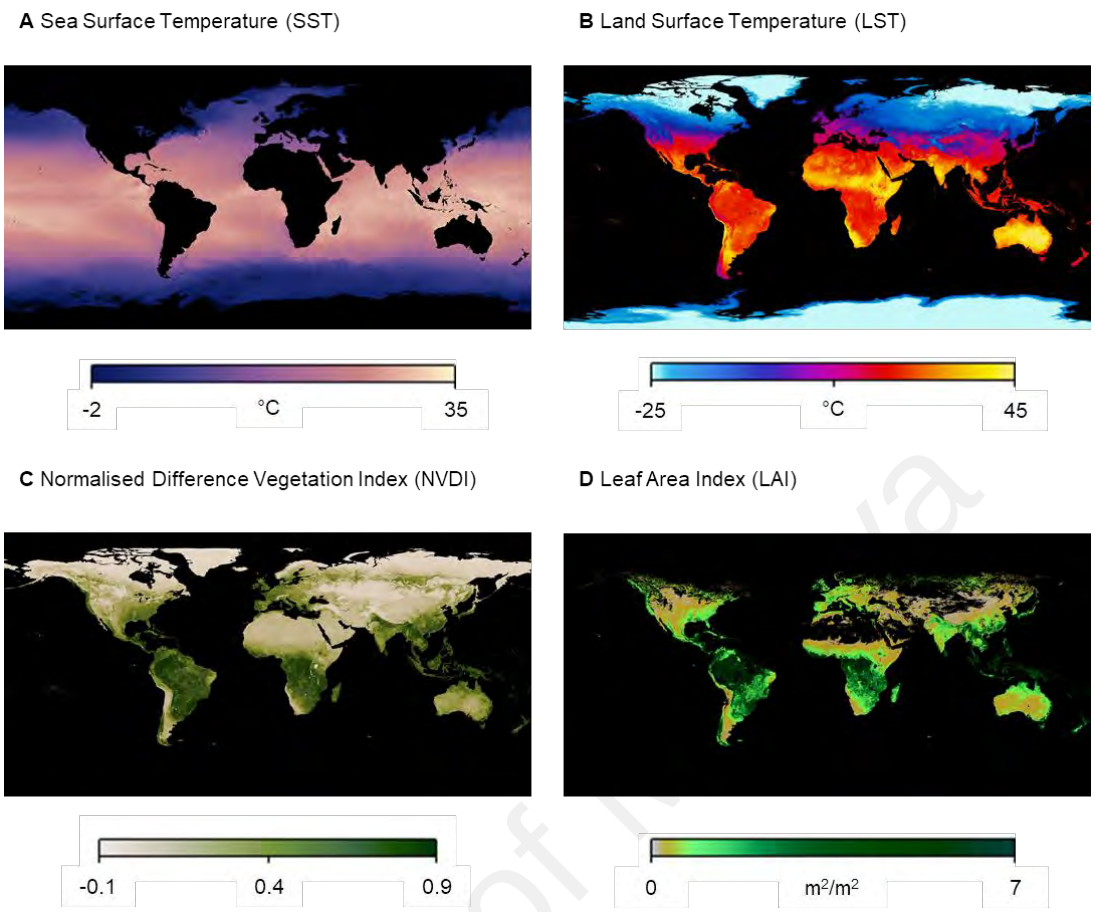

Figure 2.1: February 2017 datasets with indicators on (A) Sea Surface Temperature, (B) Land Surface Temperature, (C) Normalised Difference Vegetation Index, and (D) Leaf Area Index. Source: https://neo.sci.gsfc.nasa.gov.

The marine environment is prone to, and is already experiencing, observable symptoms of climate change. These symptoms are: (a) warming leading to increased surface temperatures, (b) increased diffusion of carbon dioxide into seawater leading to ocean acidification, (c) oxygen deprivation leading to hypoxia; and (d) nutrient enrichment from terrestrial sources leading to eutrophication (Figure 2.2). Combined effects of these could change physico-chemical characteristics of the ocean over the long term. Warming could alter stratification of the marine water column, affect seawater circulation regionally and globally, decrease solubility of gases into and in the seawater, affect metabolic rates in organisms, and increase sea levels due to melting of giant bodies of land-based ice in the Arctic and Antarctic (Pörtner, 2008; http://climate.nasa.gov).

Ocean acidification, on the other hand, could lead to inhibition of calcification in marine organisms (e.g. fishes, corals, clams). Hypoxia and eutrophication also present great threats to the marine environment– a decline in oxygen concentration and rapid enrichment of seas and/or oceans could alter food webs, encouraging proliferation of microalgae (algal blooms) with increasing seaward flux of nitrogenous nutrient sources, and promoting colonisation of fungi and other microbial groups on dying aerobic organisms on the increasingly oxygen-deprived seabed and/or ocean floor (Nagelkerken, 2009).

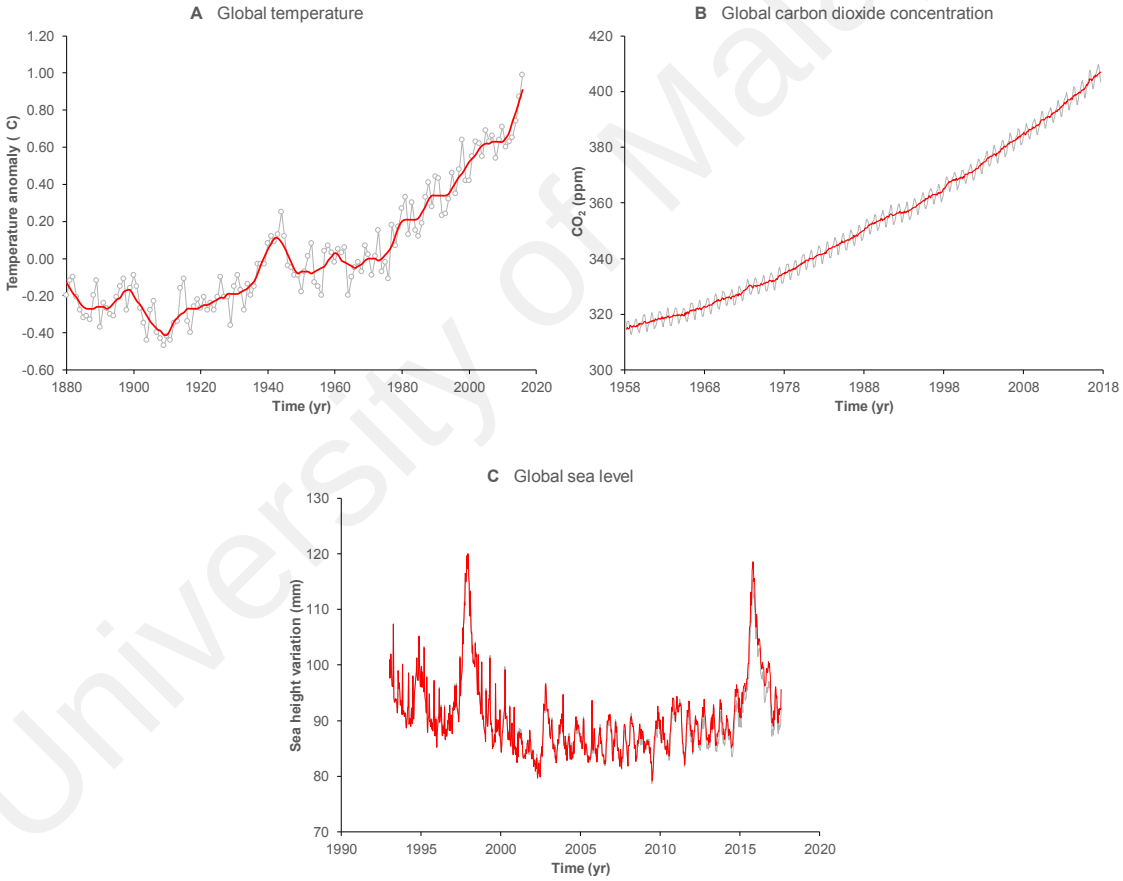

Figure 2.2: Vital signs of the Earth. (A) Global temperature anomaly from 1980 – 2016, (B) global carbon dioxide concentration from 1958 – 2017; and (C) global sea level variation from 1993 – 2017. Source: https://climate.nasa.gov.

2.2 Fungal growth in response to different environmental factors

Environmental factors such as temperature, nutrients, pH, water potential, light, and aeration affect fungal growth rates and other relevant biological processes, and act as triggers in developmental pathways.

2.2.1 Physiological responses of fungi in response to temperature, pH, water potential, aeration, and light

Temperature is the primary driver of physiological, ecological, and evolutionary processes in living systems, including in fungi. Reaction rates and properties of compounds fundamental to cellular functions are altered with changes in temperature. Lower thermal limits for microbial growth are set by the slowing down of chemical reaction rates, an increase in viscosity of cellular water at sub-zero temperatures (making cellular water less of an effective solvent to cellular solutes), and a decrease in water potential with increased accumulation of ions within and outside fungal cells. Upper thermal limits for microbial growth, on the other hand, are set by the first cellular component or process that breaks down. Due to greater complexity of biological processes, eukaryotic microorganisms generally have a lower upper thermal limit for growth than prokaryotes (Deacon, 2006). Fungi are grouped into three broad categories based on their thermal preferences for optimum growth: psychrophilic, mesosphilic, and thermophilic. An extension to this grouping convention includes other two groups: psychrotolerant and thermotolerant (Table 2.2).

Table 2.2: Thermal classification of fungi.

Category	Description	Reference
Psychrophilic	Having optimum growth at no more than 16°C and maximum growth of about 20°C.	Gounot (1986)
Mesophilic	Growing within the range of 10–40°C.	Kuehn & Gunderson (1962)
Thermophilic	Having a minimum growth temperature of 20°C or above, a maximum growth temperature of 50°C or above, and an optimum in the range of about 40–50°C.	Cooney & Emerson (1964)
Psychrotolerant	Having a minimum growth temperature of 5°C or below, maximum growth temperature of 20°C or above, and an optimum in the range of about 15–20°C.	Gounot (1986)
Thermotolerant	Having a minimum growth temperature below 20°C, a maximum growth temperature above 50°C, and an optimum growth temperature of less than 40°C.	Rosenberg (1975)

The pH is an important factor of chemical (therefore also biological) processes as it affects net charge on membrane proteins, degree of disassociation of mineral salts, and the balance between dissolved carbon dioxide (CO_2) and bicarbonate ions (HCO_3^-). Many fungi show a broad pH optima between 5.0 and 7.0. However, individual species may still vary within this range. Some fungi are acid-tolerant, such as yeasts colonising animal stomachs. Acidophilic fungi can grow down to pH 1 or 2, and are often found in acidic mine wastes. As an example, an acidophile *Acontium velatum* Morgan could initiate growth at pH 7, but it rapidly lowers the pH of culture media to about 3. Some fungal genera including *Cladosporium* Link, *Penicillium* Link, and *Fusarium* Link are alkali-tolerant, as they have been isolated from soda lakes and alkaline springs. Alkalophilic *Chrysosporium* Corda species that grow up to pH 11 have been isolated from bird nests; these are specialised degraders of keratin (Deacon, 2006).

Fungi that grow at extremes of pH are found to have an internal cytosolic pH of about 7. It has been suggested that the fungal cytosol has a strong buffering capacity, as

cytosolic pH changes, at most, about 0.2 to 0.3 units when the external pH is changed by several units. Fungal cells achieve homeostasis and tightly regulate cytosolic pH in two ways: (a) counter-pumping hydrogen ions (H^+) in acidic environments through the exchange of materials between the cytosol and acidic contents of vacuoles; and (b) interconversion of sugars and poly-alcohols (polyols) which sequester or release H^+.

Fungi can alter the ambient pH, in which the availability of nitrogen is a key factor to this adjustment. If nitrogen is supplied in the form of ammonium (NH_4^+) ions, fungal cells release H^+ in favour of NH_4^+, lowering external pH. Conversely, the uptake of nitrate (NO_3^-) rises the external pH by about one unit. Fungi also release organic acids acheiving the same effect. pH has also been found to contribute to functional efficiency of extracellular enzymes. As examples, *Metarhizium anisopliae* (Metschn.) Sorokīn increases the pH of media by releasing NH_4^+ to allow function of proteases, while *Sclerotinia sclerotiorum* (Lib.) de Bary lowers pH by secreting oxalic acid to allow function of pectinases (St. Leger *et al.*, 1999; Yamanaka, 2003; Sunitha *et al.*, 2012; Ominyi *et al.*, 2013).

All fungi need the physical presence of water for the transport of nutrients through the cell wall and cellular membrane, the release of extracellular enzymes, and relevant metabolic reactions. However, water can still be available in the environment yet bound by external forces, making it inaccessible to fungi. The sum of all forces that act on water and restrict its availability to cells is termed as water potential. Osmotic stress increases with decreasing water potential. Ultra-pure water has a potential of 0 MPa (MegaPascal), seawater of an average salinity of 29 psu has a potential of -2.8 MPa. The negative unit indicates that the environment is exerting a 'pull' on water. Fungi (except for water moulds) are highly adept at obtaining water and maintaining cell turgor, even under

significant water stress. Many fungi isolated from terrestrial environments grow readily media at -2 MPa, while some can still grow between -4 and -14 MPa (Deacon, 2006).

Fungi typically respond to low external water potential by generating an even lower internal osmotic potential. Marine fungi are known to accumulate potassium ions (K^+) in their cells over the more toxic sodium ions (Na^+), but high ionic levels may still damage the cells. Fungi and other salt-tolerant microbes counteract osmotic stress introduced by dissolved salt ions by producing osmolytes. A more common balancing method would be through the accumulation of compatible solutes consisting of sugars or sugar derivatives that do not interfere with core metabolic pathways. Glycerol is the most common compatible solute in filamentous fungi (Hocking, 1993).

Two other important factors influencing fungal growth are light and oxygen availability. Light in the near-ultraviolet (NUV) and visible parts of the spectrum, on the other hand, can stimulate pigmentation and more profoundly, fungal differentiation. NUV triggers the production of either asexual or sexual reproductive structures in many fungi. It has been found that UV-B radiation affects growth rates and conidia production in *Pseudogymnoascus* spp. Most fungi are strict (obligate) aerobes; they require oxygen in some stages of their life cycles. Some are facultative aerobes; they grow under aerobic conditions but can grow in the absence of oxygen by fermenting sugars. Very few are obligately fermentative, where their source of energy always comes from fermentation regardless of them growing with or without oxygen. Some gut microbes, especially those colonising the rumen, are obligate anaerobes; they are killed when exposed to oxygen. Likewise, fungi require carbon dioxide for carboxylation reactions that generate fatty acids, oxaloacetate, etc. However, oxygen and carbon dioxide behave differently in solution. Carbon dioxide dissolves in water to form carbonic acid (HCO_3), and this may affect fungal growth because pH of the medium is affected by this ionic disassociation.

In aerobic respiration, oxygen depletion rate is higher than carbon dioxide generation rate. Hence, fungi that grow in undisturbed water or below the surface of an agar plate may experience significant oxygen depletion. This is the reason why liquid culture media needs to be vigorously and continuously shaken and aerated (Deacon, 2006).

2.2.2 Mathematical models in microbial growth rate studies

> "Since all models are wrong the scientist cannot obtain a 'correct' one by excessive elaboration." Box (1976, p. 792).

The use of mathematical models to profile microbial growth in response to various abiotic parameters was a common practice in the field of predictive microbiology before its increasing application in ecological-based research. Growth and developmental responses of organisms to environmental factors are often varied and nonlinear (Scherm & van Bruggen, 1994). Therefore, models are applied to data to eliminate 'noise' resulting from variability in measures while extracting information that defines growth behaviour or responses of a microbial populace towards growth factors. Researchers develop mathematical models that incorporate critical growth factors such as temperature, pH and water activity (a_w) as equation parameters (singly or in combination) for many purposes.

In the field of food microbiology, the interest lies in manipulating the duration of the lag phase of a microorganism in a food system so that they can be extended or delayed indefinitely (Skinner *et al.*, 1994). In experimental microbial ecology, mathematical models are usually applied to determine specific growth rates (from the log phase), minimum and maximum thermal limits of a microorganism, and sometimes the interaction between parameters which are present in models (Scherm & van Bruggen, 1994). Predictive models generally estimate parameters such as duration of lag and log phases, generation time, specific growth rate, maximum growth rate, critical thermal

limit, and activation energy for growth (Lamb *et al.*, 1984; Baranyi & Roberts, 1995; Lactin *et al.*, 1995; Buchanan *et al.*, 1997; Brière *et al.*, 1999).

Predictive modelling is divided into four major categories: (a) simple probabilistic models, (b) regression models, (c) Arrhenius models, and (d) square root models (Skinner *et al.*, 1994). The category of models that is more useful depends on the research questions being addressed in any given study. According to Ratkowsky (1993), the best model for a dataset should be based on five selection principles:

i. Parsimony (models should contain as few parameters as possible)
ii. Parameterisation (find the one which has the best estimation properties)
iii. Range of applicability (the data should cover the full range of X and Y)
iv. Stochastic specification (the error term needs to be modelled)
v. Interpretability (parameters should be meaningful)

Simple probabilistic models are commonly used, but the amount of information obtained from such approaches is limited. Nevertheless, these models are often used as first attempts to determine general trends present in growth datasets. Regression models, on the other hand, have been developed to overcome the limitation present in simple probabilistic modelling by predicting the lag phase of a microbial population in a culture system. The lag phase has been described as the delay in the onset of exponential growth (the log phase). By taking critical growth factors into account, lag phase represents the duration of time required for microbial cells to adjust to the temperature, pH, nutrient availability and water potential of a new system. Examples of regression models include the basic logistic regression and quadratic models.

Another relevant category of nonlinear growth models is sigmoidal functions, such as the Gompertz, Richards', and Baranyi, commonly used to model microbial numbers as a

function of time (Gompertz, 1825; Richards, 1959; Baranyi & Roberts, 1995). These functions are chosen because they can estimate three phases of microbial growth, namely, the lag, the log (including rapid and slow changes in growth), and stationary phases. A useful feature of these models is that they visualise actual microbial growth rates as they are not constant over time.

2.2.3 Thermal adaptation and thermodynamics of microbial growth

Thermodynamics is a branch of physics which deals with the energy and the work system. A biological discipline that is concerned with the flow of energy from the environment into living systems, and *vice versa*, is called bioenergetics. There are three principle laws of thermodynamics (four when the zeroth law is considered) that conceptualise the transformation of energy across different systems – from a system as large as the Earth's, to one that is as microscopic as living cells. If a system has the same temperature with the other it is in contact with (e.g. the environment and a living microbial cell), both are in thermal equilibrium with each other. If they are initially at different temperatures, they will eventually achieve thermal equilibrium (this is also known as the Zeroth Law of Thermodynamics). According to the law of conservation of energy, energy can neither be created nor destroyed; it can only be transformed from one form to another e.g. potential to kinetic energy. Microbial growth reactions are spontaneous and are irreversible. Therefore, growth is coupled with a production of entropy in which microbial cells are treated as an open system (Sandler, 1991; Sandler & Orbey, 1991; Battley, 1998; von Stockar & Liu, 1999; von Stockar *et al.*, 2006; Battley, 2013).

The three laws of thermodynamics are:

i. First law of thermodynamics: When energy passes into or out of a system, the system's energy changes in accordance to the law of conservation of energy (e.g. thermal energy is converted into kinetic energy measured through biomass production within a closed culture system).
ii. Second law of thermodynamics: The entropy (disorder) of a system increases with energy input (e.g. a microbial cell reproduces more daughter cells with increasing temperature until the thermal optimum is reached).
iii. Third law of thermodynamics: The entropy of a system approaches a constant value as the temperature approaches absolute zero (0 Kelvin or -273.15°C).

Another principal theory on thermodynamics that is very relevant in our attempt to investigate thermal adaptation in biological systems from a physical perspective is the Arrhenius law. Proposed by Svante Arrhenius in 1889, the law states that the rate of reactions depended on the energy state of molecules involved in the reaction, where only those molecules above a certain energy state could complete the reaction. This energy threshold is called the activation energy (E_a); at higher temperatures, a larger proportion of molecules present are above the E_a, making reaction rates faster (Logan, 1982). The limitation of the Arrhenius law is that it assumes that only temperature changes in the system, not allowing interactions of many factors such as multiple reactions running concurrently in a cell. However, a very large body of data published over the past 100 years has demonstrated that biological rates predominantly follow the Arrhenius relationship over the normal biological temperature range (Peck, 2015). The Arrhenius law has been one of the principle theories in biochemistry, giving way to the formulation of two important hypotheses in enzymology – the lock-and-key and induced-fit hypotheses.

The thermodynamics of microbial growth has not received much research interest to date, mainly because researchers (especially chemists) believe cells are not pure, crystalline substances and therefore not susceptible to rigorous physical description. However, Battley (2013) borrowed the concept of Gibbs' free energy to apply to growth datasets of the yeast *Saccharomyces cerevisiae* (Desm.) Meyen in his long history of work on microbial growth thermodynamics from 1960 until 2013. He found that the free energy change accompanying cellular growth in an experimentally controlled system cannot be directly measured, but could be calculated using the Gibbs' equation if the heat of growth can be measured and the entropy change accompanying growth can be calculated. Even though these are difficult to quantify under a basic experimental setup, his findings prove that microbial cells are accommodating (or rather, adapting) to various energy proxies in many different energy states that are available in their surroundings, including temperature, to maintain life processes.

The relationship between growth rates and thermal adaptation can be linked based on the following premises: (i) temperature is a scale of thermal energy; (ii) temperature is a factor of growth physiology in organisms; (iii) growth rates are a physical vector of growth over time; and (iv) fungi obtain chemical energy for growth through degradation of organic substrates by EHEs (Sandler, 1991). Therefore, the synthesis for this study is that thermal adaptation in different fungal species found in specific bioclimatic regions can be unravelled by relating their growth rates to thermal energy requirement for growth under different nutrient sources. Therefore, we could infer that microbes, including fungal cells (a living powerhouse containing potential energy for growth), are adapting to the ambient temperature (a proxy to thermal energy) level typical of bioclimatic regions they predominantly occupy. Since they depend on the availability of organic substrates for growth, they may also adapt to the abundance (or lack thereof) of particulate organic matter (POM; a proxy to chemical energy) in their respective habitats (Figure 2.3).

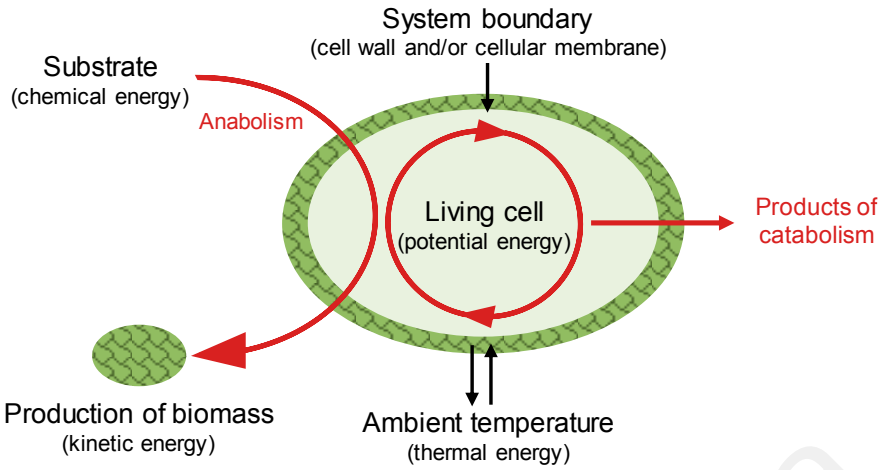

Figure 2.3: Flow of various types of energy into and out of a microbial cell.

2.3 Extracellular hydrolytic enzymes (EHEs) indicate ecological roles in fungi

Osmotrophy and chemotaxis underpin the ecology of fungi. They secrete extracellular enzymes to digest complex extracellular organic matter to simpler molecules for cellular metabolism. Extracellular enzymes are a suite of 'machineries' that fungi produce to hydrolyse high molecular weight matter to simpler molecules they can absorb for survival and proliferation. Fungi have thrived for millions of years in all ecosystems due to this feeding mechanism they share across the fungal kingdom. This results in them being either saprotrophs of detritus, parasites to infected hosts, or dependent on another organism for nutrient acquisition through symbioses. In the context of pathology, for example, extracellular hydrolytic enzymes are viewed as a virulence factor (others being antibiotics and toxins) (Åhman *et al.*, 2002).

Carbon remains the principal element in organic building blocks of microbial cells. There are now four identified major sources of carbon in the environment, namely Green, Blue, Brown, and Black Carbon (Figure 2.4). Among the four, Blue Carbon is considered the most important source of carbon on the planet due to the ocean's vast and deep surface coverage, and role as Earth's carbon sink. There are two pumping mechanisms which

govern the translocation and transformation of carbon in the marine environment: (a) the physical carbon pump, and (b) the biological carbon pump. The ocean concentrates 50 times more carbon than the atmosphere (http://thebluecarboninitiative.org/).

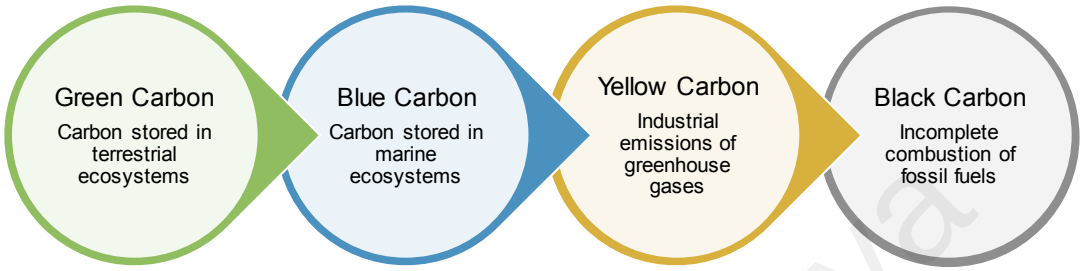

Figure 2.4: The colours of carbon. Adapted from United Nations' The Economics of Ecosystems and Biodiversity Report: Climate Issues Update, September 2009.

Fungi generally opt for chemically simpler substrates as a carbon source, preferentially utilising saccharides for metabolism over peptides and lipids (Figure 2.5). While fungi do not fix nitrogenous compounds, they still take in and assimilate simple nitrogenous molecules such as nitrate (NO_3^-) into their cells for amino and nucleic acid synthesis. Fungi thrive in nutrient-rich habitats, and they are also able to survive under oligotrophic conditions by forming cryptic biofilm-based microhabitats in substrates such as porous rocks. The extracellular matrix of the microbial biofilm 'gel' carbon sources is released by every microbial component for uptake and assimilation within the community (Selbmann *et al.*, 2005).

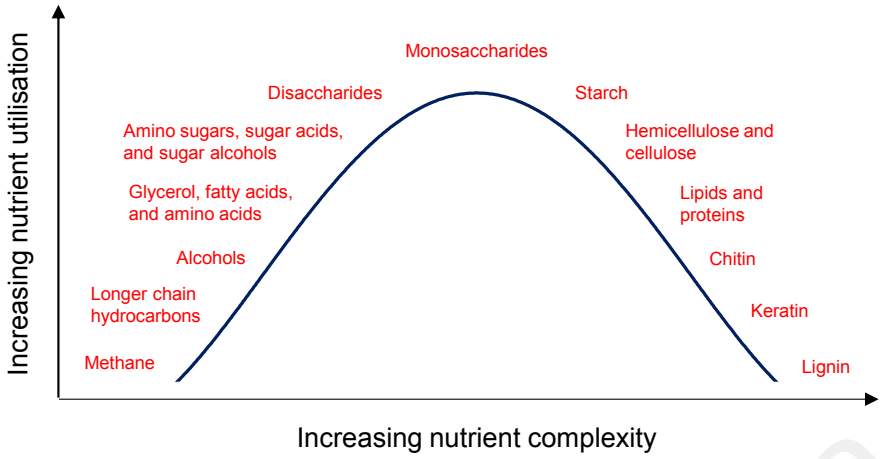

Figure 2.5: Major carbon substrates in fungi. Adapted from Deacon (2006).

Hydrolases constitute most extracellular enzymes present in the environment. They hydrolyse a complex organic matter into two chemically simpler compounds. Each of the resulting compound receive a hydroxyl ion (OH^-) and a hydrogen ion (H^+), respectively, from a water molecule. These simpler compounds are then taken into fungal cells through osmotrophy, and are further digested for cellular-level assimilation. The sequestration of chemical energy from the environment into fungal cells give fungi their roles as decomposers in biogeochemical cycles. While the role of terrestrial fungi in these processes are well-characterised, this is not the case with marine-derived and true-marine fungi (Morris *et al.*, 2010; Sowell *et al.*, 2011; Schneider *et al.*, 2012; Dong *et al.*, 2014). EHEs secreted into the marine environment are characterised by their ability to function under exposure to high salinities and pH levels, and under the anoxic conditions that may be associated with nutrient enrichment. The association of numerous fungal species with specific types of substrates and hosts may indicate fungal adaptation to various sources of nutrient availability (or lack thereof).

2.3.1 Amylases

Starch is a polysaccharide that is stored in plants as an energy source, often as semi-crystalline granules in plant tissues. A family of enzymes called the amylases catalyse

hydrolysis of starch molecules into simpler saccharides. Amylases are of great economic importance with wide application in biotechnological and remediation industries. They constitute 25% of the global enzyme market (Saranraj & Stella, 2013). Starch is a polymer of two simpler glycosylic polymers: amylose and amylopectin. Amylose is a linear polymer consisting of up to 6000 glucose units with α-1,4 glycosidic bonds. Amylopectin consists of short α-1,4 bonds linked to linear chains of 10-60 glucose units, and α-1,6 bonds linked to side chains with 15-45 glucose units. α-amylase (EC 3.2.1.1) and γ-amylase (3.2.1.3) are examples of enzymes from this family, with the former being the most widely synthesised in nature (de Souza & Magalhães, 2010). The α-amylase (α-1,4-glucan-4-glucanohydrolase) acts upon internal α-1,4-glycosidic linkages in starch between glucose, maltose, and maltotriose units.

Starch granules are generally insoluble at room temperature. When an aqueous suspension of starch is heated, however, internal hydrogen bonds weaken, water is absorbed, and starch granules swell. This process is also known as gelatinisation. Bacterial α-amylases belong to the 'liquefying' (thinning of gelatinised solution by further heating) category, while fungal α-amylase belong to the 'saccharifying' (depolymerisation of gelatinised starch into mono-, di-, and trisaccharides) category (Saranraj & Stella, 2013; Smith *et al.*, 2005). Most reports on fungal amylases have been limited to few species of mesophilic fungi, with the aim to screen for superior commercial producers of amylases. These fungal strains are usually terrestrial isolates of *Aspergillus* P. Micheli ex Haller and *Penicillium*. Fungal α-amylases are preferred over other microbial sources in commercial applications due to their *GRAS* (**G**enerally **R**ecognised **a**s **S**afe) status (Deacon, 2006).

2.3.2 Cellulases and ligninases

Cellulose is one of the planet's most important sources of carbon, with an annual biosynthesis by plants and marine algae of 0.85×10^{11} tonnes per annum. Complete enzymatic hydrolysis of cellulosic substrates requires different types of cellulases: endoglucanase (1,4-β-D-glucan-4-glucanohydrolase; EC 3.2.1.4), exocellobiohydrolase (1,4-β-D-glucan glucohydrolase; EC 3.2.1.74) and β-glucosidase (β-D-glucoside glucohydrolase; EC 3.2.1.21). The endoglucanase randomly hydrolyses the β-1,4 bonds in the cellulose molecule and the exocellobiohydrolases in most cases release one cellobiose unit in a recurrent reaction from chain extremity. Lastly, the cellobiose is converted to glucose by glucosidase (Kasana & Gulati, 2011; Payne *et al.*, 2015).

Model chemical compounds that are widely used in modern research as cellulose substitutes are the amorphous carboxylmethylcellulose (CMC) and the highly crystalline Avicel (Karlsson *et al.*, 2002; Johnsen & Krause, 2014). Amorphous cellulose can only be penetrated by an endoglucocase (attacks internal glycosidic bonds), and the crystalline cellulose an exogluconase (attacks terminal glycosidic bonds). The consequence of this being that the hydrolysis rate is much faster for amorphous cellulose than crystalline cellulose. In another natural context, other recalcitrant compounds surrounding cellulose in plants, e.g. lignin, limit binding of enzymes into the reaction sites and may slow down hydrolysis rate even more (unless degradation is aided by ligninases). Model species of fungal cellulase producers are members of the genus *Trichoderma* Pers., specifically *T. reesei* E. G. Simmons and *T. viride* Pers. These fungi are known to cause white-rot in woody substrates. As such, cellulases may be a virulent factor in fungal phytopathogenicity. Members from other fungal genera such as *Fusarium*, *Aspergillus*, and *Penicillium* are also producers of different types of cellulases (St. Leger *et al.*, 1997; Moctezuma-Zárate & Vargas-Morales, 2013).

Lignins are most often found in a type of pigment called melanins, and are bound to other natural polymers such as cellulose and hemicellulose in wood, resulting in a highly complex polymer called lignocellulose. Lignin is three-dimensional, and an optically-active phenylpropanoid polymer. Wood-rotting fungi are rampant producers of ligninases; they are classified into three specific decay groups: (a) white-rot, (b) brown-rot, and (c) soft-rot fungi. Wood-rotting fungi can degrade both lignin and cellulose, but have different degradation rates for both respective compounds, and hemicellulose. Brown-rot fungi prefer softwood, coniferous substrata (gymnosperms), while white-rot fungi prefer hardwood, deciduous substrata (angiosperms) (Rypáček, 1977; Gilbertson, 1980). Differences in structural elements building the phenylpropane backbone of the lignin component leads to the formation of hardwood (e.g. birch) and softwood (e.g. spruce). Two important enzymes in lignin biodegradation are lignin peroxidase (LiP) and manganese peroxidase (MnP) (Tuor *et al.*, 1995). Ligninolysis is an oxidative reaction, thus low nutrient nitrogen level is a prerequisite for lignin degradation (Eriksson *et al.*, 2012).

Lignocellulose is an important source of carbon in the operation of the global carbon cycle (Sjöström, 1993; Argyropoulos & Menachem, 1997; Tuomela *et al.*, 2000). Lignocellulolytic processes in fungi are thoroughly researched for this reason. To successfully degrade lignin, it is now known that fungi employ two types of extracellular enzymatic systems: (a) the hydrolytic system which is responsible in the degradation of polysaccharides naturally associated with lignin (i.e. cellulose and hemicellulose), and (b) the oxidative ligninolytic system which degrades lignin and opens phenyl rings in the compound (Sánchez, 2009).

2.3.3 Proteases

Proteases (also peptidases; EC 3.4) refer to a large group of hydrolytic enzymes that catalyse the degradation of peptide bonds in proteins. Hydrolysis of peptide bonds lead to degradation of proteinaceous substrates into constituent amino acids. Proteases are grouped into two large families of enzymes, namely endopeptidases and exopeptidases, depending on their site of action. Endopeptidases act upon inner sites of polypeptide chains, while exopeptidases act near the ends of polypeptide chains (either N or C terminus) (de Souza & Magalhães, 2010; de Souza *et al.*, 2015).

Proteases play a major role in pathophysiological processes in fungi. For example, extracellular proteases including collagenases, and chitinase are involved in nematode-cuticle penetration and host-cell digestion in nematophagous fungi (Åhman *et al.*, 2002; Huang *et al.*, 2004; Gortari & Hours, 2008). Another group of important fungal proteases is keratinases which act on the fibrous keratin present in skin, hair, nail, and quills of feathers. Important producers of keratinases are dermatophytic fungi. The majority of characterised fungal proteases reach optimal thermal stability at temperatures between 26 and 70°C.

2.3.4 Lipases

In eukaryotes, lipases are involved in various stages of lipid metabolism including fat digestion, absorption, reconstitution, and lipoprotein metabolism. Fungi able to produce lipases are found in many habitats, including soils contaminated with oils, wastes of vegetable oils, dairy product industries, seeds, and deteriorated food (Sharma *et al.*, 2001).

Lipids are a major component of the cellular membrane, and an energy reserve compound in photosynthetic organisms including microalgae. Lipids are acted upon by a group of enzymes called lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3); they

catalyse the hydrolysis of triacylglycerols to glycerol and free fatty acids. Additionally, lipases catalyse the hydrolysis and transesterification of other esters as well as the synthesis of esters, and transforming them into chirals (also known as enantiomers) (Gupta *et al.*, 2007).

2.3.5 Adaptation of enzymes to temperature

Enzymes are three-dimensional macromolecules that are made up of primary, secondary, tertiary, and quaternary structures. Among the four, the primary structure is not temperature-sensitive. The (covalent) peptide bonds linking amino acid residues are quite stable up to temperatures beyond those at which life ceases. All higher levels of protein structure are stabilised by weaker bonds (hydrogen bonds, hydrophobic interactions, van der Waals interactions, and electrostatic interactions) and are highly temperature sensitive. Secondary structures are disrupted by increase in temperature. Tertiary and quaternary structures, together forming a subunit aggregation, may be destabilised by high and low extremes of temperature.

The kinetic (catalytic and regulatory) properties of enzymes are also highly temperature sensitive. The reaction rate of enzymes generally doubles with every 10°C increase in temperature, resulting in a temperature coefficient or a Q_{10} value of 2. This is common under saturating concentrations of substrate, where an enzyme reaches its maximal velocity (V_{max}). Three critical criteria of enzymatic traits and functions are: (a) a proper degree of stability in the higher orders of enzyme structure must be maintained (structural conservation), (b) the rates of enzymatic reactions are adjusted to offset the decelerating or accelerating effects of temperature changes (activity regulation); and (c) ligand binding abilities must be strongly conserved to initiate and regulate catalysis (function specificity) (Somero, 1978).

Researchers have been studying orthologous proteins (orthologs) from differently thermally-adapted species since the mid-1960s. Differences in these orthologs are starting to be revealed. Three basic strategies have been recognised that contribute to thermal adaptation in enzymes: (a) changes in amino acid sequence that cause adaptive variation in the kinetic properties and stabilities of proteins, (b) shifts in concentrations of proteins, which are mediated through changes in gene expression and protein turnover; and (b) changes in the milieu in which proteins function, which conserve the intrinsic properties of proteins established by their primary structure and modulate protein activity in response to physiological needs (Gerday *et al.*, 2000; Somero, 2004). Hence, enzyme activity will continue to occur for as long as temperature does not cause denaturation in structural conformation and disrupt thermal response pathways in microbial cells.

CHAPTER 3: MATERIALS AND METHODS

3.1 Selection of marine-derived fungal strains

One marine-derived fungal strain from each of the tropical and polar (arctic and antarctic) latitudes was selected from the Institute of Ocean and Earth Sciences (IOES) and National Antarctic Research Centre (NARC) culture collections. Chosen strains were *Fusarium equiseti* (Corda) Sacc. 1886 (FEQ006) which was originally isolated from a tropical beach vegetated with *Vitex rotundifolia* in Peninsular Malaysia, and two strains of *Pseudogymnoascus* spp. (strains HND16 R2-1 sp. 2 and AK07KGI503 R2-1 sp. 3, respectively) Minnis and D. L. Lindner 2013 (formerly *Geomyces*) isolated from soils in maritime Antarctica and coastal sediments in High Arctic (Krishnan, *et al.*, 2011; Ali *et al.*, 2014). These strains were chosen based on the following salient features: (a) ascomycetes isolated from the marine environment; (b) strains growing as anamorphs at ambient local temperature (26 – 29°C); and (c) strains that showed highest radial growth in stock plates in comparison to other strains originating from similar biogeographical regions.

3.2 Maintenance of stock culture plates

The selected fungal strains were revived by sub-culturing into fresh Potato Dextrose Agar with an initial pH of approximately 6.8 (PDA; potato infusion 4.0 g/L, D(+)glucose 20.0 g/L, and agar-agar 15.0 g/L; Merck, Germany). After four weeks, each strain was sub-cultured again into seawater PDA plates (Crystal Sea Marinemix; USA, with 0.01% v/v chloramphenicol) with a salinity of 30 psu (3.75% w/v salt mix dissolved in distilled water, stirred overnight, and filtered through a Whatman N^{o} 1 filter paper). Subcultures of tropical strains were maintained at local ambient temperature (26 – 29˚C), while polar strains were maintained at 15˚C.

3.3 Growth of marine-derived tropical strain of *F. equiseti* and polar strains of *Pseudogymnoascus* spp. in a seawater-based solid-state culture system

3.3.1 Preparation of inoculants under different solid-state nutrient assays

Growth and extracellular hydrolytic enzyme activity (EHE) in marine-derived strains of *F. equiseti* and *Pseudogymnoascus* spp. were observed under access to three different seawater Reasoner's 2A-based (R2A; yeast extract 0.5 g/L proteose peptone 0.5 g/L, casein hydrolysate 0.5 g/L, glucose 0.5 g/L, soluble starch 0.5 g/L, sodium pyruvate 0.3 g/L, dipotassium hydrogenphosphate 0.3 g/L, magnesium sulphate anhydrous 0.024 g/L, and agar-agar 15.0 g/L; Merck, Germany) nutrient sources: skim milk (0.4% w/v skim milk in 67.4% v/v distilled water with 0.01% v/v chloramphenicol, and 32.6% v/v 92 psu artificial seawater; both fluids were autoclaved separately, and mixed immediately under sterile conditions), soluble starch (0.4% w/v soluble starch), and cellulase (0.4% w/v carboxylmethylcellulose with 0.01% w/v trypan blue) (Merck, Germany) as modified from Margesin *et al.* (2003). This medium was chosen because it was nutrient-deficient, containing trace amounts of organic matter to help initiate growth in fungi. Trial 10 d growth experiments were performed at 25°C to standardise screening time-points across all subsequent experiments due to different growth rates between the selected strains.

One mycelial plug (7 mm in diameter) from each fungal strain was inoculated into each assay plate using a sterile cork borer and a pair of N^{o} 4 forceps. Inoculated assay plates were prepared in triplicates to screen for growth in skim milk, starch, and cellulose nutrient assays before the screening of protease, amylase, and cellulase, from each assay respectively, were carried out. Screening for growth and EHE activities were carried out at temperatures of 5, 10, 15, 20, 25, 30, 35 and 40˚C during the mid-log phase of growth (D_3 of growth in *F. equiseti*, and D_5 of growth in *Pseudogymnoascus* spp., refer Figure 4.5 of Chapter 4.0) in the three selected strains.

3.3.2 Temperature-dependent relative growth rates (R_r) under different nutrient sources

Relative growth rates (R_r d^{-1}) across the experimental temperature range in the tropical strain of *F. equiseti* and polar strains of *Pseudogymnoascus* spp. were calculated using Eq. 1. Relative growth rates at different temperatures in all selected strains were calculated assuming exponential growth (Eq. 1). Calculated relative growth rates were then fitted into polynomial (Eq. 2) and Brière-2 (Eq. 3) nonlinear models to obtain best-fitting thermal growth curves of these strains growing with R2A-based skim milk, soluble starch, or carboxylmethylcellulose nutrient sources, and at temperatures of 5 to 40°C (Lamb, *et al.*, 1984; Brière, *et al.*, 1999).

$$R_r = \frac{ln(D_f) - ln(D_0)}{\Delta t} \quad \text{(Eq. 1)}$$

In Eq. 1, D_0 and D_f are the colony diameters measured before and after the experimental period, respectively, and Δt is the time span (d) of the incubation periods.

$$R_r = aT^3 + bT^2 + cT + d \quad \text{(Eq. 2)}$$

$$R_r = aT(T - T_{min})(T_{max} - T)^{1/m} \quad \text{(Eq. 3)}$$

In Eq. 2, a, b, c, and d are equation constants. In Eq. 3, a and m are equation constants and T_{min} and T_{max} the critical minimum and maximum temperatures, respectively. Curves fitted under Eq. 3 were calculated through unconstrained iterative nonlinear regression based on the Levenberg-Marquadt algorithm. Information from the raw data were used to constrain the parameters T_{min} (critical minimum temperature) and T_{max} (critical maximum temperature) of Eq. 3 (e.g. *F. equiseti* did not grow at 35°C during the 3 d incubation period). Curves fitted under Eq. 4 were calculated through constrained iterative nonlinear regression based on the quadratic sequential programming algorithm.

Curve estimates under the two nonlinear models were compared against three parameters: coefficient of determination (R^2), residual sum of squares (*RSS*), and corrected Akaike information criterion (*cAIC*) (van Boekel and Zwietering, 2007). R^2 indicates goodness-of-fit of the models against the datasets. Estimates with lower or negative *AIC*s are preferred to represent the datasets. The *AIC* was calculated using Eq. 4.

$$AIC = nln(RSS) - nln(n) + 2p \qquad \text{(Eq. 4)}$$

where n is the number of treatments, *RSS* is the residual sum of squares, and p is the number of parameters in the model.

Optimum temperature (T_{opt}) for growth in selected strains in different nutrient assays was calculated using Eq. 5 (Van Der Heide, *et al.*, 2006).

$$T_{opt} = \frac{1}{3}\left(T_{max} + T_{min} + \sqrt{T_{max}^2 - T_{min}T_{max} + T_{min}^2}\right) \qquad \text{(Eq. 5)}$$

Eq. 5 was used to calculate the growth rate at T_{opt} (R_{max} d^{-1}) by substitution of $T = T_{opt}$ as shown in Eq. 3, where a and m are equation constants following the values estimated for Eq. 3 previously. Eq. 6 was also used to manually counter-check parameter a values of Eq. 3.

$$R_{max} = aT_{opt}(T_{opt} - T_{min})(T_{max} - T_{opt})^{1/m} \qquad \text{(Eq. 6)}$$

All nonlinear regression (NLR) procedures were run on SPPS Statistics v.21 (IBM, USA).

3.3.3 Requirement of thermal energy across the experimental temperature range under solid-state culture system

To compare growth rates across the temperature series from 5 to 40°C, the temperature coefficients (Q_{10}) were calculated across the different temperature intervals (Eq. 7). R_2 is relative growth rate at higher temperature, R_1 is relative growth rate at lower temperature, T_2 is the higher temperature, and T_1 is lower temperature.

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2-T_1)} \quad \text{(Eq. 7)}$$

An Arrhenius plot was used to plot the natural log of k ($\ln k$) versus $1/T$ (k is estimated growth rate and T is absolute temperature in Kelvin) and to calculate the activation energy (E_a, J mol^{-1}) required by the three fungal strains to initiate growth at each temperature between 5 and 40°C under the different nutrient sources. E_a values could be determined from the slope ($-E_a/R$) between temperature points on the resulting plots, and by solving Eq. 9.

$$k = Ae^{-E_a/RT} \quad \text{(Eq. 8)}$$

$$E_a = -(slope)(R) \quad \text{(Eq. 9)}$$

Eq. 8 is the Arrhenius equation, in which A is pre-exponential factor, e is Euler's number (2.718), R is the gas energy constant (8.314 J mol^{-K}), and T is absolute temperature in Kelvin. Eq. 9 is derived from Eq. 8.

3.3.4 Relative activity of extracellular hydrolytic enzymes (EHE)

Extracellular hydrolytic enzyme (EHE) activity was indicated by the presence of a clear zone around the fungal colonies (colorimetric assessment). Protease and amylase activities were confirmed by flooding the nutrient assay plates with Coomasie Blue staining reagent or Lugol's solution, respectively, while cellulase activity was indicated

by decolouration of the trypan blue around fungal colonies. Diameters of fungal colonies and clear zones were measured and recorded to calculate relative enzyme activity (*RA*) indices. Values ≥ 1.0 were taken to indicate significant relative enzyme activity following Bradner *et al.* (1999). *RA* was calculated as follows:

$$RA = \frac{D_{cz} - D_f}{D_f} \quad \text{(Eq. 10)}$$

where D_{cz} is diameter of the clear zone around the fungal colony, and D_f is the diameter of fungal colony after the 3 d incubation period for the tropical strain of *F. equiseti* and 5 d for the polar strains of *Pseudogymnoascus* spp.

3.3.5 Statistical analyses of relative growth rate (R_r) and enzyme activity (*RA*)

Means and standard deviations of R_r and *RA* from all datasets were calculated. The effects of varying temperature on fungal R_r and *RA* values in the three different nutrient assays were analysed using multivariate analyses of variance (*m*ANOVA). When significant differences were detected, a *post hoc* Bonferroni test ($P < 0.05$) was applied to examine pairwise differences. Paired t-tests were applied to further examine these significant differences. All analyses were performed using SPSS Statistics v.21 (IBM, USA).

3.4 Growth of marine-derived tropical strain of *F. equiseti* and polar strains of *Pseudogymnoascus* spp. under seawater-based liquid-state culture system

3.4.1 Experimental setup and sampling regime

Each growth experiment was run for 10 d. The culture medium contained: 2.1% w/v Mueller Hinton Broth (MHB) powder (beef infusion solids, 2.0 g/L, casein hydrolysate, 17.5 g/L, and starch, 1.5 g/L; Merck, Germany) dissolved in 67.4% v/v distilled water with 0.01% v/v dissolved chloramphenicol, and 32.6% v/v 92 psu artificial seawater (25%

w/v salt mix dissolved in distilled water, stirred overnight; both fluids were autoclaved separately, and left to rest for a minimum of 24 h before mixing under sterile conditions; initial pH of approximately 6.8). This medium was chosen because it was nutrient-rich, and contained proteinaceous and carbohydrous substrates which were preferable for growth in fungi (refer Chapter 4.0 for results). Each conical flask was inoculated with 25 mL of culture medium. Forty conical flasks were prepared for each temperature experiment for each fungal strain. Of these, the planned sampling regime required 33 culture flasks to be sampled by the end of each experiment, allowing some redundancy (Krejcie & Morgan, 1970). Each culture flask was inoculated with two mycelial plugs (4 mm in diameter each) from stock culture plates. Each set of culture flasks were positioned randomly in an orbital shaking incubator set at 120 rpm. All culture flasks were assigned with random numbers generated using Research Randomizer v4.0 (Urbaniak & Plous, 2013). These numbers were randomised again, and sampling was carried out sequentially based on these randomised numbers.

Temperatures in the incubator were monitored manually and recorded daily throughout the experiments. Positions of the culture flasks were randomised again on the fifth day of every experiment to further minimise any influence of systematic variation in conditions within the incubator. Before every sampling of biomass, triplicates of 15 mL tubes containing one piece of N^{o} 1 filter paper each were labelled, and weighed on an analytical balance to obtain their initial dry biomass (in grams; later converted to milligrams). Every 24 h over the 10 d incubation, wet biomass from three randomly selected culture flasks from each set of experiments was sampled by filtering through the prepared Whatman N^{o} 1 filter papers using a vacuum pump. The samples of wet biomass were collected into the respectively labelled tubes, and stored at -80˚C before being freeze-dried for 24 h after the experiments were completed. After freeze-drying, they were weighed using an

analytical balance to obtain the final dry biomass. Filtered culture medium was sampled for pH measurements before disposal.

3.4.2 Temperature-dependent specific growth rates (R_s) under liquid conditions

Growth data of each fungal strain throughout the 10 d experiment were collected from D_0 until D_{10}. Total biomass values obtained from triplicates of samples, including that of the agar from the initial mycelial plugs, were averaged. The average biomass reading from D_0 was then used to subtract the weight of dried agar from subsequent average biomass readings collected from D_1 to D_{10}. In this way, data for dry mycelial biomass for each day t were obtained, using the following formula:

$$Mt_n = Bt_n - Bt_0 \qquad \text{(Eq. 11)}$$

where Mt_n = mycelial biomass at $t = n$, $n \leq 10$

Bt_n = mean biomass at $t = n$

Bt_0 = mean biomass at $t = 0$

Mean mycelial biomass values from each dataset were used to generate raw growth profiles and visualise growth trends. The means of mycelial biomass were transformed into natural logarithm values to evaluate exponential growth in each fungal strain at the various culture temperatures. The start of exponential growth was determined from the values given by natural-log transformations. Also, the natural log values were fitted to a straight line through a linear function ($y = mx + c$; y = value of transformed dry biomass weighed; m = slope of the straight line plot; x = the point in time expressed in unit d; and c = point of y-intercept) to obtain specific growth rate (k) values for each dataset. Goodness-of-fit for each transformed dataset could be evaluated by the value of R^2 resulting from the linear function of each transformed dataset ($R^2 \geq 0.5$). Higher values of R^2 close to 1 indicate that the values of dry biomass means fit more closely to the straight line plot.

Specific growth rate (R_s d^{-1}) values obtained from previous analysis of exponential growth in each strain at the various culture temperatures served as constants in two sigmoidal growth models, the Gompertz (Eq. 12) and Richards' logistic (Eq. 13) growth models, to obtain best-fit growth curves.

$$Y_t = Ae^{(-Be^{(-kt)})} \quad \text{(Eq. 12)}$$

In Eq. 11, where Y_t is size, biomass, etc., at time t, A is upper asymptote, B is displacement along the x-axis, k is specific growth rate, and e is Euler's number (2.7183).

$$Y_t = A + \frac{K-A}{(C+Qe^{-kt})^{1/v}} \quad \text{(Eq. 13)}$$

In Eq. 12, Y is size, biomass, etc., A is lower asymptote, K is upper asymptote, k is specific growth rate, v is the variable that fixes the inflection point, Q is the variable that controls inflection degree, and C is 1.0.

Curve estimates under the two nonlinear models were compared against three parameters: coefficient of determination (R^2), residual sum of squares (*RSS*), and corrected Akaike information criterion (*cAIC*) (van Boekel and Zwietering, 2007). R^2 indicates goodness-of-fit of the models against the datasets. Estimates with lower or negative *AIC*s are preferred to represent the datasets.

3.4.3 Requirement of thermal energy across the experimental temperature range under a liquid-state culture system

All specific growth rate estimates obtained from the previous fitting exercise using the Richards' logistic regression model were then fitted into Eq. 3, now R_r substituted with R_s as shown in Eq. 14:

$$R_s = aT(T - T_{min})(T_{max} - T)^{1/m} \quad \text{(Eq. 14)}$$

Projected R_s values calculated using Eq. 14 were then applied to Eq. 7, 8, and 9 to determine activation energy for growth in *F. equiseti* and *Pseudogymnoascus* spp. grown in liquid cultures.

3.4.4 Statistical analyses of specific growth rate (R_s)

Means, standard deviations, and standard errors of the mean of dry biomass values were calculated for each set of three flasks sampled. The effects of varying temperature on fungal growth as assessed by dry biomass data were analysed using repeated measures analysis of variance (*r*ANOVA) (IBM SPSS Statistics 21; IBM, USA). The null hypothesis (H_0) of the analysis was that there was no significant difference in the mean dry biomass sampled from the 10-d growth experiments at temperatures between 5 and 40˚C. The significance level set for the analysis was $\alpha = 0.05$.

CHAPTER 4: RESULTS

4.1 Temperature-dependent growth and activation energy (E_a) of growth based on relative growth rates (R_r)

Relative growth rates (R_r) were calculated from three replicates for each strain grown on assay plates incubated between 5 and 40°C. These calculated datasets were then fitted into third-degree polynomial and Brière-2 nonlinear temperature-dependent models. The third-degree polynomial model, while parsimonious to Brière-2, had no biologically-meaningful parameters. This model also had R^2 values consistently higher than those estimated by the Brière-2 model, but the resulting curves were not suitable to describe biological growth. This was further supported by *cAIC* values estimated by the Brière-2 model, with the majority of these being consistently lower than those estimated by the third-degree polynomial model. All the parameters estimated by these two models, and temperature-dependent curves estimated by the third-degree polynomial model are presented in Table 1 (see Appendix A).

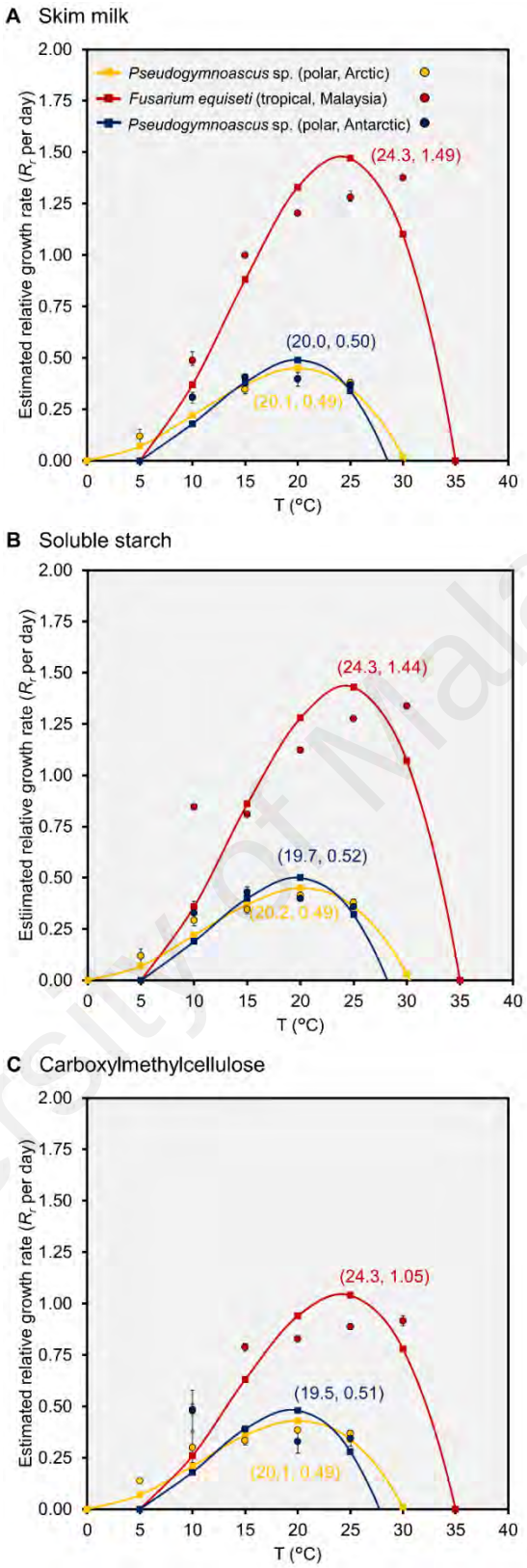

Figure 4.1: Temperature-dependent growth curves of the marine-derived arctic strain of *Pseudogymnoascus* sp. (yellow), the tropical strain of *F. equiseti* (red), and the antarctic strain of *Pseudogymnoascus* sp. (blue) inoculated into R2A-based (A) skim milk, (B) soluble starch, and (C) carboxylmethylcellulose nutrient assay plates. Coloured circles with error bars indicate mean relative growth rates with standard deviations in the three strains. Values in brackets are T_{opt} = optimum temperature for growth, and R_{max} = maximum relative growth rate, respectively.

Figures 4.1A-C show the results of curve-fitting of relative growth rates in *F. equiseti* and *Pseudogymnoascus* spp. grown under the three nutrient sources between 5 and 40°C. The growth curves estimated for *Pseudogymnoascus* spp. were similar and consistent under all three nutrient sources, with T_{opt} (mean ± standard deviation) in the arctic strain being 20°C ± 0.2 and R_{max} = 0.49 d^{-1}, and T_{opt} in the antarctic strain being 20°C ± 0.3 and R_{max} = 0.50 d^{-1} ± 0.2. These two strains had T_{max} = 30°C. Growth curves estimated for *F. equiseti* had similar shapes except when grown under carboxylmethylcellulose. The R_{max} value at T_{opt} in *F. equiseti* was three times higher than those of *Pseudogymnoascus* spp. grown under skim milk or soluble starch, and twice that under carboxylmethylcellulose. T_{opt} in *F. equiseti* as estimated by the Brière-2 model was 24.9°C, and R_{max} was the highest when grown using skim milk (R_{max} = 1.49 d^{-1}) (Figure 4.1A). R_{max} was successively lower when grown under the two remaining substrates, as shown in Figures 4.1B and 4.1C (R_{max} = 1.44 d^{-1} and 1.05 d^{-1}, respectively).

The temperature coefficient (Q_{10}) and activation energy (E_a) values were calculated using estimates by the Brière-2 nonlinear temperature-dependent model to determine the ratios of relative growth rates at a higher temperature to a lower one across the experimental temperature range. Q_{10} values could only be calculated and compared where growth occurred in the three fungal strains across the temperature series. Therefore, the data that are presented here were obtained from datasets on relative growth rates under skim milk and carboxylmethylcellulose sources, since there were marked differences in EHE activities and radial growth between the tropical *F. equiseti* and polar *Pseudogymnoascus* spp. as noted above. Calculated values of Q_{10} are presented in Table 4.1.

Table 4.1: Temperature coefficient (Q_{10}) of relative growth rates (R_r) in *F. equiseti* and *Pseudogymnoascus* spp. across five temperature series under seawater-based solid-state skim milk and carboxylmethylcellulose assays.

	Nutrient source					
	Skim milk			CM-cellulose		
	Q_{10}			Q_{10}		
Temperature series	ArcP	TropF	AntP	ArcP	TropF	AntP
A = 5 – 10°C	9.88	-	-	9.00	-	-
B = 10 – 15°C	2.83	5.66	4.46	2.94	5.87	4.69
C = 15 – 20°C	1.48	2.28	1.66	1.43	2.23	1.51
D = 20 – 25°C	0.60	1.22	0.48	0.63	1.22	0.34
E = 25 – 30°C	-	0.56	-	-	0.56	-

(ArcP) Arctic strain of *Pseudogymnoascus* sp.
(TropF) Tropical strain of *F. equiseti*
(AntP) Antarctic strain of *Pseudogymnoascus* sp.

The comparison of Q_{10} values from this point onwards would only be obtained from growth when the three strains were supplied with skim milk and carboxylmethylcellulose. This is because growth patterns in these strains under skim milk and starch were similar. Trends of Q_{10} coefficients declining with increasing temperature were consistent when grown under the skim milk and carboxylmethylcellulose nutrient assays. Progressive decrease in the Q_{10} value is clear across each temperature for all three strains and both nutrient assays (Table 4.1). Q_{10} values in *F. equiseti* were generally higher than in *Pseudogymnoascus* spp. from temperature series B through D. Using the Arrhenius plot, the activation energy (E_a) values of growth in polar *Pseudogymnoascus* spp. and tropical *F. equiseti* were estimated (Figures 4.2A and 4.2B). Figure 4.1A and 4.2B (top) show natural log values of growth rates (expressed in k) estimated using the Brière-2 model plotted against the absolute temperature scale (in Kelvin). In all strains, ln (k) peaked at $1/T = 3.40 \times 10^3$. Figure 4.2A and 4.2B (bottom) visualise energy requirements expressed in terms of E_a for these strains to grow across the experimental temperature range.

Values of E_a decreased with increasing thermal energy, obeying the Arrhenius behaviour of exothermic reactions (Sims, 2013). *Pseudogymnoascus* spp. and *F. equiseti* generally required a lower E_a when grown in plates augmented with skim milk than in plates augmented with carboxylmethylcellulose. E_a also declined with increasing temperature. A negative E_a was calculated when growth in these strains occurred, indicating absorption of less energy beyond their respective T_{opt}. Low positive E_a values at T_{opt} implies that very little E_a was required for growth because the levels of thermal energy provided by the system were sufficient to maintain optimal growth processes in fungal cells.

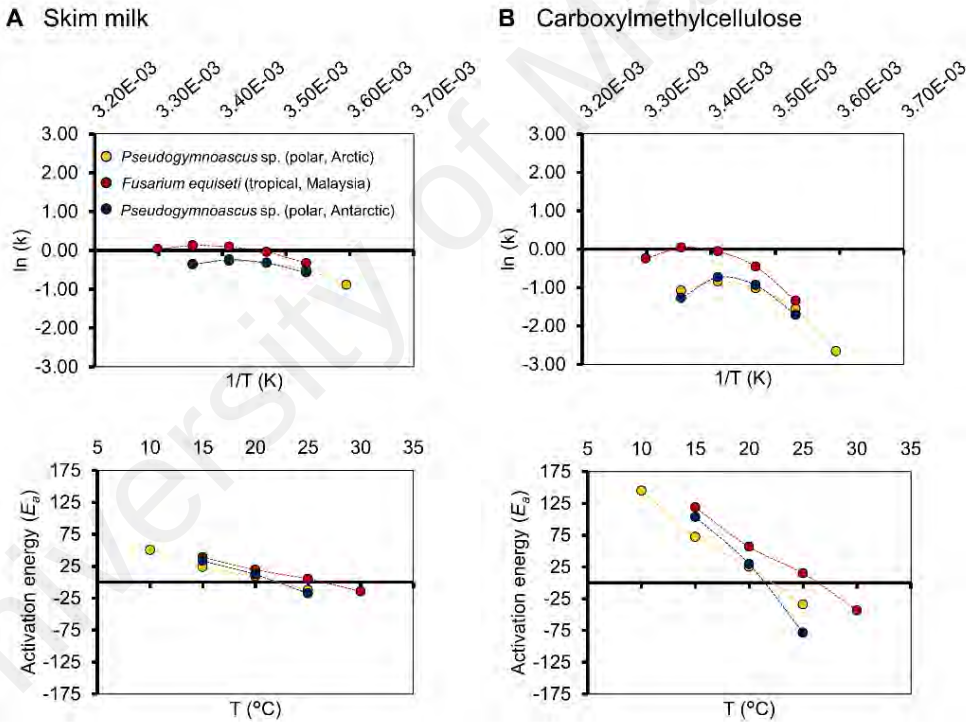

Figure 4.2: Arrhenius plots (top) of measured growth rates in the marine-derived arctic strain of *Pseudogymnoascus* sp. (yellow), tropical strain of *F. equiseti* (red), and antarctic strain of *Pseudogymnoascus* sp. (blue) inoculated onto R2A-based (i) skim milk, and (ii) carboxylmethylcellulose assay plates across the experimental temperature series between 10 and 30°C. Activation energy (E_a) of growth (bottom) in the marine-derived arctic strain of *Pseudogymnoascus* sp. (yellow), tropical strain of *F. equiseti* (red), and antarctic strain of *Pseudogymnoascus* sp. (blue) inoculated onto R2A-based (i) skim milk, and (ii) carboxylmethylcellulose plates across the experimental temperature series between 10 and 30°C.

4.2 Energy trade-off between growth and enzyme activity

Marine-derived polar strains of *Pseudogymnoascus* spp. showed only protease and amylase activities across the experimental temperature range after the 5 d incubation period when provided with skim milk or soluble starch, respectively (Figures 4.3A and 4.3B). Relative enzyme activity (*RA*) indices were the highest at temperatures at which relative growth rates in these fungal strains were the lowest or growth did not occur. These temperatures were 5°C, and between 30 and 40°C, respectively. The same pattern of enzyme activity was observed in the tropical strain of *F. equiseti* which was incubated across the full experimental temperature range for 3 d, and had the lowest relative growth rate at 5°C. Colony diameter measurements could not be carried out after *F. equiseti* was incubated at 35 and 40°C because no growth could be measured. Calculated *RA* indices for protease, amylase, and cellulase activities were low in *F. equiseti*. Cellulase activity was recorded only in *F. equiseti*, from 5 to 30°C (Figure 4.3C).

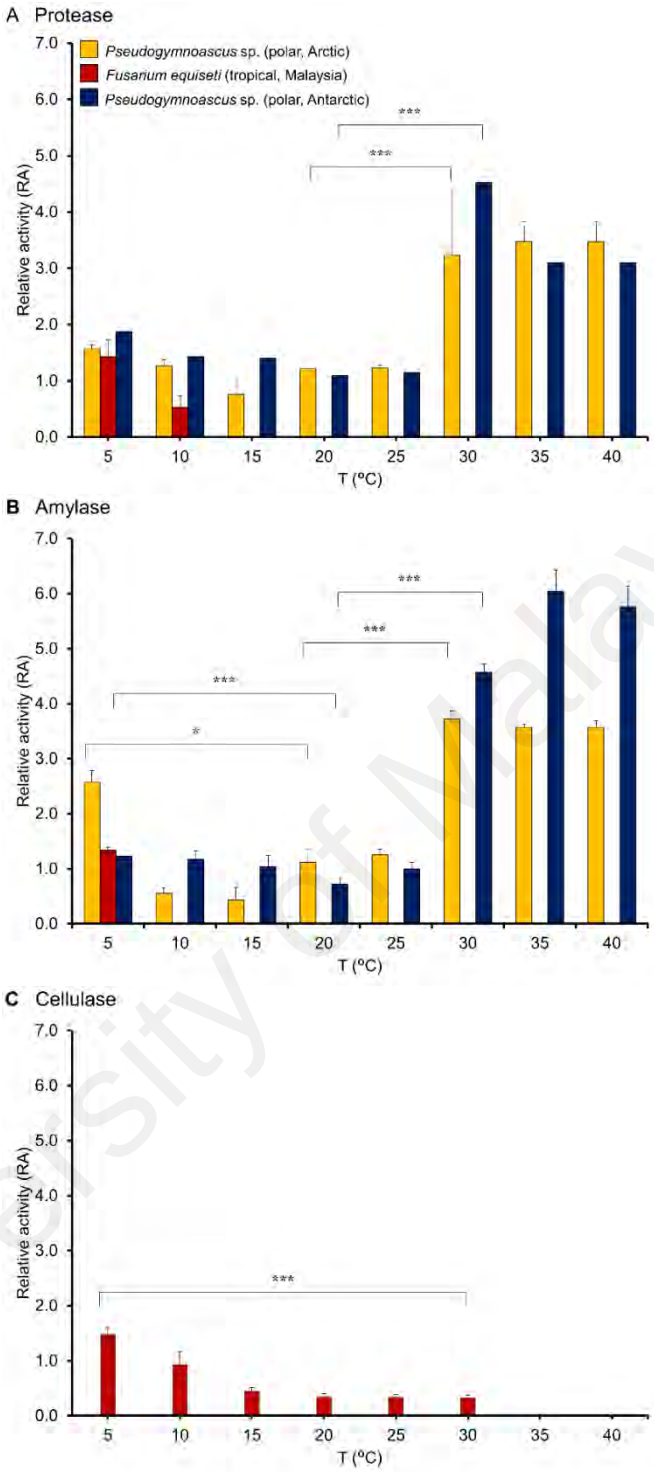

Figure 4.3: Mean relative activity of (A) protease, (B) amylase, and (C) cellulase in the marine-derived arctic strain of *Pseudogymnoascus* sp. (yellow), tropical strain of *F. equiseti* (red), and antarctic strain of *Pseudogymnoascus* sp. (blue) at temperatures between 5 and 40°C. Error bars indicate standard deviation. Horizontal brackets highlight pairwise comparison of relative enzyme activity between 5 (lowest growth rate, moderate activity) and 20°C (lowest activity, highest growth rate), and between 20 and 30°C (highest activity, no growth) among the arctic and antarctic strains in Fig 1A and 1B, and between 5 (lowest growth rate, moderate activity) and 30°C (highest growth rate, lowest activity) in the tropical strain. Significance: * - $P < 0.05$, *** - $P < 0.001$. Only positive relative activity indices are shown.

Figure 4.3 shows *RA* values of protease, amylase and cellulase in the three selected strains, and highlights important pairwise comparisons identified in the *m*ANOVA analyses. There was a significant difference in *RA* values of the three EHEs across the three selected fungal strains, $F_{56,286} = 29.21$, $P < 0.001$; Wilk's $\Lambda = 0.022$, partial $\eta^2 = 0.85$. Protease and amylase activities were higher at the maximum temperature for growth (T_{max} = 30°C) in polar *Pseudogymnoascus* spp. than at the optimum temperature (T_{opt} = 20°C) ($P < 0.001$) (Figures 4.3A and 4.3B). Note that T_{max} is a term equivalent to the upper critical limit (CL_u) used in Verant *et al.* (2012). As shown in Figure 4.3B, amylase activity at 5°C, at which the lowest growth rate occurred in both polar strains of *Pseudogymnoascus* spp., was also significantly higher than at their T_{opt} for growth ($P < 0.05$, and $P < 0.001$, respectively). A similar result was obtained when comparing cellulase activity in the tropical strain of *F. equiseti* at 5°C, and at the temperature with highest growth rate, 30°C ($P < 0.001$) (Figure 4.3C).

To obtain $RA > 0$, measures of each clear zone diameter must be larger than the fungal colony. $RA < 0$ when no EHE activity could be observed when radial growth occurred. In other words, in these instances growth occurred in the absence of activity from the EHEs of interest. Both cases were seen in the tropical strain of *F. equiseti* and polar strains of *Pseudogymnoascus* spp., respectively. *Pseudogymnoascus* spp. grew on carboxylmethylcellulose assay plates without showing any observable cellulase activity, which would be indicated by the discolouration of trypan blue. *Pseudogymnoascus* spp. might have been utilising trace amounts of starch that were available in R2A media for growth instead. Unlike *Pseudogymnoascus* spp., the tropical strain of *F. equiseti* showed protease, amylase and cellulase activities (Figure 4.4). Even though calculated *RA* indices were low between 5 and 30°C in this strain, measures of fungal colony diameter and the clear zone surrounding each were similar and consistent after the 3 d incubation period.

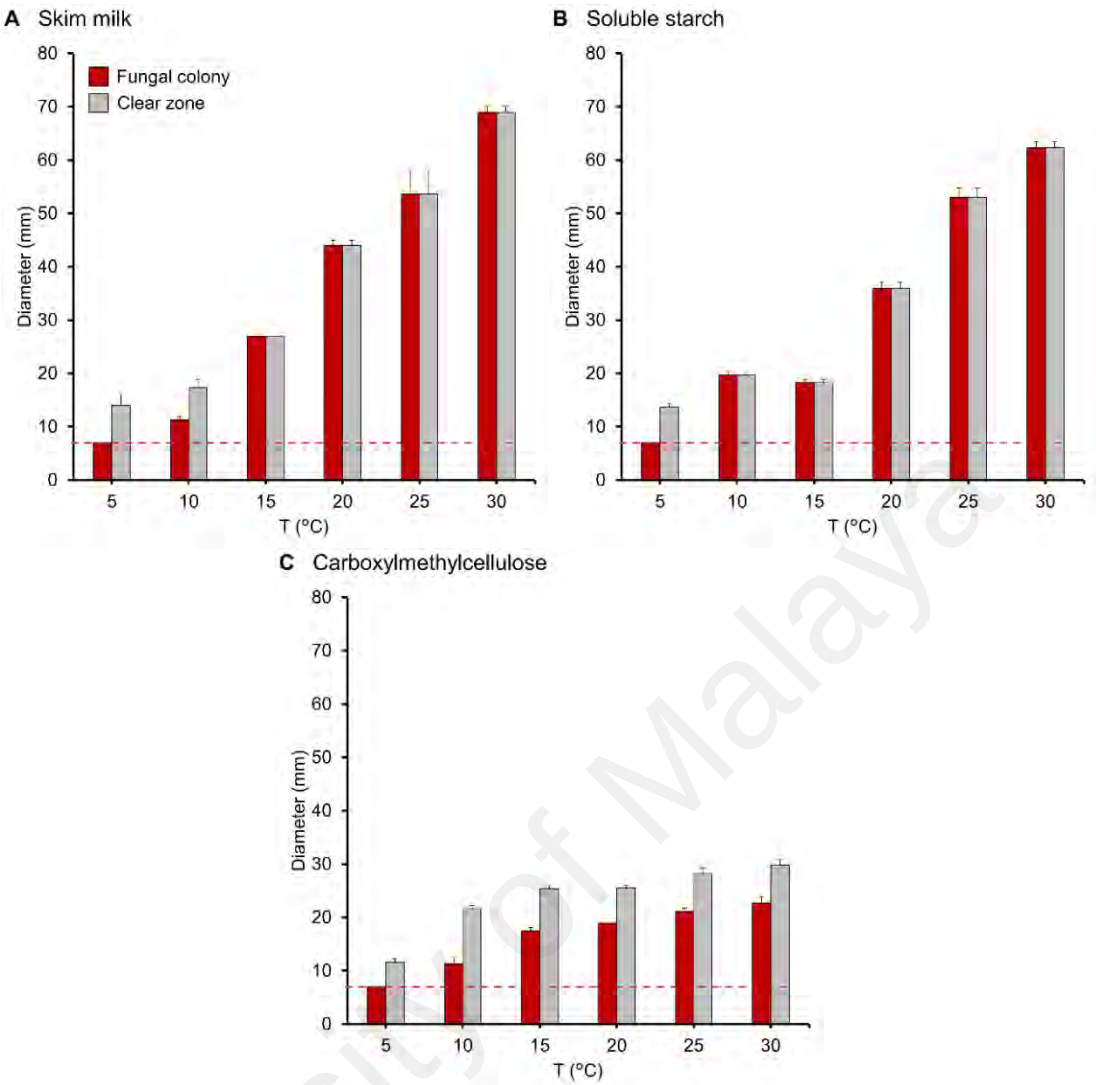

Figure 4.4: Mean colony and clear zone diameter measures in the marine-derived tropical strain of *F. equiseti* inoculated into R2A-based (A) skim milk, (B) soluble starch, and (C) carboxylmethylcellulose nutrient plates. Red dashed lines indicate initial colony diameter (7 mm). Error bars indicate standard deviation. Only temperatures at which filamentous growth occurred during the 3 d incubation period are shown.

As illustrated in Figures 4.4A and 4.4B, measures of fungal colony and clear zone diameters were similar in *F. equiseti*. Radial growth under all nutrient assays was greatest at 30°C. Growth, however, was slower than under skim milk and soluble starch assays when the strain was inoculated onto carboxylmethylcellulose plates. Measures of clear zone diameters indicating cellulase activities were also larger than fungal colonies under this assay. This resulted in $RA > 0$ in *F. equiseti* from 5 to 30°C, with the highest *RA* recorded at 5°C. Hence, our data do not permit the inference that *F. equiseti* showed no EHE activity based on the formula proposed by Bradner *et al.* (1999) alone.

4.3 Temperature-dependent growth and activation energy (E_a) of growth based on specific growth rates (R_s)

Repeated measures analyses of variance (*r*ANOVA) of all obtained dry biomass datasets were performed. Mauchly's Test of Sphericity on the dry biomass data of *F. equiseti* across the six temperatures tested indicated that the assumption of sphericity had been violated, meaning that variances of the differences between all combinations of related levels or groups were not equal ($\chi^2 = 63.17$, $P < 0.001$). Therefore, degrees of freedom were corrected using a Huynh-Feldt estimate of sphericity, $\varepsilon = 0.73$ (Mauchly, 1940 Greenhouse & Geisser, 1959; Huynh & Feldt, 1976). Results of *r*ANOVA with corrected sphericity showed that the effect of different culture temperatures on dry biomass obtained from liquid cultures of *F. equiseti* was significant, $F_{(3.66,80.54)} = 25.55$, $P < 0.001$, with a large effect size, $\eta^2 = 0.77$. Pairwise comparison between dry biomass data across the six temperatures tested for the strain showed that the means obtained were significantly different from each other, except for those obtained at 15 and 30°C ($P = 0.72$), and 20 and 25°C ($P = 1.0$).

Mauchly's Test of Sphericity on the dry biomass data of the arctic *Pseudogymnoascus* sp. again indicated that the assumption of sphericity had been violated ($\chi^2 = 55.69$, $P < 0.001$), therefore degrees of freedom were corrected using a Huynh-Feldt estimate of sphericity, $\varepsilon = 0.86$. Results of *r*ANOVA with corrected sphericity again showed that the effect of culture temperature on the dry biomass obtained from liquid cultures of *Pseudogymnoascus* sp. was significant, $F_{(4.28,94.1)} = 29.55$, $P < 0.001$, with a large effect size, $\eta^2 = 0.57$. Pairwise comparison between dry biomass data across the five temperatures tested for the strain showed that the means obtained were significantly different from each other except between 10 and 25°C ($P = 0.197$), and 15 and 20°C ($P = 0.056$), 15 and 25°C ($P = 0.404$), and 20 and 25°C ($P = 0.064$).

Mauchly's Test of Sphericity on the dry biomass data of the antarctic *Pseudogymnoascus* sp. again indicated that the assumption of sphericity had been violated (χ^2 = 46.32, P < 0.001), therefore degrees of freedom were corrected using a Huynh-Feldt estimate of sphericity, ε = 1.00. Results of *r*ANOVA with corrected sphericity again showed that the effect of culture temperature on the dry biomass obtained from liquid cultures of *Pseudogymnoascus* sp. was significant, $F_{(5.00,110.0)}$ = 96.72, P < 0.001, with a large effect size, η^2 = 0.82. Pairwise comparison between dry biomass data across the five temperatures tested for the strain showed that the means obtained were significantly different from each other except between 5 and 30°C (P = 0.668), and 15 and 20°C (P = 1.0). This indicated that the means of dry biomass of the three fungal strains obtained were not significantly different when the temperature difference was 5°C until T_{opt}.

The values of parameters and statistics to obtain best-fitting growth curves under the two sigmoidal growth models, Gompertz and Richards' logistic growth models, are shown in Table 2 and 3 (see Appendix A). All available datasets obtained from dry biomass measures of *F. equiseti* and *Pseudogymnoascus* spp. were better fitted under the Richards logistic growth model than under the Gompertz (Figures 4.5A-C) although both models gave similar results. Values of R^2 and *RSS* calculated from subtracting the mean dry mycelial mass values obtained every 24 h with the values obtained from Richards' logistic growth equation were consistently lower than that obtained with the Gompertz equation. Therefore, growth curves calculated under Richards' logistic model were chosen to profile the growth of *F. equiseti* and *Pseudogymnoascus* spp. across all completed experimental culture temperatures.

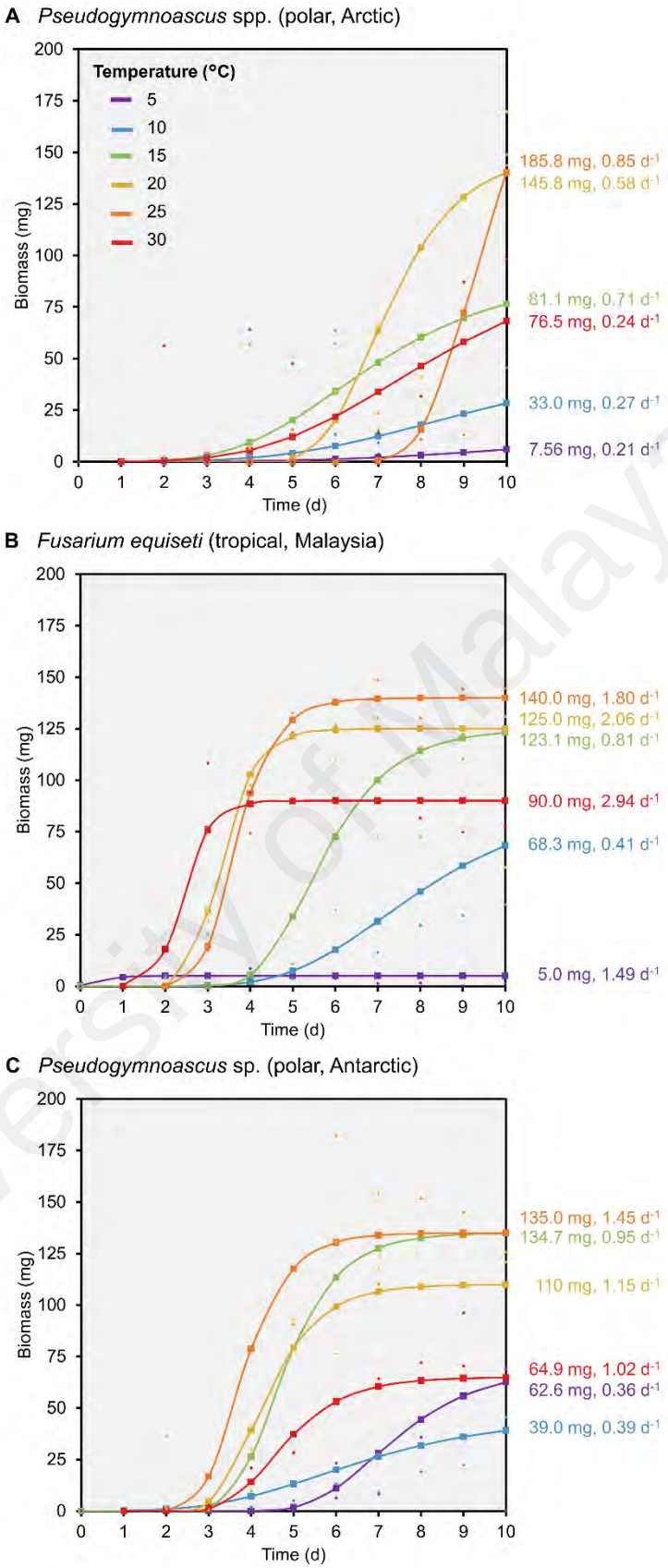

Figure 4.5: Sigmoidal temperature-dependent growth curves of the tropical strain of (A) the arctic strain of *Pseudogymnoascus* sp., (B) tropical strain of *F. equiseti*, and (C) antarctic strain of *Pseudogymnoascus* spp. calculated under Richards' logistic model. Temperature levels are indicated by colours as shown in the legend, respectively.

Figure 4.5 shows the growth profiles of *F. equiseti* (Figure 4.5B) and *Pseudogymnoascus* spp. (Figure 4.5A and 4.5B) at six experimental temperatures. The tropical strain of *F. equiseti* exhibited the highest specific growth rate at 30°C (k = 2.94 d^{-1}). Growth of *F. equiseti* at this temperature was accelerated but quickly arrested by D_2. Highest dry mycelial biomass in *F. equiseti* was observed at 25°C by D_8. of growth (k = 1.80 d^{-1}, 140.0 mg). Coupled with the result obtained from the analysis of exponential growth in this tropical strain at 25°C, the strain was yet to reach the stationary phase by the end of the 10 d experiment. Lowest specific growth rate in the strain was observed at 5°C (k = 0.41 d^{-1}). *F. equiseti* was still in exponential growth phase by D_{10} at this temperature.

Growth rate pattern in the arctic strain of *Pseudogymnoascus* sp. was similar to that of the antarctic strain. However, the log phase of growth in the arctic *Pseudogymnoascus* sp. started at around D_3 of growth. Growth rates observed were also generally lower than those of the antarctic strain. Highest growth rate was recorded at 25°C and the lowest was at 5°C (k = 0.85 and k = 0.21 d^{-1}, respectively). The antarctic strain of *Pseudogymnoascus* sp. generally underwent the log phase of growth at around D_2. The highest dry mycelial biomass in the former was produced at 25°C by D_9 before their growth declined, suggesting cell death (autolytic phase) beyond that time-point. Highest growth rate in this strain was also recorded at this temperature (k = 1.45 d^{-1}, 130.0 mg). *Pseudogymnoascus* sp. showed the lowest growth rate at 5°C (k = 0.36 d^{-1}). The strain was still undergoing exponential growth until the end of the 10 d experiment at 5°C, suggesting that they would reach the stationary phase beyond D_{10}. Specific growth rate (k = 0.36 d^{-1}) was the lowest at 5°C. The strain remained in the log phase of growth during the entire 10 d experiment at this temperature.

Figure 4.6 shows temperature-dependent growth curves of *F. equiseti* and *Pseudogymnoascus* spp. grown in liquid cultures using calculated specific growth rates (R_s) under Richards' logistic model. Maximum R_s (expressed in terms of R_{max}) of *F. equiseti* was almost twice higher (2.84 d^{-1}) than those of the arctic and antarctic *Pseudogymnoascus* spp. (0.77 and 1.40 d^{-1}, respectively). These results are consistent with our observations when these strains were grown under solid-state nutrient assays. Additionally, critical maximum temperature (T_{max}) values in each strain differed slightly from our earlier findings when grown under liquid conditions. The value of T_{max} in *F. equiseti* was 31.7°C, while values of T_{max} for arctic and antarctic *Pseudogymnoascus* spp. were 30.7 and 33.1°C, respectively. All strains exhibited growth beyond the T_{max} values that were previously estimated using R_r values from previous analysis. As a result, T_{opt} values estimated using R_s were greater than values estimated using R_r. *F. equiseti* grew optimally at 30°C while *Pseudogymnoascus* spp. grew optimally at 25°C under liquid conditions.

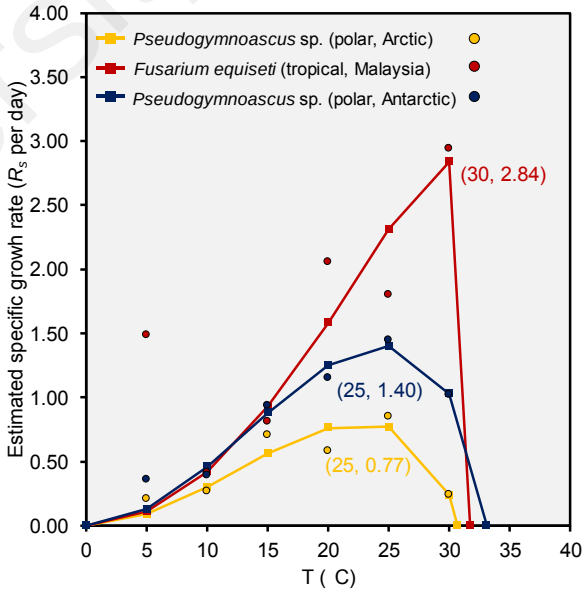

Figure 4.6: Temperature-dependent growth curves of the marine-derived arctic strain of *Pseudogymnoascus* sp. (yellow), the tropical strain of *F. equiseti* (red), and the antarctic train of *Pseudogymnoascus* sp. (blue) inoculated into seawater-based Mueller Hinton Broth. Coloured circles with error bars indicate mean specific growth rates. Values in brackets are T_{opt} = optimum temperature for growth, and R_{max} = maximum specific growth rate, respectively.

Table 4.2 shows the Q_{10} coefficients declining with increasing temperature were consistent when grown under the seawater-based Mueller Hinton Broth. Progressive decrease in the Q_{10} value is clear across each temperature for all three strains, and Q_{10} values in *F. equiseti* were generally higher than in *Pseudogymnoascus* spp. from temperature series A through E.

Table 4.2: Temperature coefficient (Q_{10}) of specific growth rates (R_s) in *F. equiseti* and *Pseudogymnoascus* spp. across five temperature series under seawater-based liquid-state culture system.

Temperature series	ArcP	TropF	AntP
A = 5 – 10°C	11.11	14.58	12.52
B = 10 – 15°C	3.48	4.90	3.66
C = 15 – 20°C	1.84	2.89	2.02
D = 20 – 25°C	1.03	2.14	1.25
E = 25 – 30°C	0.10	1.51	0.54

(ArcP) Arctic strain of *Pseudogymnoascus* sp.
(TropF) Tropical strain of *F. equiseti*
(AntP) Antarctic strain of *Pseudogymnoascus* sp.

Figure 4.7 shows the Arrhenius plot and activation energy (E_a) for growth in *F. equiseti* and *Pseudogymnoascus* spp. based on R_s values. The Arrhenius plot profiles of R_s were very similar to those calculated based on R_r from our previous analysis. Hence, the estimated E_a values follow the same pattern as previously described. However, we could estimate E_a at lower temperature levels, i.e. between 5 and 10°C, until 25°C, and could confirm that *F. equiseti* required higher energy for growth, followed by antarctic and arctic *Pseudogymnoascus* spp., respectively. Negative E_a in *Pseudogymnoascus* spp. at 25°C indicated that the fungal cells were starting to absorb less energy for growth than at 20°C. This is consistent with our E_a estimation based on R_r, implying that the cells might have experienced thermal stress when exposed to an ambient temperature beyond 20°C even though T_{opt} values were greater when calculated based on R_s.

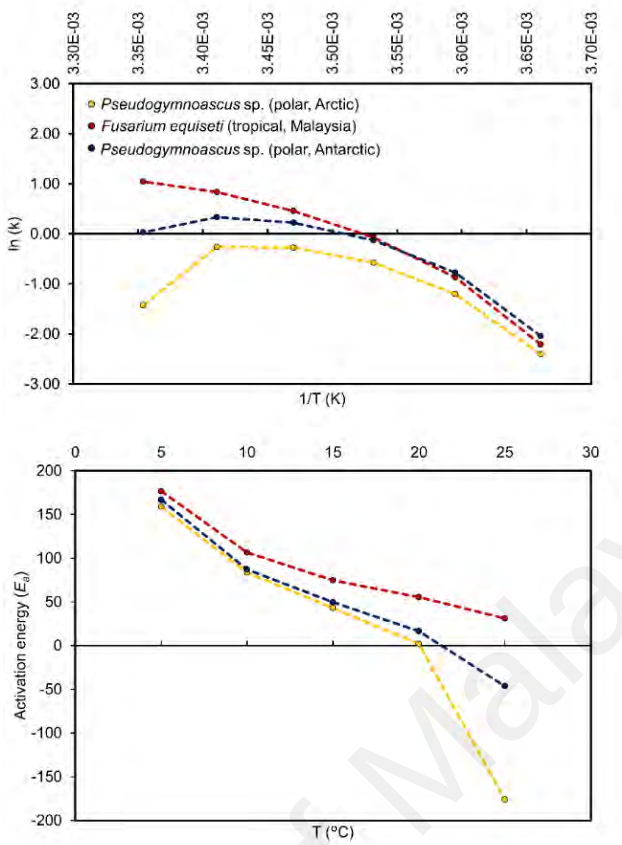

Figure 4.7: Arrhenius plot (left) of measured growth rates in the arctic strain of *Pseudogymnoascus* sp. (yellow), tropical strain of *F. equiseti* (red), and antarctic strain of *Pseudogymnoascus* sp. (blue) inoculated into seawater-based Mueller Hinton Broth across the experimental temperature series between 10 and 30°C. Activation energy (E_a) of growth (right) in the arctic strain of *Pseudogymnoascus* sp. (yellow), tropical strain of *F. equiseti* (red), and antarctic strain of *Pseudogymnoascus* sp. (blue) inoculated into seawater-based Mueller Hinton Broth.

4.4 Change in pH levels during growth

The pH levels of the seawater-based MHB culture medium throughout the 10 d growth experiment were recorded. Our preparation resulted in a slightly acidic medium on average (pH 6.5 – 7.0). However, pH levels increased with time when *F. equiseti* and *Pseudogymnoascus* spp. were grown in this medium across all culture temperatures at which growth occurred (Table 4.3).

Strain	T (°C)	Time (d) 0	1	2	3	4	5	6	7	8	9	10
1	5	6.5	6.5	7.5	7.4	6.5	6.4	6.5	6.5	6.7	6.6	6.6
	10	6.8	6.8	6.8	6.9	7.0	7.7	7.1	7.2	7.4	7.5	8.2
	15	6.8	6.8	6.8	6.9	7.0	7.2	7.3	7.8	7.2	8.3	7.4
	20	6.8	6.8	6.8	6.9	7.1	7.4	7.7	7.4	8.1	8.2	7.9
	25	6.8	6.8	6.9	7.0	7.0	7.5	7.6	7.4	8.6	8.4	8.7
	30	6.5	6.5	7.5	7.4	6.5	6.4	6.5	6.5	6.7	6.6	6.6
2	5	6.9	6.8	6.8	6.8	6.9	6.7	6.9	6.8	6.9	6.7	6.8
	10	6.9	7.0	7.0	7.0	6.9	6.9	7.0	7.6	7.8	7.5	8.2
	15	6.9	6.8	6.7	6.7	7.6	8.0	7.6	7.8	8.1	8.2	8.4
	20	6.7	6.8	7.6	7.9	8.8	8.9	8.5	8.5	8.4	8.7	8.6
	25	6.8	6.9	7.1	8.0	9.1	9.1	8.6	8.6	8.6	8.6	8.5
	30	7.1	7.8	8.6	9.0	8.5	8.3	8.5	8.4	8.5	8.6	8.6
3	5	6.8	6.8	6.7	6.8	6.8	7.0	7.1	7.0	7.4	8.0	7.8
	10	7.0	6.9	6.9	6.8	6.6	6.8	6.9	7.0	7.2	7.5	7.2
	15	6.9	7.0	7.1	6.7	7.0	7.6	8.2	8.3	8.5	8.5	8.6
	20	6.6	6.7	6.8	6.9	8.6	9.1	8.4	8.5	8.5	8.7	8.6
	25	6.9	6.8	7.0	7.8	8.5	8.6	8.1	7.3	8.1	7.6	7.0
	30	7.0	6.9	6.7	7.3	7.7	7.8	8.2	8.2	8.5	8.4	8.3

Table 4.3: Levels of pH of the culture medium during the 10 d growth experiment in (1) arctic strain of *Pseudogymnoascus* sp., (2) tropical strain of *F. equiseti*, and (3) antarctic strain of *Pseudogymnoascus* sp. under seawater-based Mueller Hinton Broth. Acidity and alkalinity of the culture medium are indicated by the red-white-blue gradation. Red indicates acidic, white neutral, and blue indicates alkaline pH levels.

The *r*ANOVA was applied to all culture medium pH datasets to determine the effect of temperature on pH levels on the culture medium over time, and to measure variability in differences in the pH measures. Mauchly's Test of Sphericity on the pH data from the tropical *F. equiseti* cultures between 5 and 30°C experimental temperatures tested indicated that the assumption of sphericity had been violated ($\chi^2 = 54.83$, $p < 0.001$). Therefore, degrees of freedom were corrected using a Huynh-Feldt estimate of sphericity, $\varepsilon = 0.99$. Results of *r*ANOVA with corrected sphericity showed that the effect of different culture temperatures on pH levels of culture medium inoculated with the tropical *Fusarium* sp. was significant, $F_{(4.95,\ 108.9)} = 1064.91$, $P < 0.001$, with a large effect size, $\eta^2 = 0.98$. Pairwise comparison between pH data across the six temperatures tested for the strain showed that the means obtained were significantly different from each other, except for those obtained at 20 and 25°C ($p = 1.0$).

Mauchly's Test of Sphericity on the pH data from the arctic *Pseudogymnoascus* sp. cultures across the six experimental temperatures tested again indicated that the assumption of sphericity had been violated ($\chi^2 = 101.49$, $p < 0.001$). Therefore, degrees

of freedom were corrected using a Huynh-Feldt estimate of sphericity, $\varepsilon = 0.99$. Results of *r*ANOVA with corrected sphericity showed that the effect of different culture temperatures on pH levels of culture medium inoculated with the arctic *Pseudogymnoascus* sp. was significant, $F_{(4.96, 109.0)} = 177.78$, $P < 0.001$, with a large effect size, $\eta^2 = 0.89$. Pairwise comparison between pH data across the six temperatures tested for the strain showed that the means obtained were significantly different from each other, except for those obtained at 10 and 15°C ($p = 0.564$).

Mauchly's Test of Sphericity performed on the pH data from the antarctic *Pseudogymnoascus* sp. also indicated that the assumption of sphericity had been violated ($\chi^2 = 91.11$, $p < 0.001$). Therefore, degrees of freedom were corrected using a Huynh-Feldt estimate of sphericity, $\varepsilon = 0.58$. Results of *r*ANOVA with corrected sphericity showed that the effect of different culture temperatures on pH levels of culture medium inoculated with the antarctic *Pseudogymnoascus* sp. was significant, $F_{(2.88, 63.4)} = 175.64$, $P < 0.001$, with a large effect size, $\eta^2 = 0.89$.

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 *Fusarium* and *Pseudogymnoascus* fungi as models in experimental studies

Fusarium and *Pseudogymnoascus* species can be used as models in relating thermal adaptation to global fungal distribution through reductionism. Mesophily and psychrophily have been observed in other fungal strains which are predominantly abundant in tropical and polar latitudes, respectively. Psychrophilic and psychrotolerant fungi are predominant in polar environmental substrates. However, polar environments also experience environmental variability due to climatic and/or seasonal variations. Environmental parameters such as temperature can reach as high as 25°C in soil microhabitats during boreal and austral summers in both Northern and Southern Hemispheres. Further, temperature can be exacerbated depending on physical features of dominant substrates making up a habitat (Convey, 1996; Krishnan *et al.*, 2011). Therefore, mesophilic fungal strains can also survive and be found in polar environments.

Fusarium and *Pseudogymnoascus* are fungal genera that are common in environmental samples originating from lower (tropical) and higher (temperate and polar) latitudes, respectively. Representatives of the two genera are widespread in marine environments, found in cryopegs and deep-sea invertebrates (Kochkina, *et al.*, 2007; Smith, 2007; Summerell, *et al.*, 2010; Batista-García, *et al.*, 2017). Some members can become pathogenic in the presence of preferred living hosts. A majority of well-characterised members of *Fusarium* (*F. oxysporum* Schltdl., and *F. graminearum* Schwabe, *F. solani* (Mart.) Saccardo, *F. equiseti* (Corda) Saccardo) are cosmopolitan plant pathogens colonising aerial and root structures in plants, and sediments/soils in which their hosts occur.

Several *Fusarium* species have been reported to grow differentially in response to specific environmental triggers such as temperature and nutrient sources; many studies report several *Fusarium* species to be dimorphic, growing as yeasts at temperatures higher than 30°C (Szécsi & Magyar, 2011). In comparison to *Fusarium*, there are currently fewer described *Pseudogymnoascus* species. However, *Pseudogymnoascus* spp. are often the most common fungal group isolated from both temperate and polar soils. The genus *Pseudogymnoascus* includes important species such as *P. pannorum* (Link) Minnis and D. L. Lindner 2013 which is a plant pathogen, and *P. destructans* (Blehert and Gargas) Minnis and D. L. Lindner 2013, the causative agent of white-nose syndrome (WNS) in bats in Europe and North America (Hayes, 2012; Verant, *et al.*, 2012).

5.2 Fungal growth rates are physical indicators of stress response and adaptation to varying environmental factors

The survival of a species in a natural habitat is ultimately dependent on its ability to grow at a rate sufficient to balance death. Microorganisms are remarkably physiologically versatile. Different environmental factors governing processes in a habitat influence physiology of organisms. Thus, the performance of a species in a culture medium does not necessarily reflect that in the natural environment. To pragmatically glimpse into the behaviour of the population in its natural habitat, however, researchers deduce ecology of a species from its behaviour in culture where environmental factors are known and controlled (Brock, 1971).

Nutrient-limited growth is a usual state for most microorganisms in the natural environment. In sediments and other solid growth substrates, organic carbon exists in recalcitrant macromolecules which are hydrolysed slowly by enzymes. In liquid growth systems, nutrients are absorbed until concentrations are decreased by microorganisms to

levels that are sufficient to maintain minimal growth. In both cases, the rate of microbial growth is set either by the transport of the nutrient of greatest need or by the rate of use of a previously stored reserve. Growth is observed by the rate of proliferation of a population, and is expressed in the change of a population over time.

Fungal thermal adaptation has also been investigated through several other approaches, including the expression of heat shock proteins (most commonly Hsp90), fluidity of the plasma membrane, production of antifreeze proteins (AFPs), trehalose and polyols including the principal sugar alcohol in fungi, ergosterol (Robinson, 2001; Niemenmaa *et al.*, 2008; Xiao *et al.*, 2010; Cowen, 2013). These are important in further understanding thermal biology in fungi. Without information on growth rates, however, complex and deeper experiments using these approaches would be difficult to develop. An investigator needs to understand growth behaviour and morphology of a fungus grown under any experimental condition.

Determining thermal energy requirements for growth in *F. equiseti* and *Pseudogymnoascus* spp. under different nutrient sources is key to understanding how these fungal strains are adapted to natural ambient temperatures, and may respond to changes in their natural habitats. Large amounts of carbon are stored as high-molecular weight (HMW) organic matter in the environment, and temperature is a primary factor driving fungal growth and hydrolytic processes of organic matter (German *et al.*, 2012). In comparison to *F. equiseti*, *Pseudogymnoascus* spp. exhibited consistently low relative growth rates when grown under different nutrient sources across the experimental culture temperatures. This gives insight to their adaptation strategy in conserving or utilising low amounts of thermal energy for growth under extreme environmental conditions.

Several studies have reported optimal temperatures for growth in *Pseudogymnoascus* spp. to range between 15 and 20°C (Zucconi *et al.*, 1996; Krishnan *et al.*, 2011; Hayes,

2012). The mesophilic marine-derived tropical strain of *F. equiseti*, on the other hand, is adapted to greater availability of thermal energy at progressively lower latitudes. Thus, it has a higher thermal optimum of approximately 25°C, and could tolerate a temperature as high as 30°C for growth. Other members of *Fusarium* have also been reported to grow and reproduce optimally between 25 and 30°C (Marin *et al.*, 1995; Doohan *et al.*, 2003). In nature, competitive interactions can restrict the growth of a fungus to a much narrower range than we find in laboratory conditions. When present, symbiotic interactions between fungi and other host organisms can also increase their tolerance to temperature stress (Redman *et al.*, 2002).

Based on the findings, polar strains of *Pseudogymnoascus* spp. required lower thermal energy than *F. equiseti* to grow optimally while accommodating to generally low temperatures and nutrient availability (as proxies for thermal and chemical energy, respectively) that are characteristic of the polar regions as temperature levels can vary dramatically both spatially and temporally in these habitats due to the interaction of various abiotic factors governed by regional climates (Convey, 1996; Nagelkerken, 2009).

Living fungal cells are in effect a system containing a mixture of biochemicals with pre-existing potential energy, whose reaction rates increase with higher input of thermal energy; this results in lower activation energy (E_a) being required to catalyse growth-related reactions in these strains (Battley, 2013; Peck, 2015). As seen in our data, marine-derived polar strains of *Pseudogymnoascus* spp. indeed grew optimally at around 20°C, and exhibited relatively high Q_{10} values for growth rates between 5 and 10°C, and lower values at higher temperatures. These data indicate that the polar strains of *Pseudogymnoascus* spp. are psychrophilic/psychrotolerant, while the possession of elevated Q_{10} values at low environmental temperatures has also been proposed as a

mechanism of stress tolerance adaptation in terrestrial biota of the polar regions (Convey, 1996).

Fungal members of other economically-important widespread genera such as *Aspergillus* and *Penicillium* have been classified as mesophiles with highest growth observed between 25 and 30°C. *Aspergillus* and *Penicillium* fungi tend to occur at lower latitudes between tropical and temperate regions, and have also been isolated from marine substrates (Meletiadis *et al.*, 2001; Larsson, 2009). Other commonly isolated cold-adapted fungal strains from higher latitudes (higher temperate and polar regions) include members of genera *Thelebolus* Tode and *Hebeloma* (Fr.) P. Kumm. Thermal growth range in these fungi fit the general definitions of psychrophily and psychrotolerance, as they can grow at temperatures as low as 4°C and exhibit growth optima at temperatures between 15 and 25°C (Bergero *et al.*, 1999; Geml *et al.*, 2012).

Fungal growth rates can be calculated using measures of hyphal length of single-spore inoculates and colony diameter in solid-state media, and wet or dry biomass under liquid-state culture conditions. Technical criteria set for these techniques are usually arbitrary, but sizes of mycelial plugs are standardised or fixed within an experimental setup (Liu *et al.*, 2008; Fuhr *et al.*, 2011). The first two techniques have their own limitation, in which measures obtained are two-dimensional. Thus, colony depth or thickness are not considered when measuring growth. However, these two techniques are not without their own merit. Fungal growth in natural environments is assessed by colony spread in order to evaluate severity of fungal infection. Biomass measures consider three-dimensional growth in fungi which otherwise would not be quantified when using solid-state culture systems. Also, this technique allows measures of changes in other parameters such as pH and salinity within a liquid culture system.

Despite the state differences of culture media in our experimental setup (solid versus liquid), our findings have shown that the profiles of E_a for growth in *Fusarium* and *Pseudogymnoascus* spp. are very similar, while also showing that the T_{opt} in these strains grown under both solid- and liquid-state culture media are consistent. These strains also showed better performance in terms of growth when grown under liquid conditions, perhaps due to continuous aeration of liquid cultures throughout the entire 10 d experiment (Casas López *et al.*, 2005; Liu *et al.*, 2008; Gomaa & El Bialy, 2009).

5.3 Activity of fungal extracellular hydrolytic enzymes (EHEs) corresponds to the composition and abundance of particulate organic matter (POM) in the marine environment

Adaptation and roles of fungal EHEs are better understood in pathogenesis than in other ecological processes, including nutrient cycling. Proteases, in particular, are common virulence factors in fungal strains preying upon nematodes and insects, and are primary biocatalysts of mycoses in animals including humans (Huang *et al.*, 2004; Yang *et al.*, 2007). Cellulases and amylases are important degradation catalysts of cellulose- and starch-based materials in the environment, which by implication are important virulence factors of fungal infection in plants (Beffa & Meins, 1996; González-Fernández *et al.*, 2010; Mathioni *et al.*, 2011).

To take advantage of various substrates available in marine environments, fungi such as those studied here may secrete a suite of EHEs that are active under the high salinity and pH levels that are characteristic of such environments. Halotolerance in EHEs from marine microbial strains has largely been identified and characterised in bacterial groups and in the marine water column, and knowledge of the expression and roles of fungal EHEs in the coastal environment is very limited at present (Arnosti *et al.*, 2014).

The data demonstrate that the *F. equiseti* and *Pseudogymnoascus* spp. strains studied can produce a range of functional EHEs that would allow utilisation of major environmental substrates. The ability of *F. equiseti* to secrete EHEs other than cellulases illustrates the versatility of this group of opportunistic microorganisms, with ability to grow under differing substrate availability. *Fusarium* fungi are most commonly associated with plant substrates, including living plant hosts as rampant pathogens. Therefore, they occur where vegetation is abundant, most often in lower latitudes and including in coastal environments (Summerell *et al.*, 2010).

St. Leger *et al.* (1997) argued that versatility in nutrient exploitation may not result from specific adaptation, and rather reflects ability to maintain the ability to exploit a wide range of substrates when they became temporarily present. However, the data indicate an absence of cellulolytic activity in marine-derived strains of *Pseudogymnoascus* spp. studied, even when the strains were growing on the R2A-based carboxylmethycellulose nutrient assay between 5 and 25°C.

Beyond upper critical temperatures for growth, enzymatic activity persisted in *F. equiseti* and *Pseudogymnoascus* spp. until 40°C. High *RA* values coupled with low fungal growth rates obtained from our experiments beyond optimum growth temperatures were possibly a result of increased substrate uptake rate by EHEs since higher thermal (higher temperature) energy was introduced into the culture system (Somero, 1978; Somero, 2004). Conversely, high *RA* values of EHEs at low temperatures were only seen in *Pseudogymnoascus* spp. This could be due to higher synthesis rates of EHEs by the fungi instead of higher enzymatic efficiency per unit of enzyme.

Psychrophilic enzymes generally show maximum activity at lower temperatures between 20 and 40°C in contrast to mesophilic enzymes, which can be chemically stable over at a higher and a wider range of temperature. Numerous mesophilic enzymes have

been recorded to show optimum activity at temperatures as high as 70°C. To compensate for the slow reaction rate, cold-adapted organisms synthesise psychrophilic enzymes having an up to ten-fold higher specificity at lower temperatures (Feller, 2013).

While there are examples of enzymes demonstrating impaired function at low temperatures, most enzymes (psychrophilic or otherwise) exhibit low-rate activity at 0 to 15°C. Enzymes from psychrophiles have thermal thresholds of inactivation at 28°C and optimum catalytic temperatures from 40 to 60°C. Maximum enzyme activity may also be observed at low temperature (5°C) but optimum temperature of activity may well be at relatively high temperatures (40 to 50°C), as in the case of polygalacturonase in snow mould *Sclerotinia borealis* (Tojo & Newsham, 2012). The relationship between enzyme kinetics and low optimum temperature in psychrophilic growth is yet to be resolved. Fungi can either be metabolically psychrophilic or mesophilic, and not necessarily be either one in terms of growth. Factors of growth at low temperatures remain unidentified and most evidence does not imply any enzyme to be sole determinant of obligate psychrophily or psychrotrophy (Gerday *et al.*, 2000).

Both *Fusarium* and *Pseudogymnoascus* species are known producers of proteases and amylases. The majority of activity of extracellular proteases and amylases has been reported in two *Pseudogymnoascus* species, *P. pannorum* and *P. destructans* (Pekkarinen *et al.*, 2000; Krishnan *et al.*, 2011; Pannkuk *et al.*, 2014; Gao *et al.*, 2016). Contradicting results between growth and cellulase activity as seen in *Pseudogymnoascus* spp. might be explained in three ways: (a) these strains were preferentially degrading minute amounts of glucose, peptone, or starch that was present in R2A due to it being a simpler organic substrate than carboxylmethylcellulose, (b) they possess cell-wall-associated cellulases (also known as endoglucanases); these enzymes are not released into the media, thus not degrading carboxylmethylcellulose present around the fungal colony, and/or (c) this

might result from adaptation through loss of function under environments that typically have limited cellulosic materials (Adams, 2004). Previous enzyme screening studies have given mixed reports on cellulase activity in *Pseudogymnoascus* spp. antarctic strains of *P. pannorum* isolated from various sites showed moderate to no cellulase activity at 25°C in non-soluble enzyme assays (Fenice *et al.*, 1997; Duncan *et al.*, 2008; Krishnan *et al.*, 2011). In contrast, *Pseudogymnoascus* strains associated with deep-sea sponges in the Irish Sea were reported to show cellulase activity (Batista-García *et al.*, 2017).

Nutrient profiles across arctic, antarctic, and tropical coastal waters are similar, in which proteins, followed by carbohydrates, and then lipids remain the largest denominators of characterised POM present in the marine water column (Fabiano & Pusceddu, 1998; Huston & Deming, 2002; Cunha *et al.*, 2010). Our data suggest that polar strains of *Pseudogymnoascus* spp. may have adapted to the scarcity of plant-based substrates such as celluloses at higher latitudes, and rather express preference for proteins and other simpler carbohydrates for extracellular digestion over celluloses. It is possible that members of *Pseudogymnoascus* prevalent at higher latitudes are under selective pressure to survive in habitats with a lower diversity of nutrient sources than is typical at lower latitudes.

Schneider *et al.* (2012) provided evidence of local temporal EHE adaptation in Austrian temperate forests, where litter nutrient content and stoichiometry of C:N:P affect the decomposer community and activity. Fungi were found to be the main producers of EHEs, and ascomycetous EHEs were succeeded by those of basidiomycetes with the seasonal transition from spring to summer as the forest floor was increasingly littered with recalcitrant lignin-containing substrates. Spatial adaptation in EHEs has also been demonstrated (German *et al.*, 2012), with cold-adapted cellulose-degrading β-glucosidase from higher latitudes being found to be more temperature-sensitive than that from lower

latitudes. Similar observations, however, are still lacking for microbial communities in coastal/marine sediments (Hyde and Lee, 2015).

The enzyme screening data obtained clearly demonstrated that the proteases and amylases produced by the tropical *F. equiseti* and polar *Pseudogymnoascus* spp. were all both cold-active (i.e. capable of functioning at low temperature) as well as thermotolerant, exhibiting activity across the experimental temperature range to as high as 40°C. The results showed that there was a trade-off between radial growth and EHE activity across the experimental temperature range from 5 to 40°C in all three strains studied. However, higher *RA* values exhibited by these strains at temperatures at which growth was lower was possibly a result of enzyme kinetics and functional efficiency rather than increased enzyme production rate by fungal cells in response to varying temperature. Production of EHEs and degradation of complex organic matter are also energy-consuming.

Hence, the association of EHEs to the fungal cell wall and secretion of EHEs outside fungal cells may be a kingdom-wide strategy to minimise energy uptake for metabolism in fungi. Fungal EHEs function independently of their original producers, and break down high molecular-weight organic matter extracellularly. Through osmotrophy, fungi do not need to absorb more energy into their cells to initiate and maintain growth-related reactions. Our data indicate a clear energy trade-off when a specific type of EHE was produced and secreted extracellularly, as seen with *F. equiseti* showing lower growth rates while exhibiting higher relative celluloytic activity in the R2A-based carboxylmethylcellulose assay than in the skim milk and starch nutrient assays (the latter screening for protease and amylase, respectively). This suggests that the production of EHEs degrading more chemically complex environmental substrates may require higher metabolic energy in fungi, diverting cellular energy resources away from growth

processes, and rewiring of the metabolic network in specific fungal strains adapted to different environments.

5.4 By-product of fungal metabolism contributes to regulation of pH levels in seawater

Ocean acidification rates are a function of the atmospheric-oceanic exchange rate of CO_2. Oceans are acidifying at a subjectively low rate presently, but the rate is predicted to increase with increasing CO_2 concentrations. The CO_2 level in the atmosphere has increased as much as 100 ppm over the past century, with recent CO_2 concentration nearing 400 ppm (Flynn *et al.*, 2015).

Fungi alter pH according to substrate availability within a culture system. St. Leger *et al.* (1999) demonstrated that the fungus *Metarhizium anisopliae* excreted ammonium ions (NH_4^+) to increase pH levels of the culture media in order to optimise protease activity under alkaline conditions. Fungal amylases show optimum activity between pH 4.0 and 7.0 in some fungal strains, while optimum activity of fungal cellulases commonly falls between pH 6.0 and 7.0 (Yamanaka, 2003; Sunitha *et al.*, 2012; Ominyi *et al.*, 2013).

Proteinaceous substrates are the most abundant particulate organic matter in the marine environment, followed by carbohydrates, and lipids. Correspondingly, proteases are found to be highly expressed in environmental samples than other subclasses of hydrolases including glycohydrolases (e.g. amylases, cellulases, and ligninases) and lipases (Dong *et al.*, 2014). Proteases generally function optimally under alkaline pH levels between 8 and 10. Some proteases can be alkalophilic, meaning they function best at pH levels between 11 and 14. Substrates in the marine environment, which are

continuously exposed to seawater temporally and/or spatially, have an average pH level of approximately 8.

This study demonstrated that *F. equiseti* and *Pseudogymnoascus* spp. altered pH levels of the seawater-modified Mueller-Hinton Broth as they continued to grow into the stationary phase. Following previously mentioned premises, it is possible that fungal components of the microbial community in marine environments are indirectly contributing to the buffering of pH levels in seawater. Microbial contribution to maintaining physico-chemical features of an ecosystem or a habitat is seldom, if ever, quantified and discussed within the context of organismal response to environmental variability.

5.5 Temperature variability impacts biotic and abiotic process in the marine environment

Natural populations are responding to global climate change by shifting their geographical distribution and timing of growth and reproduction, and these changes are, in turn, altering the composition of communities and the nature of species interactions (Walther *et al.*, 2002; Kardol *et al.*, 2015). However, the responses of many populations are likely to be inadequate to counter the speed and magnitude of climate change, leaving some organisms vulnerable to decline and extinction. Extinction can be avoided if populations move to favourable habitats, organisms successfully overcome stressful conditions via plastic changes, or populations undergo evolutionary adaptation. Fungi, being a versatile and opportunistic group, will follow suit and pose an impact to the environment in a way and at a magnitude that is challenging for us to extrapolate.

In the context of climate warming, a rise in regional surface temperatures may facilitate the spread of fungal plant and animal pathogens (Fisher *et al.*, 2012). Saprophytic fungi may become pathogenic when ambient temperature is higher, increasing incidence of pathogenicity in fungi. When no other factor is present to denature the chemical structure and function of EHEs, degradation of substrates continues until they are exhausted. Products of degraded substrates would not be sequestrated into living systems when life processes are too slow to compensate for cell death at higher temperatures. Consequently, this would the alter composition of organic matter in the environment, leading to multi-level changes within ecosystems. Some of the immediate consequences include alteration in microbial community composition and food webs; introducing shifts in physico-chemical characteristics of the environment and ultimately ecological functions and roles of fungi (Garrett *et al.*, 2006; De Bellis *et al.*, 2007; Classen *et al.*, 2015).

5.6 Future studies

The acknowledged limitation of this study may be addressed in future studies on thermal adaptation in fungi. We suggest the following:

a) Profiling growth and studying fungal growth physiology under varying pH levels;
b) Analysing the Gibbs' free energy required by fungi to grow under various environmental parameters e.g. temperature, nutrient sources, pH, ultraviolet radiation (UVR), etc.;
c) Measuring specific activity of extracellular hydrolytic enzymes (EHEs) and purifying these enzymes for structural elucidation;
d) Quantifying gene expression and mapping thermal response pathways with a focus on the expression of EHEs in selected fungal strains using transcriptomic approaches; and

e) Testing field hypotheses using environmental -omics to characterise microbial (fungal) and molecular functional groups from marine-derived substrates.

5.7 Conclusion

The results of this study have shown that fungal strains originating from different bioclimatic regions adapt to specific ranges of ambient temperature and favour chemically simpler substrates for growth. The marine-derived tropical strain of *F. equiseti* is mesophilic, growing optimally between 24.3 and 25.0°C. *Pseudogymnoascus* spp. was psychrophilic/psychrotolerant, growing optimally between 19.5 and 20.1°C. Activation energies (E_a) for growth calculated based on relative and specific growth rates (R_r and R_s, respectively) of strains were negative beyond 25°C for *F. equiseti* and 20°C for *Pseudogymnoascus* spp. *F. equiseti* requires more activation energy for growth than do the polar strains of *Pseudogymnoascus* spp., indicating that these strains adapt to ambient temperatures typical of their original habitats. Skim milk and starch are a more accessible source of nutrients for *F. equiseti* and *Pseudogymnoascus* spp. than is carboxylmethylcellulose. *F. equiseti* grew when supplied with all three substrates, and exhibits protease, amylase and cellulase activities. However, *Pseudogymnoascus* spp. only shows protease and amylase activities. Increased pH was observed during the growth (increased biomass) of *F. equiseti* and *Pseudogymnoascus* spp. in the liquid culture system, suggesting that utilisation of substrates through EHE activity (mainly protease and amylase) was facilitated by alkaline conditions.

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APPENDICES

APPENDIX A

Table 1: Descriptive statistics and parameter values for best-fit functions of relative growth rates (R_r) of *Fusarium equiseti* and *Pseudogymnoascus* spp. grown across different nutrient assays between 5 and 40°C under third-degree polynomial and Brière-2 nonlinear models.

			Nutrient		
Strain	**Model**	**Parameter**	**A**	**B**	**C**
1	Third-degree polynomial	a	6.424×10^{-5}	-8.777×10^{-6}	9.027×10^{-5}
		b	-0.005	-0.004	-0.007
		c	0.115	0.190	0.160
		d	-0.358	-0.913	-0.648
		R^2	0.83	0.82	0.91
		RSS	1.48	17.24	1.58
		cAIC	-5.52	14.14	-4.98
	Brière-2	a ($\times 10^{-4}$)	4	4	4
		T_{min}	0	0	0
		T_{max}	30.2	30.294	30.2
		m	2.09	2.11	2.09
		R^2	0.87	0.91	0.81
		RSS	3.85	11.40	10.29
		cAIC	2.15	10.83	10.02
2	Third-degree polynomial	a	6.375×10^{-5}	1.368×10^{-6}	9.660×10^{-5}
		b	-0.005	-0.004	-0.008
		c	0.116	0.184	0.168
		d	-0.366	-0.771	-0.672
		R^2	0.82	0.78	0.93
		RSS	1.50	16.43	1.66
		cAIC	-5.40	13.76	-4.57
	Brière-2	a ($\times 10^{-4}$)	10.6	10.3	7.5
		T_{min}	5	5	5
		T_{max}	35	35	35
		m	2.16	2.16	2.16
		R^2	0.80	0.80	0.74
		RSS	3.78	14.28	9.70
		cAIC	1.99	12.64	9.54
3	Third-degree polynomial	a	5.888×10^{-5}	2.679×10^{-5}	1.031×10^{-4}
		b	-0.005	-0.005	-0.008
		c	0.105	0.171	0.168
		d	-0.299	-0.753	-0.615
		R^2	0.82	0.84	0.86
		RSS	1.40	8.88	1.68
		cAIC	-5.96	8.83	-4.47
	Brière-2	a ($\times 10^{-4}$)	2.6	2.5	2.3
		T_{min}	5	5	5
		T_{max}	28.6	28.2	27.8
		m	1.14	1.09	1.03
		R^2	0.66	0.45	0.46
		RSS	3.70	15.26	5.17
		cAIC	1.83	13.17	4.51

(1) Arctic strain of *Pseudogymnoascus* sp. (A) Skim milk,
(2) Tropical strain of *Fusarium equiseti* (B) Soluble starch
(3) Antarctic strain of *Pseudogymnoascus* sp. (C) Carboxylmethylcellulose

Table 2: Descriptive statistics and parameter values for best-fit functions of biomass of *Fusarium equiseti* and *Pseudogymnoascus* spp. grown in seawater-based Mueller Hinton Broth (30 psu) between 5 and 30°C under Gompertz and Richards' logistic sigmoidal models.

			Temperature (°C)					
Strain	**Model**	**Parameter**	**5**	**10**	**15**	**20**	**25**	**30**
	Gompertz	*A*	9	50	80	150	120	100
		B	5	7	40	27	2000	4
		k	0.2083	0.2674	0.7097	0.5794	0.8452	0.2357
		R^2	0.93	34.99	0.54	0.86	0.52	0.26
		RSS	7.66	0.86	217.1	204.4	231.0	318.4
		cAIC	5.34	20.5	38.8	38.2	39.4	42.6
	Richards' logistic	*K*	20	50	90	150	230	100
1		*A*	0	0	0	0	0	0
		C	1	1	1	1	1	1
		Q	1.1	1.2	0.7	1.6	1	1.7
		v	0.1	0.1	0.1	0.007	0.001	0.1
		k	0.2083	0.2674	0.7097	0.5794	0.8452	0.2357
		R^2	0.86	0.86	0.56	0.85	0.85	0.30
		RSS	4.97	31.7	220.8	201.3	290.5	316.6
		cAIC	7.01	25.6	44.9	44.0	47.7	48.6
	Gompertz	*A*	4	130	135	120	130	90
		B	4	27	80	130	120	30
		k	1.49	0.4109	0.8118	2.0612	1.7972	2.9434
		R^2	0.07	0.87	0.95	0.84	0.94	0.90
		RSS	21.6	77.3	83.7	129.1	124.2	71.7
		cAIC	15.7	28.5	29.2	33.6	33.2	27.7
	Richards' logistic	*K*	5	90	125	125	140	90
2		*A*	0	0	0	0	0	0
		C	1	1	1	1	1	1
		Q	3	1.1	1.1	0.9	0.9	0.8
		v	0.3	0.04	0.009	0.02	0.02	0.06
		k	1.49	0.4109	0.8118	2.0612	1.7972	2.9434
		R^2	0.16	0.85	0.95	0.85	0.94	0.86
		RSS	17.8	54.7	81.4	51.4	112.7	77.1
		cAIC	19.8	31.0	34.97	30.4	38.2	34.4
	Gompertz	*A*	70	45.6	150	110	130	60
		B	10	15	80	335	40	25
		k	0.3588	0.3893	0.9946	1.1543	1.4462	1.0213
		R^2	0.74	0.86	0.96	0.96	0.90	0.53
		RSS	129.7	42.0	86.7	76.4	157.0	135.8
		cAIC	33.6	22.4	29.6	28.3	35.5	34.1
	Richards' logistic	*K*	70	45.6	135	110	135	65
3		*A*	0	0	0	0	0	0
		C	1	1	1	1	1	1
		Q	2.6	1.1	1.2	1	1.95	1
		v	0.008	0.09	0.007	0.03	0.09	0.03
		k	0.3588	0.3893	0.9446	1.1543	1.4462	1.0213
		R^2	0.84	0.84	0.96	0.96	0.90	0.53
		RSS	34.5	44.1	74.9	102.6	77.6	155.4
		cAIC	34.1	28.8	37.3	34.5	41.4	39.7

Table 3: Descriptive statistics and parameter values for best-fit functions of specific growth rates (R_s) of *Fusarium equiseti* and *Pseudogymnoascus* spp. grown in seawater-based Mueller Hinton Broth (30 psu) between 5 and 40°C under Brière-2 nonlinear models.

Strain	Parameter		Strain	Parameter		Strain	Parameter	
1	a ($\times 10^{-4}$)	4	2	a ($\times 10^{-4}$)	2.9	3	a ($\times 10^{-4}$)	5
	T_{min}	0		T_{min}	0		T_{min}	0
	T_{max}	30.7		T_{max}	31.7		T_{max}	33.1
	m	1.425		m	8.503		m	1.438
	R^2	0.90		R^2	0.75		R^2	0.97
	RSS	2.72		RSS	8.19		RSS	5.15

APPENDIX B

Repeated measures ANOVA SPSS output on biomass data from the arctic strain of *Pseudogymnoascus* spp.

Within-Subjects Factors

Measure: MEASURE_1

temp	Dependent Variable
1	mg_5
2	mg_10
3	mg_15
4	mg_20
5	mg_25
6	mg_30

Between-Subjects Factors

		N
time (d)	0	3
	1	3
	2	3
	3	3
	4	3
	5	3
	6	3
	7	3
	8	3
	9	3
	10	3

Descriptive Statistics

	time (d)	Mean	Std. Deviation	N
mg_5	0	1.8667	1.34288	3
	1	1.8333	1.90351	3
	2	3.3333	.30551	3
	3	2.4333	1.15036	3
	4	2.4333	.51316	3
	5	1.1667	.05774	3
	6	3.6333	.55076	3
	7	5.5667	1.30512	3
	8	5.9667	.23094	3
	9	6.6000	1.47309	3
	10	8.3000	1.27671	3
	Total	3.9212	2.42857	33
mg_10	0	6.7667	1.10151	3
	1	5.8667	1.60728	3
	2	4.3333	.40415	3
	3	5.7333	.64291	3
	4	30.2333	33.15755	3
	5	31.5667	13.64197	3
	6	8.9333	2.02567	3
	7	11.5333	2.29420	3
	8	129.2333	40.51448	3
	9	23.3000	11.18526	3
	10	54.2667	22.16175	3
	Total	28.3424	38.70474	33
mg_15	0	1.5667	1.20968	3
	1	.4333	.72342	3
	2	-.3000	.52915	3
	3	58.6667	53.04454	3
	4	5.3667	1.01160	3
	5	58.9000	12.42860	3
	6	17.1000	19.81994	3
	7	62.6333	58.85791	3
	8	71.7000	36.08338	3
	9	150.4667	68.93420	3
	10	62.0667	32.93969	3
	Total	44.4182	53.38557	33
mg_20	0	9.0333	2.04287	3
	1	11.8333	2.35018	3
	2	-44.1333	51.68465	3
	3	15.4667	1.80093	3
	4	-14.4667	12.26635	3
	5	43.1667	10.94182	3
	6	75.0667	24.26857	3
	7	50.3000	68.32591	3
	8	136.1667	39.29902	3
	9	178.8333	51.04570	3
	10	150.5000	27.44977	3
	Total	55.6152	75.33638	33
mg_25	0	6.1333	1.09697	3
	1	4.7333	1.24231	3
	2	10.4000	3.95095	3
	3	-3.1000	24.34399	3
	4	21.7333	15.87839	3
	5	69.8000	76.70078	3
	6	29.7667	19.65714	3
	7	17.0000	70.96189	3
	8	19.1667	32.15110	3
	9	149.9333	39.11091	3
	10	86.6333	10.41361	3
	Total	37.4727	54.34002	33
mg_30	0	59.4000	23.68882	3
	1	115.7333	38.60380	3
	2	53.4667	75.44669	3
	3	123.6333	18.18965	3
	4	107.0000	34.91461	3
	5	23.3667	44.30963	3
	6	73.5667	47.80558	3
	7	91.2000	48.70554	3
	8	146.7000	68.23994	3
	9	157.7333	66.29286	3
	10	125.3667	11.37380	3
	Total	97.9242	56.58888	33

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
temp	Pillai's Trace	.959	84.881[b]	5.000	18.000	.000	.959
	Wilks' Lambda	.041	84.881[b]	5.000	18.000	.000	.959
	Hotelling's Trace	23.578	84.881[b]	5.000	18.000	.000	.959
	Roy's Largest Root	23.578	84.881[b]	5.000	18.000	.000	.959
temp * time	Pillai's Trace	2.932	3.119	50.000	110.000	.000	.586
	Wilks' Lambda	.002	4.711	50.000	85.457	.000	.701
	Hotelling's Trace	20.874	6.847	50.000	82.000	.000	.807
	Roy's Largest Root	14.007	30.816[c]	10.000	22.000	.000	.933

a. Design: Intercept + time
Within Subjects Design: temp

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
temp	.063	55.687	14	.000	.516	.855	.200

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + time
Within Subjects Design: temp

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Sphericity Assumed	162845.448	5	32569.090	29.551	.000	.573
	Greenhouse-Geisser	162845.448	2.578	63171.128	29.551	.000	.573
	Huynh-Feldt	162845.448	4.277	38074.520	29.551	.000	.573
	Lower-bound	162845.448	1.000	162845.448	29.551	.000	.573
temp * time	Sphericity Assumed	178355.714	50	3567.114	3.237	.000	.595
	Greenhouse-Geisser	178355.714	25.778	6918.788	3.237	.000	.595
	Huynh-Feldt	178355.714	42.770	4170.094	3.237	.000	.595
	Lower-bound	178355.714	10.000	17835.571	3.237	.010	.595
Error(temp)	Sphericity Assumed	121235.517	110	1102.141			
	Greenhouse-Geisser	121235.517	56.713	2137.717			
	Huynh-Feldt	121235.517	94.094	1288.445			
	Lower-bound	121235.517	22.000	5510.705			

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	temp	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Linear	121947.749	1	121947.749	107.145	.000	.830
	Quadratic	735.842	1	735.842	.344	.563	.015
	Cubic	23933.917	1	23933.917	38.163	.000	.634
	Order 4	12862.085	1	12862.085	13.490	.001	.380
	Order 5	3365.855	1	3365.855	5.134	.034	.189
temp * time	Linear	31414.601	10	3141.460	2.760	.023	.556
	Quadratic	59357.945	10	5935.794	2.778	.022	.558
	Cubic	32476.905	10	3247.691	5.179	.001	.702
	Order 4	19681.964	10	1968.196	2.064	.075	.484
	Order 5	35424.299	10	3542.430	5.403	.000	.711
Error(temp)	Linear	25039.509	22	1138.160			
	Quadratic	46999.354	22	2136.334			
	Cubic	13797.116	22	627.142			
	Order 4	20976.403	22	953.473			
	Order 5	14423.134	22	655.597			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	394130.249	1	394130.249	434.838	.000	.952
time	198378.153	10	19837.815	21.887	.000	.909
Error	19940.470	22	906.385			

Estimates

Measure: MEASURE_1

temp	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	3.921	.189	3.529	4.313
2	28.342	3.130	21.851	34.834
3	44.418	6.203	31.554	57.283
4	55.615	6.011	43.149	68.081
5	37.473	6.394	24.213	50.733
6	97.924	8.315	80.681	115.168

Pairwise Comparisons

Measure: MEASURE_1

(I) temp	(J) temp	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-24.421*	3.117	.000	-30.885	-17.957
	3	-40.497*	6.217	.000	-53.390	-27.603
	4	-51.694*	6.058	.000	-64.257	-39.131
	5	-33.552*	6.349	.000	-46.719	-20.384
	6	-94.003*	8.296	.000	-111.208	-76.798
2	1	24.421*	3.117	.000	17.957	30.885
	3	-16.076*	6.205	.017	-28.945	-3.206
	4	-27.273*	6.589	.000	-40.937	-13.609
	5	-9.130	6.863	.197	-23.363	5.103
	6	-69.582*	9.704	.000	-89.706	-49.458
3	1	40.497*	6.217	.000	27.603	53.390
	2	16.076*	6.205	.017	3.206	28.945
	4	-11.197	5.550	.056	-22.706	.312
	5	6.945	8.167	.404	-9.992	23.883
	6	-53.506*	12.208	.000	-78.824	-28.188
4	1	51.694*	6.058	.000	39.131	64.257
	2	27.273*	6.589	.000	13.609	40.937
	3	11.197	5.550	.056	-.312	22.706
	5	18.142	9.305	.064	-1.156	37.441
	6	-42.309*	12.037	.002	-67.272	-17.346
5	1	33.552*	6.349	.000	20.384	46.719
	2	9.130	6.863	.197	-5.103	23.363
	3	-6.945	8.167	.404	-23.883	9.992
	4	-18.142	9.305	.064	-37.441	1.156
	6	-60.452*	10.322	.000	-81.859	-39.044
6	1	94.003*	8.296	.000	76.798	111.208
	2	69.582*	9.704	.000	49.458	89.706
	3	53.506*	12.208	.000	28.188	78.824
	4	42.309*	12.037	.002	17.346	67.272
	5	60.452*	10.322	.000	39.044	81.859

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.959	84.881[a]	5.000	18.000	.000	.959
Wilks' lambda	.041	84.881[a]	5.000	18.000	.000	.959
Hotelling's trace	23.578	84.881[a]	5.000	18.000	.000	.959
Roy's largest root	23.578	84.881[a]	5.000	18.000	.000	.959

Each F tests the multivariate effect of temp. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

2. time (d) * temp

Measure: MEASURE_1

time (d)	temp	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	1	1.867	.627	.567	3.167
	2	6.767	10.382	-14.764	28.298
	3	1.567	20.573	-41.100	44.233
	4	9.033	19.936	-32.311	50.378
	5	6.133	21.206	-37.845	50.112
	6	59.400	27.576	2.210	116.590
1	1	1.833	.627	.533	3.133
	2	5.867	10.382	-15.664	27.398
	3	.433	20.573	-42.233	43.100
	4	11.833	19.936	-29.511	53.178
	5	4.733	21.206	-39.245	48.712
	6	115.733	27.576	58.543	172.923
2	1	3.333	.627	2.033	4.633
	2	4.333	10.382	-17.198	25.864
	3	-.300	20.573	-42.966	42.366
	4	-44.133	19.936	-85.478	-2.789
	5	10.400	21.206	-33.579	54.379
	6	53.467	27.576	-3.723	110.657
3	1	2.433	.627	1.133	3.733
	2	5.733	10.382	-15.798	27.264
	3	58.667	20.573	16.000	101.333
	4	15.467	19.936	-25.878	56.811
	5	-3.100	21.206	-47.079	40.879
	6	123.633	27.576	66.443	180.823
4	1	2.433	.627	1.133	3.733
	2	30.233	10.382	8.702	51.764
	3	5.367	20.573	-37.300	48.033
	4	-14.467	19.936	-55.811	26.878
	5	21.733	21.206	-22.245	65.712
	6	107.000	27.576	49.810	164.190
5	1	1.167	.627	-.133	2.467
	2	31.567	10.382	10.036	53.098
	3	58.900	20.573	16.234	101.566
	4	43.167	19.936	1.822	84.511
	5	69.800	21.206	25.821	113.779
	6	23.367	27.576	-33.823	80.557
6	1	3.633	.627	2.333	4.933
	2	8.933	10.382	-12.598	30.464
	3	17.100	20.573	-25.566	59.766
	4	75.067	19.936	33.722	116.411
	5	29.767	21.206	-14.212	73.745
	6	73.567	27.576	16.377	130.757
7	1	5.567	.627	4.267	6.867
	2	11.533	10.382	-9.998	33.064
	3	62.633	20.573	19.967	105.300
	4	50.300	19.936	8.956	91.644
	5	17.000	21.206	-26.979	60.979
	6	91.200	27.576	34.010	148.390
8	1	5.967	.627	4.667	7.267
	2	129.233	10.382	107.702	150.764
	3	71.700	20.573	29.034	114.366
	4	136.167	19.936	94.822	177.511
	5	19.167	21.206	-24.812	63.145
	6	146.700	27.576	89.510	203.890
9	1	6.600	.627	5.300	7.900
	2	23.300	10.382	1.769	44.831
	3	150.467	20.573	107.800	193.133
	4	178.833	19.936	137.489	220.178
	5	149.933	21.206	105.955	193.912
	6	157.733	27.576	100.543	214.923
10	1	8.300	.627	7.000	9.600
	2	54.267	10.382	32.736	75.798
	3	62.067	20.573	19.400	104.733
	4	150.500	19.936	109.156	191.844
	5	86.633	21.206	42.655	130.612
	6	125.367	27.576	68.177	182.557

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) time (d)	(J) time (d)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	-9.2778	10.03541	1.000	-47.7274	29.1718
	2	9.6111	10.03541	1.000	-28.8385	48.0607
	3	-19.6778	10.03541	1.000	-58.1274	18.7718
	4	-11.2556	10.03541	1.000	-49.7052	27.1941
	5	-23.8667	10.03541	1.000	-62.3163	14.5829
	6	-20.5500	10.03541	1.000	-58.9996	17.8996
	7	-25.5778	10.03541	1.000	-64.0274	12.8718
	8	-70.6944*	10.03541	.000	-109.1441	-32.2448
	9	-97.0167*	10.03541	.000	-135.4663	-58.5671
	10	-67.0611*	10.03541	.000	-105.5107	-28.6115
1	0	9.2778	10.03541	1.000	-29.1718	47.7274
	2	18.8889	10.03541	1.000	-19.5607	57.3385
	3	-10.4000	10.03541	1.000	-48.8496	28.0496
	4	-1.9778	10.03541	1.000	-40.4274	36.4718
	5	-14.5889	10.03541	1.000	-53.0385	23.8607
	6	-11.2722	10.03541	1.000	-49.7218	27.1774
	7	-16.3000	10.03541	1.000	-54.7496	22.1496
	8	-61.4167*	10.03541	.000	-99.8663	-22.9671
	9	-87.7389*	10.03541	.000	-126.1885	-49.2893
	10	-57.7833*	10.03541	.000	-96.2329	-19.3337
2	0	-9.6111	10.03541	1.000	-48.0607	28.8385
	1	-18.8889	10.03541	1.000	-57.3385	19.5607
	3	-29.2889	10.03541	.438	-67.7385	9.1607
	4	-20.8667	10.03541	1.000	-59.3163	17.5829
	5	-33.4778	10.03541	.165	-71.9274	4.9718
	6	-30.1611	10.03541	.358	-68.6107	8.2885
	7	-35.1889	10.03541	.110	-73.6385	3.2607
	8	-80.3056*	10.03541	.000	-118.7552	-41.8559
	9	-106.6278*	10.03541	.000	-145.0774	-68.1782
	10	-76.6722*	10.03541	.000	-115.1218	-38.2226
3	0	19.6778	10.03541	1.000	-18.7718	58.1274
	1	10.4000	10.03541	1.000	-28.0496	48.8496
	2	29.2889	10.03541	.438	-9.1607	67.7385
	4	8.4222	10.03541	1.000	-30.0274	46.8718
	5	-4.1889	10.03541	1.000	-42.6385	34.2607
	6	-.8722	10.03541	1.000	-39.3218	37.5774
	7	-5.9000	10.03541	1.000	-44.3496	32.5496
	8	-51.0167*	10.03541	.002	-89.4663	-12.5671
	9	-77.3389*	10.03541	.000	-115.7885	-38.8893
	10	-47.3833*	10.03541	.006	-85.8329	-8.9337
4	0	11.2556	10.03541	1.000	-27.1941	49.7052
	1	1.9778	10.03541	1.000	-36.4718	40.4274
	2	20.8667	10.03541	1.000	-17.5829	59.3163
	3	-8.4222	10.03541	1.000	-46.8718	30.0274
	5	-12.6111	10.03541	1.000	-51.0607	25.8385
	6	-9.2944	10.03541	1.000	-47.7441	29.1552
	7	-14.3222	10.03541	1.000	-52.7718	24.1274
	8	-59.4389*	10.03541	.000	-97.8885	-20.9893
	9	-85.7611*	10.03541	.000	-124.2107	-47.3115
	10	-55.8056*	10.03541	.001	-94.2552	-17.3559
5	0	23.8667	10.03541	1.000	-14.5829	62.3163
	1	14.5889	10.03541	1.000	-23.8607	53.0385
	2	33.4778	10.03541	.165	-4.9718	71.9274
	3	4.1889	10.03541	1.000	-34.2607	42.6385
	4	12.6111	10.03541	1.000	-25.8385	51.0607
	6	3.3167	10.03541	1.000	-35.1329	41.7663
	7	-1.7111	10.03541	1.000	-40.1607	36.7385
	8	-46.8278*	10.03541	.007	-85.2774	-8.3782
	9	-73.1500*	10.03541	.000	-111.5996	-34.7004
	10	-43.1944*	10.03541	.016	-81.6441	-4.7448
6	0	20.5500	10.03541	1.000	-17.8996	58.9996
	1	11.2722	10.03541	1.000	-27.1774	49.7218
	2	30.1611	10.03541	.358	-8.2885	68.6107
	3	.8722	10.03541	1.000	-37.5774	39.3218
	4	9.2944	10.03541	1.000	-29.1552	47.7441
	5	-3.3167	10.03541	1.000	-41.7663	35.1329
	7	-5.0278	10.03541	1.000	-43.4774	33.4218
	8	-50.1444*	10.03541	.003	-88.5941	-11.6948
	9	-76.4667*	10.03541	.000	-114.9163	-38.0171
	10	-46.5111*	10.03541	.007	-84.9607	-8.0615
7	0	25.5778	10.03541	1.000	-12.8718	64.0274
	1	16.3000	10.03541	1.000	-22.1496	54.7496
	2	35.1889	10.03541	.110	-3.2607	73.6385
	3	5.9000	10.03541	1.000	-32.5496	44.3496
	4	14.3222	10.03541	1.000	-24.1274	52.7718
	5	1.7111	10.03541	1.000	-36.7385	40.1607
	6	5.0278	10.03541	1.000	-33.4218	43.4774
	8	-45.1167*	10.03541	.010	-83.5663	-6.6671
	9	-71.4389*	10.03541	.000	-109.8885	-32.9893
	10	-41.4833*	10.03541	.024	-79.9329	-3.0337
8	0	70.6944*	10.03541	.000	32.2448	109.1441
	1	61.4167*	10.03541	.000	22.9671	99.8663
	2	80.3056*	10.03541	.000	41.8559	118.7552
	3	51.0167*	10.03541	.002	12.5671	89.4663
	4	59.4389*	10.03541	.000	20.9893	97.8885
	5	46.8278*	10.03541	.007	8.3782	85.2774
	6	50.1444*	10.03541	.003	11.6948	88.5941
	7	45.1167*	10.03541	.010	6.6671	83.5663
	9	-26.3222	10.03541	.854	-64.7718	12.1274
	10	3.6333	10.03541	1.000	-34.8163	42.0829
9	0	97.0167*	10.03541	.000	58.5671	135.4663
	1	87.7389*	10.03541	.000	49.2893	126.1885
	2	106.6278*	10.03541	.000	68.1782	145.0774
	3	77.3389*	10.03541	.000	38.8893	115.7885
	4	85.7611*	10.03541	.000	47.3115	124.2107
	5	73.1500*	10.03541	.000	34.7004	111.5996
	6	76.4667*	10.03541	.000	38.0171	114.9163
	7	71.4389*	10.03541	.000	32.9893	109.8885
	8	26.3222	10.03541	.854	-12.1274	64.7718
	10	29.9556	10.03541	.376	-8.4941	68.4052
10	0	67.0611*	10.03541	.000	28.6115	105.5107
	1	57.7833*	10.03541	.000	19.3337	96.2329
	2	76.6722*	10.03541	.000	38.2226	115.1218
	3	47.3833*	10.03541	.006	8.9337	85.8329
	4	55.8056*	10.03541	.001	17.3559	94.2552
	5	43.1944*	10.03541	.016	4.7448	81.6441
	6	46.5111*	10.03541	.007	8.0615	84.9607
	7	41.4833*	10.03541	.024	3.0337	79.9329
	8	-3.6333	10.03541	1.000	-42.0829	34.8163
	9	-29.9556	10.03541	.376	-68.4052	8.4941

Based on observed means.

The error term is Mean Square(Error) = 151.064.

*. The mean difference is significant at the .05 level.

Repeated measures ANOVA SPSS output on biomass data from the tropical strain of *Fusarium equiseti*

Within-Subjects Factors

Measure: MEASURE_1

temp	Dependent Variable
1	mg_5
2	mg_10
3	mg_15
4	mg_20
5	mg_25
6	mg_30

Between-Subjects Factors

		N
time (d)	0	3
	1	3
	2	3
	3	3
	4	3
	5	3
	6	3
	7	3
	8	3
	9	3
	10	3

Descriptive Statistics

	time (d)	Mean	Std. Deviation	N
mg_5	0	.4000	4.01497	3
	1	6.3000	1.50000	3
	2	6.5667	1.50111	3
	3	6.8667	.80829	3
	4	10.6667	3.45012	3
	5	6.9000	1.15326	3
	6	6.0333	1.30512	3
	7	3.4667	1.85562	3
	8	3.6667	.55076	3
	9	2.3333	2.19621	3
	10	3.6000	1.17898	3
	Total	5.1636	3.20057	33
mg_10	0	8.4333	.11547	3
	1	9.1667	9.62930	3
	2	8.7333	1.45717	3
	3	12.5333	2.74287	3
	4	12.1667	3.91067	3
	5	25.8667	9.97514	3
	6	24.8000	8.59127	3
	7	38.0667	8.72716	3
	8	42.8333	37.14597	3
	9	48.0000	15.22334	3
	10	98.7333	7.22311	3
	Total	29.9394	28.47793	33
mg_15	0	.4000	.69282	3
	1	3.5667	4.55668	3
	2	26.5000	20.94588	3
	3	2.5667	.45092	3
	4	12.1000	6.50922	3
	5	38.1667	27.20925	3
	6	74.1000	60.46627	3
	7	74.0333	12.13768	3
	8	111.3667	17.27956	3
	9	132.2333	30.63598	3
	10	135.0667	45.09926	3
	Total	55.4636	55.73462	33
mg_20	0	40.9333	58.77843	3
	1	11.4667	.32146	3
	2	72.5667	27.19822	3
	3	138.6333	67.47787	3
	4	173.5333	95.74995	3
	5	151.4000	14.90403	3
	6	171.1000	4.93660	3
	7	157.1667	15.79631	3
	8	163.5667	6.76042	3
	9	98.7333	71.32863	3
	10	159.3000	17.69944	3
	Total	121.6727	67.25122	33
mg_25	0	4.3333	3.01386	3
	1	3.2000	1.22882	3
	2	21.9333	4.80867	3
	3	78.6667	37.27202	3
	4	127.6333	46.38807	3
	5	131.0000	6.39296	3
	6	153.0333	35.10304	3
	7	134.4000	28.89792	3
	8	148.6333	25.55510	3
	9	148.9667	5.76223	3
	10	190.7667	50.81204	3
	Total	103.8697	68.18854	33
mg_30	0	4.6667	.55076	3
	1	22.5333	2.45017	3
	2	113.0000	50.60079	3
	3	98.7000	8.25288	3
	4	104.7000	101.60605	3
	5	93.6333	2.73008	3
	6	93.6333	2.37136	3
	7	86.4000	3.26956	3
	8	79.4667	14.43410	3
	9	82.5000	1.87350	3
	10	92.4000	1.70587	3
	Total	79.2394	43.72255	33

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
temp	Pillai's Trace	.983	214.175[b]	5.000	18.000	.000	.983
	Wilks' Lambda	.017	214.175[b]	5.000	18.000	.000	.983
	Hotelling's Trace	59.493	214.175[b]	5.000	18.000	.000	.983
	Roy's Largest Root	59.493	214.175[b]	5.000	18.000	.000	.983
temp * time	Pillai's Trace	2.778	2.750	50.000	110.000	.000	.556
	Wilks' Lambda	.003	4.450	50.000	85.457	.000	.689
	Hotelling's Trace	21.794	7.148	50.000	82.000	.000	.813
	Roy's Largest Root	15.656	34.444[c]	10.000	22.000	.000	.940

a. Design: Intercept + time
Within Subjects Design: temp

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
temp	.043	63.166	14	.000	.450	.732	.200

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + time
Within Subjects Design: temp

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Sphericity Assumed	324100.350	5	64820.070	71.843	.000	.766
	Greenhouse-Geisser	324100.350	2.251	143959.650	71.843	.000	.766
	Huynh-Feldt	324100.350	3.661	88535.259	71.843	.000	.766
	Lower-bound	324100.350	1.000	324100.350	71.843	.000	.766
temp * time	Sphericity Assumed	166699.654	50	3333.993	3.695	.000	.627
	Greenhouse-Geisser	166699.654	22.513	7404.504	3.695	.000	.627
	Huynh-Feldt	166699.654	36.607	4553.774	3.695	.000	.627
	Lower-bound	166699.654	10.000	16669.965	3.695	.005	.627
Error(temp)	Sphericity Assumed	99246.781	110	902.243			
	Greenhouse-Geisser	99246.781	49.529	2003.803			
	Huynh-Feldt	99246.781	80.535	1232.340			
	Lower-bound	99246.781	22.000	4511.217			

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	temp	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Linear	204346.668	1	204346.668	372.929	.000	.944
	Quadratic	69412.045	1	69412.045	56.257	.000	.719
	Cubic	31115.156	1	31115.156	56.209	.000	.719
	Order 4	1635.208	1	1635.208	2.354	.139	.097
	Order 5	17591.273	1	17591.273	11.876	.002	.351
temp * time	Linear	41982.812	10	4198.281	7.662	.000	.777
	Quadratic	52708.891	10	5270.889	4.272	.002	.660
	Cubic	26171.384	10	2617.138	4.728	.001	.682
	Order 4	10697.900	10	1069.790	1.540	.191	.412
	Order 5	35138.666	10	3513.867	2.372	.044	.519
Error(temp)	Linear	12054.904	22	547.950			
	Quadratic	27144.265	22	1233.830			
	Cubic	12178.313	22	553.560			
	Order 4	15281.999	22	694.636			
	Order 5	32587.300	22	1481.241			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	859652.335	1	859652.335	1395.075	.000	.984
time	200869.838	10	20086.984	32.598	.000	.937
Error	13556.512	22	616.205			

1. time (d) * temp

Measure: MEASURE_1

time (d)	temp	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	1	.400	1.183	-2.053	2.853
	2	8.433	7.845	-7.836	24.703
	3	.400	15.876	-32.525	33.325
	4	40.933	26.889	-14.830	96.697
	5	4.333	16.470	-29.822	38.489
	6	4.667	19.998	-36.806	46.140
1	1	6.300	1.183	3.847	8.753
	2	9.167	7.845	-7.103	25.436
	3	3.567	15.876	-29.358	36.491
	4	11.467	26.889	-44.297	67.230
	5	3.200	16.470	-30.956	37.356
	6	22.533	19.998	-18.940	64.006
2	1	6.567	1.183	4.114	9.020
	2	8.733	7.845	-7.536	25.003
	3	26.500	15.876	-6.425	59.425
	4	72.567	26.889	16.803	128.330
	5	21.933	16.470	-12.222	56.089
	6	113.000	19.998	71.527	154.473
3	1	6.867	1.183	4.414	9.320
	2	12.533	7.845	-3.736	28.803
	3	2.567	15.876	-30.358	35.491
	4	138.633	26.889	82.870	194.397
	5	78.667	16.470	44.511	112.822
	6	98.700	19.998	57.227	140.173
4	1	10.667	1.183	8.214	13.120
	2	12.167	7.845	-4.103	28.436
	3	12.100	15.876	-20.825	45.025
	4	173.533	26.889	117.770	229.297
	5	127.633	16.470	93.478	161.789
	6	104.700	19.998	63.227	146.173
5	1	6.900	1.183	4.447	9.353
	2	25.867	7.845	9.597	42.136
	3	38.167	15.876	5.242	71.091
	4	151.400	26.889	95.637	207.163
	5	131.000	16.470	96.844	165.156
	6	93.633	19.998	52.160	135.106
6	1	6.033	1.183	3.580	8.486
	2	24.800	7.845	8.531	41.069
	3	74.100	15.876	41.175	107.025
	4	171.100	26.889	115.337	226.863
	5	153.033	16.470	118.878	187.189
	6	93.633	19.998	52.160	135.106
7	1	3.467	1.183	1.014	5.920
	2	38.067	7.845	21.797	54.336
	3	74.033	15.876	41.109	106.958
	4	157.167	26.889	101.403	212.930
	5	134.400	16.470	100.244	168.556
	6	86.400	19.998	44.927	127.873
8	1	3.667	1.183	1.214	6.120
	2	42.833	7.845	26.564	59.103
	3	111.367	15.876	78.442	144.291
	4	163.567	26.889	107.803	219.330
	5	148.633	16.470	114.478	182.789
	6	79.467	19.998	37.994	120.940
9	1	2.333	1.183	-.120	4.786
	2	48.000	7.845	31.731	64.269
	3	132.233	15.876	99.309	165.158
	4	98.733	26.889	42.970	154.497
	5	148.967	16.470	114.811	183.122
	6	82.500	19.998	41.027	123.973
10	1	3.600	1.183	1.147	6.053
	2	98.733	7.845	82.464	115.003
	3	135.067	15.876	102.142	167.991
	4	159.300	26.889	103.537	215.063
	5	190.767	16.470	156.611	224.922
	6	92.400	19.998	50.927	133.873

Estimates

Measure: MEASURE_1

temp	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	5.164	.357	4.424	5.903
2	29.939	2.365	25.034	34.845
3	55.464	4.787	45.536	65.391
4	121.673	8.107	104.859	138.486
5	103.870	4.966	93.571	114.168
6	79.239	6.030	66.735	91.744

Pairwise Comparisons

Measure: MEASURE_1

(I) temp	(J) temp	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-24.776*	2.433	.000	-32.781	-16.770
	3	-50.300*	4.783	.000	-66.041	-34.559
	4	-116.509*	8.217	.000	-143.552	-89.467
	5	-98.706*	4.926	.000	-114.918	-82.494
	6	-74.076*	5.916	.000	-93.545	-54.607
2	1	24.776*	2.433	.000	16.770	32.781
	3	-25.524*	4.821	.000	-41.390	-9.659
	4	-91.733*	8.682	.000	-120.305	-63.161
	5	-73.930*	4.994	.000	-90.366	-57.495
	6	-49.300*	6.523	.000	-70.766	-27.834
3	1	50.300*	4.783	.000	34.559	66.041
	2	25.524*	4.821	.000	9.659	41.390
	4	-66.209*	9.828	.000	-98.552	-33.867
	5	-48.406*	5.792	.000	-67.466	-29.346
	6	-23.776	7.583	.072	-48.729	1.177
4	1	116.509*	8.217	.000	89.467	143.552
	2	91.733*	8.682	.000	63.161	120.305
	3	66.209*	9.828	.000	33.867	98.552
	5	17.803	10.859	1.000	-17.934	53.540
	6	42.433*	12.382	.036	1.684	83.183
5	1	98.706*	4.926	.000	82.494	114.918
	2	73.930*	4.994	.000	57.495	90.366
	3	48.406*	5.792	.000	29.346	67.466
	4	-17.803	10.859	1.000	-53.540	17.934
	6	24.630*	6.296	.011	3.910	45.350
6	1	74.076*	5.916	.000	54.607	93.545
	2	49.300*	6.523	.000	27.834	70.766
	3	23.776	7.583	.072	-1.177	48.729
	4	-42.433*	12.382	.036	-83.183	-1.684
	5	-24.630*	6.296	.011	-45.350	-3.910

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.983	214.175[a]	5.000	18.000	.000	.983
Wilks' lambda	.017	214.175[a]	5.000	18.000	.000	.983
Hotelling's trace	59.493	214.175[a]	5.000	18.000	.000	.983
Roy's largest root	59.493	214.175[a]	5.000	18.000	.000	.983

Each F tests the multivariate effect of temp. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) time (d)	(J) time (d)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	.4889	8.27449	1.000	-31.2140	32.1917
	2	-31.6889	8.27449	.050	-63.3917	.0140
	3	-46.4667*	8.27449	.001	-78.1695	-14.7638
	4	-63.6056*	8.27449	.000	-95.3084	-31.9027
	5	-64.6333*	8.27449	.000	-96.3362	-32.9305
	6	-77.2556*	8.27449	.000	-108.9584	-45.5527
	7	-72.3944*	8.27449	.000	-104.0973	-40.6916
	8	-81.7278*	8.27449	.000	-113.4306	-50.0249
	9	-75.6000*	8.27449	.000	-107.3028	-43.8972
	10	-103.4500*	8.27449	.000	-135.1528	-71.7472
1	0	-.4889	8.27449	1.000	-32.1917	31.2140
	2	-32.1778*	8.27449	.043	-63.8806	-.4749
	3	-46.9556*	8.27449	.001	-78.6584	-15.2527
	4	-64.0944*	8.27449	.000	-95.7973	-32.3916
	5	-65.1222*	8.27449	.000	-96.8251	-33.4194
	6	-77.7444*	8.27449	.000	-109.4473	-46.0416
	7	-72.8833*	8.27449	.000	-104.5862	-41.1805
	8	-82.2167*	8.27449	.000	-113.9195	-50.5138
	9	-76.0889*	8.27449	.000	-107.7917	-44.3860
	10	-103.9389*	8.27449	.000	-135.6417	-72.2360
2	0	31.6889	8.27449	.050	-.0140	63.3917
	1	32.1778*	8.27449	.043	.4749	63.8806
	3	-14.7778	8.27449	1.000	-46.4806	16.9251
	4	-31.9167*	8.27449	.047	-63.6195	-.2138
	5	-32.9444*	8.27449	.035	-64.6473	-1.2416
	6	-45.5667*	8.27449	.001	-77.2695	-13.8638
	7	-40.7056*	8.27449	.004	-72.4084	-9.0027
	8	-50.0389*	8.27449	.000	-81.7417	-18.3360
	9	-43.9111*	8.27449	.001	-75.6140	-12.2083
	10	-71.7611*	8.27449	.000	-103.4640	-40.0583
3	0	46.4667*	8.27449	.001	14.7638	78.1695
	1	46.9556*	8.27449	.001	15.2527	78.6584
	2	14.7778	8.27449	1.000	-16.9251	46.4806
	4	-17.1389	8.27449	1.000	-48.8417	14.5640
	5	-18.1667	8.27449	1.000	-49.8695	13.5362
	6	-30.7889	8.27449	.065	-62.4917	.9140
	7	-25.9278	8.27449	.266	-57.6306	5.7751
	8	-35.2611*	8.27449	.018	-66.9640	-3.5583
	9	-29.1333	8.27449	.106	-60.8362	2.5695
	10	-56.9833*	8.27449	.000	-88.6862	-25.2805
4	0	63.6056*	8.27449	.000	31.9027	95.3084
	1	64.0944*	8.27449	.000	32.3916	95.7973
	2	31.9167*	8.27449	.047	.2138	63.6195
	3	17.1389	8.27449	1.000	-14.5640	48.8417
	5	-1.0278	8.27449	1.000	-32.7306	30.6751
	6	-13.6500	8.27449	1.000	-45.3528	18.0528
	7	-8.7889	8.27449	1.000	-40.4917	22.9140
	8	-18.1222	8.27449	1.000	-49.8251	13.5806
	9	-11.9944	8.27449	1.000	-43.6973	19.7084
	10	-39.8444*	8.27449	.005	-71.5473	-8.1416
5	0	64.6333*	8.27449	.000	32.9305	96.3362
	1	65.1222*	8.27449	.000	33.4194	96.8251
	2	32.9444*	8.27449	.035	1.2416	64.6473
	3	18.1667	8.27449	1.000	-13.5362	49.8695
	4	1.0278	8.27449	1.000	-30.6751	32.7306
	6	-12.6222	8.27449	1.000	-44.3251	19.0806
	7	-7.7611	8.27449	1.000	-39.4640	23.9417
	8	-17.0944	8.27449	1.000	-48.7973	14.6084
	9	-10.9667	8.27449	1.000	-42.6695	20.7362
	10	-38.8167*	8.27449	.006	-70.5195	-7.1138
6	0	77.2556*	8.27449	.000	45.5527	108.9584
	1	77.7444*	8.27449	.000	46.0416	109.4473
	2	45.5667*	8.27449	.001	13.8638	77.2695
	3	30.7889	8.27449	.065	-.9140	62.4917
	4	13.6500	8.27449	1.000	-18.0528	45.3528
	5	12.6222	8.27449	1.000	-19.0806	44.3251
	7	4.8611	8.27449	1.000	-26.8417	36.5640
	8	-4.4722	8.27449	1.000	-36.1751	27.2306
	9	1.6556	8.27449	1.000	-30.0473	33.3584
	10	-26.1944	8.27449	.246	-57.8973	5.5084
7	0	72.3944*	8.27449	.000	40.6916	104.0973
	1	72.8833*	8.27449	.000	41.1805	104.5862
	2	40.7056*	8.27449	.004	9.0027	72.4084
	3	25.9278	8.27449	.266	-5.7751	57.6306
	4	8.7889	8.27449	1.000	-22.9140	40.4917
	5	7.7611	8.27449	1.000	-23.9417	39.4640
	6	-4.8611	8.27449	1.000	-36.5640	26.8417
	8	-9.3333	8.27449	1.000	-41.0362	22.3695
	9	-3.2056	8.27449	1.000	-34.9084	28.4973
	10	-31.0556	8.27449	.060	-62.7584	.6473
8	0	81.7278*	8.27449	.000	50.0249	113.4306
	1	82.2167*	8.27449	.000	50.5138	113.9195
	2	50.0389*	8.27449	.000	18.3360	81.7417
	3	35.2611*	8.27449	.018	3.5583	66.9640
	4	18.1222	8.27449	1.000	-13.5806	49.8251
	5	17.0944	8.27449	1.000	-14.6084	48.7973
	6	4.4722	8.27449	1.000	-27.2306	36.1751
	7	9.3333	8.27449	1.000	-22.3695	41.0362
	9	6.1278	8.27449	1.000	-25.5751	37.8306
	10	-21.7222	8.27449	.850	-53.4251	9.9806
9	0	75.6000*	8.27449	.000	43.8972	107.3028
	1	76.0889*	8.27449	.000	44.3860	107.7917
	2	43.9111*	8.27449	.001	12.2083	75.6140
	3	29.1333	8.27449	.106	-2.5695	60.8362
	4	11.9944	8.27449	1.000	-19.7084	43.6973
	5	10.9667	8.27449	1.000	-20.7362	42.6695
	6	-1.6556	8.27449	1.000	-33.3584	30.0473
	7	3.2056	8.27449	1.000	-28.4973	34.9084
	8	-6.1278	8.27449	1.000	-37.8306	25.5751
	10	-27.8500	8.27449	.153	-59.5528	3.8528
10	0	103.4500*	8.27449	.000	71.7472	135.1528
	1	103.9389*	8.27449	.000	72.2360	135.6417
	2	71.7611*	8.27449	.000	40.0583	103.4640
	3	56.9833*	8.27449	.000	25.2805	88.6862
	4	39.8444*	8.27449	.005	8.1416	71.5473
	5	38.8167*	8.27449	.006	7.1138	70.5195
	6	26.1944	8.27449	.246	-5.5084	57.8973
	7	31.0556	8.27449	.060	-.6473	62.7584
	8	21.7222	8.27449	.850	-9.9806	53.4251
	9	27.8500	8.27449	.153	-3.8528	59.5528

Based on observed means.

The error term is Mean Square(Error) = 102.701.

*. The mean difference is significant at the .05 level.

Repeated measures ANOVA SPSS output on biomass data from the antarctic strain of *Pseudogymnoascus* spp.

Within-Subjects Factors

Measure: MEASURE_1

temp	Dependent Variable
1	mg_5
2	mg_10
3	mg_15
4	mg_20
5	mg_25
6	mg_30

Between-Subjects Factors

		N
time (d)	0	3
	1	3
	2	3
	3	3
	4	3
	5	3
	6	3
	7	3
	8	3
	9	3
	10	3

Descriptive Statistics

	time (d)	Mean	Std. Deviation	N
mg_5	0	.4000	4.01497	3
	1	6.3000	1.50000	3
	2	6.5667	1.50111	3
	3	6.8667	.80829	3
	4	10.6667	3.45012	3
	5	6.9000	1.15326	3
	6	6.0333	1.30512	3
	7	3.4667	1.85562	3
	8	3.6667	.55076	3
	9	2.3333	2.19621	3
	10	3.6000	1.17898	3
	Total	5.1636	3.20057	33
mg_10	0	8.4333	.11547	3
	1	9.1667	9.62930	3
	2	8.7333	1.45717	3
	3	12.5333	2.74287	3
	4	12.1667	3.91067	3
	5	25.8667	9.97514	3
	6	24.8000	8.59127	3
	7	38.0667	8.72716	3
	8	42.8333	37.14597	3
	9	48.0000	15.22334	3
	10	98.7333	7.22311	3
	Total	29.9394	28.47793	33
mg_15	0	.4000	.69282	3
	1	3.5667	4.55668	3
	2	26.5000	20.94588	3
	3	2.5667	.45092	3
	4	12.1000	6.50922	3
	5	38.1667	27.20925	3
	6	74.1000	60.46627	3
	7	74.0333	12.13768	3
	8	111.3667	17.27956	3
	9	132.2333	30.63598	3
	10	135.0667	45.09926	3
	Total	55.4636	55.73462	33
mg_20	0	40.9333	58.77843	3
	1	11.4667	.32146	3
	2	72.5667	27.19822	3
	3	138.6333	67.47787	3
	4	173.5333	95.74995	3
	5	151.4000	14.90403	3
	6	171.1000	4.93660	3
	7	157.1667	15.79631	3
	8	163.5667	6.76042	3
	9	98.7333	71.32863	3
	10	159.3000	17.69944	3
	Total	121.6727	67.25122	33
mg_25	0	4.3333	3.01386	3
	1	3.2000	1.22882	3
	2	21.9333	4.80867	3
	3	78.6667	37.27202	3
	4	127.6333	46.38807	3
	5	131.0000	6.39296	3
	6	153.0333	35.10304	3
	7	134.4000	28.89792	3
	8	148.6333	25.55510	3
	9	148.9667	5.76223	3
	10	190.7667	50.81204	3
	Total	103.8697	68.18854	33
mg_30	0	4.6667	.55076	3
	1	22.5333	2.45017	3
	2	113.0000	50.60079	3
	3	98.7000	8.25288	3
	4	104.7000	101.60605	3
	5	93.6333	2.73008	3
	6	93.6333	2.37136	3
	7	86.4000	3.26956	3
	8	79.4667	14.43410	3
	9	82.5000	1.87350	3
	10	92.4000	1.70587	3
	Total	79.2394	43.72255	33

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
temp	Pillai's Trace	.983	214.175[b]	5.000	18.000	.000	.983
	Wilks' Lambda	.017	214.175[b]	5.000	18.000	.000	.983
	Hotelling's Trace	59.493	214.175[b]	5.000	18.000	.000	.983
	Roy's Largest Root	59.493	214.175[b]	5.000	18.000	.000	.983
temp * time	Pillai's Trace	2.778	2.750	50.000	110.000	.000	.556
	Wilks' Lambda	.003	4.450	50.000	85.457	.000	.689
	Hotelling's Trace	21.794	7.148	50.000	82.000	.000	.813
	Roy's Largest Root	15.656	34.444[c]	10.000	22.000	.000	.940

a. Design: Intercept + time
Within Subjects Design: temp

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
temp	.043	63.166	14	.000	.450	.732	.200

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + time
Within Subjects Design: temp

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Sphericity Assumed	324100.350	5	64820.070	71.843	.000	.766
	Greenhouse-Geisser	324100.350	2.251	143959.650	71.843	.000	.766
	Huynh-Feldt	324100.350	3.661	88535.259	71.843	.000	.766
	Lower-bound	324100.350	1.000	324100.350	71.843	.000	.766
temp * time	Sphericity Assumed	166699.654	50	3333.993	3.695	.000	.627
	Greenhouse-Geisser	166699.654	22.513	7404.504	3.695	.000	.627
	Huynh-Feldt	166699.654	36.607	4553.774	3.695	.000	.627
	Lower-bound	166699.654	10.000	16669.965	3.695	.005	.627
Error(temp)	Sphericity Assumed	99246.781	110	902.243			
	Greenhouse-Geisser	99246.781	49.529	2003.803			
	Huynh-Feldt	99246.781	80.535	1232.340			
	Lower-bound	99246.781	22.000	4511.217			

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	temp	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Linear	204346.668	1	204346.668	372.929	.000	.944
	Quadratic	69412.045	1	69412.045	56.257	.000	.719
	Cubic	31115.156	1	31115.156	56.209	.000	.719
	Order 4	1635.208	1	1635.208	2.354	.139	.097
	Order 5	17591.273	1	17591.273	11.876	.002	.351
temp * time	Linear	41982.812	10	4198.281	7.662	.000	.777
	Quadratic	52708.891	10	5270.889	4.272	.002	.660
	Cubic	26171.384	10	2617.138	4.728	.001	.682
	Order 4	10697.900	10	1069.790	1.540	.191	.412
	Order 5	35138.666	10	3513.867	2.372	.044	.519
Error(temp)	Linear	12054.904	22	547.950			
	Quadratic	27144.265	22	1233.830			
	Cubic	12178.313	22	553.560			
	Order 4	15281.999	22	694.636			
	Order 5	32587.300	22	1481.241			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	859652.335	1	859652.335	1395.075	.000	.984
time	200869.838	10	20086.984	32.598	.000	.937
Error	13556.512	22	616.205			

1. time (d) * temp

Measure: MEASURE_1

time (d)	temp	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	1	.400	1.183	-2.053	2.853
	2	8.433	7.845	-7.836	24.703
	3	.400	15.876	-32.525	33.325
	4	40.933	26.889	-14.830	96.697
	5	4.333	16.470	-29.822	38.489
	6	4.667	19.998	-36.806	46.140
1	1	6.300	1.183	3.847	8.753
	2	9.167	7.845	-7.103	25.436
	3	3.567	15.876	-29.358	36.491
	4	11.467	26.889	-44.297	67.230
	5	3.200	16.470	-30.956	37.356
	6	22.533	19.998	-18.940	64.006
2	1	6.567	1.183	4.114	9.020
	2	8.733	7.845	-7.536	25.003
	3	26.500	15.876	-6.425	59.425
	4	72.567	26.889	16.803	128.330
	5	21.933	16.470	-12.222	56.089
	6	113.000	19.998	71.527	154.473
3	1	6.867	1.183	4.414	9.320
	2	12.533	7.845	-3.736	28.803
	3	2.567	15.876	-30.358	35.491
	4	138.633	26.889	82.870	194.397
	5	78.667	16.470	44.511	112.822
	6	98.700	19.998	57.227	140.173
4	1	10.667	1.183	8.214	13.120
	2	12.167	7.845	-4.103	28.436
	3	12.100	15.876	-20.825	45.025
	4	173.533	26.889	117.770	229.297
	5	127.633	16.470	93.478	161.789
	6	104.700	19.998	63.227	146.173
5	1	6.900	1.183	4.447	9.353
	2	25.867	7.845	9.597	42.136
	3	38.167	15.876	5.242	71.091
	4	151.400	26.889	95.637	207.163
	5	131.000	16.470	96.844	165.156
	6	93.633	19.998	52.160	135.106
6	1	6.033	1.183	3.580	8.486
	2	24.800	7.845	8.531	41.069
	3	74.100	15.876	41.175	107.025
	4	171.100	26.889	115.337	226.863
	5	153.033	16.470	118.878	187.189
	6	93.633	19.998	52.160	135.106
7	1	3.467	1.183	1.014	5.920
	2	38.067	7.845	21.797	54.336
	3	74.033	15.876	41.109	106.958
	4	157.167	26.889	101.403	212.930
	5	134.400	16.470	100.244	168.556
	6	86.400	19.998	44.927	127.873
8	1	3.667	1.183	1.214	6.120
	2	42.833	7.845	26.564	59.103
	3	111.367	15.876	78.442	144.291
	4	163.567	26.889	107.803	219.330
	5	148.633	16.470	114.478	182.789
	6	79.467	19.998	37.994	120.940
9	1	2.333	1.183	-.120	4.786
	2	48.000	7.845	31.731	64.269
	3	132.233	15.876	99.309	165.158
	4	98.733	26.889	42.970	154.497
	5	148.967	16.470	114.811	183.122
	6	82.500	19.998	41.027	123.973
10	1	3.600	1.183	1.147	6.053
	2	98.733	7.845	82.464	115.003
	3	135.067	15.876	102.142	167.991
	4	159.300	26.889	103.537	215.063
	5	190.767	16.470	156.611	224.922
	6	92.400	19.998	50.927	133.873

Estimates

Measure: MEASURE_1

temp	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	5.164	.357	4.424	5.903
2	29.939	2.365	25.034	34.845
3	55.464	4.787	45.536	65.391
4	121.673	8.107	104.859	138.486
5	103.870	4.966	93.571	114.168
6	79.239	6.030	66.735	91.744

Pairwise Comparisons

Measure: MEASURE_1

(I) temp	(J) temp	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-24.776*	2.433	.000	-32.781	-16.770
	3	-50.300*	4.783	.000	-66.041	-34.559
	4	-116.509*	8.217	.000	-143.552	-89.467
	5	-98.706*	4.926	.000	-114.918	-82.494
	6	-74.076*	5.916	.000	-93.545	-54.607
2	1	24.776*	2.433	.000	16.770	32.781
	3	-25.524*	4.821	.000	-41.390	-9.659
	4	-91.733*	8.682	.000	-120.305	-63.161
	5	-73.930*	4.994	.000	-90.366	-57.495
	6	-49.300*	6.523	.000	-70.766	-27.834
3	1	50.300*	4.783	.000	34.559	66.041
	2	25.524*	4.821	.000	9.659	41.390
	4	-66.209*	9.828	.000	-98.552	-33.867
	5	-48.406*	5.792	.000	-67.466	-29.346
	6	-23.776	7.583	.072	-48.729	1.177
4	1	116.509*	8.217	.000	89.467	143.552
	2	91.733*	8.682	.000	63.161	120.305
	3	66.209*	9.828	.000	33.867	98.552
	5	17.803	10.859	1.000	-17.934	53.540
	6	42.433*	12.382	.036	1.684	83.183
5	1	98.706*	4.926	.000	82.494	114.918
	2	73.930*	4.994	.000	57.495	90.366
	3	48.406*	5.792	.000	29.346	67.466
	4	-17.803	10.859	1.000	-53.540	17.934
	6	24.630*	6.296	.011	3.910	45.350
6	1	74.076*	5.916	.000	54.607	93.545
	2	49.300*	6.523	.000	27.834	70.766
	3	23.776	7.583	.072	-1.177	48.729
	4	-42.433*	12.382	.036	-83.183	-1.684
	5	-24.630*	6.296	.011	-45.350	-3.910

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.983	214.175[a]	5.000	18.000	.000	.983
Wilks' lambda	.017	214.175[a]	5.000	18.000	.000	.983
Hotelling's trace	59.493	214.175[a]	5.000	18.000	.000	.983
Roy's largest root	59.493	214.175[a]	5.000	18.000	.000	.983

Each F tests the multivariate effect of temp. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) time (d)	(J) time (d)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	.4889	8.27449	1.000	-31.2140	32.1917
	2	-31.6889	8.27449	.050	-63.3917	.0140
	3	-46.4667*	8.27449	.001	-78.1695	-14.7638
	4	-63.6056*	8.27449	.000	-95.3084	-31.9027
	5	-64.6333*	8.27449	.000	-96.3362	-32.9305
	6	-77.2556*	8.27449	.000	-108.9584	-45.5527
	7	-72.3944*	8.27449	.000	-104.0973	-40.6916
	8	-81.7278*	8.27449	.000	-113.4306	-50.0249
	9	-75.6000*	8.27449	.000	-107.3028	-43.8972
	10	-103.4500*	8.27449	.000	-135.1528	-71.7472
1	0	-.4889	8.27449	1.000	-32.1917	31.2140
	2	-32.1778*	8.27449	.043	-63.8806	-.4749
	3	-46.9556*	8.27449	.001	-78.6584	-15.2527
	4	-64.0944*	8.27449	.000	-95.7973	-32.3916
	5	-65.1222*	8.27449	.000	-96.8251	-33.4194
	6	-77.7444*	8.27449	.000	-109.4473	-46.0416
	7	-72.8833*	8.27449	.000	-104.5862	-41.1805
	8	-82.2167*	8.27449	.000	-113.9195	-50.5138
	9	-76.0889*	8.27449	.000	-107.7917	-44.3860
	10	-103.9389*	8.27449	.000	-135.6417	-72.2360
2	0	31.6889	8.27449	.050	-.0140	63.3917
	1	32.1778*	8.27449	.043	.4749	63.8806
	3	-14.7778	8.27449	1.000	-46.4806	16.9251
	4	-31.9167*	8.27449	.047	-63.6195	-.2138
	5	-32.9444*	8.27449	.035	-64.6473	-1.2416
	6	-45.5667*	8.27449	.001	-77.2695	-13.8638
	7	-40.7056*	8.27449	.004	-72.4084	-9.0027
	8	-50.0389*	8.27449	.000	-81.7417	-18.3360
	9	-43.9111*	8.27449	.001	-75.6140	-12.2083
	10	-71.7611*	8.27449	.000	-103.4640	-40.0583
3	0	46.4667*	8.27449	.001	14.7638	78.1695
	1	46.9556*	8.27449	.001	15.2527	78.6584
	2	14.7778	8.27449	1.000	-16.9251	46.4806
	4	-17.1389	8.27449	1.000	-48.8417	14.5640
	5	-18.1667	8.27449	1.000	-49.8695	13.5362
	6	-30.7889	8.27449	.065	-62.4917	.9140
	7	-25.9278	8.27449	.266	-57.6306	5.7751
	8	-35.2611*	8.27449	.018	-66.9640	-3.5583
	9	-29.1333	8.27449	.106	-60.8362	2.5695
	10	-56.9833*	8.27449	.000	-88.6862	-25.2805
4	0	63.6056*	8.27449	.000	31.9027	95.3084
	1	64.0944*	8.27449	.000	32.3916	95.7973
	2	31.9167*	8.27449	.047	.2138	63.6195
	3	17.1389	8.27449	1.000	-14.5640	48.8417
	5	-1.0278	8.27449	1.000	-32.7306	30.6751
	6	-13.6500	8.27449	1.000	-45.3528	18.0528
	7	-8.7889	8.27449	1.000	-40.4917	22.9140
	8	-18.1222	8.27449	1.000	-49.8251	13.5806
	9	-11.9944	8.27449	1.000	-43.6973	19.7084
	10	-39.8444*	8.27449	.005	-71.5473	-8.1416
5	0	64.6333*	8.27449	.000	32.9305	96.3362
	1	65.1222*	8.27449	.000	33.4194	96.8251
	2	32.9444*	8.27449	.035	1.2416	64.6473
	3	18.1667	8.27449	1.000	-13.5362	49.8695
	4	1.0278	8.27449	1.000	-30.6751	32.7306
	6	-12.6222	8.27449	1.000	-44.3251	19.0806
	7	-7.7611	8.27449	1.000	-39.4640	23.9417
	8	-17.0944	8.27449	1.000	-48.7973	14.6084
	9	-10.9667	8.27449	1.000	-42.6695	20.7362
	10	-38.8167*	8.27449	.006	-70.5195	-7.1138
6	0	77.2556*	8.27449	.000	45.5527	108.9584
	1	77.7444*	8.27449	.000	46.0416	109.4473
	2	45.5667*	8.27449	.001	13.8638	77.2695
	3	30.7889	8.27449	.065	-.9140	62.4917
	4	13.6500	8.27449	1.000	-18.0528	45.3528
	5	12.6222	8.27449	1.000	-19.0806	44.3251
	7	4.8611	8.27449	1.000	-26.8417	36.5640
	8	-4.4722	8.27449	1.000	-36.1751	27.2306
	9	1.6556	8.27449	1.000	-30.0473	33.3584
	10	-26.1944	8.27449	.246	-57.8973	5.5084
7	0	72.3944*	8.27449	.000	40.6916	104.0973
	1	72.8833*	8.27449	.000	41.1805	104.5862
	2	40.7056*	8.27449	.004	9.0027	72.4084
	3	25.9278	8.27449	.266	-5.7751	57.6306
	4	8.7889	8.27449	1.000	-22.9140	40.4917
	5	7.7611	8.27449	1.000	-23.9417	39.4640
	6	-4.8611	8.27449	1.000	-36.5640	26.8417
	8	-9.3333	8.27449	1.000	-41.0362	22.3695
	9	-3.2056	8.27449	1.000	-34.9084	28.4973
	10	-31.0556	8.27449	.060	-62.7584	.6473
8	0	81.7278*	8.27449	.000	50.0249	113.4306
	1	82.2167*	8.27449	.000	50.5138	113.9195
	2	50.0389*	8.27449	.000	18.3360	81.7417
	3	35.2611*	8.27449	.018	3.5583	66.9640
	4	18.1222	8.27449	1.000	-13.5806	49.8251
	5	17.0944	8.27449	1.000	-14.6084	48.7973
	6	4.4722	8.27449	1.000	-27.2306	36.1751
	7	9.3333	8.27449	1.000	-22.3695	41.0362
	9	6.1278	8.27449	1.000	-25.5751	37.8306
	10	-21.7222	8.27449	.850	-53.4251	9.9806
9	0	75.6000*	8.27449	.000	43.8972	107.3028
	1	76.0889*	8.27449	.000	44.3860	107.7917
	2	43.9111*	8.27449	.001	12.2083	75.6140
	3	29.1333	8.27449	.106	-2.5695	60.8362
	4	11.9944	8.27449	1.000	-19.7084	43.6973
	5	10.9667	8.27449	1.000	-20.7362	42.6695
	6	-1.6556	8.27449	1.000	-33.3584	30.0473
	7	3.2056	8.27449	1.000	-28.4973	34.9084
	8	-6.1278	8.27449	1.000	-37.8306	25.5751
	10	-27.8500	8.27449	.153	-59.5528	3.8528
10	0	103.4500*	8.27449	.000	71.7472	135.1528
	1	103.9389*	8.27449	.000	72.2360	135.6417
	2	71.7611*	8.27449	.000	40.0583	103.4640
	3	56.9833*	8.27449	.000	25.2805	88.6862
	4	39.8444*	8.27449	.005	8.1416	71.5473
	5	38.8167*	8.27449	.006	7.1138	70.5195
	6	26.1944	8.27449	.246	-5.5084	57.8973
	7	31.0556	8.27449	.060	-.6473	62.7584
	8	21.7222	8.27449	.850	-9.9806	53.4251
	9	27.8500	8.27449	.153	-3.8528	59.5528

Based on observed means.

The error term is Mean Square(Error) = 102.701.

*. The mean difference is significant at the .05 level.

APPENDIX C

Repeated measures ANOVA SPSS output on pH data from the arctic strain of *Pseudogymnoascus* spp.

Within-Subjects Factors

Measure: MEASURE_1

temp	Dependent Variable
1	pH_5
2	pH_10
3	pH_15
4	pH_20
5	pH_25
6	pH_30

Between-Subjects Factors

		N
time (d)	0	3
	1	3
	2	3
	3	3
	4	3
	5	3
	6	3
	7	3
	8	3
	9	3
	10	3

Descriptive Statistics

	time (d)	Mean	Std. Deviation	N
pH_5	0	6.4667	.05774	3
	1	6.4667	.05774	3
	2	7.5000	.00000	3
	3	7.4000	.10000	3
	4	6.4667	.05774	3
	5	6.4667	.05774	3
	6	6.4667	.05774	3
	7	6.4667	.05774	3
	8	6.5000	.00000	3
	9	6.5667	.05774	3
	10	6.6000	.00000	3
	Total	6.6697	.37954	33
pH_10	0	6.8000	.00000	3
	1	6.8333	.05774	3
	2	6.8000	.00000	3
	3	6.9000	.00000	3
	4	7.0000	.00000	3
	5	7.7000	.17321	3
	6	7.0667	.05774	3
	7	7.2000	.00000	3
	8	7.3667	.32146	3
	9	7.5000	.17321	3
	10	8.1667	.32146	3
	Total	7.2121	.44212	33
pH_15	0	6.7667	.05774	3
	1	6.8000	.00000	3
	2	6.8000	.10000	3
	3	6.9333	.05774	3
	4	7.0000	.10000	3
	5	7.2333	.15275	3
	6	7.3000	.30000	3
	7	7.8000	.45826	3
	8	7.2333	.05774	3
	9	8.3333	.20817	3
	10	7.4333	.30551	3
	Total	7.2394	.49746	33
pH_20	0	6.7667	.05774	3
	1	6.8000	.00000	3
	2	6.8333	.05774	3
	3	6.9000	.00000	3
	4	7.0667	.11547	3
	5	7.4333	.05774	3
	6	7.6667	.11547	3
	7	7.3667	.15275	3
	8	8.1000	.17321	3
	9	8.2333	.20817	3
	10	7.9000	.36056	3
	Total	7.3697	.54053	33
pH_25	0	6.8333	.05774	3
	1	6.8000	.10000	3
	2	6.8667	.05774	3
	3	7.0000	.00000	3
	4	7.0000	.00000	3
	5	7.5000	.36056	3
	6	7.6667	.32146	3
	7	7.4667	.23094	3
	8	8.5333	.20817	3
	9	8.4000	.51962	3
	10	8.7667	.05774	3
	Total	7.5303	.73420	33
pH_30	0	6.4667	.05774	3
	1	6.4667	.05774	3
	2	7.5000	.00000	3
	3	7.4000	.10000	3
	4	6.4667	.05774	3
	5	6.4667	.05774	3
	6	6.4667	.05774	3
	7	6.5000	.00000	3
	8	6.6667	.05774	3
	9	6.5667	.05774	3
	10	6.6000	.00000	3
	Total	6.6879	.37396	33

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
temp	Pillai's Trace	.984	221.074[b]	5.000	18.000	.000	.984
	Wilks' Lambda	.016	221.074[b]	5.000	18.000	.000	.984
	Hotelling's Trace	61.410	221.074[b]	5.000	18.000	.000	.984
	Roy's Largest Root	61.410	221.074[b]	5.000	18.000	.000	.984
temp * time	Pillai's Trace	3.591	5.609	50.000	110.000	.000	.718
	Wilks' Lambda	.000	15.535	50.000	85.457	.000	.879
	Hotelling's Trace	97.854	32.096	50.000	82.000	.000	.951
	Roy's Largest Root	67.890	149.359[c]	10.000	22.000	.000	.985

a. Design: Intercept + time
Within Subjects Design: temp

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
temp	.006	101.485	14	.000	.585	.991	.200

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + time
Within Subjects Design: temp

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Sphericity Assumed	21.216	5	4.243	177.776	.000	.890
	Greenhouse-Geisser	21.216	2.925	7.252	177.776	.000	.890
	Huynh-Feldt	21.216	4.956	4.281	177.776	.000	.890
	Lower-bound	21.216	1.000	21.216	177.776	.000	.890
temp * time	Sphericity Assumed	28.815	50	.576	24.144	.000	.916
	Greenhouse-Geisser	28.815	29.254	.985	24.144	.000	.916
	Huynh-Feldt	28.815	49.563	.581	24.144	.000	.916
	Lower-bound	28.815	10.000	2.881	24.144	.000	.916
Error(temp)	Sphericity Assumed	2.626	110	.024			
	Greenhouse-Geisser	2.626	64.360	.041			
	Huynh-Feldt	2.626	109.039	.024			
	Lower-bound	2.626	22.000	.119			

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	temp	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Linear	.652	1	.652	87.883	.000	.800
	Quadratic	16.046	1	16.046	933.448	.000	.977
	Cubic	1.295	1	1.295	70.157	.000	.761
	Order 4	3.215	1	3.215	130.790	.000	.856
	Order 5	.010	1	.010	.184	.672	.009
temp * time	Linear	1.244	10	.124	16.772	.000	.884
	Quadratic	18.508	10	1.851	107.669	.000	.980
	Cubic	2.398	10	.240	12.995	.000	.855
	Order 4	5.669	10	.567	23.067	.000	.913
	Order 5	.995	10	.100	1.925	.096	.467
Error(temp)	Linear	.163	22	.007			
	Quadratic	.378	22	.017			
	Cubic	.406	22	.018			
	Order 4	.541	22	.025			
	Order 5	1.137	22	.052			

Tests of Between-Subjects Effects

Measure: MEASURE_1
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	10032.365	1	10032.365	275507.401	.000	1.000
time	17.617	10	1.762	48.379	.000	.957
Error	.801	22	.036			

Estimates

Measure: MEASURE_1

temp	Mean	Std. Error	95% Confidence Interval Lower Bound	Upper Bound
1	6.670	.010	6.650	6.690
2	7.212	.027	7.155	7.269
3	7.239	.037	7.163	7.316
4	7.370	.027	7.314	7.426
5	7.530	.041	7.445	7.616
6	6.688	.010	6.668	6.708

Pairwise Comparisons

Measure: MEASURE_1

(I) temp	(J) temp	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
1	2	-.542*	.028	.000	-.600	-.485
	3	-.570*	.037	.000	-.647	-.492
	4	-.700*	.030	.000	-.763	-.637
	5	-.861*	.040	.000	-.943	-.779
	6	-.018*	.004	.000	-.027	-.009
2	1	.542*	.028	.000	.485	.600
	3	-.027	.047	.564	-.124	.069
	4	-.158*	.025	.000	-.209	-.107
	5	-.318*	.048	.000	-.418	-.219
	6	.524*	.029	.000	.465	.584
3	1	.570*	.037	.000	.492	.647
	2	.027	.047	.564	-.069	.124
	4	-.130*	.049	.014	-.231	-.029
	5	-.291*	.047	.000	-.388	-.194
	6	.552*	.035	.000	.478	.625
4	1	.700*	.030	.000	.637	.763
	2	.158*	.025	.000	.107	.209
	3	.130*	.049	.014	.029	.231
	5	-.161*	.052	.005	-.267	-.054
	6	.682*	.031	.000	.617	.746
5	1	.861*	.040	.000	.779	.943
	2	.318*	.048	.000	.219	.418
	3	.291*	.047	.000	.194	.388
	4	.161*	.052	.005	.054	.267
	6	.842*	.041	.000	.757	.927
6	1	.018*	.004	.000	.009	.027
	2	-.524*	.029	.000	-.584	-.465
	3	-.552*	.035	.000	-.625	-.478
	4	-.682*	.031	.000	-.746	-.617
	5	-.842*	.041	.000	-.927	-.757

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.984	221.074[a]	5.000	18.000	.000	.984
Wilks' lambda	.016	221.074[a]	5.000	18.000	.000	.984
Hotelling's trace	61.410	221.074[a]	5.000	18.000	.000	.984
Roy's largest root	61.410	221.074[a]	5.000	18.000	.000	.984

Each F tests the multivariate effect of temp. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

2. time (d) * temp

Measure: MEASURE_1

time (d)	temp	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	1	6.467	.032	6.401	6.533
	2	6.800	.091	6.611	6.989
	3	6.767	.122	6.514	7.019
	4	6.767	.089	6.581	6.952
	5	6.833	.137	6.549	7.118
	6	6.467	.032	6.401	6.533
1	1	6.467	.032	6.401	6.533
	2	6.833	.091	6.645	7.022
	3	6.800	.122	6.547	7.053
	4	6.800	.089	6.615	6.985
	5	6.800	.137	6.516	7.084
	6	6.467	.032	6.401	6.533
2	1	7.500	.032	7.434	7.566
	2	6.800	.091	6.611	6.989
	3	6.800	.122	6.547	7.053
	4	6.833	.089	6.648	7.019
	5	6.867	.137	6.582	7.151
	6	7.500	.032	7.434	7.566
3	1	7.400	.032	7.334	7.466
	2	6.900	.091	6.711	7.089
	3	6.933	.122	6.681	7.186
	4	6.900	.089	6.715	7.085
	5	7.000	.137	6.716	7.284
	6	7.400	.032	7.334	7.466
4	1	6.467	.032	6.401	6.533
	2	7.000	.091	6.811	7.189
	3	7.000	.122	6.747	7.253
	4	7.067	.089	6.881	7.252
	5	7.000	.137	6.716	7.284
	6	6.467	.032	6.401	6.533
5	1	6.467	.032	6.401	6.533
	2	7.700	.091	7.511	7.889
	3	7.233	.122	6.981	7.486
	4	7.433	.089	7.248	7.619
	5	7.500	.137	7.216	7.784
	6	6.467	.032	6.401	6.533
6	1	6.467	.032	6.401	6.533
	2	7.067	.091	6.878	7.255
	3	7.300	.122	7.047	7.553
	4	7.667	.089	7.481	7.852
	5	7.667	.137	7.382	7.951
	6	6.467	.032	6.401	6.533
7	1	6.467	.032	6.401	6.533
	2	7.200	.091	7.011	7.389
	3	7.800	.122	7.547	8.053
	4	7.367	.089	7.181	7.552
	5	7.467	.137	7.182	7.751
	6	6.500	.032	6.434	6.566
8	1	6.500	.032	6.434	6.566
	2	7.367	.091	7.178	7.555
	3	7.233	.122	6.981	7.486
	4	8.100	.089	7.915	8.285
	5	8.533	.137	8.249	8.818
	6	6.667	.032	6.601	6.733
9	1	6.567	.032	6.501	6.633
	2	7.500	.091	7.311	7.689
	3	8.333	.122	8.081	8.586
	4	8.233	.089	8.048	8.419
	5	8.400	.137	8.116	8.684
	6	6.567	.032	6.501	6.633
10	1	6.600	.032	6.534	6.666
	2	8.167	.091	7.978	8.355
	3	7.433	.122	7.181	7.686
	4	7.900	.089	7.715	8.085
	5	8.767	.137	8.482	9.051
	6	6.600	.032	6.534	6.666

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) time (d)	(J) time (d)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	-.0111	.06361	1.000	-.2548	.2326
	2	-.3667*	.06361	.000	-.6104	-.1230
	3	-.4056*	.06361	.000	-.6493	-.1618
	4	-.1500	.06361	1.000	-.3937	.0937
	5	-.4500*	.06361	.000	-.6937	-.2063
	6	-.4222*	.06361	.000	-.6659	-.1785
	7	-.4500*	.06361	.000	-.6937	-.2063
	8	-.7167*	.06361	.000	-.9604	-.4730
	9	-.9167*	.06361	.000	-1.1604	-.6730
	10	-.8944*	.06361	.000	-1.1382	-.6507
1	0	.0111	.06361	1.000	-.2326	.2548
	2	-.3556*	.06361	.001	-.5993	-.1118
	3	-.3944*	.06361	.000	-.6382	-.1507
	4	-.1389	.06361	1.000	-.3826	.1048
	5	-.4389*	.06361	.000	-.6826	-.1952
	6	-.4111*	.06361	.000	-.6548	-.1674
	7	-.4389*	.06361	.000	-.6826	-.1952
	8	-.7056*	.06361	.000	-.9493	-.4618
	9	-.9056*	.06361	.000	-1.1493	-.6618
	10	-.8833*	.06361	.000	-1.1270	-.6396
2	0	.3667*	.06361	.000	.1230	.6104
	1	.3556*	.06361	.001	.1118	.5993
	3	-.0389	.06361	1.000	-.2826	.2048
	4	.2167	.06361	.139	-.0270	.4604
	5	-.0833	.06361	1.000	-.3270	.1604
	6	-.0556	.06361	1.000	-.2993	.1882
	7	-.0833	.06361	1.000	-.3270	.1604
	8	-.3500*	.06361	.001	-.5937	-.1063
	9	-.5500*	.06361	.000	-.7937	-.3063
	10	-.5278*	.06361	.000	-.7715	-.2841
3	0	.4056*	.06361	.000	.1618	.6493
	1	.3944*	.06361	.000	.1507	.6382
	2	.0389	.06361	1.000	-.2048	.2826
	4	.2556*	.06361	.032	.0118	.4993
	5	-.0444	.06361	1.000	-.2882	.1993
	6	-.0167	.06361	1.000	-.2604	.2270
	7	-.0444	.06361	1.000	-.2882	.1993
	8	-.3111*	.06361	.004	-.5548	-.0674
	9	-.5111*	.06361	.000	-.7548	-.2674
	10	-.4889*	.06361	.000	-.7326	-.2452
4	0	.1500	.06361	1.000	-.0937	.3937
	1	.1389	.06361	1.000	-.1048	.3826
	2	-.2167	.06361	.139	-.4604	.0270
	3	-.2556*	.06361	.032	-.4993	-.0118
	5	-.3000*	.06361	.006	-.5437	-.0563
	6	-.2722*	.06361	.017	-.5159	-.0285
	7	-.3000*	.06361	.006	-.5437	-.0563
	8	-.5667*	.06361	.000	-.8104	-.3230
	9	-.7667*	.06361	.000	-1.0104	-.5230
	10	-.7444*	.06361	.000	-.9882	-.5007
5	0	.4500*	.06361	.000	.2063	.6937
	1	.4389*	.06361	.000	.1952	.6826
	2	.0833	.06361	1.000	-.1604	.3270
	3	.0444	.06361	1.000	-.1993	.2882
	4	.3000*	.06361	.006	.0563	.5437
	6	.0278	.06361	1.000	-.2159	.2715
	7	.0000	.06361	1.000	-.2437	.2437
	8	-.2667*	.06361	.021	-.5104	-.0230
	9	-.4667*	.06361	.000	-.7104	-.2230
	10	-.4444*	.06361	.000	-.6882	-.2007
6	0	.4222*	.06361	.000	.1785	.6659
	1	.4111*	.06361	.000	.1674	.6548
	2	.0556	.06361	1.000	-.1882	.2993
	3	.0167	.06361	1.000	-.2270	.2604
	4	.2722*	.06361	.017	.0285	.5159
	5	-.0278	.06361	1.000	-.2715	.2159
	7	-.0278	.06361	1.000	-.2715	.2159
	8	-.2944*	.06361	.007	-.5382	-.0507
	9	-.4944*	.06361	.000	-.7382	-.2507
	10	-.4722*	.06361	.000	-.7159	-.2285
7	0	.4500*	.06361	.000	.2063	.6937
	1	.4389*	.06361	.000	.1952	.6826
	2	.0833	.06361	1.000	-.1604	.3270
	3	.0444	.06361	1.000	-.1993	.2882
	4	.3000*	.06361	.006	.0563	.5437
	5	.0000	.06361	1.000	-.2437	.2437
	6	.0278	.06361	1.000	-.2159	.2715
	8	-.2667*	.06361	.021	-.5104	-.0230
	9	-.4667*	.06361	.000	-.7104	-.2230
	10	-.4444*	.06361	.000	-.6882	-.2007
8	0	.7167*	.06361	.000	.4730	.9604
	1	.7056*	.06361	.000	.4618	.9493
	2	.3500*	.06361	.001	.1063	.5937
	3	.3111*	.06361	.004	.0674	.5548
	4	.5667*	.06361	.000	.3230	.8104
	5	.2667*	.06361	.021	.0230	.5104
	6	.2944*	.06361	.007	.0507	.5382
	7	.2667*	.06361	.021	.0230	.5104
	9	-.2000	.06361	.259	-.4437	.0437
	10	-.1778	.06361	.581	-.4215	.0659
9	0	.9167*	.06361	.000	.6730	1.1604
	1	.9056*	.06361	.000	.6618	1.1493
	2	.5500*	.06361	.000	.3063	.7937
	3	.5111*	.06361	.000	.2674	.7548
	4	.7667*	.06361	.000	.5230	1.0104
	5	.4667*	.06361	.000	.2230	.7104
	6	.4944*	.06361	.000	.2507	.7382
	7	.4667*	.06361	.000	.2230	.7104
	8	.2000	.06361	.259	-.0437	.4437
	10	.0222	.06361	1.000	-.2215	.2659
10	0	.8944*	.06361	.000	.6507	1.1382
	1	.8833*	.06361	.000	.6396	1.1270
	2	.5278*	.06361	.000	.2841	.7715
	3	.4889*	.06361	.000	.2452	.7326
	4	.7444*	.06361	.000	.5007	.9882
	5	.4444*	.06361	.000	.2007	.6882
	6	.4722*	.06361	.000	.2285	.7159
	7	.4444*	.06361	.000	.2007	.6882
	8	.1778	.06361	.581	-.0659	.4215
	9	-.0222	.06361	1.000	-.2659	.2215

Based on observed means.
The error term is Mean Square(Error) = .006.

*. The mean difference is significant at the .05 level.

Repeated measures ANOVA SPSS output on pH data from the tropical strain of *Fusarium equiseti*

Within-Subjects Factors

Measure: MEASURE_1

temp	Dependent Variable
1	pH_5
2	pH_10
3	pH_15
4	pH_20
5	pH_25
6	pH_30

Between-Subjects Factors

		N
time (d)	0	3
	1	3
	2	3
	3	3
	4	3
	5	3
	6	3
	7	3
	8	3
	9	3
	10	3

Descriptive Statistics

	time (d)	Mean	Std. Deviation	N
pH_5	0	6.9333	.05774	3
	1	6.8333	.05774	3
	2	6.8667	.05774	3
	3	6.8000	.00000	3
	4	6.8667	.11547	3
	5	6.7333	.05774	3
	6	6.8333	.05774	3
	7	6.8000	.00000	3
	8	6.9000	.00000	3
	9	6.7333	.05774	3
	10	6.7667	.05774	3
	Total	6.8242	.07918	33
pH_10	0	6.9000	.00000	3
	1	7.0000	.00000	3
	2	7.0000	.00000	3
	3	7.0000	.00000	3
	4	6.9000	.00000	3
	5	6.9000	.00000	3
	6	7.0000	.00000	3
	7	7.6000	.00000	3
	8	7.8000	.00000	3
	9	7.5000	.00000	3
	10	8.2000	.00000	3
	Total	7.2545	.43380	33
pH_15	0	6.9000	.10000	3
	1	6.8333	.05774	3
	2	6.7333	.05774	3
	3	6.7333	.05774	3
	4	7.6667	.05774	3
	5	7.9667	.28868	3
	6	7.6333	.25166	3
	7	7.7667	.11547	3
	8	8.1000	.00000	3
	9	8.2333	.15275	3
	10	8.3667	.05774	3
	Total	7.5394	.61894	33
pH_20	0	6.7000	.00000	3
	1	6.8333	.05774	3
	2	7.5667	.28868	3
	3	7.9000	.34641	3
	4	8.8333	.23094	3
	5	8.9333	.15275	3
	6	8.5333	.23094	3
	7	8.5333	.23094	3
	8	8.3667	.05774	3
	9	8.7000	.00000	3
	10	8.6000	.10000	3
	Total	8.1364	.77654	33
pH_25	0	6.7667	.05774	3
	1	6.8667	.05774	3
	2	7.0667	.05774	3
	3	8.0000	.17321	3
	4	9.1000	.00000	3
	5	9.1000	.00000	3
	6	8.6333	.05774	3
	7	8.6333	.05774	3
	8	8.6000	.00000	3
	9	8.6000	.10000	3
	10	8.5667	.05774	3
	Total	8.1758	.84633	33
pH_30	0	7.1000	.00000	3
	1	7.8000	.00000	3
	2	8.6000	.26458	3
	3	9.0000	.00000	3
	4	8.5000	.00000	3
	5	8.3667	.05774	3
	6	8.4333	.05774	3
	7	8.4333	.05774	3
	8	8.4667	.11547	3
	9	8.6333	.05774	3
	10	8.5667	.05774	3
	Total	8.3545	.49313	33

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
temp	Pillai's Trace	.998	1946.626[b]	5.000	18.000	.000	.998
	Wilks' Lambda	.002	1946.626[b]	5.000	18.000	.000	.998
	Hotelling's Trace	540.729	1946.626[b]	5.000	18.000	.000	.998
	Roy's Largest Root	540.729	1946.626[b]	5.000	18.000	.000	.998
temp * time	Pillai's Trace	3.981	8.591	50.000	110.000	.000	.796
	Wilks' Lambda	.000	42.224	50.000	85.457	.000	.948
	Hotelling's Trace	384.196	126.016	50.000	82.000	.000	.987
	Roy's Largest Root	273.096	600.812[c]	10.000	22.000	.000	.996

a. Design: Intercept + time
Within Subjects Design: temp

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
temp	.065	54.827	14	.000	.585	.990	.200

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + time
Within Subjects Design: temp

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Sphericity Assumed	60.560	5	12.112	1064.917	.000	.980
	Greenhouse-Geisser	60.560	2.923	20.722	1064.917	.000	.980
	Huynh-Feldt	60.560	4.951	12.233	1064.917	.000	.980
	Lower-bound	60.560	1.000	60.560	1064.917	.000	.980
temp * time	Sphericity Assumed	30.458	50	.609	53.559	.000	.961
	Greenhouse-Geisser	30.458	29.225	1.042	53.559	.000	.961
	Huynh-Feldt	30.458	49.505	.615	53.559	.000	.961
	Lower-bound	30.458	10.000	3.046	53.559	.000	.961
Error(temp)	Sphericity Assumed	1.251	110	.011			
	Greenhouse-Geisser	1.251	64.296	.019			
	Huynh-Feldt	1.251	108.912	.011			
	Lower-bound	1.251	22.000	.057			

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	temp	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Linear	57.169	1	57.169	7452.571	.000	.997
	Quadratic	1.970	1	1.970	122.366	.000	.848
	Cubic	.257	1	.257	44.481	.000	.669
	Order 4	.068	1	.068	5.567	.028	.202
	Order 5	1.097	1	1.097	72.251	.000	.767
temp * time	Linear	10.267	10	1.027	133.848	.000	.984
	Quadratic	11.256	10	1.126	69.912	.000	.969
	Cubic	6.650	10	.665	114.924	.000	.981
	Order 4	.855	10	.086	7.049	.000	.762
	Order 5	1.430	10	.143	9.421	.000	.811
Error(temp)	Linear	.169	22	.008			
	Quadratic	.354	22	.016			
	Cubic	.127	22	.006			
	Order 4	.267	22	.012			
	Order 5	.334	22	.015			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	11782.580	1	11782.580	702696.012	.000	1.000
time	36.402	10	3.640	217.093	.000	.990
Error	.369	22	.017			

1. time (d) * temp

Measure: MEASURE_1

time (d)	temp	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	1	6.933	.033	6.864	7.002
	2	6.900	.000	6.900	6.900
	3	6.900	.080	6.735	7.065
	4	6.700	.111	6.471	6.929
	5	6.767	.043	6.678	6.855
	6	7.100	.055	6.986	7.214
1	1	6.833	.033	6.764	6.902
	2	7.000	.000	7.000	7.000
	3	6.833	.080	6.668	6.999
	4	6.833	.111	6.604	7.063
	5	6.867	.043	6.778	6.955
	6	7.800	.055	7.686	7.914
2	1	6.867	.033	6.798	6.936
	2	7.000	.000	7.000	7.000
	3	6.733	.080	6.568	6.899
	4	7.567	.111	7.337	7.796
	5	7.067	.043	6.978	7.155
	6	8.600	.055	8.486	8.714
3	1	6.800	.033	6.731	6.869
	2	7.000	.000	7.000	7.000
	3	6.733	.080	6.568	6.899
	4	7.900	.111	7.671	8.129
	5	8.000	.043	7.912	8.088
	6	9.000	.055	8.886	9.114
4	1	6.867	.033	6.798	6.936
	2	6.900	.000	6.900	6.900
	3	7.667	.080	7.501	7.832
	4	8.833	.111	8.604	9.063
	5	9.100	.043	9.012	9.188
	6	8.500	.055	8.386	8.614
5	1	6.733	.033	6.664	6.802
	2	6.900	.000	6.900	6.900
	3	7.967	.080	7.801	8.132
	4	8.933	.111	8.704	9.163
	5	9.100	.043	9.012	9.188
	6	8.367	.055	8.253	8.481
6	1	6.833	.033	6.764	6.902
	2	7.000	.000	7.000	7.000
	3	7.633	.080	7.468	7.799
	4	8.533	.111	8.304	8.763
	5	8.633	.043	8.545	8.722
	6	8.433	.055	8.319	8.547
7	1	6.800	.033	6.731	6.869
	2	7.600	.000	7.600	7.600
	3	7.767	.080	7.601	7.932
	4	8.533	.111	8.304	8.763
	5	8.633	.043	8.545	8.722
	6	8.433	.055	8.319	8.547
8	1	6.900	.033	6.831	6.969
	2	7.800	.000	7.800	7.800
	3	8.100	.080	7.935	8.265
	4	8.367	.111	8.137	8.596
	5	8.600	.043	8.512	8.688
	6	8.467	.055	8.353	8.581
9	1	6.733	.033	6.664	6.802
	2	7.500	.000	7.500	7.500
	3	8.233	.080	8.068	8.399
	4	8.700	.111	8.471	8.929
	5	8.600	.043	8.512	8.688
	6	8.633	.055	8.519	8.747
10	1	6.767	.033	6.698	6.836
	2	8.200	.000	8.200	8.200
	3	8.367	.080	8.201	8.532
	4	8.600	.111	8.371	8.829
	5	8.567	.043	8.478	8.655
	6	8.567	.055	8.453	8.681

Estimates

Measure: MEASURE_1

temp	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	6.824	.010	6.803	6.845
2	7.255	.000	7.255	7.255
3	7.539	.024	7.490	7.589
4	8.136	.033	8.067	8.205
5	8.176	.013	8.149	8.202
6	8.355	.017	8.320	8.389

Pairwise Comparisons

Measure: MEASURE_1

(I) temp	(J) temp	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-.430*	.010	.000	-.463	-.397
	3	-.715*	.025	.000	-.797	-.633
	4	-1.312*	.035	.000	-1.428	-1.196
	5	-1.352*	.018	.000	-1.411	-1.292
	6	-1.530*	.020	.000	-1.596	-1.465
2	1	.430*	.010	.000	.397	.463
	3	-.285*	.024	.000	-.364	-.206
	4	-.882*	.033	.000	-.992	-.772
	5	-.921*	.013	.000	-.964	-.879
	6	-1.100*	.017	.000	-1.155	-1.045
3	1	.715*	.025	.000	.633	.797
	2	.285*	.024	.000	.206	.364
	4	-.597*	.034	.000	-.710	-.484
	5	-.636*	.028	.000	-.727	-.546
	6	-.815*	.031	.000	-.917	-.713
4	1	1.312*	.035	.000	1.196	1.428
	2	.882*	.033	.000	.772	.992
	3	.597*	.034	.000	.484	.710
	5	-.039	.033	1.000	-.147	.068
	6	-.218*	.033	.000	-.326	-.110
5	1	1.352*	.018	.000	1.292	1.411
	2	.921*	.013	.000	.879	.964
	3	.636*	.028	.000	.546	.727
	4	.039	.033	1.000	-.068	.147
	6	-.179*	.022	.000	-.250	-.108
6	1	1.530*	.020	.000	1.465	1.596
	2	1.100*	.017	.000	1.045	1.155
	3	.815*	.031	.000	.713	.917
	4	.218*	.033	.000	.110	.326
	5	.179*	.022	.000	.108	.250

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.998	1946.626[a]	5.000	18.000	.000	.998
Wilks' lambda	.002	1946.626[a]	5.000	18.000	.000	.998
Hotelling's trace	540.729	1946.626[a]	5.000	18.000	.000	.998
Roy's largest root	540.729	1946.626[a]	5.000	18.000	.000	.998

Each F tests the multivariate effect of temp. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) time (d)	(J) time (d)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	-.1444	.04316	.161	-.3098	.0209
	2	-.4222*	.04316	.000	-.5876	-.2568
	3	-.6889*	.04316	.000	-.8543	-.5235
	4	-1.0944*	.04316	.000	-1.2598	-.9291
	5	-1.1167*	.04316	.000	-1.2820	-.9513
	6	-.9611*	.04316	.000	-1.1265	-.7957
	7	-1.0778*	.04316	.000	-1.2432	-.9124
	8	-1.1556*	.04316	.000	-1.3209	-.9902
	9	-1.1833*	.04316	.000	-1.3487	-1.0180
	10	-1.2944*	.04316	.000	-1.4598	-1.1291
1	0	.1444	.04316	.161	-.0209	.3098
	2	-.2778*	.04316	.000	-.4432	-.1124
	3	-.5444*	.04316	.000	-.7098	-.3791
	4	-.9500*	.04316	.000	-1.1154	-.7846
	5	-.9722*	.04316	.000	-1.1376	-.8068
	6	-.8167*	.04316	.000	-.9820	-.6513
	7	-.9333*	.04316	.000	-1.0987	-.7680
	8	-1.0111*	.04316	.000	-1.1765	-.8457
	9	-1.0389*	.04316	.000	-1.2043	-.8735
	10	-1.1500*	.04316	.000	-1.3154	-.9846
2	0	.4222*	.04316	.000	.2568	.5876
	1	.2778*	.04316	.000	.1124	.4432
	3	-.2667*	.04316	.000	-.4320	-.1013
	4	-.6722*	.04316	.000	-.8376	-.5068
	5	-.6944*	.04316	.000	-.8598	-.5291
	6	-.5389*	.04316	.000	-.7043	-.3735
	7	-.6556*	.04316	.000	-.8209	-.4902
	8	-.7333*	.04316	.000	-.8987	-.5680
	9	-.7611*	.04316	.000	-.9265	-.5957
	10	-.8722*	.04316	.000	-1.0376	-.7068
3	0	.6889*	.04316	.000	.5235	.8543
	1	.5444*	.04316	.000	.3791	.7098
	2	.2667*	.04316	.000	.1013	.4320
	4	-.4056*	.04316	.000	-.5709	-.2402
	5	-.4278*	.04316	.000	-.5932	-.2624
	6	-.2722*	.04316	.000	-.4376	-.1068
	7	-.3889*	.04316	.000	-.5543	-.2235
	8	-.4667*	.04316	.000	-.6320	-.3013
	9	-.4944*	.04316	.000	-.6598	-.3291
	10	-.6056*	.04316	.000	-.7709	-.4402
4	0	1.0944*	.04316	.000	.9291	1.2598
	1	.9500*	.04316	.000	.7846	1.1154
	2	.6722*	.04316	.000	.5068	.8376
	3	.4056*	.04316	.000	.2402	.5709
	5	-.0222	.04316	1.000	-.1876	.1432
	6	.1333	.04316	.295	-.0320	.2987
	7	.0167	.04316	1.000	-.1487	.1820
	8	-.0611	.04316	1.000	-.2265	.1043
	9	-.0889	.04316	1.000	-.2543	.0765
	10	-.2000*	.04316	.007	-.3654	-.0346
5	0	1.1167*	.04316	.000	.9513	1.2820
	1	.9722*	.04316	.000	.8068	1.1376
	2	.6944*	.04316	.000	.5291	.8598
	3	.4278*	.04316	.000	.2624	.5932
	4	.0222	.04316	1.000	-.1432	.1876
	6	.1556	.04316	.087	-.0098	.3209
	7	.0389	.04316	1.000	-.1265	.2043
	8	-.0389	.04316	1.000	-.2043	.1265
	9	-.0667	.04316	1.000	-.2320	.0987
	10	-.1778*	.04316	.025	-.3432	-.0124
6	0	.9611*	.04316	.000	.7957	1.1265
	1	.8167*	.04316	.000	.6513	.9820
	2	.5389*	.04316	.000	.3735	.7043
	3	.2722*	.04316	.000	.1068	.4376
	4	-.1333	.04316	.295	-.2987	.0320
	5	-.1556	.04316	.087	-.3209	.0098
	7	-.1167	.04316	.715	-.2820	.0487
	8	-.1944*	.04316	.010	-.3598	-.0291
	9	-.2222*	.04316	.002	-.3876	-.0568
	10	-.3333*	.04316	.000	-.4987	-.1680
7	0	1.0778*	.04316	.000	.9124	1.2432
	1	.9333*	.04316	.000	.7680	1.0987
	2	.6556*	.04316	.000	.4902	.8209
	3	.3889*	.04316	.000	.2235	.5543
	4	.0167	.04316	1.000	-.1820	.1487
	5	-.0389	.04316	1.000	-.2043	.1265
	6	.1167	.04316	.715	-.0487	.2820
	8	-.0778	.04316	1.000	-.2432	.0876
	9	-.1056	.04316	1.000	-.2709	.0598
	10	-.2167*	.04316	.003	-.3820	-.0513
8	0	1.1556*	.04316	.000	.9902	1.3209
	1	1.0111*	.04316	.000	.8457	1.1765
	2	.7333*	.04316	.000	.5680	.8987
	3	.4667*	.04316	.000	.3013	.6320
	4	.0611	.04316	1.000	-.1043	.2265
	5	.0389	.04316	1.000	-.1265	.2043
	6	.1944*	.04316	.010	.0291	.3598
	7	.0778	.04316	1.000	-.0876	.2432
	9	-.0278	.04316	1.000	-.1932	.1376
	10	-.1389	.04316	.218	-.3043	.0265
9	0	1.1833*	.04316	.000	1.0180	1.3487
	1	1.0389*	.04316	.000	.8735	1.2043
	2	.7611*	.04316	.000	.5957	.9265
	3	.4944*	.04316	.000	.3291	.6598
	4	.0889	.04316	1.000	-.0765	.2543
	5	.0667	.04316	1.000	-.0987	.2320
	6	.2222*	.04316	.002	.0568	.3876
	7	.1056	.04316	1.000	-.0598	.2709
	8	.0278	.04316	1.000	-.1376	.1932
	10	-.1111	.04316	.952	-.2765	.0543
10	0	1.2944*	.04316	.000	1.1291	1.4598
	1	1.1500*	.04316	.000	.9846	1.3154
	2	.8722*	.04316	.000	.7068	1.0376
	3	.6056*	.04316	.000	.4402	.7709
	4	.2000*	.04316	.007	.0346	.3654
	5	.1778*	.04316	.025	.0124	.3432
	6	.3333*	.04316	.000	.1680	.4987
	7	.2167*	.04316	.003	.0513	.3820
	8	.1389	.04316	.218	-.0265	.3043
	9	.1111	.04316	.952	-.0543	.2765

Based on observed means.

The error term is Mean Square(Error) = .003.

*. The mean difference is significant at the .05 level.

Repeated measures ANOVA SPSS output on pH data from the antarctic strain of *Pseudogymnoascus* spp.

Within-Subjects Factors

Measure: MEASURE_1

temp	Dependent Variable
1	pH_5
2	pH_10
3	pH_15
4	pH_20
5	pH_25
6	pH_30

Between-Subjects Factors

		N
time (d)	0	3
	1	3
	2	3
	3	3
	4	3
	5	3
	6	3
	7	3
	8	3
	9	3
	10	3

Descriptive Statistics

	time (d)	Mean	Std. Deviation	N
pH_5	0	6.8000	.10000	3
	1	6.8333	.05774	3
	2	6.6667	.05774	3
	3	6.8333	.05774	3
	4	6.7667	.05774	3
	5	6.9667	.11547	3
	6	7.1333	.15275	3
	7	7.0333	.15275	3
	8	7.3667	.05774	3
	9	8.0000	.43589	3
	10	7.7667	.11547	3
	Total	7.1061	.44084	33
pH_10	0	6.9667	.05774	3
	1	6.9000	.00000	3
	2	6.9000	.00000	3
	3	6.8000	.00000	3
	4	6.6000	.00000	3
	5	6.8000	.00000	3
	6	6.9000	.00000	3
	7	7.0000	.00000	3
	8	7.2000	.00000	3
	9	7.5000	.00000	3
	10	7.2000	.00000	3
	Total	6.9788	.23685	33
pH_15	0	6.9000	.00000	3
	1	7.0000	.10000	3
	2	7.1000	.10000	3
	3	6.6667	.05774	3
	4	7.0333	.15275	3
	5	7.5667	.35119	3
	6	8.2333	.05774	3
	7	8.3333	.11547	3
	8	8.5333	.11547	3
	9	8.5000	.00000	3
	10	8.6333	.28868	3
	Total	7.6818	.75600	33
pH_20	0	6.6667	.05774	3
	1	6.7333	.05774	3
	2	6.8333	.11547	3
	3	6.9000	.10000	3
	4	8.6000	.10000	3
	5	9.0333	.05774	3
	6	8.4333	.05774	3
	7	8.5000	.10000	3
	8	8.5333	.05774	3
	9	8.7000	.10000	3
	10	8.6000	.26458	3
	Total	7.9576	.91993	33
pH_25	0	6.9000	.00000	3
	1	6.7667	.05774	3
	2	7.0000	.10000	3
	3	7.8000	.26458	3
	4	8.5000	.00000	3
	5	8.6000	.00000	3
	6	8.1333	.15275	3
	7	7.2667	.30551	3
	8	8.1000	.10000	3
	9	7.6333	.72342	3
	10	7.0333	.61101	3
	Total	7.6121	.69136	33
pH_30	0	6.9667	.05774	3
	1	6.9000	.00000	3
	2	6.6667	.05774	3
	3	7.2667	.23094	3
	4	7.7333	.15275	3
	5	7.8333	.15275	3
	6	8.2333	.15275	3
	7	8.2000	.00000	3
	8	8.5000	.00000	3
	9	.0000	.00000	3
	10	8.3000	.10000	3
	Total	6.9636	2.31986	33

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
temp	Pillai's Trace	.994	645.539[b]	5.000	18.000	.000	.994
	Wilks' Lambda	.006	645.539[b]	5.000	18.000	.000	.994
	Hotelling's Trace	179.316	645.539[b]	5.000	18.000	.000	.994
	Roy's Largest Root	179.316	645.539[b]	5.000	18.000	.000	.994
temp * time	Pillai's Trace	4.149	10.722	50.000	110.000	.000	.830
	Wilks' Lambda	.000	61.229	50.000	85.457	.000	.963
	Hotelling's Trace	901.155	295.579	50.000	82.000	.000	.994
	Roy's Largest Root	741.402	1631.084[c]	10.000	22.000	.000	.999

a. Design: Intercept + time
Within Subjects Design: temp

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
temp	.011	91.113	14	.000	.364	.576	.200

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + time
Within Subjects Design: temp

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Sphericity Assumed	29.300	5	5.860	175.639	.000	.889
	Greenhouse-Geisser	29.300	1.821	16.086	175.639	.000	.889
	Huynh-Feldt	29.300	2.880	10.175	175.639	.000	.889
	Lower-bound	29.300	1.000	29.300	175.639	.000	.889
temp * time	Sphericity Assumed	189.888	50	3.798	113.830	.000	.981
	Greenhouse-Geisser	189.888	18.215	10.425	113.830	.000	.981
	Huynh-Feldt	189.888	28.797	6.594	113.830	.000	.981
	Lower-bound	189.888	10.000	18.989	113.830	.000	.981
Error(temp)	Sphericity Assumed	3.670	110	.033			
	Greenhouse-Geisser	3.670	40.072	.092			
	Huynh-Feldt	3.670	63.353	.058			
	Lower-bound	3.670	22.000	.167			

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	temp	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
temp	Linear	1.010	1	1.010	23.866	.000	.520
	Quadratic	18.166	1	18.166	618.009	.000	.966
	Cubic	7.158	1	7.158	382.704	.000	.946
	Order 4	2.926	1	2.926	47.690	.000	.684
	Order 5	.040	1	.040	2.648	.118	.107
temp * time	Linear	87.396	10	8.740	206.531	.000	.989
	Quadratic	57.136	10	5.714	194.380	.000	.989
	Cubic	30.256	10	3.026	161.762	.000	.987
	Order 4	12.949	10	1.295	21.101	.000	.906
	Order 5	2.153	10	.215	14.313	.000	.867
Error(temp)	Linear	.931	22	.042			
	Quadratic	.647	22	.029			
	Cubic	.411	22	.019			
	Order 4	1.350	22	.061			
	Order 5	.331	22	.015			

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	10793.695	1	10793.695	925173.857	.000	1.000
time	47.080	10	4.708	403.543	.000	.995
Error	.257	22	.012			

time (d) * temp

Measure: MEASURE_1

time (d)	temp	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	1	6.800	.094	6.606	6.994
	2	6.967	.010	6.946	6.988
	3	6.900	.093	6.708	7.092
	4	6.667	.065	6.532	6.802
	5	6.900	.183	6.520	7.280
	6	6.967	.065	6.832	7.102
1	1	6.833	.094	6.639	7.028
	2	6.900	.010	6.879	6.921
	3	7.000	.093	6.808	7.192
	4	6.733	.065	6.598	6.868
	5	6.767	.183	6.387	7.146
	6	6.900	.065	6.765	7.035
2	1	6.667	.094	6.472	6.861
	2	6.900	.010	6.879	6.921
	3	7.100	.093	6.908	7.292
	4	6.833	.065	6.698	6.968
	5	7.000	.183	6.620	7.380
	6	6.667	.065	6.532	6.802
3	1	6.833	.094	6.639	7.028
	2	6.800	.010	6.779	6.821
	3	6.667	.093	6.475	6.859
	4	6.900	.065	6.765	7.035
	5	7.800	.183	7.420	8.180
	6	7.267	.065	7.132	7.402
4	1	6.767	.094	6.572	6.961
	2	6.600	.010	6.579	6.621
	3	7.033	.093	6.841	7.225
	4	8.600	.065	8.465	8.735
	5	8.500	.183	8.120	8.880
	6	7.733	.065	7.598	7.868
5	1	6.967	.094	6.772	7.161
	2	6.800	.010	6.779	6.821
	3	7.567	.093	7.375	7.759
	4	9.033	.065	8.898	9.168
	5	8.600	.183	8.220	8.980
	6	7.833	.065	7.698	7.968
6	1	7.133	.094	6.939	7.328
	2	6.900	.010	6.879	6.921
	3	8.233	.093	8.041	8.425
	4	8.433	.065	8.298	8.568
	5	8.133	.183	7.754	8.513
	6	8.233	.065	8.098	8.368
7	1	7.033	.094	6.839	7.228
	2	7.000	.010	6.979	7.021
	3	8.333	.093	8.141	8.525
	4	8.500	.065	8.365	8.635
	5	7.267	.183	6.887	7.646
	6	8.200	.065	8.065	8.335
8	1	7.367	.094	7.172	7.561
	2	7.200	.010	7.179	7.221
	3	8.533	.093	8.341	8.725
	4	8.533	.065	8.398	8.668
	5	8.100	.183	7.720	8.480
	6	8.500	.065	8.365	8.635
9	1	8.000	.094	7.806	8.194
	2	7.500	.010	7.479	7.521
	3	8.500	.093	8.308	8.692
	4	8.700	.065	8.565	8.835
	5	7.633	.183	7.254	8.013
	6	.000	.065	-.135	.135
10	1	7.767	.094	7.572	7.961
	2	7.200	.010	7.179	7.221
	3	8.633	.093	8.441	8.825
	4	8.600	.065	8.465	8.735
	5	7.033	.183	6.654	7.413
	6	8.300	.065	8.165	8.435

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) time (d)	(J) time (d)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	.0111	.03600	1.000	-.1268	.1491
	2	.0056	.03600	1.000	-.1324	.1435
	3	-.1778*	.03600	.003	-.3157	-.0398
	4	-.6722*	.03600	.000	-.8102	-.5343
	5	-.9333*	.03600	.000	-1.0713	-.7954
	6	-.9778*	.03600	.000	-1.1157	-.8398
	7	-.8556*	.03600	.000	-.9935	-.7176
	8	-1.1722*	.03600	.000	-1.3102	-1.0343
	9	.1444*	.03600	.032	.0065	.2824
	10	-1.0556*	.03600	.000	-1.1935	-.9176
1	0	-.0111	.03600	1.000	-.1491	.1268
	2	-.0056	.03600	1.000	-.1435	.1324
	3	-.1889*	.03600	.002	-.3268	-.0509
	4	-.6833*	.03600	.000	-.8213	-.5454
	5	-.9444*	.03600	.000	-1.0824	-.8065
	6	-.9889*	.03600	.000	-1.1268	-.8509
	7	-.8667*	.03600	.000	-1.0046	-.7287
	8	-1.1833*	.03600	.000	-1.3213	-1.0454
	9	.1333*	.03600	.068	-.0046	.2713
	10	-1.0667*	.03600	.000	-1.2046	-.9287
2	0	-.0056	.03600	1.000	-.1435	.1324
	1	.0056	.03600	1.000	-.1324	.1435
	3	-.1833*	.03600	.002	-.3213	-.0454
	4	-.6778*	.03600	.000	-.8157	-.5398
	5	-.9389*	.03600	.000	-1.0768	-.8009
	6	-.9833*	.03600	.000	-1.1213	-.8454
	7	-.8611*	.03600	.000	-.9991	-.7232
	8	-1.1778*	.03600	.000	-1.3157	-1.0398
	9	.1389*	.03600	.047	.0009	.2768
	10	-1.0611*	.03600	.000	-1.1991	-.9232
3	0	.1778*	.03600	.003	.0398	.3157
	1	.1889*	.03600	.002	.0509	.3268
	2	.1833*	.03600	.002	.0454	.3213
	4	-.4944*	.03600	.000	-.6324	-.3565
	5	-.7556*	.03600	.000	-.8935	-.6176
	6	-.8000*	.03600	.000	-.9379	-.6621
	7	-.6778*	.03600	.000	-.8157	-.5398
	8	-.9944*	.03600	.000	-1.1324	-.8565
	9	.3222*	.03600	.000	.1843	.4602
	10	-.8778*	.03600	.000	-1.0157	-.7398
4	0	.6722*	.03600	.000	.5343	.8102
	1	.6833*	.03600	.000	.5454	.8213
	2	.6778*	.03600	.000	.5398	.8157
	3	.4944*	.03600	.000	.3565	.6324
	5	-.2611*	.03600	.000	-.3991	-.1232
	6	-.3056*	.03600	.000	-.4435	-.1676
	7	-.1833*	.03600	.002	-.3213	-.0454
	8	-.5000*	.03600	.000	-.6379	-.3621
	9	.8167*	.03600	.000	.6787	.9546
	10	-.3833*	.03600	.000	-.5213	-.2454
5	0	.9333*	.03600	.000	.7954	1.0713
	1	.9444*	.03600	.000	.8065	1.0824
	2	.9389*	.03600	.000	.8009	1.0768
	3	.7556*	.03600	.000	.6176	.8935
	4	.2611*	.03600	.000	.1232	.3991
	6	-.0444	.03600	1.000	-.1824	.0935
	7	.0778	.03600	1.000	-.0602	.2157
	8	-.2389*	.03600	.000	-.3768	-.1009
	9	1.0778*	.03600	.000	.9398	1.2157
	10	-.1222	.03600	.143	-.2602	.0157
6	0	.9778*	.03600	.000	.8398	1.1157
	1	.9889*	.03600	.000	.8509	1.1268
	2	.9833*	.03600	.000	.8454	1.1213
	3	.8000*	.03600	.000	.6621	.9379
	4	.3056*	.03600	.000	.1676	.4435
	5	.0444	.03600	1.000	-.0935	.1824
	7	.1222	.03600	.143	-.0157	.2602
	8	-.1944*	.03600	.001	-.3324	-.0565
	9	1.1222*	.03600	.000	.9843	1.2602
	10	-.0778	.03600	1.000	-.2157	.0602
7	0	.8556*	.03600	.000	.7176	.9935
	1	.8667*	.03600	.000	.7287	1.0046
	2	.8611*	.03600	.000	.7232	.9991
	3	.6778*	.03600	.000	.5398	.8157
	4	.1833*	.03600	.002	.0454	.3213
	5	-.0778	.03600	1.000	-.2157	.0602
	6	-.1222	.03600	.143	-.2602	.0157
	8	-.3167*	.03600	.000	-.4546	-.1787
	9	1.0000*	.03600	.000	.8621	1.1379
	10	-.2000*	.03600	.001	-.3379	-.0621
8	0	1.1722*	.03600	.000	1.0343	1.3102
	1	1.1833*	.03600	.000	1.0454	1.3213
	2	1.1778*	.03600	.000	1.0398	1.3157
	3	.9944*	.03600	.000	.8565	1.1324
	4	.5000*	.03600	.000	.3621	.6379
	5	.2389*	.03600	.000	.1009	.3768
	6	.1944*	.03600	.001	.0565	.3324
	7	.3167*	.03600	.000	.1787	.4546
	9	1.3167*	.03600	.000	1.1787	1.4546
	10	.1167	.03600	.207	-.0213	.2546
9	0	-.1444*	.03600	.032	-.2824	-.0065
	1	-.1333	.03600	.068	-.2713	.0046
	2	-.1389*	.03600	.047	-.2768	-.0009
	3	-.3222*	.03600	.000	-.4602	-.1843
	4	-.8167*	.03600	.000	-.9546	-.6787
	5	-1.0778*	.03600	.000	-1.2157	-.9398
	6	-1.1222*	.03600	.000	-1.2602	-.9843
	7	-1.0000*	.03600	.000	-1.1379	-.8621
	8	-1.3167*	.03600	.000	-1.4546	-1.1787
	10	-1.2000*	.03600	.000	-1.3379	-1.0621
10	0	1.0556*	.03600	.000	.9176	1.1935
	1	1.0667*	.03600	.000	.9287	1.2046
	2	1.0611*	.03600	.000	.9232	1.1991
	3	.8778*	.03600	.000	.7398	1.0157
	4	.3833*	.03600	.000	.2454	.5213
	5	.1222	.03600	.143	-.0157	.2602
	6	.0778	.03600	1.000	-.0602	.2157
	7	.2000*	.03600	.001	.0621	.3379
	8	-.1167	.03600	.207	-.2546	.0213
	9	1.2000*	.03600	.000	1.0621	1.3379

Based on observed means.

The error term is Mean Square(Error) = .002.

*. The mean difference is significant at the .05 level.

APPENDIX D

List of publications and presentations

Tajuddin, N., Rizman-Idid, M., Convey, P., & Alias, S. A. (2017). Thermal adaptation in a marine-derived tropical strain of *Fusarium equiseti* and polar strains of *Pseudogymnoascus* spp. under different nutrient sources. *Botanica Marina, 61*(1): 9–20.

Tajuddin, N., Rizman-Idid, M., Convey, P., & Alias, S. A. (2017). Thermal adaptation in marine-derived fungi originating from tropical and polar regions. *2nd Institute of Ocean and Earth Science (IOES) Higher Centre of Excellence Seminar 2017: Air-Ocean-Land Interactions* (p. 61). 12th September 2017. Kuala Lumpur.

Tajuddin, N., Rizman-Idid, M., Convey, P., & Alias, S. A. (2017). Temperature-dependent relative growth rates and activity of extracellular hydrolytic enzymes in marine-derived fungi from tropical and polar regions under different nutrient sources. *7th Malaysian International Seminar on Antarctica* (p. 25). 15th – 17th August 2017. Kuala Terengganu.

Tajuddin, N., Rizman-Idid, M., Convey, P., & Alias, S. A. (2016). Effects of temperature on growth and relative activity of extracellular hydrolytic enzymes in marine-derived fungal strains from different latitudes. *21st Biological Sciences Graduate Congress: Biodiversity, Ecology, and Systematics* (p. 22). 15th – 16th December 2016. Kuala Lumpur.

Tajuddin, N., Rizman-Idid, M., Convey, P., & Alias, S. A. (2016). Extracellular hydrolase enzymes (EHEs) of marine and marine-derived fungi in a warming world. *Australia-Malaysia Research Seminar Series III*. Kuala Lumpur.

Tajuddin, N., Rizman-Idid, M., Pang, K. L., Convey, P., & Alias, S. A. (2016). Effects of temperature on growth and extracellular hydrolase enzyme (EHE) relative activity in marine-derived fungi from different latitudes. *XXXIV Scientific Committee on Antarctic Research Open Science Conference 2016 with Biennial Meetings: Antarctica in the Global Earth System: From the Poles to the Tropics* (p. 632). 20th – 30th August 2016. Kuala Lumpur.

Mohamad-Fauzi, A., **Tajuddin, N.**, Halim, H., Convey, P., Rizman-Idid, M., & Alias, S. A. (2016). Diversity of polar soil fungi. *XXXIV Scientific Committee on Antarctic Research Open Science Conference 2016 with Biennial Meetings: Antarctica in the Global Earth System: From the Poles to the Tropics* (p. 632). 20th – 30th August 2016. Kuala Lumpur.