MARINE ALGAL VIRUS COMMUNITIES ALONG SOUTHERN AFRICAN COASTS

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Abstract

Southern Africa is home to highly diverse marine environments along its coasts. The main reason for the contrasting environments is the two major boundary currents, the Benguela and Agulhas currents, and their interaction around the tip of South Africa. Algal blooms are known to proliferate predominantly off the nutrient-rich west coast, however, sporadic inshore upwelling on the east coast can also illicit these events. In addition, solar salt-pans located on the coast that draw their water from the bay area are affected by bloom events. Algal viruses play a key role in regulating phytoplankton communities and modulate the dynamics of these bloom events. Identifying the viruses associated with algal blooms is the first step in determining the role they play in the bloom dynamics. Here I chose to focus on phycodnaviruses , known agents of bloom termination. Samples were taken from two specific algal blooms that occurred in 2013 in different bioregions namely Elands Bay (west coast) and Algoa Bay (east coast). Additionally the Cerebos solar salt pans located along the west coast were selected as sample sites to investigate viral composition. DNA polymerase (pol) gene fragments were amplified from environmental samples using algal-virus specific PCR primers AVS1 and POL. Amplified fragments were then sequenced. Viral sequences were identified and mapped to existing virus families. Amplicon specific primers were designed for select dominant virus group identified for both bloom events. These were used to screen across all samples to determine viral prevalence. Phylogenetic analysis of viral sequences revealed new clades of *Phycodnaviridae* in the Elands Bay and Algoa Bay regions. A bloom terminating virus, EB 1, is proposed for the Elands Bay bloom event. The Cerebos salt pans showed the greatest diversity

of all samples analysed and novel halophilic algal viruses were identified in regions with the highest salinity.

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Abbreviations

ATP – Adenosine Triphophate

bp – Base pair

DNA – Deoxyribonucleic Acid

ds – Double stranded

kb – Kilobase

Mb – Megabase

µm – Micrometer

µL – Microlitre

ss – Single stranded

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Chapter 1 – Introduction:

1.1. The virus world

1.1.1. Virus structure and characteristics

The basic structures for viruses are classed into very specific structure types (Bamford et al. 2002). All viruses consist of a genome encased in a coat of proteins called a capsid (Bamford et al. 2005). Viral genomes come in seven distinct arrangements namely Class I to VII as seen in Table 1. (Lodish H, Berk A 2000).

Table 1. Viral Genome Arrangement

Class	Nucleic acid structure	Example
Class I	dsDNA	*Phycodnaviruses*
Class II	ssDNA	*Parvoviruses*
Class III	dsRNA	*Reoviruses*
Class IV	(+)ssRNA	*Picornaviruses*
Class V	(-)ssRNA	*Rhabdoviruses*
Class VI	ssRNA-RT	*Retroviruses*
Class VII	dsDNA-RT	*Hepadnaviruses*

Proteins inside the viral capsid are also assembled into different shapes. The simplest structure is that of a helix in the example of the tobacco mosaic virus (Lodish H, Berk A 2000). Most

eukaryotic viruses have an icosahedral structure, such as the Adenovirus group (Bamford et al. 2005) and other large dsDNA viruses (Xiao & Rossman 2011). Phages have an extended icosahedron structure with a tail-like projection used for genomic insertion (Papke & Doolittle 2003). More complex viruses have neither a purely helical or icosahedral structure and incorporate a mixture of features such as the T4 bacteriophage (Miller et al. 2003). The capsid can either be naked or enveloped in an external layer of lipids. The exterior surface and structure of viruses plays an important role in infectivity and viral genomic insertion (Braunwald et al. 1985). This is because proteins on the surface are required to attach to receptors on host surface. Therefore the specificity of viral proteins and host receptors are important features for viral infection (DeAngelis 1997; Graves et al. 1999). Viral life cycles can either be lytic or lysogenic. Lytic life cycles involve viral replication in the host and viral particles escape from the host either by cell lysis or budding. Viruses with a lysogenic life cycle persist within the host and transfer themselves to daughter cells making use of host cell mitosis. These viruses then escape when required through cell lysis or budding (Lodish H, Berk A 2000).

Viruses are parasitic in nature relying entirely on a host for proliferation (Bamford et al. 2005). Viral genomic replication, depending on virus species and replication requirements, utilise host intracellular proteins and mechanisms for successful assembly of new virions (Koonin et al. 2006). The host cellular environment is therefore a vital component to understand when analysing the viral life cycle (Bandea 2009). It can then be suggested that host dynamics have an effect on viral replication efficiency. This can be determined by genomic stability, use of available amino acids of, dinucleotide bias and synonymous codon usage, therefore inadvertently influencing the evolution of viruses (Cheng et al. 2014). Viruses adapt to their

relevant host environment and not all viruses are pathogenic or cause damage to their host (Jessup & Forde 2008).

Iyer et al. (2006), described that all known viruses share two common features: intracellular parasitism and virion architecture. Other features such as genome size, mechanisms utilised for viral assembly and propagation, and virus-host interactions often differ significantly from virus to virus. This suggests multiple origins of evolution while common attributes indicate convergences linked to intracellular parasitic adaptions over time (Iyer et al. 2006a).

1.1.2. Viruses in the marine environment

Until recently the main focus of environmental microbiology was the culturing of bacteria, Archaea and Eukarya (Rohwer & Thurber 2009). Initial studies indicated that there was a low abundance of microbes thus leading to the estimation that microbial associated activities played a fairly minor role in the ecosystem and nutrient cycling (Breitbart et.al. 2005). It wasn't until 1977, when experiments using direct counts and radiolabeled compounds to assess microbial activity revealed that microbes indeed had a more significant role in global carbon cycles (Hobbie et. al. 1977). It is assumed that most virus like particles (VLPs) in the environment are phages that infect bacteria (Bergh et al. 1989).

Over the years various studies (Fuhrman 1999; Short & Suttle 2003; Angly et al. 2006) have demonstrated the worldwide distribution and abundance of viruses in marine environments. The ubiquitous nature of viruses in the ocean has been well established (Baudoux et.al. 2005)

and they have been described to contribute to the second largest biomass of any group in the ocean, being the most abundant organisms (Angly et. al. 2006). Although this may be true, few viruses have been characterised and even identified (Wilson et al. 2009). The biological properties, distribution and location of many marine viruses still remain unknown. Marine viruses have distinct features separating them from other known virus types previously characterised for terrestrial systems (Gimenes et al. 2012). High global diversity exists among marine viruses, yet niche regions are found to contain different assemblages of viruses (Angly et al. 2006). Therefore it is proposed that there are possible viral interactions with host cells that cascade into effects within specific ecosystems (Wommack & Colwell 2000). They may even affect microbial evolution through insertion of genes through phage-like mechanisms, altering the phenotype of their hosts (Jessup & Forde 2008; Rohwer & Thurber 2009). Environmental factors are involved in contributing to viral succession by external selective pressure (Angly et al. 2006). Such environmental factors include ocean currents, temperature, salinity and availability of micro- and macro-nutrients (Danovaro et al. 2011; Gimenes et al. 2012). Viral activity was shown to relate to nutrient availability (Wommack & Colwell 2000), however, Schroeder et al. (2003) showed that this was not the case when looking at the *Emiliania huxleyi* virus. Whole-community genome sequencing using metagenomics allows for characterisation of community metabolic diversity and to understand the structure of the microbial community (Angly et al. 2006).

1.1.3. Algal Blooms and viruses

Marine algal blooms are a known occurrence around the world. Increase proliferation of phytoplankton species is an important event in many ecosystems, ranging from coastal to pelagic waters (Trainer et al. 2010). Different types of blooms have been documented throughout time (Hallegraeff 1993). Some are harmless, but can become detrimental to other marine life through dense aggregations causing anoxic events (Pitcher & Probyn 2011). The more serious kind are those known to be harmful algal blooms where specific species release toxins that can impact human and/or marine life that are in close proximity (Scorzetti et al. 2009).

Unicellular algae, such as photosynthetic phytoplankton are one of the primary hosts for viral infection (Van Etten et al. 1982). Phytoplankton forms the basis of the pelagic marine food web. Changes in their dynamics and community structure can adversely affect the natural functioning of marine ecosystems (Wilhelm & Suttle 1999). Some bloom forming plankton are essential for the functioning of marine pelagic environments and regulation of global climate (Verity & Smetacek 1996). Along with mortality, cell lysis has been factored in as a major source of loss of phytoplankton biomass (Lawrence & Suttle 2004; Suttle 1994). Microbial food web structure is thus affected by viral lysis of hosts. Cell disruption and lysis, as a result of viruses, affect biogeochemical cycling as free dissolved organic matter is redistributed into the environment (Bratbak et al. 1990). It has also been described that viruses can possibly improve photosynthetic ability in certain strains of phytoplankton, (Suttle et al. 1990; Suttle 1992). Viral infection of hosts affects the natural production rates of the host, for example in the case of

Emiliania huxleyi, declining or stationary phase growth of the organism results in increased production of dimethyl sulfide (DMS) (Schroeder et al. 2003). Viruses possess possible mechanisms that control community structure and affect the ecosystem. Viral infection of hosts modifies the dynamics and diversity of the population (Baudoux et al. 2006). In phytoplankton ecology, viruses influence the community structure and species abundance leading to greater diversity within blooms (Short & Suttle 2003; Bratbak et al. 1990; Sandaa & Larsen 2006). The effect viruses have on the community structure of marine eukaryotic phytoplankton has been investigated and substantial headway has been made in this field of research with a number of algal viruses being investigated (Van Etten 2003).

Significant biomass losses have been attributed to virally induced mortality of phytoplankton during bloom events (Schroeder et al. 2003; Baudoux et al. 2006; Bratbak et al. 1993; Suttle 1994). Algal viruses are able to control host population dynamics through mortality of their phytoplankton hosts (Baudoux et al. 2005). Viruses can therefore influence population succession (Schroeder et al. 2003). The viral control of phytoplankton species diversity within a bloom has coined the term “kill the winner” (Thingstad 2000). Assessing viral infection of hosts sheds light on the vital role viruses play in the rapid population crashes observed (Wilson et al. 2009). Viral induced mortality of bloom forming algal species have been documented for almost two decades (Bratbak et al. 1996). A large proportion of this research looked at highly virulent and genetically diverse *Prasinovirus* infecting *Micromonas pusilla* (Danovaro et al. 2011).

Certain dinoflagellate bloom forming species have had lytic viruses linked to their demise. Tarutani et al. (2001) were the first to isolate and cultivate the novel dinoflagellate virus, HcV

infecting *Heterocapsa circularisquama*. These blooms have been described as harmful algal blooms and been documented as the cause of death in shellfish, specifically in the Ago Bay region in Japan. In this case, viral lysis of cells may prove to be beneficial as it can alleviate the damaging effects of the harmful algal bloom (Tarutani et al. 2001). Along with the DNA virus HcV, a single stranded RNA virus, HcRNAV, has been isolated from *H. circularisquama* blooms (Tomaru et al. 2004).

The demise of *Emiliania huxleyi* blooms have been linked to the increase in VLPs observed (Bratbak et al. 1993). As an important player in carbon and sulphur cycling in the world's oceans, investigating the virus-associated demise of *E. huxleyi* blooms will provide understanding into the potential molecular mechanisms involved in this system (Wilson et al. 2009). *E. huxleyi* specific viruses, EhV, have been isolated and intensively investigated (Schroeder et al. 2002). Studies have shown that viral infection is integral in host cell health and bloom success (Schroeder et al. 2003).

Additional research provided evidence that viral infection maintained population diversity of the harmful algae *Heterosigma akashiwo* (Nagasaki & Yamaguchi 1997). Bloom termination was documented for the *H. akashiwo virus,* HaV, by Nagasaki et al. (2005). During a particular *H. akashiwo* bloom event, Tarutani et al. (2000) noted that the number of virus infected cells were high nearing the end of the bloom. A single stranded RNA virus, HaRNAV, has also been isolated from *H. akashiwo* blooms (Tai et al. 2003). The HaRNA virus along with the HcRNAV isolated from the *H. circularisquama* blooms were the first two RNA viruses found to infect microalgae (Tomaru et al. 2004). Virus-host interactions have revealed that host specificity may be more

intricate than previously thought, and that specific strains may be virus resistant. This was indicated by the presence of certain a strain still remaining at lower abundances after the bloom was terminated (Tarutani et al. 2000).

1.1.4. Halophilic Viruses

Hypersaline environments can range from hot springs, lakes, solar salterns etc (Pietilä et al. 2013; Atanasova et al. 2015; Emerson et al. 2013). These areas are home to an array of diverse organisms highly adapted to moderate as well as extreme saline conditions (Oren & Seckbach 2011). All three domains of life; Archaea, Bacteria and Eukarya, contain halophilic organisms (Oren 2002). The majority of identified halophiles are found within Archaea and Bacteria, while Eukaryotic halophiles are rare (Ventosa et al. 2015). Many halotolerant and halophilic organisms cover a large phylogenetic range of species both Archaeal and Bacterial (Oren 2002). While Eukaryotes with halotolerant properties are rare, several algal species exist in hypersaline environments, specifically green algae such as chlorophytes and diatoms (Borowitzka 1981; Oren 2002). Vinogradova and Darienko in 2008 noted a relatively high diversity in eukaryotic algal species isolated from the Central Syvash islands. Halotolerant algal diversity, however, is not yet fully comprehensive, with a large proportion of the research centred on benthic microbial mats (Bauld 1981; Guerrero & Wit 1992). We are just starting to understand the microbial diversity within hypersaline environments, and viruses are a substantial part of that (Ventosa et al. 2015).

Viruses associated with halotolerant and halophilic organisms have been isolated through culture-based as well as sequence based investigations (Rodriguez-Brito et al. 2010; Tang et al. 2002). Research of halophilic viruses is still in the early stages (Sime-Ngando et al. 2011), however, more information is being accumulated through the increase of available sequences as well as through the development of metagenomics techniques (Ventosa et al. 2015; Rodriguez-Brito et al. 2010). Quite a large base of haloarchaeal virus diversity has already been established and reviewed by Dyall-Smith et al. (2003). While Archaea seem to dominate the hypersaline sphere, less is known about Archaeal viruses than Bacterial and Eukaryotic viruses (Atanasova et al. 2015). All haloarchaeal viruses have DNA genomes, and are typically double stranded (Porter et al. 2007), with the exception of the ssDNA virus HRPV-1. In research thus far, these viruses have exhibited unique and interesting characteristics such as morphology, genomics, life-cycles and virus-host interactions (Atanasova et al. 2015; Emerson et al. 2013; Sime-Ngando et al. 2011).

1.2. Algal virus lineages

Bratbak et al. (1995) stated that all major taxonomic classes of marine algae have had associated infections of viruses and virus-like particles (VLPs) and that reports of free form algal viruses in nature were rare. Safferman and Morris in 1964 described a virus phage infecting various blue-green algae species, LPP-1, isolated in 1963. These viruses have been identified to infect a range of blue-green algae involved in blooms. These viruses have been termed

cyanophages (Padan & Shilo 1973). Large DNA viruses such as megaviruses are also known to infect a range of algal species (Iyer et al. 2006b). Phycodnaviruses, belonging to the megavirales order, in particular have been found to infect a wide range of important marine primary producers (Short & Suttle 2002). However their ecological roles in the ecosystem are largely unknown and few virus-host systems are well understood (Sandaa 2008). In the last two decades small RNA viruses have been found to infect certain algal species (Culley et al. 2003; Tai et al. 2003; Tomaru et al. 2004)

1.2.1. Megavirales

Early comparative genomics revealed genetic similarity between viral groups suggesting the presence of conserved coding regions for integral proteins related to replication. No common gene is present in all known virus genomes, however due to this reliance on host for replication, a key group of 'viral hallmark genes' are thus shared across diverse viral families (Koonin et al. 2006). Certain assemblages of viruses known as clades within the previously assigned group of Nucleo-Cytoplasmic Large DNA Viruses (NCLDVs) have been determined by phylogenetic analysis of these hallmark genes coding for conserved protein regions (Koonin & Yutin 2010). Together with the loss and gain of genes through gene transfer (Gadelle et al. 2003; Bandea 2009) multiple NCDLV lineages may have been brought into existence (Filée et al. 2008). More recently members of this group has been formalised into the new proposed order *Megavirales* (Colson et al. 2013).

The concept of giant or mega viruses was started by the discovery of the *Mimivirus* (La Scola et al. 2003). The size alone of the first discovered *Mimivirus* caused great excitement in the virology world (La Scola et al. 2003). It has been stated by Claverie et al. (2006) that the *Mimivirus* can be perceived as an intermediary between a virus and a true cell. The *Mimivirus*, while missing ribosomal proteins, has a genome three times larger than the smallest cell and approximately four time larger than what would be necessary to maintain a normal functioning cell (Forterre 2006). However it is still classed as a virus due to its parasitic nature (Moreira & López-García 2005). Proteins genetically described within the *Mimivirus* have shown familial ties with all three domains of life (Raoult et al. 2004).

The *Megavirales* order of viruses consist of viral families infecting a range of hosts. Some of these are well-researched viruses such as *Poxviridae, Asfarviridae, Iridoviridae* and *Ascoviridae* (Williams et al. 2005; Wilson et al. 2009; Xue & Cheng 2011; Yutin & Koonin 2012). Giant viruses such as *Mimiviridae* (also referred to as *Megaviridae* in some literature (Arslan et al. 2011)), *Marseilleviridae*, and *Phycodnaviridae* are also ascribed to this order.

The largest dsDNA virus that has in 2013 caused quite a commotion is the Pandoravirus discovered by Philippe et al. (2013). In yet another recent discovery in 2014 another giant virus, *Pithovirus*, has been described by Legendre et al. (2014) through genetic analysis of a virome of Siberian permafrost. The virus isolated shares many common features such as genome structure and replication with previously characterised *Marseilleviridae* and *Iridoviridae*. Virion formation process is akin to the *Pandoravirus* (Legendre et al. 2014). Unlike *Megaviridae* that

exhibit characteristics of other large DNA viruses, *Pandoraviruses* and *Pithoviruses* are seemingly unrelated.

1.2.2. The Phycodnaviridae Family

Phycodnaviridae are a family of large dsDNA viruses that have eukaryotic algae, both fresh water and marine species, as hosts. Genetic studies of the *Phycodnaviridae* family have shown that they possess ancient genes (Mackinder et al. 2009). This suggests that they are a well-established family of viruses and have been around for a long time (Wilson et al. 2009). This corresponds with the fact that considerable differences are present between viruses within the family through evolution (Dunigan et al. 2006). Most genes found in specific viruses are unique to a particular genus. Through genetic analysis of *Phycodnaviridae* DNA polymerase genes, specifically the δ DNA polymerase gene (Dunigan et al. 2006), all large algal viruses show close evolutionary heritage (Chen et al. 1996). *Phycodnaviridae*, while being very diverse as a family, are more closely related to viruses within the family than other viruses (Dunigan et al. 2006) . Moreau et al. (2010) postulated that *Phycodnaviridae* evolved alongside their hosts, and that they could be remnants of ancient viral lineages. Although members in this family do exhibit genetic differences, they all share the same morphology and have an internal lipid membrane (Van Etten et al. 2002). Additionally their genomes are all large dsDNA molecules and were generally characterised to range between 160 and 560 kb in size (Dunigan et al. 2006).

Phycodnaviridae have a wide distribution across both saline and fresh water environments. They have been isolated in temperate waters and even in sub-Antarctic waters (Dunigan et al.

2006). The common factor is they infect algae which are immensely diverse ranging from phytoplankton all the way to kelp species. This infers the natural diversity that exists within the *Phycodnaviridae* family (Van Etten et al. 2002). Information regarding this family of algal NCLDVs has been largely acquired through studying the *Chlorovirus* and *Phaeovirus* genera. Viruses identified within these genera have been characterised as non-enveloped viruses that utilise a mechanism of inserting viral DNA into the host cell by fusing the internal lipid membrane with the host cell membrane (Van Etten et al. 2002), however this has not been true for coccolithoviruses (Mackinder et al. 2009).

Currently this family consists of six genera: *Chlorovirus*, *Prasinovirus*, *Phaeovirus*, *Coccolithovirus*, *Raphidovirus* and *Prymnesiovirus* (Dunigan et al. 2006). Each genus is recognised by the associated taxonomy of the algal host (Van Etten et al. 2002).

Hosts identified for chloroviruses are single cell green algae belonging to the phylum Chlorophyta. These viruses are unique in their production of specific enzymes possibly involved in cell wall polysaccharide degradation (Dunigan et al. 2006). They are plaque-forming viruses involved in a lytic life cycle. Cholorvirus genomes possess a high degree of variability and contain a section tolerable to deletions (Wilson et al. 2009).

Algal viruses not only infect small microscopic algae, but can infect macroalgae as well. Phaeoviruses, known for infecting brown algae, exhibit the largest range in sizes within a genus and viral species display a variety of different features (Müller et al. 1998). Pathology of infected hosts is lysogenic (Wilson et al. 2009) and also varies as infection is some shown no change in photosynthetic ability or growth in some host species but is detrimental in others

(Dunigan et al. 2006). Most comprehensively studied phaeoviruses such as Ectocarpus siliculosus Virus (EsV) have an important characteristic involved in the integration of viral genes into the host genome (Schroeder et al. 2009; Müller et al. 1990; Delaroque et al. 1999). The majority of viruses are host specific, but the EsV virus has a rare quality in its ability to infect more than one host (Müller et al. 1998). This ability can act as a mode of gene transfer from one species of algae to another within a genus (Dunigan et al. 2006).

The widespread nature of *Prasinovirus* hosts has been well established, however, prasinoviruses themselves have had very little studied about their diversity and molecular functions (Bratbak et al. 1996; Fuhrman 1999). The first and most extensively studied are the viruses infecting *Micromonas pusilla* (Mayer & Taylor 1979; Fuhrman 1999). For the *Phycodnaviridae* family, the genera that includes prasinoviruses is the least studied and this is reflected in the literature, truly indicating how little we known about these viruses (Wilson et al. 2009).

The viruses most associated to harmful algal blooms are prymnesioviruses (Hallegraeff 1993). They are known to infect algal hosts linked with the production of harmful toxins such as *Chrysochromulina* species (Van Etten et al. 2002; Baudoux & Brussaard 2005). Viral infection by prymnesioviruses have been linked to bloom crashes of *Phaeocystis* species that cause anoxia (Wilson et al. 2009). *Phaeocystis* algal species are important for cycling of sulphur and CO_2 and viral infection may have implications for climate change (Sandaa et al. 2001; Baudoux & Brussaard 2005).

Algal species forming toxic red-tides are associated to the family of *Raphidophytes* (Wilson et al. 2009). The most well understood virus infecting this genus is the *Heterosigma akashiwo virus* (Short 2012). The blooms of *Heterosigma akashiwo* have been linked to harmful algal blooms (Kudela et al. 2010) and in the past been involved in the death of fish species (Medlin et al. 2013).

1.2.3. RNA Algal Viruses

In two bloom events in Japan, small single stranded RNA viruses were identified. The first to be isolated was the HaRNA virus found in a toxic *H. akashiwo* bloom (Tai et al. 2003). In the following year Tomaru et al. (2004) described the HcRNA virus isolated from the *H. circularisquama* bloom. Since these discoveries picorna-like ssRNA viruses, RsRNAV and CtenRNAV01, have been isolated infecting diatom species *Rhizosolenia setigera* (Nagasaki et al. 2004) and *Chaetoceros tenuissimus* Meunier (Shirai et al. 2008), respectively. Further research being done on RNA viruses found in marine algal systems indicates that RNA viruses may be more abundant than previous analyses have suggested (Steward et al. 2012).

1.2.4. Methods of *Phycodnaviridae* identification

Methods of viral identification vary from virus to virus and what the researcher aim to achieve. *Phycodnavirdae* identification techniques include quantification and enumeration, as well as genetic identification through sequence analysis (Short & Suttle 2002) after DNA amplification.

Quantitative methods using probes have been used to determine viral abundance (Short et al. 2011). Direct count methods, Such as Transmission electron microscopy (TEM) and epifluorescent microscopy (EFM) Transmission electron microscopy (TEM) and epifluorescent microscopy (EFM) viral abundance can be determined without culturing hosts (Sandaa 2008, Brsheim et al. 1990; Noble & Fuhrman 1998). Recently analytical flow cytometry (AFC) and quantitative PCR (qPCR) have been introduced as a methods for enumerating viruses in environmental samples (Short 2012; Sandaa 2008).

The more commonly used technique for viral identification is genetic sequence analysis after PCR amplification of conserved genetic regions (Short & Suttle 2002). This is done using targeted primers able to amplify phycodnaviruses. Several conserved regions exist for the *Phycodnaviridae* family (Koonin et al. 2006). The major capsid protein (MCP) has been successfully used by Clerissi et al. (2014) to identify phycodnaviruses, namely prasinoviruses. This technique has also been used to identify other phycodnaviruses (Larsen et al. 2008) and viruses from the *Iridoviridae* family within the same order (Tidona et al. 1998). While the MCP has proven to be a useful genetic marker for *Phycodnaviridae* identification, most information currently available for *Phycodnaviridae* phylogeny is based on the DNA polymerase protein (Larsen et al. 2008).

The target DNA most widely used for identification of phycodnaviruses is the DNA polymerase gene (Chen et al. 1996). One of the most conserved regions within the polymerase gene is the YGDTDS catalytic site (Chen & Suttle 1995). As his is a good region to target for genetic probing of phycodnaviruses, specific algal virus primers are used; AVS1, AVS2 and POL (Schroeder et al.

2002; Sandaa et al. 2001). These primers have been designed specifically based on the unique amino acid sequence found in conserved regions within the B- family DNA polymerase, to amplify algal viruses and are less likely to amplify unknown viral DNA (Chen & Suttle 1995). Analyses of the DNA pol gene is used to investigate the *Phycodnaviridae* diversity within a sample (Filée et al. 2002). More specific primers can then be designed to amplify an individual genus of viruses, or virus of specific interest.

Future analyses will become more comprehensive through the development of improved sequencing techniques, in which whole sample metagenomics can now be done (Allen & Wilson 2008). This method is best used to illicit information regarding the whole viral community present in a sample (Angly et al. 2006). The main problem facing metagenomic analyses is the effective removal of free DNA found in environmental samples (Edwards & Rohwer 2005). This new approach to tackling viral community questions is revolutionising the virology field (Angly et al. 2006). However, this method is highly taxing on computing power and requires trained individuals to analyse data (Allen & Wilson 2008). Large amounts of data is of no use, if there is no way to interpret them. New improved bioinformatics techniques are being developed to effectively interpret viral data obtained from metagenomic analyses (Flaviani, unpublished).

1.3. Southern Africa Coastal waters

1.3.1. Southern African marine environment

The Southern African coastline encompasses three contrasting biogeographic regions; the cold-temperate west coast, the sub-tropical east coast and the south coast intermediate region. Marine biodiversity is mainly dictated by the two main boundary currents that flow on either side of the sub-continent. On the west coast, the Benguela upwelling system draws deep cold Sub-Atlantic waters up along the coast providing nutrients that give rise to plankton communities that contribute to the productive fisheries in the region. Warm tropical water is transported south-westward along the South African east coast by the Agulhas current, one of the predominant western boundary currents in the southern hemisphere (Schumann 1999; Lutjeharms et al. 2000).

1.3.2. Southern African phytoplankton blooms

Phytoplankton blooms are known to proliferate in upwelling systems and blooms in this area have a significant impact on the ecology of the coastal systems. Harmful algal blooms have been observed throughout time. Proliferations of microscopic phytoplankton, while natural in their occurrences can have negative effects on the ecosystems and human health. They are important players in the flux of organic material in and out of the environment, as well as in some cases the production of harmful compounds. Phytoplankton are at the base of the food chain and are an important food source for a variety of organisms such as filter feeders (e.g.

bivalves), larvae of most species and small pelagic fish (Hallegraeff 1993). Considering the information above, one can then understand the damage proliferation of harmful phytoplankton species can cause in human health or ecosystem functioning (Scorzetti et al. 2009).

1.3.3. Elands Bay and Harmful Algal Blooms (HABs)

Elands Bay is situated on the northern part of St Helena Bay which is located on the West coast of South Africa and forms part of the lower Namaqua shelf in the southern Benguela current. Unique to the bay is its location along the coast and the influence of the Cape Columbine upwelling plume causing the retention of high-nutrient surface waters within the bay (Pitcher & Nelson 2006). Prevailing south-easterly winds make the southern Benguela region prone to coastal upwelling of cold nutrient dense waters provides an ideal environment for the high rate of proliferation of phytoplankton (Kudela et al. 2010). Annual phytoplankton blooms are an essential part of this ecosystem as seen in figure 1. However, due to a suite of factors (many of which are unknown), certain toxic species dominate certain blooms causing negative effects on the ecosystem as well as possible human health concerns (Trainer et al. 2010). The Benguela upwelling system is susceptible to events where increased populations of one or more toxic phytoplankton species dominate causing harmful algal blooms (Pitcher & Nelson 2006).These accumulations are a widespread phenomenon causing eutrophication, anoxia (Pitcher & Probyn 2011) and other physical changes in a variety of coastal marine and fresh water environments.

Pitcher and Probyn (Pitcher & Probyn 2011) identified that in 2009 there was an anoxic event which occurred close to the coast in the southern Benguela. Low levels of oxygen have been attributed to mortalities of the West coast rock lobster, *Jasus lalandii*, as a result of dinoflagellate bloom high accumulation and decay (Trainer et al. 2010). In the Benguela, oxygen deficiency is one of the most important factors that has a significant negative effect on the functioning of the ecosystem and economically important living marine resources (Pitcher & Probyn 2011). Harmful algal blooms and phytoplankton aggregations are complex systems that have an effect on the surrounding environment both on the biogeochemical and ecosystem level (Kudela et al. 2010). Key species have been identified in several harmful algal blooms; yet the community species composition of such blooms still remains uncharacterised. It has been suggested that marine viruses are an integrated part of phytoplankton functioning and senescence of blooms (Baudoux et al. 2006).

Winds are one of the main driving forces behind the occurrence of harmful algal blooms, more importantly the relaxation of long prevailing winds leading to phytoplankton decay (Trainer et. al. 2010). Other factors include availability of macronutrients and micronutrients (Trainer et. al. 2010), as well as ocean circulation (Kudela, et. al. 2010). Knowledge of the driving forces behind harmful algal bloom initiation is incomplete. However, along with the known driving forces, there may be a molecular mechanism, i.e. viral infection of bloom forming phytoplankton, playing a role in initiation and termination of these blooms (Brussaard et al. 2005). This study used samples from a bloom event in Elands Bay to investigate viral communities.

Figure 1. Satelite image (MODIS) of phytoplankton bloom off the coast of St. Helena Bay on the 24th April 2003. Credit: Jacques Descloitres, MODIS Rapid Response Team, NASA/GSFC

1.3.4. Inshore upwelling of the Agulhas Bank near Algoa Bay

While the majority of studies on coastal upwelling have taken place on the west coast of Southern Africa due to the implications for fisheries, other upwelling sites along the coast of Southern Africa have been identified through satellite imaging and in situ observations (Schumann 1999). Upwelling has been noted inshore of the large continental shelf of the Agulhas (Probyn et al. 1995). A south-westerly wind that blows along the south coast is more prevalent during the summer months and creates Ekman drift of the surface waters, initiating upwelling at the very near shore area. The south-westerly wind only occurs intermittently

ranging in intervals from 2 to 6 days (Schumann 1999). This region is prone to a rather sharp thermocline and during these intervals the cold nutrient rich upwelled waters creates a drastic contrast (Lutjeharms et al. 2000) to the surrounding waters that can be observed via satellite (Schumann 1999).

Due to the bathymetry of the south Agulhas region, upwelling only occurs in narrow predefined sections with very little variation. Port Alfred is in the middle of this region inclined to upwelling and ranges between 85 and 300 km along the coast, including Algoa Bay region (Barlow et al. 2010). The process of upwelling in this region may significantly influence the availability of nutrients and cause phytoplankton bloom events along the eastern Agulhas bank as seen in figure 2 (Lutjeharms et al. 2000).

Figure 2. Satelite image (MODIS) of phytoplankton bloom off the coast of Algoa Bay on the 31st October 2004. Credit: Jacques Descloitres, MODIS Rapid Response Team, NASA/GSFC

The Agulhas plays an important role in industry as it is a crucial area for spawning of pelagic and demersal species that make up the largest commercial fisheries (Shannon *et al.* 2006) in South Africa and Namibia (Probyn et al. 1995). Therefore understanding phytoplankton communities in this area would be an important aspect to research. This study focused on a bloom event in the Algoa Bay region located slightly west of Port Alfred within the predefined upwelling band.

1.3.5. Salt Pans

Highly saline bodies of water are scattered around the world in the forms of salt pans, sea floor lakes and saline ground water. Despite an initial expectation of low biodiversity and lack in lifeforms within these regions, an abundance of mircoorganisms exist in this niche area (Porter et al. 2007). This group of microbial organisms that thrive under extreme saline conditions are referred to has halophilic and have developed methods of ion production inside their cells to maintain osmotic balance and prevent lysis. The majority of these organisms belong to the Archaea domain, haloarchaea, and have been focused on in scientific study for deriving novel compounds essential in industry such as protein antibiotics. While several of these compounds have been researched, few have been described in depth to fully understand molecular mechanisms (Karthikeyan et al. 2013). The presence of haloviruses infecting these haloarchaea have been noted, to date approximately 100 have been described (Pietilä et al. 2013), although very few have been isolated and studied in depth. The minority of cases that have been observed were identified in laboratory cultures and virus structure resembled that of a bacteriophage with a head-tailed morphology. Further genetic similarities with bacteriophages

suggests that gene transfer may occur in hypersaline environments (Porter et al. 2007). Viral tolerance to salinity supersedes that of its host. Effects of salinity on viral functioning is an important avenue of research to investigate as it has been discovered that salinity has an effect on the rate of host infection (Pietilä et al. 2013).

Salt pans are an essential part of the South African landscape (Smith & Compton 2004). Natural geochemical cycling of these coastal salt pans incorporates the transport of marine particles via wind and rainfall. No extensive studies have been made on this topic (Smith & Compton 2004). Studying haloviral interactions with their hosts in these areas would contribute to the understanding of salt pan ecology (Porter et al. 2007). Salt pans samples used in this study come from the Cerebos salt plant located in the Veldrift area, which feeds from the Berg River Estuary. Salt is crystalized in outdoor pans.

1.4. Aims and scope of the Study

Sample sites were selected from different regions in southern Africa. Two bloom events were sampled and one salt pan site (Figure 3).

Figure 3. Map of Sample sites. Four sites mentioned: Elands Bay (○), two at Cerebos Salt works in Veldrift (◊), Algoa Bay (□).

This study aims to expand our current knowledge of viral diversity associated with phytoplankton blooms in contrasting coastal waters and selected salt pans around South Africa. The isolation of viral sequences from this work will be compared to known algal viral sequences and potentially identify novel clades. Identification of novel viral clades will provide a significant contribution to the current database that exists for algal viruses in Southern Africa. This study used genomic (PCR-based) methods to detect selected viral groups from the host fraction and aggregated free virus particles (> 1 µm) of respective water samples. The objectives of this study include:

i.) To genetically identifying *Phycodnaviridae* present in the Elands bay and Algoa Bay bloom events.

ii.) To identify the most prevalent viral group in each respective bloom.

iii.) To compare viral groups identified between each bloom event; do they compare or are they bloom specific?

iv.) To genetically identify *Phycodnaviridae* present in the selected salt pans.

v.) To compare salt pan viruses to coastal waters.

The objectives of this study do not include analysis of the host diversity, due to time restrictions. It is important to note that this study will be greatly benefit from understanding host ecology and investigating virus-host interactions to fully understand the molecular ecology of these ecosystems.

Chapter 2 – Materials and Methods:

2.1. Sample collection and preparation

Four sampling sites were chosen for this study along the Southern African coasts (Figure 4): Elands Bay, Algoa Bay and two sites within the Cerebos salt pans, located near the Berg river mouth in Veldrift on the West coast. Sample site description is tabulated in table 2.

Elands bay. Sample site depicted in figure 4a. The field trip collection commenced after a wind-still period that lasted approximately 10 days in the Elands Bay region. Samples were taken from the visible red tide for 5 consecutive days. A total of 20 litres of seawater was sampled from the surface water (< 1m) per day. Samples were passed through a 200µm screen to exclude all zooplankton and debris from the analysis. Filtration was done at a 1 µm cut off using the $FeCl_3$ protocol as per John et al. (2011). Filters containing host fractions and aggregated free virus particles were preserved in tubes containing EtOH.

Port Elizabeth. Surface water samples (<1m) were taken approximately 1 km from the shore from a dense patch of a red tide. Location indicated in figure 4b. 125 litres were sampled for one day only.

Veldrift Cerebos Salt Pans. Samples of 25 litres were taken from pans (Figure 4c) of two different salinities, namely B5 and B25, roughly corresponding to 5 and 25 psu.

For the Port Elizabeth and Veldrift Cerebos salt pan samples, Zooplankton and debris was removed as previously described. All samples were pre-filtered through 1µm filters before using

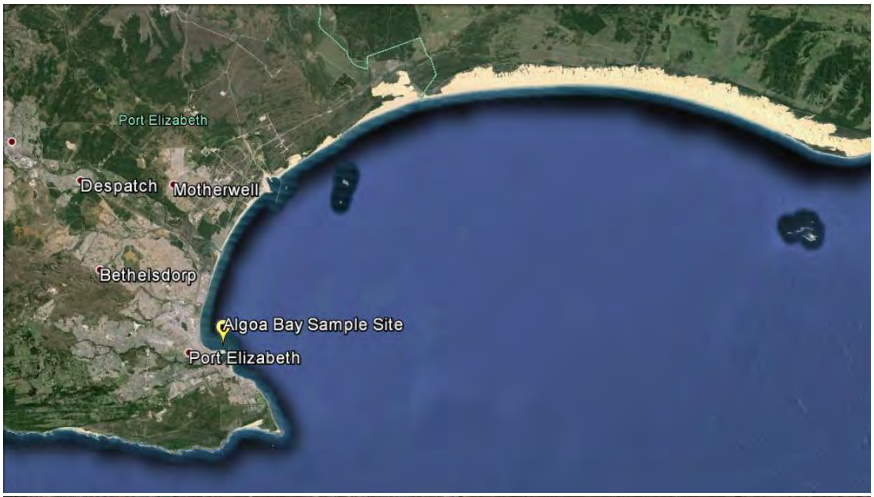
Tangenital flow filtration (TFF) (Petruševski et al. 1995). The 1µm filters were placed into 50 mL tubes and preserved in absolute EtOH. TFF was used in this instance as it was unsure what effects $FeCl_3$ would have on the results when isolating from high saline environments.

Table 2. Summary of samples sites.

Sample Site	Day	Month	Year	Station	Water depth(m)	Sample depth	Lat S	Lon E
Elands Bay	15	3	2013	1	9	< 1m	32 18.618	18 19.267
Elands Bay	16	3	2013	1	9	< 1m	32 18.618	18 19.267
Elands Bay	17	3	2013	2	26	< 1m	32 20.596	18 18.327
Elands Bay	18	3	2013	3	12	< 1m	32 23.895	18 19.569
Elands Bay	19	3	2013	4	19	< 1m	32 22.732	18 19.197
Port Elizabeth	14	4	2013	Algoa Bay	14.4	< 1m	33 57.086	25 38.249’
Cerebos – Berg River	17	4	2013	Baume 5	-	< 1m	-	-
Cerebos – Berg River	17	4	2013	Baume 25	-	< 1m	-	-

a.)

b.)



c.)

Figure 4. Maps of the sample site locations. a.) Elands bay. b.) Port Elizabeth. c.) Veldrift Cerebos salt pans

2.2. DNA extraction

Sample DNA was obtained using a protocol optimised by Schroeder et al. in 2002. The filter was transferred into a 50mL centrifuge tube and incubated overnight at room temperature in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). 10% SDS and proteinase K (10mg/mL) was then added to denature contaminating protein. Following a 30 sec Vortex the sample was then incubated overnight at 65°C at a gentle agitation. Once pre-processing has been completed, a standard phenol chloroform extraction was carried out with one round of 25:24:1 phenol:chloroform:isoamyl alcohol and at least 3 rounds of chloroform/isoamyl alcohol (24:1) until the aqueous phase was clear. All contaminating proteins were removed by precipitation. Samples were incubated at room temperature after adding 800 µL of 7.5 M ammonium acetate. Following which samples were centrifuged at maximum speed of 16 000 xg for 10 min and supernatant transferred to a new microcentrifuge tube. Remaining protein pellet was discarded. Nucleic acids were precipitated out by the additional of 100% ethanol and overnight incubation to ensure a clean product. After centrifugation at 16 000 xg for 30 min, nucleic acid pellet was washed in 200 µL of 70% ethanol with additional centrifugation and evaporation. The final step involved the re-suspending the pellet in 20 µL TE buffer.

2.3. PCR and Cloning

PCR amplification of the desired viral sequence was done using the AVS1 and POL primer set (Chen & Suttle 1995). Through a series of optimization steps the appropriate experiment parameters were determined. A 10 times dilution of the sample and PCR annealing

temperature of 56°C were used to yield the best results. Samples were run as 25µL reactions with a taq DNA polymerase concentration of 1u/µL. Results were visualised by running samples on a 1% agarose gel. The PCR product was then cleaned up using a GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich). PCR products of each sample were then cloned into a Topoisomerase I-activated pCR™2.1-TOPO® vector (K4510-20, life technologies) producing ligation mixtures for each sample. Competent *Escherichia coli* cells were then transformed with vector ligations, following the OneShot® transformation protocol. Transformations were then spread onto 15 cm Luria-Burtani (LB) agar (1.0% Tryptone, 0.5% Yeast Extract, 1.0% NaCl, pH 7.0) plates containing kanamycin and grown overnight at 37°C. Prior to spreading transformed *E. coli* cells, plates were inoculated with X-Gal to select for transformed colonies. Transformed white colonies were picked from plates, transferred to 5µL dH_2O and streaked onto a new LB agar plates to generate clone libraries. Transformed colony DNA was then further amplified by PCR using M13 forward 5'GTAAAACGACGGCCAG 3'and M13 reverse 5'CAGGAAACAGCTATGAC 3' primers. Amplified sequences were selected based on size between 200-700bp and sent off for sequencing.

2.4. Sequencing and sequence analysis

30µL of the PCR product was transferred to a 96 well plate. Sequencing was performed using Sanger Sequencing. Generated sequences were imported into BioEdit (Hall 2011). Vector sequence was removed and similar sequences were aligned and separated. Sequences were analysed using the Basic Local Sequence Alignment Tool (BLAST) algorithm provided online by

the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) in which homology searches were carried out with the blastx function. 'Blastx compares the six-frame conceptual translation products of a nucleotide query sequence' (Pirooznia et al. 2008). Bacterial DNA was removed from analysis and only virus DNA containing the pol gene was kept for further analyses.

2.5. Phylogenetic analysis

Nucleotide sequences were translated to protein using the online ExPAsy translate tool (Gasteiger et al. 2003). The output was downloaded in FASTA format and imported into BioEdit for alignment using the built in ClustalW multiple sequence alignment method (Thompson et al. 1994). The MEGA6 (Tamura et al. 2013) programme was then used to generate a phylogenetic tree of aligned protein sequences. Parameters were set for the Maximum likelihood tree at: 500 bootstrap replications, Jone-Taylor-Thornton model, Nearest-Neighbour-Interchange heuristic method. Know viral sequences of the *Poxviridae, Baculoviridae, Asfarviridae, Iridoviridae, Herpesviridae* (outgroup) and *Phycodnaviridae* families were added into the analysis. Closely related unknown sequences (Table 3) were also added to the analysis (Short & Suttle 2002; Short & Suttle 2003; Culley et al. 2009; Short et al. 2011).

Table 3. Sequences of unknown viruses added to analysis.

Name	Accession number	Isolated from	Family association	Reference
UPV(AAO11798.1)	AAO11798.1	Jericho Pier, Vancouver, Canada	*Phycodnaviridae*	(Short & Suttle 2003)
UPV(AAL02196.1)	AAL02196.1	British Columbia coast	*Phycodnaviridae*	(Short & Suttle 2002)
UPV(AAL02203.1)	AAL02203.1	British Columbia coast	*Phycodnaviridae*	(Short & Suttle 2002)
UPV(ADW08343.1)	ADW08343.1	Lake Ontario, Canada	*Phycodnaviridae*	(Short et al. 2011)
UPV(ACJ70689.1)	ACJ70689.1	O'hau, Hawaii	*Phycodnaviridae*	(Culley et al. 2009)

2.6. Targeted virus amplification

Specific primers were designed for dominant viral sequences identified within the Elands Bay and Port Elizabeth samples. The dominant viral families were established in which sequences had a similarity of more than 85%. Primers were designed and selected using Primer3 software (Koressaar & Remm 2007; Untergrasser et al. 2012), OligoAnalyzer (Owczarzy et al. 2008), as well as through manual inspection. Primer length was set at a minimum of 18 and maximum of 24 bases. Best suited primer pairs were selected based on similar GC content, low tendency for the formation of hairpin bends, low likelihood of dimer formation and with a melting temperature above 52°C (Rychlik 1995; Breslauert et al. 1986). Primer specificity was checked by doing a virtual bioinformatics check using NCBI. Primers were then tested by running a blastn analysis to validate the effectiveness at amplifying the desired sequence by PCR. 'Blastn compares a nucleotide query sequence against a nucleotide sequence database' (Pirooznia et al. 2008). The primers designed for each virus are shown in table 4. The expected product size excluding primers for virus EB1 is 369 bp and for PE1 is 177 bp.

Specific targeted viral DNA was amplified using designed primer sets. All samples diluted to a 10 times solution were screened for both the EB1 and PE1 viruses. The PCR conditions used were the same as in the previous amplification experiments described in section 2.3, with taq DNA polymerase concentration of 1u/µL and an annealing temperature of 56°C.

Table 4. Primers designed for Elands Bay virus 1 and Port Elizabeth virus 1.

PRIMER	SEQ (5' - 3')	LENGTH	Forward/Reverse	GC %	Tm (°C)
EB1 F	GGATGCTAAACCWGGWGCWCAY	22	Forward	52.3	56.9
EB1 R	TGGWAGWCGWCCATATYTMGCRCC	24	Reverse	52.1	56.3
PE1 F	GGKGTYCCMACGYTKTTRCCGAGT	24	Forward	58.3	57.8
PE1 R	RCTMCGACCYTTYGTYGTCACAGT	24	Reverse	52.1	56.5

Chapter 3 – Results:

3.1. Viral groups identified during the Elands Bay bloom 2013

Phycodnaviruses were analysed in a bloom event that occurred in Elands Bay in 2013. DNA extraction was performed on whole samples filtered onto a 1µm and used for molecular analysis. The primer set AVS1 and POL used targeted the genetic region coding for the DNA polymerase gene contained in algal viruses. Initial screening of all the Elands Bay bloom environmental samples elicited the strongest viral signal from the day 2 sample. Day 2 samples were then used for further analysis for the bloom event. Successful isolation of the gene fragment was optimised by using a PCR annealing temperature of 56°C and further amplified by transformation into competent *E. coli* cells. From the transformed cell cultures 182 colonies were picked which through gel electrophoresis yielded 79 sequences matching the suspected size range between 500-700bp, 18 of which contained the desired DNA polymerase gene. Sequences were compared to the NCBI database and top results were tabulated in table 5.

Table 5. Top five BLAST hits found on the NCBI database for each viral group identified from the Elands Bay bloom event.

Group	Nr. of Seq.	BLAST hits	Total score	Query cover	E value	Ident	Accession
EB 1.1	6	*DNA polymerase [Chrysochromulina brevifilum virus PW1]*	298	100%	1,00E-99	94%	AAB49739.1
		DNA polymerase [Phaeocystis globosa virus]	291	100%	1,00E-97	91%	ABD62757.1
		DNA polymerase [Chrysochromulina brevifilum virus PW3]	289	100%	4,00E-96	91%	AAB49740.1
		DNA polymerase [Phaeocystis globosa virus]	227	100%	6,00E-72	77%	AAR05087.1
		DNA polymerase [unknown phycodnavirus]	226	100%	9,00E-72	73%	AEE99071.1
EB 1.2	1	*DNA polymerase [Chrysochromulina brevifilum virus PW1]*	251	90%	3,00E-81	87%	AAB49739.1
		DNA polymerase [Phaeocystis globosa virus]	243	90%	7,00E-79	84%	ABD62757.1
		DNA polymerase [Chrysochromulina brevifilum virus PW3]	243	90%	9,00E-78	84%	AAB49740.1
		DNA polymerase [unknown phycodnavirus]	197	90%	3,00E-60	71%	AEE99071.1
		DNA polymerase [Phaeocystis globosa virus]	195	90%	2,00E-59	73%	AAR05087.1
EB 1.3	4	*DNA polymerase [Chrysochromulina brevifilum virus PW1]*	298	100%	1,00E-99	94%	AAB49739.1
		DNA polymerase [Phaeocystis globosa virus]	289	100%	6,00E-97	91%	ABD62757.1
		DNA polymerase [Chrysochromulina brevifilum virus PW3]	288	100%	9,00E-96	91%	AAB49740.1
		DNA polymerase [Phaeocystis globosa virus]	228	100%	3,00E-72	77%	AAR05087.1
		DNA polymerase [unknown phycodnavirus]	227	100%	8,00E-72	73%	AEE99071.1
EB 2	2	*predicted protein [Micromonas pusilla CCMP1545]*	257	100%	7,00E-77	85%	XP_003056969.1
		predicted protein [Micromonas sp. RCC299]	236	100%	2,00E-69	80%	XP_002502470.1
		DNA polymerase [Bathycoccus virus BpV161]	217	100%	7,00E-69	73%	ACP44142.1
		predicted protein [Ostreococcus lucimarinus CCE9901]	227	100%	2,00E-66	76%	XP_001416112.1
		DNA-directed DNA polymerase, family B, pol2 [Ostreococcus tauri]	226	100%	1,00E-65	76%	CEG01117.1
EB 3	1	*DNA polymerase [unknown phycodnavirus]*	103	99%	4,00E-24	45%	ACJ70680.1
		DNA polymerase [unknown phycodnavirus]	100	99%	5,00E-23	42%	ADW08310.1
		DNA polymerase [unknown phycodnavirus]	100	99%	6,00E-23	42%	ACA65633.1
		DNA polymerase [unknown phycodnavirus]	100	99%	7,00E-23	41%	ACA65626.1
		DNA polymerase [unknown phycodnavirus]	100	99%	7,00E-23	41%	ACA65627.1
EB 4	1	*DNA polymerase [unknown phycodnavirus]*	247	100%	3,00E-80	81%	ACJ70680.1
		DNA polymerase [unknown phycodnavirus]	237	100%	6,00E-76	73%	ACJ70681.1
		DNA polymerase [unknown phycodnavirus]	222	100%	3,00E-70	70%	AAL02206.1
		DNA polymerase [unknown phycodnavirus]	222	100%	4,00E-70	70%	AAL02199.1
		DNA polymerase [unknown phycodnavirus]	206	100%	3,00E-64	73%	ACJ70682.1
EB 5	1	*DNA polymerase [Ostreococcus tauri virus 2]*	271	100%	2,00E-90	93%	AFE48533.1
		DNA polymerase [unknown phycodnavirus]	273	100%	2,00E-90	95%	AAO11791.1
		DNA polymerase [Ostreococcus virus OtV09_559]	271	100%	5,00E-90	93%	AHC07952.1
		DNA polymerase [Ostreococcus tauri virus 2]	271	100%	6,00E-90	93%	ADB45835.1
		DNA polymerase [Ostreococcus virus]	271	100%	7,00E-90	93%	AHA82618.1

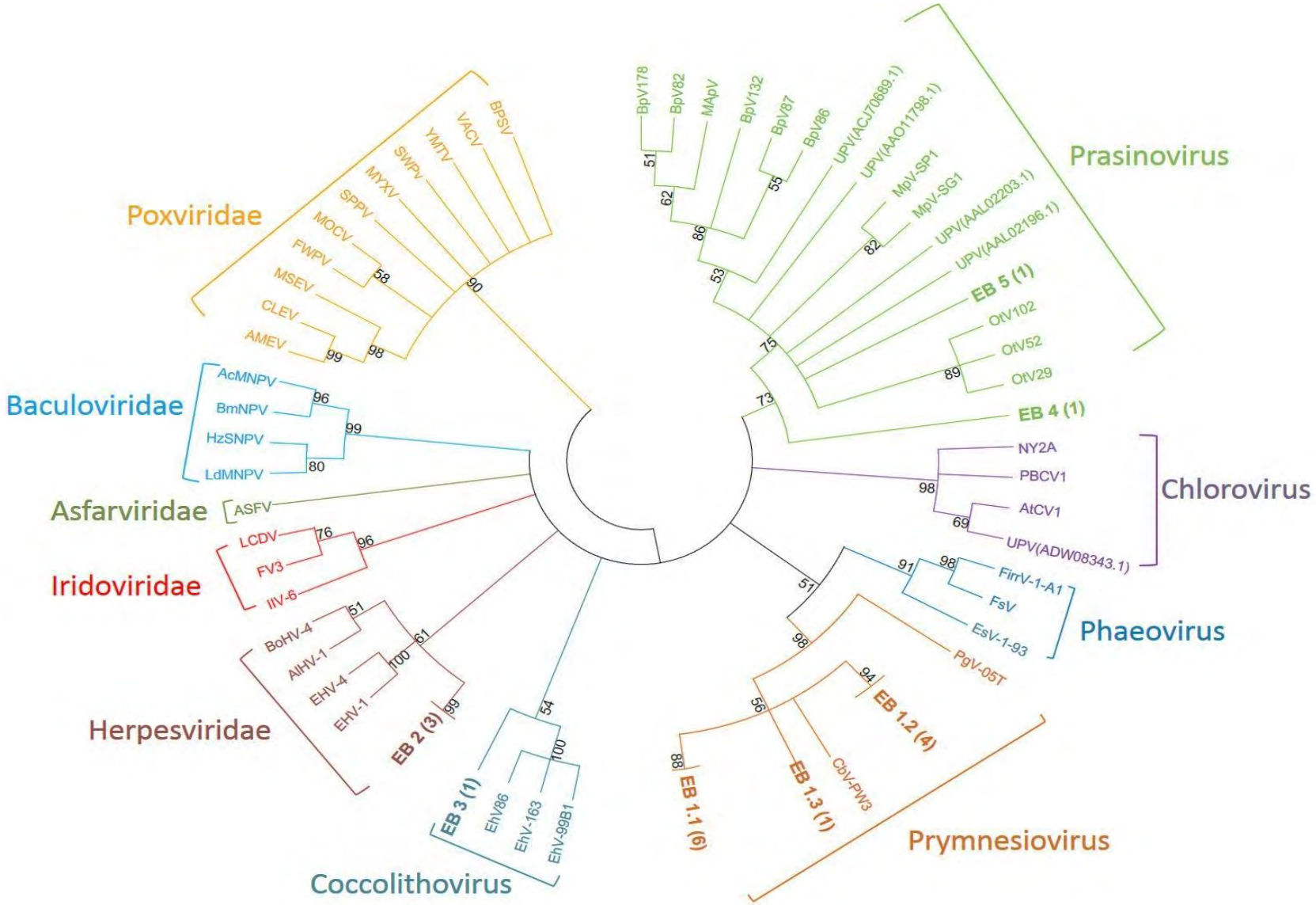


Figure 5. Figure. Molecular Phylogenetic analysis of samples collected from Elands Bay. Environmental samples are named by place of origin – C B25. Number in parenthesis refer to the number of duplicate sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al. 1992). The tree with the highest log likelihood (-2200.9892) is shown. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model. The analysis involved 110 amino acid sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

The majority of sequences were found to be within the prymnesiovirus genus, represented by EB 1.1, EB 1.2 and EB 1.3 (Figure 5). Viruses related to the coccolithovirus and prasinovirus genera were also present, EB 3 and, EB 4 and EB 5 respectively (Figure 5). Another group was identified with closest genetic resemblance to the *Herpesviridae* family, represented by EB 2 (Figure 5). Viruses EB 1.1, EB 1.2 and EB 2 show significant separation from relative groups, with bootstrapping values >90%. This indicates new virus clades of *Phycodnaviridae* identified.

Novel primers designed specifically to identify the EB1 viral group were used to cross screen all environmental samples to determine presence or absence. As seen in figure 6 the EB 1 viral subgroup was only found on days 1 to 4 of the Elands Bay bloom and not in any other samples.

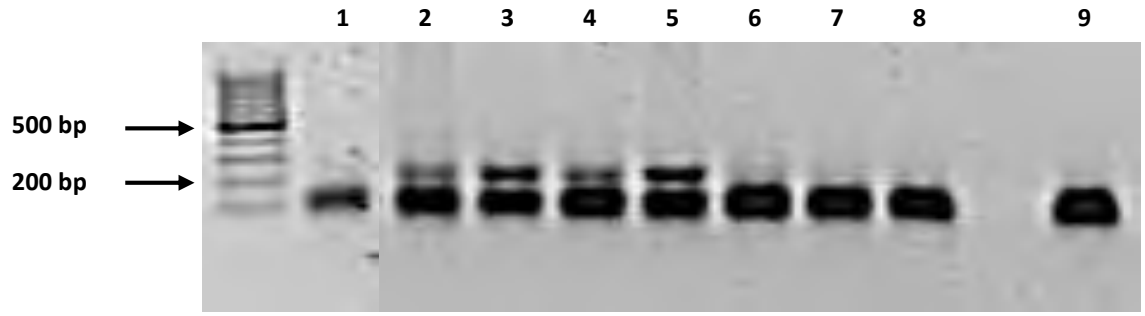


Figure 6. Image of gel electrophoresis of PCR amplified products using EB1 F and EB1 R primers to identify presence of the Elands Bay virus 1 across all samples. Lane 1 – Port Elizabeth red tide event, lane 2-6 – Elands Bay bloom event day 1-5 respectively. Lane 7 – Cerebos B5 and lane 8 – Cerebos B25. Last lane (lane 9) is the negative control.

3.2. Viral groups identified during Port Elizabeth bloom event in 2013

Viral fragments in the Port Elizabeth environmental sample were amplified using the primer set AVS1/POL and a signal was observed at the desired fragment size through electrophoresis. Of the successful transformations 88 colonies were picked and upon further analysis via gel electrophoresis 41 fragments indicated the required fragment size for additional investigations. Of the 41 fragments sequenced and compared to the NCBI database (Table 6), 18 contained the DNA polymerase gene present in dsDNA viruses of interest.

After phylogenetic analysis, the results visualized shown in figure 7, three distinct groups from the Port Elizabeth aligned specifically within the *Prasinoviridae* genus. PE 1.1, PE 1.2 and PE 2 represent the three groups identified in the environmental sample. The samples were named as such due to PE 1 analyse as a whole showing a >90% bootstrapping segregation from other known sequences. PE 1.1 and PE 1.2 are fairly similar in that their separation is at a bootstrapping value of < 70%, yet statistically there is some separation in the base-pair identification. The three sequences clustered as PE 2 show the most similarity to *Bathycoccus* viruses BpV82, BpV178 and MApV. The presence of PE 1 suggests that there is a new clade of prasinoviruses that has not been classified before.

Primers designed for amplification of the PE 1 virus did not yield conclusive results. Possible reason for inconclusive results could be that the Tm of the primers and the annealing temperature used were too similar and the primers denatured before amplification could take place. Primer design needs to be revisited and amplification protocol parameters further assessed to fully expand on this.

Table 6. Top five BLAST hits found on the NCBI database for each viral group identified from the Algoa Bay (Port Elzabeth) bloom event.

Group	Nr. of Seq.	BLAST hits	Total score	Query cover	E value	Ident	Accession
PE 1	13	*DNA polymerase [unknown phycodnavirus]*	296	100%	5,00E-99	95%	ACJ70689.1
		DNA polymerase [Bathycoccus virus BpV87]	228	100%	1,00E-72	75%	ACP44140.1
		DNA polymerase [Bathycoccus virus BpV178]	226	100%	7,00E-72	75%	ACP44143.1
		DNA polymerase [Marine aerosol phycodnavirus]	224	100%	9,00E-72	74%	AAR10815.1
		DNA polymerase [Bathycoccus virus BpV132]	225	100%	2,00E-71	74%	ACP44141.1
PE 2	3	*DNA polymerase [Marine aerosol phycodnavirus]*	272	100%	9,00E-91	98%	AAR10815.1
		DNA polymerase [Bathycoccus virus BpV178]	273	100%	2,00E-90	98%	ACP44143.1
		DNA polymerase [Bathycoccus virus BpV87]	272	100%	2,00E-90	99%	ACP44140.1
		DNA polymerase [Bathycoccus virus]	271	100%	7,00E-90	97%	AHA82625.1
		DNA polymerase [Bathycoccus virus BpV82]	271	100%	9,00E-90	98%	ACP44138.1
PE 3	1	*DNA polymerase [unknown phycodnavirus]*	268	100%	4,00E-88	95%	AAO11798.1
		DNA polymerase [Bathycoccus virus BpV178]	210	100%	8,00E-66	74%	ACP44143.1
		DNA polymerase [unknown phycodnavirus]	209	100%	2,00E-65	74%	AAO11797.1
		DNA polymerase [Bathycoccus virus BpV82]	209	100%	3,00E-65	74%	ACP44138.1
		DNA polymerase [Bathycoccus virus]	208	100%	4,00E-65	73%	AHA82625.1
PE 4	1	*DNA polymerase [Micromonas pusilla virus 38T]*	282	100%	2,00E-94	99%	AJD79102.1
		DNA polymerase [Micromonas virus MicAV11]	282	100%	3,00E-94	99%	AHC07938.1
		DNA polymerase [Micromonas virus]	282	100%	4,00E-94	99%	AHA82597.1
		DNA polymerase [unknown phycodnavirus]	283	100%	6,00E-94	99%	AAL02200.1
		DNA polymerase [unknown phycodnavirus]	282	100%	9,00E-94	99%	AAL02184.1

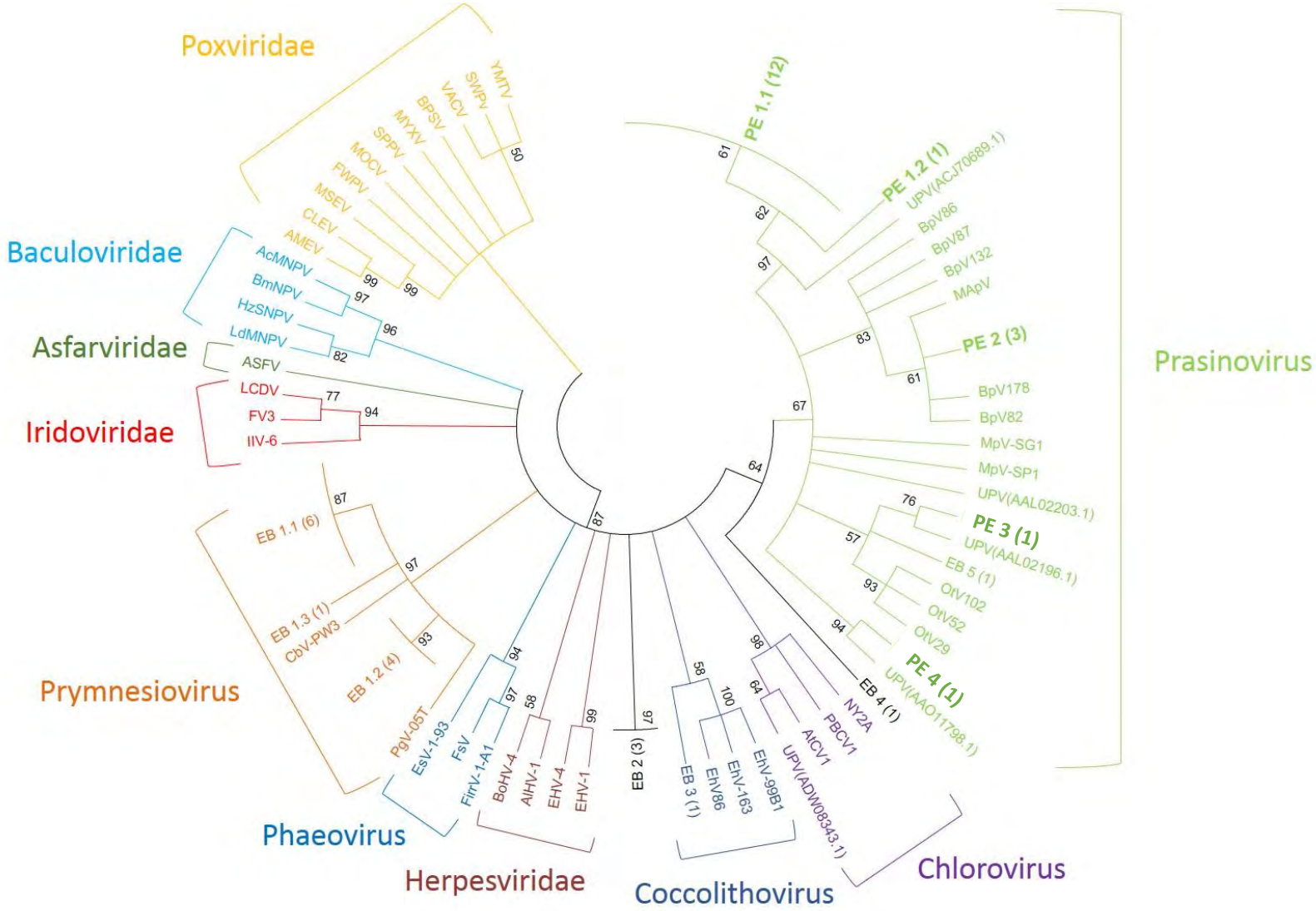


Figure 7. Figure. Molecular Phylogenetic analysis of samples collected from Port Elizabeth (Algoa Bay). Environmental samples are named by place of origin – C B25. Number in parenthesis refer to the number of duplicate sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al. 1992). The tree with the highest log likelihood (-2200.9892) is shown. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model. The analysis involved 110 amino acid sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

3.3. Salt Pan dsDNA viruses

Samples were taken from two saline pans located in the Cerebos (C) salt works in Veldrift, B5 and B25. Analysis of the C B5 samples showed a variety of dsDNA viruses present. Sequences compared to the NCBI database yielded results tabulated in table 7. Initial results indicated superficial grouping of the viruses identified. However, when applying a more stringent cut off of 50%, all C B5 environmental samples, except one, did not show significant relation to any known families displayed as seen in figure 8. The only sequence identified with significant association was C B5 7, belonging to the *Prasinoviridae* genus. Several sequences including C B5 5, 11 and 12, clustered together with the EB 2 sequence. This indicated a separate clade of viruses. Sample C B5 2 formed a cluster with PE 4 and the unknown phycodnavirus (UPV) with the accession number AAL02196.1 isolated from the coastal British Columbia (Short & Suttle 2002). Another UPV isolated from the same area with the accession number AAL02203.1 has shown to be closely related to the C B5 4 sample. The original study had associated the UPV (AAL02203.1) with viruses of the genus prasinovirus (Short & Suttle 2002).

Phylogenetic analysis of the C B25 samples produced a cluster unrelated to any of the known sequences as seen in figure 9. BLAST analysis on the NCBI website indicated the closest relatives to these sequences was that of a haloviruses (Table 8).

Table 7. Top five BLAST hits found on the NCBI database for each viral group identified from the Cerebos Salt Pan B5 in Veldrift

Group	Nr. of Seq.	BLAST hits	Total score	Query cover	E value	Ident	Accession
C B5 1	5	*DNA polymerase [Paramecium bursaria Chlorella virus 1]*	151	100%	1,00E-42	50%	AAK28921.1
		DNA polymerase [Paramecium bursaria Chlorella virus 1]	150	100%	3,00E-42	47%	AAX86472.1
		DNA polymerase [Paramecium bursaria Chlorella virus 1]	150	100%	4,00E-42	48%	AAK28920.1
		DNA polymerase [Paramecium bursaria Chlorella virus 1]	149	100%	5,00E-42	48%	AAK28932.1
		DNA polymerase [Paramecium bursaria Chlorella virus 1]	149	100%	5,00E-42	49%	AAK28919.1
C B5 2	4	*DNA polymerase [Micromonas virus MicBV10]*	284	100%	6,00E-95	99%	AHC07945.1
		DNA polymerase [Micromonas virus]	284	100%	6,00E-95	99%	AHA82607.1
		DNA polymerase [Micromonas virus]	284	100%	7,00E-95	99%	AHA82605.1
		DNA polymerase [Micromonas virus MicBV30]	283	100%	7,00E-95	99%	AHC07947.1
		DNA polymerase [unknown phycodnavirus]	280	100%	7,00E-93	97%	AAL02208.1
C B5 3	2	*DNA polymerase [unknown phycodnavirus]*	281	100%	1,00E-93	99%	AAL02203.1
		DNA polymerase [Micromonas pusilla virus 03T]	266	100%	5,00E-88	90%	AJD79090.1
		DNA polymerase [Micromonas virus MicAV16]	266	100%	5,00E-88	90%	AHC07939.1
		DNA polymerase [Micromonas pusilla virus 14T]	266	100%	5,00E-88	90%	AJD79101.1
		DNA polymerase [Micromonas pusilla virus 11T]	266	100%	5,00E-88	90%	AJD79098.1
C B5 4	2	*DNA polymerase [unknown phycodnavirus]*	266	100%	9,00E-88	93%	ACJ70691.1
		DNA polymerase [Micromonas virus MicAV8]	262	100%	1,00E-86	91%	AHC07944.1
		DNA polymerase [Ostreococcus lucimarinus virus OlV155]	262	100%	2,00E-86	88%	ADA81911.1
		DNA polymerase [Micromonas virus Mi829V1]	261	100%	3,00E-86	88%	AHC07937.1
C B5 5	2	*DNA polymerase [Bathycoccus virus BpV161]*	290	100%	2,00E-97	97%	ACP44142.1
		DNA polymerase delta catalytic subunit [Bathycoccus prasinos]	290	100%	1,00E-88	97%	XP_007514118.1
		hypothetical protein M569_02046 [Genlisea aurea]	231	100%	4,00E-70	77%	EPS72709.1
		hypothetical protein MIMGU_mgv1a0005871mg [Mimulus guttatus]	231	100%	2,00E-69	77%	EYU20644.1
		PREDICTED: DNA polymerase delta catalytic subunit [Vitis vinifera]	235	100%	6,00E-69	79%	XP_002264385.1
C B5 6	2	*hypothetical protein THAOC_13810 [Thalassiosira oceanica]*	243	100%	1,00E-76	79%	EJK65338.1
		DNA polymerase delta [Phaeodactylum tricornutum CCAP 1055/1]	247	100%	2,00E-73	83%	XP_002181135.1
		DNA polymerase [Thalassiosira pseudonana CCMP1335]	234	100%	4,00E-69	77%	XP_002291074.1
		hypothetical protein PPTG_13113 [Phytophthora parasitica INRA-310]	210	100%	6,00E-60	71%	XP_008907093.1
		hypothetical protein PHYSODRAFT_532354 [Phytophthora sojae]	209	100%	6,00E-60	72%	XP_009538490.1
C B5 7	1	*DNA polymerase [unknown phycodnavirus]*	300	100%	1,00E-100	97%	ACJ70689.1
		DNA polymerase [Bathycoccus virus BpV87]	235	100%	1,00E-75	80%	ACP44140.1
		DNA polymerase [Bathycoccus virus BpV178]	234	100%	3,00E-75	80%	ACP44143.1
		DNA polymerase [Marine aerosol phycodnavirus]	231	100%	1,00E-74	79%	AAR10815.1
		DNA polymerase [Bathycoccus virus BpV82]	233	100%	1,00E-74	80%	ACP44138.1

Table 7. Top five BLAST hits found on the NCBI database for each viral group identified from the Cerebos Salt Pan B5 in Veldrift (cont.)

Group	Nr. of Seq.	BLAST hits	Total score	Query cover	E value	Ident	Accession
C B5 8	1	*DNA polymerase [unknown phycodnavirus]*	253	100%	9,00E-83	88%	ACJ70691.1
		DNA polymerase [Micromonas virus Mi829V1]	250	100%	1,00E-81	87%	AHC07937.1
		DNA polymerase [Micromonas virus]	250	100%	1,00E-81	87%	AHA82601.1
		DNA polymerase [Ostreococcus lucimarinus virus OlV155]	248	100%	1,00E-80	85%	ADA81911.1
		DNA polymerase [Ostreococcus lucimarinus virus OlV364]	248	100%	2,00E-80	85%	ADA81896.1
C B5 9	1	*DNA polymerase [Micromonas virus MicAV8]*	133	61%	7,00E-36	77%	AHC07944.1
		DNA polymerase [unknown phycodnavirus]	132	61%	2,00E-35	77%	AAO11792.1
		DNA polymerase [Ostreococcus lucimarinus virus OlV155]	132	61%	2,00E-35	76%	ADA81911.1
		DNA polymerase [unknown phycodnavirus]	132	61%	4,00E-35	77%	AAO11794.1
		DNA polymerase [unknown phycodnavirus]	132	61%	4,00E-35	77%	AAO11795.1
		DNA polymerase [Ostreococcus lucimarinus virus OlV360]	262	100%	4,00E-86	88%	ADA81895.1
C B5 10	1	*predicted protein [Micromonas pusilla CCMP1545]*	252	100%	3,00E-75	84%	XP_003056969.1
		DNA polymerase [Bathycoccus virus BpV161]	218	100%	4,00E-69	73%	ACP44142.1
		predicted protein [Micromonas sp. RCC299]	233	100%	2,00E-68	79%	XP_002502470.1
		predicted protein [Ostreococcus lucimarinus CCE9901]	228	100%	6,00E-67	77%	XP_001416112.1
		DNA-directed DNA polymerase, family B, pol2 [Ostreococcus tauri]	228	100%	4,00E-66	77%	CEG01117.1
C B5 11	1	*predicted protein [Ostreococcus lucimarinus CCE9901]*	264	100%	1,00E-79	88%	XP_001416112.1
		DNA-directed DNA polymerase, family B, pol2 [Ostreococcus tauri]	264	100%	2,00E-79	87%	CEG01117.1
		DNA polymerase [Bathycoccus virus BpV161]	243	100%	4,00E-79	80%	ACP44142.1
		DPOD1_ORYSA DNA polymerase delta catalytic subunit gb|AAX96341.1| dna pol (ISS) [Ostreococcus tauri]	262	100%	9,00E-79	86%	XP_003075187.1
		predicted protein [Micromonas pusilla CCMP1545]	244	100%	3,00E-72	81%	XP_003056969.1
C B5 12	1	*DNA polymerase [unknown phycodnavirus]*	252	100%	5,00E-82	88%	ACA65707.1
		DNA polymerase [unknown phycodnavirus]	252	100%	6,00E-82	88%	ACA65485.1
		DNA polymerase [unknown phycodnavirus]	252	100%	6,00E-82	88%	ACA65507.1
		DNA polymerase [unknown phycodnavirus]	252	100%	6,00E-82	88%	ADW08305.1
		DNA polymerase [unknown phycodnavirus]	252	100%	6,00E-82	88%	ACA65674.1

Table 8. Top five BLAST hits found on the NCBI database for each viral group identified from the Cerebos Salt Pan B25 in Veldrift

Group	Nr. of Seq.	BLAST hits	Total score	Query cover	E value	Ident	Accession
C25 1 &2	3	*putative DNA-dependent DNA polymerase [Halorubrum phage HF2]*	149	100%	2,00E-38	55%	NP_542554.1
		DNA polymerase elongation subunit (family B) [Halovirus HRTV-5]	147	100%	5,00E-38	55%	YP_008058501.1
		DNA polymerase elongation subunit (family B) [Halovirus HRTV-8]	146	100%	1,00E-37	55%	YP_008058620.1
		DNA-directed DNA polymerase type II [Natronorubrum sulfidifaciens]	142	100%	5,00E-36	55%	WP_008160172.1
		DNA polymerase B1 [Natronorubrum bangense]	127	100%	8,00E-31	49%	WP_006065666.1
		DNA polymerase [Halovirus HSTV-2]	119	100%	6,00E-28	46%	YP_007379123.1
C25 3	1	*DNA-directed DNA polymerase type II [Natronorubrum sulfidifaciens]*	151	100%	2,00E-39	56%	WP_008160172.1
		DNA polymerase B1 [Natronorubrum bangense]	138	100%	1,00E-34	52%	WP_006065666.1
		putative DNA-dependent DNA polymerase [Halorubrum phage HF2]	128	100%	2,00E-31	50%	NP_542554.1
		DNA polymerase elongation subunit (family B) [Halovirus HRTV-5]	127	100%	5,00E-31	50%	YP_008058501.1
		DNA polymerase elongation subunit (family B) [Halovirus HRTV-8]	124	100%	4,00E-30	49%	YP_008058620.1

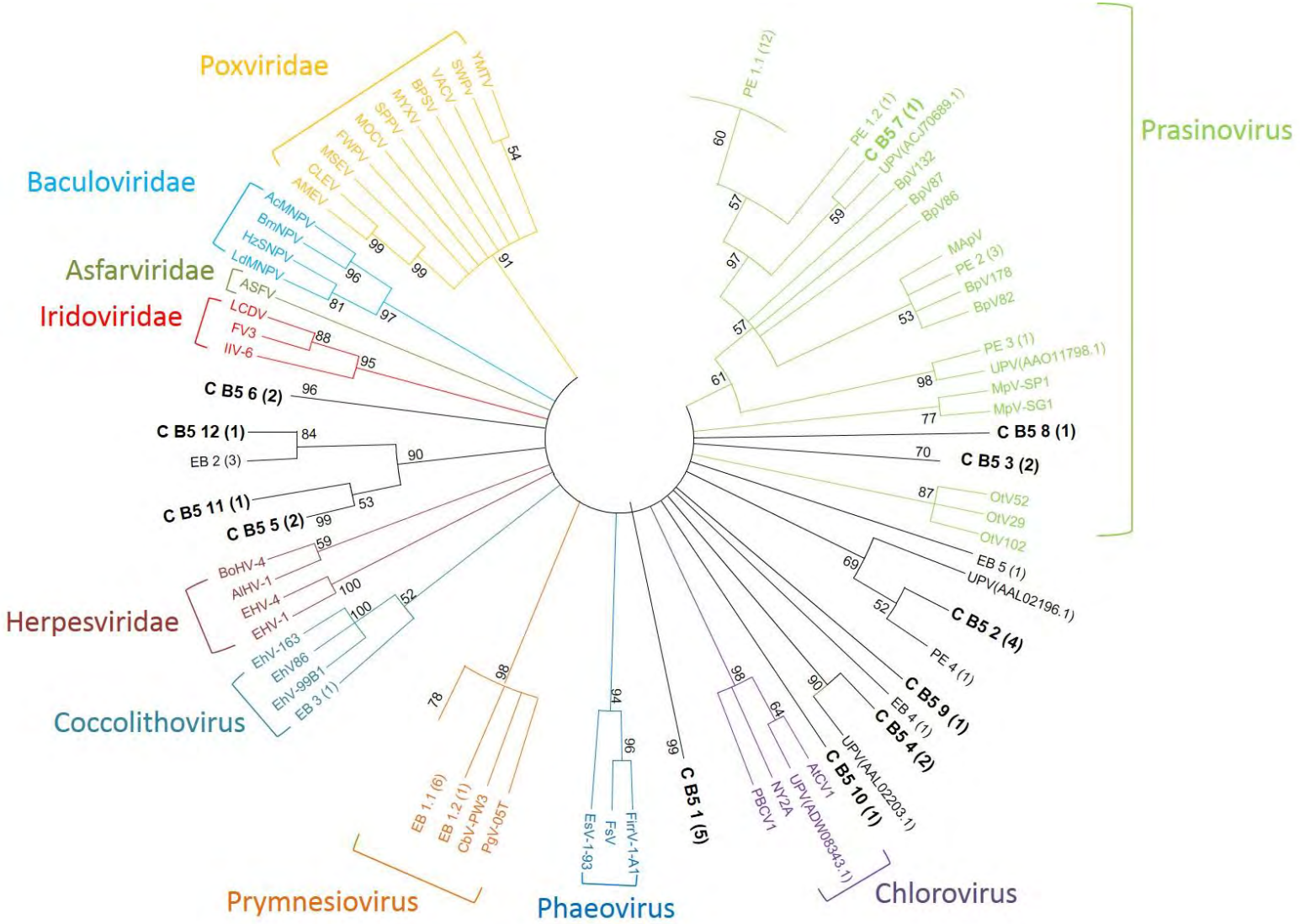


Figure 8. Figure. Molecular Phylogenetic analysis of samples collected from Cerebos salt pan baume 5. Environmental samples are named by place of origin – C B25. Number in parenthesis refer to the number of duplicate sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al. 1992). The tree with the highest log likelihood (-2200.9892) is shown. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model. The analysis involved 110 amino acid sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

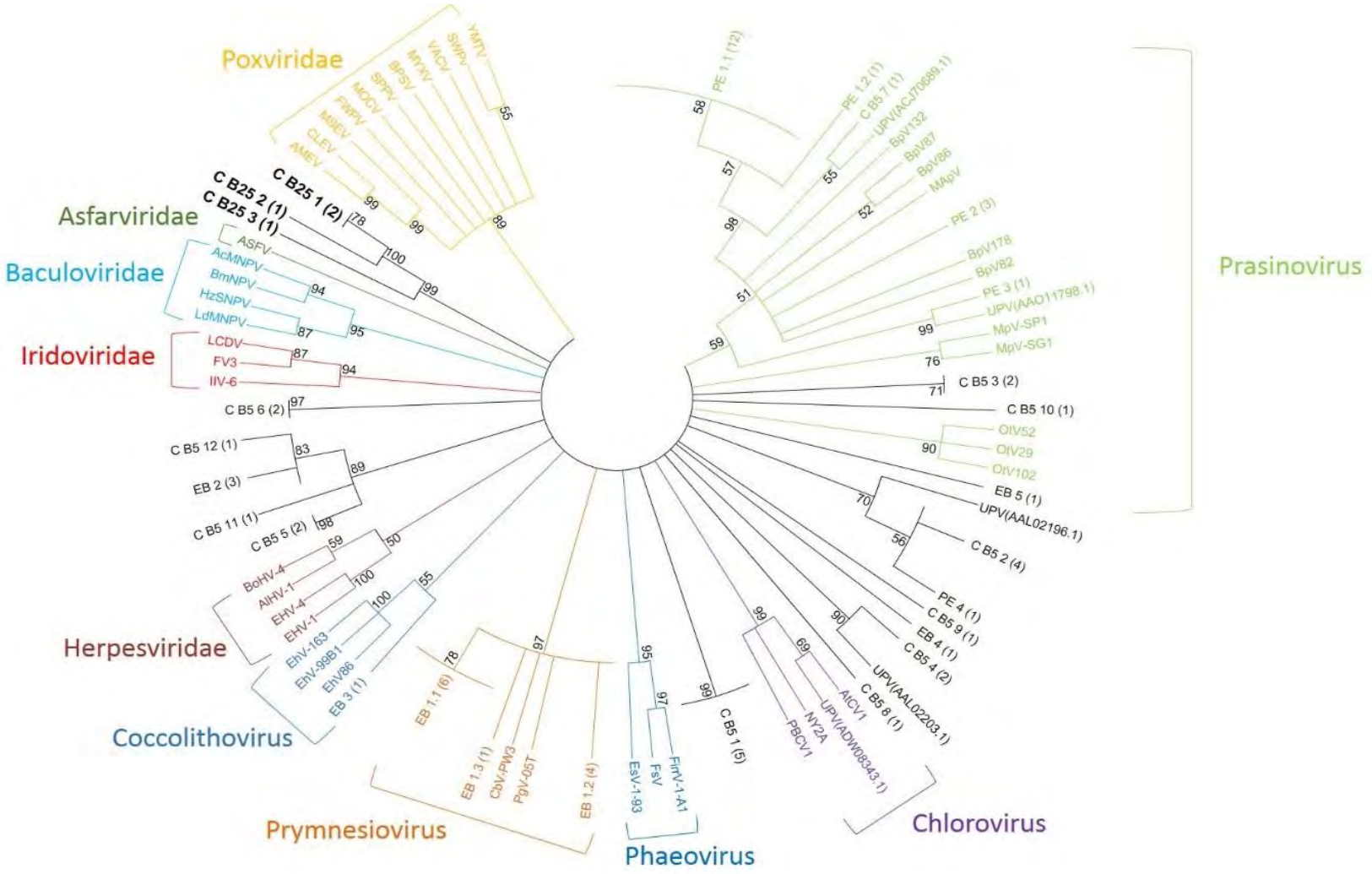


Figure 9. Figure. Molecular Phylogenetic analysis of samples collected from Cerebos salt pan baume 25. Environmental samples are named by place of origin – C B25. Number in parenthesis refer to the number of duplicate sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al. 1992). The tree with the highest log likelihood (-2200.9892) is shown. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model. The analysis involved 110 amino acid sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

Chapter 4 – Discussion:

4.1. Methods of identifying *Phycodnaviridae* from marine and hypersaline environments

Sample sites for this study were chosen based on interest and ecological importance (Pitcher & Probyn 2011; Barlow et al. 2010). Different filtration techniques were used for each sample site specifically. The TFF method was chosen for the salt pan samples based on the fact that it has been established as an effective method and increased salt levels in the samples may have an effect with the $FeCl_3$ precipitation method. Each sample site was chosen as an independent study to generate a reference database of viruses present. Initially it was not intended for a comparative study across the sites. Viral recovery using the TFF method has been noted to vary from 2% to 98% (Colombet et al. 2007; Schoenfeld et al. 2008), whereas the $FeCl_3$ precipitation method provided recovery rate of 92-95%. However, comparison of the sequences obtained is still possible as the filtration methods do not alter the ability of the viruses to be sequenced effectively (John et al. 2011).

In this study, the main objective was to identify *Phycodnaviridae* and compare sequences isolated with known *Phycodnaviridae* sequences. Understanding that a majority of genetic analysis have been successfully analysed through amplification of a fragment of the DNA polymerase gene (Chen & Suttle 1995), we decided to use the AVS1 and POL primer sets to amplify a broad range of phycodnaviruses from the samples.

Specific primers were designed for two selected viral groups identified in the Elands Bay and Algoa Bay blooms. Cross-screening analysis using the EB 1 virus primers yielded successful

results for being able to pick out EB 1 viruses from the Elands Bay bloom samples (figure 6). The corresponding analyses for the PE 1 virus, did not provide conclusive results and should be expanded on in future experiments. The annealing temperature and primer specificity should be further examined to optimise amplification of the PE 1 virus.

4.2. Isolated Algal viruses from Bloom events

Identifying algal viruses in the environment creates a starting point to work from in understanding the underlying molecular mechanisms that are involved in algal blooms. Characterisation of viral diversity provides an indication of the prominent viral clades present in a specific bloom. Using the AVS1 and POL primers algal virus specific DNA can be isolated from environmental samples, more specifically viruses belonging to the *Phycodnaviridae* family (Chen & Suttle 1995; Schroeder et al. 2002).

Phycodnaviridae were identified from the algal bloom event in Elands Bay. Viruses, EB 1.1, 1.2 and 1.3, maintained their phylogenetic position throughout all analyses. This allows, with relative certainty, to define them as prymnesioviruses. The natural progression of a bloom starts with upwelling, caused by intermittent long-shore winds (Fawcett et al. 2007). Phytoplankton then begin to proliferate until maximum abundance and then individual phytoplankter start to die off either due to depletion of nutrients or through a different termination mechanism (Smayda 1997; Ruardij et al. 2005; Philippart et al. 2000). It has been suggested that viral infection is a molecular mechanism involved in bloom termination (Bratbak et al. 1996). Identification of bloom terminating viruses is essential for understanding bloom

dynamics. Rate of viral infection is directly linked to the demise of a bloom (Bratbak et al. 1993; Nagasaki et al. 1994). It has been documented that maximal viral infection coincides with or occurs after initial decline of the phytoplankton bloom is observed (Bratbak et al. 1993; Sandaa 2008).

The PCR results for the amplification of the viral group EB 1 indicate the strongest amplification signal, i.e. thickest and most defined band observed on the gel, for day 2 of the bloom. From these results seen we can assume that the on day 2 of the bloom, EB1 viruses were present. No viral DNA was detected for day 5 of the bloom, which indicates that there were very little or no EB 1 viral groups on that day. This stands to reason as the majority of cells would have lysed at the end of the bloom. Anecdotal observations made by the collection crew suggested the demise from day 3 onwards, based on the size and colour of the bloom (unpublished data). Similarly the *E. huxleyi* virus (EhV) showed an increase in viral particles before the demise of the bloom, which has been well-established (Schroeder et al. 2003). This further supports the concept of maximal viral presence coinciding to the termination of the bloom.

With the above stated results and observations, we can postulate that the EB 1 subgroup of *Prymnesioviridae* were the involved in bloom termination for this specific bloom, however further research is needed to support this claim. Known hosts linked to these types of viruses suggest that this bloom contains *Prymnesiophyceae*. The results found in this study corroborates what has been identified previously. Prevalent harmful algal species previously documented in this area have been identified as *Dinophyceae* (Fawcett et al. 2007). More specifically in the Benguela upwelling system, Pitcher and Probyn in 2011 reported a

dinoflagellate bloom, which identified *Ceratium balechii* as the dominant species. Other dinoflagellate species have been described in harmful algal blooms across the globe (Guillou et al. 2002; Reguera et al. 2012). While dinoflagellates are found across various niche areas, many remain genetically uncharacterised (Stern et al. 2010). Identification of dinoflagellates is further complicated by contradicting morphological and genetic analyses (Lin 2011).

The identification of a new viral subgroup, namely EB 1, suggests a possible new dinoflagellate host species, as many viruses are generally host-specific (Nagasaki et al. 2005). However, there have been reports of more than one virus infecting a specific host (Baudoux & Brussaard 2005). This could signify a new virus that can infect and initiate the demise of a bloom in currently known algal host species. Further investigations into host identification and characterisation would give a more complete picture of the bloom as a whole. Another interesting finding is the possibility of a novel coccolithovirus, EB 3. This shows that there is the opportunity to isolate new viruses from this region.

Additionally in 2013, an unusual yet not uncommon occurrence was observed along the coast of Algoa Bay (Lutjeharms et al. 2000). Situated on the southern coast of South Africa and accustomed to slightly warmer waters than that present on the western coast (Pitcher & Probyn 2011), the coast is susceptible to sporadic inshore upwelling. Very little is documented on this topic compared to the well-studied Benguela upwelling system on the west coast (Nelson & Hutchings 1983), and even less is described concerning opportune algal blooms. Water samples taken from this bloom event indicated that prasinoviruses could be responsible for terminating this bloom. However, identifying bloom terminating viral species for this bloom

is more complicated as only one day of the bloom was sampled and PE 1 specific screening yielded no conclusive results. The majority of viral DNA pol amplicons resulted in the formation of a new clade of viruses within the prasinophytes. Here we can suggest that this viral clade is most likely the dominant virus in this bloom and therefore would indicate a prasinophyte host dominated bloom. Characterisation of blooms and species involved in this region have not been studied intensively. Blooms in this region are generally shorter lived than those on the west coast due to wind variability in the region (Schumann 1999).

The two blooms were separate events taking place independently from one another. Genetic analysis of the viral DNA present in the blooms support this notion as no similarity in viral groups was observed. Subgroups PE 4 and EB 4 did initially show a potential genetic similarity, however this relationship did not persist throughout the subsequent analyses. The most prevalent viral species in each bloom indicate that the dominant algal host species were different between for each bloom. This is further enforced by the fact that no EB 1 viral DNA was detected in the Port Elizabeth bloom (Figure 6). Therefore no comparison can be made from bloom to bloom with respect to viruses, indicating that the Southern African coast is home to a variety of bloom forming species.

Understanding the present literature on algal viruses and their proposed role in bloom termination, infers that viruses played a role in cell lysis and eventual demise of the two bloom events. There is not much we can determine from the Elands Bay and Algoa bay blooms as analysis of host information has not yet been completed. It is then only speculative that the hosts of the EB and PE viruses were the dominating species of phytoplankton within each

bloom respectively. Well established known bloom terminating viruses such as HaV, and EhV have been linked to host mortality (Schroeder et al. 2002; Tarutani et al. 2000). Therefore it is most likely that the EB and PE viruses caused cell lysis within their respective hosts, thereby contributing to the eventual demise of each bloom.

Bloom dynamics are influenced by a variety of factors. Viral infection can only represent one side of a multi-faceted system. The most obvious factors are temperature, wind and nutrient availability (Schumann 1999; Kudela et al. 2010). Additional factors may include alternative forms of parasitism and grazing (Montagnes et al. 2008). From the results obtained, certain links can be made and notions inferred, however all possible influences must be taken into account to fully understand the system in its entirety.

4.3. Algal viruses and halophilic viruses described in solar salt pans

Salt pans are important areas to assess microbial and viral composition as they affect the salt production and many commercially sold table salts are derived from these areas (Javor 2002). Halophilic bacteria have also been used in pharmacology research and food industry with the isolation of unique enzymes and bioactive compounds (Karthikeyan et al. 2013).

It has been naturally thought in the past that very little lives in such environments, due to their extreme conditions. However, this is not the case as seen in through various studies (Porter et al. 2007; Ventosa et al. 2015; Sime-Ngando et al. 2011) as well as the results obtained in this

study. It has been suggested by Santos et al. (2010) that hypersaline regions actually contain the most VLPs seen among plankton-rich regions.

In the first baume (B5) analysed from the Cerebos salt pans in Veldrift, a larger diversity of algal viruses was observed than the other analyses in this study. Phylogenetic analysis of the samples, demarcated as C B5 (Figure 8), showed that all except one did not statistically align to any specific genus of *Phycodnaviridae* shown. Some of the sequences, however, indicated a relationship to other environmental samples analysed. This baume (B5) is the first inlet of water from the Berg River and is the least saline in the plant. This body of water is relatively stagnant with little movement of solutes. The lack of water movement would contribute to the high diversity seen in the pan. Close proximity to the coast could account for transference of marine particles via geochemical cycling in the atmosphere (Smith & Compton 2004). This region, however, does not see much rainfall throughout the year and therefore suggests the majority of microbial and viral species present in the pan originate from the river itself.

A second sample site was chosen from the Cerebos salt works in Veldrift, baume 25 (B25). This is the most saline pan in the plant at approximately 25 psu. Three sequences: C B25 1, 2 and 3 were grouped together phylogenetically showing no relation to other samples studied. Using the NCBI database, sequences were identified to most resemble haloviruses (Table 8). These sequences identified seem to share algal virus DNA polymerase genes while exhibiting affiliation to phages-like haloviruses, this could suggest possible recombination of genes in viruses found in hypersaline environments (Tang et al. 2004). The closest relative to the C B25 viral sequences is the HF2 virus isolated from Tang et al. (2002). The mosaic genome of this

virus indicates it acts as a vector for transfer of genes. Interestingly, the HF2 virus contains genetic regions correlating to the DNA polymerase (B family) gene. The only other known haloarchaeal viruses to contain the DNA polymerase gene are *Halobacterium salinarum* strains NRC-1 and R1 (Tang et al. 2002). The use of a DNA polymerase specific primer was able to identify C B25 viruses, therefore it suggests that these virus contains the DNA polymerase gene. More closely matched sequences were that of the myoviruses HRTV-5 and HRTV-8. Researchers described these viruses to have a range of hosts (Atanasova et al. 2015). It would provide very interesting results to further analyse the C B25 viruses identified to elicit the possible hosts and viral characteristics of these viruses.

Research into solar salterns indicated a species shift relative to increase in salinity. A corresponding dominance of Archaeal viruses was seen with increasing salinity (Rodriguez-Brito et al. 2010). In the results of this study, we can clearly see a potential shift in species dominance. The lower saline environment of B5 showed a higher diversity of algal viruses. At a higher salinity in B25, the identified sequences show a greater similarity to haloarchaeal viruses, following the trend observed by Rodriguez-Brito et al. (2010). Little has been explored about halophilic viruses in Southern Africa and more in depth investigations are required to explore these viral sequences (Wommack & Colwell 2000).

4.4. Building a basis for future studies

This study achieved the required aims initially laid out. *Phycodnaviridae* sequences were identified and pieced together in phylogenetic analysis to create a basic understanding of the

algal viruses present in four distinct sites. For the Elands Bay bloom event a possible bloom terminating virus, EB 1, is proposed. Novel viral clades were identified for both bloom events expanding our current reference of available viral sequences, however more extensive genetic methods would provide more comprehensive data. These methods include viral enumeration techniques, next generation sequencing and metagenomic studies (Borsheim et al. 1990; Noble & Fuhrman 1998; Marie et al. 1999; Edwards & Rohwer 2005). These techniques could be used in future investigations, contributing to a larger frame of context to work in and therefore assess the dominant host and viral sequences present. From the results obtained in this study one can only make educated assumptions of the viruses present and their potential role in bloom termination. Investigations into the host DNA of the blooms (currently underway at the Marine Biological Association) would provide a more complete picture of the bloom composition and would allow for in depth studies on virus-host interactions and bloom dynamics.

Further research would also include better optimisation of designed primers for cross screening of environmental samples. This would then allow studies to go back to archived samples and investigate the prevalence of specific dominant viral species over time. To better understand the blooms forming on the southern coast, longer sampling periods should be established to have samples across the lifespan of the bloom from start to finish. Daily samples, as in the case with the Elands Bay bloom would give comparative data, pinpointing the viral maximum and subsequent demise of the bloom. There are several gaps in research of algal blooms and their associated viruses in the context of Southern Africa. More comprehensive studies into microbial research of these bloom events are needed (Pfaff et al. 2014). In detail research into salt pan

viral communities should be done following a section of water through its gradual progression through the baumes. This would identify the tolerance of viral species and their hosts to increased salinity. Other environmental factors should be monitored to determine microbial origin and possible transfer within the system.

References

Allen, M.J. & Wilson, W.H., 2008. Aquatic virus diversity accessed through omic techniques: a route map to function. *Current Opinion in Microbiology*, 11(3), pp.226–32.

Angly, F.E. et al., 2006. The marine viromes of four oceanic regions. *PLoS biology*, 4(11), p.e368.

Arslan, D. et al., 2011. Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae. *Proceedings of the National Academy of Sciences of the United States of America*, 108(42), pp.17486–91.

Atanasova, N. et al., 2015. Archaeal Viruses Multiply: Temporal Screening in a Solar Saltern. *Viruses*, 7(4), pp.1902–1926.

Bamford, D.H., Burnett, R.M. & Stuart, D.I., 2002. Evolution of Viral Structure. *Theoretical Population Biology*, 61(4), pp.461–470.

Bamford, D.H., Grimes, J.M. & Stuart, D.I., 2005. What does structure tell us about virus evolution? *Current Opinion in Structural Biology*, 15(6), pp.655–63.

Bandea, C.I., 2009. A Unifying Scenario on the Origin and Evolution of Cellular and Viral Domains. *Nature Precedings*, (4), pp.1–16.

Barlow, R. et al., 2010. Phytoplankton production and physiological adaptation on the southeastern shelf of the Agulhas ecosystem. *Continental Shelf Research*, 30(13), pp.1472–1486.

Baudoux, A. et al., 2006. Virally induced mortality of *Phaeocystis globosa* during two spring blooms in temperate coastal waters. *Aquatic Microbial Ecology*, 44, pp.207–217.

Baudoux, A.C. & Brussaard, C.P.D., 2005. Characterization of different viruses infecting the marine harmful algal bloom species *Phaeocystis globosa*. *Virology*, 341(1), pp.80–90.

Bauld, J., 1981. Occurrence of benthic microbial mats in saline lakes. *Developments in Hydrobiology*, 5, pp.87–111.

Bergh, Ø., BØrsheim, Knut Yngve Bratbak, G. & Heldal, M., 1989. High abundance of viruses found in aquatic environments. *Nature*, 340, pp.467–468.

Borowitzka, L.J., 1981. The Microflora. *Salt Lakes. Developments in Hydrobiology.*, 5, pp.33–46.

Bratbak, G. et al., 1995. Viral activity in relation to *Emiliania huxleyi* blooms: a mechanism of DMSP release? *Marine Ecology Progress Series*, 128, pp.133–142.

Bratbak, G. et al., 1990. Viruses as Partners in Spring Bloom Microbial Trophodynamics. *Applied and Environmental Microbiology*, 56(5), pp.1400–1405.

Bratbak, G., Egge, J.K. & Heldal, M., 1993. Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. *Marine Ecology Progress Series*, 93.

Bratbak, G., Wilson, W. & Heldal, M., 1996. Viral control of *Emiliania huxleyi* blooms? *Journal of Marine Systems*, 9, pp.75–81.

Braunwald, J., Nonenmacher, H. & Tripier-Darcy, F., 1985. Ultrastructural and Biochemical Study of Frog Virus 3 Uptake by. *Journal of General Virology*, 66, pp.283–293.

Breslauert, K.J. et al., 1986. Predicting DNA duplex stability from the base sequence. *Proceedings of the National Academy of Sciences*, 83, pp.3746–3750.

Brsheim, K.Y., Bratbak, G. & Heldal, M., 1990. Enumeration and Biomass Estimation of Planktonic Bacteria and Viruses by Transmission Electron Microscopy. , (24), pp.352–356.

Brussaard, C.P.D., Kuipers, B. & Veldhuis, M.J.W., 2005. A mesocosm study of *Phaeocystis globosa* population dynamics. *Harmful Algae*, 4(5), pp.859–874.

Chen, F. et al., 1996. Genetic Diversity in Marine Algal Virus Communities as Revealed by Sequence Analysis of DNA Polymerase Genes. *Applied and Environmental Microbiology*, 62(8), pp.2869–2874.

Chen, F. & Suttle, C.A., 1995. Amplification of DNA Polymerase Gene Fragments from Viruses Infecting Microalgae. *Applied and Environmental Microbiology*, 61(4), pp.1274–1278.

Cheng, X., Virk, N. & Wang, H., 2014. *Plant Virus–Host Interaction*, Elsevier.

Claverie, J.-M. et al., 2006. Mimivirus and the emerging concept of “giant” virus. *Virus research*, 117(1), pp.133–44.

Clerissi, C. et al., 2014. Unveiling of the diversity of Prasinoviruses (*Phycodnaviridae*) in marine samples by using high-throughput sequencing analyses of PCR-amplified DNA polymerase and major capsid protein genes. *Applied and Environmental Microbiology*, 80(10), pp.3150–60.

Colombet, J. et al., 2007. Virioplankton “pegylation”: use of PEG (polyethylene glycol) to concentrate and purify viruses in pelagic ecosystems. *Journal of microbiological methods*, 71(3), pp.212–9.

Culley, A.I., Asuncion, B.F. & Steward, G.F., 2009. Detection of inteins among diverse DNA polymerase genes of uncultivated members of the *Phycodnaviridae*. *The ISME journal*, 3(4), pp.409–18.

Culley, A.I., Lang, A.S. & Suttle, C.A., 2003. High diversity of unknown picorna-like viruses in the sea. , 424(August), pp.4–7.

Danovaro, R. et al., 2011. Marine viruses and global climate change. *FEMS Microbiology Reviews*, 35(6), pp.993–1034.

DeAngelis, P.L., 1997. Hyaluronan Synthase of Chlorella Virus PBCV-1. *Science*, 278(5344), pp.1800–1803.

Delaroque, N. et al., 1999. Persistent virus integration into the genome of its algal host , *Ectocarpus siliculosus* (*Phaeophyceae*). , pp.1367–1370.

Dunigan, D.D., Fitzgerald, L. a & Van Etten, J.L., 2006. Phycodnaviruses: a peek at genetic diversity. *Virus Research*, 117(1), pp.119–32.

Dyall-Smith, M., Tang, S.-L. & Bath, C., 2003. Haloarchaeal viruses: how diverse are they? *Research in Microbiology*, 154.4, pp.309–313.

Edwards, R.A. & Rohwer, F., 2005. Viral metagenomics. *Nature Reviews. Microbiology*, 3(June), pp.801–805.

Emerson, J.B. et al., 2013. Virus-host and CRISPR dynamics in Archaea-dominated hypersaline Lake Tyrrell, Victoria, Australia. *Archaea (Vancouver, B.C.)*, 2013, p.370871.

Van Etten, J.L. et al., 2002. Phycodnaviridae--large DNA algal viruses. *Archives of virology*, 147(8), pp.1479–516.

Van Etten, J.L., 2003. Unusual life style of giant chlorella viruses. *Annual review of Genetics*, 37, pp.153–95.

Van Etten, J.L. et al., 1982. Viruses of symbiotic Chlorella-like algae isolated from Paramecium bursaria and Hydra viridis. , 79(June), pp.3867–3871.

Fawcett, a et al., 2007. Contrasting wind patterns and toxigenic phytoplankton in the southern Benguela upwelling system. *Marine Ecology Progress Series*, 348, pp.19–31.

Filée, J. et al., 2002. Evolution of DNA polymerase families: evidences for multiple gene exchange between cellular and viral proteins. *Journal of Molecular Evolution*, 54(6), pp.763–73.

Filée, J., Pouget, N. & Chandler, M., 2008. Phylogenetic evidence for extensive lateral acquisition of cellular genes by Nucleocytoplasmic large DNA viruses. *BMC Evolutionary Biology*, 8, p.320.

Forterre, P., 2006. The origin of viruses and their possible roles in major evolutionary transitions. *Virus Research*, 117(1), pp.5–16.

Fuhrman, J.A., 1999. Marine viruses and their biogeochemical and ecological effects. *Nature*, 399, pp.541–548.

Gadelle, D. et al., 2003. Phylogenomics of type II DNA topoisomerases. *BioEssays*, 25(3), pp.232–42.

Gasteiger, E. et al., 2003. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research*, 31, pp.3784–3788.

Gimenes, M. V et al., 2012. Phylodynamics and movement of Phycodnaviruses among aquatic environments. *The ISME journal*, 6(2), pp.237–47.

Graves, M. V et al., 1999. Hyaluronan synthesis in virus PBCV-1-infected chlorella-like green algae. *Virology*, 257(1), pp.15–23.

Guerrero, M.C. & Wit, R. De, 1992. Microbial Mats in the inland Saline Lakes of Spain. *Limnectica*, 8, pp.197–204.

Guillou, L. et al., 2002. Genetic Diversity and Molecular Detection of Three Toxic Dinoflagellate Genera (*Alexandrium , Dinophysis ,* and *Karenia*) from French Coasts. *Protist*, 153, pp.223–238.

Hall, T.A., 2011. BioEdit : An important software for molecular biology. *GERF Bulletin of Biosciences*, 2(1), pp.60–61.

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia*, 32(2), pp.79–99.

Iyer, L.M. et al., 2006a. Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. *Virus Research*, 117(1), pp.156–84.

Iyer, L.M. et al., 2006b. Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. *Virus research*, 117(1), pp.156–84.

Javor, B., 2002. Industrial microbiology of solar salt production. *Industrial Microbiology and Biotechnology*, 28(1), pp.42–7.

Jessup, C.M. & Forde, S.E., 2008. Ecology and evolution in microbial systems: the generation and maintenance of diversity in phage-host interactions. *Research in microbiology*, 159(5), pp.382–9.

John, S.G. et al., 2011. A simple and efficient method for concentration of ocean viruses by chemical flocculation. *Environmental Microbiology Reports*, 3(2), pp.195–202.

Jones, D.T., Taylor, W.R. & Thornton, J.M., 1992. The rapid generation of mutation data matrices from protein sequences. *Computer Applications in the Biosciences*, 8, pp.275–282.

Karthikeyan, P., Bhat, S.G. & Chandrasekaran, M., 2013. Halocin SH10 production by an extreme haloarchaeon *Natrinema sp*. BTSH10 isolated from salt pans of South India. *Saudi journal of Biological Sciences*, 20, pp.205–212.

Koonin, E. V, Senkevich, T.G. & Dolja, V. V, 2006. The ancient Virus World and evolution of cells. *Biology direct*, 1, p.29.

Koonin, E. V & Yutin, N., 2010. Origin and evolution of eukaryotic large nucleo-cytoplasmic DNA viruses. *Intervirology*, 53(5), pp.284–92.

Koressaar, T. & Remm, M., 2007. Enhancements and modifications of primer design program Primer3. *Bioinformatics*, 23(10), pp.1289–91.

Kudela, R.M., Seeyave, S. & Cochlan, W.P., 2010. The role of nutrients in regulation and promotion of harmful algal blooms in upwelling systems. *Progress in Oceanography*, 85(1-2), pp.122–135.

Larsen, J.B. et al., 2008. Phylogenetic analysis of members of the *Phycodnaviridae* virus family, using amplified fragments of the major capsid protein gene. *Applied and Environmental Microbiology*, 74(10), pp.3048–57.

Lawrence, J. & Suttle, C., 2004. Effect of viral infection on sinking rates of *Heterosigma akashiwo* and its implications for bloom termination. *Aquatic Microbial Ecology*, 37, pp.1–7.

Legendre, M. et al., 2014. Thirty-thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology. *Proceedings of the National Academy of Sciences of the United States of America*, 111(11), pp.4274–9.

Lin, S., 2011. Genomic understanding of dinoflagellates. *Research in Microbiology*, 162(6), pp.551–69.

Lodish H, Berk A, Z.S., 2000. *Molecular Cell Biology* 4th ed. W. H. Freeman, ed., New York.

Lutjeharms, J.R.., Cooper, J. & Roberts, M., 2000. Upwelling at the inshore edge of the Agulhas Current. *Continental Shelf Research*, 20(7), pp.737–761.

Mackinder, L.C.M. et al., 2009. A unicellular algal virus, *Emiliania huxleyi* virus 86, exploits an animal-like infection strategy. *The Journal of General Virology*, 90(Pt 9), pp.2306–16.

Marie, D. et al., 1999. Enumeration of Marine Viruses in Culture and Natural Samples by Flow Cytometry. , 65(1), pp.45–52.

Mayer, J.A. & Taylor, F.J.R., 1979. A virus which lyses the marine nanoflagellate *Micromonas pusilla*. *Nature*, 281(5729), pp.299–301.

Medlin, L.K., Oce, O. & Cembella, A.D., 2013. Biodiversity of Harmful Marine Algae. *Encyclopedia of Biodiversity*, 1, pp.470–484.

Miller, E.S. et al., 2003. Bacteriophage T4 Genome. *Microbiology and Molecular Biology Reviews*, 67(1), pp.86–156.

Montagnes, D. et al., 2008. Responsibility of microzooplankton and parasite pressure for the demise of toxic dinoflagellate blooms. *Aquatic Microbial Ecology*, 53(November), pp.211–225.

Moreau, H. et al., 2010. Marine prasinovirus genomes show low evolutionary divergence and acquisition of protein metabolism genes by horizontal gene transfer. *Journal of Virology*, 84(24), pp.12555–63.

Moreira, D. & López-García, P., 2005. Comment on “The 1.2-Megabase Genome Sequence of Mimivirus.” *Science*, 308, p.1114.

Müller, D.., Kapp, M. & Knippers, R., 1998. Viruses in marine brown algae. *Advances in virus research*, 50, p.49.

Müller, D.., Kawai, H Stache, B. & Lanka, S., 1990. A Virus Infection in the Marine Brown Alga *Ectocarpus siliculosus* (*Phaeophyceae*). *Botanica Acta*, 103(1), pp.72–82.

Nagasaki, K. et al., 2005. Algal viruses with distinct intraspecies host specificities include identical intein elements. *Applied and environmental microbiology*, 71(7), pp.3599–607.

Nagasaki, K. et al., 2004. Isolation and Characterization of a Novel Single-Stranded RNA Virus Infecting the Bloom-Forming Diatom *Rhizosolenia setigera*. *Applied and Environmental Microbiology*, 70(2), pp.704–711.

Nagasaki, K. et al., 1994. Virus-like particles in *Heterosigma akashiwo* (*Raphidophyceae*): a possible red tide disintegration mechanism. *Marine Biology*, 119(2), pp.307–312.

Nagasaki, K. & Yamaguchi, M., 1997. Isolation of a virus infectious to the harmful bloom causing microalga *Heterosigma akashiwo* (*Raphidophyceae*). *Aquatic Microbial Ecology*, 13, pp.135–140.

Nelson, G. & Hutchings, L., 1983. The Benguela upwelling area. *Progress in Oceanography*, 12(3), pp.333–356.

Noble, R. & Fuhrman, J., 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquatic Microbial Ecology*, 14, pp.113–118

Oren, A., 2002. Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology & Biotechnology*, 28(1), pp.56–63.

Oren, A. & Seckbach, J., 2011. Oxygenic Photosynthetic Microorganisms in Extreme Environments. *Cellular Origin, Life in Extreme Habitats and Astrobiology*, 5, pp.3–25.

Owczarzy, R., Tataurov, A. & Al., E., 2008. IDT SciTools: a suite for analysis and design of nucleic acid oligomers. *Nucleic Acids Res.*, 36(2), p.163−169.

Padan, E. & Shilo, M., 1973. Cyanophages-Viruses Attacking Blue-Green Algae. , 37(3), pp.343–370.

Papke, R.T. & Doolittle, W.F., 2003. Phage evolution: New worlds of genomic diversity. *Current Biology*, 13(15), pp.R606–R607.

Petruševski, B. et al., 1995. Tangential flow filtration: A method to concentrate freshwater algae. *Water Research*, 29(5), pp.1419–1424.

Pfaff, M.C. et al., 2014. The Overlooked Foundation: Marine Microbes in the Oceans Surrounding South Africa. In N. Funke et al., eds. *Reflections on the State of Research and Technology in South Africa's Marine and Maritime Sectors*. The Council for Scientific and Industrial Research, pp. 100–121.

Philippart, C.J.M. et al., 2000. Long-term phytoplankton-nutrient interactions in a shallow coastal sea: Algal community structure, nutrient budgets, and denitrification potential. *Limnology and Oceanography*, 45(1), pp.131–144.

Philippe, N. et al., 2013. Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes. *Science (New York, N.Y.)*, 341(6143), pp.281–6.

Pietilä, M.K. et al., 2013. Insights into head-tailed viruses infecting extremely halophilic archaea. *Journal of vVrology*, 87(6), pp.3248–60.

Pirooznia, M., Perkins, E.J. & Deng, Y., 2008. Batch Blast Extractor: an automated blastx parser application. *BMC genomics*, 9 Suppl 2, p.S10.

Pitcher, G.C. & Nelson, G., 2006. Characteristics of the surface boundary layer important to the development of red tide on the southern Namaqua shelf of the Benguela upwelling system. *Limnology and Oceanography*, 51(6), pp.2660–2674.

Pitcher, G.C. & Probyn, T. a., 2011. Anoxia in southern Benguela during the autumn of 2009 and its linkage to a bloom of the dinoflagellate *Ceratium balechii*. *Harmful Algae*, 11, pp.23–32.

Porter, K., Russ, B.E. & Dyall-Smith, M.L., 2007. Virus-host interactions in salt lakes. *Current Opinion in Microbiology*, 10(4), pp.418–24.

Probyn, T. a., Mitchell-Innes, B. a. & Searson, S., 1995. Primary productivity and nitrogen uptake in the subsurface chlorophyll maximum on the Eastern Agulhas Bank. *Continental Shelf Research*, 15(15), pp.1903–1920.

Raoult, D. et al., 2004. The 1.2-megabase genome sequence of Mimivirus. *Science (New York, N.Y.)*, 306(5700), pp.1344–50.

Reguera, B. et al., 2012. Harmful Dinophysis species: A review. *Harmful Algae*, 14, pp.87–106.

Rodriguez-Brito, B. et al., 2010. Viral and microbial community dynamics in four aquatic environments. *The ISME Journal*, 4(6), pp.739–751.

Rohwer, F. & Thurber, R.V., 2009. Viruses manipulate the marine environment. *Nature*, 459(7244), pp.207–12.

Ruardij, P., Veldhuis, M.J.W. & Brussaard, C.P.D., 2005. Modeling the bloom dynamics of the polymorphic phytoplankter *Phaeocystis globosa*: impact of grazers and viruses. *Harmful Algae*, 4(5), pp.941–963.

Rychlik, W., 1995. *MolecularBiotechnology*,

Safferman, R.S. & Morris, M., 1964. Growth Characteristics of the Blue-Green Algal Virus LPP-1. *Journal of Bacteriology*, 88(3), pp.771–775.

Sandaa, R. a et al., 2001. Isolation and characterization of two viruses with large genome size infecting *Chrysochromulina ericina* (*Prymnesiophyceae*) and P*yramimonas orientalis* (*Prasinophyceae*). *Virology*, 290(2), pp.272–80.

Sandaa, R.-A., 2008. Burden or benefit? Virus-host interactions in the marine environment. *Research in Microbiology*, 159(5), pp.374–81.

Sandaa, R.-A. & Larsen, A., 2006. Seasonal variations in virus-host populations in Norwegian coastal waters: focusing on the cyanophage community infecting marine *Synechococcus spp*. *Applied and environmental microbiology*, 72(7), pp.4610–8.

Santos, F. et al., 2010. The metavirome of a hypersaline environment. *Environmental Microbiology*, 12(11), pp.2965–2976.

Schoenfeld, T. et al., 2008. Assembly of viral metagenomes from Yellowstone hot springs. *Applied and Environmental Microbiology*, 74(13), pp.4164–4174.

Schroeder, D.C. et al., 2002. Coccolithovirus (*Phycodnaviridae*): characterisation of a new large dsDNA algal virus that infects *Emiliana huxleyi*. *Archives of Virology*, 147(9), pp.1685–98.

Schroeder, D.C. et al., 2009. Genomic analysis of the smallest giant virus--*Feldmannia sp*. virus 158. *Virology*, 384(1), pp.223–32.

Schroeder, D.C. et al., 2003. Virus Succession Observed during an Emiliania huxleyi Bloom Virus Succession Observed during an *Emiliania huxleyi* Bloom. *Applied and Environmental Microbiology*, 69(5), pp.2484–2490.

Schumann, E.H., 1999. Wind-driven mixed layer and coastal upwelling processes off the south coast of South Africa. *Journal of Marine Research*, 57(4), pp.671–691.

La Scola, B. et al., 2003. A Giant Virus in Amoebae. *Science*, 299, p.2033.

Scorzetti, G. et al., 2009. Multiple simultaneous detection of Harmful Algal Blooms (HABs) through a high throughput bead array technology, with potential use in phytoplankton community analysis. *Harmful Algae*, 8(2), pp.196–211.

Shirai, Y. et al., 2008. Isolation and characterization of a single-stranded RNA virus infecting the marine planktonic diatom *Chaetoceros tenuissimus* Meunier. *Applied and environmental microbiology*, 74(13), pp.4022–7.

Short, C.M., Rusanova, O. & Short, S.M., 2011. Quantification of virus genes provides evidence for seed-bank populations of phycodnaviruses in Lake Ontario, Canada. *The ISME journal*, 5(5), pp.810–21.

Short, S. & Suttle, C., 2003. Temporal dynamics of natural communities of marine algal viruses and eukaryotes. *Aquatic Microbial Ecology*, 32, pp.107–119.

Short, S.M., 2012. The ecology of viruses that infect eukaryotic algae. *Environmental Microbiology*, 14(9), pp.2253–71.

Short, S.M. & Suttle, C. a., 2002. Sequence Analysis of Marine Virus Communities Reveals that Groups of Related Algal Viruses Are Widely Distributed in Nature. *Applied and Environmental Microbiology*, 68(3), pp.1290–1296.

Sime-Ngando, T. et al., 2011. Diversity of virus-host systems in hypersaline Lake Retba, Senegal. *Environmental Microbiology*, 13(8), pp.1956–72.

Smayda, T.J., 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology and Oceanography*, 42(5_part_2), pp.1137–1153.

Smith, M. & Compton, J.S., 2004. Origin and evolution of major salts in the Darling pans, Western Cape, South Africa. *Applied Geochemistry*, 19(5), pp.645–664.

Stern, R.F. et al., 2010. Environmental barcoding reveals massive dinoflagellate diversity in marine environments. *PloS one*, 5(11), p.e13991.

Steward, G.F. et al., 2012. Are we missing half of the viruses in the ocean? *The ISME Journal*, 7(3), pp.672–679.

Suttle, C., 1992. Inhibition of photosynthesis in phytoplankton by the submicron size fraction concentrated from seawater. *Marine Ecology Progress Series*, 87(JANUARY 1992), pp.105–112. A

Suttle, C. a, 1994. The significance of viruses to mortality in aquatic microbial communities. *Microbial ecology*, 28(2), pp.237–43.

Suttle, C. a., Chan, A.M. & Cottrell, M.T., 1990. Infection of phytoplankton by viruses and reduction of primary productivity. *Nature*, 347(6292), pp.467–469.

Tai, V. et al., 2003. Characterization of HaRNAV, a single-stranded RNA virus causing lysis of *Heterosigma akashiwo* (*Raphidophyceae*). *Journal of Phycology*, 39(2), pp.343–352.

Tamura, K. et al., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30(12), pp.2725–9.

Tang, S.-L. et al., 2002. HF2: a double-stranded DNA tailed haloarchaeal virus with a mosaic genome. *Molecular Microbiology*, 44(1), pp.283–296.

Tang, S.-L., Nuttall, S. & Dyall-Smith, M., 2004. Haloviruses HF1 and HF2: Evidence for a Recent and Large Recombination Event. *Journal of Bacteriology*, 186(9), pp.2810–2817.

Tarutani, K. et al., 2001. Isolation of a virus infecting the novel shellfish-killing dinoflagellate *Heterocapsa circularisquama*. *Aquatic Microbial Ecology*, 23, pp.103–111.

Tarutani, K., Nagasaki, K. & Yamaguchi, M., 2000. Viral Impacts on Total Abundance and Clonal Composition of the Harmful Bloom-Forming Phytoplankton *Heterosigma akashiwo*. *Applied and Environmental Microbiology*, 66(11), pp.4916–4920.

Thingstad, T.F., 2000. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnology and Oceanography*, 45(6), pp.1320–1328.

Thompson, J.D., Higgins, D.G. & Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), pp.4673–4680.

Tidona, C. et al., 1998. Is the major capsid protein of iridoviruses a suitable target for the study of viral evolution ? Is the Major Capsid Protein of Iridoviruses a Suitable Target for the Study of. *Virus genes*, 16(1), pp.59–66.

Tomaru, Y. et al., 2004. Isolation and characterization of two distinct types of HcRNAV , a single-stranded RNA virus infecting the bivalve-killing microalga *Heterocapsa circularisquama*. *Aquatic Microbial Ecology*, 34, pp.207–218.

Trainer, V.L. et al., 2010. The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. *Progress in Oceanography*, 85(1-2), pp.33–52.

Untergrasser, A. et al., 2012. Primer3 - new capabilities and interfaces. *Nucleic Acids Research*, 40(15), p.e115.

Ventosa, A. et al., 2015. Microbial diversity of hypersaline environments: a metagenomic approach. *Current Opinion in Microbiology*, 25, pp.80–87.

Verity, P.G. & Smetacek, V., 1996. Organism life cycles , predation , and the structure of marine pelagic ecosystems. *Marine Ecology Progress Series*, 130, pp.277–293.

Vinogradova, O. & Darienko, T., 2008. Terrestrial algae of hypersaline environments of the Central Syvash islands (Kherson Region, Ukraine). *Biologia*, 63(6), pp.813–823.

Wilhelm, S.W. & Suttle, C.A., 1999. Viruses and Nutrient Cycles in the Sea. *BioScience*, 49(10), pp.781–788.

Williams, T., Barbosa-Solomieu, V. & Chinchar, V.G., 2005. A decade of advances in iridovirus research. *Advances in Virus Research*, 65(05), pp.173–248.

Wilson, W.H., Van Etten, J.L. & Allen, M., 2009. The *Phycodnaviridae* : The Story of How Tiny Giants Rule the World. *Current Topics in Microbiology and Immunology*, 328, pp.1–42.

Wommack, K.E. & Colwell, R.R., 2000. Virioplankton : Viruses in Aquatic Ecosystems Virioplankton : Viruses in Aquatic Ecosystems. *Microbiology and Molecular Biology Reviews*, 64(1), pp.69–114.

Xiao, C. & Rossman, M.G., 2011. Structures of giant icosahedral eukaryotic dsDNA viruses. *Current Opinion in Virology*, 1(2), pp.101–109.

Xue, J.-L. & Cheng, X.-W., 2011. Comparative analysis of a highly variable region within the genomes of *Spodoptera frugiperda* ascovirus 1d (SfAV-1d) and SfAV-1a. *The Journal of General Virology*, 92(Pt 12), pp.2797–802.

Yutin, N. & Koonin, E. V, 2012. Hidden evolutionary complexity of Nucleo-Cytoplasmic Large DNA viruses of eukaryotes. *Virology journal*, 9, p.161.