

Investigating phytoplankton fluctuations and drum filter effectiveness on an abalone farm in Hermanus, South Africa.

By

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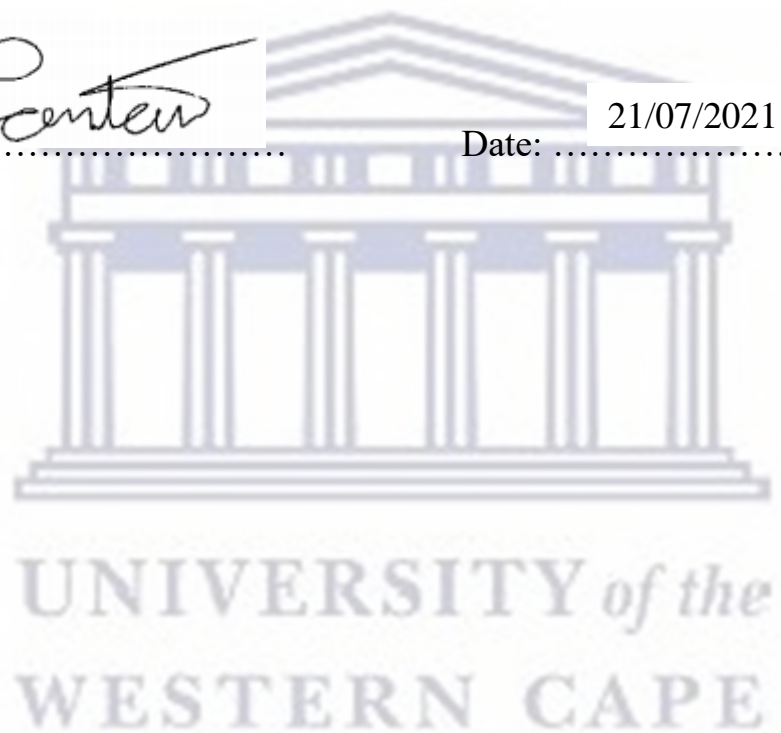
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Declaration

By submitting this thesis/dissertation electronically I declare that the entirety of the work contained therein is my own, original work, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

Aquaculture is a growing industry in South Africa, with the production of abalone, *Haliotis midae*, at the forefront. The Western Cape Province hosts 12 of the 18 abalone farms in South Africa, with a concentration of farms in the Walker Bay region of Hermanus and Gansbaai. Walker Bay is situated on the western side of the Agulhas bank, which allows warm water from the Agulhas current, as well as cold water from the Benguela current to mix. This area experiences seasonal upwelling pulses in summer, which provide the environment with a high nutrient load. This encourages the prevalence of harmful algal blooms (HAB) that can consist of toxin-producing dinoflagellate species. These species have the ability to kill organisms in the nearshore. This poses a problem for aquaculture farms situated in the area, where HAB events have caused the death of millions of abalone and has decreased productivity of farms in previous years. Farms therefore need to implement stringent phytoplankton monitoring schedules, as well as develop better filtering methods to reduce the density of phytoplankton that may flow into the farm.

This study aimed to understand the phytoplankton community assemblages that may be pumped into an abalone farm (Abagold Ltd) over a 16 month period. This was achieved by investigating how phytoplankton community metrics such as abundance, species diversity, richness and evenness fluctuated over a 16 month time period. The frequency of HABs were investigated, comparing the peaks of blooms and how they differed between seasons and the subsequent impact on monitoring activities by the farm until the bloom passes. Secondly, a study was done to determine the efficiency of drum filters to reduce the density of phytoplankton cells from the water that is sourced from the ocean and pumped through the farm.

Phytoplankton community assemblages were sampled and identified to genus level, and species level when possible, once a day for 16 months, from September 2018 to December 2019. As the risk of potential HAB formation rises, the number of sample collections increased to assist in the decision making process of the operational manager of the abalone farm to mitigate negative impacts originating from HAB events. The species richness, Shannon-Weiner diversity index and Pielou's index of evenness were calculated. The number of phytoplankton samples collected each day were tallied to understand the change in monitoring frequency with regard to HAB abundance. Phytoplankton community samples were collected before and after a 15 µm drum filter during bloom events, after which the densities were then compared.

The peak mean monthly cell density occurred in late early autumn of 2019 (March: $721\,179 \pm 226\,473$ cells/l). During this time, the diversity (Shannon-Weiner Index) of species was lower than that of mid spring and this trend is supported by literature, where a decrease in diversity occurs with an increase in HAB density. The relative abundance of species was calculated to quantify the dominant species present

over the study period. The dinoflagellate species *Lingulodinium polyedra* had the highest relative abundance, in March 2019, compared to the highest diatom relative abundance occurring in October 2018, by *Chaetoceros sp.* This is in line with the literature that states that diatoms dominate in spring. Drumfilters, placed at the beginning of farm, to remove suspended solids and material, provided a significant reduction in phytoplankton densities over the 36 day period ($p < 0.001$). The drumfilters were also able to significantly reduce *L. polyedra* densities.

HABs are a natural phenomenon that can play havoc on nearshore activities, both naturally occurring and human-related. Abalone farms in Walker Bay have faced devastating losses due to HABs, and therefore the development of better HAB management protocols are essential to ensuring production goals can be attained. The use of satellite imagery has been developed in the region, such as the National Oceans and Coastal Information Management System (OCIMS) decision-making tool, which remote-senses chlorophyll levels along the coast of South Africa. Individual farms can also make use of recirculation systems to 'recycle' water through the system, thus reducing the quantity of new water needed from the ocean during bloom periods. Recirculating water, along with multiple drumfilter lines, may provide further reductions in phytoplankton densities entering the farm water network.

This study therefore highlights the seasonal peaks of HABs during late summer and early autumn providing a temporal scale of increased risk to the aquaculture farms in the region. Furthermore, this study highlighted the effectiveness of drum filter systems in significantly reducing high phytoplankton loads during HAB events, suggesting that these filters in conjunction with other techniques could increase the ability of Abagold to provide safe, reduced densities of HAB species to its cultured animals.

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Chapter 1 : General Introduction and Literature review

The global aquaculture production in 2018 equated to approximately 114.5 million tonnes, with a market value of USD 263.6 billion (FAO, 2020). China contributed 57.93 % to of the total aquaculture production worldwide, with India in second place contributing a mere 8.61 %. Collectively, the Asian continent produced 88 % of the global aquaculture output in 2018 (FAO, 2020).

The ability of China to top the aquaculture sector globally may be attributed to how they have embraced the use of polyculture, where different taxa that function at different functional groups are cultivated together, which leads to more efficient food production (Farguhar et al., 2017). China also has a long coastline, providing a vast area for land-based cultivation of marine species to occur. Significant investment has been made into 2,000 research centres to allow for research to aid in the development of optimal production methods in the country. There exists more than 5 million jobs in the aquaculture sector in China. Other regions that contribute to global aquaculture production outside of Asia include Norway and Chile that contribute approximately 1.3 MT and 1.2 MT in 2018 towards the global aquaculture production respectively (FAO, 2020). These countries have invested in research, and developing technical training centres to achieve targets, designed by aquaculture specific policies and best practices. Within Africa, Egypt leads the charge in terms of overall aquaculture production. More than 580,000 jobs exists within the aquaculture sector in Egypt, and the industry is estimated at USD 2 billion for shellfish and cultured fish in 2014 (Shalan et al., 2018).

The promising developments within the aquaculture world, is accompanied by negative aspects, including the introduction of alien/invasive species, disease and water quality associated risks. Many regions import food species that do not naturally occur there and despite intensive measures being put in place to prevent any stock loss, individuals that manage to escape from containment into the natural environment can pose an invasive issue (Padua et al., 2017). In some cases, unexpected organisms accompany the target species when imported as parasites. For example, the fan worm, *Sabella spallanzanii* (Gmelin), accompanied live bait originating from Europe and settled in Western Australia, outcompeting many of the local species for food (Lee and Gordon, 2006). Not only do the direct fish or shellfish species that are used in farming pose a risk to local aquatic environments, but other organisms used in feed process, such as microalgae, may also pose a risk (Zhu and Ketola, 2012). With regard to a socio-economic issue, aquaculture can be the cause of a widespread loss in income for small-scale fishers and other local people, when nearshore areas have been converted into single-use resources reserved just for aquaculture (Primavera, 2006).

Aquaculture in South Africa

The first documented occurrence of aquaculture in South Africa dates back to 1673, when European settlers noticed collections of oysters being cultivated in the Western Cape by the native inhabitants they came into contact with (Haupt, 2009). It was only after 1948 that the first commercial production of oysters began in Knysna along the Southern Cape coastline (FAO, 2013), while abalone farming developed during the 1990's, after natural populations suffered severe losses through poaching and overexploitation (Cook, 2016).

Freshwater aquaculture in South Africa includes the cultivation of both brown and rainbow trout, crocodiles, Nile and Mozambique Tilapia and common carp, among others (FAO, 2013). In 2015, 152 farms cultivated freshwater species in South Africa, with the most occurring in Mpumalanga and Gauteng (DAFF, 2017). Freshwater aquaculture occurs in every province in South Africa, while marine aquaculture is obviously present in only the coastal provinces that have access to seawater, such as Northern Cape, Western Cape, Eastern Cape, and KwaZulu-Natal (DAFF, 2017). There are only 37 marine aquaculture farms in South Africa, however these farms produce 66 % of the country's aquaculture output (3 591.86 tonnes, 2015). The top producing marine crops are abalone (*Haliotis midae* (Linnaeus)) (1 479.22 tons), mussels (1 758.47 tons) and oysters (276.85 tons), with finfish contributing only 77.32 tons in 2015 (DAFF, 2017).

The coastal town of Saldanha Bay along the west coast of South Africa, supports the only production of mussel species such as *Mytilus galloprovincialis* (Lamarck), *Choromytilus meridionalis* (F. Krauss) and *Perna perna* (Linnaeus), where the sheltered bay provides protection from the large winter swells (Olivier et al., 2013; Probyn et al., 2015). Seaweed genera such as *Gracilaria* and *Ulva* require relatively sheltered conditions such as those found in Saldanha Bay, and are used within abalone aquaculture operations for animal waste removal and feed (Troell et al., 2006).

Although South Africa possesses a great diversity of marine species for aquaculture production, the sector is severely underdeveloped. Countries with similar size coastlines, such as Egypt and Thailand, produce almost 1,000 times more than South Africa (DEA, 2014). This has triggered the South African government to create "Operation Phakisa" under which the "Oceans Economy" falls, as a means of developing and building the aquaculture sector within the country. Four main enabling factors have been highlighted as quick wins to promote this development within the country (DEA, 2014). These include (1) Reforming legislation to promote aquaculture development, (2) Establishing a fund solely for aquaculture development, (3) Developing training centres and skills development to a greater number of individuals, and (4) Market-wide coordination, both locally and internationally (DEA, 2014). The South African coastline, although characterised by a high wave energy climate, has the ability to increase its aquaculture production. A flagship species for the development within the country is

Haliotis midae, known locally as perlemoen or abalone. The abalone aquaculture industry in South Africa started in the early 1990's, after an extreme decline in wild abalone populations due to overfishing and severe poaching (Minnaar et al., 2018). This sharp decline and subsequent closure of commercial abalone harvesting provided a platform for abalone aquaculture, supported by the high demand of South Africa's largest abalone species, *Haliotis midae*, by Asian countries (Britz, 1995). Initial research and development in abalone aquaculture focused on creating a thorough understanding of the life cycle and specific physical and environmental requirements through the various life stages of abalone, especially the reproductive outputs and survival of the juveniles, called spat. Understanding the nutritional requirements for the various life stages were also important to ensure maximum survival, growth and ultimate yield, and even today feed formulation is a vital aspect of R&D for aquaculture farms.

Abalone make use of broadcast spawning into the environment, where the development of a trochophore occurs after cell cleavage of an abalone zygote (Clint, 2010). The trochophore stage will last until it hatches from an egg, thus moving into the larval stage. The larvae are lecithotrophic and will therefore not feed on anything at this point in time, as no functional mouth is present. Settlement will generally occur at five days when an adequate benthic microalgal biofilm of predominantly diatoms is located (Visser-Roux, 2011). Once settled, the abalone spat will remain benthic for the remainder of the life cycle.

Cultivated abalone will be provided with a multitude of different feeds throughout its production lifetime, from settlement to a market size of 100 grams after approximately 5 years (DAFF, 2018). During the settlement phase, larvae will feed on predominantly diatoms, such as *Cocconeis*, *Amphora* and *Achnanthes*, until they reach a size of approximately 10 mm (Matthews and Cook, 1995; Nel et al., 2018). Thereafter the spat are weaned onto a mixed diet of *Ulva* and *Gracilaria*, as well as artificial feed (Bansemmer et al., 2016; Britz and Venter, 2016). The composition of the artificial feed will then vary, based on their metabolic requirements, until market size is reached (Nel et al., 2017). The inclusion of the kelp species *Ecklonia maxima* (Osbeck) Papenfuss along with a formulated feed has shown faster growth rates for *Haliotis midae*, where shell growth was better for animals with a shell length greater than 50 mm (Troell et al., 2006).

How is aquaculture supported in South Africa?

The coastline of South Africa is dominated by various environmental factors and is driven by the interplay of the warm Agulhas current along the east coast flowing towards the south and the cold Benguela current flowing along the west coast northwards (Tim et al., 2019), as seen in Figure 1.1. The west coast of South Africa is considered an eastern boundary upwelling region, where upwelling occurs mainly during summer months, when south easterly winds blow along the coast (Trainer et al., 2010).

Water temperatures can therefore drop to below 10°C (Jury, 2015). This variation in temperature influences different bioregions around the coast, directly affecting the diversity of organism that propagate in the marine environment (Smit et al., 2017), and consequently any aquaculture efforts around the coast.

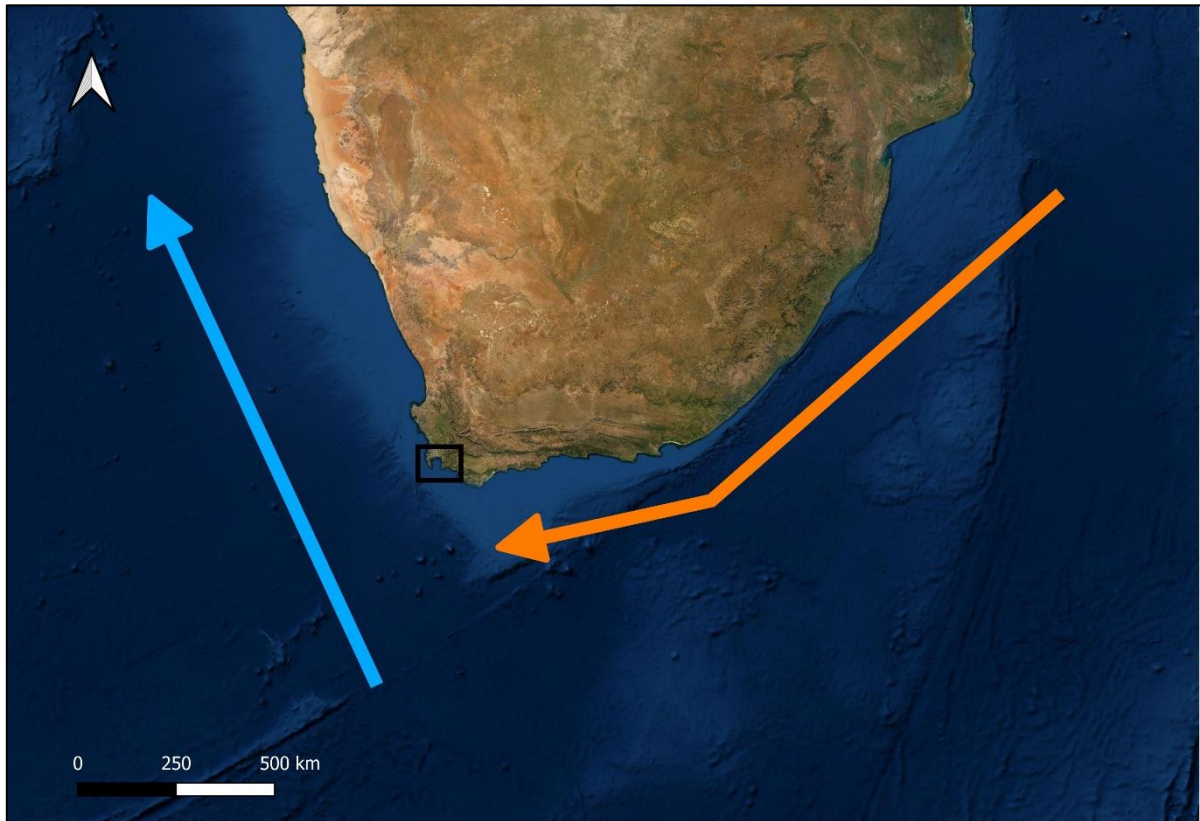


Figure 1.1 Regional map indicating the Benguela Current (blue arrow) and Agulhas Current (orange arrow) as well as a black rectangle demarcating the study area. Image acquired from Google Maps.

Majority of the farms that cultivate *Haliotis midae* are found along the south-western and west coast regions of the country, where the optimal water temperatures for abalone i.e. 12-20° C (DAFF, 2018) can be found (Smit et al., 2017). Moving towards the Eastern Cape, average water temperatures increases due to the warm Mozambique (Agulhas) current and approaches 25° C in many cases (FAO, 2013). Other aquaculture species, such as mussels, require sheltered environments, away from high wave energy sources (Haupt, 2009). Therefore mussel species such as *Mytilus galloprovincialis* are extensively grown in Saldanha Bay, with locations such as St Helena Bay and Algoa Bay also being viable options (Olivier et al., 2013; Probyn et al., 2015). Similar sheltered requirements also persist for *Gracilaria verrucosa*, a seaweed species grown along the West Coast and East Coast of South Africa (Troell et al., 2006).

The negative impact of the environment on aquaculture

The South African coastline provides an excellent platform for aquaculture to expand, and has been earmarked as an important catalyst to develop coastal communities (DEA, 2014; FAO, 2013). Optimal temperatures persist for various aquacultures species, particularly along the west coast. This region, however, is prone to seasonal temperature changes, triggered by upwelling events (Lucas et al., 2014). Upwelling events provide the nearshore with cold, nutrient-rich water that has been extracted up from the depths, as surface water is forced away from the coast by surface winds (Largier, 2020). Upwelled water still falls within the optimal temperatures range of species cultured along the coast, and therefore the temperature does not have a negative effect on facilities. These water parcels, however, provide a high nutrient load to the nearshore environment, inducing high phytoplankton productivity (Kudela et al., 2005). A high diversity of phytoplankton is found around the coast of South Africa, and during upwelling events, take advantage of the highly productive conditions. This forms part of the basis for the fisheries system that is located along the Agulhas Bank and west coast region of southern Africa (Lamont et al., 2019). However, these seasonal plankton fluctuations are not completely positive, as various harmful algal species are found within these planktonic communities (Kudela et al., 2005).

Certain phytoplankton species form Harmful Algal Blooms (HABs) when triggered by upwelling. These are harmful either through the production of biotoxins or the depletion of oxygen due to excessive bacterial decomposition (Nwankwegu et al., 2019). HABs are often dominated by dinoflagellate taxa (*Dinophysis*, *Lingulodinium*, *Prorocentrum*) as well as some diatoms (*Pseudo-nitzschia* spp.) (Rankin, 2011). Some of these species have cosmopolitan distributions across the globe, but predominate in upwelling areas (Smayda, 2010). Trainer et al. (2010) list a collection of these species found along the four main eastern boundary upwelling systems across the globe. The four systems include the California Current, the Canary Current, the Humboldt Current extending from central Chile, and the Benguela Current that this study will focus on.

Harmful algal bloom species can have devastating impacts on an ecosystem and can be a major concern to aquaculture facilities (Silva et al., 2016). Yessotoxin, found in dinoflagellates such as some *Gonyaulax* species, can kill abalone (Pitcher et al., 2019). Anoxia and hypoxia in the local environment can also be triggered during a high-biomass bloom as a consequence of high bacterial decomposition (Sellner and Rensel, 2018). The effect that HABs have on aquaculture species can cripple facilities, and cause devastation to the sector. In the late 1980s, a bloom of the dinoflagellate, *Karenia cristata* (L.Botes, S.D.Sym & G.C.Pitcher) killed approximately 40 tons of wild abalone along the southwestern coast of South Africa (Pitcher et al., 2019).

More recently, during the summer of 2017, a bloom of dinoflagellate species (*Lingulodinium polyedra* (Stein), *Gonyaulax polygramma* (Stein) and *Ceratium* spp. impacted three land-based abalone farms

situated in Walker Bay, Hermanus, South Africa (Pitcher et al., 2019). Reports suggest that several million abalone, in excess of 250 tons, died as a result of the algal bloom, providing an example at how devastating these HABs can be to a sector that is still developing (Pitcher et al., 2019). This event not only affected land-based aquaculture facilities, but had a negative impact on the natural environment with a large number of dead or dying sea urchins, sea snails and limpets observed on the nearby beach of Sandbaai, in the same bay (Pitcher et al., 2019).

The health effects caused by HABs on the abalone vary. To understand the effects that toxins had on abalone, necropsies and histological investigations were performed by abalone farms in Walker Bay, South Africa (Pitcher et al., 2019). Yessotoxins were found to be retained in abalone for several months after the algal bloom event that took place in the January of 2017, particularly concentrated in the gill tissues. Although the yessotoxin concentrations were not lethal for humans, their concentrations resulted in paralysis of the muscular foot of the abalone. Gill damage was also present in the abalone, which resulted in suffocation. These necropsies and histological data collections are commonly performed during HAB events and form part of a longstanding monitoring programme developed for bivalve related aquaculture in the region and required by legislation.

Management in response to HAB occurrence

Management of aquaculture facilities is crucial during algal bloom events, as many facets of farm production are affected. As farms develop into large-scale operations, the ability to proactively manage unprecedented events such as algal HABs become invaluable in ensuring farming continuation (Anderson, 2010). This is through the avoidance of losing large amounts of cultured crops when farms have either not had sufficient time to prepare for HABs, or develop contingency plans when they do occur, with a possible increase in occurrences expected to occur with climate change (van der Lingen et al., 2016). Various methods have been developed to minimise the effect that HABs and algal blooms in general have on the nearshore environment, focusing specifically on mariculture and land-based aquaculture facilities. In a spatial planning context, locations for new farms are being earmarked based on the prevalence of HABs in that area. Therefore stakeholders are using new and improved forecasting and historical data to allocate aquaculture farming regions that preferably do not coincide with HAB hotspots (Huber et al., 2016; Snyder et al., 2017).

Another mitigating factor to reduce the occurrence of HABs is the reduction of the nutrient load in the effluent being discharged from aquaculture and other waste water facilities. The effluent from a shrimp farm is made up of ammonium, total phosphorus, total nitrogen and chlorophyll-*a* and was higher than the farm's influent measurements for each listed (Costanzo et al., 2004). Preventing niche conditions for algal blooms to develop has become an important factor in coastal environments, where nutrient sources are not only from aquaculture facilities, but from other anthropogenic sources, such as waste

water treatments and other agricultural derivatives (Sathish Kumar et al., 2020). This, however, remains difficult to attain, as it is commonly thought that there exists high assimilation in the ocean, and high nutrient releases will simply dilute into the vast ocean, through much ignorance (Sellner and Rensel, 2018). Another step to deal with nutrient release is through the introduction of polyculture and multi-trophic integrated aquaculture (Andersen, 2005). This involves the merging of various culture species from different trophic levels, where the waste of one species provides nutrients and feed for another species. This is regularly seen in shellfish farms, where the waste flow from shellfish are redirected into seaweed culture tanks to propagate growth (Largo et al., 2016). In many cases, nutrient run-off cannot be avoided and farms have already been developed in algal bloom hotspots. In this case, mitigation methods may become the primary approach.

The ability to predict the development of an algal bloom allows management personnel to prepare for mitigation and control. Research into algal bloom forecasting has moved to the forefront for regions where coastal activities and algal bloom hotspots occur. This underpins the goal of the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB), an international, interdisciplinary venture that aims to understand the ecological and oceanographic factors that influence HAB occurrence (Glibert et al., 2018). The mechanism relies on global cooperation from regions that experience common features, i.e. upwelling areas, to share research on key species connected to harmful algal blooms (Gilbert and Pitcher, 2001). Similar to this has been the development of OCIMS, the National Oceans and Coastal Information System developed by South Africa's Council for Scientific and Industrial Research (CSIR). This is described as a decision making tool for various sectors, using advanced satellite and remote sensing technology and modelling. The decision tool provides support for various types of scenarios, from vessel tracking, flood hazards along the coastal environment, and more specifically, harmful algal blooms (Byrne et al., 2018). By integrating bloom dynamics, the impacts of hypoxia events, and the capability to monitor earth observations, colour-graded maps representing phytoplankton biomass can be developed. This extends to 50 km offshore, and provides a monitoring mechanisms for the possibility of HABs appearing (<https://www.ocims.gov.za/fisheries-and-aquaculture-tool>).

The ability to forecast when HABs and blooms in general will occur can enable facilities to prepare for the incoming threat, however once the threat arrives, mitigating actions need to be performed. To physically deal with algal blooms, a number of different methods have been used. Skimming water surfaces of algal blooms allows personnel to collect any biomass that floats on the water surface (Paerl et al., 2016). This can be useful in certain instances, however, this method is not feasible for algal blooms that cover kilometres of the coastal nearshore, or biomass occurring on a subsurface level. Therefore this method is only used for small ponds where cyanobacteria dominated harmful blooms occur (Sellner and Rensel, 2018). Flocculation is a well-known method to mitigate the development of some blooms by introducing clay into the environment (Kang et al., 2007). The clay particles clump

together with cells and become heavier, forcing the cells to sink down, or 'sweep' as they descend and then drag cells down with them (Sengco et al., 2001). Filtration involves the use of filter systems to remove particles in the water column, such as rotating screens (Anderson et al., 2001). These systems can however clog up quickly, requiring constant maintenance to prevent a reduction in flow rate. Another method includes the destruction of HAB cells by sonication. Ultrasonic waves are exposed to cells that are consequently destroyed in as quickly as two minutes (Nwankwegu et al., 2019). This method has shown to work well on genera such as *Heterosigma* and *Prorocentrum*, but only to depths of 50 cm, as found by Anderson et al. (2001). As this is not feasible for open ocean operations, conducting this method for land-based facilities may be applicable. In many cases however, more than one million litres of water can be pumped into a land-based facility, per hour, and therefore this method would not be feasible. In some cases HABs are not dominated by toxin producing phytoplankton, but rather species that just discolour the water or produce bad odours when present (Anderson et al., 2001). It is therefore important to understand the HAB assemblages present and take the necessary action based on the risk factors involved.

Impacts of aquaculture on society and the environment

Aquaculture development has the ability to improve the livelihoods of many communities situated along coastal environments, and a mechanism such as Operation Phakisa, in South Africa, aims to uplift coastal communities that have conventionally only relied on subsistence fishing for their livelihoods (DEA, 2014). Aquaculture development can however have negative effects on the environment. Habitat loss due to farm development has been a great issue in the past, such as the loss of mangrove forests due to shrimp pond introduction in countries such as India (Jayanthi et al., 2018). Another problem develops when foreign cultured stock escape aquaculture facilities into the ocean, thus creating an issue for alien species introduction, as well as the possible disease spread (De Silva et al., 2009). One study found that 55 % of salmon escapees from fish farms in Norway contributed 19 % genetic material to adult fish, just one generation later (Naylor et al., 2005). This type of interbreeding has the ability to encourage hybridisation and genetic homogenisation.

Another issue which has been widely studied as the aquaculture sector has grown in various countries, is the influence that nutrient run-off from facilities has on the natural environment (Anderson et al., 2002). Dissolved organic and inorganic matter are discharged from farms either through flow-through systems or during cleaning (i.e. husbandry practices). This release creates an environment where eutrophication can quickly develop, as certain nutrient-limited organisms are able to form algal blooms.

Operating an aquaculture facility

Aquaculture facilities must meet strict environmental criteria, animal health standards, food hygiene and safety standards, and sustained production yields. Consequently, operating a facility is therefore not easy, and a great deal of effort and money is commissioned towards the smooth running of any farm. Aquaculture facilities vary in size and shape depending on the target production species, and therefore require different capital inputs and operational requirements. A study by the Department of Agriculture, Forestry and Fisheries, South Africa, compared the varying costs and requirement of different facilities. South Africa stands as one of the top producers of abalone, and many facilities make use of flow-through systems on farms in the Overstrand region (Mau and Jha, 2018). The study found that for approximately R150 million, a flow-through abalone farm could be developed, including the purchase of land and infrastructure and equipment (DAFF, 2018). This farm would employ 82 workers, and sales would only occur after 45 months of growing (DAFF, 2018).

Abalone farms employ different combination of methods and feeds during the various stages of production. Overall however, abalone require a diet of microalgae during the settlement phase (i.e. post veliger larval stage), followed by either a mixed diet of macroalgae and formulated feeds or only formulated feeds (Bansemer et al., 2016). On many farms that incorporate hatcheries, diatoms are used as the main food source for juvenile abalone during the settlement and first 3 to 4 months of their lives (Chen, 2007). Consequently, diatom cultures are produced on-site to inoculate plates in preparation for larval settlement during the production phase. Monospecific cultures of diatoms from the *Cocconeis* and *Navicula* genus are regularly imported from registered laboratory suppliers to seed culture stocks every 3 to 6 months, usually at exorbitant prices (Correa-Reyes et al., 2001). Diatoms, however, are in abundance across the marine environment, and therefore easy to collect in the same environment where abalone grow natively. Studies have also shown that abalone are selective in their diatom feeding choice (Kawamura et al., 1995). It would therefore be in the facility's best interest to provide an abalone juvenile with biofilm that best mimics the species composition they would encounter in their natural habitat. Recent studies have also shown that bacteria found within microalgal films promote growth in post-larval abalone (Moxley and Coyne, 2020). This opens the door into understanding the fluctuations of microalgae that neighbour ocean-side abalone facilities, and how these fluctuations can, firstly, allow forecasting into possible HAB occurrences, and secondly, allow the possible utilisation of local diatom species for juvenile abalone feed, if plates can be placed to encourage the settling of the benthic diatom species.

Hermanus as a feasible abalone farming location

The Overstrand region of South Africa is situated in the south western part of the country. This is where a mixture of the two main oceans, the Atlantic (i.e. cold Benguela current) and Indian Ocean (i.e. warm

Agulhas or Mozambique current), play an important role in nutrient delivery and circulation near the southern point of the Africa continent (Jury, 2015), as seen in Figure 1.1. The Benguela current flows from the South Pole northwards along the west coast of South Africa, and injects nutrient-rich cold water into the region (Puccinelli et al., 2016). This water is regularly upwelled to the nearshore, driven by strong south-easterly winds, providing nutrients and easily triggering phytoplankton blooms in the nearshore (Lucas et al., 2014). The Agulhas current flows from the equator southwards along the eastern side of the country, and consists of warm water originating from the Western Indian Ocean (Barlow et al., 2020). This strong, narrow current consistently infiltrates the southern coastal region of South Africa, including the Overstrand region, in between Cape Point and Cape Agulhas (Boyd et al., 1985). Water stratification is strongest during upwelling events, which occur frequently during the austral summer period (i.e. November to March) (Lutjeharms and Stockton, 1991; Puccinelli et al., 2016).

The southern coastal region (i.e. Overstrand) is the epicentre for abalone aquaculture in South Africa, with several farms in close proximity of Hermanus and Gansbaai producing substantial tonnage per year for the export market to Asia (Abagold Ltd, 2019), as seen in Figure 1.2. When abalone have matured and moved from a diatom based diet, kelp and seaweed becomes the primary feed for the shellfish, supported by artificial feed (Nel et al., 2018, 2017; Troell et al., 2006). This is supported by the location of all of the abalone farms in the Western Cape, which are situated in areas with high kelp availability (Rothman et al., 2017) and optimal sea temperature for abalone (DAFF, 2018). The choice kelp species for abalone feed is *Ecklonia maxima*, which is distributed from De Hoop, west and north along the coast, and up into the high reaches of Namibia (Bolton et al., 2012). The abalone are fed freshly harvested kelp, and therefore a steady supply of the macroalgae is important. While *Ecklonia maxima* dominate the kelp biomass around the Cape Peninsula, as one moves up the west coast, north of Cape Columbine, *Laminaria pallida* (Greville) overtakes *Ecklonia maxima* as the dominant kelp species. The harvesting of *Ecklonia maxima* further up the west coast becomes more difficult as surface kelp collection is disrupted by the increased presence of *Laminaria pallida* in this region, combined with other environmental factors such as increased turbidity (Rothman et al., 2017). The west coast still provides a high biomass of harvestable kelp, but the region is also under the influence of diamond mining, where vast areas are restricted to access. The Cape Peninsula and Overstrand region provide a high biomass of *Ecklonia maxima*, and is therefore a profitable area for kelp harvesting. The Cape Peninsula however encounters higher prices for land, and is in close proximity to Table Mountain National Park (Troell et al., 2006) making it a difficult area to develop abalone farms in. This factor provides evidence as to why the Overstrand has become a favourable area for abalone aquaculture. This is supported by an average ambient temperature of approximate 14° C, with maximum temperatures reaching well into the optimal growth range for *Haliotis midae* of 12-20° C (Isaac, 1937; Muller, 2003; Nel et al., 2018).

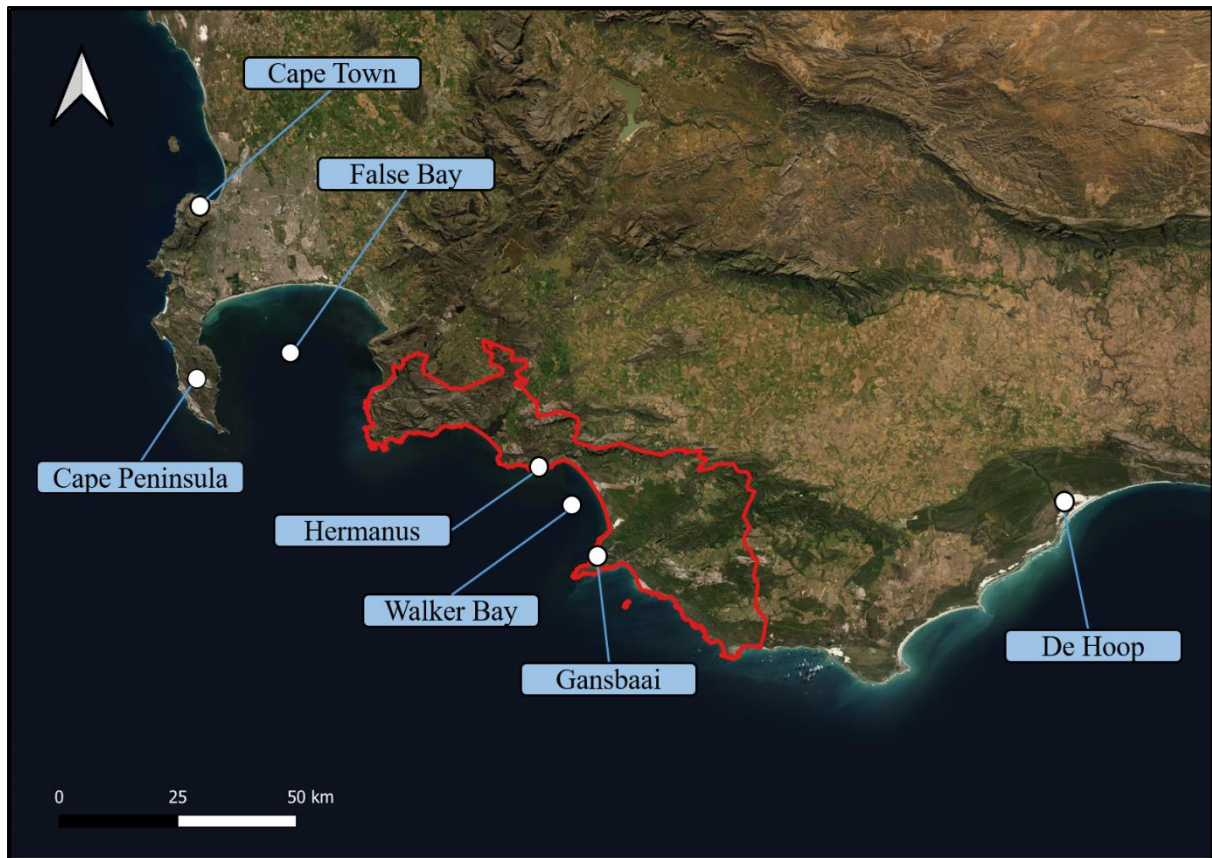


Figure 1.2 A map depicting the position of Cape Town and False Bay in relation to Walker Bay, with the aquaculture-rich towns of Hermanus and Gansbaai included. The red polygon represents the Overstrand region. De Hoop, the eastern extent of *Ecklonia maxima* distribution, is included. Image acquired from ESRI Satellite.

Phytoplankton

Functional groups

Within the aquatic environment, phytoplankton represent a diverse range of organisms, varying in both form and function (Kruk et al., 2010; Margalef, 1978). Because phytoplankton incorporates an immense array of organisms, functional groups have been developed to classify similar organisms together, and thereafter better understand how these groups interact with their environment (Wyatt, 2014). Various classifications exist to class phytoplankton in varying functional groups. On a broad scale, and like other organisms, phytoplankton can be grouped by kingdom down to species using taxonomic classifications (Wang et al., 2020). Within these groupings, life-forms also exist, and help to differentiate between groups. This is apparent for diatoms, where centric diatoms, which are usually free-floating in the water column, are circular in their shape, as opposed to pennate life-forms that are more benthic in nature and possess a more elongated shape (Taylor et al., 2007). Dinoflagellates also

possess different life forms, and in Smayda and Reynolds (2003) we see nine dinoflagellate life-forms existing based on the type of habitat characteristics present.

Another broad classification system uses the size of the taxon. Picoplankton incorporate sizes $<2\ \mu\text{m}$, and include organisms such as cyanobacteria and other bacteria (Aiken et al., 2008). Nanoplankton fall within the 2-20 μm size range, and incorporate organisms such as flagellates, including chrysophytes, cryptophytes and chlorophytes, as well as prymnesiophytes (Nair et al., 2008). The microplankton size range is made up of organisms between 20-200 μm and include diatoms and dinoflagellates (Brewin et al., 2013; Sieburth et al., 1978). This method of classification is not always accurate, as one can find diatoms within the nanoplankton size range, cryptophytes with a size $<2\ \mu\text{m}$ (Ciotti et al., 2002) and dinoflagellates $<10\ \mu\text{m}$ (Jang et al., 2017; Takahashi and Bienfang, 1983). Size-based classifications may also not be the best as they group together organisms that function differently. Functional groups can also be classified by their evolutionary history (Thomas et al., 2016) or based on their accessory pigments. Hirata et al. (2011) found that a relationship exists between chlorophyll-*a* and the pigment of phytoplankton groups. This provided evidence that the chlorophyll-*a* of the total community can be used as an index for community structure, and not only to represent phytoplankton biomass.

Environmental drivers of phytoplankton growth

Environmental conditions play an important role in the growth of phytoplankton densities. On a broad scale variables such as light, temperature, nutrients and pH influence the development of phytoplankton (Burger et al., 2020; Hansen, 2002), and their influences are outlined below. Therefore the change in these variables, often driven by seasonal changes, directly influences the change in phytoplankton community assemblages (Becker et al., 2010).

Temperature and light

Thermally stratified water in coastal environments is characterised by a top nutrient-limited layer that sits above a light-limited layer. Under these stratified conditions dinoflagellates, with their affinity to be abundant in lower nutrient environments, persist in greater numbers than diatoms (Smayda, 2010). Diatoms can be larger in size and sink into the light-limited environment easier, where they are able to persist as nutrients are higher in the bottom layer. (Mena et al., 2019).

When nutrient limitations and suboptimal temperatures are present, the stress effect is greater than the sum effect of nutrient-limitation and suboptimal temperatures on their own (Rhee and Gotham, 1981). Cell densities were observed to increase when nutrient-sufficient conditions were present in decreasing temperatures. Light also plays an important role in phytoplankton growth. Phytoplankton are photosynthetic in nature and therefore require light (López-Sandoval et al., 2014) and an increase in light has been found to correlate with an increase in phytoplankton growth (Edwards et al., 2016). A

study by Köhler et al. (2017) found that in surfaced mixed layers, phytoplankton could photosynthesis three times quicker under constant light, as opposed to fluctuating light conditions. In these types of mixed layer scenarios, not only the intensity of light, but the distribution through the mixed layer affects growth. This can have negative growth impacts on phytoplankton in scenarios when the mixed layer exceeds the depth that the euphotic zone extends to. Therefore in turbid waters, the light-limitation on phytoplankton growth has been observed (Domingues et al., 2011). This study found that diatoms were light-limited, but cyanobacteria were able to acclimate to low-light level.

Turbid waters near the coast are also often observed to show large temperature fluctuations, particularly in upwelling zones along eastern boundary upwelling systems (Trainer et al., 2010). A study by Strock and Menden-Deuer (2021) provides evidence for the growth rate acclimatisation of phytoplankton in conditions that experience temperature shifts. This acclimatisation is not dependant on the direction that the temperature shifts i.e. heating or cooling, but by the magnitude that the fluctuation occurs. Phytoplankton growth rates increased when the temperature shift magnitude was either small ($\pm 3^{\circ}\text{C}$) or large ($\pm 16^{\circ}\text{C}$), but decreased in growth rates at intermediate magnitudes ($\pm 5^{\circ}\text{C}$ to 13°C).

Nutrients

Phytoplankton growth is largely driven by the availability of phosphate and inorganic nitrate (Rhee and Gotham, 1981). A study by Agius and Jaccarini (1982) in the Mediterranean found that phosphate is the primary limiting nutrient for phytoplankton growth, followed by nitrogen irrespective of seasonal fluctuations. However, in Sakka et al. (1999) it was shown that in environments where the N:P ratio is equal to 16:1 (i.e. similar to the intracellular ratio), phytoplankton was N limited, while at an N to P ratio above 16:1, the phytoplankton become P limiting. Consequently, nitrogen and phosphorous are limiting nutrients for phytoplankton, while other macronutrients and trace metals such as iron and silicon have been shown to co-limit growth as well (Sakka et al., 1999). For example, nanoplankton have been found to be Fe-limited, and cyanobacteria requires Fe when fixing nitrogen (Hutchins et al., 1991; Rueter et al., 1990). On the other hand, silica has also been shown to be an important macronutrient that influences diatom growth. Diatoms possess a silica frustule structure and therefore require silican nutrients for growth and development (Rousseau et al., 2002). When inorganic carbon is taken up during photosynthesis, it has the potential to increase pH in the surrounding water (Hansen, 2002). This can affect growth rates of phytoplankton. When pH levels were increased to 9.4, *Thalassiosira* spp. was only able to reach a growth rate of 10 % of the maximum growth rate (Chen and Durbin, 1994).

Not only can nutrients influence the density of phytoplankton, but has a relationship with the size of phytoplankton cells. A study by Marañón et al. (2013) found that intermediately-sized phytoplankton were responsible for peak maximum growth rates and carbon-specific photosynthesis, while the conversion of nutrients into biomass for larger cells limited their growth.

Upwelling

As mentioned previously, the supply of nutrients to an environment can encourage the growth of phytoplankton. Upwelling events are a common coastal phenomenon that show evidence for providing nutrients for rapid phytoplankton growth (Lucas et al., 2014). During upwelling events along the north western coast of Spain however, high nutrients loads, especially of silica and phosphates, correspond with increased diatom biomass, such as *Skeletonema* and *Thalassiosira* (Tilstone et al., 2000). This is succeeded by diatoms that are slightly larger, such as *Cerataulina*, *Chaetoceros* and *Lauderia*, which have lower productivity rates (Margalef, 1958). An excessive increase in phytoplankton biomass due to eutrophic conditions may lead to colouration of the water and are often called red tides. Red tides or HABs are often characterised by high densities of various phytoplankton organisms, which can have detrimental effects to the environment either via the production of toxic chemicals (i.e. biotoxins) or through the depletion of oxygen in the water column (Smayda, 2000). Oxygen depletion can be a by-product of algal blooms via increase respiration rates at night or microbial decomposition of organic matter, which can create localised anoxic conditions (Seeyave et al., 2009). Therefore, if the utilisation of oxygen by phytoplankton communities is greater than the oxygen production during the day plus the air-sea exchange of oxygen into the surface layer at night, anoxic conditions can occur. This was observed by Pitcher and Probyn (2016) in St Helena Bay, South Africa, where respiration to oxygen production ratios were measured at between 0.60 – 0.73. Prolonged anoxic conditions can form ‘dead zones’ that are the direct cause of mortality for various taxa, as observed by along the Namaqua coastline of South Africa (Ndhlovu et al., 2017). Pitcher and Louw (2020) also categorise anoxic water in the Benguela upwelling system by temporal scales: (1) The northern Benguela’s bottom shelf being depleted of oxygen on a perennial basis; (2) the Namaqua shelf hosting depletion of oxygen on a seasonal basis; and (3) the coastal zone experiencing episodic anoxic conditions with the Benguela. These episodic anoxic conditions are what ‘catches’ abalone operations around the coast off-guard, which occur alongside seasonal bloom fluxes that this study will aim to summarise.

Oxygen depletion is not the only concern with excessive phytoplankton blooms. As mentioned above, some algal species in these blooms possess toxins that can be fatal to many shellfish, fish species, as well as humans (Lewitus et al., 2012). Consequently, harmful algal blooms can severely impact the aquaculture sector. Many land-based aquaculture facilities pump water directly from the ocean as a water supply for the farms and it is nearly impossible to exclude and filter large loads of algal cells when blooms occur from the intake water (Pitcher et al., 2019; Wassnig et al., 2010). Facilities that produce shellfish such as oysters and mussels are the most susceptible to biotoxin accumulation (Braga et al., 2018). Historically abalone aquaculture farms were not severely impacted by HABs until 2017 when the effects of yessotoxin on South Africa abalone was evident through mass mortalities recorded in the Hermanus node (Pitcher et al., 2019).

Role of phytoplankton in the marine environment

Phytoplankton are the main primary producers in the marine environment, and are thought to contribute up to 45 % of the net primary production worldwide (Simon et al., 2009). This positions phytoplankton at the base of the food web, and are subject to a bottom-up control from nutrients (Sinistro, 2010). Phytoplankton are able to convert inorganic nutrients, and therefore play an important autotrophic role within the aquatic environment (Mitbavkar et al., 2012). Through this conversion, consumers are able to feed on these autotrophs, and the flow of energy through the food web is sustained (Moncreiff and Sullivan, 2001). Zooplankton are usually the next stage in the food web that feed off phytoplankton, but in some cases, larger consumers feed directly on the microscopic photosynthetic organisms, such as sardines, *Sardinops sagax* (Jenyns) (van der Lingen et al., 2016). Although severe algal blooms are seen as catastrophic events for many marine species, blooms often provide pelagic fishes with a food source (Pitcher et al., 2008). This is commonly seen with the pelagic fishery on the Agulhas bank, where dinoflagellate species such as *Gonyaulax polygramma* are a good food source for pelagic fish (Pitcher et al., 2008). Filter feeders, such as shellfish, rely almost exclusively on phytoplankton within the water column as food source and actively filter the water to obtain only organic particle possible via their feeding apparatuses (Puccinelli et al., 2016).

In general, diatom communities are classified as centric (i.e. free-floating) or pennate (i.e. attached) communities (Janse van Vuuren et al., 2006). Consequently, it is not only the free-floating communities within the water column that dominates under enriched nutrient conditions, but also attached forms that can increase in biomass with elevated nutrient levels (Van Lujn et al., 1995). A study by Moncreiff and Sullivan (2001) showed the importance of epiphytic algae (i.e. attached) in seagrass meadows as a food source for the surrounding environment, and benthic microalgae are readily eaten by invertebrates, e.g. abalone spat on pennate diatoms (Chen, 2007; Kawamura et al., 1995; Matthews and Cook, 1995; Takami et al., 1997). Being photosynthetic in nature, phytoplankton, and specifically diatoms, produce an immense amount of oxygen, in turn extracting large quantities of carbon dioxide to complete the process (Barranguet et al., 1998; Strzepek and Harrison, 2004).

Phytoplankton functional groups such as diatoms and dinoflagellates have strong economic ties, in both a positive and negative manner. Diatoms have various economic uses, from the development of biofuels from lipids found in the cells, to diatomite which can be used for pest control or filtration material (Merz and Main, 2014; Schuler et al., 1991). The direct use of diatoms has become a game-changer for some aquaculture facilities, particular where juvenile ‘crops’, such as abalone, are fed diatom biofilms (Avendaño-Herrera and Riquelme, 2007). However, dinoflagellate-dominated HAB events can cause fatalities to aquaculture facilities, directly reducing profits (Abagold Ltd, 2019).

There are two aims for this study. Each aim is dealt with in separate chapters. Ultimately this study hopes to understand the fluctuations in phytoplankton abundances and diversity that an abalone farm in Hermanus, South Africa, experienced over a 16 month period, and quantify the effectiveness of drum filters in reducing phytoplankton abundances that are suspended in water that is pumped through the farm.

Aim 1: Monitor the seasonal fluctuations in phytoplankton composition and diversity over 16 months and understand the response of an abalone farm to these fluctuations.

- A) Compare the change in monthly aggregates of phytoplankton abundance, species richness, species evenness and community diversity.
- B) Determine which phytoplankton functional groups dominate during each season.
- C) Determine the dominant taxa and the change in their relative abundance during different seasons.
- D) Quantify the use of farm resources during HAB events

Aim 2: Investigate the efficiency of drum filters to reduce total biomass of phytoplankton entering the farm.

- A) Compare phytoplankton abundance before and after drum filters fitted with 15 μm screens.
- B) Investigate the change in filtering ability (percentage reduction) when fluctuating phytoplankton densities are experienced
- C) Highlight the occurrences when 15 μm screens were able to reduce phytoplankton cell densities to below the HAB threshold of 10 000 cells/l.
- D) Compare the presence and abundance of *Lingulodinium polyedra* before drum filters and after within the secondary sump prior to on-farm pumping.

Expectations and hypotheses

Aim 1

- Phytoplankton blooms will occur during summer periods, where upwelling pulses will encourage the increase in phytoplankton abundance in the nearshore environment through elevated nutrient levels and optimal irradiance levels.
- HABs (i.e. red tide events) will be dominated by dinoflagellate species, notably *Gonyaulax polygramma* and *Lingulodinium polyedra*.

- An increase in the use of farm resources (i.e. monitoring and man-hours) occur during HAB events.

Aim 2

- Phytoplankton abundance (i.e. cell density) will be significantly lower post drum filters, especially for larger dinoflagellates (>50 μm) such as *Lingulodinium polyedra*.
- A positive relationship will be seen between phytoplankton cell densities and filtering ability.
- Drum filter screens of 15 μm in size will reduce phytoplankton abundances to below the 10 000 cells/l HAB threshold.



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Chapter 2 : Seasonal fluctuations of phytoplankton and the impact of HABs on the management of an abalone farm in Hermanus, South Africa.

Introduction

Successful aquaculture farms require filtered seawater free from large organic matter to maximise productivity and profits (Magnesen et al., 2013). The quality of seawater that is pumped onto a farm will influence the growth and survivability of the culture animals, whereas water that is densely populated with organic matter or plankton can negatively influence growth and economic output (Hernandez-Vergara and Perez-Rostro, 2014). Water is sourced from the sea, for land-based facilities, through large intake pumps that can collect millions of litres of seawater per hour (S. Halse, pers. comms.). The water may consist of floating seaweed debris, suspended sediment and plankton that occurs adjacent to the farm in the nearshore.

These land-based farms are made up of various networks of holdings areas and tanks, connected by pipes, which house animals similar in size and age in the same basket. This method is opposite to *in situ* ranching techniques, where juvenile animals are seeded into the inshore region and encouraged to grow in their natural environment (Anchor Environmental Consulting, 2019). Both culture techniques can withstand general fluctuations of phytoplankton densities, but adverse coastal conditions such as HAB events may cause devastating effects to productivity.

Previous studies have investigated phytoplankton fluctuations within the Overstrand region, as this area holds important value in commercial abalone production as well as the nearshore fishing industry (Pitcher et al., 2008; van der Lingen et al., 2016). Pitcher et al. (2008) performed coastal monitoring of phytoplankton abundances for 6 months in 2007, comparing Hermanus and Gordons Bay. Similar abundances of *Gonyaulax polygramma* were observed for both localities that are situated approximately 50 km apart, with peaks in abundance observed in early autumn and spring.

The composition of the phytoplankton community in the euphotic zone usually varies seasonally, where different genera and functional groups have developed strategies to take advantage of the seasonal fluctuations in nutrients and irradiance levels (Alves-De-Souza et al., 2008). During algal blooms, the presence of certain phytoplankton taxa, particularly dinoflagellates and diatoms, contain toxins that cause diarrhetic, paralytic or amnesic effects to both humans and marine life (Díaz et al., 2019). The presence of these toxic organisms have been coined as “Harmful Algal Blooms” and not only cause great harm to the nearshore environment, but can have devastating economic effects (Anderson et al., 2000). Should a phytoplankton bloom be characterised by large scale discolouration of the water,

notable dark red to orange, it is termed a “Red Tide” (Atkins, 2017), as seen in Figure 2.1. It should be noted that not all Red Tides are toxic, and not all HABs are red tides – many chlorophyte blooms are characterised by a dark to luminescent green colour (B. Wang et al., 2019).



Figure 2.1 Water discolouration due to the presence of increased densities of phytoplankton during the red tide of 2017 off the coast of Hermanus, South Africa. Image acquired from Abagold Ltd (2017).

The aims of this study are to investigate the fluctuations of phytoplankton community aggregations on a seasonal basis and to identify dominant taxa that could impact an abalone farm in Hermanus, South Africa. This will provide a base level of knowledge for the farm to work from for future HAB monitoring, as well as the opportunity to compare findings to investigations conducted previously (Pitcher et al., 2019, 2008). The community structure of phytoplankton can be quantified by a number of metrics, such as species abundance (i.e. cell density), diversity, richness and evenness. These metrics will be supported by identifying dominant species from different phytoplankton functional groups. From a farm perspective, the study aims to quantify the resources a farm commits towards monitoring HABs. Specific objectives of this study were to determine; 1) changes in the abundance (i.e. cell density) over time; 2) changes in diversity (as calculated by the Shannon-Weiner Index), species richness and Pielou’s evenness over time; and 3) changes in the relative abundance of dominant species that occurred on a monthly basis over the sample period. Furthermore, on-farm resource demand will

be approximated by 4) monitoring the intensity of phytoplankton samples collected per day during “normal” HAB events.

Materials and Methods

Farm collection site

Abagold Limited is an abalone farm situated in Walker Bay, Hermanus, approximately 100 km from Cape Town (Figure 1.2). The Research Department at Abagold Limited perform daily phytoplankton monitoring counts as part of the Department of Agriculture, Forestry and Fisheries’ (DAFF) permit regulation for South African aquaculture farms. The staff at Abagold performed all of the sample collections, as well as conducted the identification and enumeration steps explained later on as part of their monitoring program. These data were made available by Abagold for this study, as well as the opportunity for the author to learn the sample collection and counting process. For this study, however, focus was placed on data collected over a period of 16 months from September 2018 to December 2019. Samples were collected once a day (not including weekends and public holidays), and sampling frequency increased during HAB/Red tide events for more fine-scale monitoring.

The farm consistently samples water from the Primary Sump situated before any drum filters and representing raw phytoplankton samples directly from the coastal environment. Figure 2.2 shows the flow of water (Blue arrows) entering the farm and the various holding tanks and drum filters. The red polygon signifies the Primary Sump where all samples were taken from. The purple polygon represents the drum filters that the farm uses, which flows into the Secondary Sump, the green polygon. The yellow polygon represents the swirler deck where large organic debris and seaweed fronds are pulled downwards through centrifugal force.



Figure 2.2 A map representing the flow of water through the Primary Sump, drum filter and Secondary Sump at Abagold. Image acquired from Google Maps.

Water collection and sample analysis

At the Primary sump, following Abagold standard operating procedures, 5 litres of water were collected per sampling event and filtered through a 15 μm mesh filter, followed by a 1 μm mesh filter. The remnants that did not pass through the 1 μm filter were then transferred to a 50 ml graduated cylinder and the volume was filled to 25 ml. A Sedgewick Rafter (SR) counting chamber was used for enumerating phytoplankton abundance and diversity (Steinberg et al., 2012). Phytoplankton cells were identified to species level where possible, but at least to genus level for all using literature from Lange-Bertalot and Krammer (2002). The SR counting chamber is a well-established tool to enumerate cell density in a liquid. The chamber measures 50 mm x 20 mm x 1 mm and holds a volume of 1 ml (Karlson et al., 2010). A 1 mm x 1 mm grid is engrained into the bottom of the chamber, therefore, within its dimensions provide 1,000 squares that each hold 1 μl .

The SR was placed on a flat, stable surface with the cover slip diagonally on top of the counting chamber to allow air to escape from the opposite corner to where the chamber would be filled from. Samples

were well-mixed before a 1 ml aliquot was pipetted into the chamber, carefully allowing the chamber to fill and air to escape.

Four rows were randomly selected within the chamber and 50 frames per row were selected and enumerated. All cells were counted and identified across 50 frames of each row, with a total of at least 200 frames counted per sample. Cells crossing the bottom and right frame edges were only included in the count. To calculate the total cells per litre, the formula below was adapted from Suthers and Rissik, (2009).

$$CL = \frac{C \times 1000}{F \times \frac{VF}{25}} \times 1000$$

Where:

CL = Cells per Litre

C = Cells counted

F = Frames counted

VF = Volume filtered

Data analyses

Data analyses was performed using R (R Core Team, 2019). Phytoplankton community assemblages were described by calculating four descriptors: i) richness, expressed as the number of taxa present, identified down to species level where possible; ii) evenness, expressed as Pielou's Index (J'); iii) diversity, expressed as Shannon-Weiner index (H') and iv) density, expressed as the number of cells per species per litre. H' diversity was calculated using the vegan package in R (Oksanen et al., 2019). The formula to calculate Shannon-Weiner index is:

$$H' = - \sum_{(i=1)}^s (Pi \ln(Pi))$$

Where:

H' = Shannon-Weiner Index of Diversity

Pi = Proportion of species i in total individuals

S = Number of species identified in sample

Taxa evenness was calculated as:

$$J' = \frac{H'}{\log(S)}$$

Where:

J' = Pielou's Evenness Index

H' = Shannon-Weiner Index of Diversity

S = Species Richness (i.e. number of species identified in sample)

Dominant species were calculated as the species with the highest relative abundance between taxa on a monthly level, with species with less than 1 % relative abundance excluded from all analyses. These species were compared to the DAFF's list of potential HAB species with known biotoxins to ascertain potential risk to the farms. The total number of phytoplankton samples collected per day were monitored during the sampling period as a proxy of the farm resources assigned to HAB monitoring.

Using the stats package in R (R Core Team, 2019) Kruskal-Wallis tests were performed to measure the significant differences between months and years for community assemblage descriptors such as evenness, richness and diversity (Vidal et al., 2017). Posthoc Dunn's tests, using the PMCMR package in R (Pohlert, 2020), were used to explore further significant results after the Kruskal-Wallis test was used.

Results

From September 2018 to December 2019, 459 phytoplankton samples were collected at the Primary Sump. An increase in phytoplankton abundance is seen from January 2019 to April 2019 (Austral summer to autumn, Figure 2.3). March 2019 showed the highest monthly mean abundance ($721\,179 \pm 226\,473$ cells/l), and was significantly higher than the months before and after it (February 2019: $p < 0.001$, $343\,299 \pm 91\,477$ cells/l; April 2019: $p < 0.001$, $56\,920 \pm 10\,924$ cells/l). Smaller increases in abundance are also seen in the spring of 2018 (October 2018: $88\,056 \pm 19\,690$) and spring of 2019 (November 2019: $23\,808 \pm 5\,324$).

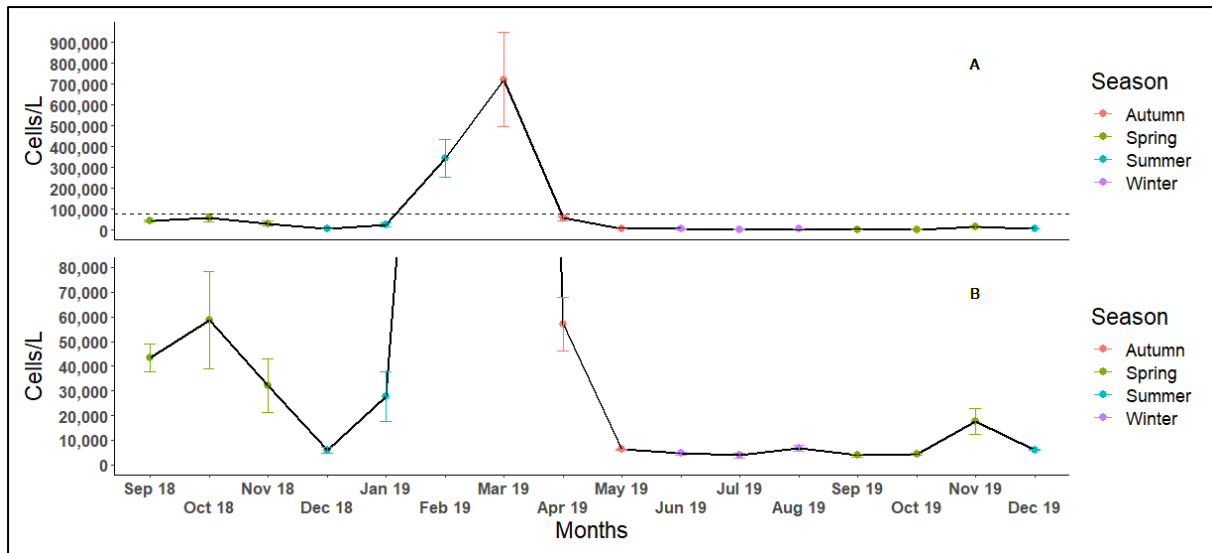


Figure 2.3 A comparison of the monthly mean phytoplankton cell counts across 16 consecutive months, represented with their standard error bars. Austral seasons are indicated by colour in the legend. Panel A represents the overall abundance, compared to panel B which represents the data below the dashed line in panel A.

A total of 90 taxa were identified over this time, with 49 dinoflagellates, 39 diatoms and 2 belonging to the functional group dictyochophyceae. One can see the overall abundance of different functional groups sampled during the 16 month time period (Figure 2.4). From September 2018 to November 2018 (Austral spring), diatoms were greater in abundance than dinoflagellates, but not to a significant degree ($p = 0.49$). Dinoflagellates then increased and remained significantly greater in abundance over diatoms ($p < 0.001$) from December 2018 to June 2019 (Austral summer to early winter). A significant dominance of diatoms over dinoflagellates then returned from July 2019 to December 2019 ($p < 0.01$).

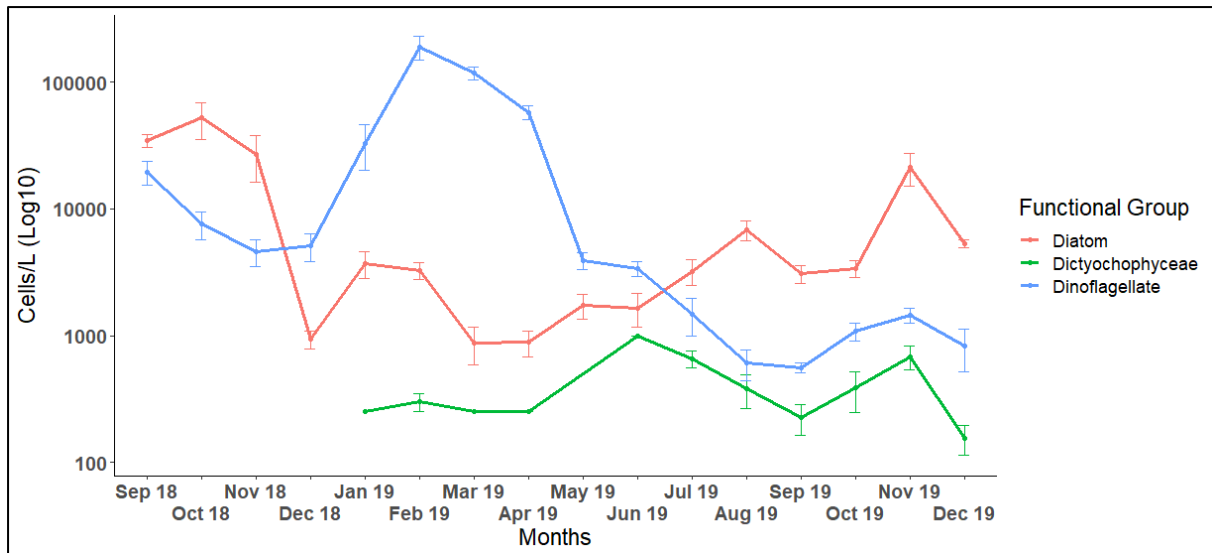


Figure 2.4 Fluctuations in the average monthly cell abundances, with their standard error bars, of phytoplankton functional groups over the 16 month study period. Cell abundances on the y-axis have been log₁₀ transformed.

One can see that dinoflagellate species such as *Lingulodinium polyedra*, *Gonyaulax polygramma* and *Prorocentrum micans* had higher relative abundances during spring, summer and autumn, compared to winter (**Table 2.1**). This is opposite of the diatom taxa such as *Chaetoceros sp.* and *Coscinodiscus granii* that were observed to be higher in relative abundance in winter and spring, compared to summer and autumn.

Table 2.1 A species list indicating the percentage relative abundance (%) that each species contributed to total phytoplankton abundance, for each specific austral season. Species were excluded from the list if their highest relative abundance contributed to <1 % of any month.

Species Name & Authority	SPRING 2018	SUMMER 2018	AUTUMN 2019	WINTER 2019	SPRING 2019	SUMMER 2019
Diatoms						
<i>Asterionellopsis glacialis</i> (Castracane)	–	0.2	–	0.9	9.9	2.7
<i>Bacillaria paxillifera</i> (O.F.Müller)	–	1.1	–	–	–	2.6
<i>Bacillaria sp.</i>	–	–	0.8	4.3	1.4	1.7
<i>Biddulphia sp.</i>	–	–	–	–	1.9	1.2
<i>Chaetoceros convolutus</i> (Castracane)	–	–	–	–	1.1	–
<i>Chaetoceros didymus</i> (Ehrenberg)	–	–	–	–	0.8	–
<i>Chaetoceros radicans</i> (F.Schütt)	–	–	–	–	0.8	–
<i>Chaetoceros similis</i> (Cleve)	–	0.9	–	–	0.7	–
<i>Chaetoceros sp.</i>	17.4	1.3	0.4	7.2	9.1	3.0
<i>Coscinodiscus granii</i> (I.F.Gough)	–	0.7	0.6	10.9	6.8	10.9
<i>Coscinodiscus sp.</i>	–	–	–	12.5	4.9	–
<i>Cylindrotheca closterium</i> (Ehrenberg)	–	–	–	–	1.0	–
<i>Ditylum brightwelli</i> (T.West) Grunow	2.4	0.5	0.2	3.2	1.3	–
<i>Eucampia sp.</i>	–	–	–	0.5	2.9	1.1
<i>Fragilaria sp.</i>	2.6	–	–	–	–	–
<i>Gomphonema sp.</i>	1.1	–	–	–	–	–
<i>Guinardia sp.</i>	–	–	–	–	1.4	–
<i>Gyrosigma sp.</i>	–	–	–	–	0.6	–
<i>Licmophora sp.</i>	–	0.6	0.3	1.2	2.3	24.2
<i>Melosira sp.</i>	–	2.7	1.1	4.8	1.6	2.3
<i>Navicula sp.</i>	7.2	–	0.6	1.5	1.8	7.1
<i>Nitzschia sp.</i>	–	0.5	0.4	0.3	1.7	2.1
<i>Pleurosigma sp.</i>	–	0.3	0.2	0.7	0.7	–
<i>Pseudo-nitzschia australis</i> (Frenguelli)	8.9	0.5	0.4	1.1	2.8	–
<i>Pseudo-nitzschia sp.</i>	–	0.5	0.8	–	1.9	1.5
<i>Rhizosolenia sp.</i>	9.9	0.8	0.4	1.4	3.0	2.1

<i>Skeletonema costatum</i> (Greville) Cleve	–	–	–	2.4	1.8	1.8
<i>Skeletonema</i> sp.	3.9	–	–	–	–	–
<i>Stephanopyxis</i> sp.	–	–	–	–	0.3	–
<i>Thalassionema</i> sp.	–	–	–	3.7	0.5	–
<i>Thalassiosira</i> sp.	5.1	–	–	1.4	7.4	3.7
<i>Triceratium</i> sp.	–	0.3	0.3	0.7	0.4	1.8
Dinoflagellates						
<i>Alexandrium</i> sp.	–	8.4	–	–	0.9	–
<i>Amphidinium carterae</i> (Hulburt)	–	–	–	–	0.4	2.3
<i>Ceratium candelabrum</i> (Ehrenberg) F.Stein	–	–	–	3.3	–	–
<i>Ceratium dens</i> (Ostenfeld & J.Schmidt)	–	0.3	0.3	0.8	–	–
<i>Ceratium furca</i> (Ehrenberg) Claparède & Lachmann	5.8	3.6	7.3	1.6	0.5	3.4
<i>Ceratium fusus</i> (Ehrenberg) Dujardin	3.7	1.0	7.5	0.5	1.0	–
<i>Ceratium horridum</i> (Cleve) Gran	–	–	–	1.0	0.7	–
<i>Ceratium lineatum</i> (Ehrenberg) Cleve	–	–	0.4	–	0.5	1.1
<i>Ceratium</i> sp.	1.1	1.3	–	–	–	–
<i>Ceratium tripos</i> (Ehrenberg) Cleve	–	0.2	0.4	1.7	–	–
<i>Dinophysis acuminata</i> (Claparède & Lachmann)	–	0.3	0.3	1.1	0.6	–
<i>Dinophysis fortii</i> (Pavillard)	9.2	0.2	–	0.6	0.4	–
<i>Dinophysis rotundata</i> (Claparède & Lachmann)	–	–	0.4	–	2.0	–
<i>Dinophysis tripos</i> (Gourret)	–	0.4	0.2	–	–	–
<i>Goniodoma polyedricum</i> (Pouchet) E.G.Jørgensen	–	–	5.9	–	–	–
<i>Gonyaulax polygramma</i> (F.Stein)	0.8	24.6	5.6	0.2	0.6	1.8
<i>Karenia cristata</i> (L.Botes, S.D.Sym & G.C.Pitcher)	–	0.6	0.3	–	0.9	–
<i>Karenia</i> sp.	–	1.0	–	–	–	–
<i>Lingulodinium polyedra</i> (F.Stein)	3.7	24.3	32.0	1.1	0.5	1.4

<i>Noctiluca scintillans</i> (Macartney) Kofoid & Swezy	–	–	0.5	0.6	1.4	1.8
<i>Noctiluca sp.</i>	2.0	0.8	0.2	–	–	–
<i>Prorocentrum gracile</i> (F.Schütt)	–	0.2	1.3	1.1	0.5	2.1
<i>Prorocentrum lima</i> (Ehrenberg) F.Stein	–	–	–	–	0.8	–
<i>Prorocentrum micans</i> (Ehrenberg)	–	2.4	19.6	1.6	1.2	–
<i>Prorocentrum minimum</i> (Pavillard) J.Schiller	–	–	2.0	13.3	1.1	1.7
<i>Prorocentrum sigmoides</i> (Böhm)	5.5	–	0.5	1.5	–	–
<i>Protoperidinium bipes</i> (Paulsen) Balech	–	–	–	–	–	1.2
<i>Protoperidinium claudicans</i> (Paulsen) Balech	–	–	–	–	0.6	1.1
<i>Protoperidinium conicum</i> (Gran) Balech	2.6	3.3	3.6	1.4	0.7	1.4
<i>Protoperidinium depressum</i> (Bailey) Balech	–	0.5	0.2	1.0	0.3	–
<i>Protoperidinium diabolium</i> (Cleve) Balech	3.7	1.8	0.7	1.0	0.8	3.4
<i>Protoperidinium divergens</i> (Ehrenberg) Balech	–	1.4	1.9	–	1.9	2.7
<i>Protoperidinium minutum</i> (Kofoid) Loeblich iii	–	–	0.4	1.1	1.2	–
<i>Protoperidinium oceanicum</i> (Vanhöffen) Balech	–	9.2	0.8	1.0	0.5	–
<i>Protoperidinium steinii</i> (E.G.Jørgensen) Balech	–	–	–	0.6	0.4	–
<i>Pyrophacus sp.</i>	3.7	0.5	–	0.7	–	–
<i>Scrippsiella sp.</i>	–	–	–	–	1.5	2.2
<i>Scrippsiella trochoidea</i> (F.Stein) A.R.Loeblich iii	–	–	–	–	0.9	–
<i>Zigabikodinium lenticulatum</i> (Loeblich & Loeblich)	–	0.4	–	0.6	–	–
<i>Zigabikodinium sp.</i>	–	0.5	–	–	–	–
<i>Dictyocha sp.</i>	–	0.2	–	0.8	0.8	1.1
<i>Octactis sp.</i>	–	0.2	0.2	2.5	1.7	1.8

The diatom taxa *Chaetoceros sp.* had the highest relative abundance during the spring of 2018 (October 2018: 34.16 %, Figure 2.5). In comparison, the dinoflagellate species with the highest relative abundance was *Dinophysis fortii* (November 2018: 12.83 %). Once the seasons changed to summer and autumn, two dinoflagellates species represented the highest relative abundance, which were *Lingulodinium polyedra* (January 2019: 39.76 %; February 19.84 %) and *Gonyaulax polygramma* (February 2019: 47.86 %). Shifting towards winter, the dominant species present was the dinoflagellate species *Prorocentrum minimum* (June 2019: 30.11 %). However, dominance in relative abundance then shifted to the diatom species *Coscinodiscus granii* for the rest of the winter season (July 2019: 24.01 %; August: 16.77 %).

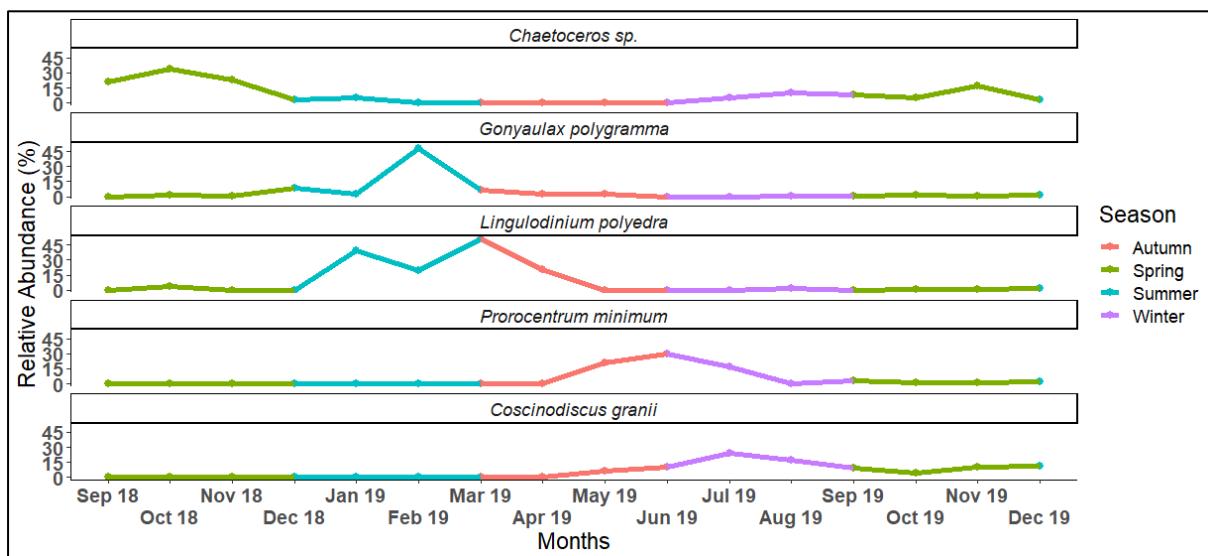


Figure 2.5 Fluctuations in the average monthly relative abundances of dominant phytoplankton species over 16 months. Austral seasons are indicated by colour in the legend

The community metrics were calculated using the phytoplankton counts (Figure 2.6). Species richness fluctuated significantly throughout the sample period ($p < 0.001$). The highest seasonal species richness was observed in the spring of 2019 (September to November 2019; 17.95 ± 4.76), and was significantly higher than the previous spring of 2018 ($p < 0.001$), which also recorded the lowest species richness for the sample period (3.6 ± 1.47).

The lowest species evenness was observed in March 2019 (0.51 ± 0.07), which coincides with the highest phytoplankton abundance over the sample period ($721\,179 \pm 226\,473$ cells/l). Spring 2019 was significantly higher in Shannon-Weiner diversity than the spring of 2018 ($p < 0.001$) and was also the highest seasonal diversity recorded over the 16 months sampled (November 2019: 2.32 ± 0.25).

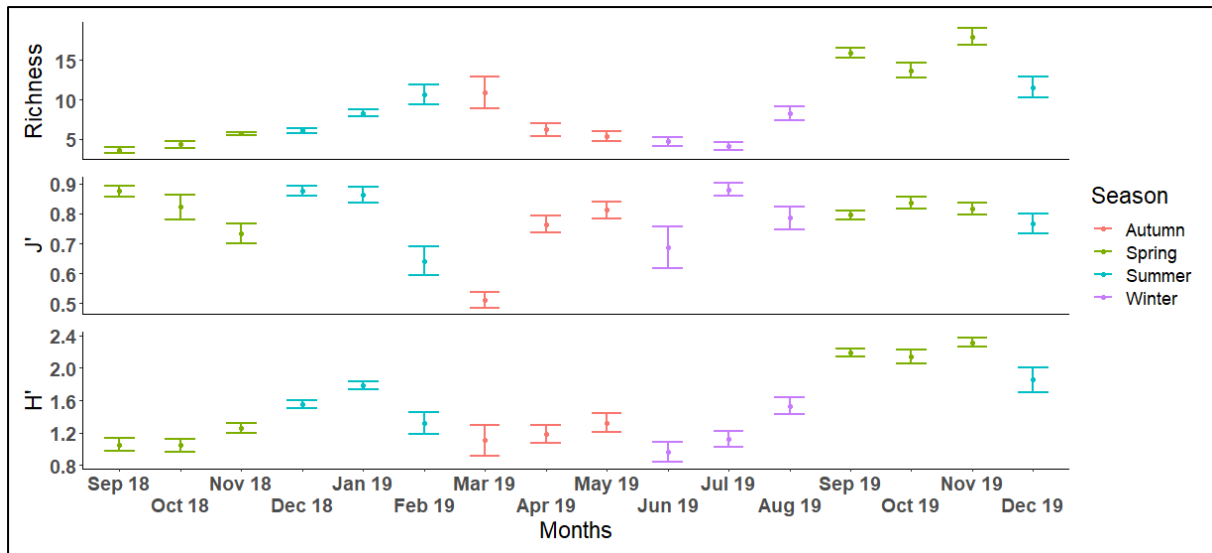


Figure 2.6 A comparison of the community assemblage descriptors during the 16 month study time period, represented with their standard error bars. Austral seasons are indicated by colour in the legend. Standard error bars represent the variability that each descriptor encountered for each respective month during the study period.

There was a significant difference in the number of samples collected per day over the 286 day study period ($p < 0.001$; Figure 2.7). The greatest number of samples collected in one day were 21 samples on the 22nd of March 2019 corresponding to the period of highest phytoplankton cell density (Figure 2.3). Two days later, on the 24th of March 2019, the highest daily averaged cell density for the entire study period was measured at 6 755 689 cells/l.

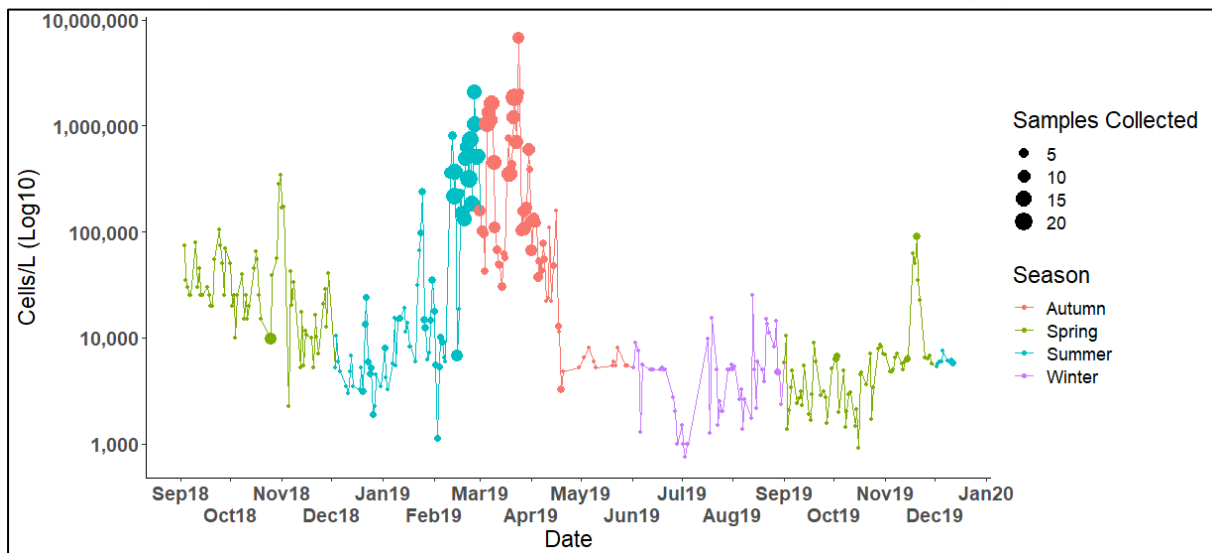


Figure 2.7 Daily sampling frequency in relation to daily averaged phytoplankton abundances experienced over the 16 month study period. Cell abundances have been log₁₀ transformed. Austral seasons are indicated by colour in the legend.

Discussion

In upwelling-prevalent regions, such as the south-west coast of South Africa, harmful algal blooms can easily develop when nutrient availability is high and a stratified water column is present (Berdalet et al., 2017). These natural phenomena can be encouraged by anthropogenic effects, and can negatively alter the productivity of coastal facilities such as aquaculture farms. With the effects of climate change, HABs are predicted to become more prevalent, where stratification may encourage a shift from a diatom community to a dinoflagellate community (Sathish Kumar et al., 2020). Dinoflagellates can access abundant light easier than non-motile diatoms, aided by a positive phototaxis (Sellner et al., 2001). Due to the negative impact of these circumstances it is becoming increasingly pertinent for aquaculture facilities, such as abalone farms, to perform fine-scale phytoplankton monitoring, such as that performed by Abagold.

As a direct result of nutrient availability, the abundance of phytoplankton in the nearshore can fluctuate over time (Largier, 2020). Low nutrient availability, such as during a delayed upwelling pulse, can result in lower productivity in the nearshore, which has been observed to lead to low recruitment of intertidal organisms, such as barnacles and mussels (Barth et al., 2007). A scenario where decreased zooplankton occurs from a short term reduction in primary productivity in the nearshore (California, USA), can have a knock-on effect through the food web, such as the reproductive failure of seabirds in an area (Sydeman et al., 2006). On the other hand, during upwelling events, higher nutrient availability leads to increased primary productivity with the possibility of HAB events occurring (Anderson and Rensel, 2016). Dormant dinoflagellate cysts lie on the ocean floor until suitable conditions develop; these cysts germinate and finally the cells reproduce through simple binary division (i.e. asexual) (Tomas, 1997). However, when conditions deteriorate (i.e. nutrient depletion), cell growth stops and sexual reproduction will commence through the formation of gametes (Persson et al., 2008). Two gametes can join to form a single cyst, which sinks to the floor for the next incoming period of adequate conditions (Kremp and Parrow, 2006). Harmful algal blooms have the ability to poison nearshore organisms, as well as cause fatalities resulting from physical effects such as gill clogging and epithelial cell sloughing (Pitcher et al., 2019). Another by-product of the presence of HABs is the reduction in oxygen availability through utilisation, leading to anoxic conditions (Pitcher and Probyn, 2016). When an increase in cell abundance of phytoplankton species occurs, nutrient and oxygen levels will decrease due to increased requirements of the living cells, as well as through the utilisation of oxygen by bacteria breaking down dead HAB cells (Altieri and Diaz, 2019). Environments can subsequently become anoxic as more oxygen is used than supplied by air-water exchange, which leads to negative impacts on other taxa within the environment (Pitcher and Probyn, 2016). A common occurrence is the 'walk out' conducted by crustaceans, such as, lobsters leaving the nearshore environment due to a lack of oxygen associated with HABs (Cockcroft et al., 2000).

Results from this study show that significant seasonal fluctuations occur in phytoplankton abundance in the study area. The highest peak in phytoplankton abundance occurred in late summer and autumn of 2019, where cell density began to increase in February and subsided in April. Teasing apart this peak and looking at a functional group level provides insight into this event, and how it fits into a global picture.

Upwelling in the region occurs during spring and summer months (September - February), when the strong south-easterly wind is present (Pitcher et al., 2010). This results in a thermal stratification that is weak in spring, but will strengthen over time into late summer and early autumn (Pitcher et al., 2010). The response of phytoplankton abundances to the physical environment will lag, where the peak phytoplankton abundances will be observed in summer and autumn (Pitcher et al., 2010). When reviewing a 16 year time series of bloom densities in False Bay (location of False Bay seen in Figure 1.2), Pitcher et al., (2008) observed a bimodal pattern of abundance in spring and late summer/early autumn, which was also observed in this study. These sites are situated approximately 50 km apart in two different bays. This bimodal trend is found to occur concurrently with the bimodal behaviour of winds that favour upwelling in the region (Pitcher et al., 1995). In general, dinoflagellate density is associated with a stratified water column and a strong thermocline, which would be present during quiescent conditions, and not during large swells where the water column is well mixed (Zonneveld et al., 2001).

The fluctuation in cell abundance is reflected in the community assemblages of phytoplankton in the nearshore. Fluctuations in the community composition vary from month to month, as well as between years where metrics such as diversity, evenness and species richness showed significant variation. Year to year differences were observed by Silva et al., (2009) in Lisbon Bay, in accordance to the severity and duration of upwelling events in the region. Other reports suggest that community assemblages would be influenced by when the samples were collected during a cyclical upwelling or downwelling event, instead of the strength of the upwelling during that specific year (Du and Peterson, 2014). This provides insight that even fine scale nutrient availability, such as what is experienced between an upwelling/ downwelling period, will directly influence the phytoplankton assemblage at that specific time (Du and Peterson, 2014). The phytoplankton assemblage is made up of different taxa that respond to different varying nutrient levels. Low nutrient levels, typical in stratified water columns, can favour dinoflagellates, while diatoms proliferate in nutrient-rich conditions where the water column is turbulent and well-mixed (Silva et al., 2009).

In this study, the highest diversity was observed during November 2019 (2.31 ± 0.246 , Figure 2.6), during a diatom rich time period, when HABs are not common. During the peak HAB event of March-April 2019, species evenness was at its lowest. Similar observations have been observed locally, where a smaller number of species dominate during HAB events. Van der Lingen et al. (2016) observed the

dominance of *Gonyaulax polygramma* dominating an HAB event off the south-western and southern coastline of South Africa during 2011, reaching > 100 000 cells/l off the Cape Peninsula, and > 220 000 cells/l near Mossel Bay. The peak abundance for all dinoflagellates during that HAB even reached approximately 300 000 cells/l. The literature points to a threshold of nutrient availability that controls the phase change from pre-bloom to bloom to post-bloom (Mishra et al., 2005). This would be the minimum nutrient concentrations that the dominant species requires to attain high quantities. In some cases, blooms consisted of only one species, such as a bloom in the Bay of Bengal during 2004 when *Asterionella glacialis* (Castracane) constituted 99.5 % of the total abundance (Mishra et al., 2005). This severe mono-species bloom was terminated when nutrients such as nitrite and silica became depleted (Mishra et al., 2005).

Dominance by a low number of species is observed during HAB events in this study, with high corresponding cells densities. Although the cysts of *Lingulodinium polyedra* have been found in the sediment of Saldanha Bay (Joyce et al., 2005), *L. polyedra* has not been a common bloom representative in the region and the first bloom of this species along the South African coast was only observed in 2014 (Bornman et al., 2014). The red tide that plagued the Walker Bay coastline in 2017 was dominated by *L. polyedra* (and *Gonyaulax spinifera* (Claparède & Lachmann) Diesing) (Pitcher et al., 2019). We therefore can see that the frequency of *L. polyedra* occurring in Walker Bay has increased. Before 2014 *L. polyedra* was non-existent in Walker Bay, and has subsequently been observed in 2017 (Pitcher et al., 2019) and in this study in 2018-2019. Another species that has become a major role-player in this study and in South African blooms is *Gonyaulax polygramma*. This species represented the second highest relative abundance during the HAB observed in this study in 2019. Previous work by van der Lingen et al. (2016) found this species to dominate a bloom that coincided with wash-ups of *Sardinops sagax* (sardines), where it was thought to cause indirect fatalities by gill irritations, rather than yessotoxin poisoning. Although first recorded in False Bay in 1962 (Grindley et al., 1964), this was the fifth biggest bloom of *G. polygramma* along the coast of South Africa, according to van der Lingen et al. (2016). During the 2017 HAB event in Walker Bay that Pitcher et al. (2019) reported on, it was interesting to note that another species within the *Gonyaulax* genus, *G. spinifera* was the dominant species present, and responsible for producing yessotoxins that ultimately caused a loss in abalone stock. We can, however, see that this species was absent during all of the seasons for the study period, while *G. polygramma* was dominant. Hallegraeff (2010) refers to the fact that with a change in ocean climate, there will be a response by phytoplankton communities and HABs. With warming seas, Hallegraeff (2010) hypothesizes that there may be a range expansion for warm-water species, with a reduction in cold water species. An alternative is that there exists interannual variability in phytoplankton community assemblages influenced by large scale circulations originating from the Agulhas Current along the Agulhas Bank (De Villiers, 1998).

A switch between unstable, varying fluctuations in community indices to stable low variance observations occurs from approximately June to July in this study (Figure 2.6). This period also links to the change of dominance of higher taxonomic levels. Diatoms, as a phytoplankton functional group, were seen to dominate from July through to the end of the year, with a changeover to dinoflagellate dominance occurring in December and January. From then, dinoflagellates would dominate the relative abundance of the phytoplankton community until June. We see this pattern followed in our diversity observations, where lower, dinoflagellate-represented diversity occurs for the first half of the year, switching over to a higher diatom-represented diversity in the second half of the year.

The proliferation of red tides and HABs in the nearshore can cause losses in many taxa within the trophic structure (Trainer et al., 2010). Species such as *Karenia brevis* (Davis) G. Hansen & Moestrup are known to kill vertebrates such as fish, while *Karenia brevisulcata* (F.H.Chang) G. Hansen & Moestrup can be toxic to *Haliotis iris* (Linnaeus), the New Zealand abalone (Brown et al., 2019; Gannon et al., 2009). It can be difficult to accurately and financially quantify the loss of organisms due to HAB events, particularly in the wild environment, but it can be easier to understand the effects of HABs on aquaculture farms (Brown et al., 2019). Aquaculture facilities are also forced to close during HAB events, which can have drastic effects on the production cycle as well as negatively affect the economic ability of the facility. Brown et al. (2019) breaks down the global economic impacts of HAB on fisheries and aquaculture facilities. A cost of between \$3-4 billion towards closing facilities just as a precautionary measure was estimated, with the EU contributing approximately \$1.2 billion and Korea, Japan and China collectively contributing approximately \$1 billion to the total (Brown et al., 2019).

On a more local scale, there has been more and more focus on HAB and their impacts on the South African aquaculture sector. During the 2017 red tide in Walker Bay, South Africa, when species such as *Gonyaulax spinifera* and *Lingulodinium polyedra* were present, land-based abalone farms lost millions of animals (Pitcher et al., 2019). Farms require a constant flow of water throughout the farm, and can pump up to 10 million litres per hour through their systems (N. Gordon, pers. comm.). When blooms occur, farms are forced to shut the flow of water off, which promotes an increase in water temperature. During this reduction in water turn-over, ammonium levels build up, and can have detrimental effects on the farmed abalone (Wassnig et al., 2010).

The 2017 red tide in Walker Bay saw the farms lose many animals, which also had a huge financial impact (Pitcher et al., 2019). Abagold Ltd lost R55 million worth of animals, which represented 30 % of their farm tonnage at that time and experienced an increase in their facility's cost of production (Abagold Ltd, 2017). Abagold have also, in the past, declared no interim dividends due to red tide effects (Abagold Ltd, 2016). Not only did the farm lose a large amount of money from animals that had died, but had to contend with reduced growth and yield from surviving stock, as well as a greater commitment of resources to monitoring red tides and mitigate against further losses (i.e. secondary

infection) (Anderson et al., 2001). During time periods when farms are not experiencing blooms, a standard frequency for phytoplankton monitoring is performed on most farms, where daily samples are taken. During the most severe HABs experienced in 2019, monitoring collections amounted to 23 samples in a day performed along multiple sections of the farm operations, i.e. intake, secondary sump, hatchery tanks, broodstock tanks & grow out tanks, with 12 samples occurring at the Primary sump (Figure 2.7). In some cases, during a previous HAB event in 2017, farms tried to increase the frequency of tanks being cleaned, or increased the flow rate through the farm, with others decreasing flow rate in an attempt to reduce the HAB from increasing in density (Pitcher et al., 2019). Broodstock were also transferred to other locations in an attempt to save the animals, e.g. Aquinion (Pty) Ltd farm (Pitcher et al., 2019). Some farms are able to breed with mature abalone, and have the entire lifecycle of the animals closed on the farm (Rhode et al., 2012). Abagold was able to produce 12.1 million spat during 2019 in order to produce enough juveniles to fill the production line post-2017 (Abagold Ltd, 2019).

The facilities that can provide other farms with spat can also be involved with abalone ranching techniques, seeding the nearshore environment with abalone spat in an effort to cultivate wild-grown abalone (DAFF, 2018). Harmful algal bloom events can cause significant mortalities to abalone spat, and can therefore affect normal settlement schedules of farms that breed with abalone, which would have had a knock-on effect as they would not be able to supply other farms, or the ranching activities (Botes et al., 2003). The farms, therefore, need to commit more resources during red tide events, which ultimately has financial impacts as labour costs and consumables increase. There is also additional testing required for animal health, i.e. histology and PCR testing, as well as marine biotoxin testing to ensure food safety and hygiene standards are met throughout (FAO, 2018). This shows that red tides not only have negative financial impacts on farms when animals are killed by blooms, but also when more resources are needed to constantly monitor the fluctuations. The 2017 red tide in Walker Bay forced farms to adjust their normal farm activities to prevent excessive losses. Two of the three farms that Pitcher et al. (2019) interviewed performed ‘pre-emptive canning’ of up to 25 tons, which allowed them to save these animals for production and sale, instead of risk the animals dying during the HAB event. One of the farms, *HIK Abalone*, which pre-emptively canned 21 tons, lost more than 1.1 million animals during the 2017 red tide (Pitcher et al., 2019). Red tides affect farms that are currently in existence, but also influence the location of new farms in the future. An important factor to consider when selecting new sites for fish farms or other aquaculture facilities is the nutrient profile of the ocean where land-based farms would draw their water from (Sellner and Rensel, 2018). Areas with high eutrophication or hypoxia levels become regions to avoid when planning a new aquaculture facility site (Sellner and Rensel, 2018).

This long-term monitoring database provided a significant look into the fluctuations in phytoplankton densities that an abalone farm encounters over a three year period. Although patterns were not always observed from year to year, similar trends in bimodal phytoplankton peaks were observed by others in

the same region (Pitcher et al., 2008). The presence of certain dinoflagellate species that were only recently recorded in the region provides an interesting insight into the possible climatic change the local environment is facing, which may be encouraging dinoflagellate growth (van der Lingen et al., 2016). To be successful in a changing environment, aquaculture facilities need to develop adaptive management strategies to deal with the dynamic nature of harmful algal blooms (Díaz et al., 2019). Continuous research can help farms understand whether their current systems are able to provide a profitable output during devastating events such as HAB. This can include the introduction of recirculating systems, or testing the effectiveness of filters in reducing phytoplankton load (Bregnballe, 2015; C. Wang et al., 2019). Currently, drum filters are used in aquaculture facilities to remove sediment and algal debris from water which provide cultured crops with better water quality to grow in, as it is pumped from the nearshore adjacent to the facility (Steynberg, 2012). By developing and improving strategies and more efficient iterations of current farm systems, an aquaculture facility can excel in a changing climate and, more importantly, be ahead of their competitors.



Chapter 3 : The efficiency of drum filters to reduce phytoplankton densities, during HAB events, on an abalone farm in Hermanus, South Africa.

Introduction

The Overstrand region in the Western Cape of South Africa provides a suitable environment to culture abalone in land-based facilities, where optimal temperatures are present, and the commercial value of the stock is high (Troell et al., 2006). A great deal of financial capital is needed to develop safe and profitable abalone farms in South Africa. A feasibility study conducted in 2018 notes that total capital expenditure for a flow-through system in the Western Cape would amount to approximately R156 million for a 600ton capacity farm, where the first abalone sales can only occur after 4 to 5 years (DAFF, 2018).

The correct water quality and feed choices play an important role in encouraging optimal farmed abalone growth (Davis and Carrington, 2005). Flow rates are adjusted throughout the various production stages to accommodate for overall size of individual animals and stocking densities per baskets and tanks (Wassnig et al., 2010). Maintaining adequate flow rates ensure temperature is regulated throughout the day, especially in warmer summer periods, and that excess nutrients and particulate organic waste is removed from the tanks to ensure adequate water quality. Aeration is also supplied to all tanks to facilitate the movement of water in the tanks, which helps to move water across the gills of abalone (Yearsley, 2007). Water flowing onto a farm usually contains seaweed debris, sediment and various species of phytoplankton that occur in the nearshore adjacent to the farm.

Aquaculture facilities commit substantial resources to mitigating and preventing the negative effects of HABs (Sellner and Rensel, 2018). Various methods are commonly used, such as flocculation, filtration, reduction in seawater abstraction and pumping, and the use of recirculation systems (Brown et al., 2019). In flocculation methods, a material such as clay is sprayed over dense HAB water parcels causing clay to clump together, either drawing cells into the clump or sweeping cells down as it sinks (Kang et al., 2007). The application of rotating drum filters are in some cases able to filter cyanobacteria, with the added support of cationic polyacrylamide flocculants (Wang et al., 2019). When farms reduce the pumping of seawater, thus minimising the abundance of phytoplankton, a build-up of free ammonia (FAN) concentrations can occur, as 'fresh' seawater is not being supplied (Ramli et al., 2017). Depending on the life stage of the abalone, it is known that certain concentrations of FAN can be lethal (Reddy-Lopata et al., 2006; Wassnig et al., 2010).

Recirculation aquaculture systems (RAS) are closed systems where outflow water from tanks are recycled on the farms through a number of filters and pumped back into the tanks (Bregnballe, 2015). Some RASs are, however, semi-closed, where recirculated water is supplemented with “fresh” seawater that is pumped from the sea and where there is some flow out of the system to the sea (Davis and Carrington, 2005). Recirculating water that has already passed through the system results in a decreased demand for this new “fresh” water (that can be dense with phytoplankton during HAB events) and decreased pumping costs (Robertson-Andersson, 2006). While these systems reduce the amount of new water needed to be pumped from the ocean, thus reducing phytoplankton loads during HABS, they come at a biological cost (Bregnballe, 2015). Similar to the method of decreasing seawater being pumped from the ocean; ammonium can increase in these systems leading to toxic conditions; CO₂ can build up and change pH levels; and retention of possible parasitic organisms and bacteria can occur, affecting animals’ health (Robertson-Andersson, 2006). The suitability of each method will depend on the infrastructure of the land-based facility, and whether it has secondary sumps or water storage units (Bregnballe, 2015). Forecasting HAB events has also become critical in facility preparedness, where satellite data and remote sensing algorithms have been developed to quantify chlorophyll concentrations (Smith and Bernard, 2020) as possible indicators of blooms.

Abagold (Pty) Limited uses a land-based flow-through system, where water is sourced from the sea via intake pipes, and millions of litres of water are pumped onto the farm (S. Halse, pers. comm.). Water flows through the primary sump, through the drumfilters and on to the Secondary sump (Figure 2.2). The role of the drum filters (shown in Purple of Figure 2.2) serves to primarily remove large debris and particulate organic matter from the intake water (Steinberg, 2012) as well as limit the introduction of nuisance species such as mussel larvae, fish larval and/or amphipods and isopods. This is done using 90 µm screens under normal and very low phytoplankton abundances that are generally <10 000 cells/l (Casper et al., 2004). This also includes material such as seaweed and sediment that has been churned up in the turbid nearshore. Although the primary role of the filters may not be to exclude phytoplankton, the farm may reduce filter sizes down to 15 µm during HAB events in an attempt to decrease algal densities in the water meant for the grow-out farms (S. Halse, pers. comm.).

This component of the study therefore aimed to investigate the efficiency of the drum filters with a 15 µm mesh in reducing phytoplankton loads and altering the phytoplankton community structure as a means to mitigate against HAB events. By analysing phytoplankton density pre- and post-drum filters and assessing the abundance of harmful species such as *Lingulodinium polyedra*, the effectiveness of a 15 µm screen will be determined. The results of this study will contribute towards improving operational contingency plans for HABs at this specific farm.

Methods and Materials

Research staff at Abagold Limited conducted phytoplankton counts from both the Primary and Secondary sump for 45 days from February to March 2019. Sample days and sample counts per site per day were dependant on phytoplankton densities, where more samples were collected during blooms to monitor incoming HAB cells. Due to the prioritisation of quantifying total phytoplankton abundance per sample and not overall community diversity, species were not always identified for each sample. Data were only included in this study if samples were collected in both the Primary and Secondary sump at the same time by the research staff from Abagold, therefore providing comparable datasets. Sample days were excluded if the number of samples collected per site per day were less than three. The outcome of these conditions provided 36 days used to investigate total phytoplankton cell densities, and 13 days to investigate the cell densities of *Lingulodinium polyedra*. Sampling methods, including water volume collected and analyses, were identical to that of the methods used in the 'Water collection method' and 'Sample analyses' section of Chapter 2.

Data analyses

Data analyses was performed using the rstatix package in R (R Core Team, 2019). Wilcoxon's paired tests were performed to measure the difference of phytoplankton densities before and after a 15 µm drum filter for each day. Wilcoxon's paired tests were also used to analyse measurements when investigating daily changes in cell density of *Lingulodinium polyedra* before and after a 15 µm drum filter.

Results

The overall mean abundance of cells over the study period was significantly lower in the Secondary sump samples ($529\,684 \pm 64\,377$ cells/l) compared to the Primary sump samples ($697\,770 \pm 134\,104$ cells/l) ($p < 0.001$). When looking at daily changes in phytoplankton density between the two sampling points, significant differences were encountered for approximately 39 % of the time (i.e. 14 days out of the 36 days) with mainly a reduction in cell density in the Secondary sump (Figure 3.1). On the 21st and 22nd of March 2019, however, significantly higher cell densities in the Secondary sump as opposed to the Primary sump were encountered.

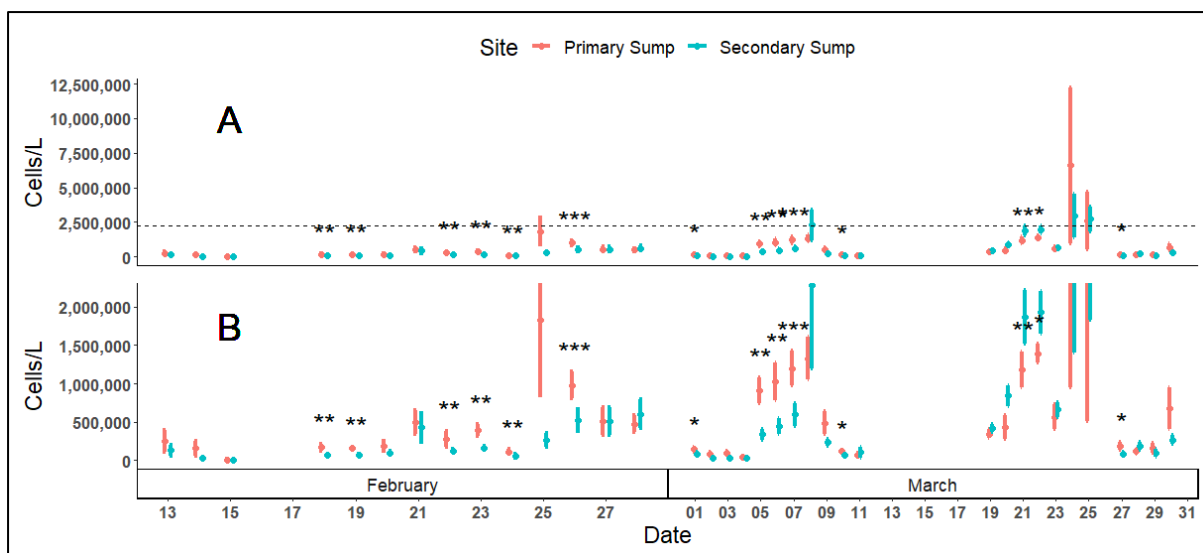


Figure 3.1 A comparison of the difference in phytoplankton abundances within the Primary and Secondary sumps, represented by their standard error bars, for 36 days in February and March 2019. Panel A represents the overall abundance, compared to panel B which further represents the data under the dashed line in panel A. The Primary and Secondary sumps are positioned before and after a 15 μm drum filter respectively. Significant differences between the sites are represented by ‘*’ labels above the date. The p-value significance is represented as follows: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

The 5th of March 2019 showed the highest significant daily average reduction in phytoplankton densities by the 15 μm drum filters over the study period, where 906 861 cells/l were reduced by 63 % to 336 278 cells/l, a total of 570 583 cells/l removed (Figure 3.2). Two days later on the 7th of March 2019, however, one can see that the greatest difference in phytoplankton densities between the two sites was achieved. On this day, a daily average of 603 631 cells/l were removed by the filters, which resulted in a significant reduction ($p < 0.001$) of 50 % of the cells measured in the Primary sump. Two dates that show the greatest significant reduction (2019-03-07: $p < 0.001$, 2019-02-26: $p < 0.001$) occur at percentages lower than the median filtering percentage of all of the points represented (52 %). A filtration success of ~50 % occurs at lower and higher densities of phytoplankton from the incoming water. In phytoplankton densities below 1 million cells/l one can see a higher filtering success of 50–60 %, where after filtering success decreases beyond 1 million cells/l.

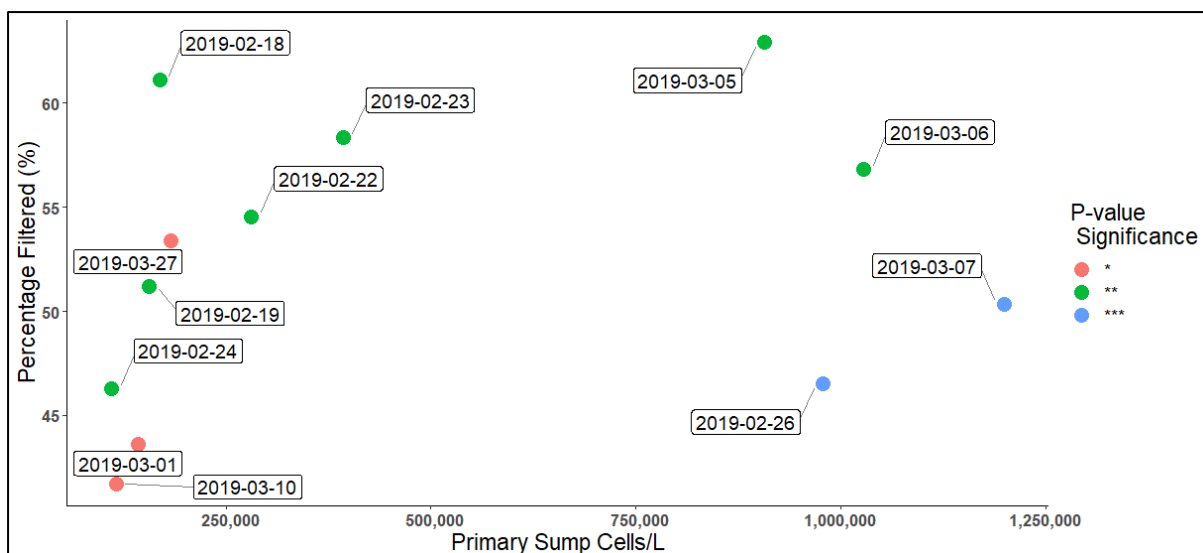


Figure 3.2 A representation of the change in filtering ability (Percentage Filtered) of the 15 μm screen as changes in the Primary sump are experienced. Only sample days when a significant reduction in phytoplankton density was observed are included. Points are indicated by the p value significance where: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

The farm uses a threshold of 10 000 cells/l as a warning density that a bloom event may occur. On nine separate instances the 15 μm filters reduced the incoming phytoplankton densities from above 10 000 cells/l in the Primary sump (PS) to below 10 000 cells/l in the Secondary sump (y-axis) (Figure 3.3).

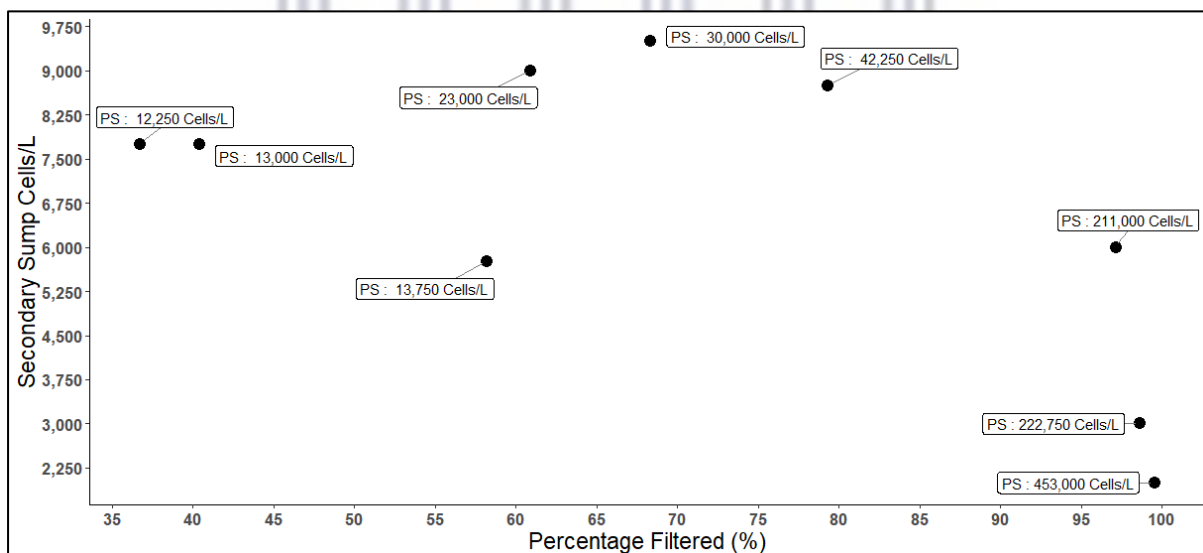


Figure 3.3 Occurrences of individual samples (from various days in the study period) when a 15 μm screen was able to reduce Primary sump phytoplankton densities from $>10\,000$ cells/l to phytoplankton densities of $<10\,000$ cells/l in the Secondary sump. The Primary sump phytoplankton densities for each occurrence are represented by the ‘PS’ labels for each point.

For the HAB species *Lingulodinium polyedra*, only two instances occurred where a significant reduction in density was observed, on the 18th of February and 27th of March 2019 (Figure 3.4). One

can, however, note that when comparing the overall differences between *L. polyedra* densities, the Secondary sump ($41\,851 \pm 6\,304$ cells/l) was significantly lower in density than the Primary sump ($56\,628 \pm 6\,072$ cells/l, $p < 0.001$), with the exception of the 25th of February and 28th of March, where cell densities were higher in the Secondary sump.

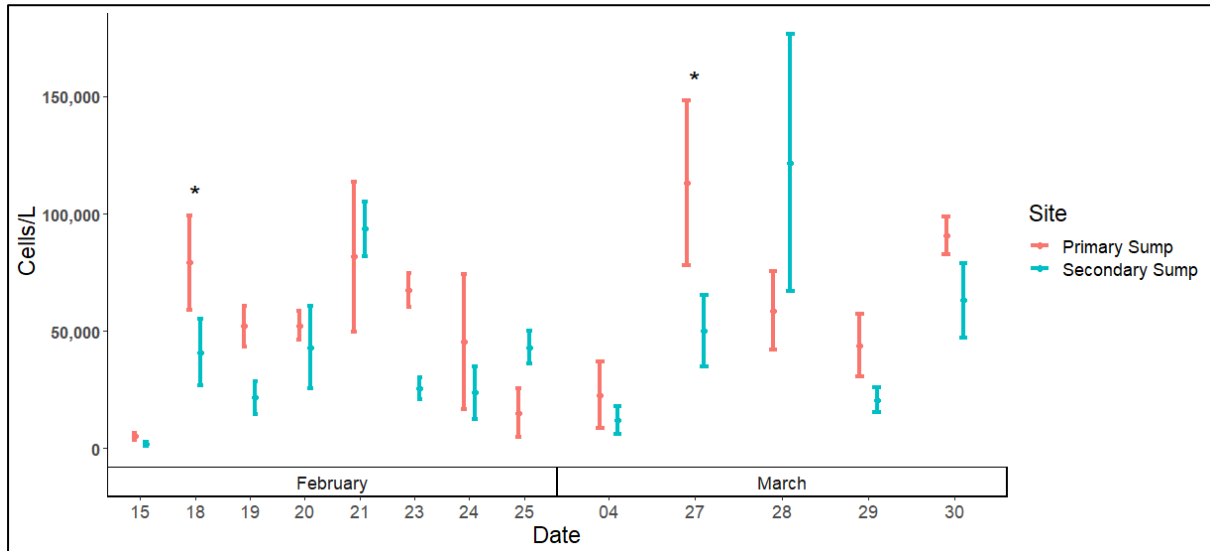


Figure 3.4 A comparison in the difference in cell density of *Lingulodinium polyedra* found within the Primary and Secondary sump, indicated by their standard error bars, for 13 days in February and March of 2019. The Primary and Secondary sumps are positioned before and after a 15 μm drum filter respectively. Significant differences between the sites are represented by ‘*’ labels above the date. The p-value significance is represented as * = $p < 0.05$

Discussion

The aim of this study was to investigate if the current drum filter system fitted with 15 μm mesh screens implemented on an abalone farm was able to reduce phytoplankton densities sufficiently during bloom conditions. The results obtained from this study show that the 15 μm filters play a significant role in filtering phytoplankton densities (i.e. cells/l). Twelve separate days over the 36 day study were observed to significantly reduce phytoplankton densities between the two sites, i.e. ~33 % filtration success. For the HAB species *Lingulodinium polyedra*, cell densities were higher before the 15 μm drum filter, and overall there was a significant reduction (~83 %) due to the presence of the drum filter.

The presence of drum filters with screens should in theory filter out all of the large algal material, sand, as well as planktonic organisms that measure larger than 15 μm . These would include harmful species such as *Lingulodinium polyedra* (40-50 μm), *Prorocentrum micans* (30-70 μm), *Gonyaulax polygramma* (35-66 μm) and species in the *Ceratium* genus such as *C. dens* (>75 μm), *C. furca* (100-200 μm), and *C. fusus* (300-600 μm) (Smayda, 2000). Although we only saw a significant reduction in density for *Lingulodinium polyedra* on two occasions, the identification of species was not always the priority of the monitoring team. We can, however, deduce that similar sized HAB species as mentioned

above would also be significantly reduced, as well as species with apical horns such as *Gonyaulax polygramma* (van der Lingen et al., 2016) that can hook onto other cells, material or the screens themselves. The smaller the filter size, the more material can be removed from the water, but at a cost. The speed of water would be influenced by the size of the filter (15 µm versus a 90 µm filter), as well as the turbidity of the water, as smaller filters would be clogged up quicker, creating a denser barrier for the water to pass through (Brinker et al., 2005) and consequently requiring cleaning or replacement more often.

Although we saw a significant reduction in densities between the sites on 12 occasions, there existed 2 days where significantly higher cells densities were measured after the drum filter. In many cases, dinoflagellates may tend to reject a colony-structured approach, preferring to move solitarily and freely through the water column, assisted by flagella (Smayda, 2010). Likewise, the ability of these dinoflagellate species to penetrate through a fine filter, such as those used by the farm during bloom events, may be explained by polymorphism as an adaptive behaviour used to exploit a niche in upwelling systems, as explain by Smayda (2010). Here the author covers the ability of some dinoflagellate species to have plastic morphological control, influenced by the environment. Warmer waters display morphologies of a *Ceratium* species with longer horns, as opposed to short-horned specimens in colder waters. Fine scale morphological differences between upwelling species were also studied by Smayda (2010). The change in ratio of swimming versus sinking can affect the survival of an organism in highly stratified water columns. By changing the geometric shape, as well as thecal wall thinning, the resistance that a cell has to sinking can be altered. What is also known is the ability of some upwelling-prevalent dinoflagellate species to produce smaller, identical cells to their larger, vegetative cells (Silva and Faust, 1995). This can occur when nutrients are not available, and these cells will occur until nutrients are in supply, where they will proliferate to normal sizes. Observations of these miniature morphologies have been seen for species such as *Lingulodinium polyedra*, which is a species that dominated during the HAB event in 2019.

The occasions where higher densities were observed after the drum filter may also be explained by phytoplankton densities experienced in the preceding days. One can see in Figure 3.4, on the 25th of February, cell densities of *L. polyedra* in the Secondary sump are higher than that of the Primary sump, but similar in density to the Primary Sump on the 24th of February. The same pattern is seen on the 28th of March, where Secondary sump densities are similar to those observed in the Primary sump on the 27th of March. This may be due to the longer time it takes for cells to move out of the Secondary sump. There may also be occurrences where staff clean the filters, especially after very high phytoplankton densities such as those experienced on the 24th and 25th of March (Figure 3.1). This occurrence of increased densities after the drum filter only occurs on a small number of occasions, and when looking at the general overview, there still exists a significant decrease in cell density after the drum filters.

It is important to provide abalone with water that is not varying from the optimal temperature range, does not exceed high ammonium or decreased oxygen levels and is not densely populated with possible harmful phytoplankton (Davis and Carrington, 2005). The ability for phytoplankton of any nature to penetrate drum filters and proliferate through the farms flow system forces the farm into a vulnerable position. The gills of abalone can be clogged by phytoplankton in the water, and reduced growth or death can occur (Dugenne et al., 2015). Abalone can also succumb to the biotoxins in some red tide species, and therefore there is a huge financial impact on abalone farms caused by HABs (Pitcher et al., 2019). Drum filters mainly reduce the biomass of vegetative macroalgae and sand from entering the water system of the farm (Bregnballe, 2015). However, results from this study show that there is a significant reduction in phytoplankton densities by drum filters on some occasions. Other strategies can be implemented if the farm is seeking to increase the number of occasions where a significant reduction is observed. Drum filters on board ships in tandem with cationic polyacrylamide as a flocculants showed significant reductions in cyanobacteria bloom densities with a flow rate of approximately 900m³/h (Wang et al., 2019).

Abagold has recently introduced a recirculating system which feeds water back into the network, and therefore allows the farm to reduce their ocean intake water needs (Abagold Ltd, 2019). While recirculating systems are useful, they can sometimes harbour and reintroduce pathogens (Gonçalves and Gagnon, 2011). Necessary treatment of recirculated water is therefore an extra step in the process, such as the use of ozone to reduced bacterial loads. The continuous monitoring system that Abagold currently employs allows the facility to understand the community assemblages that are being transported into the farm. This can be used to forecast the possible likelihood of algal blooms occurring. Indicator species observed at certain densities are widely used to forecast red tides in early warning systems (Silva et al., 2016). Future work related to this study could investigate the differences within species observed flowing onto the farm by collecting the morphometric measurements of the dominant species within samples.

By comparing the cell densities measured before and after a drum filter on an abalone aquaculture facility, we have been able to understand the high impact that these filters play in reducing phytoplankton blooms using screens of 15 µm. One of the outcomes of this investigation is that it provides the farm's management with an overview of how well the filters have behaved, and at what phytoplankton densities the filters were not sufficient reducing phytoplankton densities.

Chapter 4 : General Discussion

The southwestern and southern Cape coastline of South Africa experiences varying environmental conditions from season to season (Barlow et al., 2005). These variations will directly affect not only the phytoplankton community abundances and assemblages, but the management and monitoring behaviour of aquaculture facilities. The overall aim for this study was to investigate the seasonal fluctuations of phytoplankton community composition and abundance occurring adjacent to an abalone farm, noting the severity of harmful algal blooms (HABs) experienced by the facility, the change in behaviour regarding monitoring efforts, as well as the ability of drum filters to reduce phytoplankton densities during HABs.

Phytoplankton abundances occurring in late summer and early autumn exhibited the peak abundances over the study period, which coincided with the period in which HAB events occurred. Higher phytoplankton abundance was noted in March 2019 and was significantly higher than other months. These HAB events are a lagged phenomenon, occurring after the region has experienced the strong south-easterly wind that forces upwelling in the nearshore environment (Pitcher et al., 2010). This pattern has also been observed on a larger global scale, and is a common observation for temperate locations (Cebrián and Valiela, 1999). The variation in functional groups and their seasonal dominance can also be explained by environmental conditions. Upwelling events create a stratified water column, which compliments motile dinoflagellates over centric diatoms (Ross and Sharples, 2007). Dinoflagellates can move to access abundant light using phototaxis (Sellner et al., 2001). This is opposed to diatoms, which respond to a less stratified and more mixed water column which occurs more frequently during winter and spring (Álvarez-Salgado et al., 2008). This is seen in chapter 2, where diatom peaks occurred in the second half of each year, and dominated the phytoplankton community assemblages when the upwelling prevalent wind began to occur. The lag period occurs where water is stratified, and by late summer and autumn, conditions for a dinoflagellate dominated environment are present. The shift between dinoflagellates and diatoms may, however, change, as the environment they find themselves in is altering. Through climate change, red tides are predicted to increase in frequency (Hjerne et al., 2019). An increase in temperatures and salinity link directly to a more stratified water column, providing a better environment for dinoflagellates to proliferate (Sathish Kumar et al., 2020).

When reviewing the literature, many authors note the changes within the phytoplankton community assemblages for the region. The dinoflagellate species *Lingulodinium polyedra* was absent in the region before 2014 (Bornman et al., 2014) but has dominated the HAB event in Walker Bay in 2017 (Pitcher et al., 2019), was present in 2018 (S. Halse, pers. comm.) and dominated in this study (i.e. 2019). This is opposed to the other dominant species in this study, *Gonyaulax polygramma*, which was responsible for anoxic conditions found in False Bay in 1962 already. The apparent increase in these HAB species

abundances has, with it, economic risks to abalone farms in Walker Bay. The 2017 HAB event resulted in 250 tons of abalone being lost when abalone gills were clogged by *Lingulodinium polyedra*, and yessotoxins from *Gonyaulax spinifera* were present in the environment (Pitcher et al., 2019). Paralytic Shellfish Poisoning (PSP) toxins have been found in cultured abalone up the west coast of South Africa, 100 km from this study's site, occurring at the same time that the dinoflagellate species *Alexandrium catenella* (Whedon & Kofoid) Balech was in bloom (Pitcher et al., 2001). As mentioned above, anoxic conditions are also a common by-product of HAB events, which are known to cause reduced growth in abalone (Harris et al., 1999). Therefore various risks to the production efforts of abalone facilities exist, which can increase management costs or result in a loss of animals (Brown et al., 2019).

Farms therefore need to commit resources to prepare for and mitigate the effects of HAB events. When reviewing the ability of the current filtration systems that the farm uses in Chapter 3, one can see that the current drum filter system using a 15 µm screen during HAB events provided significant reductions in phytoplankton densities coming onto the farm. Species such as *Lingulodinium polyedra* was significantly reduced during the study. As mentioned above, a similar event occurred in Walker Bay, the same location as this study, where two species, *Gonyaulax spinifera* and *Lingulodinium polyedrum* were responsible in the 2017 HAB event (Pitcher et al., 2019). The bloom contained yessotoxins that were present in abalone being cultivated on land-based farms, which resulted in one farm losing abalone to the value of R155 million (Abagold Ltd, 2017) and two other adjacent farms with similar losses (Pitcher et al., 2019).

Phytoplankton-derived toxins affect organisms when the cells are consumed and the intracellular toxins enter the organism's system (Shelar et al., 2014). In some cases, however, toxins can exit the phytoplankton cells, and extracellular toxins can be present in the water column (Seger et al., 2015). In one study, authors investigated the intracellular and extracellular levels of paralytic shellfish poisoning toxins (PST) originating from cultured samples *Alexandrium* spp. from Sequim Bay, Washington (Lefebvre et al., 2008). Noticeable levels of both intracellular and extracellular toxins were measured in the study (Lefebvre et al., 2008). Another study has noted how the exposure to extracellular saxitoxins (STX) impaired the physiology of zebrafish larvae, where the loss of sensorimotor function occurred (Lefebvre et al., 2004). The use of flocculants have been shown to reduce extracellular toxins in the water column (Seger et al., 2015). Pierce et al. (2004) were able to remove approximately 70 % of the extracellular toxins originating from cultured *Karenia brevis* cells that were ruptured by sonication, using clay flocculation. It is therefore imperative that farms are able to quickly understand the community assemblage that makes up an HAB event, as well as the accompanied biotoxins that taxa within the bloom may produce (Stauffer et al., 2019).

Farms can also implement other mitigation methods within their facilities. The introduction of recirculating systems has become prevalent in aquaculture facilities globally (Bregnballe, 2015). By

reusing water, as opposed to pumping constantly new water from the ocean, farms can optimise the amount of water needed, leading to a reduction in water usage and as well as the amount of effluent (Gonçalves and Gagnon, 2011). This in turn allows the farm to have to use less water that is ‘contaminated’ with destructive HAB species during blooms, which therefore reduces the risk of losing culture species (Abagold Ltd, 2019; Pitcher et al., 2019). The use of recirculation comes with its own issues, such as a biosecurity threat as pathogens have the ability to remain in the system (Gonçalves and Gagnon, 2011) and therefore careful consideration with regards to the community assemblage of the HAB will influence if a facility will recirculate or not (S. Halse, pers. comm.).

Through the use of research and development, an abalone aquaculture facility can adjust and adapt to the threats that HAB events play on the facility. The findings of this study therefore provides an overview of what the farm has experienced over a 16 month period. A good indication of HAB peaks has been outlined in the results that included high phytoplankton densities, significantly greater than densities that are acceptable under normal phytoplankton conditions. Species evenness also decreased during periods of HAB events, supported by the dominance of HAB species, such as *Lingulodinium polyedra* and *Gonyaulax polygramma*. Finally, the current filtering infrastructure that the farm employs produced significant reductions in phytoplankton densities. There may however be a decrease in the filtering ability of drum filters as phytoplankton densities increase. This may allow the farm management to better understand the seasonal peaks and risks of HABs in the region, and the likely occurrence of diatoms vs. dinoflagellate blooms based on environmental divers such as spring nutrient input and calm summer conditions.

Limitations to study

Due to the nature of some of the sampling periods, as well as the fact that the farm’s main concern is abalone production and not phytoplankton monitoring, the collection of environmental data was not continuous. Therefore, months were used as a proxy for general ocean conditions, where austral summer, autumn, winter and spring months would provide a structure for when we would expect certain oceanographic events to occur. These would include events such as upwelling pulses in spring and summer, when upwelling favourable winds would blow (Jury, 2015), and increased wave heights during winter, when cold fronts were more prevalent (Coppin et al., 2020). In other cases, during the highest cell densities, individual species were not identified. The farm employs researchers to conduct phytoplankton monitoring, but during HABs when monitoring frequency increases, a trade-off between counting total cells and identifying each cell appears. Quantifying the presence of a few harmful species and the total density is more important than identifying the density of each species present and will relate directly to operational decisions.

Conclusion

This 16-month monitoring database provided a glimpse into the fluctuations in phytoplankton densities that an abalone farm encounters. Seasonal phytoplankton peaks were observed in spring as well as summer to late autumn, and similar trends in bimodal phytoplankton peaks have been observed by others in the same region (Pitcher et al., 2008). The presence of certain dinoflagellate species, such as *L. polyedra*, that were only recently recorded in the region (Bornman et al., 2014) provides an interesting insight into the possible climatic change the local environment is facing, which may be encouraging dinoflagellate growth (van der Lingen et al., 2016). The occurrence may also shed light on the possible biosecurity risk that may develop as increased shipping activities increase the likelihood of transporting invasive species into an area via ballast water (Céliavillac et al., 2013).

Long term phytoplankton measurements can be negatively affected due to data incomparability, when methodologies change from one management to another, or taxonomic anomalies that cannot be investigated using light microscopy (Vidal et al., 2017). These long-term phytoplankton series can, however, provide a history of algal variations in certain regions, and provide an aquaculture facility with cues to look out for during bloom-favourable conditions.

The farm has provided an interesting and extensive database of phytoplankton counts over a 16-month period, where peaks and troughs of phytoplankton density can be easily observed. Linked to these counts is the availability of satellite imaging that assesses the likelihood of red tides by measuring chlorophyll density along the coast via remote sensing. A decision tool has been developed which merges the use of satellite chlorophyll-*a* levels and *in situ* colour optics to provide the aquaculture industry with a decision tool to monitor the likelihood of HAB events (<https://www.ocims.gov.za/fisheries-and-aquaculture-tool/>). Another avenue to follow may be measuring the cell size of species in each sample, which would allow researchers to understand the different morphologies of specific species, and if cell size and life stage plays a role in which species are present during blooms.

Analysing the phytoplankton counts from the farm has allowed us to see how the need for more fine-scale monitoring during HAB events has increased resource needs. More researchers are required to deal with a higher frequency of monitoring counts during these blooms, to better understand if densities are subsiding and whether the farm can possibly return to a normal water flow and operations. This study has also highlighted the need for finer scale temperature, nutrient, pH and dissolved oxygen monitoring, which are the key drivers in bloom development (Burger et al., 2020). The more data that can be collected, the better one can possibly fit classification models and build risk assessment procedures, which can predict and allow greater preparedness for aquaculture farms in the region.

Chapter 5 : References

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