A CRITICAL ANALYSIS OF MICROBIOTA ASSOCIATED WITH THE PRODUCTION OF SHELLFISH IN SALDANHA BAY, SOUTH AFRICA

By

Likentso Sylvia Shuping

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Supervisors: Prof IS Human

Co-Supervisors: Prof JFR Lues and Dr AN Paulse

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DECLARATION

I, **Likentso, Sylvia Shuping**, hereby declare that the thesis submitted for the degree, Doctor of Philosophy in Environmental Health, at the Cape Peninsula University of Technology is my original work and has not previously been submitted to any other institution of higher learning towards any qualification. Furthermore, it represents my opinions and not the opinions of the Cape Peninsula University of Technology.

November 2023

SIGNATURE OF STUDENT DATE

DEDICATION

I dedicate this work to my loved ones and friends in appreciation of their encouragement and support. My husband for his continuous encouragement and support. My mother for being the pillar of my strength. This journey was a demonstration to my daughters Remofilwe and Tshimologo that everything is achievable with enough willpower. May this serve as motivation for them to put up their best effort. I would like thank the almighty God for the strength, perseverance and for making it possible for me to finish this task.

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ABSTRACT

Aquaculture is part of the blue economy and is entrusted to fulfil some of the United Nations' sustainable development goals. The aquaculture industry in South Africa is steadily growing, which comes with added responsibility to ensure sustainable aquaculture. There is an increasing concern globally about food accessibility and security for a growing population. It is estimated that global population growth will reach 9 billion by 2050. The development of sustainable marine aquaculture farming is attributed as the solution to food security.

Marine bivalve molluscs such as mussels, oysters and cockle are harvested commercially from various parts of the world for human consumption as part of aquaculture. They are grown in freshwater ecosystems, brackish or coastal waters, and their cultivation areas must be approved by relevant authorities to ensure food safety requirements are adhered to. Bivalve molluscs are filter feeders by nature. They bioaccumulate and retain contaminants available in their growing waters and, therefore, are regarded as a major contributor to foodborne outbreaks worldwide, as they are eaten raw or partially cooked.

Bivalve mollusc production areas are often exposed to various types of pollutants, including the disposal of treated and untreated sewage. Sewage contains a wide diversity and concentration of microorganisms. The primary concern is the contamination of bivalve molluscs produced by pathogenic microorganisms (bacteria, viruses, and protozoa), creating a public health threat.

A critical analysis of microbiota associated with the primary production of shellfish in Saldanha Bay in South Africa was investigated. The aims of this study were to investigate the sources of potential contaminations and microbiota during the primary production of mussels and oysters and to develop practical ways to eliminate or reduce risks related to sources of contamination and associated hazards to acceptable limits.

The methodology used involved sample collection and analysis of mussels, oysters, and seawater samples using conventional culture methods to isolate *Salmonella*, *Vibrio* species in mussels, oysters and seawater samples. Serotyping of microorganisms detected was done using the Vitek compact 2 instrument. The following microorganisms were identified: *Enterobacter cloacae complex; Citrobacter freundii; Klebsiella pneumoniae; Aeromonas sobria; Vibrio alginolyticus;* and *Sphingomonas paucimobilis*. Most Probable Number analysis technique was used for detection and enumeration of faecal coliforms and *Escherichia coli*.

The findings from this study included the need for consistency in the microbiological monitoring programme as well as the development of a microbiota database for the identification of possible pathogenic bacteria which were excluded from the commonly known foodborne disease-causing bacteria. Regulatory gaps, where some gaps included duplication of regulations by various departments and lack of a centralised Competent Authority for aquaculture activities. In conclusion, it was recommended that further studies need to be conducted on the bacterial species identified and the application of One Health approach to prevent the occurrence of public health threats due to contaminated shellfish by pathogenic bacteria.

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GLOSSARY

Acronyms	Definition/ explanation
CPUT	Cape Peninsula University of Technology
DAFF	Department of Agriculture, Forestry and Fisheries
DAL-RRD	Department of Agriculture Land and Rural Reform Development
DEFF	Department of Environment, Forestry and Fisheries
DSP	Diarrhetic Shellfish Poisoning
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopy
EU	European Union
FAO	Food and Agriculture Organisation
FIBs	Faecal indicator bacteria
F-RNA	Fluoro-Ribonucleic acid
GI	Geno group one
HAV	Hepatitis A virus
HMM	Higher Molecular Mass
IARC	International agency for research on cancer
ISO	International standardisation organisation
LMM	Lower Molecular Mass
MLRA	Marine Living Resources Act
MPN	Most Probable Number
NCTC	National Collection of Type Cultures
NLV	Norwalk-like virus
NoV	Norovirus
NOAA	National oceanic and atmospheric administration
NSP	Neurotoxic Shellfish Poisoning
NSSP	National Shellfish Sanitation Program

ORF	Open reading frame
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
SAMSM&CP	South African Molluscan Shellfish Monitoring and Control Programme
SPP	Species
STEC	Shiga-toxin-producing *Escherichia coli*
US	United States
USEPA	United States Environmental Protection Agency
WWTW	Wastewater treatment work

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND AND JUSTIFICATION

There is an increasing concern globally about food accessibility and security for a growing population. It is estimated that global population growth will reach 9 billion by 2050. Scientists are therefore concerned with the provision of an adequate amount of food for the growing population (Venugopal and Gopakumar, 2017; Marriott et al., 2020). The development of sustainable marine aquaculture farming is attributed as the solution to food security. Marine bivalves fall under the commonly used term “shellfish” which includes molluscs, crustaceans and echinoderms (Da Silva et al*.,* 2015). Total production of fisheries and aquaculture reached a record of 179 million tonnes in 2018 and decreased slightly to 178 million tonnes in 2020 Globally. Production was dominated by finfish reaching 57.5 million tonnes in 2020 from inland aquaculture and 17.7 million tonnes of bivalve molluscs. Asia dominated world aquaculture production by producing 91.6 percent of aquatic animals and algae in 2020. Following Asia, Africa has the second-largest volume of inland water captured by region. African aquaculture decreased slightly in 2020 by 1.2 percent compared to 2019, because of decline in production in Egypt, which is Africa’s major producer. Production declined in Nigeria, which is the largest producer in Sub-Saharan Africa, by 9.6 percent. The remainder of the continent saw a production growth of 14.5 percent reaching 396 700 tonnes in 2020 from 346 400 tonnes in 2019 (FAO, 2022).

Marine bivalves such as mussels (*Mytilus galloprovincialis, Choromytilus meridionalis, and Perna perna*), oysters (*Crassostrea gigas* and *Ostrea edulis*), clams (*Chamelae gallina, Ruditapes philippinarum, Curbicula fluminea, Ruditapes decussatus, Tellina crassa,* and *Dosinia exoleta*) and Cockle (*Cerastoderma edule*) are harvested commercially from various parts of the world for human consumption (Mesquita et al., 2011; Rubini et al., 2018). They are all characterised by having two valved shells hinged together by an elastic ligament (Potasman et al., 2002). Their sources of food are zooplankton, phytoplankton and organic particulate matter. They are grown in freshwater ecosystems, brackish or coastal waters, and their cultivation areas must be approved by relevant authorities. Marine bivalves are regarded as a major contributor to foodborne disease outbreaks worldwide due to their natural characteristics such as filter feeding, and their growing areas, which are often contaminated with sewage, heavy metals, algal biotoxins, pathogenic bacteria, and viruses. These become a public health threat as they are consumed raw or partially cooked (Cruz et al., 2015; Polo et al., 2015; Woods et al., 2016; Marceddu et al., 2017; Xu et al., 2017; Sferlazzo et al., 2018; Walker et al., 2018).

In South Africa, marine bivalve aquaculture occurs mostly on the coasts of the Western Cape, Northern Cape, and Eastern Cape. Mussel farming is confined to Saldanha Bay in the Western Cape of South Africa; two species are farmed, indigenous black Cape mussels (*Choromytilus meridionalis*) which are not a preferred species for farming due to the dark flesh colour of the female species, and exotic Mediterranean mussels (*Mytilus galloprovincialis*) which were introduced in South Africa in the 1970s (Pavlov et al., 2015). Mediterranean mussels are a preferred species for culture, due to their quick adaptation nature. They are more abundant than the indigenous black Cape mussels and have proven to be commercially viable (Bezuidenhout et al., 2020). The mussels are sold locally and exported to neighbouring countries such as Namibia and Mauritius. Production systems such as longlines and rafts are used, where mussels are grown on ropes hanging from a floating platforms as shown in Figure 1.1. Mussels reach market size within seven months. Harvesting occurs every three months, and only mussels and oysters weighing more than 50g are harvested (Sanitary Survey Report, 2018). The oysters are placed in mesh bags or perforated plastic trays or on suspended floating ropes.

Figure 1.1 *Rafts and longlines for rope suspension. (Adapted from Farmers weekly, 2016)*

Currently, Pacific oysters (*Mogallana gigas*) shown in Figure 1.2, are the only species farmed along the coast of the Northern Cape, Western Cape, and Eastern Cape. It is an introduced species native to the pacific coast of Asia. The farmed pacific oysters are sold locally and exported internationally to Hong Kong, China and Taiwan in Asia and Zambia in Africa. The spat (seed oysters) are imported, but small quantities are produced in Kleinzee in the Northern Cape. Oysters reach marketable size in 10 -12 months (Ferreira, 2016). Pacific oysters (*Crassostrea gigas*) have proven to be the only commercially viable species of oysters cultivated in Saldanha Bay (Heinecken et al., 2017).

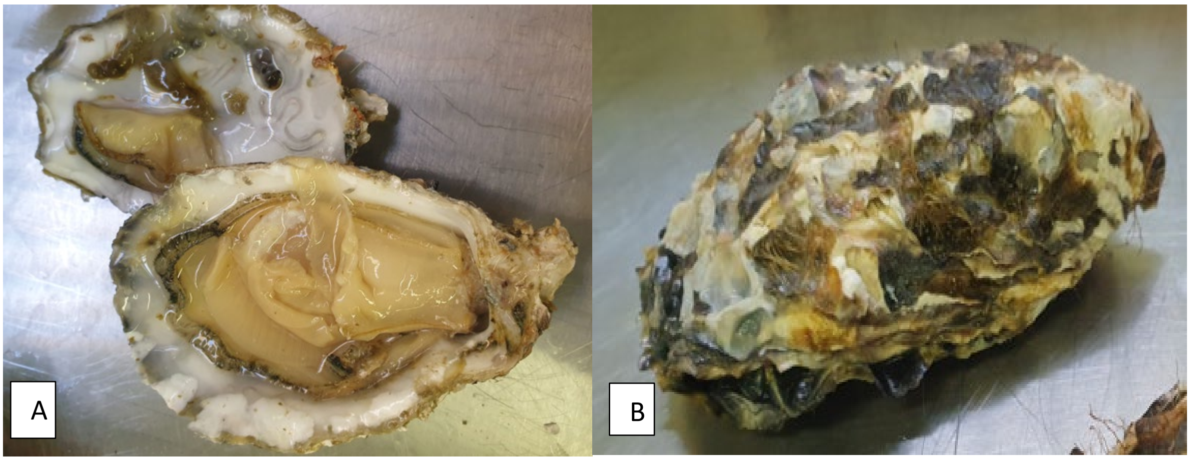

Figure 1.2 *(A) The flesh of the Pacific oyster (Crassostrea gigas), (B) outer protective 2-valve shell*

1.2 PRIMARY PRODUCTION

Saldanha Bay, situated on the West Coast of South Africa is the only naturally sheltered embayment found in South Africa (Clark et al., 2018). It comprises approximately 9500 ha and is estimated to be 12 km wide and 11 km deep with its mouth facing in a South-Westerly direction. It has 884 ha of sea space, divided into three sections: Small Bay, Big Bay, and Outer Bay. Mussels and oyster farming already occupy half of the sea space. Plans are underway to establish new aquaculture which will be facilitated by the Department of Agriculture, Forestry and Fisheries under Operation Phakisa Delivery Unit, using the remaining sea space (Clark et al., 2018). Saldanha Bay is unique in global terms as it offers good protection for mussels and is one of the most nutritious sea environments in the world, with the Benguela current system offering the most nutritious water (Olivier et al., 2013). In aquaculture, the quality of the site depends on the quality of the water, protection from currents, absence of pollutants and good water circulation (Ferreira, 2016). An example of mussels (*Mytilus galloprovincialis*) farmed in Saldanha Bay is shown in figure 1.3.

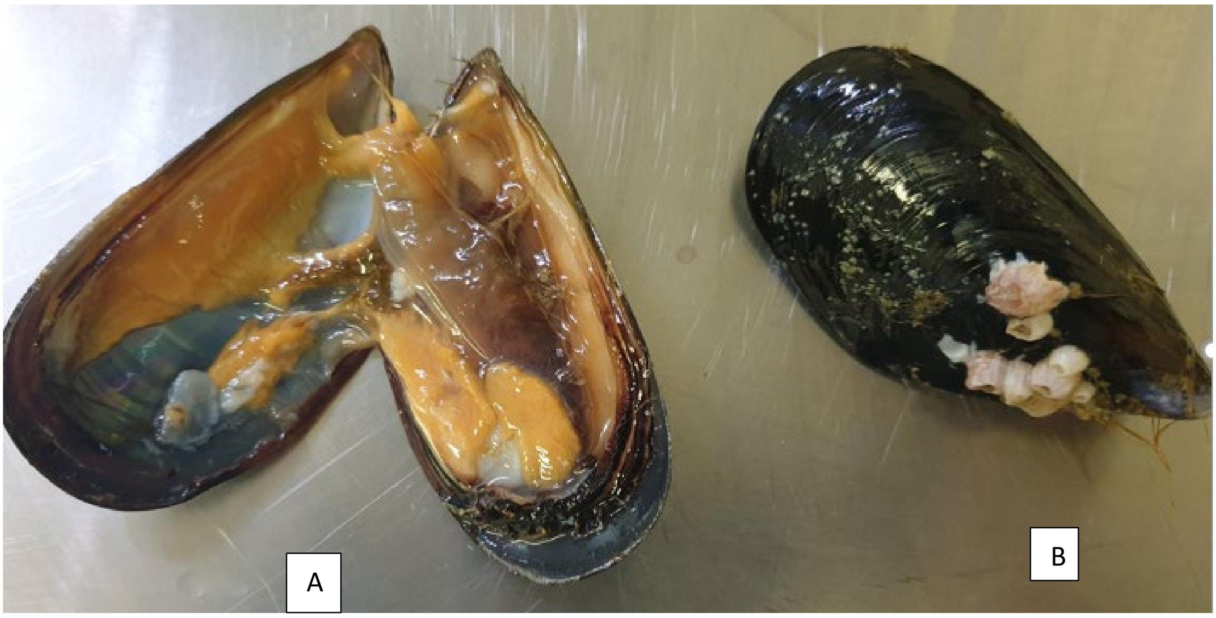

Figure 1.3 *(A) The flesh of the Mediterranean mussel (Mytilus galloprovincialis), (B) outer protective 2-valve shell*

Saldanha Bay has been classified as sheltered in terms of waves, speed and direction, hence the development of shellfish farming. Rainfall is mostly during the winter season (Firth et al., 2019).

The authorities conduct sampling throughout the year to test for heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyl (PCB). Tests for radionuclides are conducted every three years. The Saldanha Bay Mussel and Oyster Association conduct joint microbiological monitoring which tests for microorganisms such as *Escherichia coli, Salmonella, Vibrio parahaemolyticus and Vibrio cholerae*. Samples are collected throughout the year from selected sampling sites located in Small Bay, Big Bay, and Outer Bay. The presence of *Escherichia coli* is tested on a weekly basis for sampling sites located in Small Bay due to the close proximity to pollutant sources and then monthly at sampling sites located in Big Bay and Outer Bay. The presence of *Salmonella, Vibrio parahaemolyticus,* and *Vibrio cholerae* are tested for and monitored on a monthly basis. In addition, samples are collected on days after heavy rainfalls (>20mm rain) from all sites, and tested for *Escherichia coli* over a span of a few days following heavy rainfall (DFFE, 2021). On certain occasions, mussel tissue samples on which *Escherichia coli* analyses were conducted, exceeded the regulatory limit (230 MPN/100g) stipulated in the South African Molluscan Shellfish Monitoring and Control Programme. The maximum level on average was 1300 MPN/100g in 2016 and 3500 MPN/100g in 2018, resulting in a temporary closure of the affected farms (Sanitary Survey Report 2016, 2018). Pathogenic virus monitoring is not documented. Figure 1.4 indicates Saldanha Bay harbour, surrounding developments and conservation areas.

1.3 PROBLEM STATEMENT

Saldanha Bay is regarded as one of the areas highly favourable for the mariculture of shellfish such as oysters and mussels due to its natural characteristics (Olivier et al., 2013). The coastal upwellings bring cold nutrient-rich water to the surface during summer seasons that provide an environment favourable for phytoplankton growth and various pollutants that may be readily available to the bivalves. Oysters and mussels are filter-feeders and when grown in contaminated waters, might bioaccumulate human pathogens, thereby posing a potential risk of infection to consumers, especially when eaten raw or undercooked (Dabrowski et al., 2014; Olalemi et al., 2016; Campos et al., 2017; Fusco et al., 2017; De Sousa et al., 2018).

Figure 1.4 *Regional map of Saldanha Bay, Langebaan Lagoon and Danger Bay showing development (grey shading) and conservation areas (adapted from Clark et al., 2018)*

The challenges faced by the mariculture in Saldanha Bay are activities such as a large Port for the export of iron ore, ship traffic, ballast water discharge, oil storage and gas transport, dredging, several sewage pump stations, stormwater drains, industrial effluent (brine, cooling water discharges), biotoxins or algal blooms, and wastewater treatment works that discharge sewage a few kilometres from where the Bok River meets the Bay (Clark et al., 2018). For this study, potential threats in terms of microbiota due to sewage disposal were investigated. The shellfish farmers in Saldanha Bay intend to expand production and export their products to other regions internationally. For the exporting of products to be successful, these products will have to meet regional and international standards for food safety. One of the challenges that shellfish farmers are faced with is microbiological monitoring which indicates high concentrations of *E. coli* during some sampling occasions (Sanitary Survey Report, 2016). High concentrations of *E. coli,* which is used as an indicator organism for faecal contamination, render shellfish harvested in these production areas unsafe for human consumption (Lunestad et al., 2016). Therefore, investigations need to be conducted to determine the cause of contamination and the control measures that need to be taken to eliminate or reduce contamination by pathogenic microorganisms to acceptable levels.

1.3.1 Research question

What impact will the presence of pathogenic bacteria and viruses have on the primary production of mussels and oysters in Saldanha Bay?

1.3.2 Aim of the research

This study aims to investigate the sources of potential contaminants (microbiota) in particular during the primary production of mussels and oysters and to develop practical ways to eliminate or reduce risks related to sources of contamination and associated hazards to acceptable limits.

1.3.3 The specific objectives were to:

- Investigate the potential sources of contamination and associated hazards in Saldanha Bay.
- Evaluate the existing microbiological monitoring programme for *Escherichia coli* from 2017 to 2020.
- Assess the prevalence of bacteria related to the primary production of mussels and oysters.

- Conduct a systematic review of viruses related to the primary production of mussels and oysters.
- Conduct a gap analysis on compliance related to the primary production process for mussels and oysters based on national and/or international standards.
- Identify and recommend interventions to the industry towards compliance and risk mitigation.

1.4 ETHICAL CONSIDERATION

Permission to undertake the study was obtained from the Cape Peninsula University of Technology (CPUT) ethics committee (Appendix D). Permission was also obtained from the Department of Environment, Forestry and Fisheries (DEFF) (Appendix C).

1.5 DELINEATION OF THE RESEARCH

The study will only focus on seawater and shellfish (oysters and mussels) found in the three sections of Saldanha Bay harbour.

1.6 SIGNIFICANCE OF THE STUDY AND EXPECTED OUTCOMES

This study will be the first to focus on both bacterial and viral contaminants associated with shellfish production in Saldanha Bay. Furthermore, the study will provide a significant contribution related to the presence of human pathogenic microorganisms. It will serve as a risk assessment tool for the Department of Environment, Forestry and Fisheries. Section 1.6.1 shows the chapter layout of the study, with four articles to be published in peer-reviewed, internationally accredited journals.

1.6.1 Layout of the chapters

- CHAPTER 1: Introduction (brief historical overview and backdrop, problem statement, aims and objectives).
- CHAPTER 2: Literature review: Sources of contamination, hazards and applicable legislation impacting on the primary production of shellfish in the Saldanha Bay area, South Africa: A review.

- CHAPTER 3: Microbiological monitoring from 2017 - 2020 in Saldanha bay shellfish farms: an assessment of *Escherichia coli* prevalence in mussels and oysters.
- CHAPTER 4: The prevalence of bacteria commonly related to the primary production of mussels and oysters in Saldanha Bay. This chapter has been published in Aquaculture Research Journal as a research article.
- CHAPTER 5: The prevalence of viruses related to the production of mussels and oysters in Saldanha Bay: A systematic review. This chapter has been published in Aquaculture Journal as a review article.
- CHAPTER 6: South African and International aquaculture legislation and standards: A comparative gap analysis.
- CHAPTER 7: Conclusions and recommendations.
- Appendices (photographs, diagrams, letters, source documents).

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CHAPTER 2

SOURCES OF CONTAMINATION, HAZARDS AND APPLICABLE LEGISLATION IMPACTING ON THE PRODUCTION OF SHELLFISH IN THE SALDANHA BAY AREA, SOUTH AFRICA: A REVIEW

ABSTRACT

For successful aquaculture, the ideal site depends on the productivity and quality of the water, protection from ocean currents, the absence of pollution, and good water circulation. Saldanha Bay on the west coast of South Africa offers an ideal environment for the culture of shellfish as it is naturally sheltered from strong ocean currents and is highly productive owing to its linkage to the coastal upwelling system of the Benguela current. However, it is also surrounded by residential areas, industrial activities, and a wastewater treatment plant. This review addresses the challenges shellfish production in Saldanha Bay faces, which might affect the safety and suitability of shellfish for human consumption. Furthermore, it assesses water quality management and the proximity of production areas to sources of pollution or contamination. The primary concern is contamination by pathogenic microorganisms (bacteria, viruses, and protozoa) creating public health threats. It also considers the occurrence of chemical contaminants including heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and pesticides as well as biotoxin contaminants based on their poisoning symptoms (Diarrhetic Shellfish Poisoning, Amnesic Shellfish Poisoning, Paralytic Shellfish Poisoning, and Neurotoxic Shellfish Poisoning). Lastly, it explores the types of regulations pertinent to shellfish and shellfish production areas. The review indicates that, in the South African context, further research is required to attain the level of developed countries in managing shellfish production areas effectively.

Keywords: contaminants, shellfish, aquaculture, biotoxins

2.1 INTRODUCTION

Saldanha Bay is a naturally sheltered embayment on the west coast of South Africa thereby offering good protection for shellfish culture. It is also a highly productive system owing to its link to the coastal upwelling system of the Benguela current thereby providing an optimal environment for the farming of shellfish (Pitcher and Calder, 1998; Pitcher et al., 2015; Probyn et al., 2015). In aquaculture, the suitability of the site usually depends on good water circulation and protection from wind and waves, and the quality of the water, including the absence of pollution (Olivier et al., 2013). Unfortunately, Saldanha Bay is compromised by sewage pump stations and disposal, storm water discharge points, occasional dredging around the iron ore jetty, and residential and industrial activities. Such activities introduce contaminants including pathogenic microorganisms (bacteria, viruses, and protozoa), harmful chemicals (heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and pesticides). In addition, marine biotoxins that are produced by harmful phytoplankton species (mostly dinoflagellates) that occur naturally within the waters of the Benguela upwelling system. The primary concern in this review is the contaminants such as pathogenic microorganisms that could create public health threats. It has been shown that bivalves bioaccumulate and bioconcentrate suspended materials available in their growing waters, including pathogenic microorganisms (Kim et al., 2017; Vinothkannan et al., 2022). This could result in outbreaks of foodborne infections due to the consumption of contaminated shellfish, which is well documented in developed countries and other developing countries (Elbashir et al., 2018; Razafimahefa et al., 2019; Sarmento et al., 2020). No such studies seem to have been conducted on bivalve molluscs in Saldanha Bay. A microbiological monitoring programme has however, been run by the Department of Forestry, Fisheries and Environment (DFFE), testing for the prevalence of *Escherichia coli* (*E. coli)*, which is used as an indicator species for faecal contamination. The objective of this review is to identify possible contaminants in Saldanha Bay, their sources, and how it is dealt with in legislation governing food safety in South Africa and in other parts of the world. Ultimately, the aim is to expand the knowledge resulting from this study so that it will assist in developing solutions related to water quality management for the future expansion of the aquaculture industry.

2.1.1 Aquaculture

Seafood constitutes 16% of all global animal protein consumption, with an estimated 4.5 billion people relying on it as their main source of protein (Little et al., 2016). In Africa, fish consumption constitutes 36% of the protein intake, making it the most critical animal protein on the continent. It also supports the livelihoods of 12.3 million people, mainly within the low-income bracket (Chan et al., 2019). The top aquaculture producers in Africa are considered to be Egypt; Nigeria; Uganda; Ghana; Tunisia; Kenya; Zambia; Madagascar; Malawi; and South Africa (Adeleke et al., 2020). Aquaculture is considered the fastest-growing food production technology and is estimated to reach 93.6 million metric tons globally, in 2030 (Anderson et al., 2018).

The same trend has been recorded in South Africa as there has been an increase in shellfish production from 4808 tonnes in 2013 to 5418 tonnes in 2015, with abalone contributing 31%; mussels 23.2%; and oysters 5.8% (Adeleke et al., 2020). This steady growth in aquaculture globally has improved the quality of life for low to middle-income communities by supplying a cheaper source of high-quality protein as well as job opportunities (Toufique and Belton 2014; Fiedler et al., 2016; Ogello and Munguti 2016; Olaganathan and Kar Mun 2017; Mkuna et al., 2020; Reksten et al., 2020). The National Oceanic and Atmospheric Administration (NOAA) defines aquaculture as "the breeding, rearing, and harvesting of fish, shellfish, algae and other organisms in all types of water environment" (NOAA, 2021). The methods of aquaculture differ from species to species, on land in water tanks; freshwater ponds; in rivers; and in the open ocean. Furthermore, there are two types of aquacultures namely, freshwater aquaculture and marine aquaculture. This review is focusing on marine aquaculture as applied in Saldanha Bay. The bivalves are produced using suspension methods where seeded mussel ropes and oyster containers are suspended from longlines or rafts moored in a fixed position (Heinecken et al., 2017). However, inadequate location selection, leading to establishing farms near pollution sources, and ensuing ecological impacts may outweigh the benefits of such projects (Jeamsripong et al., 2018; Safford et al., 2019). Worldwide, the health threats related to the consumption of contaminated shellfish are of serious concern (Rees 2010; Goblick et al., 2011; Pouillot et al., 2015; Fauvel et al., 2017; Jeamsripong and Atwill 2019; Safford et al., 2019).

2.2 MATERIALS AND METHODS

This literature review was conducted using the Cape Peninsula University of Technology in Cape Town's facilities, South Africa's electronic database including Web of Science, Google Scholar, and Pubmed, and using the following keywords in accredited journal articles from 2010 to 2022. Information pre-2010 was however included concerning disease outbreak events. Keywords included shellfish production area contamination, bioaccumulation of pathogens by bivalve mollusks, and *E. coli* prevalence in mussel and oyster production areas, pathogen detection in shellfish and theoretical framework, pathogens associated with shellfish foodborne outbreaks, physicochemical parameters and shellfish production, Hepatitis A in mussels and oysters, food pathogens, norovirus in mussels and oysters, sewage and shellfish growing areas, marine biotoxins. These were classified according to the following headings and sub-headings: aquaculture, pathogenic microorganisms, viruses, bacteria, protozoa, chemicals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides, marine biotoxins, diarrhetic shellfish poison, paralytic shellfish poison, amnesic shellfish poison, neurotoxic shellfish poison, sources of contaminants, human activities and industrial activities, sewage discharges, regulations governing shellfish and shellfish production areas, EU shellfish regulations, US shellfish regulations and South African legislation.

2.3 RESULTS AND DISCUSSION

The next section elaborates on contaminants including pathogenic microorganisms; chemical pollutants; marine biotoxins; sources of these contaminants; and regulations governing shellfish and shellfish production areas. Table 2.1 shows the type of hazards associated with bivalve shellfish consumption.

Table 2.1: *Hazards to human health associated with the consumption of shellfish*

Type of hazards	Causative agent	Contaminants	Human health effects	References
Infections	Bacteria	*Salmonella spp., Shigella spp., Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae, Campylobacter spp., Listeria monocytogenes*	Abdominal cramps, diarrhoea.	(Arab et al. 2020)
	Viruses	Norovirus, Hepatitis *A*	Gastrointestinal infection, liver damage, abdominal cramps.	
Intoxication	Chemicals	Heavy metals: Mercury, Cadmium, Lead	Cardiovascular diseases, DNA damage.	(Baeyens et al. 2019)
		Organics: dioxins, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pesticides	Cancers, congenital disabilities, dysfunctional immune and reproductive system.	(Chang 2018)
	Biotoxins	Paralytic shellfish poisoning Diarrheic shellfish poisoning Amnesic shellfish poisoning Neurotoxic shellfish poisoning	Diarrhoea, short-term memory loss, respiratory distress, cardiac arrhythmia, paralysis of the chest and abdomen muscles leading to death.	(Mafra Jr et al. 2019)

2.3.1 Pathogenic microorganisms

Foodborne illnesses caused by microbiological hazards are a growing public health problem and of great concern to scientists as some illnesses may lead to the loss of human life (Li et al. 2020). Foodborne illnesses result from consuming food containing microbial agents such as bacteria, viruses, and parasites. Table 2.2 indicates the documented incidences of infectious diseases due to shellfish consumption. It is also important to classify pathogenic microorganisms as zoonotic, environmental, and contaminants to determine the levels of risk to consumers, which in turn would require different mitigation measures to render seafood safe.

Table 2.2: *A summary of microbial agents of foodborne diseases associated with shellfish consumption*

Microbial agent	No. of Outbreaks	No. of Cases	Geographical Location	Shellfish type	References
*Plesiomona Shigelloides***	1	36	US- North Carolina	Roasted oysters	(Rutala et al. 1982)
*Listeria monocytogenes***	1	4	New Zealand	Smoked mussels	(Brett et al. 1998)
*Salmonella enteritis***	4	106	New York	Oysters, mussels	(Wallace et al. 1999)
*Vibrio spp. ***	1	204	New York	Oysters	(Graczyk and Schwab 2000)
*Hepatitis A **	8	± 294 000	US, Italy, China, Australia	Clams, oysters, mussels	(Potasman et al. 2002)
*Norovirus***	1 1	202 127	Italy France	Oysters	(Le Guyander et al. 2006)
Vibrio parahaemolyticus	1	64	Spain	Oysters	(Martinez-Urtaza et al. 2016)
Vibrio parahaemolyticus	1	82	Canada	Oysters	(Taylor et al. 2018)
Norovirus	2	449	Canada	Oysters	(Meghnath et al. 2019)

*** Zoonotic pathogen, * Environmental pathogen*

2.3.1.1 Viruses

Viruses are tiny entities, ranging from 0.02 to 0.4 micrometres in diameter and are smaller than bacteria and parasites. Viruses can only reproduce within living host cells and therefore not in processed foods (Maalouf et al., 2010). However, some viruses remain infectious outside the host and are thus transported through the food chain. The most common foodborne viruses associated with shellfish consumption are the norovirus (NoV), (formerly known as Norwalk-like viruses (NLVs), and the hepatitis *A* virus *(*Hodgson et al., 2017). The NoV is a genus in the family *Caliciviridae* of single-stranded polyadenylated RNA viruses. They are classified into genogroups GI – GVI and possibly seventh genogroup GVII. Genogroups GI and GII are known to infect humans (Younger et al., 2020). These genogroups are further divided into 38 genotypes (Kroneman et al., 2013). The NoV genome contains three open reading frames (ORF1, ORF2, and ORF3) (Koo et al., 2017). A new novel strain of NoV namely GII.17 Kawasaki, which has replaced GII.4 Sydney 2012, has been reported in several countries, including Japan, Hong Kong, Taiwan, North America, Australia, France, Italy, the Netherlands, New Zealand, and Russia (De Graaf et al., 2015; Lu et al., 2015; Medici et al., 2015; Lee et al., 2015; Koo et al., 2017; La Rosa et al., 2017; Chhabra et al., 2019; Chan et al., 2019).

The NoV is transmitted through person-to-person contact and consumption of contaminated food and drinking water (Campos et al., 2017b; Hassard et al., 2017). In the United States of America (USA), NoV is reported to be responsible for 58% of foodborne illnesses (Woods et al., 2016). In the United Kingdom (UK), 76% of oysters sampled from commercial productions tested positive for the presence of NoV, according to a study conducted by Campos et al. (2017a). These authors (EFSA 2012; Polo et al., 2015) reported that NoV does not grow *in vitro*, and its detection in food matrices is based on molecular techniques. The laboratory detection methods available include Electron Microscopy (EM) and an enzyme-linked immunosorbent assay (ELISA). However, these can detect clinical samples but not environmentally contaminated samples. The reverse transcription-polymerase chain reaction (RT-PCR) is the only published assay that may detect NoVs directly from environmental samples such as bivalve contamination (Izumiya et al., 2011). Norovirus is persistent in shellfish tissues and cannot be effectively removed by depuration (Rodriguez-Souto et al., 2017; Brake et al., 2014; Yu et al., 2015; La Bella et al., 2017; Sferlazzo et al., 2018). The prevalence of viral pollutants in Saldanha Bay, is unknown as monitoring of viruses is not conducted (Sanitary survey report, 2018).

Hepatitis *A* (HAV) belongs to the *Picornaviridae* family and can be classified into six genotypes. It has a single-stranded positive-sense RNA genome of 7 to 8kb and is responsible for acute human viral hepatitis. Genetic heterogeneity of HAV allows classifying HAV strains into seven different genotypes, namely: I-VII. Genotypes I and III have been further divided into sub-genotypes A and B (Goblick et al., 2011). Outbreaks caused by HAV are reported less frequently, but the effect caused by the disease is more severe. The illness has a long incubation period (2 to 8 weeks) which can make the identification of the source of infection difficult (Iwamoto et al., 2010). High-risk factors for HAV infection are cold weather (low seawater temperatures) and a lack of ultraviolet (UV) light (Wang and Deng 2016; Biswas et al., 2018). Hepatitis *A* can persist in estuaries, marine waters, and in shellfish from a few days to several months (Goblick et al., 2011; Fusco et al., 2017). Field data on the time that HAV persists in shellfish as well as for environmental spread are limited. The same applies to the information on HAV contamination for different types of shellfish species, water bodies, and environmental conditions (Brake et al., 2018). Hepatitis *A* is found in coastal waters as a result of the release of human faecal pollutants caused by disrupted wastewater treatment works or the leaching of agricultural soil treated with sewage sludge (Adefisoye et al., 2016). As with the NoV, HAV does not grow *in vitro,* and its detection in food matrices is based on molecular techniques. It is less common in countries with a high standard of hygiene (Polo et al., 2015).

Detection of these viruses in shellfish remains challenging despite *E. coli* and faecal coliforms being used as indicator species for contamination of bacteria and viruses. As bacterial standards do not always reveal the presence of viruses, it has been proposed that indicators of viral faecal pollution be improved for effective microbiological control of shellfish production. The use of bacteriophages as a potential indicator of infectious viruses has been documented by various researchers (Doré et al., 2000; Formiga-Cruz et al., 2003; Da Silva et al., 2015; Hartard et al., 2016; Olalemi et al., 2016; Woods et al., 2016; Fauvel et al., 2017; Hodgson et al., 2017). Several studies have used bacteriophages to model viral removal from shellfish during depuration. Some authors found that the F-RNA bacteriophages are removed from the digestive tract of contaminated shellfish at a slower rate than *E. coli*. The slow elimination kinetics appears to be representative of the elimination kinetics of human enteric viruses (Hartard et al., 2016; Hodgson et al., 2017). This demonstrates the significance of having viral pollutant indicators as viruses take longer to be purged compared to bacteria.

2.3.1.2 Bacteria

Bacteria are single-celled microorganisms with a cell wall but no nucleus. They exhibit a variety of shapes, types, and properties. In contrast to viruses, bacteria can be seen with a conventional microscope. Bacterial cells multiply when each cell divides into two. These grow to full size and split into two again (two-fold division/binary fusion). Unlike viruses or parasites, bacteria can multiply in or on food. The most common foodborne bacteria associated with shellfish consumption include *Escherichia coli, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae, Salmonella spp., Campylobacter spp., Plesiomonas spp., Aeromonas spp., Listeria monocytogenes, and Klebsiella spp.* (Pereira et al., 2015; Raszl et al., 2016; Walker et al., 2018).

Escherichia coli is commonly used as indicator species for faecal contaminants present in bivalve growing waters (Doré et al., 2000; Kim et al., 2017) and recreational waters (Baliere et al., 2016; Lunestad et al., 2016). It is also found in the lower intestine of warm-blooded organisms as well as the digestive organs of bivalves (Lunestad et al., 2016). Shiga-toxin-producing *E. coli* (STEC) is a bacterium that can cause severe foodborne diseases (Lee and Yoon, 2021). Primary sources of STEC outbreaks are raw or undercooked ground meat products, raw milk, and vegetables. Five major serotypes responsible for most cases and outbreaks worldwide, include 0157:H7; 026:H11; 0103:H2; 0111:H8, and 0145:H28. *Escherichia coli* 0157:H7 is the STEC serotype most critical for public health (Baliere *et al.*, 2016). However, while the serotype 0157:H7 has previously been detected in shellfish, the other four STECs have not yet been reported as being involved in shellfish-borne outbreaks (Baliere et al., 2015). The Saldanha Bay microbiological monitoring programme follows the European

Union regulations and therefore uses *E. coli* as an indicator species for faecal contamination.

The genus *Vibrio* is a group of gram-negative bacteria with more than 30 species. They are ubiquitous in aquatic environments. At least 12 *Vibrio* species are pathogenic to humans while *Vibrio cholerae, Vibrio parahaemolyticus,* and *Vibrio vulnificus* have been associated with foodborne diseases due to consumption of raw or undercooked shellfish (Izumiya et al., 2011; Raszl et al., 2016; Xu et al., 2017). *Vibrio cholerae* consists of two serotypes, namely 01 and 0139, responsible for cholera, which is an acute intestinal infection caused by the ingestion of contaminated food or water. *Vibrio vulnificus*, another pathogenic species found in seawater, can cause gastrointestinal diseases and septicaemia. It severely affects children and immune-compromised patients (Izumiya et al., 2011; Xu et al., 2017). *Vibrio spp.* are responsible for numerous human cases of seafood-borne illnesses in many Asian countries and the USA (Elbashir et al., 2018). The consumption of raw or undercooked seafood is responsible for 95% of all seafood-related deaths in the USA (Scro et al., 2019), and in Italy, Spain, and France it is also considered a growing public health threat (Sferlazzo et al., 2018; Lamon et al., 2020).

Vibrio parahaemolyticus is a halophilic bacterium. It is naturally present in areas used to grow and harvest shellfish and is considered part of the autochthonous microflora in estuarine and coastal environments (Cruz et al., 2015; Raszl et al., 2016). Its prevalence and abundance are often influenced by two significant factors; temperature and salinity (Mannas et al., 2014). It has not yet been detected during microbiological monitoring in Saldanha Bay. Clinical characteristics are abdominal cramps, diarrhoea, nausea, headaches, and fever (Raszl et al., 2016). The presence of this bacterium is also influenced by climate change as seawater temperatures have increased; its adaptation to cooler water; the emergence of new strains; and a typical distribution due to ballast water from one area to another (Cruz et al., 2015). Although scant data exist regarding *Vibrio parahaemolyticus* infections in humans, a notable increase in worldwide incidences have been reported. In Asia, *Vibrio parahaemolyticus* is a common cause of foodborne disease, responsible for 20 to 30% of cases, and in

European countries, several outbreaks have also been reported (Terio et al., 2014). It is a leading cause of gastroenteritis in the USA. However, no foodborne incidences due to the consumption of contaminated shellfish by *Vibrio* species have been reported in South Africa. The challenge is whether shellfish harvested is free of contaminants or there is a problem of under-reporting or non-reporting of cases.

The genus, *Salmonella,* consists of gram-negative bacilli and belongs to the Enterobacteriaceae family. *Salmonella* is a ubiquitous and hardy bacterium that can survive several weeks in a dry environment and several months in water. However, it is not considered a natural inhabitant of marine and estuarine environments but is often reported in high numbers in coastal waters because of sewage discharge (Gyawali and Hewitt, 2020). Although *Salmonella spp*. is considered one of the most common causes of human gastroenteritis, the risk of foodborne diseases associated with mollusc consumption is low compared to viruses and naturally occurring *Vibrios* in marine environments. Seafood can become contaminated with *Salmonella* during storage and processing. Increased *Salmonella* infections have been reported since 1980, and many of the incidents have been associated with the consumption of raw or undercooked shellfish (Lunestad et al., 2016; Campos et al., 2017; Fusco et al., 2017). *Salmonella* is also a significant pathogen that causes acute foodborne outbreaks worldwide. In the EU, 88,715 people were hospitalised due to salmonellosis in 2014 (Gao et al., 2018). Within two species, *Salmonella bongori* and *Salmonella enterica*, over 2500 different serotypes or serovars have been identified to date. *Salmonella enterica* has more than 99% of the serotypes, some of which are pathogenic to humans and cause most infections (Hariharan and Amadi 2016; Gao et al., 2018; Rubini et al., 2018). *Salmonella enterica* serotype *typhi* is associated with outbreaks of seafood illnesses linked to polluted waters (Rubini et al., 2018).

2.3.1.3 Protozoa

Protozoan parasites are single-celled microorganisms without a rigid cell wall but with an organized nucleus. They are larger than bacteria and viruses, with dimensions usually greater than 10 micrometres (µm). Like viruses, they do not multiply in foods but only in their hosts. Protozoa associated with food and water-borne infections include *Entamoeba histolytica, Toxoplasma gondii, Giardia lamblia, Cryptosporidium parvum, Giardia enterica,* and *Cyclospora cayatenensis.* These parasites only cause infection, not toxicity. *Cryptosporidium parvum*, *Giardia enterica,* and *Toxoplasma gondii* have been found in shellfish (Ghozzi et al., 2017; DeMone et al., 2020) and can survive in seawater for six months up to two years (Tedde et al., 2019). *Cryptosporidiosis*, *Toxoplasmosis*, and *Giardiasis* cause gastrointestinal disease and could result in severe symptoms in children and immunocompromised individuals. Transmission occurs through the faecal-oral route resulting from the consumption of contaminated shellfish (DeMone et al., 2021). Even though outbreaks have also been reported in several countries worldwide (Cong et al., 2021), underreporting of mild cases; difficulty in determining the source of foodborne illness; and lack of standardised screening methods remain a challenge (Ree 2010; Moratal et al., 2020).

2.3.2 Chemicals

2.3.2.1 Heavy metals

The following heavy metals are essential for a living organism's growth and become toxic only above a certain threshold level: copper (Cu); chromium (Cr); zinc (Zn); and iron (Fe). By contrast, metals such as mercury (Hg); arsenic (As); cadmium (Cd); and lead (Pb) are highly toxic (Milun et al., 2016). That is the case even though metal toxicity levels differ for different living organisms. Metals that are considered essential to one aquatic organism might be toxic to another even at low concentrations (Delgado-Alvarez et al., 2019). Heavy metals such as arsenic (As); copper (Cu); chromium (Cr); mercury (Hg); and zinc (Zn) as well as nickel (Ni) and silver (Ag) occur naturally in the earth's crust. Their abundance in the marine environment and availability due to anthropogenic activities, however, raises concerns due to possible toxic effects on filter-feeding organisms and subsequent threats to human health. Although there are no known short-term health effects due to the consumption of contaminated shellfish, the possible long-term health consequences, including cancer, should not be overlooked despite a lag in symptom presentation and challenges with tracing it to the source (Baeyens et al., 2019). Semi-enclosed embayments such as Saldanha Bay, are exposed to various pollutants, including heavy metals. Such heavy metals are introduced into the bay through sources such as industrial effluent; urban land run-off;

mining operations; atmospheric emissions; stormwater drainage input; and increasing human population. The local authority, the Saldanha Bay Municipality, conducts annual heavy metal sampling through the Department of Environmental Health for among others, lead (Pb); mercury (Hg); and cadmium (Cd) (Sanitary survey report, 2018). Firth et al. (2019) conducted a study in Saldanha Bay which raised concerns about several heavy metal-related issues. This includes fewer studies being conducted in the area; seasonal variations in the accumulation of heavy metals by mussels farmed in the area; high concentrations of aluminium and cadmium, above the permissible limits compared to international studies.

2.3.2.2 Polycyclic aromatic hydrocarbons (PAHs)

In simple terms, polycyclic aromatic hydrocarbons (PAHs) are chemicals produced when coal, oil, gas, and wood (to mention a few) are burned. El Nemr et al. (2016) defined PAHs as organic compounds resulting from the combustion of organic matter, oil, and its sources. Ofori et al. (2020) in their study, classified PAHs as Lower Molecular Mass (LMM) PAHs and Higher Molecular Mass (HMM) PAHs. They further indicated the adverse effects and bioaccumulation effects of lower molecular PAHs by marine organisms; the hazardous and carcinogenicity of HMM PAHs to humans; wildlife; and its persistence in marine environments. Furthermore, PAH adsorption into suspended particulate matter is facilitated by mechanically dispersed oil in the marine environment (El Nemr et al., 2016; Replinger et al., 2017; Ofori et al., 2020). Although widely distributed, these days, PAHs are primarily found in marine environments due to anthropogenic sources around the coastal environments (Akhbarizadeh et al., 2019) and have been listed by the United States Environmental Protection Agency (USEPA), as priority pollutants. They are insoluble in water; non-biodegradable; and may lead to toxic bioaccumulation; and biomagnification in aquatic ecosystems (Ramesh et al., 2011). High PAH levels have been found in Saldanha Bay and are speculated to be the result of the mining of zircon; rutile; ilmenite; steel manufacturing; and crude oil imports surrounding the bay (Firth et al., 2019). The PAHs that have been detected in mussels farmed in Saldanha Bay include fluorene, fluoranthene, benzo(a)anthracene, pyrene, and benzo(a)pyrene. Compared to the other compounds, fluorene showed the highest concentrations. The source is suspected to be petrogenic due to the ship traffic in the area (Rufina et al., 2017; Firth et al., 2019).

2.3.2.3 Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls are part of toxic organic pollutants. They are persistent in the environment, they have a high affinity for sediments in the marine environment (Arblaster et al., 2015; Qian et al., 2017) and have been detected in plastic pellets washed onto beaches (O'Donovan et al., 2018). They are classified as hazardous waste elements found in urban and industrial waste and are deemed poisonous and infectious. Prolonged exposure can have chronic health effects including cancer, birth defects, and dysfunctional immune and reproductive systems (Olenycz et al., 2015; Fergusson, 2017; Milun et al., 2020). Some studies have documented changes such as inflammation; degenerative lesions; and reduced fecundity in the internal organs of aquatic animals due to exposure to PCBs (Oosthoek, 2015; Debipersadh et al., 2018). They are hydrophobic in nature, they mix with organic particles suspended in water columns and are deposited in sea sediments (O'Donovan et al., 2018). Marine bivalves' filter-feeding nature makes them susceptible to various chemicals in the water, including PCBs (Ghribi et al., 2020). Firth et al. (2019) detected ten PCBs within the flesh of mussels farmed in Saldanha Bay. However, the concentrations were below the maximum limits of the EU and the US standards. Seafood consumption is regarded as the main route of exposure for humans (Qian et al., 2017; Habibullah-Al-Mamun et al., 2019; Tsutsumi et al., 2021). South Africa is among 152 signatories of the Stockholm convention adopted in 2001, which banned the production and use of PCBs. There is progress in this regard as the government gazetted a regulation that prohibited the use of PCB materials after the year 2023 (Regulation R549, 10 July 2014). This is expected to reduce the organic pollution loads and assist in ensuring sustainable aquaculture in Saldanha Bay and the country.

2.3.2.4 Pesticides

Pesticides are present in marine environments as a cocktail of hazardous chemicals (Cuevas et al. 2018). What adds to the complexity of pesticide pollution is the combined effects of this concoction being worse than each compound on its own. Insufficient and ineffective monitoring of pesticides in coastal waters is amplified by factors such as the natural biological and physicochemical complexity of transition ecosystems; multiple sources of contaminants; and pesticides' ability to mix with other pesticides and form a highly toxic compound (Cuevas et al., 2018). Pesticides are classified into five categories: organochlorine; organophosphate; carbamate; pyrethrin; and pyrethroid (Hassaan and El Nemr 2020). Organochlorine pesticides (OCPs) such as Aldrin, chlordane, dieldrin, and dichlorodiphenyltrichloroethane (DDT) in particular, are persistent and resistant to degradation. Due to their lipophilic and hydrophobic nature, they bioaccumulate in living and dead material; marine organisms; and seafood (Saber et al., 2018; Li et al., 2021). The concern is that these chemicals can cause adverse health effects such as endocrine disruption, neurotoxicity, and cancer in humans and animals (Chang, 2018).

2.3.3 Marine biotoxins

It is known that shellfish accumulate and retain contaminants, including biotoxins such as Azaspiracid (AZA); Domoic acid (DA); and Okadaic acid (OA) to name a few, in their bodies. Climate change and increased amounts of nutrients in the sea are raising concerns regarding biotoxins in shellfish-growing waters. According to Lee et al. (2016) and Wang et al. (2022), an increase in toxic microalgal species has been observed in temperate areas due to rising ocean temperatures and eutrophication. Gvozdenovic et al. (2015) define natural biotoxins as planktonic algae products produced in defence against predators. Because of their filter-feeding nature, bivalve shellfish can accumulate biotoxins in large amounts, providing early warnings of harmful algae bloom toxins. As a result, they are ideal candidates for biotoxin monitoring programmes (Grizzle et al., 2018). The concentrations of biotoxins in shellfish are observed during algal blooms, which often pose a health risk to humans and the rest of the food chain. This can lead to a temporary closure of farms leading to financial losses (Schmidt et al., 2018). Based on their chemical structures, marine biotoxins are classified into eight groups, namely, Azaspiracid (AZA); Brevetoxin (BTXs); Cyclic imine (SPX); Domoic acid (DA); Okadaic acid (OA); Pectenotoxin (PTX); Saxitoxin (STX); and Yessotoxin (YTX) (Abraham et al. 2021). When classified according to their poisoning symptoms, biotoxins are classified as toxins causing diarrhetic shellfish poison (DSP); amnesic shellfish poison (ASP); paralytic shellfish poison (PSP); and neurotoxic shellfish poison (NSP) (Farabegoli et al., 2018). Saldanha Bay falls within the Benguela upwelling system that is prone to a high incidence of harmful algal blooms (Pitcher and Louw, 2021). The greatest concerns are the PSP caused by *Alexandrium catenella*, DSP caused by Dinophysis species and *Protoceratium reticulatum* blooms causing YTXs (Pitcher et al., 2020). A well-established marine biotoxin monitoring program exists in Saldanha Bay coupled with substantial research being conducted (Pitcher and Jacinto, 2019; Tamele et al., 2019; Pitcher et al., 2020; Smith and Bernard, 2020; Pitcher and Louw, 2021).

2.3.3.1 Diarrhetic shellfish poisoning (DSP)

Diarrhetic shellfish poisoning is a gastrointestinal disorder caused by the consumption of seafood contaminated with okadaic acid (OA) and dinophysistoxins (DTXs) (Dominguez et al., 2010; Lee et al., 2016). It is caused by several species of dinoflagellates belonging to the genera *Dinophysis* and *Prorocentrum* (Campos et al., 2020). Its occurrence is increasing worldwide, rendering it a threat to public health and a risk to food security (Braga et al., 2018). According to Emery et al. (2021) cooking and freezing do not affect the structural integrity of these biotoxins. This is a concern even though no fatalities have been recorded to date. Furthermore, affected people present with symptoms such as diarrhoea; nausea; vomiting; and abdominal pain (Mafra Jr et al., 2019). Okadaic acid and DTXs are the most widespread and have been implicated in poisoning outbreaks worldwide (Hinder et al., 2011; Ciminiello et al., 2014). The outbreaks have occurred in Europe, North America, South Africa, Asia, Australia, and New Zealand (Lee et al., 2016; Young et al., 2019; Campos et al., 2020).

2.3.3.2 Paralytic shellfish poisoning (PSP)

Paralytic shellfish poisoning is caused by a group of more than fifty marine toxins produced by dinoflagellates of the genus *Alexandrium*, *Gymnodinium,* and *Pyrodinium* with saxitoxin (STX) as a parent compound *(*Arnich and Thébault 2018; Silva et al., 2018; Dell'Aversano et al., 2019). Symptoms vary from vomiting, nausea, abnormal speech, difficulty in swallowing, numbness in the extremities, muscular paralysis, and tingling in the face, to respiratory distress and are often fatal in humans (Knaack et al., 2016; Turner et al., 2019; Raposo et al., 2020). Like other biotoxins, consumption of contaminated shellfish results in intoxication, not an infection (Farabegoli et al., 2018).

Outbreaks have been reported worldwide, triggering the need to strengthen statutory safety requirements for environmental and human protection (Burrell et al., 2016).

2.3.3.3 Amnesic shellfish poisoning (ASP)

The causative toxin in amnesic shellfish poisoning is domoic acid (DA), produced by *Pseudo-nitzchia*. It is characterised by symptoms such as vomiting, abdominal pain, diarrhoea, memory loss, and death (Johnson et al., 2016; Lopes et al., 2018). Domoic acid intoxication is not limited to humans. It has also been detected in seabirds, fish, and mammals in coastal waters (Rowland-Pilgrim et al., 2019). Outbreaks have been reported in Canada and North America and has led to the closure of shellfisheries in the United Kingdom (Johnson et al., 2016; Rowland-Pilgrim et al., 2019).

2.3.3.4 Neurotoxic shellfish poisoning (NSP)

Neurotoxic shellfish poisoning is caused by the consumption of shellfish contaminated with brevetoxins (BTXs) produced by dinoflagellate *Karenia brevis*. The exposure to humans is mostly through contaminated oysters, mussels, and clams (Farabegoli et al., 2018). Patients present with symptoms such as nausea; diarrhoea; vomiting; myalgia (muscle pain); seizures; and ataxia (loss of coordination). Despite reports of outbreaks worldwide, there is very little epidemiological data on NSP available, possibly because of under-reporting or misdiagnosing of patients (Abraham et al., 2021). South Africa uses the EU and the US regulatory standards for biotoxin monitoring, and the regulatory limits are listed in Table 2.3.

Table 2.3: *Regulatory limits for biotoxins in bivalve molluscs, adapted from EU and USA (EFSA, 2008)*

Toxin Group	EU Limits	US Limits
Diarrhetic shellfish poison (DSP)	160 mg/kg SM	0.16 mg/kg
Paralytic shellfish poison (PSP)	800 mg/kg SM	0.16 mg/kg
Amnesic shellfish poison (ASP)	20 mg/kg SM	≥ 20ppm
Neurotoxic shellfish poison (NSP)	0.8 mg/kg SM	20MU/100g

SM = shellfish meat

2.3.4 Sources of contaminants

2.3.4.1 Human activities and industrial activities

Saldanha Bay has seen an increase in industrial activities, commercial activities, and tourism since the beginning of the 20th century. Presently, Saldanha Bay is recognized as an international port that accommodates ore carriers and deep-sea trawlers. Metal ores that are exported include iron; lead; copper; zinc; and manganese (Henrico and Bezuidenhout, 2020). This has been accompanied with a rise in the human population figures at a growth rate of 2.7% per annum from 2001 to 2011, well above the average influx rate for a small town (Olivier et al., 2013). In turn, with population growth increased development has been observed, including private and industrial building infrastructure; roads; rail networks; commercial and recreational fishing; and tourism amenities. This has added significant pressure to the provision of municipal services and infrastructure such as wastewater treatment and stormwater drainage systems. Naturally, this has affected marine resources. Pollution levels in the bay have escalated for several reasons, for instance, ballast water discharges, oil spills, and increased sewage discharge from households and surrounding industries. While fish processing plants dispose of brine, cooling water discharges and fish factory effluent in the bay, the growth of mariculture farms, and the upgrade and extension of the iron ore jetty have also had an impact on the situation (Firth et al., 2019). Furthermore, dredging the bay has severely impinged on suspended feeding organisms. When sunlight penetration becomes limited, algae; phytoplankton, and other particles that have settled with sediment become readily available for bioaccumulation by suspended feeders. The result is the die-off of fish (Clark et al., 2020).

2.3.4.2 Sewage discharges

Globally, it is well documented that shellfish grown in seawater where sewage disposal occurs, poses a human health threat (Lees 2000; EFSA 2012; Wang and Deng 2016; Campos et al., 2017a; Campos et al., 2017b; Koo et al., 2017). Factors that lead to this type of pollution include the type of location, levels of treatment at sewage plants, and the quantity of sewage. It is exacerbated by malfunctions at wastewater treatment works (WWTW) and heavy rainfall causing an overload of the system resulting in pre-treatment sewage discharge and stormwater overflow (Lunestad et al., 2016). Also, WWTW in Saldanha Bay, as in the rest of South Africa, are currently not designed to remove viruses during the treatment process (Maalouf et al., 2010; Hassard et al., 2017). Investigations into outbreaks associated with shellfish conducted internationally and reviewed by Bellou et al. (2013), classified foodborne outbreaks as follows: 83.7% were caused by NoV; 12.8% by hepatitis A; and 58% were due to the consumption of contaminated oysters. Shellfish poisoning outbreaks have often been attributed to water contaminated by sewage (Lunestad et al., 2016; Olalemi et al., 2016; Strubbia et al., 2016; Fusco et al., 2017; Hodgson et al., 2017; Sferlazzo et al., 2018; Walker et al., 2018).

In 2012, the EFSA concluded that the most effective control measure is to produce shellfish in waters that are not faecally contaminated. However, this is not feasible for many countries involved in shellfish farming including South Africa. Saldanha Bay is exposed to sewage discharge and wastewater generated by food processing waste; brewing and distillation waste; paper pulp milling waste; and chemical industry waste. Wastewater effluents have high concentrations of nutrients such as nitrates and phosphates. These nutrients stimulate the growth of algae such as phytoplankton which could ultimately lead to oxygen depletion in receiving waters (EFSA, 2012).

Moreover, Saldanha Bay receives treated effluent from two wastewater treatment works, one in Saldanha, and the other in neighbouring Langebaan, close to the mouth of the bay. According to the South African National Water Act no. 36 of 1998, Saldanha Bay is licensed to treat effluent not exceeding 958 000 m^3 per annum. However, in 2008 the daily average volume was 2625 m^3 with a gradual increase recorded at 3452 m^3 in 2014 (Wright et al., 2018; Clark et al., 2020). On occasion, up to 16 sewage pump stations have been reported to overflow due to malfunctions or power failures (Clark et al., 2020). In such cases, raw sewage was released directly into the bay, compromising the water quality needed for aquaculture. More recently, the Saldanha Bay municipality, in collaboration with local businesses, has started recycling treated effluent. In June 2018, most of the treated effluent from the treatment works was being used for irrigation of sports grounds and a golf course, while excess is discharged into the Bok River which ultimately joins the bay (Clark et al., 2020).

2.3.5 Regulations governing shellfish and shellfish production areas

Countries may opt to subscribe to international standards and/or organisations that develop acceptable standards for all member countries. Member countries, as well as other countries, wanting to import and export products from such regions are obliged to comply with these policies. Each country also sets national standards and regulations such as the national acts and regulations. South Africa is also governed by regional standards and regulations for food safety and the aquaculture industry.

The following international standards have been developed by the Codex Alimentarius and facilitate international trade for shellfish amongst countries and regions: 1)The standard for live and raw bivalve molluscs – Code Stan 292- 2008; 2) Standard for crackers from marine and freshwater fish, crustaceans and molluscan shellfish – Code Stan 222- 2001; 3) Guidelines on the application of general principles of food hygiene to the control of pathogenic vibrio species in seafood – CAC/GL – 73- 2010; 4) Guidelines on the application of general principles of food hygiene to the control of viruses in food – CAC/GL – 79- 2012; 5) Guidelines on the application of general principles of food hygiene to the control of foodborne parasite – CAC/GL – 88 - 2016; 6) General principles of food hygiene- CXC – 1 -1969; and 7) Code of practice for fish and fishery products – CXC – 52 – 2003 (Torok et al., 2018; Kapthouang Tchatchouang et al., 2020).

Worldwide, countries involved in mariculture and aquaculture set regulations for microbiological monitoring of production areas. However, for import and export purposes, EU and USA standards and regulations are predominantly used as a benchmark. These standards provide a measure for the sanitary quality of the shellfish production areas and the classification of shellfish growing areas. The EU countries make use of *E. coli* as an indicator species, whereas the USA uses faecal coliforms as indicator species. The latter also prohibits the disposal of sewage adjacent to the production areas, specifying a buffer zone of 1000:1 dilution of estuarine water to treated effluent (Campos et al., 2017). However, as mentioned earlier, *E. coli* and faecal coliforms have been proven as efficient indicators of viral contamination hence the momentum building to develop standards to address viral pathogen indicators (Lees, 2010; EFSA, 2012; Hellmér et al., 2014; Strubbia et al., 2016; Campos et al., 2017).

2.3.6 European Union shellfish regulations

European legislation classifies the molluscan shellfish harvesting areas into three categories based on *E. coli* level safety for human consumption (De souza et al., 2019). Class A shellfish is safe to be consumed directly after harvesting. Class B shellfish with a sample not exceeding the limits of a five-tube, three-dilution MPN test of 4 600 *E. coli* per 100g of flesh and intravalvular liquid must undergo a relay treatment before human consumption. Class C shellfish, relaying for an extended period is needed before humans are allowed to consume it. In this case a sample may not exceed the limits of a five-tube, three-dilution MPN test of 46 000 *E. coli* per 100g of flesh and intravalvular liquid (Commission and (EU)) (EU 854/2004). Table 2.4 shows the classification of shellfish growing areas and permissible limits.

Table 2.4: *European Union classification of shellfish harvesting areas*

Indicator species	**Growing area Category**	**Permissible limits**
E. coli	Class A	≤230 *E. coli* MPN/100g of flesh and intravalvular liquid in 80% of samples. The remaining 20 % must not exceed 700 *E. coli* MPM/100g of flesh and intravalvular liquid.
	Class B	≤4,600 *E. coli* MPN/100g of flesh and intravalvular liquid in 90% of samples. The remaining 10% must not exceed ≤46,000 *E. coli* MPN/100g of flesh and intravalvular liquid.
	Class C	Maximum ≤46,000 *E. coli* MPN/100g of flesh and intravalvular liquid.

(Adopted from De Souza et al. 2019 and modified)

2.3.7 United States of America shellfish regulations

In the US, the national shellfish sanitation program (NSSP) provide guidelines for controlling molluscan shellfish contamination in shellfish growing areas; the classification of growing areas; and the bacteriological water quality limits, as shown in Table 2.5. The US Food and Drug Administration (FDA) recommends a minimum of 1000:1 dilution for shellfish growing area classification adjacent to Wastewater Treatment Works discharges. Harvesting shellfish from areas with a dilution of less than 1000:1 should be prohibited (Goblick et al., 2011).

Table 2.5: *United States criteria for the classification of shellfish harvesting areas*

Indicator species	Growing areas categories	Permissible limits
Faecal coliform	Approved areas	<230 / 100ml water
	Restricted areas	<2300 / 100ml water
	Prohibited areas	harvesting not permitted

(Source: NSSP, 2017)

2.3.8 South African legislation

Various Departments that govern food safety in this country apply national and provincial legislation, regulations, and municipal by-laws. South African shellfish production partially follows EU standards for microbiological monitoring of shellfish and the water for growing purposes. Production areas are classified as Approved areas (class A); Restricted areas (class B); Prohibited areas (class C); and Conditional areas (temporary class A or B) (DAFF, 2018). To determine the suitability of a production site for harvesting, a sanitary survey must be conducted before approval for a new production area is given. The sanitary survey assesses the suitability and safety of the area for shellfish production.

Shellfish harvested from a class A growing area may not exceed the limit of 230 *E. coli* per 100g of flesh and intravalvular liquid in 80% of the samples. Shellfish growing in a class B area may not exceed 4 600 *E. coli* of flesh and intravalvular liquid in 90% of samples. Class C samples may not exceed 14 000 *E. coli* per 100g of flesh and intravalvular liquid. Shellfish harvested from classes B and C must undergo relaying and depuration before human consumption (SAMSM&CP, DAFF, 2012). Table 2.6 lists South African legislation relevant to shellfish production and safety for human consumption.

Table 2.6: *List of South African legislation for shellfish safety*

- Marine Living Resources Act, 1998 (Act No.18 of 1998).
- Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972).

  Regulation R638 of 2018, promulgated under Government Gazette No.41730, 22 June 2018.
- National Regulator for Compulsory Specifications Act, 2008 (Act No. 5 of 2008).

  Compulsory Specification for the Manufacture, Production, Processing and Treatment of Canned Fish, Canned Marine Molluscs and Canned Crustaceans. Regulation 790, 19 July 2004, in terms of the National Regulator for Compulsory Specifications Act (Act No. 5 of 2008).

  Compulsory Specification for Frozen Fish, Frozen Marine Molluscs, and Frozen Products derived therefrom. Regulation 979, 4 July 2003, in terms of the NRCS Act, 2008 (Act No. 5 of 2008).
- Municipal Structures Act, 1998 (Act No. 117 of 1998).
- Trade Metrology Act, 1973 (Act No. 77 of 1973).

(Source: SAMSM&CP, 2016)

2.4 CONCLUSION

The aquaculture industry in Saldanha Bay faces challenges of contaminant disposal close to the shellfish production farms despite being considered highly suitable for shellfish production. The advantages of being protected from ocean currents and having high nutritional levels may be outweighed by the prevalence of contaminants such as bacteria, viruses, heavy metals, and marine biotoxins, which remain a major concern for human health. This problem requires up-to-date and appropriate information in managing the microbial contamination of shellfish farms. This is, however, not the case for aquaculture in Saldanha Bay. Interventions such as collaboration between government departments, academic institutions, and shellfish farmers to facilitate rigorous investigation and information sharing and a thorough understanding to reach the reporting levels of developed countries will be beneficial. This will ensure safety of the shellfish produced in the bay and create a sustainable shellfish farming industry that benefits the people and the economy of South Africa.

2.4.1 Questions for further research

- It is evident from the literature that microbial contamination of shellfish is a global challenge that has been studied extensively. However, information specifically focused on South Africa and Saldanha Bay is limited. Will the shellfish industry in Saldanha Bay ever reach a level to be on par with other shellfish producers globally? Alternatively, what can be done to enhance collaboration between stakeholders to ensure global compliance?
- Even though outbreaks of foodborne diseases due to consumption of contaminated shellfish are widely reported globally, very little information related to shellfish products from Saldanha Bay has been documented. Follow–up studies into the reporting protocols are key, as it cannot be assumed that this lack of information is the result of effective monitoring.

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CHAPTER 3

MICROBIOLOGICAL MONITORING FROM 2017 - 2020 IN SALDANHA BAY SHELLFISH FARMS: AN ASSESSMENT OF *ESCHERICHIA COLI* PREVALENCE IN MUSSELS AND OYSTERS

ABSTRACT

Microbiological monitoring programmes aim to ensure compliance with regulations by shellfish farmers. The objective of this study was to evaluate the trends of *Escherichia coli (E. coli)* prevalence in Saldanha Bay shellfish farms using the microbiological sampling data provided by the Department of Agriculture, Forestry and Fisheries (DAFF) analysed between 2017 and 2020. Most of the samples were collected in the Small Bay farms (N= 420), followed by Outer Bay (N=89) and Big Bay (N=59). Pearson's correlations were performed to test the relationship between *E. coli* counts and temperature and *E. coli* counts and rainfall. No significant difference was observed between these variables. Mean *E. coli* comparisons were performed between sampling locations and mussels and oysters. A significant difference was observed between the mean *E. coli* comparisons from various sampling points in Small Bay (f=1.960, df= 13.406, p=0.023) with spikes that exceeded the South African Molluscan Shellfish Monitoring and Control Programme limit for Class A production areas (230 *E. coli* per 100g). The findings from this study recommend the need for consistency in the microbiological monitoring programme for future sampling runs to identify the spikes' sources. The study forms a baseline for future studies in this area.

KEYWORDS: shellfish, Most Probable Number (MPN), accumulate, microbial pollutants.

3.1 INTRODUCTION

Shellfish farming, which forms part of seafood production, has increased exponentially worldwide. It provides relief on food security for the world's growing population. Seafood is a general term for animals from the sea that can be consumed and includes shellfish. Shellfish consists of two main groups of invertebrates, namely, crustaceans and molluscs. Crustaceans have segmented bodies protected by a hard shell and include shrimps, lobsters, crayfish, prawns, crab, and krill. Molluscs have soft bodies with a foot and a visceral part. Phylum Mollusca can be divided into several classes, one of them being Class Bivalvia (mussels, oysters, clams, and scallops).

Seafood is a good source of protein, essential nutrients, and minerals including amino acids, vitamin D, selenium, iodine, and polyunsaturated fatty acids (Venugopal and Gopakumar, 2017; Elbashir et al., 2018; Ding et al., 2022; Vinothkannan et al., 2022). These invertebrates are inhabitants of the ocean. Along the coastlines, where human settlements and industrial activities are commonly observed, the ocean is the ultimate dumping area of pollutants including sewage disposal. Some of the pollutants of concern include microplastics, bacteria, viruses, heavy metals, and biotoxins. Microplastic pollution has recently been studied in-depth to understand the source and the risk of human exposure. They are ubiquitous in the marine environment and are bioavailable for marine organisms as they could easily be confused with plankton that marine organisms feed on due to the size and structure which is similar to plankton. Human exposure results from the ingestion of shellfish contaminated with microplastic (Ding et al., 2022). Pollution due to bacteria and viruses is mainly caused by the disposal of sewage into the marine environment. Some of the bacteria are found naturally in the marine environment. The presence of these microorganisms in high numbers results in intoxication and infection of the shellfish. Bivalve molluscs are known to filter large quantities of water in their environment (Potasman et al., 2002). This results in bioaccumulation and bioconcentration of bacteria and viruses in their digestive systems. The consumption of raw or partially cooked bivalve molluscs create a public health threat as they are eaten with their digestive system. The foodborne outbreaks reported between the years 1980 to 2012 implicated norovirus and hepatitis A as the greatest public health threat with norovirus being responsible for 87% of disease outbreaks and hepatitis A responsible for 12.8% with oyster consumption being the source of the outbreaks (Torok et al., 2018). Other pollutants of global concern are algal biotoxins which occur naturally in the marine environment but have certain species including paralytic shellfish poison, diarrhetic shellfish poison and amnesic shellfish poison causing shellfish poisoning through the consumption of biotoxin-contaminated shellfish (Davidson et al., 2021; Mudadu et al., 2022).

The occurrence of harmful algal blooms results in the temporary closure of shellfish farms which could lead to economic losses for farmers. Other pollutants of global concern are heavy metals even though some are part of the earth’s crust, and some are essential trace elements in living organisms. Industrialisation and urbanisation along the coastlines have contributed to excessive amounts of heavy metals in marine environments as they are characterised as non-biodegradable, easily accumulated in shellfish tissues, and ecologically toxic. Human exposure is through the consumption of contaminated shellfish (Jia et al., 2018; Fan et al., 2020; Liu et al., 2020). The water quality of shellfish production areas is monitored through enumeration of the faecal indicator bacteria (FIBs), *Escherichia coli* (*E. coli*), faecal coliforms, and enterococci (Rince et al., 2018).

Escherichia coli is a rod-shaped, gram-negative bacterium that belongs to the *Enterobacteriaceae* family (Jang et al., 2017). It can proliferate under optimal growth conditions and is frequently used as an indicator species for various bacteriological tests (Walker et al., 2018). *Escherichia coli* is excreted with the faeces of warm-blooded animals and humans and is frequently discharged into the environment through wastewater effluent in areas where there is proper wastewater infrastructure (Lunestad et al., 2016; Winterbourn et al., 2016). *Escherichia coli* serotype 0157:H7 is well known for causing outbreaks of water and food-borne diseases worldwide (Ribeiro et al., 2016).

The European Food Safety Authority (EFSA) suggests the most effective control measure in preventing contamination of shellfish by faeces is having production areas in waters that are not faecally contaminated (Hazards, 2012). However, it is a significant challenge to meet this control measure as sewage effluent is commonly discharged along the coastlines in South Africa. At the same time, most shellfish production areas are mainly located along these coastlines. Saldanha Bay, one of the shellfish production areas on the west coast, is a nutrient-rich location favourable for aquaculture. Being in a semi-enclosed embayment protected from strong ocean currents, it is one of the four shellfish farming sites in South Africa used for the production of mussels (*Mytilus galloprovinciallis*) and oysters (*Crassostrea gigas*). Like other coastal areas, Saldanha Bay has a sewage discharge point close to the shellfish farms. It is well documented that oysters and mussels are filter feeders, as they filter large quantities of water in their growing environments (Suffredini et al., 2012; Dabrowski et al., 2014; Lunestad et al., 2016; Strubbia et al., 2016). Therefore, any contaminants present in the water bodies can bioaccumulate and bioconcentrate within the bivalves. Contaminants can pose a serious health threat as the bivalves are eaten raw or partially cooked (Henigman et al., 2015; Polo et al., 2015; Fusco et al., 2017; La Bella et al., 2017; Marceddu et al., 2017). According to the National Shellfish Sanitation Program (NSSP, 2017) guide for controlling molluscan shellfish, a sanitary survey of growing areas should be conducted every twelve years or less. The growing area classification should be reviewed every three years at least.

The South African Molluscan Shellfish Monitoring and Control Programme (SAMSM&CP, 2012) classifies the shellfish production areas as APPROVED areas (Class A), where harvesting for direct human consumption is allowed and RESTRICTED areas (Class B), where harvesting for immediate human consumption is not permitted, as bivalves will be subjected to depuration or relaying. In PROHIBITED areas (Class C), shellfish may not be harvested for immediate human consumption, depuration, relaying, or further processing. The conditional areas (TEMPORARY Class A or B) refer to areas where shellfish that do not meet Class A compliance limits are subjected to depuration. Depuration utilises bivalve molluscs' natural filtering system to purge contaminants in controlled clean seawater (Martinez-Albores et al., 2020).

This study investigated the microbiological analysis of *E. coli* in mussels and oysters harvested in Saldanha Bay from January 2017 to July 2020, identifying the trends in the prevalence of *E. coli* from different sampling locations as well as the correlation between seasonality and rainfall. The data used was provided by the Department of Agriculture Forestry and Fisheries (DAFF), now known as the Department of Forestry, Fisheries, and Environment (DFFE). The goal is to contribute to the body of knowledge on microbial pollutants and their sources in Saldanha Bay and find a solution to mitigate and reduce pollutants to permissible limits. Compliance conditions and limits are described in more detail in Table 3.1.

Table 3.1: *Classification of shellfish production areas - compliance conditions and limits*

Shellfish production areas classification	Compliance conditions and Limits
APPROVED AREA (Class A)	• The *E. coli* count (MPN) may not exceed 230 *E. coli* per 100g of 100g of flesh and intravalvular liquid in 80% of the samples. No sample may exceed 700 *E. coli* per 100g of flesh and intravalvular liquid. • Shall not contain hazardous concentrations of toxic substances that exceed the regulatory limits. • Harvesting for direct human consumption may take place at any time in an approved area provided a temporary closure is not in effect due to adverse pollution or biotoxin events.
RESTRICTED AREA (Class B)	• The *E. coli* count (MPN) may not exceed 4 600 *E. coli* per 100g of flesh and intravalvular liquid in 90% of the samples. No sample may exceed 14 000 *E. coli* per 100g of flesh and intravalvular liquid. • No shellfish may be harvested for direct human consumption from restricted areas at any time. Shellfish from restricted areas can only be harvested for depuration or relaying.
PROHIBITED AREA (Class C)	• Shellfish shall not be harvested from prohibited areas for either direct human consumption, depuration, relaying or further processing.
CONDITIONAL AREA (TEMPORARY Class A or B)	• Conditional areas are subject to intermittent microbiological pollution events but may be classified as conditionally approved or conditionally restricted if they meet the relevant criteria for a reasonable and predictable period.

(Source: The SAMSM&CP, 2012)

3.2 MATERIALS AND METHODS

3.2.1 Sampling

As per the monitoring programme conducted by DFFE, five hundred and sixty-eight samples of mussels and oysters were collected from the shellfish farms in Saldanha Bay from January 2017 to July 2020. The sample sizes comprised N=384 mussels (*Mylitus galloprovinciallis)* and N=184 oysters (*Crassostrea gigas*). Each sample, containing 18 - 20 pooled individual bivalves was stored in a cooler box and transported to the laboratory at a temperature of less than 4°C. Upon arrival at the laboratory, the bivalves were analysed within 24 hours after sampling.

3.2.2 Sampling sites

Samples were collected from three respective sites (Figure 3.1), namely the Small Bay (33°00’30.90” S, 17°58’15.21” E), Big Bay (33°1’39.54” S, 18°1’14.94” E), and Outer Bay (33°2’1.42” S, 17°56’43.32” E) areas. In the Small Bay area, samples were collected from several mussel and oyster farms. This site receives treated effluent discharged into the Bok River and runoff from the roads and stormwater drainage. The Big Bay site is separated from the Small Bay site by an Iron-ore jetty, which is used to accommodate capsized bulk ore carrier ships exporting iron ore from the country. Outer Bay, the third sampling site, is located near the harbour entrance and was selected as it appeared to be the least contaminated site as it is far from the possible contaminants found in the other sites (DFFE, 2021). The total number of samples collected from January 2017 until July 2020 where *E. coli* was detected was (N=568). Most samples were collected on farms within Small Bay with a total number of mussel samples (n=297) and oyster samples (n=123) and Outer Bay with mussels (n=87) and oysters (n=2) and Big Bay with only oysters (n=59) as mussels were not available to sample, as shown in Table 3.2.

Table 3.2: *Mussels and oysters samples collected from the three sampling sites (n=568)*

Sampling site	Mussels (n)	Oysters (n)
Small Bay	297	123
Big Bay	Not available	59
Outer Bay	87	2

3.3 MICROBIOLOGICAL ANALYSIS

Samples were analysed for the presence of *E. coli* (N=568), with each sample consisting of the flesh and intravalvular fluid. The enumeration was conducted using the 5-tube dilution Most Probable Number (MPN) procedure based on ISO 16649 – 3 (ISO, 2005) (Donovan et al., 1998; Bazzardi et al., 2014; Tabanelli et al., 2017; Walker et al., 2018). The Most Probable Number technique is widely used in South Africa and can be used in a variety of laboratory settings as it is a simple step-by-step method to follow, and results are easy to analyse. It is widely used in developing countries where affordability plays a significant role in the choice of sampling techniques (Abioye and Okoh, 2018; Mkhungo, Oyedeji and Ijabadeniyi, 2018; Walker et al., 2018). It is also an approved methodology according to the South African National Standard (SANS 21528-1, 2005).

Figure 3.1 *Regional map of Saldanha Bay, Langebaan lagoon, and Danger Bay showing development (grey shading) and conservation areas (source: Clark et al., 2020)*

3.4 METEOROLOGICAL DATA

The average daily maximum and minimum temperature (°C) data were obtained from the South African Weather Services station (0061298 8) – Langebaanweg measured at 08:00, from January 2017 through July 2020. The lowest minimum temperature was in July 2017 (5.1°C), August 2018 (6.2°C), and July 2020 (6.7°C). The highest maximum temperatures were in January 2016 (31.4°C); and January 2020 (30.4°C). On average, February was the hottest month and June the coldest (Du Plessis and Schloms, 2017; Mchunu et al., 2018; Van der Walt and Fitchett, 2020).

3.5 STATISTICAL ANALYSIS

Statistical analyses were performed using IBM SPSS version 28.0.0.0. (190) software. Mean comparisons were conducted between the three sampling locations, the shellfish type (mussels and oysters), and the various sampling points at the Small Bay sampling location. Pearson correlation tests were performed to determine the relationships between rainfall and *E. coli* concentrations as well as temperature and *E. coli* concentrations. Lastly, the trend analysis was performed between (1) the summer period and *E. coli* concentrations and (2) the winter period and *E. coli* concentrations from 2017 until 2020. The p values of ($P \leq 0.05$) were considered statistically significant.

3.6 RESULTS AND DISCUSSION

The microbiological monitoring programme conducted by (DAFF) during the 2017 - 2020 period demonstrated consistency in sampling every month but not consistency in the number of samples collected at selected sampling sites. Park et al. (2018) emphasised the importance of intensive and continuous monitoring to detect the presence and patterns of microbial contaminants. The routine monitoring programme was compliant with the SAMSM&CP standard operating procedure during the abovementioned sampling period as samples were collected every month.

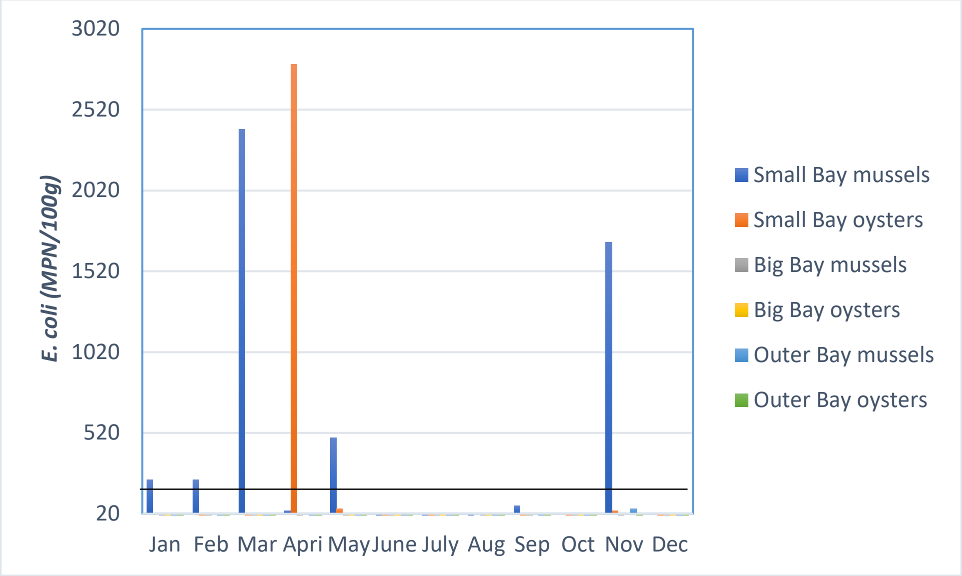

Figure 3.2 *Comparison of E. coli concentrations between the three sampling locations in mussels and oysters from January to December 2017. The black horizontal line shows the limit for Class A production area of 230 microorganisms/100g for E. coli*

In 2017, comparisons between the three sampling locations showed Small Bay with high levels of *E. coli* in mussels and extremely high levels of *E. coli* in oysters on a single occasion compared to the other two locations (Figure 3.2). The spikes were observed (during March 2017- *E. coli* count of 2400 microorganisms/100g), April 2017 (*E coli* count of 2800 microorganisms/100g), and November 2017 (*E. coli* count of 1700 microorganisms/100g), respectively. These spikes exceeded the permissible limit for class A production areas, and therefore purification treatment is required where permissible limits are exceeded. A study conducted in Italy (Sferlazzo et al., 2018; Lamon et al., 2020) reported a positive reduction of *E. coli* loads after purification treatment.

In 2018*, E. coli* was not detected in Big Bay and North Bay locations. In addition, *E. coli* was not detected in oysters from the Small Bay sampling location, while *E. coli* counts in mussels were at the permissible limit in May and June. A spike in the *E. coli* concentration of 3500 microorganisms/100g was observed in August (winter and rainy period), which is significantly above the permissible limit for Class A production area (Figure 3.3).

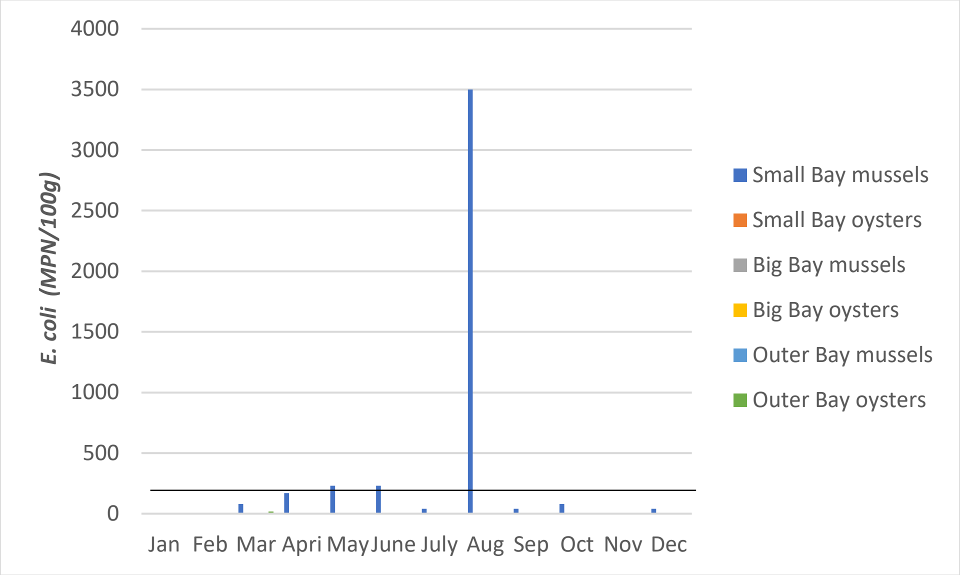

***Figure 3.3** Comparison of E. coli concentrations between the three sampling locations in mussels and oysters from January 2018 to December 2018. The black horizontal line shows the limit for Class A production area of 230 microorganisms/100g for E. coli*

In the 2019 comparisons between sampling locations, *E. coli* concentrations were detected below the permissible limit for a Class A production area for the two sampling locations, with no *E. coli* detected in the Big Bay sampling location. However, even though the *E. coli* concentrations were below permissible limits, a slight spike was observed in August (winter and rainy period) with an *E. coli* concentration of 110 microorganisms/100g (Figure 3.4).

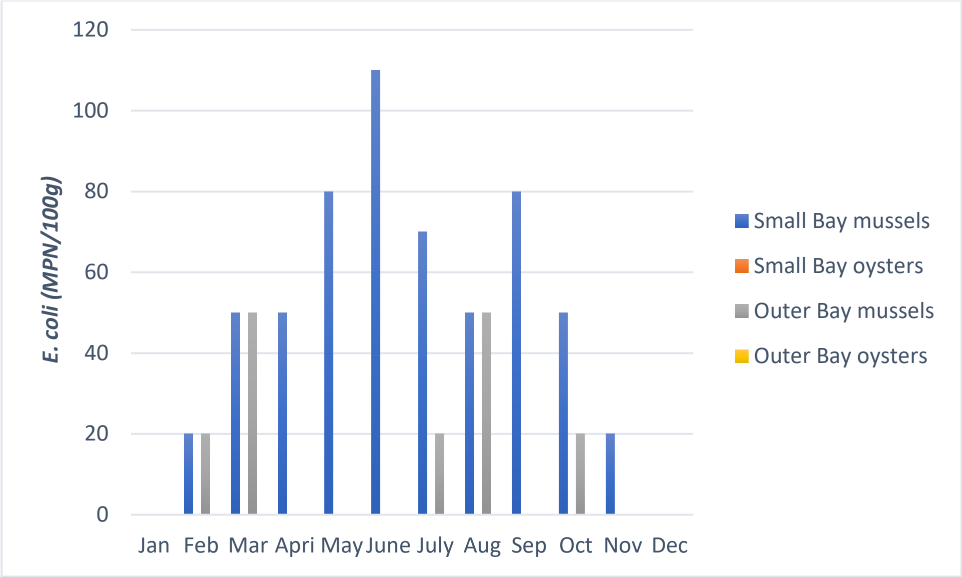

***Figure 3.4** Comparison of E. coli concentrations between the two sampling locations in mussels and oysters from January to December 2019. The E. coli concentrations were below the permissible limit of 230 microorganisms/100g for E. coli*

In 2020, *E. coli* was not detected in Big Bay and Outer Bay sampling locations in mussels and oysters. In the Small Bay sampling location, a peak in *E. coli* concentrations of 490 microorganisms/100g in mussels was observed in June (winter month), which was above the permissible limit for a Class A production area. No *E. coli* was detected in oysters for this period (Figure 3.5).

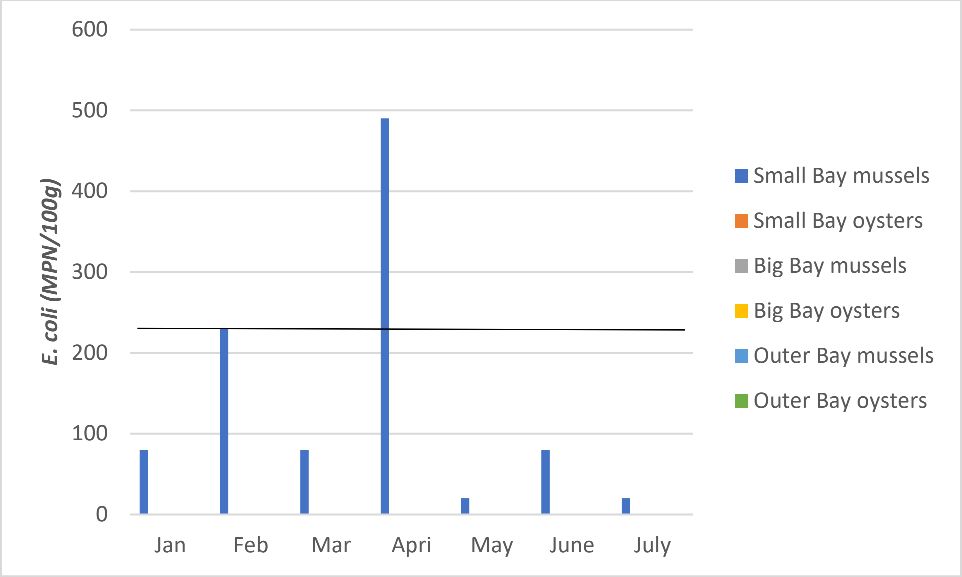

Figure 3.5 *Comparison of E. coli concentrations between the three sampling locations in mussels and oysters from January to July 2020. The black horizontal line shows the limit for Class A production area of 230 microorganisms/100g for E. coli*

The mean comparisons of *E. coli* concentrations between the three sampling locations are as follows: Four hundred and twenty samples of both mussels and oysters (n=420) were collected from Small Bay, fifty-nine samples (n=59) from Big Bay, and eighty-nine (n=89) from Outer Bay. The mean *E. coli* concentrations in Small Bay were (80.65± 823.43 microorganisms/100g), Big Bay was (1.69± 5.6 microorganisms/100g), and Outer Bay (3.26±10.31 microorganisms/100g). The mean *E. coli* concentrations between the three sampling sites were not significantly different (f=0.662, df=2.565, p= 0.516). In the Small Bay sampling location, the total number of oysters collected was N=123, and the number of mussels was n=297. The mean *E. coli* concentrations in oysters (33.44±254.12 microorganisms/100g) did not differ significantly from the mean *E. coli* concentrations in mussels (100.20±965.33 microorganisms/100g) (t=-0.756, df=418, p=0.450). It was clear that the Small Bay area had the most spikes and therefore further statistical analysis was conducted for this sampling site specifically (Table 3.3). However, there was a significant difference in mean *E. coli* concentrations between the various sampling points in the Small Bay sampling location (f=1.960, df= 13.406, p=0.023), as shown in table 3.3.

In the Big Bay sampling location, 59 oyster samples were collected. Two oyster (n=2) samples and eighty-seven (n=87) mussels were collected in Outer Bay. The mean *E. coli* concentration of oysters (10.00±14.14 microorganisms/100g) did not differ significantly from the mean *E. coli* concentration in mussels (3.10±10.27 microorganisms/100g) (t=0.935, df= 87, p=0.353).

Table 3.3: *ONEWAY ANOVA analysis of mean E. coli concentrations for sampling points in Small Bay*

Sampling point	No. of Samples	Mean	Std. Deviation
SP9_O	7	.00	.000
SP9_M	25	71.20	339.415
SP8_M	15	66.67	145.291
SP7_O	40	99.00	441.999
SP7_M	23	901.30	3332.867
SP3_M	99	12.22	32.468
SP2_O	37	.08	.493
SP2_M	104	46.15	343.292
SP15_O	1	.00	.
SP13_O	18	5.00	12.948
SP12_O	20	3.00	7.327
SP12_M	4	12.50	25.000
SP11_M	9	2.22	6.667
SP10_M	18	9.44	31.524
Total	420	80.65	823.430

*SP=sampling point, O=oyster, M= mussel

A correlation analysis test was performed to determine the relationship between rainfall, temperature, and *E. coli* concentrations. No relationships were evident between these variables. Temperature and rainfall (r=-0.015, N= 568, p= 0.729), rainfall and *E. coli* (microorganisms/100g) (r= 0.002, N= 568, p= 0.959), temperature and *E. coli* (microorganisms/100g) (r=0.031, N=468, p= 0.460) are shown in Table 3.4.

Table 3.4: *Pearson correlation analysis between temperature, rainfall and E. coli concentrations*

		Average temperature (°C)	Rainfall (mm)	*E. coli* (microorganisms/100g)
Average temperature (°C)	Pearson Correlation	1	-.015	.031
	Sig. (2-tailed)		.729	.460
	N	568	568	568
Rainfall	Pearson Correlation	-.015	1	.002
	Sig. (2-tailed)	.729		.959
	N	568	568	568
E. coli (microorganisms/100g)	Pearson Correlation	.031	.002	1
	Sig. (2-tailed)	.460	.959	
	N	568	568	568

Most of the months the counts for all three locations were below limit for Class A production area requirement of 230 *E. coli* per 100g of flesh and intravalvular liquid. Small Bay mussels were mostly affected by spikes exceeding the Class A limits, with August 2018 mussels samples (3 500 E. coli / 100g) close to Class B maximum limit of 4 600 E. coli per 100 of flesh and intravalvular liquid. The Small Bay sampling site is near businesses, residential areas, and the mouth of the Bok River, where the sewage discharge point is located. The proximity of shellfish production areas to residential areas runs the risk of faecal-borne pathogen contamination if the sewage treatment processes are ineffective.

During the rainy season, the possibility of sewage overflow and runoff from stormwater drains could bring faecal-borne pathogens into the production areas (Hassard et al., 2017; Jeamsripong et al., 2018; Campos et al., 2020). The Department of Agriculture, Forestry, and Fisheries (DAFF) listed the pollution sources, and the sampling points close to the pollution sources they have identified in the sanitary survey report (DAFF, 2016).

The mean *E. coli* concentrations revealed that SP7M, SP7O, SP8M, SP9M, and SP2M were affected by the pollution sources close to the Small Bay sampling location, which corresponds with the DAFF sanitary survey report. Many countries, including the United States of America (USA), have taken precautionary measures by prohibiting shellfish harvesting near the sewage treatment works discharge point. It was also recommended that a buffer zone of 1:1000 dilution of estuarine water to treated effluent be established (ISSC, 2015). The European Union has no legislation addressing sewage outfall and proximity to shellfish production areas. However, some member countries have implemented the closure of areas around sewage outfalls (Campos et al., 2017). Water temperature is the most critical factor known to influence *E. coli* survival and growth in the environment (Jang et al., 2017). Several studies observed results where an increase in *E. coli* concentrations occurred with an increase in temperatures (Derolez et al., 2013; Park et al., 2018). Studies conducted by Campos et al. (2013), Jeamsripong et al. (2018) and Younger et al. (2018), on the contrary, found that sunlight, which is high in the summer months, increases bacterial inactivation. In this study as seen in Table 3.4, there was no clear indication to support or disagree with the observations above, as some peaks were observed during summer months with the average maximum temperatures reaching 28°C and winter months with the average maximum temperatures reaching 18°C. There was also no relationship between rainfall and *E. coli* concentrations and temperature and *E. coli* concentrations.

Summer holidays also attract tourists to the West Coast, during which the Bay provides the ideal environment for recreational activities. Zormati et al. (2018) reported tourist activities during summer as directly linked to higher concentrations of *E. coli* in production areas of the Sfax region of Southern Tunisia. According to Tabanelli et al. (2017), seasonal variables such as seasonal changes in the human population and environmental parameters such as seawater temperature influence *E. coli* concentration levels. A spike in *E. coli* concentrations was observed in November 2017 (Figure 3.2) in Small Bay. However, it is difficult to draw a conclusion as high *E. Coli* concentrations were not observed throughout summer months and subsequent years. In addition, higher seawater temperature and rainfall patterns favour the multiplication of *E. coli* as sewage discharges, and stormwater runoffs introduce pollutants into the sea (Park et al., 2018). A similar conclusion was reached by Campos et al. (2013) and

Chinnadurai et al. (2016), who discovered that seasonality influenced *E. coli* counts, as these counts increased during the rainy season. Lee and Silk (2013) agreed with the above-mentioned studies and suggested that frequent monitoring at individual sites is necessary to detect seasonal effects and unpredicted sources of contamination. Furthermore, concluded that geographical location, sampling date, and species sampled were the most significant variable sources. This study showed a similar observation as the samples collected from the Small Bay sampling site had higher concentrations of *E. coli* than the Big Bay and Outer Bay areas. In addition, mussels also had higher concentrations of *E. coli* compared to oysters.

Additionally, the bird population is a significant concern in Saldanha Bay. Food availability is the main attraction for the migration of the bird population to these farms. Birds feed on farm waste, spilled feed, faeces, or deadstock (Barrett et al., 2019). Callier et al. (2018) reviewed the effects of attraction and repulsion of wild animals to or from bivalve aquaculture but acquired little information on the long-term impact of these interactions on the entire marine ecosystem. Saldanha Bay comprises of sections that are regarded as marine protected areas. Migratory birds and other bird populations inhabit these areas. Derolez et al. (2013), in their study, suspected faecal material from birds as the source of *E. coli* in dry weather conditions. As a result, one could not overrule the possibility of contamination due to the presence of bird populations on the islands found in the Bay and on the rafts used for mussel and oyster production.

As previously mentioned, Saldanha Bay production areas are near businesses and residential areas. Jeamsripong and Atwill (2019) investigated the use of environmental parameters such as ambient air temperature, relative humidity, wind speed, geographical location, and climate change as part of a rapid warning system that could be used to predict levels of *E. coli* before the harvesting of oysters. It was concluded that both environmental parameters and testing of indicator species' concentrations could help farmers identify the proper time to harvest. It was also discovered that temperature and precipitation are significant ecological parameters that are reliable and have been supported by several studies (Chinnadurai et al., 2020).

Where food safety is concerned, monitoring procedures play an integral part in ensuring that food produced or manufactured is of good quality and safe for human consumption (Mkhungo et al., 2018). Younger et al. (2018), in their study on shellfish production areas across the United Kingdom, highlighted the importance of identifying pollution sources and their impacts on the accurate interpretation of *E. coli* detected during routine monitoring programmes and identified risk management measures that may be applied. Furthermore, a study conducted by Gourmelon et al. (2006), investigated the presence of Shiga toxin-producing *E. coli* (STEC) and *E. coli* O157:H7 in shellfish from French coastal environments. The study recommended the tracking of pollutant sources situated upstream of shellfish farming areas including agricultural and urban sources. This is also applicable to the Saldanha Bay situation, where the observed spikes need to be evaluated and the basis of origin needs to be investigated.

The International Organisation for Standardisation (ISO) 22000 (2018) defines food safety as a concept in which food will not cause harm to the consumer when it is prepared and/or when eaten according to its intended use. However, various studies on shellfish production emphasise the public health risks associated with the consumption of raw or partially cooked shellfish (especially oysters) and the possible foodborne-related disease occurrences (Potasman et al., 2002; Lees, 2010; La Bella et al., 2017). Food safety addresses food safety hazards (biological, chemical, physical, allergens) that could occur at any stage of production; hence, the importance of having systems such as the Hazard Analysis Critical Control Point (HACCP) system in place. In Addition, food safety standards such as ISO 22000:2018 also required that producers and manufacturers must consider the place of origin (provenance) of a raw material and the method of production.

Microbiological monitoring of shellfish production areas ensures that microbiological hazards that could cause harm or render shellfish unsafe for human consumption are eliminated or reduced to an acceptable level. Therefore, the development of regulations and standards to govern shellfish production (Hazards, 2012) have been instituted. The Standard operating procedure: Molluscan Shellfish Microbiological Action Plan for Saldanha Bay Aquaculture Facilities (2017) and Control Program

(SAMSM&CP, 2012) stipulates that routine microbiological sampling would be undertaken once a month as well as a day after every high rainfall (> 20mm rain) for microbial contamination using *E. coli* as an indicator species (DAFF, 2012).

3.7 CONCLUSION

The routine monitoring procedure complied with the standard operating practice of the SAMSM&CP (2012) for microbiological monitoring, which stipulates that Class A and restricted production areas should be tested once a month for microbial contamination. Samples were therefore taken every month. The only problem observed was the consistency of sampling per individual sampling site. Samples collected varied from month to month, which was contrary to what Lee and Silk (2013) suggested. According to Lee and Silk (2013), frequent monitoring at individual sites is necessary to detect seasonal effects and unpredicted sources of contamination.

Microbiological analysis of *E. coli* concentrations was mainly detected below the permissible limit for a Class A shellfish production area, except for a few occasions where concentration spikes were observed. Spikes occurred in March and April 2017, as well as August 2018. April and August fall within the rainy season of the West Coast. This observation is similar to the majority of studies conducted globally, which found that environmental factors such as season, rainfall, and changing tidal currents directly influence *E. coli* levels.

Therefore, the findings of this study could be used as a baseline for in-depth research investigating sources of microbial pollutants. Consistency in routine microbiological monitoring will make it easier to identify trends and sources thereof, enabling the authorities to make informed decisions and efficiently manage shellfish production areas. This study, as presented, provided baseline research that can be used as a reference for future studies. A follow-up study is planned to investigate the bacterial and virus communities present in Saldanha Bay shellfish production farms.

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CHAPTER 4

THE PREVALENCE OF BACTERIA COMMONLY RELATED TO THE PRODUCTION OF MUSSELS AND OYSTERS IN SALDANHA BAY

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ABSTRACT

Bivalve molluscs are a good source of high-quality protein and perform important ecological functions. Their ability to bioaccumulate materials in their soft tissues makes them suitable aquatic species for biomonitoring of environmental conditions. The discharge of treated and untreated sewage into the bivalve-growing areas is a concern. The study aimed to investigate the prevalence of bacterial microbiota in shellfish farms in Saldanha Bay harbour using pathogens commonly associated with shellfish-related foodborne disease outbreaks. Seawater and mussel samples were collected from five sampling points located in three sampling locations. Oyster samples were collected from the harbour deck immediately after harvesting by the farmers. The Most Probable Number (MPN) method was used to enumerate *E. coli* and faecal coliforms. Cultural methods were used for the detection of *Salmonella* and *Vibrio* spp. The *E. coli* concentrations were measured on 15 March and 14 July (<0.18 MPN/100ml) at all sampling sites and on 25 August (<0.18 MPN/100ml) for all sampling sites except sampling site SP2 (0.2 MPN/100ml). Spikes were observed in the total MPN counts in winter. *Salmonella* and *Vibrio* spp. were not detected. However, other bacterial species were identified through their phenotypic profile using the Vitek 2 system. Based on the low *E. coli*- MPN concentrations, the study concluded that the molluscs were safe for human consumption. Further studies need to be conducted on the bacterial species identified.

KEYWORDS: Bivalve molluscs; antimicrobial resistant; shellfish; permissible limits; high-priority pathogens

4.1 INTRODUCTION

Shellfish farming is becoming an important sector for the South African government as it creates much-needed job opportunities for the coastal communities. The sustainability and safety of shellfish growing areas are essential in terms of protection from contaminants and preventing contaminants from reaching the bivalves produced (Liu et al., 2021). Bivalve molluscs play important ecological functions in aquatic ecosystems as well as being highly nutritious. They can be found at the bottom of the sea or attached to hard surfaces or on one another. Their filter-feeding nature assists in purifying the surrounding waters and increases the penetration of sunlight (Cravo et al., 2022).

Furthermore, they provide micronutrients to other marine organisms increasing primary production and nutrient recycling, coastal habitat conservation, and restoration (Vaughn and Hoellein, 2018; Silvestre et al., 2021). These characteristics and the ability to bioaccumulate materials in their soft tissues make the bivalve molluscs suitable aquatic species for biomonitoring of environmental conditions. Bioaccumulation of materials is not selective, as both beneficial and harmful materials are equally accrued (Ghribi et al., 2020). Besides being highly nutritious compared to beef, chicken, and pork, bivalves are highly perishable and require proper handling from farm to fork. Failure to adhere to food safety best practices could lead to an increased risk of illness from pathogens, including bacteria, viruses, and protozoa (Wright et al., 2018; Theuerkauf et al., 2022).

Aquatic environments are home to various microbiota, some indigenous and some introduced through anthropogenic activities around these environments. The presence of pathogens in aquatic ecosystems is a risk to the shellfish production industry and poses a public health threat. Several foodborne outbreaks have been reported globally due to the consumption of contaminated shellfish (Xu et al., 2017; Walker et al., 2018; Aminharati et al., 2019). Zgouridou et al. (2022) in their study indicated that mussels of the genus *Mytilus* are primarily the bivalve species that are a public health risk to consumers, as well as oysters (*Ostrea edulis*) and clams (*Venus verrucosa*). Bioaccumulation and bioconcentration of pathogens vary according to host species and seasonality. Both mussels and oysters can concentrate pathogens in their body tissues. However, oysters are an important medium for infecting humans with these pathogens as they are eaten raw or partially cooked (Destoumieux-Garzón et al., 2020). Several studies have been conducted worldwide to determine the bacterial prevalence in shellfish-growing waters (Bazzardi et al., 2014; Arab et al., 2020; Antony et al., 2021). To date, similar studies have not been conducted in Saldanha Bay except for the microbiological monitoring undertaken by the South African Department of Forestry, Fisheries, and Environment. This created a need to investigate the bacterial communities, especially the disease-causing ones that may be present in this Bay.

This study investigated pathogens commonly associated with shellfish-related foodborne disease outbreaks, such as *Salmonella, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholera,* and the prevalence of *Escherichia coli* as an indicator species. The results did not conform to prior expectations, as bacteria such as the *Enterobacter cloacae complex; Citrobacter freundii; Klebsiella pneumoniae* spp. *pneumoniae; Aeromonas sobria; Vibrio alginolyticus*; and *Sphingomonas paucimobilis* were confirmed through biochemical characterisation.

The study applied an experimental design, and interpretations were formed using a multimethod, quantitative strategy for three sampling occasions. The researcher collected samples during warm, cold, and rainy periods as informed by obtained literature. Data collection and analysis techniques are detailed under materials and methods (Saunders et al., 2015).

4.2 MATERIALS AND METHODS

4.2.1 Study area

Saldanha Bay harbour on the West Coast of South Africa (Latitude: -33.027699, Longitude: 17.917631) houses the biggest port in Southern Africa operating as an international port for the export of iron ore. The Bay's water depth is approximately 23.7m. Construction of a 4km long iron ore jetty has divided the Inner bay into Small Bay and Big Bay (Henrico and Bezuidenhout, 2020), and a 1.7km long breakwater separates the Inner bay from the Outer bay. Small Bay is sheltered from offshore swells and has constrained water circulation while Big Bay is semi-exposed to wave energy with better circulation compared to Small bay. The outer Bay which is located at the mouth of the Bay is regarded as the less polluted site (DAFF, 2018). The Bay is exposed to the disposal of treated and untreated sewage from the nearby wastewater treatment plant which discharges into the Bok River. Several sewage pumps, ballast water, dredging, stormwater discharge and ship traffic are some of the sources of pollutants close to Small Bay. Two mussel species are farmed, the indigenous black mussels (*Choromytilus meridionalis*), which is not a preferred species for farming due to the dark flesh colour of the female species and the exotic Mediterranean mussels

(*Mytilus galloprovincialis*), while the Pacific oysters (*Crassostrea gigas*) are also farmed. Figure 4.1 shows five sampling points where mussels and seawater were collected.

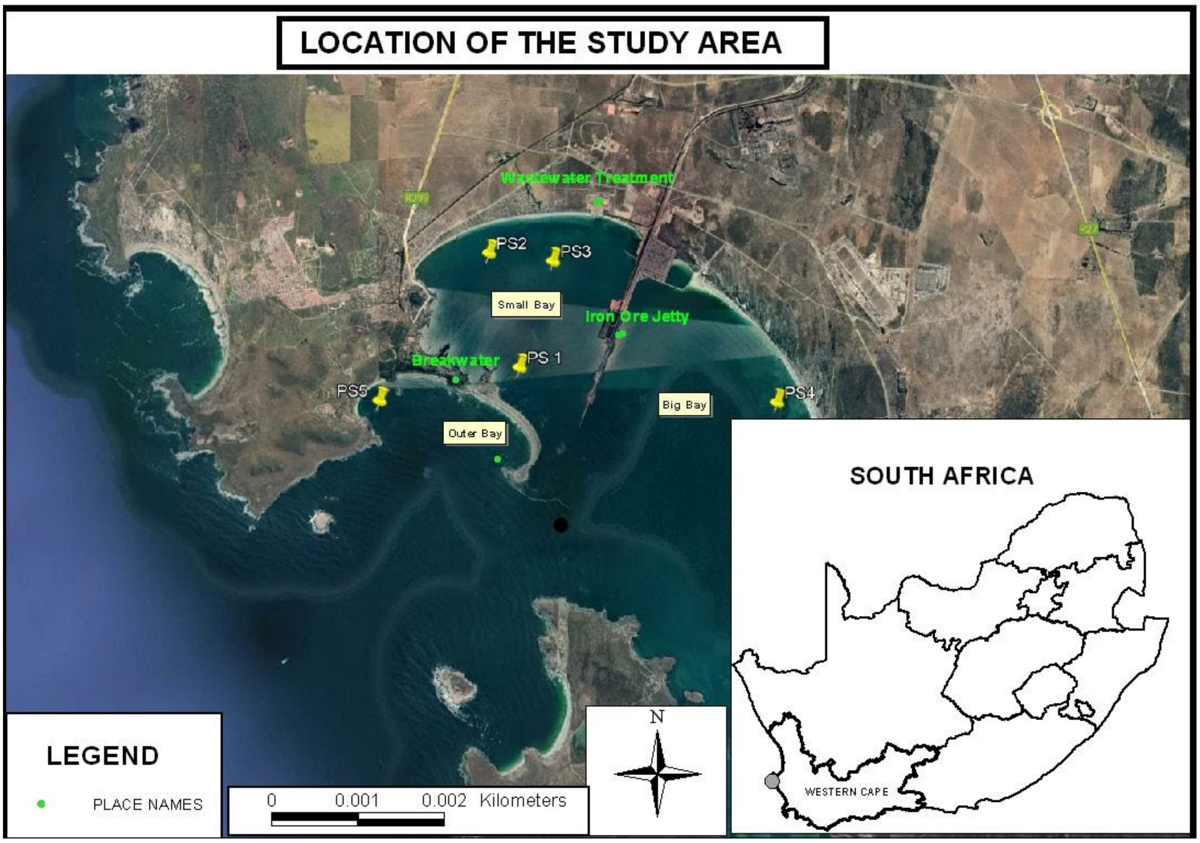

Figure 4.1 *Aerial photograph depicting Saldanha Bay Harbour and Sampling Points (source: Arcview 3.3 version. Data source: Google Earth Satellite, 2022)*

4.2.2 Sample collection

A total of 27 shellfish and seawater (mussels (n=12); seawater (n=13); and oysters (n=2)) samples were collected from various sampling sites. Samples were collected in the morning between 8:00 am and 11:00 am during low tides to reach all sites especially the offshore ones. Oysters were collected from the harbour deck immediately after harvesting by the farmers. Five sampling points were used for the collection of seawater and mussels. Three of them were located in Small Bay (SP1, SP2, SP3), one in Big Bay (SP4) and the last one in Outer Bay (SP5) (Figure 4.1). Samples were collected in March (warm period), July (winter-before heavy rainfall), and August (winter-after heavy rainfall). During sampling, 30 oysters and 30 mussels were hand-picked and stored in sterile whirl-pack bags (Nasco, USA). Seawater samples were collected (2 meters below the surface) in 1-litre sterile Schott bottles (Schott, UK) and mussels from a hanging rope. Physico-chemical parameters (i.e., water temperature (°C), salinity (psu), and dissolved oxygen (ppm) were measured during sampling at each sampling point using a Hanna HI9810-6 multimeter. Samples were transported to the laboratory in a cooler box packed with ice packs, maintaining a temperature between 2 - 8°C, within 2 hours and microbiological analyses were performed immediately.

4.2.3 Sample preparation

Upon arrival at the laboratory, the mussel and oyster samples were scrubbed under running tap water to remove shell debris, and attached algae and the shells were opened aseptically with a sterile chucking knife. Approximately 300g of flesh and intravalvular liquid of mussels and oysters were stored in 500g sterile beakers, then transferred aseptically into stomacher bags (circulator 400, Seward, Worthing, UK). Samples were homogenised with 200ml sterile phosphate water at 230rpm speed for 2 minutes.

4.2.4 Most Probable Number (MPN) (mussel, oyster and seawater samples) - *Escherichia coli*

Lauryl Tryptose broth (LTB) (Merck, Germany), Brilliant Green broth (BGBB) (Merck, Germany), and Tryptone Water (TW) (Merck, Germany) were prepared following the manufacturers' instructions. The method was conducted using the method described by Leuta (2015) (Figure 4.2 a, b, c). Concentrated mussel and oyster homogenate extracted from the mussel samples was used as stock to conduct the five-tube MPN technique. Serial dilutions of 10^{-1} to 10^{-5} of the mussel and oyster homogenate, and seawater samples, respectively, were performed before inoculation of 1ml of each diluted sample into LTB tubes containing Durham tubes. Durham tubes provide a visual indication of gas production. The inoculated test tubes were incubated for 48 hours at 37°C (indicating all gas-producing organisms). All tubes showing gas formation after a 48-hour incubation period were regarded as a positive presumptive test, and the presumptive total MPN count was read off De Man's tables (De Man, 1983). For each positive presumptive LTB tube, a 10ml Brilliant Green Bile Broth (BGBB) tube and a 10ml Tryptone Water (TW) tube were prepared. One hundred microliters (µl) of the sample from each positive LTB tube was re-inoculated into BGBB and TW tubes, respectively, according to the guidelines set out by the South African Bureau of Standards (SABS, 1984). These guidelines also incorporate the standard methods, set out by the American Public Health Association, for the examination of seawater and shellfish as well as the methods for the examination of water and wastewater (American Society for Microbiology, 1970; American Society for Microbiology, 1995). These tubes were incubated in a 44.5°C water bath for 24 hours (44°C – 44.5°C) which has the specific advantage of detecting *E. coli*, as it is the only faecal coliform present in water capable of producing indole at this temperature. With the observation of positive gas production in the BGBB tubes [indicating faecal coliforms (FC)] after 24 hours, a few drops of Ehrlich's reagent (LabChem, USA) were added to the corresponding TW tubes. The presence of *E. coli* was confirmed with a colour change from clear to pink or red in the tryptone water tubes.

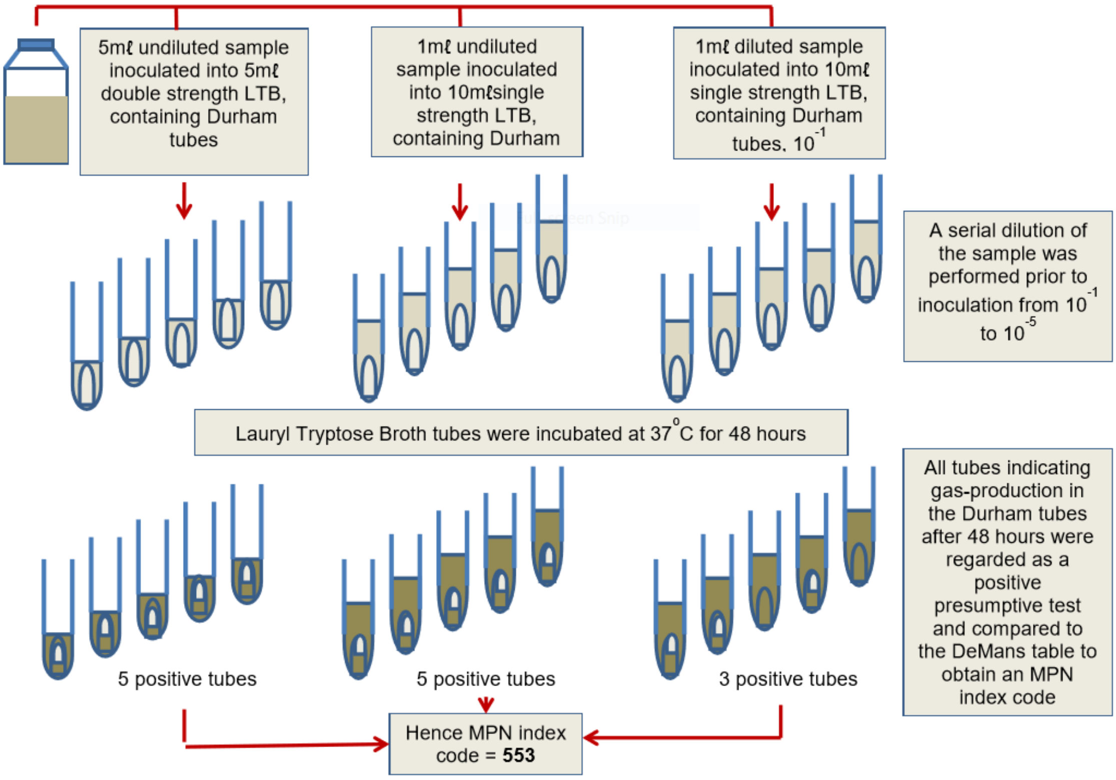

Figure 4.2a *Inoculation of undiluted and diluted samples into LTB tubes to obtain positive presumptive test (Adopted and adapted from Leuta, 2015)*

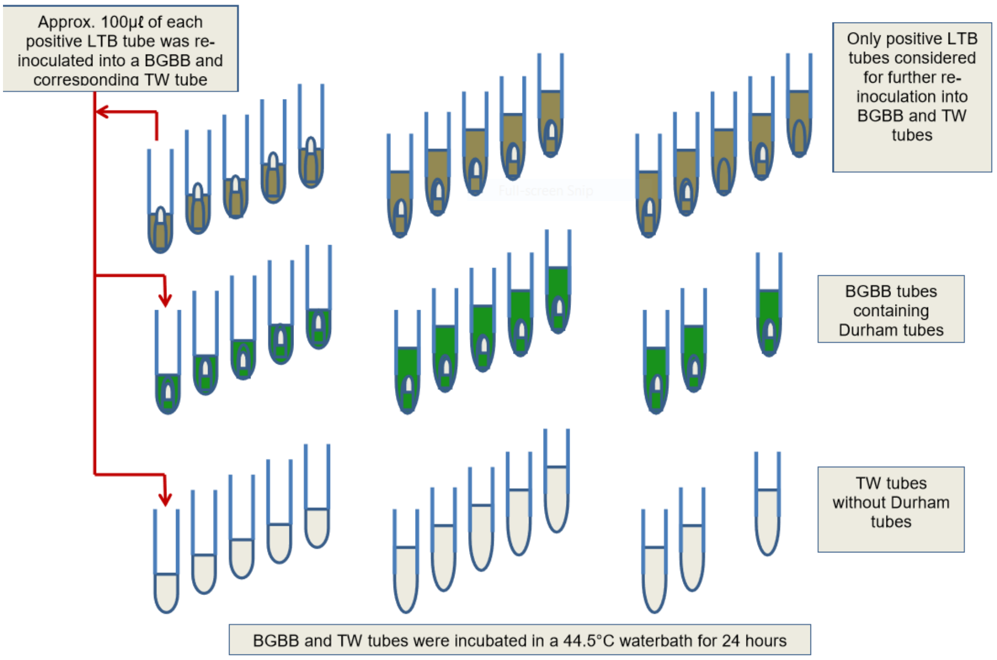

***Figure 4.2b** Re-inoculation of positive LTB tubes into BGBB and TW tubes (Adopted and adapted from Leuta, 2015)*

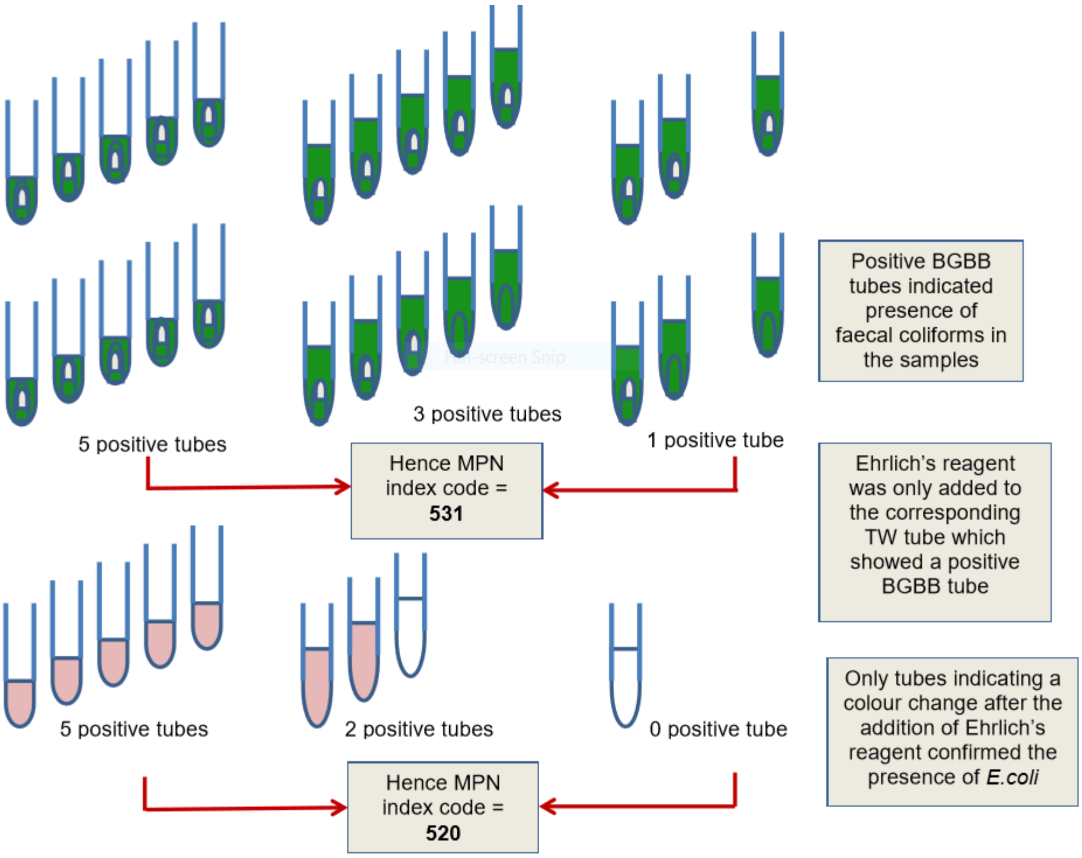

***Figure 4.2c** Enumeration of faecal coliforms (from BGBB tubes) and E. coli (from TW tubes) in water samples (Adopted and adapted from Leuta, 2015)*

4.2.5 Detection and isolation of *Salmonella*

Salmonella spp. was detected according to the protocol based on ISO 6579-1:2007 (ISO,2007). Buffered Peptone Water (BPW) (Merck, Germany), Selenite Cysteine broth (SCB) (Merck, Germany), and *Salmonella shigella* agar (SS agar) (Oxoid, Uk) were prepared according to the manufacturers' instructions. Twenty-five grams (25g) of mussels and oyster's homogenate were aseptically weighed and placed into a 225ml sterile BPW to prepare a pre-enrichment culture. The mixture was then incubated at 37°C for 16hrs to 20hrs. After incubation, the sample was gently mixed, and 1ml of the BPW was added into a sterile McCartney bottle. Ten millilitres (10ml) of SCB were added to the sample to prepare an enrichment culture and incubated at 37°C for 24hrs. After the 24hr incubation period, the enriched culture was streaked onto SS agar and incubated inverted at 37°C for 24hrs. The plates were examined (typical pinkish-red colonies) for the absence or presence of *Salmonella* spp. Subsequently, Gram stains were performed on the obtained colonies. The observation of Gram-negative, non-spore-forming colonies confirmed the presence of *Salmonella*, while biochemical identification was carried out using Vitek 2 compact gram-negative (GN) ID cards (bio Mérieux, France). *Salmonella typhimurium* (NCTC 12023) strain was used as a positive control.

4.2.6 Detection and isolation of *Vibrio cholerae* and *Vibrio parahaemolyticus*

Vibrio spp. were detected according to the protocol based on ISO/TS 21872-1:2007 (ISO,2007). Alkaline Salt Peptone Water (ASPW) (Oxoid, Uk), Thiosulphate Citrate Bile Sucrose agar (TCBS) (oxoid, Uk), and Saline nutrient agar (SNA) (Oxoid, Uk) were prepared according to the manufacturers' instructions. Twenty-five grams (25g) of mussel and oyster homogenate was aseptically weighed and placed into a 225ml sterile ASPW to prepare a pre-enrichment culture. The mixture was then Incubated at 41.5°C for 6hrs (41.5°C is recommended for fresh products). After incubation, a loopful (1µl) of the enriched sample was inoculated into fresh 10ml ASPW (secondary enrichment) and incubated at 41.5°C for 18hrs. After the 18hrs of incubation, the enriched culture was streaked onto a selective plating medium (TCBS) and incubated inverted at 37°C for 18hrs to 24hrs. The plates were examined (suspected *V. cholerae* colonies would appear yellow, flat, and 2-3mm in diameter; suspected *V. parahaemolyticus* colonies would appear blue/green and 2-5mm in diameter) for the absence or presence of Vibrio spp. Five representative colonies were streaked from each plate onto SNA and incubated at 37°C for 24hrs, after which Gram stains and oxidase tests were performed on the colonies isolated onto SNA. Typical *Vibrio* colonies were oxidase positive; isolates were identified using Vitek 2 compact gram-negative (GN) ID cards (bio Mérieux, France). *Vibrio furnissii* (NCTC 11218) was used as a positive control for *Vibrio cholerae,* and *V. parahaemolyticus* (NCTC 109030) as a positive control for *V. parahaemolyticus.*

4.2.7 Statistical analysis

A Pearson correlation was conducted to determine the relationship between seawater samples, shellfish samples, physico-chemical parameters (temperature, salinity, and dissolved oxygen). For all the tests, the criterion for statistical significance was $p < 0.05$. The researcher performed statistical analysis using the statistical package IBM SPSS v28.0.0.0 (190).

4.3 RESULTS AND DISCUSSION

4.3.1 Physico-chemical parameters

Physico-chemical parameters recorded in Table 4.1 did not show a significant variation, where the recorded temperature ranged between 12°C to 19°C, salinity ranged between 33.91 psu to 35.45 psu, and dissolved oxygen between 0.71 to 2.96 ppm showing prevailing hypoxic conditions which are often associated with pollution due to anthropogenic activities (Kuk-Dzul and Díaz-Castaṅeda, 2016). Seawater temperature, salinity, pH, dissolved oxygen, turbidity, and organic matter becomes water quality stressors when available in excessive amounts (Steeves et al., 2018). These stressors may influence the survival, health, and growth of shellfish which depend on the water quality of their growing environments. Poor water quality increases the risk of shellfish contamination with disease-causing pathogens (Li et al., 2017).

An increase in salinity during warm periods and a decrease during cold periods were observed throughout the study and correlated with similar findings reported by Lamine et al. (2019). The lowest rainfall was observed in July and the highest rainfall in August. Colaiuda et al. (2021) found in their study that the amount of rainfall and the increased *E. coli* concentrations in shellfish depend on the specific area where the samples were collected. Chahouri et al. (2022) and Padovan et al. (2020) found that high precipitation increases levels of faecal coliform.

In this study, no clear indication of the influence of rainfall on *E. coli* levels was detected. Similar results were observed by Sampson et al. (2006), where no association was found between precipitation and bacterial concentrations. Tabanelli et al. (2017) included the influence of the flow rate of the river feeding into the coastal area of their study and concluded that meteorological events could bring a substantial amount of contaminated fresh water into coastal water. This could be the case with the Bok River which feeds into Saldanha Bay. During heavy rainfall, an increase in the Bok River flow rate increase is suspected, which could wash down all the runoff from upstream agricultural areas and runoff from roads and residential areas (Chahouri et al., 2022).

4.3.2 Prevalence of faecal coliforms and *Escherichia coli* in mussels and oysters

Oyster harvesting did not take place during the March sampling occasion. Sampling sites SP4 (during March and July) and SP5 (during March) could not be reached due to high tides.

Table 4.1: *Physico-chemical parameters of shellfish production areas and rainfall in Saldanha Bay*

Sampling site	Sampling date	Temperature (degree Celsius) °C	Salinity (PSU)	Dissolved oxygen (ppm)	Monthly average rainfall (mm)	Samples
Sp1	15 March 2021	17	35	2.2	54.4	Mussels
Sp2	15 March 2021	19	35.45	2.90	54.4	Mussels
Sp3	15 March 2021	18	35.36	2.96	54.4	Mussels
Sp1	14 July 2021	18	35.38	0.85	5.6	Mussels
Sp2	14 July 2021	12	35.23	0.73	5.6	Mussels
Sp3	14 July 2021	12.5	35.30	0.71	5.6	Mussels
Sp4	14 July 2021	12.5	35.36	0.82	5.6	Water
Harbour Deck	14 July 2021	-	-	-	5.6	Oysters
Sp1	25 August 2021	14.25	34.04	0.85	61.4	Mussels
Sp2	25 August 2021	14.74	33.91	1.59	61.4	Mussels
Sp3	25 August 2021	14.97	33.95	1.5	61.4	Mussels
Sp4	25 August 2021	14.40	33.64	1.71	61.4	Water
Sp5	25 August 2021	13.90	34.11	0.95	61.4	Mussels
Harbour Deck	25 August 2021	-	-	-	61.4	Oysters

In addition, no mussels were available during August at the SP4 site (Table 4.2). The total MPN counts per 100ml of mussel samples were between 4.9 and 4700 microorganisms/100ml, and for oysters they were 18 and 1000 microorganisms/100ml in (July and August), respectively. Increased total MPN counts of 400 microorganisms/100ml were observed in mussel samples collected at SP1 in July. Of the recorded total MPN count at this site, FC and *E. coli* counts of <0.18 microorganisms/100ml, respectively, were observed. In the August sampling run, mussels collected at SP2 recorded a total MPN count of 4700 microorganisms/100ml, while a total MPN count of 1000 microorganisms/100ml) was recorded in oyster samples at the Harbour Deck site. In comparison, the FC and *E. coli* concentrations at these respective sites were 0.2 microorganisms/100ml, respectively (mussels), and <0.18 microorganisms/100ml, respectively (oysters).

Mussels and oysters can accumulate and retain suspended particles of phytoplankton size and pathogenic microorganisms in their bodies due to their filter-feeding nature (Rubini et al., 2018; Jeamsripong and Atwill, 2019). This creates a public health concern, especially for oysters, as oysters are consumed raw or partially cooked (Campos et al., 2017; Fusco et al*.,* 2017). The spikes observed in mussels and oysters suggest possible contamination due to heavy rainfall or pollution sources, including the sewage pump stations, stormwater drains, and a sewage discharge point that is located close to the affected sampling sites (Campos et al., 2013; Henigman et al., 2019).

Saldanha Bay Municipality recently made remarkable improvements to their sewage treatment plants and diverted the majority of treated effluent for the irrigation of sports grounds and use by interested local businesses. However, the small quantity that is being discharged together with effluent from fish factory industries, untreated stormwater discharge and ballast water, should not be underestimated. According to Clark et al. (2020), the shipping traffic has increased in the harbour, which brings large volumes of ballast discharge. All of these need to be monitored closely. Several studies in various parts of the world seem to agree on the fact that microbial contaminants are the result of treated and untreated sewage being discharged into shellfish growing waters, sewage overflow during rainfall periods, and runoff from agricultural areas (Keller et al*.,* 2019; Durand et al., 2020). Sewage is loaded with nutrients that, in excessive amounts, could stimulate microbial growth, production of harmful algal blooms, and eutrophication, ultimately affecting the viability of shellfish mariculture (Webber et al., 2021). Even though oyster samples were not taken from the farm but at the loading area of the harbour, i.e., the Harbor Deck, the samples came from the same farming area as the mussels.

Table 4.2: *Faecal coliforms and Escherichia coli counts in mussel and oyster homogenate*

Sample date	Sample point	Total MPN count (microorganisms/100ml)	Faecal coliforms (microorganisms/100ml)	*E.coli* (microorganisms/100ml)
15 March 2021	SP1	4.9	<0.18	<0.18
15 March 2021	SP2	4.9	<0.18	<0.18
15 March 2021	SP3	33	<0.18	<0.18
14 July 2021	SP1	400	<0.18	<0.18
14 July 2021	SP2	13	<0.18	<0.18
14 July 2021	SP3	24	<0.18	<0.18
14 July 2021	SP4	13	<0.18	<0.18
14 July 2021	Harbour Deck (oyster)	18	<0.18	<0.18
25 August 2021	SP1	7.9	<0.18	<0.18
25 August 2021	SP2	4700	0.2	0.2
25 August 2021	SP3	40	<0.18	<0.18
25 August 2021	SP5	60	<0.18	<0.18
25 August 2021	Harbour Deck (oyster)	1000	<0.18	<0.18

4.3.3 Prevalence of faecal coliforms and *Escherichia coli* in seawater

Sampling site SP5 could not be reached due to high tides in March and July (Table 4.3). The total MPN count/100ml in seawater ranged from <0.18–1.3 microorganisms/100ml, with a high spike recorded at SP2 in August (2400 microorganisms/100ml). Faecal coliforms and *E. coli* concentrations were the same (<0.18 microorganisms/100ml) at all sampling sites. The high increase in the total MPN count observed in SP2 in the seawater sample correlates with a spike in mussels collected during the same period. This could be attributed to heavy rainfall, stormwater drain discharges and sewage discharges, and the location and proximity of the sampling site to pollution sources. Sampling site SP2 is located in Small Bay which is subjected to various sources of pollution including a sewage discharge outfall. Understanding the causes of faecal contamination in areas where shellfish are grown is essential for assessing the associated health risks and determining the way forward to address the problem (Florini et al., 2020).

During high tide episodes, pollutants can be transported rapidly from the areas where they are highly concentrated through advection, mixing, dispersion, and dilution of sewage (Cravo et al., 2022). The sampling sites in Small Bay, sheltered from the sea swells and close to the sewage discharge point, sewage pump stations, and stormwater drains may not benefit from this natural process and therefore presented higher contamination levels. These natural processes are also evident in the analysis results of the Big Bay and Outer Bay sampling sites where lower contamination levels were observed. Both sites are semi-exposed to the sea swells, explaining the relative improvement in water quality. In other words, the possibility of having shellfish farms far away from sewage discharge points could eliminate the microbial contamination problem. Similarly, Florini et al. (2020) reported a decrease in the concentrations of faecal indicator species with an increase in distance from sewage discharge points. The low concentration results were ascribed to possible dilution and die-off effects.

Contamination of water bodies by wastewater is a fundamental problem worldwide. The bacteria, parasites, and viruses from animals and humans reach the oceans through runoff from roads, agricultural areas, and sewage discharges (Chinnadurai et al., 2020). In addition, heavy rainfall may cause sewage overflows and drain leakages (Lunestad et al., 2016). As mentioned, faecal coliform and *Escherichia coli* are indicators of water quality. The presence of these organisms is undesirable in areas used for shellfish farming.

Table 4.3: *Prevalence of faecal coliforms and Escherichia coli in seawater samples*

Sample date	Sample point	Total MPN count (microorganisms/100ml)	Faecal coliforms (microorganisms/100ml)	*E. coli* (microorganisms/100ml)
15 March 2021	SP1	0.2	<0.18	<0.18
15 March 2021	SP2	0.2	<0.18	<0.18
15 March 2021	SP3	1.3	<0.18	<0.18
15 March 2021	SP4	0.2	<0.18	<0.18
14 July 2021	SP1	0.2	<0.18	<0.18
14 July 2021	SP2	0.2	<0.18	<0.18
14 July 2021	SP3	0.2	<0.18	<0.18
14 July 2021	SP4	<0.18	<0.18	<0.18
25 August 2021	SP1	<0.18	<0.18	<0.18
25 August 2021	SP2	2400	<0.18	<0.18
25 August 2021	SP3	<0.18	<0.18	<0.18
25 August 2021	SP4	<0.18	<0.18	<0.18
25 August 2021	SP5	<0.18	<0.18	<0.18

No correlation could be drawn between the total MPN count in water (microorganisms/100ml) and shellfish (microorganisms/100g) samples, physico-chemical parameters, as well as between rainfall patterns and MPN counts in water and shellfish ($p>0.05$) (Table 4.4). As the total MPN count in water samples increased, the total MPN count in shellfish samples increased ($r = 0.997$, $n = 11$, $p = <0.001$).

Table 4.4: *Correlations between physico-chemical parameters, rainfall, and total MPN count in seawater and shellfish*

		Shellfish (MPN count 100ml)	Water (MPN count 100ml)
Shellfish (MPN count/100ml)	Pearson Correlation	1	.997**
	Sig. (2-tailed)		<.001
	N	14	11
Water (MPN count/100ml)	Pearson Correlation	.997**	1
	Sig. (2-tailed)	<.001	
	N	11	13
Temperature (°C)	Pearson Correlation	-.029	-.048
	Sig. (2-tailed)	.933	.881
	N	11	12
Dissolved oxygen (ppm)	Pearson Correlation	.030	.042
	Sig. (2-tailed)	.931	.897
	N	11	12
Salinity (psu)	Pearson Correlation	-.442	-.357
	Sig. (2-tailed)	.174	.254
	N	11	12
Average monthly rainfall (mm)	Pearson Correlation	.245	.243
	Sig. (2-tailed)	.467	.446
	N	11	12

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

4.3.4 Bacterial species isolated from selected sample sites

Salmonella spp., *Vibrio cholerae* and *Vibrio parahaemolyticus* were not detected. Bacterial species identified included the *Enterobacter cloacae complex; Citrobacter freundii; Klebsiella pneumoniae* spp. *pneumoniae; Aeromonas sobria; Vibrio alginolyticus;* and *Sphingomonas paucimobilis* (Table 4.5). These microorganisms may be grouped into pathogens that are often present in aquatic environments (e.g.

Klebsiella pneumoniae spp.; Aeromonas sobria; Vibrio alginolyticus; and *Sphingomonas paucimobilis).* These microorganisms are pathogens of high priority as some are antimicrobial resistant and may cause illnesses in humans. In addition, pathogens naturally present in human beings and animals *(e.g., Citrobacter freundii and Enterobacter cloacae complex),* are also high-priority pathogens and their presence should not be taken lightly (Pot et al., 2021). The *Enterobacter cloacae complex* has also proved to be abundant in aquatic environments (Zhou et al., 2018).

Table 4.5: *Bacterial species isolated from mussels and oysters sampling points*

Samples and Sampling point	Sampling Date	Growth Media	Isolated organisms	Vitek probability
SP1 – mussel	14 July 2021	SS Agar	*Enterobacter cloacae complex*	99 %
Sp2 – mussel	14 July 2021	SS Agar	*Enterobacter cloacae complex*	99 %
Sp3-mussel	14 July 2021	SS Agar	*Enterobacter cloacae complex*	99 %
HD-oyster	14 July 2021	TCBS	*Citrobacter freundii*	95 %
HD-oyster	14 July 2021	SS Agar	*Klebsiella pneumoniae spp*	95 %
Sp1-mussel	25 August 2021	SS Agar	*Aeromonas sobria*	87 %
Sp2-mussel	25 August 2021	TCBS	*Vibrio alginolyticus*	90 %
Sp3-mussel	25 August 2021	TCBS	*Sphingomonas paucimobilis*	86 %
Sp5-mussel	25 August 2021	SS Agar	*Aeromonas sobria*	89 %
HD-oyster	25 August 2021	SS Agar	*Klebsiella pneumoniae spp*	91 %

* SS Agar (*Salmonella shigella* agar), TCBS (Thiosulphate citrate bile sucrose agar), HD (harbour Deck)

4.4 CONCLUSION

The study used conventional culture methods to isolate *Salmonella* and *Vibrio spp.* in mussels, oysters, and seawater samples obtained from the Saldanha Bay Harbour. The Most Probable Number (MPN) analysis technique was used for detecting and enumerating faecal coliforms and *E. coli* in the obtained samples. The identification of species was conducted using the Vitek 2 automated system, which successfully identified species such as *Enterobacter cloacae complex; Citrobacter freundii; Klebsiella pneumoniae* spp*. pneumoniae; Aeromonas sobria; Vibrio alginolyticus;* and *Sphingomonas paucimobilis*. The findings observed with correlations between MPN counts in seawater, mussels and oysters, and correlations between physico-chemical parameters and rainfall, did not show any significant difference. However, total MPN count spikes were observed in mussels, oysters, and seawater, which could be ascribed to the rainfall period and winter season although the spikes did not have a significant impact on the *E. coli* concentrations, as the concentrations were below the permissible limits. This information may be used as a basis to conduct an in-depth investigation of sources of pollutants. Further studies need to be conducted on the bacterial species identified to determine their prevalence and assess the probability of their presence becoming a public health threat to shellfish consumers.

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CHAPTER 5

THE PREVALENCE OF VIRUSES RELATED TO THE PRODUCTION OF MUSSELS AND OYSTERS IN SALDANHA BAY: A SYSTEMATIC REVIEW

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ABSTRACT

The disposal of treated and untreated sewage near shellfish harvesting areas is a global concern. Discharged sewage may be contaminated with enteric viruses present in human faeces. Bivalve molluscs, in turn, act as vectors for enteric viruses through bioaccumulation and retention of these viruses during the filter-feeding process, resulting in outbreaks of infections due to the consumption of contaminated shellfish. This review was conducted using peer-reviewed articles published from 2012 until September 2022, obtained from online databases such as Google Scholar, Scopus and Science Direct, highlighting the challenges that the shellfish industry is faced with concerning pollutants ending up in the shellfish production areas. Developed countries have made some advancements by upgrading sewage infrastructures, which reduced viral loads in sewage. However, it is difficult to measure the significance of these improvements, as there are no regulations in place which stipulate the permissible limits for viruses. In most developing countries, including South Africa, there is a lack of effective management plans for virus monitoring in shellfish harvesting areas. The findings of this study indicated a need for extensive research on the origin of viruses, their interactions with other organisms within the marine ecosystem, the quantification of viruses within the Saldanha Bay harbour, and the development of virus management plans which currently are non-existent.

KEYWORDS**:** shellfish, production areas, norovirus, hepatitis A, enteric viruses.

5.1 INTRODUCTION

The shellfish farming industry has seen exponential growth worldwide, providing an alternative source of protein needed for the growing world population (Chan et al., 2019; Willer and Aldridge, 2019). Shellfish farming is conducted in rivers, estuaries, and open seas. In open seas, the ideal location is a sheltered embayment, often surrounded by residential and industrial areas. However, the challenge is the protection of shellfish farms or production areas from contamination (McLeod et al., 2017). Production areas are subjected to the discharge of treated and untreated sewage, which may be loaded with bacteria and viruses, as well as wastewater disposal from industries nearby, which may be loaded with chemicals and heavy metals (Freeman et al., 2019; Todd et al., 2019; Bashir et al., 2020). Enteric viruses are widely spread in soil, water and food. Viruses are also excreted in large quantities in the faeces of infected individuals (up to 10^{11} viral particles per gram of stool) (Brake et al., 2014), and are persistent in the environments where they are found (Purpari et al., 2019). Some of the enteric viruses of concern include norovirus Genogroup I and Genogroup II (NoV GI and GII); hepatitis A (HAV); rotavirus (RV), astrovirus (AsV); sapovirus (SaV), adenovirus (AdV), aichivirus (AiV) and Hepatitis E (HEV). The SaV, AiV, and HEV are regarded as emerging viruses (Fusco et al., 2019; Fiorito et al., 2021). Norovirus (NoV) is regarded as the most common cause of acute and nonbacterial gastroenteritis in humans and is implicated in most shellfish-borne viral outbreaks (Strubbia et al., 2016). Furthermore, NoV is reported as the most causative agent in foodborne outbreaks in European countries after *Salmonella* bacteria. It was also associated with 457 outbreaks in 2019 in Europe (Savini et al., 2021), with oysters being the most implicated bivalve molluscs in foodborne outbreaks (Le Guyader et al., 2008; Bellou et al., 2013; Woods et al., 2016; Meghnath et al., 2019).

Hepatitis A (HAV) has also been reported as a cause of foodborne diseases (Richards, 2016), and a common cause of acute hepatitis infections worldwide with mild illnesses (Yan et al., 2022). It is transmitted through the faecal-oral-route from person to person or through consumption of contaminated food or water. The biggest outbreak of HAV was in Shanghai in China in 1988, which was attributed to the consumption of raw clams (Yan et al., 2022). Improved hygiene conditions and the introduction of the Hepatitis A vaccine caused a decline in cases until recently, in 2020 when an outbreak occurred in Yantai, which also implicated the consumption of contaminated shellfish. Other foods such as frozen berries have been implicated in an outbreak of HAV in Italy in 2013 -2014 (Scavia et al., 2017). Hepatitis E virus is a major cause of acute hepatitis in humans. Approximately 17 outbreaks have been reported in Africa annually since 1979 resulting in 650 deaths (Modiyinji et al., 2021). Pregnant women are at an increased risk of developing complications with HEV infection that could lead to fulminant hepatic failure (Simani et al., 2022).

Bivalve molluscs, including oysters and mussels, feed on suspended materials in their growing waters. They filter large quantities of water subsequently accumulating and retaining available contaminants that are bound to the suspended materials (Fiorito et al., 2021). Shellfish, apart from being a good source of protein and rich in micronutrients, play an important role in the marine environment as they purify water, thereby reducing eutrophication (Ahmed et al., 2019). The shellfish's nature of filter feeding is a public health concern as contaminants such as viruses can pose a health threat to shellfish consumers. Moreover, disease outbreaks where shellfish contaminated with enteric viruses are consumed, are exacerbated by the norm of eating shellfish raw or partially cooked (Hassard et al., 2017; Baptista et al., 2020; Gyawali and Hewitt, 2020).

Sources of contaminants including sewage outfall, illegal wastewater discharge, stormwater discharge, heavy rainfall, agricultural runoffs, overboard disposal of faeces from boats, river flows, informal settlement dwellers, and natural disasters such as hurricanes and flooding increase the risk of contamination of shellfish growing areas (Campos and Lees, 2014; Mok et al., 2018; Trottet et al., 2022; Vinothkannan et al., 2022). Even though the available literature is mainly from other parts of the world, 17 studies were conducted on enteric viruses in shellfish over the past two decades in Africa (Upfold et al., 2021). In South Africa, studies were conducted on the contamination of rivers by enteric viruses (Lin and Singh, 2015; Adefisoye et al., 2016; Marie and Lin, 2017; Potgieter et al., 2020). Few studies have been conducted on the contamination of shellfish by viruses and none so far explored virus contamination in Saldanha Bay (Onosi et al., 2019). Contaminants affecting Saldanha Bay shellfish farms are primarily from port operations (export of metal ores), shipping traffic, ballast water discharge, oil spills, municipal sewage discharge, industrial effluent (brine, cooling water discharges, fish factory effluent) and stormwater discharge which may contain enteric viruses (Clark et al., 2020).

5.1.1 Impact of enteric virus-contaminated shellfish on public health

Norovirus has been implicated in 14-23% of global infections due to the consumption of contaminated shellfish. It is also responsible for 125 million foodborne illnesses and 35 000 deaths in a year (Hassard et al., 2017). Norovirus has six genogroups (GI – GVI), with genogroups I, II, and IV associated with human gastroenteritis infections. In the United Kingdom (UK), approximately 74 000 NoV cases due to the consumption of contaminated foods and 11 800 due to the consumption of contaminated oysters are reported annually (Gyawali et al., 2019a). Clinical symptoms include headache, fever, diarrhoea, abdominal cramps, and nausea. Clinical symptoms related to HAV infections include jaundice, diarrhoea, abdominal pain, fever, malaise, and loss of appetite, with 1.4 million cases reported globally per annum (Elbashir et al., 2018). It can also cause acute liver failure which may be fatal (Randazzo and Sánchez, 2020). Studies conducted on HAV-contaminated foodstuffs have only contributed to 2-7% of all the food-related outbreaks worldwide, and are also reported as less common in bivalve molluscs compared to NoV (Randazzo and Sánchez, 2020; Suffredini et al., 2020).

Hepatitis E is receiving attention in food contamination as it affects 20 million people per annum with 56 600 deaths (Rivadulla et al., 2019). It is regarded as an emerging zoonotic foodborne threat (Romalde et al., 2018). Shellfish consumption is implicated in transmitting HEV in Asian countries such as China, Japan, Korea, and Thailand. In Vietnam, it has been detected in shellfish samples (Suffredini et al., 2020). A few cases have also been reported in European countries including, as well as Netherlands, Spain and the UK (Fusco et al., 2019; Suffredini et al., 2020; Prabdial-Sing et al., 2021). In Italy, HEV has not been detected in shellfish approved harvesting areas (Suffredini et al., 2020). In the African continent, HEV is a waterborne disease that is transmitted through faecal contamination of water and poor sanitation and hygiene. The lack of specific HEV surveillance in most African countries makes it difficult to monitor trends in disease incidence and to identify outbreaks (Bagulo et al., 2020; Prabdial-sing et al., 2021; Simani et al., 2022).

Aichivirus was reported in 1989 in Japan as the cause of oyster-associated nonbacterial gastroenteritis (Le Guyader et al., 2006). Sapovirus was first detected in an outbreak which occurred at an orphanage in Japan in 1977 and was detected in 3 out of 11 outbreaks in Japan which occurred between 2002 and 2006, due to the consumption of contaminated oysters (Nakagawa-Okamoto et al., 2009; Varela et al., 2016). Outbreaks are also common among children (Varela et al., 2016). Rotavirus causes acute diarrhoea in children under 5 years of age, with clinical symptoms including diarrhoea, anorexia, dehydration, and occasional vomiting (Richards, 2016). Clinical symptoms relating to Astrovirus infections include occasional vomiting, fever, abdominal pain, and anorexia. It has been detected in mussels and oysters, even though fewer outbreaks have been reported (Vasquez-García et al., 2022). Rotavirus is one of the main causes of childhood diarrhoea worldwide. It has not been linked to gastroenteritis outbreaks related to seafood; neither has adenovirus (Keller et al., 2019). Table 5.1 shows some of the documented foodborne disease outbreaks due to viral infections.

Table 5.1: *Enteric viruses associated with shellfish disease outbreaks*

Enteric virus	**Country and year**	**Bivalve mollusc**	**Reference (s)**
Norovirus (GI and GII)	France, 2012	Oyster	(Loury et al., 2015)
	Canada, 2016	Oyster	(Meghnath et al., 2019)
	US, 2009 – 2014	Oyster	(Woods et al., 2016)
	South Korea, 2013	Oyster	(Cho et al., 2016)
	France, 2019	Oyster	(Fouillet et al., 2020)
Hepatitis A	Hawaii, 2016	Scallops	(Viray et al., 2019)
	China, 1988	Clams	(Yan et al., 2022)
	China, 2020	Oyster	(Yan et al., 2022)
	Italy, 1997 – 2004	Raw shellfish	(Purpari et al., 2020)
	Australia	Oysters	(Richards, 2016)
Aichivirus	Japan, 1989	Oyster	(Le Guyander et al., 2008)
Sapovirus	Japan, 1977	Oyster	(Nakagawa-Okamoto et al., 2009)
Astrovirus, Adenovirus, Rotavirus		The rapid increase of cases but not frequently implicated in outbreaks occurrence (Fusco et al., 2019; Vasquez-García et al., 2022)	

Apart from the contaminants mentioned above, there is currently contaminants described as emerging contaminants (pharmaceutical products, personal care products, antibiotics, surfactants, artificial sweeteners, flame retardants, cleaning solvent, sun protection products etc.), including microplastics. Their presence in the marine ecosystem is a concern for the health of marine organisms. They are aggressive in nature, have toxic effects and the ability to bioaccumulate and are not effectively removed from sewage by the traditional wastewater treatment methods (Rathi et al., 2021). Microplastics may act as vectors for the spread of antimicrobial-resistant genes, and harmful microorganisms, and as a microcosm for gene exchange between bacteria (Ahmad et al., 2020; Li et al., 2021; Moresco et al., 2021).

The microbiological monitoring programme in Saldanha Bay is well established for the monitoring of bacterial contamination, but lacking for virus contamination monitoring. This review aims to gather epidemiological data available on virus contamination at shellfish harvesting areas. The existing data could assist with information relevant for policy-makers to consider when developing policies and strategies in managing virus contamination in Saldanha Bay.

5.2 MATERIALS AND METHODS

For this desktop review, all literature on virus contamination in shellfish harvesting areas worldwide was included. The literature search was conducted using online electronic databases such as Science Direct, Scopus and Google Scholar with a timeframe of 2012 and 2022 to ensure that the latest information was included. However, there were no timeframe restrictions on the introduction and discussion sections. The Boolean logical connectors (‘AND’ and ‘OR’) and truncation were applied in the search strategy. The search terms were mussels and oysters AND hepatitis A and “norovirus GI and GII” AND production areas OR shellfish farms OR growing waters AND sources. The following inclusion and exclusion criteria were used: the original articles written in English from peer-reviewed journals and the prescribed timeframes were included. Unpublished papers, theses, non-peer-reviewed articles, articles on mussels and oysters from wholesale and retail markets, food processing, post-harvest treatments, book chapters and abstracts were excluded. The exclusion and inclusion criteria were strictly followed to reduce the risk of biases. Two reviewers worked together to screen records and retrieve reports. A report was retrieved from the Scopus database. Reports were not available for retrieval from Google Scholar and Science Direct. Records screened from Google Scholar and Science Direct that met the inclusion criteria were saved in the Google Scholar library of one of the reviewers. The screening, evaluation and identification of records for eligibility were conducted following the Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) flow diagram (Figure 5.1) (Zelalem et al., 2019).

5.3 RESULTS

A total of 181 records were identified through a database search. After screening the records and looking at titles, abstracts and duplicates, 147 records were excluded. Full-texts of the remaining 29 were scrutinised for eligibility and 10 records were excluded. Nineteen records were retained for qualitative synthesis (Figure 1).

Table 5.2 summarises the findings and recommendations of the studies that were included in this review. Most of the studies implicated sewage discharge in shellfish growing areas as the source of enteric virus contamination. Therefore, recommended the development of effective management plans for shellfish harvesting areas, and the development of virus compliance limits as well as virus indicator species, since the use of *E. coli* has proved to be ineffective in detecting viral contaminants.

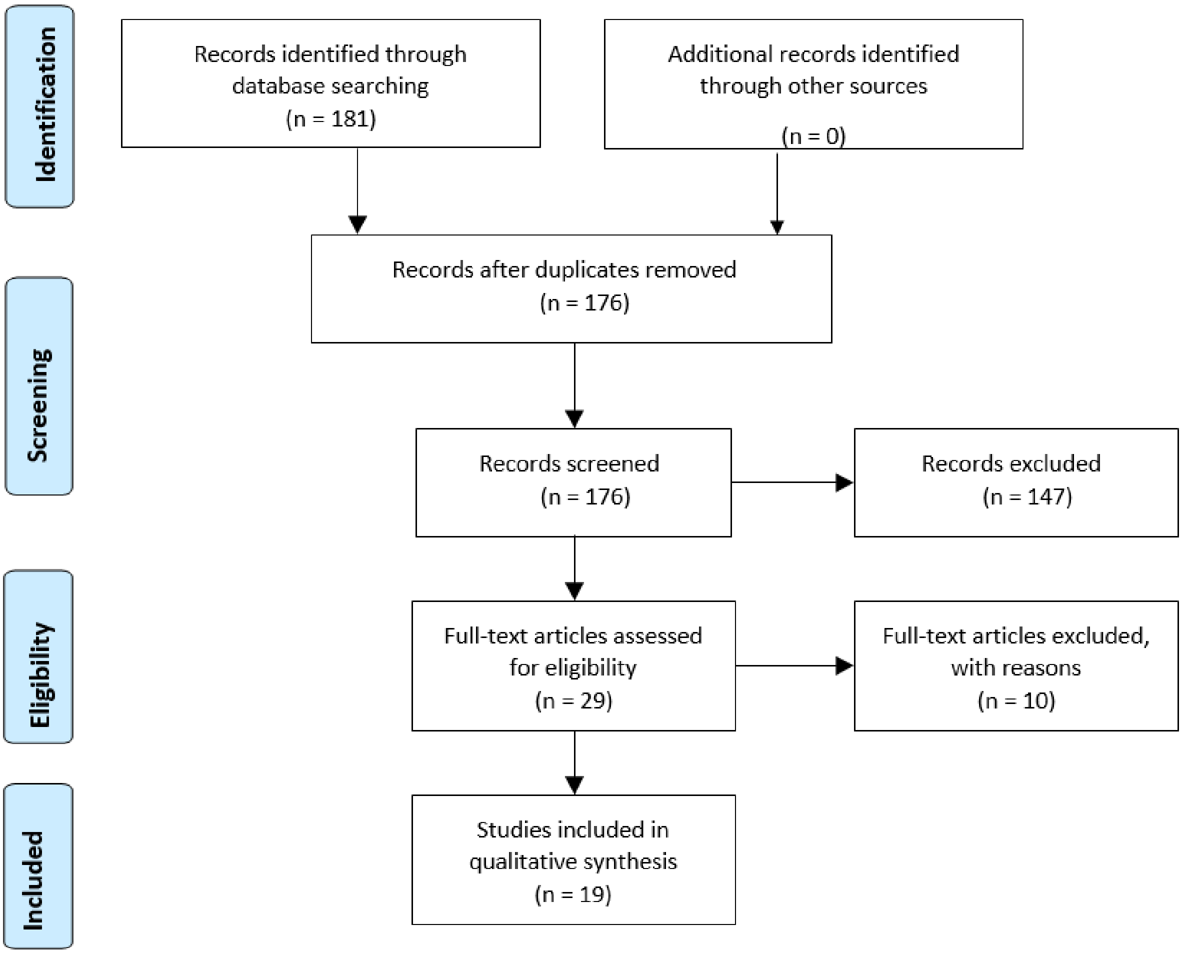

***Figure 5.1** PRISMA methodology of literature search (available from: http://www.prisma- statement.org, [6 September 2022]*

Table 5.2 represents the literature available on enteric viruses' contamination in shellfish harvesting areas and the studies conducted according to the search keywords used per country. The data search was from 2012 until September 2022, the studies conducted per country are as follows: Britain (1); Italy (6); Australia (1); England and Wales (1); Montenegro Coast (1); Brazil (3); France (1); Argentina (1); Spain (1); Poland (1); Korea (1) and Morocco (1).

Table 5.2 *Summary of studies included in the review that met all the criteria specified*

Study area	**Aim of the study**	**Findings**	**Recommendations**	**References**
Poole Harbour, Britain	1) To determine the impact of geographical and temporal changes on *E. coli* and norovirus in mussels. 2) Influence of environmental factors.	1) High concentrations of NoV on sampling sites close to the sewage discharge point. 2) Higher concentrations of *E. coli* in mussels than in seawater.	Geographical and temporal variations must be considered in harvest area monitoring programmes and most importantly incorporated into food safety regulations and harvest area management plans.	(Strubbia et al., 2016)
Syracuse, Italy	Assessment of enteric viruses including HAV and NoV in bivalve molluscs.	Detection of enteric viruses indicates they are widely distributed in aquatic environments.	1) Development of strategies and methodologies to assess public health risks. 2) Systemic controls for viral pathogens to protect consumers. 3) An integrated surveillance system reporting food safety, environmental exposure, and clinical cases.	(Purpari et al., 2019)
Gulf of Pozzuoli, Italy	Determine existence of a relationship between viral and chemical contamination.	Oysters demonstrated a possible link between chemical pollutants and the bioaccumulation of multiple enteric viruses.	Efficient monitoring of harvest areas to determine a correlation between environmental pollutants and foodborne viruses.	(Fiorito et al., 2021)
Sardinian shellfish farms, Italy	1) Detection of *E. coli,* HAV and NoV in shellfish samples. 2) Assessment of possible relationships between pathogens and seasonality.	Norovirus was prevalent in all areas during the winter season.	1) Stringent shellfish monitoring programmes. 2) Inclusion of environmental parameters. 3) Improved monitoring plans could lead to better management of shellfish harvest areas.	(Bazzardi et al., 2014)
Baltic sea, Poland	Evaluate NoV and HAV occurrence in wild Baltic mussels and possible inclusion as indicator species for viruses.	Underreporting of viruses' occurrence in wild shellfish could limit insight into the role they play in environmental viral transmission.	The development of viral indicators such as *E. coli* is not effective in the prediction of viral pollution.	(Bigoraj et al., 2014)

Australia, New South Wales, Tasmania and South Australia	Assess the occurrence of NoV and *E. coli* in pacific oysters and Sydney rock oysters.	The human population around the shellfish growing areas is lower in Australia compared to other countries.	An increase in viral contaminations calls for control measures specific to virus pollution in shellfish-growing areas.	Brake et al., 2014
Commercial harvesting areas- coast of England and Wales	Determine the possible predictor variables for NoV in oysters.	Heavy rainfall was implicated in high concentrations of *E. coli* levels.	Viruses-specific control measures are required for monitoring harvesting areas. Improvement of sewage infrastructure and improvement of risk management measures.	(Campos et al., 2017a)
Campania region, Italy	To establish a relationship between environmental parameters and viral contamination.	The presence of chemical contaminants influenced viruses' bioavailability.	Integration of microbiological and chemical monitoring programmes as an effective warning system.	(Fiorito et al., 2019)
Gulf of Naples, Italy	Evaluate the effects of the different enteric viruses within bivalve molluscs.	Confirmed circulation of multiple enteric viruses in bivalves.	Consumer awareness campaigns on the importance of cooking seafood thoroughly.	(Fiorito et al., 2019)
Montenegro Coast	To determine the distribution rate of NoV in mussels along the coast.	Seasonality and the human population contribute to the prevalence and spread of viruses.	1) Improvement of shellfish monitoring programmes. 2) Development of virus-specific criteria.	Iliac et al., 2017
Mangrove estuary, Brazil	Quantify enteric pathogenic viruses.	1) High concentrations of HAV, NoV and AdV were detected during winter months in water, mussels, and oyster samples. 2) Estuary should be classified as a prohibited zone for bivalve harvesting for human consumption.	Improvement in shellfish monitoring programmes.	(Keller et al., 2019)
Atlantic Coast Lagoon, France	NoV contamination and gastroenteritis outbreaks.	Seasonality and high prevalence of NoV in oysters.	1) Prevention of contamination. 2) Development and implementation of an outbreak alert system.	(Le Mennec et al., 2017)
Production in Sicily, Italy	To develop a routine monitoring plan for enteric viruses.	Prevalence of enteric virus.	Development of a monitoring system for viruses, using data from epidemiological studies and existing molecular methods.	(Macaluso et al., 2021)

Southern Coast of Buenos Aires province, Argentina	To determine the presence of NoV and RvA in oysters.	Detected NoV in RvA in samples and resistance to heat treatment.	Understanding viruses' circulation to improve existing surveillance plans.	(Mozgovoj et al., 2022)
Galicia, Spain	Compile a database on the prevalence of enteric viruses in shellfish.	1) Harvest areas affected by close proximity to pollutants discharge points and dilution factors. 2) Role of bivalves as a vector of zoonotic diseases.	A better understanding of emerging enteric viruses' behaviour and pathogenicity.	(Romalde et al., 2018)
Jinhae Bay, Korea	Investigate the contamination status of the oyster growing area by NoV and the circulation of NoV genotypes.	NoV GII strains are the most prevalent in oysters and elimination can be difficult.	1) No commercial harvesting in polluted areas. 2) Prevent faecal contamination in production areas.	(Shin et al., 2013)
Florianópolis, on the coast of Santa Catarina, Brazil	To perform a thorough diagnosis of the shellfish growing areas.	1) Contamination by various pathogens due to sewage discharge. 2) Complexities of microbiological and chemical compound contaminants.	Control measures are necessary to ensure the safety of produce.	(Souza et al., 2012)
Lagunar complex Cananẻia, Brazil	Analysed the presence of Astrovirus and NoV in mussels and oysters.	Mussels and oysters tested positive for the studied viruses.	Mapping out the pollution sources, using the findings to develop risk assessment and control measures of pathogen contamination.	(Vasquez-García et al., 2022)
Oualidia lagoon, Morocco	Evaluate NoV frequency in bivalve shellfish harvested at Oualidia lagoon.	Risk of contamination in oysters.	Importance of implementing a national survey plan and sanitary control for the viral risk, including NoV in bivalve molluscs.	(El Moqri et al., 2019)

These studies included Hepatitis A, norovirus, and other emerging viruses, with a focus on contamination in harvesting areas. Most of the studies conducted were on oyster contamination with the most implicated virus being norovirus.

5.4 DISCUSSION

Contamination of bivalve molluscs with viruses is a global concern. Vulnerable people such as the elderly and immunocompromised individuals die when foodborne outbreaks occur (Hassard et al., 2017). According to Table 5.2, only one study was conducted on the African continent. Urgent interventions are needed for the continent to develop food safety in the shellfish industry to the same level as other developed and developing countries. Detection of HAV and NoV indicates that these agents are widely distributed in aquatic environments (Cooper et al., 2018; Mannion et al., 2020). This correlates with global trends where sewage is discharged along the coastal areas, including African countries and South Africa in particular. The findings and recommendations from the nineteen studies listed in Table 5.2 could be a starting point for the development of management plans for virus detection and monitoring in Saldanha Bay.

The summary of Table 5.2 findings and recommendations are as follows: High concentrations of NoV at harvesting areas or sampling sites close to the sewage discharge point were mentioned by most of the studies. Disposal of sewage whether treated or untreated is a major challenge facing the shellfish industry globally (Campos and Lees, 2014; Polo et al*.,* 2015; Hassard et al., 2017; Tan et al., 2018; Gyawali et al., 2019b; Sharp et al., 2021; Pouillot et al., 2022). Sewage discharge points near shellfish harvesting areas, give rise to the challenge of eliminating viruses by bivalve molluscs. Pouillot et al. (2022) demonstrated in their study how oysters bioaccumulated viral particles in high concentrations within a short period. They also added that through the bioaccumulation process, virus concentrations in oyster tissues exceeded the virus concentrations in the water. Similarly, Sharp et al. (2021) observed high concentrations of NoV in mussels within 3 hours of exposure to water contaminated with treated and untreated sewage. Souza et al*.* (2018) added that viral particles may be 100 times more concentrated in bivalve molluscs' organs than in contaminated seawater. To prevent contamination of commercially harvested bivalves, the United States Food and Drug Administration (USFDA) determined a prohibition zone, where bivalves may not be harvested for commercial purposes, and decided on the dilution criteria of 1000 parts of receiving waters to every part of effluent (1000:1) (NSSP, 2019). These were determined through comparisons of studies such as

USFDA hydrographic dye dispersion and numerical hydrodynamic models to track the discharge of wastewater. The 1000:1 dilution criterion is equated to waters within a distance of 3.77 km from the sewage outfall (True, 2018). Schaeffer et al. (2018) also agree with having a buffer zone whereby shellfish harvesting is prohibited if contamination cannot be prevented.

Chemical contaminants from industrial activities and runoffs from land also pose a challenge and a public health risk. The risk for consumers differs depending on the chemical compounds formed by different chemicals. For example, Arsenic (As) in its inorganic form may alter tumor-suppressing genes and has been classified by the international agency for research on cancer (IARC) as cancer-causing in humans (Battistini et al., 2021). Fiorito et al. (2019) in their studies observed a correlation between chemical pollutants and viral contaminants in mussels. The mussels that tested positive for viral presence exhibited higher levels of chemical pollutants than the mussels that tested negative for viral contaminants. The higher the exposure to chemical agents, the weaker the immune system and thus the higher the uptake of microbial agents (Ukwo et al., 2019). The effects of having both biological and chemical contaminants need to be explored further to ascertain a possible link between chemical pollutants and the bioaccumulation of multiple enteric viruses in bivalve molluscs (Fiorito et al., 2019).

Adding to the burden of chemical contaminants are Microplastics, which are considered emerging pollutants. Owing to their small size, they can be mistaken for food by bivalves. Some studies demonstrated that mussels and oysters ingest microplastic particles from the surrounding seawater (Bowley et al., 2021). Although the potential for human viruses to attach to microplastics and to persist in the plastisphere is unclear (Bowley et al., 2021), Moresco et al. (2021) indicated that biofilm on surfaces of plastics could provide a platform for viral attachment. Yamada et al. (2020) agree with the suggestion that Microplastics could be potential adsorbents for viruses. However, they added that more research is needed on the influence viruses have when attached to biotic and abiotic particles in marine ecology, as well as the extent to which these attached viruses are infective to their hosts. On the other hand, it is well established that microplastics can provide a habitat for the colonization and enrichment of Vibrio species and vectors of pathogenic bacteria including *Pseudomonas* and *Acinetobacter* (Junaid et al., 2022). The danger lies in the possibility of microplastics being a vehicle for the spread of infection in aquaculture which could have a detrimental economic impact (Ahmad et al., 2020).

Bivalve production sites are mostly situated along the coastal areas (Mok et al., 2018; Park et al., 2018; Theuerkauf et al., 2022), where possible exposure to sewage contaminated with human enteric viruses is high. Bivalves act as zoonotic disease vectors through the bioaccumulation process when filtering the water in their growing areas. The concentration and retention of enteric viruses for long periods pose a public health threat as bivalves are commonly eaten raw or partially cooked, particularly oysters (Gyawali et al., 2019). Coastal areas are becoming more attractive for residential developments and tourism (which is important for the coastal economy) as some are secluded and provide peace and quality of life away from densely populated suburban metropolitan areas (Guo et al*.,* 2017). Such developments bring an increase in activities such as boating, snorkelling, fishing and many more activities which may introduce pollution into the ocean near the developments (Kyzar et al., 2021). To meet the demands of the human population increase, agricultural lands are converted into residential and industrial developments, leading to altered water quantities and quality as well as altered sediment which ultimately lands in the ocean, increasing faecal microbial loads and nutrients (Freeman et al., 2019). El Mahrad et al. (2020) reported and described changes that are brought about by human influx in a coastal city in Morocco, including alteration of sedimentary forms especially the dunes on the east side of the lagoon. Furthermore, changes in ocean chemistry may cause ocean acidification, changes in precipitation, rise of sea levels, and seawater pH (He and Silliman, 2019). Increased inputs of nutrients could trigger harmful algal blooms (Landrigan et al., 2020).

A summary of the recommendations are as follows:

Geographical and temporal variations must be considered in monitoring programmes of harvesting areas and incorporated into regulations or harvesting area management plans. The environmental factors within the harvesting areas play a role in microbial pollutants' prevalence (Bazzardi et al., 2014). Strubbia et al. (2016) observed high microbiological contamination at a site that was receiving large amounts of sewage discharge and concluded that such events must be taken into consideration for monitoring harvesting areas. Microbial pollutants are not the only aspect affected by geographical and temporal variation, marine biotoxins also show similar trends. Goya et al. (2020) identified temporal variability with paralytic shellfish poisoning toxins which were higher in summer months, lower in winter and lowest in autumn. According to Wu et al. (2018) regional and seasonal differences were observed, where some biotoxins bloomed during the upwelling season and some during the downwelling season. Spatial and temporal variability could also be due to coastal natural processes and local changes in environmental conditions (Rufino et al., 2018).

There seems to be a consensus about the lack of effective management plans or strategies in shellfish harvesting areas (Bazzardi et al., 2014; Strubbia et al*.,* 2016; Ilic et al., 2017; Romalde et al., 2018; Fiorito et al., 2021). Some researchers also highlighted the need to have an integrated surveillance system that will enable simultaneous reporting on environmental exposure and clinical cases to facilitate tracking and correlation of occurrences of clinical cases with environmental factors in harvesting areas (Purpari et al., 2019). La Rosa et al. (2020) agreed with the concept of an integrated surveillance system and added that it could be useful to monitor changes in viral patterns and could offer a warning of contamination in advance. Some researchers also recommended the inclusion of environmental parameters in shellfish management plans for the correlation between meteorological events and the prevalence of viruses. Furthermore, the development of viral indicators was recommended (Olalemi et al., 2016), as *E. coli* is not effective in the prediction of viral pollution since it has lower environmental resistance than viral pathogens (Strubbia et al. 2016). Sharp et al. (2021) demonstrated in their study how oysters accumulated *E. coli* and norovirus in their bodies and how both microorganisms responded to depuration, with *E. coli* being successfully purged from the shellfish tissues within 72 hours but failed to remove norovirus from the shellfish tissues. Several studies proposed the use of bacteriophages as indicator species for viral contamination. Others proposed the use of norovirus as an indicator species (Brake et al., 2014;

Campos and Lees, 2014; Winterbourn et al., 2016; Ilic et al., 2017; Sharp et al., 2021). The traditional way of eating bivalve molluscs, i.e. raw or undercooked, appears to be a health risk and consumer awareness is necessary. Consumers are perceived to be aware of chemical hazards in food as they cause long-term adverse effects including cancer and are less concerned about microbial hazards (Venugopal and Gopakumar, 2017). Ricardo et al. (2017) added that it is important to precisely know the bivalve origin and the sources of pollutants considering their sedentary lifestyle and feeding behaviour. They bioaccumulate pollutants, which is exacerbated by how they are eaten (raw or undercooked), leading to the risk of transmission of infections to humans. More studies on foodborne outbreaks associated with shellfish consumption will increase consumer awareness (De Silva et al*.,* 2021). Crovato et al. (2019) evaluated studies that conducted research on shellfish consumption and reported that consumers were confused about the risks and benefits of bivalve molluscs. They concluded that accurate information is necessary for helping consumers to make informed choices. In addition, Fiorito et al. (2019) highlighted the importance of awareness campaigns to inform consumers of the thorough cooking of seafood. This remains a challenge as people prefer eating oysters raw and mussels lightly cooked (Crovato et al., 2019).

Evaluation of sources of pollutants is the first step that may lead to effective management of shellfish harvesting areas. Knowing what pollutants are present, where they come from and their composition will enable policymakers to develop management plans and monitoring programmes accordingly (Vasquez-García et al., 2022). For example, Mok et al. (2016) evaluated the bacteriological seawater quality and oyster quality from a coastal area in Japan, where they conducted a sanitary survey to analyse all the possible sources of pollutants. The study concluded that faecal coliform levels were high after heavy rainfall events compared to other sampling occasions. Therefore, it was reported that oysters are safe to be consumed raw during dry seasons based on their bacterial water quality. Chinnadurai et al. (2020) similarly evaluated seasonal occurrences of faecal coliforms and found elevated levels during monsoon periods, and therefore suggested that authorities develop sanitary control in line with international standards, as no microbiological standards existed. The prevalence of viruses is influenced by seasonality. Several studies reported high concentrations of NoV during the winter season when heavy rainfall occurred (Maalouf et al*.*, 2010; Le Mennec et al*.*, 2017; Rincé et al*.*, 2018; Tan et al*.*, 2018; Sarmento et al*.*, 2020; da Silva et al*.*, 2022; Pouillot et al*.*, 2022). Lower concentrations were measured in summer months with low rainfall, which is suggested to be due to the inactivation of viruses by sunlight and viral clearance because of the high metabolic rate in summer (Savini et al*.,* 2021). A sanitary survey process identifies and evaluates all environmental factors and actual and potential sources of pollution that could harm the water quality of shellfish growing waters (Chinnadurai et al*.*, 2020).

The National Shellfish Sanitation Program (NSSP, 2019) outlines what a sanitary survey must entail which includes conducting a shoreline survey, and a survey of the microbiological quality of the water. In addition, an evaluation of the effect of any meteorological, hydrodynamics and geographic characteristics on the growing areas will assist in determining the appropriate growing areas' classification. Once a sanitary survey exists, it must be updated or reevaluated every three years. An annual review based on meteorological and hydrodynamics should also be performed to ensure that conditions are unchanged. Regulations have been developed internationally, regionally, and locally to specify acceptable levels of bacterial pathogens in shellfish tissues or in water where shellfish are grown. This has led to the classification of production areas for shellfish harvest fit for human consumption (Hodgson et al., 2017). Standards are available for the detection of HAV and NoV in some food groups, which would eventually lead to the establishment of regulations where set compliance limits for enteric viruses in shellfish are specified (ISO 15216 – 1: 2017 +1:2021).

Emerging viruses must also be prioritized due to the rapidly increasing number of cases associated with them (Cooper et al*.*, 2018). As mentioned, the presence of viruses in food is a global concern, with viruses such as norovirus and Hepatitis A causing foodborne outbreaks (Fusco *et al.*, 2019). Further studies are needed on the occurrence, behaviour, and genotype distribution of emerging enteric viruses in the environment, their epidemiology, temporal and geographical distribution, environmental stability, and potential health risks to humans (Kitajima and Gerba, 2015; Ilic et al*.*, 2017).

Effective risk management strategies need to focus on the prevention of contamination (Hodgson et al., 2017). The European Food Safety Authority (EFSA) recommended that contamination should be prevented in the first instance, which might be achieved by ensuring viral treatment of sewage or growing oysters sufficiently distant from sewage outflows (European Food Safety, 2019) (EFSA, 2019). Shin et al. (2013) are of the same view that the most effective control measure is to prevent faecal contamination in oyster production areas or to restrict commercial harvesting from contaminated production areas. Campos et al. (2017) emphasised that if this recommendation was to be successful or effective, it would require a good understanding of the environmental factors influencing the movement of NoV and other enteric viruses within the hydrological catchments and integration of microbiological and chemical monitoring programmes for effective warning systems. Written management plans are required for each shellfish growing area. Detailed reports on characteristics of effluent discharged near shellfish growing areas, dilution factors depending on the classification of growing areas, determining the distance from the pollution sources to the growing areas, and the impact of each source on the growing area should be compiled (NSSP, 2019). Several studies have shown that world population growth and urban developments have a detrimental impact on the microbiological quality of freshwater ecosystems and marine ecosystems (Winterbourn et al., 2016; Campos et al., 2017b; Garbossa et al., 2017; Freeman et al., 2019; Younger et al., 2022). Webber et al. (2021) in their study detected the presence of NoV, AdV, and RT in shellfish farms and found that the treatment of sewage did not provide effective removal or elimination of pollutants. Garbossa et al. (2017) also reported in their study that shellfish farms are at risk of being contaminated with enteric viruses which are transported to the farms through sewage discharge and indicated that the current wastewater treatment plants are not designed to effectively remove enteric viruses. Trottet et al. (2022) and Hassard et al. (2017), recommended upgrades of existing wastewater treatment plants, effluent pipes or shellfish bed relocation based on principles of dilution factors' zoning. Campos et al. (2020) tested the effectiveness of faecal coliform load reduction after the improvement of a sewage treatment plant's infrastructure and observed a reduction in *E. coli* concentrations with the greatest reduction identified at a site more distant from sewage discharges.

The available literature is mostly from developed countries and some developing countries (Table 5.2). The available literature on the African continent shows that only 17 studies were conducted in the past two decades in detecting enteric viruses in Africa (Upfold et al., 2021). In Saldanha Bay, on the West Coast of South Africa, where both mussels (*Mylitus galloprovincialis*) and oysters (*Crassostrea gigas*) are farmed, monitoring of bacteriological contamination is conducted, while monitoring of enteric viruses is lacking. The findings of this review highlight the importance of monitoring enteric viruses for the safety of public health, and therefore, may serve as a baseline for the development of a management plan for enteric viruses in Saldanha Bay, Western Cape, South Africa.

5.5 CONCLUSION

This review provides information on the global prevalence of enteric viruses in studies conducted between 2012 and 2022 in shellfish production areas. The findings confirm that shellfish contamination happens in harvesting areas that are subjected to the discharge of treated and untreated sewage continuously or intermittently. Several studies are of the view that the effective control measure for viral contamination is to harvest shellfish in areas that are not faecally contaminated and encourage the development of effective viral management plans for shellfish harvest areas. Quantification of the virus community in Saldanha Bay Harbor is crucial as well as quantification of existing pollutants and emerging pollutants to develop comprehensive strategies in the management of viruses as contaminants for bivalve molluscs. This study will serve as a baseline for the shellfish industry in South Africa to consider monitoring viruses and developing management plans for viruses. Before the development of management plans for viruses, extensive research is needed in understanding viruses’ origins, the influence brought by interaction with other organisms within the marine ecosystem and the potential risks to consumers.

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CHAPTER 6

SOUTH AFRICAN AND INTERNATIONAL AQUACULTURE LEGISLATION AND STANDARDS: A COMPARATIVE GAP ANALYSIS

ABSTRACT

Aquaculture is part of the blue economy and is entrusted to fulfil some of the United Nations' sustainable development goals. The aquaculture industry in South Africa is steadily growing which comes with added responsibility to ensure sustainable aquaculture. Proper policies and planning supported by an enabling environment as well as proper infrastructure, technical expertise, and investment are needed to promote marine aquaculture. This brought about a need to evaluate the existing regulatory framework alignment with international standards and regulations of potential export countries. A comparison of South African, European Union, and Codex Alimentarius standards on live bivalve molluscs was conducted using the Fishbone Gap Analysis technique. The focus was on regulations governing the live bivalve molluscs harvesting areas. Regulatory gaps were identified, including duplication of regulations between departments and a lack of a centralised Competent Authority for aquaculture activities. Recommendations made included the establishment of a one-stop-shop unit for aquaculture governance with a centralized competent authority.

KEYWORDS: aquaculture, gap analysis, bivalve molluscs regulations, policies, competent authority

6.1 INTRODUCTION

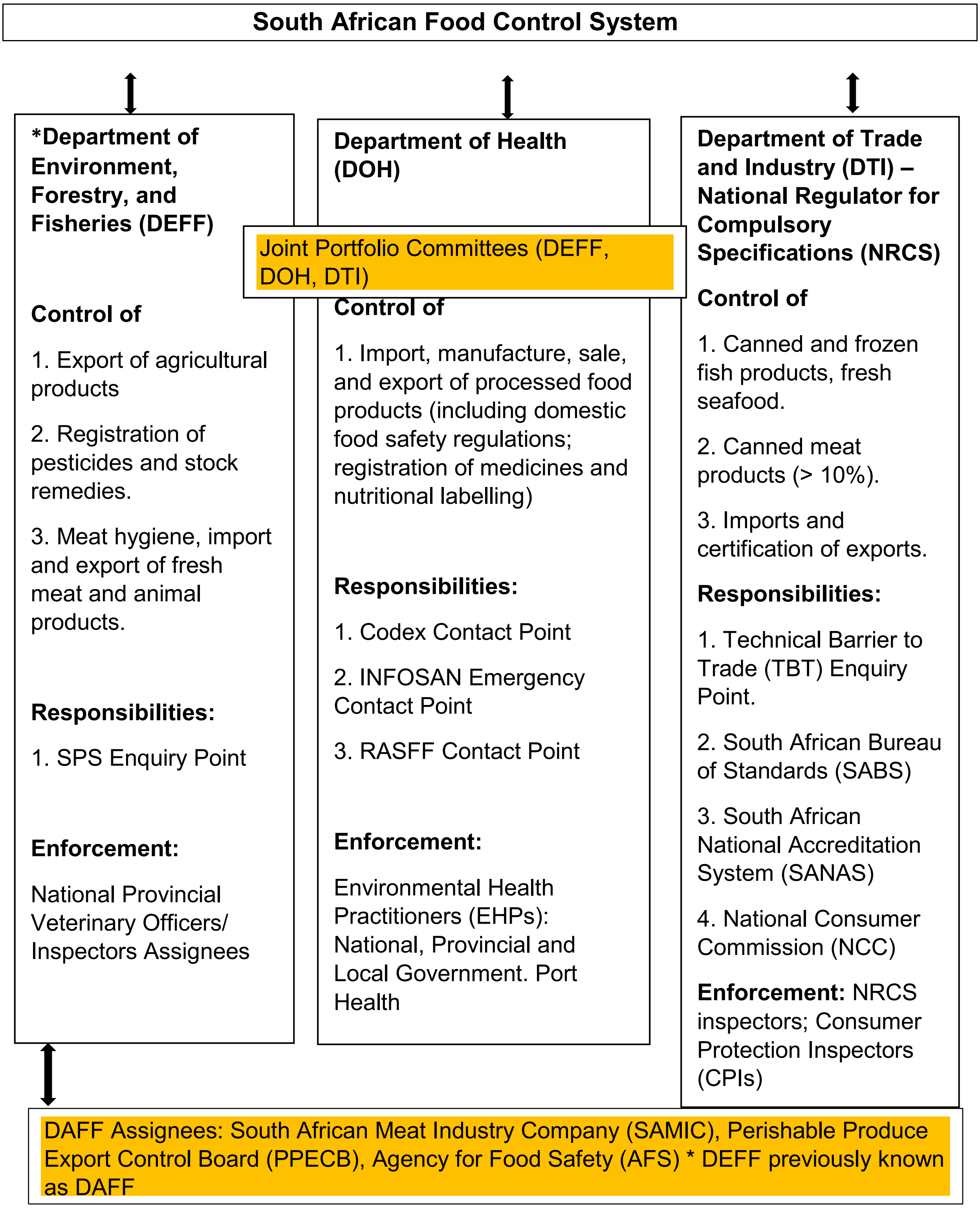
Aquaculture is the fastest-growing food production sector in the world. It is recognised for the essential contribution to food security and nutrition for the growing global population (Ahmed et al., 2019; FAO, 2022). Total production of fisheries and aquaculture reached a record of 179 million tonnes in 2018 and decreased slightly to 178 million tonnes in 2020. Production was dominated by fin fishing, reaching 57.5 million tonnes in 2020 from inland aquaculture and 17.7 million tonnes of bivalve molluscs. Asia dominated world aquaculture production by producing 91.6% of aquatic animals and algae in 2020. Following Asia, Africa has the second-largest volume of inland water captured by region (FAO, 2022). African aquaculture decreased slightly in 2020 by 1.2% compared to 2019, due to a decline in production in Egypt, which is Africa's major producer. Production also declined by 9.6% in Nigeria, the largest producer in Sub-Saharan Africa. The remainder of the continent saw a production growth of 14.5% reaching 396 700 tonnes in 2020 from 346 400 tonnes in 2019 (FAO, 2022).

Aquaculture, as part of Blue Economy/Blue Growth, is regarded as part of a solution to sustainable development goals, in particular, Goal 2 (ending hunger, achieving food security and improving nutrition, as well as promoting sustainable agriculture) and Goal 14 (conserving and sustainably using the oceans, seas, and marine resources for sustainable development) (Ababouch and Fipi, 2015).

Challenges which aquaculture may be facing range from water quality management; lack of available space in coastal areas; harmful algal blooms; climate change, and burdensome policies and regulations (Brown et al., 2020). The challenge brought by aquaculture regulatory frameworks and policies hinders progress in the aquaculture industry on the African continent and South Africa in particular. Many countries across the globe have developed their expertise and support institutions for marine and coastal aquaculture, while most African countries still lag behind (Madibana et al., 2020; Brugene et al., 2021). Proper policies and planning, supported by an enabling environment in support of infrastructure, technical expertise and investment are needed to promote marine aquaculture in Africa (FAO, 2022). Even though regulatory challenges are far from being resolved, Mapfumo (2022), when reviewing the status and trends in aquaculture development in Sub-Saharan Africa, applauded South Africa for having an advanced regulatory system for monitoring fish standards compared to other countries in the region. This was possible due to the work of the National Regulator for Compulsory Specifications (NRCS), which is recognised as a Competent Authority for food safety (Boatemaa et al., 2019). In the aquaculture sector, NRCS conduct monitoring and surveillance of factories, processes, sampling of aquaculture products to ensure safety and compliance with local and international standards and issuance of health guarantees for exported products.

There is consensus that for aquaculture to be successful, policy developers must adopt an ecosystem approach, whereby integration of the social, economic, and ecological aspects; as well as coordination between different institutions in policy formulation and implementation are observed (Suplicy et al., 2017; Colonna et al., 2020; Garza-Gil and Perez-Perez, 2020). Multiple entities with their own roles and objectives, dealing with numerous policies and regulations regarding marine and coastal management, environmental protection, food safety, or animal health among others, and at different institutional levels may complicate or discourage the implementation of more sustainable aquaculture practices (Gismervik et al., 2020; Regueiro et al., 2021).

South African regulations, which stipulate requirements for bivalve molluscs are derived from three national government departments, i.e., the Department of Health (DOH); the Department of Forestry, Fisheries and Environment (DFFE); and the Department of Trade and Industry (DTI) (Hofherr et al., 2016), followed by provincial and local government. The DOH is at the local level, represented by an Environmental Health Practitioners (EHPs), who are responsible for enforcing the Foodstuffs, Cosmetics, and Disinfectants Act, Act 54 of 1972 (FCDA). The Act controls the sale, manufacture and importation of foodstuffs, cosmetics, and disinfectants. In addition, incidental matters and regulations promulgated under this Act also include Regulation R638 of 2018, which governs general hygiene requirements for food premises, the transport of food and related matters (Schőnfeldt et al., 2018). The National Department of Agriculture, Forestry, and Fisheries which is now divided into National Departments (Department of Agriculture Land Reform and Rural Development (DALRRD), and Department of Forestry, Fisheries and Environment (DFFE). The former, at provincial level is represented by the assignees who are responsible for enforcing the Meat Safety Act, Act 40 of 2000, and Agricultural Product Standards Act, Act 119 of 1990. The latter (DFFE) enforces the Marine Living Resources Act (MLRA), Act 18 of 1998. The DTI assigned the National Regulator for Compulsory Specifications (NRCS) to enforce compulsory specifications for canned, frozen fish, and fresh seafood products under the Compulsory Specifications Act, Act 5 of 2008. The Figure 6.1. gives a detailed summary of the functions of the different National Departments as briefly described above.



South African Food Control System

***Department of Environment, Forestry, and Fisheries (DEFF)**

Control of

1. Export of agricultural products

2. Registration of pesticides and stock remedies.

3. Meat hygiene, import and export of fresh meat and animal products.

Responsibilities:

1. SPS Enquiry Point

Enforcement:

National Provincial Veterinary Officers/ Inspectors Assignees

Department of Health (DOH)

Joint Portfolio Committees (DEFF, DOH, DTI)

Control of

1. Import, manufacture, sale, and export of processed food products (including domestic food safety regulations; registration of medicines and nutritional labelling)

Responsibilities:

1. Codex Contact Point

2. INFOSAN Emergency Contact Point

3. RASFF Contact Point

Enforcement:

Environmental Health Practitioners (EHPs): National, Provincial and Local Government. Port Health

Department of Trade and Industry (DTI) – National Regulator for Compulsory Specifications (NRCS)

Control of

1. Canned and frozen fish products, fresh seafood.

2. Canned meat products (> 10%).

3. Imports and certification of exports.

Responsibilities:

1. Technical Barrier to Trade (TBT) Enquiry Point.

2. South African Bureau of Standards (SABS)

3. South African National Accreditation System (SANAS)

4. National Consumer Commission (NCC)

Enforcement: NRCS inspectors; Consumer Protection Inspectors (CPIs)

DAFF Assignees: South African Meat Industry Company (SAMIC), Perishable Produce Export Control Board (PPECB), Agency for Food Safety (AFS) * DEFF previously known as DAFF

Figure 6.1 *South African Food Control system showing three tiers of government (Boatemaa et al., 2019)*

The aim of this study was to compare the South African food safety legislation for aquaculture, particularly the bivalve molluscs, with the EU bivalve molluscs regulations (as the shellfish farmers in Saldanha Bay would like to export their products to the EU countries and compliance with their legislation is crucial) and Codex Alimentarius standards, in order to identify gaps that might be beneficial to incorporate into the existing bivalve molluscs regulations.

6.2 MATERIALS AND METHODS

The gap analysis study focused on aquaculture and harvesting and excluded processing, packaging and further processes down the supply chain line. This gap analysis study is presented in two-fold. Firstly, a comparison was made of the interdepartmental SA legislation governing live bivalve mollusc-harvesting areas followed by a comparison of SA legislation with the EU regulations and the Codex Alimentarius Codes of Practices and Standards governing live bivalve mollusc-harvesting areas. Secondly, A fishbone analysis technique (Figure 6.2) was used to visualise the identified gaps between the above-mentioned regulations focusing on selected main categories. Causes are grouped into categories to identify the overall sources of the effect.

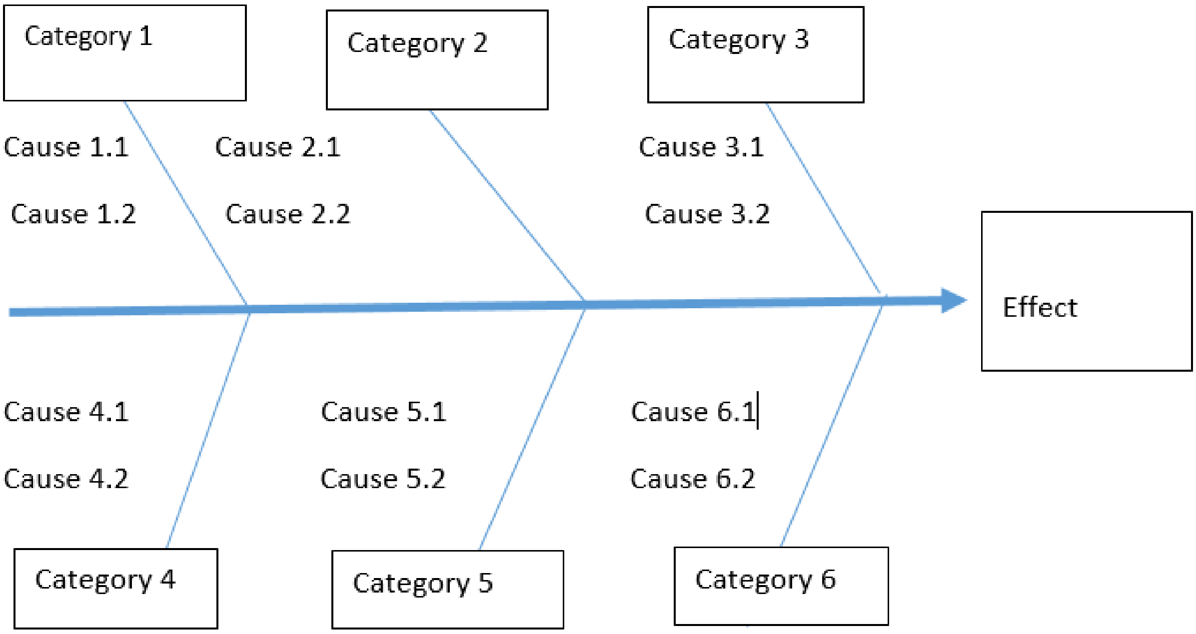


***Figure 6.2** Fishbone diagram (adopted from Coccia, 2020)*

6.3 RESULTS

The GAP analysis was conducted by firstly, providing a synopsis of the relevant legislation to bivalve molluscs, secondly, by applying the Fishbone Strategy to identify shortcomings, and thirdly, to propose strategies and interventions for improvement. The synopsis of relevant legislation was done by categorising and tabulating relevant requirements and aligning it with existing legislation. This presented an at-a-glance overview of the South African regulations for live bivalve molluscs (Table 6.1), SA vs EU regulations for live bivalve molluscs (Table 6.2), and the SA vs Codex Alimentarius codes of practices and standards (Table 6.3). The tables significantly aided the integration of variables to facilitate the consequent GAP analysis and enable effective responsive interventions.

The South African regulations governing food safety including live bivalve harvesting, listed in Table 6.1, show duplication of regulatory requirements which is attributed to the lack of interactions across levels of government and between departments. Food safety and quality legislation are the responsibility of the Department of Health, the Department of Environment, Forestry and Fisheries and the Department of Trade and Industry.

A comparison between SA and EU regulations showed a lack of stand-alone live bivalve mollusc regulations in South Africa (Table 6.2). The requirements listed under EU regulations were compared with the requirements listed under the SASM&CP guideline developed by DFFE. The SA microbiological criteria for *E. coli* (i.e., absence in 10g of a sample) differed from the EU criteria limit (i.e., 230 total MPN/100g). Where the requirements for *Salmonella* were concerned, SA regulations correlated with that of the EU with no *Salmonella* detected in 25g of a sample.

A comparison between SA and Codex Alimentarius codes of practices and standards showed a lack of stand-alone live bivalve mollusc regulations in SA (Table 6.3). The requirements specific for live bivalve molluscs, listed in codex standards and practices, were compared to the requirements listed under the SASM&CP guideline.

Microbiological criteria for *E. coli* in SA (i.e., absence in 10g of a sample) differed from the one prescribed by the Codex standards (i.e., no more than 700 *E. coli*/100g). Alignment of live bivalve mollusc regulations in South Africa with the Codex Alimentarius codes of practices and standards is necessary for participation in global trade. An overview of the SA interdepartmental legislation for live bivalve molluscs (Table 6.1), SA vs EU regulations for live bivalve molluscs (Table 6.2), and the SA vs Codex Alimentarius codes of practices and standards (Table 6.3) follows.

Table 6.1 *South African interdepartmental legislation governing food safety from primary production to consumers*

Key features	DOH (Food Control)	DFFE	DTI (NRCS)	Gaps
Factory construction, layout and conditions	Government notice R638 (government gazette No 41730) of 22 June 2018: 5 (2) Location, design, construction, finish. 6 (1) Equipment	None related to the food premises and food safety requirements	Government notice R1076 (government gazette No 25245) of 1 August 2003: 3.2 Factory construction, layout and conditions. 3.3 Equipment 3.3.1 Layout, installation, design, construction and usage. 3.4 Water 3.5 Hygiene operating requirements 10 Labelling and marking	SA: lack of interactions across levels of government to avoid duplication of regulations. Lack of a forum to handle matters of common interest.
Labelling of foodstuffs	R146 (government gazette No 32975) of 1 March 2010: 2-6 General provisions 9 Identification 10 Country of origin 11 Batch identification 12 Date marking			
Requirements for food handlers	Government notice R638 (government gazette No 41730) of 22 June 2018: 11 (1), (3) Duties of food handler.		R979 (government gazette No 25172) of 4 July 2003 3.5 Requirements for employees engaged in the handling, preparation, processing, packaging and storage of products.	

All legislation used are cited in the table and included in the reference list at the end of the chapter.

Table 6.2 *South Africa vs European Union legislation governing shellfish harvesting*

Key features	SA regulations	EU regulations	Gaps
Live bivalve molluscs	Regulation 73 of the Marine Living Resources Act (Government notice R1111) government gazette 19205 of 2 September 1998: 73 (1) “No person shall establish a mariculture facility in any area contaminated with toxic substances, faecal matter, human pathogens or marine biotoxins, to the extent that the cultivated fish pose a health risk to consumers”. South African Shellfish Monitoring and Control Programme (SASM&CP): issue 8 of January 2021: 8 Classification of shellfish production areas. 9 Monitoring of shellfish production areas after classification. 10 Microbiological monitoring. 11 Monitoring of Environmental and veterinary drug residues. 12 Biotoxin monitoring 13 Phytoplankton monitoring	Regulation (EC) No 854/2004 Article 6 –Annex. II, Chapter II: official controls concerning live bivalve molluscs from classified production areas: A. classification of production and relaying areas. B. Monitoring of classified relaying and production areas. C. Decisions after monitoring. D. Additional monitoring requirements. E. Recording and exchange of information. F. Food business operator’s own checks.	No legislation specific for Live bivalve molluscs in South Africa.
Hygiene requirements for production / harvesting areas	Regulation 73 of the Marine Living Resources Act (Government notice R1111) government gazette 19205 of 2 September 1998: 73 (3) “Harvesting from actual and potentially affected growing waters may be restricted during public health emergencies such as marine biotoxins events, oil spills and sewage contamination”.	Regulation (EC) No 853/2004 Article 15 – Annex.1 2. Live bivalve molluscs 2.1 Bivalve molluscs 2.2 Marine biotoxins 2.3 Conditioning 2.4 Gatherer 2.5 Production areas: means any sea, estuarine or lagoon area, etc. 2.6 Relaying area 2.7 Dispatch centre	SA: no legislation specific for hygiene requirements for production or harvesting areas.

	SASM&CP issue 8 of January 2021: 14 Requirements for harvesting and transport of live shellfish 15 Sampling and transport of samples 16 Requirements for relaying of shellfish 17 Depuration 18 Wet storage 19 Requirements for dispatch centres 20 Feed management 21 Drug management 22 Samples and sample taking 23 Laboratory responsibility	2.8 Purification centre 2.9 Relaying Chapter II: Hygiene requirements for the production and harvesting of live bivalve molluscs A. Requirements for production areas. B Requirements for harvesting and handling following harvesting. C. Requirements for relaying live bivalve molluscs.	
Microbiological criteria for live bivalve	Government notice R692 of 16 May 1997: 6(1) (a) uncooked oysters, mussels *Salmonella*, *Shigella*, *Vibrio cholerae* and *Vibrio parahaemolyticus*: absence in 25g (e) Presumptive *E. coli* absence in 10g (g) oysters, mussels, faecal coliforms shall not exceed 500/100g when harvested from approved shellfish harvesting area	Commission Regulation (EC) No 2073/2005 / Commission Regulation (EU) 2015/2285 Live bivalve molluscs *E. coli* limit: 230 MPN/100g of flesh and intra-valvular liquid. Live bivalve molluscs Salmonella limit: absence in 25g	SA requirements for microbiological criteria for live bivalves are similar to EU requirements

All legislation used are cited in the table and included in the reference list at the end of the chapter.

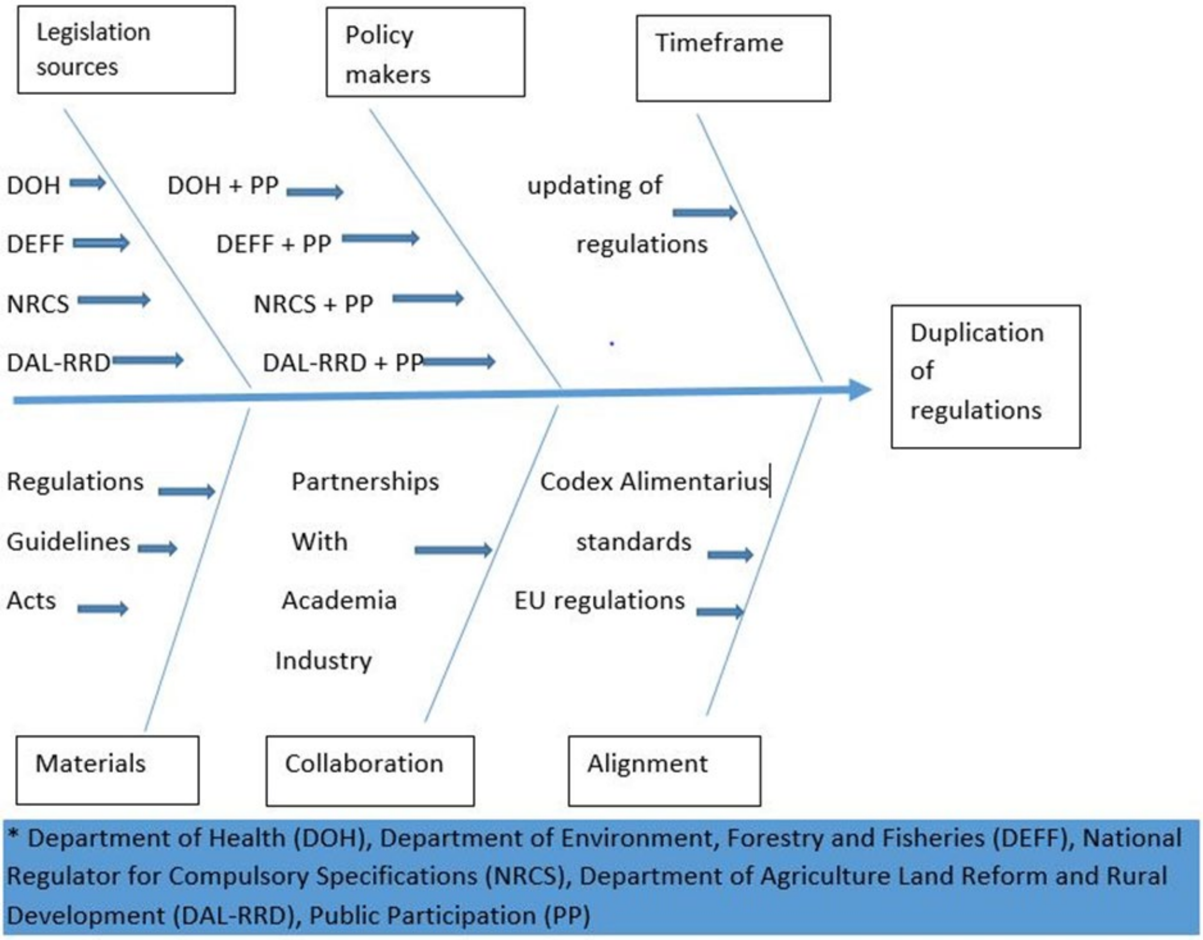
Table 6.3 *South African vs Codex Alimentarius codes of practices and standards governing shellfish harvesting*

Key features	SA regulations	Codex standards	Gaps
Live bivalve molluscs	Regulation 73 of the Marine Living Resources Act (Government notice R1111) government gazette 19205 of 2 September 1998: 73 (1) "No person shall establish a mariculture facility in any area contaminated with toxic substances, faecal matter, human pathogens or marine biotoxins, to the extent that the cultivated fish pose a health risk to consumers". South African Shellfish Monitoring and Control Programme (SASM&CP): issue 8 of January 2021: 8 Classification of shellfish production areas. 9 Monitoring of shellfish production areas after classification. 10 Microbiological monitoring. 11 Monitoring of Environmental and veterinary drug residues. 12 Biotoxin monitoring 13 Phytoplankton monitoring	CXC-52-2003 Code of practice for fish and fishery products: Section 7: Processing of live and raw bivalve molluscs. 7.2 Classification and monitoring of growing area (potential hazards) 7.2.1 Classification of growing areas 7.2.2 Monitoring of growing areas 7.2.2.1 *E. coli*/faecal coliforms/ total coliforms 7.2.2.2 Pathogens monitoring 7.2.2.3 Marine biotoxin control 7.2.2.4 Marine biotoxin test methods 7.2.2.5 Chemical contaminants	No specific regulations for live bivalve molluscs in South Africa.
Hygiene requirements for production / harvesting areas	Regulation 73 of the Marine Living Resources Act (Government notice R1111) government gazette 19205 of 2 September 1998: 73 (3) "Harvesting from actual and potentially affected growing waters may be restricted during public health emergencies such as marine biotoxins events, oil spills and sewage contamination". SASM&CP issue 8 of January 2021: 14 Requirements for harvesting and transport of live shellfish 15 Sampling and transport of samples 16 Requirements for relaying of shellfish 17 Depuration 18 Wet storage 19 Requirements for dispatch centres	7.3 Harvesting and transportation of live bivalve molluscs 7.4 Relaying 7.5 Depuration	South African Regulation 73 of the MLRA should be updated and broadened in terms of the scope of work.

	20 Feed management 21 Drug management 22 Samples and sample taking 23 Laboratory responsibility		
Microbiological criteria for live bivalve	Government notice R692 of 16 May 1997: 6(1) (a) uncooked oysters, mussels *Salmonella*, *Shigella*, *Vibrio cholerae* and *Vibrio parahaemolyticus*: absence in 25g (e) Presumptive *E. coli* absence in 10g (g) oysters, mussels, faecal coliforms shall not exceed 500/100g when harvested from approved shellfish harvesting area	Standard for live and raw bivalve molluscs (CXS 292-2008) MPN Method ISO 16649-3 *E. coli* n=5, c=1, m=230, M=700 In analysis involving five (5) 100g samples of the edible parts, none may contain more than 700 *E. coli* and not more than one (1) of five (5) samples may contain 230 and 700 *E. coli*	SA regulations for microbiological criteria for live bivalves, *E. coli* criteria need to be updated to align with international standards.

All legislation used are cited in the table and included in the reference list at the end of the chapter.

The fishbone cause and effect diagram used in Figure 6.3 adopted by the researcher illustrates the roles the various government departments play in enforcing various Acts and Regulations that may overlap. This may include policymakers from various departments working in silos; a lack of lasting partnerships and collaboration with industry and academic institutions; and difficulties in aligning Acts and Regulations with international standards and regional regulations for export purposes. These departments are playing catch-up due to the long timeframes and processes of amending Acts and Regulations. The main effect becomes the duplication of regulations due to a lack of interaction across levels of government and between various departments.



* Department of Health (DOH), Department of Environment, Forestry and Fisheries (DEFF), National Regulator for Compulsory Specifications (NRCS), Department of Agriculture Land Reform and Rural Development (DAL-RRD), Public Participation (PP)

Figure 6.3 *Fishbone diagram of causes and effect of South African Legislation gaps*

6.4 DISCUSSION

Brugere et al. (2021) demonstrated how a lack of policy coherence at a national level could jeopardise the progress and development of mariculture. The duplication of regulations and other causes and effects derived from the main factors are discussed in the next section.

The following gaps were identified during the comparison of the regulatory frameworks from SA, EU, and Codex Alimentarius standards:

- Duplication of regulations by different departments was observed in SA regulations, i.e., Regulation R638 and Regulation R1076 in terms of requirements for food factory layout, design and construction, duties of employees, and protective clothing.
- Specific regulations for bivalve molluscs are lacking in SA regulations compared to the EU regulations and the Codex Alimentarius codes of practices and standards.
- Classification of production areas: not addressed in SA regulations, as is the case with EU and Codex Alimentarius codes of practices and standards.

6.4.1 Duplication of regulatory requirements

Lack of coordination between the departments, fragmented policy framework, overlap of regulations and confusion about who is responsible for what, weaken the South African food control system (Boatemaa et al., 2019). This is evident through examples presented in Table 6.1. Authorities may address the lack of harmonisation of regulations by deciding on one regulation that covers a specific requirement and other departments may refer to it. For example, when the NRCS refer to the hygiene requirements, they may rather refer to Regulation R638 of 2018, which deals with requirements related to hygiene requirements for food premises in South Africa. This will eliminate duplication of regulatory requirements in South Africa. This practice is similar to EU regulations and Codex codes of practices and standards, where the users of Codex CXC-52-2003 are referred to CXC 1-1969 on matters related to food hygiene requirements. Alexander et al. (2015) in their study observed a similar challenge whereby aquaculture is governed by various institutions and recommended that specific functions be identified in fulfilling roles such as governance structure (e.g., who will be responsible for aquaculture), planning (e.g. authorisation/licensing, access to land and water), and operation (e.g. water and wastewater, fish movement and disease control, and food safety). Furthermore, it is recommended that regulations be simplified in order not to encumber farmers. Once again, the encumber farmers must have the opportunity to partake in discussion and polity development.

This could be achieved through the development of a centralised one-stop-shop licensing unit as proposed by Madibana et al. (2020). Suplicy et al. (2017) concurred with the proposed recommendation and emphasised the importance of coordination from various departments during the policy formulation phase and implementation to overcome challenges. Ruff et al. (2019) compared the mariculture governance of Norway and the United States of America (USA). It was discovered that, even though both countries have extensive political and regulatory requirements for offshore mariculture operations, Norway's production growth surpassed the USA despite their ecological capabilities. Ruff et al. (2019) further discovered that Norway centralised the governance of offshore mariculture while the USA mariculture is governed by multiple federal and state agencies. While governance can be an enabling force for mariculture, it can also hinder growth through too many regulations and complex permitting processes. In Cuba, the authorisation of all aquaculture activities (licenses and permits) is issued by one institution, the Ministry for Food Industry (Ruff et al., 2019). This makes compliance and permit acquisitions simpler and cheaper. Love et al. (2017) observed the disconnection of policies from the health and fisheries department in Brazil and concluded that aligning fisheries and health policies is imperative to improve food security.

Coordination between different departments responsible for developing and implementing shellfish policies is essential to avoid contradictory policy outcomes and duplication of work (Cavallo et al., 2016). In addition, it also promotes the strengthening of partnerships, that bring together multilevel and diverse resources, knowledge, and commitment from all stakeholders (Guerrero and Fernandez, 2018). A positive outcome of such partnerships is observed in Santa Catarina in Brazil, which resulted in the establishment of a laboratory for marine molluscs at the Federal University of Santa Catarina, where researchers grew juvenile oyster seeds and offered them to shellfish farmers at a low price. In addition, they also provided production-focused science and commercially valuable technical assistance to farmers (Safford et al., 2019).

6.4.2 Specific regulations for live bivalve molluscs

The classification of production areas is listed in the SAMSM&CP manual developed by DFFE. Even though the requirements listed in this manual address the provision of section 73 of the Marine Living Resources Act, No.18 of 1998, the manual is not a legal document or law. One would suggest that these requirements be incorporated into the regulations as is the case with the EU regulations and Codex Alimentarius codes of practices and standards.

6.4.3 Prospects of Bivalve molluscs exportation

Shellfish farmers in Saldanha Bay are exploring the possibility of exporting farmed and wild molluscs (mussels, oysters, and abalone) and attempting to meet the EU requirements for food safety and animal health conditions for these products. For international trade, compliance with regional and international regulations is crucial. Countries that desire to export bivalve molluscs to EU countries need to demonstrate that their legislative framework is equivalent to that of the EU for public health protection (Pinn et al., 2023). This is achieved through assessments of relevant laws and regulations. In South Africa, the assessment of regulations towards EU compliance for the export of farmed molluscs from South Africa was conducted by Trade Forward Southern Africa (TFSA, 2022) in which the following gaps were identified:

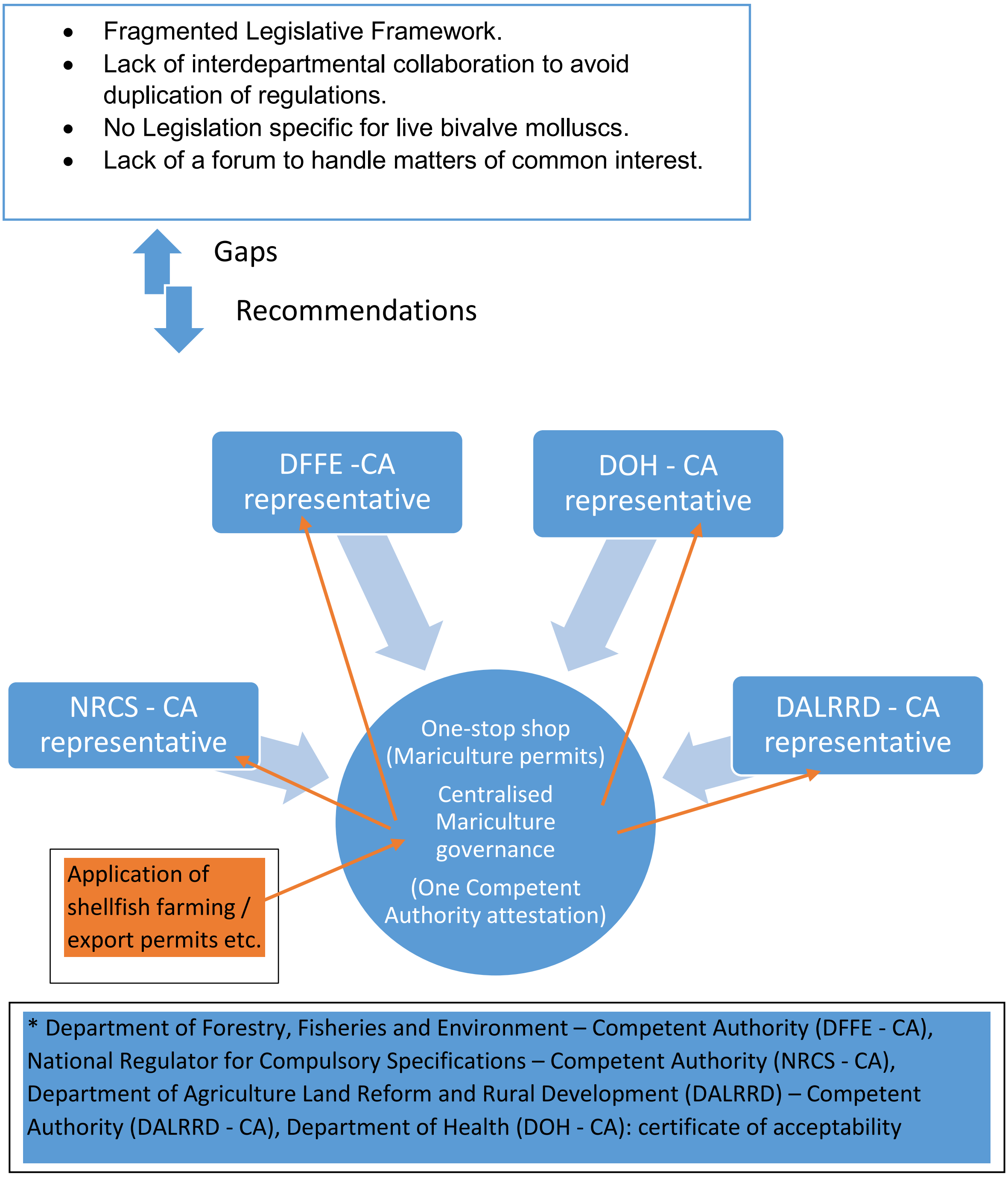
- Lack of coordination in some of the control measures.
- No official monitoring of veterinary residues in aquaculture products and aquatic animal health control systems equivalent to those of the EU.

- No central competent authority (CA) with lawfully mandated regulatory powers.
- No system for addressing non-compliance resulting in a compliant sector.
- Marine biotoxins and microbiological criteria for molluscan shellfish are not equivalent to that of the EU.
- Veterinary medicine monitoring and control, and animal health for molluscs and crustaceans lacking a control system equivalent to the EU.

South Africa met the regulatory framework requirements for conditions relating to the general food safety of fishery products and is therefore one of the countries authorised to supply the EU with certain fishery products (TFSA, 2022). Currently, in South Africa three National Departments serve as Competent Authorities (CA) for assigned functions. For example, DFFE is a Competent Authority for microbiological and marine biotoxin monitoring, while the Department of Agriculture, Land Reform and Rural Development (DAL-RRD) is a Competent Authority for animal health and veterinary residue matters, and the NRCS is a Competent Authority for DTI general food safety assurances. Once again, a further fragmentation, and the role of local municipalities is excluded in this process which is the first point of reference for ensuring that bivalve molluscs processing plants comply with the food hygiene requirements and issue a certificate of Acceptability for compliance. The TFSA report recommended having a central CA who will undertake final attestation on behalf of all departments (TFSA, 2022).

6.4.4 Centralised one-stop-shop office for aquaculture

In the South African context, The NRCS is an internationally recognised body for food safety (SASM&CP, 2021). If the authorities were to adopt a centralised one-stop-shop office, the NRCS could function as the CA mandated with final attestation for issuing aquaculture and export permits. The benefit of a centralised office is that all the relevant departments' representatives are under one roof, and any documentation needed by farmers is acquired from one office (Madibana et al., 2020). A one-stop-shop-office could develop a digital system that could work as follows: when new applications are received, the system alert the representatives of DFFE, DAL-RRD and NRCS of a new application, the officials are given a timeframe to process the application as per their mandate. Officials submit their reports digitally for final approval by the appointed Competent Authority for a final decision after consolidating all the reports. Figure 6.4 illustrates the process flow from the gaps identified and the recommendations for a one-stop-shop office to improve the current legal frameworks.



- Fragmented Legislative Framework.
- Lack of interdepartmental collaboration to avoid duplication of regulations.
- No Legislation specific for live bivalve molluscs.
- Lack of a forum to handle matters of common interest.

* Department of Forestry, Fisheries and Environment – Competent Authority (DFFE - CA), National Regulator for Compulsory Specifications – Competent Authority (NRCS - CA), Department of Agriculture Land Reform and Rural Development (DALRRD) – Competent Authority (DALRRD - CA), Department of Health (DOH - CA): certificate of acceptability

Figure 6.4 *Gaps and recommendations for South African Mariculture operations and governance (Orange arrows shows new incoming applications captured and distributed to officials for processing. Blue arrows shows reports being send to the appointed Competent Authority for final approval.)*

6.5 CONCLUSION

Aquaculture is the fastest-growing food production sector in the world. It is acknowledged for its critical role in ensuring food security and promoting nutrition for the growing global population. The obstacle posed by regulations and regulatory frameworks pertaining to aquaculture prevents the sector from developing further on the African continent, specifically in South Africa. The identified Challenges or gaps include a fragmented legal framework, a lack of interdepartmental collaboration to avoid duplication of requirements, a lack of legislative framework specific to the production of live bivalve molluscs, and a lack of a forum to manage matters of common interest. To minimise inconsistent policy outcomes and duplication of work, coordination between multiple National Departments responsible for establishing and executing shellfish policies is necessary. We recommend that these gaps be addressed as a solid legislative framework is critical for the sustainability of the sector. In addition, the system must be configured to enable the development of a one-stop-shop-office with a single designated competent authority for attestation or final approval.

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 INTRODUCTION

Aquaculture might be a solution to unemployment and food security in coastal areas. The shellfish industry has created job opportunities for communities in these areas, which in turn improved the livelihoods of these communities. Added benefits of seafood products are the provision of high-quality animal protein, which is low in fat, high in omega-3 fatty acids, vitamins and minerals, and environmental benefits. Bivalve molluscs contribute to the environment by regulating marine and freshwater ecosystems through their diet, which also contributes to nutrient availability for other organisms found in these environments. As the popularity of seafood increases, the protection of consumer health becomes crucial. Seafood as part of food products, traded globally, is susceptible to contamination by physical, chemical, and microbiological contaminants (Olaganath and Kar Mun, 2017; Norman et al., 2019). The shellfish farming industry in Saldanha Bay is steadily growing. With the growth and anticipated export of products across the globe, compliance with local and international food safety standards and regulations applies. This study attempted to assess the prevalence of contamination by pathogenic bacteria in the production area and possible control measures to eliminate or reduce the risk to acceptable levels. Chapter 1 of this study looked at the background overview of shellfish farming and the problems or challenges that lead to this study. Saldanha Bay shellfish farms are exposed to the following pollution sources: wastewater treatment plant discharge, sewage pump stations, stormwater drains discharge, fish factory discharge (brine, cooling water discharges), industrial effluent, occasional dredging around the iron ore jetty, ship traffic, ballast water discharge, and oil storage and gas transportation. Nevertheless, it is regarded as an ideal location for shellfish farming.

7.2 CONCLUSIONS

The literature reviewed in Chapter 2 focused on the sources of contamination, hazards, and legislation affecting the primary production of shellfish. For successful and sustainable aquaculture, the shellfish production areas should have effective water quality management; should be protected from winds and ocean currents; should be free from pollution; and have good water circulation. Shellfish farmers and the government authorities should consider the following:

- Effective water quality management: aquaculture should provide a balance between sustainability and profitability of production for producers, prioritise the health and safety of consumers, and focus on the conservation of the environment (Figure 7.1) (Ahmed et al., 2020). Regarding research capacity, governance, and regulation, the aquaculture sector is still in its infancy. Outside of seasonal cycle changes, factors such as climate change impacts the surroundings, which affects water quality. Dissolved oxygen, water temperature, pH, salinity, and total ammonia and nitrogen levels are important water quality parameters that provide insight into physical, chemical, and biological water quality. These assist in the initial scoping of the farming site and are important as part of routine monitoring throughout farming site operations (Schmidt et al., 2018; You et al., 2021).
- Protection from winds and ocean currents: wind direction and speed affect water quality; such impacts are noticed during monsoons in areas that experience monsoon temperatures and rainfall, which add to non-point source pollution (You et al., 2021).
- Absence of pollution: According to Schmidt et al. (2018), “A viable shellfish industry depends on productive waters that are free from pollution”.
- Good water circulation provides low risks of organic enrichment. This is evident in shellfish harvest areas, i.e., deeper water and faster currents, which assist in clearing organic material away (Gentry et al., 2016). Figure 7.1 shows that when water quality in shellfish harvesting areas is managed effectively, it promotes a safe environment for various marine organisms, and leads to sustainable aquaculture where there are controls to prevent over-exploitation of marine resources with greater benefits for farmers and bivalve molluscs.

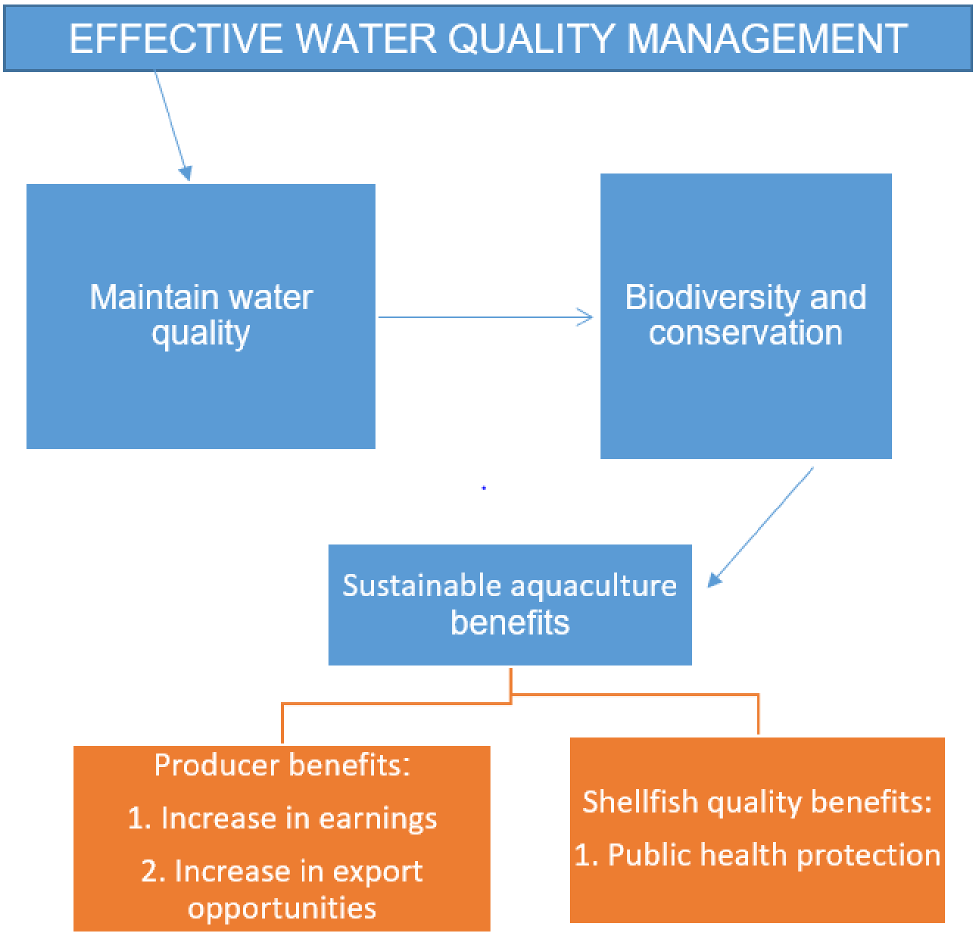

Figure 7.1 *Illustration of benefits of effective water quality management*

In addition, the lag in research when it comes to microbiological impacts, prevalence, and sources is problematic. A substantial amount of research has been conducted on marine biotoxins and heavy metal pollution in Saldanha Bay harbour. However, no significant research studies have been conducted on microbial contamination except the routine microbiological monitoring conducted by the Department of Environment, Forestry and Fisheries (DFFE) and the National Regulator for Compulsory Specifications (NRCS). Microbiological pollution refers to the presence of viruses or bacteria in shellfish that could pose a public health threat to shellfish consumers.

In chapter 3, an overview of the *E. coli* trends in samples analysed between 2017 and 2020 was provided. The correlation analysis conducted to test the relationships between *E. coli*, rainfall, and temperature did not show any significant difference. However, there was a significant difference between mean *E. coli* and sampling points in Small Bay, with spikes that exceeded the permissible limits for a Class A production area. Another finding was the inconsistency in sampling at individual sampling sites conducted by the Department of Environment, Forestry, and Fisheries. On some sampling occasions, samples were collected from all sampling sites, while on other occasions samples were collected from fewer sampling sites. This makes it difficult to identify trends and sources of contamination.

Chapter 4 investigated pathogens commonly associated with shellfish-related foodborne disease outbreaks, such as *Salmonella, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae,* and the prevalence of *Escherichia coli* as an indicator species. *Escherichia coli* concentrations were below the permissible limit (230 MPN/ 100g). *Salmonella* and *Vibrio spp.* were not detected. No correlation could be drawn between the total MPN count in water (microorganisms/100ml) and shellfish (microorganisms/100g) samples, physico-chemical parameters, as well as between rainfall patterns and MPN counts in water and shellfish ($p>0.05$) (Table 4.4). As the total MPN count in water samples increased, the total MPN count in shellfish samples increased ($r = 0.997$, $n = 11$, $p = <0.001$).

The results did not conform to prior expectations, as bacteria such as the *Enterobacter cloacae complex; Citrobacter freundii; Klebsiella pneumoniae* spp*; Aeromonas sobria; Vibrio alginolyticus*; and *Sphingomonas paucimobilis* were isolated instead. This shows aquatic environments are home to various microbiota, some indigenous and some introduced through anthropogenic activities around these environments. The presence of pathogens in aquatic ecosystems is a risk to the shellfish production industry and poses a public health threat. *Klebsiella pneumoniae spp.* is associated with sewer overflow discharge and is responsible for one-third of hospital infections caused by gram-negative bacteria (Durand et al., 2020; Håkonsholm et al., 2020). *Vibrio alginolyticus* poses a public health threat as it has caused *vibriosis* outbreaks in mariculture throughout Europe and South Asia (Destoumieux-Garzón et al., 2020; Scro et al., 2019).

Chapter 5 was a systematic review that followed a PRISMA methodology of literature search, which focused on the literature related to virus contamination in shellfish harvesting areas between 2012 and 2022. The majority of studies implicated sewage discharge in shellfish growing areas as the source of enteric virus contamination. Viruses are excreted in large quantities from the faeces of infected individuals and are persistent in the environments where they are found. Moreover, the few people infected in a given population may shed an enormous charge of viral particles, in other words, infections can spread quickly (Fusco et al., 2019), hence, the importance of having a sanitary survey and management plans for virus monitoring. The available post-harvest treatments such as the depuration of bivalve molluscs before placement on commercial markets have been reported as ineffective in removing or reducing viruses to acceptable levels (El Moqri et al., 2019). Routine virus monitoring requires the use of both mussels and oysters as there are strain-specific variations in binding patterns, for example, it is reported that norovirus genogroup I bind to the midgut digestive diverticula of oysters which makes it difficult to remove from oysters (Younger et al., 2020). It is therefore advised that authorities responsible for microbiological monitoring in Saldanha Bay shellfish farms, consider using both oysters and mussels when implementing a virus sanitary survey.

Lastly, in Chapter 6, the gaps between South African legislation governing shellfish production were compared to the Codex Alimentarius and the European Union (EU) regulations (since the farmers are considering expanding their exports into EU countries). Gaps were identified in the South African regulations governing shellfish harvesting and were listed in Tables 6.1, 6.2, and 6.3. Furthermore, an evaluation of the cause-and-effect phenomena illustrated by a fishbone diagram in chapter 6, Figure 6.1, was also applied. The causes were the various government departments that are involved in policy-making for the shellfish production industry. Departments working in silos or a lack of collaboration between government departments resulted in duplication of requirements stipulated in regulations. Regardless of the different institutions having different key roles to play in ensuring the safety of bivalve molluscs produced, the lack of harmonisation of regulations will make compliance impossible for shellfish farmers. In South Africa, there is a lack of stand-alone regulations specific to shellfish production compared to the EU regulations. For example, classification of production areas: South African requirements are not incorporated in the Marine Living and Resources Act or Regulations promulgated under this Act, whereas these requirements are incorporated in EU regulations and Codex codes of practices. Another key finding was a lack of a central Competent Authority (CA), which will be lawfully mandated in issues of certification, permits, and liaison with international bodies in addressing food safety issues in mariculture.

7.3 RECOMMENDATIONS

- Chapter 2: interventions were recommended such as collaborations between government departments, academia, and industry to facilitate investigations and knowledge sharing. These included upscaling of research on microbial contamination of shellfish to keep up with the rest of the world.
- There should be consistency in conducting sanitary survey updates and re-evaluations every three years as practised globally. An annual review based on meteorological and hydrodynamics should be performed to ensure that conditions are unchanged (NSSP, 2019).
- Chapter 3: it is evident that sewage disposal at coastal areas used for shellfish harvesting is a global problem. To control or minimise the risk of shellfish contamination, other countries decided to use shellfish harvesting exclusion zones. There are different types, which include geographical-proximity-based zoning, dilution-based zoning, and shellfish sampling-based zoning (Hassard et al., 2017). The United Nations National Shellfish Sanitation Program makes use of dilution-based zoning of 1000:1 proximity of harvesting areas to the discharge outfall. Campos et al. (2017) emphasised that the implementation of a buffer zone needs a thorough understanding of the dispersion and dilution of sewage effluent. The European Union has not regulated the buffer zone concept; however, some member states apply a strict measure of closure of areas around sewage outfalls (Campos et al., 2017). Some countries implemented the closure of harvesting areas around sewage outfalls. The shellfish industry and

government policymakers need to understand that a viable shellfish industry depends on production waters that are free from pollution (Schmidt et al., 2018).

- Chapter 4: the list of isolated bacteria in Table 4.2 shows that aquatic environments are home to various microbiota, of which some are found naturally in these environments or introduced through anthropogenic activities. Intensive and continuous monitoring of these microorganisms over and above the standard faecal indicator bacteria or commonly known foodborne disease-causing bacteria is crucial in the identification of their origin, prevalence, and trends (Chinnadurai et al., 2020).
- Chapter 5: recommendations for the development of effective management plans for shellfish harvesting areas were highlighted. Development of virus compliance limits, as well as virus indicator species such as the use of *E. coli* has proved to be ineffective in detecting viral contaminants. Written management plans are required for each shellfish growing area. Detailed reports on characteristics of effluent discharged near shellfish growing areas, and dilution factors, depending on the classification of growing areas should be provided (NSSP, 2019).
- Chapter 6: the development of a one-stop-shop office with a single designated competent authority for attestation or final approval in aquaculture related matters was recommended.
- Coordination between different departments responsible for developing and implementing shellfish policies is essential to avoid contradicting policy outcomes and duplication of work. In addition, it will strengthen partnerships that bring together multilevel and diverse resources, knowledge, and commitment from all stakeholders.

7.4 LIMITATIONS TO THE STUDY

The sampling sites are located offshore of Sandanha Bay harbour. Sampling runs were dependent on weather conditions. On some occasions, some sampling points could not be reached due to sudden weather changes. This affected sample collection at a site located in Outer Bay which is close to the mouth of the bay and highly exposed to ocean currents.

The country lock-down due to Covid-19 affected sampling collection in 2020. As a result, fieldwork was confined to 2021, leading to changes to the study's structure.

7.5 FUTURE RESEARCH

The results of the study identified the following areas for future research:

- Further studies on possible *E. coli* contamination due to bird populations on shellfish farms.
- Longitudinal studies on bacterial species identified to develop a database on the bacteria of concern in Saldanha Bay harbour.
- The systematic review on virus contamination presented a summarised global perspective on virus contamination. An experimental study on virus prevalence would provide an indication of the status of Saldanha Bay in terms of virus contamination, what viruses are present, their prevalence and trends, and control measures to apply.
- Investigation of virus presence in all shellfish farms and boat workstations and development of a virus database.
- Seasonal impacts of physico-chemical parameters (pH, salinity, temperature, and dissolved oxygen), concerning virus and bacteria prevalence in oysters and mussels.

7.6 LIST OF PUBLICATIONS AND CONFERENCE PRESENTATIONS:

Articles published:

Chapter 4: The prevalence of bacteria commonly related to the production of mussels and oysters in Saldanha Bay. (2023). *Aquaculture Research*, Vol 2023. Article ID 7856515, 10 pages. https://doi.org/10.1155/2023/7856515.

Chapter 5: The prevalence of viruses related to the production of mussels and oysters in Saldanha Bay: A systematic review. (2023). *Aquaculture Journal*, 90-106. https//doi.org/10.3390/aquacj3020009.

Conference:

South African Institute of Environmental Health (SAIEH) - 21st National Environmental Health Conference. The conference theme: “One Health- an Environmental Health Perspective”. Dates: 16-19 November 2018. Location: Cape Town, South Africa. Oral presentation. Title: A critical analysis of contaminants associated with the production of shellfish in Saldanha Bay, South Africa.

Cape Peninsula University of Technology Post graduate research conference: Dates: 01 March 2023. Location: SARATEC, Bellville Campus. Oral presentation. Title: The prevalence of bacteria commonly related to the production of mussels and oysters in Saldanha Bay.

South African Association for Food Science & Technology 25th biennial congress: Dates: 28 – 30 August 2023. Location: Cape Town International Convention Centre. Poster presentation. Title: Microbiological monitoring from 2017 - 2020 in Saldanha bay shellfish farms: an assessment of *Escherichia coli* prevalence in mussels and oysters

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This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has therefore been unavoidable including references cited.

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APPENDIX A:

Images of sample collection and mussel and oyster samples collected

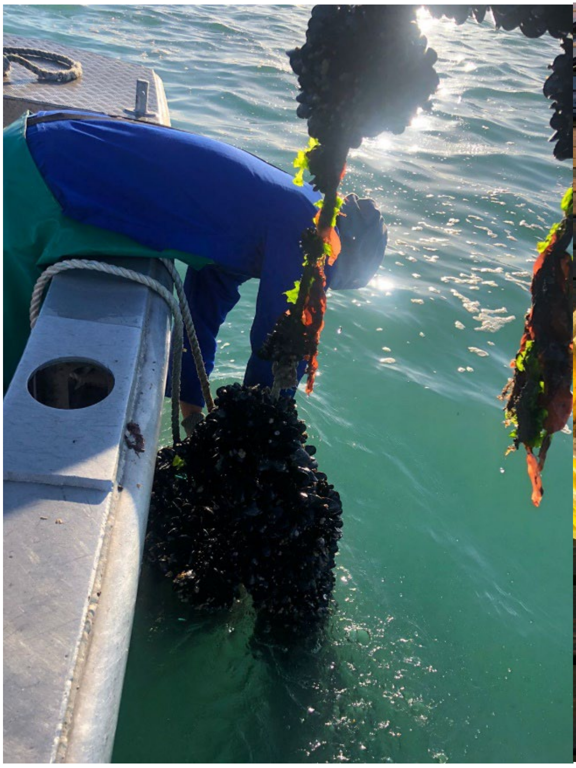


Figure 1 *Mussels grown on a rope*

Figure 2 *Oysters before removal of dirt*

Figure 3 *Oysters after removal of dirt*

***Figure 4** Cage used for growing oysters*

***Figure 5** Hose used for taking water samples*

***Figure 6** Documenting temperature and pH readings with a multimeter*

APPENDIX B:

Images of presumptive test results on culture media

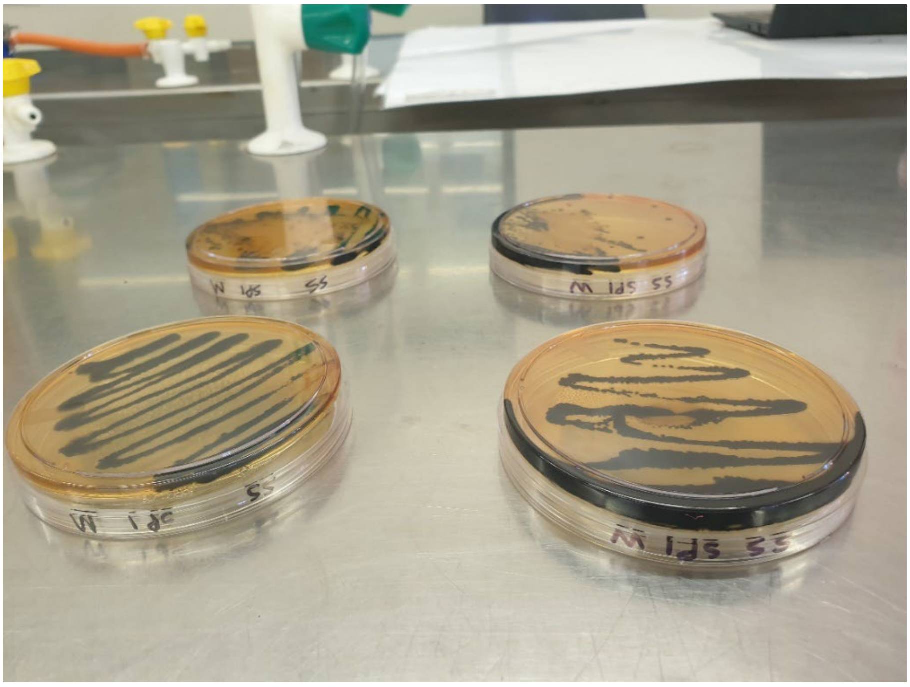


Figure 7 *Presumptive test results on Salmonella using Salmonella Shigella Agar*

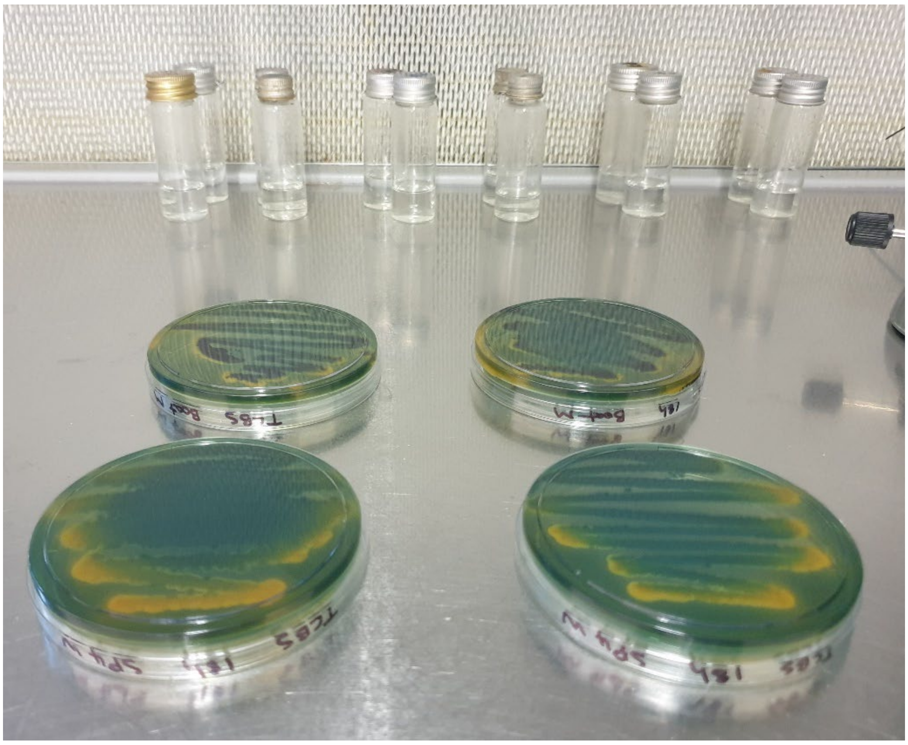


Figure 8 *Presumptive test results on Vibrio spp. using Thiosulphate Citrate Bile Sucrose Agar and Saline Nutrient Agar*

APPENDIX C:

Permission obtained from Department of Agriculture, Forestry and Fisheries (DAFF) to access the sampling area

agriculture,
forestry & fisheries
Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate: Sustainable Aquaculture Management, Department of Agriculture, Forestry and Fisheries

Private Bag X2, Roggebaai, Cape Town, 8012

Enquiries: Mr John Foord, Tel: (021) 430 7065, E-mail: JohnF@daff.gov.za

NRF

THUTHUTKA APPLICATIONS 2019

Dear Ms Sir/ Madam,

ACCESS APPROVAL FOR PROJECT: MRS LIKENTSO SHUPING

The Bivalve Shellfish Association of South Africa and the Department of Agriculture, Forestry and Fisheries (DAFF) has given Mrs Likentso Shuping's permission to access the aquaculture production facilities in Saldanha Bay to take water and shellfish samples for microbiological research purposes as part of her PhD.

Do not hesitate to contact me should you need any additional information.

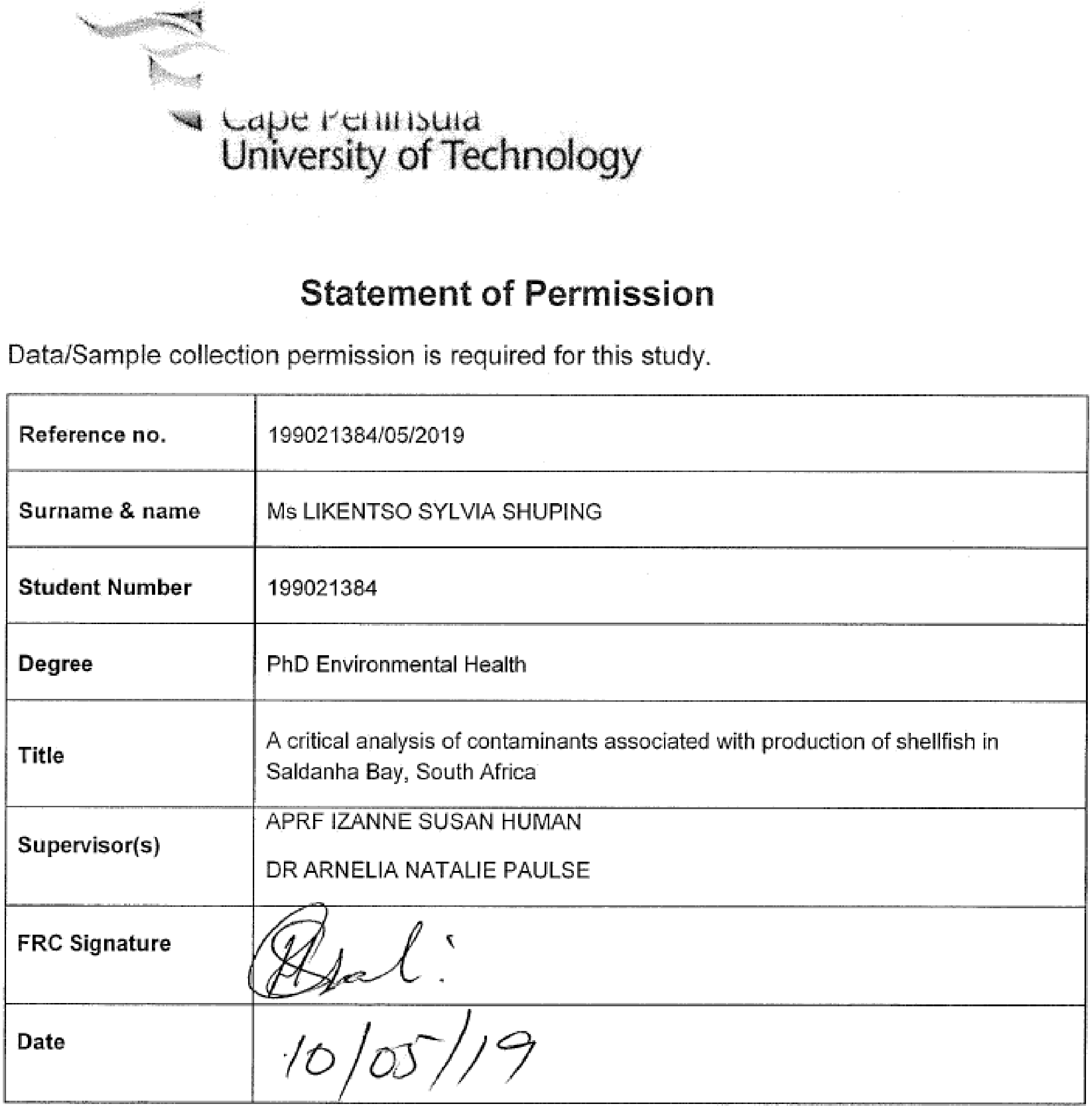
Yours faithfully.

MR JOHN FOORD

ENVIRONMENTAL OFFICER SPECIALISED PRODUCTION AQUACULTURE AND ECONOMIC DEVELOPMENT DATE: 21/05/2018

APPENDIX D:

Research Ethical clearance compliance form



Cape Peninsula
University of Technology

Statement of Permission

Data/Sample collection permission is required for this study.

Reference no.	199021384/05/2019
Surname & name	Ms LIKENTSO SYLVIA SHUPING
Student Number	199021384
Degree	PhD Environmental Health
Title	A critical analysis of contaminants associated with production of shellfish in Saldanha Bay, South Africa
Supervisor(s)	APRF IZANNE SUSAN HUMAN DR ARNELIA NATALIE PAULSE
FRC Signature	
Date	10/05/19

Cape Peninsula
University of Technology

P.O. Box 1906 □ Bellville 7535 South Africa □Tel: +27 21 953 8677 (Bellville), +27 21 460 4213 (Cape Town)

Office of the Chairperson **Research Ethics Committee**	**Faculty of Applied Sciences**
Reference no.	**199021384/05/2019**

The Faculty Research Committee, in consultation with the Chair of the Faculty Ethics Committee, has determined that the research proposal of **Ms Likentso Shuping** for research activities related to the: PhD Environmental Health to be undertaken at the Cape Peninsula University of Technology does not require ethical clearance.

Proposed title of dissertation/thesis	A critical analysis of contaminants associated with production of shellfish in Saldanha Bay, South Africa

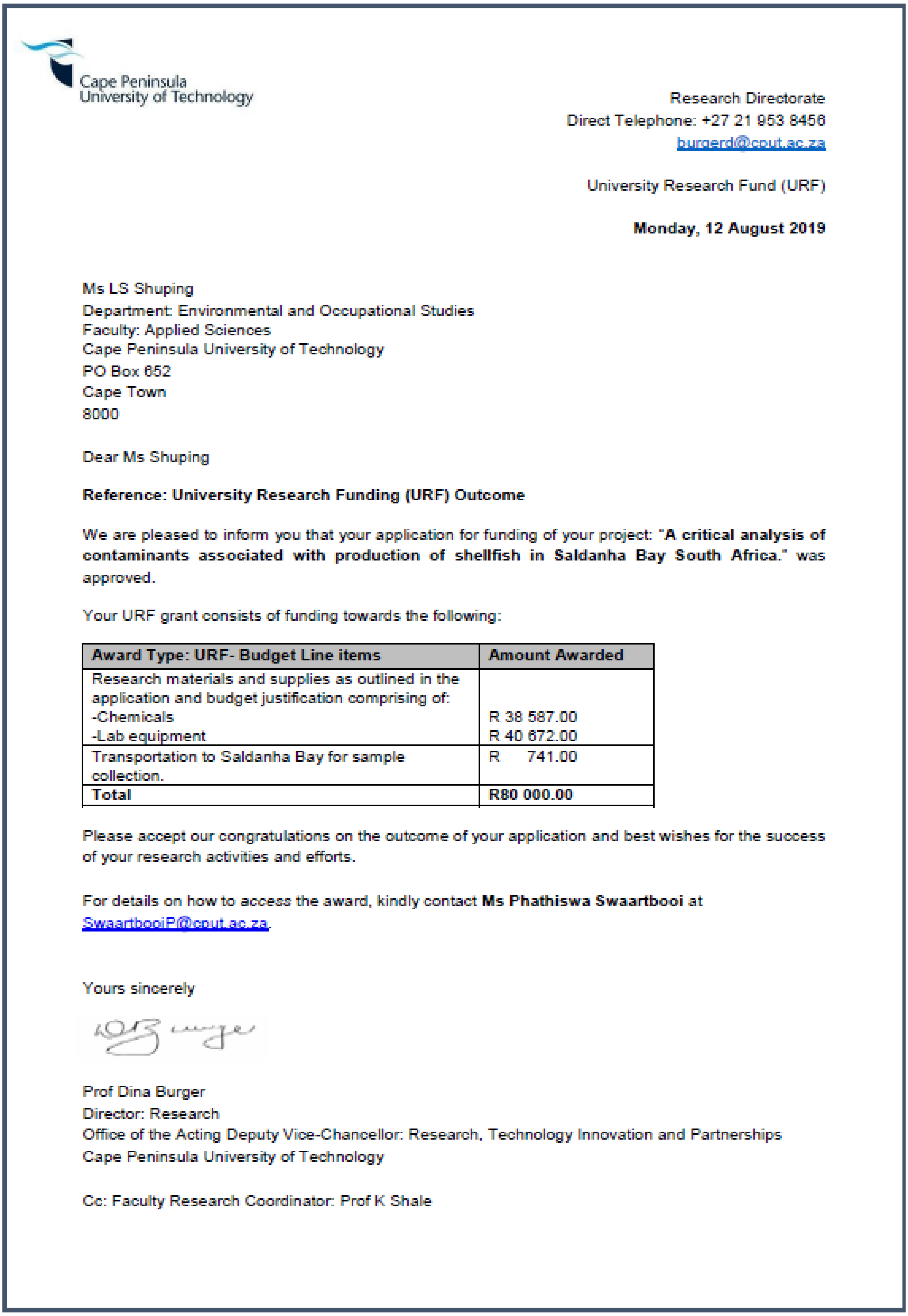
Comments (Add any further comments deemed necessary, eg permission required)

Research activities are restricted to those detailed in the proposal. Access to external research sites would have to be obtained by the researchers. This permission is granted for the duration of the study

Signed: Acting Chairperson: Research Ethics Committee	**02/05/2019** **Date**

APPENDIX E:

Funding received for study



Cape Peninsula
University of Technology

Research Directorate
Direct Telephone: +27 21 953 8456
burgerd@cput.ac.za

University Research Fund (URF)

Monday, 12 August 2019

Ms LS Shuping
Department: Environmental and Occupational Studies
Faculty: Applied Sciences
Cape Peninsula University of Technology
PO Box 652
Cape Town
8000

Dear Ms Shuping

Reference: University Research Funding (URF) Outcome

We are pleased to inform you that your application for funding of your project: "**A critical analysis of contaminants associated with production of shellfish in Saldanha Bay South Africa.**" was approved.

Your URF grant consists of funding towards the following:

Award Type: URF- Budget Line items	**Amount Awarded**
Research materials and supplies as outlined in the application and budget justification comprising of: -Chemicals -Lab equipment	 R 38 587.00 R 40 672.00
Transportation to Saldanha Bay for sample collection.	R 741.00
Total	**R80 000.00**

Please accept our congratulations on the outcome of your application and best wishes for the success of your research activities and efforts.

For details on how to access the award, kindly contact **Ms Phathiswa Swaartbooi** at SwaartbooiP@cput.ac.za.

Yours sincerely

Prof Dina Burger
Director: Research
Office of the Acting Deputy Vice-Chancellor: Research, Technology Innovation and Partnerships
Cape Peninsula University of Technology

Cc: Faculty Research Coordinator: Prof K Shale