Temporal and inter-species variations in the proximate and contaminant compositions of farmed mussels, *Choromytilus meridionalis* and *Mytilus galloprovincialis*, from Saldanha Bay, South Africa

DEBORAH CAITLIN FIRTH

Thesis presented in fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN ANIMAL SCIENCE

in the Faculty of AgriSciences at Stellenbosch University

Supervisor: Prof Louwrens C. Hoffman
Co-supervisors: Dr Bernadette O'Neill
Dr Khalid Salie

March 2018

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2018

SUMMARY

Seafood is known to be a healthy source of protein, minerals and omega-3 fatty acids, but trace metal and persistent organic pollution in the ocean can threaten the health of seafood consumers through the bioaccumulation of contaminants in the marine food chain. Mussel aquaculture in Saldanha Bay creates essential local employment and provides ~1600 tonnes of seafood per annum for local consumption and export, but is threatened by the expansion of heavy industry, shipping traffic and a growing human population. Research into the impact of marine pollution in aquaculture is necessary to protect farmers and consumers, and to establish baseline data for future pollution studies.

To assess temporal and inter-specific variations in the proximate and contaminant compositions of the farmed mussels from Saldanha Bay, native *Choromytilus meridionalis* and invasive *Mytilus galloprovincialis*, samples were collected from commercial mussel rafts at the centre of Small Bay at two-month intervals over two-years. Mussels were analysed for differences in morphometry, whole weights, meat weights, percentage meat yields, moisture, protein, fat, ash, trace metals (determined via ICP-MS) and a range of persistent organic pollutants (determined via GC-MS/MS), including organochlorine pesticides, polyaromatic hydrocarbons and polychlorinated biphenyls (PCBs). Significant temporal variations in whole weight, meat weight, percentage meat yield, proximate composition and contaminant compositions (Fe & As in *C. meridionalis*, Fe, As, Hg & Pb in *M. galloprovincialis*, and cis-permethrin, trans-permethrin, PCB 118 & PCB 149 in both species) were linked to changes brought on by the gametogenic cycle and storm water runoff during the winter rainfall period. Morphometrically, *M. galloprovincialis* was found to have a longer, wider and more ventrally flattened shell than *C. meridionalis*, and both species had similar moisture, protein, fat and ash contents. Species-related differences were observed for Al, Cr, Fe, Zn, Cd, Pb and PCBs 118 and 149 (higher in *M. galloprovincialis*), and Cu and Mn (higher in *C. meridionalis*). While concentrations of all persistent organic pollutants within the mussels were determined to be well below international regulatory limits, As concentrations exceeded the South African maximum limit (3mg/kg wet weight) once in each species, and Pb concentrations in *M. galloprovincialis* exceeded local regulations for fish (0.5mg/kg w.w.) on more than one occasion, but did not exceed EU regulatory limits for bivalves (1.5mg/kg w.w.).

Overall, both farmed mussel species from Saldanha Bay were determined to be healthy sources of protein and essential trace metals for consumers, with the added benefit of low fat contents. Apart from As and Pb, the samples were relatively uncontaminated with trace metals or persistent organic pollutants and likely do not pose a significant human health risk. Additional research into pollution sources in Saldanha Bay and bivalve-specific contaminant regulations are suggested to support the future growth of the aquaculture industry in South Africa. The information from this study will be useful to farmers and consumers, and as baseline data for future research.

OPSOMMING

Seekos is bekend daarvoor dat dit 'n gesonde bron van proteïene, minerale en omega-3 vetsure is, maar die voorkoms van spoormetaal en organiese besoedeling in die oseaan kan egter die gesondheid van seekos verbruikers bedreig deur middel van bioakkumulasie van kontaminante in die mariene voedselketting. In Saldanhabaai, skep mossel-akwakultuur noodsaaklike plaaslike indiensneming en lewer ~1600 ton seekos per jaar vir plaaslike verbruik en uitvoer. Dit word egter bedreig deur die uitbreiding van swaar nywerhede, skeepsverkeer en 'n toename in die menslike bevolking. Navorsing oor die impak van mariene besoedeling in akwakultuur is noodsaaklik om ten einde boere en verbruikers te beskerm maar ook vir die bepaling van basislyn data vir toekomstige besoedelingstudies.

Om die tydelike en interspesifieke variasies in die proksimale en kontaminante komposisies van die gekweekte mossels van Saldanhabaai, inheemse *C. meridionalis* en indringende *M. galloprovincialis*, te evalueer, was monsters van kommersiële mosselvlotte by die middelpunt van Kleinbaai elke twee maande geoes, oor 'n tydperk van twee jaar. Mossels was ontleed om verskille in morfometrie, heelgewig, vleisgewig, persentasie vleisopbrengs, vog, proteïen, vet, as, spoormetale (bepaal met ICP-MS) en 'n reeks organiese besoedelstowwe (bepaal met GC-MS/MS), insluitend organochlorine plaagdoders, poli-aromatiese koolwaterstowwe en poli-chloor bifenyls (PCBs) te bepaal. Beduidende tydelike variasies in heelgewig, vleisgewig, persentasie vleisopbrengs, proksimale komposisie en kontaminante komposisies (Fe en As in *C. meridionalis*, Fe, As, Hg en Pb in *M. galloprovincialis*, en cis-permetrien, trans-permetrien, PCB 118 en PCB 149 in albei spesies) was gekoppel aan veranderinge wat deur die gametogene siklus en stormwaterafloop, tydens die winterreënvalperiode, veroorsaak is. Morfometries is daar gevind dat *M. galloprovincialis* 'n langer, wyer en meer ventrale afgeplatte dop het as dié van *C. meridionalis*. Beide spesies het soortgelyke vog-, proteïen-, vet- en asinhoude gehad. Spesies-verwante verskille is waargeneem vir Al, Cr, Fe, Zn, Cd, Pb en PCBs 118 en 149 (hoër in *M. galloprovincialis*), en Cu en Mn (hoër in *C. meridionalis*). Alhoewel die konsentrasies van al die organiese besoedelstowwe binne die mossels vasgestel was om vêr onder internasionale regulatoriese perke te wees, het As konsentrasies die Suid-Afrikaanse maksimum limiet (3mg/kg natgewig) een keer in elke spesie oortref en Pb konsentrasies in *M. galloprovincialis* plaaslike regulasies oortref vir vis (0.5mg/kg natgewig) by meer as een geleentheid, maar dit het nie die EU-regulatoriese perke vir tweekleppiges (1.5mg/kg natgewig) oorskry nie.

Algeheel was dit vasgestel dat beide gekweekte mosselspesies van Saldanhabaai gesonde bronne van proteïene en noodsaaklike spoormetale vir verbruikers is, met die addisionele voordeel van 'n lae vetinhoud. Afgesien van As en Pb, was die monsters relatief onbesmet met spoormetale of organiese besoedelstowwe en hou nie 'n gesondheidsrisiko vir die mens in nie. Daar word voorgestel dat addisionele navorsing op die besoedelings bronne in Saldanhabaai en tweekleppige-spesifieke besoedelings regulasies gedoen word om die toekomstige groei van die akwakultuurbedryf in Suid-Afrika te ondersteun. Die inligting uit hierdie studie sal nuttig wees vir boere en verbruikers, en as basislyn data vir toekomstige navorsing.

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisors, Prof Louwrens C. Hoffman, Dr Bernadette O'Niell and Dr Khalid Salie, for their encouragement, guidance and patience throughout this two-year learning experience. This process has changed the way I approach and appreciate research, and it would not have been possible without the support of my supervisors.

I would also like to thank the staff and students of the Department of Animal Science at the University of Stellenbosch, specifically Alretha van Heerden and Mari van Aswegen for volunteering your time to help me, and Gail Jordaan for making statistics enjoyable!

To my best friend and favourite human, Clive Cronje. Thank you for your tireless support, encouragement and love, for helping me become a better person, and always reminding me to "just keep swimming".

To my parents, who have stood by me throughout my entire life, and through all the rough patches on my scientific journey. Thank you for believing in me, funding my never-ending academic career and for always being there with a hug and kind words. I would not be where I am today without your love and support.

Lastly, to my brother, Mark Jones. Though you have been gone from our lives for almost 10 years now, your passion and enthusiasm for life continue to inspire me to this day. Thank you for teaching me what it means to be bold, fearless and unapologetic.

I would also like to express my sincerest appreciation for the following institutions for their research and financial support:

This research is supported by the South African Research Chairs Initiative (SARChI) and funded by the South African Department of Science and Technology (UID: 84633), as administered by the National Research Foundation (NRF) of South Africa. The financial assistance of the NRF towards this research is hereby acknowledged. The opinions expressed, and conclusions arrived at, in this thesis are those of the authors and are not necessarily to be attributed to the NRF.

NOTES

The language and style of this thesis is in accordance with the requirements of The South African Journal of Animal Science. This thesis represents a compilation of manuscripts where each chapter is an individual entity and therefore some repetition between chapters, especially in the Materials and Methods section, was unavoidable.

Results of this study have been presented at the following conference:

Firth, D.C., Hoffman, L.C., Salie, K., and O'Neill, B. 2017. Investigating seasonal variability in the contaminant composition of the commercially cultured native black mussel (*Choromytilus meridionalis)* and invasive Mediterranean mussel (*Mytilus galloprovincialis*) in Saldanha Bay, South Africa. World Aquaculture 2017. June 2017. Cape Town, South Africa. Poster presentation.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AOAC	Association of Analytical Communities
CAC	Codex Alimentarius Commission
cm	Centimetre
DAFF	Department of Agriculture, Forestry and Fisheries (South Africa)
°C	Degrees Celsius
DEA	Department of Environmental Affairs (South Africa)
DOH	Department of Health (South Africa)
DDT	Dichlorodiphenyltrichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDD	Dichlorodiphenyldichloroethane
DHA	Docosahexaenoic acid
d.w.	Dry weight
EU	European Union
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organisation of the United Nations
GC-MS	Gas Chromatography-Mass Spectrometry
g	Grams
HCB	Hexachlorobenzene
HMW	High Molecular Weight
HCl	Hydrochloric acid
ICP/MS	Inductively Coupled Plasma Mass Spectrometry
LMW	Low Molecular Weight
$MgSO_4$	Magnesium sulfate
ML	Maximum Limit
m	Metre
µg/day	Micrograms per day
µg/kg	Micrograms per kilogram
µl	Microlitre
mg/kg	Milligrams per kilogram
ml	Millilitre
mm	Millimetre
ng/g	Nanograms per gram
HNO_3	Nitric acid
OCP	Organochlorine Pesticide

OPP	Organophosphate Pesticide
ppm	Parts per million
ppb	Parts per billion
%	Percentage
POP	Persistent Organic Pollutants
pg/g	Picograms per gram
PAH	Polyaromatic Hydrocarbons
PCB	Polychlorinated Biphenyls
PTWI	Provisional Tolerable Weekly Intake
NaCl	Sodium chloride
QuEChERS	Quick, Easy, Cheap, Efficient, Rugged and Safe
Rpm	Rotations per minute
ZAR	South African Rand
SASSI	South African Sustainable Seafood Initiative
TEQ	Toxic Equivalency
UNEP	United Nations Environment Programme
USA	United States of America
USEPA	United States Environmental Protection Agency
w.w.	Wet weight
WWF	World Wildlife Fund
WHO	World Health Organisation
WWTW	Waste Water Treatment Works
PCB 18	2,2',5-Trichlorobiphenyl
PCB 28	2,4,4'-Trichlorobiphenyl
PCB 44	2,2',3,5'-Tetrachlorobiphenyl
PCB 52	2,2',5,5'-Tetrachlorobiphenyl
PCB 110	2,3,3',4',6-Pentachlorobiphenyl
PCB 118	2,3',4,4',5-Pentachlorobiphenyl
PCB 138	2,2',3,4,4',5'-Hexachlorobiphenyl
PCB 149	2,2',3,4',5',6-Hexachlorobiphenyl
PCB 153	2,2',4,4',5,5'-Hexachlorobiphenyl
PCB 180	2,2',3',4,4',5,5'-Heptachlorobiphenyl

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

Fish is known to be a good source of protein, essential minerals, and healthy polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for the human diet (Raatz *et al.*, 2013; Bongiorno *et al.*, 2015; Fernández *et al.*, 2015). Fish consumption per capita has doubled worldwide in the past 50 years, with the majority of this increase in demand being supplied by the growing aquaculture industry, as capture fisheries have struggled to sufficiently increase their catch rates (FAO, 2014a).

In Africa, aquaculture has been identified as one of the many routes through which poverty and food insecurity can be alleviated, whether through the direct production of food fish for poorer communities, or through the generation of jobs in the feed, grow-out and post-processing industries (Hishamunda & Ridler, 2006). In South Africa, aquaculture production is dominated by the culture of marine species (mariculture), with abalone (*Haliotis midae*) and mussel (*Choromytilus meridionalis* and *Mytilus galloprovincialis*) farms making up 25% and 32%, respectively, of all aquaculture production in 2014 (DAFF, 2016). All mussel culture in the country currently occurs within the relatively calm waters of Saldanha Bay, which is the largest semi-enclosed bay on the West Coast of South Africa (DAFF, 2016). The mussel aquaculture industry in the Small Bay of Saldanha Bay produced over 1600 tonnes of seafood in 2014, and employed over 100 residents in the farming and processing procedures (DAFF, 2016). Researchers have stated that the high primary productivity levels of the bay, due to it being a part of the Cold Benguela Upwelling system, could support a 28-fold increase in the size of the mussel aquaculture industry (Olivier *et al.*, 2013), and there are plans in place for the large-scale expansion of aquaculture activities in the area, with the government proposal of a sea-based Aquaculture Development Zone (Clark *et al*., 2016).

Unfortunately, Saldanha Bay is also currently home to a growing human population, is used as a large international deep-water shipping port for crude oil and iron and lead ores, has three small craft harbours, and the surrounding areas are home to heavy industry in the form of a steel manufacturing plant, mining operations and several fish processing facilities (Clark *et al.*, 2002, 2014). Plans are in place for the expansion of many of the heavy industries in Saldanha Bay (Clark *et al*., 2016), but industrial development could pose a major threat to the water quality of the bay through the modification of water circulation patterns and the introduction of organic waste, heavy metals, hydrocarbons, untreated sewage and storm water runoff (Clark *et al.*, 2002). The overburdening and underperformance of Saldanha Bay's Waste Water Treatment Works is a source of serious pollution concerns, as these facilities receive large quantities of municipal and industrial waste water, which is often released untreated into Small Bay (Clark *et al.*, 2014). On top of these worrying contamination sources, the iron ore jetty which bisects Saldanha Bay has caused greatly decreased rates of water replacement (Weeks *et al.*, 1991), allowing potentially harmful pollutants to remain within the bay for extended periods of time and accumulate in sediments and biota (Clark *et al.*, 2014).

Mussels are known bioaccumulators of a wide range of environmental pollutants, making them effective biomonitors of marine contamination (Farrington *et al.*, 1983, 2016), but also resulting in them posing serious health risks to human consumers if they accumulate toxic pollutants beyond safe concentrations (Jović & Stanković, 2014). In order to protect human consumers, strict regulations have been set by the South African government's Department of Health (DOH), international governments and regulatory bodies such as the World Health Organisation on the maximum limits of environmental pollutants within the flesh of fish and bivalves (JECFA FAO/WHO, 1982; DOH, 2004; European Commission, 2006; FDA, 2008). The majority of quality control tests on products from aquaculture facilities in South Africa are, however, performed by government institutions, meaning that this data is not available to the general public (Wepener & Degger, 2012). Additionally, following the concerning general trend of an overall decrease in marine contaminant-monitoring studies in South Africa (Wepener & Degger, 2012), prior public research on trace metal and persistent organic pollutant (POP) contamination in Saldanha Bay is minimal. The Saldanha State of the Bay report, which monitors and publishes on a wide variety of pollutants including trace metals, ceased the monitoring of hydrocarbons (a common POP) in 1999 (Clark *et al.*, 2002, 2014), and only one study on POPs has occurred since (Degger *et al.*, 2011a).

The small number of publicly available studies on contaminants in mussels have found a wide variety of trace metals, polychlorinated biphenyls and polyaromatic hydrocarbons to be present in the waters of Saldanha Bay, with these contaminants at times exceeding regulatory limits within the flesh of both farmed and wild mussels (Degger *et al.*, 2011a; b; Clark *et al.*, 2014; Pavlov *et al.*, 2015). Considering that the contamination of the waters in the bay is already at concerning levels, the expansion of human populations and heavy industry within Saldanha Bay could, therefore, threaten the future of the aquaculture sector if they result in increased pollution entering the water column and the mussels themselves (Olivier *et al.*, 2013; Clark *et al.*, 2014, 2016). Up-to-date research is therefore necessary to create baseline data for future pollution studies, and help protect the aquaculture industry, and human consumers, from potential negative pollution impacts that may be caused by the expansion of heavy industry and human population growth.

In Saldanha Bay, *M. galloprovincialis* is the focus species of mussel aquaculture, due their superior growth rates and greater investment in flesh weight when compared to *C. meridionalis* (Van Erkom Schurink & Griffiths, 1992; van Erkom Schurink & Griffiths, 1993). Unfortunately, public information on the biometrics and proximate composition of the farmed mussels in Saldanha Bay (*C. meridionalis* and *M. galloprovincialis*) is either limited or over 30 years old, with the majority of studies on these species having been last performed in the 1980's, and with those studies focusing primarily on ecophysiology and reproductive biology (Griffiths, 1980; van Erkom Schurink & Griffiths, 1991). Current information on the proximate compositions of *C. meridionalis* and *M. galloprovincialis*, and how season affects these compositions, would be useful to farmers and the public alike, as it can give an indication of the health of the mussels, as well as their potential health benefits to seafood consumers.

The aims of this study were therefore to add to the expansion of current knowledge and create baseline data by:

1) Investigating seasonal and inter-species changes in the proximate composition of farmed *Choromytilus meridionalis* and *Mytilus galloprovincialis* from Saldanha Bay,
2) Determining the extent of trace metal and persistent organic pollutant contamination within farmed *Choromytilus meridionalis* and *Mytilus galloprovincialis* from Saldanha Bay, and whether these pose a health risk to human consumers or the mussels themselves,
3) Investigating seasonal and inter-species changes in the trace metal and persistent organic pollutant contamination of farmed *Choromytilus meridionalis* and *Mytilus galloprovincialis*.

1.1. REFERENCES

Bongiorno, T., Iacumin, L., Tubaro, F., Marcuzzo, E., Sensidoni, A., & Tulli, F. 2015. Seasonal changes in technological and nutritional quality of *Mytilus galloprovincialis* from suspended culture in the Gulf of Trieste (North Adriatic Sea). Food Chem. 173, 355–362 https://doi.org/10.1016/j.foodchem.2014.10.029.

Clark, B., Laird, M., Hutchings, K., Liebau, V., Biccard, A., Turpie, J., & Parker-Mallick, N. 2014. The state of Saldanha Bay and Langebaan Lagoon 2013/2014. Technical Report. Report No. 1581/1 prepared by Anchor Environmental Consultants for the Saldanha Bay Water Quality Trust.

Clark, B. M., Massie, V., Hutchings, K., Laird, M., Biccard, A., Brown, E., Duna, O. O., & Turpie, J. 2016. The State of Saldanha Bay and Langebaan Lagoon 2016. Technical Report. Report No AEC 1691/1 prepared by Anchor Environmental Consultants (Pty) Ltd for the Saldanha Bay Water Quality Forum Trust.

Clark, B. M., Lane, S., Turpie, J., van Niekerk, L., & Morant, P. D. 2002. South Africa National Report Phase 1: Integrated Problem Analysis. Report prepared for GEF MSP Sub-Saharan Africa Project (GF/6010-0016), Cape Town.

DAFF. 2016. Aquaculture Yearbook 2015. The Department of Agriculture, Forestry and Fisheries, Cape Town.

Degger, N., Wepener, V., Richardson, B. J., & Wu, R. S. S. 2011a. Brown mussels (*Perna perna*) and semi-permeable membrane devices (SPMDs) as indicators of organic pollutants in the South African marine environment. Mar. Pollut. Bull. 63, 91–97 https://doi.org/10.1016/j.marpolbul.2011.04.024.

Degger, N., Wepener, V., Richardson, B. J., & Wu, R. S. S. 2011b. Application of artificial mussels (AMs) under South African marine conditions: A validation study. Mar. Pollut. Bull. 63, 108–118 https://doi.org/10.1016/j.marpolbul.2011.04.040.

DOH. 2004. Regulation relating to maximum levels for metals in foodstuffs (No. R.500 30 April 2004). Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). Department of Health, South Africa.

European Commission. 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Communities L364, 5–24 https://doi.org/10.2203/dose-response.06-012.Hanekamp.

FAO. 2014. Value Statistics Database Updated to 2012. Food and Agriculture Organisation of the United Nations, Rome.

Farrington, J. W., Bowen, V. T., Goldberg, E. D., Risebrough, R. W., & Martin, J. H. 1983. U.S. 'Mussel Watch' 1976-1978: An Overview of the Trace-Metal, DDE, PCB, Hydrocarbon, and Artificial Radionuclide Data. Environ. Sci. Technol. 17, 490–496 https://doi.org/10.1021/es00114a010.

Farrington, J. W., Tripp, B. W., Tanabe, S., Subramanian, A., Sericano, J. L., Wade, T. L., & Knap, A. H. 2016. Edward D. Goldberg's proposal of 'the Mussel Watch': Reflections after 40 years. Mar. Pollut. Bull. 110, 501–510 https://doi.org/10.1016/j.marpolbul.2016.05.074.

FDA. 2008. Compliance Policy Guide [CPG Sec. 575.100 Pesticide Chemical Residues in Food – Enforcement Criteria (CPG 7141.01)]. U.S. Department of Health and Human Services, Food and Drug Administration, Maryland.

Fernández, A., Grienke, U., Soler-Vila, A., Guihéneuf, F., Stengel, D. B., & Tasdemir, D. 2015. Seasonal and geographical variations in the biochemical composition of the blue mussel (*Mytilus edulis* L.) from Ireland. Food Chem. 177, 43–52 https://doi.org/10.1016/j.foodchem.2014.12.062.

Griffiths, R. J. 1980. Ecophysiology of the Black Mussel *Choromytilus meridionalis* (Krauss). PhD, University of Cape Town, Cape Town.

Hishamunda, N., & Ridler, N. B. 2006. Farming fish for profits: A small step towards food security in sub-Saharan Africa. Food Policy 31, 401–414.

JECFA FAO/WHO. 1982. Evaluation of certain food additives and contaminants. Twenty-Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series 683, 30.

Jović, M., & Stanković, S. 2014. Human exposure to trace metals and possible public health risks via consumption of mussels *Mytilus galloprovincialis* from the Adriatic coastal area. Food Chem. Toxicol. 70, 241–251 https://doi.org/10.1016/j.fct.2014.05.012.

Olivier, D., Heinecken, L., & Jackson, S. 2013. Mussel and oyster culture in Saldanha Bay, South Africa: Potential for sustainable growth, development and employment creation. Food Secur. 5, 251–267 https://doi.org/10.1007/s12571-013-0244-1.

Pavlov, D. F., Bezuidenhout, J., Frontasyeva, M. V, & Goryainova, Z. I. 2015. Differences in Trace Element Content between Non-Indigenous Farmed and Invasive Bivalve Mollusks of the South African Coast. Am. J. Anal. Chem. 6, 886–897 https://doi.org/10.4236/ajac.2015.611084.

Raatz, S. K., Silverstein, J. T., Jahns, L., & Picklo, M. J. 2013. Issues of fish consumption for cardiovascular disease risk reduction. Nutrients 5, 1081–1097 https://doi.org/10.3390/nu5041081.

van Erkom Schurink, C., & Griffiths, C. L. 1991. A comparison of reproductive cycles and reproductive output in four southern African mussel species. Mar. Ecol. Prog. Ser. 76, 123–134 https://doi.org/10.3354/meps076123.

van Erkom Schurink, C., & Griffiths, C. L. 1992. Physiological energetics of four South African mussel species in relation to body size, ration and temperature. Comp. Biochem. Physiol. -- Part A Physiol. 101, 779–789 https://doi.org/10.1016/0300-9629(92)90358-W.

van Erkom Schurink, C., & Griffiths, C. L. 1993. Factors affecting relative rates of growth in four South African mussel species. Aquaculture 109, 257–273 https://doi.org/10.1016/0044-8486(93)90168-X.

Weeks, S. J., Boyd, A. J., Monteiro, P. M. S., & Brundrit, G. B. 1991. The currents and circulation in Saldanha Bay after 1975 deduced from historical measurements of drogues. South African J. Mar. Sci. 11, 525–535 https://doi.org/10.2989/025776191784287475.

Wepener, V., & Degger, N. 2012. Status of marine pollution research in South Africa (1960-present). Mar. Pollut. Bull. 64, 1508–1512 https://doi.org/10.1016/j.marpolbul.2012.05.037.

CHAPTER 2

Literature Review

2.1. THE STATUS OF WORLDWIDE AQUACULTURE

Fish, aquatic invertebrates and seaweeds are generally known to be a healthy source of important proteins, essential fatty acids, vitamins and minerals in the human diet (Jović & Stanković, 2014). Over the last five decades, due to population growth, increasing incomes and more efficient fish production and distribution chains, the world's per capita fish consumption per annum has increased from 9.9kg to 19.2kg on average (FAO, 2014b).

Though traditionally most fish protein was supplied by wild capture fisheries, many of the species that these fisheries rely on are either at the limits of their sustainable harvest or have been overexploited to the point where the fishery has collapsed or been closed (FAO, 2014a). Despite the stagnation of wild capture fisheries, food fish supply has increased annually by 3.2% on average, exceeding the world population growth rate of 1.6% per annum, and this increased demand has largely been supplied by the growing aquaculture industry (FAO, 2014b). Aquaculture has been defined as the farming of aquatic organisms, with farming implying a level of intervention in the rearing process such as feeding, regular stocking, and protection from predators, in order to enhance production, and which are harvested and owned by a particular individual or corporate body (FAO, 2014a). In 2012 the total global production of marine and freshwater species reached 158 million tonnes, with 91.3 million tonnes coming from capture fisheries and 66.6 million tonnes coming from aquaculture production (FAO, 2014a). In the last two decades, the annual growth of the aquaculture industry has far outpaced that of the capture fisheries industry, with growth between 2002 and 2012 remaining consistently at 6.1% per annum, resulting in aquaculture now supplying almost half of all fish consumed worldwide (Figure 2.1).

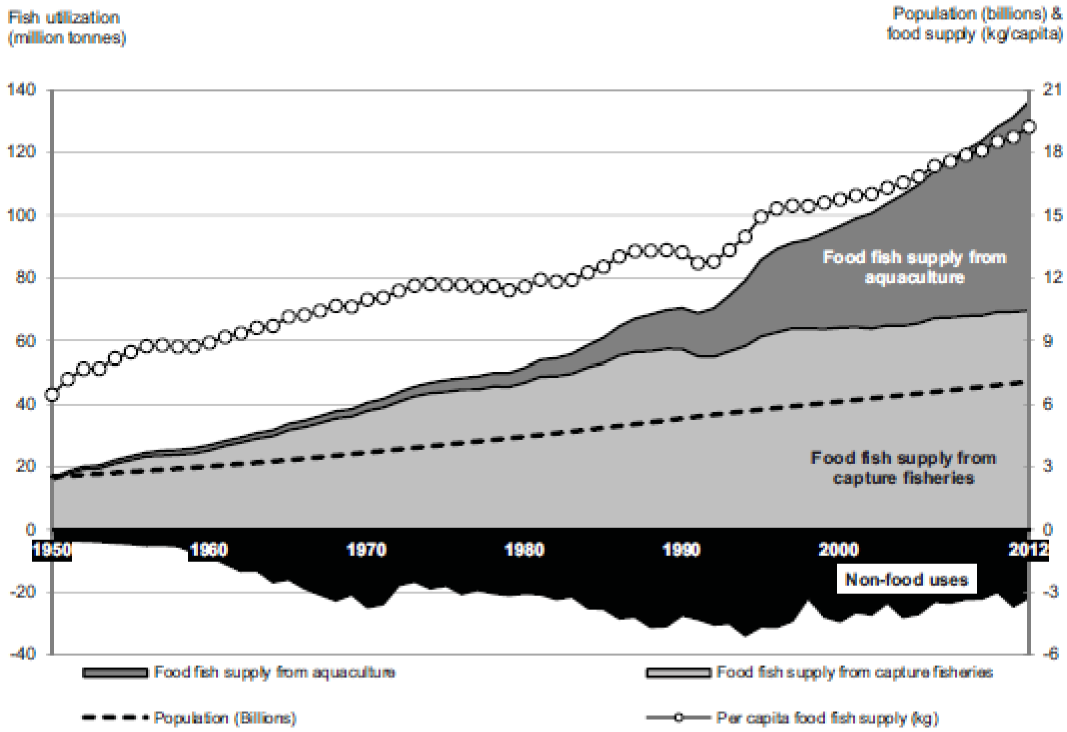


Figure 2.1 Human population growth, worldwide per capita food fish supply, the proportion of food fish supplied by capture fisheries and aquaculture, and the amount of fish used for non-food purposes (FAO, 2014a)

The highest contributing sector to food fish aquaculture worldwide is finfish, at 66% (44 million tonnes) of total production, followed by molluscs at 23% (15.2 million tonnes), crustaceans at 10% (6.4 million tonnes) and a combination of all other aquatic animal species at 1% (FAO, 2014b). Currently inland (or freshwater) aquaculture production, estimated at 42 million tonnes per annum, exceeds that of marine aquaculture (mariculture) at 24.7 million tonnes per annum (FAO, 2014b). This is likely because of the higher costs associated with mariculture, due to the need to farm in rough waters or build complicated land-based aquaculture facilities for sensitive and high-value species, while freshwater aquaculture can occur in simple ponds with more hardy or tolerant fish species that require far less capital input (Brummett *et al.*, 2008).

2.2. BIVALVE AQUACULTURE

Mussels belong to the Phylum Mollusca (including bivalves, gastropods and cephalopods) and the Class Bivalva, which includes oysters, scallops and clams (Gosling, 2004). Bivalve aquaculture is typically a form of non-fed aquaculture, as they are filter-feeders which obtain their nutrients from the water column in which they live. Feed is currently considered one of the main limiting factors to the expansion of aquaculture in developing countries (FAO, 2016), as it can often make up more than 50% of the running costs of a fish farm, which highlights the advantage of non-fed aquaculture species such as bivalves. While non-fed aquaculture has been identified as largely underdeveloped in Africa, it offers potential through species diversification, improvement of food security and nutrition, and a smaller impact on marine and freshwater environments than fed aquaculture (FAO, 2016). Though species from the Molluscan Phylum made up approximately 23% of total aquaculture in 2012 (FAO, 2014b), this group includes many marine species, and bivalve aquaculture forms a small subsector. In 2013, molluscs comprised 9.8% of all aquatic trade by value and 10.4% by live weight, while the subsector of bivalve trade worldwide made up 3% of all aquatic animal species traded by value, and 5.6% by live weight (FAO, 2016). In 2014, the total world production of aquaculture mussels was 16 113 thousand tonnes, with the top producers in the world being (FAO, 2016):

1. China (13 418 thousand tonnes),
2. Japan (376 thousand tonnes)
3. Republic of Korea (359 thousand tonnes)
4. Chile (246 thousand tonnes)
5. Spain (225 thousand tonnes)
6. Thailand (209 thousand tonnes)
7. Vietnam (198 thousand tonnes)
8. USA (160 thousand tonnes)
9. France (154 thousand tonnes)
10. Taiwan Province of China (99 thousand tonnes)

According to Gosling (2003) there are nine commercially important mussel species fished and farmed worldwide (summarized in Table 2.1). The most widely farmed species of mussels are *Mytilus edulis* and *Mytilus galloprovincialis*; these species originated in Europe and the Mediterranean, respectively, but are highly invasive and are found and farmed in both the Northern and Southern Hemisphere (Gosling, 2004).

Table 2.1 The commercially important mussel species found worldwide and the countries in which they are fished or farmed (adapted from Gosling, 2003)

Species	Northern Hemisphere	Southern Hemisphere
Mytilus edulis	Great Britain, Western & Northern Europe, Scandinavia, Eastern USA	Chile, Argentina, New Zealand
Mytilus galloprovincialis	Eastern & Western Europe, Mediterranean, North Africa (Morocco, Algeria, Tunisia), Western USA, Eastern China, Japan	Australia, New Zealand, South Africa, Namibia
Mytilus trossulus	Eastern and Western USA & Canada, Baltic Sea, Western Russia, Northern Japan	
Mytilus californianus	Western coasts of Mexico, USA and Canada	
Aulacomya ater		Peru, Chile, Argentina, Uruguay, Brazil, New Zealand
Perna perna		Brazil, Venezuela, Mauritius, South Africa, Angola, Tanzania, Kenya, Somalia, Morocco, Western Sahara, Mauritania
Perna viridis	Persian Gulf, Pakistan, India, Thailand, South-eastern China, Philippines	New Guinea, Indonesia
Choromytilus chorus		Chile
Perna canaliculus		New Zealand

2.2.1. Farming Methods

Mussels are farmed in a number of different ways; the most common methods of mussel farming are on rafts and longline systems, but a number of other farming techniques are used around the world.

2.2.1.1. Rafts

Rafts typically consist of a wooden framework which contains floats made of cylindrical fiberglass or steel. They are secured using a heavy concrete anchor on the seabed, and between 200-700 ropes of approximately 9m in length are secured along the length of the raft. These rafts are usually spaced 80-100m apart from one another, and a large group of rafts is referred to as a park (Gosling, 2004). The culture of mussels can be divided into five phases:

1. Collecting seed (6-8mm in length)
2. Attaching seed to the ropes
3. Thinning the ropes
4. On-growing
5. Harvesting

In some cases, spat settles naturally out of the water column onto the ropes, and in other cases the ropes must be seeded by collecting juveniles from their natural habitat and attaching them to the ropes in biodegradable cotton socks or water-soluble rayon netting (Gosling, 2004). After 5-6 months when the mussels are half-grown (4-5cm shell length), they are removed, cleaned and redistributed onto two or three new ropes (thinning), which is done to decrease risk of the mussels falling off in clumps, and to increase their growth rate. Mussels can reach marketable size of 8-10cm within 13-16 months, and this growth rate can be increased if densities are kept low (Gosling, 2004).

2.2.1.2. Longline systems

Longline systems are used in the UK, Ireland and New Zealand. They consist of ~60m-long surface lines with flotation drums spaced out at 6m intervals, and large anchors in the seabed at each end of the line (Gosling, 2004). Vertical ropes or mesh-stocking collectors are hung at ~50cm intervals, and the practices of seed-collection, thinning and grow-out are much like those of the raft system. Longline systems are, however, cheaper in general than raft systems to construct and more durable for harsh winter storms (Gosling, 2004).

2.2.1.3. Bouchot

Spat is collected using natural settlement out of the water onto parallel rows of poles with horizontal ropes, which are situated offshore. Once the spat is a few months old it is removed from the ropes and placed into mesh tubes. Oak poles of between 4-7m in length and 12-25cm in diameter are embedded halfway into the seabed in the intertidal zone, and the mesh tubes containing the spat are wound around these poles for grow-out. These mussel farms normally consist of 15 000-20 000 poles, and are family run in areas like France with *Mytilus edulis* (Gosling, 2004). They are generally not sufficient to supply large markets as they have a slow grow-out rate, with harvest occurring at sizes over 4cm, which takes 12-18months (Gosling, 2004).

2.2.1.4. Bamboo poles

In Thailand and the Philippines, bamboo poles of 6-8metres in length are driven into the sea bed in a circle and tied together to form a “teepee” shape. Both spat collection and grow-out occurs on these poles, mainly for *Perna viridis* (Gosling, 2004). Mussels are ready for harvest comparatively early, at between 6-10months, when they have reached a 5-10cm shell length (Gosling, 2004).

2.3. SOUTH AFRICAN AQUACULTURE

Large scale fish culture in Africa began in Kenya with tilapia (*Oreochromis niloticus*) in 1924, and since then aquaculture in Africa has spread to over 42 countries producing 65 species (Brummett & Williams, 2000). Despite this growth in the industry, Africa still supplies less than 3% (1.5 million tonnes per annum) of world aquaculture production, with over a million tonnes coming exclusively from Egyptian aquaculture production facilities (FAO, 2014b). It is important to note, however, that in 2012 African aquaculture was experiencing the highest growth rate of any continent, at 11.7 percent per annum (FAO, 2014b).

In South Africa, as in many African countries, aquaculture has been practiced on an extensive, small-scale basis for many years, but large-scale commercial aquaculture is relatively new to the country. Freshwater aquaculture in South Africa began with the import of trout (*Oncorhynchus mykiss*) ova in 1896, mainly encouraged by the recreational trout-fishing industry, while mariculture in South Africa began in 1948 with the farming of oysters in Knysna (Hecht & Britz, 1992; Shipton & Britz, 2007). Since then the South African aquaculture industry has grown steadily and in 2011 was already worth approximately 379 million ZAR, and employed approximately 1 600 people (DAFF, 2014).

The marine species currently farmed in South Africa include finfish such as dusky kob (*Argyrosomus japonicus*) and Atlantic salmon (*Salmo salar*), invertebrates such as abalone (*Haliotis midae*), mussels (*Mytilus galloprovincialis* and *Choromytilus meridionalis*) and oysters (*Crassostrea gigas*), and seaweeds such as *Ulva* and *Gracilaria* species (DAFF, 2016). In 2011, abalone mariculture was the most valuable marine aquaculture subsector in South Africa, with total production at 1 036 tonnes (55% of total mariculture); this was followed by mussel and oyster aquaculture at 570 tonnes (30%) and 69 tonnes (14%), respectively (DAFF, 2014). Three years later, however, South Africa's total marine aquaculture output increased dramatically from 1 883 to 3 417 tonnes, with mussel production experiencing major growth and exceeding that of abalone production, though abalone remains the most valuable sub-sector overall (DAFF, 2014, 2016). Mussel production in 2014 reached 1 682 tonnes (50% of total mariculture), while abalone aquaculture produced 1 306 tonnes (39%), and oyster and finfish production produced 266 (6.25%) and 161 tonnes (4.82%) respectively (DAFF, 2016).

Though most mussels produced in South Africa are sold in the local market, in 2014, 515 tons of mussels were exported to foreign markets, at a value of ~309 million ZAR (DAFF, 2016). Mussels were exported to 18 countries worldwide including African countries such as Namibia, Botswana, Ghana and Mozambique (Table 2.2). The majority of exports, however, went to Hong Kong (65%), Taiwan (20%), and Japan (3%) (Table 2.2). At the same time, 114 tonnes of mussels were imported into South Africa, with most of it coming from China (86%). The total value of mussel imports was approximately R2.59 million (DAFF, 2016).

Table 2.2 Top six countries to which South African mussels were exported, and from which foreign mussels were imported, in 2014 (compiled from DAFF, 2014)

Country	Import/Export	Quantity (Tonnes)	Value (million ZAR)	Average price/kg (ZAR)
Hong Kong	Export	337.81	196.97	617.07
Taiwan	Export	103.51	43.71	427.92
Japan	Export	26.58	38.16	514.35
Singapore	Export	18.91	17.55	822.22
China	Export	15.42	7.76	487.45
Malaysia	Export	5.49	4.99	1306.42
China	Import	98.05	1.11	N/A
Namibia	Import	12.55	0.945	N/A
Portugal	Import	2.03	0.15	N/A
New Zealand	Import	0.75	0.385	N/A
Mozambique	Import	0.54	0.0008	N/A
South Korea	Import	0.01	0.001	N/A

The simultaneous import and export of mussels in South Africa is largely due to the production of South African mussels falling well below the local demand until very recently, and therefore foreign mussels were imported to satisfy the market (DAFF, 2014). South Africa has only begun exporting mussels within the past three years, due to the recent growth in the industry in Saldanha Bay (DAFF, 2016). Saldanha Bay is currently the only location in South Africa where mussels are farmed on a commercial basis, as there are few other sheltered bays of similar size, and the rough waters along the exposed coastline of the country make mariculture impossible in the open ocean (Olivier *et al.*, 2013).

2.4. SALDANHA BAY

Saldanha Bay is situated on the West Coast of South Africa, approximately 110km north of Cape Town. It is a highly productive economic area of South Africa; home to a rapidly growing human population, and containing a large shipping port, massive industrial development in the form of a steel manufacturing plant and iron ore terminal, large scale fish production and processing operations, and the nearby Langebaan Lagoon is also a valuable tourist site (Clark *et al.*, 2014). Saldanha Bay is the only natural sheltered embayment along South Africa's rough coastline, and the relatively calm nature of the waters in the bay allow for the raft culture of mussels and oysters (Stenton-Dozey *et al.*, 2001). Currently, due to the rough conditions of the South African coastline, all mussel production in South Africa occurs here. Commercial mussel aquaculture in Saldanha Bay began with Seafarm in 1984, in an enclosed seawater dam, and in 1987 Atlas Sea Farms and Sea Harvest Corporation began open water cultivation of mussels using the longline and raft rope culture systems (van Erkom Schurink & Griffiths, 1990). Currently there are seven mariculture operators active in Saldanha Bay, producing either mussels, oysters or seaweeds, with plans for large-scale expansion of the aquaculture industry in the form of a sea-based Aquaculture Development Zone (Clark *et al.*, 2016).

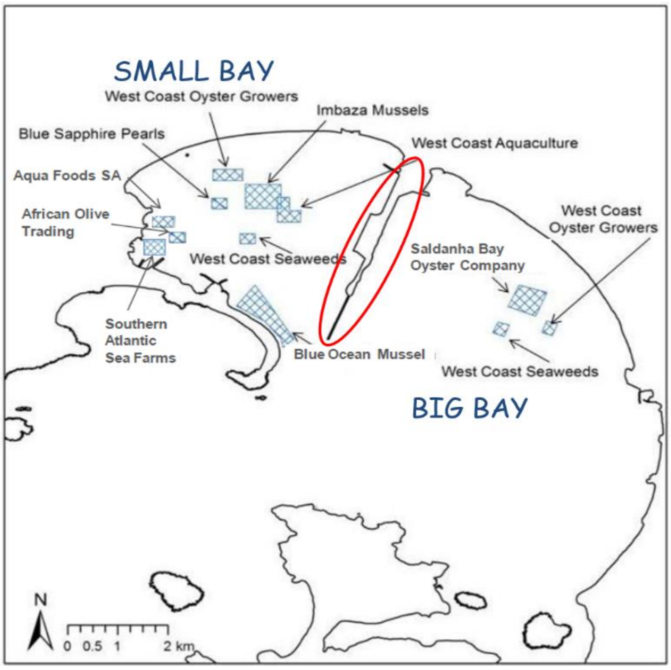


Figure 2.2 The Saldanha Bay allocation of mariculture operation concession areas, with the iron ore terminal which bisects Small and Big Bay circled in red (Image modified from Clark, *et al*., 2014)

Two species of mussel are farmed year-round in different quantities in Saldanha Bay; the native black mussel *Choromytilus meridionalis*, and the invasive Mediterranean mussel *Mytilus galloprovincialis*. These two species will be discussed in greater detail in later sections of this Chapter. The majority of mussels in Saldanha Bay are currently farmed in Small Bay (see Figure 2.2) on rafts of similar design to the wooden rafts used elsewhere in the world, but which have instead been made with large high-density polyethylene (HDPE) pipes (Figure 2.3). The harvest and processing system and facilities of one such mussel farm, Imbaza Mussels, are shown in Figure 2.3.

Figure 2.3 Images of A) Black HDPE rafts on which the mussels are grown, B) Mussel ropes attached to smaller HDPE pipes at regular intervals, C) Processing raft, with equipment used to clean and size-grade harvested mussels, D) Mussel ropes being pulled onto the deck of the processing raft for harvest, E) Farm workers sorting and size-grading mussels by hand, F) Crates of cleaned and harvested mussels as they are shipped to shore.

For some time now concerns over water quality and environmental pollution due to anthropogenic activities in the area have been raised by locals and government officials. This is due to the fact that Saldanha Bay is surrounded by a growing human population, is used as a port for large shipping vessels which pump their ballast water into the bay, has an iron ore terminal (circled in red in Figure 2.2) with plans for future expansion, three small craft harbours, numerous aquaculture farms and several fish processing facilities (Clark *et al.*, 2014). While the port was initially intended to be used as an export facility for iron ore, it has since expanded to accommodate the import of crude oil and export of heavy minerals and steel products (Clark *et al.*, 2002). Though Olivier *et al*. (2013) state that mussel culture in the bay could be safely increased by 10- to 28-fold without environmental harm, the input of pollutants into the system from anthropogenic activities in the area is a cause for concern, as it could lead to food safety issues for humans who consume the mussels grown in Saldanha Bay.

A serious pollution concern in Saldanha Bay is the issue of waste water treatment. Though there are no major river inputs bringing anthropogenic waste into Saldanha Bay, there are two waste water treatment facilities in Saldanha and Langebaan which release treated effluent from residential and industrial sources into the waters of the bay (Clark *et al.*, 2014). The Saldanha Waste Water Treatment Works (WWTW) treats water from numerous industrial sources, including waste from fish processing facilities and the Transnet Port

Authority, and these industrial wastes often put the facility under significant strain, resulting in effluent of substandard quality being released into the bay (Clark *et al.*, 2014). From 2008-2012 the Saldanha WWTW exceeded its average daily volume limit of 2 625m^3 consistently throughout the winter months (Clark *et al.*, 2014). Though this treatment system underwent upgrades in 2012 to increase capacity and reduce the occurrence of malfunctions which result in the release of raw sewage into the bay, in 2014 the Saldanha WWTW was releasing 3 363m^3 of waste water into the bay, far exceeding the daily limit, and the Langebaan WWTW was found to be illegally releasing waste into the Langebaan Marine Protected Area (Clark *et al.*, 2014). This issue is, unfortunately, likely to increase in severity as the communities of Saldanha and Langebaan continue to grow, and the WWTWs continue to receive and release more effluent than they can effectively process.

For many years mussels have been used for the biomonitoring of trace metals and pollution in marine environments due to their sedentary nature, resistance to pollutants, extensive distribution and their ability to concentrate environmental pollutants by factors of greater than a thousand (Watling & Watling, 1976; Sparks *et al.*, 2014). This ability to accumulate pollutants is of particular concern to humans, as mussels are consumed in large quantities worldwide and could therefore pose a serious risk to human health if the environments in which they are grown become polluted (Sparks *et al.*, 2014), as is the concern in Saldanha Bay.

There are a number of contaminants assimilated by mussels in polluted waters that could be of concern to human consumers; these include inorganic compounds (heavy metals such as lead, mercury, cadmium and arsenic), organic compounds (pesticides/insecticides), potentially infectious microbes and many other harmful substances such as antibiotic and drug residues (Stanković & Jović, 2012). Clark *et al*. (2014) found that trace metal concentrations in marine filter feeders where the mussel farms are located were not of immediate concern, but the concentrations of trace metals in mussels that grow along the shoreline frequently exceeded guidelines for marine foodstuffs. This is cause for concern, as these mussels are harvested by local recreational fisherman, and it gives an indication that pollution levels in the bay have the potential to exceed health limits. The report speculated the food safety of the mussel farms could have been superior due to the rafts location in the center of the bay increasing their exposure to uncontaminated water from upwelling events along the West Coast.

The causeway and iron ore terminal, which bisect the bay (circled in red in Figure 2.2), have already reduced rates of flushing and water replacement within the whole of Saldanha Bay, with the largest impact being on Small Bay (Clark *et al.*, 2014). The reduced rate of water replacement creates increased concerns over pollution, as contaminants may be allowed to remain in the bay for extended periods of time, causing greater harm, if they are not removed fast enough by the now-weakened ocean currents (Clark *et al.*, 2014). With the current and planned expansion of heavy industry in Saldanha Bay and the concerning issue of inadequate waste water treatment, it is important to establish baseline studies of pollution levels in order to ensure that these expansions do not negatively impact the health of the bay and in turn the health of the aquaculture production facilities. Previous studies on the contamination levels of trace metals in farmed and wild mussels from Saldanha Bay have repeatedly found that contamination levels are greatly elevated in wild, shore-collected mussels when compared to samples from aquaculture facilities (Clark *et al.*, 2014; Pavlov *et al.*, 2015). These differences have been attributed to the fact that mussels which grow along the shore frequently experience far lower rates of water replacement than the aquaculture facilities in Small Bay, which are intentionally located in areas with increased water circulation (Clark *et al.*, 2014; Pavlov *et al.*, 2015). The mussels along the shoreline are therefore in contact with pollutants in the water, as well as potentially polluted sediments, for far longer periods than those on the rafts, thereby allowing more time for passive diffusion of contaminants into the flesh and higher overall contaminations levels (Clark *et al.*, 2014; Pavlov *et al.*, 2015).

Due to their ubiquity along the South African coastline and their importance to mussel aquaculture in the country, numerous studies in South Africa have focused on the growth, reproduction, biology and more recently the contamination levels of a variety of pollutants in the invasive *M. galloprovincialis* (Sparks, 2012; Clark *et al.*, 2014; Kampire *et al.*, 2015; Pavlov *et al.*, 2015). In *C. meridionalis*, however, the majority of research done on this species occurred in the 1980's (Griffiths, 1980a; b, 1981; Griffiths & Buffenstein, 1981) and centered around ecophysiology, growth and reproductive biology, with only two studies assessing the accumulation rates of trace metals (Watling & Watling, 1976; Orren *et al.*, 1980). Previous studies have found different species of mussels to accumulate trace metals and other contaminants at varying rates (Usero *et al.*, 2005; Dahms *et al.*, 2014), and the same could hold true for the two farmed species of Saldanha Bay, *Choromytilus meridionalis* and *Mytilus galloprovincialis*, which will be discussed in depth below.

2.5. *MYTILUS GALLOPROVINCIALIS*

2.5.1. Origin, identification and invasion of South Africa

Mytilus galloprovincialis originates from the coasts of the Mediterranean, Adriatic and Black Seas (GISD, 2015). Its shell is brown to dark blue or black in colour, ventrally flattened and broad in cross-section (Figure 2.4.), and the gonad of the female is typically yellow, while the gonad of the male varies from off-white to yellow and orange (van Erkom Schurink & Griffiths, 1990; Stanković & Jović, 2012). It is listed on the "100 of the World's Worst Invasive Alien Species" database, which is compiled by the Invasive Species Specialist Group of the World Conservation Union (Lowe *et al.*, 2000). It has invaded numerous countries worldwide, including Australia (McDonald *et al.*, 1991), Hong Kong (Morton, 1987), Japan (Wilkins *et al.*, 1983), Hawaii (Apte *et al.*, 2000) and the Pacific coasts of North America and Canada (Wonham, 2004). *Mytilus galloprovincialis* tends to invade areas with temperate climates and large shipping ports, commonly spreading from its point of introduction and expanding its range, confirming it to be an effective and concerning invasive species (Branch & Steffani, 2004).

Figure 2.4 Image of the inside and outside of the shell of a *M. galloprovincialis* mussel

Mytilus galloprovincialis was first identified in South African waters in 1985 by a study which determined that the species must have been recently introduced (within the last 2 decades) by humans, though whether this introduction was done intentionally or inadvertently could not be discerned (Grant & Cherry, 1985). It is likely that these mussels were introduced to Southern Africa by ships sailing from European and Mediterranean waters, as mussels are known to accumulate on the hulls of ships and could have broken off or spawned in southern African waters (Grant & Cherry, 1985). The introduction of *M. galloprovincialis* into South African waters went unnoticed for some time, due to it being confused with the local mussels, *C. meridionalis* and *P. perna* (Griffiths *et al.*, 1992). By the time it was discovered it had already invaded and dominated the coastline of southern Africa from Cape Point all the way up the West coast to Lüderitz in southern Namibia (Griffiths *et al.*, 1992). This species has been found to dominate the mid- to low-intertidal zones previously occupied by the indigenous, ribbed mussel *Aulacomya ater*, at the expense of the local species (Griffiths *et al.*, 1992). It has not, however, had the same effect on most *C. meridionalis* populations as they tend to occur in subtidal areas which experience high levels of abrasion and sand cover; these sites are unfavourable conditions for *M. galloprovincialis* growth and survival (van Erkom Schurink & Griffiths, 1990; Griffiths *et al.*, 1992).

There are multiple reasons why *M. galloprovincialis* has been so successful in its invasion of South Africa. Studies have shown that the invasive mussel outcompetes local mussels like *C. meridionalis*, *A. ater* and *P. perna* in all biological aspects; the invasive mussel has a superior growth rate across a wide range of temperatures, a higher fecundity than all other mussels except *A. ater*, and a greater ability to maintain normal growth rates and low mortalities when exposed to aeration and desiccation (van Erkom Schurink & Griffiths, 1991, 1993; Griffiths *et al.*, 1992). The invasion of *M. galloprovincialis* has had very complex repercussions on the local species. The Mediterranean mussel is known to form denser beds than local mussel species, which has not only displaced some species, but has also resulted in an increase in the overall biomass of the mid and upper rocky intertidal shores (Griffiths *et al.*, 1992). An example of this complex situation comes from the invasion of this species in Saldanha Bay; local *A. ater* and limpet (*Patella granularis*) adults are out-competed for primary rock space, but the increased shelter of the denser mussel beds has resulted in the presence of invertebrate infauna being doubled, and overall limpet density in fact increases due to an increase in recruitment sites for juvenile limpets (Griffiths *et al.*, 1992).

2.5.2. Growth and reproduction

Mytilus galloprovincialis can reach a maximum size of up to 150mm and experiences growth rates of up to 40mm per annum in ideal water conditions, with the species preferring temperatures >15°C (van Erkom Schurink & Griffiths, 1993; Stanković & Jović, 2012). Despite water temperatures in Saldanha Bay (which is part of the cold Benguela Upwelling System) often falling below their ideal temperature range, the scope for growth of *M. galloprovincialis* in this area has been determined to remain high (Griffiths *et al.*, 1992; van Erkom Schurink & Griffiths, 1993). They feed on organic matter and phytoplankton on the water column, and a 50mm individual has the ability to filter up to 5litres of water per hour (Stanković & Jović, 2012). It has the highest feeding and metabolic rate of the four common mussel species in South Africa (the others being *A. ater*, *P. perna* and *C. meridionalis*), with filtration rate increasing as water temperatures increase from 10-20°C (Van Erkom Schurink & Griffiths, 1992).

Mytilus galloprovincialis gametogenic cycle in the Northern Hemisphere generally begins in early autumn (though this can vary greatly according to location), as reserves which were built up over the summer are converted to gonadal tissue (Villalba, 1995). Mass spawning occurs once or twice a year, in spring and again at a lower intensity in late summer or autumn, depending on whether environmental conditions are favourable (Villalba, 1995; Stanković & Jović, 2012). A study on the reproductive cycles of *M. galloprovincialis* in South Africa found slightly different results, with the data showing two protracted spawning seasons in both summer and winter, with spawning intensity and timing varying annually (van Erkom Schurink & Griffiths, 1991). This study also found that *M. galloprovincialis* invest vast amounts of energy in their reproductive process, and at 65mm, their dry flesh weight can vary 3-fold between ripe (pre-spawned) and spawned conditions. The spat generally settle out of the water column onto the mussel culture ropes in Saldanha from summer to autumn, and form dense beds composed of multiple layers of mussels (van Erkom Schurink & Griffiths, 1991; Griffiths *et al.*, 1992). The ability of this species to form such thick beds (unlike other South African mussel species which grow in a single layer), and survive in harsh conditions non-favourable to local mussels, has increased both the vertical extent and overall standing stock of mussel beds along the West Coast of South Africa (Griffiths *et al.*, 1992).

2.5.3. Commercial aquaculture

Mytilus galloprovincialis is cultured extensively throughout countries surrounding the Mediterranean Sea, such as Spain, Italy and Turkey, and is valued by consumers in these countries due to its competitive pricing when compared to other bivalves (oysters, etc.) and its organoleptic properties, which are retained post-processing (Orban *et al.*, 2002; Babarro *et al.*, 2003; Çelik *et al.*, 2012).

The factors which allow *M. galloprovincialis* to outcompete local species in South Africa also make this species an ideal candidate for aquaculture in the country (van Erkom Schurink & Griffiths, 1993). Currently in South Africa, the large-scale commercial harvest of wild mussels does not occur, and the only source of *M. galloprovincialis* is from the aquaculture facilities in Saldanha Bay (DAFF, 2016). The mussel aquaculture farms in Saldanha rely on spat to settle out of the water column onto the ropes, and while they cannot influence which species settle out of the water, the target species of the facilities is the invasive *M. galloprovincialis*, and in general this species makes up over 80% of the mussels harvested off the ropes (*pers. comm.* Vos Pienaar, Imbaza Mussels, 30th May 2016).

2.6. *CHOROMYTILUS MERIDIONALIS*

2.6.1. Origin and identification

Choromytilus meridionalis, otherwise known as the South African black mussel, inhabits both the intertidal and sublittoral zones of the South African coastline (van Erkom Schurink & Griffiths, 1990). It has a patchy distribution along the coastline, and tends to occur either in dense beds or very low concentrations otherwise (Griffths, 1977). The shell is normally smooth, black in colour (or sometimes a very dark brown; Figure 2.5) and as a whole is far thinner than that of other mussel species when looked at in cross-section (van Erkom Schurink & Griffiths, 1993). The females can be easily distinguished from other mussel species found in South Africa, as the gonad colour is chocolate brown, while the males gonad colour ranges from yellow to off-white (Griffiths *et al.*, 1992).

Figure 2.5 Image of the inside and outside of the shell of a *C. meridionalis* mussel

2.6.2. Growth and reproduction

Choromytilus meridionalis reaches an approximate maximum size of 90mm in the intertidal and 150mm in the sublittoral zones respectively (van Erkom Schurink & Griffiths, 1990). The growth rate of *C. meridionalis* has been found to be highly variable, depending on water temperatures, food availability and competition for space (Griffths, 1977). In optimal cool water conditions (<15°C), it can attain a growth rate of 38mm per year, and prefers areas of high water circulation rather than those of low water circulation (van Erkom Schurink & Griffiths, 1993). Of the four common mussel species in South Africa, *C. meridionalis* was found to have the second fastest feeding and metabolic rates (after *M. galloprovincialis*), and the filtration rate was found to decrease as the water temperature was increased from 10 to 20°C, confirming it to be a cold-water species (Van Erkom Schurink & Griffiths, 1992).

The spawning season of *C. meridionalis* in South Africa differs according to different scientific papers; while some state that spawning occurs throughout spring and summer (August – February) (Griffths, 1977; Griffiths, 1981b), others found that the species generally has two protracted spawning seasons in summer and winter (van Erkom Schurink & Griffiths, 1991). These studies were conducted on samples from False Bay and Bloubergstrand, using similar methodologies, which demonstrates how highly variable the spawning seasons of this species could be between different years. *Choromytilus meridionalis*' dry flesh weight can vary by up to 2.8-fold throughout a year (van Erkom Schurink & Griffiths, 1991).

Settlement of larvae occurs in sublittoral and lower littoral areas of the rocky shore, and has been found to occur at irregular intervals every 4-6years (Griffiths, 1981b). Juvenile *C. meridionalis* tend to settle at the base of large adults, or on any clear rock surface, and as they grow they have been found to push the old cohort of adults off the rock within a year of settlement, and initially this caused researchers to underestimate the maximum sizes of this species (Griffiths, 1981b). *Choromytilus meridionalis* has been found to prefer settling in areas where the rock substrate is covered in sand or silt, and in fact experiences accelerated growth rates in these areas, which are typically avoided by other mussel species in South Africa (van Erkom Schurink & Griffiths, 1993).

2.6.3. Commercial aquaculture

Saldanha Bay, South Africa is the only place in the world where *C. meridionalis* is farmed, and even here it is only farmed because the spat settles naturally out of the water column onto the ropes; the focus species of the Saldanha aquaculture facilities is rather on the invasive *M. galloprovincialis* (van Erkom Schurink & Griffiths, 1990).

According to a commercial mussel farmer in Saldanha Bay, though both species are found on the ropes, *M. galloprovincialis* is found in much higher abundance than *C. meridionalis* (in general the ratios are 80%:20% respectively), and consumers tend to prefer the invasive species over the local species (*pers. comm.* Vos Pienaar, Imbaza Mussels, 30th May 2016). This preference is possibly due to *C. meridionalis* females turning a grey-brown colour when sexually mature; apparently the taste of this brown gonad does not compare favourably with the taste of sexually mature *M. galloprovincialis* (*pers. comm.* Vos Pienaar, Imbaza Mussels, 30th May 2016), although Van Erkom Schurink and Griffiths (1990) state that both species are equally palatable, and the local species is simply not marketed due to the unusual female gonad colour.

The proximate composition of mussels can have a significant impact on their flavour profile, technological aspects and palatability (Orban *et al.*, 2002), as well as the rate of contaminant accumulation within their flesh (Bayarri *et al.*, 2001), and is therefore useful information for mussel consumers, farmers, processors and researchers alike.

2.7. PROXIMATE COMPOSITION OF MUSSELS

Mussels are known to be a highly nutritive source of proteins, vitamins, minerals, glycogen and polyunsaturated fatty acids in the human diet (Bongiorno *et al.*, 2015; Fernández *et al.*, 2015; Irisarri *et al.*, 2015). The proximate composition and meat yield of farmed mussel species is therefore important to both mussel farmers and consumers, as it can give an idea of the nutritional and commercial quality of farmed mussels, as well as their marketability and taste (Orban *et al.*, 2002; Fuentes *et al.*, 2009; Irisarri *et al.*, 2015). The proximate condition of farmed mussels has been found to change both seasonally and spatially due to fluctuations in water temperature, food availability and the gametogenic cycle causing build ups and depletions of reserves in the mussel tissue (Pieters *et al.*, 1980; Bressan & Marin, 1985; Çelik *et al.*, 2012; Martínez-Pita *et al.*, 2012; Irisarri *et al.*, 2015; Azpeitia *et al.*, 2016). Monitoring these changes in the farmed mussels of Saldanha Bay could help farmers make important decisions related to the harvest, processing and marketing of their stock, and consumers in determining the health benefits of incorporating mussels into their diets.

Due to its extensive commercial culture in many European countries, a significant amount of research has been done on the nutritional quality and seasonal changes thereof for the Mediterranean mussel, *M. galloprovincialis* (Fuentes *et al.*, 2009; Bongiorno *et al.*, 2015; Azpeitia *et al.*, 2016). Unlike the Mediterranean mussel, however, *C. meridionalis* has had little to no research done on its nutritional composition or the effect that season may have on this, as it is only found in Southern Africa and is not a targeted aquaculture species. Research is therefore necessary in order to fill such knowledge gaps, because despite these mussels not being the targeted species of aquaculture in Saldanha Bay they are still harvested in low quantities and sold to consumers.

2.7.1. Meat Yield

Meat yield gives an idea of the mussel condition, and is calculated as:

$$Meat\ yield = \frac{Wet\ meat\ weight\ (g)}{Total\ weight\ (g)} x100$$

Çelik *et al*. (2012) found that meat yield in *M. galloprovincialis* varied seasonally between 17.43-26.69%, and was influenced by both environmental factors and the reproductive cycle. Meat yield was found to decrease rapidly at the start of the spawning period, and recover quickly afterwards due to an abundant food supply in autumn and winter, and subsequent gametogenesis (Çelik *et al.*, 2012). Another study on *M. galloprovincialis* in Turkey found that meat yield varied significantly between sites, from 26 to 34% within the same season (Fuentes *et al.*, 2009). A study on the same species in Italy found that meat yield ranged between 25 to 40% both seasonally (minima in September and December) and between sites, and concluded that the differences between sites were linked to phytoplankton availability which consequently affected the gametogenic cycle of the mussels (Orban *et al.*, 2002). An Italian study on *M. galloprovincialis* in the Venice Lagoon found that flesh weight (and therefore meat yield) decreases due to spawning were so drastic that commercial mussel harvest was suspended in the post-spawn season (Bressan & Marin, 1985). A study in South Africa found that the dry flesh weights of *C. meridionalis* and *M. galloprovincialis* dropped by up to 2.8 and 3-fold, respectively, after spawning (van Erkom Schurink & Griffiths, 1991), indicating that drastically decreased flesh weights post-spawn could be a concern for local mussel farmers.

2.7.2. Protein

Numerous studies on mussels have found that protein is essential in the process of gametogenesis, or gonadal maturation, and therefore it can be expected that the protein content of mussels will fluctuate seasonally according to their reproductive cycle (Çelik *et al.*, 2012; Bongiorno *et al.*, 2015; Fernández *et al.*, 2015). Protein content in *M. galloprovincialis* from a study in Spain ranged between 7.53-11.08% of wet weight, with summer maxima and winter minima coinciding with accumulation and depletion of lipid reserves (Azpeitia *et al.*, 2016). Comparable results were found with the same species in the Adriatic Sea where protein content of the mussels varied between 7.5-11.6% wet weight, with autumn maxima and winter minima coinciding with winter spawning (Bongiorno *et al.*, 2015). Higher protein contents of ~55-75% dry weight were found in *M. galloprovincialis* samples from the Black Sea, with maxima in winter when food was abundant, and low protein in summer coinciding again with the spring spawning period (Çelik *et al.*, 2012). High protein values were also found in *M. edulis* mussels from Ireland, in which protein constituted 43.4-62.9% on an ash-free dry weight basis, with the lowest levels of protein coinciding with spawning (Fernández *et al.*, 2015). Protein content in *M. galloprovincialis* mussels from the Venice Lagoon was found to fluctuate throughout the year between 31-52% of dry flesh weight, with no identifiable seasonal trend (Bressan & Marin, 1985). The conditions of the Lagoon of Venice are unique, however; the water temperature and phytoplankton availability remain relatively stable throughout the year, which means the mussels can continually feed and replace the reserves lost to spawning.

Protein has also been found to vary between locations, as well as seasons. Protein content ranged between 42.4-56.2% wet weight in two Italian study sites, while seasonal changes in protein content were only significant in one of the sites (Orban *et al.*, 2002), and in two sites in Spain, protein content was significantly different, varying between 6.5-10% of wet weight (Fuentes *et al.*, 2009). Though these two studies do not identify why these differences occur, it is suggested that they are due to the sessile nature of mussels making them reliant on their immediate food source, therefore any differences in food availability or seston composition between sites could result in differences in protein content.

2.7.3. Lipids

Similar to protein, lipids have also been identified as essential for gametogenesis in mussels, and therefore are also expected to fluctuate with the gametogenic cycle and food availability. In Turkey, a study on *M. galloprovincialis* found that lipid content peaked in winter, at 18% of dry weight, with minimum values of 6% in summer (Çelik *et al.*, 2012). Lipids were noted to increase as gametogenesis progressed and lipid reserves were built up in the eggs, and to decrease rapidly after spawning. Equivalent results have been found by two Italian studies where the lipid content of *M. galloprovincialis* ranged from 5.8-13.4% (Orban *et al.*, 2002) and 1-2.2% wet weight (Bongiorno *et al.*, 2015). Peak lipid content in both studies occurred pre-spawn in summer, with lowest lipids coinciding with the winter spawning period and reduced phytoplankton availability. This pattern has also been confirmed in other mussel species; the lipid content in *M. edulis* in Ireland varied between 5.0-7.9% on an ash-free dry weight basis, with season having a significant effect, and the lipid content peaking in winter and spring (Fernández *et al.*, 2015). The above-mentioned studies occur over a 1-year period and therefore do not represent the inter-annual variability of the lipid content of mussels. A 3-year study on the lipid content of *M. galloprovincialis* in the Venice Lagoon showed that it peaked in early spring and summer at ~14% of dry flesh weight, and dipped to winter minima of ~2%, with consistent patterns between the study years (Bressan & Marin, 1985). The seasonal pattern was attributed to the reproductive cycle of the mussels; the accumulation of lipid reserves occurred during the mussels' summer sexual resting phase when phytoplankton was abundant, and the winter minima were due to an autumn spawning period, when these reserves were used in gametogenesis (gamete production). This study was unique in that the conditions for mussel growth are optimal throughout the year in the Lagoon of Venice, with stable, elevated water temperatures and raised nutrient availability resulting in comparatively high phytoplankton levels year-round, meaning that changes in proximate conditions could be more clearly linked to reproductive cycle, and not food availability.

Fuentes *et al*. (2009) showed that site also has a significant effect on the lipid content of *M. galloprovincialis* mussels; their study found that lipids varied from 1.4 to 2.10% wet weight between three different sites in Spain, and the researchers concluded that site affected both the size and chemical composition of the mussels.

2.7.4. Moisture & Ash

Alike to lipid and protein content, moisture and ash are also linked to food availability and the gametogenic cycle of mussels, although in a reverse manner. While lipids and proteins peak with high food availability and as reserves are built up pre-spawn, ash and moisture contents tend to peak in the mussels during their spent (or post-spawn) stage, when there is less of a dilution factor.

Moisture content in *M. galloprovincialis* has been found to vary between 79-83.8% in Spain (Fuentes *et al.*, 2009), 78.7-87.3% in the Adriatic Sea (Bongiorno *et al.*, 2015), 78-86% of total weight on the Turkish coastline (Çelik *et al.*, 2012) and 83.3-89% in the Bay of Biscay (Azpeitia *et al.*, 2016). Bongiorno *et al*. (2015) noted that the minimum moisture values in spring coincided with the buildup of reserves and gametogenesis, and that moisture values were highest post-spawn, once mussels were in their spent stage.

Ash content in *M. galloprovincialis* in Italy was found to fluctuate between 11-21% dry weight (Orban *et al.*, 2002) and 3.3% wet weight throughout the year (Bongiorno *et al.*, 2015), with peak ash content in both studies coinciding with minimum lipid contents which occurred post-spawn. Another study on Spanish *M. galloprovincialis* mussels noted that ash content ranged between 2.4-4.2% of total weight, and were at a maximum during winter, when the condition of mussels was at its lowest (Azpeitia *et al.*, 2016). This study also recommended that mussels not be harvested during this season, due to them being in such poor condition. In contradiction to these studies, ash content in *M. edulis* samples from Wales ranged from 8.8-17.2% dry weight, with peak values in spring coinciding with peak protein and minimum values found post-spawn (Dare & Edwards, 1975). Significant differences in ash content of *M. galloprovincialis* were also found between two study sites, with values ranging between 2.2-3.38% (Fuentes *et al.*, 2009).

2.8. CONTAMINANTS IN MUSSELS

Being filter feeders, mussels have the potential to accumulate and concentrate trace elements and other contaminants found in their diets and the aquatic environment (Bongiorno *et al.*, 2015). Ocean currents and the constant movement of water in marine environments makes it very difficult to monitor pollution levels through sediments or analyses of the water itself; studying the accumulation of polluting substances in bivalves may give a better idea of pollution levels in marine environments and can also give researchers an idea of the bioavailable fraction of environmental pollutants (Baumard *et al.*, 1998). The ability of mussels to accumulate and concentrate toxic metals such as lead, mercury, cadmium and arsenic, as well as persistent organic pollutants such as organochlorine pesticides and compounds, in the water column can, unfortunately, also result in them posing a serious health threat to human consumers (Baumard *et al.*, 1998; Bayarri *et al.*, 2001; Orescanin *et al.*, 2006; Stanković & Jović, 2012). Concerns have been raised on the water pollution levels in Saldanha Bay due to both the heavy industries which surround the bay and anthropogenic pollution sources (Clark *et al.*, 2014), and it is therefore important to assess the levels of potential contaminants in the farmed mussels in order to ensure they do not pose health risks to consumers. Assessing current pollution levels in the farmed mussels can also form baseline data for future studies on the health of the aquaculture facilities in Saldanha Bay, as planned expansion of the industry and ever-expanding human populations pose even more dire future pollution concerns.

2.8.1. Trace metals

It is well known that marine invertebrates accumulate both essential and toxic metals into their flesh, and that this poses health risks to the humans who consume them, as well as to the animals themselves (Rainbow, 2007; Stanković & Jović, 2012). Due to this ability, the United States Mussel Watch Program was initiated in 1976, which aimed to improve pollution monitoring techniques using mussels as biomonitors (Goldberg *et al.*, 1983). Since then, mussels have been used worldwide as biomonitors to indicate levels of marine pollution (Orescanin *et al.*, 2006; Przytarska *et al.*, 2010; Brooks *et al.*, 2015), as well as being assessed for the potential deleterious impacts of their accumulated contaminants on human consumers (Claisse *et al.*, 2001; Stanković *et al.*, 2011; Spada *et al.*, 2013; Maar *et al.*, 2015). Due to this wide-ranging body of research, numerous studies have investigated the effects of species, depuration, size (or age), season and sex on the levels of trace metal contamination or bioaccumulation in bivalves in order to improve the accuracy of using mussels as biomonitors and to more effectively protect human consumers, and their findings are discussed below.

2.8.1.1. Species

When comparing two mussel species from the same areas along the Namibian coastline, it was found the bioaccumulation of certain heavy metals differed between *P. perna* and *C. meridionalis*, with levels of cadmium, chromium and zinc being significantly higher in the former (Dahms *et al.*, 2014). Similarly, when trace metal concentrations were tested in two Spanish clams from the same location, *Donax trunculus* and *Chamelea gallina*, it was found that concentrations of mercury, arsenic, zinc, lead, cadmium and chromium were significantly higher in *D. trunculus* (Usero *et al.*, 2005).

2.8.1.2. Depuration

Certain studies using mussels as bioindicators will depurate their live mussels in seawater for approximately 24 hours before they are processed so that the contents of the mussels' stomach, which have not yet been assimilated into the flesh, do not influence the overall concentration of the metals within their samples (Usero *et al.*, 2005; Przytarska *et al.*, 2010; Sparks *et al.*, 2014). A study on the effect of depuration on *M. edulis* found that depuration caused a reduction in manganese concentrations, but also resulted in an unexplained increase in copper and nickel concentrations within the samples (Latouche & Mix, 1982). A potential explanation for this effect was that the depuration process caused increased stress levels within the mussels, which resulted in the expenditure of energy and affected the overall concentrations of certain trace metals. Latouche and Mix (1982) therefore suggested that, if researchers would like to exclude the gut contents of mussels in their study, they should rather dissect away the gut, as depuration can result in unexpected changes in metal concentrations. In human exposure studies, however, the gut is included in analyses as mussels are generally cooked and eaten whole (Stanković *et al.*, 2011; Spada *et al.*, 2013; Maar *et al.*, 2015).

2.8.1.3. Size

One study found that cadmium concentrations within the bodies of sexually mature *M. edulis* were significantly lower and varied considerably more than those of sexually immature mussels, and their findings were attributed to the biochemical changes that occur within adult mussels' sexual cycles (Cossa *et al.*, 1979). Comparable results were found with cadmium, lead and zinc concentrations in *P. viridis* and this reduction in contamination levels with increasing size was attributed to larger mussels possessing a lower filtration rate per unit bodyweight, and therefore their uptake of trace metals was decreased per unit bodyweight (Yap *et al.*, 2009). Another study found that while trace metal concentrations did decrease with increasing mussel dry weight, smaller individuals (<55m) had much higher levels of inter-individual variability than larger ones (Richir & Gobert, 2014), contradicting the results of Cossa *et al*. (1979). Therefore, while some studies suggest that marine pollution monitoring studies should use exclusively sexually immature mussels to increase accuracy (Cossa *et al.*, 1979), others recommend that future studies should rather focus on using animals of a similar size (Richir & Gobert, 2014). When investigating human exposure to trace metals through mussel consumption, however, the importance falls rather on assessing the contamination levels within the range of harvestable size (or edible size) mussels that consumers would be eating (Irisarri *et al.*, 2015).

2.8.1.4. Season

Seasonal changes in the composition of mussel tissues are mainly due to their sexual cycle and gametogenesis, though food availability also plays a role (Çelik *et al.*, 2012; Bongiorno *et al.*, 2015; Fernández *et al.*, 2015). Gametogenesis is linked closely to seasonal cycles, as annual changes in food availability and photoperiod often determine the onset and intensity of their reproductive period (Gosling, 2004). Certain studies suggest that the annual reproductive cycle of mussels does not influence trace metal concentrations between different body compartments, but that massive increases in total flesh weight due to gamete build-up could potentially cause an overall dilution of trace metals in the mussel's tissue (Yap *et al.*, 2009; Richir & Gobert, 2014). In a review of historical Cape Town Mussel Watch Programme data from 1985-2008 it was found that the concentrations of all tested metals (cadmium, copper, lead, zinc, mercury, iron and manganese) in *M. galloprovincialis* fluctuated significantly between different seasons and years (Sparks *et al.*, 2014). The fluctuations observed in this study were linked to changes in metal concentrations in coastal waters rather than gametogenesis. Other studies state that manganese, magnesium, nickel, iron and copper showed significantly higher concentrations in the tissue of *M. galloprovincialis* during late winter and spring, just before spawning occurred (Orescanin *et al.*, 2006; Bongiorno *et al.*, 2015), suggesting a link between metal concentrations and annual gametogenesis.

Seasonal changes in water quality linked to the Benguela Upwelling System, rainwater input and water temperature could also potentially impact the metal concentrations in the water column and therefore the farmed mussels in Saldanha Bay (Orren *et al.*, 1980; Lares *et al.*, 2002). Prior studies on upwelling systems have found that concentrations of cadmium and iron are significantly higher in the water column during upwelling events, and that aluminium concentrations in the water are linked to precipitation events (Lares *et al.*, 2002; Ruttenberg & Dyhrman, 2005; Nakayama *et al.*, 2010). Seasonal variations in the trace metal content of *C. meridionalis* mussels were suggested to be linked to changes in water temperature, as increased water temperatures caused increased metabolic activity and therefore raised metal excretion rates (Orren *et al.*, 1980). A study on the uptake of copper, cadmium, chromium, lead and zinc concentrations in *P. perna* mussels from harbours around the South African coastline (Saldanha Bay, Cape Town, Port Elizabeth and Richards Bay) found considerable seasonal variations in metal concentrations for all sites (Degger *et al.*, 2011b). The study associated these seasonal changes with a number of drivers including upwelling events, urban run-off (linked to precipitation) and dredging of the harbours (resulting in re-suspension of particulate matter into the water).

2.8.1.5. Sex

The concentrations of certain trace metals in mussels have been found to differ between the sexes. In *C. meridionalis*, the concentration of metals in mature females was found to be approximately twice that of mature males (Watling & Watling, 1976; Orren *et al.*, 1980) and in *M. galloprovincialis*, 13 of 19 trace elements analysed showed significantly higher concentrations in female mussels than in males (Richir & Gobert, 2014). Similarly, in *M. edulis*, manganese, cadmium and zinc were found to occur in higher concentrations in female mussels than males (Latouche & Mix, 1982), though none of the abovementioned studies were able to clearly explain the cause of these sexual differences.

2.8.2. Persistent organic pollutants

Persistent organic pollutants (POPs) are chemical compounds produced as intentional products or unintentional by-products of human industry, which do not easily degrade through natural processes and are therefore persistent in the natural environment (Tesar, 2000). They are of particular concern as they can cause a variety of negative side effects in animals (specifically humans), resulting in allergies, reproductive disorders, birth defects, disruption of the immune and nervous systems, cancer and mortalities (Tesar, 2000). There are a wide range of POPs that pose a threat to human health and these include organophosphate pesticides or insecticides (OPPs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), each of which are produced by diverse industries and have differing toxicities. All of the abovementioned POPs will be discussed in further detail below.

2.8.2.1. Organophosphate Pesticides (OPPs)

Organophosphate pesticides are used in homes, gardens, veterinary practices and agriculture, and prior to the 21st century they were the most widely used and available pesticides (Roberts & Reigart, 2013). Organophosphates cause mortality in insects (as well as birds, amphibians and mammals) by phosphorylation of the acetylcholinesterase enzyme at nerve endings, thereby disrupting the nervous system (Roberts & Reigart, 2013), and have been infamously used in chemical warfare under the name “sarin gas”. The use of OPPs has decreased in recent years due to their acute toxicity to humans; if inhaled, ingested or exposed to the skin they can result in headaches, nausea, diarrhea, tachy- and bradycardia, respiratory arrest and seizures, and certain OPPs have the ability to be stored in fat, causing delayed toxicity (Roberts & Reigart, 2013).

2.8.2.2. Organochlorine Pesticides (OCPs)

Organochlorine pesticides include insecticides such as dichlorodiphenyltrichloroethane (DDT), aldrin, dieldrin, mirex, toxaphene, chlordane and hexachlorobenzene (HCB). Due to their high toxicity and fat solubility the majority of OCPs have been banned in America and other first-world countries (Roberts & Reigart, 2013). DDT is the most infamous of the OCPs; it has been used worldwide to protect humans from insect-borne diseases like malaria, as well as being used as an agricultural insecticide (Tesar, 2000). It is a highly persistent pollutant; up to 50% can remain in soil after 15 years, and DDT residues can still be detected in food today, even though many countries banned it in the 1970’s after it was found to cause eggshell-thinning in birds of prey (Tesar, 2000). Aldrin, dieldrin, endrin, mirex, toxaphene and chlordane are OCPs which were used to control termites and other agricultural pests, and are all regarded as highly toxic to birds, frogs, fish and humans (Tesar, 2000). The ingestion of food items is considered to be the main pathway for the aforementioned OCPs to impact humans and other organisms (Tesar, 2000). HCB was initially used as a fungicide in food crops, but is also a by-product of the manufacture of certain industrial chemicals, and grain treated with this chemical has been linked to serious adverse health effects in humans (Tesar, 2000). These OCPs are all included on the “Dirty Dozen” list, which consists of 12 POPs which the United Nations Environment Programme (UNEP) has targeted for elimination, and have therefore largely been banned worldwide (Tesar, 2000). DDT is also on this list, but unlike the other POPs it has been targeted for restricted use rather than outright elimination, as certain malaria-stricken areas would not be able to effectively control outbreaks of the disease without it. DDT is still in use in the KwaZulu Natal, Limpopo and Mpumalanga provinces of South Africa, as it is one of the countries which has been given an exemption for the continued use of this product in malaria vector control to minimize human suffering (Schlenk *et al.*, 2005; DEA, 2012).

Dichlorodiphenyldichloroethylene (DDE), a metabolite of DDT, was found to be more prevalent in higher trophic level marine species like mackerel (*Scomber scombrus*), which possess higher fat contents, when compared to filter feeders at the bottom of the food chain, e.g. *M. galloprovincialis* mussels (Bayarri *et al.*, 2001). In addition, an increase in OCPs (HCB, DDT and DDE) in marine waters and species has been directly related to increasing human population densities and industry, indicating the unequivocal effect of human settlements on OCP prevalence and concentrations (Bayarri *et al.*, 2001; Kljaković-Gašpić *et al.*, 2010).

2.8.2.3. Polyaromatic Hydrocarbons (PAHs)

Polyaromatic hydrocarbons can be formed either naturally, through seeping of petroleum, forest fires and volcanism, or through industrial processes like the burning of fossil fuels in power plants and vehicles (Perugini *et al.*, 2007), and are known to be highly toxic, carcinogenic and mutagenic (Maliszewska-Kordybach, 1999). Once released into marine environments their low water solubility results in rapid absorption into suspended particles and sediments, where they become bioavailable to marine organisms at different concentrations depending on their trophic level, and persist in the tissues due to their lipophilic nature (Baumard *et al.*, 1998; Maliszewska-Kordybach, 1999; Perugini *et al.*, 2007).

Due to concerns over their toxicity, and their inclusion in the list of pollutants to monitor in the worldwide Mussel Watch Programme, which began in the 1970's, PAHs in marine environments have been studied relatively extensively worldwide (Goldberg, 1975; Goldberg *et al.*, 1983). The concentrations of PAHs in marine species have been found to be affected by trophic level, habitat and feeding behavior, with organisms living in the sediment having much higher exposure to PAH contamination than those living in the water column (Baumard *et al.*, 1998). A European study found that *M. galloprovincialis* mussels from France and Spain accumulated more, heavier molecular weight PAHs than fish species, due to fish possessing a higher capacity for biotransformation and elimination of PAHs (Baumard *et al.*, 1998). Contrary to these results, overall PAH contamination levels in *M. galloprovincialis* and other invertebrates from the Adriatic Sea were on average three times lower than those in the studied vertebrates (Perugini *et al.*, 2007). The notable difference between these studies was the collection site of the mussels; in France and Spain the mussels were collected wild from the rocky shore, while in the Adriatic Sea (Italy) they were collected from the rafts of a mussel farming operation (Baumard *et al.*, 1998; Perugini *et al.*, 2007). The differences between the contamination levels of *M. galloprovincialis* in the two studies could therefore be explained by the statement by Baumard *et al*. (1998) that organisms living closer to the sediment would have higher levels of exposure to PAHs; the rope-grown mussels would therefore be likely to have lower contamination levels as they have less contact with sediment than wild mussels growing on rocks near the ocean floor.

Polyaromatic hydrocarbon contamination levels in Brazilian *P. perna* mussels were found to increase with increasing human population densities and anthropogenic input (Yoshimine *et al.*, 2012), confirming once again that humans have a direct influence on PAH contamination levels. In South Africa, a PAH contamination study of *P. perna* showed that these pollutants are widespread along the coastline, and identified Saldanha Bay as the most contaminated of all studied harbours, including larger ports like Cape Town and Port Elizabeth (Degger *et al.*, 2011a). Despite the levels of PAHs in South African waters being of concern due to diminished marine monitoring programmes (Degger *et al.*, 2011a), they were found to be orders of magnitude lower than contamination levels found in highly industrialised and urbanized places like Hong Kong.

2.8.2.4. Polychlorinated Biphenyls

Polychlorinated biphenyls are produced for use as heat-exchange fluids in capacitors and electric transformers, as well as additives in paint and plastics, and are listed as one of the "Dirty Dozen" (Tesar, 2000). PCBs can have half-lives of up to 10 years, are known to cause reproductive disruptions and mortalities in fish, and have been identified as potential carcinogens in humans, where exposure to these pollutants comes largely through the diet (Tesar, 2000). Studies on PCB contamination levels in Europe have found that concentrations of these pollutants increase with increasing human population density, but that the contamination levels in *M. galloprovincialis* are currently so low that they do not currently pose a threat to consumers (Licata *et al.*, 2004; Kljaković-Gašpić *et al.*, 2010). In South African waters, PCB levels were found to be highest in Cape Town harbour, though the mussels from Saldanha Bay had the highest variety of PCBs (Degger *et al.*, 2011a).

2.9. CONCLUSION

Mussel aquaculture is a thriving industry worldwide, and in South Africa it has been growing steadily into the largest marine aquaculture subsector, by weight. The industry has the potential to at least triple in size in Saldanha Bay, and research into the meat quality of the mussels will help farmers make decisions on potential expansion and harvest procedures and will help consumers by informing them of the nutritional value of mussels as a food source. Meat yield and proximate analyses give an idea of the nutritional quality and value of the mussels, and monitoring seasonal changes in these parameters could be useful to consumers and producers alike. Due to Saldanha Bay being the only sheltered embayment in South Africa where mussel aquaculture can occur, the health of the waters in the bay are essential to the health of the mussels grown there, but water quality in the bay is negatively affected by numerous industrial and anthropogenic activities in the area. Since mussels are established bio-accumulators of a wide range of environmental pollutants, regular monitoring of the concentrations of contaminants in the mussels themselves needs to occur in order to prevent consumers from the potential health risks of eating contaminated seafood. Monitoring the levels of contamination from trace metals and persistent organic pollutants is essential to determining whether the farmed mussels pose a health risk to consumers. The above review also demonstrates that, while *M. galloprovincialis* has had an extensive amount of research done on its proximate condition, metal contamination and persistent organic pollutant accumulation levels, there is a dearth of information in these same fields for *C. meridionalis*. Baseline studies such as this one are essential to the aquaculture industry as they expand knowledge and understanding of the farmed mussels in Saldanha Bay, aid in assessing whether mussels are safe and healthy seafood sources for human consumers, and may help protect the aquaculture industry from the future expansion of industrial and other anthropogenic activities and the potential pollutants these release into the bay.

2.10. REFERENCES

Apte, S., Holland, B. S., Godwin, L. S., & Gardner, J. P. A. 2000. Jumping ship: a stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment. Biol. Invasions 2, 75–79 https://doi.org/10.1023/A.

Azpeitia, K., Ferrer, L., Revilla, M., Pagaldai, J., & Mendiola, D. 2016. Growth, biochemical profile, and fatty acid composition of mussel (*Mytilus galloprovincialis* Lmk.) cultured in the open ocean of the Bay of Biscay (northern Spain). Aquaculture 454, 95–108 https://doi.org/10.1016/j.aquaculture.2015.12.022.

Babarro, M. F., Labarta, U., & Jose, M. 2003. Growth patterns in biomass and size structure of *Mytilus galloprovincialis* cultivated in the Ria de Arousa (north-west Spain). J. Mar. Biol. Assoc. UK 83, 151–158 https://doi.org/10.1017/S002531540300691Xh.

Baumard, P., Budzinski, H., Garrigues, P., Sorbe, J. C., Burgeot, T., & Bellocq, J. 1998. Concentrations of PHAs (Polycyclic Aromatic Hydrocarbons) in various marine organisms in relation to those in sediments and to trophic level. Mar. Pollut. Bull. 36, 951–960 https://doi.org/10.1016/S0025-326X(98)00088-5.

Bayarri, S., Baldassarri, L. T., Iacovella, N., Ferrara, F., & Domenico, A. Di. 2001. PCDDs, PCDFs, PCBs and DDE in edible marine species from the Adriatic Sea. Chemosphere 43, 601–610 https://doi.org/10.1016/S0045-6535(00)00412-4.

Bongiorno, T., Iacumin, L., Tubaro, F., Marcuzzo, E., Sensidoni, A., & Tulli, F. 2015. Seasonal changes in technological and nutritional quality of *Mytilus galloprovincialis* from suspended culture in the Gulf of Trieste (North Adriatic Sea). Food Chem. 173, 355–362 https://doi.org/10.1016/j.foodchem.2014.10.029.

Branch, G. M., & Steffani, C. N. 2004. Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). J. Exp. Mar. Bio. Ecol. 300, 189–215 https://doi.org/10.1016/j.jembe.2003.12.007.

Bressan, M., & Marin, M. G. 1985. Seasonal variations in biochemical composition and condition index of cultured mussels (*Mytilus galloprovincialis* Lmk) in the Lagoon of Venice (North Adriatic). Aquaculture 48, 13–21 https://doi.org/10.1016/0044-8486(85)90048-1.

Brooks, S. J., Harman, C., Hultman, M. T., & Berge, J. A. 2015. Integrated biomarker assessment of the effects of tailing discharges from an iron ore mine using blue mussels (*Mytilus* spp.). Sci. Total Environ. 524–525, 104–114 https://doi.org/10.1016/j.scitotenv.2015.03.135.

Brummett, R. E., Lazard, J., & Moehl, J. 2008. African aquaculture: Realizing the potential. Food Policy 33, 371–385 https://doi.org/10.1016/j.foodpol.2008.01.005.

Brummett, R. E., & Williams, M. J. 2000. The evolution of aquaculture in African rural and economic development. Ecol. Econ. 33, 193–203.

Çelik, M. Y., Karayücel, S., Karayücel, I., Öztürk, R., & Eyüboğlu, B. 2012. Meat yield, condition index, and biochemical composition of mussels (*Mytilus galloprovincialis* Lamarck, 1819) in Sinop, south of the Black Sea. J. Aquat. Food Prod. Technol. 21, 198–205 https://doi.org/10.1080/10498850.2011.589099.

Claisse, D., Cossa, D., Bretaudeau-Sanjuan, J., Touchard, G., & Bombled, B. 2001. Methylmercury in molluscs along the French Coast. Mar. Pollut. Bull. 42, 329–332 https://doi.org/10.1016/S0025-326X(01)00036-4.

Clark, B., Laird, M., Hutchings, K., Liebau, V., Biccard, A., Turpie, J., & Parker-Mallick, N. 2014. The state of Saldanha Bay and Langebaan Lagoon 2013/2014. Technical Report. Report no. 1581/1 prepared by Anchor Environmental Consultants for the Saldanha Bay Water Quality Trust.

Clark, B. M., Massie, V., Hutchings, K., Laird, M., Biccard, A., Brown, E., Duna, O. O., & Turpie, J. 2016. The State of Saldanha Bay and Langebaan Lagoon 2016. Technical Report. Report No AEC 1691/1 prepared by Anchor Environmental Consultants (Pty) Ltd for the Saldanha Bay Water Quality Forum Trust.

Clark, B. M., Lane, S., Turpie, J. ., van Niekerk, L., & Morant, P. D. 2002. South Africa National Report Phase 1: Integrated Problem Analysis. Report prepared for GEF MSP Sub-Saharan Africa Project (GF/6010-0016), Cape Town.

Cossa, D., Bourget, E., & Piuze, J. 1979. Sexual maturation as a source of variation in the relationship between cadmium concentration and body weight of *Mytilus edulis* L. Mar. Pollut. Bull. 10, 174–176 https://doi.org/10.1016/0025-326X(79)90425-9.

DAFF. 2014. Aquaculture Yearbook 2014. The Department of Agriculture, Forestry and Fisheries, Cape Town.

DAFF. 2016. Aquaculture Yearbook 2015. The Department of Agriculture, Forestry and Fisheries, Cape Town.

Dahms, S., van der Bank, F. H., & Greenfield, R. 2014. A baseline study of metal contamination along the Namibian coastline for *Perna perna* and *Choromytilus meridionalis*. Mar. Pollut. Bull. 85, 297–305 https://doi.org/10.1016/j.marpolbul.2014.05.037.

Dare, P. J., & Edwards, D. B. 1975. Seasonal Changes in Flesh Weight and Biochemical Composition of Mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales. J. Exp. Mar. Bio. Ecol. 18, 89–97.

DEA. 2012. National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants. Department of Environmental Affairs, South Africa.

Degger, N., Wepener, V., Richardson, B. J., & Wu, R. S. S. 2011a. Application of artificial mussels (AMs) under South African marine conditions: A validation study. Mar. Pollut. Bull. 63, 108–118 https://doi.org/10.1016/j.marpolbul.2011.04.040.

Degger, N., Wepener, V., Richardson, B. J., & Wu, R. S. S. 2011b. Brown mussels (*Perna perna*) and semi-permeable membrane devices (SPMDs) as indicators of organic pollutants in the South African marine environment. Mar. Pollut. Bull. 63, 91–97 https://doi.org/10.1016/j.marpolbul.2011.04.024.

FAO. 2014a. The state of world fisheries and aquaculture. Food and Agriculture Organisation of the United Nations, Rome.

FAO. 2014b. Value Statistics Database Updated to 2012. Food and Agriculture Organisation of the United Nations, Rome.

FAO. 2016. The state of world fisheries and aquaculture. Food and Agriculture Organisation of the United Nations, Rome.

Fernández, A., Grienke, U., Soler-Vila, A., Guihéneuf, F., Stengel, D. B., & Tasdemir, D. 2015. Seasonal and geographical variations in the biochemical composition of the blue mussel (*Mytilus edulis* L.) from Ireland. Food Chem. 177, 43–52 https://doi.org/10.1016/j.foodchem.2014.12.062.

Fuentes, A., Fernández-Segovia, I., Escriche, I., & Serra, J. A. 2009. Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins. Food Chem. 112, 295–302 https://doi.org/10.1016/j.foodchem.2008.05.064.

GISD. 2015. Global Invasive Species Database. http:/www.iucngisd.org/gisd/species.php?sc=102#. Date accessed: 12th July 2017.

Goldberg, E. D. 1975. The mussel watch - A first step in global marine monitoring. Mar. Pollut. Bull. 6, 111 https://doi.org/10.1016/0025-326X(75)90271-4.

Goldberg, E. D., Koide, M., Hodge, V., Flegal, A. R., & Martin, J. 1983. U.S. Mussel Watch: 1977-1978 results on trace metals and radionuclides. Estuar. Coast. Shelf Sci. 16, 69–93 https://doi.org/10.1016/0272-7714(83)90095-1.

Gosling, E. M. 2004. Bivalve molluscs. First Edit. Fishing News Books, a division of Blackwell Publishing, Cornwall.

Grant, W. S., & Cherry, M. I. 1985. *Mytilus galloprovincialis* Lmk. in Southern Africa. J. Exp. Mar. Bio. Ecol. 90, 179–191.

Griffiths, R. J. 1980a. Natural Food Availability and Assimilation in the Bivalve *Choromytilus meridionalis*. Mar. Ecol. Prog. Ser. 3, 151–156.

Griffiths, R. J. 1980b. Ecophysiology of the Black Mussel *Choromytilus meridionalis* (Krauss). PhD, University of Cape Town, Cape Town.

Griffiths, R. J. 1981. Population Dynamics and Growth of the Bivalve *Choromytilus meridionalis* (Kr.) at Different Tidal Levels. Estuar. Coast. Shelf Sci. 12, 101–118 https://doi.org/10.1016/S0302-3524(81)80120-X.

Griffiths, R. J., & Buffenstein, R. 1981. Aerial Exposure and Energy Input in the Bivalve *Choromytilus meridionalis*. J. Exp. Mar. Bio. Ecol. 52, 219–229.

Griffiths, C. L., Hockey, P. A. R., Van Erkom Schurink, C., & Le Roux, P. J. 1992. Marine invasive aliens on South African shores: implications for community structure and trophic functioning. South African J. Mar. Sci. 12, 713–722 https://doi.org/10.2989/02577619209504736.

Griffths, R. J. 1977. Reproductive Cycles in Littoral Populations of *Choromytilus meridionalis* (Kr.) and *Aulacomya ater* (Molina) with a Quantitative Assessment of Gamete Production in the Former. J. Exp. Mar. Bio. Ecol. 30, 53–71.

Hecht, T., & Britz, P. J. 1992. The current status, future prospects and environmental implications of mariculture in South Africa. Suid-Afrikaanse Tydskr. vir Wet. 88, 335–342.

Irisarri, J., Fernández-Reiriz, M. J., & Labarta, U. 2015. Temporal and spatial variations in proximate composition and Condition Index of mussels *Mytilus galloprovincialis* cultured in suspension in a shellfish farm. Aquaculture 435, 207–216 https://doi.org/10.1016/j.aquaculture.2014.09.041.

Jović, M., & Stanković, S. 2014. Human exposure to trace metals and possible public health risks via consumption of mussels *Mytilus galloprovincialis* from the Adriatic coastal area. Food Chem. Toxicol. 70, 241–251 https://doi.org/10.1016/j.fct.2014.05.012.

Kampire, E., Rubidge, G., & Adams, J. B. 2015. Distribution of polychlorinated biphenyl residues in sediments and blue mussels (*Mytilus galloprovincialis*) from Port Elizabeth Harbour, South Africa. Mar. Pollut. Bull. 91, 173–179 https://doi.org/10.1016/j.marpolbul.2014.12.008.

Kljaković-Gašpić, Z., Herceg-Romanić, S., Kožul, D., & Veža, J. 2010. Biomonitoring of organochlorine compounds and trace metals along the Eastern Adriatic coast (Croatia) using *Mytilus galloprovincialis*. Mar. Pollut. Bull. 60, 1879–1889 https://doi.org/10.1016/j.marpolbul.2010.07.019.

Lares, M. L., Flores, M. G., & Lara-Lara, R. 2002. Temporal variability of bioavailable Cd, Hg, Zn, Mn and Al in an upwelling regime. Environ. Pollut. 120, 595–608.

Latouche, D. Y., & Mix, M. C. 1982. The Effects of Depuration, Size and Sex on Trace Metal Levels in Bay Mussels. Mar. Pollut. Bull. 13, 27–29.

Licata, P., Trombetta, D., Cristani, M., Martino, D., & Naccari, F. 2004. Organochlorine compounds and heavy metals in the soft tissue of the mussel *Mytilus galloprovincialis* collected from Lake Faro (Sicily, Italy). Environ. Int. 30, 805–810 https://doi.org/10.1016/j.envint.2004.01.007.

Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. 2000. 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission SSC of the World Conservation Union (IUCN), 12pp.

Maar, M., Larsen, M. M., Tørring, D., & Petersen, J. K. 2015. Bioaccumulation of metals (Cd, Cu, Ni, Pb and Zn) in suspended cultures of blue mussels exposed to different environmental conditions. Estuar. Coast. Shelf Sci., 1–13 https://doi.org/10.1016/j.ecss.2015.10.010.

Maliszewska-Kordybach, B. 1999. Sources, Concentrations, Fate and Effects of Polycyclic Aromatic Hydrocarbons (PAHs) in the Environment. Part A: PAHs in Air. Polish J. Environ. Stud. 8, 131–136.

Martínez-Pita, I., Sánchez-Lazo, C., Ruíz-Jarabo, I., Herrera, M., & Mancera, J. M. 2012. Biochemical composition, lipid classes, fatty acids and sexual hormones in the mussel *Mytilus galloprovincialis* from cultivated populations in south Spain. Aquaculture 358–359, 274–283 https://doi.org/10.1016/j.aquaculture.2012.06.003.

McDonald, J. H., Seed, R., & Koehn, R. K. 1991. Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Mar. Biol. 111, 323–333.

Morton, B. 1987. Recent Marine Introductions into Hong Kong. Bull. Mar. Sci. 41, 503–513.

Nakayama, Y., Kuma, K., Fujita, S., Sugie, K., & Ikeda, T. 2010. Temporal variability and bioavailability of iron and other nutrients during the spring phytoplankton bloom in the Oyashio region. Deep. Res. Part II Top. Stud. Oceanogr. 57, 1618–1629 https://doi.org/10.1016/j.dsr2.2010.03.006.

Olivier, D., Heinecken, L., & Jackson, S. 2013. Mussel and oyster culture in Saldanha Bay, South Africa: Potential for sustainable growth, development and employment creation. Food Secur. 5, 251–267 https://doi.org/10.1007/s12571-013-0244-1.

Orban, E., Di Lena, G., Nevigato, T., Casini, I., Marzetti, A., & Caproni, R. 2002. Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites. Food Chem. 77, 57–65 https://doi.org/10.1016/S0308-8146(01)00322-3.

Orescanin, V., Lovrencic, I., Mikelic, L., Barisic, D., Matasin, Z., Lulic, S., & Pezelj, D. 2006. Biomonitoring of heavy metals and arsenic on the east coast of the Middle Adriatic Sea using *Mytilus galloprovincialis*. Nucl. Instruments Methods Phys. Res. Sect. B Beam Interact. with Mater. Atoms 245, 495–500 https://doi.org/10.1016/j.nimb.2005.11.050.

Orren, M. J., Eagle, G. A., Hennig, H. F. K. O., & Green, A. 1980. Variations in Trace Metal Content of the Mussel *Choromytilus meridionalis* (Kr.) with Season and Sex. Top. Catal. 11, 253–257 https://doi.org/10.1016/0025-326X(80)90313-6.

Pavlov, D. F., Bezuidenhout, J., Frontasyeva, M. V, & Goryainova, Z. I. 2015. Differences in Trace Element Content between Non-Indigenous Farmed and Invasive Bivalve Mollusks of the South African Coast. Am. J. Anal. Chem. 6, 886–897 https://doi.org/10.4236/ajac.2015.611084.

Perugini, M., Visciano, P., Giammarino, A., Manera, M., Di Nardo, W., & Amorena, M. 2007. Polycyclic aromatic hydrocarbons in marine organisms from the Adriatic Sea, Italy. Chemosphere 66, 1904–1910 https://doi.org/10.1016/j.chemosphere.2006.07.079.

Pieters, H., Kluytmans, J. H., Zandee, D. I., & Cadée, G. C. 1980. Tissue Composition and Reproduction of *Mytilus edulis* in Relation to Food Availability. Netherlands J. Sea Res. 14, 349–361.

Przytarska, J. E., Sokołowski, A., Wołowicz, M., Hummel, H., & Jansen, J. 2010. Comparison of trace metal bioavailabilities in European coastal waters using mussels from *Mytilus edulis* complex as biomonitors. Environ. Monit. Assess. 166, 461–476 https://doi.org/10.1007/s10661-009-1015-5.

Rainbow, P. S. 2007. Trace metal bioaccumulation: Models, metabolic availability and toxicity. Environ. Int. 33, 576–582 https://doi.org/10.1016/j.envint.2006.05.007.

Richir, J., & Gobert, S. 2014. The effect of size, weight, body compartment, sex and reproductive status on the bioaccumulation of 19 trace elements in rope-grown *Mytilus galloprovincialis*. Ecol. Indic. 36, 33–47 https://doi.org/10.1016/j.ecolind.2013.06.021.

Roberts, J. R., & Reigart, J. R. 2013. Recognition and Management of Pesticide Poisonings. Sixth Edit. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington.

Ruttenberg, K. C., & Dyhrman, S. T. 2005. Temporal and spatial variability of dissolved organic and inorganic phosphorus, and metrics of phosphorus bioavailability in an upwelling-dominated coastal system. J. Geophys. Res. 110, 1–22 https://doi.org/10.1029/2004JC002837.

Schlenk, D., Sapozhnikova, Y., & Cliff, G. 2005. Incidence of organochlorine pesticides in muscle and liver tissues of South African great white sharks *Carcharodon carcharias*. Mar. Pollut. Bull. 50, 208–211 https://doi.org/10.1016/j.marpolbul.2004.11.032.

Shipton, T., & Britz, P. J. 2007. A study on the status of aquaculture production and trade in South Africa. Volume 1: Industry Status and Diagnostic Report. A report for the Department of Trade and Industry produced by Enviro-Fish Africa (Pty.) Ltd. 90pp.

Spada, L., Annicchiarico, C., Cardellicchio, N., Giandomenico, S., & Di Leo, A. 2013. Heavy metals monitoring in mussels *Mytilus galloprovincialis* from the Apulian coasts (Southern Italy). Mediterr. Mar. Sci. 14, 99–108 https://doi.org/10.12681/mms.323.

Sparks, C. 2012. Metal Contamination and Antioxidant Responses of *Mytilus galloprovincialis* Along the West Coast of the Cape Peninsula. DTech, Cape Peninsula University of Technology, Cape Town.

Sparks, C., Odendaal, J., & Snyman, R. 2014. An analysis of historical Mussel Watch Programme data from the west coast of the Cape Peninsula, Cape Town. Mar. Pollut. Bull. 87, 374–380 https://doi.org/10.1016/j.marpolbul.2014.07.047.

Stanković, S., & Jović, M. 2012. Health risks of heavy metals in the mediterranean mussels as seafood. Environ. Chem. Lett. 10, 119–130 https://doi.org/10.1007/s10311-011-0343-1.

Stanković, S., Jović, M., Milanov, R., & Joksimović, D. 2011. Trace elements concentrations (Zn, Cu, Pb, Cd, As and Hg) in the Mediterranean mussel (*Mytilus galloprovincialis*) and evaluation of mussel quality and possible human health risk from cultivated and wild sites of the southeastern Adriatic Sea, Montenegro. J. Serbian Chem. Soc. 76, 1725–1737 https://doi.org/10.2298/JSC110420095S.

Stenton-Dozey, J., Probyn, T., & Busby, A. 2001. Impact of mussel (*Mytilus galloprovincialis*) raft-culture on benthic macrofauna, in situ oxygen uptake, and nutrient fluxes in Saldanha Bay, South Africa. Can. J. Fish. Aquat. Sci. 58, 1021–1031 https://doi.org/10.1139/cjfas-58-5-1021.

Tesar, C. 2000. POPs: What they are; how they are used; how they are transported. North. Perspect. 26, 1–20.

Usero, J., Morillo, J., & Gracia, I. 2005. Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. Chemosphere 59, 1175–1181 https://doi.org/10.1016/j.chemosphere.2004.11.089.

van Erkom Schurink, C., & Griffiths, C. L. 1990. Marine mussels of Southern Africa - their distribution patterns, standing stocks, exploitation and culture. J. Shellfish Res. 9, 75–85 https://doi.org/10.2983/035.029.0302.

van Erkom Schurink, C., & Griffiths, C. L. 1991. A comparison of reproductive cycles and reproductive output in four southern African mussel species. Mar. Ecol. Prog. Ser. 76, 123–134 https://doi.org/10.3354/meps076123.

van Erkom Schurink, C., & Griffiths, C. L. 1992. Physiological energetics of four South African mussel species in relation to body size, ration and temperature. Comp. Biochem. Physiol. -- Part A Physiol. 101, 779–789 https://doi.org/10.1016/0300-9629(92)90358-W.

van Erkom Schurink, C., & Griffiths, C. L. 1993. Factors affecting relative rates of growth in four South African mussel species. Aquaculture 109, 257–273 https://doi.org/10.1016/0044-8486(93)90168-X.

Villalba, A. 1995. Gametogenic cycle of cultured mussel, *Mytilus galloprovincialis*, in the bays of Galicia (N.W. Spain). Aquaculture 130, 269–277.

Watling, H. R., & Watling, R. J. 1976. Trace metals in *Choromytilus meridionalis*. Mar. Pollut. Bull. 7, 91–94 https://doi.org/10.1016/0025-326X(76)90149-1.

Wilkins, N. P., Fujino, K., & Gosling, E. M. 1983. The mediterranean mussel *Mytilus galloprovincialis* Lmk. in Japan. Biol. J. Linn. Soc. 20, 365–374 https://doi.org/10.1111/j.1095-8312.1983.tb01597.x.

Wonham, M. J. 2004. Mini-review: Distribution of the Mediterranean mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae) and hybrids in the Northeast Pacific. J. Shellfish Res. 23, 535–543.

Yap, C. K., Ismail, A., & Tan, S. G. 2009. Effect of Body Size on Heavy Metal Contents and Concentrations in Green-Lipped Mussel *Perna viridis* (Linnaeus) from Malaysian Coastal Waters. Pertanika J. Sci. Technol. 17, 61–68.

Yoshimine, R. V., Carreira, R. S., Scofield, A. L., & Wagener, A. L. R. 2012. Regional assessment of PAHs contamination in SE Brazil using brown mussels (*Perna perna* Linnaeus 1758). Mar. Pollut. Bull. 64, 2581–2587 https://doi.org/10.1016/j.marpolbul.2012.07.013.

CHAPTER 3

Seasonal and species-related differences in the proximate composition of *Mytilus galloprovincialis* and *Choromytilus meridionalis*

3.1. ABSTRACT

The mussel aquaculture industry in South Africa has the potential for massive growth in Saldanha Bay, with appropriate support from research and targeted investments. Inter-specific and temporal variabilities in morphology, weight and proximate composition were assessed in *Mytilus galloprovincialis* and *Choromytilus meridionalis*, the two commercially cultured mussel species within Saldanha Bay. *Mytilus galloprovincialis* was found to have a significantly larger, wider and more ventrally flattened shell than *C. meridionalis*, while both species showed similar whole weights (23.9g vs. 26.2g, respectively), meat weights (11.1g vs. 13.2g), percentage meat yields (44.4% vs. 50%), moisture (87.4% vs. 86.2% wet weight), ash (2.6% vs. 2.5% w.w.), fat (2.1% vs. 1.8% w.w.) and protein contents (10.3% vs. 9.6% w.w.). The whole and meat weights, percentage meat yield and proximate compositions of both species were found to experience significant temporal variation. Overall, peaks in whole weights, meat weights, percentage meat yields, protein and fat in both species were found prior to spawning events in spring and summer, while peak moisture and ash contents tended to coincide with post-spawn periods, as did minimum whole and meat weights, indicating that the flesh weights and proximate composition of the mussels are closely linked to their annual reproductive cycles. Due to their low fat content, high protein content and the environmentally friendly nature of the mussel aquaculture industry, both species of farmed mussel in Saldanha Bay were determined to be a healthy and sustainable source of seafood to human consumers.

3.2. INTRODUCTION

The Imbaza Mussel Farming Empowerment Project (a.k.a. Imbaza Mussels), which was initiated by the Blue Ocean Mussel company in 2012, is the first commercial, black-owned mussel farm in South Africa, providing much needed employment and economic upliftment to Saldanha Bay locals (Anonymous, 2017). The farm began with six employees and co-owners, and currently directly (production) and indirectly (processing factories) employs 18 and 80 people, respectively (*pers. comm*. Vos Pienaar, Imbaza Mussels, 30th May 2016). Studies on the potential aquaculture production of Saldanha Bay suggest that the highly productive nature of the bay could support huge expansion of the mussel and oyster aquaculture industries, as the phytoplankton growth in the bay currently far exceeds the current needs of the farms (Heasman *et al.*, 1998; Olivier *et al.*, 2013). The Benguela Large Marine Ecosystem, of which Saldanha Bay is a part, has been estimated to have 30-65 times the primary productivity of the world's oceans on a unit area basis (Waldron & Probyn, 1992), making it the ideal location for commercial mussel aquaculture (Pitcher & Calder, 1998). A 2013 study on the mussel and oyster farming activities in Saldanha Bay estimated that the aquaculture of these two species has the potential to grow by up to 28-fold, creating up to 2 500 jobs for locals (Olivier *et* *al.*, 2013), though the farmers in Saldanha Bay believe this to be an overestimation (*pers. comm*. Vos Pienaar, Imbaza Mussels, 30th May 2016). Whether or not these estimates are realistic, there are plans in place for the expansion of the aquaculture facilities in Saldanha Bay (Clark *et al*., 2016), and therefore baseline studies (on morphology, nutritional composition and potential pollutant contamination) are required for later comparison and to aid in the informed expansion of aquaculture activities.

Meat yields and proximate analyses of mussels are commonly used worldwide to determine variations in their nutritional value to humans (Fuentes *et al.*, 2009). The moisture, ash, fat and protein contents of mussels have been found to fluctuate temporally (Bongiorno *et al.*, 2015; Fernández *et al.*, 2015), and since their composition determines both their nutritional value and organoleptic properties this information is important to consumers and farmers alike (Orban *et al.*, 2002). There is a drive in modern day society for consumers to eat healthier, which includes initiatives to either eat less animal fats or to alter the composition of the meat they consume to favour healthier, unsaturated fats (Filer, 1974). Mussels are known to possess low fat contents, in the ranges of 5.8-13.4% (Orban *et al.*, 2002) and 1.4-2.10% wet weight (Fuentes *et al.*, 2009), with high polyunsaturated fatty acid ratios (Bongiorno *et al.*, 2015; Fernández *et al.*, 2015) which are reported to be beneficial to human health (Raatz *et al.*, 2013). They therefore have the potential to be a healthier protein source than other livestock such as pigs, where the fat content can reach 25% of the whole carcass composition (Filer, 1974).

Proximate analyses can also be used to track the changes in mussel tissue which are associated with their yearly reproductive cycles (Bressan & Marin, 1985; Irisarri *et al.*, 2015). In-depth knowledge of the reproductive output and timing of farmed mussels is highly valuable to the local aquaculture industry because the release of gametes has been found to have a significant impact on the meat yield of mussels in South Africa, with *C. meridionalis* and *M. galloprovincialis* experiencing 2.8- and 3-fold reductions in dry flesh weight post-spawn, respectively (van Erkom Schurink & Griffiths, 1991). Mussel farmers are paid by processing facilities according to the total weight of their harvest, and drastic changes in the meat weight of the farmed mussels would therefore likely result in decreased profit for the farmers, as well as smaller mussels which could be less appealing to consumers. Proximate analyses can therefore aid farmers in determining when the mussels in Saldanha Bay are spawning, as well as the effect that spawning has on the mussels' nutritional content, as previous studies seem to disagree on the timing of the reproductive cycles in South Africa (Griffths, 1977; van Erkom Schurink & Griffiths, 1991). Proximate analyses can also help understand species and seasonal changes in potential pollutant data, as certain pollutants have been found to be deposited in the fat of mussels while others may accumulate or be diluted when the mussels are building up gametogenic reserves (Cossa *et al.*, 1979; Bayarri *et al.*, 2001; Richir & Gobert, 2014). The nutritional quality of mussels, as well as any potential harmful substances stored within the mussels' tissue, is of increasing importance to informed consumers.

While *C. meridionalis* is the native large black mussel species, the invasive *M. galloprovincialis* is the targeted species of aquaculture in Saldanha Bay. This is partially because there is a perception among farmers that the brown female gonad of *C. meridionalis* compares unfavourably in colour and taste to that of the male *C. meridionalis* and the male and female *M. galloprovincialis*, and partially because *M. galloprovincialis* naturally occurs in greater abundance on the culture ropes of the mussel farm due to a higher reproductive output (*pers. comm*. Vos Pienaar, Imbaza Mussels, 30th May 2016). Though an extensive base of knowledge from experience exists in the mussel farming community of Saldanha Bay, there is often a lack of published scientific research to support this knowledge. There is also a serious disparity in the volume of information collection for the two species over the years; while *M. galloprovincialis* has had numerous studies done on every aspect of its biology (due to it being a commercially cultured species in Europe), there is far less information available for *C. meridionalis*, with the majority of research occurring over 20 years ago. It is advantageous to have up to date information on the current commercial species in Saldanha Bay, in order to inform farmers of the current state of the mussels and recommend best practices going forward (with respect to harvest times and focal species), and which could be used by consumers wishing to make informed decisions on their food choices.

The aim of the research in this Chapter was to determine the extent of inter-specific and temporal variability in morphology, weight, meat yield and proximate compositions of the two commercially cultured mussel species (*M. galloprovincialis* and *C. meridionalis*) within Saldanha Bay. This information can potentially aid farmers in making harvest decisions, help future aquaculture expansion projects in the planning of their production systems and aid consumers who want to make informed decisions about the foods they eat.

3.3. MATERIALS AND METHODS

3.3.1. Study location

All samples were harvested from the grow-out rafts of Imbaza Mussels within the Small Bay of the Saldanha Bay Municipality (33°01’97” S, 18°19’03” E). The farm consists of 24 rafts, laid out in a six-by-four pattern in the centre of Small Bay. Each raft has approximately 800, 6-metre long ropes attached to the frame, where the mussels are grown. The bay forms a sheltered part of the Benguela Large Marine Ecosystem that runs along most of the West coast of South Africa, Namibia and Angola. This ecosystem is characterised by high primary productivity levels; strong offshore winds cause the regular upwelling of cold, nutrient-rich waters and promote elevated levels of phytoplankton growth which, when combined with the sheltered nature of the bay, allow for the culture of oysters (*Crassostrea gigas*) and mussels (Olivier *et al.*, 2013).

3.3.2. Sampling

Harvesting of 200-300 mussels of each species (*M. galloprovincialis* and *C. meridionalis*) occurred approximately every two months for two years (Table 3.1). Once harvested the mussels used for this study were transported to the Blue Ocean Mussel processing facilities in Velddrif and frozen, before being transported to the Wild Peacock facilities in Stellenbosch. Samples were then collected and transferred to the -20°C freezer in the meat lab of the Stellenbosch University Department of Animal Sciences, where they were stored until analyses occurred. Sample collection began in March 2015 and ended in February 2017, with the exception of January, March and May 2016 (Table 3.1). Sampling did not occur during these three months because there was a toxic algal bloom in Saldanha Bay and mussel farmers had to halt harvest until the mussels were deemed safe for human consumption. Samples from June 2016 were taken (in lieu of May 2016) once harvest began again in order to minimise the gap in data collection. Samples were collected late in December 2016 and February 2017 (in lieu of November 2016 and January 2017), due to a complete absence of *C. meridionalis* on the culture rafts which were being harvested at the time. The comparative nature of this study required that the sample only be taken once both species were found upon the same raft at the same time. Table 3.1 also defines the seasons in which mussels were harvested, due to the fact that in the discussion section the date of collection will be discussed according to season, and the dates of the seasons in South Africa (and the whole southern Hemisphere) are as follows:

- Summer: 1 December – 28/29 February
- Autumn: 1 March – 31 May
- Winter: 1 June – 31 August
- Spring: 1 September – 30 November

Table 3.1 Sampling dates, batch numbers and missed samples for both species

Date Collected	Batch Number & Size	Season
March 2015	1: 200-300 *M. galloprovincialis* & *C. meridionalis*	Autumn
May 2015	2: 200-300 *M. galloprovincialis* & *C. meridionalis*	Autumn
July 2015	3: 200-300 *M. galloprovincialis* & *C. meridionalis*	Winter
September 2015	4: 200-300 *M. galloprovincialis* & *C. meridionalis*	Spring
November 2015	5: 200-300 *M. galloprovincialis* & *C. meridionalis*	Spring
January 2016	No harvest or sampling due to toxic algal bloom	Summer
March 2016	No harvest or sampling due to toxic algal bloom	Autumn
May 2016	No harvest or sampling due to toxic algal bloom	Autumn
June 2016	6: 200-300 *M. galloprovincialis* & *C. meridionalis* (in lieu of May 2016)	Winter
July 2016	7: 200-300 *M. galloprovincialis* & *C. meridionalis*	Winter
September 2016	8: 200-300 *M. galloprovincialis* & *C. meridionalis*	Spring
December 2016	9: 200-300 *M. galloprovincialis* & *C. meridionalis* (in lieu of November 2016)	Summer
February 2017	10: 200-300 *M. galloprovincialis* & *C. meridionalis* (in lieu of January 2017)	Summer

All mussels harvested for this study were of commercial sale size i.e. >60mm in length. All mussels in this study were sexually mature to reduce variation caused by the changes which occur before and after sexual maturity, such as different resource apportioning for gamete production instead of growth (Griffiths, 1981a; Gosling, 2004). For this study, mussels were not divided into size classes as they were all of commercial production size for sale and were rather analysed for their potential benefits and risks to consumers, who may prefer larger mussels but generally do not discriminate on size basis.

Mussels destined for consumer markets are also transported to the processing facilities in Velddrif, where they are cooked at 90°C for 2.5 minutes. Next, they are manually cleaned by removing the beard, scrubbing the shell and removing one half of the shell. They are then placed into a freezing tunnel at -48°C until they reach a core temperature of between -18 to -24°C, after which they are packaged and sold (*pers. comm.* Francois Dunn, Factory Manager at Blue Ocean Mussels processing factory).

It is noteworthy that there were unavoidable gaps in sample collection for January, March and May 2016 due to a large toxic algal bloom along much of the West Coast. As a result, maximum and minimum whole weights, meat weights, percentage meat yields and proximate data for 2016 may not be a true representation of the actual maxima and minima, due to the unfortunate gap in data collection. This study will not attempt to extrapolate data for this period of missing information, as yearly differences in ocean temperature, patterns in phytoplankton biomass and variations in spawning period mean that changes in the parameters in 2016 would not necessarily follow the pattern of those changes in 2015.

3.3.3. Species identification

Mytilus galloprovincialis and *C. meridionalis* can be reliably distinguished from one another in a number of ways. Firstly, the shells are different shapes; *C. meridionalis* is typically much thinner than *M. galloprovincialis* in cross section/breadth (van Erkom Schurink & Griffiths, 1990). Secondly, when looking on the inside of the shell, *M. galloprovincialis* has a pitted resilial ridge (the hinge which connects the two shell halves) and an anterior (where the shell tapers to a point) adductor muscle, while the resilial ridge of *C. meridionalis* is smooth and lacking this muscle (van Erkom Schurink & Griffiths, 1990). Lastly, the gonad colour of *C. meridionalis* females is a dark, chocolate brown, while the gonad colour of *M. galloprovincialis* females is orange, and the gonad colour of the males of both species varies from yellow to off-white (van Erkom Schurink & Griffiths, 1990).

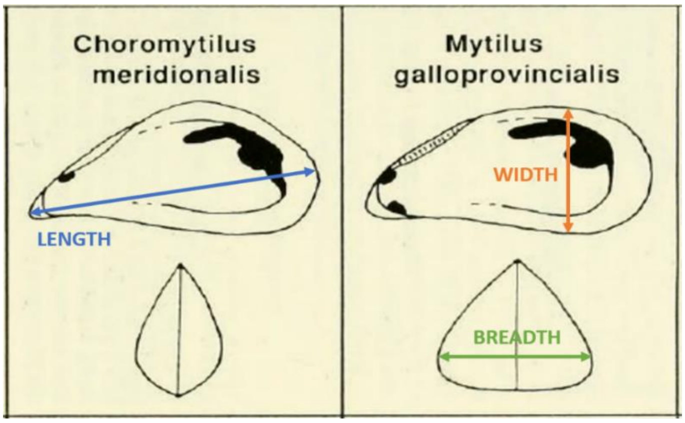


Figure 3.1 The morphometric differences between *C. meridionalis* and *M. galloprovincialis*, with indications of where length, width and breadth measurements were taken for this study (Image modified from Van Erkom Schurink & Griffiths, 1990)

3.3.4. Sample preparation and processing

Mussels were removed from the -20°C freezer ~24 hours prior to analysis and defrosted. Once fully defrosted, whole mussel weight was recorded (in grams, to two decimal places). Length, breadth and width measurements were taken (in millimetres, to two decimal places) using a Vernier calliper, with the location of the measurements taken being specified in Figure 3.1. Mussels were then "shucked" (flesh removed from shell) by severing the adductor muscles which connected the flesh to the shell. The wet weight of the flesh and shell were then recorded, and the shell was discarded. Due to the fact that an individual mussel is too small for multiple proximate analyses, and in order to decrease the inter-individual variability of the samples, the flesh of multiple mussels was grouped into pooled samples with a total flesh weight of approximately 100grams wet weight per pooled sample (Orban *et al.*, 2002; Bongiorno *et al.*, 2015; Irisarri *et al.*, 2015). Six pooled samples (repeats) were created per species and per time period, which resulted in a total of 120 samples for the entire study. After processing and data collection, the newly created pooled samples and unused mussels were refrozen at -20°C until further analysis.

3.3.5. Proximate analyses: Sample preparation

Initially samples were to be homogenized for analysis using liquid nitrogen, but this method was determined to be both time-consuming and ineffective. Subsequently it was decided that freeze drying the samples and then blending them to a powder would produce a more homogenous sample for analysis. This method was previously used in multiple studies on mussels (Bongiorno *et al*. 2015, Fernandez *et al*. 2015). Samples were freeze dried in batches of ~15, with freeze drying taking 72-96 hours to dry sufficiently. Whole samples weights were recorded before and after freeze drying in order to calculate moisture loss in the samples.

3.3.6. Proximate analyses

3.3.6.1. Moisture

Though most of the water in the samples was removed during the initial freeze-drying process, residual moisture was also analysed in order to determine total moisture of the samples. The moisture content was determined according to the methodology laid out in the AOAC official method 934.01 (AOAC International, 2002). Clean, empty porcelain crucibles were dried at 100°C for 2 hours, before being cooled in a desiccator for 30 minutes. Moisture analyses were done, in duplicate, by placing 1gram (value B) of powdered sample into the pre-weighed porcelain crucibles (value A). The sample and crucible were then dried at 100°C for 24 hours, allowed to cool in a desiccator, and re-weighed (value C).

The percentage moisture in the samples was then calculated as:

$$\%\,moisture = \frac{[(A+B)-C]}{B}x100$$

The total percentage moisture was then calculated by combining the percentage moisture from the analysed samples with the percentage moisture which was lost during freeze-drying.

3.3.6.2. Ash

Ash content of samples was determined according to the AOAC official method 942.05 (AOAC International, 2002). The samples previously used for moisture determination, with known sample and crucible weights (value A), were used for ash content analyses in duplicate. The crucibles with the moisture-free samples were placed into a furnace, and ashed at 500°C for 6 hours. Once ashed, the sample was allowed to cool and was then re-weighed (value D).

The percentage ash was then calculated as:

$$\%Ash = \frac{D-A}{B}x100$$

3.3.6.3. Total Fat

Total fat in the samples was determined in duplicate using methodology determined for fish samples (Lee *et al.*, 1996b). One gram of powdered sample was weighed off into a clean, dry glass beaker. To this, 50ml of a chloroform:methanol solution (2:1) and 4ml of deionised water were added, before blending for 30 seconds with a Bamix stick blender. The blended mix was then filtered through Whatman No.1 filter paper into a separation funnel and allowed to stand until all of the solution had passed through it. The filters were then removed and discarded. Subsequently, 20ml of a 0.5% NaCl solution in distilled water was added to the separation funnel, and the solution was shaken four times, before being allowed to separate for one hour. Once the separation of the NaCl (top layer) and chloroform:methanol (bottom layer) was clear, the bottom layer was tapped off into an Erlenmeyer flask. Clean, dry fat beakers were placed into a desiccator to cool for one hour before being weighed. Five millilitres of this solution was then pipetted into the pre-weighed fat beakers. The fat beakers were then placed onto a heated sand plate (70°C) for one hour to allow for the evaporation of the chloroform:methanol. Once evaporated, fat beakers were placed into a desiccator to cool, and then re-weighed.

Fat percentage was then calculated as follows:

$$\% \, Fat = \frac{(Fat\ beaker + fat) - (fat\ beaker)}{sample\ mass} \, x \, \frac{Chloroform\ volume}{5} \, x \, 100$$

3.3.6.4. Protein

Protein was determined according to the Dumas combustion method 992.15 (AOAC International, 2002). The freeze-dried, homogenized samples were dried in an oven at 60°C for a minimum of 2 hours before analysis in order to ensure the samples were moisture-free. Once dried, approximately 0.15grams of sample was weighed off (in duplicate) into Leco tin foil cups, which were twisted closed and placed into the Leco protein analyser (LECO FP-528) for analysis. Values obtained from the analysis were in % nitrogen and were converted to % protein by multiplying the given value by 6.25. The Leco was recalibrated after every 30 samples to maintain high accuracy and recovery rates, using Leco EDTA Reference Material (Nitrogen 9.56 ±0 .04).

3.3.6.5. Conversion of data to wet weight

All data obtained from the proximate analyses was according to the dry weight percentages, and in order to find the true percentage composition of the mussels the obtained data for protein, fat and ash were converted to percentage wet weights using the formula below:

$$Ash \, \% \, wet\ weight = \% \, ash\ dry\ weight\ (A) \, x \, \frac{Whole\ sample\ (A)\ dry\ weight *}{100}$$

*the dry weight of the sample as a whole was determined using the freeze-drying weight loss data

3.3.7. Statistical Analyses

The experimental design was a completely randomised design, with samples being collected every two months from Imbaza Mussels' rafts during normal harvesting processes. The two main effects were collection date and species. All data was analysed in Microsoft Excel using the additional statistical analysis software XLSTAT Premium (Annual Version 19.4.45237), developed by Addinsoft.

One-way ANOVAs were performed on shell length, width and breadth to determine morphometric differences between the two species. Variations in shell length, width and breadth were not analysed statistically for seasonal differences because this study was not a growth trial and each batch of mussels varied in size, as they were harvested at random from the mussel rafts and the researchers did not have control over the production parameters for harvest (precedent for this was set by Irisarri *et al*. 2015). Two-way ANOVAs were performed on whole weight, meat weight, percentage meat yield and all proximate analysis data (moisture, ash, fat and protein). Collection date and species were selected as the main effects, and an interaction term was included. Post-hoc Bonferroni pairwise comparison tests were then performed on all data to determine where significant differences were present. The assumptions of normal distribution and homogeneity of variances were checked, and if heterogeneity of variances was found, Welch's statistic was included in the ANOVA procedure to ensure heteroscedasticity did not influence the accuracy of the analysis. Pearson's correlation matrices were created for all variables in each section (biometric and proximate data) in order to examine the linear relationships between all variables. For this study, though the Pearson's test itself identified many of the correlations as statistically significant, they were only considered to be biologically significant if the correlation coefficient (R value) equalled or exceeded 0.7.

3.4. RESULTS

3.4.1. Morphometric analyses

The shells of farmed *M. galloprovincialis* specimens were found to be significantly ($p<0.0001$) longer, wider and to have a greater breadth than those of *C. meridionalis* (Table 3.2). In *C. meridionalis*, shell length and width were found to have significant ($p<0.05$) positive correlation with one another, as were shell length and breadth and shell width and breadth (Table 3.3). Shell length and width were found to correlate significantly ($p<0.05$) and positively with one another in *M. galloprovincialis*, as were shell length and shell breadth, and shell width and breadth (Table 3.3).

3.4.2. Whole Weight, Meat Weight and Meat Yield Analyses

Significant interactions ($p<0.0001$) were found between the main variables of season and species for whole weight, meat weight and percentage meat yield, and therefore main effects could not be discussed separately. The significant interaction term between the main variables of season and species was not unexpected, due to the fact that the two species have been observed to possess different spawning periods in Saldanha Bay, with *M. galloprovincialis* spawning in early summer and *C. meridionalis* spawning in late summer (*pers*. *comm*. Vos Pienaar, Imbaza Mussels, 30th May 2016). Spawning has been found to significantly affect the whole weights of numerous mussel species (van Erkom Schurink & Griffiths, 1991, 1993; Orban *et al*., 2002; Çelik *et al*., 2012; Bongiorno *et al*., 2015), and therefore the interaction term between species and season for whole weight, meat weight and meat yield was not surprising, as the two species did not follow the same patterns of accumulation and depletion over the sampling periods.

Both season and species were found to significantly affect ($p<0.0001$) the whole weights, meat weights and percentage meat yields of the mussels (with the exception of the species effect on whole weight, where $p=0.012$). The combined temporal and species-related changes in whole weight, meat weight and percentage meat yield are depicted in Figure 3.2, with different letters from the post-hoc Bonferroni denoting significant differences. In general, *C. meridionalis* individuals were found to have significantly higher percentage meat yields overall than *M. galloprovincialis* (Table 3.2, Figure 3.2). No significant ($p\leq0.0$) inter-annual differences were identified by the Bonferroni tests (Figure 3.2).

The seasonal differences in maxima and minima for whole weights, meat weights and percentage meat yields were identified as statistically significant for both species by the post-hoc Bonferroni tests (Figure 3.2). The whole weights, meat weights and percentage meat yields of *C. meridionalis* in 2015 peaked in May (31.0 ± 1.26g, 16.3 ± 0.73g and 52.2 ± 0.55%, respectively) and July (30.9 ± 0.70g, 16.3 ± 0.44g and 52.4 ± 0.45%, respectively). In 2016, peak whole and meat weights occurred again in July (34.0 ± 1.27g and 18.1 ± 0.83g) while the percentage meat yield peaked in June (54.5 ± 0.41%) and December (53.1 ± 0.42%). The December peak was unexpected and in contrast to the general trend, but a closer look at the data showed that this drastic increase in percentage meat yield (in contrast to the drops in whole and meat weights experienced at the same time) was caused by the batch being comprised of particularly small individuals with high meat weights, which caused a spike in meat yield. Minimum whole and meat weights for *C. meridionalis* occurred in September 2015 (21.2 ± 0.54g and 10.8 ± 0.33g) and November (21.2 ± 0.48g and 10.2 ± 0.31g), with lowest percentage meat yield occurring in November (47.2 ± 0.58%). The minimum whole and meat weights occurred in December 2016 (16.5 ± 0.23g and 8.8 ± 0.16), with percentage meat yield reaching a minimum in September (45.7 ± 0.39%). For *M. galloprovincialis*, the maxima for whole weight, meat weight and percentage meat yield occurred in May 2015 (33.9 ± 1.40g, 17.8 ± 0.88g and 52.0 ± 0.80%, respectively) and July 2016 (37.5 ± 1.51g, 20.0 ± 0.99g and 52.6 ± 0.81%). The minima for whole and meat weights occurred in September 2015 (16.3 ± 0.40g and 6.9 ± 0.24, respectively) with minimum percentage meat yield in November (40.5 ± 0.46%), while minima for all parameters were found in December 2016 (18.4 ± 0.35g, 7.1 ± 0.23g and 38.3 ± 0.65%).

Whole weight in *C. meridionalis* was found to have biologically significant positive correlations with shell length, shell width, shell breadth and meat weight (Table 3.3). Meat weight was also found to correlate significantly and positively with shell length and width. In *M. galloprovincialis*, whole weight had a significant positive correlation with shell length, width, breadth and meat weight. Meat weight was found to correlate significantly with percentage meat yield and whole weight.

3.4.3. Proximate analyses

Significant interaction terms ($p \leq 0.05$) were found between the explanatory variables of season and species for moisture, protein, fat and ash. As explained previously (in Section 3.4.2. Whole Weight, Meat Weight and Meat Yield Analyses), the likely reason for this interaction term is the difference in spawning period between the two species, as spawning in mussels has previously been found to significantly affect their proximate condition (Okumus & Stirling, 1998; Çelik *et al.*, 2012; Bongiorno *et al.*, 2015; Fernández *et al.*, 2015). Season and species were both found to have a significant ($p<0.001$) impact on the moisture, ash, fat and protein contents of the mussels, with the models having substantial explanatory power (R^2 = 88%, 64%, 75% and 92%, respectively).

Table 3.2 Number of individuals, mean (± standard error) and range of morphometric, weight and yield data collected for *C. meridionalis* and *M. galloprovincialis*

	Number of individuals		**Mean (± standard error)**		**Range**	
	C. meridionalis	*M. galloprovincialis*	*C. meridionalis*	*M. galloprovincialis*	*C. meridionalis*	*M. galloprovincialis*
Shell length (mm)	768	934	76.6 ± 0.26	78.8 ± 0.22	61.38 - 103.65	59.05 - 102.07
Shell width (mm)	768	934	36.8 ± 0.11	39.4 ± 0.11	21.83 - 47.45	24.03 - 51.60
Shell breadth (mm)	704	871	23.4 ± 0.11	25.3 ± 0.09	16.57 - 32.65	18.31 - 37.82
Whole weight (g)	768	934	26.2 ± 0.30	23.9 ± 0.30	12.17 - 60.62	8.43 - 75.71
Meat weight (g)	768	934	13.2 ± 0.17	11.1 ± 0.19	3.06 - 31.20	2.88 - 45.06
Percentage meat yield (%)	768	934	50.0 ± 0.21	44.4 ± 0.24	24.13 - 63.40	24.81 - 64.44

Table 3.3 Pearson's correlation matrix for morphometric, weight and yield measurements of *C. meridionalis* and *M. galloprovincialis*

Species	*C. meridionalis*					*M. galloprovincialis*				
	WW (g)	**SL**	**SW**	**SB**	**MW**	**WW**	**SL**	**SW**	**SB**	**MW**
Shell length (mm)	0.88*					0.78*				
Shell width (mm)	0.78*	0.83*				0.70*	0.78*			
Shell breadth (mm)	0.71*	0.79*	0.72*			0.76*	0.82*	0.68*		
Meat weight (g)	0.95*	0.77*	0.70*	0.59*		0.97*	0.67*	0.60*	0.67*	
Percentage Meat Yield (%MY)	0.21*	-0.04	0.00	-0.14*	0.48*	0.63*	0.28*	0.22*	0.31*	0.79*

Abbreviations: WW = whole weight, SL = shell length, SW = shell width, SB = shell breadth, MW = meat weight
*Values marked with a * are statistically significantly different from zero at p≤0.05*

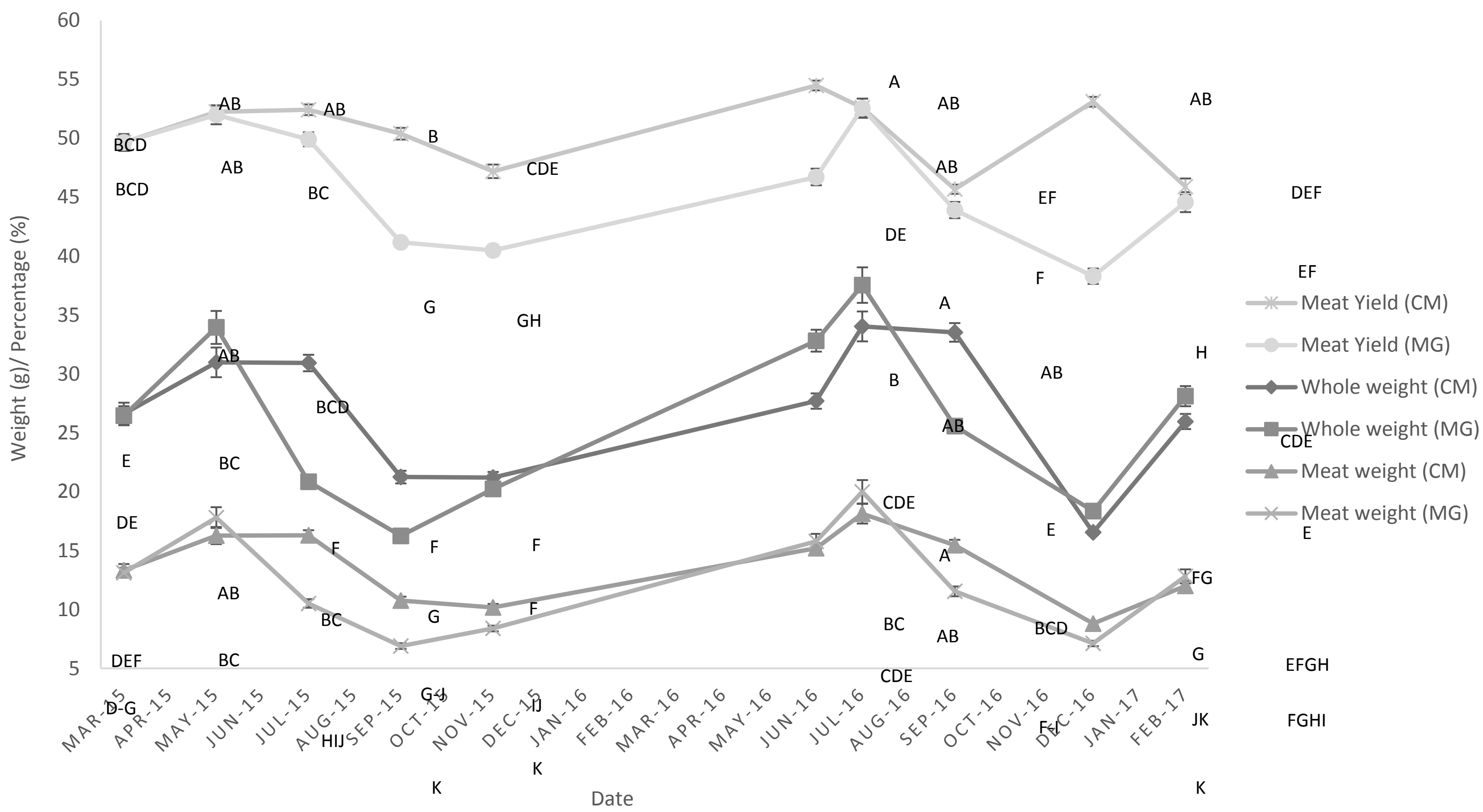


Figure 3.2 Temporal changes in the whole weight, meat weight and percentage meat yield of *C. meridionalis* (CM) and *M. galloprovincialis* (MG) over a two-year period, with different letters denoting significant differences at p≤0.05

The temporal and species-related changes in moisture, ash, fat and protein are depicted in the graphs of Figure 3.3. The moisture content of *C. meridionalis* fell to a minimum in July 2015 (85.2 ± 0.44%) and June 2016 (79.8 ± 0.38%), with peaks observed in September 2015 and 2016 (93.7 ± 1.24% and 88.3 ± 0.44% respectively). Ash content was lowest in May 2015 (2.4 ± 0.12%) and June and September 2016 (2.3 ± 0.02% and 2.3 ± 0.03%), with peaks in March and November 2015 (2.7 ± 0.05% and 2.6 ± 0.03% respectively), and July and December 2016 (2.4 ± 0.05% and 2.5 ± 0.02%). Fat content of *C. meridionalis* dropped to a minimum in May 2015 (1.0 ± 0.12%) and September 2016 (1.4 ± 0.06%), while maxima occurred in November 2015 (1.9 ± 0.23%) and June and July 2016 (3.0 ± 0.19% and 2.9 ± 0.32%). Protein content peaked in July 2015 and 2016 (11.7 ± 0.17% and 14.1 ± 0.28%) and fell to a minimum in September 2015 (7.6 ± 0.22%), and December 2016 (9.9 ± 0.15%).

In *M. galloprovincialis* the minimum moisture contents occurred in March 2015 (83.7 ± 0.36%) and June 2016 (83.2 ± 0.51%), with peaks in September 2015 and 2016 (91.7 ± 0.42% and 90.3 ± 0.24%). Minimum ash content was found in March and May 2015 (2.6 ± 0.04% and 2.5 ± 0.04% respectively) and June 2016 (2.4 ± 0.06%), with maxima in September 2015 (2.9 ± 0.04%) and December 2016 (2.6 ± 0.04%). Fat content was observed to be lowest in March 2015 (1.5 ± 0.07%) and July 2016 (1.7 ± 0.19%) and peaked in November 2015 (3.1 ± 0.08%) and June and September 2016 (2.5 ± 0.16% and 2.4 ± 0.11% respectively). The protein content rose to a peak in July 2015 (11.1 ± 0.20) and June 2016 (11.5 ± 0.28%), while the lowest values occurred in September 2015 (8.3 ± 0.07) and December 2016 (8.4 ± 0.19%). The post-hoc Bonferroni test confirmed that the differences between minimum and maximum moisture, ash, fat and protein values were significant for both species (Figure 3.3), and the proximate composition of the two species were observed to follow similar seasonal trends for moisture, ash and protein content. The post-hoc Bonferroni identified significant inter-annual variability in *C. meridionalis* proximate composition, with higher moisture content in 2015, and higher protein and fat contents in 2016.

The protein content of *C. meridionalis* was found to have a biologically significant negative correlation with the moisture content of the mussels, and a positive correlation with the fat content (Table 3.5). No biologically significant correlations were found between proximate variables for *M. galloprovincialis*.

Table 3.4 Number of individuals, mean (± standard error) and range of proximate data (moisture, protein, fat and ash) for *C. meridionalis* and *M. galloprovincialis* (wet weight)

Chemical	**Number of observations**		**Mean (± standard error)**		**Range** (min – max)	
Component	*C. meridionalis*	*M. galloprovincialis*	*C. meridionalis*	*M. galloprovincialis*	*C. meridionalis*	*M. galloprovincialis*
Moisture (%)	60	60	86.2 ± 0.50	87.4 ± 0.37	78.55 - 96.29	81.60 - 93.67
Protein (%)	60	60	10.3 ± 0.28	9.6 ± 0.18	6.67 - 14.90	7.09 - 12.23
Fat (%)	60	60	1.8 ± 0.09	2.1 ± 0.07	0.66 - 3.73	1.04 - 3.44
Ash (%)	60	60	2.5 ± 0.02	2.6 ± 0.02	1.81 - 2.88	2.21 - 2.98

Table 3.5 Pearson's correlation matrix for morphometric, weight and yield measurements of *C. meridionalis* and *M. galloprovincialis*

	Species					
	C. meridionalis			***M. galloprovincialis***		
	Fat (%)	**Ash (%)**	**Protein (%)**	**Fat (%)**	**Ash (%)**	**Protein (%)**
Ash (%)	-0.20			-0.03		
Protein (%)	0.70*	-0.33*		0.08	-0.38*	
Moisture (%)	-0.46*	0.34*	-0.76*	0.13	0.50*	-0.52*

*Values marked with * are significantly different from zero at p≤0.05*

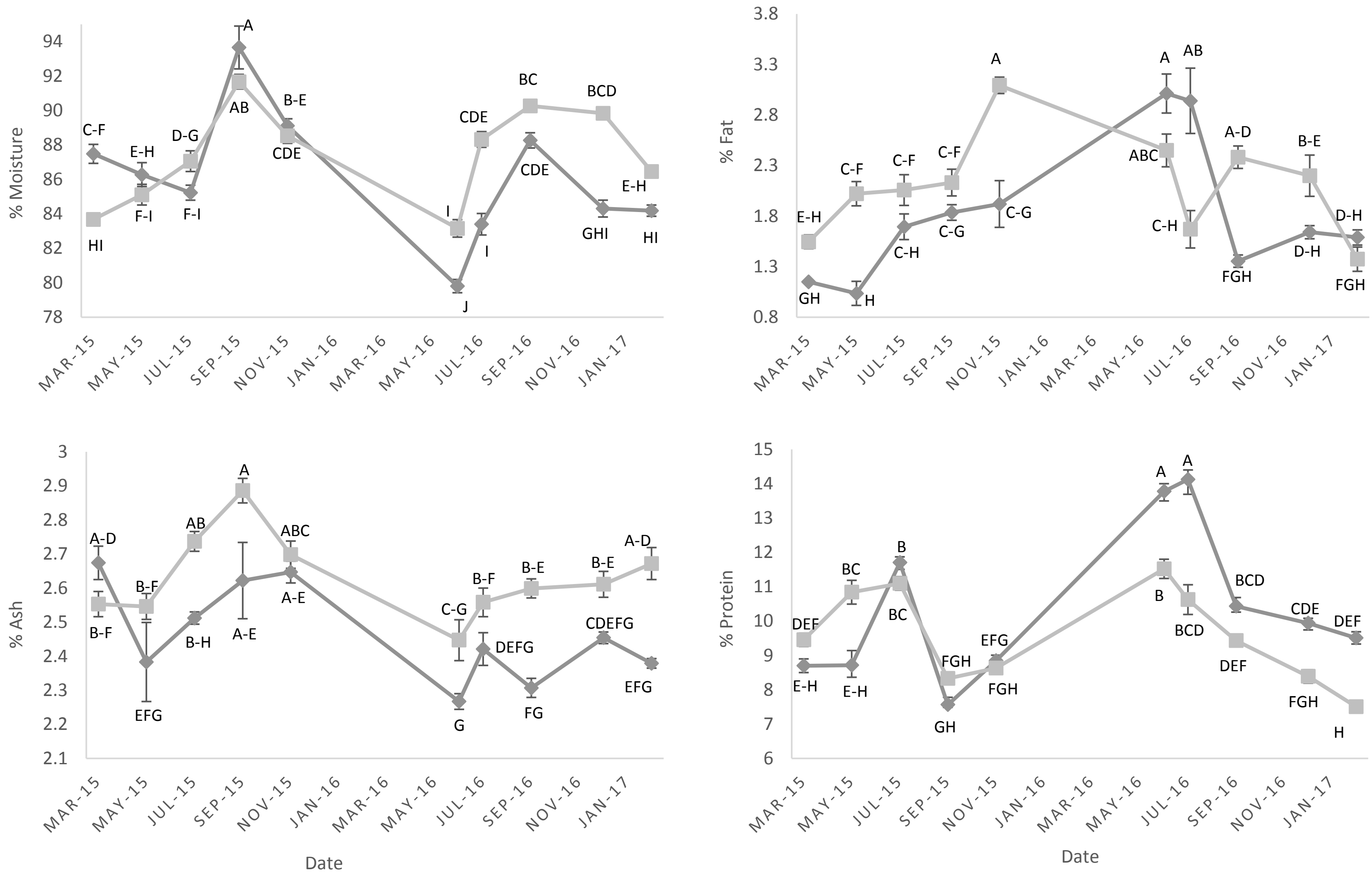


Figure 3.3 Temporal changes in % moisture, ash, fat and protein (wet weight) of *C. meridionalis* (diamond marker) and *M. galloprovincialis* (square marker), with different letters denoting significant differences at p≤0.05

3.5. DISCUSSION

The morphometric measurements of the two species confirm the statement of previous papers; that *M. galloprovincialis* is generally larger, wider and ventrally flatter (breadth) than *C. meridionalis*, and that these characteristics can be used to aid in identification of the species (van Erkom Schurink & Griffiths, 1990, 1993). In this study, shell width and breadth were found to increase linearly with increasing shell length in both species. Comparable results have been found previously in Saldanha Bay; when increases in shell length were regressed against shell breadth and width, R^2 values of ~97% and ~95%, respectively, were found for *C. meridionalis*, and ~96% for both parameters in *M. galloprovincialis* (van Erkom Schurink & Griffiths, 1993). Increases in shell length, width and breadth were also all found to correlate significantly with increased whole weight in both species. Van Erkom Schurink and Griffiths (1993) found shell length to be positively correlated (R^2=98%) to whole weight in both *C. meridionalis* and *M. galloprovincialis* in Saldanha Bay, but did not investigate correlations between whole weight, breadth and width. These findings make biological sense, as a positive linear relationship between length, width, breadth and whole weight is surely linked to uniform shell growth and larger mussels possessing heavier shells and greater meat weights than smaller individuals. Meat weight was only found to have a biologically significant positive correlation with shell length and width in *C. meridionalis*. This result is somewhat unexpected, as previous studies on *C. meridionalis* have shown that flesh weights in sexually mature mussels fluctuate drastically due to annual gametogenesis and spawning, while shell length increases linearly over time, meaning that it is unlikely for them to be easily correlated with one another (van Erkom Schurink & Griffiths, 1993).

The whole weights, meat weights and percentage meat yields fluctuated significantly both between the two species and over the two-year period of the study. Due to the high linear correlation between the whole and meat weights of both species, and the fact that these two variables follow a similar pattern throughout the study period, only the changes in whole weight and percentage meat yield of the mussels will be discussed to reduce repetition. While previous studies have stated *M. galloprovincialis* to have considerably higher flesh yields than *C. meridionalis* (van Erkom Schurink & Griffiths, 1991, 1993), the whole weights and percentage meat yields of *C. meridionalis* in this study were found to be equal to or greater than those of *M. galloprovincialis*. A potential reason for this could be that adjustments were not made to control for size differences in this study, as was the case in the studies by van Erkom Schurink and Griffiths (1991, 1993). Mirroring these prior studies, the perception of the farmers in Saldanha Bay is that *M. galloprovincialis* is far larger, heavier and possessing a greater meat yield than *C. meridionalis* (*pers. comm*. Vos Pienaar, Imbaza Mussels, 30th May 2016), but the results of this study contradict this. Perceived superior weights and meat yields are, however, not the only reasons for the farms' focus on the invasive *M. galloprovincialis* over the native *C. meridionalis*. The most significant factor determining the focus species of aquaculture in Saldanha Bay is likely the reproductive output of the species; *M. galloprovincialis* has the second highest investment in gonadal material of the four common South African mussels (after *Aulacomya ater*, the ribbed mussel), releasing far more gametes into the water column than *C. meridionalis* (van Erkom Schurink & Griffiths, 1991). The farms in Saldanha Bay rely on the natural settlement of mussel seed onto the ropes (van Erkom Schurink & Griffiths, 1990), and the higher reproductive output of *M. galloprovincialis* is observable in the fact that ~80% of the mussels growing on the culture ropes are *M. galloprovincialis*, while *C. meridionalis* makes up ~20% of the total mussel biomass (*pers. comm*. Vos Pienaar, Imbaza Mussels, 30th May 2016). Of the four common mussel species in South Africa, *M. galloprovincialis* also has the fastest growth rate, lowest mortality and has been found to be the species best suited to aquaculture in Saldanha Bay (Van Erkom Schurink & Griffiths, 1992; van Erkom Schurink & Griffiths, 1993). As a result, this species has continued to be the focal species of aquaculture operations in Saldanha Bay. Despite the results of this study suggesting that *C. meridionalis* could potentially be a more viable aquaculture species than previously suggested, if the farms were to focus on the native species they would have to actively promote the settlement of *C. meridionalis* spat onto their ropes, which would cause increased production costs and complexities. They would also have to invest in increased marketing and promotion of the product, as the perception is currently that the flesh of the female *C. meridionalis* is less palatable than that of *M. galloprovincialis* (van Erkom Schurink & Griffiths, 1990), though this has not been scientifically confirmed and warrants further research. In comparison, *M. galloprovincialis* has a well-established market in Mediterranean countries (Orban *et al.*, 2002; Irisarri *et al.*, 2015), and therefore local farmers likely find this widely recognisable species far easier to market and export.

Significant seasonal differences in the whole weights and meat yields were found for both species in this study. Previous studies on the flesh weight and meat yield of *C. meridionalis* and *M. galloprovincialis* state that these parameters fluctuate significantly on an annual basis, with the majority of studies linking these changes to gametogenic cycles (van Erkom Schurink & Griffiths, 1991, 1993; Orban *et al.*, 2002; Çelik *et al.*, 2012; Bongiorno *et al.*, 2015). The build-up of reserves for reproduction and the conversion of these reserves into gametes results in massive weight gain in the gonadal tissues of the mussels (Çelik *et al.*, 2012), and weight losses post-spawn have been registered in the ranges of 2.8- and 3-fold decreases in flesh weight in *C. meridionalis* and *M. galloprovincialis*, respectively (van Erkom Schurink & Griffiths, 1991). Studies from the Cape Peninsula state the spawning periods of both species to occur in spring/early summer and again in autumn (van Erkom Schurink & Griffiths, 1991). In Saldanha Bay, *M. galloprovincialis* has been noted to spawn in early summer, prior to *C. meridionalis*, which spawns in mid-summer (*pers. comm*. Vos Pienaar, Imbaza

Mussels, 30th May 2016). In this study both species were found to follow similar patterns in the timing of seasonal minima and maxima, but reductions in whole weight and percentage meat yield occurred first in *M. galloprovincialis*, followed by *C. meridionalis* approximately 2 months later. This pattern fits the information provided by the mussel farmers of Saldanha Bay, with the invasive mussel spawning and experiencing subsequent weight losses first, and the local mussel following shortly after. Reductions in the whole weight and percentage meat yield of both species in 2015 did not align with the stated spawning periods, occurring in early spring rather than summer, but in 2016 massive reductions in flesh weight and percentage meat yield were observed in summer, coinciding with the aforementioned spawning periods. The slight mismatch of weight reductions with the predicted spawning period in 2015 is very likely evidence that spawning occurred earlier than expected, rather than showing that weight changes are not linked to gametogenesis. This is because *M. galloprovincialis* and *C. meridionalis* experience high inter-annual variability in their spawning periods, as their timing is affected by exogenous environmental cues such as water temperature and food availability, which vary annually (Griffths, 1977; van Erkom Schurink & Griffiths, 1991; Gosling, 2004). It is therefore possible that both species experienced an earlier spawning period in 2015 due to changes in environmental parameters (which were not measured by this study). The results from the present study suggest that changes in the whole weights and percentage meat yields of both *C. meridionalis* and *M. galloprovincialis* are linked to the gametogenic cycle, as minimum values for both parameters were experienced in spring and summer, when the mussels are known to spawn. Evidence that the weight changes are linked to gametogenesis rather than food availability can also be found in the fact that in this study the whole weights of *M. galloprovincialis* fluctuated over a greater range than those of *C. meridionalis*. Previous studies on these species state *M. galloprovincialis* to experience a more intense spawning period, releasing a greater number of gametes into the water column than *C. meridionalis*, which are represented in greater weight losses post spawn (van Erkom Schurink & Griffiths, 1991), as is the case in this study.

Seasonal dependant changes in whole weight are a useful parameter for mussel farmers because a good harvestable mussel weight is approximately 30-40g per mussel (25-30 mussels per kg), and changes in the weight of the mussels will affect the amount of money that the farmers receive from processing companies which buy their stock. However, the current study observed that mussels only equalled or exceeded the preferred 30gram per mussel threshold in May 2015 and June and July 2016 for *M. galloprovincialis*, and May and July 2015 and July and September 2016 for *C. meridionalis*. This information can therefore help inform farmers on the best collection times according to the desired mussel weight, thereby saving both time and resources (such as the cost of running a boat and running processing equipment). Traditionally mussel harvest in Saldanha Bay ceased for almost a month in summer when the mussels were post-spawn, due to low flesh condition (Heasman, 1996), as is common practice on European mussel farms (Bressan & Marin, 1985). Currently, however, mussel harvest continues throughout the year despite their acknowledgement of severe weight loss in spring and summer; the Blue Ocean Mussels company, a major mussel processor and distributor for Saldanha Bay aquaculture, notes that mussels from spring and summer have a smaller meat size due to spawning, and advise consumers who prefer "meatier" mussels to buy frozen products instead (Anonymous, 2017).

Changes in the percentage meat yield are important to farmers and consumers alike, as meat yield is considered to be an important aspect of the marketability of mussels (Okumus & Stirling, 1998; Fuentes *et al.*, 2009). Though no literature could be found directly linking consumer preference to larger, fatter mussels, this researcher's personal experience, combined with the above stated importance of meat yield, suggests that consumers are likely to prefer a greater meat weight. The reason for this is undoubtedly that mussel shells are inedible and therefore if a client were to purchase a pack of mussels a reduced meat yield would result in the consumer paying proportionally more for shell than edible flesh weight. This situation would surely be unappealing to cost-sensitive clientele. The average meat yields of *C. meridionalis* and *M. galloprovincialis* mussels within this study were greater than the maximum meat yields found for *M. galloprovincialis* (~33% and ~34%) in the Gulf of Trieste (Bongiorno *et al.*, 2015) and Spain (Fuentes *et al.*, 2009), respectively, and *M. edulis* (38%) in Scotland (Okumus & Stirling, 1998). This is likely due to the highly productive natures of the waters in Saldanha Bay, which exceed the maximum food requirements for mussels throughout the year (Heasman, 1996; Olivier *et al.*, 2013), resulting in local mussels not experiencing the same depletion of resources experienced by foreign aquaculture operations (Dare & Edwards, 1975; Çelik *et al.*, 2012; Irisarri *et al.*, 2015; Azpeitia *et al.*, 2016). Mussels grown in Saldanha Bay are currently exported to numerous countries worldwide (DAFF, 2016); with plans for massive expansion of the mussel aquaculture industry, the observed superior meat yield of local over foreign mussels could be a valuable competitive advantage in marketing strategies promoting the export of Saldanha Bay mussels. The difference in spawning periods between Northern and Southern hemisphere mussels could also be exploited by companies wishing to export local mussels. In the Northern hemisphere, *M. galloprovincialis* is known to spawn in spring and summer (Orban *et al.*, 2002; Bongiorno *et al.*, 2015), which coincides with the peak meat yields of Saldanha Bay mussels in autumn and winter. Local producers could therefore guarantee high percentage meat weights from mussels in Saldanha Bay while those in the northern hemisphere are in their "spent" or post-spawn stage, potentially creating a large European market for South African mussels.

High inter-seasonal variability was observed in the moisture, protein, fat and ash contents for both mussel species. The moisture contents of both species were found to peak in spring 2015 and 2016, coinciding with the post-spawn phases and lowest protein contents of the mussels. Minimum moisture values occurred in autumn 2015 for *M. galloprovincialis* and winter 2015 for *C. meridionalis*, and winter 2016 for both species, coinciding with maximum protein contents for both species. Previous studies on *M. galloprovincialis* have found moisture content to fluctuate seasonally between 83.3 - 89% (Azpeitia *et al.*, 2016), 78 – 86% in Turkey (Çelik *et al.*, 2012) and 78.7 – 87.3% in the Adriatic Sea (Bongiorno *et al.*, 2015), with highest moisture contents often occurring when mussels were in their "spent" or post-spawn stage (Çelik *et al.*, 2012; Bongiorno *et al.*, 2015). The relative moisture content of mussels increases within this spent stage because lipid and protein reserves, which were built up for egg development pre-spawn, have been released and the percentage moisture content therefore increases relative to the organic constituents of the mussels (Fernández *et al.*, 2015). Moisture content has also been associated with the juiciness and texture of mussels, and is therefore an important technological aspect of meat quality and should be considered in the processing phase (Fuentes *et al.*, 2009; Bongiorno *et al.*, 2015).

The mussels in this study were found to accumulate protein to peak concentrations in winter of both years, pre-spawn, with rapid decreases in protein content as spring 2016 occurred. Previous studies on *M. galloprovincialis* found that protein content of the mussels to fluctuate seasonally from 6.5 – 10% (Fuentes *et al.*, 2009), 7.5 – 11.6% (Bongiorno *et al.*, 2015) and 7.5 – 11% (Azpeitia *et al.*, 2016), linking these changes with both gametogenesis and food availability (Orban *et al.*, 2002; Irisarri *et al.*, 2015; Azpeitia *et al.*, 2016). High protein levels have been associated with the build-up of reserves for gametogenesis, with low protein levels regularly being found in mussels post-spawn (Çelik *et al.*, 2012; Bongiorno *et al.*, 2015; Fernández *et al.*, 2015); this is supported by the fact that *M. edulis* eggs are composed of 53% protein and 21% fat (Pieters *et al.*, 1980). For both *M. galloprovincialis* and *C. meridionalis*, protein and whole weight followed very similar trends of accumulation and depletion. Though the changes in protein content cannot be inextricably linked to gametogenesis or food availability, *M. galloprovincialis* and *C. meridionalis* are known to spawn in early summer and mid-summer, respectively, and the decrease in total protein content of the mussels in spring and summer would suggest that the protein is being utilised in gametogenesis and the sharp drop-off is indeed caused by spawning. Protein was found to correlate both significantly and negatively with moisture content of *C. meridionalis* and positively with fat content, as has been the case in previous proximate analyses studies of *Mytilus* species (Okumus & Stirling, 1998; Fuentes *et al.*, 2009; Bongiorno *et al.*, 2015; Azpeitia *et al.*, 2016). The negative correlation between protein and moisture is evident in moisture content peaking in September and November of 2015, when protein content of the mussels was lowest, and in 2016 when moisture fell to a minimum in June, when protein was at a maximum. This relationship is likely due to moisture increasing proportionally in the mussels as the protein content drops post-spawn (Irisarri *et al.*, 2015). In 2016, coincidental peaks and dips in the protein and fat content in *C. meridionalis* indicate that there was a massive build-up of gamete reserves and subsequent large spawning event. This pattern follows with the information from the mussel famers in Saldanha Bay and published studies on this species; that *C. meridionalis* experiences vastly different spawning intensities in different years (Griffths, 1977; van Erkom Schurink & Griffiths, 1991). The whole weight, fat and protein data combined suggests that *C. meridionalis* did spawn at the end of 2015, but that it was a far less intense spawning than that of 2016. This cannot be conclusively stated, however, as gonadal smears to determine the state of gametogenesis within the mussels (which were not within the scope of this study) would be necessary to confirm exact spawning periods.

Fat content of *C. meridionalis* peaked in spring 2015, with a significantly greater peak in winter 2016, and fell to lowest values in autumn 2015 and spring 2016. In *M. galloprovincialis* the seasonal changes follow a less clear trend, with post-spawn peaks in the fat content in spring 2015 and 2016, and an unexpected drop in fat content in winter 2016, which is quickly recovered by spring. The less clear pattern evident in the fat content of the mussels could potentially be due to the unfortunate gaps in data collection which occurred over the study period; more even collection intervals may have presented a clearer seasonal pattern. Fat contents of *M. galloprovincialis* mussels are known to range temporally between 0.74 – 1.51% of wet weight in Northern Spain (Azpeitia *et al.*, 2016), 1 – 2.2% in the Gulf of Trieste (Bongiorno *et al.*, 2015) and 1.4 – 2.1% in Spain (Fuentes *et al.*, 2009), with seasonal changes in fat content found in numerous studies on mussels and other bivalves worldwide (Bressan & Marin, 1985; Chu *et al.*, 1990; Orban *et al.*, 2002; Prato *et al.*, 2010; Çelik *et al.*, 2012; Bongiorno *et al.*, 2015; Fernández *et al.*, 2015; Irisarri *et al.*, 2015). Studies on lipid contents of mussels have repeatedly linked the build-up and depletion of lipids to gametogenesis and the reproductive cycle (Bressan & Marin, 1985; Çelik *et al.*, 2012), although the periods of maxima and minima differ between sites; some state maxima in summer (Orban *et al.*, 2002; Bongiorno *et al.*, 2015) while others state autumn and winter maxima (Çelik *et al.*, 2012; Irisarri *et al.*, 2015). All papers agree, though, that lipids increase in the mussel tissue before spawning and are greatly depleted post-spawn. The peaks in fat content of *M. galloprovincialis* occurred in spring and summer, when the winds which cause upwelling are strong and the phytoplankton count in Saldanha Bay would be high, thereby allowing intensive feeding and weight gain. The cause of the sharp dip and recovery in fat content of *M. galloprovincialis* in winter 2016 (and the second dip in summer 2017) could potentially be due to the fact that this species has been found to spawn in early spring and quickly recover the gonadal tissue for a second spawning event later in spring or summer (Villalba, 1995). The pattern of lipid accumulation and depletion in *C. meridionalis* was discussed above in relation to protein content, due to the high correlation of the two variables.

Maximum ash contents occurred in spring 2015 and summer 2016 for both species, with minima in autumn 2015 and winter 2016, following a similar seasonal pattern to that of moisture. Studies assessing the ash content of mussels have found significant temporal variation between 2.2 – 3.3% (Bongiorno *et al.*, 2015) and 2.4 – 4.2% of wet weight of *M. galloprovincialis* (Azpeitia *et al.*, 2016), 11-21% (Orban *et al.*, 2002) and 4.2 – 14% of dry weight in *M. galloprovincialis* and *M. edulis* respectively (Okumus & Stirling, 1998). The ash content represents the amount of inorganic substances present within the mussels and therefore would be expected to increase proportionally as biochemical reserves (protein and lipids) are depleted in gametogenesis (Fernández *et al.*, 2015), but highest ash contents have been found to coincide with highest moisture contents in certain studies (Fuentes *et al.*, 2009; Bongiorno *et al.*, 2015), highest protein and fat contents in others (Okumus & Stirling, 1998; Azpeitia *et al.*, 2016), and lowest protein contents in some (Çelik *et al.*, 2012). Though no biologically significant relationships were observed between ash and the other measured proximate variables in this study, ash contents did appear to follow a somewhat reverse pattern when compared to that of protein, in both species.

The moisture, protein, fat and ash contents of the mussels within Saldanha Bay generally fell within the ranges found by previous studies. Previous studies have found that the proximate composition of mussels is significantly impacted by the composition of the phytoplankton biomass on which the mussels feed, with significant variation being found between separate locations in Spain at the same time of the year (Fuentes *et al.*, 2009; Irisarri *et al.*, 2015). This could explain why the mussels in this study experienced higher overall fat and protein contents than prior studies; the differences are potentially due to different compositions of the phytoplankton biomass on which the mussels feed, in the northern and southern hemispheres. Further research would be necessary to confirm this, however.

What does this information mean for the consumer? Mussels have been found to be a good source of essential minerals, high biological value proteins, non-cholesterol sterols and omega-3 polyunsaturated fatty acids (Karakoltsidis *et al.*, 1995; España *et al.*, 2007; Ventrella *et al.*, 2008). In comparison to previous studies on *Mytilus* species, both mussel species from the aquaculture facilities in Saldanha Bay were found to be relatively high in fat and protein contents. Modern human diets, specifically in Western culture, have been found to be high in carbohydrates and omega-6 fatty acids, but low in protein and severely lacking in omega-3 fatty acids, and this diet structure is being increasingly linked to coronary heart disease, diabetes and obesity (Simopoulos, 2002). Progressively, humans are being encouraged to incorporate more healthy proteins and fatty acids into their diets, and the low fat content of mussels (when compared to beef or pork), combined with their high omega-3 polyunsaturated fatty acid composition (Orban *et al.*, 2002; Martínez-Pita *et al.*, 2012; Bongiorno *et al.*, 2015; Fernández *et al.*, 2015; Azpeitia *et al.*, 2016), could make these bivalves a healthy dietary choice for modern consumers.

There is a drive worldwide by sustainable seafood initiatives to encourage consumers to become more aware of where their seafood comes from and how it is caught or farmed, in order to preserve the stocks of wild fish and to ensure intensive aquaculture operations do not negatively impact on the environment (WWF, 2014). Therefore, another benefit of eating farmed, South African mussels is that both *C. meridionalis* and *M. galloprovincialis* from mussel aquaculture in Saldanha Bay are listed as "green" by the WWF-SASSI (World Wildlife Fund – South African Sustainable Seafood Initiative) list, meaning they are a sustainably managed aquaculture industry and making them a guilt-free farmed seafood choice for informed consumers (WWF-SASSI, 2017).

3.6. CONCLUSION

The aim of this study was to assess morphometric variations between the two farmed mussel species of Saldanha Bay, as well as temporal and inter-species differences in whole weight, meat weight, meat yield and proximate composition in *C. meridionalis* and *M. galloprovincialis*. The morphometric dimensions of the shells were found to be significantly different, with *M. galloprovincialis* individuals being larger, wider and more ventrally flattened than *C. meridionalis*. The whole weights, meat weights, percentage meat yields and proximate composition of *C. meridionalis* and *M. galloprovincialis* were found to experience significant seasonal changes, but species-related differences were not clear over the study period. Temporal variability in the biometrics and proximate composition of both species were linked to the reproductive cycles of the mussels, with peaks in whole weight, protein and fat (associated with gamete build-up) generally occurring before the spring and summer spawning periods for both species. Peak moisture and ash content were generally found post-spawn, following a reverse pattern to that of fat and protein content. This data fills gaps in the knowledge of the proximate composition of *C. meridionalis*, and the changes in proximate condition experienced by both species in Saldanha Bay. This research could potentially be used to aid farmers in determining 'off' seasons, when the weights and overall condition of these mussels are below the minimum threshold that consumers would enjoy. The data from this study also shows that mussels from Saldanha Bay have low overall fat content, and a superior protein content when compared to European *Mytilus* species mussels, meaning that they are a healthy source of high quality nutrition to consumers.

3.7. REFERENCES

Anonymous. 2017. Blue Ocean Mussels. http://blueoceanmussels.com. Date accessed: 15th August 2017.

AOAC International. 2002. Official methods of analysis. 17th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Azpeitia, K., Ferrer, L., Revilla, M., Pagaldai, J., & Mendiola, D. 2016. Growth, biochemical profile, and fatty acid composition of mussel (*Mytilus galloprovincialis* Lmk.) cultured in the open ocean of the Bay of Biscay (northern Spain). Aquaculture 454, 95–108 https://doi.org/10.1016/j.aquaculture.2015.12.022.

Azpeitia, K., Ríos, Y., Garcia, I., Pagaldai, J., & Mendiola, D. 2017. A sensory and nutritional validation of open ocean mussels (*Mytilus galloprovincialis* Lmk.) cultured in SE Bay of Biscay (Basque Country) compared to their commercial counterparts from Galician Rías (Spain). Int. Aquat. Res. 9, 89–106 https://doi.org/10.1007/s40071-017-0159-0.

Bayarri, S., Baldassarri, L. T., Iacovella, N., Ferrara, F., & Domenico, A. Di. 2001. PCDDs, PCDFs, PCBs and DDE in edible marine species from the Adriatic Sea. Chemosphere 43, 601–610 https://doi.org/10.1016/S0045-6535(00)00412-4.

Bongiorno, T., Iacumin, L., Tubaro, F., Marcuzzo, E., Sensidoni, A., & Tulli, F. 2015. Seasonal changes in technological and nutritional quality of *Mytilus galloprovincialis* from suspended culture in the Gulf of Trieste (North Adriatic Sea). Food Chem. 173, 355–362 https://doi.org/10.1016/j.foodchem.2014.10.029.

Bressan, M., & Marin, M. G. 1985. Seasonal variations in biochemical composition and condition index of cultured mussels (*Mytilus galloprovincialis* Lmk) in the Lagoon of Venice (North Adriatic). Aquaculture 48, 13–21 https://doi.org/10.1016/0044-8486(85)90048-1.

Çelik, M. Y., Karayücel, S., Karayücel, I., Öztürk, R., & Eyüboğlu, B. 2012. Meat yield, condition index, and biochemical composition of mussels (*Mytilus galloprovincialis* Lamarck, 1819) in Sinop, south of the Black Sea. J. Aquat. Food Prod. Technol. 21, 198–205 https://doi.org/10.1080/10498850.2011.589099.

Chu, F. L. E., Webb, K. L., & Chen, J. 1990. Seasonal changes of lipids and fatty acids in oyster tissues (*Crassostrea virginica*) and estuarine particulate matter. Comp. Biochem. Physiol. - Part A Physiol. 95, 385–391 https://doi.org/10.1016/0300-9629(90)90237-M.

Clark, B. M., Massie, V., Hutchings, K., Laird, M., Biccard, A., Brown, E., Duna, O. O., & Turpie, J. 2016. The State of Saldanha Bay and Langebaan Lagoon 2016. Technical Report. Report No AEC 1691/1 prepared by Anchor Environmental Consultants (Pty) Ltd for the Saldanha Bay Water Quality

Forum Trust.

Cossa, D., Bourget, E., & Piuze, J. 1979. Sexual maturation as a source of variation in the relationship between cadmium concentration and body weight of *Mytilus edulis* L. Mar. Pollut. Bull. 10, 174–176 https://doi.org/10.1016/0025-326X(79)90425-9.

DAFF. 2016. Aquaculture Yearbook 2015. The Department of Agriculture, Forestry and Fisheries, Cape Town.

Dare, P. J., & Edwards, D. B. 1975. Seasonal Changes in Flesh Weight and Biochemical Composition of Mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales. J. Exp. Mar. Bio. Ecol. 18, 89–97.

España, M. S. A., Rodríguez, E. M. R., & Romero, C. D. 2007. Comparison of mineral and trace element concentrations in two molluscs from the Strait of Magellan (Chile). Arch. Environ. Contam. Toxicol. 52, 519–524 https://doi.org/10.1007/s00244-006-0104-5.

Fernández, A., Grienke, U., Soler-Vila, A., Guihéneuf, F., Stengel, D. B., & Tasdemir, D. 2015. Seasonal and geographical variations in the biochemical composition of the blue mussel (*Mytilus edulis* L.) from Ireland. Food Chem. 177, 43–52 https://doi.org/10.1016/j.foodchem.2014.12.062.

Filer, L. J. 1974. Fat Content and Composition of Animal Products. Page 250 in Board on Agriculture and Renewable Resources; Commission on Natural Resources; Food and Nutrition Board; Assembly of Life Sciences; National Research Council, Washington, D.C.

Fuentes, A., Fernández-Segovia, I., Escriche, I., & Serra, J. A. 2009. Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins. Food Chem. 112, 295–302 https://doi.org/10.1016/j.foodchem.2008.05.064.

Gosling, E. M. 2004. Bivalve molluscs. First Edit. Fishing News Books, a division of Blackwell Publishing, Cornwall.

Griffiths, R. J. 1981. Population Dynamics and Growth of the Bivalve *Choromytilus meridionalis* at Different Tidal Levels. Estuar. Coast. Shelf Sci. 12, 101–118.

Griffths, R. J. 1977. Reproductive Cycles in Littoral Populations of *Choromytilus meridionalis* (Kr.) and *Aulacomya ater* (Molina) with a Quantitative Assessment of Gamete Production in the Former. J. Exp. Mar. Bio. Ecol. 30, 53–71.

Heasman, K. G. 1996. The Influence of Oceanographic Conditions and Culture Methods on the Dynamics of Mussel Farming in Saldanha Bay, South Africa. MSc, Rhodes University, Rhodes.

Heasman, K. G., Pitcher, G. C., McQquaid, C. D., & Hecht, T. 1998. Shellfish mariculture in the Benguela system: Raft culture of *Mytilus galloprovincialis* and the effect of rope spacing on food extraction, growth rate, production, and condition of mussels. J. Shellfish Res. 17, 33–39.

Irisarri, J., Fernández-Reiriz, M. J., & Labarta, U. 2015. Temporal and spatial variations in proximate composition and Condition Index of mussels *Mytilus galloprovincialis* cultured in suspension in a shellfish farm. Aquaculture 435, 207–216 https://doi.org/10.1016/j.aquaculture.2014.09.041.

Karakoltsidis, P. a., Zotos, A., & Constantinides, S. M. 1995. Composition of the commercially important Mediterranean finfish, crustaceans, and molluscs. J. Food Compos. Anal. 8, 258–273 https://doi.org/10.1006/jfca.1995.1019.

Lee, C. M., Trevino, B., & Chaiyawat, M. 1996. A Simple and Rapid Solvent Extraction Method for Determining Total Lipids in Fish Tissue. J. AOAC Int. 79, 487–492.

Martínez-Pita, I., Sánchez-Lazo, C., Ruíz-Jarabo, I., Herrera, M., & Mancera, J. M. 2012. Biochemical composition, lipid classes, fatty acids and sexual hormones in the mussel *Mytilus galloprovincialis* from cultivated populations in south Spain. Aquaculture 358–359, 274–283 https://doi.org/10.1016/j.aquaculture.2012.06.003.

Okumus, I., & Stirling, H. P. 1998. Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. Aquaculture 159, 249–261 https://doi.org/10.1016/S0044-8486(97)00206-8.

Olivier, D., Heinecken, L., & Jackson, S. 2013. Mussel and oyster culture in Saldanha Bay, South Africa: Potential for sustainable growth, development and employment creation. Food Secur. 5, 251–267 https://doi.org/10.1007/s12571-013-0244-1.

Orban, E., Di Lena, G., Nevigato, T., Casini, I., Marzetti, A., & Caproni, R. 2002. Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites. Food Chem. 77, 57–65 https://doi.org/10.1016/S0308-8146(01)00322-3.

Pieters, H., Kluytmans, J. H., Zandee, D. I., & Cadée, G. C. 1980. Tissue Composition and Reproduction of *Mytilus edulis* in Relation to Food Availability. Netherlands J. Sea Res. 14, 349–361.

Pitcher, G. C., & Calder, D. 1998. Shellfish mariculture in the Benguela system: Phytoplankton and the availability of food for commercial mussel farms in Saldanha Bay, South Africa. J. Shellfish Res. 17, 15–24.

Prato, E., Danieli, A., Maffia, M., & Biandolino, F. 2010. Lipid and fatty acid compositions of *Mytilus galloprovincialis* cultured in the Mar Grande of Taranto (Southern Italy): Feeding strategies and trophic relationships. Zool. Stud. 49, 211–219 https://doi.org/10.1007/s11745-997-0127-8.

Raatz, S. K., Silverstein, J. T., Jahns, L., & Picklo, M. J. 2013. Issues of fish consumption for cardiovascular disease risk reduction. Nutrients 5, 1081–1097 https://doi.org/10.3390/nu5041081.

Richir, J., & Gobert, S. 2014. The effect of size, weight, body compartment, sex and reproductive status on the bioaccumulation of 19 trace elements in rope-grown *Mytilus galloprovincialis*. Ecol. Indic. 36, 33–47 https://doi.org/10.1016/j.ecolind.2013.06.021.

Shipton, T., & Britz, P. J. 2007. A study on the status of aquaculture production and trade in South Africa. Volume 1: Industry Status and Diagnostic Report. A report for the Department of Trade and Industry produced by Enviro-Fish Africa (Pty.) Ltd. 90p.

Simopoulos, A. P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother. 56, 365–379 https://doi.org/10.1016/S0753-3322(02)00253-6.

van Erkom Schurink, C., & Griffiths, C. L. 1990. Marine mussels of Southern Africa - their distribution patterns, standing stocks, exploitation and culture. J. Shellfish Res. 9, 75–85 https://doi.org/10.2983/035.029.0302.

van Erkom Schurink, C., & Griffiths, C. L. 1991. A comparison of reproductive cycles and reproductive output in four southern African musel species. Mar. Ecol. Prog. Ser. 76, 123–134 https://doi.org/10.3354/meps076123.

van Erkom Schurink, C., & Griffiths, C. L. 1992. Physiological energetics of four South African mussel species in relation to body size, ration and temperature. Comp. Biochem. Physiol. -- Part A Physiol. 101, 779–789 https://doi.org/10.1016/0300-9629(92)90358-W.

van Erkom Schurink, C., & Griffiths, C. L. 1993. Factors affecting relative rates of growth in four South African mussel species. Aquaculture 109, 257–273 https://doi.org/10.1016/0044-8486(93)90168-X.

Ventrella, V., Pirini, M., Pagliarani, A., Trombetti, F., Manuzzi, M. P., & Borgatti, A. R. 2008. Effect of temporal and geographical factors on fatty acid composition of *M. galloprovincialis* from the Adriatic Sea. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 149, 241–250 https://doi.org/10.1016/j.cbpb.2007.09.012.

Villalba, A. 1995. Gametogenic cycle of cultured mussel, *Mytilus galloprovincialis*, in the bays of Galicia (N.W. Spain). Aquaculture 130, 269–277.

Waldron, H. N., & Probyn, T. A. 1992. Nitrate supply and potential new production in the Benguela upwelling system. South African J. Mar. Sci. 12, 29–39 https://doi.org/10.2989/02577619209504688.

WWF. 2014. From boat to plate. https://doi.org/10.13140/RG.2.1.2814.0967. Date accessed: 12th October 2017.

WWF-SASSI. 2017. The SASSI List. http://wwfsassi.co.za/sassi-list/. Date accessed: 11th November 2017.

CHAPTER 4

Bioaccumulation of trace metals in *Mytilus galloprovincialis* and *Choromytilus meridionalis* from aquaculture facilities in Saldanha Bay, South Africa

4.1. ABSTRACT

Mussels are a healthy source of protein, minerals and healthy fats, but can also pose health risks to consumers via the accumulation and concentration of excess trace metals from the marine environment into their flesh. Saldanha Bay is the location of heavy industry and international shipping activities, resulting in concerns over pollution levels within the waters of the bay for aquaculture facilities. To investigate seasonal and inter-species variations in trace metal content of the two farmed mussels, *C. meridionalis* and *M. galloprovincialis*, samples were collected from commercial mussel rafts located in the centre of Saldanha Bay over a two-year period and analysed for trace metal content using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Variability in trace metal accumulation rates was observed between the two species; Al, Cr, Fe, Zn, Cd and Pb were higher in *M. galloprovincialis*, while *C. meridionalis* accumulated Cu and Mn to a greater extent. Species-related differences in trace metal accumulation were attributed to variations in filtration rate and feeding strategies (regarding particle selection processes). Temporal fluctuations were noted in Fe and As concentrations in *C. meridionalis*, and Fe, As, Hg and Pb in *M. galloprovincialis*. Both species were determined to be healthy sources of essential metals (Mn, Fe, Cu, Zn and Cr), and toxic Hg and Cd were below regulatory limits. Arsenic exceeded the South African regulatory limit (3mg/kg) at one time period in each species (Max = 3.4mg/kg w.w.). Although the Pb concentrations in *M. galloprovincialis* were consistently within EU regulatory limits for bivalves (1.5mg/kg), they exceeded the South African regulatory limits for fish (0.5mg/kg) at four time-periods (winter and spring). Improperly treated waste, storm and ballast waters, as well as the export of multiple ores, were determined to be concerning sources of As and Pb. Overall, the farmed mussels from Saldanha Bay were determined to be largely safe for human consumption, with some caution required in relation to As and Pb concentrations. It is important for the South African government to establish bivalve-specific regulations to support the growth of the mussel and oyster aquaculture industry, and to closely monitor the expansion of heavy industry growth and pollution in Saldanha Bay, which are expected to increase in the near future.

4.2. INTRODUCTION

Mussels are generally considered to be a good source of protein, minerals, vitamins and healthy fats, particularly polyunsaturated fatty acids such EPA and DHA (Bongiorno *et al.*, 2015; Fernández *et al.*, 2015), which have been linked to decreased cardiovascular disease in humans (Raatz *et al.*, 2013).

Unfortunately, due to their filter-feeding nature and aquatic lifestyle, mussels are exposed to a variety of marine pollutants and contaminants which they have the potential to accumulate and concentrate in their flesh (beyond the ambient water concentrations), and can therefore pose a health risk to human consumers (Stanković & Jović, 2012).

A group of common and well-studied pollutants in marine environments and molluscs are the trace metals. Trace elements are those which are present in all living organisms in extremely small amounts, and a wide variety of trace metals are found in living tissue (Reilly, 2004). Trace metals are divided into three groups: essential trace metals, non-essential trace metals and toxic trace metals. Essential metals include boron (B), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn). These metals are essential to the healthy functioning of biological organisms, but can also become toxic if present in excess (Pais & Benton-Jones, Jr., 1997). Non-essential metals are those that play no known biological role in living organisms, but also do not have specific toxicities. Toxic trace metals are those which have high toxicity at very low doses, including arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg) (Stanković & Jović, 2012).

While a portion of trace metals are introduced into the marine coastal environment naturally, the majority of trace metal pollution occurs through anthropogenic sources such as industrial effluent, urban run-off, mining operations and atmospheric emissions (Sunlu, 2006). Anthropogenic sources of trace metal pollution are of particular concern in coastal zones, and elevated trace metal concentrations have been linked to increasing human population densities, heavy industrial activity and river or storm water inputs (Chase *et al.*, 2001; Kljaković-Gašpić *et al.*, 2010). Trace metals enter the marine food chain either through direct contact (trace metals in sediments in which the organisms live) or through their incorporation into the algae upon which low-trophic level animals feed (Förstner & Wittmann, 1979), and trace metal bioaccumulation in mussels has been identified as a serious concern for both the health of mussels and the humans who may consume them, as well as a useful tool for monitoring the health of marine ecosystems (Goldberg, 1975; Gosling, 2004; Stanković & Jović, 2012). It is therefore imperative for aquaculture operations to regularly monitor the contamination levels of the marine environment and the mussels living within it in order to ensure the safety of consumers and monitor the impact human pollution is having on the oceans (Goldberg, 1975; Stanković & Jović, 2012).

In order to protect human consumers from excess trace metals in their foodstuffs, the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) have developed maximum levels (MLs) and Provisional Tolerable Weekly Intakes (PTWI) for metals of concern (CAC, 1995). MLs are established for specific foods or products through which humans are likely to be exposed to toxic metals, and PTWIs are set for "metal contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods", and are typically used for trace heavy metals which are known to accumulate in the human body (CAC, 1995, 2011). The international and local ML's for some of the most common toxic trace metals in bivalves and fish are summarised in Table 4.1.

Table 4.1 Local and international maximum limits for toxic and essential metals (mg/kg wet weight)

Metal	ML	Regulatory Body	Specifications
Arsenic	3.0	South Africa, 1994	Shellfish (including cephalopods) and shellfish products
	3.0	DOH, 2004	Fish and processed fish
Cadmium	3.0	South Africa, 1994	Shellfish (including cephalopods) and shellfish products
	3.0	DOH, 2004	Shellfish and shellfish products
	1.0	EC, 2006	Bivalve molluscs
	2.0	GAIN, 2014	Bivalves (viscera removed)
Chromium	2.0	GAIN, 2014	Fish and shells
Copper	50.0	South Africa, 1994	Shellfish (including cephalopods) and shellfish products
	30.0	JECFA, 1982	Based on PTWI of 0.5 mg/kg bodyweight
Lead	4.0	South Africa, 1994	Shellfish (including cephalopods) and shellfish products
	0.5	DOH, 2004	Fish and processed fish
	1.5	EC, 2006	Bivalve molluscs
Mercury	1.0	South Africa, 1994	Shellfish (including cephalopods) and shellfish products
	0.5	DOH, 2004	Shellfish and shellfish products
	0.5	EC, 2006	Fishery products
Zinc	300.0	South Africa, 1994	Shellfish (including cephalopods) and shellfish products
	50.0	USDA, 2003	Fish

Concerns over the health and safety of the waters in Saldanha Bay have been raised by residents and seafood farmers alike, particularly in light of the fact that there are plans for expansion of many of the heavy industries in the area (Olivier *et al.*, 2013; Clark *et al.*, 2014). Originally Saldanha Bay was one large, open bay, but in 1975 the building of an iron ore jetty and the Marcus Island causeway (Figure 4.1) bisected the bay and drastically changed the hydrographic properties of the area (Weeks *et al.*, 1991). This constriction of the seawater flow into Small Bay could potentially be resulting in harmful pollutants remaining in the bay for extended periods of time, and regular monitoring of metal contamination levels has been suggested to ensure the safety of their products (Olivier *et al.*, 2013). Since the construction of the iron ore jetty and Marcus Island causeway, Saldanha Bay has become an international deep-water shipping port for iron, lead, copper, zinc and manganese ores, steel and other industrial products (Clark *et al.*, 2014). Subsequent to the building of this iron ore terminal, overall trace metal concentrations within Small Bay were found to increase drastically, likely due to dust from the ore piles entering the marine ecosystem through storm water or wind (Clark et al. 2017).

The number of ships visiting Saldanha Bay has also doubled in the last decade, and these ships are known to regularly release ballast water (~24 million tonnes in 2016) into Saldanha Bay which exceeds the regulations set by the Southern African government for cadmium, zinc, lead, chromium and nickel (Clark *et al.*, 2016). These large ships, combined with recreational ships and yachts from Small Bay, have also been stated to be a concerning source of cadmium and copper in the area due to their use of antifouling paints (Clark *et al*. 2017). In addition to this, the Saldanha Bay Waste Water Treatment Works, which treats the sewage water from Saldanha, as well as effluent water from abattoirs, hotels, numerous fishing companies, and the mining and steel industries, regularly fails to meet water quality standards for waste water (Clark *et al.*, 2014). This results in water loaded with nutrients and trace metal pollutants being released into Small Bay; a serious concern for the health of the bay. Lastly, a seasonal pollution concern is the untreated, unmanaged storm water runoff which enters the bay during the winter rainfall period and introduces pesticides, hydrocarbons and trace metals into the marine environment (Clark *et al.*, 2014).

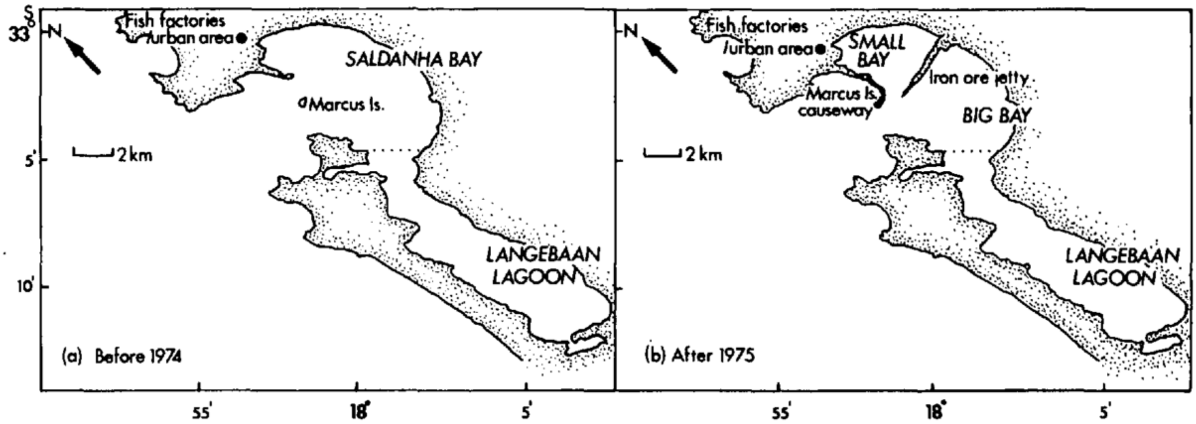


Figure 4.1 Before and after the building of the iron ore jetty and Marcus Island causeway in Saldanha Bay, illustrating the constriction of water flow into Small Bay (Weeks *et al.*, 1991)

Due to the serious threat that even low concentrations of toxic metals pose to human consumers, and the success of worldwide Mussel Watch Programmes, metal concentrations have been assessed numerous times in various mussel species and environments worldwide (Watling & Watling, 1976; Orescanin *et al.*, 2006; Przytarska *et al.*, 2010; Degger *et al.*, 2011b; Dahms *et al.*, 2014; Richir & Gobert, 2014; Sparks *et al.*, 2014; Maar *et al.*, 2015). Many of these studies have identified seasonal trends in the metal contamination levels of farmed and wild mussel species, linking the phases of accumulation and depletion to gametogenic cycles, water temperatures and precipitation events, and the majority of research agrees that trace metal accumulation in bivalves is strongly impacted by proximity to anthropogenic pollution sources such as human settlements and industry.

Recent studies on *M. galloprovincialis* mussels collected from varying locations in Saldanha Bay determined that wild, shore-collected mussels consistently exceeded the local and international MLs in foodstuffs for lead, cadmium and zinc (Clark *et al.*, 2014; Pavlov *et al.*, 2015). Samples from the aquaculture rafts, however, were rarely found to exceed maximum allowable limits, possibly due to their increased exposure to fresh water when compared to shoreline specimens (Clark *et al.*, 2014; Pavlov *et al.*, 2015). Another study using transplanted *Perna perna* mussels in Saldanha Bay determined concerning levels of arsenic accumulating within the mussel tissue (Degger, 2010). In addition to this, samples of smoothhound shark (*Mustelus mustelus*) in Langebaan Lagoon, adjacent to Saldanha, were found to regularly exceed MLs for mercury and arsenic, indicating that these are pollutants of concern in the area (Bosch *et al*., 2016). Despite these concerning findings, mussel farms in Saldanha Bay are only required to test the metal contamination levels of their product twice a year (*pers*. *comm*. Vos Pienaar, Imbaza Mussels, 30th May 2016). Considering that metal concentrations in *M. galloprovincialis* and other mussels species have significant intra- and inter-annual fluctuations (Casas *et al.*, 2004; Orescanin *et al.*, 2006; Przytarska *et al.*, 2010; Degger *et al.*, 2011b; Sparks *et al.*, 2014), biannual testing of samples from the aquaculture facility may be insufficient, potentially resulting in an incorrect representation of metal contamination levels.

Aside from a handful of studies on mussels in Saldanha Bay and Cape Town, there has been an overall decline in marine pollution studies in South Africa since the 1980's, and currently the minimal monitoring of the metal contamination levels of marine fauna are performed by the government and not released to the public (Wepener & Degger, 2012). Researchers in the marine pollution field have suggested a desperate need for increased studies on trace metals and other pollutants in marine environments in order to create baseline studies and facilitate a greater understanding of their sources and patterns of accumulation (Degger, 2010; Wepener & Degger, 2012; Sparks *et al.*, 2014). Metal contamination studies on farmed mussels are likewise important for both farmers and consumers, as they allow consumers to determine the health of their food sources, and farmers to better understand levels of essential and toxic trace metals in their samples, which can effect both consumers and the health of the mussels themselves (Dahms *et al.*, 2014).

The aims of this research are therefore to assess the trace metal contamination levels within the farmed mussels from Saldanha Bay, create baseline data for future studies on the health of the bay, and gain an understanding of patterns of accumulation and depletion of trace metals in both *C. meridionalis* and *M. galloprovincialis* to aid farmers and consumers alike with regards to healthy mussel production and consumption.

4.3. MATERIALS AND METHODOLOGY

For study location, sampling methods and sample preparation, please refer to Chapter 3.

4.3.1. Sample analysis

The dried, homogenised samples (n=30 per species) were used for trace metal analyses via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The concentrations of 21 metals [Lithium (Li), Beryllium (Be), Boron (B), Aluminium (Al), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Strontium (Sr), Molybdenum (Mo), Cadmium (Cd), Antimony (Sb), Barium (Ba), Mercury (Hg) and Lead (Pb)] were assessed in *M. galloprovincialis* and *C. meridionalis*.

In order to solubilise the acid-extractable elemental content of the sample, digestion was performed on a MARS microwave digestor, using ultra-pure HNO_3 and HCl, at elevated temperature and pressure. After a cooling period, the extractant was made up to 50ml volume with deionised water, then analysed by ICP-MS for the selected analytes.

Trace elements were analysed on an Agilent 7900 quadrupole ICP-MS. Samples are introduced via a 0.4ml/min micromist nebulizer into a peltier-cooled spraychamber at a temperature of 2°C. The instrument was optimised for analysis in HMI mode, where all samples and standards are diluted with argon gas to minimise matrix load to the analyser. Oxide formation was less than 0.3%. 3 Replicate measurements with appropriate dwell times were done on each analyte. All elements were measured in He-collision mode. An internal standard (ISTD) solution containing scandium (Sc), yttrium (Y), germanium (Ge), rhodium (Rh) and Indium (In) was introduced online to monitor instrument drift and correct for matrix differences between samples and standards. Appropriate ISTD's were matched to analytes according to their proximity in mass and ionisation potential. The instrument was calibrated using NIST traceable standards from Inorganic Ventures (INORGANIC VENTURES, 300 Technology Drive, Christiansburg, VA 24073) to quantify selected elements. NIST-traceable quality control standards at high and low concentration levels, from De Bruyn Spectroscopic Solutions, Bryanston, South Africa, were analysed to verify the accuracy of the calibration before sample analysis, as well as every 12 samples to monitor drift. During the course of the analysis, ISTD recovery was between 90 and 110% for all samples, and recovery for drift monitor standards between 95 and 105%. Results from the ICP-MS analyses were given in µg/kg dry weight and converted to mg/kg wet weight (wet weight conversion equation stated in Chapter 3) for statistical analysis and to allow comparison to legal maximum limits.

<u>Please Note:</u>

The accumulation of certain trace metals has been found to differ significantly between the sexes in both *C. meridionalis* and *M. galloprovincialis* (Watling & Watling, 1976; Richir & Gobert, 2014). This study did not assess sex-related differences in trace metal content because humans consume mussels irrespective of their sex and therefore overall species nutritional composition and toxicity is more important than sex-specific levels of contaminants (Watling & Watling, 1976; Orren *et al.*, 1980). It is assumed that the random sub-sample used for this study is representative of the natural ratio of males to females.

Despite previous studies recommending depuration of mussels prior to analysis to eliminate gut contents (Orren *et al.*, 1980), mussels in this study were not depurated because the processing factory in Saldanha Bay (Blue Ocean Mussels) does not depurate their products before sale. The intention of this study was to evaluate the health risk that mussels could pose to consumers, and since consumers eat the whole mussel (gut included) the decision was made to keep the samples intact.

4.3.2. Statistical analysis

The experimental design was a completely randomised design, with samples being collected every two months from Imbaza Mussels' rafts during normal harvesting processes. The two main effects were collection date and species. All data was analysed in Microsoft Excel using the additional statistical analysis software *X*LSTAT Premium (Annual Version 19.4.45237), developed by Addinsoft.

The normality and homoscedasticity of the data was checked, and data points contributing to high variability were removed when necessary (i.e. when they were determined to be due to experimental error or when their removal significantly decreased the heteroscedasticity of the data). Two-way ANOVAs were then performed, with collection date and species selected as the main effects, and an interaction term was included. Where data was determined to be heteroscedastic, Welch's t-test statistics were included to ensure heteroscedasticity did not affect the accuracy of the analysis. Post-hoc Bonferroni pairwise comparison tests were performed on all data to determine where significant differences were present. Pearson's correlation matrices were created for all metals (including proximate analyses data from Chapter 3) in order to examine potential linear relationships. For this study, though the Pearson's test itself identified many of the correlations as statistically significant, they were only considered to be biologically significant if the correlation coefficient (R value) equalled or exceeded 0.7.

4.4. RESULTS

Though a total of 21 metals were analysed in the samples, the Results and Discussion sections of this Chapter will focus on 10 essential and toxic elements which were chosen for further investigation and discussion due either to their frequently assessed status in mussels, or because they have previously been identified as trace metals of concern to human health in Saldanha Bay (Degger, 2010; Clark *et* *al.*, 2014; Bosch *et al.*, 2016). The ranges, means and standard errors of the 11 metals not discussed below are listed in Addendum A.

4.4.1. Species-related differences:

The average concentrations of Al, Cr, Fe, Zn, Cd and Pb were significantly higher in *M. galloprovincialis*, while *C. meridionalis* had higher than average concentrations of Mn and Cu (Table 4.2).

Table 4.2 Range and mean trace metal compositions (mg/kg wet weight) of *C. meridionalis* and *M. galloprovincialis*, in increasing order of composition

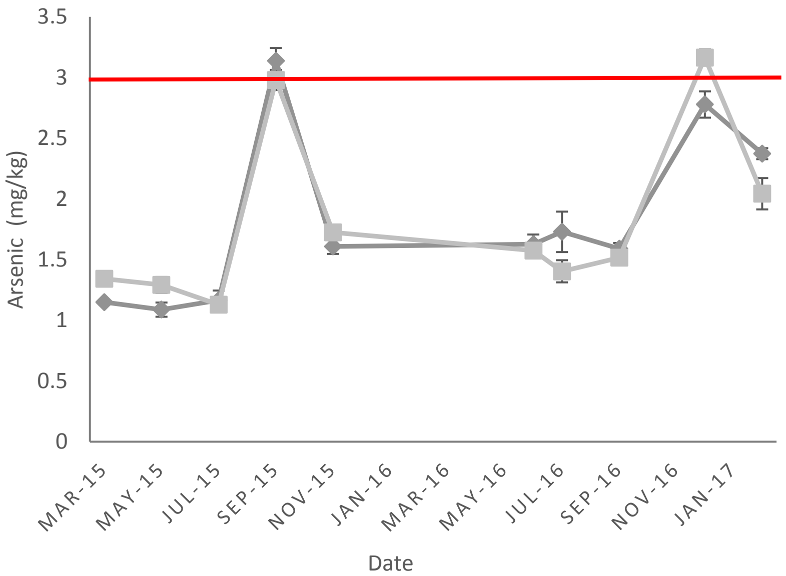
Trace Metal	**Mean (± std. error)**		**Range** (min - max)	
	C. meridionalis	*M. galloprovincialis*	*C. meridionalis*	*M. galloprovincialis*
Hg	0.003 ± 0.0001	0.004 ± 0.0001	0.002 - 0.01	0.002 - 0.005
Cr	0.1 ± 0.01	**0.2 ± 0.01**	0.03 - 0.32	0.10 - 0.38
Pb	0.2 ± 0.01	**0.5 ± 0.02**	0.10 - 0.36	0.26 - 0.79
Cd	0.4 ± 0.03	**1.0 ± 0.03**	0.16 - 0.98	0.57 - 1.40
Cu	**1.2 ± 0.03**	0.7 ± 0.02	0.69 - 1.69	0.35 - 1.01
Mn	**1.6 ± 0.04**	0.5 ± 0.01	0.95 - 2.15	0.39 - 0.94
As	1.8 ± 0.09	1.8 ± 0.09	0.82 - 3.40	0.96 - 3.37
Al	6.4 ± 0.42	**17.3 ± 0.82**	0.62 - 14.01	6.62 - 32.25
Fe	11.3 ± 0.42	**25.1 ± 0.76**	5.10 - 18.02	15.30 - 35.87
Zn	15.5 ± 0.32	**25.9 ± 0.52**	10.15 - 22.25	18.45 - 37.44

*means in bold denote significant species differences at $p \leq 0.0001$

4.4.2. Temporal trends:

There were no clear, biologically significant seasonal trends observable for Al, Cr, Mn, Cu, Zn, Cd, Hg and Pb for *C. meridionalis*, though both Mn and Cu showed significantly higher concentrations in 2016 than 2015. In *C. meridionalis*, Fe dropped to a minimum in spring 2015 and summer 2016, when As concentrations peaked. Arsenic concentrations showed substantial peaks in spring 2015 and summer 2016, exceeding the South African ML in spring 2015 (Figure 4.2).

No temporal patterns were observable in the concentrations of Al, Cr, Mn, Cu, and Zn for *M. galloprovincialis*. In *M. galloprovincialis* samples, Fe was found to increase gradually from winter to spring/summer in both 2015 and 2016. As concentrations showed extreme peaks in spring 2015 and summer 2016 (exceeding the South African ML in spring 2016; Figure 4.2), with Hg concentrations showing a similar pattern, though the build-up to the peaks was more gradual. Cd concentrations peaked in autumn 2015, and winter and spring 2016. Lead concentrations peaked, exceeding the South African MLs, in spring 2015 and 2016 (Figure 4.2).



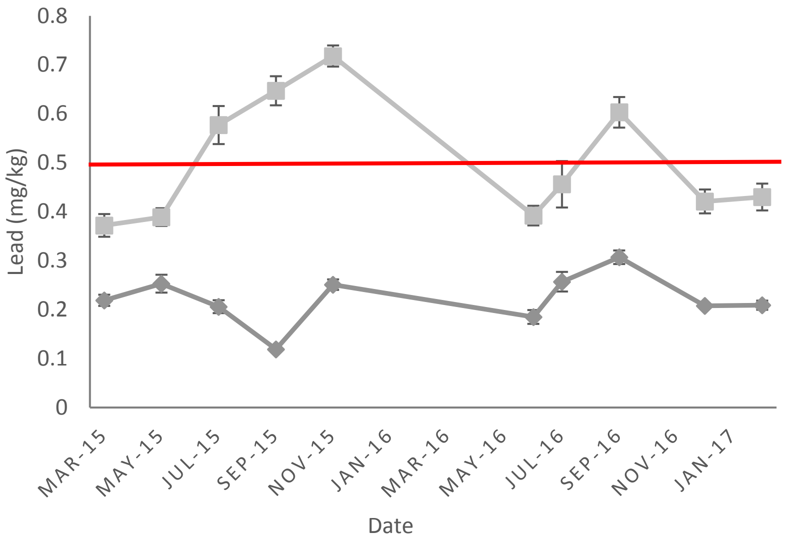


Figure 4.2 Temporal variation in the As and Pb concentrations (±standard error) of farmed *C. meridionalis* (diamond marker) and *M. galloprovincialis* (square marker) from Saldanha Bay, with red lines denoting maximum legal limits for fish (all values in wet weight)

4.4.3. Correlations:

The correlation tables were not included in this Chapter, as the only metals found to correlate with one another were Al and Fe in both *C. meridionalis* (Correlation coefficient = 0.89, R^2 = 0.79) and *M. galloprovincialis* (Correlation coefficient = 0.84, R^2 = 0.70).

4.5. DISCUSSION

Metals which are essential or possess low toxicities will be discussed first, followed by potentially toxic metals, followed by metals which exceeded regulatory limits within the study period (MLs), and lastly, species-related differences and temporal variations in trace metal contents will be covered.

4.5.1. Aluminium (Al)

Aluminium is the most abundant of all trace elements, and while dietary intakes vary between 2.5-11mg per day, it can become toxic to humans if more than 5g is ingested at one time (Pais & Benton-Jones, Jr., 1997; Reilly, 2004). The main probable sources of Al in Saldanha Bay area are likely the steel production facilities and potentially the use of Al-alloy sacrificial anodes to minimise marine corrosion of harbour equipment and ships (Mao *et al.*, 2011). Aluminium concentrations are not frequently assessed in mussels, as it is seldom an element of concern in marine pollution studies (Richir & Gobert, 2014), and is not considered toxic in low doses like lead or mercury. The average Al concentrations of *C. meridionalis* and *M. galloprovincialis* (6.4 and 17.3mg/kg wet weight, respectively; Table 4.2) within this study were found to be considerably higher than values observed for *C. meridionalis* in Namibia (Dahms *et al.*, 2014), and far lower than values obtained for *M. galloprovincialis* in Corsica (Richir & Gobert, 2014) and *P. perna* in Saldanha Bay (Degger, 2010).

At the time of writing this thesis, the South African government had not established guidelines or MLs for Al in seafoods. The FAO and WHO, however, have set a PWTI of 1mg/kg bodyweight for Al (CAC, 1995), meaning an adult with a body weight of 60kg could safely consume foods containing a total of 60mg Al per week. A 60kg adult would therefore have to eat in excess of 3kg of *M. galloprovincialis* from the aquaculture facilities in Saldanha Bay per week to exceed the PWTI, which is unlikely. While the Al concentrations within the mussels do not pose a current threat to human consumers, excess levels in the seawater could cause decreased filtration and result in stunted mussel growth (Mao *et al.*, 2011). This trace metal should therefore be included into programmes which monitor long term changes in trace metal contamination of the waters of the bay, such as the State of Saldanha and Langebaan Lagoon report (Clark *et al*. 2016).

4.5.2. Manganese (Mn)

Manganese is essential to both plants and animals, with human daily dietary intake ranging between 0.4-10mg, and Mn deficiencies resulting in reproductive and skeletal development issues (Pais & Benton-Jones, Jr., 1997). While it is considered toxic to industrial workers exposed to Mn fumes, oral intake of this trace metal has very low toxicity, due to low absorption and rapid excretion by the human body (Reilly, 2004). Likely due to this low toxicity in mammals, no MLs for Mn concentrations had been set for seafood by the South African government at the time of writing this thesis (Pais & Benton-Jones, Jr., 1997).

The Mn concentrations of *C. meridionalis* and *M. galloprovincialis* (1.6 and 0.5mg/kg w.w., respectively; Table 4.2) within this study were found to be considerably lower than those of *Mytilus* species from the EU coastline (Przytarska *et al.*, 2010) and *M. galloprovincialis* from Cape Town and Saldanha Bay (Sparks *et al.*, 2014; Pavlov *et al.*, 2015), but similar to values found for *Mytilus edulis* in Oregon (Latouche & Mix, 1982), *M. galloprovincialis* in Corsica and the Adriatic Sea (Orescanin *et al.*, 2006; Richir & Gobert, 2014) and *C. meridionalis* from Saldanha Bay, Cape Town and Namibia (Watling & Watling, 1976; Orren *et al.*, 1980; Dahms *et al.*, 2014). Manganese concentrations within both species in this study were low, and similar to previous studies which determined samples to be unpolluted; the farmed Saldanha Bay mussels can therefore be considered to be a healthy source of essential manganese in the human diet.

4.5.3. Iron (Fe)

Iron is an essential trace metal in the human body; 60-70% of Fe is found in the haemoglobin in the bloodstream, and deficiencies can result in serious health issues such as anaemia (Pais & Benton-Jones, Jr., 1997). Unfortunately, because humans do not have a homeostatic mechanism for Fe excretion it can also accumulate to toxic levels within the body, resulting in cirrhosis, liver cancer, diabetes and arthritis (Reilly, 2004), and therefore Fe intake in humans needs to be regulated. Iron is a major constituent of the lithosphere (Pais & Benton-Jones, Jr., 1997) and normally the majority of Fe is introduced into marine environments through natural weathering of rocks releasing Fe into rivers and thereafter into the ocean (Sparks *et al.*, 2014). In Saldanha Bay, however, the major source of Fe in the marine environment is likely the iron ore used for steel production at the ArcelorMittal Saldanha Steel works, which is stored in large stockpiles near the harbour (Clark *et al.*, 2014). The dust from these stockpiles is known to disperse into the surrounding areas and the ubiquity of Fe ore dust in Saldanha is evidenced by the reddish-brown colouring of many of the structures and roads in the town (Clark *et al.*, 2014).

Despite species-related differences between *C. meridionalis* and *M. galloprovincialis* (11.3 and 25.1mg/kg w.w., respectively; Table 4.2), Fe concentrations in the mussels were low when compared to previous studies on wild *C. meridionalis*, *P. perna* and *M. galloprovincialis* in Saldanha Bay (Watling & Watling, 1976; Degger, 2010; Clark *et al.*, 2014), suggesting that the location of the Imbaza Mussels rafts (in the centre of the Small Bay) has relatively low levels of exposure to the high Fe levels generally experienced in the area, potentially due to higher rates of water circulation. This is in line with studies by Clark *et al*. (2014) and Pavlov *et al*. (2015) in Saldanha Bay, which confirmed that wild mussels from the shore had far higher concentrations of all trace metals, due to these areas experiencing lower water circulation rates than mussel farm rafts. Another potential reason for lower levels of Fe accumulation in the raft-grown mussels is that they experience higher growth rates than wild, shore-grown mussels and therefore have less time for the accumulation of contaminants (Clark *et al*., 2016). Iron levels in both species were found to be similar to those in *C. meridionalis* in Cape Town (Orren *et al.*, 1980), higher than values registered for the same species in Namibia (Dahms *et al.*, 2014), and very low relative to studies on *M. galloprovincialis* in Corsica, Croatia, Cape Town and varied coastal waters of the EU (Orescanin *et al.*, 2006; Przytarska *et al.*, 2010; Richir & Gobert, 2014; Sparks *et al.*, 2014). The relatively low levels of Fe within the mussels from Saldanha Bay was somewhat unexpected, as previous studies have linked high Fe concentrations within mussels to close proximity to industrial pollution sources such as mining, steel production and port activities (Orescanin *et al.*, 2006; Przytarska *et al.*, 2010). While the South African government has not established MLs for Fe, toxic Fe intake for humans is ~100mg (Pais & Benton-Jones, Jr., 1997), and a consumer would therefore have to eat 9kgs of *C. meridionalis* or 4kgs of *M. galloprovincialis* in one sitting to exceed the toxic threshold, which is highly unlikely. The mussels in Saldanha Bay can therefore be considered a healthy source of the essential trace metal Fe for human consumers.

4.5.4. Copper (Cu)

Copper is an essential trace metal for both plants and animals (Pais & Benton-Jones, Jr., 1997) and plays a role in the formation of haemoglobin, the immune system and bone health, and has an antioxidant function when combined with zinc (Reilly, 2004). Copper deficiencies have been linked to increases in low-density-lipoprotein cholesterol, cardiac issues, immunological problems and osteoporosis, while excess Cu in the diet can result in nausea, vomiting, diarrhoea and eventual death (Sandstead, 1995; Reilly, 2004). Previous studies on Cu accumulation in mussels have found elevated levels of Cu in samples from areas close to high population densities and industrialisation (Kljaković-Gašpić *et al.*, 2010), particularly in mussel samples collected from harbours, where copper-based anti-fouling paints are commonly used on ship's hulls (Kljaković-Gašpić *et al.*, 2010; Dahms *et al.*, 2014). The proximity to heavy industry and the presence of an international port therefore make Saldanha Bay an area that should be monitored closely for Cu pollution.

Copper concentrations in *C. meridionalis* and *M. galloprovincialis* tested within this study were consistently low (1.2 and 0.7mg/kg w.w., respectively; Table 4.2), and similar to those found for *P. perna* in Saldanha Bay (Degger, 2010), *C. meridionalis* from Namibia (Dahms *et al.*, 2014), Cape Town (Orren *et al.*, 1980) and Saldanha Bay (Watling & Watling, 1976), and *M. galloprovincialis* from Corsica (Richir & Gobert, 2014), the Adriatic Sea (Orescanin *et al.*, 2006), Cape Town (Sparks *et al.*, 2014), Turkey (Sunlu, 2006), Montenegro (Stanković *et al.*, 2011) and Italy (Spada *et al.*, 2013). Previous studies have also found lower Cu concentrations in Italy (Licata *et al.*, 2004) and far higher concentrations (369mg/kg dry weight) in *M. galloprovincialis* from Croatia (Kljaković-Gašpić *et al.*, 2010). Copper concentrations in *C. meridionalis* and *M. galloprovincialis* from this study were far lower than expected considering the concerning levels of Cu pollution of Saldanha Bay sediments, and the high Cu concentrations found in mussels from the shoreline of Small Bay (Clark *et al.*, 2014, 2017). There are three potential explanations for this. Firstly, the generally low levels of Cu within mussel tissue have been attributed to their ability to regulate the Cu concentrations within their flesh, excreting excess Cu when necessary (Rainbow, 1995; Przytarska *et al.*, 2010). Secondly, Cu is an element that has been found to have a high biosediment accumulation factor (BSAF), which is the ratio between the metal concentrations in organisms and the sediment in which they live (Usero *et al.*, 2005; Sparks, 2012; Maar *et al.*, 2015). This means that mussels in contact with marine sediments generally have far higher concentrations of Cu due to the high bioavailability of this metal in sediment, and could explain why raft-grown mussels, which never encounter the ocean floor, have far lower concentrations of Cu in their tissues than shore-grown mussels (Maar *et al.*, 2015). Lastly, as mentioned for Fe, the higher growth rates of raft-grown mussels could result in decreased contaminant accumulation due to decreased exposure times (Clark *et al*., 2016). The Cu concentrations in the tissues of both species were, however, orders of magnitude lower than the 50mg/kg limit set for shellfish by the South African government (DOH, 1994). Farmed mussels from Saldanha Bay can therefore be considered a healthy source of essential Cu, with mussels posing no threat of copper overdose to human consumers.

4.5.5. Zinc (Zn)

Zinc is an essential trace element in humans, playing important roles in antioxidation and metabolism, and human RDAs range between 5-11mg per day (Reilly, 2004). Zinc is not considered a highly toxic element in humans, as it is generally well-controlled by homeostasis, but regular excessive consumption can lead to decreased absorption of other essential minerals such as copper and iron (Reilly, 2004). Zinc deficiencies, however, can result in serious problems with the skin, immune system, skeleton, gastrointestinal system and central nervous system (Reilly, 2004). Zinc is commonly the trace metal with the highest concentration in mussels (Usero *et al.*, 2005; Sunlu, 2006; Sparks, 2012; Spada *et al.*, 2013) and filter-feeding marine mussels are known to have the highest Zn concentrations of all marine species (Eisler & ScienceDirect, 2010), despite the fact that bivalves have the ability to partially regulate the concentrations of Zn within their bodies (Przytarska *et al.*, 2010). *M. galloprovincialis* samples from Saldanha Bay have previously been found to exceed the 300mg/kg^{-1} ML for Zn in mussels (Pavlov *et al.*, 2015), and Zn could therefore be a trace metal pollutant of concern to the aquaculture facilities in the bay.

The Zn levels of *C. meridionalis* and *M. galloprovincialis* (15.5 and 25.9mg/kg w.w., respectively; Table 4.2) in this project were found to be lower than concentrations found in previous studies on *Mytilus* species from the coastline of the EU (Przytarska *et al.*, 2010), Croatia (Kljaković-Gašpić *et al.*, 2010), Montenegro (Stanković *et al.*, 2011), Denmark (Maar *et al.*, 2015) and the Adriatic Sea (Orescanin *et al.*, 2006), and considerably higher than levels for *C. meridionalis* in Namibia (Dahms *et al.*, 2014). Similar Zn concentrations were found for *C. meridionalis* in Cape Town (Orren *et al.*, 1980) and Saldanha Bay (Watling & Watling, 1976), *P. perna* from Saldanha Bay (Degger *et al.*, 2011b), and *M. galloprovincialis* from Cape Town (Sparks *et al.*, 2014), Italy (Licata *et al.*, 2004; Spada *et al.*, 2013) and Turkey (Sunlu, 2006). The South African government has set a ML of 300mg/kg for Zn in shellfish and shellfish products (SA, 1994), and the maximum Zn content of the mussels from Saldanha Bay was seven times less than this limit. It can therefore be concluded that farmed mussels from Saldanha Bay are safe source of essential Zn for consumers.

4.5.6. Chromium (Cr)

Chromium is an essential trace element in humans, playing a role in carbohydrate and lipid metabolism, but can become toxic if more than 200mg is ingested at one time (Pais & Benton-Jones, Jr., 1997). In previous studies, elevated levels of Cr have been found in *M. galloprovincialis* samples from harbours and marinas (Kljaković-Gašpić *et al.*, 2010), as well as areas with high exposure to river discharge from urban and industrial areas, and elevated Cr in marine species can also be due to natural enrichment from sediment (Spada *et al.*, 2013).

The average Cr values found in *C. meridionalis* and *M. galloprovincialis* (0.1 and 0.2mg/kg w.w., respectively; Table 4.2) from this study were low in comparison to those found in *M. edulis* from southern Italy (Spada *et al.*, 2013) and *M. galloprovincialis* in Croatia (Kljaković-Gašpić *et al.*, 2010), and similar to concentrations found in *M. galloprovincialis* from Corsica and the Adriatic Sea (Orescanin *et al.*, 2006; Richir & Gobert, 2014), *C. meridionalis* from Saldanha Bay and Namibia (Watling & Watling, 1976; Dahms *et al.*, 2014) and *P. perna* from Saldanha Bay (Degger *et al.*, 2011b). While MLs for Cr in seafood have not been set by the South African government at the time of writing this Chapter, the Chinese government has set a 2mg/kg wet weight Cr limit for fish and aquatic animals (GAIN, 2014). The highest chromium concentrations found in either species in this study were at least five times below this legal limit, and therefore Cr concentrations within the flesh of *C. meridionalis* and *M. galloprovincialis* can be considered no threat to human consumers.

4.5.7. Mercury (Hg)

Mercury is a non-essential, bio-accumulative trace metal which has extremely high toxicity for both plants and animals (Pais & Benton-Jones, Jr., 1997). Excess Hg in the human diet is introduced into the environment and food chain through volcanic activity, coal combustion, smelting and pesticide use (Pais & Benton-Jones, Jr., 1997). Mercury occurs in a number of forms, but the highly toxic methylmercury is formed mostly in aquatic environments, and therefore the major route of Hg exposure in humans is through consumption of fish and terrestrial animal products which have been fed fish meal (Pais & Benton-Jones, Jr., 1997; CAC, 2011). Methylmercury was first identified in the 1950's as the cause of the catastrophic mass-poisoning of Japanese residents in Minamata Bay, which caused impairment of cerebral functions, paralysis and eventual death (Förstner & Wittmann, 1979). Subsequent to this, Hg has been implicated in numerous mass-poisonings and deaths, and is the most infamous of the trace metals (Förstner & Wittmann, 1979), with strict guidelines set by the FAO and WHO to prevent future disasters (CAC, 2011). The South African government has established a 0.5mg/kg ML for total Hg in shellfish (DOH, 2004), which is in line with the limits set by the European Union for all fish products (European Commission, 2006).

At the time of writing, minimal published research could be found on the mercury status of South African or Mediterranean mussel species in Saldanha Bay, with Hg monitoring of the mussels in Saldanha Bay occurring for the first time in 2016 (Clark *et al*., 2016). A study on the smoothhound shark (*M. mustelus*) from Langebaan Lagoon identified levels of Hg (with toxic methylmercury as the dominant species) which exceeded the 1mg/kg ML set for fish by the South African government (DOH, 2004; Bosch *et al*., 2016), indicating that metal pollution in the area could be a potential concern for human consumers. Despite this, overall Hg levels in *C. meridionalis* and *M. galloprovincialis* from Saldanha Bay were found to be extremely low (0.003 and 0.004mg/kg w.w., respectively; Table 4.2), with even maximum concentrations falling 10 times below the legal ML of 0.5mg/kg (DOH, 2004). This low overall Hg content is in line with the findings of Clark *et al*. (2016) for mussel farms in Saldanha Bay, and is likely due to both the location of the farms, and the fact that mussels form a part of the lower trophic levels in most ecosystems, and therefore are the first step in the bioaccumulation process (Claisse *et al.*, 2001). The Hg content of mussels within this study was found to be far lower than previous studies found for *M. galloprovincialis* samples from the coastlines of Montenegro (Stanković *et al.*, 2011), Italy (Licata *et al.*, 2004; Spada *et al.*, 2013) and Croatia (Kljaković-Gašpić *et al.*, 2010), and far below the 0.23mg/kg dry weight level which would indicate concerning levels of environmental pollution (Cantillo, 1998). These results indicate that Hg pollution levels within the aquaculture mussels from Saldanha Bay are not of concern to human consumers.

4.5.8. Cadmium (Cd)

Cadmium is a non-essential, highly toxic and carcinogenic trace metal with the ability to accumulate in the food chain due to a long biological half-life, therefore making it a metal of serious concern to human health (Pais & Benton-Jones, Jr., 1997; CAC, 2011). Cadmium occurs naturally in very small amounts in the earth's crust, but it's prevalence is greatly increased through anthropogenic activities (Pais & Benton-Jones, Jr., 1997; CAC, 2011). The main route of exposure to Cd for humans is through the diet, and bivalves are a known accumulator of Cd which need to be closely monitored in order to prevent human health issues (España *et al.*, 2007; CAC, 2011). Previous studies on *M. galloprovincialis* have found that Cd concentrations within the tissues of the mussels increased with proximity to high levels of industrial, harbour and urban wastes (Kljaković-Gašpić *et al.*, 2010). The Cd contents of *M. galloprovincialis* mussels from both the shoreline and one set of mussel rafts in Saldanha Bay have been found to frequently exceed the 3mg/kg^{-1} ML set by the South African government for shellfish (DOH, 2004; Clark *et al.*, 2014). This indicates that Saldanha Bay is an area of concern for Cd pollution, and aquaculture products from the bay should therefore be closely monitored in order to protect human consumers.

The Cd concentrations of *C. meridionalis* and *M. galloprovincialis* (0.4 and 1.0mg/kg w.w., respectively; Table 4.2) in this study were found to be less than half the ML of 3mg/kg, and were similar to concentrations found for *M. galloprovincialis* in Turkey (Sunlu, 2006), Italy (Spada *et al.*, 2013), Corsica (Richir & Gobert, 2014), Croatia (Kljaković-Gašpić *et al.*, 2010) and Montenegro (Stanković *et al.*, 2011) and *P. perna* in Saldanha Bay (Degger *et al.*, 2011b), and low in comparison to samples from Cape Town, where Cd concentrations were found to exceed legal MLs (Sparks *et al.*, 2014). Lower concentrations for Cd have been determined in *C. meridionalis* from Namibia (Dahms *et al.*, 2014), Cape Town (Orren *et al.*, 1980) and notably, Saldanha Bay in the 1970's (Watling & Watling, 1976), indicating that Cd contamination levels in the bay may be increasing, albeit slowly.

Despite previous studies finding concerningly high levels of Cd in mussels from Saldanha Bay (Clark *et al.*, 2014), the Cd concentrations in mussels from this study were low; this could be due to three reasons. Firstly, the location of the Imbaza Mussels rafts could potentially be in the ideal location for water circulation, resulting in the mussels having reduced exposure to polluted waters of the bay and therefore accumulating less of the toxic Cd. Secondly, Cd has been found to have a high BSAF (Sparks, 2012), and since the mussels have no contact with the ocean floor their contact with cadmium-enriched sediments is minimised (Maar *et al.*, 2015). Lastly, bivalves have a metallothionein protein which has the ability to safely bind to Cd and aid in its excretion, thereby reducing Cd concentrations within their bodies in relation to environmental concentrations (Choi *et al.*, 2007). Potentially due to a combination of these factors, the Cd concentrations in the mussels from this study were found to be lower than both the government regulations and therefore can be considered safe for human consumption. It should be noted, however, that on a dry weight basis the average Cd concentration of *M. galloprovincialis* samples exceeded the 3.7mg/kg limit which would indicate concerning levels of environmental pollution (Cantillo, 1998), and therefore sources of Cd pollution in the area should be closely monitored in future.

4.5.9. Arsenic (As)

Arsenic is a trace metalloid which is essential to humans in small doses (12-25µg/day), but easily becomes poisonous and carcinogenic if consumed in excess (Pais & Benton-Jones, Jr., 1997), and seafood is known to be one of the major dietary sources of As for humans (Stanković & Jović, 2012). Arsenic is widely distributed in marine sediments and waters, but can also be enriched in marine environments through industrial processes such as ore smelting and coal burning, and through the use of agricultural pesticides (Reilly, 2004; CAC, 2011). Currently, the majority of arsenic pollution originates from arsenic in lead alloys, batteries, paints and pharmaceuticals which enter the marine environment through sewage and storm water (Sciortino & Ravikumar, 1999). Namakwa Sands, situated near Saldanha Bay harbour, is an iron ore smelting and mining operation which could also potentially be contributing to elevated As pollution levels in Saldanha Bay (Gous, 2006). Previous studies on mussels (*P. perna*) from Saldanha Bay and smoothhound sharks (*M. mustelus*) from Langebaan found the tissue concentrations of As in these species to exceed the limit of 3mg/kg set by the South African government for fish and shellfish (DOH, 2004), which is a cause for concern for human consumers.

The maximum As concentrations within both species (3.4mg/kg w.w.; Table 4.2) in this study were found to be lower than values for previous studies on *M. galloprovincialis* from the coastlines of Italy (Spada *et al.*, 2013), Corsica (Richir & Gobert, 2014) and the Adriatic Sea (Orescanin *et al.*, 2006) and *P. perna* from Saldanha Bay (Degger, 2010), and similar to those found for *M. galloprovincialis* in Montenegro (Stanković *et al.*, 2011). The average concentrations of As within both farmed mussel species from Saldanha Bay were well below the legal ML of 3mg/kg (Table 4.2), but tissue concentrations in both species were found to peak drastically in spring 2015 and summer 2016 (Figure 4.2). These concentrations exceeded the legal limit in *C. meridionalis* in spring 2015 (Avg. 3.14mg/kg; n=6) and *M. galloprovincialis* in summer 2016 (Avg. 3.16mg/kg). While it could be considered concerning that the samples exceeded the safe limits set by the government, it is important to note that As occurs in many different forms in the marine environment, with each form possessing different toxicities (CAC, 2011). The principal form of As in mussels is usually arsenobetaine (~80%), which is highly stable and easily broken down by the human body, and therefore not considered toxic to humans (Lorenzana *et al.*, 2009; CAC, 2011; Taylor *et al.*, 2017). Inorganic As, the form of As known to be toxic and carcinogenic to humans, forms a very small part of the total arsenic in bivalve tissues (Taylor *et al.*, 2017). The PWTI's set by the WHO and FAO for As stipulate that these standards are in relation to inorganic arsenic exclusively, and that As speciation in fish and bivalves is necessary to determine their actual risk to human consumers (CAC, 2011). Therefore, in order to determine the realistic potential threat these mussels pose to human consumers, As speciation would have to be performed to identify the major forms of As in the farmed Saldanha Bay mussels. However, considering the findings of previous studies on As speciation in mussels and fish, it is safe to assume that, despite both *M. galloprovincialis* and *C. meridionalis* samples each exceeding the regulatory limits at one point in the study period, the actual amount of toxic inorganic As is expected to be low overall, likely making the mussels safe for human consumption (though this warrants further research).

A metal contamination study by Usero *et al*. (2005) also states that "only if the lowest level of the range is greater than the maximum level in the legislation is it certain "beyond reasonable doubt" that the sample concentration of the analyte is greater than required by the legislation", due to potential measurement errors and expanded uncertainties. Upon closer inspection of the data, the lowest As concentrations for *C. meridionalis* in spring 2015 (2.89mg/kg) and *M. galloprovincialis* in summer 2016 (2.90mg/kg) were found to be below the legal limit. It therefore cannot be said, with absolute certainty, that the samples are not fit for human consumption, as the minimum values were within the permissible legal limits set by the South African government. This study does, however, confirm that As is a trace metal of concern in Saldanha Bay and needs to be carefully monitored going forward to prevent potential negative health impacts for human consumers of the aquaculture products grown in the bay.

4.5.10. Lead (Pb)

Lead is a non-essential, toxic trace metal which has the ability to accumulate in the food chain and can cause cancers, reproductive disorders, cardiovascular issues and neurological development problems in young children (Pais & Benton-Jones, Jr., 1997; CAC, 2011). Lead is used and produced in large amounts in human industrial processes such as mining, smelting and steel-making (CAC, 2011), and human exposure to Pb comes mostly through polluted air, water and food sources (Stanković & Jović, 2012). Increased Pb concentrations in mussel tissues have been linked to proximity to heavily industrialised areas and municipal waste water outflows (Chase *et al.*, 2001; Orescanin *et al.*, 2006; Kljaković-Gašpić *et al.*, 2010). Saldanha Bay, which has mining, smelting, ore-processing and steel-making industry in close proximity to the bay, as well as the presence of an international shipping port (where ships are known to release ballast water which exceeds regulatory guidelines for trace metals) and underperforming waste water treatment works, is an area of serious concern for Pb pollution (Clark *et al.*, 2014). Another source of Pb pollution could also be from storm water runoff, which could transport terrestrial Pb from road surfaces directly into the bay (Kljaković-Gašpić *et al.*, 2010). The storm water runoff in Saldanha Bay has been found to greatly exceed South African guideline limits for metals (Clark *et al*., 2016). Previous studies on mussels from the shoreline and mussel rafts in Saldanha Bay have found samples to frequently drastically exceed the 0.5mg/kg^{-1} ML set by the South African government (DOH, 2004), though overall concentrations of Pb are stated to be decreasing (Clark *et al.*, 2014).

Average Pb concentrations in the *M. galloprovincialis* samples from Saldanha Bay (0.5mg/kg w.w.; Table 4.2) were found to be low in comparison to values found for the same species from the coastlines of Montenegro (Stanković *et al.*, 2011), Turkey (Sunlu, 2006), Croatia (Kljaković-Gašpić *et al.*, 2010) and the Adriatic Sea (Orescanin *et al.*, 2006), but similar to samples from the coastlines of Italy (Spada *et al.*, 2013) and the EU (Przytarska *et al.*, 2010), and high in comparison to samples from Corsica (Richir & Gobert, 2014) and Lake Faro (Licata *et al.*, 2004). Average Pb concentrations in *C. meridionalis* samples from this study (0.2mg/kg w.w.; Table 4.2) were found to be similar to previous studies on this species in Saldanha Bay (1970's), Cape Town and Namibia (Watling & Watling, 1976; Orren *et al.*, 1980; Dahms *et al.*, 2014). This is either an indication that *C. meridionalis* has the ability to regulate Pb levels within its flesh or that Pb pollution levels in Saldanha Bay have not increased since the 1970's.

The mean and maximum concentrations of Pb within *C. meridionalis* were below the 0.5mg/kg ML set by the South African government (DOH, 2004), while the mean Pb concentration of *M. galloprovincialis* samples equalled to the ML, and maximum Pb concentrations exceeded the regulations four times; in winter and spring (both September and November) of 2015 and again in spring 2016 (Table 4.2). Previous studies on Pb in mussel samples from the West Coast of South Africa likewise found concentrations to be higher in spring than winter, and postulated that this was either due to the mussels being in a post-spawn condition or because the colder temperatures of the upwelled spring waters reduced the metabolic rate of excretion of certain metals (Orren *et al.*, 1980). Whatever the reason for the raised spring Pb concentrations in the *M. galloprovincialis* samples, it is very concerning for farmers and consumers alike that they exceeded the 0.5mg/kg ML set by the South African government for fish (DOH, 2004), as well as exceeding the 3.2mg/kg dry weight limit which indicates concerning levels of contamination (Cantillo, 1998). These results indicate that sources of Pb pollution within Saldanha Bay are of serious concern to the mussel farmers and human consumers, and need to be better regulated and monitored. No PTWI could be calculated for Pb from the Saldanha Bay mussels in the human diet, because the previous WHO and FAO PWTI of 25µg/kg bodyweight was withdrawn after it was noted to not sufficiently protect consumers from the adverse effects of lead poisoning (CAC, 2011). It is noteworthy, though, that the European Commission, which set a 0.3mg/kg ML for fish, has set a separate and elevated ML of 1.5mg/kg for Pb in bivalve molluscs (European Commission, 2006). The mussel samples from this study which exceeded the local ML do not exceed this raised ML set specifically for bivalves by the European Commission. The South African government has not set a specific ML for Pb in bivalves, and the industry therefore goes by the limits set for fish samples, but the higher limit set by the EC indicates that lead contamination rates in mussels differ significantly from those in fish and that somewhat more lenient regulations could be necessary to promote growth in the South African bivalve aquaculture industry.

4.5.11. Species-related differences in trace metal content

Of the measured elements, Al, Cr, Zn, Cd, Fe and Pb concentrations were found to be significantly higher in *M. galloprovincialis* than *C. meridionalis*, while Mn and Cu were found to be higher in *C. meridionalis* samples. Similar species-related differences have been found in previous bivalve studies, attributing differences to species-related variability in trace metal accumulation rates (Usero *et al.*, 2005; España *et al.*, 2007; Dahms *et al.*, 2014).

The identified differences could be due to species-related variations in food preference and selection, as mussels exposure to trace metals is known to be linked to the trace metal concentrations of their food sources, as well as other factors (Förstner & Wittmann, 1979; Sunlu, 2006). Mussels feed by using their gills to filter suspended particles from the environment in which they live, but because not all substances filtered from the water column are nutritive (e.g. silt) they have the ability to select for more nutritive particles such as algae, while rejecting non-nutritive particles (Gosling, 2004). The effectiveness of this particle selection has been shown to vary between bivalve species (Kiørboe & Møhlenberg, 1981), and due to *C. meridionalis* adaptation to areas with high silt loads (van Erkom Schurink & Griffiths, 1993), it is likely that the two farmed species from Saldanha Bay have different selection efficiencies and particle preferences due to different lifestyle adaptations; this could potentially result in different rates of accumulation of trace metals from their diets. It should also be noted that *M. galloprovincialis* has greater filtration capacity, growth rates and investments in reproductive tissue and flesh weight than *C. meridionalis* (Van Erkom Schurink & Griffiths, 1992; van Erkom Schurink & Griffiths, 1993), which could also result in increased sequestration of certain trace metals in the invasive species.

Previous studies have suggested *C. meridionalis* to accumulate certain trace metals at slower rates than *P. perna* and *Mytilus* species (Watling & Watling, 1976; Dahms *et al.*, 2014), with the exception of Mn. The species-related differences could therefore be connected to *C. meridionalis* ability to sequester and excrete metals, or to an overall lower accumulation rate; no published work could be found on the relative ability of *C. meridionalis* to control the metal concentrations within its flesh when compared to other mussel species, but studies have found bivalves to be able to control Cu and Zn levels within their tissues (Przytarska *et al.*, 2010), and therefore the local species could potentially have an untested ability at removing a greater variety of trace metals from the body.

4.5.12. Temporal variations in trace metal content

Temporal patterns of accumulation and depletion were evident in the concentrations of Fe and As in *C. meridionalis*, and the concentrations of Fe, As, Hg and Pb in *M. galloprovincialis*. Seasonal fluctuations in trace metal content are common in the literature for both species (Orren *et al.*, 1980; Latouche & Mix, 1982; Richir & Gobert, 2014; Sparks *et al.*, 2014). Trace metal accumulation rates have been linked to the gametogenic cycle of mussels, as the mass-production of metal-poor gonadal tissue can result in the dilution of total trace metal content in the mussel tissues (Cossa *et al.*, 1979; Latouche & Mix, 1982). The majority of trace metals have been found to accumulate in the somatic tissues of mussels, such as the gills and hepatopancreas (Richir & Gobert, 2014), and therefore when mussels are in their post-spawn state the relative trace metal concentrations are increased. Trace metal accumulation rates have also been linked to water temperatures; studies have found that trace metal contents in mussels are lower when food availability and temperature are higher, because these factors result in higher metabolic and excretion rates (Bryan, 1973).

In *M. galloprovincialis*, gradual increases in Fe content were visible from winter to summer in both 2015 and 2016. This pattern was antagonistic to the pattern seen in whole weight of the mussels (Chapter 3), suggesting that the relative metal content of the mussels increases post-spawn, which is common in the literature (Watling & Watling, 1976; Orren *et al.*, 1980; Latouche & Mix, 1982; Richir & Gobert, 2014). In the upwelling regime experienced by Saldanha Bay, water temperatures are often colder in summer than winter, which may have slowed the metabolism of *M. galloprovincialis*, a species known to grow faster in slightly warmer water (van Erkom Schurink & Griffiths, 1993), thereby resulting in relatively higher concentrations of Fe in the tissues in summer (Orren *et al.*, 1980). Less clear seasonal trends were visible for *C. meridionalis*, where Fe levels peaked in both winter and summer months, with drops in Fe concentrations coinciding with spawning and post-spawn periods, suggesting that *C. meridionalis*, unlike other mussels, has fairly high concentrations of Fe within the gonadal tissue. This is in contradiction to previous literature on this species (Orren *et al.*, 1980), which states that Fe concentrations in *C. meridionalis* specimens increased considerably post-spawn.

Arsenic concentrations in both species peaked in spring 2015 and summer 2016, with Hg concentrations in *M. galloprovincialis* following a similar pattern (though less drastic). While the peaks in As concentration coincide somewhat with the post-spawn period of the mussels (lowest whole weights, Chapter 3), the identical and drastic nature of the peaks in both species suggest that As concentrations could be linked rather to environmental parameters than the gametogenic cycle of the mussels, as they have different spawning times. These peaks could have been caused by either large, repeated pollution events or due to the increased accumulation of As into the algae upon which the mussels feed (Taylor *et al.*, 2017). Arsenic speciation would need to be performed on the mussels, their food sources and the waters of Saldanha Bay, in order to determine the source of the As peaks (Taylor *et al.*, 2017). Mercury, however, increased gradually with highest concentrations coinciding clearly with post-spawn periods, in line with the literature on trace metal accumulation. While previously Hg concentrations have been shown to be linked to the fat content of mussels (Kljaković-Gašpić *et al.*, 2010), no similarities were found between the patterns of accumulation and depletion of Hg and fat in *M. galloprovincialis* from Saldanha Bay.

The highest Cd concentrations in *M. galloprovincialis* were found in autumn and winter of 2015 and 2016, with lowest values found in post-spawn periods. These patterns are contradictory to the expected temporal changes, as Cd has been shown to be concentrated in the somatic tissues (specifically the gills and hepatopancreas) of *M. galloprovincialis* and other *Mytilus* species (Latouche & Mix, 1982; Richir & Gobert, 2014), and would therefore be expected to increase in relative concentration in post-spawn mussels. This data therefore suggests that Cd concentrations in the mussels may be linked to environmental levels of Cd within Saldanha Bay, and since peak concentrations were found in the mussels in autumn and winter (the rainfall period for the Western Cape), it is possible that this trace metal was introduced into the marine environment through excess, untreated storm water runoff during heavy winter rains, a known issue in the area (Clark *et al.*, 2014). A study by Orescanin *et al*. (2006) found the concentrations of certain trace metals to be linked to spring rains, as these rains washed pollutants, naturally weathered trace metals and nutrients into nearby marine waters, thereby increasing the trace metal content of the waters and the mussels.

Lead concentrations in *M. galloprovincialis* increased gradually from winter to spring in both 2015 and 2016. While peak concentrations coincided somewhat with post-spawn periods, it is more likely that fluctuations in the Pb composition of the mussels were linked to environmental changes in the Pb content of the marine environment. This is because the Pb composition of the mussels was found to increase throughout the winter rainfall period, when Pb ore from the multipurpose quay and Pb residues from road and building surfaces were likely washed into the bay with untreated storm water runoff (Clark *et al.*, 2014).

4.6. CONCLUSION

In conclusion, *M. galloprovincialis* appeared to accumulate a range of trace metals (Al, Cr, Fe, Zn, Cd and Pb) to a higher concentration than *C. meridionalis*, while Cu and Mn were present in higher concentrations within the flesh of *C. meridionalis*. Reasons for these differences are potentially due to different food selection processes, filtration and growth rates, excretion rates and *C. meridionalis* having lower overall trace metal accumulation rates. Concentrations of Fe and As in *C. meridionalis*, and Fe, As, Hg and Pb in *M. galloprovincialis*, were found to fluctuate temporally. Increases in total content of Fe and Hg were found post-spawn in *M. galloprovincialis*, likely due to massive decreases in the spawned metal-poor gonad tissue causing a relative increase in these trace metals, which are stored in the somatic tissue. Raised concentrations of Cd and Pb could potentially be linked to winter rainfall periods when storm water runoff introduces excess, untreated water into the bay. Overall, mussels were found to be a healthy source of essential metals such as Mn, Fe, Cu, Zn and Cr, and levels of the toxic metal Hg were found to be extremely low across the entire study period. Cadmium was consistently below MLs set by the South African government, but should be monitored going forward. Arsenic and Pb were both found to exceed the South African MLs for these toxic trace metals on one or more of the 10 sampling dates. While As in the mussels does not pose a serious threat to human consumers, due to the majority of As in seafood occurring in the non-toxic arsenobetaine form, the Pb composition of the *M. galloprovincialis* samples exceeded the South African ML four times within the study period and is concerning for human consumers. This study does note, however, that the South African ML for Pb is set for "fish", not specifically for bivalves (like those for Cd, Cu and Hg), and the European Commission has set a far higher ML for Pb in bivalves, which the samples from this study do not exceed. Consideration should therefore be given to establishing a specific ML for Pb in bivalves in order to promote the growth of the South African mussel industry, but it is also imperative to better control and regulate sources of Pb pollution within Saldanha Bay.

4.7. REFERENCES

Bongiorno, T., Iacumin, L., Tubaro, F., Marcuzzo, E., Sensidoni, A., & Tulli, F. 2015. Seasonal changes in technological and nutritional quality of *Mytilus galloprovincialis* from suspended culture in the Gulf of Trieste (North Adriatic Sea). Food Chem. 173, 355–362 https://doi.org/10.1016/j.foodchem.2014.10.029.

Bryan, G.W. 1973. The occurrence and seasonal variation of trace metals in the scallops *Pecten maximus* (L.) and *Chlamys opercularis* (L.) J. mar. biol. Ass. UK. 53, 145-166.

Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E., & Hoffman, L. C. 2016. Heavy metal accumulation and toxicity in smoothhound (*Mustelus mustelus*) shark from Langebaan Lagoon, South Africa. Food Chem. 190, 871–878 https://doi.org/10.1016/j.foodchem.2015.06.034.

CAC. 1995. Codex General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995). Codex Alimentarius Commission, Food and Agricultural Organisation of the United Nations, World Health Organisation.

CAC. 2011. Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods. Codex Alimentarius Commission, Food and Agricultural Organisation of the United Nations, World Health Organisation. The Hague, The Netherlands.

Cantillo, A. Y. 1998. Comparison of results of Mussel Watch programs of the United States and France with Worldwide Mussel Watch studies. Mar. Pollut. Bull. 36, 712–717 https://doi.org/10.1016/S0025-326X(98)00049-6.

Casas, S., Cossa, D., Gonzalez, J. L., Bacher, C., & Andral, B. 2004. Modeling trace metal accumulation in the Mediterranean mussel, *Mytilus galloprovincialis*. Rapp. Comm. int. Mer. Medit. 37, 306–308.

Chase, M., Jones, S., Hennigar, P., Sowles, J., Harding, G. C., Freeman, K., Wells, P., Krahforst, C., Coombs, K., Crawford, R., Pederson, J., & Taylor, D. 2001. Gulfwatch: Monitoring Spatial and Temporal Patterns of Trace Metal and Organic Contaminants in the Gulf of Maine (1991–1997) with the Blue Mussel, *Mytilus edulis* L. Mar. Pollut. Bull. 42, 490–504 https://doi.org/10.1016/S0025-326X(00)00193-4.

Choi, H. J., Ji, J., Chung, K. H., & Ahn, I. Y. 2007. Cadmium bioaccumulation and detoxification in the gill and digestive gland of the Antarctic bivalve *Laternula elliptica*. Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 145, 227–235 https://doi.org/10.1016/j.cbpc.2006.12.005.

Claisse, D., Cossa, D., Bretaudeau-Sanjuan, J., Touchard, G., & Bombled, B. 2001. Methylmercury in molluscs along the French Coast. Mar. Pollut. Bull. 42, 329–332 https://doi.org/10.1016/S0025-326X(01)00036-4.

Clark, B., Laird, M., Hutchings, K., Liebau, V., Biccard, A., Turpie, J., & Parker-Mallick, N. 2014. The state of Saldanha Bay and Langebaan Lagoon 2013/2014. Technical Report. Report no. 1581/1 prepared by Anchor Environmental Consultants for the Saldanha Bay Water Quality Trust.

Clark, B. M., Massie, V., Hutchings, K., Laird, M., Biccard, A., Brown, E., Duna, O. O., & Turpie, J. 2016. The State of Saldanha Bay and Langebaan Lagoon 2016. Technical Report. Report No AEC 1691/1 prepared by Anchor Environmental Consultants (Pty) Ltd for the Saldanha Bay Water Quality Forum Trust.

Clark, B. M., Massie, V., Hutchings, K., Brown, E., Biccard, A., Laird, M., Harmer, R., Makhosonke, A., Wright, A. & Turpie, J. 2017. The State of Saldanha Bay and Langebaan Lagoon 2017, Technical Report. Report No. AEC 1741/1 prepared by Anchor Environmental Consultants (Pty) Ltd for the Saldanha Bay Water Quality Forum Trust, October 2017.

Cossa, D., Bourget, E., & Piuze, J. 1979. Sexual maturation as a source of variation in the relationship between cadmium concentration and body weight of *Mytilus edulis* L. Mar. Pollut. Bull. 10, 174–176 https://doi.org/10.1016/0025-326X(79)90425-9.

Dahms, S., van der Bank, F. H., & Greenfield, R. 2014. A baseline study of metal contamination along the Namibian coastline for *Perna perna* and *Choromytilus meridionalis*. Mar. Pollut. Bull. 85, 297–305 https://doi.org/10.1016/j.marpolbul.2014.05.037.

Degger, N. 2010. The application of passive artificial devices for monitoring of metallic and organic pollutants along the South African coastline. MSc, University of Johannesburg, Johannesburg.

Degger, N., Wepener, V., Richardson, B. J., & Wu, R. S. S. 2011. Application of artificial mussels (AMs) under South African marine conditions: A validation study. Mar. Pollut. Bull. 63, 108–118 https://doi.org/10.1016/j.marpolbul.2011.04.040.

DOH. 1994. Regulations relating to metals in foodstuffs (No. R.1518 9 September 1994). Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). Department of Health, South Africa.

DOH. 2004. Regulations relating to maximum levels for metals in foodstuffs (No. R.500 30 April 2004). Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). Department of Health, South Africa.

Eisler, R., & ScienceDirect. 2010. Compendium of trace metals and marine biota. 1st Edition. Elsevier, Amsterdam.

España, M. S. A., Rodríguez, E. M. R., & Romero, C. D. 2007. Comparison of mineral and trace element concentrations in two molluscs from the Strait of Magellan (Chile). Arch. Environ. Contam. Toxicol. 52, 519–524 https://doi.org/10.1007/s00244-006-0104-5.

European Commission. 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Communitites L364, 5–24 https://doi.org/10.2203/dose-response.06-012.Hanekamp.

Fernández, A., Grienke, U., Soler-Vila, A., Guihéneuf, F., Stengel, D. B., & Tasdemir, D. 2015. Seasonal and geographical variations in the biochemical composition of the blue mussel (*Mytilus edulis* L.) from Ireland. Food Chem. 177, 43–52 https://doi.org/10.1016/j.foodchem.2014.12.062.

Förstner, U., & Wittmann, G. T. W. 1979. Metal Pollution in the Aquatic Environment. Springer-Verlag, Berlin Heidelberg.

GAIN. 2014. China’s Maximum Levels of Contaminants in Foods (GB 2762-2012). Global Agricultural Information Network, Beijing.

Goldberg, E. D. 1975. The mussel watch - A first step in global marine monitoring. Mar. Pollut. Bull. 6, 111 https://doi.org/10.1016/0025-326X(75)90271-4.

Gosling, E. M. 2004. Bivalve molluscs. First Edit. Fishing News Books, a division of Blackwell Publishing, Cornwall.

Gous, M. 2006. An overview of the Namakwa Sands ilmenite smelting operations. J. South. African Inst. Min. Metall. 106, 379–384.

Kiørboe, T., & Møhlenberg, F. 1981. Particle Selection in Suspension-Feeding Bivalves. Mar. Ecol. Prog. Ser. 5, 291–296 https://doi.org/10.3354/meps005291.

Kljaković-Gašpić, Z., Herceg-Romanić, S., Kožul, D., & Veža, J. 2010. Biomonitoring of organochlorine compounds and trace metals along the Eastern Adriatic coast (Croatia) using *Mytilus galloprovincialis*. Mar. Pollut. Bull. 60, 1879–1889 https://doi.org/10.1016/j.marpolbul.2010.07.019.

Latouche, D. Y., & Mix, M. C. 1982. The Effects of Depuration, Size and Sex on Trace Metal Levels in Bay Mussels. Mar. Pollut. Bull. 13, 27–29.

Licata, P., Trombetta, D., Cristani, M., Martino, D., & Naccari, F. 2004. Organochlorine compounds and heavy metals in the soft tissue of the mussel *Mytilus galloprovincialis* collected from Lake Faro (Sicily, Italy). Environ. Int. 30, 805–810 https://doi.org/10.1016/j.envint.2004.01.007.

Lorenzana, R. M., Yeow, A. Y., Colman, J. T., Chappell, L. L., & Choudhury, H. 2009. Arsenic in seafood: Speciation issues for human health risk assessment. Hum. Ecol. Risk Assess. 15, 185–200 https://doi.org/10.1080/10807030802615949.

Maar, M., Larsen, M. M., Tørring, D., & Petersen, J. K. 2015. Bioaccumulation of metals (Cd, Cu, Ni, Pb and Zn) in suspended cultures of blue mussels exposed to different environmental conditions. Estuar. Coast. Shelf Sci., 1–13 https://doi.org/10.1016/j.ecss.2015.10.010.

Mao, A., Mahaut, M. L., Pineau, S., Barillier, D., & Caplat, C. 2011. Assessment of sacrificial anode impact by aluminum accumulation in mussel *Mytilus edulis*: A large-scale laboratory test. Mar. Pollut. Bull. 62, 2707–2713 https://doi.org/10.1016/j.marpolbul.2011.09.017.

Olivier, D., Heinecken, L., & Jackson, S. 2013. Mussel and oyster culture in Saldanha Bay, South Africa: Potential for sustainable growth, development and employment creation. Food Secur. 5, 251–267 https://doi.org/10.1007/s12571-013-0244-1.

Orescanin, V., Lovrencic, I., Mikelic, L., Barisic, D., Matasin, Z., Lulic, S., & Pezelj, D. 2006. Biomonitoring of heavy metals and arsenic on the east coast of the Middle Adriatic Sea using *Mytilus galloprovincialis*. Nucl. Instruments Methods Phys. Res. Sect. B Beam Interact. with Mater. Atoms 245, 495–500 https://doi.org/10.1016/j.nimb.2005.11.050.

Orren, M. J., Eagle, G. A., Hennig, H. F. K. O., & Green, A. 1980. Variations in Trace Metal Content of the Mussel *Choromytilus meridionalis* (Kr.) with Season and Sex. Top. Catal. 11, 253–257 https://doi.org/10.1016/0025-326X(80)90313-6.

Pais, I., & Benton-Jones, Jr., J. 1997. The Handbook of Trace Elements. St. Lucie Press, Boca Raton, Florida.

Pavlov, D. F., Bezuidenhout, J., Frontasyeva, M. V, & Goryainova, Z. I. 2015. Differences in Trace Element Content between Non-Indigenous Farmed and Invasive Bivalve Mollusks of the South African Coast. Am. J. Anal. Chem. 6, 886–897 https://doi.org/10.4236/ajac.2015.611084.

Przytarska, J. E., Sokołowski, A., Wołowicz, M., Hummel, H., & Jansen, J. 2010. Comparison of trace metal bioavailabilities in European coastal waters using mussels from *Mytilus edulis* complex as biomonitors. Environ. Monit. Assess. 166, 461–476 https://doi.org/10.1007/s10661-009-1015-5.

Raatz, S. K., Silverstein, J. T., Jahns, L., & Picklo, M. J. 2013. Issues of fish consumption for cardiovascular disease risk reduction. Nutrients 5, 1081–1097 https://doi.org/10.3390/nu5041081.

Rainbow, P. S. 1995. Biomonitoring of heavy metal availability in the marine environment. Mar. Pollut. Bull. 31, 183–192 https://doi.org/10.1016/0025-326X(95)00116-5.

Reilly, C. 2004. The Nutritional Trace Metals. Blackwell Publishing Ltd, Pondicherry, India.

Richir, J., & Gobert, S. 2014. The effect of size, weight, body compartment, sex and reproductive status on the bioaccumulation of 19 trace elements in rope-grown *Mytilus galloprovincialis*. Ecol. Indic. 36, 33–47 https://doi.org/10.1016/j.ecolind.2013.06.021.

Sandstead, H. H. 1995. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. Am. J. Clin. Nutr. 61.

Sciortino, J. A., & Ravikumar, R. 1999. Fishery Harbour Manual on the Prevention of Pollution. Bay of Bengal Programme for Fisheries Management (BOBP/MAG/22) and Food and Agriculture Organisation of the United Nations. Madras, India.

Spada, L., Annicchiarico, C., Cardellicchio, N., Giandomenico, S., & Di Leo, A. 2013. Heavy metals monitoring in mussels *Mytilus galloprovincialis* from the Apulian coasts (Southern Italy). Mediterr. Mar. Sci. 14, 99–108 https://doi.org/10.12681/mms.323.

Sparks, C. 2012. Metal Contamination and Antioxidant Responses of *Mytilus galloprovincialis* Along the West Coast of the Cape Peninsula. DTech, Cape Peninsula University of Technology, Cape Town.

Sparks, C., Odendaal, J., & Snyman, R. 2014. An analysis of historical Mussel Watch Programme data from the west coast of the Cape Peninsula, Cape Town. Mar. Pollut. Bull. 87, 374–380 https://doi.org/10.1016/j.marpolbul.2014.07.047.

Stanković, S., & Jović, M. 2012. Health risks of heavy metals in the mediterranean mussels as seafood. Environ. Chem. Lett. 10, 119–130 https://doi.org/10.1007/s10311-011-0343-1.

Stanković, S., Jović, M., Milanov, R., & Joksimović, D. 2011. Trace elements concentrations (Zn, Cu, Pb, Cd, As and Hg) in the Mediterranean mussel (*Mytilus galloprovincialis*) and evaluation of mussel quality and possible human health risk from cultivated and wild sites of the southeastern Adriatic Sea, Montenegro. J. Serbian Chem. Soc. 76, 1725–1737 https://doi.org/10.2298/JSC110420095S.

Sunlu, U. 2006. Trace metal levels in mussels (*Mytilus galloprovincialis* L. 1758) from Turkish Aegean Sea coast. Environ. Monit. Assess. 114, 273–286 https://doi.org/10.1007/s10661-006-4780-4.

Taylor, V., Goodale, B., Raab, A., Schwerdtle, T., Reimer, K., Conklin, S., Karagas, M. R., & Francesconi, K. A. 2017. Human exposure to organic arsenic species from seafood. Sci. Total Environ. 580, 266–282 https://doi.org/10.1016/j.scitotenv.2016.12.113.

Usero, J., Morillo, J., & Gracia, I. 2005. Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. Chemosphere 59, 1175–1181 https://doi.org/10.1016/j.chemosphere.2004.11.089.

van Erkom Schurink, C., & Griffiths, C. L. 1993. Factors affecting relative rates of growth in four South African mussel species. Aquaculture 109, 257–273 https://doi.org/10.1016/0044-8486(93)90168-X.

van Erkom Schurink, C., & Griffiths, C. L. 1992. Physiological energetics of four South African mussel species in relation to body size, ration and temperature. Comp. Biochem. Physiol. -- Part A Physiol. 101, 779–789 https://doi.org/10.1016/0300-9629(92)90358-W.

Watling, H. R., & Watling, R. J. 1976. Trace metals in *Choromytilus meridionalis*. Mar. Pollut. Bull. 7, 91–94 https://doi.org/10.1016/0025-326X(76)90149-1.

Weeks, S. J., Boyd, A. J., Monteiro, P. M. S., & Brundrit, G. B. 1991. The currents and circulation in Saldanha Bay after 1975 deduced from historical measurements of drogues. South African J. Mar. Sci. 11, 525–535 https://doi.org/10.2989/025776191784287475.

Wepener, V., & Degger, N. 2012. Status of marine pollution research in South Africa (1960-present). Mar. Pollut. Bull. 64, 1508–1512 https://doi.org/10.1016/j.marpolbul.2012.05.037.

CHAPTER 5

Bioaccumulation of organic pollutants in *Choromytilus meridionalis* and *Mytilus galloprovincialis* from aquaculture facilities in Saldanha Bay, South Africa

5.1. ABSTRACT

Persistent organic pollutants (POPs) such as polyaromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are lipophilic, anthropogenic pollutants which have the potential to accumulate in marine food chains and cause health issues for human consumers. Farmed *C. meridionalis* and *M. galloprovincialis* samples were collected from aquaculture facilities in Saldanha Bay and analysed for POP contaminants, which were extracted using the QuEChERS method and analysed via GC-MS/MS. The eight detected OCPs (trans-permethrin > cis-permethrin > dieldrin > chlordane > chloro-benzilate > endosulfan > nonachlor > DDD) were the highest contributors to POP contamination, with means from 0.7 - 48ng/g dry weight. This was followed by the five detected PAHs (flourene > pyrene > fluoranthene > benzo(a)pyrene > benz(a)anthracene) where mean values fell between 1 – 13.4ng/g d.w., and ten PCBs (180 > 149 > 28 > 110 > 153 > 52 > 18 > 44 > 118 > 138) with means between 0.3 – 3.2ng/g d.w. Species-related differences in contaminant accumulation were minimal. Temporal differences were detected in both species for cis- and trans-permethrin, and PCBs 18, 118 and 149, with seasonal changes linked to the fat content and gametogenic cycle of the mussels. All contaminants within the mussels were below the maximum limits set by international regulatory bodies, and both species can therefore be considered safe for human consumption. This study establishes baseline data which can be used by future POP monitoring studies in Saldanha Bay to assess the impacts of planned expansions in heavy industry and urban growth.

5.2. INTRODUCTION

In some parts of the modern world, the health benefits of consuming seafood are currently being outweighed by the potential health risks, due to anthropogenic pollution of marine ecosystems resulting in elevated levels of harmful pollutants in the flesh of edible marine species. A concerning and well-studied group of marine contaminants are the Persistent Organic Pollutants (POPs), which include pesticides such as DDT (dichlorodiphenyltrichloroethane), fungicides such as hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) previously used in the electrical industry, and dioxins and furans produced unintentionally during industrial processes (Tesar, 2000). POPs are introduced into marine environments through a variety of sources; the increased presence of persistent organic pollutants such as PCB's, dichlorodiphenyltrichloroethane (DDT) and polyaromatic hydrocarbons (PAHs) in marine coastal areas has been linked to proximity to industrial and urban areas, with harbours and rivers often being a major input of these pollutants (Soclo *et al.*, 2000;

Perugini *et al.*, 2007; Campillo *et al.*, 2017). The reason these chemicals pose serious human and ecosystem health risks is their general hydrophobic and lipophilic natures, meaning they have a high bioavailability to plants and animals, resulting in them bioaccumulating in the fat of living creatures and biomagnifying up the food chain (Bayarri *et al.*, 2001; Abdel-Shafy & Mansour, 2016).

The Stockholm Convention on Persistent Organic Pollutants was created by the United Nations Environmental Programme (UNEP) to control and phase out 12 priority POPs which are hazardous to human and ecosystem health, after it was discovered that these chemicals are transported around the world via oceans and the atmosphere (Olenycz *et al.*, 2015), and therefore the commitment of all countries was necessary to effectively control these substances (Tesar, 2000). South Africa entered into the Stockholm Convention on POPs, in an international binding agreement, in September 2004 (DEA, 2012). South Africa has since implemented various programmes to identify, control and dispose of hazardous POPs, but continues to use DDT for malaria vector control (DEA, 2012) and is known to be one of the largest importers of pesticides in sub-Saharan Africa, due to the extent and variety of crops grown in the country (Quinn *et al.*, 2011).

Despite strict legislation on the limits of metals and other contaminants in human and animal food, South Africa does not have specific legislation governing the maximum limits of POPs in foodstuffs at the time of writing. This study will therefore use the limits set by the European Commission and the United States of America, summarised in Table 5.1 below, to assess the consumer safety of the investigated mussels.

Table 5.1 Maximum limits for persistent organic pollutants in fish and shellfish (wet weight)

Pollutant	ML	Regulatory body	Specifications
Benzo(a)pyrene	5µg/kg	(European Commission, 2006)	Bivalve molluscs (fresh, chilled or frozen)
Σ benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene	30µg/kg	(European Commission, 2006)	Bivalve molluscs (fresh, chilled or frozen)
Σ Dioxins (PCDD/F) and dioxin-like PCBs	6.5pg/g	(European Commission, 2006)	Muscle meat of fish and fishery products
Σ 6 indicator PCBs	75ng/g	(European Commission, 2006)	Muscle meat of fish and fishery products
DDT	5ppm	(USEPA, 2000)	Fish
Chlordane	0.3ppm	(FDA, 2008)	Fish
Dieldrin	0.3ppm	(FDA, 2008)	Fish

Due to the inclusion of POPs in the worldwide Mussel Watch Programme proposed by Edward Goldberg in the 1970's (Goldberg, 1975), the concentrations of a variety of organic contaminants have been assessed in mussel and fish species in many countries. Mussels have been found to effectively accumulate OCP's, PAH's and PCB's, but typically to a lower extent than species higher up the food chain which possess higher fat contents (Bayarri *et al.*, 2001; Perugini *et al.*, 2007). Overall, PAH concentrations in mussels are generally lower than in fish species, but because of their low biotransformation capabilities when compared to vertebrates, mussels tend to accumulate greater concentrations of carcinogenic high molecular weight (HMW) PAH's (Baumard *et al.*, 1998; Jonsson *et al.*, 2004; Perugini *et al.*, 2007; Degger, 2010; Kljaković-Gašpić *et al.*, 2010). Numerous studies have also found mussels to preferentially accumulate the specific hexachlorobiphenyl PCB congeners 138 and 153, two of the six indicator congeners commonly used in PCB contamination studies (Okay *et al.*, 2009; Degger *et al.*, 2011a; Kampire *et al.*, 2015). Lastly, temporal variations in the concentration of persistent organic pollutant in mussel and fish tissues are common in the literature; these fluctuations have been attributed to the lipophilic nature of organic contaminants resulting in their concentrations being dependent on the fat content of the mussels, which in turn varies with gametogenesis and food availability (Herceg-Romanić *et al.*, 2014; Olenycz *et al.*, 2015).

Despite the analysis of POPs in South African marine species occurring on a far less frequent basis than in more developed countries, some literature does exist on the contamination levels within local coastal waters and animals. A variety of POPs have been assessed in blacktail (*Diplodus sargus capensis*), hottentot (*Pachymetopon blochii*), yellowfin tuna (*Thunnus albacares*), yellowtail (*Seriola lalandi*), South African snoek (*Thyrsites atun*), soupfin shark (*Galeorhinus galeus*), Blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*) and smoothhound shark (*Mustelus mustelus*), and DDT concentrations were determined in the great white shark (*Carcharodon carcharias*) (Schlenk *et al.*, 2005; Chukwumalume, 2016). Low to moderate PAH and DDT contamination levels were determined around the South African coastline, which were linked to input sources (such as current DDT use in KwaZulu Natal), feeding habit, trophic level and lipid content (Schlenk *et al.*, 2005; Chukwumalume, 2016). The majority of PAHs identified were LMW, and the concentrations of the indicator PAH, benzo(a)pyrene, exceeded the maximum limits set by the EU in a variety of species and locations, including Saldanha Bay (Chukwumalume, 2016). The only other published research on PAH and PCB contamination levels was performed by Degger *et al*. (2011) using *Perna perna* mussels, where PAH's were found to be ubiquitous along the South African coastline, with Saldanha Bay having the highest levels of contamination of the four harbours analysed. Mussels from Saldanha Bay were also found to possess the greatest variety of PCB congeners, despite Cape Town harbour having the highest levels of PCB contamination (Degger *et al.*, 2011a).

It is therefore evident from the literature that Saldanha Bay is a potential area of concern for persistent organic pollution, which is unsurprising due to the nature of the bay; as the only deep-water port in South Africa, and the site of heavy industries such as mining and steel manufacturing, it is naturally an at-risk area for anthropogenic pollution (Clark *et al.*, 2002). The prevalence of both PCBs and PAHs in sediments and mussels has been linked directly to large harbours, petroleum pollution and heavy industry in numerous studies (Soclo *et al.*, 2000; Kampire *et al.*, 2015; Olenycz *et al.*, 2015), and the observed PCB contamination levels of *M. galloprovincialis* samples from Port Elizabeth Harbour were attributed directly to industrial activities and heavy shipping traffic (Kampire *et al.*, 2015). Unfortunately, despite strict programmes monitoring the trace metal and biological contamination (*Escherichia coli*, Paralytic Shellfish Poisoning, etc.) levels of farmed and wild mussels within Saldanha Bay, no consistent monitoring of POP pollution has occurred in the area since the Saldanha State of the Bay report declared hydrocarbon pollution to be of no concern in 1999 (Clark *et al.*, 2002, 2014, 2016).

This lack of POP monitoring is a trend within the country; there are currently very few published POP studies on marine species in South Africa, and because government run initiatives such as the Mussel Watch Programme [conducted by Marine and Coastal Management (MCM) and the Council for Scientific Research (CSIR)] do not release their data to the public, the burden has fallen onto universities and private researchers to investigate these topics (Wepener & Degger, 2012). It has also been noted that, since the 1980's, there has been an overall decrease in the number of pollution studies in South Africa, and that most published data focuses on metal contamination rather than organic pollutants, likely due to high analysis costs (Wepener & Degger, 2012). This highlights the necessity for new organic pollution studies to assess current contamination levels along the South African coastline, and to establish baseline data which will allow for future comparative studies and could help protect both seafood consumers and the marine environment in the long term.

The aims of this study are therefore to determine the levels of POPs present in the farmed mussels of Saldanha Bay, *M. galloprovincialis* and *C. meridionalis*, in order to create a baseline dataset for future researchers, determine seasonal fluctuations in total POP content, and to use this information to advise consumers on the health of their seafood.

5.3. MATERIALS AND METHODOLOGY

For study location, sampling methods and sample preparation, please refer to Chapter 3.

5.3.1. Materials

Six-point GC calibration curves were established using Restek Calibration Standards, at concentrations of 0.5, 1, 5, 10, 50 and 100 µl. The PAH standard consisted of 16 PAHs [naphthalene, acenaphthylene, acenapthene, flourene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b,k)flouranthracene, benzo(a)pyrene, indeno(1,2,3)pyrene, dibenzo(a,h)anthracene and benzo(ghi)perylene] at 200 µg/mL, in methanol: benzene: methylene chloride (80:1.25:18.75), the multiresidue organophosphate pesticide (OPP) standard consisted of 6 pesticides (diazinon, chlorpyfiros, fenitrothion, quinalphos, pyrazophos, pyraclofos) at 100 µg/mL in toluene, the PCB standard consisted of 11 PCB's (congeners 18, 28, 44, 52, 70, 110, 118, 138, 149, 153 and 180) at 500 µg/mL in acetone, and the OCP standard consisted of 23 pesticides (chloroneb, hexachlorobenzene, alpha-, beta-, gamma- and y-lindane, heptachlor, acetochlor, aldrin, heptachlor epoxide, alpha-chlordane, trans-nonachlor, endosulfan I and II, endosulfan sulfate, 4,4-DDT, DDE and DDD, dieldrin chlorobenzilate, endrin and cis- and trans-permethrin) at 500 µg/mL in acetone.

Limits of quantification and detection were established using the calibration curves, and detection limits for all analytes are summarised in Table 5.2.

5.3.2. Sample analysis

The dried, homogenised samples (n=30 per species) were used in the Quick, Easy, Cheap, Efficient, Rugged and Safe (QuEChERS) method of organic pollutant analysis. Approximately one gram of sample was weighed into plastic 50ml QuEChERS Cleanert MAS-Q tubes. Nine ml of Milli-Q water were added to the dry sample, which was vortexed for 10 seconds to ensure mixing. Subsequently, 10ml acetonitrile and 100µl of internal standard was added. The first QuEChERS solution (1.5g NaCl + 6g $MgSO_4$) was then added to the tubes before 30 minutes of sonication in a water bath. Once sonicated, samples were centrifuged for 5 minutes (14°C, 7600rpm) in an Eppendorf Centrifuge 5430R until clear separation of the acetonitrile layer was visible. Five millilitres of the acetonitrile layer were then transferred into the 15ml QeEChERS tubes which contained a "cleaning" solution (300mg PSA, 150mg C18 + 900mg $MgSO_4$). The tubes were vortexed and centrifuged for 5 minutes. One millilitre of the top layer was pipetted into 5ml Eppendorf tube and centrifuged until dry. After drying, the sample was reconstituted with 1 ml toluene and placed in a GC vial for GC-MS/MS analysis; 1µl of the sample was injected on a Thermo TSQ 8000 triple quadrupole MS [operated in a Selected reaction monitoring (SRM) mode]. Separation of the POPs was performed using a Rxi 1310 gas chromatograph coupled with a non-polar Rxi-5Sil MS with integra-Guard (15 m, 0.25 mm ID, 0.25 µm film thickness) capillary column. The ionization source temperature was set at 250 °C and emission current of 50 µA was used with Argon collision. Results were presented in parts per billion dry weight (or ng/g d.w.).

Table 5.2 The full names and abbreviations of analytes and GC-MS/MS detection limits for PCBs, OCPs, OPPs and PAHs

Analyte	Abbreviation	Detection limit (ppb)	Analyte	Detection limit (ppb)
2,2',5-Trichlorobiphenyl	PCB 18	0.5	Chlorobenzilate	0.5
2,4,4'-Trichlorobiphenyl	PCB 28	0.5	Endrin	0.5
2,2',3,5'-Tetrachlorobiphenyl	PCB 44	0.5	Cis-/Trans-permethrin	0.5
2,2',5,5'-Tetrachlorobiphenyl	PCB 52	0.5	Diazinon	0.5
2,3,3',4',6-Pentachlorobiphenyl	PCB 110	0.5	Chlorpyfiros	0.5
2,3',4,4',5-Pentachlorobiphenyl	PCB 118	0.5	Fenitrothion	0.5
2,2',3,4,4',5'-Hexachlorobiphenyl	PCB 138	0.5	Quinalphos	0.5
2,2',3,4',5',6-Hexachlorobiphenyl	PCB 149	0.5	Pyrazophos	0.5
2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153	0.5	Pyraclofos	0.5
2,2',3',4,4',5,5'-Heptachlorobiphenyl	PCB 180	0.5	Naphthalene	0.5
Cloroneb		0.5	Acenaphthylene	0.5
Hexachlorobenzene	HCB	0.5	Accenapthene	0.5
α-, β-, γ-Lindane		0.5	Flourene	0.5
Heptachlor		0.5	Phenanthrene	0.5
Acetochlor		0.5	Anthracene	0.5
Aldrin		0.5	Flouranthene	0.5
Heptachlor epoxide		0.5	Pyrene	0.5
α-Chlordane		0.5	Chrysene	0.5
Trans-nonachlor		0.5	Benz(a)anthracene	0.5
Endosulfan I/II/sulfate		0.5	Benzo(b,k)flouranthracene	0.5
4,4-Dichlorodiphenyltrichloroethane	DDT	0.5	Benzo(a)pyrene	0.5
4,4-Dichlorodiphenyldichloroethylene	DDE	0.5	Indeno(1,2,3)pyrene	0.5
4,4-Dichlorodiphenyldichloroethane	DDD	0.5	Dibenzo(a,h)anthracene	0.5
Dieldrin		0.5	Benzo(ghi)perylene	0.5

5.3.2. Statistical analysis

The experimental design was a completely randomised design, with samples being collected every two months from the Imbaza Mussels rafts during normal harvesting processes. The two main effects were collection date and species. All data was analysed in Microsoft Excel using the additional statistical analysis software *X*LSTAT Premium (Annual Version 19.4.45237), developed by Addinsoft.

For the purpose of descriptive statistics and statistical analyses all non-detectable (ND) values were removed from the dataset. For POPs where >25% of the values were non-detectable (n=60), the data was considered insufficient for reliable or accurate statistical analysis, and therefore was not subjected to the ANOVA procedure. The normality and homoscedasticity of the data were checked, and where necessary data was squared or log-transformed. Two-way ANOVAs were then performed, with season and species selected as the main effects, and an interaction term was included. Where data was determined to be heteroscedastic, Welch's statistics were included to ensure heteroscedasticity did not affect the accuracy of the analysis. Post-hoc Bonferroni pairwise comparison tests were performed on all data to determine where significant differences were present. If normality of the data could not be achieved through transformation (as was the case for PCB 28 and 110, flourene and benzo(a)anthracene), Kruskal-Wallis non-parametric tests were performed, with post-hoc Steel-Dwass-Critchlow-Fligner multiple pairwise comparisons.

Pearson's correlation matrices were created for all POPs with sufficient data points (<25% of non-detectable values, n=30) and relevant total fat percentages of corresponding samples (sample processing and analysis detailed in Chapter 3) in order to examine potential linear relationships. For this study, though the Pearson's test itself identified many of the correlations as statistically significant, they were only considered to be biologically significant if the correlation coefficient (R value) equalled or exceeded 0.7.

5.4. RESULTS

Though a wide range of POPs were analysed in the samples, the Results and Discussion sections of this Chapter will focus on the POPs which were present or detectable within the samples, which included five PAHs [flourene, fouranthene, benzo(a)antracene, pyrene and benzo(a)pyrene], eight OCPs (4,4-DDD, cis-permethrin, trans-permethrin, alpha chlordane, trans-Nonachlor, dieldrin, chlorobenzilate, and endosulfan II) and 10 PCBs (congeners 11, 28, 44, 52, 110, 118, 138, 149, 153 and 180). No OPPs were detected in any of the samples. Due to the extremely low levels of POPs detected within the samples, values are not reported in wet weight unless necessary in the Discussion section for comparison to regulated maximum limits.

5.4.1. Species-related differences

Despite the contamination levels of *C. meridionalis* and *M. galloprovincialis* differing for a number of POPs (Table 5.3), statistical analyses only determined significant species variations (*M. galloprovincialis* > *C. meridionalis*) in PCB 118 and PCB 149.

5.4.2. Temporal trends

There was only sufficient data for temporal analyses in six PCB congeners (18, 28, 110, 118, 153 and 180), three PAHs [flourene, fluoranthene and benz(a)anthracene] and three OCPs (DDD, cis- and trans-permethrin). Significant temporal differences in contamination levels were found for PCB 18 in *C. meridionalis* (Figure 5.1), as well as PCBs 118 and 149 (Figure 5.2) and cis- and trans-permethrin (Figure 5.1) in both species, with a significant ($p \leq 0.05$) interaction term for all contaminants except PCB 149.

In *C. meridionalis*, both cis- and trans-permethrin peaked in May 2015 and June 2016, with trans-permethrin experiencing an additional peak in November 2015. The minimum seasonal values for both cis- and trans-permethrin occurred in July 2015, while in 2016 minimum values were reached in July for trans-permethrin and December for cis-permethrin. In *M. galloprovincialis* both cis- and trans-permethrin experienced maxima in November 2016 and September 2016, with minima occurring in July 2015 and December 2016.

In *C. meridionalis*, PCB 18 showed maxima in July 2016 and minima in September 2016, with no significant temporal differences identified in 2015. In 2015, PCBs 118 and 149 levels followed the same pattern, with maxima in November and minima in July, while in 2016 PCBs 118 and 149 peaked in June and September, respectively, with lowest values occurring in December and June, respectively. In *M. galloprovincialis* samples from 2015, the PCB congeners 118 and 149 reached maxima in September and November, respectively, with minima in May. In 2016, maximum values for PCB 118 and 149 occurred in June, with minimum values recorded in July.

5.4.3. Correlations

In *C. meridionalis*, moderate positive associations were found between fluoranthene and benz(a)anthracene ($r=0.66$) and PCB 118 and percentage fat ($r=0.64$), and a moderate negative correlation was found between PCB 110 and percentage fat ($r=-0.62$). In *M. galloprovincialis*, moderate positive correlations were detected between cis- and trans-permethrin and percentage fat ($r=0.65$ and $r=0.59$, respectively), between cis- and trans-permethrin ($r=0.77$), and PCB 118 and 149 ($r=0.59$).

Table 5.3 The means (±standard error) and ranges of persistent organic pollutants detected in *C. meridionalis* and *M. galloprovincialis* samples from Saldanha Bay (ng/g dry weight)

		Mean ± Std. Error		Range	
		C. meridionalis	*M. galloprovincialis*	*C. meridionalis*	*M. galloprovincialis*
Organochlorine Pesticides	Trans-Permethrin	38.6 ± 5.95	48.0 ± 8.14	ND – 95.67	1.54 - 162.53
	Cis-Permethrin	33.7 ± 6.03	35.6 ± 6.23	ND – 124.12	1.61 - 121.60
	Dieldrin	4.4 ± 1.29	3.2 ± 1.34	ND - 9.85	ND - 8.46
	Alpha chlordane	2.3 ± 0.39	1.2 ± 0.16	ND - 6.67	ND - 3.93
	Chlorobenzilate	1.5 ± 0.003	1.5 ± 0.002	ND - 1.47	ND - 1.46
	Endosulfan II	1.4 ± 0.02	1.5 ± 0.09	ND - 1.67	ND - 2.84
	Trans-Nonachlor	1.2 ± 0.002	1.2 ± 0.002	ND - 1.67	ND - 1.25
	4,4-DDD	0.7 ± 0.009	0.7 ± 0.009	0.66 - 0.84	0.67 - 0.86
Polyaromatic Hydrocarbons	Flourene	13.4 ± 2.91	11.2 ± 1.97	0.58 - 53.62	4.86 - 49.76
	Pyrene	4.7 ± 1.73	7.4 ± 3.81	ND - 26.29	ND - 55.23
	Benzo(a)pyrene	3.2 ± 0.65	2.1 ± 0.68	ND - 5.74	ND - 6.73
	Fluoranthene	3.1 ± 0.97	3.1 ± 1.49	ND - 16.82	ND - 28.99
	Benz(a)anthracene	1.0 ± 0.05	1.1 ± 0.10	ND - 1.88	ND - 3.49
Polychlorinated Biphenyls	PCB 18	0.5 ± 0.005	0.5 ± 0.005	0.49 - 0.60	0.49 - 0.60
	PCB 28	1.6 ± 0.005	1.6 ± 0.007	1.53 - 1.66	1.57 – 1.70
	PCB 44	0.4 ± 0.009	0.5 ± 0.01	ND - 0.50	ND – 0.53
	PCB 52	1.2 ± 0.003	1.2 ± 0.001	ND – 1.24	ND – 1.25
	PCB 110	1.5 ± 0.007	1.5 ± 0.005	ND – 1.59	ND – 1.55
	PCB 118	0.3 ± 0.008	0.3 ± 0.006	ND – 0.38	ND – 0.40
	PCB 138	0.3 ± 0.001	0.3 ± 0.016	ND – 0.28	ND – 0.33
	PCB 149	1.8 ± 0.004	1.8 ± 0.007	ND – 1.82	1.76 – 1.90
	PCB 153	1.4 ± 0.04	1.4 ± 0.05	ND – 1.89	ND – 1.86
	PCB 180	3.2 ± 0.01	3.2 ± 0.004	ND – 3.28	ND – 3.23
	Σ PCB's	6.9 ± 0.36	6.7 ± 0.60		

*ND = not detected

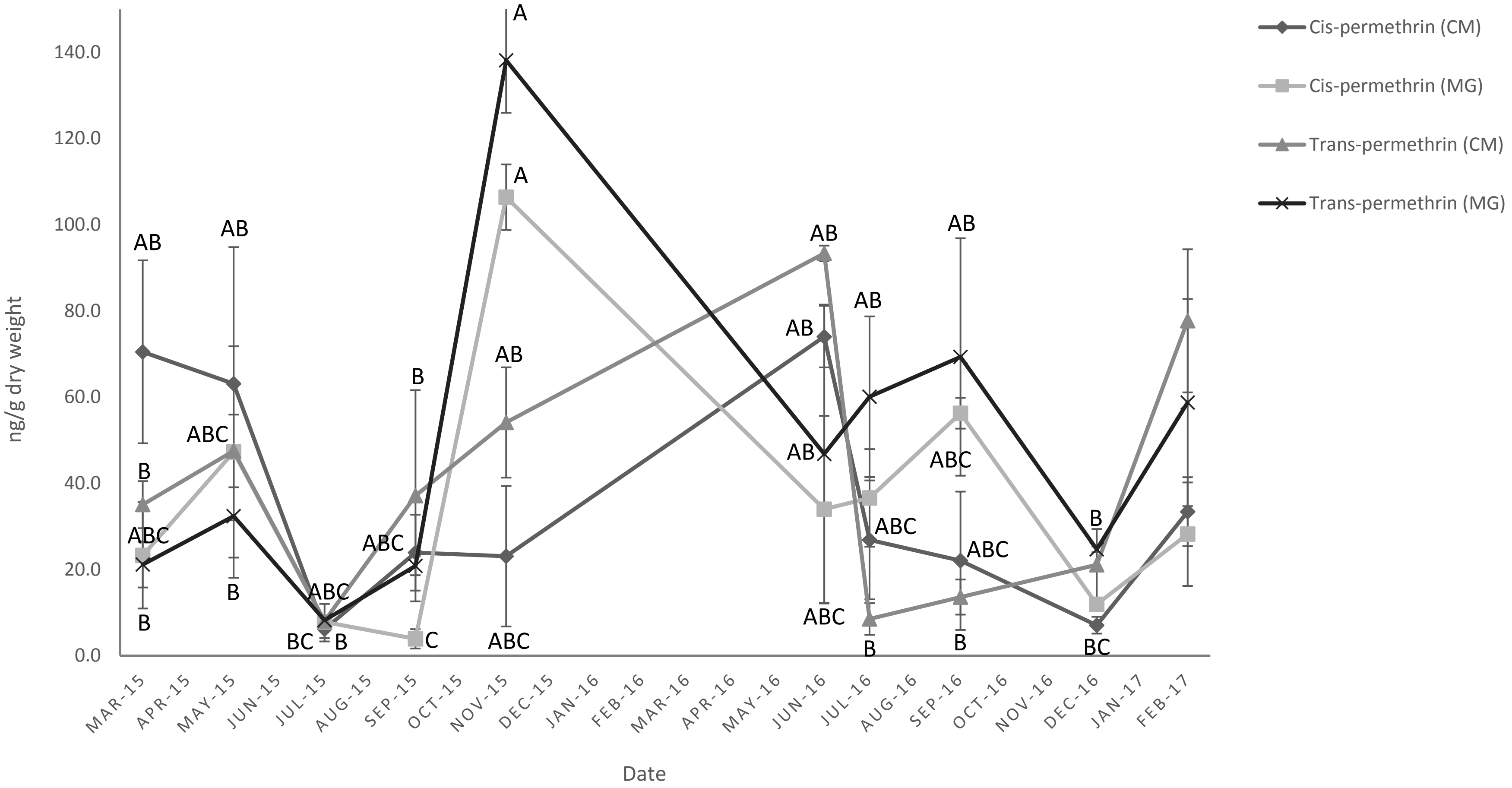


Figure 5.1 Temporal changes (±standard error bars) in the content of cis- and trans-permethrin in *C. meridionalis* (CM) and *M. galloprovincialis* (MG), with different letters denoting significant differences at p≤0.05

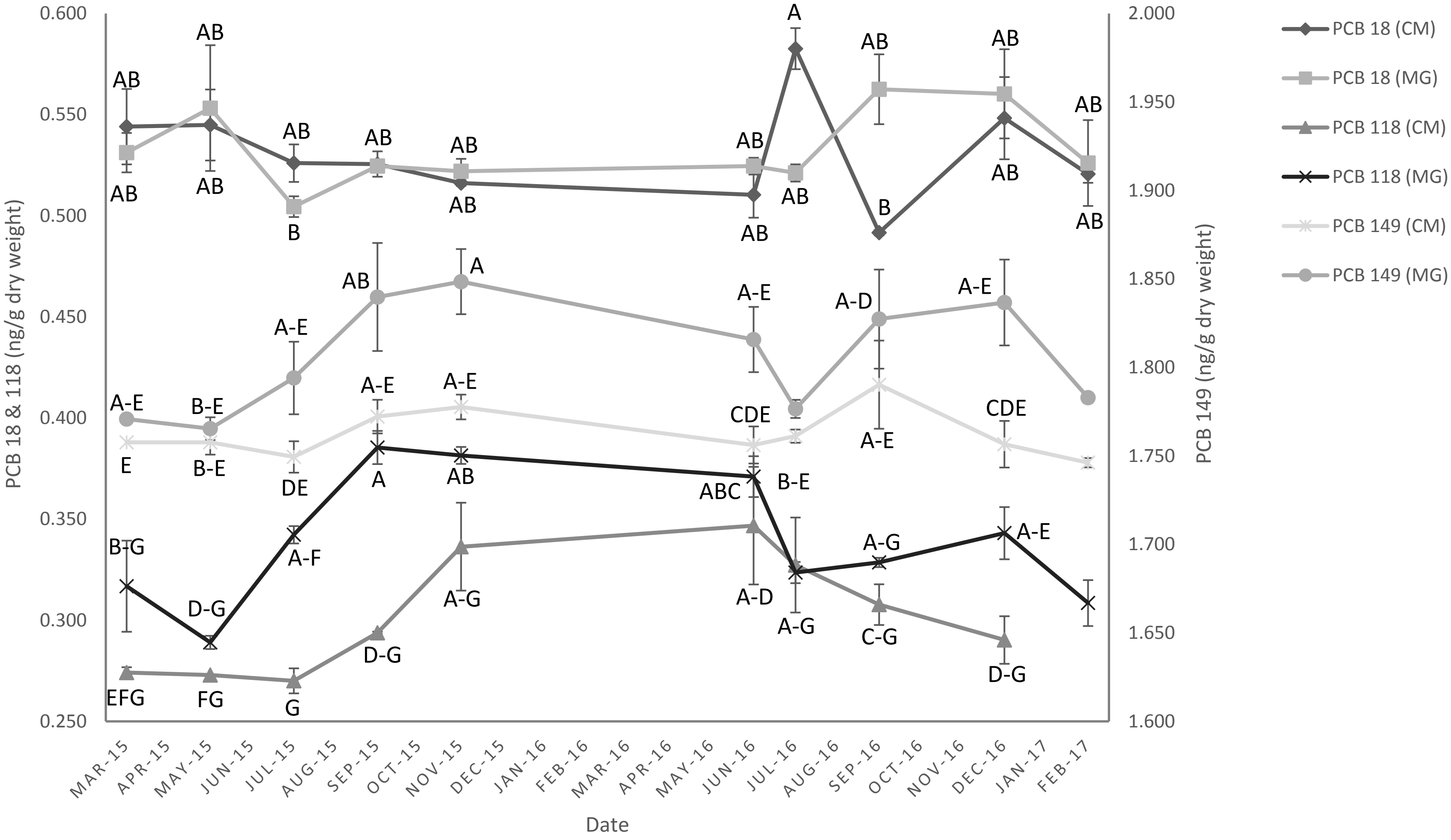


Figure 5.2 Temporal changes (±standard error bars) in the content of PCBs 18, 118 and 149 in *C. meridionalis* (CM) and *M. galloprovincialis* (MG), with different letters denoting significant differences at p≤0.05

5.5. DISCUSSION

5.5.1. Organochlorine Pesticides (OCP's)

Organochlorines are a popular type of pesticide used in agriculture and domestic residences worldwide, and the presence and concentration of OCPs in marine environments has been linked directly to nearby human settlements (Bayarri *et al.*, 2001; Kljaković-Gašpić *et al.*, 2010). Due to the wide variety of crops grown across the country, each with specific pesticide needs, South Africa is one of the largest importers of pesticides in sub-Saharan Africa, with over 500 registered pesticides (Quinn *et al.*, 2011). Pesticides are known to be the main source of POPs in sub-Saharan Africa (DEA, 2012), and the ability of these contaminants to accumulate in lipid tissue and bio-magnify up the food chain is well-known and a cause for concern to human health, as diet is one of the main routes of human exposure to these chemicals (Tesar, 2000).

Eight OCPs were detected within the farmed mussels of Saldanha Bay, and total OCPs were found to be the greatest contributor to total POP contamination in both species. In this study, cis- and trans-permethrin were the OCPs with the highest concentration in *C. meridionalis* and *M. galloprovincialis*, as well as the highest overall concentrations of all measured POPs. Permethrin is a pyrethroid insecticide commonly used for the control of mosquitoes and headlice, and while this chemical is also used in agriculture, over 70% is used for non-agricultural (domestic and industrial) purposes (USEPA, 2012). Pyrethroids are lipophilic compounds which can accumulate in the food chain and have the potential to disrupt the normal functioning of the human endocrine system (Sun *et al.*, 2007). They are also currently being investigated for their potential use in marine antifouling (as an alternative to more toxic chemicals) and have been found to significantly decrease the settlement of *Balanus albicostatus* barnacles on ships' hulls (Feng *et al*., 2009). This is concerning for mussel farmers in Saldanha Bay, as a heavy presence of pyrethroids in the water could reduce settlement rates of mussels on the culture ropes, and the presence of pyrethroids in the mussel samples should therefore be closely monitored. The peak concentrations of cis- and trans-permethrin coincided with peak fat concentrations (determined in Chapter 3) in both species in spring 2015 and 2016 (Figure 5.1), despite correlation tests not determining a significant linear relationship between these variables. The reason for the lack of correlation was likely that, other than maxima, cis- and trans-permethrin did not follow the same temporal trends as those seen for fat content. Seasonal fluctuations in OCP concentrations in mussel tissue are common, with studies linking these changes to the gametogenic cycle and lipid content (Lee *et al.*, 1996a; Kljaković-Gašpić *et al.*, 2010; Suárez *et al.*, 2013; Herceg-Romanić *et al.*, 2014).

Considering that Saldanha Bay does not have any large river inputs that would transport this chemical into the marine environment from agricultural sources (Clark *et al.*, 2002), it is likely that the majority of cis- and tans-permethrin entered Small Bay through storm water runoff or inefficiently treated waste water, a common issue in the area (Clark *et al.*, 2014). Regulations on the acceptable limits for permethrin in food samples were not available at the time of writing, but this chemical is generally accepted to have low toxicity to humans (USEPA, 2012). Permethrin is, however, known to be acutely toxic to aquatic species (USEPA, 2012), and therefore should be monitored in future to ensure there are no increases in permethrin pollution which could result in adverse health effects on the farmed mussels.

A number of other OCPs were also detected in the mussel samples, including alpha-chlordane, trans-nonachlor, dieldrin, chlorobenzilate and endosulfan II. Alpha chlordane and trans-nonachlor (hereafter referred to as chlordane and nonachlor) are two chemicals which form a part of "technical chlordane", an insecticide mostly used for the control of termites (NPIC, 2000), and chlordane is one of the "Dirty Dozen" targeted by the Stockholm Convention on POPs for complete elimination worldwide (Tesar, 2000). Chlordane has been used as a broad spectrum insecticide in South Africa since the 1940's, but the import or use of this chemical in agriculture has been prohibited since 2005 (DEA, 2012). Chlordane is a potential human carcinogen (Tesar, 2000) and is currently listed as a "Group 1" hazardous substance in South Africa, meaning that importers of this insecticide would need to obtain a license which specifies the strict conditions for use (DEA, 2012). Dieldrin, another of the "Dirty Dozen", has been used for the control of soil insects in South Africa since the 1950's, but was prohibited for import or use in agriculture in 1983 (DEA, 2012). While regulations on the limits of these pesticides in fish do not currently exist in South Africa, the Food and Drug Administration (FDA) of the United States of America recommends chlordane and dieldrin concentrations in fish are kept below 0.3 parts per million (ppm) or 300ng/g to protect human consumers (FDA, 2008). The mean levels of chlordane, dieldrin and nonachlor detected within both *C. meridionalis* (2.3, 4.4 and 1.2ng/g d.w., respectively) and *M. galloprovincialis* (1.2, 3.2 and 1.2ng/g d.w.), even when considered cumulatively, are far below the FDA recommendations, and therefore do not pose a threat to human consumers.

Endosulfan and chlorobenzilate are two agricultural pesticides which were deregistered or withdrawn from use in South Africa in the 1970's (Naidoo & Buckley, 2003). While no maximum allowable limits could be found for chlorobenzilate or endosulfan in fish, the FAO and WHO recommend an acceptable daily intake of 0.02mg/kg bodyweight for chlorobenzilate in the human diet (FAO/WHO, 1969). This acceptable daily intake limit equates to 1.2mg per day for a 60kg adult; considering that both chlorobenzilate and endosulfan levels were miniscule in both *C. meridionalis* (1.5 and 1.4ng/g d.w.) and *M. galloprovincialis* (1.5 and 1.5ng/g d.w.), the number of mussels a human would have to eat to reach this limit is inconceivable, and the levels of these OCPs in mussels can therefore be considered to pose no threat to consumers.

The use of the pesticide DDT is highly controversial, due to its high rate of persistence in terrestrial and marine environments, and the fact that it has devastating effects on populations of large birds of prey and can cause chronic illness in humans (Tesar, 2000). Though it is listed as one of the “Dirty Dozen” POPs targeted for immediate action by UNEP, as well as being a “Group 1” hazardous substance prohibited for general use in South Africa since 1983, the country continues to use DDT for malaria vector control in the KwaZulu Natal, Mpumalanga and Limpopo provinces (DEA, 2012). The Stockholm Convention on POPs includes the exemption of DDT use for malaria vector control in various countries worldwide due to the fact that it continues to be the cheapest, easiest and most effective form of malaria control, and is therefore essential for the prevention of human suffering caused by this disease (Tren & Bate, 2004).

In previous studies, the presence of DDT has been linked specifically to riverine sources, due to either the remobilization of toxic DDT from sediments or the continued use of DDT in agricultural practices or malaria vector control (Herceg-Romanić *et al.*, 2014; Campillo *et al.*, 2017). Considering this fact, Saldanha Bay is unlikely to be heavily contaminated by DDT as it has no large river inputs (Clark *et al.*, 2002) and is situated on the West Coast of South Africa, far from the provinces where DDT is still in use. The results of this study show that neither DDT nor its metabolite DDE were detectable in any of the samples, with only DDD metabolite residues present in the mussels. The ratio of DDE and DDD to DDT is indicative of the level of degradation of the DDT in the environment, giving an idea of whether or not new DDT has been released into the system in recent years (Ballschmiter & Wittllnger, 1991; Carro *et al.*, 2014). It is generally accepted that a (DDE+DDD)/DDT ratio <1 is indicative of recent inputs of DDT into an ecosystem (Ballschmiter & Wittllnger, 1991; Shi *et al.*, 2013; Carro *et al.*, 2014; Campillo *et al.*, 2017). The fact that no DDT was detectable within the mussels therefore suggests that there have been no recent inputs of DDT into the marine ecosystem. The DDD residues detected could either be from former local sources (if DDT was used in the area prior to 1983), or potentially from oceanic or atmospheric transport of this persistent chemical from local or international sources; DDT has been shown to be present in locations as remote as the Arctic, giving evidence to its persistence and potential for long-range transport (Tesar, 2000).

The levels of DDD within the mussels from Saldanha Bay (0.7ng/g d.w. for both species) was found to be low in comparison to wild *M. galloprovincialis* samples collected from harbours and industrial areas in Croatia (Kljaković-Gašpić *et al.*, 2010) and Spain (Campillo *et al.*, 2017), and similar to those found for a mussel farm situated along the Croatian coastline (Herceg-Romanić *et al.*, 2014). The South African government does not have regulations in place for DDT residues or metabolites in fish, but the United States Environmental Protection Agency suggests an “action level” of 5ppm (500ng/g), and the contamination levels within the mussels of this study fall far below this limit. The farmed mussels of Saldanha Bay can therefore be considered safe for human consumption with regard to DDT residues or metabolites. Campillo *et al*. (2017) state that concentrations of DDT and its metabolites are decreasing in many parts of the world due to strict controls of this pesticide's sale and usage, and the results of this study confirm that this is likely the case in Saldanha Bay. A study on the DDT content of plastic pellets from a beach 40km South of Saldanha Bay found total DDT concentrations to decrease from 1 281ng/g in 1989 to 31ng/g in 2008 (Ryan *et al.*, 2012), supporting the conclusion that DDT concentrations are decreasing due to a lack of new input into the ecosystem. Aside from the study by Ryan *et al*. (2012), however, no other studies have been performed on the long-term changes of POPs in the Saldanha Bay area and this study cannot, therefore, decisively conclude whether overall permethrin, chlordane, nonachlor, dieldrin, endosulfan, chlorobenzilate and DDT residues in Saldanha Bay are in fact decreasing from historic levels, though evidence suggests that they are.

5.5.1. Polyaromatic Hydrocarbons (PAH's)

Polyaromatic hydrocarbons are produced mainly through the incomplete combustion of organic materials (coal, oil and wood) in human industry, but can also be formed naturally through forest fires, volcanism and petroleum seepage (Perugini *et al.*, 2007; Abdel-Shafy & Mansour, 2016). These chemicals are hydrophobic and lipophilic and therefore accumulate readily in sediments and onto dissolved organic matter in the water column (Abdel-Shafy & Mansour, 2016). They are known to be both toxic and carcinogenic to humans, and the main routes of human exposure are through the atmosphere and diet (Abdel-Shafy & Mansour, 2016). PAH contamination levels in marine environments have been linked to large harbours and urban areas affected by petroleum pollution (Chase *et al.*, 2001; Degger *et al.*, 2011b; Galgani *et al.*, 2011; Yoshimine *et al.*, 2012; Olenycz *et al.*, 2015). In a prior study on PAH levels in mussels from harbours around South Africa, Saldanha Bay harbour was identified as the most PAH-contaminated harbour in the country (Degger *et al.*, 2011a), and is therefore an area of concern for PAH pollution.

Five PAHs were detected in the farmed *C. meridionalis* and *M. galloprovincialis* samples from Saldanha Bay, namely flourene, fluoranthene, benz(a)anthracene, pyrene and benzo(a)pyrene (Table 5.3). The PAHs with the highest concentrations were flourene and pyrene, which made up 53 and 19% of the total PAHs in *C. meridionalis* and 45 and 30% in *M. galloprovincialis*, respectively. The predominance of flourene, a 2-ring, low molecular weight (LMW) PAH, suggests that the source of PAHs in Saldanha Bay is petrogenic (Yoshimine *et al.*, 2012; Stogiannidis & Laane, 2015). Petrogenic PAHs are introduced into marine environments through municipal and urban runoff, oil spills and routine tanker operations (Zakaria *et al.*, 2002), all of which occur in Saldanha Bay. Though the deep water harbour of Saldanha Bay was initially designed exclusively for the export of iron ore, it is currently used for the importation of crude oil (Clark *et al.*, 2002), and this is likely a major source of the PAH contamination in the farmed mussels. While flourene was the most abundant in both species, a wider variety of high molecular weight (HMW) PAHs were detected within the samples; namely fluoranthene, benz(a)anthracene, pyrene and benzo(a)pyrene. This is line with the literature, as mussels are known to possess limited biotransformation capabilities, unlike vertebrates, meaning they struggle to transform and excrete the higher weight, more hydrophobic PAHs which then accumulate in their flesh (Baumard *et al.*, 1998; Jonsson *et al.*, 2004).

At the time of writing, South Africa did not have regulations on the maximum limits of PAHs in mussels or fish, and therefore the EU maximum limits of 5µg/kg (5ng/g) for benzo(a)pyrene and 30µg/kg for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene in bivalve molluscs were used instead to assess the health and food safety of the mussels (European Commission, 2006). The levels of benzo(a)pyrene in both the farmed *C. meridionalis* and *M. galloprovincialis* samples (3.2 and 2.1ng/g d.w., respectively) were found to be significantly lower than the limit set by the EU for bivalve molluscs (European Commission, 2006). The sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene (ND) and chrysene (ND) in *C. meridionalis* and *M. galloprovincialis* (3.2 and 2.1ng/g, respectively) also fell considerably below the EU limit set for these PAHs in bivalves (European Commission, 2006). The farmed mussels from Saldanha Bay can therefore be considered safe for human consumption, but monitoring of these POPs should continue in future to ensure that there are no increases in contamination as the size and uses of the harbour increase.

5.5.3. Polychlorinated Biphenyls (PCB's)

Polychlorinated biphenyls were initially produced for use as heat-exchange fluids in the electrical industry, but their production has been banned in many countries since the 1970's after it was realised that they were a potential carcinogen with serious negative impacts on the human immune system (Tesar, 2000). There are 209 different types of PCBs, with each congener possessing different levels of toxicity to humans and other animals (Tesar, 2000). South Africa has never been a producer of PCBs and, in accordance with the Stockholm Convention on POPs, is currently in the process of phasing out all PCB oils and oil-containing equipment, with imports of PCBs classified as "Prohibited and Restricted" (DEA, 2012). The major sectors which still use PCB-containing equipment in South Africa are the energy, mining, transport, chemical manufacturing and petrochemical industries (DEA, 2012), many of which are present in Saldanha Bay. Studies on PCB contamination in mussels have repeatedly linked the levels of contamination to the proximity of the samples from industrial zones, rivers which run through urban areas, and harbours or heavy shipping routes (Burt & Ebell, 1995; Bayarri *et al.*, 2001; Okay *et al.*, 2009; Kampire *et al.*, 2015; Olenycz *et al.*, 2015). A prior study on PCB contamination in South African harbours found that, while mussels situated in Cape Town harbour presented the highest concentrations, Saldanha Bay harbour had the highest PCB congener variety (Degger *et al.*, 2011a), indicating that Saldanha Bay may be an area of concern for PCB pollution.

In this study, 10 PCB congeners were detected within the flesh of both farmed mussel species (Table 5.3). The PCB's with the highest concentrations were congeners 28, 52, 110, 149, 153 and 180, with PCB 180 being present in the samples at far higher rates than any other congener (Table 5.3). Previous studies on mussels from harbours in South Africa and around the world have found similar results, with higher chlorinated biphenyls such as congeners 138, 153 and 180 often being the major contributors to PCB contamination in mussels (Bayarri *et al.*, 2001; Okay *et al.*, 2009; Degger, 2010; Kampire *et al.*, 2015; Olenycz *et al.*, 2015). This is likely because higher chlorinated PCBs possess lower water solubility and volatilizations rates, and higher bioaccumulation rates in fat, meaning they persist in sediments and marine environments (WHO, 1993). Mono-, di- and trichlorinated biphenyls (PCBs 18 & 28) are also broken down rapidly by microorganisms in the water column, while tetrachlorinated biphenyls (PCBs 44 & 52) are broken down slowly, and higher chlorinated biphenyls are resistant to breakdown (WHO, 1993). The results of this study are therefore in line with the literature, with lipophilic, breakdown-resistant PCBs present in higher concentrations within the flesh of the mussels. The PCB congeners 118 and 149 were determined to be significantly greater in *M. galloprovincialis* than *C. meridionalis*, but whether these differences are biologically significant is debatable, as the concentrations were so low (parts per billion). Other than the species-specific differences in these two congeners, the minimal variation in mean PCB contents between the two species (Table 5.2) suggests that the bioaccumulation and excretion of PCBs occurs at a similar rate in *C. meridionalis* and *M. galloprovincialis*.

Three of the studied PCBs, namely the congeners 18, 118 and 149, were determined to experience significant seasonal fluctuations throughout the two-year study period (Figure 5.2). PCB 18 showed seasonal differences exclusively for *C. meridionalis* in 2016; the winter maxima and spring minima of PCB 18 concentrations corresponded with minimum and maximum fat contents for 2017 (Chapter 3), suggesting a link between the fat content of the mussels and the PCB contamination level, which is common in the literature (Bayarri *et al.*, 2001; Olenycz *et al.*, 2015). In *C. meridionalis* in 2015, PCB congeners 118 and 149 were at a minimum in winter, slowly increasing until peaking in spring. In 2016, however, concentrations of PCB 118 and 149 followed an opposite pattern; maxima arose in winter and spring, respectively, with minima occurring in summer. With the exception of the 2016 minima, the accumulation and depletion patterns of PCB 118 are similar to those of fat content in *C. meridionalis* (Chapter 3), suggesting accumulation of this PCB congener within the fat of the mussels, while the temporal changes in PCB 149 are less clear. This result is supported by the correlation tests, which found moderate correlation between PCB 118 and fat content of the mussels. In *M.*

galloprovincialis in 2015, PCB 118 and 149 experienced seasonal minima in autumn, with maxima in spring. In 2016, minima occurred in winter, with maxima for PCB 118 found in late winter and maxima for PCB 149 found in summer. Both PCB 118 and 149 follow a pattern similar to that of fat content in *M. galloprovincialis* for the two-year study period, despite correlation tests not determining a significant linear relationship between these variables. Prior studies on the seasonal fluctuations of PCB contaminants within the flesh of mussels have repeatedly found changes to correlate with the fat content of the mussels, which is in turn dependent on their gametogenic cycle (Olenycz *et al.*, 2015).

The PCB contamination levels of the mussels in Saldanha Bay were low in comparison to *M. galloprovincialis* samples from Croatia (Kljaković-Gašpić *et al.*, 2010; Herceg-Romanić *et al.*, 2014), Spain (Campillo *et al.*, 2017), the Adriatic Sea (Bayarri *et al.*, 2001) and Turkey (Okay *et al.*, 2009), and *P. perna* mussels from Port Elizabeth harbour in South Africa (Kampire *et al.*, 2015). While South Africa does not have regulations for PCBs in fish, the European Commission has set a maximum limit of 75ng/g w.w. for the six indicator PCBs (namely PCB congeners 28, 52, 101, 138, 153 and 180) which are recommended by the European Union for assessing contamination levels and which generally comprise >50% of total PCBs (European Commission, 2006; Kampire *et al.*, 2015; Olenycz *et al.*, 2015). The sum of all detectable PCBs in *C. meridionalis* and *M. galloprovincialis* samples (6.9 and 6.7ng/g d.w., respectively, or 1.4ng/g w.w.), which included five of the six recommended indicator PCBs, was found to be lower than the limit set by the EC. The only dioxin-like PCB identified in the samples was PCB congener 118, and the maximum values (*C. meridionalis*: 1.9ng/g w.w. or 0.000057ngTEQ/g, ; *M. galloprovincialis*: 0.3ng/g w.w. or 0.000009ngTEQ/g) did not exceed the 6.5pg/g WHO-TEQ (World Health Organisation-Toxic Equivalency Factor) wet weight limit set by the EU for the sum of dioxins and dioxin-like PCBs in fish and fishery products (European Commission, 2006). Considering these findings, the farmed mussels from Saldanha Bay do not currently pose a human health risk to consumers and can be considered safe. It is noteworthy, however, that PCBs tend to bioaccumulate through the food chain, and mussels, as a low trophic species, are often the least contaminated animals in the marine environment (Bayarri *et al.*, 2001). Future research should therefore investigate the PCB contamination of higher trophic level species in Saldanha Bay area to ensure they too are safe for human consumption.

5.6. CONCLUSION

The ability of certain pesticides to bioaccumulate in living tissue and bio-magnify up the food chain makes them a serious concern for wildlife and human health, and the monitoring of their presence in human food species is therefore essential. A wide variety of POP sources exist in Saldanha Bay, including heavy industry, crude oil shipping activities, storm water runoff and improperly treated waste water (Clark *et al.*, 2014), all of which make the sheltered Small Bay where most aquaculture facilities are located an at-risk area for POP pollution. This study detected eight OCPs (trans-permethrin, cis-permethrin, dieldrin, chlordane, chlorobenzilate, endosulfan, nonachlor and DDD), five PAHs (flourene, pyrene, fluoranthene, benzo(a)pyrene and benz(a)anthracene), and 10 PCBs (180, 149, 28, 110, 153, 52, 18, 44, 118 and 138) within the flesh of farmed *C. meridionalis* and *M. galloprovincialis* from Small Bay. Species-related differences in contamination were minimal, and where seasonal trends could be established, they were mainly linked to changes in total fat content of the mussels, and therefore their gametogenic cycle. Despite the detection of a variety of POPs in the mussels, overall pollution levels were low compared to those in developed countries, and consistently below maximum limits set by the EU and USA for fish and bivalve molluscs. The results of this study show that farmed mussels from Small Bay are safe for human consumption with respect to POPs, and that Saldanha Bay does not currently suffer from significant inputs of persistent organic pollution, or alternatively that the mussel farm is in an area with sufficient water circulation to minimise contact with POPs. Unfortunately, due to a lack of prior data or research, this study cannot conclude whether persistent organic pollution levels are increasing or decreasing in Saldanha Bay. Future monitoring of these substances in the water and farmed mussels should be performed regularly, as imminent increases in industrialisation and urbanization of the Saldanha Bay area could increase the overall POP load within Small Bay and in turn threaten mussel aquaculture facilities through decreased mussel settlement rates and the bioaccumulation of toxic substances in their edible product.

5.7. REFERENCES

Abdel-Shafy, H. I., & Mansour, M. S. M. 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egypt. J. Pet. 25, 107–123 https://doi.org/10.1016/j.ejpe.2015.03.011.

Ballschmiter, K., & Wittllnger, R. 1991. Interhemisphere Exchange of Hexachlorocyclohexanes, Hexachlorobenzene, Polychlorobiphenyls, and 1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane in the Lower Troposphere. Environ. Sci. Technol. 25, 1103–1111 https://doi.org/10.1021/es00018a014.

Baumard, P., Budzinski, H., Garrigues, P., Sorbe, J. C., Burgeot, T., & Bellocq, J. 1998. Concentrations of PHAs (Polycyclic Aromatic Hydrocarbons) in various marine organisms in relation to those in sediments and to trophic level. Mar. Pollut. Bull. 36, 951–960 https://doi.org/10.1016/S0025-326X(98)00088-5.

Bayarri, S., Baldassarri, L. T., Iacovella, N., Ferrara, F., & Domenico, A. Di. 2001. PCDDs, PCDFs, PCBs and DDE in edible marine species from the Adriatic Sea. Chemosphere 43, 601–610 https://doi.org/10.1016/S0045-6535(00)00412-4.

Burt, J. S., & Ebell, G. F. 1995. Organic pollutants in mussels and sediments of the coastal waters off Perth, Western Australia. Mar. Pollut. Bull. 30, 723–732 https://doi.org/10.1016/0025-326X(95)00063-S.

Campillo, J. A., Fernández, B., García, V., Benedicto, J., & León, V. M. 2017. Levels and temporal trends of organochlorine contaminants in mussels from Spanish Mediterranean waters. Chemosphere 182, 584–594 https://doi.org/10.1016/j.chemosphere.2017.05.025.

Carro, N., Cobas, J., García, I., Ignacio, M., & Mouteira, A. 2014. Distribution and trend of organochlorine pesticides in galicia coast using mussels as bioindicator organisms. Possible relationship to biological parameters. Chemosphere 102, 37–47 https://doi.org/10.1016/j.chemosphere.2013.12.010.

Chase, M., Jones, S., Hennigar, P., Sowles, J., Harding, G. C., Freeman, K., Wells, P., Krahforst, C., Coombs, K., Crawford, R., Pederson, J., & Taylor, D. 2001. Gulfwatch: Monitoring Spatial and Temporal Patterns of Trace Metal and Organic Contaminants in the Gulf of Maine (1991–1997) with the Blue Mussel, *Mytilus edulis* L. Mar. Pollut. Bull. 42, 490–504 https://doi.org/10.1016/S0025-326X(00)00193-4.

Chukwumalume, R.-M. C. 2016. Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticide residues in selected marine fish species along the coast of South Africa. PhD, Stellenbosch University, Stellenbosch.

Clark, B., Laird, M., Hutchings, K., Liebau, V., Biccard, A., Turpie, J., & Parker-Mallick, N. 2014. The state of Saldanha Bay and Langebaan Lagoon 2013/2014. Technical Report. Report no. 1581/1 prepared by Anchor Environmental Consultants for the Saldanha Bay Water Quality Trust.

Clark, B. M., Massie, V., Hutchings, K., Laird, M., Biccard, A., Brown, E., Duna, O. O., & Turpie, J. 2016. The State of Saldanha Bay and Langebaan Lagoon 2016. Technical Report. Report No AEC 1691/1 prepared by Anchor Environmental Consultants (Pty) Ltd for the Saldanha Bay Water Quality Forum Trust.

Clark, B. M., Lane, S., Turpie, J., van Niekerk, L., & Morant, P. D. 2002. South Africa National Report Phase 1: Integrated Problem Analysis. Report prepared for GEF MSP Sub-Saharan Africa Project (GF/6010-0016), Cape Town.

DEA. 2012. National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants. Department of Environmental Affairs, South Africa.

Degger, N. 2010. The application of passive artificial devices for monitoring of metallic and organic pollutants along the South African coastline. MSc, University of Johannesburg, Johannesburg.

Degger, N., Wepener, V., Richardson, B. J., & Wu, R. S. S. 2011a. Brown mussels (*Perna perna*) and semi-permeable membrane devices (SPMDs) as indicators of organic pollutants in the South

African marine environment. Mar. Pollut. Bull. 63, 91–97 https://doi.org/10.1016/j.marpolbul.2011.04.024.

Degger, N., Wepener, V., Richardson, B. J., & Wu, R. S. S. 2011b. Application of artificial mussels (AMs) under South African marine conditions: A validation study. Mar. Pollut. Bull. 63, 108–118 https://doi.org/10.1016/j.marpolbul.2011.04.040.

European Commission. 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Communities L364, 5–24 https://doi.org/10.2203/dose-response.06-012.Hanekamp.

FAO/WHO. 1969. 1968 Evaluations of some Pesticide Residues in Food: The Monographs. Food and Agriculture Organisation of the United Nations (FAO/PL:1968/M/9/1), World Health Organisation (WHO/FOOD ADD./69.35), Geneva.

FDA. 2008. Compliance Policy Guide [CPG Sec. 575.100 Pesticide Chemical Residues in Food – Enforcement Criteria (CPG 7141.01)]. U.S. Department of Health and Human Services, Food and Drug Administration, Maryland.

Feng, D., Ke, C., Li, S., Li, C., & Guo, F. 2009. Pyrethroids as Promising Marine Antifoulants: Laboratory and Field Studies. Mar. Biotechnol. 11, 153-160 https://doi.org/10.1007/s10126-008-0130-9.

Galgani, F., Martínez-Gómez, C., Giovanardi, F., Romanelli, G., Caixach, J., Cento, A., Scarpato, A., Benbrahim, S., Messaoudi, S., Deudero, S., Boulahdid, M., Benedicto, J., & Andral, B. 2011. Assessment of polycyclic aromatic hydrocarbon concentrations in mussels (*Mytilus galloprovincialis*) from the Western basin of the Mediterranean Sea. Environ. Monit. Assess. 172, 301–317 https://doi.org/10.1007/s10661-010-1335-5.

Goldberg, E. D. 1975. The mussel watch - A first step in global marine monitoring. Mar. Pollut. Bull. 6, 111 https://doi.org/10.1016/0025-326X(75)90271-4.

Herceg-Romanić, S., Kljaković-Gašpic, Z., Klinčić, D., & Ujević, I. 2014. Distribution of persistent organic pollutants (POPs) in cultured mussels from the Croatian coast of the Adriatic Sea. Chemosphere 114, 69–75 https://doi.org/10.1016/j.chemosphere.2014.04.017.

Jonsson, G., Bechmann, R. K., Bamber, S. D., & Baussant, T. 2004. Bioconcentration, biotransformation, and elimination of polycyclic aromatic hydrocarbons in sheepshead minnows (*Cyprinodon variegatus*) exposed to contaminated seawater. Environ. Toxicol. Chem. 23, 1538–1548 https://doi.org/10.1897/03-173.

Kampire, E., Rubidge, G., & Adams, J. B. 2015. Distribution of polychlorinated biphenyl residues in sediments and blue mussels (*Mytilus galloprovincialis*) from Port Elizabeth Harbour, South Africa. Mar. Pollut. Bull. 91, 173–179 https://doi.org/10.1016/j.marpolbul.2014.12.008.

Kljaković-Gašpić, Z., Herceg-Romanić, S., Kožul, D., & Veža, J. 2010. Biomonitoring of organochlorine compounds and trace metals along the Eastern Adriatic coast (Croatia) using *Mytilus galloprovincialis*. Mar. Pollut. Bull. 60, 1879–1889 https://doi.org/10.1016/j.marpolbul.2010.07.019.

Lee, K. M., Kruse, H., & Wassermann, O. 1996. Seasonal fluctuation of organochlorines in *Mytilus edulis* L. from the South West Baltic Sea. Chemosphere 32, 1883–1895 https://doi.org/10.1016/0045-6535(96)00103-8.

Naidoo, V., & Buckley, C. 2003. Survey of Pesticide Wastes in South Africa and Review of Treatment Options. Report to the Water Research Commission (WRC Report No. 1128/1/03), 82pp.

NPIC. 2000. Chlordane Technical Fact Sheet. National Pesticide Information Center, United States Environmental Protection Agency, Oregon.

Okay, O. S., Karacik, B., Başak, S., Henkelmann, B., Bernhöft, S., & Schramm, K. W. 2009. PCB and PCDD/F in sediments and mussels of the Istanbul strait (Turkey). Chemosphere 76, 159–166 https://doi.org/10.1016/j.chemosphere.2009.03.051.

Olenycz, M., Sokotowski, A., Niewińska, A., Wołowicz, M., Namieśnik, J., Hummel, H., & Jansen, J. 2015. Comparison of PCBs and PAHs levels in European coastal waters using mussels from the *Mytilus edulis* complex as biomonitors. Oceanologia 57, 196–211 https://doi.org/10.1016/j.oceano.2014.12.001.

Perugini, M., Visciano, P., Giammarino, A., Manera, M., Di Nardo, W., & Amorena, M. 2007. Polycyclic aromatic hydrocarbons in marine organisms from the Adriatic Sea, Italy. Chemosphere 66, 1904–1910 https://doi.org/10.1016/j.chemosphere.2006.07.079.

Quinn, L. P., de Vos, J., Fernandes-Whaley, M., Roos, C., Bouwman, H., Kylin, H., Pieters, R., & van den Berg, J. 2011. Pesticide use in South Africa: one of the largest importers of pesticides in Africa, Pesticides in the Modern World – Pesticides Use and Management, Margarita Stoytcheva (Ed.), ISBN: 978-953-307-459-7.

Ryan, P. G., Bouwman, H., Moloney, C. L., Yuyama, M., & Takada, H. 2012. Long-term decreases in persistent organic pollutants in South African coastal waters detected from beached polyethylene pellets. Mar. Pollut. Bull. 64, 2756–2760 https://doi.org/10.1016/j.marpolbul.2012.09.013.

Schlenk, D., Sapozhnikova, Y., & Cliff, G. 2005. Incidence of organochlorine pesticides in muscle and liver tissues of South African great white sharks *Carcharodon carcharias*. Mar. Pollut. Bull. 50, 208–211 https://doi.org/10.1016/j.marpolbul.2004.11.032.

Shi, S., Huang, Y., Zhou, L., Yang, W., Dong, L., Zhang, L., & Zhang, X. 2013. A preliminary investigation of BDE-209, OCPs, and PAHs in urban road dust from Yangtze River Delta, China. Environ. Monit. Assess. 185, 4887–4896 https://doi.org/10.1007/s10661-012-2910-8.

Soclo, H. H., Garrigues, P., & Ewald, M. 2000. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: Case studies in Cotonou (Benin) and Aquitaine (France) Areas. Mar. Pollut. Bull. 40, 387–396 https://doi.org/10.1016/S0025-326X(99)00200-3.

Stogiannidis, E., & Laane, R. 2015. Source Characterization of Polycyclic Aromatic Hydrocarbons by Using Their Molecular Indices: An Overview of Possibilities. Rev. Environ. Contam. Toxicol. 234, 38 https://doi.org/10.1007/978-3-.

Suárez, P., Ruiz, Y., Alonso, A., & San Juan, F. 2013. Organochlorine compounds in mussels cultured in the Ría of Vigo: Accumulation and origin. Chemosphere 90, 7–19 https://doi.org/10.1016/j.chemosphere.2012.02.030.

Sun, H., Xu, X. L., Xu, L. C., Song, L., Hong, X., Chen, J. F., Cui, L. B., & Wang, X. R. 2007. Antiandrogenic activity of pyrethroid pesticides and their metabolite in reporter gene assay. Chemosphere 66, 474–479 https://doi.org/10.1016/j.chemosphere.2006.05.059.

Tesar, C. 2000. POPs: What they are; how they are used; how they are transported. North. Perspect. 26, 1–20.

Tren, R., & Bate, R. 2004. South Africa's War against Malaria: Lessons for the Developing World. Policy Anal. 513, 1–20.

USEPA. 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 2: Risk Assessment and Fish Consumption Limits, 3rd Edition. United States Environ. Prot. Agency, Washington, DC 1, 823-NaN-00–008 https://doi.org/EPA 823-B-00-008.

USEPA. 2012. Permethrin Facts (EPA 738-F-09-001). Prevention, Pesticides and Toxic Substances (7508P), United States Environmental Protection Agency.

Wepener, V., & Degger, N. 2012. Status of marine pollution research in South Africa (1960-present). Mar. Pollut. Bull. 64, 1508–1512 https://doi.org/10.1016/j.marpolbul.2012.05.037.

WHO. 1993. Polychlorinated Biphenyls and Terphenyls (second edition). International Programme on Chemical Safety, World Health Organisation, Geneva.

Yoshimine, R. V., Carreira, R. S., Scofield, A. L., & Wagener, A. L. R. 2012. Regional assessment of PAHs contamination in SE Brazil using brown mussels (*Perna perna* Linnaeus 1758). Mar. Pollut. Bull. 64, 2581–2587 https://doi.org/10.1016/j.marpolbul.2012.07.013.

Zakaria, M. P., Takada, H., Tsutsumi, S., Ohno, K., Yamada, J., Kouno, E., & Kumata, H. 2002. Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in Rivers and Estuaries in Malaysia: A

Widespread Input of Petrogenic PAHs. Environ. Sci. Technol. 36, 1907–1918 https://doi.org/10.1021/es011278.

CHAPTER 6

General Conclusions and Recommendations

Aquaculture is a growing industry in South Africa, with the potential to increase food security and create much-needed jobs for residents (Olivier *et al.*, 2013). Mussel aquaculture in Saldanha Bay is an example of this, but the expansion of heavy industry and human populations in and around the bay could threaten the future of the mussel and oyster farms through pollution of the waters in which the mussels are grown (Clark *et al.*, 2014), due to their propensity for accumulating and concentrating contaminants in the water column. The aim of this project was to conduct research into the seasonal changes in proximate and contaminant composition of the farmed mussels in Saldanha Bay, *C. meridionalis* and *M. galloprovincialis*, in order to assess differences in proximate composition and contaminant bioaccumulation between the two species, support and protect both the aquaculture industry and human consumers, and create baseline data for future pollution research and management.

In line with previous morphological studies, shell shape was found to differ between species, with *M. galloprovincialis* individuals having significantly longer, broader and more ventrally flattened shells than *C. meridionalis*, which is useful for identification and differentiation of the two species. Aside from meat yield, none of the biometric or proximate compositions differed significantly between the two species. Significant temporal variations were found in both *C. meridionalis* and *M. galloprovincialis* for whole weights, meat weights, percentage meat yields and proximate compositions (moisture, protein, fat and ash). These fluctuations were associated with the reproductive cycle of the mussels, due to the spring and summer spawning periods of the two species coinciding with highest moisture and ash percentage compositions, and the pre-spawn periods (during gamete build-up) coinciding with whole weight, and peak protein and fat percentage compositions.

The overall bioaccumulation of trace metals was found to be higher in *M. galloprovincialis* for Al, Cr, Fe, Zn, Cd and Pb, while *C. meridionalis* accumulated Cu and Mn to greater concentrations. These species-related differences could be attributed to differences in accumulation and excretion patterns, filtration and growth rates, and food selection processes. Temporal fluctuations were found for Fe and As concentrations in *C. meridionalis*, and Fe, As, Hg and Pb concentrations in *M. galloprovincialis*. For Fe these changes were linked to spawning (the release of metal-poor gonadal tissue into the water causing relative increases in metal content of the flesh), while for Cd and Pb concentrations were found to be higher during winter rainfall periods (when untreated storm water runoff volumes are high). While toxic Hg concentrations in the mussels were extremely low, and Cd was also found to be below maximum limits for bivalves, toxic As and Pb concentrations at times exceeded local maximum limits within the flesh of the mussels. Arsenic was determined to likely be of low risk to human consumers, as the majority of As in mussels occurs in the non-toxic form arsenobetaine, and Pb concentrations were found to be below the bivalve-specific limit set by the European Commission, despite exceeding local guidelines. Overall, mussels were found to accumulate essential trace metals such as Mn, Fe, Cu, Zn and Cr at concentrations not exceeding recommended limits for human consumption, and can therefore be considered healthy sources of these essential elements.

Both mussel species were found to accumulate a range of POPs, including eight OCPs (trans-permethrin, cis-permethrin, dieldrin, chlordane, chlorobenzilate, endosulfan, nonachlor and DDD), five PAHs (flourene, pyrene, fluoranthene, benzo(a)pyrene and benz(a)anthracene), and 10 PCBs (18, 28, 44, 52, 110, 118, 138, 149, 153 & 180). Species-related differences were determined for PCB 118 and 149, and temporal fluctuations were found for PCB 18 in *C. meridionalis*, and PCBs 118 and 149, as well as cis-and trans-permethrin, in both species. These seasonal changes were mostly connected to changes in fat composition of the mussels caused by spawning, and were therefore linked to the gametogenic cycle. Despite Saldanha Bay being one of the largest ports along the South African coastline, POP contamination levels were found to be relatively low within the flesh of the mussels, and consistently below maximum limits set by the EU and the USA, meaning they are safe for human consumption. A potential reason for the low contamination levels is the ideal location of the aquaculture facilities; located in the centre of the bay, the rafts would experience high rates of water replacement, thereby minimising the mussels contact with harmful contaminants within the water column. Due to the lack of previous POP research in South Africa in general, and Saldanha Bay specifically, this study could not determine whether POP pollution levels were increasing or decreasing.

The data from this study fills or expands on knowledge gaps in species-related differences and temporal changes in the proximate and contaminant compositions of farmed mussels from Saldanha Bay aquaculture facilities, and could be useful to farmers and consumers of mussels alike. Despite species-related differences in rates of contaminant accumulation, the results of this study suggest that farmed both *C. meridionalis* and *M. galloprovincialis* mussels from Saldanha Bay are a healthy source of protein and essential minerals for moderate shellfish consumers, with low overall fat and contaminant compositions.

A notable limitation experienced within this study was the gaps in data collection caused by either the absence of *C. meridionalis* samples on the rafts, or by the cessation of harvest activities during the harmful algal bloom (*Dinophysis acuminata*) event in 2016. Future studies conducted on the mussel farms in Saldanha Bay should ensure that a backup sample collection method is in place in order to avoid the gaps in data collection incurred by harmful algal blooms, which are a frequent issue along the Western coastline of South Africa (Shipton & Britz, 2007). As all harvest on the mussel farms is required by law to stop until samples have been sent away and ensured to be safe for human consumption, it is not possible to get the farm-owned boats to head out to sea during these periods, and the gaps this cause in data collection can make interpretation of the results difficult.

An aspect which warrants further research is the monitoring of the temperature, salinity, chlorophyll and contaminant concentrations of the water column in which the mussels are growing, as well as determining the proximate glycogen or carbohydrate composition of the mussels. This could aid researchers in conclusively determining whether seasonal changes in the meat weights, meat yield, proximate composition and contaminant accumulation of the mussels are indeed due to their gametogenic cycle or changes in environmental parameters and pollution inputs (Dare & Edwards, 1975; Bressan & Marin, 1985; Okumus & Stirling, 1998; Çelik *et al.*, 2012; Martínez-Pita *et al.*, 2012; Azpeitia *et al.*, 2016). Research is also currently lacking on the fatty acid compositions of mussels from Saldanha Bay; the fatty acid composition of *C. meridionalis* has not yet been published, and despite numerous studies being performed on this aspect of *M. galloprovincialis* in Europe, their research found fatty acid compositions to vary significantly between locations and seasons (Ventrella *et al.*, 2008; Fuentes *et al.*, 2009; Prato *et al.*, 2010; Bongiorno *et al.*, 2015; Fernández *et al.*, 2015; Azpeitia *et al.*, 2016), meaning that the composition of South African *M. galloprovincialis* cannot be extrapolated from prior studies overseas. To support this research, the fatty acid composition of the phytoplankton in the water column could also be investigated, as mussels do not have the capacity to synthesize omega-3 fatty acids and therefore rely entirely on their food sources for this (Ventrella *et al.*, 2008). Future research could also be conducted upon the sensory attributes of the two mussel species to determine whether the farmers' perception, that *C. meridionalis* female gonads have an unappealing flavour in comparison to those of *M. galloprovincialis*, is in fact accurate. Previous sensory trials have also been used to identify whether seasonal changes in the proximate composition and meat quality of the mussels are noticeable by consumers (Azpeitia *et al.*, 2017), and could be useful to compare local and exotic mussels to determine whether mussels from Saldanha Bay would be appealing to European consumers. This information would be useful to local farmers wishing to export their products overseas. A trial to determine consumers preferential mussel meat yield would also be beneficial to the mussel aquaculture industry, as it could help determine a minimum threshold below which consumers no longer enjoy the product.

While the levels of trace metal and POP contamination were generally found to be low, the fact that As and Pb at times exceeded local government regulations, that such a wide variety of POPs were detected within the samples, and that contamination levels cannot be determined to be increasing or decreasing due to a lack of prior studies, underpins the requirement for future research into the marine pollution of Saldanha Bay. Monitoring of harmful contaminants within the mussels and oysters grown in Saldanha Bay should occur regularly in order to determine whether the future expansion of the human population and heavy industry causes increased levels of pollution to enter the waters of the bay, thereby threatening the consumer safety of the raft-grown mussels. This researcher also recommends that either the South African government or private researchers should investigate the current maximum limits of contaminants within fish and bivalves, as these are at times non-specific to bivalve aquaculture (most are set for "fish") and can differ greatly from the strict European and American standards. Research is needed in order to establish bivalve-specific contamination limits which both protect human consumers but also do not hamper the growth of the aquaculture industry in Saldanha Bay.

6.1. REFERENCES

Azpeitia, K., Ferrer, L., Revilla, M., Pagaldai, J., & Mendiola, D. 2016. Growth, biochemical profile, and fatty acid composition of mussel (*Mytilus galloprovincialis* Lmk.) cultured in the open ocean of the Bay of Biscay (northern Spain). Aquaculture 454, 95–108 https://doi.org/10.1016/j.aquaculture.2015.12.022.

Azpeitia, K., Ríos, Y., Garcia, I., Pagaldai, J., & Mendiola, D. 2017. A sensory and nutritional validation of open ocean mussels (*Mytilus galloprovincialis* Lmk.) cultured in SE Bay of Biscay (Basque Country) compared to their commercial counterparts from Galician Rías (Spain). Int. Aquat. Res. 9, 89–106 https://doi.org/10.1007/s40071-017-0159-0.

Bongiorno, T., Iacumin, L., Tubaro, F., Marcuzzo, E., Sensidoni, A., & Tulli, F. 2015. Seasonal changes in technological and nutritional quality of *Mytilus galloprovincialis* from suspended culture in the Gulf of Trieste (North Adriatic Sea). Food Chem. 173, 355–362 https://doi.org/10.1016/j.foodchem.2014.10.029.

Bressan, M., & Marin, M. G. 1985. Seasonal variations in biochemical composition and condition index of cultured mussels (*Mytilus galloprovincialis* Lmk) in the Lagoon of Venice (North Adriatic). Aquaculture 48, 13–21 https://doi.org/10.1016/0044-8486(85)90048-1.

Çelik, M. Y., Karayücel, S., Karayücel, I., Öztürk, R., & Eyüboğlu, B. 2012. Meat yield, condition index, and biochemical composition of mussels (*Mytilus galloprovincialis* Lamarck, 1819) in Sinop, south of the Black Sea. J. Aquat. Food Prod. Technol. 21, 198–205 https://doi.org/10.1080/10498850.2011.589099.

Clark, B., Laird, M., Hutchings, K., Liebau, V., Biccard, A., Turpie, J., & Parker-Mallick, N. 2014. The state of Saldanha Bay and Langebaan Lagoon 2013/2014. Technical Report. Report no. 1581/1 prepared by Anchor Environmental Consultants for the Saldanha Bay Water Quality Trust.

Dare, P. J., & Edwards, D. B. 1975. Seasonal Changes in Flesh Weight and Biochemical Composition of Mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales. J. Exp. Mar. Bio. Ecol. 18, 89–97.

Fernández, A., Grienke, U., Soler-Vila, A., Guihéneuf, F., Stengel, D. B., & Tasdemir, D. 2015. Seasonal and geographical variations in the biochemical composition of the blue mussel (*Mytilus edulis* L.) from Ireland. Food Chem. 177, 43–52 https://doi.org/10.1016/j.foodchem.2014.12.062.

Fuentes, A., Fernández-Segovia, I., Escriche, I., & Serra, J. A. 2009. Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins. Food Chem. 112, 295–302 https://doi.org/10.1016/j.foodchem.2008.05.064.

Martínez-Pita, I., Sánchez-Lazo, C., Ruíz-Jarabo, I., Herrera, M., & Mancera, J. M. 2012. Biochemical composition, lipid classes, fatty acids and sexual hormones in the mussel *Mytilus galloprovincialis* from cultivated populations in south Spain. Aquaculture 358–359, 274–283 https://doi.org/10.1016/j.aquaculture.2012.06.003.

Okumus, I., & Stirling, H. P. 1998. Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. Aquaculture 159, 249–261 https://doi.org/10.1016/S0044-8486(97)00206-8.

Olivier, D., Heinecken, L., & Jackson, S. 2013. Mussel and oyster culture in Saldanha Bay, South Africa: Potential for sustainable growth, development and employment creation. Food Secur. 5, 251–267 https://doi.org/10.1007/s12571-013-0244-1.

Prato, E., Danieli, A., Maffia, M., & Biandolino, F. 2010. Lipid and fatty acid compositions of *Mytilus galloprovincialis* cultured in the Mar Grande of Taranto (Southern Italy): Feeding strategies and trophic relationships. Zool. Stud. 49, 211–219 https://doi.org/10.1007/s11745-997-0127-8.

Shipton, T., & Britz, P. J. 2007. A study on the status of aquaculture production and trade in South Africa. Volume 1: Industry Status and Diagnostic Report. A report for the Department of Trade and Industry produced by Enviro-Fish Africa (Pty.) Ltd. 90p.

Ventrella, V., Pirini, M., Pagliarani, A., Trombetti, F., Manuzzi, M. P., & Borgatti, A. R. 2008. Effect of temporal and geographical factors on fatty acid composition of *M. galloprovincialis* from the Adriatic Sea. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 149, 241–250 https://doi.org/10.1016/j.cbpb.2007.09.012.

ADDENDUM A

Table 1 The Range and mean trace metal compositions (mg/kg wet weight) of *C. meridionalis* and *M. galloprovincialis*

Element	Mean (± standard error)		Range (min-max)	
	C. meridionalis	*M. galloprovincialis*	*C. meridionalis*	*M. galloprovincialis*
Lithium	0.01 ± 0.004	0.1 ± 0.004	0.004 - 0.16	0.02 - 0.16
Boron	4.7 ± 0.05	4.7 ± 0.05	3.41 - 5.51	4.00 - 5.71
Vanadium	0.4 ± 0.09	0.7 ± 0.19	0.03 - 3.24	0.05 - 5.98
Cobalt	0.02 ± 0.001	0.02 ± 0.0004	0.01 - 0.03	0.02 - 0.03
Nickel	0.2 ± 0.02	0.2 ± 0.03	0.07 - 1.46	0.07 - 1.08
Selenium	0.4 ± 0.01	0.6 ± 0.01	0.26 - 0.59	0.42 - 0.76
Strontium	7.4 ± 0.13	8.2 ± 0.16	4.97 - 9.76	5.96 - 10.96
Molybdenum	0.6 ± 0.13	0.7 ± 0.18	0.07 - 3.83	0.07 - 5.05
Antimony	0.001 ± 0.0001	0.003 ± 0.0002	0.001 - 0.00	0.001 - 0.01
Barium	0.2 ± 0.02	0.3 ± 0.02	0.04 - 0.47	0.06 - 0.71
Beryllium	ND	ND	ND	ND