The microbial quality of locally harvested snoek (*Thyrsites atun*) as influenced by the current supply chain management

by

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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.

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Opsomming

Met die doel om die effek van die ongereguleerde prosesseringsketting op die kwaliteit van plaaslik gevangde snoek *Thyrsites atun* te bepaal, is mikrobiese en patogeniese (fekale kontaminasie indikators) teenwoordigheid en insidensie, spier pH en temperatuur tesame met heersende temperatuur geassesseer op verskeie punte van prosessering, van vang tot gebruiker gemeet. Twee duidelik onderskeibare metolodigeë; totale lewensvatbare tellings (TLT) in kolonievormende-eenhede (KVEs/cm^2) en polimerase kettingreaksie (PKR) was gebruik om bakteriële kontaminasie en die teenwoordigheid/afwesigheid van vier spesifieke indikator organismes (*Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* en *Vibrio parahaemolyticus*) in vyf verwante eksperimente te bepaal.

Die resultate wys dat meeste besmetting ontstaan rondom die stoorplekke aanboord die visskuite. Die aas- en goinglappe waarop die aas in kleiner stukkies gesny is, is geïdentifiseer as addisionele bronne van besmetting met 4.69 en 6.92 $\log_{10}$ KVE/cm^2, onderskeidelik. Daar is tot die slotsom gekom dat die vissermanne se hande verantwoordelik was vir die oordrag van menslik-verwante patogene *E. coli* en *S. aureus* nadat die snoek beide van die skuit en bakkie afgelaai is. Daar is waargeneem dat deur snoek op ys te hou kon temperature van onder 10˚C gehandhaaf word vir ongeveer 10-12 stoor-ure nadat die skuit afgelaai is. Die verskillende behandelings gebruik (ys en skoonmaak) is waargeneem om nie ’n aansienlike invloed op bakteriële ladings te hê nie, terwyl ys waargeneem is om die afname in spier pH-vlakke sowel as die vertraging in bakteriële groei te handhaf.

Herhaalde en gerepliseerde metings van hierdie studie word vereis om die verskeie bronne en weë van kontaminasie te verken. Dit sal meer verteenwoordigend wees van hanterings- en sanitasie prosedures wat tans in plek is vir die snoekvoorsieningsketting. Die samevatting van hierdie studie dui op ‘n aanbeveling dat die standaard operasionele prosedures (SOPs) afgedwing en uitgevoer moet word deur kommersiële vissermanne aanboord, tydens die vervoer van die vis op land en dan uiteindelik tot en met die verkoopspunt.

Summary

In order to determine the effect of the unregulated supply chain on the quality of locally caught snoek *Thyrsites atun*, microbial and pathogen (faecal contamination indicators) presence and prevalence, muscle pH and temperature and ambient temperature were assessed at various points of processing, from catch to consumer. Two distinct methodologies; total viable counts (TVC) in colony forming units (CFUs/cm²) and polymerase chain reaction (PCR), were used for determining the bacterial contamination and the presence/ absence of four specific indicator organisms (*Escherichia coli, Salmonella enterica, Staphylococcus aureus* and *Vibrio parahaemolyticus*) in five interlinked experiments.

The results show that most contamination originated from the storage holds on-board the fishing vessel. The bait and hessian cloth on which the bait was cut into smaller pieces were identified as additional sources of contamination with 4.69 and 6.92 $\log_{10}$CFU/cm², respectively. It was concluded that the hands of fishermen were responsible for the transmission of human related pathogens *E. coli* and *S. aureus* on-board to snoek both post-vessel (after snoek are offloaded from the fishing vessel) and post-bakkie (after transportation and offloading of snoek from the bakkie) offload. It was observed that keeping snoek under ice, maintained temperatures to below 10°C up to approximately 10-12 hours storage time, post-vessel offload. The different treatments used (ice and cleaning) were observed to have no considerable effect on bacterial load, however ice was observed to maintain the reduction in muscle pH levels as well as delay bacterial growth.

Repeated and replicated measures of this study are required to explore various other sources and routes of contamination. This would ensure that the supply chain has been thoroughly analysed such that good manufacturing practices (GMPs) and standard sanitation operating procedures (SSOPs), can be effectively implemented. It is therefore recommended that standard operating procedures (SOPs) be enforced and practiced by commercial fishermen on-board, to transporting fish on land and finally at the selling point.

Dedication

I dedicate this thesis to my mother, father and grandfather who have been and still continue to be my source of endless inspiration. You make me brave.

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Notes

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Animal Science*.

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Abbreviations

µl	microlitre
AIDS	Acquired Immune Deficiency Syndrome
ANC	African National Congress
APC	Aerobic Plate Count
ATP	Agreement on the international carriage of perishable foodstuffs
BAM	Bacterial Analytical Manual
bp	Base pairs
CAC	Codex Alimentarius Commission
CCP	Critical Control Point
CDC	Centres for Disease Control and Prevention
CFU	Colony Forming Units
CTAB	Cetyl-Trimethyl-Ammonium-Bromide
DAFF	Department of Agriculture, Forestry and Fisheries
DEAT	Department of Environmental Affairs and Tourism
DNA	Deoxyribonucleic acid
dNTPs	Deoxy-Nucleotide-Tri-Phosphates
D-value	Decimal reduction time
EDTA	Ethylene-Diamine-Tetracetic-Acid
E_h	Electro harmonix
EHEC	Enterohaemorhagenic
EIEC	Enteroinvasive
EMB	Eosin Methylene Blue agar
EPEC	Enteropathogenic
ETEC	Enterotoxigenic
C_2H_6O	Ethanol

EU	European Union
FAO	Food Agriculture Organization of the United Nations
FDA	Food and Drug Administration
GDP	Gross Domestic Product
GHP	Good Hygiene Practice
GMP	General Management Practice
GRAS	Generally Recognised As Safe
HACCP	Hazard Analysis Critical Control Point
ICMSF	International Commission on Microbiological Specifications for Foods
IOM	Institute of Medicine
ISO	International Organization for Standardization
kg	kilogram
km	kilometre
log	logarithm
m	meter
$MgCl_2$	Magnesium Chloride
Milli-Q	Ultrapure water
ml	millilitre
MSC	Marine Stewardship Council
NaCl	Sodium Chloride
NDA	National Department of Agriculture
NH_4A_c	Ammonium acetate
NRC	National Research Council
NRCS	National Regulator for Compulsory Specifications
NZQA	New Zealand Qualifications Authority
PCA	Plate Count Agar

PCR	Polymerase Chain Reaction
PSO	Physiological Saline Solution
rpm	Revolutions Per Minute
SABS	The South African Bureau of Standards
SANAS	South African National Accreditation System
SEVAG	Chloroform-isoamyl-alcohol
SOP	Standard Operating Procedure
spp.	Species
SSOP	Standard Sanitation Operating Procedure
t	tonne
Taq	Thermostable DNA polymerase
TES (buffer)	Tris- Ethylene-diamine-tetra-acetic-acid-Sodium chloride
TFTC	Too Few To Count
TMTC	Too Many To Count
TVC	Total Viable Count
U.S. A.	United States of America
UNEP	United Nations Environment Programme
VTEC	Verocytoxin
WHO	World Health Organization
ZAR	South African Rand

Chapter 1

Introduction

Snoek (*Thyrsites atun*), a commercially important species for the low-income coastal communities in the Western Cape of Southern Africa, is currently facing a few challenges in terms of reaching full market potential (Isaacs, 2013). One of the challenges involves the re-allocation of fishing rights. Fishing rights have been allocated in 2007 by the African National Congress (ANC) government according to all sorts of fishers with an emphasis on transformation, with a limit on boat numbers and crew numbers (DAFF, 2012). These rights were due for re-allocation in 2014 (Isaacs, 2013), but the outcome has been challenged successfully as procedures were not followed. As in most fisheries around the world the crew gets a share depending on the number of fish they catch (DAFF, 2012). The boat gets around 50% of the daily total. From that the skipper needs to buy fuel and maintain the rig (boat, trailer and bakkie). All fishers depend therefore on the langanas (fish buyers/ sellers) as they control the price (DAFF, 2012).

Furthermore, the contribution of *T. atun* to food security in South Africa is crippled by a similar species of different origin, the New Zealand 'barracouta'. Local suppliers of *T. atun* are now competing with imported New Zealand barracouta, incorrectly labelled as 'snoek' by local retailers (West Cape News, 2012). The local supermarket chains have limited confidence in the locally caught *T. atun* whereas the imported *T. atun* is widely accepted by the South African food industry (West Cape News, 2012). This is due to the New Zealand stock being considered of superior quality, fully traceable and a consistent fish supply can be guaranteed (Isaacs, 2013).

Catch records reveal that the landed value of hand line-caught snoek from the Western Cape in 1982 was R 3.3 million, and R 21.3 million was the value for the demersal fishery of South Africa (Anon., 1983). In 2010, total barracouta imported from New Zealand was recorded 5 690 968 kg, while the total line-fish landed in South Africa was 6 638 139 kg (Warman, 2011). Ten years prior to this record, imported barracouta was at 2 759 858 kg, however records for line-fish landings were not available. Lack of consistent historical catch records for snoek make it difficult to assess the potential market for it, especially since stock numbers fluctuate due to seasonal and migratory patterns (Palmer *et al*., 2008).

The quality of snoek and other commercially important fishes [*Merluccius capensis* (South African hake), *Sardinops ocellatus* (South African pilchard), and *Thyrsites atun* (South African snoek)] are primarily threatened by a parasite (*Kudoa thyrsites* or *Kudoa paniformis*) which can be associated with accelerated muscle-degradation, also known as post-mortem myoliquefaction (Moran *et al.,* 1999; Henning e*t al*., 2013), locally referred to as 'pap snoek', in the case of snoek. According to Tsuyuki *et al.* (1982), the parasite releases proteolytic enzymes that degenerate muscle tissue causing an undesirable soft and pasty flesh. The quality of snoek can vary due to the parasite's prevalence whilst inconsistencies in handling and processing can exacerbate the condition. *K. thyrsites* is still under further research as it has great implications to marine and aquaculture industries (Stehr *et al*., 1986). Suggestions have been made to look into manipulating post-mortem pH and temperature control to destroy or inhibit the photolytic activity of the parasite's enzyme (Henning e*t al*., 2013).

Furthermore, as with any food processing establishment, hygiene and the procurement of safe and reliable food made available to consumers is a requirement of all food industries (Grigoryan *et al.,* 2010). The snoek supply chain is a food operating chain which is lacking investments in the cold chain for keeping catch fresh by the use of ice and cold store facilities (Isaacs, 2013).

An additional important aspect is enforcing sanitation and hygiene procedures for providing food which is safe for human consumption. In terms of sanitation, hygiene and food preservation, microorganisms play an important role in the spoilage of seafood and food borne illnesses worldwide (FDA, 2001). Their presence or absence on food is a function of the harvest environment, sanitary conditions, and activities that are associated with equipment and personnel in the processing environment (FDA, 2001; Huss, 2003). Studies by Pether and Gilbert (1971) and Scott and Bloomfield (1990) looking into contamination of food borne pathogens through handling, found that *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus* together with other organisms survived for hours to days on utensils, cloths and hands from initial contact with the organism. Having a science-based understanding of their growth and activity can help develop preservation techniques and subsequently, reduce product loss caused by spoilage (Gram & Dalgaard, 2002).

In light of the catch statistics, which reveal that although a consistent supply of fish, is not guaranteed on a monthly basis, South African line-fish (snoek) can alone ensure a supply large enough for the local markets to support. Therefore, focus can be shifted to the second reason behind the lack of local support. This follows a current lack of scientific information available on the handling nature and microbiological status of seafood from the time it is harvested up until the time it reaches the consumer particularly that linked to an informal practice. Studies based on seafood contamination only have reports on work done from the receiving point of the processing chain.

This would be one of few studies monitoring the cold chain (by temperature and pH measurements) as well as evaluating the general microbiological quality of fresh and handled snoek, which can be relative to all fish in general.

The aim of this study is therefore to determine the microbial quality of fresh, handled snoek and evaluate the microbial load from catching to the informal marketing, so as to determine where contamination occurs and if the prevalence of bacterial microorganisms increases with the progression of the value chain. The presence of specific food borne pathogens were explored at each stage by means of conventional and molecular methods. Contamination sources were identified as well as their pathways of transmission. Potential ways to minimise contamination and spoilage were also explored, and recommendations made as to the use of standard operating procedures (SOPs), which may contribute positively to the fish food industry through consumer confidence.

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Chapter 2

Literature Review

Snoek biology and ecology

South African snoek are classified as Ostichthyes (bony fish), order Perciformes, family Gempylidae and genus and species *Thyrsites atun* (Burton *et al.,* 2002). It is also commonly known in New Zealand and Australia as 'barracouta', Chile as 'sierra', Japan as 'ooshibikamasu' and Russia as 'snek' (FAO, 1993). It is a medium-size pelagic predator with a maximum weight of 9 kg (Nepgen, 1979) where 50% maturity is attained at 73.0 cm fork length (3 years). The majority of the body is silver with pale dark strands running along its sides while the dorsal section is bluish-black in colour (Figure 2.1).

Figure 2.1 Snoek, *Thyrsites atun* (Euphrasen, 1791).

Snoek are distributed (Figure 2.2) in temperate coastal waters (50-68°F; 10-20°C) in the Southern Hemisphere (Southern Africa to Tristan da Cunha, Argentina, Chile, New Zealand and Australia) (Burton *et al*., 2002). They travel in large schools and generally inhabit the mid-waters but can also be found from the ocean surface to depths of up to 550 m (Kailola *et al*., 1993). South African snoek mainly feed on sardine (*Sardinops sagax*), South African pilchard (*Sardinops ocellatus*), South African mackerel (*Trachurus trachurus*) and pelagic crustaceans such as krill (*Ephausia* and *Nyctiphanes*) (Burton *et al*., 2002). However, their feeding pattern is seasonal and generally ceases prior to the spawning season in the austral winter and spring (Burton *et al*., 2002).

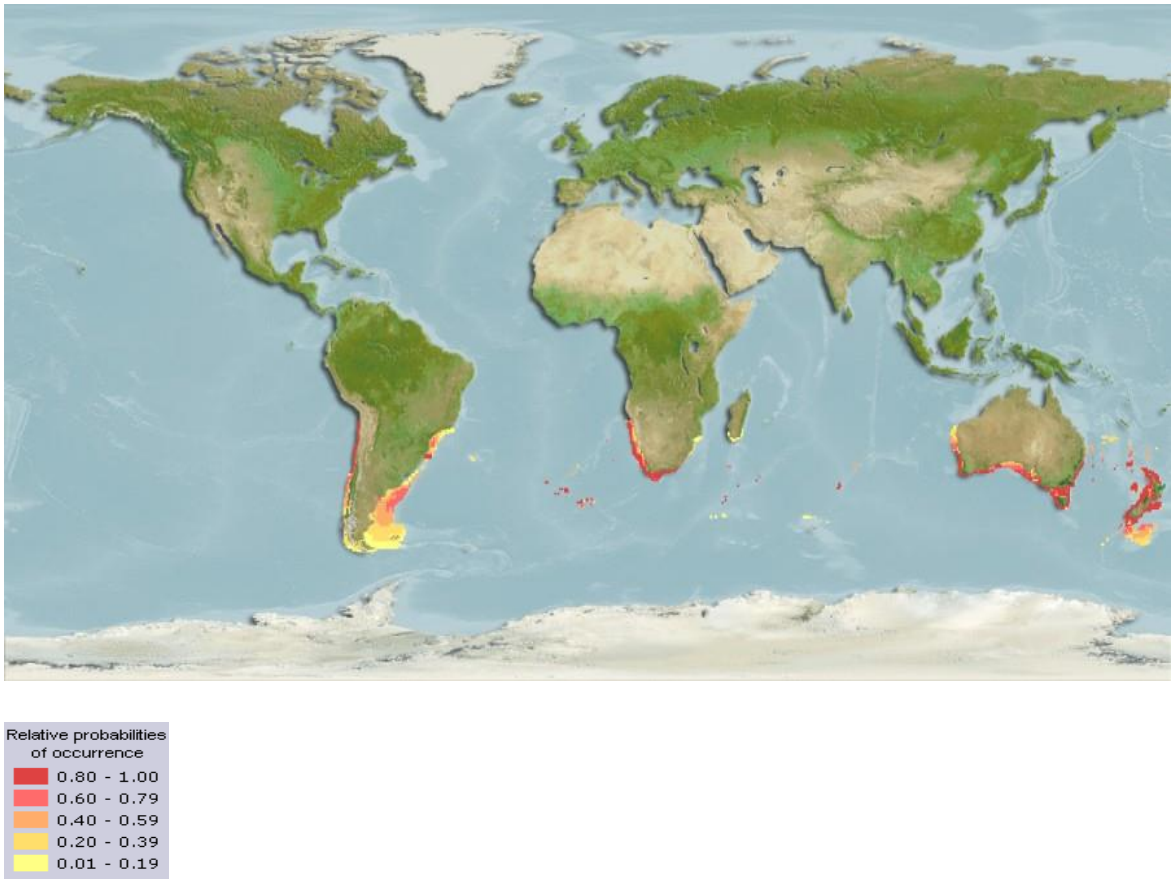

Figure 2.2 Distribution map for *Thyrsites atun* (snoek) (Anon., 2000).

Global fisheries

Global fish production is currently outpacing world population growth (1.6% annually) by 3.2% annually (FAO, 2014). According to FAO statistics, the average world fish consumption per capita, increased from 9.9 kg during the 1960s to 19.2 kg in 2012 (FAO, 2014). China is the largest exporter of fish and fishery products (greatly influenced by aquaculture) worldwide, providing a total of 13 869 604 tonnes in marine capture fisheries in 2012 (Table 2.1), followed by Indonesia and the United States of America (FAO, 2014).

Table 2.1 Marine capture fisheries: 18 major producer countries.

2012 Ranking	Country	Continent	Tonnes (t) 2003	2011	2012	Variation (%) 2003-2012	2011-2012
1	China	Asia	12 212 188	13 536 409	13 869 604	13.6	2.4
2	Indonesia	Asia	4 275 115	5 332 862	5 420 247	27.0	1.7
3	United States of America	Americas	4 912 627	5 131 087	5 107 559	4.0	-0.5
4	Peru	Americas	6 053 120	8 211 716	4 807 923	-20.6	-41.5
5	Russian Federation	Asia/ Europe	3 090 798	4 005 737	4 068 850	31.6	1.6
6	Japan	Asia	4 626 904	3 741 222	3 611 384	-21.9	-3.5
7	India	Asia	2 954 796	3 250 099	3 402 405	15.1	4.7
8	Chile	Americas	3 612 048	3 063 467	2 572 881	-28.8	-16.0
9	Viet Nam	Asia	1 647 133	2 308 200	2 418 700	46.8	4.8
10	Myanmar	Asia	1 053 720	2 169 820	2 332 790	121.4	7.5
11	Norway	Europe	2 548 353	2 281 856	2 149 802	-15.6	-5.8
12	Philippines	Asia	2 033 325	2 171 327	2 127 046	4.6	-2.0
13	Republic of Korea	Asia	1 649 061	1 737 870	1 660 165	0.7	-4.5
14	Thailand	Asia	2 651 223	1 610 418	1 612 073	-39.2	0.1
15	Malaysia	Asia	1 283 256	1 373 105	1 472 239	14.7	7.2
16	Mexico	Americas	1 257 699	1 452 970	1 467 790	16.7	1.0
17	Iceland	Europe	1 986 314	1 138 274	1 449 452	-27.0	27.3
18	Morocco	Africa	916 988	949 881	1 158 474	26.3	22.0
Total 18 major countries			**58 764 668**	**63 466 320**	**60 709 384**	**3.3**	**-4.3**
World total			**79 674 875**	**82 609 926**	**79 705 910**	**0.0**	**-3.5**
Share 18 major countries (percentage)			**73.8**	**76.8**	**76.2**		

Source: Adapted from FAO (2014)

South Africa is recognised as one of the larger fishing nations in Africa (annual catch of 643 812 tonnes in 2000) (FAO, 2010) ranging from small-scale to industrial-scale fisheries targeting a broad range of species (WWF, 2011). According to FAO statistics, Morocco shows to be the largest in Africa in terms of fishing with 1 158 474 t recorded in 2012. Approximately 43 000 people are employed (both land-based and sea-going) within the South African commercial fisheries both directly and indirectly, with the industry valued at approximately ZAR12 billion annually which contributes (the industry in itself) minimally to national GDP (FAO, 2010).

Over-exploitation and high fishing intensity has increased over the last decade and has been mainly driven by an increasing demand for fish food due to population increases, higher consumer rates and emergent export markets and tourism (Sherman, 2003). As a result, a steady depletion of fish stocks beginning with large, high-value species, and progressing towards small, low-value species is evident (Jennings & Kaiser, 1998). Snoek is recognised as one of the low-value species in South Africa targeted by small-scale commercial fishermen (Attwood, 1999).

The South African commercial line-fishery

South Africa's coastline spans two ecosystems over a distance of 3 623 km which extends from the Orange River in the west on the border with Namibia (Figure 2.3), to Ponta do Ouro east on the Mozambique border (FAO, 2010). The commercial fishery has its highest productivity on the western coastal shelf (FAO, 2010). Snoek is targeted by the line-fishery but caught as by-catch by the trawl fisheries and sold via formal markets or via an informal marketing system (Isaacs, 2013).

South African has three commercial fishing sectors which include the traditional line, hake-hand line and the tuna-pole fishery (Mann, 2013). The term 'line-fishing', defines the capture of fish by hook and line exclusively boat-based, and excludes the use of long lines (DAFF, 2012). The line-fishery (three sectors; commercial, recreational, and subsistence) targets between 95 and 200 of the 2 200 marine fish species of South Africa (DAFF, 2012). The line-fish sector is one of the biggest in terms of area fished and numbers of fishermen involved (FAO, 2010).

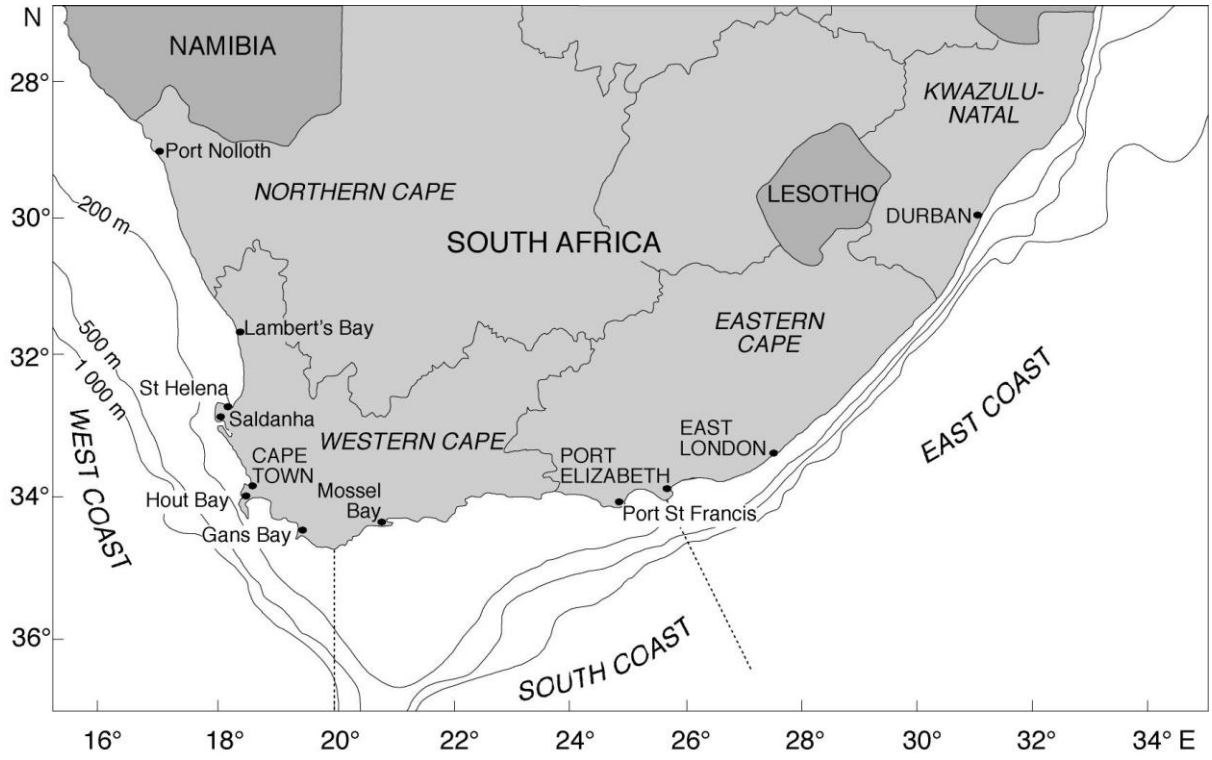

Figure 2.3 South African coastline showing main fishing harbours (adapted from Branch *et al*., 1994).

The commercial line-fish sector primarily target yellowtail (*Seriola lalandi*) and snoek (*Thyrsites atun*) species (Winker *et al*., 2012). Snoek (predominantly and traditionally a hand-line fishery) is considered an important species caught on the west coast of South Africa, particularly for the small-scale fishermen (Table 2.2) (Griffiths, 2002).

The hand line-fishery operates based on small-craft boats (ski-boats) (Dudley, 1987), which include: Harbour based resident fleets, boats ranging from two-man dinghies, through to six- to ten-man open motor boats with a maximum size of 10 m (Dudley, 1987).

The ski-boats are largely operated by artisanal fishermen and are powered by an outboard engine (Mann, 2013). Although many larger sea going vessels contain cold room facilities, the ski-boats generally do not due to space constraints on the boat (Mann, 2013). Some small-scale fishers have begun to incorporate ice into their daily fishing operations but implementation is overall limited (Isaacs, 2013).

Most of the commercial fish landings takes place at designated fishing harbours (FAO, 2010). The main landing sites for the line-fishery are harbours and slipways situated around the entire coast. The main sites in the Western Cape Province for snoek are Lamberts Bay, St. Helena Bay, Saldanha, Hout Bay, Gans Bay (Figure 2.3), Yzerfontein, Table Bay, Miller's Point, Kalk Bay, Gordon's Bay, Struis Bay and Arniston (FAO, 2012 & DAFF, 2012).

South African fisheries with special emphasis on snoek

The origin of the snoek line-fishery dates back to Dutch colonization of the Cape in 1652 (Isaacs, 2013). During early colonization, fishing for snoek as well as southern mullet (*Liza richardsonni*), hottentot

(*Pachymetopon blochii*), white steenbras (*Lithognathus lithognathus*), galjoen (*Dichistius capensis*), dusky kob (*Argyrosomus japonicus*) was permitted by all persons including indigenous workers and slaves, which provided the locals with an easily accessible source of protein (Isaacs, 2013). The British took occupation of the Cape in 1795, banning slave trade and introducing the commercial fishing industry in 1801 (van Sittert, 1993). Consequently, merchants were allowed to ship dried snoek from South Africa to Mauritius after 1856 (van Sittert, 1993). Snoek has since been a delicacy and an important protein source for many poor households in the Western Cape Province to date (Isaacs, 2013). Furthermore, this provided the poorer coastal communities with food security through the provision of income and the availability of a nutritional food source (Isaacs, 2013).

Throughout its distribution, *T. atun* supports moderate fisheries (<1000 metric tonnes [t]/yr.) in southern Australia, Chile, and Tristan de Cunha, and substantial fisheries (>10,000 [t]/yr.) in New Zealand and Southern Africa (FAO, 1997). The snoek fishery is a popular and important small-scale line-fishing industry (since the early 1800s) within Southern Africa (Isaacs, 2013) and this fishery constitutes 70-96% of line-caught fish in the Western Cape (Griffiths, 2002; DAFF, 2012). Snoek is caught throughout the year in Southern Africa, however the peak fishing season is generally from May to August (Isaacs, 2013).

High inter-annual fluctuations exist for snoek landings made by the line-fishery in South Africa which may reflect true natural fluctuations in population size. However, as the range of the small crafts is limited, fluctuations are also a function of inshore-offshore movement of this highly nomadic species.

Recent records reveal that snoek landings still remain above the competitive number of barracouta imported from New Zealand (Table 2.2).

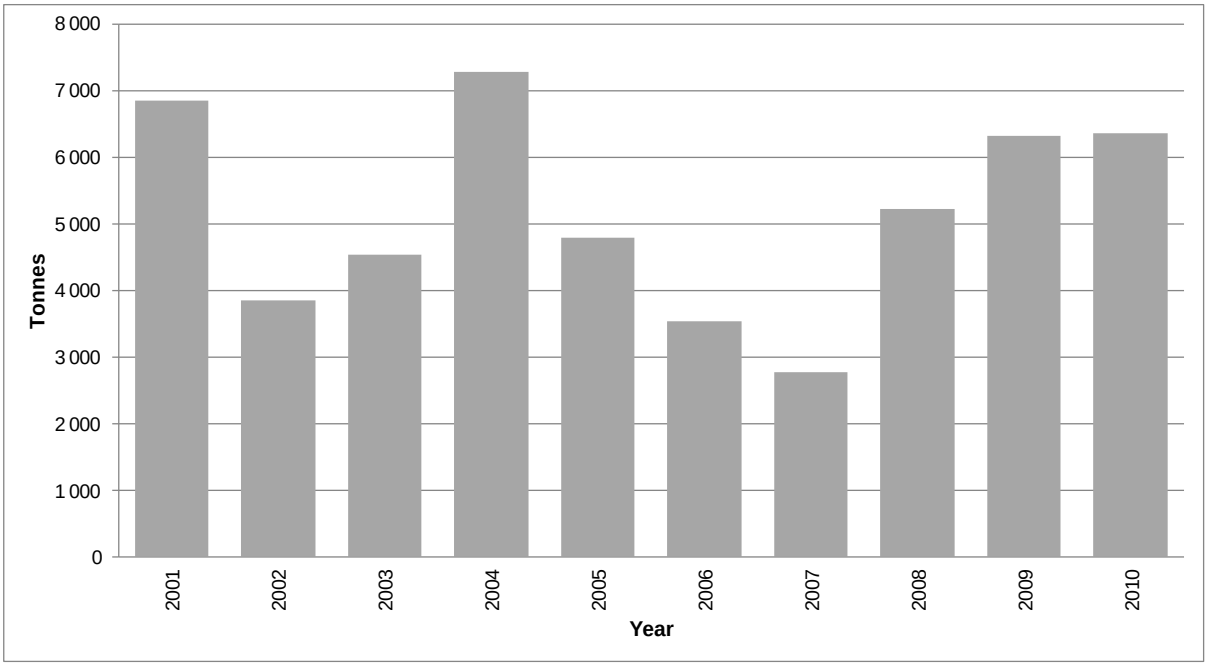

Figure 2.4 Total annual snoek catches for South Africa from 2001 to 2010 (DAFF, 2012).

Despite a thriving national snoek fishery (Figure 2.4), a strong import trade exists with New Zealand providing more than 5 000 tonnes (2010) of imported barracouta, labelled misleadingly as snoek (Table 2.2)

(Warman, 2011; Isaacs, 2013). Local retailers are reported to prefer stocking and selling of imported barracouta (New Zealand) rather than snoek (South African) mainly due to the assurance of consistent quality, traceability and a sufficient constant supply (Isaacs, 2013).

Table 2.2 South African snoek landings, and imports (t) from New Zealand 2010 (Warman, 2011).

Sources of snoek	Tonnes (t)
Line-fish	6 638
Deep sea hake trawl	3 650
Hake longline	3.5
Inshore trawl	0.709
New Zealand barracouta (imports)	5 691

Source: *Fishing Industry Handbook* (2011).

A number of large supermarket chains have voiced their willingness to sell local South African snoek, however they insist processing methods must first be met according to legislation set out by international standards (West Cape News, 2012). Standard operating procedures (SOPs) have been outlined for the safe practice and handling of fish products in New Zealand (NZQA, 2014) which have not been observed/adopted by the South African snoek industry. Objectives set in place to meet the standards outlined by the EU regulation [((EC) No852/2004)] (Amos, 2007) for exported foods, can ensure maximum approval of South African snoek by large supermarket chains with the potential for future export of snoek. For artisanal fishermen, particularly within developing countries, having access to adequate operational facilities remains an added challenge to providing consistently good quality fish (Isaacs, 2013).

Factors affecting quality and quality changes in fish

The quality of fish can be described by assessing the nutritional, microbiological, biochemical and physiochemical characteristics (FAO, 1988). These characteristics can vary for a number of reasons which include species type, environmental factors and the chemical and processing procedures which they undergo (Gonçalves *et al.,* 2007). The precise processing strategy is dependent on the fish's nutritional status, the fishing operation, the fish's biological state when caught and the temperature of storage (Gonçalves *et al.,* 2007).

Environmental, chemical, biological and processing factors affecting snoek quality

Chemical composition of fish meat varies not only between but also within species. It is dependent on a number of factors. In snoek, the following factors have been found to influence the composition: age, sex, environment and season (FAO, 1995). During energy demanding phases (spawning and migration) fish can display a distinct loss of body condition (body mass, body fat reserves and immunity) while heavy feeding can replenish the protein and lipids lost; overall increasing the fish condition (FAO, 1995). Seasonal variation in the condition of both the South African and New Zealand snoek populations has been documented to

generally decrease in spring and increase in summer (Gilchrist, 1914; Hart, 1946; Biden, 1948; Davies, 1954).

One of the current major environmental factors affecting snoek involves a marine parasite. The parasite *Kudoa thyrsites* accelerates spoilage in snoek aided by warm sea temperatures, which requires that snoek be rapidly chilled once caught (Moran *et al*., 1999; Isaacs, 2013). The presence and prevalence of *K. thyrsites* is of major concern due to the adverse effects (myoliquefaction) it has on a number of commercial marine fishes such as Cape hake (*Merluccius capensis)*, Cape dory (*Zeus capensis*), South African pilchard as well as Cape snoek (Henning *et al*., 2013). Myoliquefaction is essentially the breakdown and softening of fish tissue due to the release of proteolytic (protein breakdown) enzymes (Stehr *et al.,* 1981; An *et al*., 1994) by the *K. thyrsites* parasite and is often referred to as 'pap' snoek in South Africa (Stehr *et al.,* 1981; An *et al*., 1994; Henning *et al*., 2013). The majority of locally caught snoek is sold to consumers within 24 hours of catch however, the effect of *K. thrysites* only starts to become evident 38-56 hours post-mortem. The delay in reaction and lack of detection prior to selling can have serious implications on consumer trust, appeal and reaction affecting product sale as national and international markets require a product of consistent quality (Isaacs, 2013).

Although seasonal differences for these population groups are so distinct, further research into the prevalence of the parasitic nature (*K. thyrsites*) in relation to season can assist fishermen to catch better conditioned fish, improving not only the quantity (more flesh by weight for the same number of fish caught) but also the quality of the catch (fat fish preferred over thin fish, including *T. atun* in South Africa) (Blackburn, 1959).

Icing as a preservation method for snoek

A number of preservation methods such as smoking, drying, freezing, chilling, brining, canning and fermentation are used within the food industry to extend the shelf-life of seafood and other meat products (Ghaly *et al*., 2010). Low temperature storage (fridge / freezer) is however one of the most common methods currently used (Kitinoja, 2013) due to its efficiency in rapid preservation and shelf-life extension (Johnston *et al*., 1994). These methods and investing in fast cold post-harvest technologies can avoid significant waste and loss of revenue for the snoek industry (Henning *et al*., 2013). Simple methods such as keeping ice or even shading fish have been found to improve the fish quality to a great extent (Eyo & Ita, 1977).

Ice is commonly used to maintain a low fish temperature for a limited period of time or until frozen storage is made available. According to Rand and Pivarnik (1992), ice has two main functions: (a) maintaining uniform low storage temperatures, (b) reducing autolysis and bacterial degradation. Icing is a widely utilised method for fish preservation in developing countries particularly for small-scale fisheries because of its low cost and effectiveness for maintaining low fish temperature while fishing, after landing, during transport and selling (Kitinoja, 2013). Four different types of ice can be utilised for chilling fish: liquid ice, flake-ice, tube-ice, and block ice (Quang *et al.,* 2005). The cooling rate varies among these ice types (Quang *et al.,* 2005). Therefore, choice of ice is important when considering cooling/chilling fish.

The cold chain and implementation of a Hazard Analysis Critical Control Points (HACCP) plan in a fish processing establishment

According to FAO (1995), fish quality can be defined as the visual appearance and freshness or the extent of spoilage. The processing chain involves a number of steps which can affect shelf-life and product quality of fish (Kreyenschmidt *et al*., 2013). These processing methods include the fishing method, on-board handling, transfer method, fish cleaning, further processing (salting and drying), transportation time and storage (on board fishing boat and vendors storage facility) (Kreyenschmidt *et al*., 2013). Therefore, in order to ensure a high quality product and minimal degradation each step in the processing chain requires constant attention and standardised protocols (Jordaan, 2006). Figure 2.5 illustrates an example of the different steps involved in the processing of white fish once landed (UNEP, 2000). These steps can be recommended for the snoek fishery.

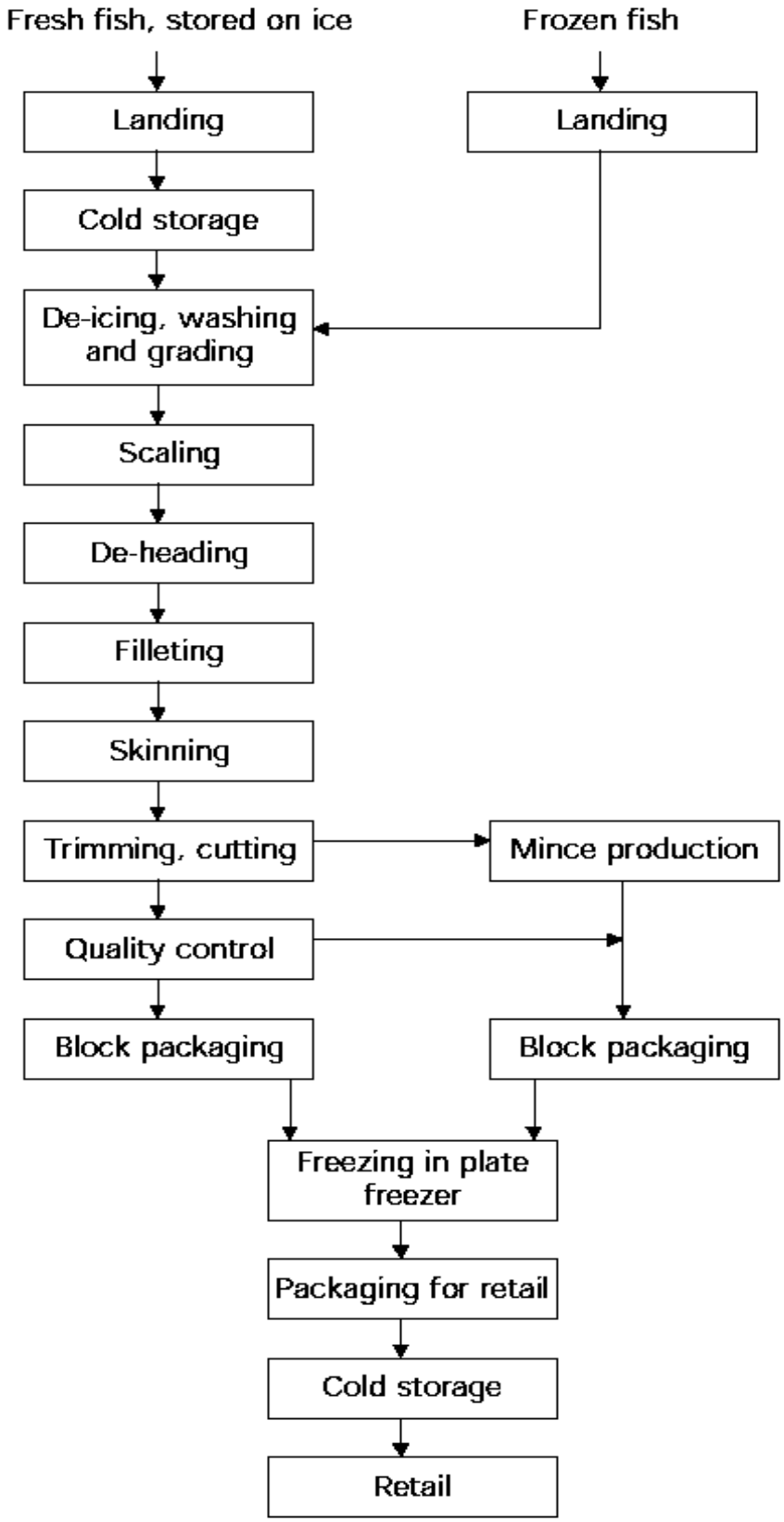

Figure 2.5 Process flow diagram for the processing of white fish (adapted from UNEP, 2000).

The Hazard Analysis Critical Control Points (HACCP) system has been implemented internationally within the fishing and other food industries to enhance the quality and safety of food products (Buchanan, 1990).

The definition for HACCP by Pearson and Dutson (1995) reads as follows: “HACCP is an industry driven concept that provides a preventative system for hazard control. It is based upon a systematic approach to define hazard and critical control points based on scientific evidence within the system. It requires both industry and government participation, with the industry’s role primarily being to design and execute the system, and the governments’ role being that of approving the industry system design, verification and technical assistance.”

When applying the HACCP system to a food processing establishment, a logical sequence of steps (Figure 2.6) must be applied to each point within the food supply chain (WHO, 1997).

Step	Completed Y/N?
1. Identify and list all of the products you process	
2. Complete a Product Description for each product	
3. Develop Process Flow Diagrams for these products‡	
4. Verify the Flow Diagram	
5. Complete a Hazard Analysis	
5.1 List processing steps	
5.2 Identify and list all potential hazards (chemical, physical and biological) that can be reasonably expected to occur at each processing step	
5.3 Identify and record significance of hazard § (This may be documented in a Hazard Analysis Table)	
5.4 Provide justification for inclusion or exclusion as a significant hazard (why is it significant or not significant?)	
5.5 Identify and list Control Measures – i.e. what can be done to prevent the hazard from occurring	
5.6 Determine whether the step is a Critical Control Point (CCP) for food safety	
6. Complete a HACCP table	
6.1 List the Critical steps	
6.2 List the potential hazards	
6.3 Establish the critical control point/s (factor/s) and critical limits and record them on the HACCP table	
6.4 Validate Critical Limits and record them on the HACCP Table or in a separate document	
6.5 Establish a monitoring system for each CCP and record it on the HACCP Table	
6.6 Establish Corrective Actions and record them on the HACCP Table or in a separate document	
6.7 Establish Verification procedures and record them on the HACCP Table or in a separate document	
7. Keep Records	

Figure 2.6 HACCP checklist (adapted from HACCP guideline, 2005)[1].

[1]‡ Some products can be covered by the same process flow diagram for example, if you process more than one species of frozen fish, you may not require a separate process flow diagram for each species
§ Significant hazard means a hazard (or a hazard in combination with other hazards) that is of such a nature that its elimination, control or reduction to an acceptable level is essential to the production of a safe food. (Subclause 3.9 of Schedule 2 of the Orders).

The implementation of HACCP within the fish industry varies. Large commercial fishing fleets generally incorporate HACCP, however, small-scale fisheries tend to have limited standard operating procedures (SOPs) and abstain from HACCP guidelines, mainly due to the lack of information and technological development and advances (Mlolwa, 2000). As a result, the HACCP plan overlooks the competitive nature of non-mechanised, labour intensive factories and processing facilities in developing countries (Mlolwa, 2000).

Microbial quality of fish and fishery products

Indicator microorganisms

A microbiological indicator can be defined as a single or group of microorganisms, or a metabolic product, whose presence in a food or the environment at a given level is indicative of a potential quality, hygiene, and/or safety issue (Ravaliya *et al.,* 2013). Some of the most commonly used indicators include aerobic plate count (APC), coliforms, faecal coliforms, *Enterobacteriaceae* and *Escherichia coli* (Ravaliya *et al.,* 2013). The acceptance or rejection of fishery products for human consumption is determined through plate counts which are separated into different levels of microbiological quality (ICMSF, 1986). Acceptable standards for fish products include plate counts below 5×10^5 colony forming units (CFUs) while counts between 5×10^5 and 10^7 are marginally accepted and plate counts at or above 10^7 are considered unacceptable (ICMSF, 1986). By implementing systems whereby each step within the processing of food is consistently monitored, the survival of microorganisms can be prevented or limited (IOM/ NRC, 2003).

Natural and pathogenic bacteria: sources and routes of contamination

Microorganisms occur naturally within the aquatic and terrestrial environments. Once fish are caught, the outer surface is free from microbial activity until the immune system shuts down allowing bacteria to proliferate freely on the skin and between scale pockets (Huss, 1995). According to Huss (1995), an increase in microbial growth and spoilage of fish meat increases with an increase in microbial load on the surface of the fish which may be a result of bacterial enzymes diffusing into the flesh. Such an increase in microbial load can occur in fish stored in both iced and ambient temperatures (Huss, 1995).

Pathogenic microorganisms can be transferred through several vectors such as humans, water, soil, animals and air (Ashie *et al.,* 1996). The microbial quality and survival of spoilage microorganisms during storage depend on the nature of microorganisms and fish species, life history of the fish, harvesting method and handling, storage procedures and processing time while aboard the fishing vessel (Ashie *et al*., 1996). When sanitation protocols are not in place (unwashed / non-sanitised surfaces) on-board fishing vessels and throughout the fish supply chain, contamination can occur which can reduce the shelf-life properties of fish (Huss *et al*., 1974).

The design of fish storage holds on board fishing vessels play a significant role in terms of hygiene. Purge (drip loss) provides conditions favourable for bacterial growth, therefore the hold design should allow for easy collection of purge (Hermansen, 1983). In addition, fish landing sites are prone to bacterial contamination (Amos, 2007) due to dirty, unclean and non-disinfected contact surfaces (Huss *et al*., 1974). In the line-fishery fish are purchased at off-loading by factory agents and other small-scale middlemen (owners of bakkies) (Amos, 2007) while quality checks prior to purchase rarely take place.

The lack of hygiene SOPs and HACCP methodology implementation at various stages in the processing chain can result in human contamination of potentially serious pathogens such as:

1. *Vibrio parahaemolyticus* (marine related pathogen) found on raw fish caused by near-shore harvest water contamination, poor sanitary practices on the harvest vessel and poor aquaculture practices (FDA, 2011).
2. *Campylobacter* species, *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus* and *Listeria monocytogenes*, are important food pathogens known to cause gastroenteritis in humans when ingested (Prescott *et al*., 2002).

Gram-negative pathogens affecting fish

Escherichia coli

Escherichia coli is a common aerobic organism found in the gastrointestinal tract of humans and animals (Varnam, 1991) and its presence in food can indicate faecal contamination (Varnam, 1991). Five strains of diarrhoea producing *E. coli* are known (Varnam, 1991), four of which are of most concern are indicated in Table 2.3. For the purpose of this study, only enterohaemorhagenic *E. coli* (EHEC) will be discussed further.

Table 2.3 Four main diarrheagenic strains of *E. coli* (Varnam, 1991).

E. coli	**Description**
Enteropathogenic (EPEC)	Class I and II (includes strains referred to as 'attaching-effacing *E.coli*')
Enterotoxigenic (ETEC)	
Enteroinvasive (EIEC)	*Shigella* – like *E. coli*'; 'Dysentery-like *E. coli*'
Enterohaemorhagenic (EHEC)	Verocytoxin-producing *E. coli* (VTEC); 'colohaemorrhagic *E.coli*'

The diarrheagenic strains of *E. coli* are largely of human origin, except for enterohaemorhagic strains (found in the intestinal contents of some cattle) (Kaper *et al*., 2004). In the early 1890s, *E. coli* was associated with animal disease, while a similar association with disease in children was confirmed in the late 1940s. *E. coli* can inhabit living hosts without adversely affecting host health, however should the host's immune system be compromised; infection and disease can occur (Kaper *et al*., 2004). *E.coli* has in recent years been recognised as a specific pathogen in both intestinal and extra intestinal disease (Varnam, 1991).

E. coli as well as coliforms, *Staphylococcus* and enterococci species are commonly used as indicators of fish processing quality as they are not commonly present in freshly caught fish (Chattopadhyay, 2000). However, contamination and transmission of *E.coli* in fish has been linked to food handlers and inappropriate sanitation practices during the processing chain (Ayulo *et al.,* 1994).

E. coli can grow at temperatures between 7 and 48°C, while optimal growth occurs at 37°C. There are important exceptions for different pathogenic strains as some have optimum growth temperatures as low as 30°C. The D-value heat resistance for *E. coli* is 5 minutes at 55°C and 0.1 minutes at 60°C. Furthermore, *E. coli* also grow at pH levels ranging between 4.4 and 9.0 (ICMSF, 1980).

Salmonella enterica

Salmonella spp., just like *E. coli*, also belong to the family *Enterobacteriaeceae*. *Salmonella* are gram-negative, facultative anaerobic, rod-shaped bacteria. Peritrichous flagella aid motility of *Salmonellas*, and members of this genus are responsible for human and animal diseases. Only two species in the genus *Salmonella (Salmonella enterica* and *Salmonella bonyori)* have been recorded based on the classification scheme used by the United States Centres for Disease Control and Prevention (CDC), World Health Organization (WHO) and some journals. Both belong to *S. enterica* subsp. *enteriaca* and affect the aquatic and general environment through faecal contamination of man and animals (including birds) (Pelzer, 1989).

Salmonella remains one of the most primary causes of food poisoning (Varnam & Evans, 1991) with reported cases of isolates found in fish, shellfish and other seafood (Aissa *et al.,* 2007 and Kumar *et al.,* 2008). This pathogen is found in seafood as a result of concentrated intake of the pathogen by filter feeders (oysters, mussels, etc.) from contaminated waters (Martinez-Urtaza *et al*., 2003). Normal heat processing of raw foods can destroy the organism however, cross-contamination from raw to processed food due to improper handling can compromise safe consumption (Varnam & Evans, 1991).

Vibrio parahaemolyticus

The genus *Vibrio* belongs to the family *Vibrionaceae*. These bacteria are gram-negative, straight or curved rod-shaped and are capable of fermentative and respiratory metabolism. *Vibrio* species inhabit both high and low salinity waters (Baumann *et al*., 1984; Colwell, 1984). In addition, *Vibrio* is found on marine plants and animals and is part of the natural flora of the digestive tract of marine animals (Baumann *et al*., 1984; Bergh *et al*., 1994; Sakata, 1990).

Vibrio parahaemolyticus infections are associated with the consumption of raw (particularly oysters) under cooked, or cooked, and contaminated fish and shellfish. With 40-70% of food poisoning cases reported, *V. parahaemolyticus* is known to have been pandemic in Japan (Hackney & Dicharry, 1988). Ingesting food contaminated with the *V. parahaemolyticus* bacterium can cause visible symptoms after an incubation period of 9 to 12 hours. However, symptoms can appear earlier (two hours post ingestion) and later (96 hours post ingestion) than the normal incubation period (Varnam, 1991).

Symptoms include diarrhoea (predominant), accompanied by abdominal cramps and nausea. Other significant but less common symptoms include vomiting, headache, low-grade fever and chills (Fujino *et al*., 1974).

The temperature growth range for *V. parahaemolyticus* is between 5 and 43°C, with an optimum growth temperature of 37°C (Varnam & Evans, 1991). Enteropathogenic vibrios are easily destroyed by cooking; however, *V. cholera* has higher heat resistance than *V. parahaemolyticus* (Table 2.4). In general, the growth of vibrios is retarded at pH levels below 7.0, however, *Vibrio parahaemolyticus* has shown to grow at 4.8 (ICMSF, 1980).

Table 2. 4 Thermal resistance of *Vibrio cholerae* and *Vibrio parahaemolyticus* (adapted from Varnam & Evans, 1991).

Species	Temperature (°C)	Denaturation (D) value (min)
V. cholerae[1]	49	8.15
	60	2.65
	71	0.3
V. parahaemolyticus[2]	49	0.7
	51	0.54
	53	0.31
	55	0.24

[1] (Varnam, 1991); heating menstruum crab slurry
[2] (Varnam, 1991); heating menstruum clam slurry

Gram-positive pathogens affecting fish

Staphylococcus aureus

Staphylococcus aureus derives its name from its spherical-ovoidal and grape-like formed cells when viewed under a microscope (Adams & Moss, 2000). This organism is catalase-positive, oxidase-negative and a facultative anaerobe.

Staphylococci normally inhabit the skin and the mucous membranes of warm blooded animals. Of the genus Staphylococcus, there are currently 27 species and 7 subspecies in existence where *S. aureus* is one of the principal enterotoxin producing species (Adams & Moss, 2000). *S. aureus* has also been isolated from faeces, environmental sites such as soil, marine and freshwater, plant surfaces, dust and air (Adams & Moss, 2000). *S. aureus* is an opportunistic pathogen and can infect exposed skin and immune-compromised hosts (Adams & Moss, 2000).

Enterotoxigenic strains of staphylococcus produce enterotoxins which are responsible for poisoning when contaminated food is ingested (Kérouanton *et al*., 2007; Le-Loir *et al*., 2003). Cases of staphylococcal food poisoning are under-reported due to its mild and short-lived effects as an illness.

During the period of January 2008 to April 2009, a significant portion (~25%) of retail purchased fish products in Galicia were contaminated with *S. aureus* (Vázquez-Sánchez *et al*., 2012). Of these products, fresh fish had the highest yield while other products included smoked fish, surimis, fish roe and other ready-to-eat products which did not comply with enforced legal limits (Vázquez-Sánchez *et al*., 2012). This indicated that processing and handling methods of fish products required investigation and the implementation of management practices that would regulate contamination (Vázquez-Sánchez *et al*., 2012). The enterotoxin-producing staphylococci are proteolytic and heat-stable which makes its presence in food a significant safety risk (Omoe *et al*., 2005). Table 2.5 indicates some of the conditions favourable for *S. aureus* growth.

Table 2.5 Factors and ranges which enhance growth and enterotoxin production of *Staphylococcus aureus* (Adapted from Adams & Moss, 2000).

	Growth		***Enterotoxin Production***	
Factor	***Optimum***	***Range***	***Optimum***	***Range***
Temperature °C	35-37	7-48	35-40	10-45
pH	6.0-7.0	4.0-9.8	[2]Ent. A. 5.3-6.8 other 6-7	4.8-9.0
NaCl	0.5%-4.0%	0-20%	0.5%	0-20%
Water activity	0.98->0.99	0.83->0.99	>0.99	0.86->0.99
Atmosphere	Aerobic	Aerobic-Anaerobic	5-20% DO_2	Aerobic-Anaerobic
[1]E_h	>+200mV	< - 200 to >+200mV	>+200mV	?

[1]E_h – oxidation-reduction or redox potential
[2]Ent. A. – Enterotoxin A (*Staphylococcus* spp. strain)

Isolation and detection methods for bacterial contaminants

Conventional method – Total viable counts (TVCs)

The total viable counts (TVCs) also referred to as viable plate count is one of the most commonly used methods for the enumeration of bacteria (Sutton, 2011). This method relies on enrichment and isolation procedures of presumptive bacterial colonies for target microorganisms grown on solid media (Sutton, 2011).

Although not considered reliable as indicators of product stability, TVCs are useful in determining the general microbiological quality of food and encompass all microorganisms which grow aerobically at 30ºC (Sutton, 2011). TVCs serve as indicators of potential pathogenic contamination and also provide information on the microbial quality of the production process (Sutton, 2011). The current acceptable limit of TVCs in raw meat is 5×10^6 CFU/g [(EU: Reg. (EC) 2073/2005 and CAC/GL 21-1997)].

The disadvantage to utilising this method includes labour intensity, time required to obtain results and chemical, physical and physico-chemical changes that require the presence of large numbers of bacterial cells present in order for the reaction to take place (McMeekin & Ross, 1996).

Molecular based detection method – Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a molecular based method and a highly sensitive means by which DNA can be amplified to detect specific microorganisms. Kary B. Mullis (Mullis & Faloona, 1987) invented the PCR technique in 1985 paving the way to its use in science for obtaining millions of copies of limited samples of DNA, detecting the AIDS virus in human cells, and in criminology in which trace samples could be linked back to suspects or victims from a crime scene (Saiki *et al*., 1985; Mullis & Faloona, 1987). Trace amounts of biological matter from viruses, spores and bacteria have also been detected through this technique.

The PCR reaction is composed of three steps (Figure 2.7) each performed at different temperatures:

1. Denaturation
    - DNA is extracted from the sample and transferred into a test tube. The test tube is then exposed to a high temperature (one minute at 95ºC) that causes the two target DNA strands to separate into single strands (Smart, 2008).

2. Primer annealing
    - During this step a primer binds to one of the single DNA strands while the other binds to the complimentary strand. The primers have specific annealing sites which will prime DNA synthesis for the target region of interest. This process takes place at a lower temperature (one minute between 45ºC and 60ºC) than the denaturation step.

3. Polymerase extension
    - Once the primer molecules have annealed with their target duplexed genes, DNA polymerase will have been activated by Mg^{2+} ions and will zero in their preferred substrates which are the primer/ target duplexes. Polymerase will bind nucleotides (A, C, T, and G) to the end of the hybridized primer and extend the DNA sequence (in the 5'→3' direction) creating a complement to the target strand (IDT, 2005 & 2011). Dehybridization of the newly formed strands doubles the number of strands to four and this is repeated until the 30^{th} cycle (Figure 2.8).

      A thermal cycler is required when running a PCR and controls the temperature of samples throughout the process.

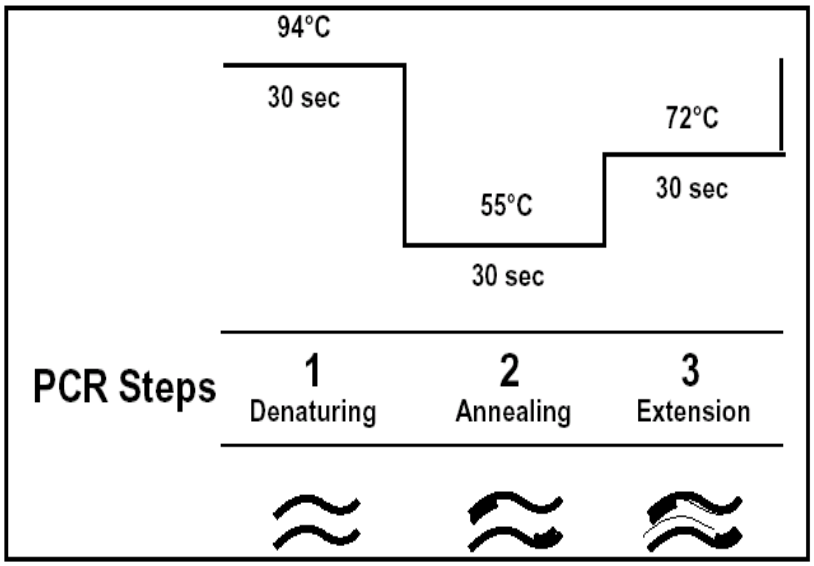

Figure 2.7 A generic three-step PCR cycle profile (adapted from Andy Vierstraete, 1999).

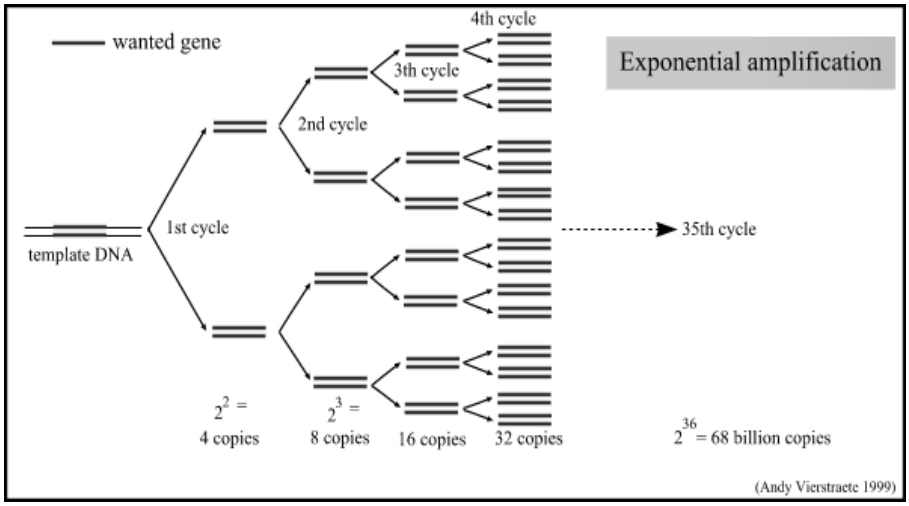

Figure 2.8 Exponential amplification of DNA by repetitions of primer annealing and extension. This process results in 4 strands of DNA, then 8 strands, then 16. 30 cycles yield over one billion copies of the desired region (adapted from Andy Vierstraete, 1999).

Limitations to PCR

Because the PCR process is so sensitive to detecting even trace amounts of DNA, using very small amounts of the sample in the reaction can result in obtaining false positives as a result of contamination. Sources of contamination vary and include the researcher performing the experiment, the tubes, enzymes and buffers used for the reaction (Mullis & Faloona, 1987).

Conclusion

South Africa is a major fishing nation with highly industrialised fisheries and processes. In some fisheries for some products there are high standards such as the hake and tuna fishery (certified with credible standards by the Marine Stewardship Council (MSC), but for the line-fishery which mainly targets snoek, this seems to not be the case. From the literature gathered on the snoek fishery, it appears there are some concerns in terms of missing protocols, unregulated and unmonitored practices. These concerns may lead to a decline in the market for local snoek products, opting for the alternative better quality New Zealand barracouta. In terms of food security and job opportunities, this could negatively affect poor local communities in the Western Cape that rely on a cheap but nutritional source of protein.

Further research into the processing protocols involved in the snoek supply chain is required in order to identify flaws in the existing practices involved. This will enable the development and application of standard operating procedures (SOPs) for this supply chain.

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Chapter 3

Microbial quality and variation in total viable counts and food related pathogen presence (catch to post-vessel to post-bakkie offload) in the snoek value chain

Summary

A number of studies (pilot and standard experiments) were implemented to investigate potential bacterial contamination sources and critical points in the snoek processing chain. The study identified possible sources of contamination by examining fish contact surfaces (storage holds, cutting boards, bakkie, tarpaulin, etc.). A simulated storage experiment was conducted to identify the present level of vessel related contamination whilst also identifying optimal short-term (< 12 hours on board vessel) storage conditions for snoek. In-built vessel storage holds were cleaned with generally recognised as safe (GRAS) and South African Bureau of Standards (SABS) approved detergents whilst additionally comparing ice and no ice treatments in which pH and temperature were monitored. Microbial analysis was carried out using conventional methods (plate count agar; CFU/cm²) and molecular polymerase chain reaction (PCR) methods. Overall results were variable however, it was observed that ice delayed bacterial growth and kept fish temperatures at acceptable storage conditions (< 2°C). The storage point in the processing chain was identified as the highest point of contamination with bacterial counts greater than 5 $\log_{10}$ CFU/cm², even after cleaning. Bacterial counts were higher on snoek removed from “cleaned” holds compared to the unclean holds, suggesting that either cleaning was not executed well or contamination may have originated from a secondary source. Hands were identified as the primary transport medium of four food borne pathogens detected (*Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*). It is recommended that due to the small sample size obtained in this study, that the experiment be repeated and replicated in order to have substantive and representative results. Contamination may be an issue in this supply chain therefore training and the development of standard operating procedures (SOPs) to improve current handling and storage conditions is beneficial, as it would lead to a better quality snoek with potential to enhance the market value and scope of markets that the local small-scale fishermen can reach.

Introduction

Snoek (*Thyrsites atun*) is a commercially exploited line-fish, predominantly targeted by boat based commercial fishers within the Western Cape, South Africa. The peak season for snoek ranges from May to August (Isaacs, 2013). However, it is also caught throughout the year, albeit inconsistently due to the fish's nomadic nature (DEAT, 2005). Snoek is caught by baited saury (*Scomberesox* saurus) or sardine (*Sardinops sagax*) hand lines. Once caught, the snoek are handled quickly and carefully due to the risk of causing excessive bleeding (haemo-toxin in its teeth) when its handler is bitten (Griffiths, 2002).

Hand-line fishing is physically demanding due to low cost equipment and minimal facilities available for the fishers both on-board and at the harbours. Furthermore, as a result of inadequate facilities and hygiene and sanitation procedures, the quality of fresh snoek being sold can vary which is likely due to inconsistencies in handling and processing strategies. It has been observed (Isaacs, 2013) that many boats and "bakkies" (pick-up trucks) used by hawkers (also referred to as 'langanas'; fish buyers or sellers) do not employ stringent sanitation protocols and cooling methods (lack of investment in this supply chain) when handling snoek which may result in contamination and premature spoilage of the fish (Isaacs, 2013). Spoilage in fish is greatly influenced by temperature which plays an important role in its quality and safety (Main *et al*., 2009). Temperature fluctuations accelerate the activity and growth of specific spoilage microorganisms, including foodborne pathogens which grow best in temperatures between 5 to 60 °C (41 to 140 °F) (Jol *et al*., 2005; Raab *et al*., 2008). According to Gonzalez (1999), the accepted initial total viable count of fresh, whole and un-gutted fish is $10^2 – 10^3$ $\log_{10}$ CFU/cm². This bacterial range is considered indicative of high fish quality and good manufacturing practice. The microbiological upper limit for fresh fish is a count of 7 $\log_{10}$ CFU/cm², however this is normally reached between 10-18 days under aerobic refrigerated storage when maximum spoilage is stimulated, depending on the fish species (ICSMF, 1986). Spoilage in food is a health risk and leads to decreased value and loss of revenue to the food industry. Therefore, the identification of contamination sources, pathways and potential ways to minimising contamination and spoilage can have positive effects for the industry (Ward & Beyens, 2012; Smartfish paper No 001).

The overall aim of this study was therefore to assess and describe the microbiological quality of snoek from the harvesting point to its point of sale and determine the effects of various storage treatments on snoek quality.

The objectives were:

1. To describe the typical snoek supply chain for commercial line-fishing,
2. To identify contamination sources at various stages in the processing chain,
3. To determine the effect of storage and handling on microbial load, fish body temperature and pH on-board the vessel and during land transportation,
4. To identify the presence or absence of four foodborne pathogens on snoek as a result of contamination.

Materials and methods

The snoek processing chain involves a series of processes from harvesting the fish to eventually reaching the consumer (sale point) (Figure 3.1). The various stages (assessed separately in five different experiments) of possible microbial contamination are summarised as follows:

1. Fish tackle and baiting
    a. Bait and hand lines were prepared for fishing.
        i. The bait was identified as a possible contaminant and was swabbed.
2. Harvest and slaughter
        i. The hands were identified as a possible contaminant and were swabbed.
        ii. The fish were swabbed once caught in order to quantify initial bacterial load prior to storage and further handling.
3. Storage on boat
    a. Fishers stand within built-in fibre glass sheeted holds (2 m^3) whilst fishing from the vessel. These holds are simultaneously used for storing all fish caught until offloading at the harbour. Additionally, fishermen regularly fillet fish for personal consumption on board using cutting boards and hessian cloths. The hessian cloths were also used to cover fish within the holds on hot days.
        i. The holds and cutting boards were swabbed; a sample from the hessian cloth was collected and stored in a sterile vial for testing.
4. Post-vessel offload
    a. Once the vessel returned to the harbour, fish were auctioned. Fish were offloaded from the boat onto the ground or directly into a box or bakkie depending on the buyers (bakkie owners, wholesalers, private customers).
        i. The fish were swabbed subsequent to offloading from the boat.
        ii. The hands of fishermen were swabbed after offloading fish from the vessel.
5. Transport via bakkie of whole fish from hawker site
    a. Snoek were loaded and transported whole via bakkies to a road side selling point, wholesale buyer, local fish and chips outlet, or other retailers. Ice was used by some bakkie owners during transportation however, this was dependant on ice availability (some harbours have ice plants that supply crushed ice) and cost. A tarpaulin was used as coverage for the snoek during transportation.
        i. The bakkie and tarpaulin were swabbed.
6. Post-bakkie offload (sale)
    a. The snoek were sold informally along a roadside (usually highway). Here the snoek are sold either whole, filleted and/or salted or dried depending on the customer's preference. Once the snoek had reached their destination, they were offloaded
        i. The fish were swabbed as they were offloaded from the bakkie.
        ii. The food handler's hands and knife used for filleting were swabbed.

A number of experiments were carried out in this study in order to gain a better understanding of the process chain and to identify ways of improving it. Each of the experimental aims were as follows:

1. Experiment 1:

   Determine the effect of on-board storage (ice and no ice holds) on the microbial load (CFUs/ cm^2) of snoek at different anatomical sites (head, middle, tail).

2. Experiment 2:

   Determine the effect of on-land transportation (bakkie) on the microbial load (CFUs/ cm^2) of snoek.

3. Experiment 3:
   a. Deteremine the effect of on-land transportation (bakkie) on the microbial load (CFUs/ cm^2) of snoek,
   b. Determine the presence or absence of possible foodborne pathogens from on-land transportation (*E. coli, S. enterica S. aureus,* and *V. parahaemolyticus*) by molecular methods (PCR),
   c. Assess the variation of fish muscle pH and temperature during on-land transportation (post-vessel offloading to post-bakkie offloading).

4. Experiment 4:
   a. Determine the effect of cleaning and sanitation procedures, and the use of ice on the microbial load (CFUs/cm^2) of snoek stored in the on-board fish holds,
   b. Identify sources of contamination from and associated with a fishing vessel,
   c. Determine the presence or absence of possible foodborne pathogens during on-board transportation (*E. coli, S. enterica S. aureus,* and *V. parahaemolyticus*) by molecular methods (PCR),
   d. Assess the variation of fish muscle pH and temperature during on-board transportation (post-catch to post-vessel offload).

5. Experiment 5:
   a. Determine the effect of the South African Bureau Standards (SABS) cleaning protocol on foodborne pathogen load (CFUs/cm^2) within the storage holds, chopping boards on-board the boat and on fishers' hands post-vessel offload.

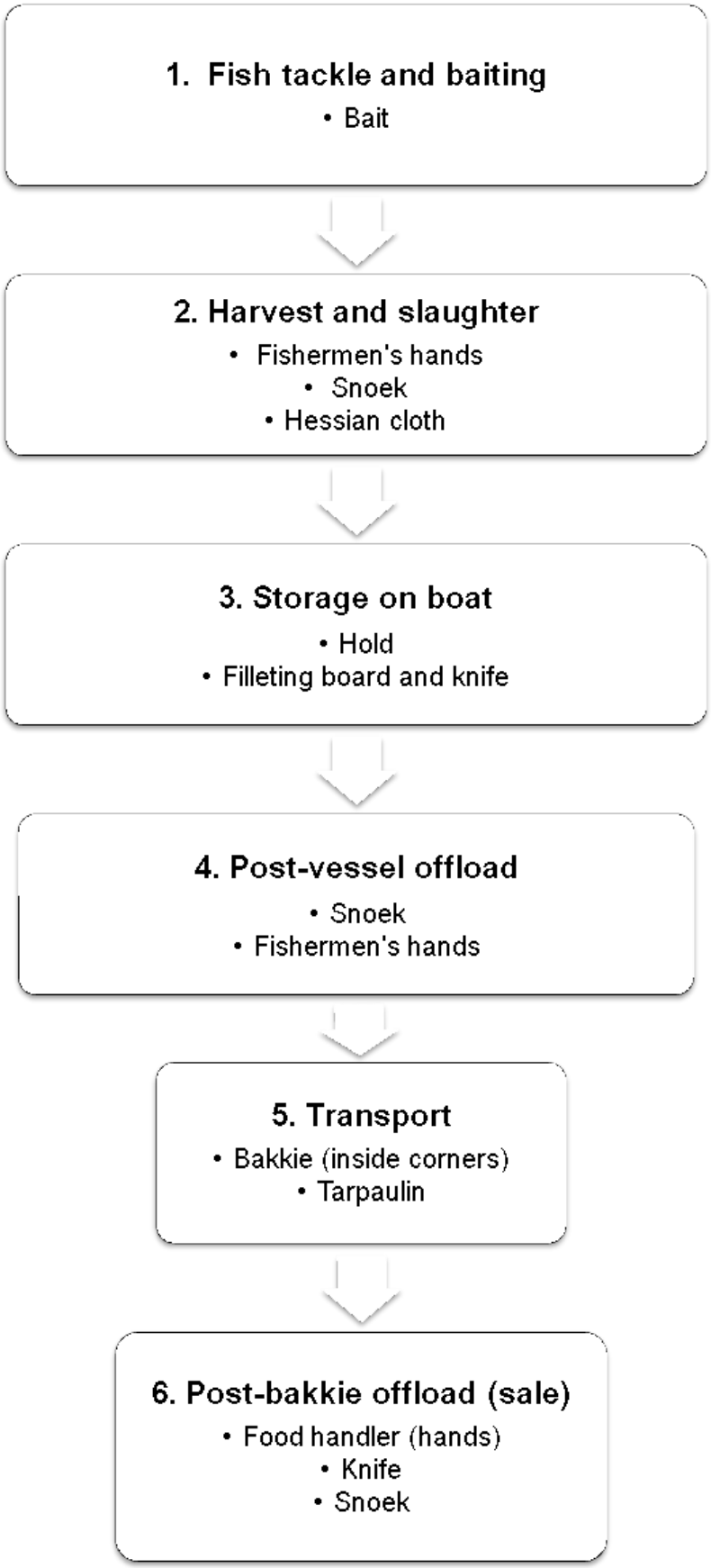

Figure 3.1 Summary of the process flow of snoek from catch to consumer (● represent contaminant surfaces swabbed during the experiments).

Five experiments (Table 3.1) were carried out (three conducted on the fishing vessel of which one was a pilot study; two were conducted on a bakkie of which one was a pilot study).

Table 3.1 Dates and locations (launch sites/ harbours) of each experiment conducted.

Experiment	Location	Date
1. Vessel pilot study	Yzerfontein	04 December 2013
2. Bakkie pilot study	Miller's Point	27 November 2013
3. Bakkie	St. Helena Bay	10 June 2014
4. Vessel	St. Helena Bay	08 July 2014
5. Vessel	Yzerfontein	31 August 2014

A visual of the fishing vessel holds and experimental layout of holds used is presented in Figure 3.2.

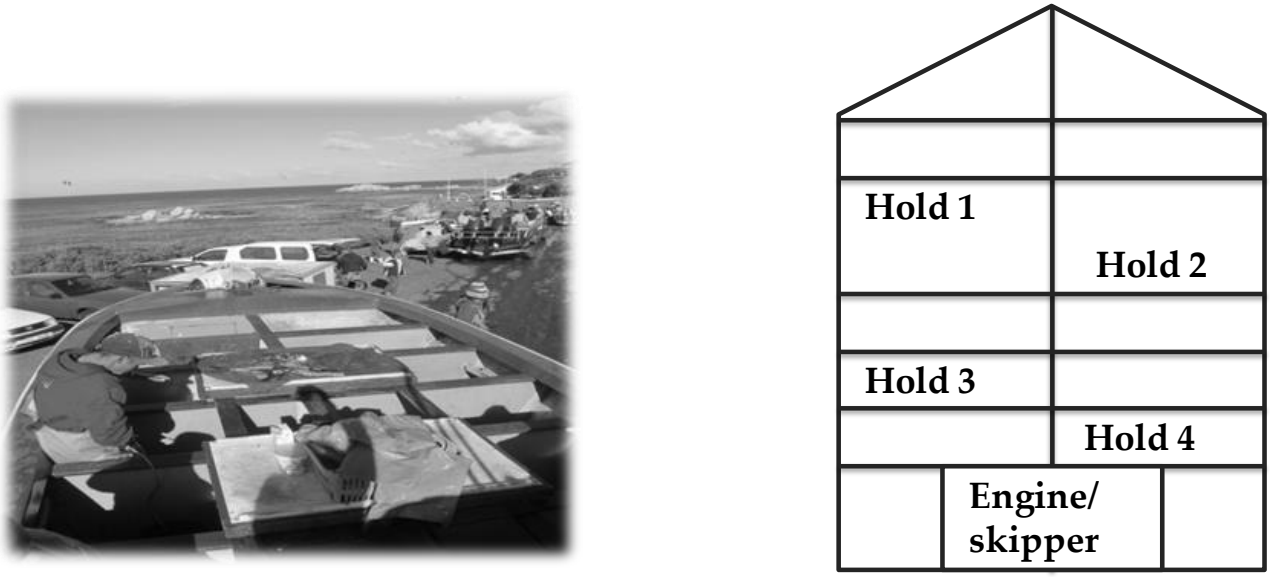

Figure 3.2 Vessel, holds and experimental layout on-board the fishing vessel *Lady M* (experiments 1 and 4).

Two pilot studies were used to preliminary investigate the current processing practice and potential contamination sources along the snoek supply chain, from boat to land. The subsequent trials (three experiments) formed more in-depth studies on contamination sources, pathogen presence and how the process chain affected the snoek physiology (pH and temperature) at various stages of the process chain: harvest, storage, at vessel offloading, bakkie offloading and the snoek selling point. The fishing vessel, crewed by the skipper and six experienced fishers, was launched from the slipway at approximately 06h00 for each sampling trip. Materials and consumables (including pre-labelled sterilized swabs and tags) were prepared in advance for all trips and loaded onto the vessel at the time of launching. Fishing ceased in the

late afternoon where the related time depended on the day's fishing success. Temperature (muscle and ambient) and pH (muscle) were measured and monitored during the various experiments. In Experiments 1, 3 and 4, crushed ice was used as it was the most accessible and economical form of ice available. When open and available, ice was obtained from an ice plant located near St. Helena Bay harbour (Experiment 1 & 4), alternatively ice was brought directly from Stellenbosch University laboratories (Experiment 4). Cleaning procedures in Experiment 4 were carried out prior to launching the fishing vessel.

Upon arrival at Stellenbosch University's meat science lab, swab samples were refrigerated overnight at 4ºC and analysed the following morning. Swab samples were taken in duplicate from the surface of the fish. Half the swab samples were aseptically transferred into a 2 ml eppendorf tube with nutrient broth (1 ml) and incubated at 30ºC for 24h, for the molecular detection of microorganisms. This served as the enrichment phase for the molecular detection of foodborne pathogens in Experiments 3 and 4. Conventional methods for total viable count (TVC - CFU/cm²) (microbial load) were used for the remaining swab samples (Experiments 1, 2, 3, and 4). Foodborne pathogens were quantified (CFU/cm²) using conventional methods in Experiment 5 (ISO 4833).

Conventional detection of microorganisms

Total Viable Count

Serial dilutions were carried out on the homogenised suspension [(physiological saline solution (PSO) - Sigma S588G) and swab)], in which swabs were gently vortexed for +/- one minute, to remove bacteria from the swabs, for the total viable count (TVC). Solid plate count agar (PCA) - (Sigma 70152) was spread with 0.1 ml of the inoculant for each dilution (e.g.10^{-1} to 10^{-2}) and incubated overnight at 30ºC for 24-72h. This was carried out in accordance with ISO (4833) methods. The results were expressed as $\log_{10}$ CFU/cm² (ISO 17604). Plates which yielded more than 250 or 300 colonies could not be counted and were designated too many to count (TMTC). Those with less than 25 colonies were too few to count (TFTC). Viable counts are usually expressed as colony forming units (CFUs) per unit volume, rather than number of microbes (Collins *et al.,* 1995). The US Food and Drug Administration, *Bacterial Analytical Manual (BAM)* recommends a countable range of 25-250 CFU/ plate (Sutton, 2011).

Detection of the food borne pathogens by conventional methods

Escherichia coli

The presence of *E. coli* was assessed by transferring 0.1 ml of pre-enrichment suspension to 10 ml of physiological saline solution (PSO). Triplicate eosin methylene blue agar (EMB) (Merck 1.01347.0500) was prepared according to the product manufacturer's instructions. Solid agar was spread with 0.1 ml of the inoculant for each dilution (10^{-1} to 10^{-2}) and incubated overnight at 35ºC for 18-24h (ISO 7251).

Salmonella enterica

Salmonella samples were analysed by transferring and spread plating 0.1 ml of inoculated solution to triplicate *Salmonella-Shigella* (SS) agar (Sigma 85640) and incubated at 35ºC for 18-24h (ISO 6579).

Staphyloccous aureus

Serial dilutions (10^{-1} to 10^{-2}) were prepared with a physiological saline solution. From these solutions, 0.1 ml aliquots were spread plated on triplicate Baird-Parker agar (Sigma 11705) plates supplemented with egg yolk and Polymyxin B Selective Supplement (Sigma 17148-1VL and P9602) to obtain dominant *S. aureus* isolates and incubated at 35ºC for 48h (ISO 6888-3).

Note: *V. parahaemolyticus* was not assessed by conventional methods.

Molecular detection of indicator organisms

PCR preparation

DNA was extracted from the pre-enrichment swab sample suspension for the detection of *E. coli, S. enterica, S. aureus* and *V. parahaemolyticus*, following the Möller *et al.* (1992) extraction method. Swabs were aseptically added to sterile eppendorf tubes (2 ml) with 1 ml nutrient broth (pre-enrichment) (Sigma 70122) and incubated overnight at 30ºC. Overnight incubated swab samples were homogenised and the suspension (1.5 ml) transferred to eppendorf tubes with sterilized glass beads and 500 µl TES buffer. These were vortexed at maximum speed (13 000 rpm) for 5 min. Five µl of Proteinase K was added to each tube and incubated at 60ºC for 60 min. This was followed by adding 140 µl NaCl and 65 µl CTAB (in this order) and incubating samples at 65ºC for 10min. Seven hundred µl of SEVAG was subsequently added and placed on ice for 30 min. Tubes were then centrifuged at maximum speed for 10 min after which the supernatant was carefully removed and transferred to a new sterilized 1.5 ml eppendorf tube. 225 µl of 5M NH_4A_c was added, gently mixed and incubated on ice for 30 min. Samples were centrifuged again at maximum speed for 5 min. This was followed by transferring 500 µl of the supernatant to a new and sterilized 1.5 ml eppendorf tube. To precipitate the DNA, equal volumes of Isopropanol was added.

Samples were then placed on ice to incubate for 15-30 min and centrifuged at maximum speed for 10min. The resulting supernatant was removed carefully and discarded. The remaining pellet was washed twice with 500 µl of cold ethanol (70% C_2H_6O). The pellet was dried and 50 µl of Milli-Q was added to the pellet. Samples were stored in the refrigerator (4ºC) before loading and running the sample on an electrophoresis gel. The mixture was allowed to cool to room temperature and 1 µl of the DNA extract was used for PCR amplification. This DNA extraction protocol was the same for all four food borne pathogens concerned.

PCR conditions and DNA analysis

The same protocol was carried out for all PCR reactions with respective primer sets and annealing temperatures (Table 3.2) as follows:

The PCR reaction mixture (10 µl) contained 0.25 µl of forward and reverse primer each, 5 µl Readymix (PCR buffer, $MgCl_2$, dNTPs and Taq polymerase) 4.25 µl sterile dH_2O, and 0.2 5 µl of crude DNA extract. Reactions for *E. coli* were performed in a Thermo Hybaid PCR Express thermal cycler with an initial denaturation at 94ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 60ºC for 45 s and extension at 72ºC for 50 s (Bayardelle *et al.,* 2002). The amplified products were analysed by agarose gel electrophoresis on a 1% agarose gel stained with ethidium bromide (3 µl/ 40 ml). The reactions

for *S. eneterica, S. aureus* and *V. parahaemolyticus* were performed with an initial denaturation at 94ºC for 30 s, followed by 30 cycles of denaturation at 94 ºC, annealing at 55ºC for 30 s and extension at 72ºC for 30 s. (Cheng *et al*., 2012).

Materials and methods and results for each experiment

Experiment 1 - Vessel pilot study

The experiment was conducted on the *Lady M*, an eight meter commercially licensed line-fishing vessel. The vessel operated from Yzerfontein where it left the harbour at 07h00 for a period of seven hours. Fishing commenced on anchor close to Dassen Island (33° 25’ 7 42”S; 018° 06’ 133”E) at 07h45. Eight snoek (Table 3.3), *T. atun,* were caught via hand line, immobilised by breaking their necks, and immediately transferred to two holds; at which time ice was added to one hold (a) and not added (b) to the other hold (Figure 3.3). A total of eight snoek were caught and tagged (4 per hold) and the temperature of each snoek was monitored continuously from capture to offloading. The muscle pH for each snoek was recorded at capture and post-vessel offload. Four of the eight fish (n=2 per hold) were assessed for microbial contamination immediately after capture and again during offload. Details of the swabbing and the temperature and pH measuring procedures are described in detail below.

Table 3.2 Primer sets for the different indicator organisms tested.

Species	**Forward**		**Reverse**		**Annealing temp**	**Fragment size**	**Gene region**	**Reference**
Staphylococcus aureus	SAF	5'-CATATCGCTAATGGCTCTAACCC-3'	SAR	5'-ACAAATTACAACGTGTTGAAGACC-3'	55°C	112 bp	SAOUHSC_02297	Cheng, Huangh, Chiu, Liou, Chiangh, Huang. (2012).
Salmonella enterica	invAF	5'-ACGCCAAGTTTGTCCCCAATAG-3'	invAR	5'-CGCCAAACGTCACGTAGAATTATC-3'	55°C	114bp	invA	
Vibrio parahaemolyticus	tlhAF	5'-ACATTAGATTTGGCGAACGAGAAC-3'	tlhAR	5'-ATGCGTTAAAGATGTTGCCTGTATC-3'	55°C	171bp	tlhA	
E. coli	ECA1	5′-GGTGTTCGGCAAGCT TTATCTCAG-3′	ECA2	5′-GGTTAAATTGGGGCTGCCACCACG-3′	60°C	763bp	rfe	Bayardelle Zafarullah, (2002).
E. coli (nested)	KA7	5′-GCGGCCATTAATGCGTTCAAC-3′	K6	5′-ATTGCGAGGCTGGTTTGCC-3′	55°C	122 bp	rfe	

Table 3.3 Summary of data collected in Experiment 1 (Total number of fish evaluated = 8).

Hold number	Treatment	Number of fish	Temperature and pH (Crison PH25)	Trajectory Temperature (Loggers)	Number of fish swabbed ([3]TVC)
[1]H1	With Ice	4	4	4	2
[2]H2	Without Ice	4	4	4	2

[1]H1 – Hold number 1;
[2]H2 – Hold number 2
[3]Total plate count (TVC) – CFUs/cm²

Sterile, cotton-tipped microbial swabs (stored in sterile sampling tubes) were pre-moistened with 0.9% physiological saline solution prior to aseptic swabbing. Four corners and the centre of each hold on the boat were swabbed (one swab per corner and centre) prior to fishing, in accordance with the International Organization for Standardization methods (ISO 7218:2007). Immediately after fish were captured post mortem, swabs were taken from the head, middle and tail regions of the fish (swab area = 2 cm²) (Figure 3.4). The swabs were placed back into their tubes and stored in sterile plastic bags in a foam box with ice to keep the samples cool. Swab samples can be kept refrigerated (at 2ºC) for a maximum of two days.

After swabbing, internal muscle temperature and pH were recorded for the eight fish caught by inserting a Crison PH25 pH meter probe into the dorsal section of the fish, just behind the head (Figure 3.5). One snoek did not have its neck snapped immediately as all others, but was delayed for ten minutes prior to neck snapping (Table 3.4). This was done to assess if delayed mortality affected the muscle pH and temperature. Trex-8 remote probe temperature loggers (recording internal temperature over the duration of the fishing trip) were inserted 4-6 cm posterior to the head and secured via a cotton string. Once inserted, loggers were activated for the duration of the fishing trip (from catch to post-vessel offloading). The loggers were programmed to record fish body temperature every ten minutes. Microbial surface swabs of four fish (two from each hold) were obtained during vessel offloading at the offloading site (Figure 3.6).

(a) (b)

Figure 3.3 Snoek in the boat hold stored with (a) and without ice (b).

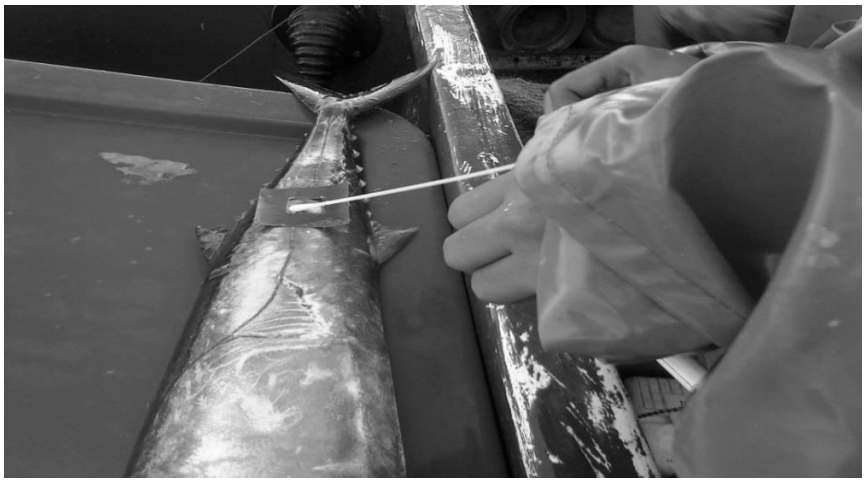

Figure 3.4 Snoek skin surface area swabbed aseptically using a 2 cm² measuring template.

Figure 3.5 Recording of pH and temperature and typical insertion of a temperature probe.

Figure 3.6 Yzerfontein catch offloading site where the auctioning of snoek takes place. Snoek are then loaded onto bakkies and transported to various end points.

Results – Experiment 1

Snoek were captured from 06H00 to 10H40 and offloaded at 14H00 resulting in a minimum and maximum exposure time of five hours and eight hours, respectively.

Figure 3.7 below provides a summary of the average muscle pH and temperature of eight snoek at catch and offloading, at five and eight hours respectively, post-catch and post-offloading.

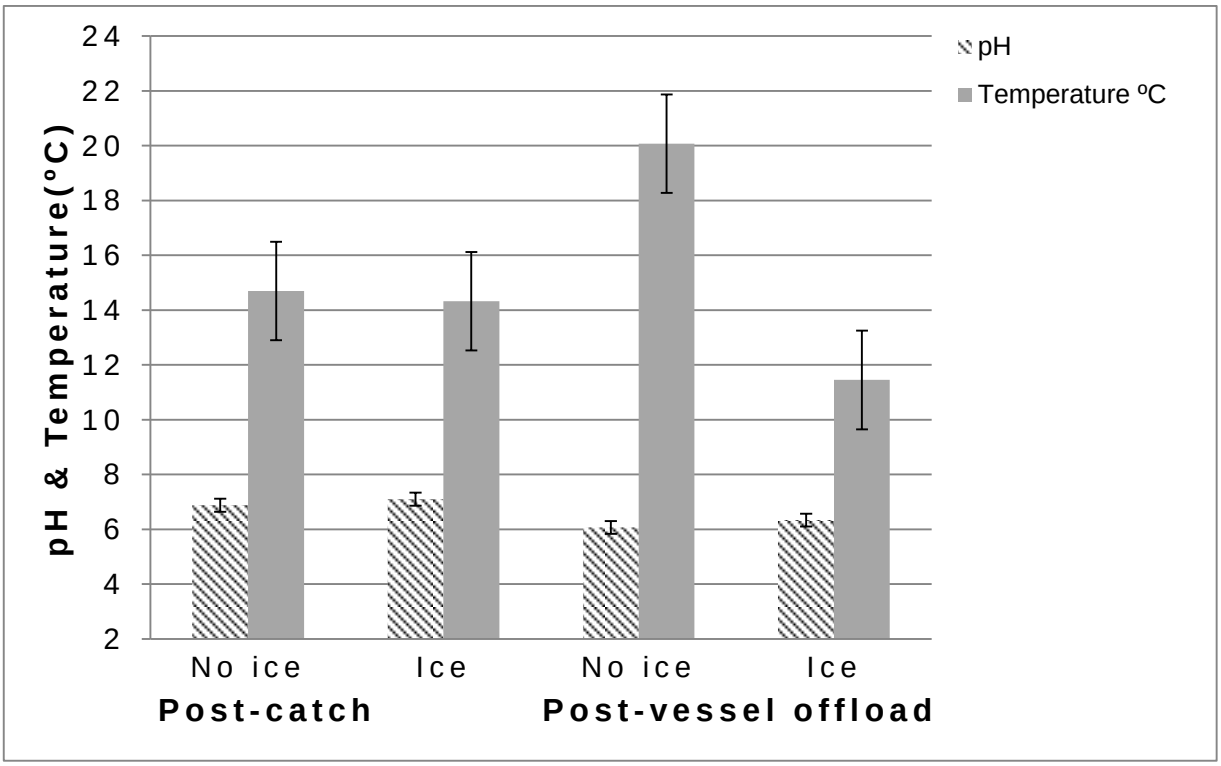

Figure 3.7 Average muscle temperature and pH of eight fish treated with and without ice immediately post catch and post offloading at harbour. Error bars represent the standard error.

The muscle temperature in snoek treated with ice decreased by 2.88°C (from 14.33°C to 11.45°C) during the duration of the experiment whilst those snoek treated without ice had an increased muscle temperature of 5.38°C (from 14.7°C to 20.08°C) (Figure 3.7). It was observed that the final muscle pH in snoek treated with ice was higher (6.34) than snoek treated without ice (6.07) whilst the snoek which experienced a ten minute delay in snapping its neck, displayed the lowest muscle pH of 5.97 (Table 3.4).

Table 3.4 Muscle temperature and pH of eight snoek at catch and offload using Crison PH25 pH meter.

Fish no	Time caught	Time offload	Treatment	Temperature °C		pH	
				Catch	Offload	Catch	Offload
1	06:15	14:06	ICE	13.50	8.50	7.90	6.61
2	06:35	14:00	ICE	13.00	12.90	6.86	6.21
3	09:40	14:07	ICE	14.80	13.20	6.77	6.19
4	10:40	14:08	ICE	16.00	11.20	6.86	6.33
1	06:38	14:21	NO ICE	13.20	19.00	7.26	6.10
2	08:12	14:20	NO ICE	13.70	18.80	6.81	6.06
3	09:23	14:19	NO ICE	14.60	19.50	6.96	6.13
4	10:00	14:17	NO ICE	17.30	23.00	6.46	5.97

Samples used for microbial analysis
10-minute delay in killing

The bacterial counts varied considerably between the three fish surface areas (Figure 3.8). Post off-loading from the vessel, the snoek treated without ice had lower bacterial counts (1.56 $\log_{10}$ CFU/cm², head and 1.46 $\log_{10}$ CFU/cm², middle) compared to snoek treated with ice (2.16 and 3.30 $\log_{10}$ CFU/cm², head and middle). It was observed that bacterial counts from the middle region decreased (instead of a logical increase) from 2.92 $\log_{10}$ CFU/cm² to 1.46 $\log_{10}$ CFU/cm². Of the three snoek surfaces assessed, the tail region had the highest number of CFUs whilst those treated with ice had slightly lower CFUs (4.36 $\log_{10}$ CFU/cm²) compared to snoek treated without ice (4.77 $\log_{10}$ CFU/cm²). The highest overall number of CFUs were observed in the on-board fish holds, where both the iced (5.78 $\log_{10}$ CFU/cm²) and un-iced (5.83 $\log_{10}$ CFU/cm²) holds had comparable levels.

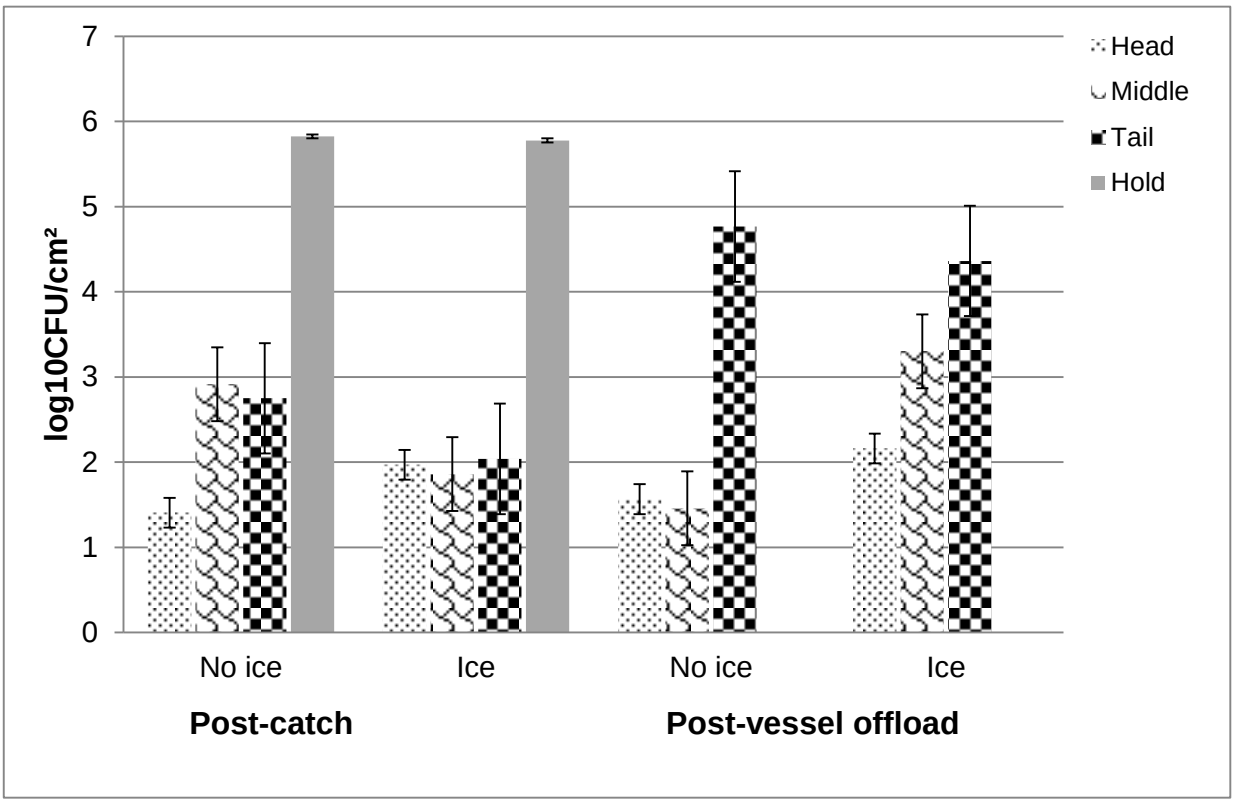

Figure 3.8 Average log_{10}CFU/cm² on two snoek (sampled from the head, middle and tail skin surface) treated with and without ice immediately post-catch and post-vessel offload at the harbour. The graph displays CFU/cm² for two holds prior to fish storage. Error bars represent standard error.

Experiment 2 – Bakkie pilot study

Ten snoek were randomly sampled off a bakkie at a 'Snoek Wholesalers' in Phillipi, Cape Town (Table 3.5). The snoek were caught off Miller's Point, 20 km away from Phillipi. Details on the fishing duration (arrival at harbour) and time of harbour departure are undocumented. Upon arrival at the wholesaler (15h00), the snoek were offloaded from the bakkie and microbial swabs were taken in the same manner as previously outlined (Experiment 1). Separate sections were not assessed in the current experiment and one swab was used to collectively sample the three regions on each fish (head, middle, tail). Samples were transported to Stellenbosch University lab and refrigerated at 4ºC overnight for analysis the following morning. Samples were analysed in triplicate for total colony forming units (CFUs) on PCA agar.

Table 3.5 Summary of data collected in Experiment 2.

Treatment	Number of fish sampled	Temperature and pH (Crison PH25)	Trajectory temperature (Loggers)	Number of fish swabbed (*TVC)
None	10	Not recorded	Not recorded	10

* Total plate count (TVC) – CFUs/cm²

Results – Experiment 2

The results from this experiment provide a snapshot of the bacterial load on snoek post-bakkie transportation (Figure 3.9). Comparisons across studies (Experiments 1 and 2) showed that bacterial counts from this study (2.83 $\log_{10}$ CFU/cm^2) were relatively comparable to the results obtained in experiment 1 (2.94 $\log_{10}$ CFU/cm^2). The comparison across the two studies demonstrates the quantification of bacterial growth during the supply chain progression.

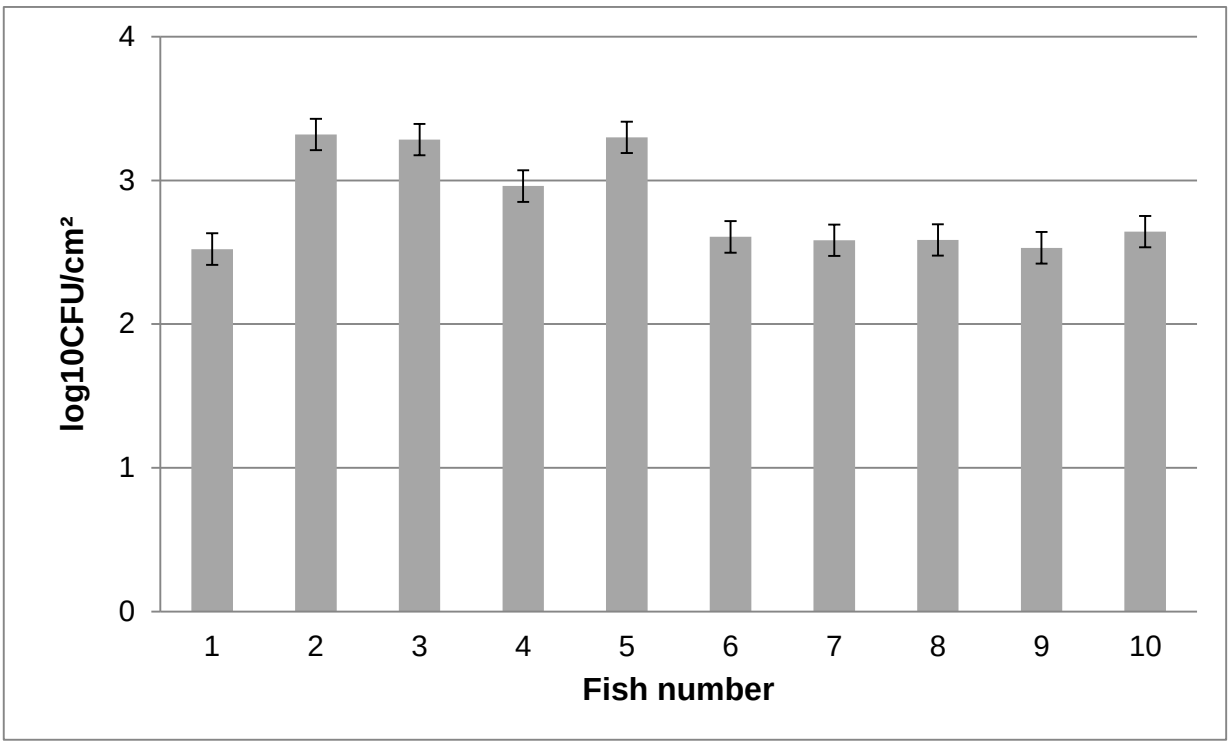

Figure 3.9 Average $\log_{10}$CFU/cm² obtained from snoek skin surface (n=10 fish) post-bakkie transportation. Error bars represent standard error.

Experiment 3 – Bakkie

The most probable sources of contamination from vessel offloading to bakkie transportation were identified and quantified in this experiment, whilst Experiments 4 and 5 aimed to expand on this topic further. Microbial swab samples were collected aseptically from a bakkie (corners were swabbed) and a tarpaulin (4 random surfaces swabbed) prior to snoek offloaded from the fishing vessel onto the bakkie (Table 3.6). It is typical for the bakkie owners to have a tarpaulin on the bottom of the bakkie, the fish are then placed inside this tarpaulin (sometimes with ice) and then the ends of the tarpaulin are folded over the fish to cover the fish. Ten snoek were swabbed (across the length of each fish) upon arrival at St. Helena harbour (time: 17h00). Post swabbing, the muscle temperature and pH probe (recorded in intervals; post-vessel offload and post-bakkie offload) and temperature loggers were inserted roughly 5 cm into the dorsal musculature of fish as indicated in Table 3.6. Temperature loggers logged internal muscle temperature over twenty-one hours (transportation and storage time). Crushed ice was added to the bakkie and the fish were subsequently transported from St. Helena Bay to Belhar in Cape Town. An extra quantity of ice was added to the fish during overnight storage on the bakkie. Fish were offloaded onto a second bakkie the next day (14h00) from

which they were sold locally (Belhar). Identical swabbing procedures, temperature and pH recordings employed at the harbour were obtained at the point of final sale of snoek. Two additional potential contamination sources were also swabbed at the point of sale: the knife used to fillet the fish at the sale site as well as the hands of the food handler.

Table 3.6 Summary of data collected in Experiment 3.

Treatment	No of fish sampled	Temperature and pH (Crison PH25)	Trajectory temperature (Loggers)	No of fish swabbed (*TVC & PCR each)	Bakkie swabs (TVC & PCR each)	Tarpaulin and hand swabs (TVC & PCR each)	Knife swabs (TVC & PCR each)
None	10	10	7	[1]9	4	5	1

[1] Only nine swabs analysed; 10th swab was faulty
* Total plate count (TVC) – CFUs/cm²

Samples were transported back to Stellenbosch University, refrigerated (4°C) overnight and tested the following day. Microbial analysis (total plate count) was conducted using conventional (PCA) and molecular methods (PCR). DNA was extracted from duplicated swabs for polymerase chain reaction (PCR) analysis with species specific primers. PCR products were run through gel electrophoresis. PCR was carried out as a presence-absence test for the following bacteria: *Escherichia coli, Staphyloccocus aureus, Salmonella enterica,* and *Vibrio parahaemolyticus*.

Results – Experiment 3

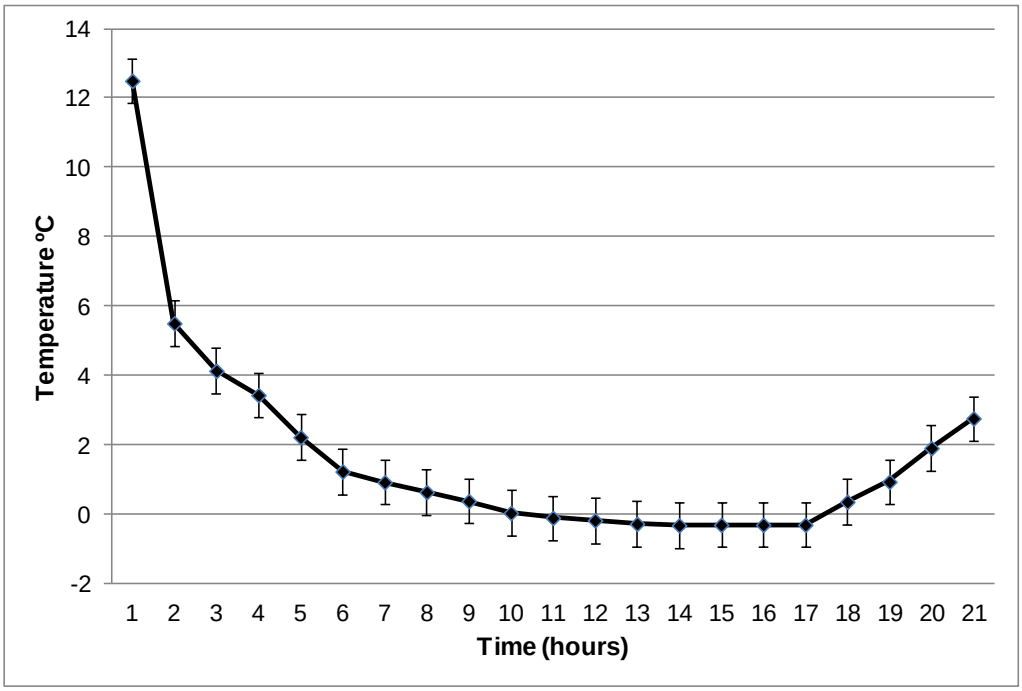

Figure 3.10 Average muscle temperature of seven snoek over a 21 hour (18h00-14h00) overnight holding period post-vessel offload to post-bakkie offload. Error bars represent standard error.

Snoek muscle temperature declined sharply in the first hour of transportation from 12.5°C to 5.5°C by 7°C. Subsequent to the initial hour, a gradual decrease was observed until time 11h00 (-0.3°C) at which time the muscle temperature gradually increased to 2.8°C at the time of consumer sale (Figure 3.10). The muscle pH in snoek decreased considerably over the 21 hour experiment, from post-vessel offload to point of sale in Belhar (Figure 3.11). At the point of post-vessel offloading the pH levels in the snoek muscle were higher in the current experiment compared to experiment 1 (6.21).

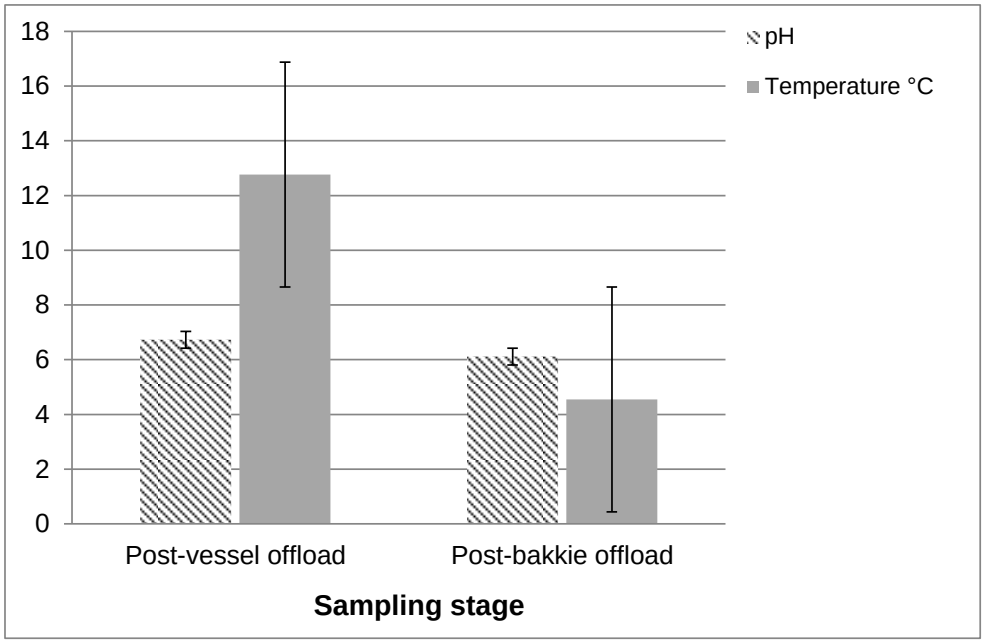

Figure 3.11 The average muscle temperature and pH of ten snoek post-vessel offload and post-bakkie offload (post-21 hours of ice storage). Error bars represent standard error.

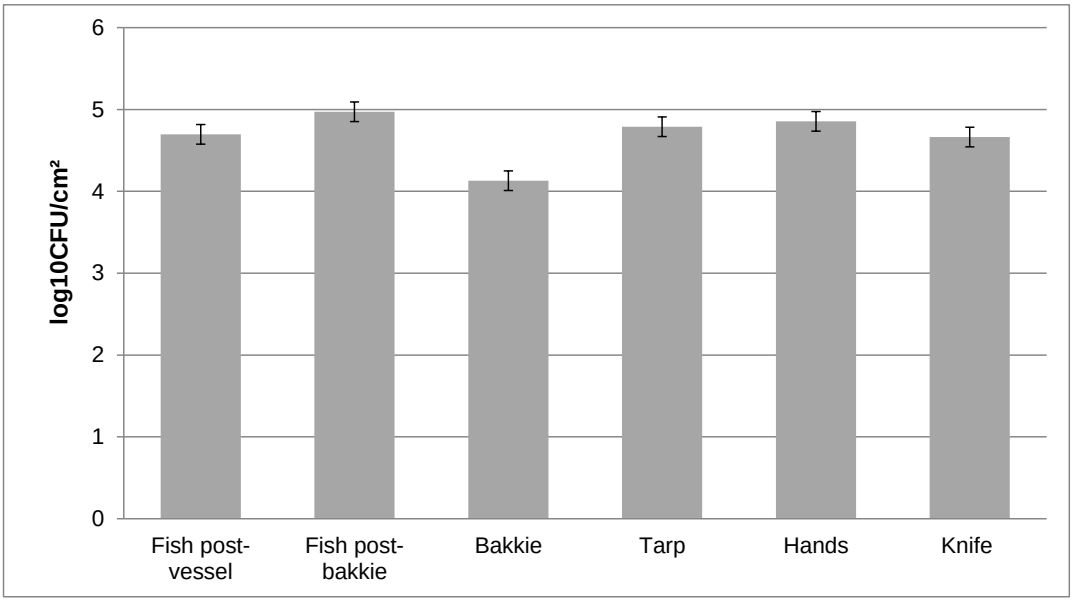

Figure 3.12 Average log_{10}CFUs/cm² of fish post-vessel offload (Fish A) and post-bakkie offload (Fish B) (n=9), the bakkie surface, tarpaulin cover, fisher's hands (n=5) and knife (n=1) were swabbed. Error bars represent standard error.

Bacterial counts on snoek increased by 0.28 log_{10} CFU/cm² from 4.69 to 4.97 log_{10} CFU/cm² over the experimental duration (Figure 3.12). The bacterial counts observed from the hands of the food handler, the tarpaulin, the knife and bakkie were 4.86 log_{10} CFU/cm^2, 4.79 log_{10} CFU/cm^2 4.66 log_{10} CFU/cm^2 and 4.13 log_{10} CFU/cm^2, respectively. When compared to the final bacterial counts observed on fish post-vessel offload and post-bakkie offload in the previous two experiments, bacterial counts were found to be exponentially greater in this experiment (> 4.00 log_{10} CFU/cm^2).

E. coli was detected on two of the swabs collected from the bakkie. *S. enterica* and *V. parahaemolyticus* were each detected on two of the swabs collected from the fish skin. *E. coli* was also detected on nine of the ten fish sampled (Table 3.7). *S. aureus* tested negative for all swab samples.

Table 3.7 Presence or absence of indicator organisms detected on the four contaminant sources and the 10 fish post-vessel offload.

No.	Sample	No of swabs	Results			
			E. coli	***V. parahaemolyticus***	***S. enterica***	***S. aureus***
1	Bakkie	6	2	0	0	0
2	Tarp	3	0	0	0	0
3	Hand	1	0	0	0	0
4	Knife	1	0	0	0	0
5	Fish skin	10	9	2	2	0

Experiment 4 – Vessel

Experiment 4 was carried out on the *Lady M* commercial fishing vessel, which launched in St. Helena Bay at approximately 06h00. Fishing commenced at approximately 07h00. In order to determine the effect of ice

and vessel hold cleaning on snoek quality (microbial load and muscle temperature), four distinct treatments were evaluated:

1. Hold A represented the hold under normal working conditions (control)
2. Hold B was cleaned using sanitising products
3. Hold C contained ice only
4. Hold D was cleaned using sanitising products and contained ice.

Holds B and D were cleaned and sanitized with generally recognised as safe (GRAS) chemicals: Hygen F29 (detergent). The detergent was prepared according to the manufacturer's instructions. Hold B and D were rinsed with fresh water and the detergent was subsequently added and the holds were scrubbed in order to remove as much dirt and biofilms (immobilised bacteria enclosed in an organic polymer matrix; Jessen & Lammert, 2003) present as possible. Following scrubbing the holds were rinsed with fresh water. The holds were further sanitized with Kemklor, a hypochlorite based sanitizer, prepared according to instructions by the manufacturer, which remained in the hold for a minimum of ten minutes at which point it was rinsed with sea water (only water available at time of rinsing).

Microbial swabs were collected post cleaning (where cleaning was employed) from each of the four holds to assess the bacterial load and pathogen presence for each treatment. Twenty fish were caught in total and five fish were allocated per hold (Table 3.8). Microbial swab samples were collected (as in Experiment 3) aseptically from each fish immediately after catch and subsequently placed in the appropriate holds/treatments. Muscle temperature and pH measurements were taken in the same manner as described in Experiment 3. Temperature loggers recording muscle temperature were faulty (intended initially for ten fish) for the duration of the experiment. Hence, the results from five fish treated with ice were used (Table 3.8). Ambient air temperature was recorded by two air loggers placed in two holds (shelved above the fish on the inside of the hold) used in the experiment (one containing ice whilst the other containing none). Additional points of contamination were identified (bait, hessian sack and fishers hands) and swabbed (Table 3.8). The bait (sardine – *Sardinops sagax*) is generally cut into pieces prior to being attached to the hand-line hook. As the bait is not eviscerated, the cutting causes stomach contents to pour out and this can become a potential contamination risk. Swabs were taken off the knife used and also off the bait (area where the bait was cut; cross-section). The hands of the fishermen were swabbed (prior to and at the end of the fishing trip) by rubbing the swab gently over their fingers and palms. Thirdly, a portion of the hessian sack (used often to cover fish on hot days) was swabbed for microbial analysis. All swab samples were collected in duplicate for the assessment of total plate count and pathogen analysis. A number of PCRs were run to detect the following pathogens: *Escherichia coli, Staphyloccocus aureus, Salmonella enterica,* and *Vibrio parahaemolyticus*.

Table 3.8 Summary of data collected in Experiment 4.

Hold number	Treatment	No of fish used	Temperature and pH (Crison PH25)	Trajectory temperature (Loggers)	No of fish swabbed (*TVC & PCR each)	Bait swabs (TVC & PCR each)	Hands swabs (TVC & PCR each)	Cloth sample (TVC & PCR each)
A	Control	5	5	5	5	5	4	1
B	Cleaned	5	5		5			
C	Ice	5	5		5			
D	Cleaned + ice	5	5		5			

* Total plate count (TVC) – CFUs/cm²

Results – Experiment 4

Snoek muscle temperature (transported in ice on bakkie) experienced a decrease in temperature between 20 and 80 minutes from 13.40°C to 4.10°C, increasing to 4.55°C after 120 minutes at the time of offloading snoek from the vessel (Figure 3.13).

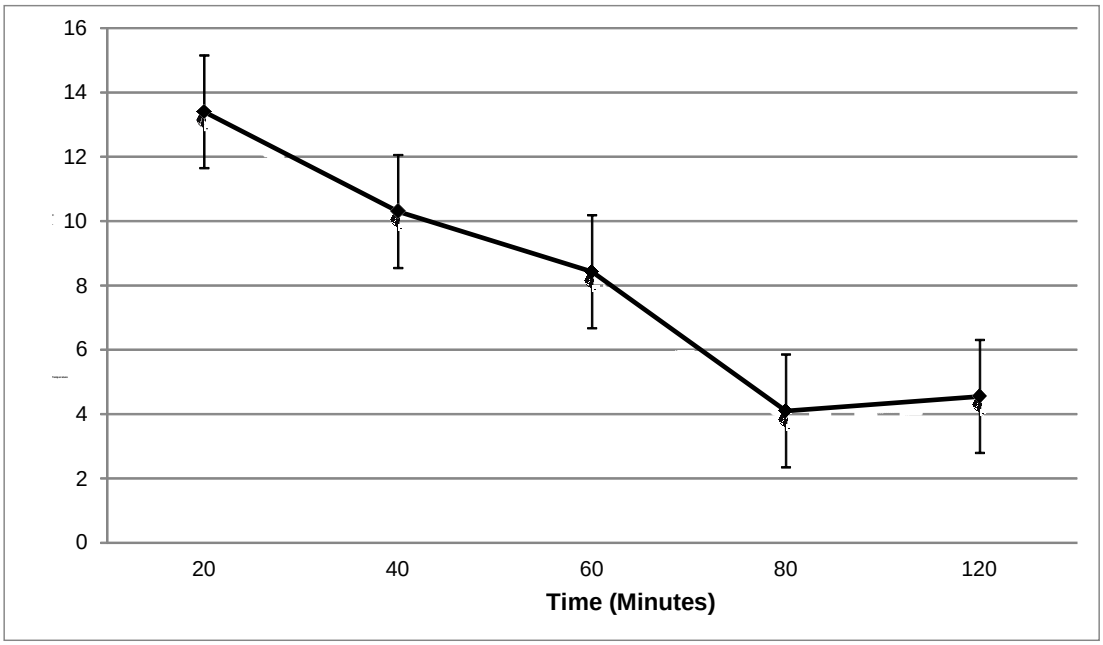

Figure 3.13 Average muscle temperature of five snoek over 120 minutes of on-board ice storage (14h30-16h30). Error bars represent standard error.

The ambient temperature in both holds (ice and without ice) gradually increased from 9.36°C and 9.46°C to 15.99°C to 14.80°C for the hold containing ice and no ice respectively (Figure 3.14). An unexpected increase in temperature from the hold with ice was observed between 2.5 and 5 hours of storage from 10.83°C to 15.72°C.

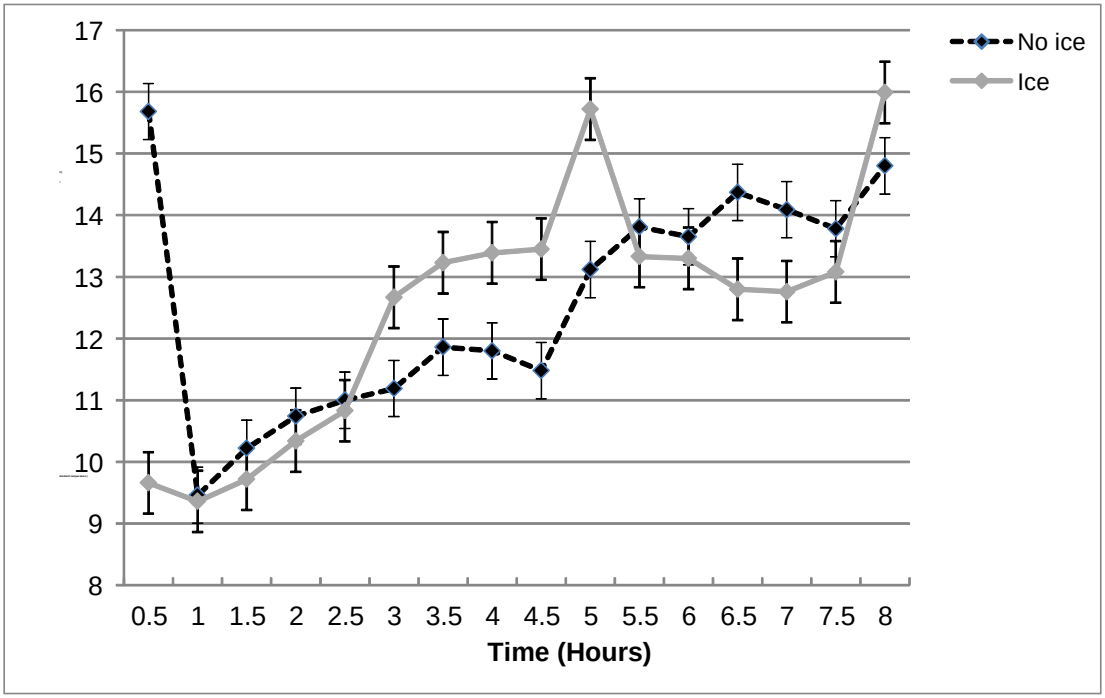

Figure 3.14 Ambient temperature within two fish holds (one containing ice and the other not containing ice) during the on-board storage of snoek (n=8 hours). Error bars represent standard error.

The muscle temperature in snoek treated with ice showed a marked decrease in temperature from post-catch to post-vessel offloading. On average, the fish treated with ice were 7.98ºC lower than those not treated with ice post-vessel offloading (Figure 3.15). The muscle pH of fish within all treatments remained above 6.00, with the lowest pH value post-vessel offload recorded in snoek treated without ice (6.64). However, the lowest pH value in the current study is higher than the pH values in snoek muscle observed in Experiments 1 and 3.

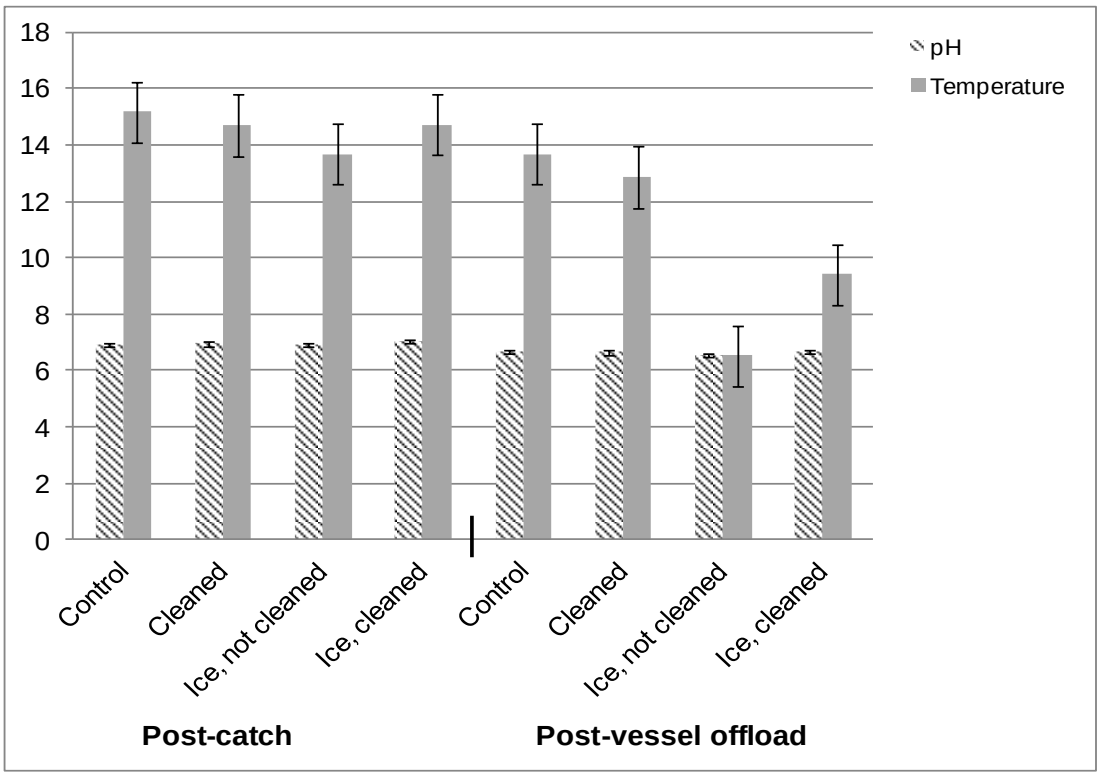

Figure 3.15 Average temperature and pH of snoek (n=20) post-catch and post-vessel offload, treated with or without ice. Error bars represent standard error.

The overall bacterial count on snoek increased from post-catch to post-vessel offload (Figure 3.16). The snoek post B (cleaned holds) and D (ice and cleaned holds) treatment displayed bacterial counts of 2.46 and 2.41 $\log_{10}$ CFU/cm^2, respectively post-vessel offload, compared to snoek post A (control) and C (ice and not cleaned holds) treatment (2.16 and 1.69 $\log_{10}$ CFU/cm^2). The bacterial counts for fish post-offload from hold B, were higher compared to the other fish post-vessel offload, having increased from 1.22 to 2.46 $\log_{10}$ CFU/cm^2 (by 0.93 $\log_{10}$ CFU/cm^2). Holds B and D which were cleaned displayed bacterial counts (5.98 and 6.27 $\log_{10}$ CFU/cm^2, respectively) higher than holds A and C, respectively) (5.77 and 6.35 $\log_{10}$ CFU/cm^2 which were not cleaned (Figure 3.17; Table 3.9).

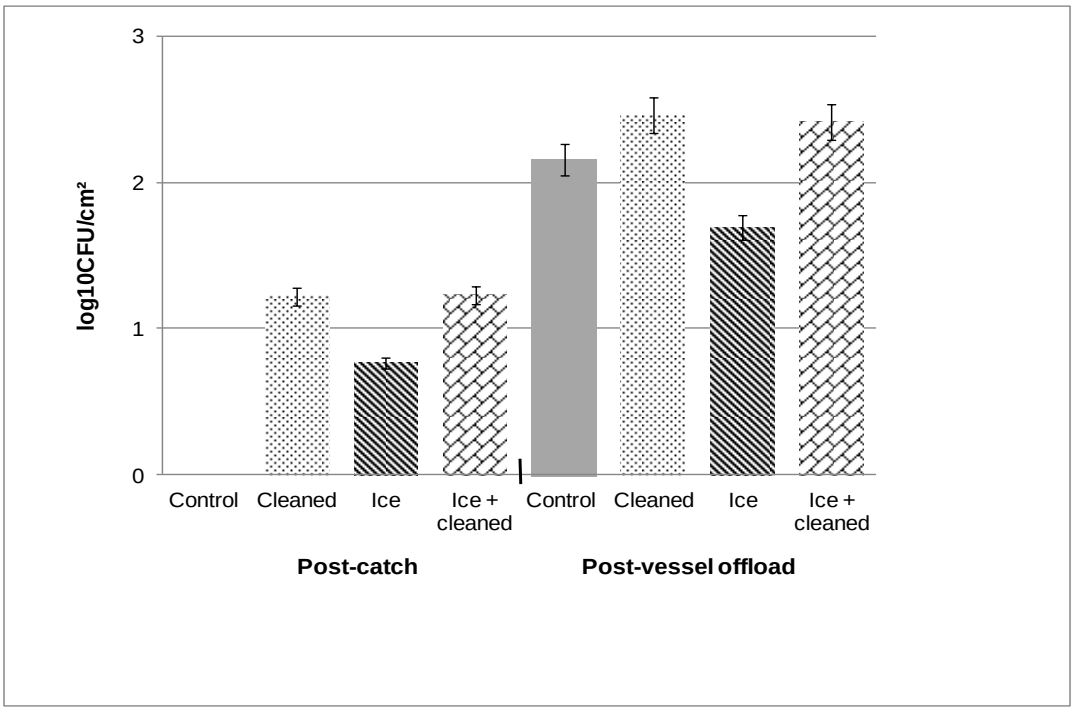

Figure 3.16 Average log_{10}CFUs/cm² from the skin surface of snoek (n=20) both post-catch and post-vessel offload with respect to four various treatments used (A - control, B - cleaned, C – ice, not cleaned and D – ice, cleaned). Error bars represent standard error.

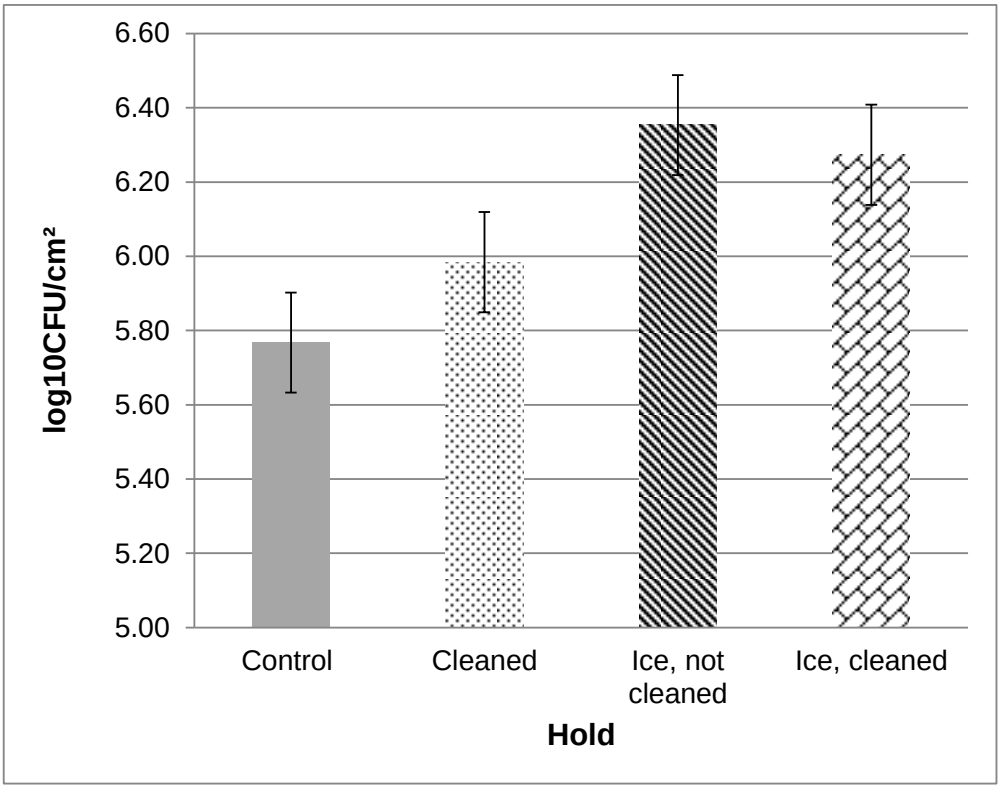

Figure 3.17 Average log_{10} CFU/cm² from four storage holds post-treatment (A, B, C, and D). Error bars represent standard error.

Table 3.9 Summary of LS Means ± standard error of log_{10} CFU/cm² present on snoek (n=20) from four distinct treatments at post-catch and post-vessel offload.

Treatment	Post -catch (CFU/ cm²)	Post-vessel offload (CFU/ cm²)	p-value post-catch	p-value post-vessel offload
Control (A)	0.00 ± 0.00	2.16 ± 2.14	0.03	0.01
Clean (B)	1.21 ± 2.00	2.46 ± 3.19	0.43	0.35
Ice, not cleaned (C)	0.76 ± 1.86	1.69 ± 2.23	0.47	0.38
Ice, not cleaned (D)	1.23 ± 2.00	2.41 ± 3.12	0.43	0.35

The bait, fisher's hands and hessian cloth sampled had on average 4.69 log_{10} CFU/cm (lowest), 4.92 log_{10} CFU/cm² and 6.92 log_{10} CFU/cm² (highest), respectively (Figure 3.18).

All pathogens were detected on the bait and hands of fishers post-catch (Table 3.10), while only two of the four pathogens (*E. coli* and *V. parahaemolyticus)* were detected in hold B (cleaned).

Post off-loading fish held in hold B (cleaned) and D (cleaned and iced) tested positive for *E. coli and V. parahaemolyticus* whilst no other pathogen was detected in either of the two remaining holds (A & C).

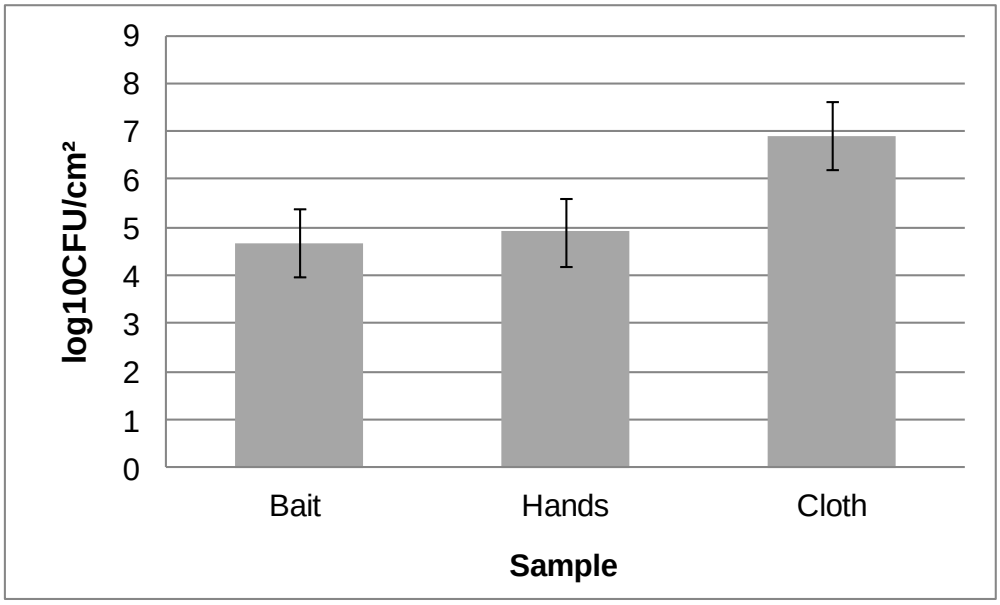

Figure 3.18 Average log_{10} CFUs/cm² from bait (n=5), fisher's hands (fishermen=4), and a hessian cloth section (n=1). Error bars represent standard error.

Table 3.10 Summary of the presence/ absence of foodborne pathogens from 11 sampling points where A, B, C and D represents the control, clean, ice, not clean, and ice, clean holds, respectively.

S.No.	Sample	No. of swabs	Result			
			E. coli	*V. parahaemolyticus*	*S. enterica*	*S. aureus*
Samples collected on-board (Post-catch)						
1	Hold A	**1**	0	0	0	0
2	Hold B	**1**	1	1	0	0
3	Hold C	**1**	0	0	0	0
4	Hold D	**1**	0	0	0	0
5	Hands	**4**	1	1	1	1
6	Bait	**5**	3	3	2	2
7	Cloth	**1**	0	0	0	0
Samples collected post-vessel offoad						
8	Fish (A)	**5**	0	0	0	0
9	Fish (B)	**5**	1	1	0	0
10	Fish (C)	**5**	0	0	0	0
11	Fish (D)	5	1	1	0	0

Experiment 5 – Vessel

The initial aim for Experiment 5 was to determine the effect of various on board treatments (a replica of Experiment 4) on pathogen counts. However, without prior notice, the *Lady M* fishing vessel (which was utilised in all boat based experiments) underwent a standard cleaning and sanitation procedure as per requirement by the South African Bureau of Standards (SABS) and therefore replication was not possible. In addition, no replacement vessel was allocated. Therefore, the aim of the experiment was altered. The aim thus became to determine the efficacy of the SABS approved cleaning procedure in eliminating pathogens and bacteria from the boat.

Sixteen holds were swabbed in total (Table 3.11); eight were swabbed prior to fishing, whilst another eight were swabbed at landing/offloading. Three chopping boards were swabbed prior to launching and four of the fishermen's hands were swabbed after offloading fish from the vessel (Table 3.11). Although snoek were the target species, only yellowtail (*Seriola lalandi*) were caught. These fish were not sampled due to handling preferences of the skipper. With respect to the pathogens tested for in this experiment, *V. parahaemolyticus* was omitted due to the growth media being unavailable at the time.

Table 3.11 Summary of data collected in Experiment 5.

Holds	Treatment	Number of fish sampled	Temperature and pH (Crison PH25)	Trajectory temperature (Loggers)	Hand swabs	Chopping board
16	Cleaned with SABS approved detergent (Shipgaurd)	None	Not recorded	Not recorded	*4	*3

* Total plate count (TVC) – CFUs/cm²

Results – Experiment 5

Table 3.12 Summary of mean pathogen counts (log_{10} CFU/cm²) from 16 holds (8 swabbed separately prior to vessel launch, 8 swabbed separately post-vessel offload), 3 filleting boards (swabbed prior to vessel launch) and 4 hands (swabbed post-vessel offload).

	Hold		Hands	Filleting Board
	Prior launching (8)	**Post-offloading (8)**	**(4)**	**(3)**
Escherichia coli	1.44	1.64	0.00	0.00
Salmonella enterica	2.12	1.66	0.00	0.00
Staphylococcus aureus	0.52	0.00	0.52	0.00

S. enterica counts were higher (2.12 log_{10} CFU/cm²) than *E. coli* (1.44 log_{10} CFU/cm²) both prior to launching and post-offloading from the 8 holds (Table 3.12). *S. aureus* appeared lowest in 8 of the holds prior to launching and on the hands of 4 fishermen post-vessel offload (0.52 log_{10} CFU/cm²). Pathogen counts for *S. enterica* were low (1.66 log_{10} CFU/cm²) in the 8 holds post-offloading compared to the 8 holds prior to launching (log_{10} CFU/cm²). No *E. coli* and *S. enterica* were detected on the food handler's hands and filleting boards while *S. aureus* was also not detected on the filleting boards.

Discussion

A number of contamination sources were identified in the traditional South African snoek processing chain (catch to consumer). The current study found that bacteria and food borne pathogens were present early in the snoek processing chain at harvest (Experiments 1 and 4), with bacterial counts generally increasing at each step of the flow chain (Table 3.13) (Experiments 2 and 3).

During the harvesting phase of snoek on-board the fishing vessel, the initial and likely contamination sources identified included the storage holds, fishers breaking the snoek neck, hands, knives, bait, hessian cloth and the chopping boards. The average initial total viable count on snoek post-catch was between 1-3 log_{10} CFU/cm². Counts exceeding this range were most probably associated with cross-contamination of snoek samples during the harvesting process. The bait was identified as the primary source of contamination, followed by the hands, identified as the secondary source of contamination, transferring bacteria onto the snoek and subsequently to food contact surfaces (handling of bait, knives, cutting boards and the hessian cloth) and/or vice versa by hands. Samakupa *et al*. (2003) indicated that during handling and preparation, bacteria are transferred by the hands of food handlers to food and subsequently to other surfaces. Furthermore, according to observations that were done, the fishermen operate whilst standing inside the storage holds on-board, with their feet (boots) in direct contact with the fish, making the transmission of (faecal) bacteria possible. This is an important contamination point to be considered for possible further research. The results obtained from the harvesting stage on the snoek post-vessel offload are higher than those found by Gonzalez (1999) (10^2-10^3 log_{10} CFU/cm²), indicative of high fish quality and good manufacturing processes. Total viable counts from the contamination sources exceeded 10^4 log_{10} CFU/cm², which suggests that proper cleaning and sanitation procedures need to be applied in order to avoid or eliminate further contamination. The cleaning and use of ice in Experiment 4 resulted in bacterial counts that were unexpectedly higher in storage holds (> 5 log_{10} CFU/cm²) post-cleaning and on snoek post-vessel

offload (> 2.40 $\log_{10}$ CFU/cm²), compared to bacterial counts observed from the holds not cleaned and only containing ice. However, the increase in bacterial counts on snoek post-ice treatment was smaller compared to snoek post-cleaning and no ice treatment. This may be due to the presence of psychrophilic bacteria (cold-loving) which can grow under low temperatures however, their growth rate is decreased with decreasing temperatures (Chytiri *et al*., 2004). Hence, ice can be used to minimise bacterial growth. Alternatively, it could be that the ice itself was contaminated – this is an aspect that warrants further research.

The results in this study correlate with those by Chytiri *et al.* (2004), reporting a bacterial count of 2.5 $\log_{10}$ CFU/cm² in farmed whole rainbow trout after 1 day of storage in ice. Concerning the bacterial count observed in storage holds after cleaning and sanitation, the contact time allowed for the detergent and the efficacy of the cleaning procedure to destroy biofilms may have been insufficient. Frank and Koffi (1990) indicated that bacteria in biofilms may express an increased resistance to disinfectants. Another study by Anwar *et al.* (1990) indicated that the resistance is more severe in older biofilms (24 hrs of accumulation of algae, diatoms, and bacteria build-up on a fishing vessel without any cleaning) than in younger ones. Similarly, the results obtained in Experiment 5 which correspond with those obtained by Olgunoglu (2010) show that *E. coli*, *S. aureus* and *S. enterica* were present after the application of SABS cleaning procedures. Either the efficacy of cleaning was not optimal or contamination may have occurred from an external source, including contamination during the preparation of growth media in the lab. The chemicals used may also not have been active against the microorganisms with the possibility of biofilms protecting the organisms. According to Olgunoglu (2010), foodborne pathogens in food ready for consumption can be destroyed through exposure to heat and proper cooking methods.

The results obtained post-vessel offload after roughly 10 hrs post-catch (Experiment 3), show that the bacterial count on snoek had doubled. Sufficient time was given for bacterial growth to take place under no ice storage. According to FAO (1995), bacteria will grow with a doubling time of approximately 1 day during ice storage. The results from snoek post-bakkie offload, during 21 hrs ice storage, show that bacterial counts only experienced a slight increase. This suggests that overnight storage in ice during a very cold winter night impedes or inactivates bacterial growth. Similarly to results obtained from the harvesting stage, contaminant sources identified include the bakkie (transport medium), tarpaulin and the knife used for filleting fish at the sale point. The hands of the food handler were once more identified as the primary source of contamination. The results obtained from contaminant sources post-vessel offload, are above 10^4 CFU/cm² and slightly higher than results obtained by Olgunoglu (2010) for food contact surfaces and processing equipment.

The overall temperature for snoek stored in ice maintained levels below 16°C; even reaching temperatures as low as -0.3°C when properly layered with ice. According to the Agreement on the International Carriage of Perishable Foodstuffs (ATP Agreement, 2013), it is specified that the maximum temperature for the transportation of fish is 2°C. Results from snoek stored in ice on a bakkie comply with the requirements of the ATP agreement. The reduced bacterial counts observed during ice storage suggest that maintaining low temperatures can improve the microbial quality of snoek over long periods of storage.

E. coli is an indicator of faecal contamination (human and animal) while *S. aureus* and *S. enterica* are indicators of poor sanitation (Samakupa *et al.*, 2003). *V. parahaemolyticus* is an indicator of faecal

contamination originating from marine mammals and plants (Baumann *et al*., 1984). All four indicators were detected during the harvesting stage on the hands of fishermen and from the bait (Table 3.14). The repeated presence of these organisms on fish post-vessel offload proves that contamination originated from the harvesting stage, caused by insufficient sanitation applications.

With reference to the issue of myoliquefaction in snoek, a study by Funk *et al.* (2008) reported that distinct cathepsin L proteases (protein-degrading enzyme) derived from the parasite *K. thyrsites*, are responsible for the myoliquefaction in infected fish. The maximum activity of this enzyme was observed at pH 5.5. Fish muscle pH drops from 7.0 to 6.5 during early post-mortem storage (Chéret *et al*., 2007; Françoise, 2010). The pH results observed from snoek stored in ice correlate with these findings suggesting that ice is not an optimal method for improving the occurrence of myoliquefaction in fish. However, it can be noted that the neck snapping method does reduce the amount of lactic acid generated in the fish muscle post-mortem. This was observed in a snoek with a 10-minute delay in neck snapping, displaying a pH value of 5.97. According to Samaranayaka *et al*. (2006), this reduction in lactic acid consequently results in the reduced activity of cathepsin L proteases released from the parasite.

Fish should either be prepared immediately or frozen. In addition, from the ambient temperature results provided in Experiment 4, it was observed that the use of ice in the storage holds provide a cool environment for the snoek suggesting that it can assist in the preservation of snoek for longer hours. Therefore, shading of the holds can be recommended protecting the fish from direct exposure to sunlight. Bacterial contamination originates primarily from the vessel holds, followed by the hessian cloth/ sack. This point in the chain can be identified as the critical control point where corrective action would need to take place in terms of sanitation and hygiene, and enforcing SOPs.

Some of the limitations to this study include the small sample size in each experiment, and the lack of replicated data that could be more representative of general hygiene. The maximum potential and execution of the study was limited by circumstances beyond control; having to rely on the availability and willingness of fishermen and langanas to accommodate this research. Despite these limitations, basic but valuable information was obtained about the general processes and procedures involved in the local trade of snoek in the Western Cape of South Africa. Further research is required to determine the level of food borne pathogens present at the end of the processing chain when snoek reaches the consumer in its whole and processed (salted, etc.) state.

Table 3.13 Summary of the mean bacterial counts (log_{10} CFU/cm^2) on various contaminant surfaces on a fishing boat, post-catch and post vessel offload to post-bakkie offload).

	Fish	Bait	Hold	Filleting board	Knife	Hessian sack	Hands	Bakkie	Tarpaulin	Average TVC at processing point
Harvest	3.38	4.71	-	-	-	-	4.59	-	-	4.23
Storage	-	-	5.55	-	-	5.28	-	-	-	5.42
Post-vessel offload	5.07	-	-	-	-	-	-	4.61	3.19	4.29
Sale point (Post-bakkie offload)	5.32	-	-	-	4.66	-	4.86	-	-	4.95
Average TVC for surface	4.59	4.71	5.55	-	4.66	5.28	4.73	4.61	3.19	

Table 3.14 Summary of pathogen prevalence (*Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus)* on snoek and contaminant surfaces at each processing point in the snoek supply chain. The prevalence of pathogens post-SABS cleaning procedures are also displayed.

Microorganism	**Harvest (Post-catch)**		**Storage (Holds post-treatment; prior to storing fish)**					**Bakkie offload**	**Sale point (Post-bakkie offload)**	**Fish (post-vessel offload) from different treatments in experiment 4**			
	Hands	*Bait*	*A (Control)*	*B (Cleaned)*	*C (Ice, not cleaned*	*D (Ice, cleaned)*	*SABS*	*Fish post-bakkie offload*		*A*	*B*	*C*	*D*
Escherichia coli	33.33%[1] (3/9)[2]	25.00% (1/4)	0.00% (0/1)	100.00% (1/1)	0.00% (0/1)	0.00% (0/1)	25.00% (4/16)	90.00% (9/10)	0.00% (0/5)	0.00% (0/5)	20.00% (1/5)	0.00% (0/5)	20.00% (1/5)
Salmonella enterica	22.22% (2/9)	25.00% (1/4)	0.00% (0/1)	0.00% (0/1)	0.00% (0/1)	0.00% (0/1)	31.25% (5/16)	22.22% (2/9)	0.00% (0/5)	0.00% (0/5)	20.00% (1/5)	0.00% (0/5)	20.00% (1/5)
Staphylococcus aureus	22.22% (2/9)	25.00% (1/4)	0.00% (0/1)	0.00% (0/1)	0.00% (0/1)	0.00% (0/1)	12.50% (2/16)	0.00% (0/9)	0.00% (0/5)	0.00% (0/5)	20.00% (1/5)	0.00% (0/5)	20.00% (1/5)
Vibrio para-haemolyticus	33.33% (3/9)	25.00% (1/4)	0.00% (0/1)	100.00% (1/1)	0.00% (0/1)	0.00% (0/1)	-	22.22% (2/9)	20.00% (1/5)	0.00% (0/5)	20.00% (1/5)	0.00% (0/5)	20.00% (1/5)

[1] Percentage of positive samples at each point
[2] Number of samples tested at each point that was positive

Conclusion

In conclusion, the microbial load does increase as the supply chain progresses (harvest, post-vessel offload and post-bakkie offload identified as critical points). Crushed ice can be recommended as a suitable method for maintaining the microbial quality of snoek. The results from contaminant sources, suggests there are currently no standard methods and management procedures in place to identify and regulate contamination throughout the process chain. Therefore, the implementation of standard operating procedures (SOPs) for good manufacturing practices (GMPs) is vital for the snoek supply chain. The SABS cleaning procedure used in experiment five is effective in terms of reducing and avoiding bacterial contamination during on-board storage and transport. It is advisable that the SABS procedure be followed through on a regular basis (weekly, or at least twice a month).

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Chapter 4

Theoretical development of an "ideal" HACCP plan for the local snoek supply chain

Summary

Prior to developing a HACCP plan, GMPs, and other standards and regulations must be in place. This follows standard sanitation operating procedures (SSOPs), and then HACCP. The snoek supply chain in the Western Cape harbours do not have ideal standard operating procedures (SOPs) in place, except for Kalk Bay. However, if SOPs were in place, or rather assuming they were in place, one can then develop a theoretical example that simulates what would be an ideal HACCP plan for this supply chain. Therefore, assuming the snoek supply chain has GMPs and SSOPs in place, and assuming the whole process (supply chain) takes place under one umbrella, the aim of this study was to develop an "ideal", theoretical example of a HACCP plan for the artisanal small-scale snoek supply chain in South Africa.

Introduction

The South African Bureau of Standards (SABS) was developed as a legal measure and requirement to ensure that locally manufactured or imported products into South Africa or exported from South Africa meet the minimum requirements for health and safety (Wilson *et al*., 2003). These standards currently function under the terms of the Standards Act (Act No. 29 of 1993) and have been accredited by the South African National Accreditation System (SANAS) (Wilson *et al*., 2003). SABS functions through a team of inspectors visiting importers, retailers and manufacturers to monitor their compliance with the compulsory specifications. The fisheries sector in Southern Africa is well regulated, mainly by SABS, however the Department of Agriculture, Forestry and Fisheries (DAFF) and the Department of Environmental Affairs and Tourism (DEAT), also contribute to these regulations (Wilson *et al*., 2003). SABS follows a "continuous inspection" system for high risk and imported fishery products whereby raw materials and products are subjected to physical and sensory examinations (Wilson *et al*., 2003). Compliance with SABS specifications are enforced through surveillance and conformity assessment (Wilson *et al*., 2003). According to van de Venter (2003), SABS, now referred to as the National Regulator for Compulsory Specifications (NRCS), is recognized by the European Commission as the competent authority in South Africa responsible for the verification and certification of fishery products with requirements of Directive 91/493/EEC.

SABS exercises control over three categories:

1. -60°C freezer vessels (evisceration may be executed),
2. Factory freezers (undertake complete processing e.g. processing and packaging fillets),
3. Ice boats transporting chilled fish to the factory.

The EU does, however, not allow imports of South African aquaculture and mariculture products such as bivalve or marine molluscs due to mercury content issues during the early 1990s (Wilson *et al*., 2003). Aquaculture which contributes to fish produce and is still a growing industry requires a sophisticated monitoring infrastructure making South Africa in this regard unable to meet international requirements (Wilson *et al*., 2003). In a case relating to these requirements, it was reported that Spain had rejected South African white fish exports in 2001 due to a parasite infestation problem (Wilson *et al*., 2003). Issues lie with the processing methods which need to be critically analysed in order to avoid hazards (foodborne pathogens) from entering food products.

In an effort to prevent hazards in processing plants, the Hazard Analysis Critical Control Points (HACCP) plan was developed and has been applied to artisan fisheries in Africa (Mlolwa, 2000). The main advantage of implementing a HACCP plan is that it can direct the energy and resources in areas where food is at risk of contamination (Pearson & Dutson, 1995). This plan is accomplished by identifying the potential hazards in each step of the process chain: harvesting, processing, storing and distribution to the consumer (Scott, 1993). The HACCP system has been implemented by the food industry (especially the seafood industry) in South Africa for two main reasons:

1. To meet requirements for the export of food,
2. It is mandatory for foods destined for local consumption.

With regards to food produced for domestic consumption (e.g. snoek), implementing HACCP with a “boat to throat” approach through the food chain is challenging due to the diverse food regulatory authorities situated within several government departments and statutory bodies within the three tiers of government – national, provincial and local (Van de Venter, 2003). This has led to the circulation of numerous sets of legislation, resulting in fragmentation, overlapping and uncertainty in some areas (Van de Venter, 2003). Suggestions have been made for the introduction of a new, rationalised, national food control system which can potentially rectify these problems.

With reference to a typical food processing factory, it is essential that the managers of such an industry seek to initially establish, assemble and train a HACCP team consisting of members from each department in the processing chain and a knowledgeable food technology and safety consultant (Tompkin, 1990). This will enable a sense of ownership of the plan by workers and can provide success in the application of this programme once the whole workforce and the industry itself acknowledges the importance of the HACCP plan in a production system (Mlolwa, 2000).

Describing the product is the first task for the team and this includes the environment and temperature of the product (Corlett, 1998). The intended use of the product will be subsequently established e.g. who the consumer is and whether any special allergenic or religious condition of the food needs to be specified (Corlett, 1998). The steps both prior to and after processing will be outlined through a typical clear flow diagram (Figure 2.6; Chapter 2). Subsequently, the accuracy of the operation should be confirmed on site and once the team is satisfied with these first steps, the following seven HACCP principles can be applied (Corlett, 1998):

1. **List of the potential hazards**

A hazard can be defined as a biological, chemical or physical agent in, or a condition of food with the potential of causing adverse health effects (HACCP, 2005). The three categories of hazards include:

1. Biological

- Microbiological
  - Bacterial e.g. *E. coli*
  - Viral e.g. Hepatitis A
  - Fungal e.g. moulds
- Parasites

2. Chemical

- Naturally occurring toxins e.g. ciguatera
- Heavy metal e.g. cadmium
- Cleaning/ maintenance chemicals
- Food additives e.g. SO_2 from Sodium metabisulphite
- Agricultural and veterinary chemicals e.g antibiotics

3. Physical

- Foreign objects such as hooks, shell fragments, seaweed etc.
- Splinters, paint flakes, fibreglass fragments, rust

2. **Determine Critical Control Points (CCPs)**

A Critical Control Point (CCP) is the step in which if no control measure is applied, the food is likely to be unsafe for consumption (HACCP, 2005).

3. **Establish critical limits for each CCP**

The critical limit is the limit to which the hazard must be controlled in order to prevent, control, eliminate or reduce the occurrence of the hazard to an acceptable level (e.g. chilled fish or fish product must be stored between -1°C and 5°C (HACCP, 2005).

4. **Establish a monitoring system for each CCP**

The monitoring system includes a planned sequence of observations or measurements to assess whether the CCP is under control (e.g. operator monitoring temperature or pH to measure acidity or alkalinity of a product (HACCP, 2005).

5. **Establish corrective actions**

Corrective actions ensure the safety and suitability of a fish or fish product by re-visiting an operation that may have gone outside of critical limits. This involves identifying and fixing the problem, deciding what to do with the non-conforming product and investigating measures to avoid the problem from re-occurring (HACCP, 2005).

6. **Establish verification procedures**

   Verification involves applying methods, procedures, tests, and other evaluations including monitoring which will determine whether a requirement is complied with (HACCP, 2005).

7. **Establish documentation and record keeping**

   Accurate record keeping is an essential aspect of a HACCP programme (Mlolwa, 2000). This step is carried out for verification and auditing purposes and also to determine whether the operating system conforms to the initial plan (HACCP, 2005).

Development of a theoretical HACCP plan with reference to the snoek supply chain in the Western Cape, South Africa (Chapter 3)

There is a lack of regulations for the processing and supply chain of locally caught snoek in South Africa. Figure 4.1 presents a typical supply chain of freshly caught snoek, including contaminant sources identified as capable of introducing potential hazards within the chain. The HACCP plan can be applied at various stages as outlined in the HACCP hazard analysis [table worksheets (Tables 4.1 and 4.2) with reference to the process flow in Figure 4.1]. The critical control points displayed in Table 4.3, may not be ideal logistically in a snoek supply chain, however, these points can be applied through regular inspections made by health inspectors.

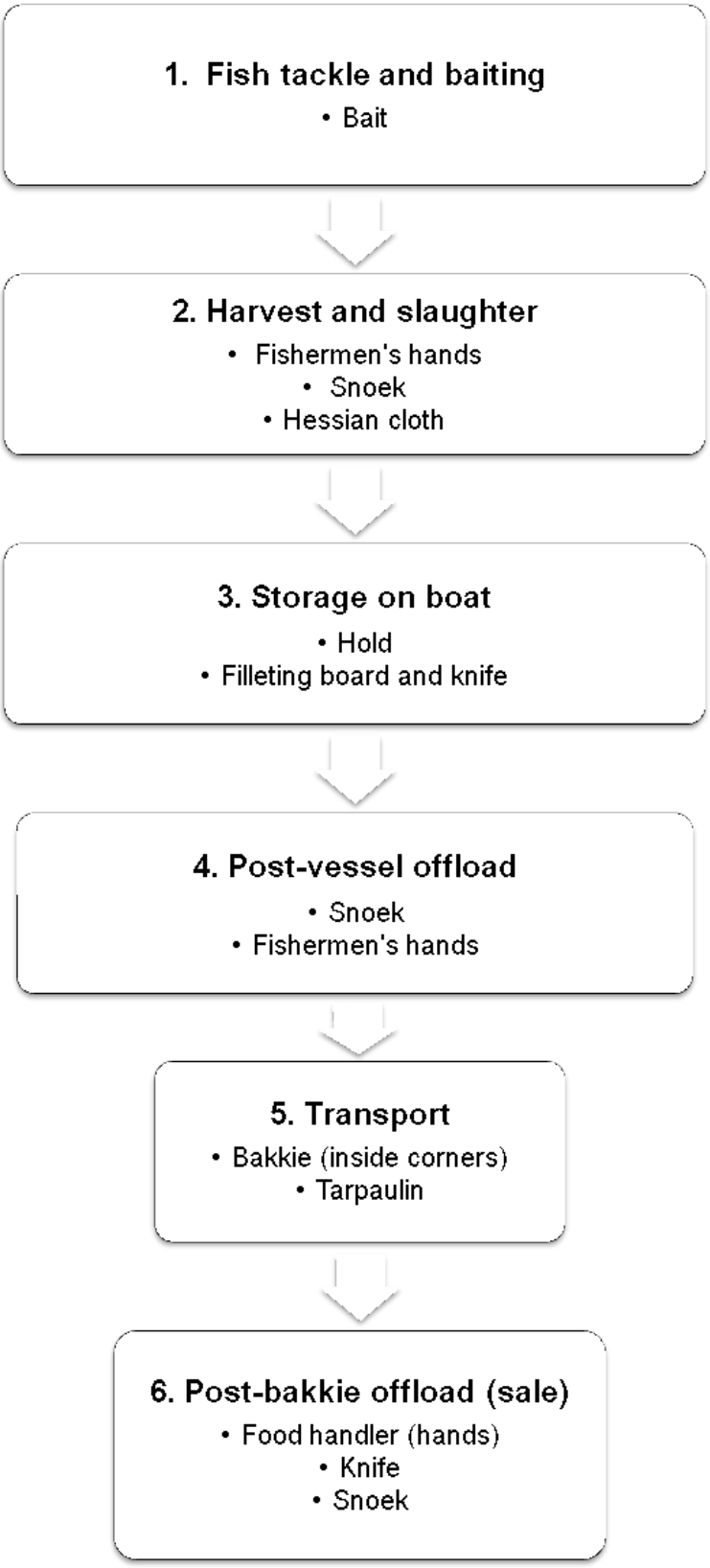

Figure 4.1 Summary of the process flow of snoek from catch to consumer (● represent contaminant sources swabbed during the experiments).

***Lady M* fishing vessel**

Fresh snoek

Table 4.1 HACCP product description of freshly caught snoek (*T. atun*).

Product description	
Product raw material	Snoek (*Thyrsites atun)*
Source of raw materials	Atlantic ocean- Western Cape
Ingredients added(including food additives)	None
Processing/preservation method	None
Final product	Fresh
Intended use	Domestic consumption
Packaging	None
Transport	Transportation by road on an open bakkie lined with a tarpaulin
Storage	None
Finished products requirement South African/ European Union	Initial product for further processing depending on customer or vendor, etc. E.g for smoked snoek, follow National Regulator for Compulsory Specifications (NRCS). VC 8021. STANDARDS ACT, *1962.* (Act No. 33 of *1962).*

Step	Process
1 Fish tackle and baiting	Saury (*Scomberesox* saurus) or sardine (*Sardinops sagax*) used as bait (cut in half).
↓	
2 Harvest and slaughter	Fish caught by hand line. Fish is killed by grasping head under the arm and snapping the neck; main arteries were severed that bled out.
↓	
3 Storage on boat	Fish placed into fishing vessel hold where stored until offloading.
↓	
4 Post-vessel offload	Fish are offloaded onto the back of a plastic lined bakkie; or (filleted &whole) sold as offloading takes place.
↓	
5 Transport:	Fish transported to local wholesalers, long term storage facility (sale off season) or sales point along the road.
↓	
6 Post-bakkie offload (sale):	Fish normally sold along the road from the back of a bakkie.

Figure 4.2 Process flowchart.

Hazard Analysis Worksheet: Fresh snoek

Table 4.2 Hazard Analysis Worksheet for fresh snoek.

1. Processing step	**2. Potential hazard**	**3. Is the potential hazard significant? (Yes/No)**	**4. Justification for inclusion/ exclusion as a significant hazard**	**5. What can be done to prevent hazard in column 2 from occurring?**	**6. Critical Control Point for food safety? Yes/No**
1. Fish tackle and baiting	Biological *E. coli* *S. aureus* *S. enterica* *V. parahaemolyticus.* Chemical Sanitizers & detergents	 No No No No No	 Product is not ready to eat. Product is intended to be fully cooked prior to consumption. Pathogens can be avoided or reduced with GMPs/SSOPs in place. Residuals unlikely to occur due to use of GRAS approved chemical.		
2. Harvest and slaughter	*E. coli* *S. aureus* *S. enterica* *V. parahaemolyticus* Histamine/ scombrotoxin	No No No No No	Pathogens can be destroyed through cooking. Potential for temperature abuse at this step not reasonably likely to occur due to brief duration.		
3. Storage on boat	*E. coli* *V. parahaemolyticus* Histamine/ scombrotoxin	No No Yes	Pathogen will be destroyed through cooking. Scombrotoxin producing bacteria grow in absence of effective refrigeration. High temperatures likely during storage.	Temperature should be kept low with ice.	Yes
4. Post-vessel offload	*E. coli* *S. aureus* *S. enterica* *V. parahaemolyticus* Histamine/ scombrotoxin	No No No No No	Potential for bacterial growth is unlikely to occur due to brief duration of this step. Period of time at this stage is short. if product remains under low temperature, bacterial growth not		

			reasonably likely to occur.		
5. Transport	*E. coli* Histamine/ scombrotoxin	No Yes	Pathogen will be destroyed through cooking. Exposure to high temperatures likely during this stage.	Temperatures should be kept low with ice.	Yes
6. Post-bakkie offload (sale)	*E. coli* *S. aureus* *S. enterica* Histamine/ scombrotoxin	Yes Yes Yes Yes	Food pathogens can heavily contaminate fish due to high moisture levels if not thoroughly dried. Exposure to high temperatures (sun) likely during this stage.	Maintaining sanitation at landing sites adequately prior to consumption can prohibit the growth of these pathogens. Fish should be properly cooked prior to consumption. Temperatures should be kept low with ice or frozen as soon as possible.	Yes

Critical Control Points (CCPs)

Table 4.3 HACCP plan form with exemplary realistic critical control points from the processing points in Figure 4.1.

Critical Control Point (CCP)	Hazard(s) to be Addressed in HACCP Plan	Critical Limits for Each Control Measure	Monitoring				Corrective Action	Verification Activities	Record-keeping Procedures
			What	How	Frequency	Who			
3. Storage	Histamine/ scombrotoxin	Product adequately iced.	Presence of ice.	Visual.	Every hour.	Assigned fisherman.	Add ice; check product internal temperature if possible. If internal temperature exceeds 7.2°C, or above 4.4ºC > 4 hours, reject or conduct histamine test.	Quarterly tests can be run for verification of histamine test method.	Cooler log.
5. Transport	Histamine/ scombrotoxin	Internal fish temperature must not be higher than 10°C and gut cavity properly iced. Harvest record must show fish cooled to 10°C within six hours of death.	Internal fish temperature. Time-temperature record of harvest. Harvest vessel records.	Thermometer or visual check (icing).	Every hour.	Assigned fisherman.	Reject or test for histamine; reject if exceeds 50 PPM.	Quarterly tests can be run for verification of histamine test method.	Temperature log. Receiver's log.

6. Sale (Post-bakkie offload)	*E. coli* *S. aureus* *S. enterica*	Fish should not be exposed to ambient temperatures above 4.4°C for more than 8 hours, cumulatively, as long as no portion of that time is at temperatures above 21°C.	Length of time fish are exposed to unrefrigerated conditions (i.e., above 4.4°C, and the ambient temperatures during the exposure periods.	Visual observations of length of exposure to unrefrigerated conditions.	At least every two hours.	Trained quality controller.	For fresh fish, [(AOAC 977.13 (1990)] reject if: *E. coli* (Type 1) - is > 10 per 100 grams, *S. enterica* – is present in 20 grams, *V. parahaemolyticus* – is present in 20 grams.	Periodically measure internal temperatures of fish (if possible) to ensure that the ice or cooling media is sufficient to maintain product temperatures at 4.4°C, or less.	Time and temperature logs.
	Histamine/ scombrotoxin						Reject or test for histamine; reject if exceeds 50 PPM.		

Discussion

The current study identified potential contamination sources within the traditional snoek processing chain and determined possible ways of mitigating contamination through a number of feasible procedures. The snoek fishery, presently described as informal, has no proper cold chain maintenance which has an impact on sanitation and product quality as observed in Ocean View (Western Cape) by Isaacs (2003). The HACCP plan discussed in this chapter provides a formal outline of procedures recommendable to local fishermen in the commercial long line fisheries. A study by Quang (2005) investigated the procedures used in Vietnam from catching fish at sea, to transporting it to a processing plant. The activities present themselves similar to those of the snoek supply chain. Standard operating procedures (SOPs) were implemented according to standards required by Iceland (importing country). Similarly, the critical points outlined in the HACCP plan aim to highlight the need for quality control in the supply chain, and make recommendations based on SABS regulations.

Overall, the main problem observed was contamination originating from the preparation phase. The presence of *E. coli* with each of the critical control points identified indicates that faecal contamination is a potential problem and handling methods need to be rectified. Several hours spent at sea with no toilet and hand washing facilities result in faecal contamination (*E. coli* and *S. aureus*). Encouraging a hand washing protocol on board with mild, environmental soap and seawater, can help reduce or eliminate contamination from hands. The presence of *S. enterica* indicates that working surfaces both on-board the vessels through to transporting snoek on a bakkie are not regularly cleaned. The equipment used on-board (knives, cutting boards, hessian cloth) easily harbour bacteria that multiply and develop antiviral defence mechanisms over time. When regular cleaning and sanitizing of equipment is not maintained, bacteria are not easily destroyed. With regards to the boots fishermen wear, faecal matter can accumulate between the grooves of the boot bottom and easily contaminate fish whilst standing amongst them in the storage holds. A cleaning and scrubbing protocol of equipment and boots is encouraged both before and after each fishing trip.

With regards to chilling snoek, temperature needs to be maintained around - 2ºC to 4°C throughout the supply chain (ATP, 2011). The use of ice on board was observed to be a challenge. Space is limited on board fishing vessels as fishermen optimise on man power for a larger catch.

Employing standard protocols for hygiene and sanitation are essential for food and personal health. A clean environment is comfortable and pleasant to work in, and attractive. South African standards (SABS) are available for cleaning procedures including guidelines for the microbiological safety of fish products. The SABS procedure showed to be effective from the results obtained in Chapter 3 (Experiment 5). However, the lack of enforcing this as a standard operating procedure remains questionable. Possible questions relating to this observation may include the following:

1. Are the fishermen too tired at the end of a fishing trip?
2. Is it too costly to look into the use of cleaning detergents and sanitizers?

3. Is there any payment involved for following through with these standards and carrying out tests to see if they comply with the standards?

It can be proposed that the buyers (local retailers, etc.) invest in this supply chain by having trained inspectors follow through with the required standards for such an establishment. Other future improvements in this fishery include the use of mechanised handling (e.g. trolleys) for saving exposure time of fish and human labour at landing. Insulated containers need to replace the wooden deck surface contacting fish at landing in order to keep hygiene and sanitation.

Conclusion

Although the South African traditional, boat-based line-fishery is stable in terms of fish resource availability, the competitiveness of the industry may be restricted by the lack to comply with prerequisite programmes, infrastructure, supporting industries and trained human resources, compared to a complying New Zealand barracouta fishery. Snoek meat quality may be inconsistent, mostly due to the uncontrollable effects of parasite infestation; however microbial quality can be controlled. Handling and hygiene seem to be an underestimated problem in this supply chain and awareness of its importance should be prioritised. GMPs and SSOPs must take priority in this supply chain; example training of handlers in Kalk Bay (CPUT/ DAFF, 2013).

The basics of food safety and hygiene may improve the snoek industry on a competitive basis. Government needs to assist in this area such that the industry complies with export and food regulatory pre-requisite programmes.

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