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Systematic review of *Halioticida noduliformans* infection in South African abalone (*Haliotis midae*)

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Supervisor: Prof. PN Thompson

Co-supervisor: Dr JD Grewar

Declaration

I declare that this is my own work, that **Systematic review of *Halioticida noduliformans* infection in South African abalone (*Haliotis midae*)**, which I hereby submit for the degree MSc (Veterinary Epidemiology) at the University of Pretoria, has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name: Shuichi Tabei___________ Date: 08/10/2022 ____________

Signed__________________________________

List of abbreviations

AbHV	Abalone herpesvirus
AVG	Abalone viral ganglioneuritis
ATM	Abalone Tubercle Mycosis
DAFF	Department of Agriculture, Forestry and Fisheries
DFFE	Department of Forestry, Fisheries and the Environment
DOAR	Directory of Open Access Repositories
DOI	Digital Object Identifier
FAO	Food and Agriculture Organization
GMS	Grocott methenamine silver
H&E	Hematoxylin & eosin
OIE	Office International des Epizooties
PCR	Polymerase chain reaction
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
REC	Research Ethics Committee
USD	United States dollar
UNEP-WCMC	United Nations Environment Programme World Conservation Monitoring Centre
WOAH	World Organisation for Animal Health

Summary

Systematic review of *Halioticida noduliformans* infection in South African abalone (*Haliotis midae*)

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Abstract

The South African abalone (*Haliotis midae*) industry is a growing and significant market in terms of export potential as well as job creation. The present abalone industry in South Africa is made up of a single species, *Haliotis midae. Halioticida noduliformans* infection has been associated with elevated mortalities in South African abalone, marked by necrosis of the epithelium and superficial muscle tissues. The disease condition itself has been termed abalone tubercle mycosis (ATM). There is a perception that the disease can have a substantial impact on producers, but there appears to be little published information on this. There is also a lack of cohesive information regarding its control. Through the use of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines, this systematic review aimed: 1) to provide a clear and comprehensive overview of available information on *Halioticida noduliformans* infection in South Africa, from sources including scientific and grey literature, technical reports and legislation; 2) if possible, to produce summary estimates of relevant epidemiological parameters; and 3) to identify deficiencies in research which will guide future investigations by formulating relevant research questions. Relevant legislation and surveillance policy and practices were also summarised. The amount of primary literature was found to be very limited with the same sources being cited repeatedly. Where quantitative data was found, it was traced to a single source. Although the available information regarding this organism covers pathology and diagnostic test ability, there are no reports of prevalence, risk factors, assessment of treatments or intervention, and numerous areas for further research were identified.

Keywords: Abalone, Disease, Tubercle mycosis, Peronosporomycete, *Halioticida noduliformans*

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Chapter 1 Introduction

1.1 Abalone farming in South Africa

The perlemoen / abalone or South African abalone (*Haliotis midae*) industry is a growing and significant market in terms of export potential as well as job creation. By 2006, South Africa was already the largest producer outside of Asia (Troell et al., 2006). It is a relatively young industry with the first farms starting production in the 1990's. The export market is made up of land-based culture facilities, with wild abalone fisheries or ranching facilities being scarcer (Troell et al., 2006). It has the potential to alleviate poverty and improve the livelihoods of marginalised communities by promoting socio-economic growth (Christison, 2019).

In 2020, the global fisheries and aquaculture industry recorded 178 million tonnes of production. The international trade of these products totalled 151 billion USD (FAO, 2022). The global abalone industry accounts for less than 200 000 tonnes of production, with China making up almost 90% of that figure. Although the production of abalone make up a small proportion of the aquaculture industry, it is a sought after product of high value (Cook, 2016). The following species of cultured abalone can be found worldwide: *Haliotis laevigata* (Australia), *H. ruber* (Australia), *H. rufescens* (Chile and USA), *H. discus hannai* (Chile, China and South Korea), *H. midae* (South Africa) (Hoshino, Gardner, Jennings, & Hartmann, 2015).

The present abalone industry in South Africa is made up of a single species, *Haliotis midae* (Troell et al., 2006). This is the only species of abalone that is registered, produced, and exported in South Africa. *Haliotis midae* is endemic to South Africa, with most wild populations reported to occur in the south of the Western Cape between Cape Columbine and Cape Agulhas (UNEP-WCMC, 2010). The wild population is shown to be in decline due to illegal harvesting, which is believed to be one of the main reasons why a fishery collapses when poaching is present (Cook & Gordon, 2010). The total allowable catch in commercial zones for *H. midae* was significantly reduced between 1996 and 2008 but it had no impact on the growing trade of the illegal harvest (UNEP-WCMC, 2010).

Farming operations include hatchery (Figure 1) and grow-on facilities where animals are grown to market size (Sales & Britz, 2001). This size varies based on the demand, but it generally takes four years to produce abalone of market size (Troell et al., 2006). The majority of the farms with cultured abalone can be found in the Western Cape, west of Cape Agulhas, the southernmost tip of Africa, extending along the West Coast all the way up to Port Nolloth (Figure 2). There is currently one farm located in the Eastern Cape province. There are currently fourteen sites registered with the Department of Forestry, Fisheries and the Environment. A recent survey conducted by an industry service provider, which included seven sites, revealed that a site can have, on average, 200 tonnes of stock at any given time (Amanzi Biosecurity, unpublished data, 2021), with around 150 tonnes removed annually for export purposes. This industry accounts for almost 30% of the total aquaculture production in South Africa.

Figure 1 Abalone spat in the hatchery https://www.globalseafood.org/wp-content/uploads/2018/10/Abagold_ABALONE_Spat.jpg

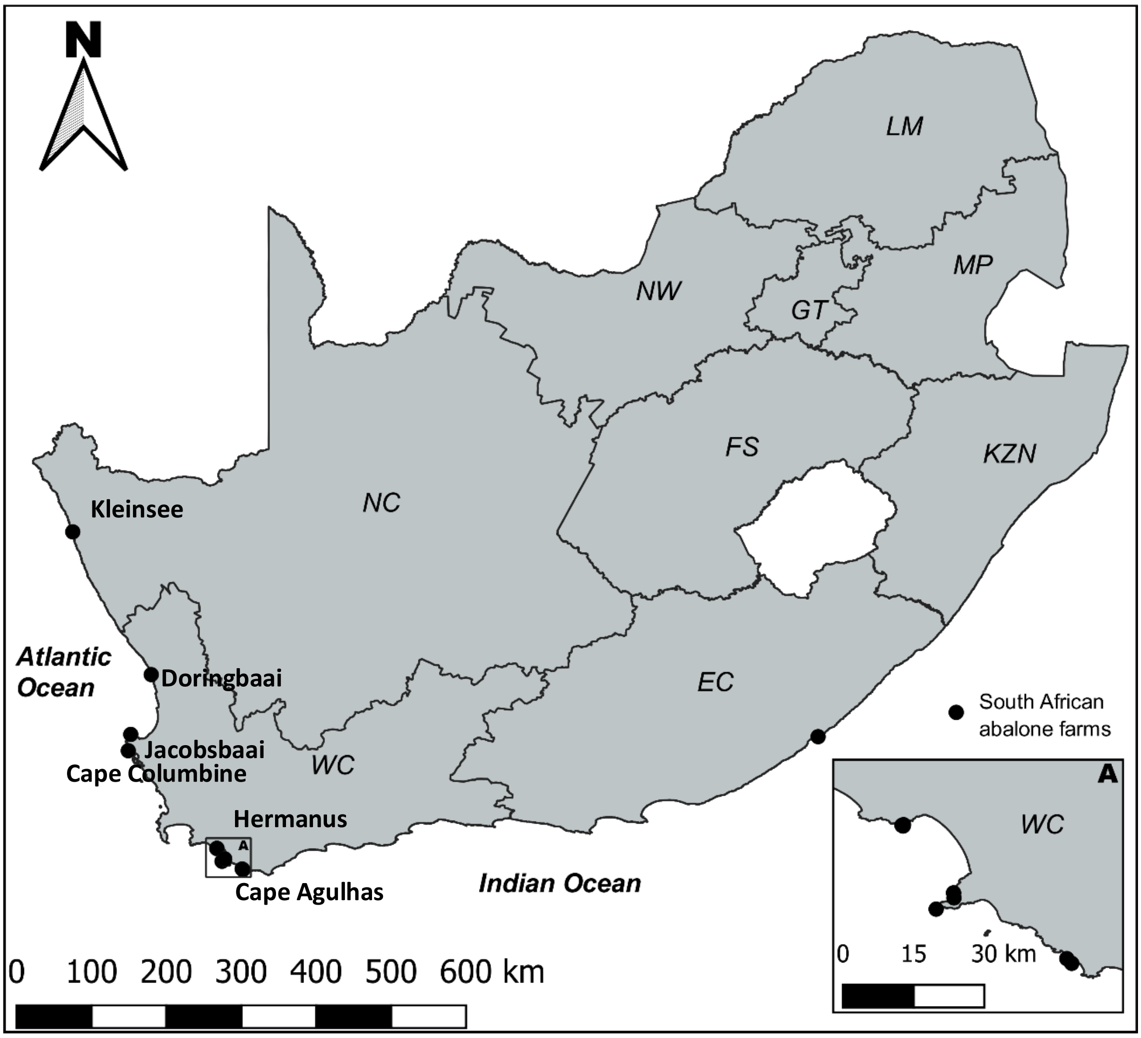

Figure 2. Map of abalone farms in South Africa

1.2 Production systems

There are two main production systems that are utilised in the South African abalone industry: the flow-through and recirculation systems. Even though both flow-through and recirculation systems employ different approaches, they remain intensive farming systems with high stocking rates. The impact of stocking rates on the health status of the animals must be considered (Mouton, 2010). An epidemiological unit with a high population density will negatively impact the water quality which in turn has a negative impact on the growth and performance of the stock, while also making the stock susceptible to disease (Mouton, 2010).

Flow-through system

The flow-through system (Figure 3) is used by pumping seawater into a main holding tank. Thereafter, the water is distributed to the abalone holding tanks. This requires large amounts of water to be pumped to constantly provide good quality water to the system. As a result of this, it can result in high costs (Vivanco-Aranda, Gallardo-Escarate, & Rio-Portilla, 2011). Each tank contains baskets which are lined with rows of plastic substrate for the abalone to adhere to. This increases the surface area for each basket and allows for a better distribution of stock. The aeration of each tank is done by air blowers which maintain the constant circulation of the water. The water exiting the tanks is joined to a common outlet pipe which returns to the ocean.

Recirculation system

The recirculation system employs a closed system where water is treated and reused. There is a low replacement of water compared to the flow-through system (Vivanco-Aranda et al., 2011). In such cases, a large emphasis is placed on water quality monitoring. This system has the benefit of being able to increase water temperature as well as limiting the introduction of seawater containing toxic algae in the case of harmful algal blooms (Pitcher et al., 2019). The disadvantage would be the accumulation of particulate matter and bacteria if the system is not maintained properly (Troell et al., 2006). Similar to a flow-through system, water will enter and exit a tank. It is necessary to treat the water as the water exiting a tank can contain elevated suspended solids, ammonia and carbon dioxide. This is done by mechanical and biological filtration (Mouton, 2010). Mechanical filters can remove larger particles while biological filtration relies on bacteria for the conversion of toxic ammonia to nitrates for example. The oxygen levels of the water will also have to be restored before it can be used again as factors such as high stocking and elevated ammonia levels can deplete the dissolved oxygen within the water.

Figure 3 Typical flow-through system https://cisp.cachefly.net/assets/articles/images/resized/0000524549_resized_aquacultureherm anus1022.jpg

1.3 Diseases in abalone

Apart from *Halioticida noduliformans* infection, diseases of importance that have been reported in abalone culture facilities are abalone herpesvirus (AbHV) infection; withering syndrome caused by *Xenohaliotis californiensis*; *Haliphthorous milfordensis*; and *Perkinsus* spp. infection (S. M. M. Bower, S.M., 2018; OIE, 2019). Abalone herpesvirus (AbHV) infection, withering syndrome and *Perkinsus* spp. infection are of export relevance but have never been reported or diagnosed in *Haliotis midae* in South Africa (Christison, 2019), and the perlemoen abalone (*Haliotis midae*) has not been listed as a susceptible host species for any of these diseases (OIE, 2021).

1.3.1 Abalone Herpesvirus

Haliotid herpesvirus-1 (HaHV-1) is the aetiological agent of abalone viral ganglioneuritis (AVG), a contagious disease of abalone species (Hooper, Hardy-Smith, & Handlinger, 2007). The species that are known to be susceptible are the greenlip abalone (*Haliotis laevigata*) and the blacklip abalone (*Haliotis rubra*) as well as hybrids of these two species (Chang et al., 2005; Corbeil, 2020).

Reported outbreaks of AVG in Australia were found to affect all age classes in both farmed and wild abalone. The mortality rates were as high as 90% in greenlip and blacklip abalone (Hooper et al., 2007). In Chinese Taipei, AVG was reported in *H. diversicolor supertexta* (variously coloured abalone) with mortalities exceeding 70%. Japanese black abalone exposed to infected variously coloured abalone remained unaffected (Chang et al., 2005).

The mortalities have been reported to occur three days after the onset of clinical signs. The clinical signs associated with AVG include: swelling and protrusion of the mouth parts; an unsymmetrical peripheral elevation of the foot muscle, not adhering to the substrate within a housing unit; the eversion of the radula; decreased motion in the pedal muscle; increased mucus production; and the absence of a foot extension when trying to correct it position when turned upside down (Chang et al., 2005). The main lesion found using histopathology is in animals affected with AVG is ganglioneuritis: inflammation confined to neural tissue. The transmission is horizontal via the faeco-oral route though no direct contact is necessary (Chang et al., 2005). Transmission within and between facilities is through seawater containing the infectious material.".

1.3.2 Withering syndrome

Withering syndrome is a disease caused by an intracellular prokaryote, *Xenohaliotis californiensis*, presenting the morphological characteristics of the class Proteobacteria, order Rickettsiales and family Rickettsiaceae (Friedman et al., 2000). The species that are known to be susceptible are black (*Haliotis cracherodii*), red (*Haliotis rufescens*), white (*Haliotis sorenseni*), pink (*Haliotis corrugata*), green (*Haliotis fulgens*), and European (*Haliotis tuberculata*) abalone (Crosson et al., 2014).

Xenohaliotis californiensis has been reported in Baja California, Mexico and in California, USA but the geographical distribution may be broader because infected abalone were transported during the period of the outbreak (Wetchateng et al., 2010). The red abalone found in California have been transported to Chile, China, Iceland, Japan, Spain and Thailand (Wetchateng et al., 2010).

It is primarily a wasting disease in abalone and is associated with mass mortalities. The incubation period is 3 months but can extend to 7 months (Friedman et al., 2000). It infects the gastrointestinal epithelial cells of the posterior oesophagus, digestive gland and, to a lesser extent, the intestine. The bacteria can be transmitted via the water column and no direct contact is required between infected and uninfected abalone. The disease development is accelerated by elevated temperatures (Wetchateng et al., 2010). At 18-20°C, death usually occurs within one month of the appearance of the clinical signs. The transmission occurs between abalone exposed to the same seawater which contains the infectious material (OIE, 2019).

1.3.3 *Perkinsus olseni*

Perkinsus olseni is a protistan parasite with a phylogenetic positioning between Apicomplexa and dinoflagellates. The geographical distribution is widespread and has been reported in Australia, Korea, New Zealand, Japan, China, Portugal, Spain, Italy and Uruguay (OIE, 2019).

Heavy infections appear to cause retarded growth and delayed gamete maturation resulting in altered population dynamics and stability. In spite of a high prevalence, mortalities were not recorded in Australia (Lester, 2005). Transmission of this parasite occurs directly between individual molluscs (Lester, 2005). *Perkinsus olseni* produces pustules (abscesses containing a caseous creamy-brown fluid) in the foot and mantle of abalone (S. M. Bower, 2003).

1.3.4 *Haliphthorous milfordensis*

There is another oomycete disease affecting abalone that has not been recorded in South Africa. It is not a listed disease by the WOAH. The *Haliphthorous milfordensis* infection limited to culture facilities in Japan and New Zealand (Hatai, 2012). This disease presents with similar tubercle-like swellings on the mantle and foot of culture abalone. More uncommonly, shell mycosis can also be present, affecting the inner shell surface and extending to the foot-muscle attachment site. The host species include *Haliotis sieboldii*, *Haliotis iris*, *Haliotis australis* and *Haliotis virginea virginea* (S. M. Bower, 2003). Though it has not been isloted from Halio

There are long-term implications due to the chronic, progressive nature of the mycosis. Oomycete hyphae can be observed histologically and can be isolated via various culture techniques (Macey, Christison, & Mouton, 2011). Polymerase chain reaction can be used to distinguish between *Haliphthorous milfordensis and Halioticida noduliformans* infection (Macey et al., 2011)

1.4 *Halioticida noduliformans* infection in *Haliotis midae*

Halioticida noduliformans infection has been associated with elevated mortalities marked by necrosis of the epithelium and superficial muscle tissues. As a result, the affected areas of necrosis are visible on the surface of the abalone (Figure 4). The disease condition itself has been termed abalone tubercle mycosis (ATM) (Macey et al., 2011). ATM is not considered a common disease. It is neither listed by WOAH nor is it a notifiable disease in South Africa (Department of Environment Forestry and Fisheries (South Africa), 2019; OIE, 2019). Following the initial outbreaks in South African abalone farms in 2006 (Macey et al., 2011), there have not been any more published reports of ATM outbreaks.

Figure 4 Abalone Tubercle Mycosis: well circumscribed areas of muscle necrosis affecting the foot, epipodium and mantle (picture supplied by Amanzi Biosecurity)

1.4.1 Disease agent characteristics

The causative agent was found to be an peronosporomycete during outbreaks in recirculation systems in Japan (Muraosa, Morimoto, Hatai, Sano, & Nishimura, 2009). Similarly to algae and diatoms, peronosporomycetes belong to the phylum *Heterokontophyta* (Muraosa et al., 2009).

This is an obligate marine fungus – it requires seawater to grow, and it cannot propagate in estuarine or fresh water (Atami, Muraosa, & Hatai, 2009). Under laboratory conditions, growth was found to occur between 10 and 25°C and no growth occurred in the absence of salt (Macey et al., 2011). The survival time in the environment is unknown (Greeff, 2012).

There was speculation that *Halioticida noduliformans* is ubiquitous to the environment, but further research is required (Macey et al., 2011). Therefore, the organism may be present everywhere in the environment which may result in increased exposure of the organism to abalone. Following the outbreaks in South African abalone facilities, *Halioticida noduliformans* was isolated and was found to produce zoospores which indicates that it has the ability to spread between animals in close proximity in seawater (Macey et al., 2011). The transmission is through direct contact, abalone within the same housing unit may become infected if a positive case is present (Muraosa et al., 2009).

Infection with *Halioticida noduliformans* has also been confirmed in the gills of wild mantis shrimp (*Oratosquilla oratoria*) in Japan (Atami et al., 2009). It has also been confirmed that there was an occurrence of infection in the gills and eggs of European lobsters (*Homarus gammarus*), resulting in pathology of the eggs, likely reducing fecundity (Holt et al., 2018). The effect of this infection on the reproductive ability of abalone was not investigated. Transmission between various species of *Haliotis* was confirmed (Muraosa et al., 2009). ATM has been recorded in the following abalone: *H. midae*, *H. rufescens*, *H. sieboldii* and *H. rubra* (Macey et al., 2011).

1.4.2 Incubation period

Production losses of up to 90% in spat and 30% in older animals were recorded in South African culture facilities in 2006 (Macey et al., 2011). ATM was found to have an incubation period of three to six days in *Haliotis midae* (Macey et al., 2011). Its relevance lies in the fact that direct financial losses have been attributed to disease outbreaks. Veterinary and mitigation strategy costs as well as production losses are some of the major factors implicated.

1.4.3 Pathology and progression

Macroscopically, infected abalone were found to have well circumscribed areas of muscle necrosis affecting the foot, epipodium and mantle. The affected tissue extends through the epithelium, connective tissue and muscle fibres (Macey et al., 2011). The presence of multifocal white nodules on the mantle has also been identified in infected abalone (Muraosa et al., 2009).

Histologically, it is characterised by highly branched hyphae that penetrate the superficial muscles of the foot, epipodium and mantle with the absence of an inflammatory response. These findings are visualised with the use of a Grocott methenamine silver (GMS) stain as opposed to the conventional haematoxylin and eosin (H&E) stain widely used for diagnostic histopathology. (Macey et al., 2011).

No information regarding the pathogenesis of ATM could be found which affects the understanding of its progression.

1.4.4 Diagnostic methods

Real-time quantitative polymerase chain reaction (PCR) is used to confirm a diagnosis of ATM (Greeff, Christison, & Macey, 2012). The benefits of this diagnostic method are a faster turnover time compared to histopathology as well as increased sensitivity (Greeff et al., 2012). Previously, macroscopic lesions and histopathology were used to diagnose ATM. PCR has to be used to confirm ATM as gross lesions and histopathology findings are not pathognomonic. This can be confused with lesions found on abalone with *Haliphthorous milfordensis* infection. The mantle, epipodium or foot tissue containing the area of necrosis or white nodule are usually sampled for PCR which includes the epithelium, underlying muscle fibres and connective tissue. (Macey et al., 2011). The possibility of *Halioticida noduliformans* being ubiquitous to the environment has been indicated but the effect of how it may influence a PCR test due to its ubiquitous nature, is unknown.

1.4.5 Factors associated with infection

ATM has been regarded and managed as a disease which is exacerbated in conditions where abalone are under stress due to environmental factors such as poor water quality and inadequate husbandry (Macey et al., 2011). High population density in intensive aquaculture facilities also contributes to stress and sub-optimal conditions, as well as the transmission of the organism (Mouton, 2010).

Recirculating seawater systems were found to be a higher risk due to the organism remaining within the system as opposed to flow-through systems which would eliminate infected water immediately (Macey et al., 2011).

The understanding and establishment of risk factors for disease threats in aquaculture needs to be refined (Christison, 2019). An intensive environment needs to be managed correctly because disease can easily spread rapidly if introduced, as was evident in farms that suffered devastating losses to abalone herpesvirus and withering syndrome (*Xenohaliotis californiensis*) (Crosson et al., 2014).

Control measures include culling of affected stock, disposal of in-contact biological filter material and sterilization of fomites while observing suitable fallowing periods (Macey et al., 2011). These strategies have been successful at managing the risk for spreading and establishment of this disease in culture facilities.

1.4.6 Surveillance in South Africa

Surveillance is the main method used to collect information on diseases in aquatic animals as a tool to understand the pattern of disease within a population (Cameron, 2002). The information gathered includes all cases of disease. The government employs an active and passive surveillance programme which comprises of targeted surveillance for diseases listed by the World Organisation for Animal Health (WOAH), stock inspections and disease reporting. However, ATM is not a disease that is listed by the WOAH.

Passive surveillance implies that the users of the information make no active effort to collect the disease information (Cameron, 2002). The government are dependent on receiving recorded investigations of disease events from the abalone producers within the country throughout the year. The users of the information would be the Department of Forestry, Fisheries and the Environment in South Africa (Department of Environment Forestry and Fisheries (South Africa), 2019).

Active surveillance makes use of surveys of a small, representative sample of the population so that information can be gathered about that population. The quality of the information collected is better than reports from the passive system and it is easier to evaluate but it only represents a small fraction of the population. It results in a more accurate estimate of a population parameter which makes this type of surveillance useful for prevalence studies, but it is generally unsuitable for monitoring an entire population for emerging pathogens.

Passive surveillance is beneficial in monitoring the farms for emerging or exotic diseases as all cases of disease events are recorded. A larger portion of the population is covered under passive surveillance as opposed to a smaller representative sample seen with active surveillance.

In South Africa, each farm is required to test their stock for abalone herpesvirus, withering syndrome and *Perkinsus olseni* on an annual basis (Department of Environment Forestry and Fisheries (South Africa), 2019). ATM is not required to be tested as part of active surveillance in South Africa. Three stock inspections are conducted by private veterinarians as well as state officials annually.

As part of the active surveillance programme, stock inspections are a clinical inspection of the farmed abalone. It is primarily done to assess the health status of the abalone as well as risk factors that may contribute to the establishment of disease or indicate an ongoing disease present in the stock. The inspection comprises the visual examination of one basket per tank, with the basket of abalone assumed to be a representative sample of the whole tank. There are 165 baskets selected out of all baskets present on the farm to be examined throughout the inspection. This ensures with 95% confidence that diseased animals will be detected in a basket provided a disease is present in 2% or more of the baskets on a farm. (Department of Environment Forestry and Fisheries (South Africa), 2019).

1.5 Systematic and scoping reviews

Systematic reviews, scoping reviews and meta-analysis are transparent and repeatable methods to summarise the available research so that an important topic can be addressed such as clinical or public health issues (Sargeant & O'Connor, 2020).

The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) is a statement which is used by systematic reviewers to objectively report why the review was done, what was done by the authors and what the findings of the review were. The statement outlines a reporting system which reflects methods to identify, select, appraise and synthesise articles (Page, McKenzie, et al., 2021).

Systematic and scoping reviews both contain structured processes but differ in the rationale for why they are used. Where the results from multiple studies are combined to answer the research question that addresses the reliability of an outcome measure, a systematic review should be used. The risk of bias is only considered in a systematic review as the scoping review does not aim to produce results that will be appraised or answer a specific research question. In the absence of quantitative data, the risk of bias will not be evaluated.

1.5.1 Systematic reviews

Systematic reviews are used to address a specific question. The types of questions that can arise, may address topics such as prevalence, incidence, aetiology and diagnostic test accuracy for example (Sargeant & O'Connor, 2020). These questions become applicable to a disease which has not been comprehensively covered in literature.

The types of questions that can be formulated are descriptive, exposure, diagnostic test accuracy and intervention. Descriptive questions cover estimate parameters such as incidence and prevalence from individual to farm level. Exposure questions relate to aetiology or exposure. Diagnostic test accuracy questions determine the sensitivity, specificity, and likelihood ratios of the available diagnostic techniques. The intervention questions assess the efficacy of intervention, treatment and control measures.

This follows rigorous process to ensure that the results of the synthesis for which relevant evidence is obtained for a specific research question, are reliable and applicable (Munn et al., 2018). It is used to assess current practices, inform prospective areas of research, guide policy decisions and explore international evidence (Moher et al., 2015). There are multiple reviewers in some cases in order to reduce the potential for bias. The search for literature is followed by the extraction of data relevant to the question being addressed by the systematic review.

1.5.2 Meta-analysis

A meta-analysis is a statistical synthesis that involves combining the quantitative results of two or more studies that estimate one or more parameters, e.g. prevalence of a condition (estimated as a proportion), or the association of a factor with an outcome (estimated as an odds ratio or risk ratio) (Page, Moher, et al., 2021). It is the analytical component of the systematic review.

The sample size and statistical power from various studies can be increased when pooling quantitative data, depending on the degree of heterogeneity present (Page, Moher, et al., 2021). This will result in an improvement to the estimates of the size of the effect and it will give insight to the pooled effect of the studies. Groups from different studies are generally weighted according to sample size. A quantitative summary estimate, with confidence interval, is produced from the study effect estimates and their variances across studies. The heterogeneity (variation in effect estimates between studies) can be quantified to assess the reliability of the effect size estimate across various studies (Sargeant & O'Connor, 2020). This can be evaluated with Cochrane's Chi squared test with the null hypothesis that the same effect is being evaluated by the articles (Muka et al., 2019). The estimates for heterogeneity are obtained by using the Higgins I^2 statistic (Higgins, Thompson, Deeks, & Altman, 2003).

Studies that report prevalence can be assessed with a meta-analysis through the double arcsine transformation. This can be implemented using the MetaXL 5.3 add-in for Excel (Barendregt, Doi, Lee, Norman, & Vos, 2013) in an inverse variance heterogeneity model.

The most common models used are fixed and random effects models. The fixed-effect model will assume any observed variation between studies is random variation, where only one true outcome exists (Brusselaers, 2015). A fixed effects model is appropriate to use when the true effect size is assumed to be the same common value. If there is a significant variety of study designs, a random effects model must be considered as it allows the true effects underlying the studies to differ and thus accounts for unexplained heterogeneity between studies. It is used to assess both intra-study and inter-study variance (Muka et al., 2019).

1.5.3 Scoping reviews

Scoping reviews are ideal for determining and organising the amount of available literature and studies on a given topic as well as giving an overview of its focus (Munn et al., 2018). Where systematic reviews will address specific queries, scoping reviews offer a broader approach for examining evidence when clear questions still cannot be framed for systematic reviews (Sargeant & O'Connor, 2020). Scoping reviews are used where the purpose of the review is to identify knowledge gaps, scope a body of literature, clarify concepts or to investigate research conduct (Munn et al., 2018). This is involves an extensive search through the available literature followed by a structured mapping of the results (Sargeant & O'Connor, 2020).

1.6 Study Rationale

The South African abalone (*Haliotis midae*) industry is a growing and significant market in terms of export potential as well as job creation. Due to the intensive farming system required for land-based culture facilities, the health status of the animals must be considered. High population density will negatively impact the water quality which in turn has a negative impact on the growth and performance of the stock, while also making the stock susceptible to disease. ATM is a disease that was reported to cause widespread mortalities in South African abalone farms during 2005. (Greeff et al., 2012). Due to the mortalities associated with the reported outbreaks, ATM is considered a health and economic threat to the abalone industry. Literature shows that the presence of *Halioticida noduliformans* infection has been confirmed in culture facilities (Macey et al., 2011). There is interest from industry to identify epidemiological and legislative information pertaining to the disease that could aid in preventative and mitigation strategies. There is a perception that the disease can have a substantial impact on producers, but there appears to be little published information on this. There is also a lack of cohesive information regarding its control. This systematic review will provide a source of consolidated information for producers. This review aims to provide a clear and comprehensive overview of available information on *Halioticida noduliformans* infection in South Africa., if possible, to produce summary estimates of relevant epidemiological parameters, and to identify deficiencies in research which will guide future investigations by formulating relevant research questions.

1.7 Aim and objectives

The aim of the study was to conduct a systematic review of *Halioticida noduliformans* infection in South African abalone (*Haliotis midae*).

The specific objectives of the study were:

1. To conduct a systematic review of the literature relating to *Halioticida noduliformans* infection in South African abalone (*Haliotis midae*) including grey literature, technical reports, and legislation
2. Where quantitative data are present, for example on risk factors associated with infection, prevalence data, treatment options or incubation period, to conduct a meta-analysis in order to produce summary estimates.
3. To identify deficiencies in knowledge and surveillance of *H. midae* infection in SA and recommend areas for future research.

Chapter 2 Materials and Methods

2.1 Study design

The methodology for this systematic review was based on the guidelines as set out in the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement and explanation. The PRISMA extension for systematic reviews was recently revised and provides standardised definitions and guidelines for systematic reviews (Page, McKenzie, et al., 2021).

The PRISMA methodology was an aid for reviewers to transparently report the reason for the review, the activity of the authors and the findings. The guidelines outlined by the PRISMA methodology have been listed below to emphasise the order in which the methods were followed for the review process:

- The title, abstract, rationale, objective of the review must be stated.
- The information sources should include all sources listed.
- The full search strategy for all databases and websites – including filters and limits must be stated.
- The collection of data from reports was influenced by all the variables for which data is sought.
- The report selection should describe results of the search and selection process including the number of reports in the form of a flow diagram.
- The criteria for inclusion must be stated and the findings for the included study must be presented. The collected data must be prepared and tabulated.
- The discussion should include an interpretation of results while discussing the limitations of the information found and how the review processes; can inform future policy research.

2.2 Literature search and data collection

2.2.1 Databases of scientific literature

Three major veterinary databases were used to obtain publications for this study namely:

- Science Direct - https://www.sciencedirect.com/ (multidisciplinary, 1823 - present)
- PubMed - https://pubmed.ncbi.nlm.nih.gov/ (multidisciplinary, 1892 - present)
- ISI Web of Science - https://www-webofscience-com.uplib.idm.oclc.org/wos/cabi/basic-search (multidisciplinary, 1900 - present).

No language, date, subject, or type filters were used during the searches, which allowed for a comprehensive search and reduced limitations on publications obtained. All databases were searched using the topic or subject search function. The search included the titles, abstracts and keywords of publications found on each respective database from its inception to the present-day A base search string was developed using terms based on the population group (host) and outcome of interest (pathogen or disease).

The following terms were used in topic or subject headings:

I. (*Haliotis midae*” OR "*H. midae*” OR abalone OR gastropod OR perlemoen) AND

II. (“H. noduliformans” OR “*Halioticida noduliformans*” OR “ATM” OR “Abalone tubercle mycosis” OR mycosis OR peronosporomycete OR fungal OR fungus)

2.2.2 Grey literature

Grey literature searches were done for the selection of information that is outside of traditional publishing and distribution channels. This can include but is not limited to reports, government documents and newsletters. The same base search string was used for the grey literature searches. The searches were done on:

- Google Scholar (https://scholar.google.com/),
- OpenDOAR: The Directory of Open Access Repositories (https://v2.sherpa.ac.uk/opendoar/),
- Eldis (https://www.eldis.org/)
- OpenGrey: System for Information on Grey Literature in Europe (http://www.opengrey.eu/).

An interview was conducted with a government official from the Department of Forestry, Fisheries and the Environment to identify the relevant legislative documents.

2.2.3 Other internet sources

The internet (Google search engine), as well as the following disease reporting databases were used to identify reports, conference papers and legislative documents.

- World Organisation for Animal Health (https://www.oie.int/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/)
- Department of Forestry and Fisheries and the Environment (https://www.environment.gov.za/legislation/guidelines)
- Department of Agriculture, Land Reform and Rural Development (https://www.dalrrd.gov.za/)

Backward citation searching was applied to all articles from South African authors or articles that dealt specifically with indigenous species of South African abalone. This included searching the reference lists of identified articles for any relevant references which could be used to identify articles that were not found during the primary database searches.

2.2.4 Citation management and data extraction

The data were extracted in tabular format within Microsoft Access. Every source found was contained within its own row. Columns were created for each of the following: Authors, title, journal, year, Digital Object Identifier, reference, reference type, database search, eligibility screening criteria and comments (Page, McKenzie, et al., 2021). The eligibility screening criteria included the reason for inclusion or exclusion.

2.3 Screening of articles

2.3.1 Duplicate removal

All of the sources, including the duplicates, obtained by the various database searches were manually exported from each database and imported into a Microsoft Access table. The elimination of duplicate sources was automated through Microsoft Access before the first stage of screening took place. Duplicates were identified through titles and DOI (Digital Object Identifier) numbers. Where there were minor differences in the title, the duplicate record was manually removed following the screening of both abstracts.

2.3.2 Inclusion and exclusion criteria

The articles eligible for selection were subject to a multistage selection process. The first stage involved identifying articles from screening titles and abstracts. The records which did not contain the inclusion criteria were excluded.

For inclusion, a record had to include information on the species of interest, namely abalone within the *Haliotis* genus. The records also had to include the pathogen of interest, *Halioticida noduliformans*, or the name of the disease as a result of its infection in abalone, namely Abalone Tubercle Mycosis. No exclusion criteria were defined.

2.3.3 Eligibility Assessment

The full text of the records that remained after applying the inclusion criteria were assessed for eligibility to be included in the final review. The assessment of the included records would report on the kind of information present. Mortality rates, incubation period and prevalence rates were recorded when present.

The risk factors were recorded as a contributing factor that would increase the susceptibility of the abalone to ATM. These would include: farming systems (flow-through or recirculation systems); changes to water quality; stressor events such as grading and cleaning procedures; and the introduction of new abalone onto the farm

Table 1 Assessment criteria for final review of articles

Assessment criteria	**Description**
Manuscript focus	Review, research paper, outbreak investigation, policy document, surveillance report or conference presentation
Manuscript type	Scientific, legislative or grey literature
Country of origin	Location of study
Region	Local or international
Prevalence	Inter-farm, intra-farm or n/a
Mortality rates	Reported or not
Incubation period	Reported or not
Risk factors	Reported or not
Control measures	Reported or not
Method of diagnosis	Reported or not

The record selection process was presented in the form of the PRISMA 2020 flow diagram (Page, Moher, et al., 2021).

2.4 Statistical analysis

Any articles or reports that contained quantitative information on prevalence of infection, association of factor(s) with outcome(s) or effect of treatment or intervention were identified for possible inclusion in a meta-analysis.

2.5 Ethical Considerations

Approval for the project was obtained from the Research Ethics Committee, Faculty of Veterinary Science, University of Pretoria (Protocol no. REC135-20). This study only required access to publicly available databases.

Chapter 3 Results

The identification of articles via databases resulted in 1880 records from 3 databases (Figure 5). The removal of duplicate records before screening resulted in 188 records being removed. The articles eligible for selection were subject to a multistage screening process. The first stage involved identifying articles from screening titles and abstracts. Most records were excluded on the basis that the outcome of interest was not measured or recorded. There were 1686 records which did not meet the inclusion criteria and were excluded. Thereafter, full text article screening was performed for eligibility with the remaining 6 records.

The identification of reports via other methods included searching for grey literature through Google Scholar, Eldis, Open Grey and OpenDOAR, which added 3 additional documents. Backward citation referencing included searching the reference lists of identified articles for any relevant references which could be used to identify articles (n = 1) that were not found during the primary database searches. Other internet sources and an interview were also used to find the relevant legislation, contained in 4 additional documents. Together, these methods produce 8 additional reports and documents which could be included in the review, resulting in a total of 14 documents. The results are presented in the form of the PRISMA 2020 flow diagram for new systematic reviews (Figure 2).

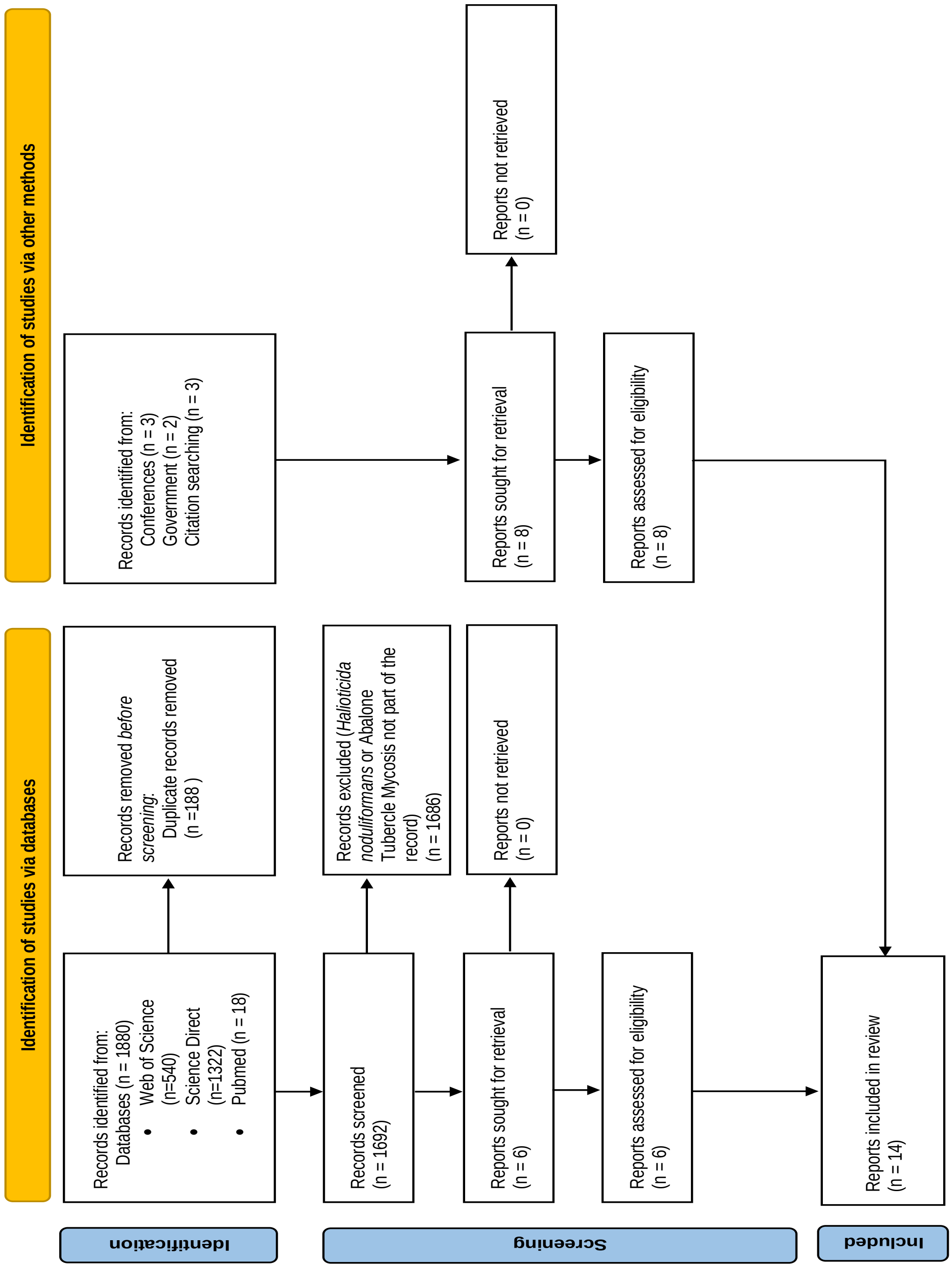

Figure 5 PRISMA 2020 flow diagram for a systematic review of Halioticida noduliformans infection in Haliotis midae which included searches of databases and other sources (Page, Moher, et al., 2021)

3.1 Categorisation of articles

Based on the database searches, 6 articles were assessed for full-text eligibility, all of which were included in the review as they met the requirements for final inclusion. The articles were all scientific literature. 2 articles were written in South Africa by local researchers. The rest of the articles were international publications.

3 legislative documents, 3 reviews, 1 conference presentation and 1 scientific paper were identified through other methods. 2 documents contained no direct relevance to the outcome of interest yet were conditionally included on the basis that they contained legislation which would impact the management of such a disease event (Government of South Africa, 1998) (Department of Agriculture Forestry and Fisheries (South Africa), 2018). All 8 documents were assessed for eligibility and subsequently included. 7 of the assessed reports were written by South African authors with a manuscript focus on South Africa.

The final number of documents reviewed totalled 14, of which 6 were scientific, 5 were grey and 3 were legislative documents. Table 2 lists the articles retrieved by their source; Table 3 lists the categorisation of articles following eligibility assessment; and Table 4 lists the categorisation of articles assessing the type of information reported for ATM.

Table 2 Eligible articles and records retrieved by their source

Record ID	Title	Author	Source	Citation
1	Development and preliminary evaluation of a real-time PCR assay *for Halioticida noduliformans* in abalone tissues	Greeff, M. R.; Christison, K. W.; Macey, B. M.	Scientific database	(Greeff et al., 2012)
2	*Halioticida noduliformans* isolated from cultured abalone (*Haliotis midae*) in South Africa.	Macey, B. M.; Christison, K. W.; Mouton, A.	Scientific database	(Macey et al., 2011)
3	*Halioticida* infection found in wild mantis shrimp *Oratosquilla oratoria* in Japan.	Atami, H.; Muraosa, Y.; Hatai, K.	Scientific database	(Atami et al., 2009)
4	A new peronosporomycete, *Halioticida noduliformans* gen. et sp. nov., isolated from white nodules in the abalone *Haliotis* spp. from Japan.	Muraosa, Y.; Morimoto, K.; Sano, A.; Nishimura, K.; Hatai, K.	Scientific database	(Muraosa et al., 2009)
5	Emerging oomycete threats to plants and animals.	Derevnina, L.; Petre, B.; Kellner, R.; Dagdas, Y. F.; Sarowar, M. N	Scientific database	(Derevnina et al., 2016)
6	*Halioticida noduliformans* infection in eggs of lobster (*Homarus gammarus*) reveals its generalist parasitic strategy in marine invertebrates.	Holt C, Foster R, Daniels CL, van der Giezen M, Feist SW, Stentiford GD, Bass D.	Scientific database	(Holt et al., 2018)
7	Aquaculture Development Bill	Department of Agriculture Forestry and Fisheries (South Africa)	Legislation	(Department of Agriculture Forestry and Fisheries (South Africa), 2018)
8	Health Management Procedures For South African Abalone Produced for Export.	Department of Environment Forestry and Fisheries (South Africa)	Legislation	(Department of Environment Forestry and Fisheries (South Africa), 2019)
9	Epidemiology and management of *Halioticida noduliformans* infections on South African abalone.	Christison, K. W.	Grey (Google Scholar)	(Christison, 2011)
10	The Development, Optimisation and Evaluation of Molecular Methods to Diagnose Abalone Tubercle Mycosis (ATM) Caused by *Halioticida Noduliformans* in South African Abalone, *Haliotis Midae*	Greeff, M. R.; Christison, K. W.; Macey, B.M.	Dissertation	(Greeff, 2012)
11	Marine Living Resources Act	Government of South Africa	Legislation	(Government of South Africa, 1998)
12	Abalone tubercle mycosis an emerging disease in the South African abalone industry.	Christison, K. W.; Macey, B.M.; Mouton, A.	Grey (Google Scholar)	(Christison, Macey, & Mouton, 2009)
13	Diseases of Fish and Shellfish Caused by Marine Fungi	Hatai, K.	Grey (Google Scholar)	(Hatai, 2012)
14	Control measures for abalone tubercle mycosis	Amanzi Biosecurity	Grey (internal company document)	(Amanzi Biosecurity, 2014)

Table 3 Categorisation of articles following eligibility assessment

Record ID	Region covered by manuscript	Manuscript type	Manuscript focus
1	South Africa	Scientific	Diagnostic test development
2	South Africa	Scientific	Outbreak investigation
3	Japan	Scientific	Outbreak investigation
4	Japan	Scientific	Outbreak investigation
5	Global	Grey	Review
6	Global	Scientific	Review
7	South Africa	Legislative	Policy document
8	South Africa	Legislative	Policy document
9	South Africa	Grey	Outbreak investigation
10	South Africa	Scientific	Diagnostic test development
11	South Africa	Legislative	Policy document
12	South Africa	Grey	Outbreak investigation
13	Global	Grey	Review
14	South Africa	Grey	Review

Table 4 Categorisation of articles assessing the type of information reported for ATM

Record ID	Quantitative/ Qualitative	Diagnostic method	Risk factors reported	Prevalence reported	Incubation period reported	Mortality reported
1	Qualitative	rPCR	-	-	-	30-90%*
2	Qualitative	rPCR and histopathology	Intensive farming. Recirculation seawater systems. Ubiquitous to the marine environment	-	3 days	30-90%*
3	Qualitative	rPCR and histopathology	Same pathogen isolated from shrimp	-	-	NA
4	Qualitative	Isolation and molecular phylogeny	Transmission between species in *Haliotis* genus	-	-	1% daily mortality rate in stocked abalone from which isolates were collected on various farms
5	NA	qPCR	Ability to infect multiple species of *Haliotis*	-	-	30-90%*
6	Qualitative	rPCR and histopathology	Intensive aquaculture systems and hatcheries. Same pathogen isolated in lobster eggs	-	-	NA
7	NA	NA	-	-	-	NA
8	NA	NA	ATM notifiable with positive cases	-	-	NA
9	NA	Clinical signs and histopathology	Recirculation systems, stock movements. Outbreaks occur within two weeks of grading/sorting	-	-	NA
10	Qualitative	Histopathology, PCR and fungal culture	-	-	-	30-90%*
11	NA	NA	-	-	-	NA
12	NA	Pure cultures isolated from tissue samples of infected abalone	-	-	-	30-90%*
13	NA	NA	Intensive farming with high stocking rates	-	-	1% daily mortality*
14	NA	Confirmation by culture or PCR	Subclinical cases can be present	-	-	30-90%*

*Cited value from a reference article

3.2 Manuscript type

The most common type of source found (9/14; 64%) was scientific articles. The grey manuscript types consisted of an unpublished review, conference presentations and textbook chapters. The unpublished review was an internal company document that was prepared for the Abalone Farmers Association of South Africa (Amanzi Biosecurity, 2014). The 3 legislative documents were all South African policy documents found through the interview with the government official. Relevant legislative documents in other countries which directly referenced ATM could not be found.

3.3 Manuscript focus

There were no surveillance reports found. 5/14 (36%) of articles and reports reviewed dealt with outbreak investigations and only 2/14 (14%) contained diagnostic test development as the manuscript focus. 4 of the 14 documents (29%) were reviews. 9 of the 14 articles and reports (64%) reported on risk factors relating to ATM.

2 of the 14 articles dealt with the development of a PCR assay for ATM, but 1 was a dissertation on which the second article was based (Greeff, 2012).

3.4 Quantitative data

Prevalence and incubation period information was required for a meta-analysis. However, no such articles were found. No prevalence estimates or reports of treatment effects or measures of association were found, which precluded any meta-analysis. Mortality figures could not be used for a meta-analysis because the figure most cited by the studies could not be traced back to an original study.

Mortality data were recorded in 2 scientific articles. The other articles used cited values from these 2 scientific articles. Severe production loses of up to 90% in spat and 30% in older animals were recorded (Macey et al., 2011). The mortality data reported in this article (Macey et al., 2011) were not cited from another source, but no explanation is given on how these data were obtained from the reported outbreaks in South African abalone farms, and this appears to be the original source of this information. This was reported in 6/14 (43%) articles as a cited value.

A daily mortality of 1% was reported in 1 article. This was recorded in experimentally stocked abalone and not as part of an outbreak investigation on an abalone farm (Muraosa et al., 2009).

Only 1/14 article reported the incubation period. The scientific article involved the isolation of the organism in clinically infected abalone in South Africa. There were no previous sources cited for the incubation period.

Chapter 4 Discussion

This systematic review aimed to collate all the epidemiological and legislative information available relating to *Halioticida noduliformans* infection in South African abalone (*Haliotis midae*). Since very little quantitative information was found on any epidemiological parameters, risk factors, treatment or control measures, no meta-analysis was possible. Many deficiencies were found in the available information regarding the disease and in the legislation relevant to its control.

4.1 Agent and host range

The nature of the pathogen was reported in the majority of articles and reports, revealing its morphological characteristics, transmission, and pathology. *Halioticida noduliformans* is shown to be an obligate marine fungus which cannot propagate in estuarine or fresh water. Laboratory experiments revealed the optimal temperature for growth was found to be ~20°C. No growth was observed below 5°C and above 30°C (Atami et al., 2009). During the summer months, seawater temperatures can rise up to 20°C and above. This will create the ideal growth conditions demonstrated under laboratory conditions.

Its survival time in the environment under natural conditions is unknown. This is dependent on environmental reservoirs; whether the oomycete is an obligate pathogen and whether the oomycete undergoes repeated zoospore emergence or polyplanetism. As a result of this, when positive cases are found and quarantined on a farm, there is uncertainty with regards to how long the organism can persist in the environment before the quarantine can be lifted. This poses a risk to the healthy animals that may be exposed to previously contaminated tanks and equipment though this risk can be mitigated through disinfection and desiccation of equipment.

The same isolate was found in wild mantis shrimp (*Oratosquilla oratoria*) and European lobster (*Homarus gammarus*). This may imply cross-species transmission which will require further research. Although the European lobster is not native to South Africa, there are various species of lobster found in colder, coastal waters which abalone farms use for their flow-through systems. It is unknown whether one of the local species of lobster can be a carrier of *Halioticida noduliformans*. This may be a potential biosecurity risk as the organism can be introduced onto the farm through the incoming seawater, though on-farm reservoirs also pose a risk to the establishment of the pathogenic agent within the farm.

More work is required to highlight the extent of its host range and subsequent effects on the aquaculture industry. *Halioticida noduliformans* has been documented in other species which highlights that challenge experiments are needed to confirm the host susceptibility and host range across various marine invertebrate taxa. The organism has been shown to infect various species of abalone kept in the same tank (Muraosa et al., 2009). The abalone industry in South Africa farms exclusively with the local *Haliotis midae* which is endemic to the coastal waters in South Africa. It is less likely that another species of abalone will introduce *Halioticida noduliformans* to a farm, but this does not rule out other aquatic life in the coastal waters as potential carriers or intermediate hosts.

The sub-clinical nature of ATM has not been extensively researched. This creates uncertainty with regards to the disease status of stock even if a passive surveillance programme is implemented by the farm to identify and record events of disease.

4.2 Epidemiological parameters and risk factors

The incubation period was only reported in 1 paper as part of an experimental infection in abalone (Macey et al., 2011), which estimated it at 3 days. Knowledge of the incubation period will aid producers in determining the potential risk to exposed abalone and subsequently influence mitigation strategies as the isolation of diseased stock is required. For example, if lesions are found on an abalone, then any stock that had been in contact within the previous 3 days should also be isolated and monitored. That is not limited to other abalone within the same tank but to recent grading and handling procedures on the farm.

It becomes difficult to track the initial point of exposure as well as the transmission points with other animals and people if the incubation period is unknown. The ubiquitous nature of the organism may complicate establishing the incubation period as the organism may always be present in the environment.

The type of farming system being utilised can likely also influence prevalence as flow-through and recirculation systems vary in their operation. Flow-through systems can potentially pose a risk in bringing unknown organisms through continual incoming seawater. Recirculation systems limit the intake of incoming seawater, and the replacement of water happens at a slower rate than the flow-through systems (Mouton, 2010). The incidence and prevalence information for wild, coastal abalone as well as farmed abalone is unknown and should be considered an area for further research.

The lack of prevalence data available between and within farms will affect surveillance targets and establishing underlying likely prevalence levels for a given population. As a result of this, freedom of disease surveys cannot be conducted and scientifically justified as these characteristics are required when calculating sample size as well as evaluating surveillance programmes. Without knowing the estimated number of existing cases that may be present, it becomes difficult to secure the correct number of resources and establish the extent of the mitigation strategies as well as obtain funding.

The specific climate associated with each region can influence the prevalence as well because the optimal growth temperature of the organism was found to be ~20°C (Atami et al., 2009).

Mortality rates were reported as 90% in spat and 30% in older animals (Macey et al., 2011) and a daily mortality of 1% in experimentally stocked abalone (Muraosa et al., 2009). These findings were based on early outbreaks and may need to be revised with additional information from more outbreak investigations and surveillance studies.

4.3 Diagnostic tools

It is evident that steps have been taken to investigate the nature of this disease as well as develop a reliable diagnostic tool for surveillance purposes. The aquaculture industry will benefit from more diagnostic data on sub-clinical cases using real-time PCR. This information can be used to determine a prevalence for sub-clinical cases in abalone farms and determine when it poses a significant risk.

The turnaround time of histology and real-time PCR investigations may necessitate the development of a “pen-side” field diagnostic tool so that the appropriate actions can be taken sooner at the culture facilities. Due to the apparent rare nature of this disease, the provision of resources for the diagnostic tool may not be realistic as the last recorded outbreaks in literature are from 2006 (Macey et al., 2011).

The current passive surveillance systems on the farm will only ensure that abalone with characteristic lesions for ATM are isolated while samples are submitted for confirmation by PCR.

Further emphasis on the development of diagnostic capacity and research is essential to mitigate the detrimental impacts of other infectious and emerging diseases on aquatic animal health and production (Christison, 2019).

4.4 Surveillance

The *Health Management Procedures for South African Abalone Produced for Export* (Department of Environment Forestry and Fisheries (South Africa), 2019) is a document that explains the passive and active surveillance components that abalone producers should adhere to. As no outbreak of documented abalone diseases listed by the WOAH has ever occurred in South Africa, the document established its guidelines focusing on diseases that affect other species of abalone in the *Haliotis* genus (Christison, 2019).

ATM is not on the official list of controlled and notifiable diseases in South Africa which excludes ATM from the associated legislation regarding surveillance and control of the disease (Department of Environment Forestry and Fisheries (South Africa), 2019). However, the *Health Management Procedures for South African Abalone Produced for Export* makes provision for this exclusion by classifying diseases in abalone into four categories: infections or conditions that occur in the abalone population, without production losses or a public health risk such as ATM; diseases listed by the WOAH; emerging diseases; and diseases of national significance associated with production losses that are not controlled at an international level.

The Department of Forestry, Fisheries and the Environment (DFFE) requires that permit holders for abalone culture facilities report certain diseases. Infection with *Halioticida noduliformans* (ATM) must be reported to the DFFE under current export permit conditions of the *Health Management Procedures for South African Abalone Produced for Export* (Department of Environment Forestry and Fisheries (South Africa), 2019).

The *Health Management Procedures for South African Abalone Produced for Export* only covers the grow-on section of a farm which is the post-weaned stock destined for the export market. The other sections of the farm that are not under a compulsory health programme are the hatchery and broodstock sections. As a result of this, the health status of abalone in those sections is effectively unknown until they reach the grow-on section of their respective farms. Each farm has its own hatchery and produces spat on-site for the grow-on section so the abalone that are ultimately tested can be traced back to their respective broodstock and hatcheries.

As part of the active surveillance programme for ATM, physical stock inspections are performed by private and state veterinarians. There are three stock inspections performed altogether throughout the year, but a more robust passive surveillance component may be necessary for a disease of this nature given the lack of knowledge of epidemiological parameters such as prevalence. The three annual inspections may not be enough to detect ATM as only a subset of population are examined. The number of baskets (165) examined for the inspection equate to a small fraction of the population. The inspection is based on the assumption that 2% of the baskets will contain diseased abalone if ATM is present on the farm but seeing that the true prevalence for ATM is unknown the absence of macroscopic lesions may result in positive cases being missed during inspections.

Targeted surveillance for export purposes is done through PCR and this does not include screening for ATM. The diseases listed by WOAH are required to be tested for by PCR on an annual basis to maintain an export permit. ATM is not prioritised in this regard as it is classified as a disease without significant production losses or public health risk.

4.5 Control measures

ATM is regarded by South African regulatory authorities as a contagious disease (Greeff et al., 2012). The control measures listed in South African literature include effective containment with destocking; sterilisation of equipment with fallowing; and the removal and decontamination of biological filter material (Macey et al., 2011). The most effective compounds that can be used for sterilisation will require more research as this has not been reported in the literature. This is important as it has been reported that the organism may be ubiquitous in the environment (Holt et al., 2018). In the event of an outbreak, the tanks with positive abalone will require an effective sterilisation following destocking (Christison, 2011). The growth of the organism can become challenging to control due to the wide range of temperatures that it can grow within (Muraosa et al., 2009). As a result of this, the other factors on a farm that influence the health of an abalone such as feeding, cleaning and handling will need to be considered when implementing preventative measures.

4.6 Legislation

The Animal Diseases Act (Act 35 of 1984) (Government of South Africa, 1984) has long been used as the standard for the agriculture industry. Since shellfish are invertebrates, the Act excludes them and thereby restricts its application to vertebrate aquatic animals which constitute ~70% of the biomass produced by the aquaculture sector in South Africa (Christison, 2019). As a result of this, the Aquaculture Development Bill has been drafted to provide for the development of a national aquatic animal health programme relating to health, welfare, safety and quality of aquatic organisms and products (Department of Agriculture Forestry and Fisheries (South Africa), 2018). Even though there are not any diseases of invertebrates listed as controlled or notifiable diseases in terms of the Animal Diseases Act (Act 35 of 1984), the processes are in place for the correct reporting of such under the marine aquaculture permit which is issued by the governing authorities (formerly the Department of Agriculture, Forestry and Fisheries, now the Department of the Forestry, Fisheries and the Environment) under the Marine Living Resources Act (Act 18 of 1998) and the *Health Management Procedures for South African Abalone Produced for Export* (Department of Environment Forestry and Fisheries (South Africa), 2019). The *Health Management Procedures for South African Abalone Produced for Export* states that positive cases of ATM should be notifiable.

ATM is not explicitly mentioned in the Aquaculture Draft Bill. The bill does state that any pest or pathogen that is listed by WOAH or of national significance can be declared as notifiable if it poses a risk to aquatic organisms, consumers of aquaculture products or the aquatic environment (Department of Agriculture Forestry and Fisheries (South Africa), 2018). The bill also makes provision for the development of a national aquatic animal health and disease programme which includes its regulation and management. If ATM does begin to pose a significant risk to farmed abalone, the Aquaculture Development Bill can be used for the control of such diseases once it has been declared as a disease of significance by the governing authorities.

Relevant legislative documents in other countries which directly referenced ATM could not be found. The lack of information from other countries cannot inform control measures for outbreak events. The lack of legislative documents could also indicate that this disease is not seen as a high priority in any other country.

4.7 Scientific literature base

The identification of articles via databases did not yield as many articles as expected. The types of articles from the database searches consisted of 4/6 outbreak investigations and 1/6 diagnostic test development. Only 2/6 articles were from South Africa. There were no articles that addressed prevalence, risk factors, assessment of treatments or intervention. Only 3/6 articles addressed abalone (*Haliotis* species) and *Halioticida noduliformans*.

2 of the 6 articles addressed *Halioticida noduliformans* with the focus on mantis shrimp and lobster, respectively. These articles focused on outbreak investigation and the isolation of the organism. There was no additional information regarding ATM in abalone that had not already been reported in the other articles found.

The articles found had each referenced at least 1 of the other articles from our database searches. The amount of primary literature was found to be limited in that regard with only 3 articles found that comprise the current knowledge on which the disease is based (Muraosa et al., 2009; Macey et al., 2011; Greeff et al., 2012). The information from the other articles came from other scientific articles which focused on *Halioticida noduliformans* but not on abalone. A superficial review (Derevnina et al., 2016) was found which focused on emerging oomycete threats to plants and animals.

In summary, the retrieval of information resulted in a very limited body of primary research with the same sources being cited repeatedly. Where quantitative data were found, it was traced to a single source. This would indicate the need for specific research projects concentrating on aspects of epidemiological data such as incidence, prevalence, mortality rate, incubation period and risk factors associated with infection.

4.8 Areas for further research

The following deficiencies or gaps in the knowledge of ATM as well as shortcomings in legislation or practice were identified:

- The molecular characterisation of the oomycete and its pathogenicity
- Environmental conditions for an outbreak of ATM, including whether water quality, husbandry and farming systems play a significant role in increasing the susceptibility of abalone to *Halioticida noduliformans* infection
- Determining the tissue distribution of *Halioticida noduliformans* in infected abalone
- Whether PCR can detect subclinical carriers of *Halioticida noduliformans*
- Challenge experiments to test the pathogenicity and host susceptibility of *Halioticida noduliformans* in shrimp and abalone. Both of which have had *Halioticida noduliformans* isolated.
- The extent of the host range which are susceptible to infection
- Possible co-infection with pathogenic organisms
- The survival of the organism outside the host in the environment and its possible ubiquitous nature. As well as the role of carriers and vectors
- The mode of transmission of *Halioticida noduliformans* in an abalone culture facility and how feeding, handling and cannibalism may influence the transmission
- Range of incubation period for *Halioticida noduliformans* infection
- Establishing prevalence data between and within farms to aid surveillance programs and freedom of disease surveys
- Treatment options for infected abalone
- Effective disinfection methods for contaminated equipment following an ATM outbreak
- The development of a health management programme that address animal health in hatchery spat and broodstock abalone. The current programme only applies to abalone in the grow-on section of the farm.
- The effectiveness of three stock inspections per year as part of an active surveillance programme for ATM

- The lack of legislation for invertebrate marine animals as the Animal Diseases Act (Act 35 of 1984) does not make provision for it. It may be necessary to explore international legislation of invertebrate marine animals.
- Whether it is necessary to make ATM a notifiable disease in legislation if there is a lack of knowledge regarding the possibility of significant production losses or a public health risk

4.9 Conclusion

This systematic review aimed to collate all the epidemiological and legislative information available relating to *Halioticida noduliformans* infection in South African abalone (*Haliotis midae*). The amount of primary literature was found to be limited in that regard with only 3 articles found that can cover the current knowledge on which the disease is based. Although the available information regarding this organism covers pathology and diagnostic test ability, there were no reports identified which discussed prevalence, risk factors, assessment of different treatments or interventions Further research is urgently required. Even though there are not any invertebrate diseases listed as controlled or notifiable diseases in terms of the Animal Diseases Act (Act 35 of 1984), the processes are in place for the correct reporting of such under the marine aquaculture permit which is issued by the governing authorities.

Chapter 5 References

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Appendix

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee

14 January 2022

CONDITIONALLY APPROVAL

Ethics Reference No	**REC135-20**
Protocol Title	**A systematic review of Haliotoida noduliformans infection in South African abalone (Haliotis midae)**
Principal Investigator	**Dr S Tabel**
Supervisors	**Prof PN Thompson**

Dear Dr S Tabel,

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC135-20) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

Conditionally approved. NOTE: Researcher agreements - Only a postgraduate student can be granted the rights to publish a dissertation/thesis.

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

100 YEARS

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