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An Epidemiological Study of Parasites Infecting The South African Abalone (*Haliotis Midae*) In Western Cape Aquaculture Facilities

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SUMMARY CHAPTER

Global growth in aquaculture, referred to as the blue revolution, is seen by many to be the solution to future food scarcity. However, this growth has been accompanied by disease emergence. Disease emergence is inevitable when host populations are concentrated and densities exceed the threshold value for occurrence of outbreaks. Abalone farming is a relatively recent development and diseases of abalone are not well characterised. There have been relatively few systematic surveys of abalone diseases in the world. Much of the available information resulted from investigations of specific disease outbreaks, such as withering syndrome in California. The outstanding example of a formal survey of abalone health was conducted in Australia. A methodical survey of abalone health, encompassing all farms and including wild abalone, has never been done in South Africa. However, South Africa has for many years had a herd health program for abalone producers and this has generated the largest body of data on abalone disease occurrence in the world. Although these data have some shortcomings, it was felt that analysis could provide insights into the epidemiology of parasites in farmed *Haliotis midae*, as well as informing better surveillance techniques for the future.

Data for abalone submitted from nine farms as part of the herd health management program during the period 1 January 2000 to 31 December 2004 were analysed. No wild abalone were included in these data and the only abalone species considered was the South African abalone or perlemoen, *Haliotis midae*. Data on the age of the abalone and their diet were obtained from the farms. The abalone originated from either flow through or recirculation systems. Each animal was weighed and measured at the laboratory. A standard set of tissue sections was fixed and processed for histology. Presence of parasites was recorded, as well as the sex and degree of gonad development.

Once data had been captured in Excel, a series of tables was constructed from counts of infected and non infected abalone for all host and environmental factors contained in the data set. Charts of the tables were drawn. Where host and environmental factors appeared to interact, these data were also tabulated and charted. Statistical analyses of the data in Excel followed. All analyses were performed for sessile ciliates, renal coccidia, gut protozoa, digestive gland

protozoa and rickettsia like prokaryotes. The rarity of trematode infections made meaningful analyses difficult. The chi square test, effect sizes and odds ratios were used to seek significant associations. When confounding and interaction were suspected, stratum specific odds ratios were calculated. The summary odds ratio used in this study was the Mantel Haenszel summary odds ratio. The Breslow Day test for interaction was performed when necessary. Confidence intervals were determined using the method of Woolf.

The overall prevalence of the various parasites was very variable. Sessile ciliates were the most common, with a prevalence of 68.3%. Rickettsia like prokaryotes were found in 13.1% of animals. The other gut associated parasites were more scarce, with prevalences under five percent. Renal coccidia affected less than two percent of animals. Trematode infections were extremely rare, at a prevalence of 0.05%.

The results of the chi square test showed a significant association between age and parasite prevalence for all parasites tested. Odds ratios were calculated comparing animals of 24 months and younger to those older than 24 months. In all cases, except trematode and left kidney coccidian infections, risk of parasite infection tended to increase with increasing age. For left kidney coccidian infections, risk of infection decreased with increasing age. Trends for body mass were similar to those for age, which is expected, as animals generally become larger with increasing age. A significant association between growth rate and parasite prevalence existed for some parasites. The chi square test showed a significant association between condition index and parasite prevalence for all parasites tested. A significant association between sex and parasite prevalence was found for all parasites tested. A significant association also existed between parasite prevalence and gonad development for sessile ciliates, renal coccidia and gut protozoa.

There were significant differences in parasite prevalences between farms for all parasites tested. The South and West coasts were next compared using the chi square test. There were significant differences in parasite prevalences between coasts for only three of the parasites tested, namely renal coccidia, gut protozoa and rickettsia. Crude odds ratios showed that, with the exception of left kidney coccidia and trematodes, there was a greater risk of parasite infection on the West than the South coast. For left kidney coccidia, the risk was greater on the

South coast. A chi square test was performed to examine the relationship between parasite prevalence in Hermanus and other areas. A significant difference was found for renal coccidia and gut associated parasites. The crude odds ratios for parasite prevalence in other areas compared to Hermanus were calculated. With the exception of sessile ciliates and trematodes, there was a greater risk of parasite infection in areas other than Hermanus. In the case of sessile ciliates, there was a greater risk within Hermanus than in other areas.

Unfortunately, it was almost impossible to determine whether a seasonal effect exists for parasite prevalence from the available data. This was shown to be partly due to the effect of prevalence on individual farms.

Significant differences in parasite prevalences between diets for all parasites tested were shown using the chi square test. To further test the strength of the association, odds ratios were calculated comparing only kelp and artificial feed. For right kidney coccidia and gut associated parasites, the odds ratios indicated a significantly increased risk of infection in animals receiving kelp compared to those on artificial feed. There was no difference in risk for sessile ciliates and left kidney coccidia.

The majority of animals originated in flow through systems. The chi square test showed significant differences in parasite prevalences between systems for sessile ciliates, renal coccidia and rickettsia like prokaryotes. Odds ratios showed a significantly greater risk of sessile ciliate infections, but a smaller risk of left kidney coccidia, in animals in flow through systems when comparing only kelp fed animals.

Age is likely to lead to increased prevalences if the risk of infection is constant over time and also if infections are retained. Physiological changes in the animals may also affect their risk of infection. A further important aspect of age in abalone relates to changes in husbandry. Increasing age may be the underlying reason for some of the prevalence patterns seen with mass and sex. The origin of the sample population was considered in terms of farm of origin as well as geographic area. Abalone production in the study area was highly concentrated, with approximately two thirds coming from six farms situated within ten kilometres of each another on the South coast and almost forty percent from Hermanus alone. The present study found no

evidence of increased parasite prevalence in areas where abalone farming is concentrated. It was felt that this study could not generate much insight into seasonal occurrence, due to uneven distribution of variables between months. The relationship between diet and parasite prevalence was perhaps the most interesting aspect of this study and possible reasons for the association are explored.

Lastly, the results indicated that parasite buildup in recirculation systems was not as problematical as may be expected. It is possible that the increased prevalence of left kidney coccidia in recirculation systems is linked to the resistance of the host population rather than to the dynamics of the actual system. The very low prevalence of sessile ciliates in recirculation systems could not be explained by examination of any other variable considered in this study.

Overall, the prevalences of soft tissue parasites in *Haliotis midae* compared favourably with those found for parasites of other abalone species abroad. Measures which would tend to reduce parasite prevalence include separation of age groups and maintaining a relatively young population on the farm. Culling of underperforming animals is recommended. Kelp should not be used in animals of two years or younger. In older animals, there was still a greater risk associated with kelp than with artificial feed, but it was not as marked. Recirculation systems proved to be less associated with increased parasite prevalence than one may expect. The other major findings of this study did not lend themselves to practical application.

Chapter 1
Introduction

1.1 Background

In 2004, aquaculture and capture fisheries supplied approximately 106 million tonnes of food to the human population of the world. Aquaculture accounted for roughly 43% of this total. Production from aquaculture increased from 36 million tonnes in 2000 to 46 million tonnes in 2004. This represents growth of about 7% per year. Annual growth in aquaculture from 1970 onwards has been closer to 9% per year, compared to less than 3% for terrestrial animal production. These figures (FAO, 2006), commonly quoted by both the media and scientists, will be familiar to anyone reading about aquaculture. Reference is made to the blue revolution, optimistically predicting that development of aquaculture will be the solution to future food scarcity (Sachs, 2007). However, it is necessary to moderate this enthusiasm with some consideration of the realities and risks associated with aquaculture.

The world organisation for animal health is the Office International des Epizooties (OIE). Its purpose is to facilitate international trade in animals and animal products whilst avoiding the associated risk of spreading diseases. Besides publishing generic recommendations on disease control, the OIE also lists specific diseases of concern and provides guidelines on their diagnosis and prevention (Bernoth, 2006). In 1960, the OIE recognised the needs of the growing aquaculture sector and founded the Fish Diseases Commission. Invertebrates were added to the ambit of this Commission in 1988 and it was renamed the Aquatic Animal Health Standards Commission in 2003 (OIE, 2007). The Commission listed a total of twenty four diseases in 1995, of which six were mollusc diseases (Håstein, 1996). By 2007, this had grown to thirty four, of which nine were mollusc diseases. The additional three diseases are all of abalone (OIE, 2006a; OIE, 2006b).

It is inevitable that growth and intensification of aquatic animal production will lead to disease emergence and an increasing number and severity of disease outbreaks. The reasons are very clearly presented in a paper by Reno (Reno, 1998). He describes the epidemiological principles governing the spread of disease in fish populations, but his findings are equally applicable to

other aquatic animals, including molluscs. Models of disease are largely based on density parameters, in other words the number of individuals per unit area, because of the critical importance of frequency of contact between infectious and susceptible individuals in disease outbreaks. This applies both to direct contact and shedding of infectious material in the water. Introduction of a pathogen to a host population will only result in a disease outbreak if the host density is greater than a specific threshold value. Obviously, intensive animal production, which has the primary objective of increasing animal densities, creates ideal conditions for disease outbreaks.

There are also factors other than density which increase the risk of disease in aquaculture. Movement of animals and their products, especially on an international level, has led to several highly significant disease outbreaks and is one of the reasons why an organisation like the OIE exists (Bernoth, 2006; Subasinghe and Bondad-Reantaso, 2006). On a more localised scale, disease in aquaculture is frequently linked to changes in, most often deterioration of, the aquatic environment. Poor water quality is a constant threat and one of the underlying aims of intensive aquaculture is to determine the minimum acceptable water quality for animal production, as any measures to improve water quality invariably have associated costs (Branson, 1993; Wedemeyer, 1996). Aquatic animals may have poor disease resistance for other reasons, including acute and chronic stress, shortcomings in nutrition, and selective breeding leading to inbreeding (Southgate, 1993). It is possible to continue the list, but hopefully a sufficient argument has been provided. As long as aquaculture exists, disease emergence and outbreaks of infectious disease will continue.

1.2 Infectious diseases of abalone

Anyone seeking information on this topic is invariably drawn to the excellent review provided by Bower and McGladdery on their website, Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish (Bower and McGladdery, 2003). This is one of the very few reasonably complete sources on abalone diseases. Abalone are newcomers to the world of aquaculture and their diseases have not yet enjoyed the same attention from researchers as those of bivalves, notably oysters. A brief overview of known problems will be presented here, with reference to the South African situation.

Abalone viral mortality has been much in the news due to the ongoing outbreak in Australia (Sexton, 2007). This follows on the Asian outbreaks reported some years earlier (Wang *et al*, 2004; Zhang *et al*, 2004). It is not currently known whether the aetiological agents are the same, or similar, and what the relationships, if any, are between the outbreaks. It has been problematical to obtain good information on the Asian outbreaks, especially as much of the work done so far has not been published in English. It would appear that mortalities have occurred in cultured *Haliotis discus hannai*, or ezo awabi, in northern China since the early 1990's. At the peak of the epidemic, losses of up to 90% of spat and 30% of older abalone were experienced, as well as mass mortalities of wild animals. The outbreak declined by around 1997, partly due to improved husbandry practices. Use of hybrids derived from *H. discus hannai* and *H. discus discus* seemed to provide added resistance (Handlinger and Zhengli, 2004; Zhang *et al*, 2004).

The major abalone species cultured in southern China is *Haliotis diversicolor aquatilis*, previously *supertexta*, or tokobushi. It originates from Taiwan. A major epidemic affected these animals in the late 1990's, causing up to 100% mortality on many farms in the southern provinces (Wang *et al*, 2004). The outbreak spread to Taiwan in 2003, costing the industry USD 11.5 million, according to the Taiwanese government (Yu-Tzu, 2003). At the time of the Fifth International Abalone Symposium, held in China later that year, it was reported that many farms in southern China and Taiwan were empty, due to failure of spat production and poor survival of stock. Viral particles were found in both the northern and southern outbreaks, but have not been completely characterised. Clinical signs are non specific, as tends to be the case with abalone diseases. More interesting is the histological finding of inflammation associated with the nerve ganglia. This was also characteristic of the Australian outbreak.

Mortalities in farmed abalone in the Australian state of Victoria were first seen in late 2005. The affected species were *Haliotis laevigata*, or greenlip abalone, *H. ruber*, or blacklip abalone, and hybrids of the two (Hardy-Smith, 2006a). By the middle of 2006, two abalone farms and two cage culture operations had been destocked and the disease was spreading through the wild population. It is still doing so now. With typical efficiency, the Australians rapidly identified a herpes like virus as the aetiological agent. On farms, the virus caused up to 90% mortality in affected tanks (Hardy-Smith, 2006b). The disease has had a significant financial impact on both the aquaculture and commercial fisheries sectors. Exact figures are not yet available, but suffice

it to say that Victoria's AUD 60 million commercial abalone fishery is threatened. Further research is being done to characterise the virus and attempt comparison with those found in Asia (DPIVic, 2007).

Moving on to Japan, one finds amyotrophia affecting *Haliotis discus*. The disease was first recognised in the early 1980's in cultured abalone, with evidence of a viral aetiology. A retro like virus was isolated from affected animals, but could not reproduce the disease, thereby failing to fulfill Koch's postulates. Affected animals suffer severe muscle atrophy, eventually leading to death. Mass mortalities have been reported in spat at several facilities on the west coast of Japan (Bower and McGladdery, 2003; Hara *et al*, 2004).

To date, there have been no viral diseases of abalone documented from other countries and none seen in South Africa. This will no doubt change as new diseases emerge and diagnostic techniques improve. The diagnosis of viral diseases in abalone is complicated by a lack of isolation methods, which forces reliance on electron microscopy for initial identification. Use of molecular methods is growing, but is at present more useful for research than routine diagnostics. As a result, viruses are generally only found when actively sought as potential causes of outbreaks. Routine surveillance has not detected any viruses of abalone and is not likely to do so anytime soon.

Bacterial diseases are as common in abalone as in any other farmed animal. The literature contains numerous papers on various bacterial infections affecting abalone, generally caused by bacteria in the genus *Vibrio* or closely related to this group (Elston and Lockwood, 1983; Nicolas *et al*, 2002; Li *et al*, 1998). These are almost exclusively opportunistic. The exception is withering syndrome. Withering syndrome results from infection by an intracellular bacterium, *Xenohaliotis californiensis*, of the Rickettsia family (Friedman *et al*, 2000; Moore *et al*, 2001; OIE, 2006). It was first discovered during the investigation of mass mortalities of *Haliotis cracherodii*, or black abalone, along the coast of California. Complete loss of black abalone populations occurred in some areas. The abalone show gradual atrophy of the foot and other organs, eventually leading to extreme weakness and inability to adhere to substrates. Bacterial inclusions are present in the gastrointestinal epithelium, including the digestive gland, and can be readily detected using standard histological sections (Friedman *et al*, 1997; Gardner *et al*, 1995). Various molecular techniques are available both for primary surveillance and confirmation

purposes (Andree *et al*, 2000 ; OIE, 2006). Withering syndrome was also found to affect a wide range of other abalone species, including *H. rufescens*, the red abalone, both in the wild and on farms. It has spread, apparently due to translocation of red abalone for culture purposes, to countries as far apart as Chile and Iceland, and now enjoys the status of an OIE listed disease (OIE, 2006). Thanks to the work of Friedman and others, *Xenohaliotis californiensis* is fairly well understood, facilitating efforts at control, at least in farmed abalone, and attempts to prevent further international spread. When the disease was first recognised in the early 1990's, it was coupled to high water temperatures and low food availability associated with the El Niño weather pattern. High water temperatures have since been found to be a prerequisite for the development of clinical disease in infected individuals. It is not known whether *Xenohaliotis californiensis* was previously present in the wild abalone population, emerging as a pathogen during El Niño conditions, or whether it was introduced from elsewhere (Friedman *et al*, 1997). To date, this disease has not been found in South Africa, although *H. midae*, the South African abalone, frequently contains intracellular inclusions of an unrelated bacterium (Mouton, 2000). Rickettsia like inclusions are common in molluscs and usually considered incidental when encountered during surveys. The example of withering syndrome suggests that it may be prudent to take these inclusions more seriously.

Fungal diseases of abalone may be broadly divided into shell and systemic infections. Shell infections occur primarily in New Zealand, affecting *Haliotis iris*, or black paua, and were the subject of a dissertation by Grindley (1997)(Grindley *et al*, 1998). Thus far, shell fungi have not impacted on abalone culture. Systemic infections, on the other hand, have caused significant mortalities in abalone held in recirculating systems. Abalone tubercule mycosis was described from *H. sieboldii*, or megai, in a holding facility in Japan and ascribed to *Haliphthoros milfordiensis*. *H. milfordiensis* appears to be something of a marine opportunist, having been isolated from various diseased crustaceans and other invertebrates (Hatai, 1982; Sparks, 1985). Another fungus, *Atkinsiella awabi*, has also been found to cause tubercule mycosis (Kitancharoen *et al*, 1994) and it is likely that further research will reveal more potential agents of mycoses. For the past two years, tubercule mycosis has been troublesome in South African abalone culture, primarily associated with recirculating systems. Affected animals show typical multiple areas of superficial necrosis on the foot and mantle. Mortalities may reach 90% in spat and 30% in older abalone. Outbreaks have been responsive to destocking and decontamination of systems.

A variety of protozoan parasites are known to occur in abalone, some associated with disease. Their taxonomy embraces change and no attempt at order is made in this overview. Instead, a geographic approach will be taken. Starting with the Americas, renal coccidia of abalone were first described from California (Friedman, 1991). These organisms were initially suspected to be the cause of withering syndrome in black abalone, before *Xenohaliotis californiensis* was recognised (Friedman *et al*, 1997; Gardner *et al*, 1995). At the time, the coccidia from California were described as *Pseudoklossia haliotis*, but have since been renamed *Margolisiella haliotis* (Desser and Bower, 1997). Similar organisms are found in almost all abalone growing countries, including South Africa, and there are differing opinions on their significance. Friedman considered renal coccidia in *Haliotis cracherodii* benign (Friedman *et al*, 1997). On the other hand, at the Sixth International Abalone Symposium held in Chile in 2006, workers from this country ascribed mortalities and poor production in cultured abalone to renal coccidiosis (Godoy and Aedo, 2006).

Labyrinthuloides haliotidis holds partial responsibility for failure of the only commercial abalone farming venture in Canada, during the 1980's. The parasite caused up to 100% mortality in cultured juvenile *Haliotis kamtschatkana*, or pinto abalone. Abalone larger than 25 mm were found to be resistant to infection (Bower, 1987a; Bower, 1987b). *Labyrinthuloides haliotidis* has not been reported from any other country and is not known to occur in South Africa. From the other side of the world, a similar tale emerges, but with a different parasite at centre stage. A haplosporidian infecting a commercial abalone farm in New Zealand caused mortalities of up to 90% in juvenile black paua over a sixth month period. This outbreak was reported in 2002 (Diggles *et al*, 2002). Subsequently, the haplosporidian has disappeared and surveys of cultured and wild abalone have failed to find it. For the time being, the outcome is more favourable than in the case of *Labyrinthuloides haliotidis*, but it is not possible to predict whether the haplosporidian will return or, perhaps, appear in a different country.

Although *Perkinsus olseni* (*P. atlanticus*) (Murrell *et al*, 2002) has a widespread distribution which includes Europe and Asia, it has only been reported as causing disease to abalone in Australia. *Perkinsus* is associated with clams in Asia, apparently not affecting abalone there. In Australia, *Perkinsus* primarily infects wild abalone and is associated with formation of pustules in the flesh, rendering the meat unfit for consumption. Declines in abalone populations in certain areas have been blamed on *Perkinsus*, notably greenlip abalone in South Australia. Blacklips show some

resistance, but act as carriers. The greater prevalence in wild than cultured abalone is an unusual feature of this disease. There have been only two outbreaks on farms, both due to the introduction of infected blacklip abalone. These passed the parasite to greenlips, with resulting mortalities of up to 40% (Handlinger *et al*, 2006). Perkinsosis of oysters, caused by *P. marinus*, deserves a brief mention. It has devastated culture of the Eastern oyster, *Crassostrea virginica*, in the United States of America, subsequently giving rise to a massive body of research and greatly contributing to our understanding of mollusc pathology in general (Ford and Tripp, 1996). No species of *Perkinsus* has ever been found in South Africa, in either abalone or bivalves, but, given the wide distribution of the genus and the dearth of local surveillance data, this does not conclusively exclude its presence.

There are a variety of other protozoa known to infect abalone, but which have not been associated with disease. Sessile peritrichous ciliates are frequently seen by all abalone pathologists, exciting little interest and few publications (Bower and McGladdery, 2003). These organisms are considered to be ectocommensals rather than true parasites, although an increase in their numbers may be a warning of deteriorating environmental conditions. This has been reported for fish (Lom, 1995). The sessile ciliate affecting abalone in South Africa, both *Haliotis spadicea*, the siffie, and *H. midae*, was the subject of a masters' dissertation by Heléne Botes (Botes, 1999). Perhaps the most remarkable feature of this ciliate, named *Mantoscyphidia midae*, is that it is itself parasitised by a ciliate, *Caliperia perlemoenae*. Whilst *Mantoscyphidia* uses the abalone as a platform for attachment, *Caliperia* attaches to *Mantoscyphidia*, exercising a stranglehold around the body of its host. Both ciliates filter feed. This association is described merely because it is wonderful, but it has no real relevance to issues of abalone disease.

Abalone are also infected by uncharacterised protozoa, encountered mostly during surveys. Protozoan parasites in the gastrointestinal system, including the digestive gland, are found in several countries, but have not been formally described. The most complete summary of the occurrence and distribution of these parasites is included in the final report of the Australian national survey of abalone health (Handlinger *et al*, 2006). It states that gut protozoa, which are cryptosporidial in appearance on histological examination, have been seen in both North and South America, as well as Australia and, of course, South Africa. A different protozoan, infecting the digestive gland, has been observed in Australia, New Zealand and South Africa. The report also mentions a third protozoan, infecting the oesophageal diverticula, which may be unique to

Australia. However, pathologists from other countries, such as the United States of America, have made mention of gregarines in the oesophagus, possibly representing the same or a closely related organism. None of these gastrointestinal parasites are associated with clinical signs of disease, but, when one considers the impact of similar parasites on terrestrial animal production, their presence merits further investigation. For example, coccidiosis, caused by protozoa of the phylum Apicomplexa, is considered one of the most important poultry diseases worldwide. The cost to the poultry industry of anticoccidial medication is approximately US$ 60 million per year in the United States of America alone (Jordan, 1990). Other economic impacts of the disease are more difficult to quantify.

Abalone are not greatly afflicted by multicellular parasites. Trematodes, seen from time to time in all molluscs, have been reported in abalone from various localities. In the Australian survey, a high prevalence of trematodes was found in Western Australia, but not in other areas. The trematodes caused some localised tissue reaction related to their migration, apparently without significant impact on the overall health of the host (Handlinger *et al*, 2006). Heléne Botes discusses trematodes in her dissertation, as trematode infection was highly prevalent in her study populations of *Haliotis spadicea*. However, she does not attempt a formal description and the species identity remains unknown (Botes *et al*, 1999). Although Botes did not find trematodes in her samples of *H. midae*, infections do occur, as will be seen.

The shells of abalone are as subject to infestation by parasites as are the coats of mammals. The main difference is that shell parasites do not generally impact directly on the soft tissues of the host. Most shell parasites only utilise the shell as a home and are perhaps more correctly described as ectocommensals. These include the sabellid polychaete, *Terebrasabella heterouncinata*, originally from South Africa and a famous export (McBride, 1998; Simon *et al*, 2004), now found in many abalone growing regions of the world, including the Americas. Molluscs in general are prone to shell damage caused by boring polychaetes and abalone are no exception. Shell borers have been a problem in abalone culture in places as diverse as South Africa (Simon *et al*, 2006), Australia (Lleonart *et al*, 2003) and Chile (Aviles *et al*, 2006). Abalone shell conditions were not included in the present study, but a large body of data exists and could perhaps form the basis for future epidemiological analysis.

1.3 Surveys of abalone diseases

There have been relatively few systematic surveys of abalone diseases in the world. Much of the information summarised above resulted from investigations of specific disease outbreaks. In at least two cases, surveillance was a fortunate byproduct of the outbreak. The significant decline in black abalone populations in California in the late 1980s led to sampling and examination of a large number of abalone in the search for a cause. In the process, a new disease, withering syndrome, and its aetiological agent, *Xenohaliotis californiensis*, were described (Friedman *et al*, 1997; Moore *et al*, 2001). Serendipitously, renal coccidia of abalone were also discovered and characterised (Friedman, 1991). Subsequently, much work has been done on withering syndrome and this has included ongoing surveillance of black and other abalone, in the wild and on farms. The haplosporidian outbreak in New Zealand led to extensive surveillance, in an attempt to identify infected populations and possible reservoirs. Although the parasite has eluded pathologists, the surveys have contributed important data on the health of New Zealand abalone, both farmed and wild (Diggles and Oliver, 2005).

Modest surveys have been reported from other abalone growing countries. Workers in Baja California, which lies in Mexico, just south of California, examined 51 abalone and 313 empty shells from a farm. The farm held red abalone which were progeny of animals imported from California (Cáceres-Martínez and Tinoco-Orta, 2001). Increasing culture of abalone in Chile has been accompanied by systematic surveillance of farms (Godoy and Munoz, 2003). Although many of the results were presented at the Sixth International Abalone Symposium, held in Chile in 2006, the proceedings have not yet been produced. The situation is similar for most abalone producing countries, especially those in Asia. Data have been generated from investigations of disease outbreaks and do not form part of a formal survey, but nonetheless contribute to our knowledge of abalone pathology, or would, if published in accessible form.

The outstanding example of a formal survey of abalone health was conducted in Australia with funding from the Fisheries Research and Development Corporation, under their abalone aquaculture subprogram. The final report of this survey was published in 2006 and contains detailed results, as well as excellent information on findings from other countries. Wild and farmed abalone were systemically sampled in all states where they occur. A total of 3163 abalone were examined and invaluable capacity built in the process. Besides the obvious benefit

of establishing the health status of abalone, an important outcome of the survey included familiarisation of a large number of pathologists with normal and pathological anatomy of abalone. This makes it more likely that sampling by farmers and divers will continue in future, as they have a realistic expectation of a useful result. It was also hoped that creation of capacity would encourage growers to set up herd health programs, thereby also maintaining an informal surveillance system (Handlinger *et al*, 2006).

A formal survey of abalone health, encompassing all farms and including wild abalone, has never been done in South Africa. One is currently underway, funded by the Department of Science and Technology and administrated by Marine and Coastal Management. However, South Africa has for many years had a herd health program for abalone producers and this has generated the largest body of data on abalone disease occurrence in the world (Mouton, 2000). Unfortunately, as a surveillance mechanism, the program has several shortcomings. Participation is voluntary, therefore coverage is patchy. Farms in more remote areas are very poorly represented in the data set. In addition, the aim of the program is to promote herd health in the sense of optimising production, not to generate epidemiological data, and this is reflected in the sampling strategy. In spite of this, it was felt that analysis of the data set could provide some insights into the epidemiology of parasites in farmed *Haliotis midae*, as well as informing better surveillance techniques for the future. Although this work deals specifically with data obtained from farmed abalone, insight into the disease status of wild abalone would have been helpful. Data on parasite prevalence in wild abalone could inform interpretation of farm data, for example when comparing parasite prevalence amongst areas.

1.4 Abalone farming in South Africa

Abalone farming is the success story of South African aquaculture. Much has been said about the potential for aquaculture development in South Africa, but in truth it is always likely to be limited. The country is relatively arid and demands on available freshwater resources are high, which is not encouraging for freshwater aquaculture. Farming of valuable species such as trout is economically feasible, but environmental conditions dictate that the industry, although well established, will remain small. The climate is better suited to warmwater species such as sharptoothed catfish. Unfortunately, locally produced catfish cannot compete on price in the international market and there is no domestic market. More examples can be sited, but perhaps it is sufficient to state that the entire sub Saharan African region contributes 0.16% of the world's aquaculture production by quantity. This figure includes marine aquaculture (FAO, 2006). The coastline of South Africa is not ideal for aquaculture, offering very few sheltered sites. Invariably, there are competing uses for these sites, notably from shipping and recreation. Marine aquaculture is currently focussed on land based operations, which are capital intensive, technologically demanding and expensive to run. Economics dictate that such farms must produce high value species, of which abalone are the prime example. It is interesting that the sub Saharan African region contributes 0.16% of the world's aquaculture production by quantity, but 0.36% by value. The only mention of South Africa in the whole of the World Review of Fisheries and Aquaculture is in relation to abalone (FAO, 2006).

South Africa is the largest producer of farmed abalone in the world outside Asia. This production is entirely based on one species, *Haliotis midae*. There are approximately sixteen farms and their output is rapidly approaching 1000 tonnes per year, which will generate around ZAR 173 million in income at farmgate. Virtually all this abalone is exported to Asia, either live or in processed form, of which the most important is canned abalone. According to a recent study, the abalone industry employs 1386 people directly and many more in related industries, such as feed production and supply. Many abalone farms are in underdeveloped coastal areas and their impact on local economies is greater than may be expected judging from employment figures alone (Troell *et al*, 2006). The abalone industry is officially represented by the Abalone Farmers' Association of South Africa, or AFASA. AFASA acts as spokesbody to government, more specifically Marine and Coastal Management of the Department of Environmental Affairs and

Tourism. AFASA has also been active in promoting research and other activities which benefit industry. Amongst these is the abalone health management program, available to all members.

As mentioned before, the abalone health management program focuses on herd health with the aim of improving production. However, it was originally started as a surveillance program. The abalone industry wished to enter the European market and required surveillance data for meeting export requirements. The program was originally based at the Onderstepoort Veterinary Institute in Pretoria and the first samples were received in 1998. It was managed by the author, who also viewed all histological sections and communicated results to the farms. In 2001, the author and the program moved to the western Cape and joined the Department of Agriculture. This improved access to participating farms, which are concentrated in the western Cape. During the following year, the abalone industry made the decision to adopt the program in partnership with Marine and Coastal Management. Inevitably, there was some disruption associated with each move and this is reflected as gaps in the data set generated by the program.

Although there have been changes in production technology over the years, the basics of abalone farming have remained constant. Abalone farming in South Africa is almost exclusively land based. The abalone are housed in tanks on land and seawater is pumped from the ocean through the tanks. Tanks contain baskets, premoulded or made from oyster mesh, in which the abalone are confined. As abalone adhere to substrate, the available surface area in the baskets is enlarged by provision of plastic inners. The majority of tanks are aerated by blowers connected to airlines. Aeration helps to ensure good water circulation. In flow through systems, there is constant replacement of water in the tanks with new seawater. Water leaving the tanks returns to the sea. Recirculation systems have minimal water replacement and rely on treatment and reuse of water. Treatment is necessary to restore oxygen levels, and to remove suspended matter and dissolved toxic metabolic byproducts such as carbon dioxide and ammonia. Removal of particulates can be done by various mechanical filters, whereas dissolved compounds are taken out by mechanical and biological filtration. Biological filtration relies on the action of bacteria to convert toxic ammonia to relatively harmless nitrates. Most of the South African farms use flow through systems.

There are essentially two feed options open to abalone farmers, namely artificial feed or a natural diet. Artificial feed is produced locally in the form of Abfeed and can also be imported from Australia or Taiwan. Abfeed is by far the most commonly used artificial feed in South Africa. Wild abalone eat macroalgae and many farms rely on a natural diet, primarily of *Ecklonia maxima*, commonly known as kelp. This is harvested from the sea. It is possible to culture certain types of macroalgae and some abalone farms make extensive use of cultured seaweeds, either *Ulva* or *Gracillaria*, as feed. This practice originated on the East coast, where kelp does not occur and high temperatures preclude the use of artificial feed in summer. Although farms in the western Cape have now also started using cultured seaweeds, this was not prevalent in the period reflected in the current study.

The essentials of abalone husbandry are feeding, cleaning and splitting. Feeding frequency, amount and system are all affected by type of diet. In short, kelp is fed less often, in larger amounts and straight into the basket. As kelp is harvested, availability may be affected by weather conditions at certain times of the year. Whereas abalone can climb onto the kelp to eat it, artificial feed must be presented on some form of horizontal surface or placed into the bottom of the basket. In contrast to macroalgae, which continues to live in the basket, artificial feed spoils fairly rapidly, necessitating smaller feeds at shorter intervals. It also has a higher energy density, so animals consume less. The perception that artificial feed causes a deterioration in water quality has led to more regular cleaning of tanks where animals receive artificial feed than those with macroalgae.

Cleaning of abalone is simple. The tank is drained, rinsed and refilled. This is usually accompanied by brushing down the sides and base and, in some cases, spraying off the baskets of animals. Some farms move baskets from tank to tank when cleaning, as it is easier to clean an empty tank than one containing baskets. It also helps to reduce the time that the abalone are out of the water. On the other hand, it requires availability of empty tanks and increases opportunities for transfer of potential pathogens. Splitting refers to reduction in density of abalone in a basket. The animals in a basket are removed and redistributed, so that there are fewer in each basket. This may be accompanied by size grading or sorting, which separates animals of different sizes to reduce competition and facilitate husbandry. Although abalone grow relatively slowly, splitting is necessary roughly every four to six months. If splitting is not done regularly, the animals become overcrowded, growth slows and shell quality deteriorates.

All farmed abalone in South Africa are captive bred. Most of the parent stock are wild caught and some have been on the farms since inception. There is some replacement of brood stock every year, but animals which perform well will tend to remain on the farm. Although the hatchery production of abalone spat is a fascinating subject, it will not be discussed here, having little relevance to the subject of this dissertation. The important point is that none of the animals considered in this study would ever have spent time in the wild. Most farms have their own hatcheries and produce their own spat. However, there are some which rely on purchases. A continual discrepancy between hatchery supply and farm demand on various farms has led to much buying and selling of abalone, both spat and older animals. Stock movements have become more frequent as the industry grows and virtually all farms can be said to have bought in animals at some stage.

Abalone farming is not a seasonal activity. Animals are produced continuously in the hatchery and move every month from the nursery to the ongrowing section of a farm. Harvesting generally occurs every week and is partly driven by market demand. Harvesting for processing relies less on market demand than harvesting for live exports.

1.5 Objectives of this study

The abalone health management program has gathered data on the occurrence of several parasites infecting South African abalone, together with information on host and environmental factors. It was felt that this data could be usefully analysed. The objectives of this study were:

1. to describe the prevalence of these parasites on farms participating in the abalone health management program

2. to determine the relationship between infestation with soft tissue parasites of *Haliotis midae* and the following factors:

- age
- mass
- growth rate
- condition index
- sex and degree of gonad development
- origin
- seasonality
- diet
- system

3. to establish a baseline for the health of South African abalone on farms participating in the health management program

4. to identify risk factors for parasite infection and formulate recommendations for mitigation

5. to identify areas for further research into the biology of parasites.

Chapter 2
Materials and Methods

2.1 Data collection

The samples from each farm were selected to include the various age groups present on the farm in ongrowing facilities. Abalone in the hatchery and nursery phase of production were not included in these data. As abalone typically remain in the hatchery and nursery phase until they are approximately 15 mm in shell length, the youngest abalone submitted were eight months old. In most cases, a group of ten animals were sent for each cohort that was sampled. Cohorts were generally sampled repeatedly during their lifetimes.

Data for abalone submitted as part of the abalone health management program during the period 1 January 2000 to 31 December 2004 were analysed. There were ten abalone farms participating in the program during this time, but data from one were excluded because the farm could not provide correct ages. The ages were identified as incorrect due to a large disparity between the stated age and the measured size of the abalone. The remaining nine abalone farms are not equally represented in the data set. These farms are located on the southern and western coasts, as shown in figure 2.1.1.

Figure 2.1.1:
Position of abalone farms sampled for this study. Cape Town is given as reference point.

Abalone were submitted live. The abalone were placed in plastic bags with added oxygen and then packed in polystyrene boxes. Ice sheets were included in the boxes to maintain a low temperature. Boxes were transported to the laboratory and animals dissected within two to 36 hours of collection. In almost all cases, the animals were alive when received for dissection. Animals that were dead and showing significant decomposition were not included in these data. Losses were almost exclusively due to poor packaging or problems with couriers.

A sample of abalone received from a single culture facility on one day was assigned a unique case number which identified the entire sample. Each individual abalone was also numbered. Therefore, all data relating to a single animal, including data pertaining to the sample to which it belonged, carried a unique combination of case and animal number. Data were recorded on standard forms by laboratory personnel or in laboratory notebooks by the pathologist.

The shell length of each abalone was measured using a vernier caliper and the value recorded to 0.1 mm. The animal was then weighed to 0.1 g. Thereafter, the abalone was shucked, in other words, removed from its shell, using an oyster shucker. A shucked abalone is illustrated in figure 2.1.2, which demonstrates the standard set of five tissue sections collected from each abalone. This set will provide adequate representation of all the major organ systems. Shell mass was determined to 0.1 g.

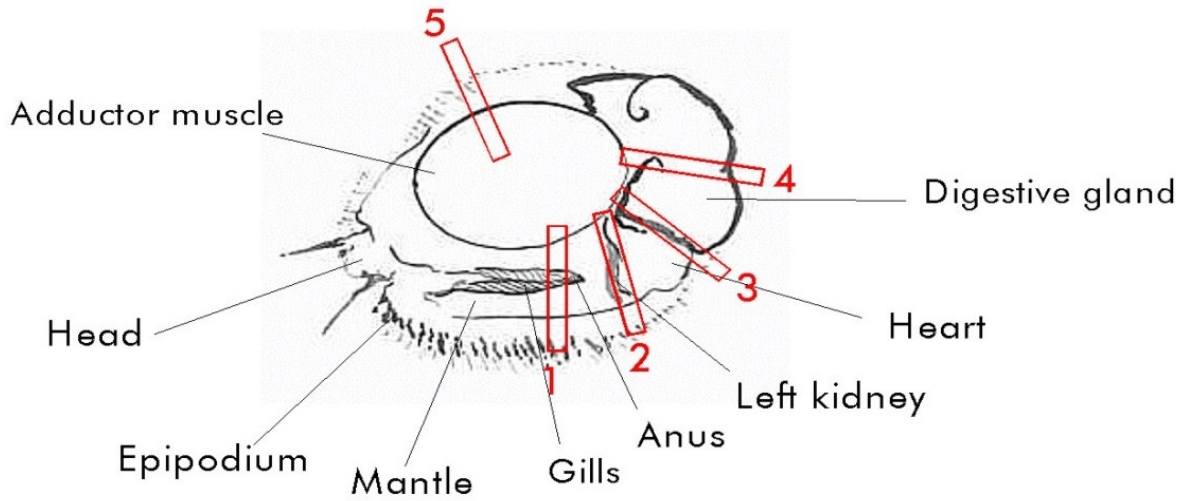

Figure 2.1.2:

Shucked abalone showing five standard tissue sections collected for histological analysis

The tissue sections were placed in plastic histological cassettes and fixed in Davidson's solution for marine invertebrates. After fixing for 36 to 48 hours, the cassettes were removed and postfixed in 70% ethanol. The samples were then processed by serial dehydration through graded ethanol concentrations and clearing with xylene or toluene, before impregnation with paraffin wax. Impregnated samples were embedded in paraffin wax prior to sectioning. These are standard histological techniques (Austin and Austin, 1989).

Tissue sections of 5 to 6 µm were cut with a microtome and adhered to glass slides. All sections were stained with Harris's haematoxylin and eosin. No other stains were used for this study. The sections were examined under light microscopy to determine the presence or absence of soft tissue parasites, illustrated in figures 2.1.3 to 2.1.11. In most cases, all the tissue samples from an individual abalone are fitted onto one section. Very large abalone may require tissue samples to be placed in two or three cassettes, resulting in two or three sections. The entire section is examined. The parasites are relatively large and easily visible at 100x to 200x magnification.

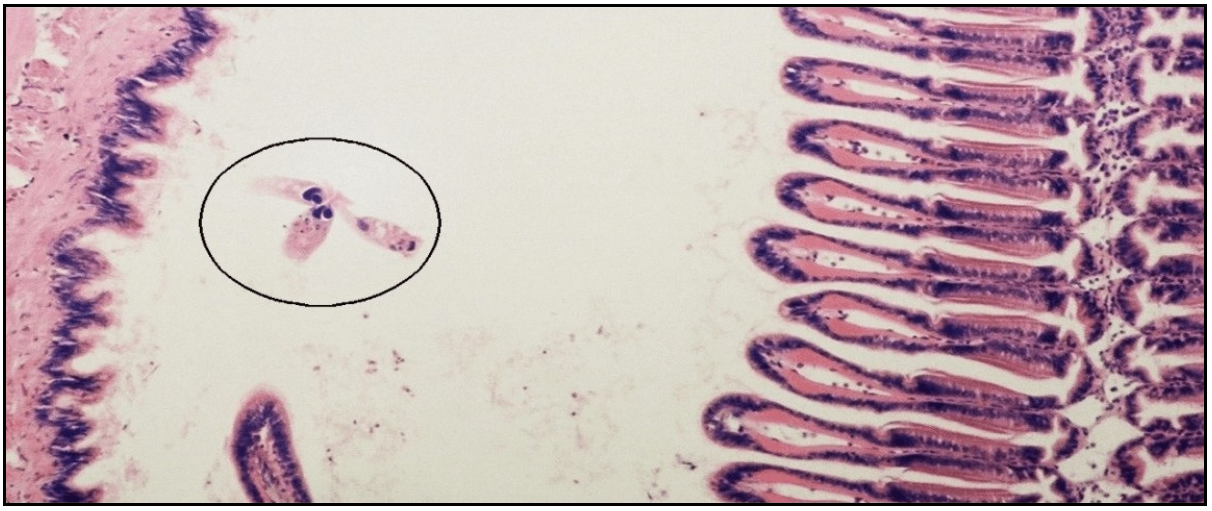

Figure 2.1.3: Sessile ciliates in mantle cavity of abalone. Circle includes both *Mantoscyphidia midae* and *Caliperia perlemoenae*

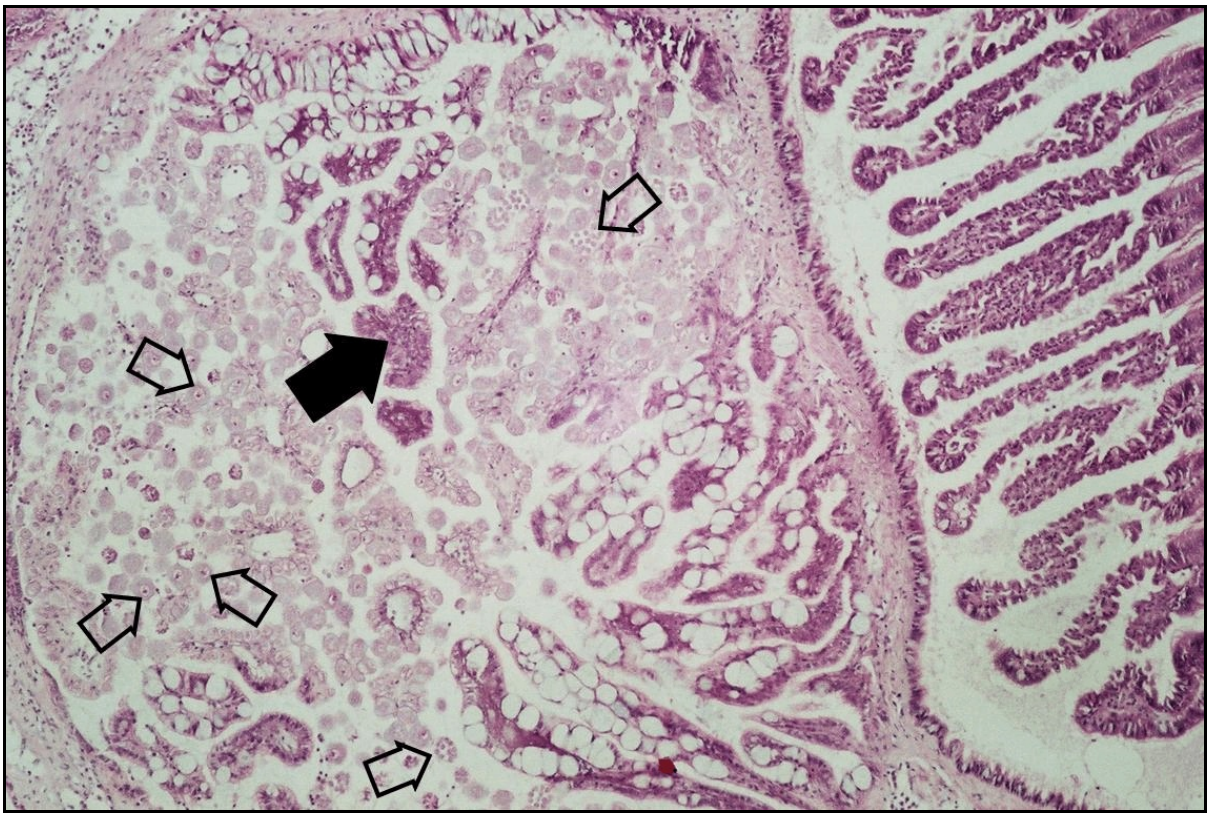

Figure 2.1.4: Renal coccidia in left kidney of abalone. Open arrows point to examples of parasites and solid arrow indicates remaining normal kidney tissue. Mantle cavity with gills lie to right.

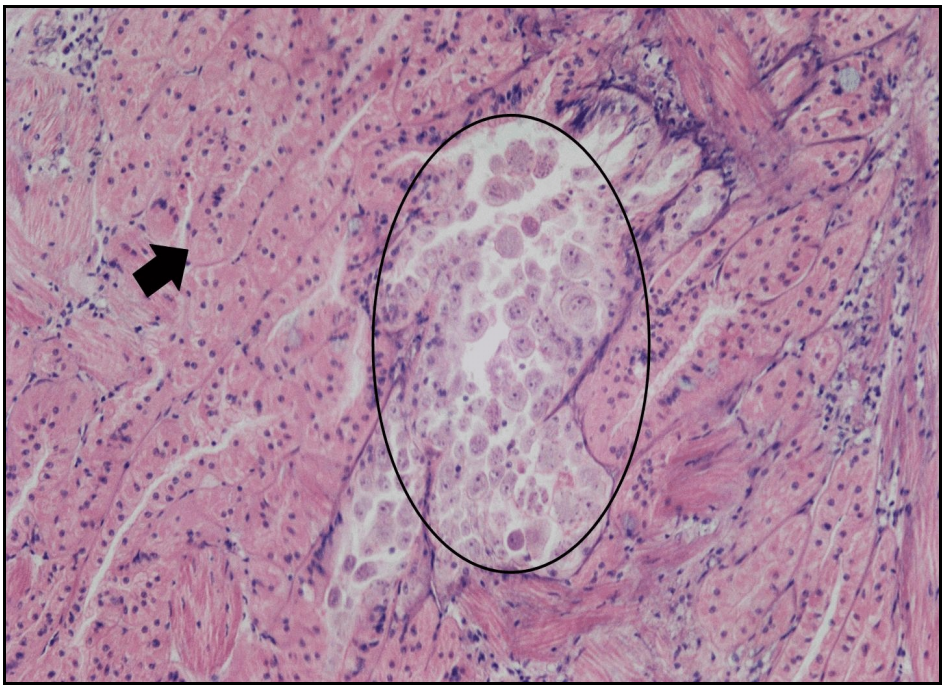

Figure 2.1.5: Renal coccidia in right kidney of abalone. Parasites are circled and arrow indicates normal uninfected tubule.

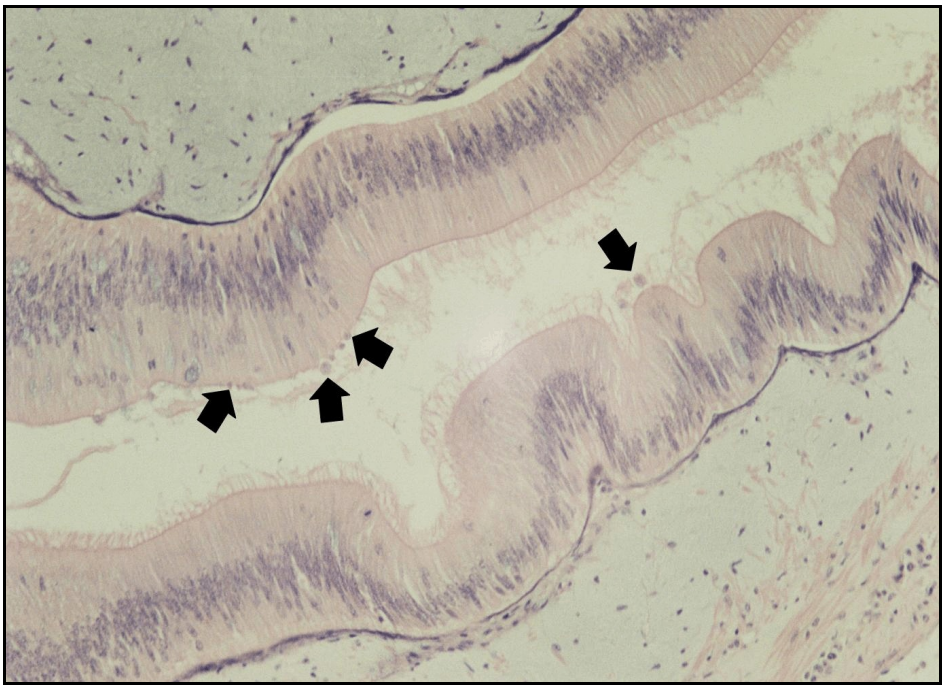

Figure 2.1.6: Small protozoa attached to intestinal epithelium of abalone. These are believed to be infective stages.

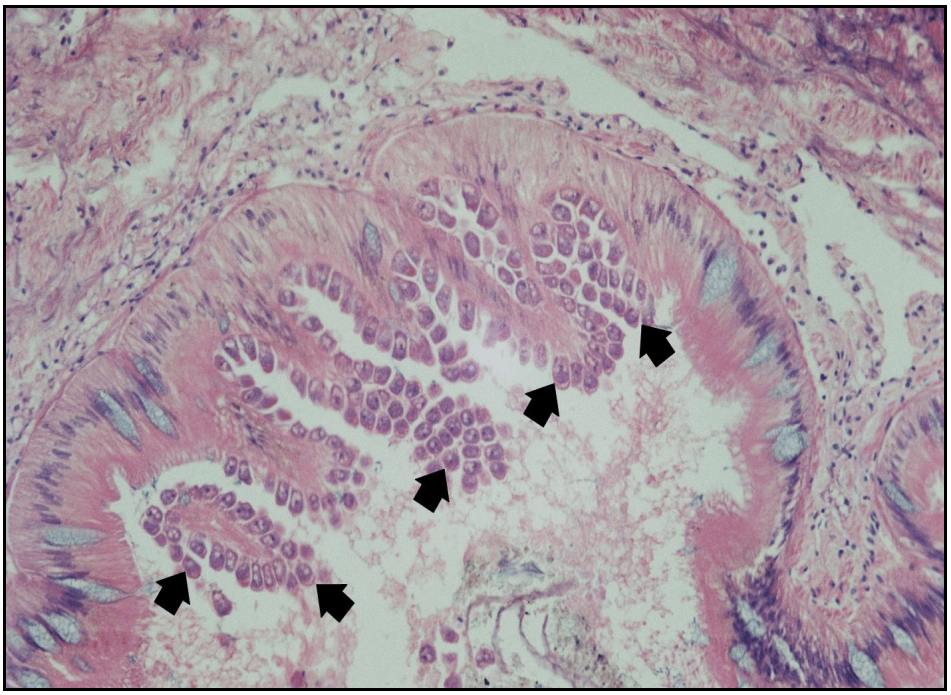

Figure 2.1.7: Gut protozoa attached to abalone intestinal epithelium. These are believed to be the same parasites as in the previous figure.

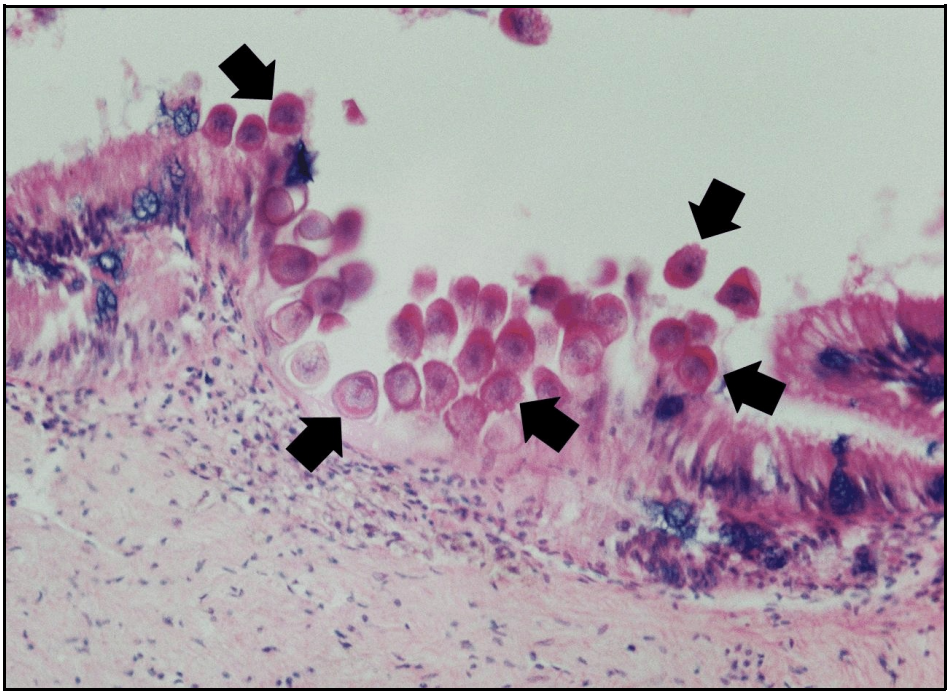

Figure 2.1.8: Large protozoa attached to abalone intestinal epithelium. These are believed to be the mature parasites.

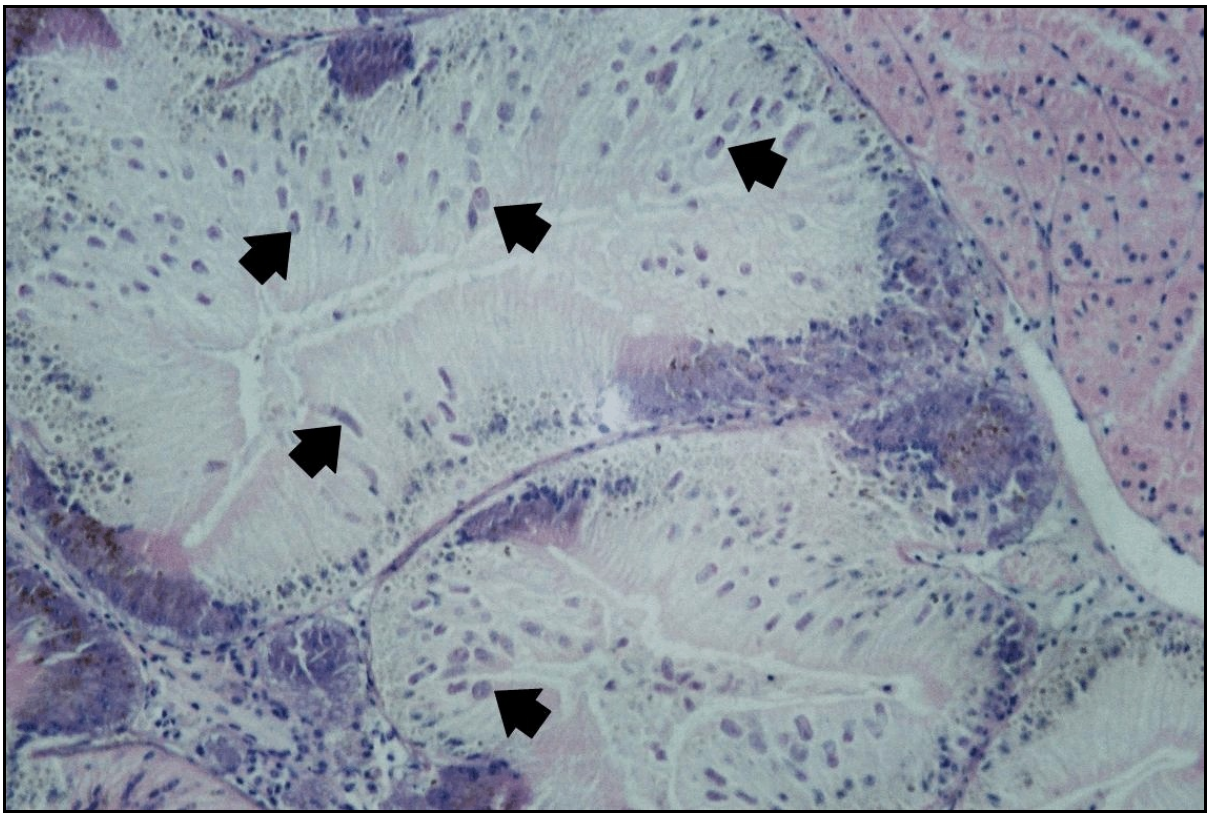

Figure 2.1.9: Protozoan parasites in digestive gland of abalone. Arrows indicate examples of parasitic cells.

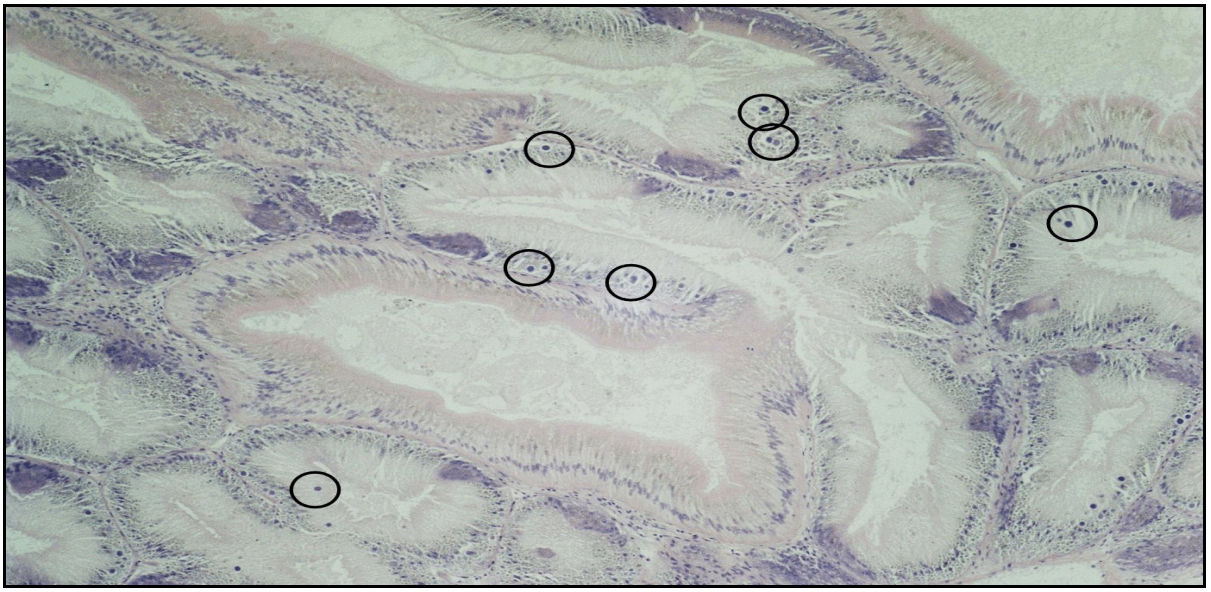

Figure 2.1.10: Rickettsia like prokaryotes in digestive gland of abalone.

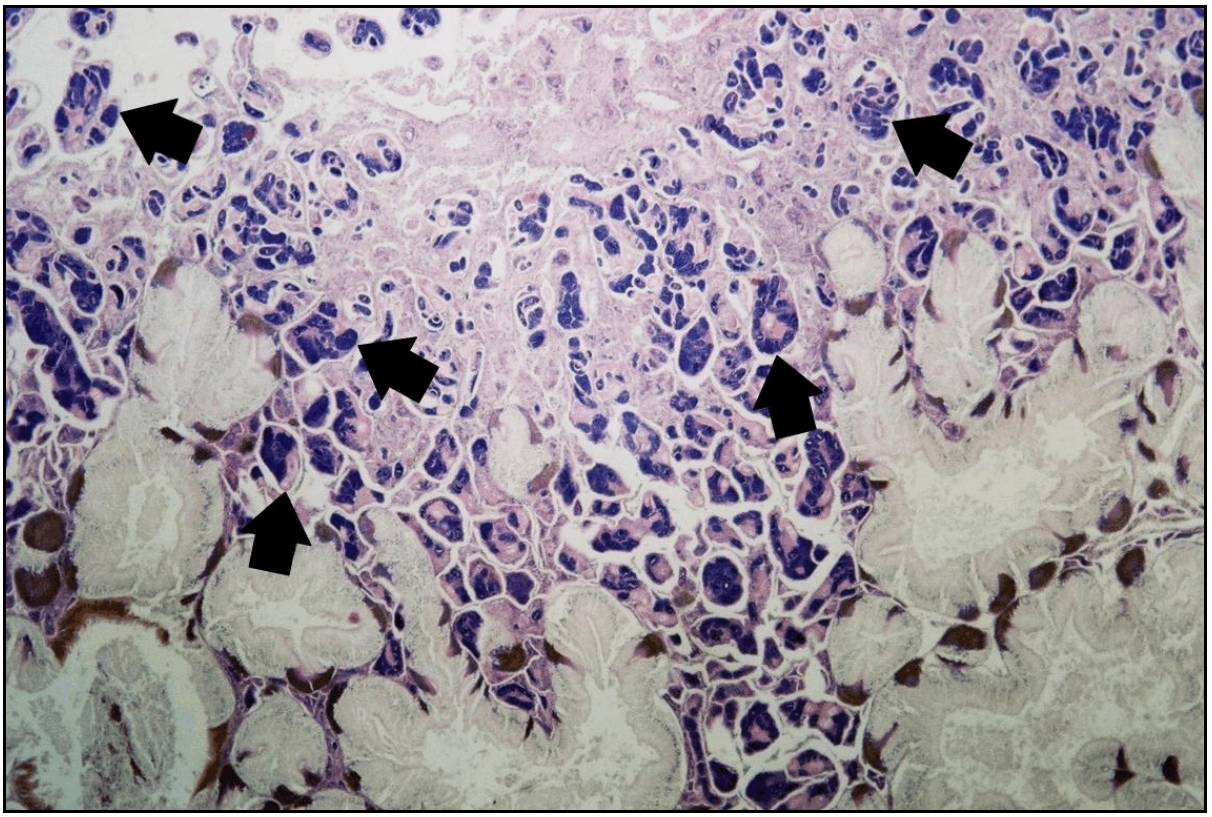

Figure 2.1.11: Trematodes replacing abalone digestive gland tissues. Arrows indicate examples of individual parasites.

The sex and degree of gonad development were recorded, when gonad development was present. Gonad development was scored according to the number of mature sex cells relative to immature sex cells, as shown in figures 2.1.12 to 2.1.15. In each case, male gonad is depicted on the left and female gonad to the right.

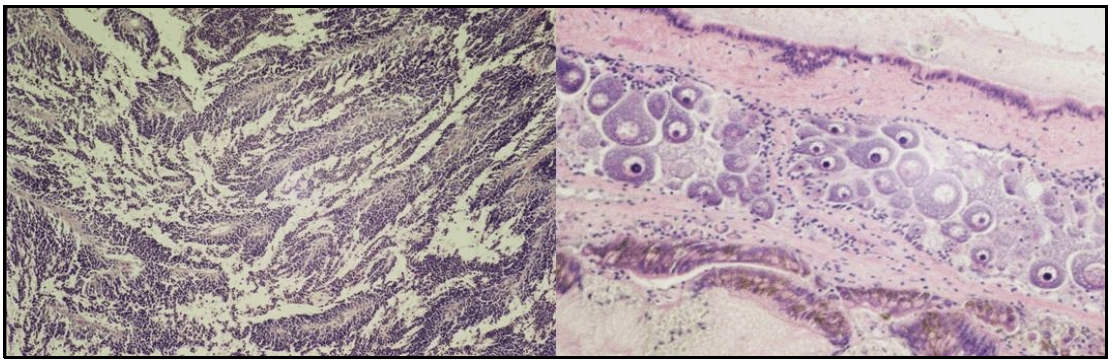

Figure 2.1.12: Immature or stage 1 gonads

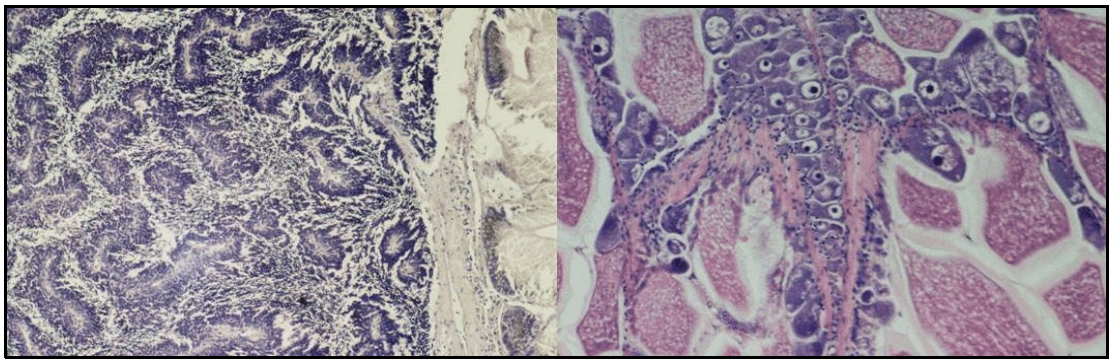

Figure 2.1.13: Moderately developed or stage 2 gonads

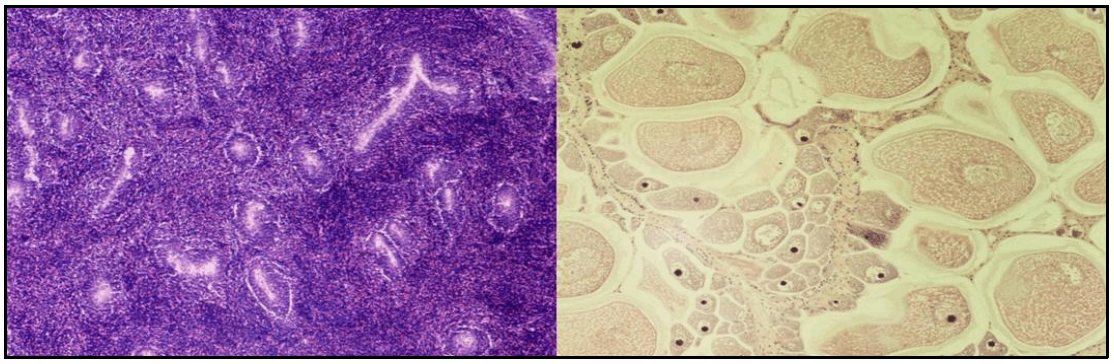

Figure 2.1.14: Moderately developed or stage 3 gonads

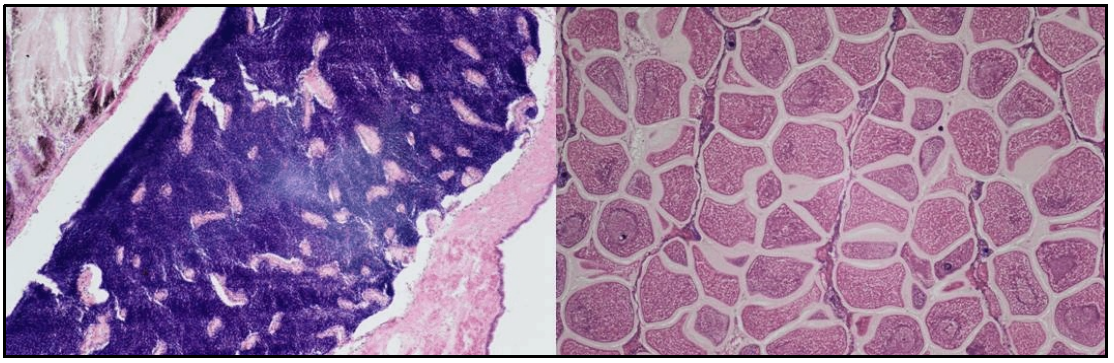

Figure 2.1.15: Mature or stage 4 gonads

The only abalone species considered in this study was the South African abalone or perlemoen, *Haliotis midae*. All abalone were received from abalone culture facilities. No wild abalone were included in these data. Data on the age of the abalone were obtained from the farms. In some cases, the exact date of spawning is known for a cohort. In other cases, the age is estimated from the date when the abalone left the nursery to enter the ongrowing phase of production. All ages were converted to a number of months, with fractions rounded up to whole months. Information on the diet was also obtained from the farm. Diets were categorised as artificial feed, kelp or a mixed diet containing both. A small number of animals received other macroalgae and these were classed as kelp for the purposes of the analyses.

All abalone included in this study were cultured in land based systems. Seawater is supplied to these systems by pumping from the ocean. The majority of the abalone were kept in flow through systems, in which the seawater in the tanks is continuously replaced by new seawater. Some farms utilised recirculation systems. In recirculation, a large portion of the water is treated to remove abalone waste products and is then reused. Treatment generally entails a combination of biological filtration and physical processes such as foam fractionation. As the type of system may affect parasite prevalence, this was included in the data set.

The data for each individual abalone were captured in an Excel (Microsoft Corporation) spreadsheet under the headings shown in table 2.1.1. Initially, data from 18 693 animals were captured. All animals for which the data were incomplete were then removed. The majority of these were animals of unknown age or diet. The relationships between the presence of the various tissue parasites and host and environmental factors were then investigated in the remaining 12 950 abalone.

Table 2.1.1: Data fields

Host factors	Environmental factors	Parasites
Age in months	Farm of origin	Sessile ciliates
Shell length	Date of sampling	Renal coccidia
Total mass	Diet	Gut protozoa
Shell mass	Recirculation or flow through	Digestive gland protozoa
Sex		Rickettsia like prokaryotes
Gonad development		Trematodes

2.2 Methods of data analysis

Once data had been captured in Excel, a series of tables was constructed. Essentially, these comprised counts of infected and non infected abalone for all the host and environmental factors listed above. Where appropriate, these factors were stratified. Charts of the tables were drawn using Excel. Several charts were constructed for each variable with the aim of finding the simplest chart, usually with the fewest data categories, that clearly illustrated the prevalence pattern. This made the data more accessible and facilitated identification of trends. Where host and environmental factors appeared to interact, these data were also tabulated and charted. Statistical analyses of the data in Excel followed. All analyses were performed for sessile ciliates, renal coccidia, gut protozoa, digestive gland protozoa and rickettsia like prokaryotes. For renal coccidia, data on left kidney and right kidney infestations were analysed separately. This was not done because of any evidence to suggest that coccidia infecting left and right kidneys are not the same species. Rather, it was to investigate striking differences in prevalence patterns noticed during routine viewing of abalone sections. Trematode infections are rare. Only seven cases were identified in this study. As the chi square test is not considered valid when expected frequencies are less than 1.0, as in the case for trematode infections, trematodes were omitted from this analysis. Overall parasite prevalences were calculated by dividing the total number of positive animals by the entire sample population.

To investigate the associations between parasite prevalences and host and environmental factors, either the chi square test or odds ratios may be utilised. Both were employed in this study. The chi square test is used to determine whether two random variables are independent of each other. It does not provide a measure of the extent of the association, if one exists. Where sample sizes are large, significant chi square values may be found even with weak associations (Gummow, 2000). Effect sizes with confidence intervals were calculated from significant chi square values to provide some indication of the strength of associations (Steyn, 2005). Effect sizes of approximately 0.1 were considered small, 0.3 medium and 0.5 large (Steyn, 2005).

Odds ratios have the advantage of both identifying significant associations and giving an indication of the strength of the association. However, odds ratios can only be applied to variables which may be presented as a two by two table. When confounding and interaction

were suspected, it was necessary to calculate stratum specific odds ratios. To do this, the data were stratified and odds ratios calculated for each stratum according to the same formula that is used to calculate the crude odds ratio. Where the stratum specific odds ratios differed from the crude odds ratio, a summary odds ratio was calculated to reduce the potential effect of confounding variables. The summary odds ratio used in this study was the Mantel Haenszel summary odds ratio. As stratum specific odds ratios for certain parasites not only differed from the crude odds ratio, but also from one another, the Breslow Day test for interaction was performed. Confidence intervals were determined using the method of Woolf (Gummow, 2003; Thompson, 2004).

The association between growth rate and parasite infection was further investigated by comparing growth rate in animals with and without parasites. Growth rate was treated as a continuous variable. As size was known to impact on both growth rate and parasite prevalence, these analyses were also performed separately for animals up to 24 months of age and those older. When the data was normally distributed with equal variances, a two sample t-test was used to compare means. When data was not normally distributed or variances were unequal, the nonparametric Kolmogorov-Smirnov test was used (Hintze, 2001). These analyses were conducted using NCSS software (NCSS, Kaysville, Utah).

2.2.1 Host factors

2.2.1.1 Age

The population was divided into various age categories to chart parasite prevalence according to age. Parasite prevalences were determined for each soft tissue parasite in animals of 9 months and less and classes of three months thereafter, up to a maximum of 75 months. This was repeated for larger intervals, with the greatest being one year, to find the simplest representation of the data. Final parasite prevalence was determined for animals of 12 months and less, 13 to 24 months, 25 to 36 months, 37 to 48 months, and 49+ months. A similar approach was used throughout this study for stratification of variables.

In each case, prevalence was calculated as

$$\frac{\text{number of infected animals in age class}}{\text{total number of animals in age class}}$$

and expressed as a percentage.

Charts were constructed in Excel from the resulting tables. The relationship was explored further using both the chi square test and odds ratios. Effect sizes were calculated for all significant chi square tests.

To examine the data using odds ratios, it was necessary to divide the population in two age categories. An odds ratio was calculated for parasite prevalence in animals of 24 months and younger, and those older than 24 months. This was then repeated for animals of 36 months and younger, and those older than 36 months. Confidence intervals were determined for all odds ratios. As larger odds ratios were obtained for the 24 month partitioning, this was used for all subsequent calculations.

2.2.1.2 Size

2.2.1.2.1 Total mass

Abalone size can be measured as either total body mass and shell length. Total body mass includes shell. The population was divided into various mass categories to chart parasite prevalence according to size. Parasite prevalences were determined for each soft tissue parasite in animals of 0 to 5 g and classes of five grams thereafter, up to 90 g. Animals of more than 90 g were grouped in one class. This was repeated for larger class intervals, with the greatest being 15 g. In other words, parasite prevalence was determined for animals of 0 to 15 g, 16 to 30 g, 31 to 45 g, and so forth, up to 75 g. Charts were constructed in Excel from the resulting tables. Chi square testing was done to test the significance of the associations between parasite prevalences and total mass.

2.2.1.2.2 Growth rate

Shell length is commonly used to calculate growth rate, using the formula:

$$\frac{\text{shell length in mm}}{\text{age in months - 1}}$$

The resulting figure of mm per month is used to measure performance throughout the abalone industry. The population was divided into various growth rate categories to chart parasite prevalence according to performance. Parasite prevalences were determined for each soft tissue parasite in animals of 0.4 mm per month up to 3.2 mm per month, in increments of 0.1. This was repeated using increments of 0.4. Charts were constructed in Excel from the resulting tables. The significance of the association between parasite prevalence and growth rate was evaluated using the chi square test. Effect sizes were calculated for all significant associations. Growth rates of animals with and without parasites were also compared using either a two sample t-test or the Kolmogorov-Smirnov test.

2.2.1.2.3 Condition index

The relationship between condition index and parasite prevalence was examined. The condition index used was calculated according to the formula:

$$\frac{\text{total mass in g}}{\text{shell mass in g}}$$

The population was divided into condition index classes with intervals of 1.0, starting at less than 3.0 and progressing to greater than 7.0. The prevalence of each soft tissue parasite was charted according to condition index. As condition index is affected by age and diet, this was also charted. The significance of the association between parasite prevalence and condition index was evaluated using the chi square test. Condition index is known to be affected by age. A x y scatter plot was created to investigate the association. The trend line and R^2 value were generated by Excel. A chart was also included showing condition index distribution for animals receiving different diets.

2.2.1.3 Sex

Abalone were classified according to gender as male, female or none, where none were those animals showing either no gonad development or insufficient development to allow differentiation of sex. Animals with gonad development were scored as shown in table 2.2.1.3.1. None in this context refers to none detectable histologically. Results of classification of sex and gonad development of the sample population were presented as pie charts.

Table 2.2.1.3.1: Scoring system for gonad development based on histological appearance

Score	Label	Description
0	None	No visible gonad
1	Immature	Gonad has no mature sex cells
2	Moderate	Gonad has few mature sex cells, but immature cells predominate
3	Moderate	Gonad has predominantly mature sex cells, but immature cells are still present
4	Mature	Gonad filled with mature sex cells

The prevalence of soft tissue parasites in different sexes and stages of gonad development was determined from the data and charted. On histological examination, it may be more difficult to distinguish an immature male gonad than an immature female gonad, leading to the inclusion of immature male animals in the none category and a relative overestimation of the number of female animals. Therefore, a separate analysis was performed with all stage 1 gonads included with nones as immature animals. Stages 2 and 3 were combined in the moderate category and stage 4 retained as mature.

The relationship between sex and parasite prevalence was explored using chi square testing. Chi square values were calculated by cross tabulating sex and presence or absence of parasites. The analyses were repeated comparing mature gonads in both sexes to other stages, as it is possible that animals with mature gonads are more prone to parasite infestation. Effect sizes were calculated for all significant chi square values.

2.2.2 Environmental factors

2.2.2.1 Origin

To protect the confidentiality of the farms, each was assigned a letter. The prevalence of each parasite was first charted per farm and a chi square test was then performed to test the significance of observed differences. Effect sizes were calculated for significant chi square values.

As can be seen from figure 2.1.1, the abalone farms sampled in this study fall in two broad geographic zones, the southern and western parts of the southwestern Cape coast. These areas differ due to variations in oceanic currents and climate. Therefore, the prevalence of each parasite was charted according to South and West coasts and the chi square test performed to test the significance of the observed differences. Effect sizes were calculated where appropriate. A chart was included showing the prevalences of parasites where differences exist between coasts. Odds ratios for parasite prevalence on the West coast compared to the South coast were calculated.

For reasons to be discussed, the difference between parasite prevalence on farms in Hermanus and those in other areas were investigated. This was done using the chi square test and effect sizes. Significant prevalences were illustrated by charts. Odds ratios were also calculated.

2.2.2.2 Seasonality

Parasite prevalence was determined per month for the 60 month period covered by the data set. The prevalences were then charted per month for each year, per month for all years combined, and per year. The age structure of the sample population at various times was examined, as was the effect of origin on apparent temporal fluctuations. Chi square testing was used to demonstrate the significance of the association between parasite prevalence and year or month. For reasons to be discussed later, the association between parasite prevalence and seasonality was not further explored using statistical testing.

2.2.2.3 Diet

Parasite prevalences in abalone receiving kelp, artificial feed or a mixed diet were compared. The data were first used to construct charts and the relationship between diet and parasite prevalence was then explored further using the chi square test and effect sizes. As odds ratios rely on the construction of two by two tables, only abalone receiving artificial feed or kelp were considered and those on a mixed diet were excluded. A subsample of 10 882 abalone received either artificial feed or kelp. The crude odds ratio for parasite prevalence on artificial feed and kelp was calculated.

Unfortunately, the data set did not contain equal representation of abalone of the various age categories on different diets. This was further complicated by different diet usage in different age categories in different areas. Diet use in different age groups was charted. A crude odds ratio was determined for artificial feed and kelp usage in different age groups. The data was then stratified by age and odds ratios determined for parasite prevalence in abalone fed artificial feed and kelp. The Mantel Haenszel summary odds ratio and confidence intervals were calculated, and the Breslow Day test for interaction was performed, for relevant parasites.

Diet use in different locations was explored, firstly by use of charts. The crude odds ratio for parasite prevalence in Hermanus and other areas was previously calculated as mentioned in section 2.2.2.1. An odds ratio for artificial feed and kelp use in Hermanus and other areas was now calculated. The data were then stratified according to area and odds ratios calculated for different diets in different areas. The Mantel Haenszel summary odds ratio and confidence intervals were calculated, and the Breslow Day test for interaction was performed, for relevant parasites.

Diet use in different age groups in different localities was illustrated by means of a chart. To gain a better understanding of the associations between age, diet and area, odds ratios were calculated. An odds ratio was constructed for age and area and charts of these data included. Finally, as seasonal diet use was not constant over the entire period, this was also charted.

2.2.2.4 System

Parasite prevalences in recirculation systems were compared to those in flow through systems using a set of two by two tables and charts. The significance of the association between parasite prevalence and system was determined using the chi square test and effect size for each parasite. Age distribution and diet use was compared between systems by means of charts. As the recirculating systems used only kelp, odds ratios were constructed for parasite prevalence in kelp fed animals comparing flow through systems to recirculation. It is important to note that these analyses are not the same as those comparing farms. Although not all farms had recirculating systems, all farms utilising recirculating systems also had tanks on flow through.

Chapter 3

Results

3.1 Host factors

3.1.1 Age

The age distribution of the sample population is given in figure 3.1.1.1. The age range of the sample was 8 to 75 months. It can be seen that there are relatively few animals in the first year class and that the majority of animals are one to four years of age.

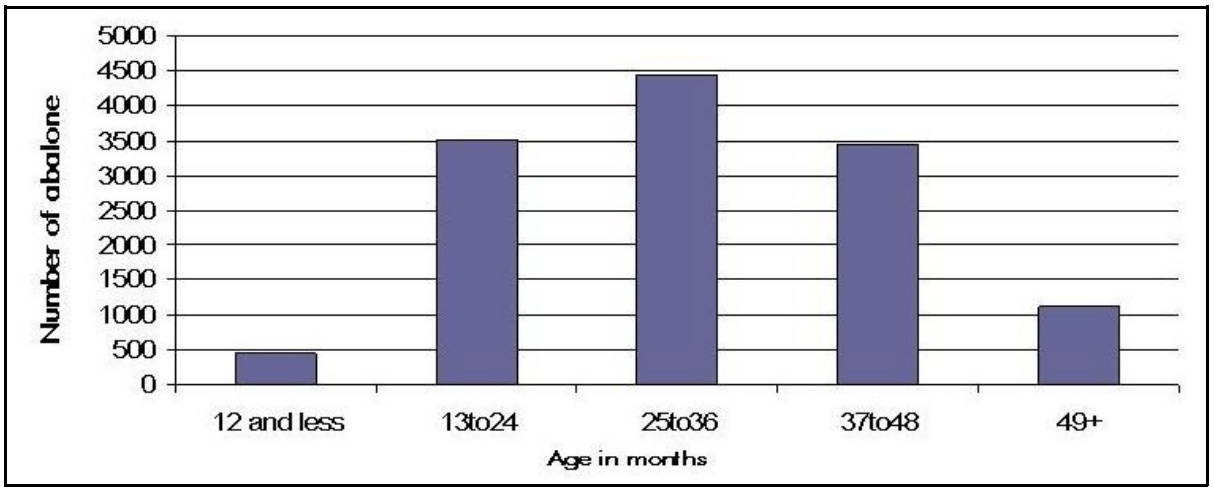

Figure 3.1.1.1: Age distribution of sample population

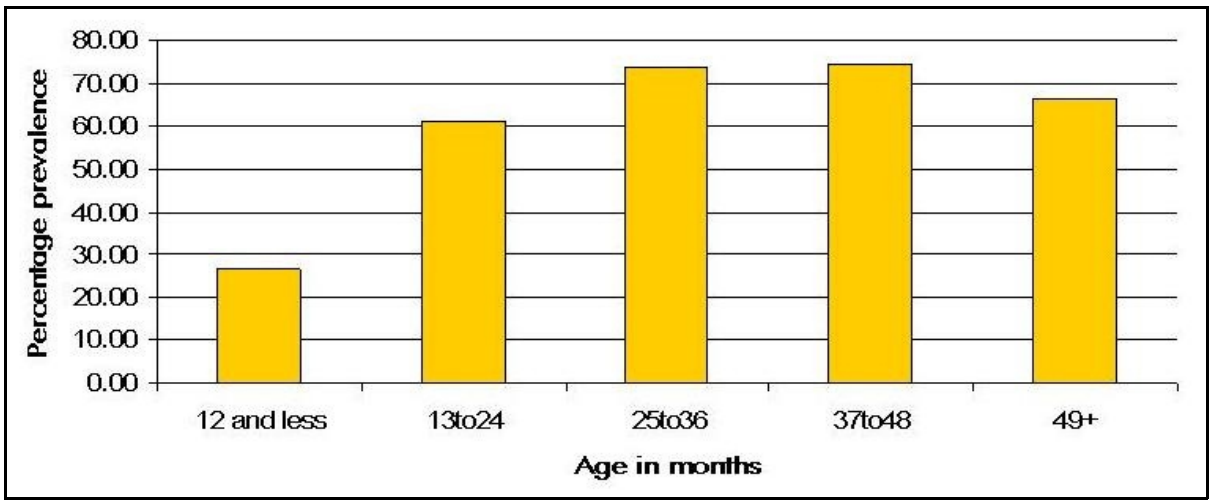

Figure 3.1.1.2: Prevalence of sessile ciliates by host age

The prevalence of the various parasites is given in figures 3.1.1.2 to 3.1.1.5. Note that the scale of the Y axis is not the same on the various charts.

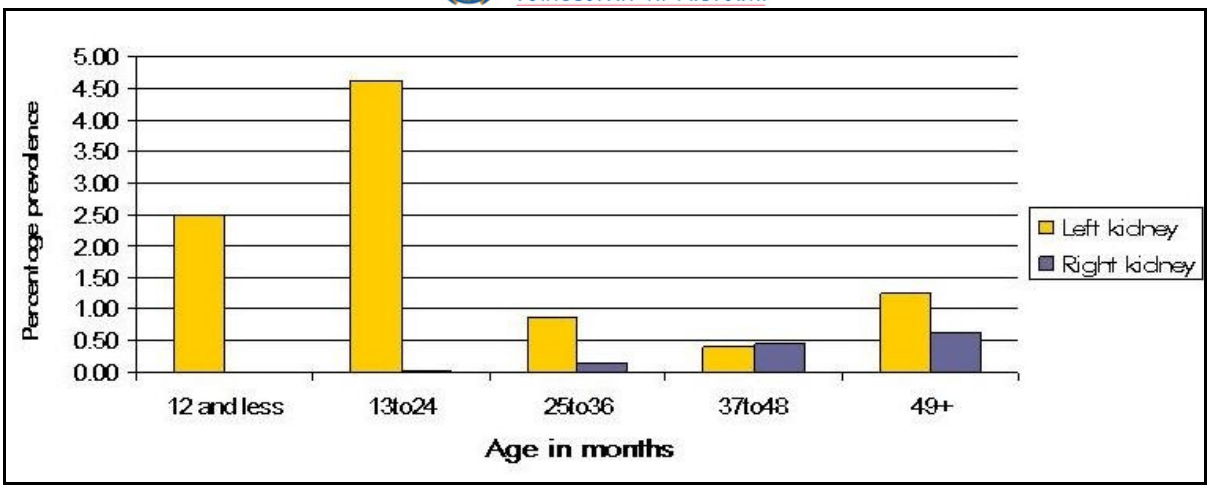

Figure 3.1.1.3: Prevalence of renal coccidia by host age

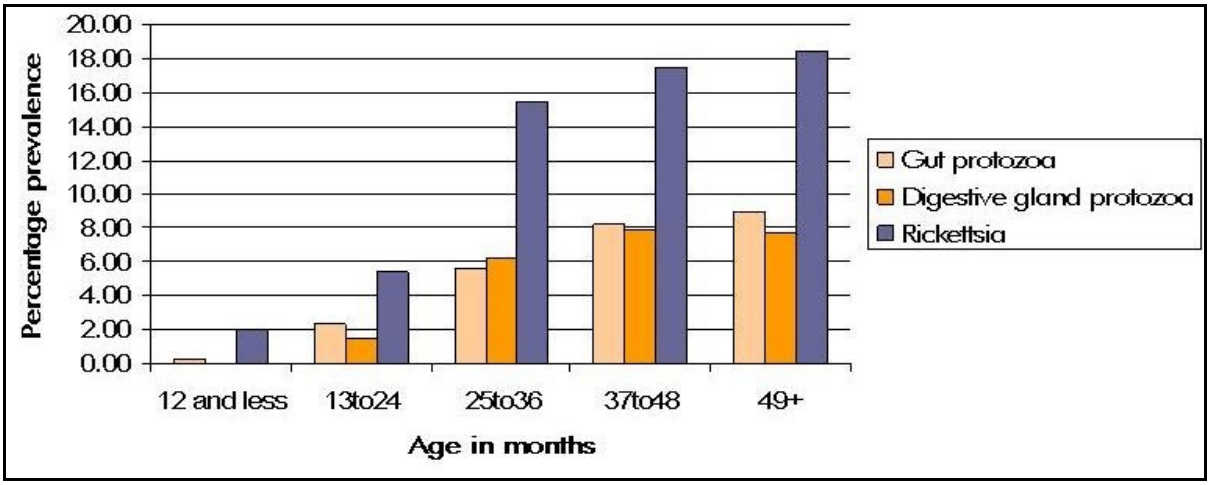

Figure 3.1.1.4: Prevalence of gut associated parasites by host age

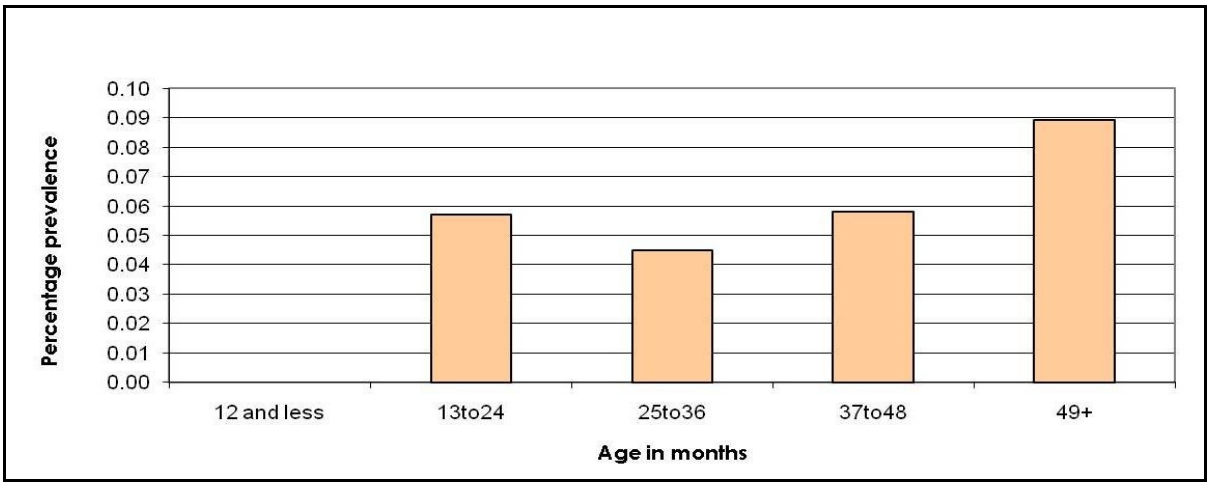

Figure 3.1.1.5: Prevalence of trematodes by host age

The results of the chi square test are summarised in table 3.1.1.1. It can be seen that a significant association between age and parasite prevalence exists for all parasites tested. Effect sizes of approximately 0.1 are considered small and 0.3 medium, so it can be seen that the effect sizes for age are modest. Only in the case of sessile ciliates is a medium effect size approached.

Table 3.1.1.1: Results of chi square test for relationship between parasite prevalence and host age

Parasite	Value of X^2	Df	P	Effect size	95% confidence interval
Sessile ciliates	559.97	4	0.005	0.21	0.19 to 0.22
Renal coccidia	238.98	8	0.005	0.10	0.12 to 0.15
Gut protozoa	163.67	4	0.005	0.11	0.09 to 0.13
Digestive gland protozoa	190.15	4	0.005	0.12	0.10 to 0.14
Rickettsia like prokaryotes	336.21	4	0.005	0.16	0.14 to 0.18

To further test the strength of the association between parasite prevalence and host age, odds ratios were calculated, as summarised in table 3.1.1.2. In each case, the odds ratio gives the risk to animals older than 24 months compared to those of 24 months and younger. This age was chosen because it gave larger odds ratios than 36 months. There is also evidence to suggest that 24 months is an important physiological milestone for abalone.

Table 3.1.1.2: Odds ratios for parasite prevalence in animals older than 24 months compared to those of 24 months and younger

Parasite	Odds ratio	Confidence interval
Sessile ciliates	2.03	1.87 to 2.19
Renal coccidia left kidney	0.16	0.12 to 0.22
Renal coccidia right kidney	12.78	1.74 to 93.89
Gut protozoa	3.45	2.74 to 4.33
Digestive gland protozoa	5.54	4.18 to 7.35
Rickettsia like prokaryotes	3.73	3.20 to 4.35
Trematodes	1.10	0.21 to 5.67

In all cases, except trematode and left kidney coccidian infections, risk of parasite infection tends to be greater in older animals. The association between trematode infection and age was not significant, as the confidence interval for the odds ratio includes 1.0. In the case of left kidney coccidian infections, the odds ratio is smaller than 1.0, indicating a greater risk of infection in animals of two years and younger. In other words, for left kidney coccidian infections, risk of infection decreases with increasing age.

3.1.2 Size

3.1.2.1 Total mass

The mass distribution of the sample population is given in figure 3.1.2.1.1. The mass range of the sample was 0.16 to 240.21 g. It can be seen that there are relatively few animals larger than 45 g in the sample population.

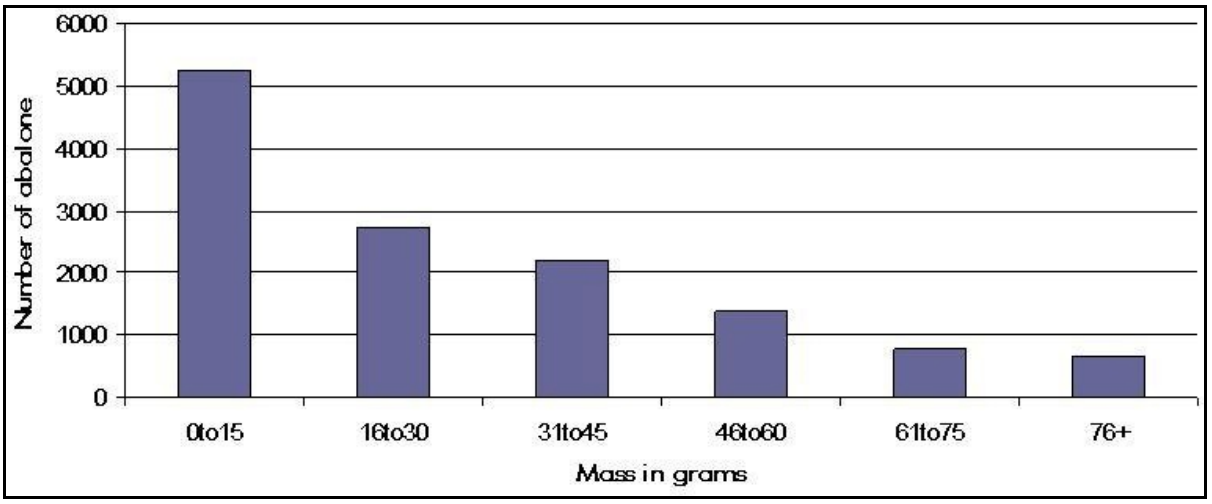

Figure 3.1.2.1.1: Mass distribution of sample population

The prevalence of the various parasites is given in figures 3.1.2.1.2 to 3.1.2.1.5. Note that the scale of the Y axis is not the same on the various charts.

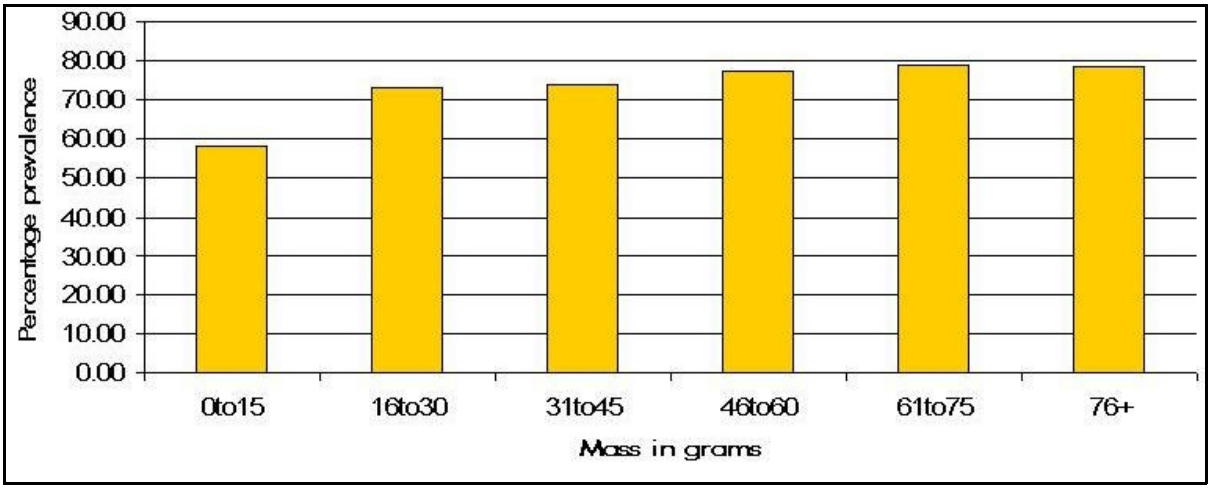

Figure 3.1.2.1.2: Prevalence of sessile ciliates by host mass

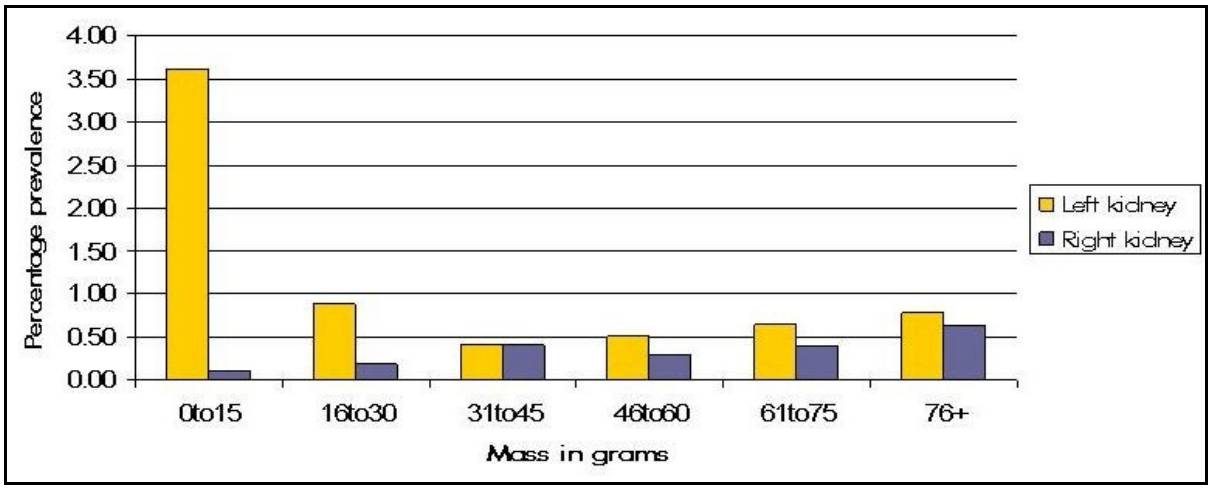

Figure 3.1.2.1.3: Prevalence of renal coccidia by host mass

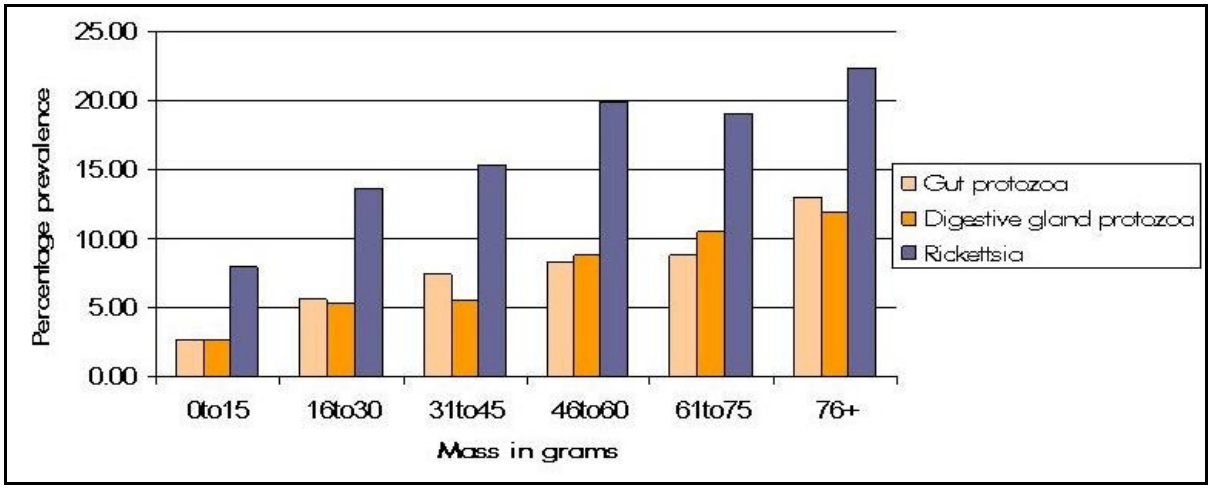

Figure 3.1.2.1.4: Prevalence of gut associated parasites by host mass

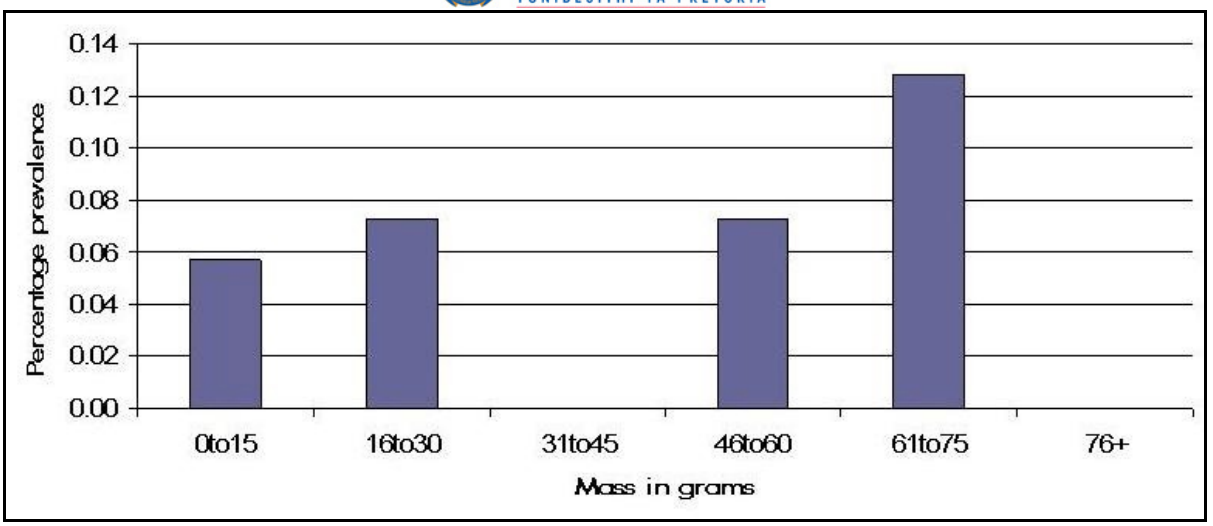

Figure 3.1.2.1.5: Prevalence of trematodes by host mass

The charts show similar trends to those for age, which is expected, as animals generally become larger with increasing age.

The results of the chi square test are summarised in table 3.1.2.1.1. It can be seen that a significant association between total mass and parasite prevalence exists for all parasites tested.

Table 3.1.2.1.1: Results of chi square test for relationship between parasite prevalence and host mass

Parasite	Value of X^2	Df	P	Effect size
Sessile ciliates	425.91	4	0.005	0.18
Renal coccidia	164.42	8	0.005	0.11
Gut protozoa	202.32	4	0.005	0.12
Digestive gland protozoa	205.70	4	0.005	0.13
Rickettsia like prokaryotes	261.33	4	0.005	0.14

The results of the chi square test indicated a significant association between total mass and the prevalence of all parasites. Effect sizes of approximately 0.1 are considered small and 0.3 medium, so it can be seen that the effect sizes for mass are modest.

3.1.2.2 Growth rate

The growth rate distribution of the sample population is given in figure 3.1.2.2.1. The growth rate range of the sample was 0.36 to 3.24 mm per month. The labels on the X axes of the charts refer to upper limits of each growth rate category. Animals most commonly grew at 1.3 to 2.0 mm per month.

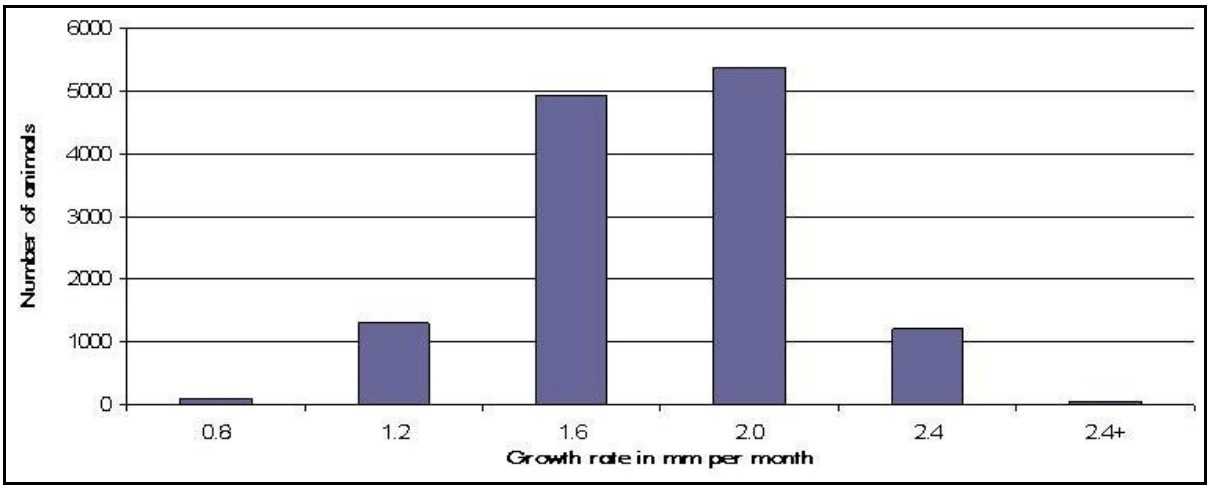

Figure 3.1.2.2.1: Growth rate distribution of sample population

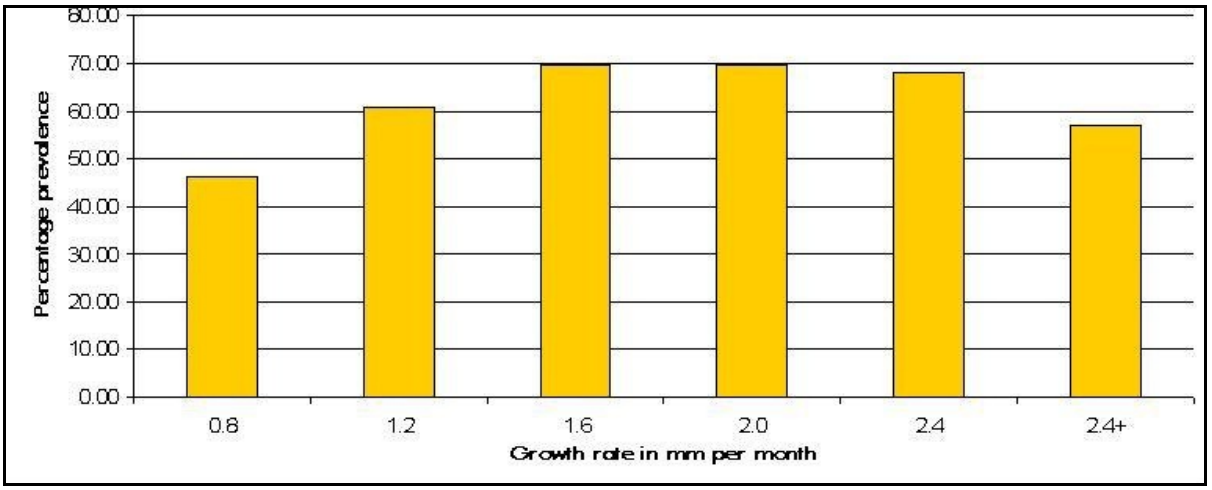

Figure 3.1.2.2.2: Prevalence of sessile ciliates by host growth rate

Prevalences of various parasites are shown in figures 3.1.2.2.2 to 3.1.2.2.5. The scale of the Y axis is different on different charts.

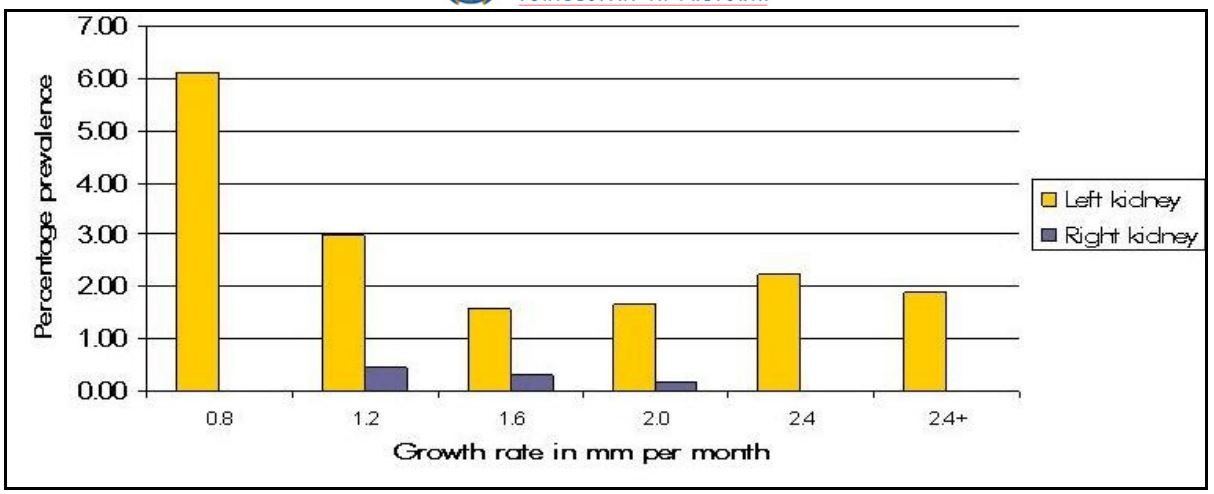

Figure 3.1.2.2.3: Prevalence of renal coccidia by host growth rate

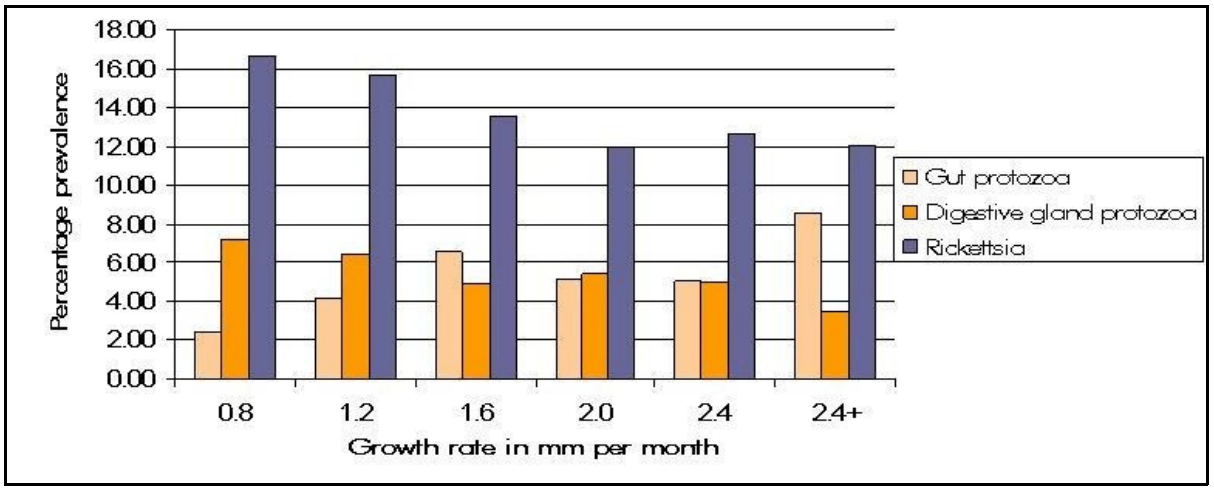

Figure 3.1.2.2.4: Prevalence of gut associated parasites by host growth rate

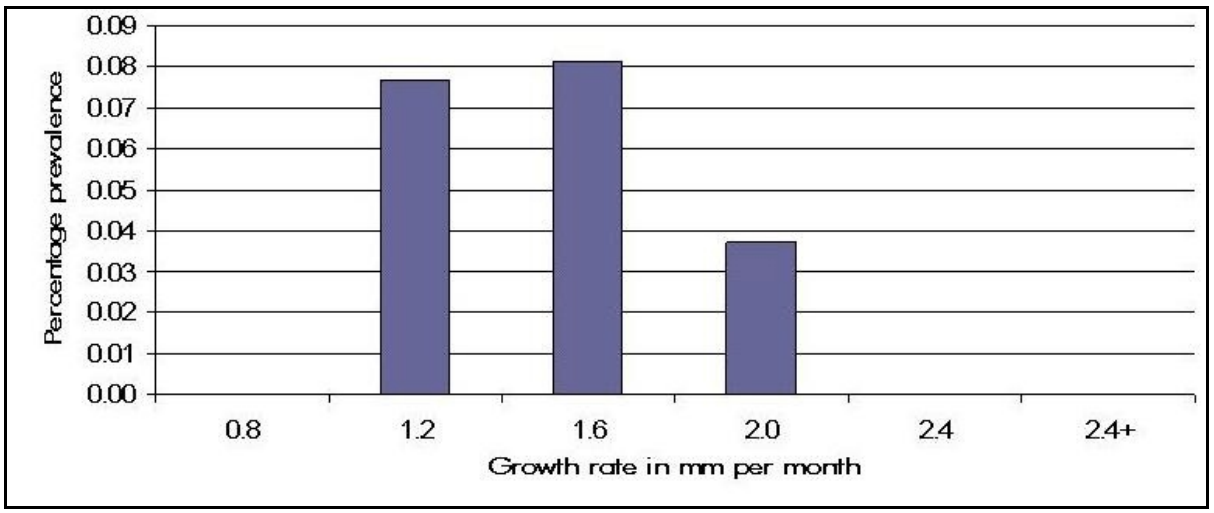

Figure 3.1.2.2.5: Prevalence of trematodes by host growth rate

The results of the chi square test are summarised in table 3.1.2.2.1. It can be seen that a significant association between growth rate and parasite prevalence exists for some parasites. However, the effect sizes are very small, indicating that the association is unlikely to be of great importance.

Table 3.1.2.2.1: Results of chi square test for relationship between parasite prevalence and host growth rate

Parasite	Value of X^2	Df	P	Effect size	95% confidence interval
Sessile ciliates	67.98	8	0.005	0.07	0.05 to 0.09
Renal coccidia	30.08	16	0.025	0.03	0.01 to 0.05
Gut protozoa	21.77	8	0.01	0.04	0.01 to 0.05
Digestive gland protozoa	10.46	8	0.5	na	na
Rickettsia like prokaryotes	16.80	8	0.05	na	na

Figure 3.1.2.2.6 shows that growth rate tends to decline as abalone age. Because of this association, it was considered worth investigating parasite prevalence at various growth rates for individual age groups. The results for renal coccidia in animals 24 months and less of age are charted in figure 3.1.2.2.7. Charts for prevalence of renal coccidia in other age groups were similar and are not shown.

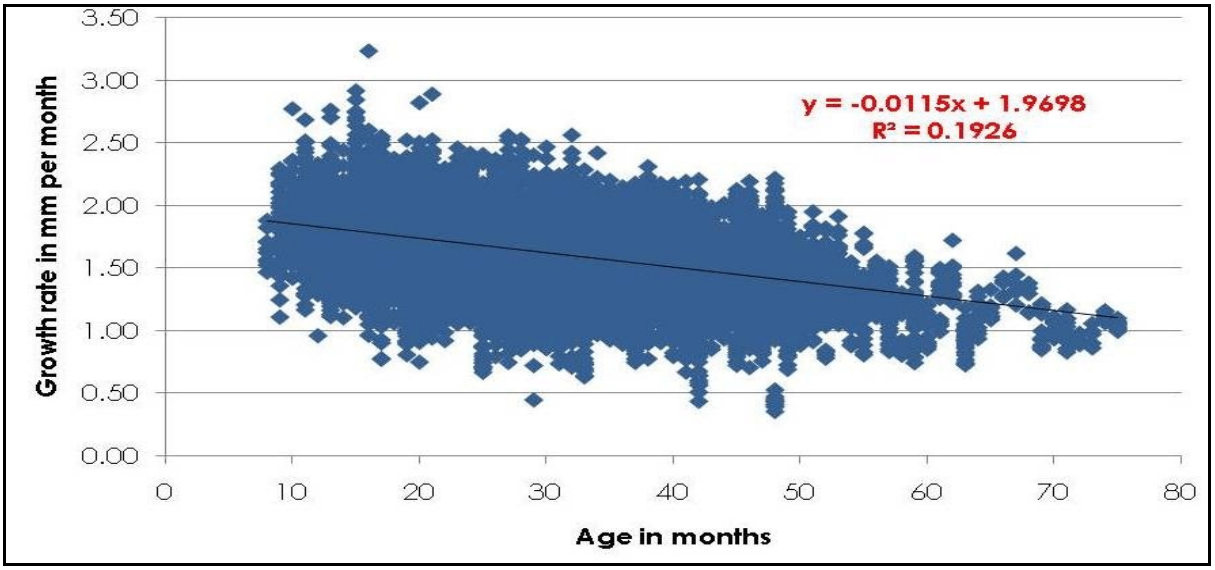

Figure 3.1.2.2.6: Growth rate by host age

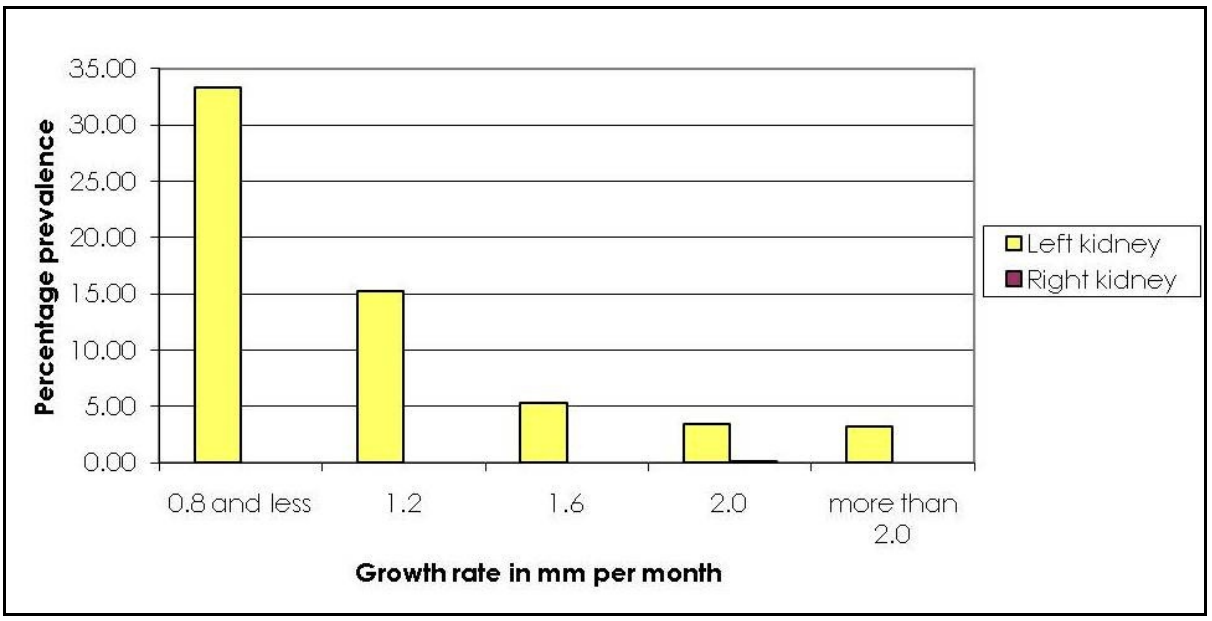

Figure 3.1.2.2.7: Prevalence of renal coccidia by host growth rate in animals of 24 months and less of age

The results of comparison of growth rate in abalone with and without various parasites are shown in tables 3.1.2.2.2 to 3.1.2.2.6. It can be seen that apparent association between sessile ciliate infestation and growth rate is lost when the data are stratified according to age. In contrast, abalone with renal coccidia have lower growth rates than those without and this association is not linked to age. This result is similar to that obtained using other statistical methods as reported above. There was no significant difference in growth rate between animals with and without gut protozoa or rickettsia like prokaryotes. For digestive gland protozoa, a difference was found for animals up to 24 months of age. However, the test showed this to be a difference in the shape of the distributions rather than an actual association.

Table 3.1.2.2.2: Results of comparison of growth rate in abalone with and without sessile ciliates

Age group	Test used	Dmn/p	Decision
All ages	Kolmogorov-Smirnov	0.576	Reject H_0
Up to 24 months	Kolmogorov-Smirnov	0.000	Accept H_0
Older than 24 months	Kolmogorov-Smirnov	0.010	Accept H_0

Table 3.1.2.2.3: Results of comparison of growth rate in abalone with and without renal coccidia

Age group	Test used	Dmn/p	Decision
All ages	Kolmogorov-Smirnov	0.364	Reject H_0
Up to 24 months	Kolmogorov-Smirnov	0.364	Reject H_0
Older than 24 months	T-test	0.001	Reject H_0

Table 3.1.2.2.4: Results of comparison of growth rate in abalone with and without gut protozoa

Age group	Test used	Dmn/p	Decision
All ages	Kolmogorov-Smirnov	0.152	Accept H_0
Up to 24 months	Kolmogorov-Smirnov	0.133	Accept H_0
Older than 24 months	T-test	0.994	Accept H_0

Table 3.1.2.2.5: Results of comparison of growth rate in abalone with and without digestive gland protozoa

Age group	Test used	Dmn/p	Decision
All ages	Kolmogorov-Smirnov	0.202	Reject H_0
Up to 24 months	Kolmogorov-Smirnov	0.272	Reject H_0
Older than 24 months	Kolmogorov-Smirnov	0.051	Accept H_0

Table 3.1.2.2.6: Results of comparison of growth rate in abalone with and without rickettsia like prokayotes

Age group	Test used	Dmn/p	Decision
All ages	Kolmogorov-Smirnov	0.162	Accept H_0
Up to 24 months	Kolmogorov-Smirnov	0.172	Accept H_0
Older than 24 months	Kolmogorov-Smirnov	0.000	Accept H_0

3.1.2.3 Condition index

The condition index distribution of the sample population is given in figure 3.1.2.3.1. The condition index range of the sample was 1.0 to 27.3. The mode for conditon index is 5.0, which can be considered normal.

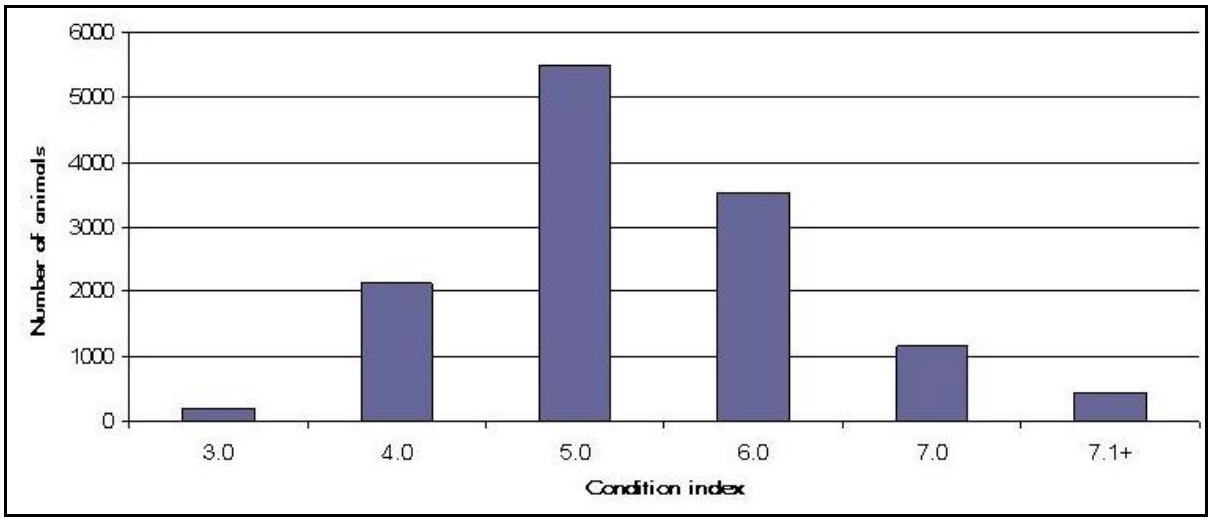

Figure 3.1.2.3.1: Condition index distribution of sample population

The prevalences of the various parasites are given in figures 3.1.2.3.2 to 3.1.2.3.5. The scale of the Y axes of the different charts are different.

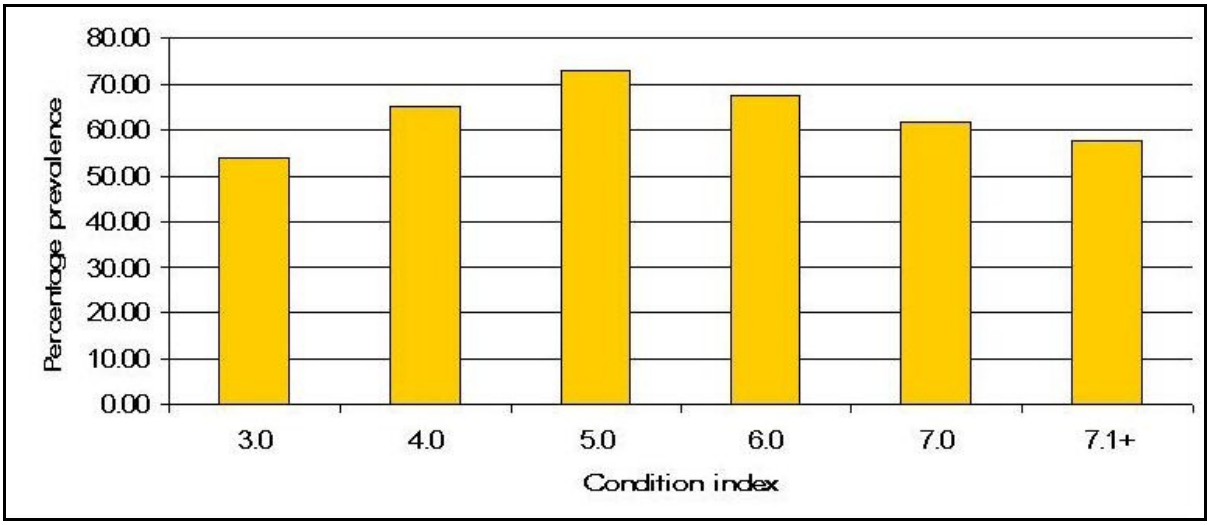

Figure 3.1.2.3.2: Prevalence of sessile ciliates by host condition index

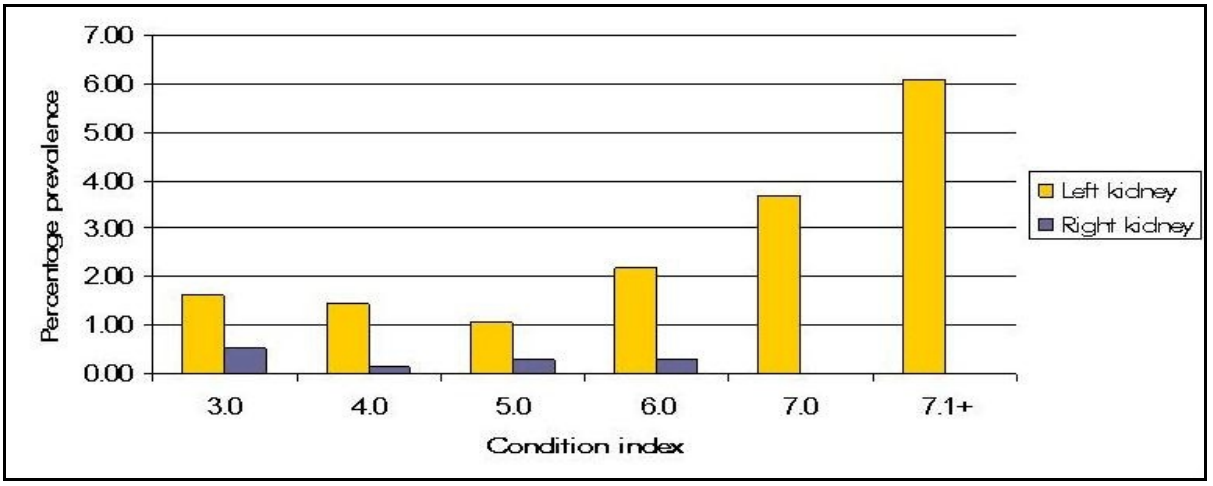

Figure 3.1.2.3.3: Prevalence of renal coccidia by host condition index

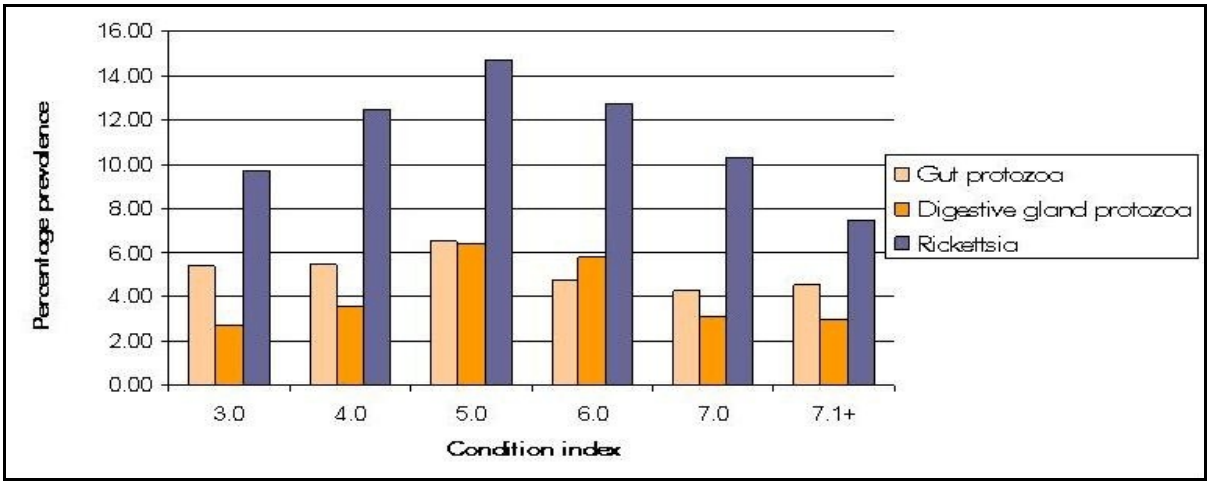

Figure 3.1.2.3.4: Prevalence of gut associated parasites by host condition index

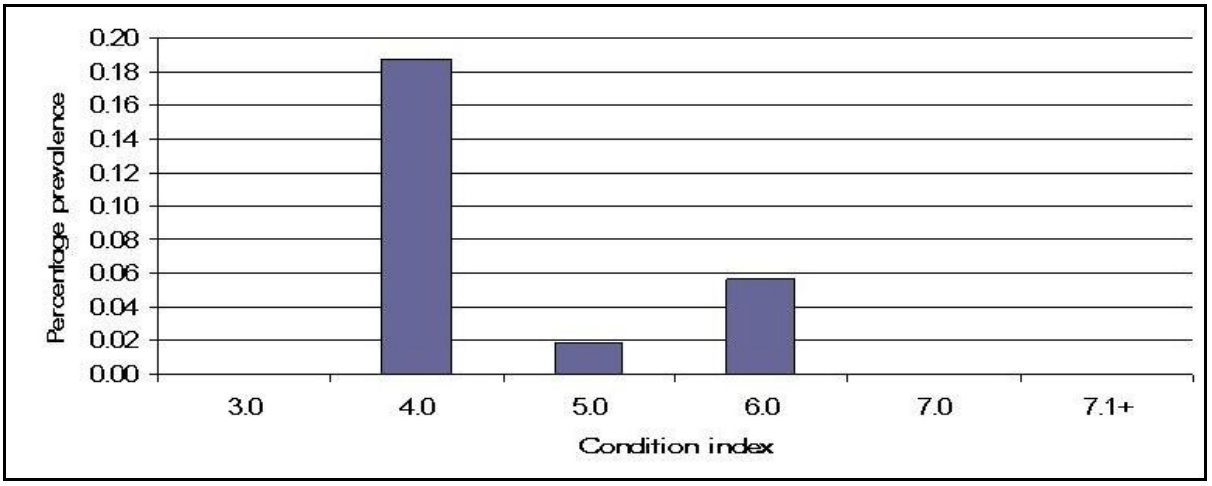

Figure 3.1.2.3.5: Prevalence of trematodes by host condition index

The results of the chi square test are summarised in table 3.1.2.3.1. It can be seen that a significant association exists between condition index and parasite prevalence for all parasites tested. However, the effect sizes are small.

Table 3.1.2.3.1: Results of chi square test for relationship between parasite prevalence and host condition index

Parasite	Value of X^2	Df	P	Effect size	95% confidence interval
Sessile ciliates	130.98	8	0.005	0.10	0.08 to 0.12
Renal coccidia	105.52	16	0.005	0.06	0.07 to 0.10
Gut protozoa	20.21	8	0.01	0.04	0.01 to 0.05
Digestive gland protozoa	47.30	8	0.005	0.06	0.04 to 0.07
Rickettsia like prokaryotes	36.70	8	0.005	0.05	0.03 to 0.07

The association between age and condition index is shown in figure 3.1.2.3.6. It can be seen that a portion of the variation in condition index is explained by age, with condition index tending to decrease with increasing age. In other words, the shell accounts for a larger portion of total body mass in older animals than in younger ones.

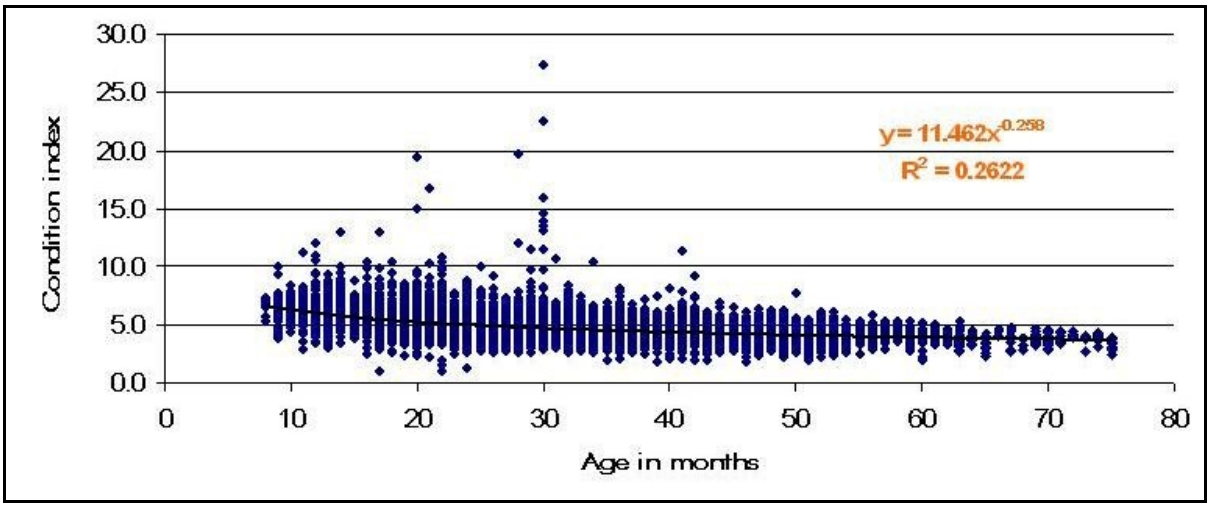

Figure 3.1.2.3.6: Condition index by host age

The relationship between condition index and diet is shown in figure 3.1.2.3.7. The percentage of animals falling in each condition index category has been calculated for each diet. It can be seen that the distribution is not the same for the different diets. Animals on artificial feed tend to have lower indices than those on diets containing kelp.

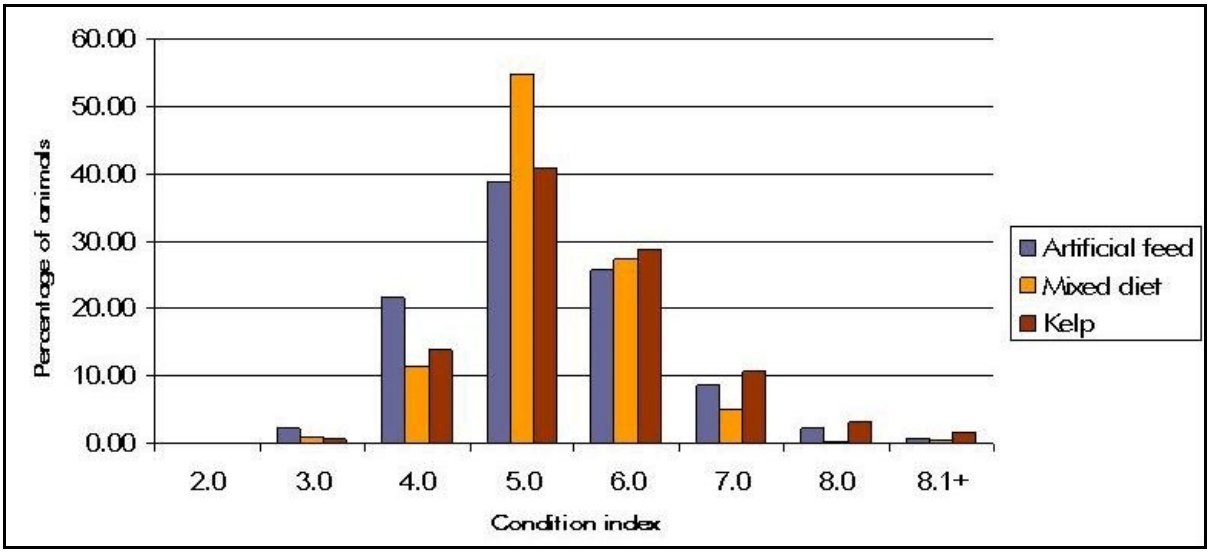

Figure 3.1.2.3.7: Condition index distribution of animals on different diets

These results should be interpreted with consideration of the physiological meaning of condition index. Whereas low condition index is clearly the result of low soft tissue mass, above average condition index is often due to erosion or breakage of the shell. Consequently, both low and high condition indices may be undesirable. This is discussed further in the next chapter.

3.1.3 Sex

The sex composition of the sample population is given in 3.1.3.1.

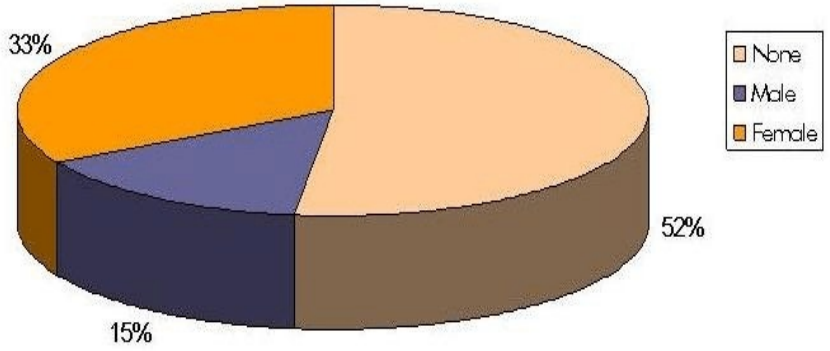

Figure 3.1.3.1: Sex distribution of sample population

Although it would appear that the ratio of female to male is approximately 2 to 1, this is probably not actually the case. On the histological sections, three quarters of the females show immature gonad development, as opposed to a fifth of the males. It is more difficult to identify immature male gonads histologically and these animals are likely to be included in none. It can be seen from figure 3.1.3.2 that animals with undifferentiated gonads comprise more than half of the sample population and those with first stage development account for nearly a third.

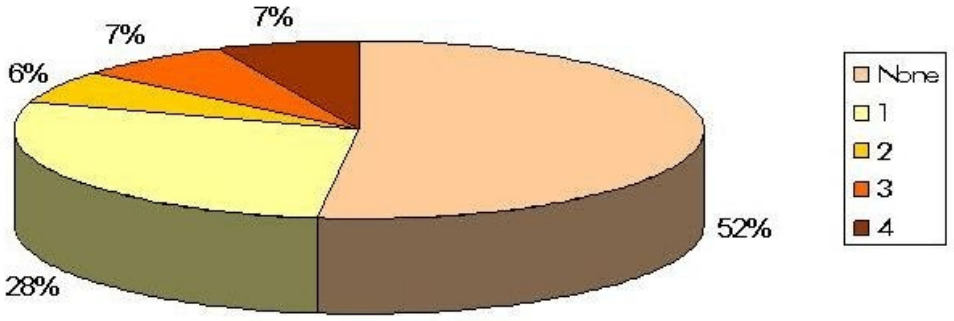

Figure 3.1.3.2: Gonad development scores of sample population

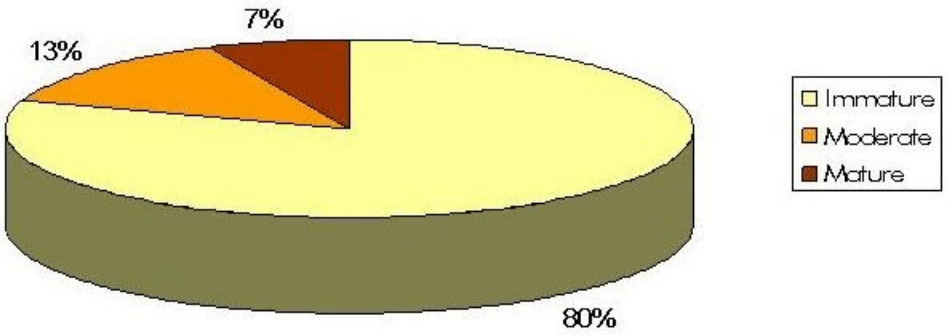

Figure 3.1.3.3: Gonad development distribution of host population

To attempt differentiation of gonad development which was not biased by sex, gonads were also classed as immature, moderate and mature, as shown in figure 3.1.3.3.

The prevalences of the various parasites according to sex are shown in figures 3.1.3.4 to 3.1.3.7.

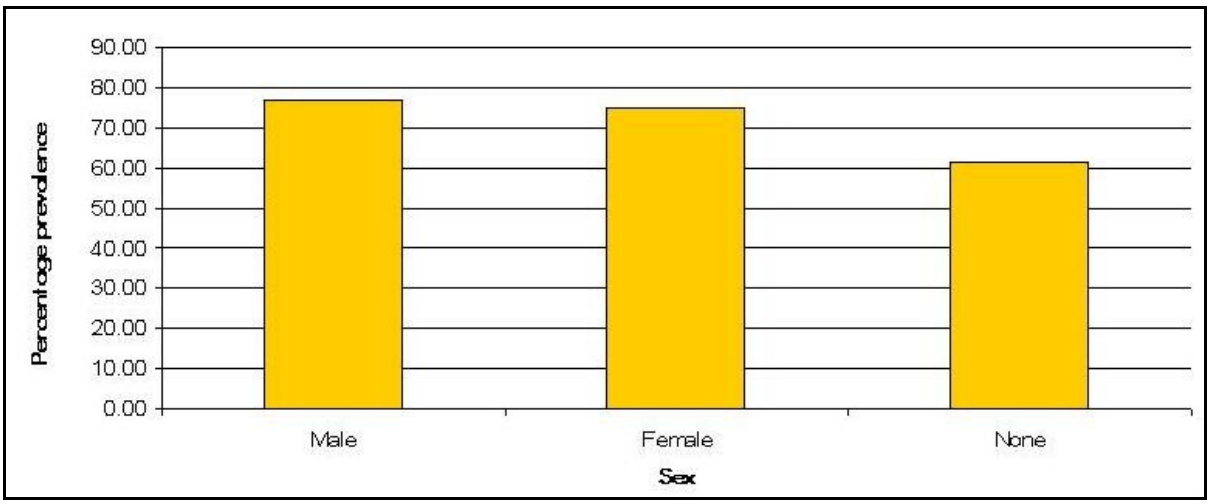

Figure 3.1.3.4: Prevalence of sessile ciliates by host sex

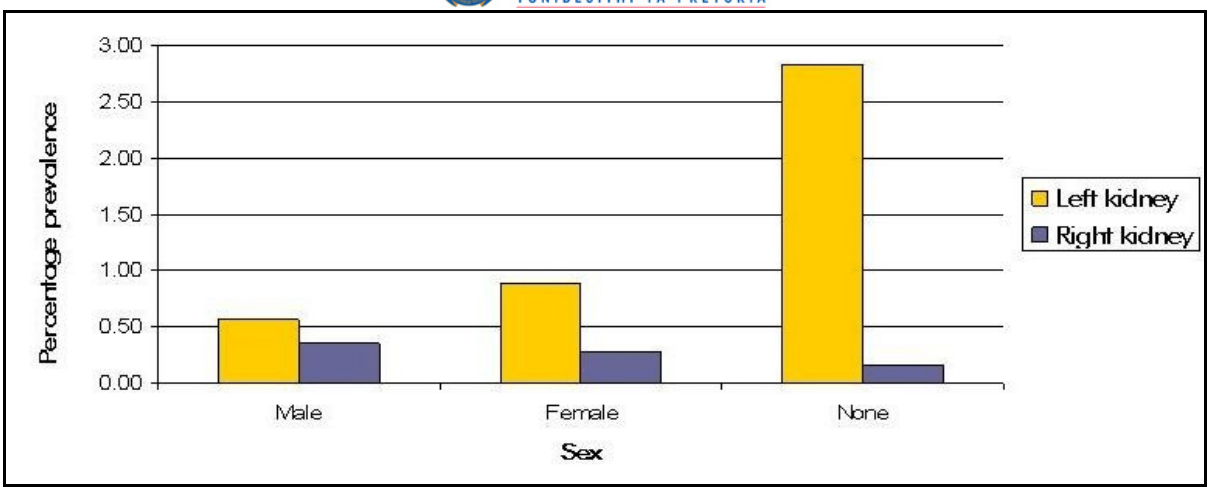

Figure 3.1.3.5: Prevalence of renal coccidia by host sex

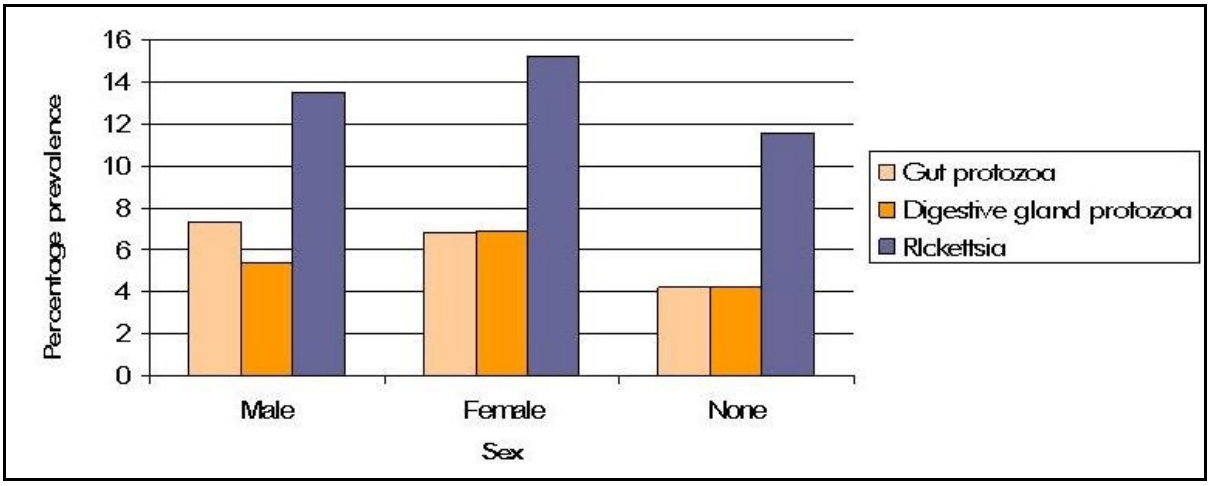

Figure 3.1.3.6: Prevalence of gut associated parasites by host sex

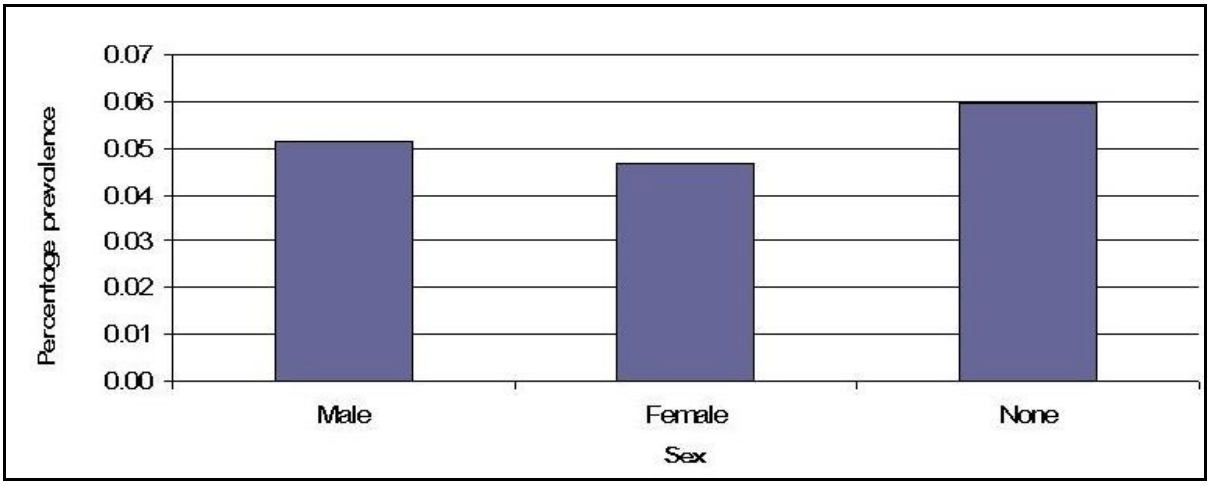

Figure 3.1.3.7: Prevalence of trematodes by host sex

The results of the chi square test for sex are summarised in table 3.1.3.1. It can be seen that a significant association between sex and parasite prevalence exists for all parasites. However, the only even moderately sized effect is for sessile ciliates.

Table 3.1.3.1: Results of chi square test for relationship between parasite prevalence and host sex

Parasite	Value of X^2	Df	P	Effect size	95% confidence interval
Sessile ciliates	302.03	2	0.005	0.15	0.14 to 0.17
Renal coccidia	78.36	4	0.005	0.08	0.06 to 0.09
Gut protozoa	47.88	2	0.005	0.06	0.04 to 0.08
Digestive gland protozoa	35.62	2	0.005	0.05	0.03 to 0.07
Rickettsia like prokaryotes	30.61	2	0.005	0.05	0.03 to 0.07

When assessing these results, the relationship between sex and age, shown in figure 3.1.3.8, should be kept in mind. It has already been shown that parasite prevalence is associated with age. The determination of an animal's sex is also associated with age. Therefore, it can be concluded that the apparent difference in parasite prevalence is in fact due to age and not gender.

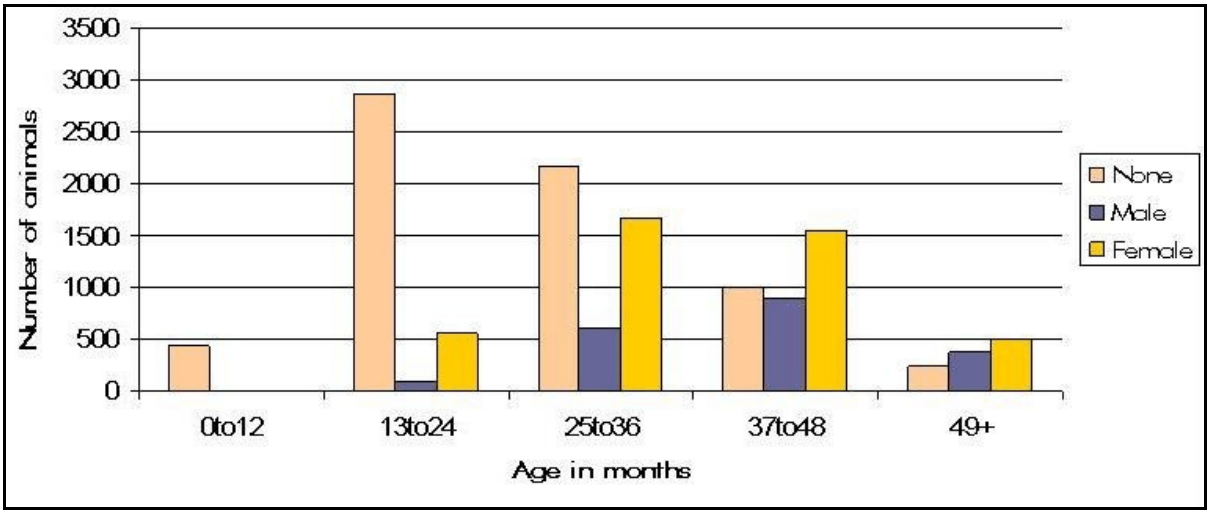

Figure 3.1.3.8: Sex distribution in different age classes of sample population

Parasite prevalences according to gonad development are shown in figures 3.1.3.9 to 3.1.3.12.

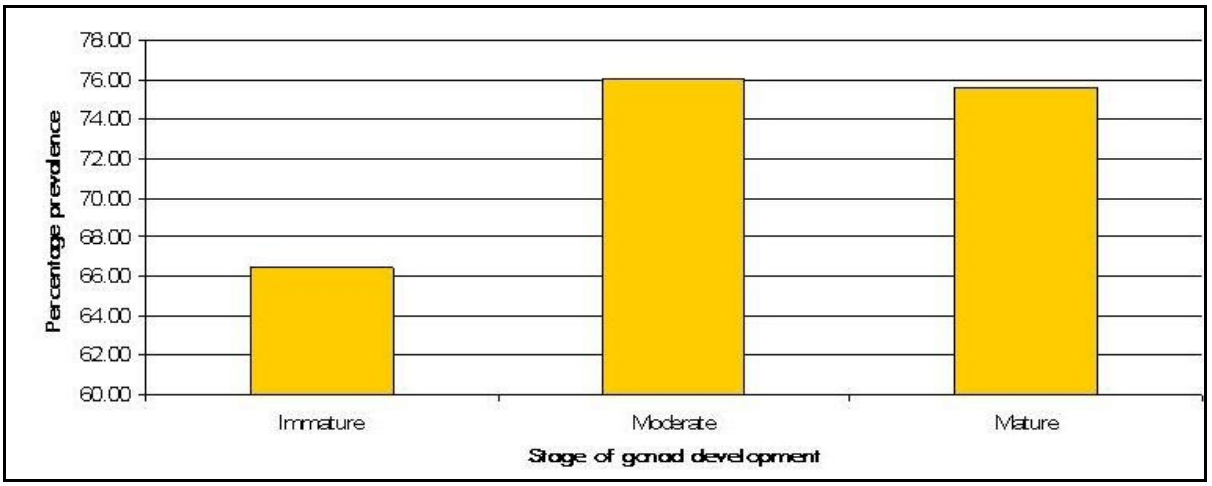

Figure 3.1.3.9: Prevalence of sessile ciliates by gonad development of host

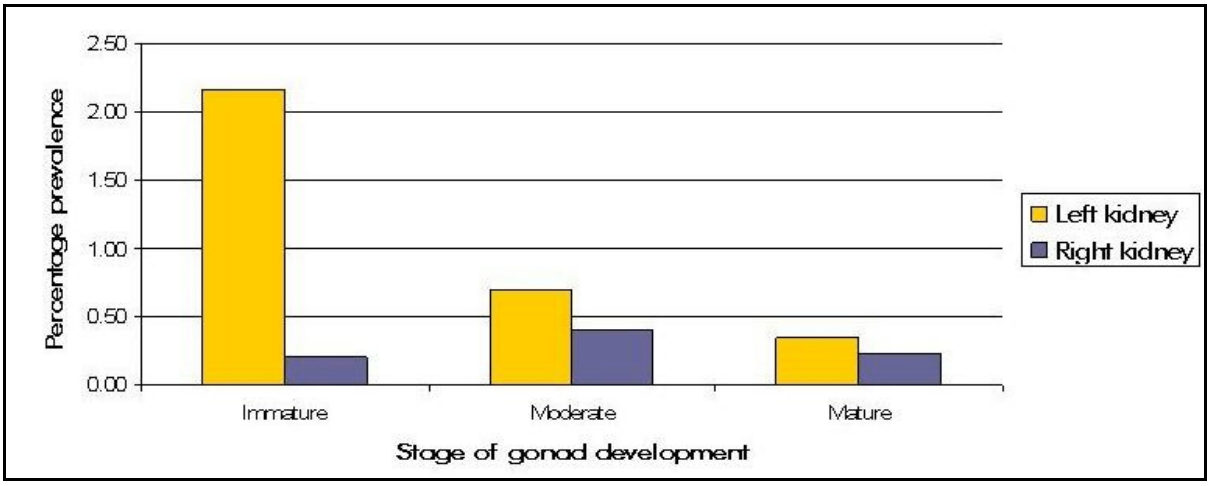

Figure 3.1.3.10: Prevalence of renal coccidia by gonad development of host

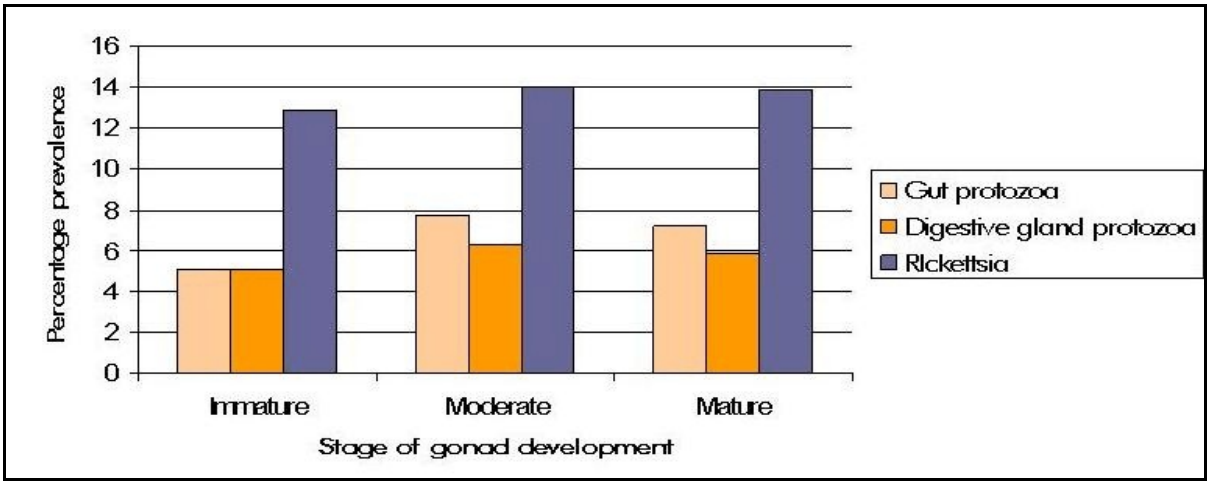

Figure 3.1.3.11: Prevalence of gut associated parasites by gonad development of host

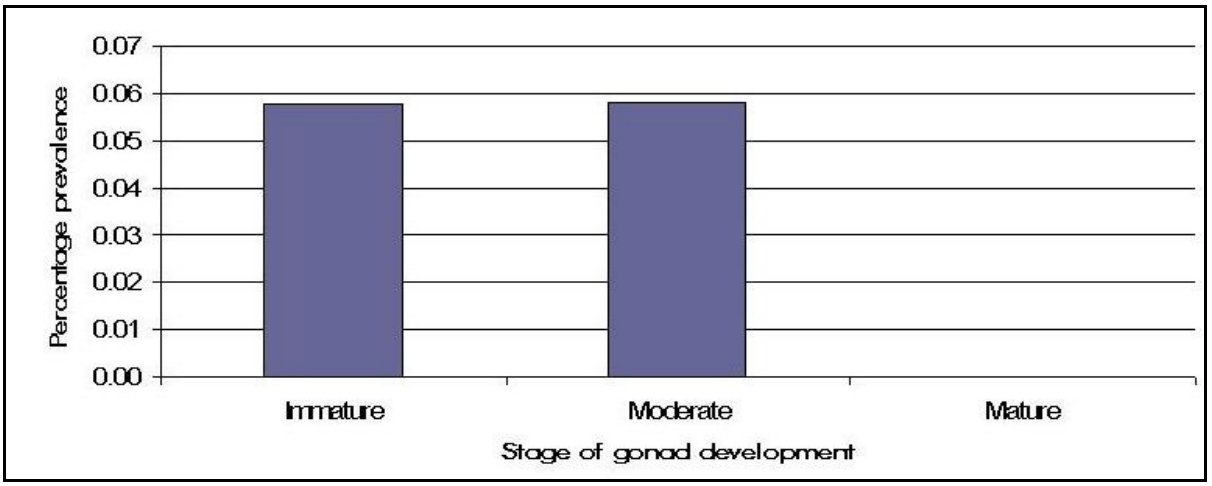

Figure 3.1.3.12: Prevalence of trematodes by gonad development of host

The results of the chi square test for degree of gonad development, classed as immature to mature, are summarised in table 3.1.3.2. It can be seen that a significant association between parasite prevalence and gonad development exists for some parasites, but the effect sizes are very small. The above comment of considering the effect of age on sex also applies here. The apparent relationship between parasite prevalence and gonad development is due to age and not the actual level of sexual maturity. This relationship is shown in figure 3.1.3.13.

Table 3.1.3.2: Results of chi square test for relationship between parasite prevalence and host gonad development

Parasite	Value of X^2	Df	P	Effect size	95% confidence interval
Sessile ciliates	85.53	2	0.005	0.08	0.06 to 0.10
Renal coccidia	31.48	4	0.005	0.05	0.03 to 0.06
Gut protozoa	25.95	2	0.005	0.04	0.03 to 0.06
Digestive gland protozoa	4.77	2	0.1	na	na
Rickettsia like prokaryotes	2.32	2	0.9	na	na

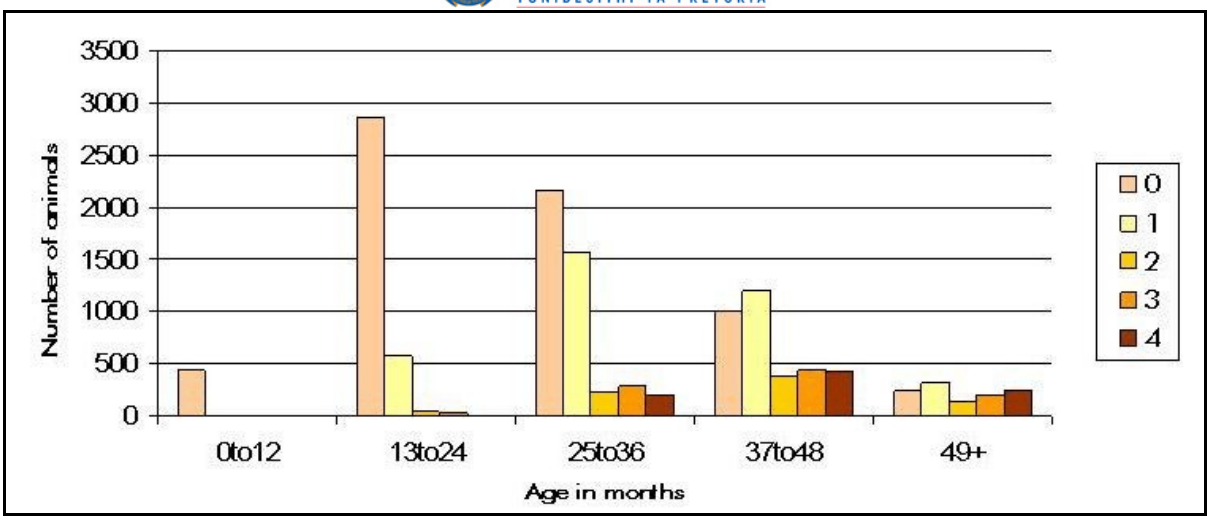

Figure 3.1.3.13: Gonad development in different age classes of sample population

The results of the chi square test for gonad development score are very similar to those for sex and are not shown.

3.2 Environmental factors

3.2.1 Origin

The sample population was not evenly distributed among the nine farms, as can be seen from figure 3.2.1.1. Farm b submitted the largest number of samples, 3133, whereas the fewest abalone were received from farm a, 545. Not all farms participated in the health management program for the entire period. In addition, some farms had greater data removal due to incomplete information than other farms.

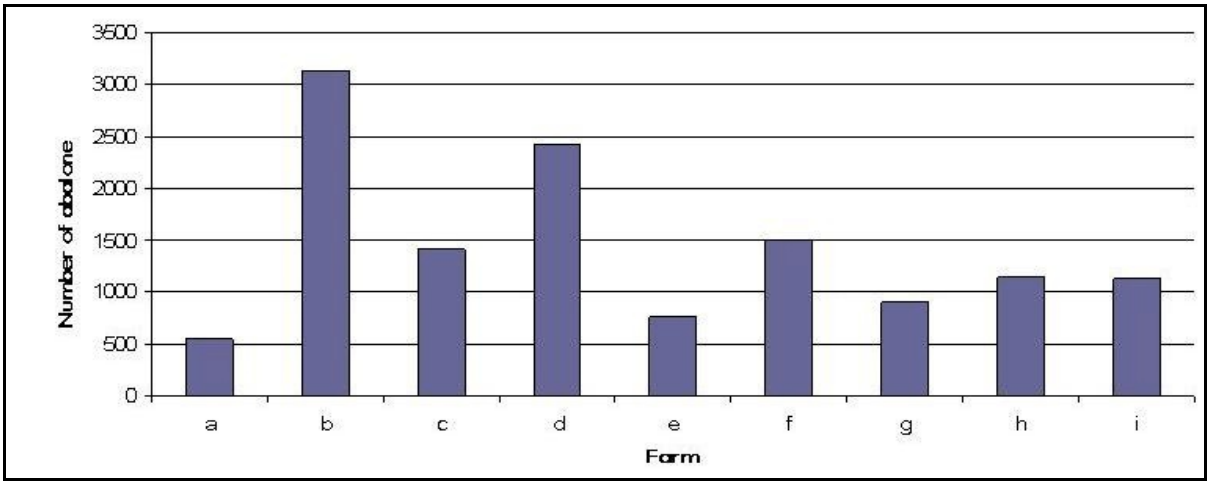

Figure 3.2.1.1: Number of animals from each farm included in study

The prevalences of the various parasites are given in figures 3.2.1.2 to 3.2.1.5. Note that the scale of the Y axis is not the same on the various charts.

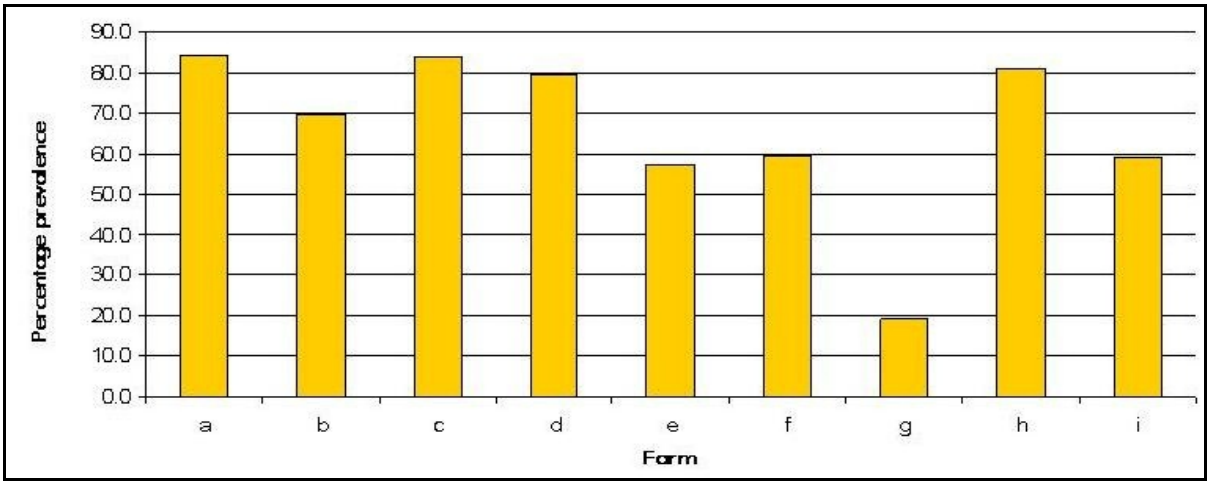

Figure 3.2.1.2: Prevalence of sessile ciliates on each farm

Sessile ciliates were by far the most common parasite identified during this study and it can be seen from figure 3.2.1.2 to be common on the majority of farms, with the notable exception of farm g. In contrast, this farm had the highest prevalence of left kidney coccidian infections, as shown in figure 3.2.1.3.

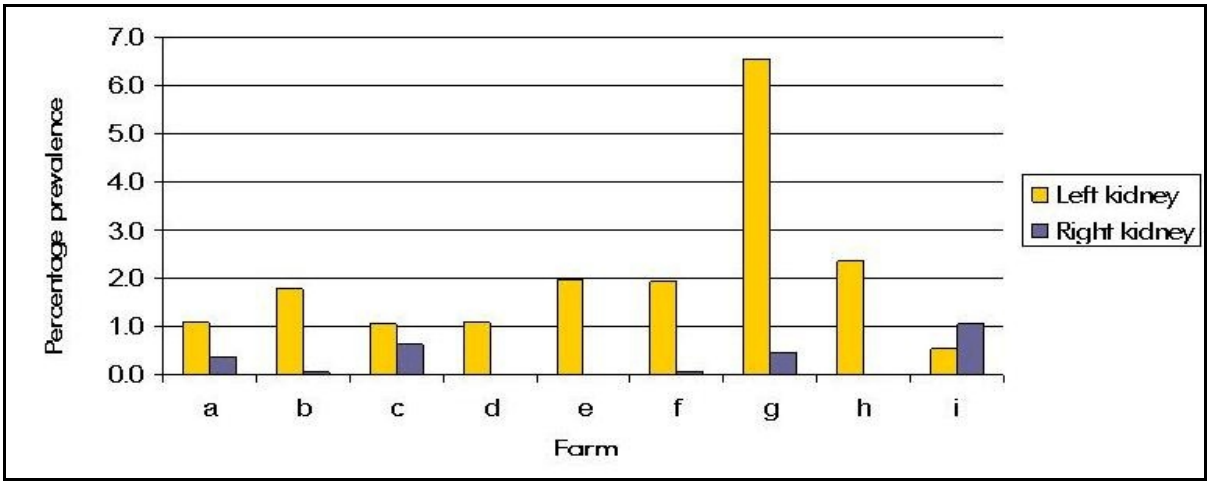

Figure 3.2.1.3: Prevalence of renal coccidia on each farm

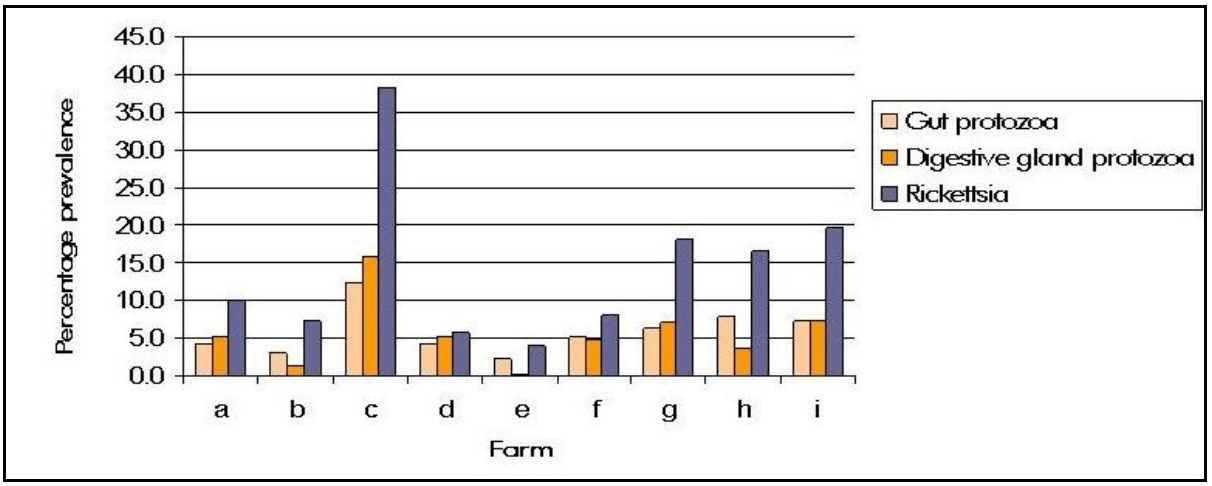

Figure 3.2.1.4: Prevalence of gut associated parasites on each farm

Rickettsia like prokaryotes were markedly more prevalent on farm c than on other farms. The other farms seemed to fall roughly into two categories, with farms a, b, d, e and f having comparable, relatively low prevalences. Farms g, h and i were comparable with intermediate prevalences. It is interesting to note that farms g, h and i also used nearly exclusively kelp as diet. The prevalence of gut associated protozoa was also higher on farm c than other farms.

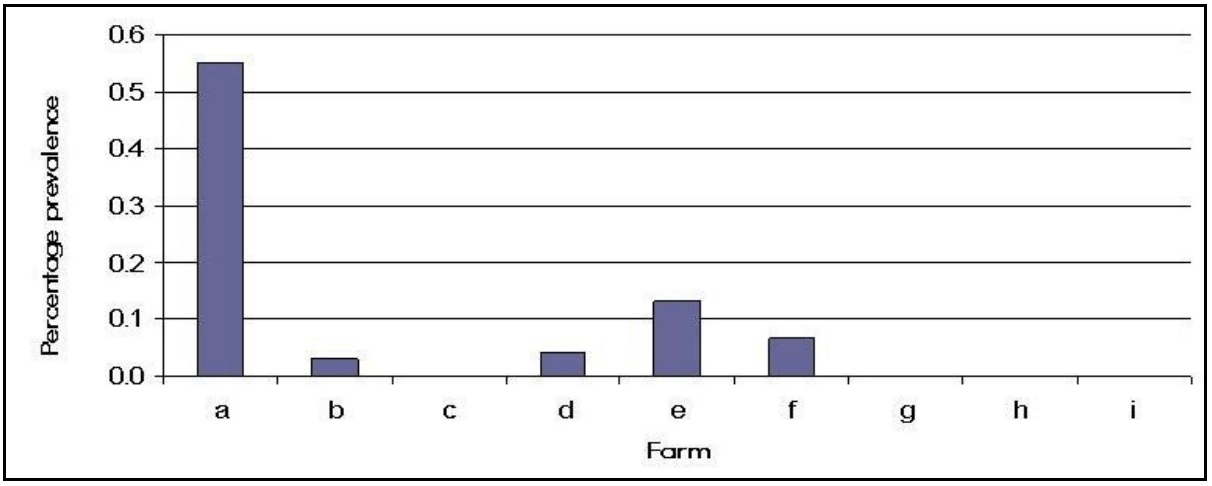

Figure 3.2.1.5: Prevalence of trematodes on each farm

The results of the chi square test are summarised in table 3.2.1.1. It can be seen that there are significant differences in parasite prevalences between farms for all parasites tested. The effect sizes for most parasites are small, except in the case of sessile ciliates and rickettsia like prokaryotes, where medium effect sizes are found.

Table 3.2.1.1: Results of chi square test for relationship between parasite prevalence and farm of origin

Parasite	Value of X^2	Df	P	Effect size	95% confidence interval
Sessile ciliates	1582.82	8	0.005	0.35	0.33 to 0.37
Renal coccidia	198.60	16	0.005	0.12	0.10 to 0.14
Gut protozoa	208.40	8	0.005	0.13	0.11 to 0.14
Digestive gland protozoa	471.1	8	0.005	0.19	0.17 to 0.21
Rickettsia	1166.32	8	0.005	0.30	0.28 to 0.32

The South and West coasts were next compared. On figures 3.2.1.2 to 3.2.1.5 above, farms a to g are on the South coast and farms h and i are on the West coast. The crude odds ratios for parasite prevalence between coasts are given in table 3.2.1.2. It can be seen that, with the exception of left kidney coccidia and trematodes, there is a greater risk of parasite infection on the West than the South coast. For left kidney coccidia, the risk is greater on the South coast. This may be largely due tot the effect of the high prevalence of left kidney coccidian infections on farm g. As no trematodes were found on the West coast, an odds ratio could not be calculated.

Table 3.2.1.2: Odds ratios for parasite prevalence in animals on the West coast compared to those from the South coast

Parasite	Odds ratio	Confidence interval
Sessile ciliates	1.32	1.19 to 1.46
Renal coccidia left kidney	0.65	0.44 to 0.95
Renal coccidia right kidney	3.91	1.75 to 8.72
Gut protozoa	2.22	1.83 to 2.69
Digestive gland protozoa	1.30	1.06 to 1.60
Rickettsia like prokaryotes	2.15	1.89 to 2.45

The Hermanus farms are indicated by d to f on figures 3.2.1.2 to 3.2.1.5 above. Hermanus farms were considered separately for two reasons. One is that it appeared from the routine examination of sections that parasite prevalence in Hermanus may differ from other areas. The other is the high concentration of farmed abalone in Hermanus and its potential impact on occurrence of parasites.

The crude odds ratios for parasite prevalence in other areas compared to Hermanus are given in table 3.2.1.3. It can be seen that, with the exception of sessile ciliates and trematodes, there is a greater risk of parasite infection in areas other than Hermanus. In the case of sessile ciliates, there is a greater risk within Hermanus than in other areas. These differences most probably relate to other variables and will be revisited below.

Table 3.2.1.3: Odds ratios for parasite prevalence in animals outside Hermanus compared to those from within Hermanus

Parasite	Odds ratio	Confidence interval
Sessile ciliates	0.85	0.79 to 0.93
Renal coccidia left kidney	1.53	1.14 to 2.05
Renal coccidia right kidney	15.75	2.13 to 116.67
Gut protozoa	1.43	1.19 to 1.71
Digestive gland protozoa	1.29	1.07 to 1.56
Rickettsia like prokaryotes	2.71	2.35 to 3.12
Trematodes	0.91	0.20 to 4.07

3.2.2 Seasonality

The temporal distribution of the samples is given in figure 3.2.2.1. To make these data more accessible, the cumulative distributions per year, for all months combined, figure 3.2.2.2, and, per month for the entire five year period, figure 3.2.2.3, are also shown. It can be seen that the distribution is not even. More samples were received over winter months than in summer and more samples were received in 2000 and 2004 than in other years. Winter is generally accepted to fall between March and May, and summer between December and February, although seasonal changes in water temperatures and weather conditions tend not adhere to these time frames.

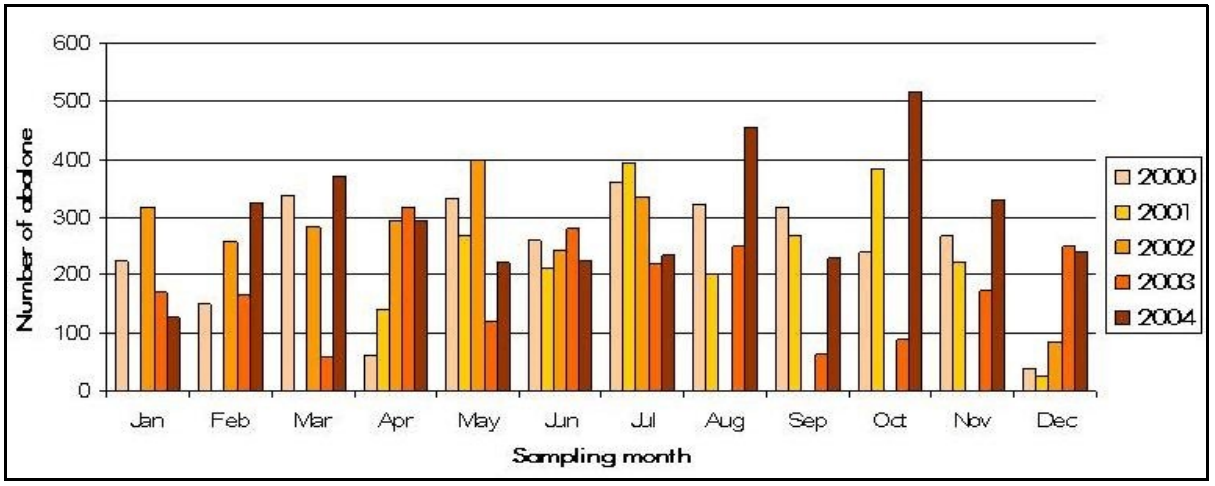

Figure 3.2.2.1: Temporal distribution of sample population

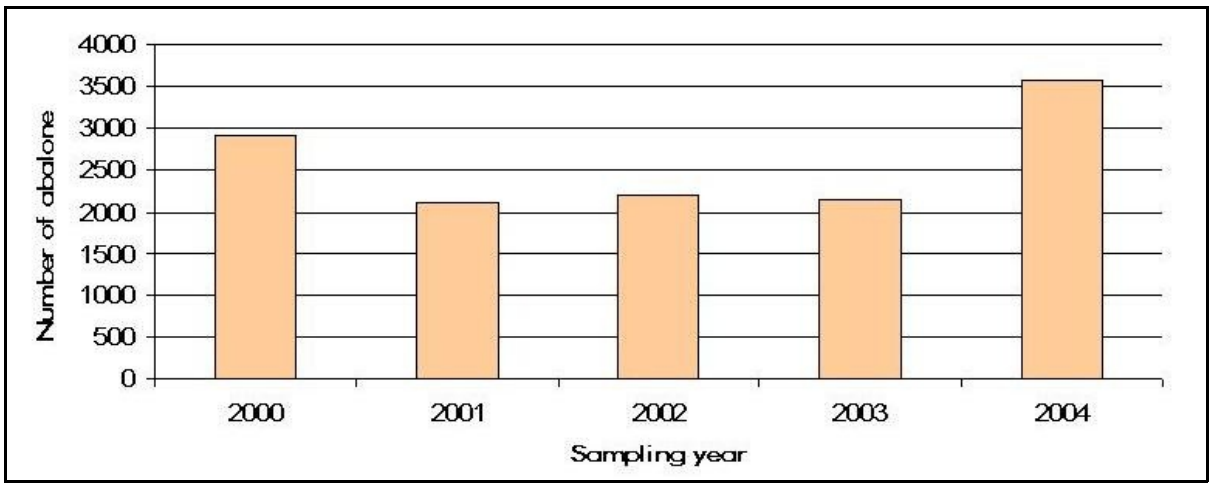

Figure 3.2.2.2: Distribution of sample population per year

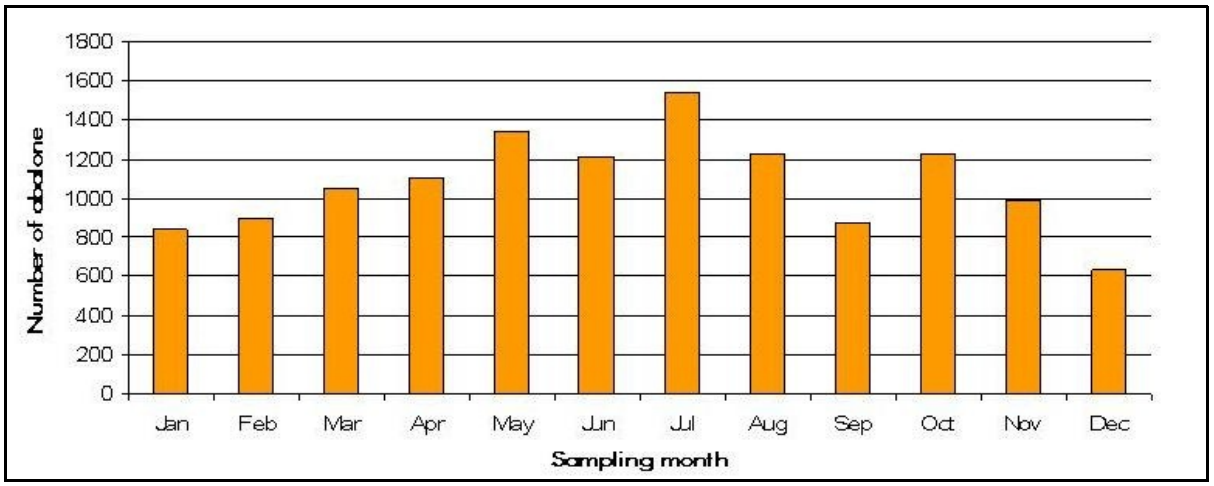

Figure 3.2.2.3: Distribution of sample population by month for all years combined

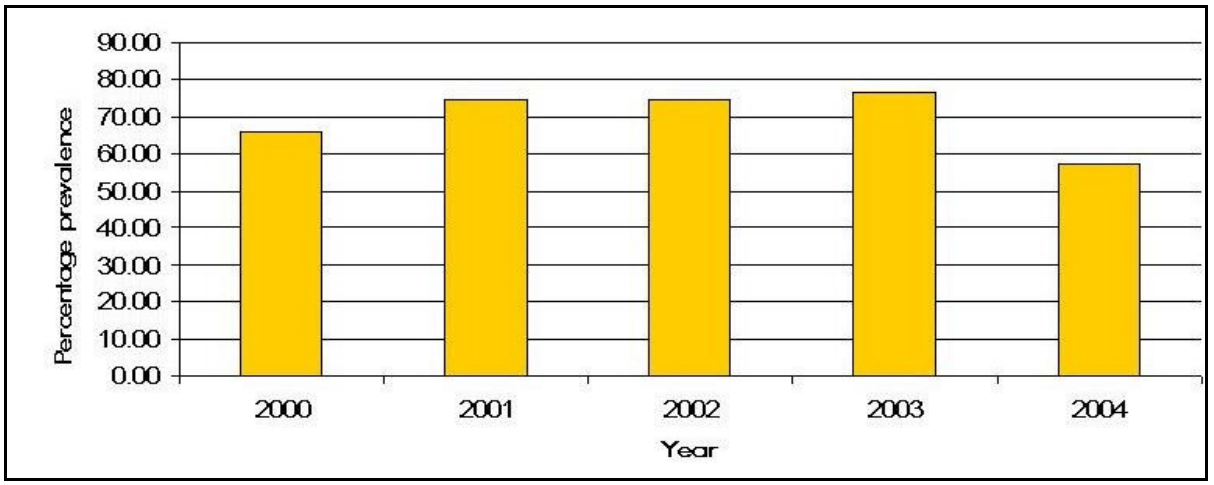

Figure 3.2.2.4: Prevalence of sessile ciliates by year

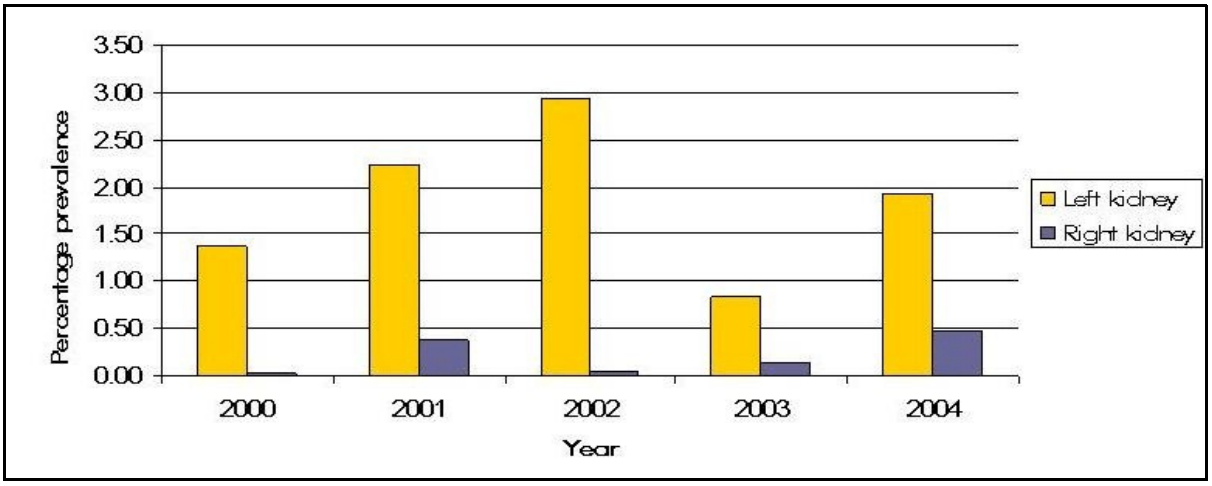

Figure 3.2.2.5: Prevalence of renal coccidia by year

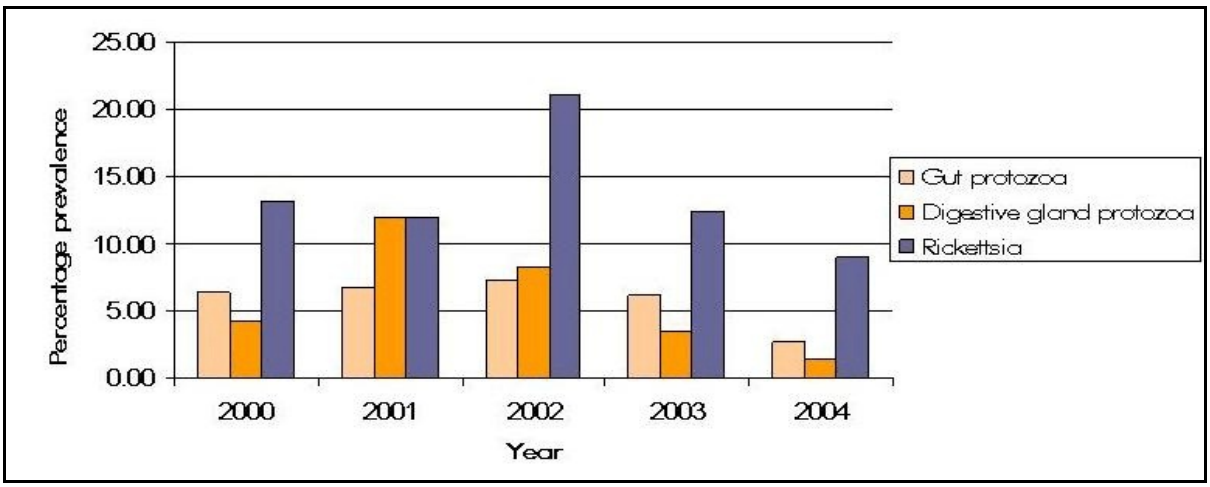

Figure 3.2.2.6: Prevalence of gut associated parasites by year

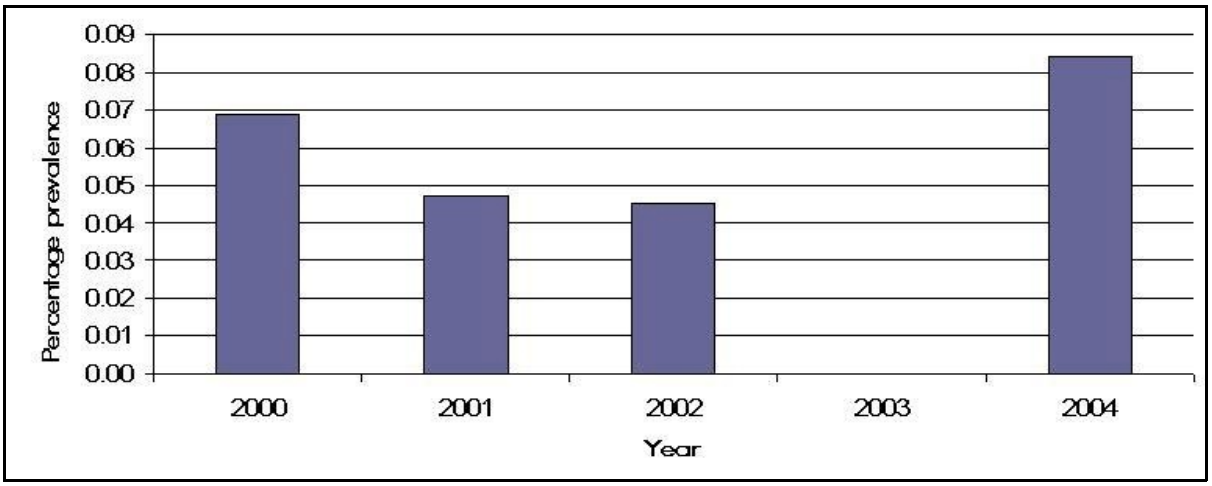

Figure 3.2.2.7: Prevalence of trematodes by year

The prevalences of the various parasites per year are given in figures 3.2.2.4 to 3.2.2.7. Note that the scale of the Y axis is not the same on the various charts.

Table 3.2.2.1: Results of chi square test for relationship between parasite prevalence and year

Parasite	Value of X^2	Df	P
Sessile ciliates	350.09	4	0.005
Renal coccidia	33.13	4	0.005
Gut protozoa	76.06	4	0.005
Digestive gland protozoa	354.36	4	0.005
Rickettsia	183.71	4	0.005

It can be seen from table 3.2.2.1 that there was a significant difference in the prevalence of all parasites in various years.

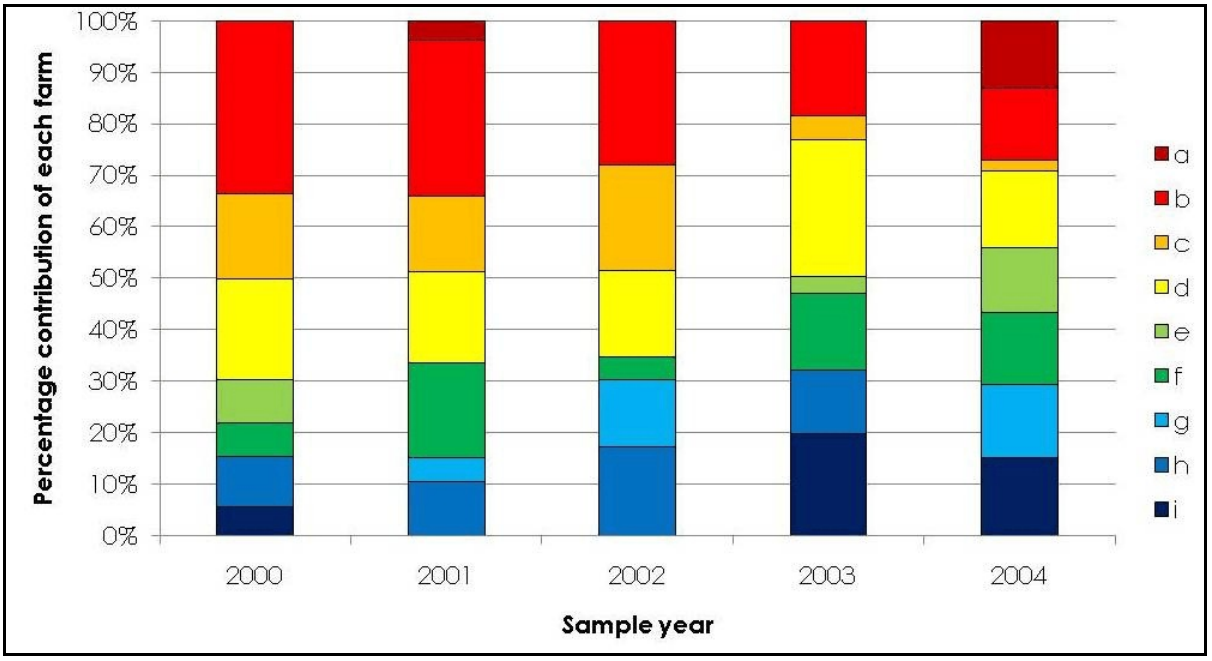

Figure 3.2.2.8: Percentage contribution of each farm to the total samples for every year

When considering parasite prevalence per year, the percentage contribution of each farm to the samples for that year, shown in figure 3.2.2.8, must be taken into consideration. For example, it was shown in 3.2.1 that farm g had by far the highest prevalence of renal coccidia. It is interesting to see that in 2003, when renal coccidia were at low levels, farm g was not included in the sample set. Similarly, farm c had very high numbers of animals infected with rickettsia. Farm c contributed a higher percentage of the total samples in the years 2000 to 2002 than in 2003 to 2004. Farms g, h and i also had elevated rickettsia prevalences relative to other farms. It can be seen from figure 3.2.2.8 that there was considerable variation in the contribution of these farms, but that their greatest combined contribution to total sample numbers was in 2002, which also saw a peak in rickettsia prevalence.

The prevalences of the various parasites per month are given in figures 3.2.2.9 to 3.2.2.12. Note that the scale of the Y axis is not the same on the various charts.

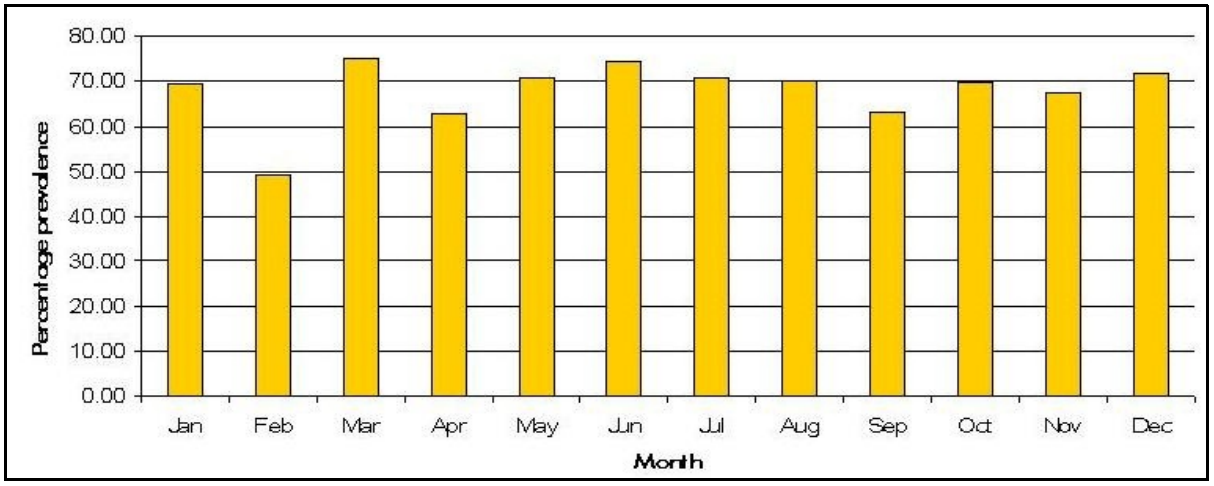

Figure 3.2.2.9: Prevalence of sessile ciliates by sampling month

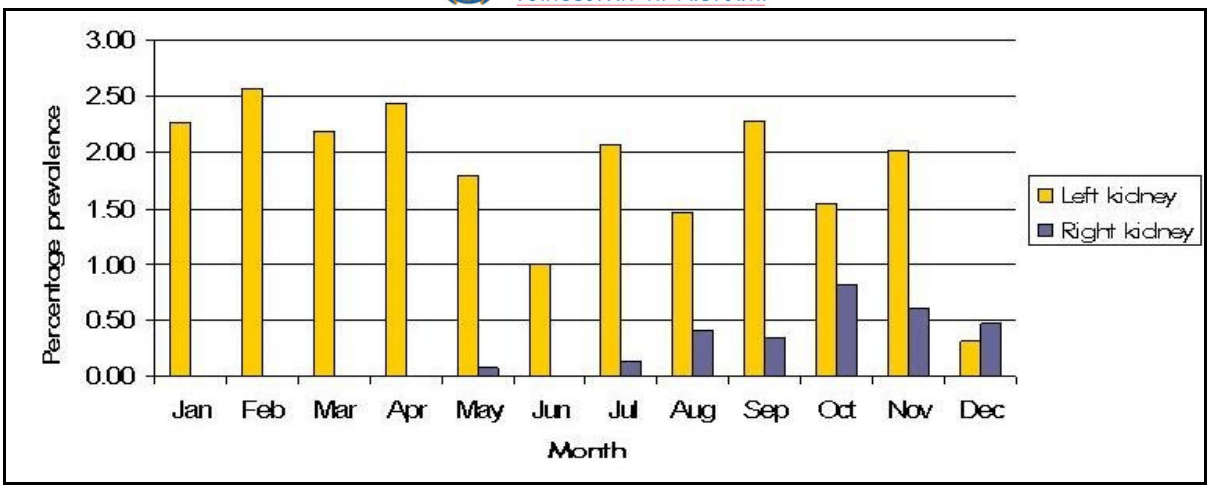

Figure 3.2.2.10: Prevalence of renal coccidia by sampling month

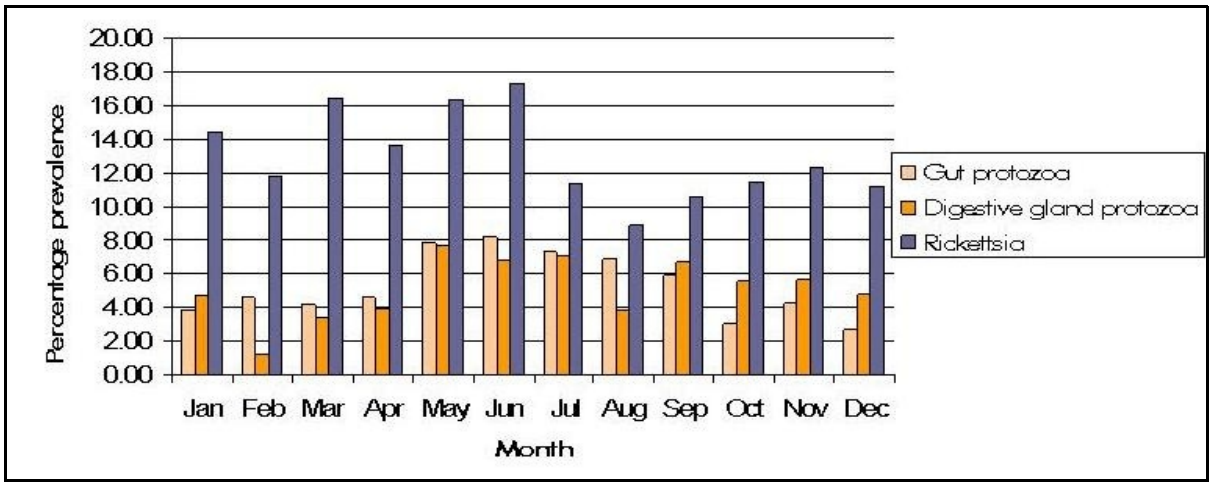

Figure 3.2.2.11: Prevalence of gut associated parasites by sampling month

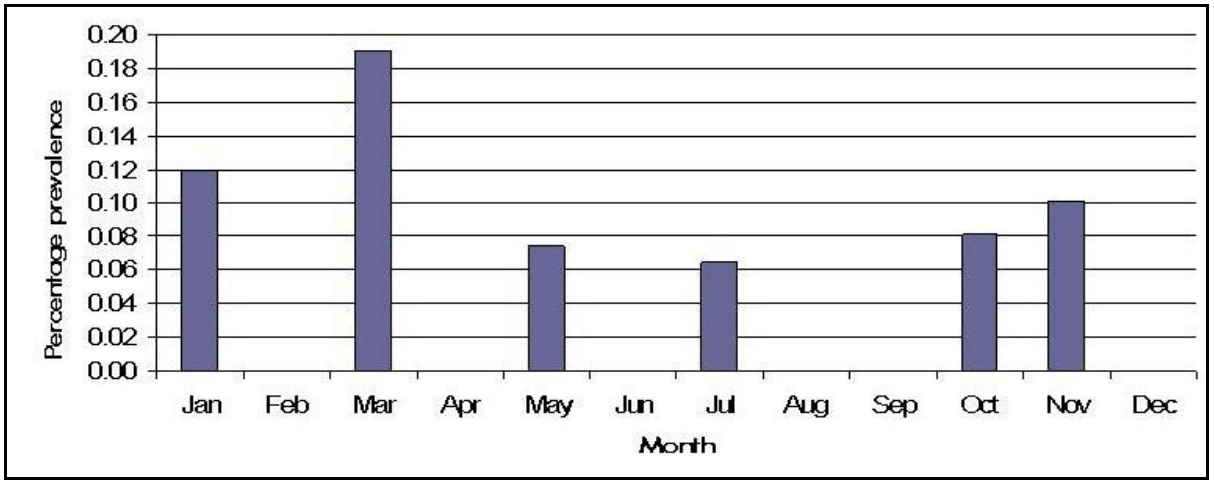

Figure 3.2.2.12: Prevalence of trematodes by sampling month

Table 3.2.2.2: Results of chi square test for relationship between parasite prevalence and month

Parasite	Value of X^2	Df	P
Sessile ciliates	235.06	11	0.005
Renal coccidia	63.92	22	0.005
Gut protozoa	81.95	11	0.005
Digestive gland protozoa	82.10	11	0.005
Rickettsia	78.44	11	0.005

It can be seen from table 3.2.2.2 that there was a significant difference in the prevalence of all parasites over various months.

Unfortunately, it is almost impossible to determine whether a seasonal effect exists for parasite prevalence from the available data. Some of the reasons will be discussed later. However, one of them is the uneven distribution of samples from individual farms in different months. For example, it can be seen from figure 3.2.1.3 that farm g has the highest prevalence of renal coccidia of all farms sampled. Figure 3.2.2.13 shows the percentage of samples for each month contributed by farm g. In both February and September, farm g contributed more than ten percent of the total samples. It is interesting that prevalence of renal coccidia, charted in figure 3.2.2.10, also shows a peak in February and a lesser peak in September.

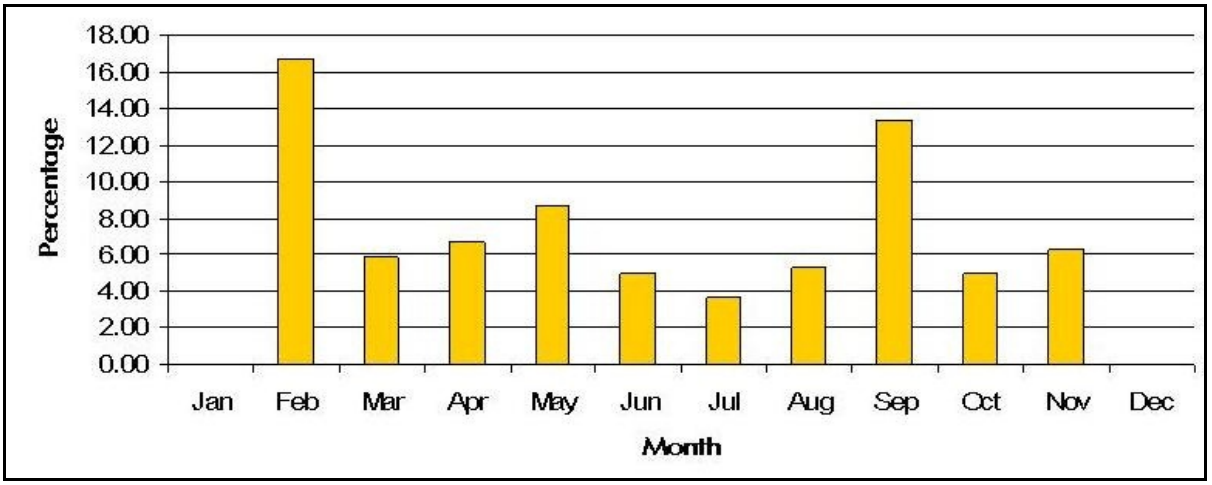

Figure 3.2.2.13: Percentage of total samples contributed by farm g in each month

It is also worth considering farm c. Figure 3.2.1.4 shows that the prevalence of gut associated parasites is highest on farm c. The percentage samples contributed by farm c and the total prevalence of rickettsia are shown in figure 3.2.2.14. There does appear to be a degree of correlation between the two variables, suggesting that the farm effect would tend to mask any true seasonal effect.

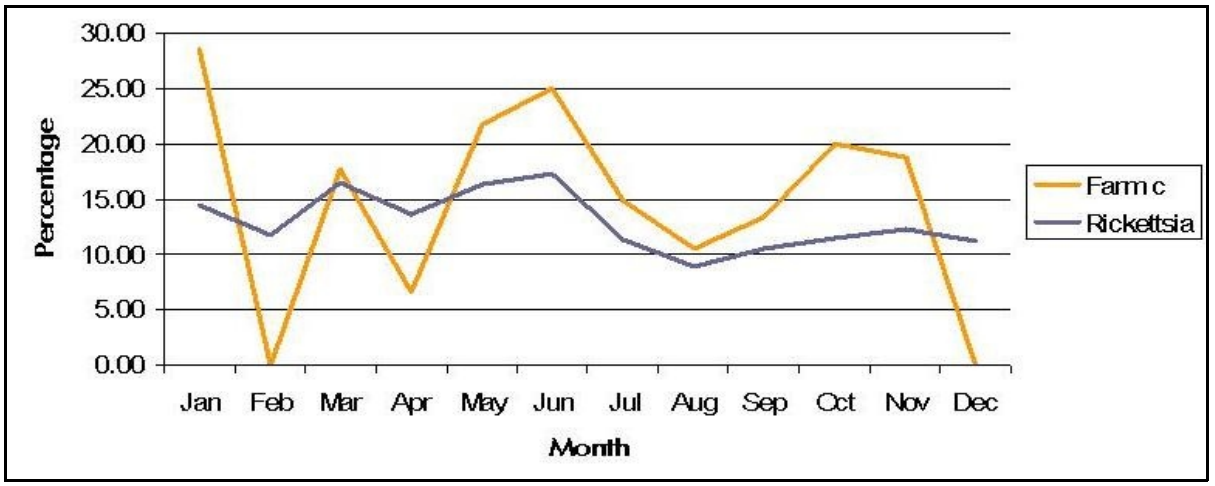

Figure 3.2.2.14: Percentage of total samples contributed by farm c compared to prevalence of rickettsia in each month

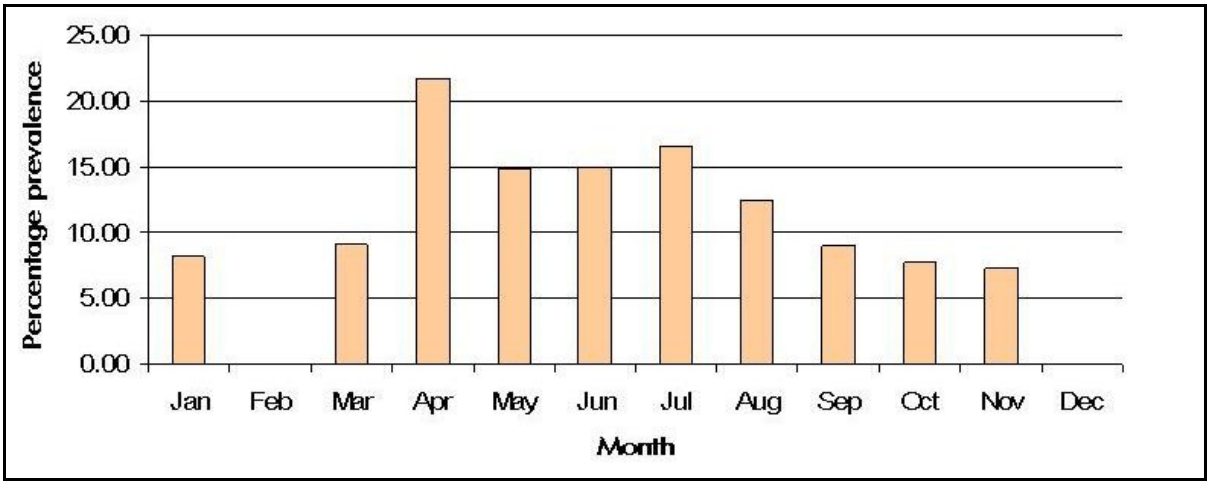

Figure 3.2.2.15: Prevalence of gut protozoa on farm c by month

It may be possible to examine seasonality by looking at events on individual farms. Figure 3.2.2.15 illustrates monthly prevalence of gut protozoa on farm c. The pattern is roughly similar to that for overall monthly prevalence of gut protozoa shown in figure 3.2.2.11, suggesting that the increase in prevalence over the winter months may be a true reflection of seasonality. However, other confounding variables also play a role, as will be shown below.

In addition to the effect of individual farms, the age profile of the sample population was not constant, as can be seen from figures 3.2.2.16 and 3.2.2.17. However, this does not relate in an obvious way to parasite prevalence.

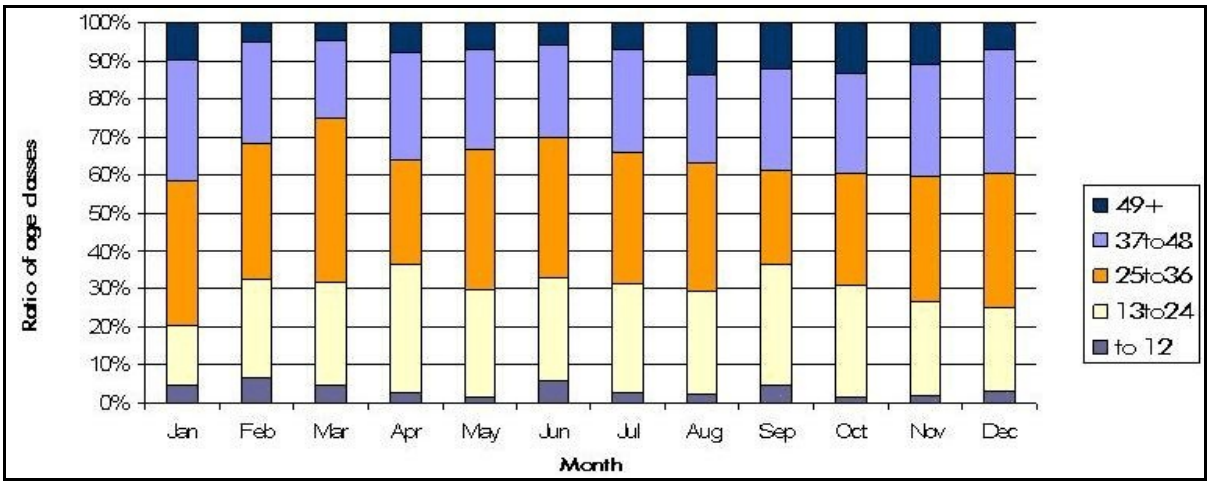

Figure 3.2.2.16: Age distribution of samples in each month

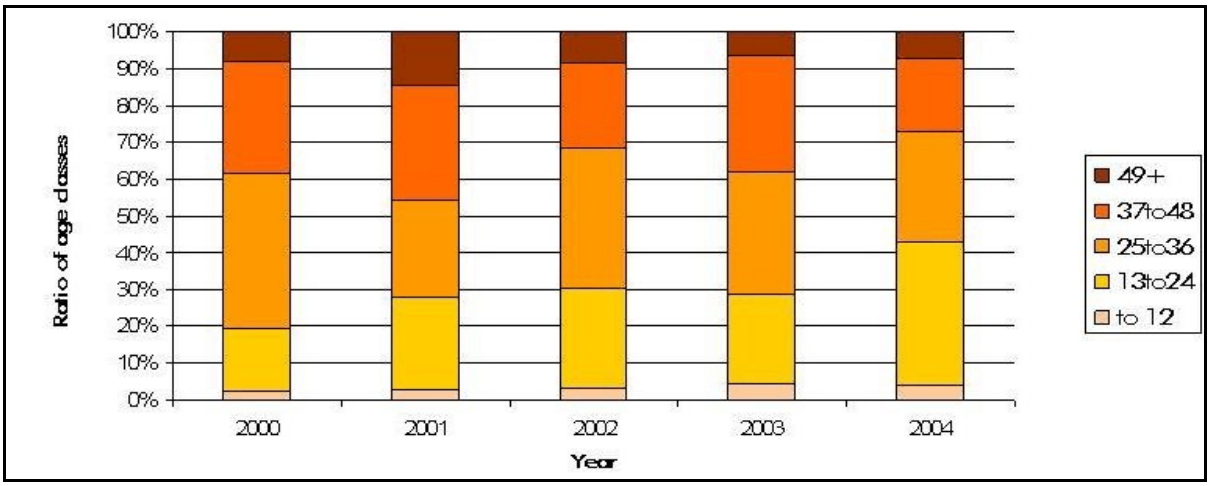

Figure 3.2.2.17: Age distribution of samples in each year

Lastly, the use of diet varied substantially over the sample period, as shown in figures 3.2.2.18 and 3.2.2.19. It will be shown in section 3.2.3 that diet use affects parasite prevalence in some cases. The amount of artificial feed in the diet varied from approximately a quarter in December to as much as half in March. There was also a reduction in the use of artificial feed from 2000 to 2003, followed by an increase in 2004.

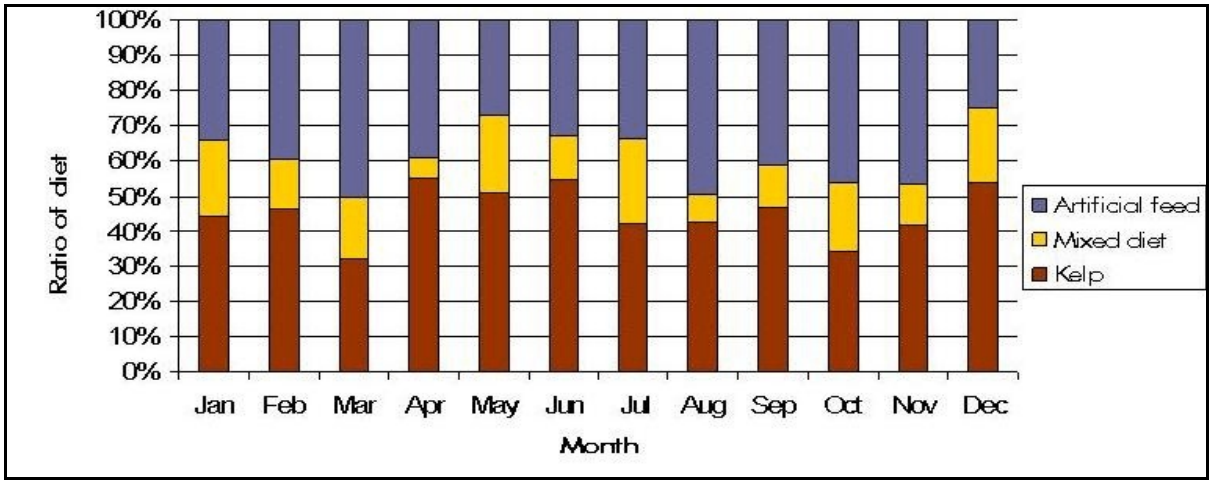

Figure 3.2.2.18: Diet use in sample population by month

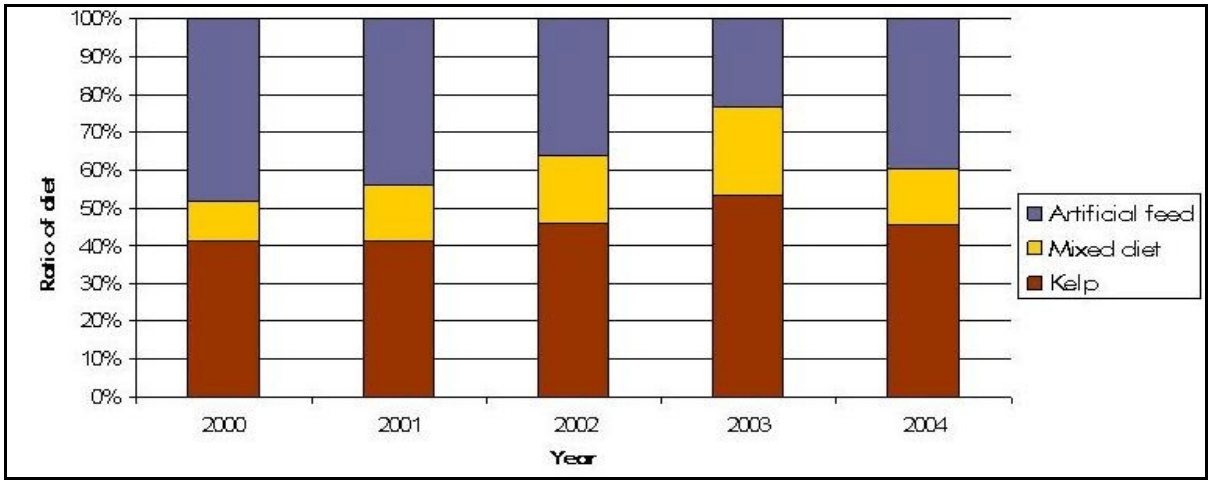

Figure 3.2.2.19: Diet use in sample population by year

3.2.3 Diet

Diet use in the sample population is shown in figure 3.2.3.1. The mixed diet is one containing both artificial feed and macroalgae, fed either simultaneously or as a rotational diet with a cycle of less than one month.

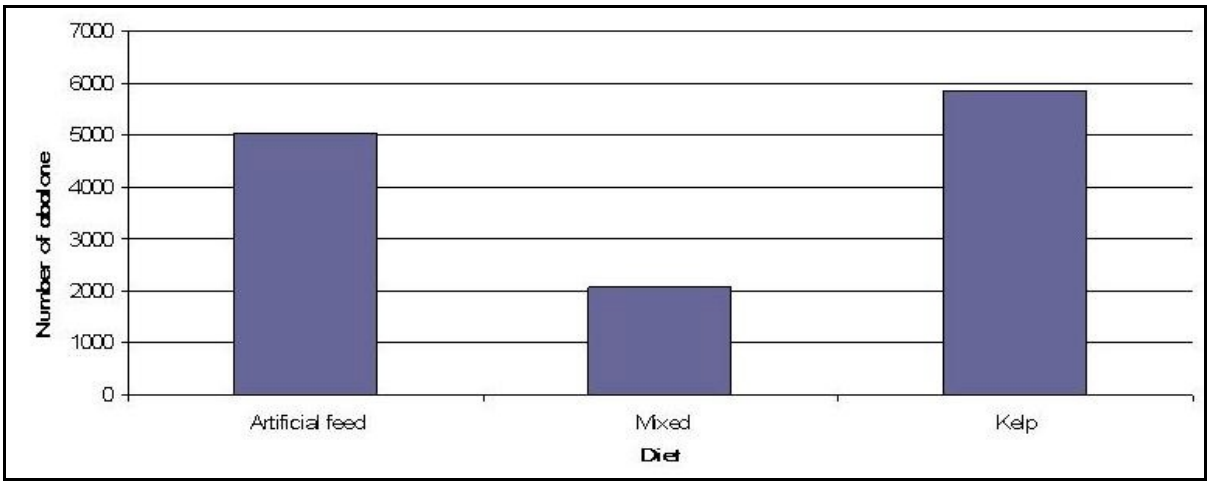

Figure 3.2.3.1: Diet use in sample population

The prevalences of the various parasites are given in figures 3.2.3.2 to 3.2.3.5. Note that the scale of the Y axis is not the same on the various charts.

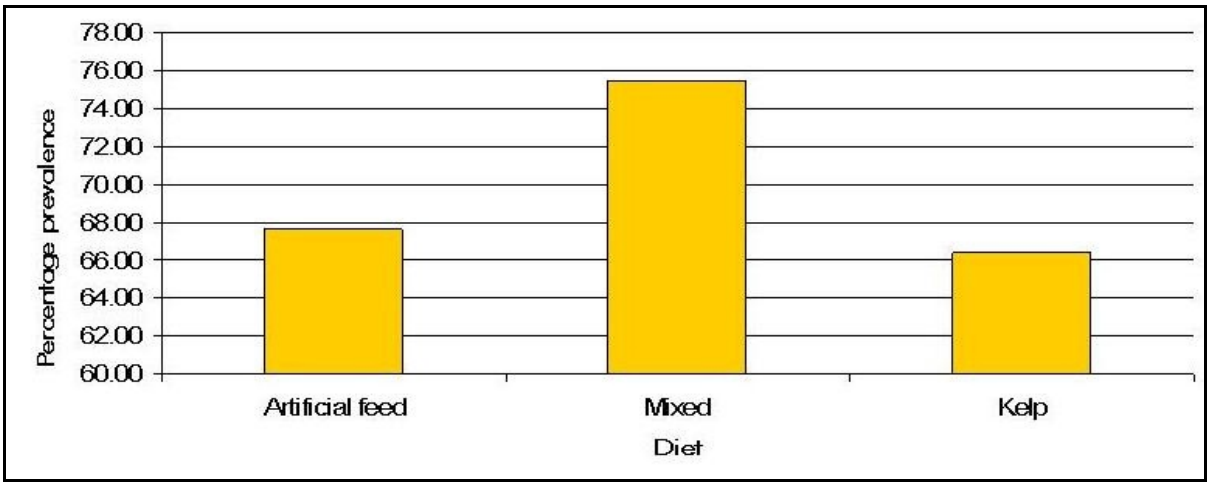

Figure 3.2.3.2: Prevalence of sessile ciliates in host population receiving different diets

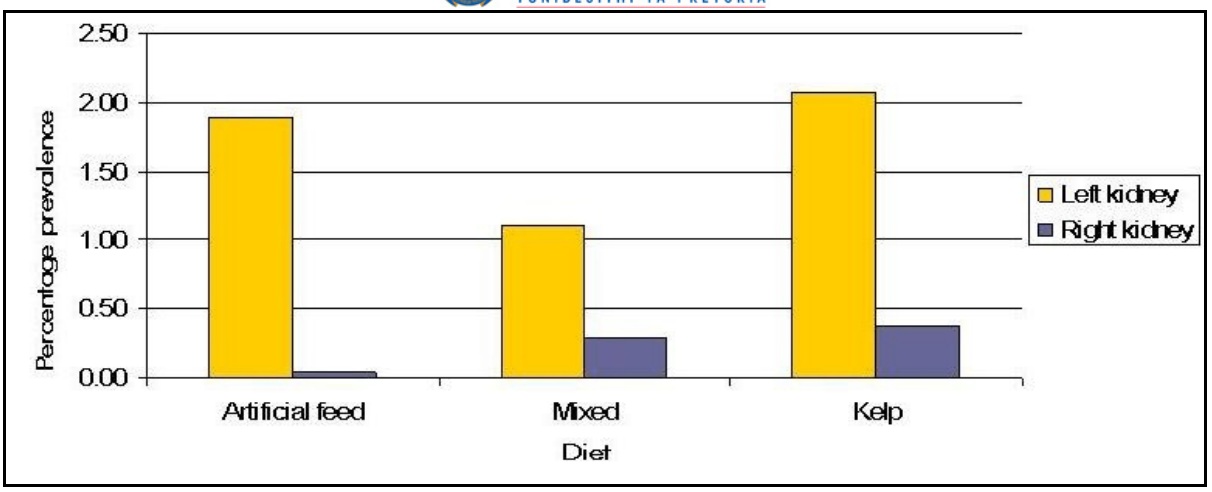

Figure 3.2.3.3: Prevalence of renal coccidia in host population receiving different diets

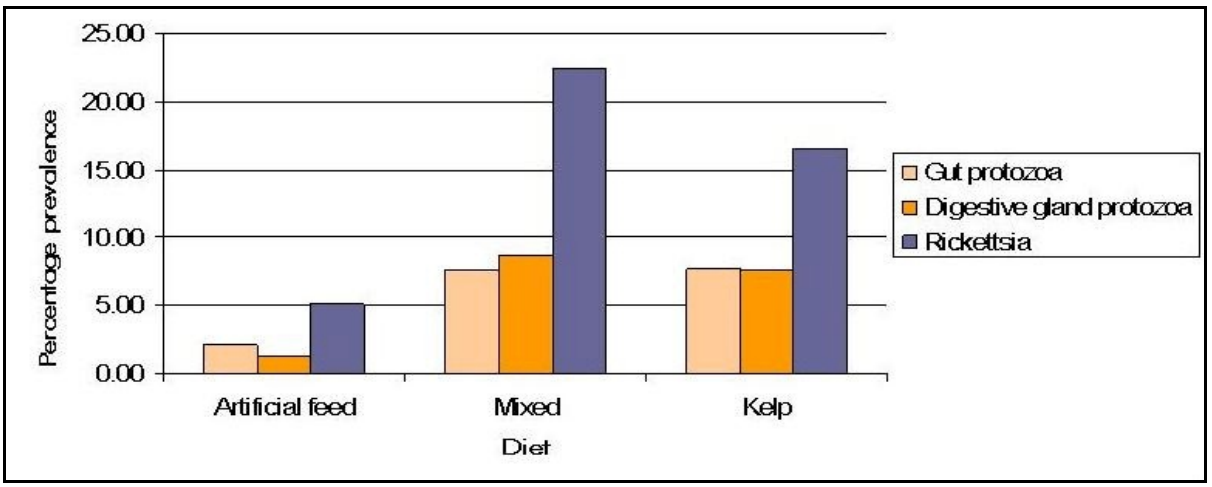

Figure 3.2.3.4: Prevalence of gut associated parasites in host population receiving different diets

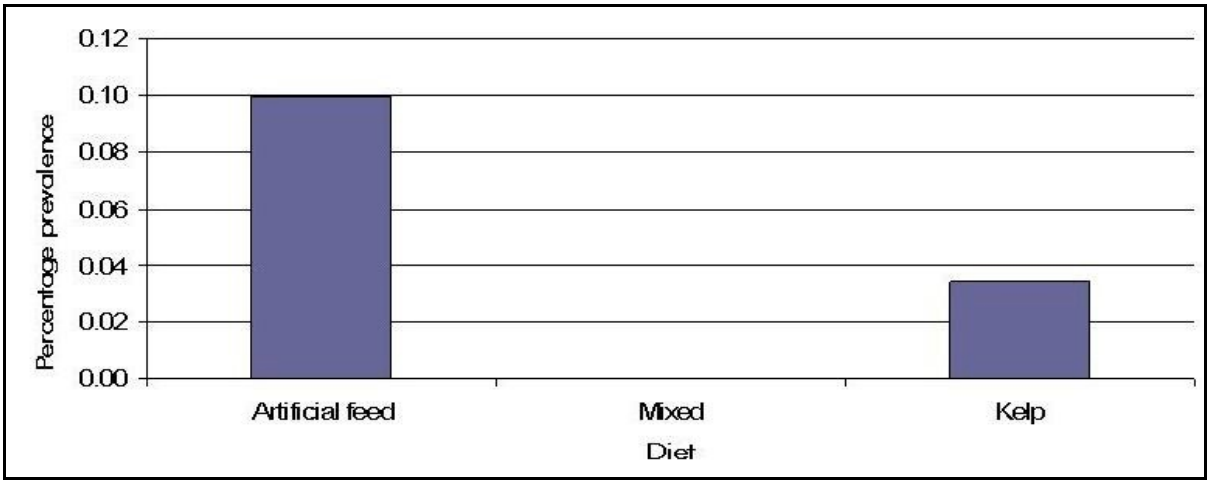

Figure 3.2.3.5: Prevalence of trematodes in host population receiving different diets

The results of the chi square test are summarised in table 3.2.3.1. It can be seen that there are significant differences in parasite prevalences between diets for all parasites tested. The effect sizes are small in all cases.

Table 3.2.3.1: Results of chi square test for relationship between parasite prevalence and host diet

Parasite	Value of X^2	Df	P	Effect size	95% confidence interval
Sessile ciliates	59.94	2	0.005	0.07	0.05 to 0.08
Renal coccidia	21.42	4	0.005	0.04	0.02 to 0.06
Gut protozoa	177.45	2	0.005	0.12	0.10 to 0.13
Digestive gland protozoa	270.42	2	0.005	0.14	0.13 to 0.16
Rickettsia like prokaryotes	508.03	2	0.005	0.20	0.18 to 0.22

The results of the chi square test indicate a significant association between diet and the prevalence of certain parasites. To test the strength of the association, odds ratios were calculated, as summarised in table 3.2.3.2. As calculation of odds ratios requires the construction of two by two tables, mixed diets were omitted and only kelp and artificial feed compared.

Table 3.2.3.2: Odds ratios for parasite prevalence in animals receiving kelp compared to those on artificial feed

Parasite	Odds ratio	Confidence interval
Sessile ciliates	0.95	0.87 to 1.03
Renal coccidia left kidney	1.10	0.84 to 1.44
Renal coccidia right kidney	9.50	2.23 to 40.41
Gut protozoa	3.75	3.03 to 4.63
Digestive gland protozoa	6.43	4.93 to 8.40
Rickettsia like prokaryotes	3.71	3.22 to 4.29

For right kidney coccidia and gut associated parasites, the odds ratios indicate a significantly increased risk of infection in animals receiving kelp compared to those on artificial feed. There was no difference in risk for sessile ciliates and left kidney coccidia.

It has already been shown in section 3.1.1 that there is an association between age and parasite prevalence for all of the above, except trematodes. The age distribution of animals on different diets is shown in figure 3.2.3.6. There is clearly a difference in diet use between older and younger animals, with more kelp being fed to older animals. The relationship between parasite prevalence, age and diet was explored using odds ratios. As it is necessary to construct a two by two table for calculation of odds ratios, the animals were divided into two age classes, one for animals of 24 months and younger, and another for older animals.

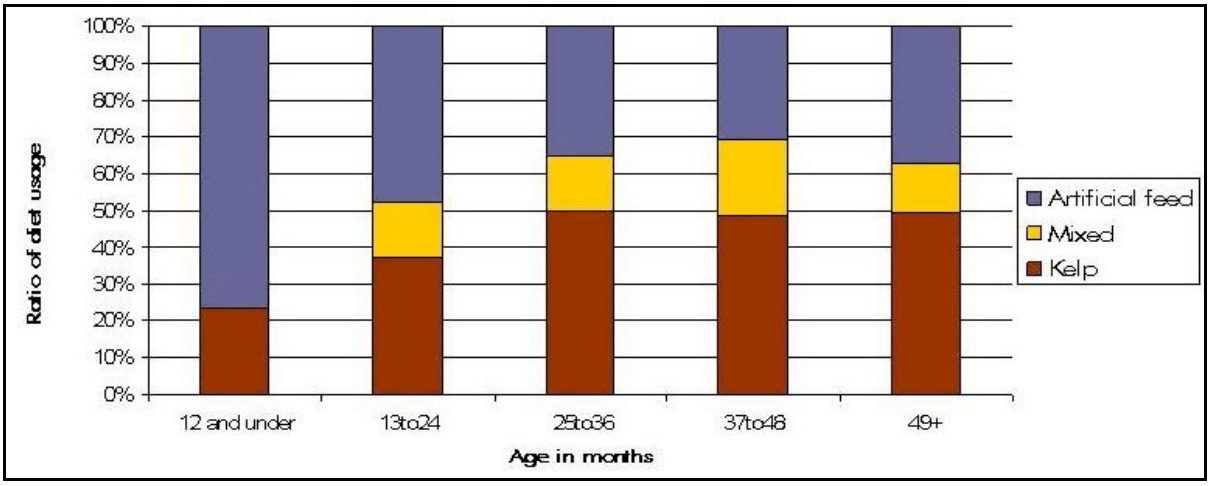

Figure 3.2.3.6: Comparison of diet use in different age classes of sample population

The crude odds ratio for use of kelp compared to use of artificial feed, in animals older than 24 months, compared to younger animals, is 2.09 (confidence interval 1.93 to 2.27). In other words, there is a significantly greater probability that animals older than 24 months will receive a kelp diet than those that are younger.

The stratum specific odds ratios for parasite prevalence on kelp compared to artificial feed are summarised in table 3.2.3.3 for animals of 24 months and younger, and in table 3.2.3.4 for older animals. No animals of 24 months and younger receiving artificial feed and infected with right kidney coccidia or digestive gland protozoa were present and odds ratios could not be calculated for these groups. For gut protozoa and rickettsia like prokaryotes, the risk of infection was greater on kelp for both age groups.

Table 3.2.3.3: Odds ratios for parasite prevalence in animals of 24 months and younger, receiving kelp compared to those on artificial feed

Parasite	Odds ratio	Confidence interval
Renal coccidia right kidney	-	-
Gut protozoa	12.87	6.17 to 26.85
Digestive gland protozoa	-	-
Rickettsia like prokaryotes	5.38	3.77 to 7.66

Table 3.2.3.4: Odds ratios for parasite prevalence in animals older than 24 months, receiving kelp compared to those on artificial feed

Parasite	Odds ratio	Confidence interval
Renal coccidia right kidney	7.19	1.68 to 30.67
Gut protozoa	2.71	2.16 to 3.38
Digestive gland protozoa	4.53	3.46 to 5.94
Rickettsia like prokaryotes	3.00	2.56 to 3.52

As the stratum specific odds ratios differ from the crude odds ratios, the presence of confounding is suspected. The stratum specific odds ratios also differ from each other, indicating the presence of interaction. It was not possible to explore the relationship further for right kidney coccidian and digestive gland protozoan infections, as odds ratios could not be calculated for the younger animals.

For gut protozoa, a summary odds ratio of 3.25 (confidence interval 2.49 to 3.82) was calculated using the Mantel Haenszel technique. The summary odds ratio differs by approximately 15.3 percent from the crude odds ratio, indicating that the crude odds ratio is not an accurate estimate in this case. The Breslow Day test for interaction yielded a value of 15.81 (1 degree of freedom), which is significant (p=0.005).

In the case of rickettsia, a summary odds ratio of 3.30 (confidence interval 2.86 to 3.82) was calculated using the Mantel Haenszel technique. The summary odds ratio differs by approximately 11.2 percent from the crude odds ratio, again indicating that the crude odds ratio is not an accurate estimate. The Breslow Day test for interaction yielded a value of 8.66 (1 degree of freedom), which is significant (p=0.005).

For both gut protozoa and rickettsia, the risk of infection when receiving kelp was greater in younger than in older animals. It is possible to explore this relationship further by stratifying the data according to diet and then calculating odds ratios comparing the different age groups. This is summarised in tables 3.2.3.5 and 3.2.3.6. It can be seen that, when animals receive artificial feed, the older animals have a greater risk of infection with gut protozoa and rickettsia compared to the younger animals, than when both groups are fed kelp.

Table 3.2.3.5: Odds ratios for parasite prevalence in animals fed kelp, comparing animals older than 24 months, to animals of 24 months and younger

Parasite	Odds ratio	Confidence interval
Renal coccidia right kidney	6.68	0.90 to 49.74
Gut protozoa	1.83	1.41 to 2.38
Digestive gland protozoa	2.56	1.90 to 3.45
Rickettsia like prokaryotes	2.05	1.69 to 2.47

Table 3.2.3.6: Odds ratios for parasite prevalence in animals fed artificial feed, comparing animals older than 24 months, to animals of 24 months and younger

Parasite	Odds ratio	Confidence interval
Renal coccidia right kidney	-	-
Gut protozoa	8.71	4.23 to 17.93
Digestive gland protozoa	-	-
Rickettsia like prokaryotes	3.66	2.61 to 5.14

For gut protozoa, a summary odds ratio of 2.52 (confidence interval 1.72 to 2.82) was calculated using the Mantel Haenszel technique. The summary odds ratio differs by approximately 48.8 percent from the crude odds ratio, indicating that the crude odds ratio is not an accurate estimate in this case. The Breslow Day test for interaction yielded a value of 15.81 (1 degree of freedom), which is significant (p=0.005).

In the case of rickettsia, a summary odds ratio of 2.38 (confidence interval 1.99 to 2.77) was calculated using the Mantel Haenszel technique. The summary odds ratio differs by approximately 55.9 percent from the crude odds ratio, again indicating that the crude odds ratio is not an accurate estimate. The Breslow Day test for interaction yielded a value of 8.98 (1 degree of freedom), which is significant (p=0.005).

In addition to the effect of age, an association between coast and parasite prevalence was demonstrated in section 3.2.1 for gut associated parasites. The distribution of animals from different coasts on different diets is shown in figure 3.2.3.7 and from different areas in figure 3.2.3.8. There is clearly a difference in diet use between coasts and areas.

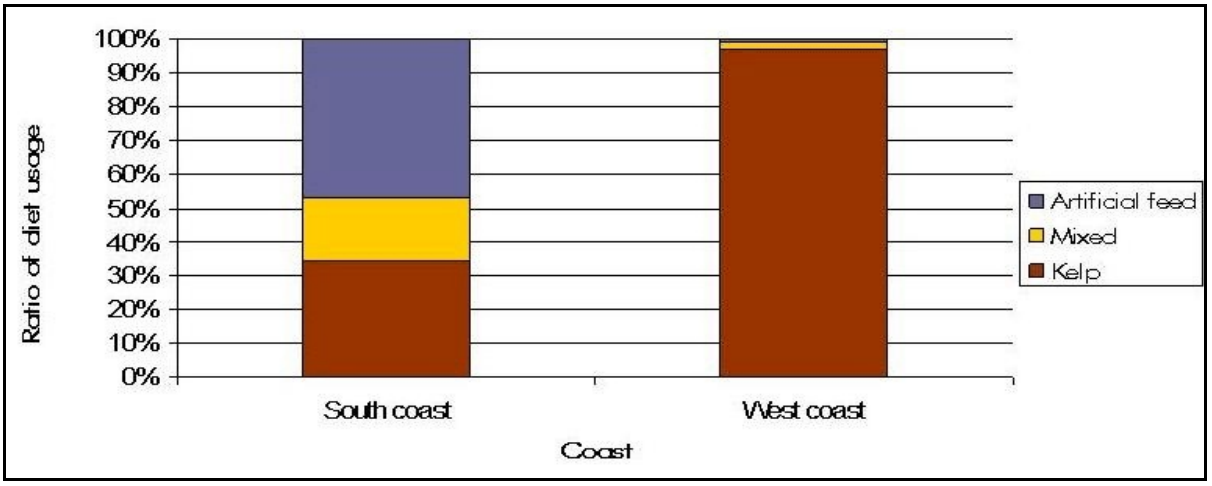

Figure 3.2.3.7: Comparison of diet usage in sample population from different coasts

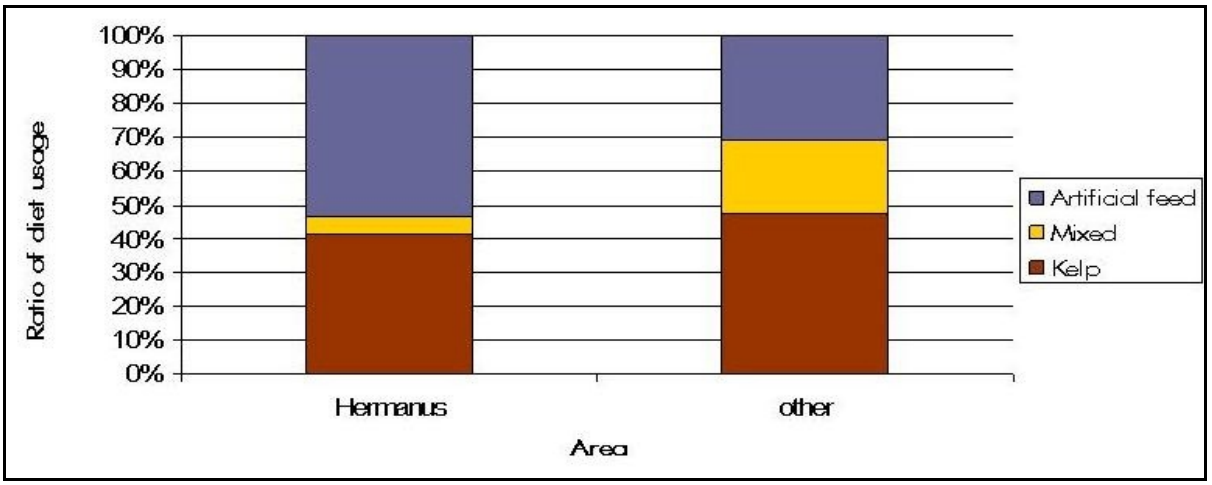

Figure 3.2.3.8: Comparison of diet usage in sample population from different areas

The relationship between parasite prevalence, area and diet was explored using odds ratios. Although there is also a difference in diet use between coasts, this was not explored, as only 20 animals receiving artificial feed were present in the entire West coast sample. Consequently, no significant odds ratios were obtained for parasite prevalences in West coast animals when the data were stratified.

The crude odds ratio for use of kelp compared to use of artificial feed, in animals outside Hermanus, compared to those in Hermanus, is 1.98 (confidence interval 1.83 to 2.14). In other words, there is a significantly greater probability that animals outside Hermanus will receive a kelp diet than artificial feed.

The stratified odds ratios for parasite prevalence on kelp compared to artificial feed are summarised in table 3.2.3.7 for animals in other areas, and in table 3.2.3.8 for animals from Hermanus. No animals from Hermanus receiving artificial feed and infected with right kidney coccidia were present and an odds ratio could not be calculated for this group.

Table 3.2.3.7: Odds ratios for parasite prevalence in animals from outside Hermanus, comparing animals fed kelp to those receiving artificial feed

Parasite	Odds ratio	Confidence interval
Renal coccidia right kidney	6.86	1.61 to 29.29
Gut protozoa	2.59	2.01 to 3.34
Digestive gland protozoa	3.82	2.81 to 5.18
Rickettsia like prokaryotes	3.04	2.57 to 3.59

Table 3.2.3.8: Odds ratios for parasite prevalence in animals from Hermanus, comparing animals fed kelp to those receiving artificial feed

Parasite	Odds ratio	Confidence interval
Renal coccidia right kidney	-	-
Gut protozoa	6.55	4.45 to 9.64
Digestive gland protozoa	19.00	10.54 to 34.26
Rickettsia like prokaryotes	4.26	3.20 to 5.66

As the stratum specific odds ratios differ from the crude odds ratios, the presence of confounding is suspected. The stratum specific odds ratios also differ from each other, indicating the presence of interaction. It was not possible to explore the relationship further for right kidney coccidian infections, as an odds ratio could not be calculated for the Hermanus animals.

For gut protozoa, a summary odds ratio of 3.49 (confidence interval 2.77 to 4.23) was calculated using the Mantel Haenszel technique. The summary odds ratio differs by approximately 7.4 percent from the crude odds ratio, indicating that the crude odds ratio is a reasonable estimate in this case. The Breslow Day test for interaction yielded a value of 15.50 (1 degree of freedom), which is significant (p=0.005).

For digestive gland protozoa, a summary odds ratio of 6.03 (confidence interval 4.09 to 7.03) was calculated using the Mantel Haenszel technique. The summary odds ratio differs by approximately 6.6 percent from the crude odds ratio, indicating that the crude odds ratio is a reasonable estimate in this case. The Breslow Day test for interaction yielded a value of 22.47 (1 degree of freedom), which is significant (p=0.005).

In the case of rickettsia, a summary odds ratio of 3.30 (confidence interval 2.87 to 3.83) was calculated using the Mantel Haenszel technique. The summary odds ratio differs by approximately 12.4 percent from the crude odds ratio, indicating that the crude odds ratio is not an accurate estimate. The Breslow Day test for interaction yielded a value of 4.03 (1 degree of freedom), which is significant (p=0.05).

The data appear to show that there is a greater risk of infection with gut associated parasites when animals are fed kelp in Hermanus than in other areas. However, when stratum specific odds ratios are calculated for diet use and age in Hermanus and other areas, it can be seen that there is a significant difference between the age profile of animals on the various diets in different areas. This is shown in figure 3.2.3.9 and summarised in table 3.2.3.9.

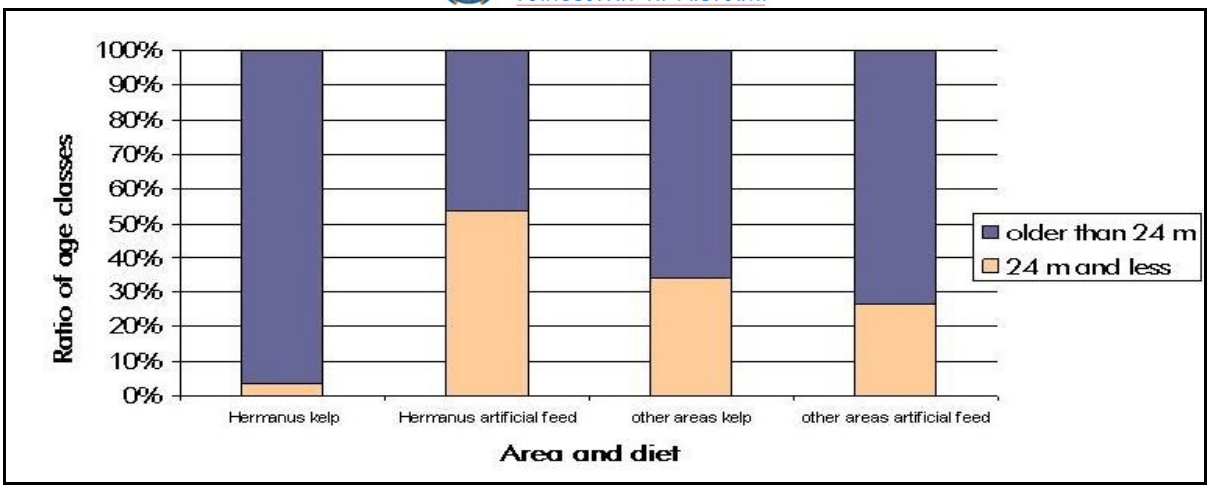

Figure 3.2.3.9: Diet use in different age groups in different areas

Table 3.2.3.9: Odds ratios for use of kelp in animals from in and outside Hermanus, comparing animals of older than 24 months to those of 24 months and younger

Area	Odds ratio	Confidence interval
Hermanus	30.87	24.01 to 39.69
other areas	0.69	0.62 to 0.77

There is a significantly greater probability that animals older than 24 months in Hermanus will receive kelp than artificial feed. In other areas, there are actually more older animals which are fed artificial feed than ones on kelp. Whereas diet confounds the association between area and parasite prevalence, age confounds the stratum specific odds ratios for diet and parasite prevalence in different areas.

Finally, the relationship between age and area was examined. The age distribution of the sample population by area is shown in figure 3.2.3.10. It can be seen that similar numbers of abalone from the various age classes were sampled in Hermanus and other areas. This is also true when comparing coasts, as illustrated in figure 3.2.3.11. The odds ratio comparing different age classes in other areas to Hermanus is 1.04 (confidence interval 0.95 to 1.13), which is not significant. The odds ratio comparing different age classes on the West coast to the South coast is 1.11 (confidence interval 1.00 to 1.23), which is also not significant.

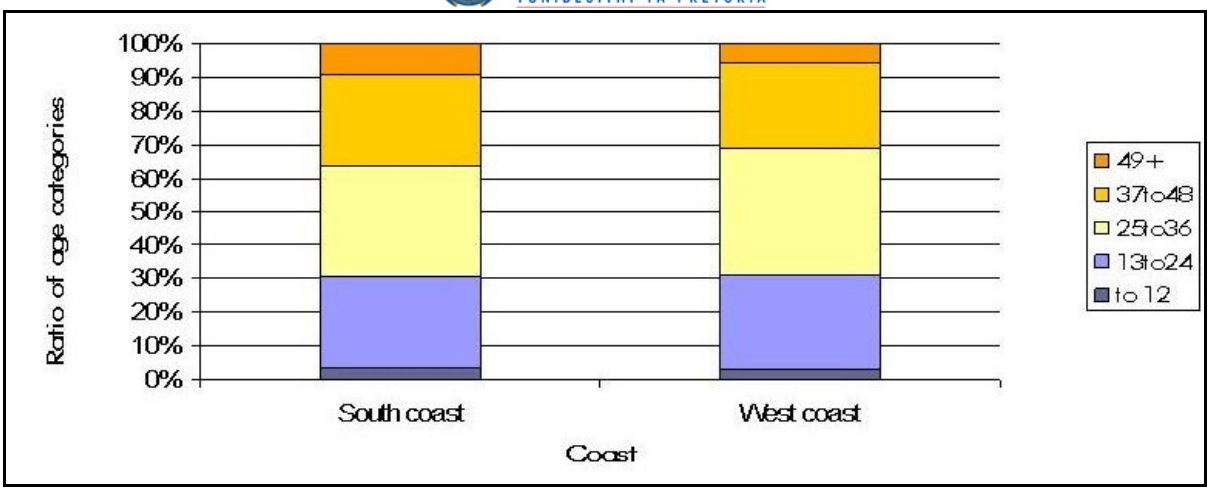

Figure 3.2.3.10: Age distribution of sample population from different coasts

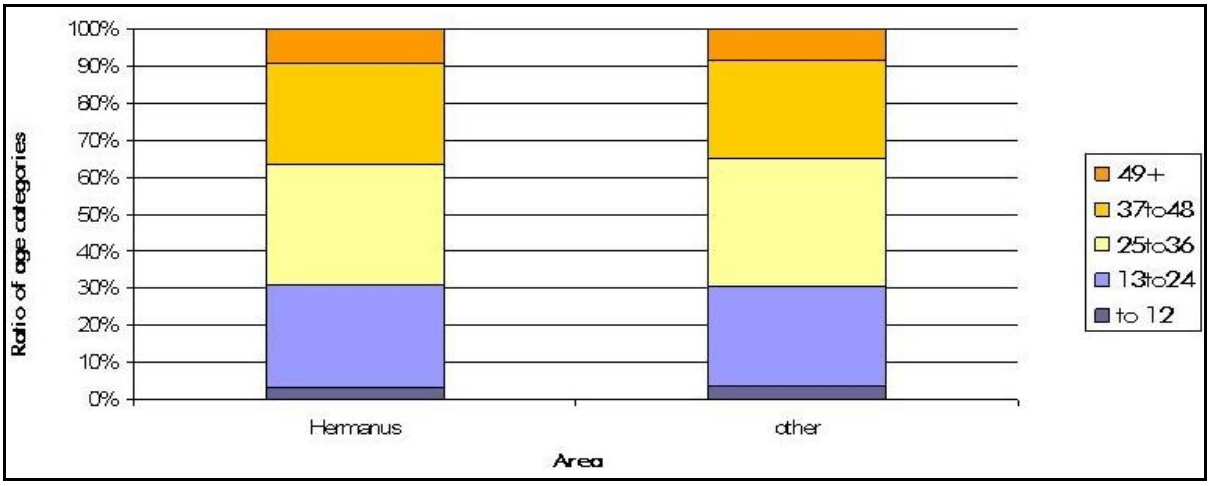

Figure 3.2.3.11: Age distribution of sample population from different areas

3.2.4 System

The distribution of the sample population between flow through and recirculation systems is shown in figure 3.2.4.1. It can be seen that the majority of animals originated in flow through systems.

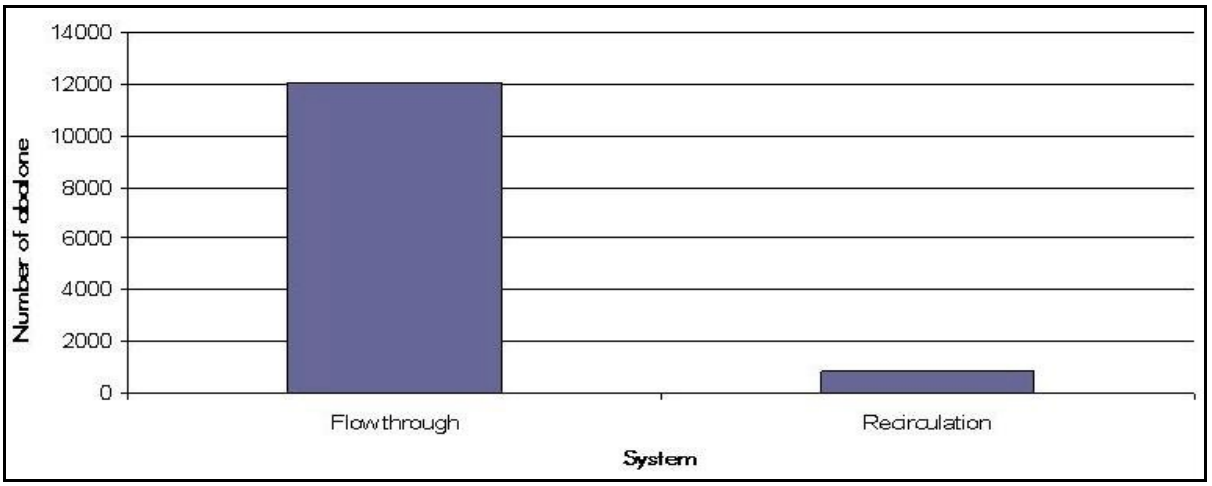

Figure 3.2.4.1: System distribution of sample population

The prevalences of the various parasites are given in figures 3.2.4.2 to 3.2.4.5. Note that the scale of the Y axis is not the same on the various charts.

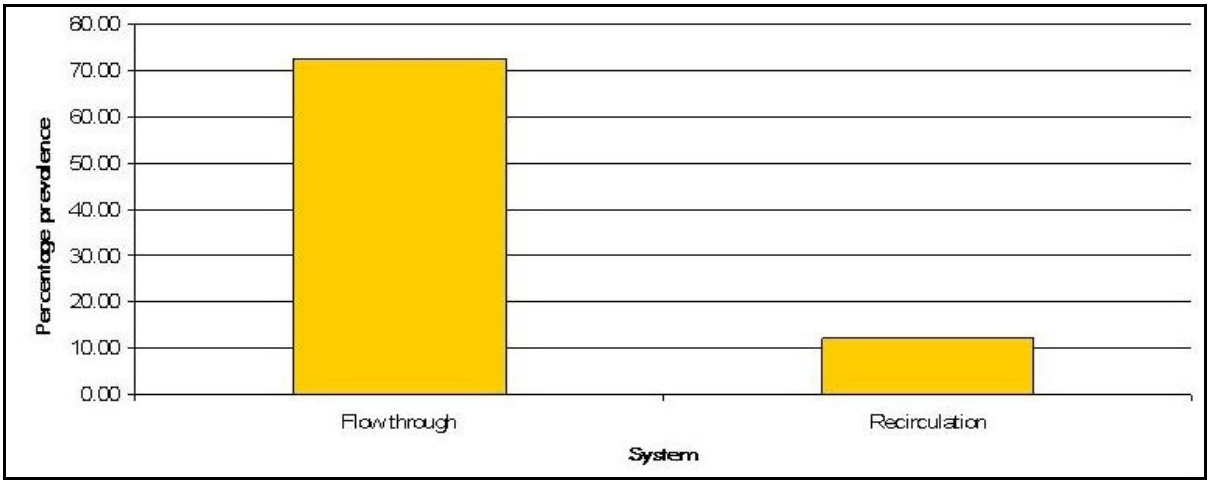

Figure 3.2.4.2: Prevalence of sessile ciliates in host population from different systems

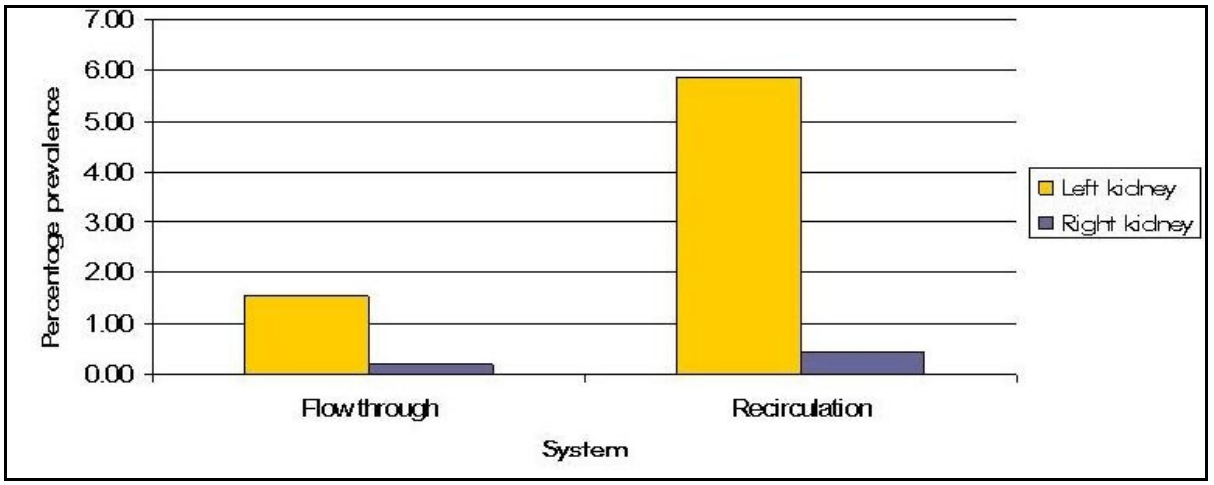

Figure 3.2.4.3: Prevalence of renal coccidia in host population from different systems

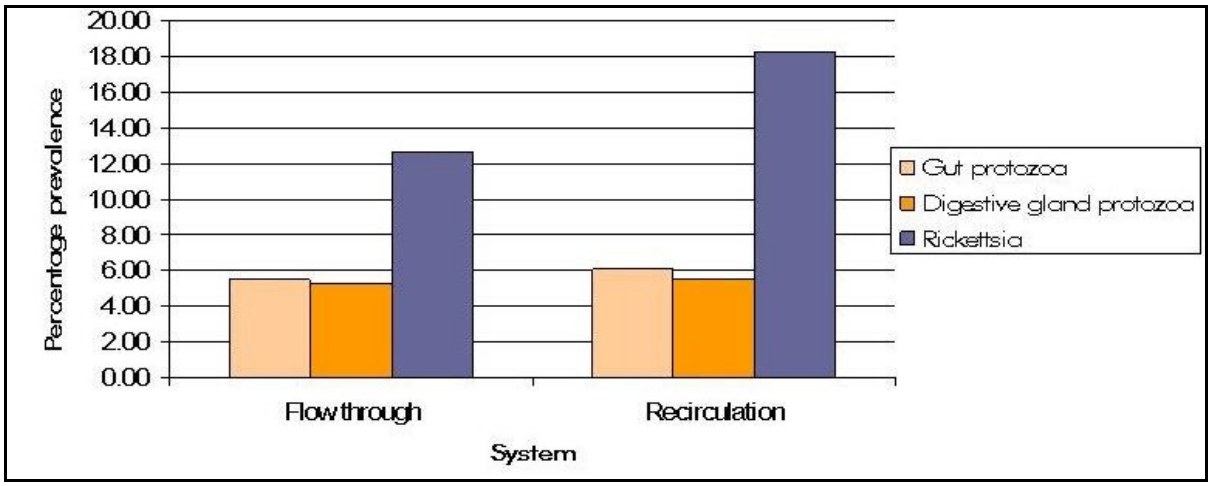

Figure 3.2.4.4: Prevalence of gut associated parasites in host population from different systems

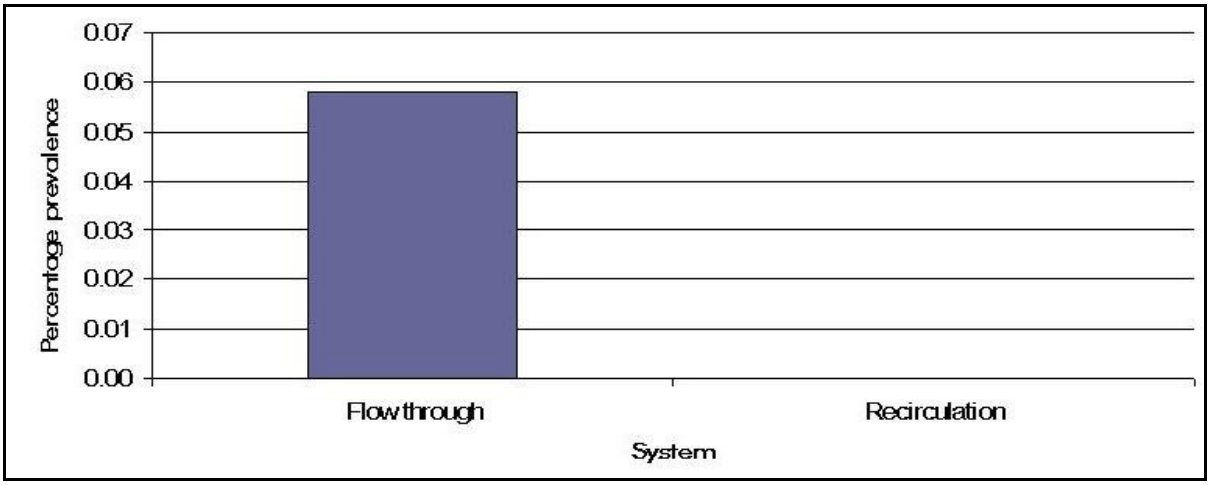

Figure 3.2.4.5: Prevalence of trematodes in host population from different systems

The results of the chi square test are summarised in table 3.2.4.1. It can be seen that there are significant differences in parasite prevalences between systems for sessile ciliates, renal coccidia and rickettsia like prokaryotes.

Table 3.2.4.1: Results of chi square test for relationship between parasite prevalence and system

Parasite	Value of X^2	Df	P	Effect size	95% confidence interval
Sessile ciliates	1384.47	1	0.005	0.33	0.31 to 0.34
Renal coccidia	86.79	2	0.005	0.08	0.06 to 0.10
Gut protozoa	0.52	1	0.5	na	na
Digestive gland protozoa	0.11	1	0.9	na	na
Rickettsia like prokaryotes	22.50	1	0.005	0.04	0.02 to 0.06

As it has been shown that parasite prevalence is affected by age, the age distribution of animals in flow through and recirculation systems were compared, as shown in figure 3.2.4.6. It can be seen that the age distributions are very similar.

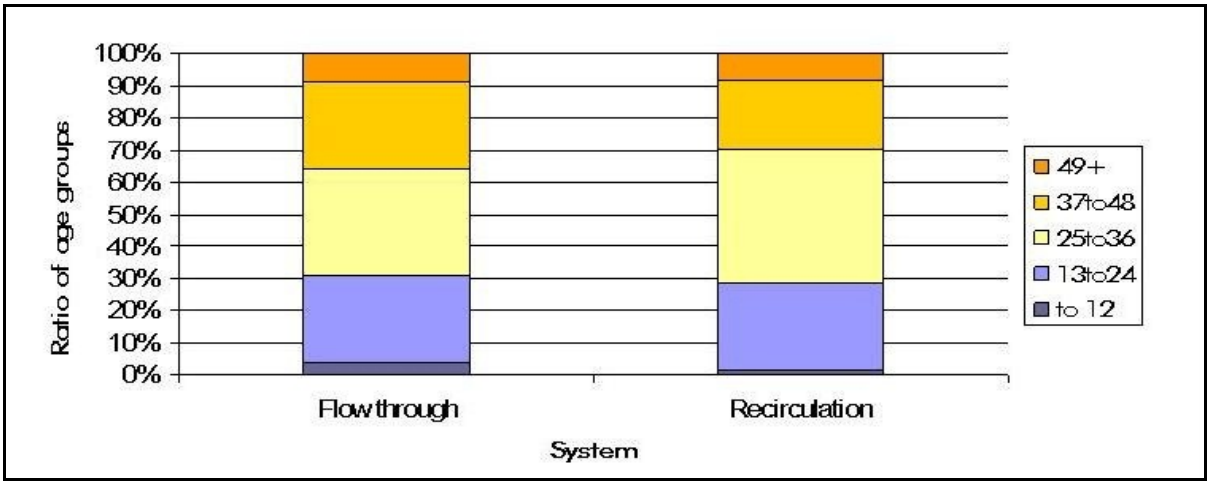

Figure 3.2.4.6: Distribution of age groups in sample population from different systems

Diet was considered next. The proportions of animals on various diets in the different systems are charted in figure 3.2.4.7. All the animals in the recirculation systems received kelp, whereas the animals in the flow through systems were also fed artificial feed or a mixed diet.

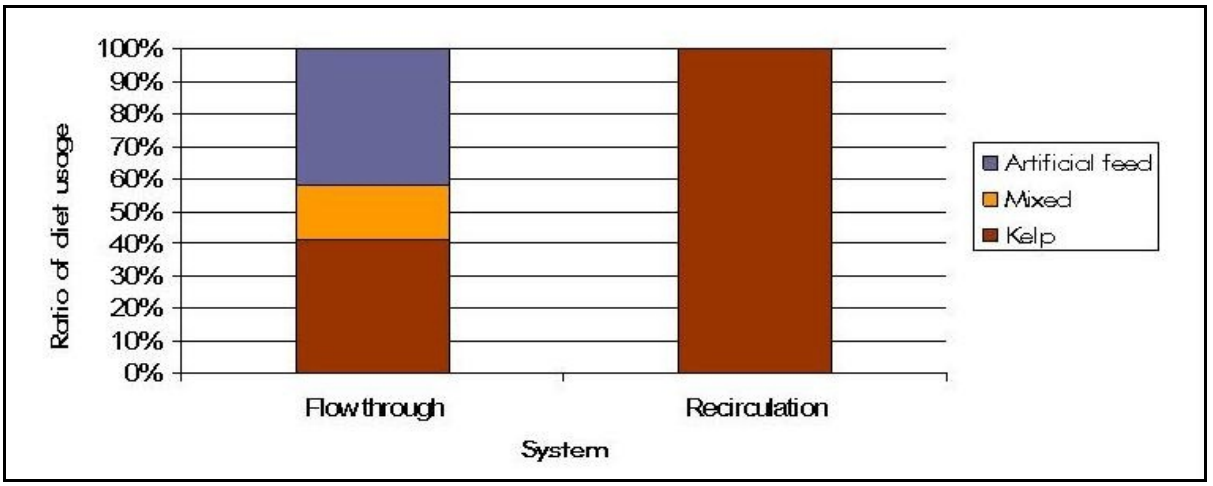

Figure 3.2.4.7: Diet usage in sample population from different systems

Odds ratios were constructed for certain parasites in animals receiving kelp only, in flow through systems compared to recirculation systems. As shown in table 3.2.4.2, when animals on the same diet are compared, there are no significant differences in risk of infection with right kidney coccidia, gut protozoa or rickettsia between systems. Interestingly, there is a difference between systems for digestive gland protozoa, with a greater risk of infection in flow through systems. This was not shown by the chi square test.

Table 3.2.4.2: Odds ratios for parasite prevalence in animals receiving kelp, comparing animals from flow through systems to those in recirculation

Parasite	Odds ratio	Confidence interval
Sessile ciliates	22.77	18.44 to 28.12
Renal coccidia left kidney	0.23	0.16 to 0.33
Renal coccidia right kidney	0.80	0.27 to 2.38
Gut protozoa	1.35	1.00 to 1.81
Digestive gland protozoa	1.47	1.08 to 1.99
Rickettsia like prokaryotes	0.87	0.72 to 1.05

The increased prevalence of sessile ciliates in flow through systems cannot be explained by diet, as there is a significantly greater risk of ciliate infection in animals in flow through systems even when comparing only kelp fed animals. Left kidney coccidian infections are not associated with diet and it is not surprising that the increased risk in recirculation systems is still observed when only animals on kelp are compared.

3.3 Overall prevalence

The overall prevalence of the various parasites is summarised in table 3.3.1.

Table 3.3.1: Parasite prevalence for entire sample population

Parasite	Prevalence as % infected	95% confidence interval
Sessile ciliates	68.33	67.53 to 69.13
Renal coccidia left kidney	1.85	1.61 to 2.08
Renal coccidia right kidney	0.23	0.15 to 0.31
Gut protozoa	5.54	5.15 to 5.94
Digestive gland protozoa	5.27	4.89 to 5.66
Rickettsia	13.07	12.49 to 13.65
Trematodes	0.05	0.01 to 0.09

Chapter 4

Discussion

4.1 Materials and methods

4.1.1 Sampling strategy

The work described in this dissertation is an example of a retrospective study. A drawback of retrospective studies is the use of historical data which was not originally collected for the purpose of the study. The current data set comes from the abalone health management program, a herd health program designed to assist abalone farmers in optimising the health of their stock. Participating farms are sampled at set intervals throughout the year. The aim is to sample cohorts which represent all age groups present on the farm. Typically, a cohort will be sampled repeatedly for several years, from leaving the nursery until the time it is harvested. During the course of a year, some cohorts are harvested and lost, to be replaced by new ones entering the ongrowing section of the farm. The age and size distribution of the animals included in this study should therefore be reasonably representative of the farmed abalone population. However, the farmed abalone population is extremely large. At the time this data were collected, each participating farm had on average around 3 million abalone. The abalone health program is based on the assumption that this population will be relatively uniformly affected by system and husbandry and that the existing sampling strategy is adequate to identify areas for improvement in technology and management. It hardly needs saying that one cannot assume that parasite prevalence is evenly distributed across a farm, affected only by the few variables included in this study. There are likely to be differences in prevalence between baskets in a tank, between tanks, within cohorts, and between cohorts of similar ages. This would be especially true in the case of parasites which are directly transmitted from one abalone to another. It is not possible to examine these relationships using the current data set.

A further problem with use of historical data is incomplete records. Nearly a third of the abalone sampled during the period covered by this study had to excluded from the final data analyses due to incomplete or incorrect records. Of the ten farms which had participated in the health program, one was excluded when it was found to be unable to supply correct ages for its animals. This was discovered by comparing the size of the animals, measured in the laboratory,

with the stated age provided by the farm. Age data were also not available for certain cohorts received at various times from other farms. This was sometimes a consequence of farms buying animals from other farms. Diet was the other variable which was poorly recorded. As will be described, many farms experimented with both artificial feed and kelp during the study period. Any samples where there was uncertainty about which diet they received were excluded from the data set. For much of the study period, most samples were collected by farms and sent to the laboratory using courier services. Although farms were given data submission forms on which to record variables such as age and diet, compliance was imperfect. At present, nearly all samples for the abalone health management program are collected by laboratory staff. A detailed data sheet is completed at the time of collection, including fields for stocking densities, flow rates and dates of husbandry interventions such as grading. It is hoped that future analyses of these data will provide some answers not obtained from the present study.

The sampling strategy described above leads to clustering of data. Abalone from the same cohort or farm could have more similar parasite prevalences than those from different cohorts or farms, because they share a common environment and are in close contact. The data set used in this study is hierarchical. The first level is abalone from the same cohort and which are sampled from the same basket in the same tank. The second level is abalone which originate from the same farm and at the third level are abalone which originate from farms in the same area. Spatial clustering of the data can also play a role due to the relative position of abalone on a farm and of farms in an area. Clustering affects both outcome and predictor variables. Predictor variables used in this study, such as age, size, growth rate, diet and origin, are all clustered, mainly due to the practice of sampling ten abalone per cohort.

The implication of clustering is that it invalidates the assumption of independence inherent in most statistical methods. One of the effects of ignoring clustering is that the standard errors of parameter estimates will tend to be too small, leading to confidence intervals that are narrower than is truly the case. It can also lead to standard errors that are too large. Another effect is that analyses that is not adjusted for clustering can allow disproportional contribution by certain groups to the outcome. For example, farms which submitted very large numbers of abalone can affect the prevalence patterns of certain parasites. This is illustrated in the results and discussion of temporal patterns, where farms with very high prevalences of certain parasites caused spikes

in prevalence during the months when their samples represented a large portion of the total. To fully account for the effects of clustering when considering binary data, such as presence or absence of parasites, together with discrete and continuous predictor variables, it is necessary to use generalised linear mixed models (Dohoo *et al*, 2003). These statistical methods are beyond the scope of this study.

Lastly, the data set would appear to lend itself to analysis by logistical regression. When this was attempted, it was found that the available input variables explain only a neglible portion of the variation in parasite prevalence. This was also borne out by the small effect sizes obtained in nearly all the chi square tests described above. It was felt that the predictive power of a logistical regression model generated from the current data set would be so small as to render the exercise essentially futile.

4.1.2 Methods of diagnosis

Histology was the only method of diagnosis used in this study. With the exception of *Mantoscyphidia midae*, the sessile ciliate, none of the parasites examined have been formally described. Therefore, they lack names and are designated by descriptive terms. The assumption has been that parasites which look the same, are the same. Most of the parasites in this study do not always look the same and exhibit different stages of development. These stages are described for renal coccidia (Bower and McGladdery, 2003) and intracellular bacteria such as the rickettsia like prokaryotes (Garrity, 2005). In the case of other parasites, development is inferred by seeing incremental changes when viewing a large number of sections. The most appropriate analogy would be seeing all stages of growth of an oak from an acorn to a tree, thus allowing identification of both acorn and tree as oaks. However, it is recognised that this may lead to misidentification by grouping parasites with similar appearances as one. This is a problem inherent in any identification method which relies on appearance only.

Lack of comparative tests is a further problem when dealing with poorly known parasites for which histology is the only diagnostic method. As there are no other tests, it is not possible to estimate the sensitivity or specificity of the histology. It can be assumed that sensitivity will be less

than optimal, because the parasites have an irregular distribution in the tissues in which they occur and only a small sample of the tissues are examined. Fortunately, the parasites are all easily seen at relatively low magnifications of 100x to 200x. It is not unusual to find very few parasites on a section, sometimes only one, but it is not possible to know what the likelihood is of missing an infection when it is present at very low intensity. Specificity is likely to be very good, except for the potential problem of occurrence of very similar parasites infecting the same tissues, discussed above. In summary, it is safe to assume that prevalences reported from this study underestimate true prevalence.

Routine viewing of sections and analysis of data as part of the abalone health program service indicated some trends in parasite prevalence which were then included for further exploration during this study. The low prevalence of parasites in Hermanus, seemingly contradicting the expected relationship of increased parasite occurrence in areas of high abalone density, was one such a trend. Another was the difference in occurrence of left and right kidney coccidian infections. There is no evidence to suggest that more than one species of coccidia are involved. The left and right kidneys of abalone are considered morphologically and functionally separate organs. Although right kidney coccidian infections were comparatively rare in this data set, they attracted interest and additional analyses due to their distinctive behaviour.

4.2 Host factors

4.2.1 Age

Age was found to be significantly associated with prevalence for nearly all parasites examined in this study. The results of the chi square test, given in table 3.1.1.1, showed highly significant associations except in the case of trematodes, where the small number of infected animals limits application of the test. However, the effect sizes were small. Odds ratios were calculated comparing animals of two years and younger to older animals. As can be seen from table 3.1.1.2, all were significant, except the odds ratio for trematodes. There are many aspects of age which can be expected to impact on prevalence. Although age is usually considered a host characteristic, it has implications for environmental factors as well as interacting with the parasite life cycle.

At the most basic level, age may be defined as the length of time an animal has lived. If this animal has a constant risk of parasite infection, one may assume that, the older it is, the greater the probability that it is infected. Should animals, once infected, tend to retain the parasite, prevalence in the population would be expected to increase over time. In parasites with a simple life cycle and direct transmission between hosts, this increase may be exponential rather than linear. Plainly put, if animals are infecting their contacts, more infected animals must lead to more infected animals. In other words, the risk of parasite infection is no longer constant, but increases as the number of infected animals increases. Animals may, for various reasons, lose their infections over time and could then be candidates for reinfection, or not. Much depends on parasite characteristics. Unfortunately, there is little known about the parasites examined in this study.

It can be seen in figure 3.1.1.2 that the prevalence of sessile ciliates increases rapidly with age, with the chart suggestive of an exponential rather than a linear process. *Mantoscyphidia midae* is known to divide by binary fission whilst on the host. Once a host is infected, there is likely to be little chance of losing the ciliates. Sessile ciliates are able to form motile stages or telotrochs, enabling them to infect new hosts. Telotroch formation is stimulated by stress (Botes, 1999) and may potentially be induced by routine husbandry procedures on farms. Over time, handling of abalone may contribute to new ciliate infections as a result of telotroch formation. The decrease in prevalence after 48 months of age may be related to diet and will be discussed further under 4.3.3.

Parasite prevalence also increases with age in the case of gut associated parasites, illustrated in figure 3.1.1.4. This increase is approximately linear for gut protozoa. A sharp increase in prevalence occurs after 24 months of age for both digestive gland protozoa and rickettsia like prokaryotes. This may be partly explained by diet. Although the life cycles of the gut associated parasites have not been described, something may be learned from their histological appearance, as shown in figures 2.16 to 2.1.10. All these parasites show distinct developmental stages, of which the final one may be seen in the lumina of the digestive gland tubules and gut. This suggests a life cycle of which some part takes place outside the host, freeing the host of infection, possibly only temporarily. Individuals sometimes show parasites in different stages of development, perhaps due to infection having occurred more than once or to autoinfection. This

is most common in rickettsial infections and could contribute to the higher prevalence of these parasites. Digestive gland protozoa show a decrease in prevalence after 48 months similar to that seen for sessile ciliates.

The above discussion considers age in the context of time passing, but of course it is more than that. The abalone changes physiologically with age. In the current study, all abalone were essentially juveniles or subadults. Abalone are long lived and, in the wild, considered sexually mature at eight to ten years. As shown in figure 3.1.1.1, the majority of animals in the current study were between two and three years, with the oldest individual just over six. Cultured abalone spend their first six to nine months in the hatchery and nursery, which were not sampled for the purposes of this study, leading to poor representation of very young animals in the sample population. Harvest of abalone commences at approximately three years of age and most farms have relatively few animals over forty two months old. In general, an animal over forty two months old that is still on the farm is there because it has failed to reach a marketable size. Whatever the reasons for its lack of performance, it is safe to say that such an animal is not directly comparable to those in the younger classes. This is especially true for animals older than four years. Up to three years of age, all animals are included in the sample population. Thereafter, progressively more slow growers are being sampled.

Twenty four months appears to be an important milestone in the development of the young abalone. The physiology of abalone has not been well studied, so most of the evidence for this statement is anecdotal. Abalone farmers report a decline in growth rates at two years of age, apparently unrelated to changes in husbandry or diet. It is certainly so that abalone have increased gonad development from two years onwards, although there is substantial individual variation. This aspect will be discussed further when sex is considered in 4.2.3.

As mentioned above, there is a sharp increase in the prevalence of rickettsia like prokaryotes and digestive gland protozoa after twenty four months. The increase in prevalence for gut protozoa, although not as marked, is also greater after twenty four months than at other times. This can be largely explained by changes in diet, but it is possible that physiological changes also contribute to increased prevalence. The trend for renal coccidia is very much the opposite to that seen for other parasites. Left kidney coccidian infections are by far more common in

animals up to two years of age than in older ones, as shown in figure 3.1.1.3. Older animals have a very low prevalence of renal coccidian infections, but right kidney infections occur almost exclusively in abalone over twenty four months. In older animals, it is interesting to note that right kidney infections increase with increasing age and left kidney infections, although comparatively rare, occur most frequently in animals over four years of age.

Immune function in abalone is poorly understood. It is not possible to say what contribution resistance makes to changes in parasite prevalence. However, it is tempting to speculate that the marked decline in left kidney coccidian infections after two years is due to development of resistance. The left kidney in abalone is known to be immunologically active, containing a large number of phagocytic cells which filter foreign material and potential pathogens, primarily bacteria. Observations of histological sections indicate tissue changes associated with coccidian infections in certain individuals, including increased mucous cells. Right kidney coccidian infections are sometimes accompanied by a moderate foreign body reaction. More usually, coccidian infections do not appear to elicit obvious inflammatory or other changes. Histological examination of large numbers of abalone for herd health purposes seems to indicate that animals under stress are those which suffer right kidney coccidian infections. This could be explained by a breakdown in resistance under stressful conditions and presents interesting avenues for future research.

A further important aspect of age in abalone relates to changes in husbandry. It has already been mentioned that abalone move from the nursery to grow out systems in their first year of life. Thereafter, they are subject to various changes, some gradual and others abrupt. The most obvious gradual change is a steady increase in population density. In a study of management practices conducted for the Abalone Farmers' Association of South Africa (Mouton, 2006) it was found that, although the number of animals per basket declined as the animals became older, the total mass of animals per basket increased. So, whereas animals in their first year may be stocked at 1250 per basket and animals of three years old at 190, the mass of animals per basket increases from approximately 3 kg in the first year to more than 8 kg by the third year. In addition, the water flow rate relative to the animals decreases from just over 60 litres per kg per hour in the first year to less than 20 litres per kg per hour by the third year. For parasites with a direct life cycle, the increased concentration of abalone may facilitate spread and this

could be a contributing factor in the higher prevalences seen in older animals for most parasites. However, improved understanding of the life cycles of these parasites is necessary before the relationship between prevalence and density can be investigated.

The possible role of handling in stimulating transmission of sessile ciliates has been mentioned. It is not known what the effect, if any, of handling would be on other parasites. Handling becomes more intensive on many farms as animals near harvest and are graded into the various size classes required by clients.

The only parasite which did not show very interesting trends relating to age was the trematode. Although figure 3.1.1.5 indicates a possible increase in prevalence with age, this is not nearly as outspoken as the patterns seen with other parasites. It could be that the rarity of trematodes on farms is due to the relatively young abalone population, but it is far more likely that the farm environment is not conducive to infections.

4.2.2 Size

When considering size, it may appear so closely related to age as to render analysis of both host factors unnecessary. However, it can be seen from figure 3.1.2.1.1 that the size distribution does not follow directly from the age distribution, as given in figure 3.1.1.1. The reason lies in growth rate variation. Growth rate and condition index may both be used as measures of production performance and could be interpreted as indicators of general health. This will be discussed later.

Nearly eighty percent of animals included in this study weighed 45g or less. The average abalone will have reached 45g by its third year and animals gain weight very rapidly thereafter. Most will surpass 100g before four years of age. Although some farms sell abalone in the 40 to 50g size class, this is not standard practice and the majority of abalone will be marketed at sizes closer to 90g. The samples for the health program focus on animals which are not yet marketable, as these have a longer period remaining on the farm and there is thus greater opportunity to make positive interventions in their management.

The prevalences of sessile ciliates and gut associated parasites, charted in figures 3.1.2.1.2 and 3.1.2.1.4, increased with increasing mass. This increase occurs earlier and more rapidly in the case of sessile ciliates than with gut associated parasites. For all of these, the progression is somewhat uneven. This may be due to the considerable variation in age within the different size categories. Renal coccidia also showed similar trends in prevalence with mass, shown in figure 3.1.2.1.3, as with age. Smaller animals were most affected by left kidney coccidian infections, whereas right kidney infections were more common in larger animals. The rarity of trematode infections again complicated interpretation of prevalence. Prevalence for trematodes is illustrated in figure 3.1.2.1.5. Data on trematode occurrence in abalone over 100g would be interesting.

Increasing age may, of course, be the underlying reason for some of the prevalence patterns seen with mass. However, size in itself could affect prevalence. For sessile ciliates, which are surface associated parasites, larger animals may provide more opportunities for attachment. The mechanisms of transmission for the internal parasites are not known, but it is conceivable that size may play a role in facilitating infection. For example, a larger animal eats more and, if parasites are ingested, this may lead to higher incidences. Unfortunately, such speculation will achieve little until information on parasite biology becomes available.

Abalone farmers are naturally much concerned with the growth rates of their stock. This may be calculated from the total shell length and the age of the animal and is expressed in mm per month. Unfortunately, abalone do not grow in the direction in which they are measured. Their shells are flat spirals and they grow accordingly. Therefore, rate of length growth tends to decrease as the animal becomes larger and the spiral wider. This is reflected in decreasing growth rates as animals age. The determination of growth rate is further complicated by the use of length mass conversion curves. Most farmers do not measure the abalone, they weigh them and then convert the mass into length mathematically. The length mass curve is not linear and a good fit is not obtained when using the same curve for animals of widely differing sizes. Data gathered from farm records for 2004 indicate an average growth rate of 1.85 mm per month (Mouton, 2008). The average growth rate obtained in the current study was somewhat lower, at 1.60 mm per month. This difference is probably at least partly due to improvements in abalone husbandry as the industry develops. Average growth rates for 2007 were 1.94 mm per month (Mouton, 2008). The figures are also not entirely comparable because farm data on

growth rates may rely on calculations which were not used on the current data set. The distribution of growth rates is shown in figure 3.1.2.2.1. Growth rate depends on a great many factors, most of which are management related. However, the possibility of associations between growth rate and parasite prevalence was considered worth exploring.

The results of the chi square test, summarised in table 3.1.2.2.1, showed a significant association between growth rate and parasite prevalence for sessile ciliates, renal coccidia and gut protozoa. In the case of sessile ciliates, illustrated in figure 3.1.2.2.2, prevalence was highest at average growth rates. The lowest prevalence of ciliates was found at the lowest growth rates. This may be due to the large number of animals from recirculation systems that have low growth rates, as sessile ciliates are nearly absent from recirculation systems. This will be discussed further when system effects are considered under 4.3.4. The reduced prevalence of sessile ciliates at high growth rates could be an effect of age, as younger animals tend to have both higher growth rates and fewer ciliates. When the data were stratified into two age classes and the growth rates of animals with and without sessile ciliates compared using the Kolmogorov-Smirnov test, no association was found.

For renal coccidia, it is interesting to note a reduction in left kidney infections with increased growth rates, shown in figure 3.1.2.2.3. As left kidney infections are most common in animals of two years and younger, one would expect more infections at higher growth rates. Even when data for various age groups are considered separately, this trend remains very obvious. Animals with the lowest growth rates have much higher prevalences of left kidney coccidian infections. This is especially marked in animals younger than three years. The association was further confirmed by the results of the Kolmogorov-Smirnov test. It is not possible to state the nature of the relationship between growth rate and left kidney coccidian infections. Left kidney infections may contribute to reduced growth, or reduced growth may predispose animals to left kidney infections, or the same factors that lead to slow growth may also lead to left kidney coccidian infections. The same relationship is not seen for right kidney infections. Very few right kidney infections are represented in this data set and none of these infections were even moderate in intensity, as opposed to left kidney infections which were frequently severe. It is possible that right kidney coccidian infections have little impact on the abalone. It is also possible that the current data set is not optimal for investigation of the relationship between right kidney infections

and growth rate.

The relationship between gut associated parasites, charted in figure 3.1.2.2.4, and growth rate is difficult to interpret.There is no significant association between prevalence of digestive gland protozoa and growth rate. The difference found with the Kolmogorov-Smirnov test relates to the shape of the distributions and not their means. In the case of gut protozoa, a significant association was found with the chi square test, but not with the Kolmogorov-Smirnov and t-tests. For rickettsias like prokaryotes, prevalence appears higher at lower growth rates, but the association was not significant. Analysing each age class separately only serves to confuse the issue further and the results are not shown. Unlike the case with renal coccidia, there is no uniform trend and it would appear that analysis of the current data set is unlikely to add much to understanding these relationships, if indeed there are any relationships to understand. There was no significant association between prevalence of trematodes, charted in figure 3.1.2.2.5, and growth rates.

Condition indices are commonly used in mollusc culture in an attempt to determine the amount of soft tissue or meat present in an individual, relative to shell length or mass (Shumway, 1996). The general feeling is that more meat and less shell are desirable, but this is not necessarily always the case, as will be explained. Shell deposition in molluscs is complex and the factors affecting growth are poorly understood (Cerrato, 2000; Lewis and Cerrato, 1997). The rate of length increase, usually referred to as growth rate, is highly dependent on environment and management. Rapid growth is associated with stable, favourable, water temperatures, vigorous aeration, and rapid water flow through tanks. Growth spurts are known to occur after animals have been handled and when a kelp diet is first introduced. No doubt growth is also affected by many other variables. On the other hand, the shell increases in thickness at a constant rate, apparently unrelated to length increase. Shell thickness is primarily a function of age, although it may conceivably be influenced by diet and other elements (Day et al, 2000).

When farming abalone, negative shell growth or loss of shell is an important consideration. The two principal causes of shell loss are erosion and breakage. Abalone shell consists mainly of calcium carbonate and will erode or dissolve under conditions of reduced pH and high levels of carbon dioxide (Spotte, 1979). This is a problem in intensive abalone culture, especially in

recirculating systems. Breakage results from handling and, unlikely as it may sound, from abalone fighting. Abalone show aggressive behaviour by attempting to push other abalone away, often accompanied by rapid swivelling of the shell. Where shells come into contact, fragments break off and are lost.

It has been shown for other molluscs that shell growth and soft tissue deposition are not directly linked (Lewis and Cerrato, 1997). Ongoing research indicates that the same is true for abalone (Laas, 2006). This is clearly seen when abalone are exposed to an environment that promotes shell growth, but are not provided with adequate nutrition. The animals will generally become very flat and have small, watery bodies, even when the shells grow rapidly. Nutrition appears to be the most important determinant of soft tissue deposition, but has relatively little effect on shell growth. Soft tissue deposition is also likely to be affected by temperature, sexual activity and chronic disease. Abalone are able to resorb foot muscle and other tissues, not including the shell, when nutrient limited and can survive long periods of starvation. This is a necessary adaptation for an animal which relies on irregular food supplies in its natural environment.

The most commonly used condition indices in molluscs work with ratios of total mass, or soft tissue mass, and either shell length or shell height (Lawrence and Scott, 1982). Unfortunately, the ratio of length to mass in abalone is not constant over different size classes and, consequently, different ages. This is generally the case for all animals, that larger animals have less surface in relation to their volume than smaller ones. In analysing the data for this project a condition index based on the ratio of total mass to shell mass was used. It can be seen from figure 3.1.2.3.6 that this too is affected by age, but much less than ratios of length to mass. The decrease in condition index with increasing age is unexpected, as older animals should be larger and have smaller shells relative to their volume, which represents their mass. Increasing shell thickness over time no doubt partly accounts for this anomaly. In other words, an older animal would have a thicker, and therefore heavier, shell than a younger animal.

Figure 3.1.2.3.1 shows that the majority of animals have a condition index centred around 5.0. This means that approximately 20% of their total mass is made up of shell. A decreasing condition index results from a relative decrease in soft tissue mass. Theoretically, if an animal had no soft tissue at all, it would have an index of 1.0. It is easy to see that a low condition index is

undesirable and anything below 4.0 could be considered a sign of emaciation. However, as previously mentioned, more is not necessarily better. Condition indices above 6.0 frequently represent animals which have suffered shell loss due to breakage, often precipitated by severe polychaete infestations. The effect of age should be kept in mind, as animals younger than two years tend to have higher than average condition indices whereas those older than five years are nearly all below 5.0.

Diet usage in different condition index classes is shown in figure 3.1.2.3.7. In the data for this study, there was greater use of artificial feed at lower condition indices. Kelp usage was more prevalent at higher condition indices. This finding is not consistent with current beliefs about abalone culture. It has generally been held by abalone farmers that animals which receive artificial feed are heavier for their size than those which are fed kelp. This is supported by production data from farms as well as by canning yields, which take into account shell mass. In addition, most of the animals in the current data set which were fed kelp were older than two years and would be expected to have lower condition indices. Close to two thirds of animals two years and younger were fed artificial feed and would be expected to have higher condition indices. There is clearly a discrepancy between the results obtained from this data set and those from farms.

Using the chi square test, a significant association was found between prevalence and condition index for all parasites tested. Effect sizes are very small. Results are shown in table 3.1.2.3.1. The prevalence of sessile ciliates, charted in figure 3.1.2.3.2, is greatest at a condition index of 5.0 and decreases in both directions away from 5.0. A similar pattern is seen for gut associated parasites, illustrated in figure 3.1.2.3.4. It is most marked in the case of rickettsia like prokaryotes. Possible reasons for this relationship are not readily apparent. Age would be expected to lead to greater prevalences at lower condition indices, but not at higher ones.

In the case of gut associated parasites, the relatively greater use of artificial feed at lower condition indices could be expected to contribute to lower prevalences. It is possible that a combination of these factors is influencing the results. It is also likely that there are other elements at work, not considered in this data analysis. Whatever the case, condition index does not

appear to be negatively affected by the presence of sessile ciliates or gut associated parasites.

Left kidney coccidian infections are more prevalent at higher condition indices, most probably due to younger age groups having higher indices. However, the peak prevalence occurs at 7.1 and greater, which can be considered an abnormally high condition index, associated with shell loss. On the other hand, right kidney coccidian infections occur in lower condition indices and, again, this may be connected to age and the occurrence of right kidney infections in older animals. These relationships are shown in figure 3.1.2.3.3. The apparent association between kidney coccidia and condition index would be an interesting area for further investigation, especially as renal function in abalone has been poorly researched.

It is not possible to draw strong conclusions about trematode infections and condition index, especially as a significant association could not be shown. Based on macroscopic and histological examination, many infected animals do show varying degrees of emaciation. Trematode infections are usually very severe and the tissues of the abalone are subject to the presence of massive numbers of developing parasites. A relative decrease in soft tissue mass would be expected under such circumstances, but examination of a larger number of infected animals is needed to clarify the pathogenesis of this disease. Figure 3.1.2.3.5 does to some extent indicate more infections at a lower condition index.

4.2.3 Sex

The sexuality of abalone is not considered an important aspect of their production, with the exception of those individuals held specifically for use as broodstock. Abalone do not mature sexually before they reach a marketable age. It can be seen from figure 3.1.3.1 that approximately half the animals in the current data set show no gonad development at all. Figure 3.1.3.2 shows that eighty percent have either no gonad development or gonads without any mature sex cells. Less than a tenth of the abalone in this data set have gonads which would be capable of spawning, represented by the mature category in figure 3.1.3.3. Although these are classified as mature gonads, the gonads are substantially smaller than would be seen in an adult abalone. Abalone on farms have been known to spawn at four to five years of age, and viable

offspring are reported to have resulted from such spawnings, but the number of gametes produced is small relative to adult spawnings.

Adult male abalone spawn more readily in captivity than females and appear to live longer. Adult females develop very large gonads and often lose condition over time when spawning regularly. They sometimes become weak and die during spawning. Male and female abalone in ongrowing are not known to show differences in either production performance or general behaviour. In this study, both sex and gonad development were examined for relationships with parasite prevalence. Sex was not expected to have any connection to prevalence. However, the physiological changes and energetic costs associated with gonad development were considered to be potentially conducive to parasite infection.

The results of the chi square test, given in table 3.1.3.1, show a significant difference in parasite prevalence between sexes for all parasites except trematodes. Effect sizes were very small. As discussed below, this is more likely to represent the difference in prevalence between those animals which show gonad development and those which are immature, than a true difference between males and females. The results of the chi square test for degree of gonad development, presented in table 3.1.3.2, show a significant difference in parasite prevalence for sessile ciliates, renal coccidia and gut protozoa. Effect sizes were again very small.

The prevalence of sessile ciliates was similar in males and females, charted in figure 3.1.3.4. It was also similar in animals with moderate and mature gonads, shown in figure 3.1.3.9. The lower prevalence in animals showing no gonad development and immature gonads is predictable, given that these animals would be younger than those with more advanced gonad development. A similar pattern, demonstrated in figures 3.1.3.6 and 3.1.3.11, is seen for gut associated parasites. The trend is reversed for left kidney coccidian infections, as expected from the greater prevalence of left kidney coccidian infections in younger animals. Right kidney infections show no differences. Prevalences of renal coccidia are presented in figures 3.1.3.5 and 3.1.3.10. Sex and gonad development relative to age are illustrated in figures 3.1.3.8 and 3.1.3.13. It can be seen that differentiation of the sexes only becomes possible in the second year of life and mature gonads are seen in the third year.

Significant associations between sex or gonad development and trematode infections were not found. Prevalences are shown in figures 3.1.3.7 and 3.1.3.12. The small number of animals in this data set infected with trematodes makes meaningful analysis of their epidemiology difficult. It is interesting to note the absence of trematodes in animals with mature gonads. This may be a chance event, but the phenomenon of parasitic castration by trematodes has been well described in molluscs. As already mentioned, the collection of data on trematode infections in the wild abalone population is likely to provide greater insights into the biology of these parasites and their effect on the host population.

4.3 Environmental factors

4.3.1 Origin

The origin of the sample population can be considered in terms of farm of origin as well as geographic area. Abalone farms differ in their management practices, even when not different in location. In addition, management practices on any given farm will change with time, sometimes due to natural evolution and at other times due to external factors. It was not possible to examine the relationship between parasite prevalence and most management factors in this study, but some potentially important management practices will be briefly discussed where appropriate. There are several reasons why it is difficult to investigate the role of management factors. To illustrate this one can use the example of flow rates. Although most farms can provide a rough estimate of average water flow rates based on their pump capacity and reticulation system, this tells one very little about delivery to individual tanks. Water flows to tanks varies considerably depending on the position of tanks on the farm. In addition, consistency of flow is critical, but farms seldom account for this. Consistency of flow is affected by a wide range of factors, including fouling of the reticulation system, downtime for maintenance and repairs, and use of additional water for tank cleaning. It can safely be said that accurate data on management factors such as flow rates need to be collected when sampling, as they are not available from the farm. Other factors, such as dates of grading or splitting, can be obtained from the farms, but generally only for as long as the cohort of interest is still on the farm. The problem of incomplete data in retrospective studies has been previously discussed. Another problem is confidentiality of data. Farms are often not prepared to permit the publicising of their

data in any way that allows identification with their farm, especially if they feel it portrays them negatively, for example by showing that they have particularly high parasite prevalences. They are also very hesitant about sharing any information that they believe gives them a competitive advantage.

It can be seen from figure 3.2.1.1 that all farms were not equally represented in the data. This is partly because not all farms participated in the health program for the same number of years. The ones contributing the fewest abalone were some of those that only belonged to the program for a short time. In other cases, farms submitted a large number of abalone, but the age and diet are not known, so these animals could not be included in the data analysis. Nine farms are represented. A tenth farm was completely excluded from the study after it was found that the ages of the animals were supplied incorrectly.

There are significant differences in parasite prevalence between farms for all parasites tested, as shown in table 3.2.1.1 and illustrated by figures 3.2.1.2. to 3.2.1.5. Effect sizes range from small to moderate. It was not considered useful to determine differences between individual farms or to rank them according to prevalence, as it would not be possible to examine the underlying causes from the available data. For example, stocking densities and flow rates are likely to be important determinants of incidence for certain parasites. The intricacies of stocking densities and flow rates are of consuming interest to all abalone farmers and most, if not all, aquaculturists. It is appreciated that this interest may be less developed in others, so only a brief discussion will be provided here.

The simplest definition of stocking density is the number of animals per unit volume. On an abalone farm, that translates into the number of abalone per basket. Unfortunately, the reality is not so simple. Abalone are not free swimming, but adhere to surfaces. Baskets for abalone contain inner structures to increase surface area for adherence. Most stocking densities for abalone are based on surface area coverage, in other words, the percentage of total available surface area in the basket that is covered by abalone. Obviously, large abalone take up more space than small ones, so small animals are stocked in higher numbers per basket than large ones. On the other hand, small animals weigh substantially less than large ones, and the total mass of abalone per basket is far higher for large animals than small ones, as previously pointed

out. Stocking densities vary from farm to farm, as do basket design and the number of baskets per tank.

Stocking density in itself affects the abalone, as they are somewhat aggressive and compete for food and favoured positions in a basket. Density will also impact on water quality. Water quality in the majority of abalone tanks is primarily maintained by continuous replacement. Replacement rate is determined by the size of the tank and the water flow rate. Fresh incoming seawater provides oxygen, removes or dilutes waste products such as ammonia, and serves to maintain water temperature. Clearly, flow rate should be related to stocking density, as more animals per tank will lead to more rapid deterioration in water quality. In practice, as mentioned previously, the amount of water per kg of abalone decreases dramatically the larger the animals become. Production performance declines when densities are too high and flow rates too low. On the other hand, there are economic implications to lowering stocking densities and providing more water. Successful abalone farming depends on finding the correct balance and farms have different philosophies as to the optimum strategy.

Population density can be critical in determining the spread of infection for parasites with a direct life cycle (Reno, 1998). The life cycles of most of the parasites included in this study are not known, so it is difficult to predict which are likely to be affected by densities. However, it is interesting to note the very high prevalence of rickettsia like prokaryotes on farm c. This farm has always maintained a system of very high densities coupled to very high water flows. Rickettsia like prokaryotes have been shown to be directly transmitted in other abalone species (Friedman *et al*, 2002). Should the same be true of the South African organism, it is feasible that a combination of high population densities and rapid water flows facilitates transmission and increases overall prevalence. Farm c also had higher prevalences of other gut associated parasites, of which the life cycles and mode of transmission are not known.

It is clear that density and flow rate may impact prevalence by affecting direct transmission. High flow rates could also conceivably help flush parasites from tanks, although particulates tend to settle out below the baskets. Abalone tanks are periodically cleaned to remove faeces, uneaten feed and other detritus from the bottom. If parasites have a life cycle which is direct, but requires a period of development outside the host, increased cleaning frequency may be

expected to lower prevalence, by removing parasites before infective stages develop. Again, it is interesting to speculate. The prevalence of renal coccidia on farm g is notably higher than on other farms. This farm runs almost exclusively on recirculation and tanks are not regularly cleaned as on other farms. Allowing time for parasite development outside the host may be contributing to high prevalence in this case. In contrast, farm g has by far the lowest prevalence of sessile ciliates, as these parasites do not seem to thrive in recirculation systems. This aspect is discussed further in section 4.3.4.

Management of stock on abalone farms is also dictated by circumstances. Any event which slows the flow of stock through or off the farm will lead to increased densities. This tends to suppress growth rates and exacerbate the stock flow problem. It is easy to see how other factors can then contribute to increased parasite prevalence, such as increased age of the population. All farms have experienced such problems at one time or another, making it difficult to compare them over a five year period, as in the present study. To generalise, it is probably fair to say that good husbandry, by leading to increased growth rates and a lower average age of the abalone population, will help to decrease the prevalence of many parasites. Good husbandry would tend to include good hygiene and practices such as separation of age groups. Lastly, diet use accounts for some of the variation in prevalence between farms, and will be dealt with under 4.3.3.

The distribution of abalone farms along the South African coast is discussed in the Introduction. The present study only dealt with farms in the southwestern Cape. It is unfortunate that abalone production is highly concentrated, with approximately two thirds coming from six farms situated along roughly ten kilometres of coastline. To place this in perspective, in Norway, the minimum distance between salmon farms is ten kilometres. Almost forty percent of South African abalone production takes place in Hermanus, with the three responsible farms sharing boundaries and, in some cases, intakes. It is inevitable that the effluents of some farms are pumped in by others from time to time. There are also a large number of abalone processors in close proximity to the farms and these received both farmed and wild abalone from all over the country. Considering the potential for infectious disease outbreaks under such circumstances, parasite prevalence was compared geographically.

Seven of the nine farms included in this study are situated on the South coast and the other two

are on the West coast. The two farms on the West coast are not in close proximity to each other. It can be seen from table 3.2.1.2 that there was a significant difference using odds ratios in parasite prevalence between the South and West coasts all parasites, except trematodes, which did not occur in the West coast samples. In the case of the gut associated parasites, prevalence was higher on the West coast, which suggests that proximity of farms and resulting increased geographical density is not driving the association. Diet, which is discussed later, may be playing a role, as the prevalence of gut associated parasites is higher on kelp based diets and the two farms from the West coast feed almost exclusively kelp. The odds ratios were calculated with a subset of data containing only animals on artificial feed and kelp.

Three of the nine farms included in this study are situated in Hermanus, the rest are elsewhere on the South coast or on the West coast. When comparing parasite prevalence in Hermanus to that in other areas, it can be seen from table 3.2.1.3 that significant differences are obtained with the odds ratios for renal coccidia and gut associated parasites. As was found when comparing coasts, prevalence is not higher in areas where more abalone farming occurs. Diet could again be contributing to this pattern. Age distribution of the sample population is similar for both coasts and for Hermanus and other areas. There may of course be many other variables affecting parasite prevalence in different areas, some of which may be masking an association. However, the present study found no evidence of increased parasite prevalence in areas where abalone farming is concentrated.

4.3.2 Seasonality

Seasonal patterns of disease occurrence are common and caused by a variety of factors, often related to the life cycle of the infectious agent. A typical example is fluctuation in prevalence of parasites with indirect life cycles, due to seasonal abundance of vectors such as insects. These diseases tend to disappear at the time of year when the vector populations are suppressed by unfavourable weather, usually either too cold or too dry or both. Host activities can also be seasonal and contribute to changes in disease prevalence. The majority of aquatic animals are ectothermic, in other words their metabolic rate depends on the temperature of their environment. It has been shown that immune function in fish is suppressed during very cold periods. When water warms, microorganisms generally adapt more rapidly than the fish and opportunistic infections

take place (Wedemeyer, 1996). Abalone may or may not be similar. In addition, abalone may be adversely affected by periods of excessively warm water, which frequently lead to secondary bacterial infections and sometimes mortalities. There are many other potential drivers of seasonal changes in parasite prevalence, ranging from variation in diet quality and availability, when kelp is fed, to increased problems with water supply in winter months due to power outages and rough seas.

Although the samples analysed for this study were collected over a five year period, not every month is represented in every year. This can be seen from figure 3.2.2.1. In an attempt to compensate for this problem and to make the data more accessible, data were pooled by year, shown in figure 3.2.2.2, and by month, see figure 3.2.2.3. Most samples were received in July and fewest in December. The most samples per year were received in 2004. In both 2001 and 2002, the sampling year was incomplete due to interruption and translocation of the program. Samples that were received but not used for the study due to incomplete data also affect the seasonal sample distribution. Of the abalone processed for the health program, approximately a third had incomplete or unreliable data and could not be included in this study.

The prevalences of the various parasites by year were charted in figures 3.2.2.4 to 3.2.2.7. Monthly prevalences are illustrated in figures 3.2.2.9 to 3.2.2.12. The results of the chi square test for the relationships between parasite prevalence and year are given in table 3.2.2.1 and for parasite prevalence and month in table 3.2.2.2. These show significant temporal associations. Prevalences were not otherwise analysed, because it was felt that this study could not generate much insight into seasonal occurrence.

Annual patterns of prevalence are of interest when considering the overall disease risk in the abalone industry. The industry has been expanding rapidly for several years, accompanied by increased numbers of abalone on farms as well as larger abalone populations in areas where farms are concentrated. This creates a danger of parasite accumulation in such areas, due to the creation of ever more favourable conditions for transmission, including cycling of parasites within a farm or between the immediate ocean and the farm. There is also the threat of parasite adaptation to the farm environment, which may lead to greater prevalence. A pattern of increased annual prevalence would tend to suggest that one of these undesirable events is underway.

Fortunately, no such conclusion can be drawn from the data. The opposite appears to be occurring, with lower prevalences in 2004 than in previous years for most parasites. As discussed below for monthly prevalences, sampling of different farms in different years will affect prevalence. The contribution of different farms to the sample population for various years is charted in figure 3.2.2.8. As previously discussed, the proportion of farms with higher than average rickettsial prevalence in certain years is reflected in elevated total rickettsial prevalence for those years. Age distribution is probably also contributing to annual variation. It can be seen from figure 3.2.2.17 that less than 20% of the animals sampled in 2000 were two years of age or younger, compared to more than 40% in 2004. The figure was intermediate at roughly 30% in the other years. This may account for the low prevalence of sessile ciliates and gut associated parasites in 2004. It may also partially explain the greater prevalence of left kidney coccidia in 2004 compared to 2003. However, it does not account for increased left kidney coccidian infections from 2000 to 2002, or the sharp decrease in 2003. The absence in 2003 of samples from farm g, which has by far the highest prevalence of left kidney coccidia, may be the reason for the scarcity of coccidia that year.

The annual use of diets is shown in figure 3.2.2.19. It can be seen that kelp use increased until 2003, thereafter declining in 2004, possibly partly due to the age profile of the samples in 2004. The relationship between age and diet will be discussed further under diet. Mixed diets show a similar pattern. Lower prevalences of sessile ciliates and gut associated parasites in 2004 may be related to diet as well as age. Variation in other years is less readily explained, especially the high prevalences of left kidney infections in 2002. Longer term analysis of data may contribute more towards understanding annual fluctuations in prevalence.

When attempting to find seasonal patterns, one of the problems with the data set is uneven distribution of variables between months. In 2000, participating farms were sampled monthly, but this frequency soon declined and by 2004, farms were sampled every second month. As there are relatively few farms in the data set and, as has been shown in the section on origin, some have widely varying prevalences of certain parasites, their sampling intervals can potentially affect the seasonal pattern of prevalence. The contribution of farm g to the total sample population is illustrated in figure 3.2.2.13. Farm g may, for example, explain the higher prevalence in certain months, as shown in figure 3.2.2.10, for left kidney coccidia. In the case of sessile ciliates, charted in figure 3.2.2.9, monthly prevalence is almost certainly affected by farm

of origin. The prevalence of sessile ciliates on farm g is approximately 20%, compared to an average for all farms of close to 70%. It can be expected that the presence of samples from farm g during any given month will tend to depress the prevalence for that month. When this relationship is examined further, it is found that, in February, farm g sampling was 16.67% of total sampling, compared to the average contribution of 6.19% for farm g. February has the lowest prevalence of sessile ciliates. A similar pattern is evident for September, but not for April. The contribution of farm c is also considered. As shown in figure 3.2.2.14, the exceptionally high prevalence of rickettsia like prokaryotes on this farm is likely to affect the overall monthly prevalence depending on the percentage of the total sample population originating from farm c.

Having said this, it is entirely feasible that seasonal patterns of prevalence exist for certain parasites. Revisiting figure 3.2.2.10 on monthly prevalence of left kidney coccidia and ignoring the bimonthly fluctuation, there does appear to be an increased prevalence in spring and autumn. It is also interesting to note that right kidney coccidia were only found from May to December, with a peak in October, thereby also showing an apparent relationship to spring. Water temperature data kept by abalone farms over many years show that temperatures tend to increase from late spring, which is around October, to peak in late December or early January.

The effects of wind and upwelling during the summer months can lead to profound fluctuations in water temperatures, often as much as 10°C in a day. On the other hand, absence of wind results in very warm water, above the comfort zone of abalone. Average temperatures gradually drop off from January and by late autumn or early winter have stabilised. The period between about late May to early October is characterised by cooler water, generally in the preferred range for abalone, with little temperature change. This pattern varies slightly from year to year and also between farms, depending on their location and the position of their inlet. The most significant difference is between the South and West coasts, with the West coast being on average two to three degrees colder. To return to the original point of lower left kidney coccidian infections in winter, it is possible that the reason, at least partly, may be the favourable and stable water temperatures. One may speculate that more stressful conditions at other times of the year contribute to greater susceptibility of the abalone to left kidney coccidian infections. The very low prevalence of left kidney coccidian infections in December is less readily explained, but the farms with high prevalences of left kidney coccidia were very poorly represented in the December

sample.

The monthly distribution of gut associated parasites is hard to interpret. When considering rickettsia like prokaryotes, shown in figure 3.2.2.11, the absence of samples from farm c in February and December must be kept in mind, as previously discussed. There appears to be a decrease in rickettsia June to August, but there is also a decrease in the number of samplings at farm c from June to August and the same correlation is seen thereafter with the increased prevalence. In a further attempt to identify a seasonal trend in rickettsia prevalence, data from farm c was examined separately. Unfortunately, hardly any animals of two years or older were sampled from farm c in 2003 and 2004, potentially affecting the calculation of prevalence in those years. Therefore, it was decided to also examine the data when excluding these years. The resulting chart is unimpressive and not shown. There appears to be an increase in prevalence from January to April, a general drop off towards September, and then another increase peaking in November, or possibly December, as data is not available for December. This is somewhat similar to the trend for the entire population, but not very convincingly so.

The picture for the gut associated protozoa is less ambiguous, although still far from clear. Gut protozoa become more prevalent from May onwards, with a slight peak in June and slow reduction thereafter to reach a low point in December. Separate analysis of the data from farm c, which has the highest prevalence of gut protozoa, shows a similar pattern, illustrated in figure 3.2.2.15, but with a sharp increase in April. This increase is also present for rickettsia and is probably related to age. The animals sampled from farm c in April were all older than two years with an average age of 35 months. There is more variation in the prevalence of digestive gland protozoa, notably in February and August. Otherwise, the picture is broadly similar to that for gut protozoa, with an apparent reduction in prevalence during the warmer summer months. The reason for this trend, assuming that it is real, is not known. However, some conclusions can be drawn from the seasonal distribution of other variables.

When looking at the age profile of the sample population by month, as shown in figure 3.3.2.15, no obvious relationship emerges. On the other hand, use of diets, charted in figure 3.3.2.17 may explain some of the variation in prevalences. April, May, June and December were the months with the greatest portion of kelp in the diet. April, May and June also see an increase in rickettsia like prokaryotes. This is not seen in December, but, as has already been pointed out, there is poor

representation of farms with high prevalences of rickettsia in December. It is also interesting that there is an increase in gut associated protozoa from May onwards, apparently starting to decline after July. Again, the same is not true for December, but here too there is poor representation of farms with high prevalences. If this pattern is a true reflection of seasonal changes in prevalence, it may indicate that rickettsia like prokaryotes develop more rapidly and are recognisable histologically at an earlier time post infection than gut associated protozoa.

The above discussion highlights two problems with the current study. Firstly, the variables nearly all interact in some way or another. Monthly prevalence is affected not only by seasonality, it is affected by which farms were sampled in any given month, the age of animals in some samples, the diet used, and so on. This topic will be revisited at length in the following section on diet. The second problem is specifically related to analyses of temporal data. It is perhaps the most important reason why the current data set does not yield much of use regarding seasonal patterns of parasite prevalence. This problem is the absence of data on incidence. To understand seasonal changes in parasite prevalence, incidence data is needed, or, at the very least, some idea of the duration of infections. The data in this study was all generated by histological analysis, which is destructive testing and yields a result of infected or not. There are no tests by which a live animal may be monitored over time to determine when it becomes infected, thereby leading to identification of periods when animals are more at risk. In addition, it is not known whether infections are permanent or are lost after a time. If infections are lost, how long does this take? When would reinfection be expected, if this occurs? Until such information becomes available, discussion of seasonality will remain at best highly speculative.

4.3.3 Diet

Feeding practices on abalone farms have changed radically over the years and continue to evolve. Virtually all the artificial feed used by industry is manufactured by one supplier. Although the use of alternatives does occur, it cannot be considered significant, especially not on the farms included in this study. Kelp is by far the dominant macroalgae used as abalone feed. Use of other types are increasing, especially those which may be grown by the farms on site, but this practice was not common at the time of data collection. The major shift which occurred during the five years covered by this study was greater use of artificial feed, notably in older animals. This trend started towards the end and was to some extent preceded by a move away from artificial feed. To understand why, it is necessary to learn more about the sabellid polychaete.

The sabellid polychaete *Terebrasabella heterouncinata*, commonly referred to as the sabellid, lives in tunnels in the shells of abalone and some other molluscs. Sabellids are filter feeders and have no impact on abalone soft tissues. Although the effect of sabellids is much debated, the worms are universally detested by abalone farmers, who hold sabellids responsible for slow growth of their abalone, brittle and eroded shells, and infections by shell boring polychaetes belonging to other genera. Much research has been conducted on the sabellid, mostly funded by the abalone industry. This has shown, amongst other things, that sabellids prefer artificial feed to other diets (Simon *et al*, 2004). In addition, anecdotal accounts from farms suggest that abalone fed kelp show more rapid shell deposition. Expansion of the abalone industry has led to massive sabellid infestations on many farms. Sabellids produce crawling larvae which frequently settle only a short distance away from the parent, so infected shells tend to become worse over time. Older animals tend to have higher prevalences and intensities of sabellid infestations, from there the reluctance of farms to use artificial feed in older abalone. This trend peaked in 2003, as can be seen from figure 3.2.2.19.

However, artificial feed has benefits over kelp. Consistent supply and ease of feeding are among these. The most important benefit is a much higher nutrient density than kelp. Kelp is approximately 90% water, whereas artificial feed is a dry formulated pellet. The abalone must graze far more kelp to obtain the same quantity of protein and energy as it would when eating artificial feed. It is debatable whether it is physically possible for the animal to ingest volumes

of kelp that are nutritionally equivalent to artificial feed. To make matters worse, kelp produces antinutritional substances in the form of polyphenols at certain times of the year (Tugwell and Branch, 1992). The result is that animals on artificial feed tend to show superior mass gain, export performance and canning yields to those which are fed kelp. There is a strong incentive for farms to use good hygiene practices to control sabellids, to allow feeding of artificial feed to as many animals as possible. This trend towards more artificial feed has been ongoing for several years, strengthened by erratic kelp availability. Some farms still prefer to use kelp in animals approaching export size, as they believe kelp fed abalone to have a better taste than those on artificial feed. In addition, kelp is used almost exclusively on the West coast and in recirculation systems.

The relationship between diet and parasite prevalence is perhaps the most interesting aspect of this study. Unfortunately, it is also complex and hard to unravel. Starting with figure 3.2.3.1, it can be seen that the majority of animals in the sample population received kelp. A smaller, but comparable, number received artificial feed and approximately 16% were fed a mixed diet of artificial feed and macroalgae. Animals are not necessarily fed the same diet throughout their lives, in fact, the opposite is more likely. Data was not available on the dietary history of animals and it is not known to what extent changes in diet affect parasite prevalence.

A significant difference existed for parasite prevalences on the various diets for all parasites tested. The results of the chi square test are given in table 3.2.3.1. Effect sizes are small. Sessile ciliates were most common in animals on a mixed diet, as shown in figure 3.2.3.2. In contrast, left kidney coccidia were least present in the mixed diet group and most common in those fed kelp. Right kidney coccidia were almost absent in animals on artificial feed, as illustrated in figure 3.2.3.3. The gut associated parasites were also much less prevalent in animals receiving artificial feed and those on a mixed diet had more rickettsia like prokaryotes than those on kelp, charted in figure 3.2.3.4. Trematode prevalence was higher on artificial feed according to figure 3.2.3.5. It was felt that odds ratios would be useful in quantifying these differences, but, as odds ratios require construction of two by two tables, mixed diets were not considered. Odds ratios for the relationship between parasite prevalences and diet were not significant for left kidney coccidia and sessile ciliates, as shown in table 3.2.3.2. For right kidney coccidia and gut associated parasites, there was a significantly greater risk in animals fed kelp

than those on artificial feed.

Diet use in different age categories is given in figure 3.2.3.6 and a crude odds ratio for use of kelp in older animals given. Put plainly, younger animals were fed more artificial feed whereas older ones received mostly kelp. An animal older than 24 months is twice as likely to eat kelp as artificial feed. The relationship between age and parasite prevalence has been previously discussed. The problem which now emerges is that of association between age and diet. This was explored by stratifying the data, as shown in tables 3.2.3.3 and 3.2.3.4, for those parasites shown to have significant crude odds ratios in table 3.2.3.2. Unfortunately, no animals of two years or younger receiving artificial feed and infected with right kidney coccidia or digestive gland protozoa were present in the data set. For older animals, the stratum specific odds ratio yielded similar information to the crude odds ratio, showing that animals on kelp have a greater risk of parasite infestation than those on artificial feed. In the case of rickettsia like prokaryotes and gut protozoa, it was shown that the crude odds ratio is not an accurate estimate, and that both confounding and interaction are present. The data was also stratified by diet and the calculations repeated, this time comparing age groups. The results are shown in tables 3.2.3.5 and 3.2.3.6.

Essentially, the calculations confirm that older animals are more likely to be infected with certain parasites and show that this is partly an effect of older animals being fed more kelp. However, regardless of diet, older animals have greater prevalences of these parasites than younger animals. The difference in risk between older and younger animals is much less when receiving kelp than artificial feed. Stratum specific odds ratios for age demonstrate greater risk for younger animals when fed kelp compared to older animals. In other words, age both affects prevalence in itself and modifies the relationship between diet and prevalence. At the same time, age is also a confounder, because it affects both the use of diet and the prevalence of parasitic infection.

The possible effect of age on parasite resistance in abalone has already been discussed under the section on age and the speculative nature of such discussion should again be stressed. Yet, the finding that age modifies the relationship between diet and parasite prevalence would tend to lend at least some credibility to such speculation. It seems plausible that younger animals are

more susceptible to infection and that they are more at risk than older animals when fed kelp, which is the diet associated with a greater risk of parasite infection.

It was shown that there is a significant association between area of origin and parasite prevalence. Unfortunately, there is also an association between origin and diet, as can be seen from figures 3.2.3.7 and 3.2.3.8. This is most marked when comparing the South and West coasts, as the farms on the West coast use virtually only kelp. Traditionally, the reason has been cited as colder water temperatures leading to reduced growth rates and consequent increased risk of sabellid infestation. The large difference between South and West coasts in terms of diet is probably partly responsible for the difference between Hermanus and other areas. Hermanus can be seen to use more artificial feed than other areas.

It can be seen from tables 3.2.1.2 and 3.2.1.3 that a significant association between prevalence of sessile ciliates exits for coast and area of origin. However, in both cases, this odds ratio is close to 1.0 and the difference may be considered marginal. The same may be said of the odds ratio for digestive gland protozoa on the South and West coasts. Odds ratios were also small in some other cases, including digestive gland protozoa in and outside Hermanus.

When examining the relationship between diet and area, it was found that animals in Hermanus are more likely to receive artificial feed than those outside Hermanus. Knowing the relationship between diet and parasite prevalence, this creates an expectation that animals in Hermanus will have fewer sessile ciliates, right kidney coccidian infections and gut associated parasites. This is supported by the odds ratios, except in the case of sessile ciliates, where a greater risk occurred in Hermanus. Stratified odds ratios for parasite prevalence on different diets in Hermanus and other areas provide further insight.

It can be seen from tables 3.2.3.7 and 3.2.3.8 that the relationship between diet and prevalence of gut associated parasites was stronger in Hermanus than in other areas. The odds ratio for right kidney coccidian infections could not be calculated, as there were no infected animals in Hermanus receiving artificial feed. This appears to show that, although Hermanus has a lower prevalence overall, there is in fact greater risk of infection with gut associated parasites when animals are fed kelp in Hermanus than in other areas. Perhaps fortunately, as illustrated in figure 3.2.3.9, very few animals of 24 months and younger receive kelp in Hermanus. It is of course possible that

the greater risk associated with kelp in Hermanus is actually reflecting the age profile of the animals receiving kelp. Table 3.2.3.9 gives the odds ratios for kelp usage in older animals compared to younger ones. Older animals in Hermanus are far more likely to receive kelp than younger ones. It may be that, were more younger animals in Hermanus fed kelp, parasite prevalences would increase. The age profiles of samples from different places, shown in figures 3.2.3.10 and 3.2.3.11, were not different, so it is safe to say that the larger numbers of certain parasites on the West coast and outside Hermanus is not the result of an older population in these areas.

Although the relationship between diet and parasite prevalence is complicated by the various interactions and confounding discussed above, it is clear that there is an association. Of course, the nature of this association cannot be determined by the available data or through the methodology of descriptive epidemiology. Having said this, it is interesting to consider some of the potential reasons for the increased prevalence of certain parasites in animals fed kelp. Kelp is harvested from the ocean and arrives on the farm together with a large collection of epifauna. These range from minute crustaceans to molluscs such as limpets. It is entirely feasible that some of these creatures may be either intermediate hosts or carriers of parasites. No work has been done to examine this possibility. Furthermore, it is conceivable that infective stages of parasites may be attached to kelp. Wild abalone populations are found in kelp beds and one can imagine that they may shed parasites which then attach to kelp, awaiting ingestion by aquatic grazers, much as is seen with some parasites of terrestrial grazers. Artificial feed clearly lacks these features, being made in a factory and delivered in plastic containers.

The dynamics and management of tanks where kelp is fed may also play a role. Kelp in a tank acts as a substrate for attachment of abalone. It creates habitat. Animals literally sit on their food and graze, as opposed to artificial feed, which is offered to them at a feeding station. One could compare this to terrestrial animals being on a pasture compared to eating from a trough. Perhaps there is more opportunity for ingestion of excreted parasites in a kelp system. As a generalisation, kelp tanks are also cleaned less frequently than tanks where artificial feed is used. The theory is that kelp, a living macroalgae, improves water quality, whereas artificial feed deteriorates fairly quickly, increases the particle and dissolved nutrient load in the water, and creates a substrate for bacterial overgrowth. Typically, kelp tanks will be cleaned fortnightly, as opposed to weekly for tanks receiving artificial feed. It may be that there is more accumulation of infective

stages of parasites in kelp tanks, or it may be that certain parasites require a period outside the host to become infective, which is available to them in kelp tanks. Entertaining though it may be to speculate, life cycle and other work on these parasites are needed before the relationship between their prevalence and the diet of the abalone is ever to be understood.

4.3.4 System

By far the majority of abalone farms utilise flow through systems. As can be seen from figure 3.2.4.1, most of the samples came from these systems. Flow through systems rely on continual replacement of water in the tanks to maintain water quality. Depending on the farm, the water in a tank will be displaced anything from once every two hours to once every five hours. More rapid turnover carries the benefits of cleaner water, less temperature fluctuation and higher dissolved oxygen levels. It is also more expensive, as it requires larger volumes of water to be pumped through the farm. Recirculation systems are seen as an alternative to flow through. There is very little replacement of water in recirculation systems and various combinations of mechanical and biological filtration are used to preserve water quality. The advantages and disadvantages of flow through and recirculation systems are hotly debated in aquaculture circles. Only aspects of potential relevance to parasite prevalence will be considered here.

It is obvious that continual replacement is a simpler and probably more effective way of keeping water clean than filtration, provided that the incoming water is of adequate quality. Experience has shown that recirculation systems are hard to manage and prone to sudden deterioration of water quality when the biofilter fails. Biofiltration also leads to a gradual decline in pH, necessitating the regular addition of chemicals to counteract this process. It is not known to what extent the composition of the seawater in these systems changes over time. Temperature control is often given as a benefit of recirculation systems over flow through. In fact, it is extremely difficult to control the temperature in recirculation systems as they tend to acquire the same temperature as the surrounding air and are not usually housed in structures which allow modification of air temperature. It is common for recirculation systems to become too hot or cold during the peaks of summer and winter. In short, abalone are more likely to encounter stressful environmental conditions in recirculation systems than in flow through. Potential problems in flow through are overstocking, equipment failure and the risk of an incoming pollutant, all of which

may also affect recirculation systems.

The most obvious advantage of flow through systems from the point of view of parasite reduction is flushing. The continual displacement of water will tend to remove a portion of any parasites shed by the tank inhabitants, whereas recirculation systems retain parasites. In addition, the tanks in flow through systems are cleaned regularly. This involves draining the tank and spraying off the sides and base to remove any faeces and uneaten food. Many farms also spray the baskets of abalone to rid them of particulates. The tank is then rapidly refilled with clean water and resumes its normal turnover. Recirculation tanks are cleaned infrequently, often in response to failure of the biofilter. It is a reasonable assumption that recirculation systems offer more opportunities for the buildup of parasites and would tend to support higher incidences than flow through systems.

Proponents of recirculation systems claim that pathogens are excluded because the system is closed. It should be noted that systems in use in South Africa are only partially closed and rely on constant replacement of a relatively small percentage of the total volume. Incoming water could theoretically be treated to eliminate microorganims, although this is difficult to implement effectively. Just to start, one would need to know characteristics such as the size and resistance of infective agents. Of course, recirculation systems can offer no benefits over flow through systems when potential pathogens are introduced by routes other than water, for example with feed or by buying in abalone.

The results of this study only partly confirm the above assumption that recirculation systems favour higher parasite prevalences. The results of the chi square test are shown in table 3.2.4.1. A significant association between parasite prevalence and system was found for half of the parasites examined, these being sessile ciliates, renal coccidia and rickettsia like prokaryotes. In the case of sessile ciliates, the prevalence in recirculation systems is far lower than in flow through, with a moderate effect size. Renal coccidia and rickettsia are more prevalent in recirculation than flow through systems, but effect sizes were very small. Trematodes were not found in the recirculation systems, but the small sample of infected animals precludes obtaining a significant difference. Prevalences are charted in figures 3.2.4.2 to 3.2.4.5.

As age and diet are known to affect parasite prevalence, these were considered in relation to

system. It can be seen from figure 3.2.4.6 that the age profile of animals sampled from different systems is similar. However, as shown in figure 3.2.4.7, diet was not. The animals in the recirculation systems received exclusively kelp, as opposed to those in flow through which may also be fed artificial feed or a mixed diet. Based on this, the prevalence of parasites in flow through and recirculation systems were compared for only animals which receive kelp. Using odds ratios, given in table 3.2.4.2, significant differences were found for sessile ciliates, left kidney coccidian infections and digestive gland protozoa. Farm g, which had a very low prevalence of sessile ciliates and a high prevalence of renal coccidia, functioned primarily on recirculation.

The very low prevalence of sessile ciliates in recirculation systems cannot be explained by examination of any other variable considered in this study and speculation is again tempting. Sessile ciliates are the only parasites which are surface associated, the others are all internal. Water quality may potentially have a far greater impact on these ciliates than on the other parasites. Changes in pH, chemical control of pH, and differences in mineral levels are some possible causes which come to mind.

The significant difference in the prevalence of rickettsia like prokaryotes in the two systems found with the chi square test was not reflected in the odds ratios, most likely due to the strong association of rickettsial infection with diet. A similar pattern would be expected for gut protozoa, but this is not seen. It is likely that other variables are at play. In the case of digestive gland protozoa, no association is detected with the chi square test, but the odds ratio is significant, although not large. These results indicate that parasite buildup in recirculation systems is not as problematical as may be expected. Possible reasons include entrapment of infective stages in the biofilter or removal by foam fractionation.

Left kidney coccidian infections were more prevalent in the recirculation systems than in flow through. These parasites are not associated with diet and are more common in younger animals. As many younger animals in flow through systems receive artificial feed, comparison of kelp fed animals only could result in bias of the odds ratio. However, the same result was obtained with the chi square test. Renal coccidia and digestive gland protozoa are the only parasites of South African abalone for which some evidence exists of a relationship between occurrence and stress, based on histological findings from the health management program. The general performance of animals in recirculation systems would support an argument that conditions in these systems is

less favourable than in the average flow through system. It is possible that the increased prevalence of left kidney coccidia in recirculation systems is linked to the resistance of the host population rather than to the dynamics of the actual system. Seemingly contradicting this, the odds ratio shows a lower risk of digestive gland protozoan infection in abalone in recirculation systems. Much more research is needed before this association can be understood.

4.4 Overall prevalence

It can be seen from table 3.3.1 that there are large differences in the prevalences of the various parasites found in South African abalone. As little is known about these parasites, any discussion of the reasons for variation in prevalence tends to be highly speculative. The only one which has been named is *Mantoscyphidia midae* (Botes, 1999), the sessile peritrichous ciliate found on gills and external epithelia of *Haliotis midae*. No attempt has been made to describe the renal coccidia and research on the gut associated parasites is still ongoing. Identification of the trematodes infecting *Haliotis spadicea* (Botes *et al*, 1999) would probably shed some light on those in *Haliotis midae*, but has not been done.

In the current study, sessile peritrichous ciliates were present in 68.33% of abalone, making them by far the most common parasites of South African abalone. *Mantoscyphidia midae* divides by binary fission. Ciliates would generally remain on an individual host, increasing in numbers over time (Botes, 1999). It is likely that the high prevalence is largely the result of persistent infections. Epithelial ciliates are found in abalone and other aquacultured species worldwide, but do not receive much attention from pathologists, as they are considered to be ectosymbionts rather than true parasites (Botes, 1999; Bower and McGladdery, 2003).

Renal coccidia have been reported from several countries. A national survey of abalone in Australia unearthed a single infected wild animal (Handlinger *et al*, 2006). In contrast, examination of wild abalone in California yielded a prevalence of 69% (Friedman *et al*, 1997). A survey of farmed abalone in Baja California, in Mexico, found prevalences of up to 72% (Cáceres-Martínez and Tinoco-Orta, 2001). Renal coccidia are comparatively rare in *Haliotis midae*. Left kidney infections were found in only 1.85% and right kidney infections in 0.23% of animals in this study. Infections were limited to either the left or the right kidney, whereas renal

coccidia from other abalone species occur in both kidneys, generally at very high intensities (Friedman, 1991; Godoy and Aedo, 2006). The reasons for these differences are not known.

Protozoan parasites of the gut and digestive gland have also been reported from other countries. In the Australian survey, protozoan parasites closely resembling those from South Africa were found in wild and farmed abalone. Unfortunately, the prevalence is not given, but is stated to be low overall (Handlinger *et al*, 2006). Pathologists working in California, Baja California and Chile likewise make mention of these parasites (Aviles *et al*, 2006b). In the current study, gut protozoa were seen in 5.54% and digestive gland protozoa in 5.27% of South African abalone. The life cycles of these parasites are not known, but a faecal oral route of infection is likely. In South African culture systems, abalone are effectively separated from their faeces, thereby possibly limiting new infections with gut associated parasites.

Inclusions of rickettsia like prokaryotes are often found in marine molluscs and are not usually associated with disease (Bower and McGladdery, 2003). Such inclusions were considered rare in abalone examined as part of the Australian survey (Handlinger *et al*, 2006). In other parts of the world, rickettsia like prokaryotes of abalone are associated with withering syndrome. Prevalences of up to 100% have been reported from areas within California where withering syndrome occurs (Friedman *et al*, 1997). On the other hand, a prevalence of 1.84% was claimed for red abalone, *Haliotis rufescens*, from Chile (Godoy and Aedo, 2006). Rickettsia like prokaryotes were the second most common parasites of South African abalone in the current study, at a prevalence of 13.07%. Histological sections of infected digestive gland tissue usually show rickettsial colonies in different stages of development, suggesting that autoinfection may occur. In addition, far more organisms are shed from infected tissue than are seen with the gut associated protozoan parasites, which may indicate greater infection pressure. These observations may partly explain the higher prevalence of rickettsia like prokaryotes.

Trematodes were the rarest parasites in this study, at a prevalence of 0.05%. In *Haliotis spadicea*, collected from the wild, trematodes were found in 75.19% of animals sampled over a period of five years (Botes, 1999). It is possible that the prevalence in wild *Haliotis midae* may also be higher, as conditions on farms might not be favourable for infection of abalone. Trematodes generally have complex life cycles and the abalone is most likely acting as an intermediate host. The farm environment will tend to prevent exposure of abalone to other hosts in the cycle. Data

from the Australian survey showed trematodes to be more common in the wild than on farms (Handlinger *et al*, 2006). The low prevalence relative to *Haliotis spadicea* may also indicate that *Haliotis midae* is not a primary host for these parasites.

Chapter 5
Conclusion

In the usual course of events, the epidemiology of parasites is examined after they have been described and something learnt of their life cycles. It is very difficult to interpret the findings of the current study when so little is known of the parasites. However, in spite of these limitations, the results may serve to inform decisions on husbandry practices in abalone culture and, perhaps, assist those working on parasite biology in the future.

It is almost certain that the current study underestimates parasite prevalence. None of these parasites are evenly distributed in their various target organs. Infections of especially gut protozoa tend to be very focal. As only a portion of the animal's tissues are sampled for histology and only a fraction is represented on the sections, it is inevitable that some infections will be missed. At present diagnosis of infection relies solely on histology and there are no other tests available, so it is not possible to estimate the percentage of false negatives or the true prevalence. On the other hand, the parasites, when seen, are unmistakable and false positives should not occur, except perhaps through errors in data entry. This does not negate comparison with results from other countries, as these too were generated from histological examination of animals.

Overall, the prevalences of soft tissue parasites in *Haliotis midae* compare favourably with those found for parasites of other abalone species abroad. *Mantoscyphidia midae*, the local sessile ciliate, was the only common parasite in this study and can be considered an ectosymbiont rather than a true parasite. The others, which are of greater concern, were all relatively rare. One can cast these results in a positive light by stating that cultured *Haliotis midae* appear to enjoy good health. Future surveillance work is necessary to ascertain whether this will continue. In the interim, some measures are suggested below which may contribute to maintaining low prevalences of certain parasites.

Separation of age groups as a method for lowering disease incidence is well established in terrestrial animal culture. The increased prevalence of most parasites in older abalone indicates that separation of age groups may also be usefully applied to them. This practice is already

successfully implemented to control sabellid polychaetes. If other parasites are found to have direct life cycles and transmission on farms is important in establishing new infections, separation of age groups is a logical management intervention. However, should the primary source of infection be associated with kelp or incoming seawater, success is less likely.

Another aspect of the effects of age relates to the age distribution of the abalone population on a farm. Given that most parasites are more prevalent in older animals, it becomes desirable to maintain as few older animals on the farm as possible. This can only be achieved by increasing growth rates so that animals may be marketed at the earliest opportunity. Good husbandry underlies superior growth and has the added benefit of decreasing risk in all areas of farming. Even the best performing group of abalone will always contain slow growers and runts, which can potentially contribute to an increased average age of the population. These individuals should be identified and culled at the earliest opportunity, yet another device which has proven effective in both terrestrial and aquatic animal culture.

The differences in parasite prevalences between various farms indicate that management is already playing a role in disease control, but current understanding of parasite biology is not sufficient to allow confident identification of best practices. Epidemiological analysis of specific factors such as flow rates and stocking densities could be helpful and may be worth considering for future research.

The relationship between diet and the prevalence of gut associated parasites was perhaps the most interesting finding of this study. Unfortunately, the reason for the association between diet and prevalence is not known. It is entirely possible that different reasons exist for the various parasites. However, there is undoubtedly an increased risk of parasite infection for younger animals when fed kelp. The logical conclusion is that kelp should not be used in animals of two years or younger. In older animals, there is still a greater risk associated with kelp than with artificial feed, but it is not as marked. One would prefer to also see older animals on artificial feed, but these animals may have some degree of resistance to parasite infection that younger abalone lack. There is a growing trend in the industry to culture seaweeds on the farm, in farm effluent, for use as feed. One interpretation of the results of this study is that such seaweed is not suitable for younger abalone, especially not when grown in effluent from tanks containing

older abalone.

Recirculation systems proved to be less associated with increased parasite prevalence than one may expect. In the case of sessile ciliates, there is a far lower prevalence in recirculation than in flow through systems. If this is due to abnormal characteristics of the water, lower prevalence may be undesirable. Left kidney coccidia, which were more common in recirculation systems, could be stress related. Left kidney coccidian infections are more prevalent at lower growth rates. On the other hand, digestive gland protozoa were less prevalent in recirculation systems. None of this indicates a causal relationship between system and parasite prevalence, except perhaps for sessile ciliates.

The other major findings of this study do not lend themselves to practical application. The associations between sex and gonad development and parasite prevalence are most likely due to age and size. Differences in prevalence between various areas may be largely ascribed to feeding practices, although it is possible that true differences do exist. Even if so, existing farms are not going to relocate on the basis of parasite prevalence, unless overtaken by a major disease outbreak. Seasonal patterns of parasite occurrence would be best examined when there is a means of determining incidence, preferably through non destructive sampling.

The current study deals with data for the years 2000 to 2004. The abalone health management program has continued past this period and further data are now available. Very preliminary analysis of these data points towards changing patterns of prevalence and may cast some light on past results. Lessons learnt from this study have since been applied to data collection, so that less data is lost through incomplete recording of details such as diet. There is much exciting epidemiological work that lies ahead. In parallel to the data analysis, work is being carried out on characterising the parasites of South African abalone. No doubt the results of the parasitological research will contribute to better understanding of the epidemiology. Ultimately, it may prove that none of the parasites of *Haliotis midae* have serious pathogenic potential and our work have no more than curiosity value. Ironically, this outcome is to be sincerely desired.

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