The occurrence of gut associated parasites in the South African abalone, *Haliotis midae*, in Western Cape aquaculture facilities

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abstract

Abalone have been cultured in South Africa for seventeen years. The growing industry has led to increased intensification. Farms are concentrated in certain areas, notably Hermanus on the South coast, and may be close to wild abalone populations and processing facilities. These factors contribute to increased risk of disease emergence. Data on parasite prevalence generated from the abalone health management program between 2000 and 2004 was analysed for trends. Abalone were sampled systematically from participating farms and subjected to gross and histological examination. Data on age, size, gonad development, diet and type of system were recorded. This paper presents the most significant results for gut protozoa, digestive gland protozoa and rickettsia like prokaryotes, which are all gut associated. Prevalence was found to increase with increasing age and size. Higher parasite prevalences were found on the West coast than on the South coast, and outside Hermanus compared to within Hermanus, suggesting that concentration of farms is not leading to increased prevalence. Gut associated parasites were significantly more prevalent in animals fed on kelp than artificial feed. It was found that animals younger than 24 months are more at risk of infection when fed kelp than older animals. The results indicate that separation of age groups, removal of poor performers and use of artificial feed, especially in younger animals, are likely to reduce risk of infection with gut associated parasites.

Keywords: Abalone, Haliotis midae, Epidemiology, Gut protozoa, Digestive gland protozoa, Rickettsia like prokaryotes

1. Introduction

The South African abalone or perlemoen, *Haliotis midae*, is known to be infected with a number of parasites. Of these, only *Mantoscyphidia midae*, a sessile peritrichous ciliate infesting the gills, mantle cavity and external epithelia, has been formally described (Botes, 1999). Research is ongoing to characterise the others, which include protozoan parasites of the gut and digestive gland, rickettsia like prokaryotes of the digestive gland, and renal coccidia. To date there is no evidence that any of these parasites cause clinical disease or mortalities, but the possibility exists that they may emerge as significant pathogens in the future, due to the increasing growth and intensification of the abalone farming industry.

The South African abalone farming industry is entirely based on *H. midae. H. midae* has been commercially cultured for about seventeen years. During this time, total production has reached approximately 1000 t per annum,nearly all from intensive pump ashore systems. There are several factors which favour disease emergence in the South African abalone industry. The concentration of ever increasing numbers of abalone on farms is one and the tendency for farms to cluster in certain areas is another. The majority of farms are in the Western Cape (Fig. 1). Approximately two thirds of the total abalone production is from six farms situated along ten kilometres of coastline around Walker Bay on the South coast. Almost forty percent of South African abalone production takes place in the small town of Hermanus, with the three responsible farms sharing boundaries and, in some cases, intakes. It is inevitable that farm effluent is pumped in from time to time. There are also a large number of abalone processors in close proximity to the farms and these receive both farmed and wild abalone from all over the country.

Other factors which may lead to disease emergence on farms include the presence of wild abalone populations near most culture facilities. This provides the opportunity for cycling of potential pathogens between populations. In addition, many abalone farms feed kelp, primarily *Ecklonia maxima*, which is harvested from the sea. The majority of kelp beds contain wild abalone and are also very close to farms. Artificial abalone feed is available in South Africa and provides an alternative to a natural diet. Seaweeds

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may also be cultured on the farm, but as this is usually done in effluent water, it increases rather than mitigates disease risk.

On average, *H. midae* takes 42 to 54 months to reach a marketable size. The implication is that abalone farms do not have the option of practising an all in all out management system. It is inevitable that an abalone culture facility will hold animals of varying ages and, once begun, production is continuous, without intervals of fallowing. Parasite transmission between age groups and increasing prevalence over time may create conditions which are conducive to the selection of more pathogenic strains of microorganisms.

South African abalone farmers have had, since 1998, the option of participating in a health management program for their stock (Mouton, 2000). The primary focus of the program is prevention of disease and production losses. It attempts to identify stressors such as poor environmental conditions or deficiencies in husbandry and feeding practices, thereby avoiding circumstances conducive to disease emergence and outbreaks, as far as is possible. Farms belonging to the health management program are sampled on a regular basis and animals subjected to gross and histological examination. This has, over the years, generated a large body of data on parasite prevalence. It was felt that analysis of the data set using epidemiological techniques could be useful in understanding which factors increase parasite prevalence and thereby contribute to the risk of disease emergence. This paper presents some of the results obtained for gut associated parasites.

2. Materials and methods

Data for abalone submitted as part of the abalone health management program during the period 1 January 2000 to 31 December 2004 were analysed. The abalone came from a total of nine abalone farms, but not all farms are equally represented in the data set. These farms are located on the southern and western coasts, as shown in Fig. 1. The samples from each farm were selected to include the various age groups present on the farm in on growing facilities. Abalone in the hatchery and nursery phase of production were not included in these data Asabalone 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.

Data on the age and diet of the abalone were obtained from the farms. 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 by pumping from the ocean. The majority of the abalone were kept in flow through systems.

Abalone were submitted live. The date of collection, farm of origin, shell length and total mass were recorded for each individual. It was then shucked, shell mass determined and a standard set of five tissue sections collected. This set will provide adequate representation of all the major organ systems. The tissue sections were processed using standard histological techniques (Austin and Austin, 1989). Histological sections were stained with Harris's haematoxylin and eosin and examined under light microscopy to determine the presence or absence of soft tissue parasites.

The data for each individual abalone were captured in an Excel (Microsoft Corporation, 2003) spreadsheet. Initially, data from 18693 animals were captured. All animals for which the data set was 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 12950 abalone.

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, the chi square test and odds ratios were used. Due to the large sample sizes, it was possible that significant chi square values were found although associations were weak (Knapp and Miller, 1992). 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 are significant when not equal to 1.0 and the size of the odds ratio is a direct measure of the strength of an association. When confounding and interaction were suspected, it was necessary to calculate stratum specific odds ratios. 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 (Dohoo et al., 2003).

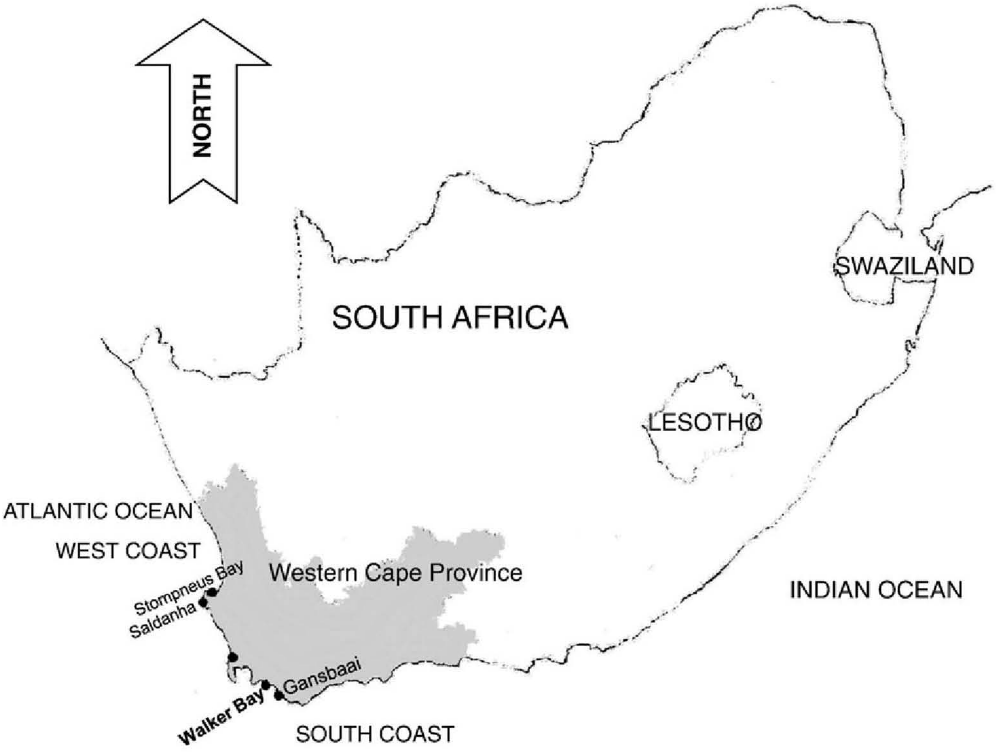

Fig. 1. The location of the study area within South Africa.

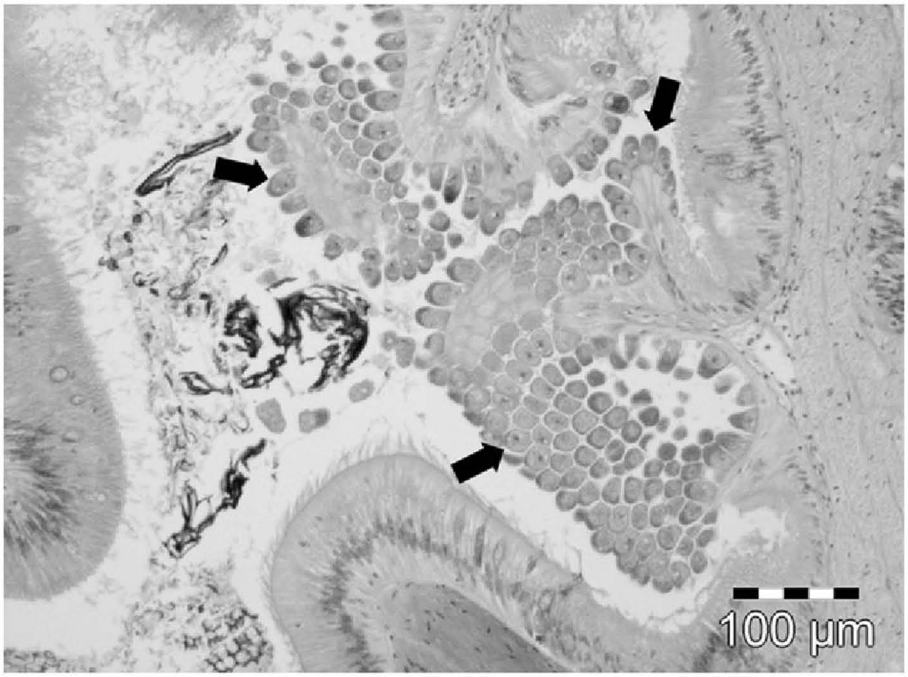

Fig. 2. Light micrograph showing gut protozoa attached to intestinal border. Examples of typical parasites indicated by arrows.

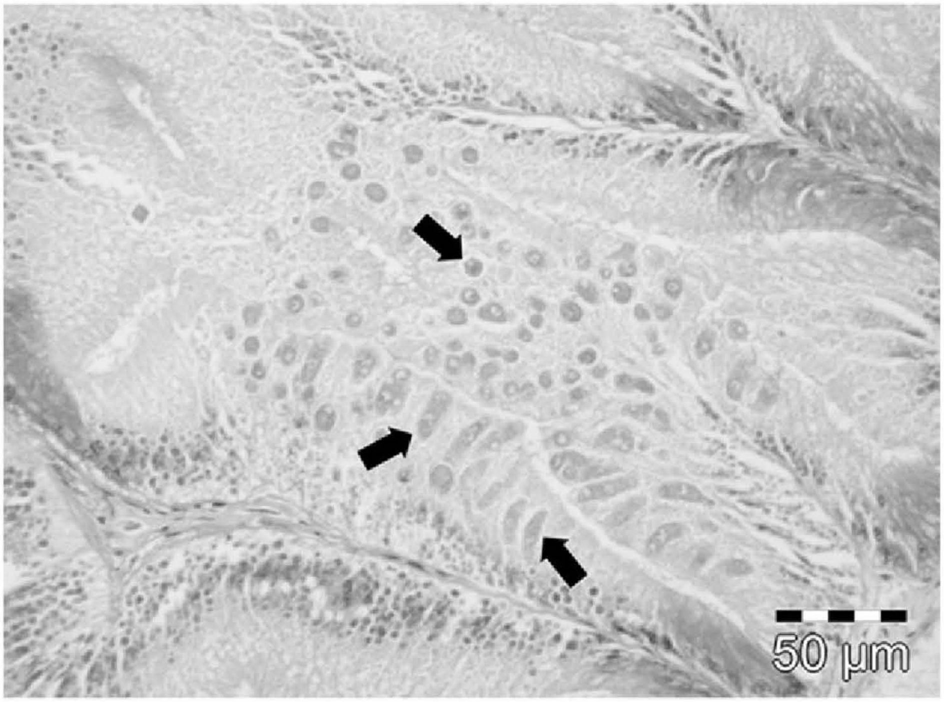

Fig. 3. Light micrograph showing digestive gland protozoa. Examples of typical parasites indicated by arrows.

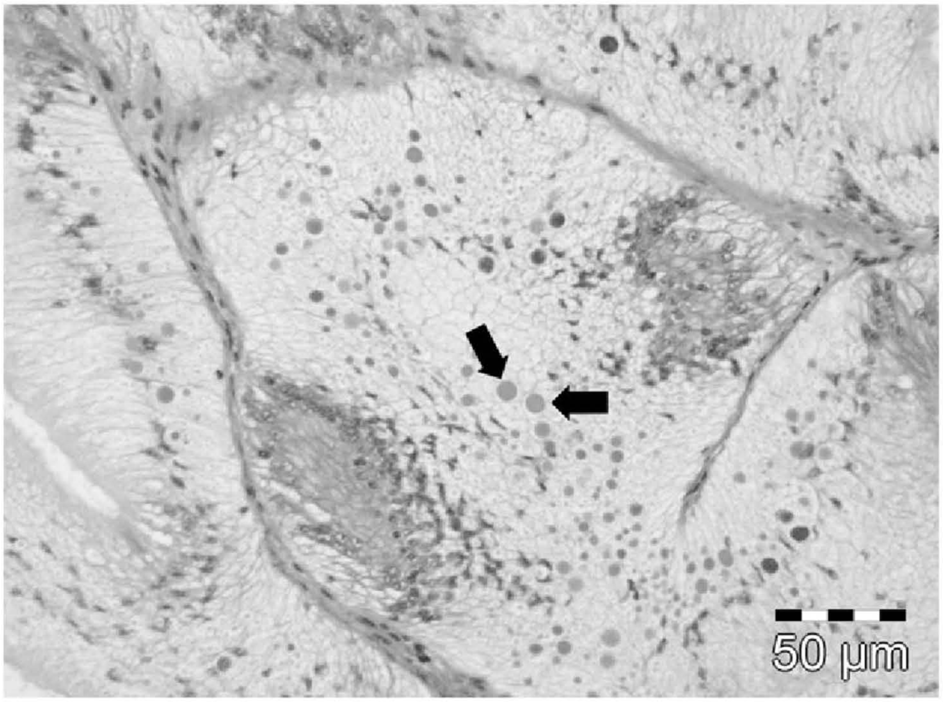

Fig. 4. Light micrograph showing colonies of rickettsia like prokaryotes in digestive gland. Examples of typical parasites indicated by arrows.

3. Results

The histological appearance of the various gut associated parasites is shown in Figs. 2, 3 and 4. The overall prevalences of the various gut associated parasites are summarised in Table 1.

The age of the animals ranged from 8 to 75 months. There were relatively few animals in the first year class and the majority of animals were 13 to 48 months of age. The prevalences of the various parasites all increased with host age. The chi square test showed a significant association (pb0.005) between age and parasite prevalence, but the effect sizes were modest. To further test the strength of the association between parasite prevalence and host age, odds ratios were calculated. In all instances the odds of parasite infestation are significantly greater in animals older than 24 months compared to those of 24 months and younger. This effect was most marked for digestive gland protozoa (OR=5.54), followed by rickettsia like prokaryotes (OR=3.73) and gut protozoa (OR=3.45).

The mass range of the samples was 0.16 to 240.21 g. There were relatively few animals larger than 45 g in the sample population. The results for the relationship between parasite prevalence and mass were similar to those for age. This is expected, as animals generally become larger with increasing age.

Seven farms were on the South coast and two on the West coast. When parasite prevalence on the South and West coasts were compared, significant differences were demonstrated for gut protozoa and rickettsia like prokaryotes using the chi square test (pb0.005). The effect sizes were very small in both cases. Crude odds ratios for parasite prevalence between coasts showed that there is a greater risk of parasite infection on the West than the South coast. This was most marked for gut protozoa (OR=2.22), followed by rickettsia like prokaryotes (OR=2.15) and digestive gland protozoa (OR=1.30).

There were three farms in Hermanus. A significant difference existed for prevalence of gut associated parasites between Hermanus and other areas (pb0.005). The effect sizes were small. The differences, in fact, relate to other environmental factors and will be further explored below. Crude odds ratios for parasite prevalence in other areas compared to Hermanus show that there is a greater risk of parasite infection in areas other than Hermanus. Odds ratios were greatest for rickettsia like prokaryotes (OR=2.71), followed by gut protozoa (OR=1.43) and digestive gland protozoa (OR=1.29).

Approximately 45.2% of the sample population received kelp, 38.9% received artificial feed and 16.0% were on a mixed diet. The mixed diet contained both artificial feed and macroalgae, fed either simultaneously or as a rotational diet with a cycle of less than one month. The chi square test showed significant differences in parasite prevalences between diets for all parasites tested (pb0.005). The effect sizes were modest. To further test the strength of the association, odds ratios were calculated, comparing only kelp and artificial feed. These indicated a significantly increased risk of infection in animals receiving kelp compared to those on artificial feed. This was most marked for digestive gland protozoa (OR=6.43), followed by gut protozoa (OR=3.75) and rickettsia like prokaryotes (OR=3.71).

It has already been shown that there is an association between age and prevalence of gut associated parasites. Unfortunately, there is a difference in diet use between older and younger animals, with more kelp being fed to older animals. 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. The relationship between parasite prevalence, age and diet was explored using odds ratios and comparing animals of 24 months and younger with older animals.

The stratum specific odds ratios for parasite prevalence on kelp compared to artificial feed were calculated for animals of 24 months and younger, and for older animals. No animals of 24 months and younger receiving artificial feed and infected with digestive gland protozoa were present and odds ratios could not be calculated for this group. For gut protozoa and rickettsia like prokaryotes, the risk of infection was greater on kelp for both age groups, but this effect was larger in younger animals. The disparity in odds ratios for different age classes was more marked for gut protozoa (OR=12.87 for 24 months and younger and OR=2.71 for older animals) than for rickettsia like prokaryotes (OR=5.38 for 24 months and younger and OR=3.00 for older animals).

As the stratum specific odds ratios differed from the crude odds ratios, the presence of confounding was suspected. The stratum specific odds ratios also differed from each other, indicating the presence of interaction. 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% 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% 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

Table 1
Prevalence of gut associated parasites in South African abalone.

Parasite	Prevalence as % infected	95% confidence interval
Gut protozoa	5.54	5.15 to 5.94
Digestive gland protozoa	5.27	4.89 to 5.66
Rickettsia like prokaryotes	13.07	12.49 to 13.65

diet and then calculating odds ratios comparing the different age groups. When animals receive artificial feed, the older animals have a greater risk of infection with gut protozoa (OR=8.71) and rickettsia like prokaryotes (OR=3.66) compared to the younger animals, than when both groups are fed kelp (OR=1.83 for gut protozoa and OR=2.05 for rickettsia like prokaryotes).

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% 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% 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 area and parasite prevalence was demonstrated. As there is a difference in diet use between 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, for animals from Hermanus and for those from other areas show that animals in Hermanus have a greater risk of infection when fed kelp than those in other areas. The data appear to demonstrate a greater risk of infection with gut associated parasites when animals are fed kelp in Hermanus than in other areas. However, stratum specific odds ratios for diet use and age in Hermanus and other areas show that there is a significant difference between the age profile of animals on the various diets in different areas. 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.

4. Discussion

Age was found to be significantly associated with prevalence for the gut associated parasites examined in this study. The increase in prevalence with age may be partly explained by changes in diet, as older animals were more likely to receive kelp. However, there are many aspects of age which can be expected to impact on prevalence. These include greater length of exposure in older animals, physiological development and changes in husbandry. Older animals are typically held at higher densities with relatively less water flow than younger ones (Mouton, 2005).

Increased parasite prevalence in larger sizes may be directly related to age. However, size in itself could affect prevalence. For example, a larger animal eats more and, if parasites are ingested, this may lead to higher incidences.

The present study only dealt with farms in the Western Cape. As mentioned above, the concentration of abalone farms increases the potential for infectious disease outbreaks. 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. Prevalence of gut protozoa and rickettsia like prokaryotes was found to be significantly higher on the West than the South coast. This suggests that proximity of farms and resulting increased geographical density has not affected parasite prevalence. Diet 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.

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. For reasons previously discussed, conditions in Hermanus are theoretically favourable for increased parasite prevalence. However, when comparing parasite prevalence in Hermanus to that in other areas, prevalence in Hermanus is significantly lower. As was found when comparing coasts, prevalence is not higher in areas where more abalone farming occurs.

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. 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. Odds ratios demonstrated a significantly greater odds of infection in animals fed kelp than those on artificial feed. Analyses of diet use in different age categories showed that younger animals were fed more artificial feed whereas older ones received mostly kelp. An animal older than 24 months had twice the odds of eating kelp compared to 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. 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 should be considered, although such discussion is highly speculative. The finding that age modifies the relationship between diet and parasite prevalence would tend to suggest that resistance changes with age. 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. This is most marked when comparing the South and West coasts, as the farms on the West coast use virtually only kelp. 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.

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 gut associated parasites. This is supported by the odds ratios. The relationship between diet and prevalence of gut associated parasites was stronger in Hermanus than in other areas. 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. 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, as 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 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. Some of the potential reasons for the increased prevalence of gut associated parasites in animals fed kelp include presence of vectors in harvested kelp and less frequent cleaning of tanks in which kelp is fed.

Protozoan parasites of the gut and digestive gland of abalone have been reported from other countries. In a national 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., 2006). In the current study, gut protozoa were seen in 5.54% and digestive gland protozoa in 5.27% of South African abalone.

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 most common gut associated parasite of South African abalone in the current study, at a prevalence of 13.07%.

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.

5. Conclusion

Based on the findings of this study, some measures are suggested 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.

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.

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