The use of physiological parameters in the management of the South African abalone (*Haliotis midae*) aquaculture systems in South Africa.

André Laas

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in Zoology of The North-West University.

Promoter:	Dr. A. Vosloo
Co-promoter:	Dr. A. Mouton

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Preface

This thesis represents the results of research work conducted at the North-West University, Potchefstroom Campus in collaboration with members of the Abalone Farmers Association of South Africa (AFASA). The research was performed under supervision of Dr. André Vosloo, North-West University and co-supervision by Dr. Anna Mouton, AFASA. The study was conducted from 2003 to 2005.

The results from the research conducted in fulfilment of the requirements of this thesis are presented as a compilation of scientific papers. Papers included in this thesis have been written in whole by the author of the thesis, the co-author only being responsible for assistance with final editing. Due to the nature of the study, some overlapping of data between research papers were unavoidable. The research presented is the original work of the author, and has not been previously submitted for degree purposes to any other university. Permission by the co-author of the papers used in the study has been included.

References cited in this thesis have been listed in the Harvard style. References for the respective papers were written according to the guide to authors of the journals where the manuscripts were or will be submitted to. The guides to authors for each journal have been included.

The text in the thesis is based on the following papers, which will be referred to by Roman numerals in the text:

I. LAAS, A. & VOSLOO, A. 2006. Internationally published abalone research: National and international trends. (Submitted to *African Journal of Aquatic Science*)

II. LAAS, A. & VOSLOO A. 2006. Towards using physiological parameters in the management of South African abalone (*Haliotis midae*) mariculture: Exploration of basic physiology. (Submitted to *Aquaculture*)

III. LAAS, A. & VOSLOO, A. 2006. Advances in the use of physiological parameters in the management of South African abalone (*Haliotis midae)* mariculture: Seasonal physiological changes on three geographically separated farms. (Submitted to *Aquaculture*)

IV. LAAS, A. & VOSLOO, A. 2006. Advances in the use of physiological parameters in the management of South African abalone (*Haliotis midae)* mariculture: Correlating export performance to physiological parameters. (To be submitted to *Aquaculture*)

For the ease of marking this thesis, the following deviations were made from the guide to authors for the various journals:

1. Figures and tables were put in line with the text, and not as separate attachments.
2. Figures and tables were not downscaled.
3. Line numbers were omitted.

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Dedicated to my loving, devoted Father

and Mother, but above all to my

Magnificent Father in Heaven

Abstract

The aquaculture of the South African abalone (*Haliotis midae*) is the most lucrative and fastest growing division of Southern African mariculture. This industry is driven by a demand far exceeding supply, and natural stocks at the brink of depletion. A study was launched to gather clues from the basic physiological constituents in abalone, which will help in the management of abalone farms in South Africa.

In 2003 an intensive literature study was launched to assess the availability of literature on abalone research, and to find research trends in published literature. From 2003 to 2005, basic physiological constituents (including glucose, glycogen, proteins and lipids) were studied in abalone from six farms in South Africa. A two phased approach was followed, the first of which was an exploratory phase (2003 - 2004) where physiological constituents were studied six-weekly in the muscle tissue and digestive gland of abalone, in two size classes (±50 mm and 70 mm shell length) and four feeding regimes (natural, artificial and two rotational feeds), from one farm. At the end of Phase one, a single live export simulation trial was conducted following standard farm protocols. In Phase two (2004 - 2005), physiological constituents were studied seasonally in the muscle tissue and haemolymph of abalone, in two size classes (±50 mm and 70 mm shell length) and on two dietary regimes (natural and artificial feed), from five abalone farms, of which two had to withdraw from the project. Live export simulations were conducted seasonally in phase two.

South Africa is one of the world leaders in publishing abalone research. The main focus of research in South Africa is on the development and enhancement of artificial diets. There is, however, a need for abalone research in South Africa to be diversified.

Owing to the variety of functions of the digestive gland, physiological constituents studied in this organ was too variable to be useful for the purposes of this project. It was concluded that digestive gland tissue were not practical to study for farm management practices. Muscle tissue yielded 0.65 – 1.72 $g \cdot kg^{-1}$ glucose, 11.04 – 88.35 $g \cdot kg^{-1}$ glycogen, ± 0.08 $g \cdot kg^{-1}$ haemolymph, 15.99 – 31.64 $g \cdot kg^{-1}$ lipids and 28.83 – 52.85 $g \cdot kg^{-1}$ proteins. On average, abalone lost ± 15% of their body mass during simulated export.

Season was the most important parameter in the regulation of physiological constituents, and in mass loss experienced during simulated export trials. Different feeding regimes had limited effect on physiological constituents and on mass loss. Animal size influenced mass loss, with small animals being more prone to mass loss than large animals, but did not have pronounced effects on physiological constituents. The results obtained for animals from different farms did not differ significantly. Correlations of physiological constituents with export mass loss indicated that muscle glucose was the only constituents with predictive powers in terms of predicting mass loss during export.

Physiological constituents, studied in the muscle tissue, are useful indicators of abalone condition in the aquaculture environment. The artificial feed currently employed by South African farmers is not producing optimal results, and the formulation could be improved to harness the full potential of an optimally balanced diet. The most important factors affecting mass loss during simulated export are animal size and season. By selecting larger animals for export, and by limiting exports during summer months, mass loss during simulated export can be significantly reduced, which will significantly reduce corresponding losses of revenue.

Opsomming

Die akwakultuur van die Suid-Afrikaanse perlemoen (*Haliotis midae*) is die mees winsgewende en vinnigste groeiende afdeling van die Suid-Afrikaanse marikultuur bedryf. Die industrie word gedryf deur 'n behoefte wat lewering by verre oorskry, en natuurlike hulpbronne wat op die rand van uitputting wankel. 'n Studie van die basiese fisiologiese komponente in perlemoen is geloods om inligting te versamel wat van waarde sal wees vir die bestuur van perlemoenplase in Suid-Afrika.

Vanaf 2003 is 'n intensiewe literatuurstudie onderneem om die beskikbaarheid van, en algemene neigings in, perlemoen navorsingsliteratuur te ondersoek. Vanaf 2003 tot 2005 is basiese fisiologiese komponente (glukose, glukogeen, proteïene en lipiede) bestudeer in perlemoen vanaf ses plase in Suid-Afrika. Die projek is in twee fases aangepak waarvan die eerste fase (2003 - 2004) verkennend van aard was. In Fase een is fisiologiese komponente ses-weekliks gemeet in die spierweefsel en spysverteringsklier van perlemoen in twee grootte-klasse (±50 mm en 70 mm skulp-lengte) en vier dieetbehandelings (natuurlik, kunsmatig en twee roterende diëte) vanaf een plaas. Aan die einde van Fase een is 'n enkele gesimuleerde lewendeuitvoerproef onderneem volgens die standaard protokol op die plaas. In Fase twee (2004 - 2005), is fisiologiese komponente seisoenaal bestudeer in die spierweefsel en hemolimf van perlemoen in twee grootte klasse (±50 mm en 70 mm skulp-lengte) en twee dieetbehandelings (natuurlike en kunsmatige diëte), vanaf vyf plase, waarvan twee onttrek het. Lewendeuitvoersimulasies in Fase twee is seisoenaal uitgevoer.

Suid-Afrika is een van die wêreldleiers in die publisering van perlemoen-navorsing. Die hoof-fokus van navorsing in Suid-Afrika is op die ontwikkeling en verbetering van kunsmatige voere, maar daar is 'n behoefte om perlemoen-navorsing uit te brei.

Weens die verskeidenheid funksies wat deur die verteringsklier verrig word, was die variasie van fisiologiese komponente in hierdie orgaan te groot om van nut te wees in hierdie studie. Die gevolgtrekking is gemaak dat die bestudering van die verteringsklier nie prakties is vir plaasbestuurdoeleindes nie. Spierweefsel se samestelling was 0.65 – 1.72 $g \cdot kg^{-1}$ glukose, 11.04 – 88.35 $g \cdot kg^{-1}$ glikogeen, 15.99 – 31.64 $g \cdot kg^{-1}$ lipiede en 28.83 – 52.85 $g \cdot kg^{-1}$ proteïene. Die gemiddelde massa-verlies tydens gesimuleerde uitvoere was ongeveer 15% van die liggaamsmassa in 36 uur.

Seisoen is die enkele faktor wat belangrikste rol gespeel het in die variasie van fisiologiese komponente en massaverlies tydens gesimuleerde uitvoer. Verskillende dieetbehandelings het

beperkte invloed gehad op beide fisiologiese komponente en massaverlies. Die grootte van die diere het massaverlies beïnvloed - soos verwag was klein diere meer vatbaar vir massaverlies as groot diere. Diergrootte het nie ‘n merkbare invloed gehad op fisiologiese komponente nie. Die resultate van verkillende plase het nie van mekaar verskil nie. Korrelasies van fisiologiese komponente met uitvoer-massaverlies het getoon dat spier-glukose die enigste komponent was wat massa verlies tydens uitvoer kon voorspel.

Fisiologiese komponente wat in die spierweefsel bestudeer word is nuttige aanduiders van die kondisie van perlemoen in die akwakultuur-omgewing. Die kunsmatige voer wat tans gebruik word lewer nie optimale resultate nie, en die formulering daarvan kan verbeter word. Die belangrikste faktore wat massa-verlies tydens uitvoer bepaal is diergrootte en seisoen. Deur groter diere te kies vir uitvoer, en uitvoere tot die winter te beperk, kan massaverlies tydens uitvoere betekenisvol verminder word, wat die ooreenstemmende verlies aan inkomste sal verminder.

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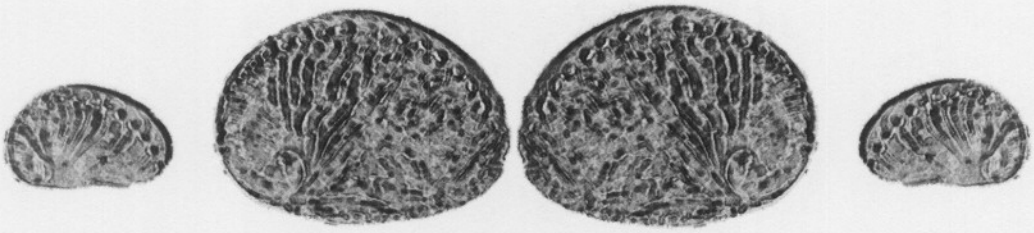
Chapter 1:

Introduction



1.1. Abalone biology

Abalone are marine molluscs that belong to the largest and most diverse class, the Gastropoda. (Purchon, 1968; Hickman *et al.*, 1997; Leighton, 2000; Pechenik, 2000). There are more than 70 extant haliotid species, all of which are marine (Stevens, 2003). Abalone are found throughout the majority of the world's temperate oceans, living on firm substrates of rock and sometimes coral and are browsers or grazers of algae (Purchon, 1968; Branch *et al.*, 1994; Hickman *et al.*, 1997; Leighton, 2000; Stevens, 2003).

Abalone have an ear-shaped shell that is coiled starting from the apex and spiralling around the central axis or columella (Hickman *et al.*, 1997). The columellar muscle plays an important role in most major body movements. The visceral mass sits atop a muscular foot, and is protected by the shell (Pechenik, 2000). Respiration is carried out by two ctenidia (gills) located in the mantle cavity of the abalone (Hickman *et al.*, 1997).

Abalone feed mainly on a diversity of macro-algae, and their digestive system is anatomically and biochemically suited to feed on such food. The rhipidoglossate radula of *Haliotis spp.* is well structured to crop microflora, and in adults to reduce macro-algae to pieces that can be consumed easily. The elongated intestine is typical of herbivorous species. Their digestive system uses enzymes (produced abundantly, chiefly by the digestive gland) to break down structural polysaccharides of algae and to hydrolyse algal proteins (Leighton, 2000; Tarr, 2000a; García-Carreño *et al.*, 2003; Viera *et al.*, 2005). Polysaccharides comprise the bulk of the organic matter in marine macro-algae and consequently the nutrition of abalone is strongly carbohydrate based (Leighton, 2000; Picos-García *et al.*, 2000; Gómez-Montes *et al.*, 2003). Abalone do not have a need for proteins and lipids in bulk, but rather for the correct balance of certain essential amino and fatty acids (Mai *et al.*, 1995a; Mai *et al.*, 1995b; King *et al.*, 1996; Bautista-Teruel & Millamena, 1999; Leighton, 2000; Shipton & Britz, 2001; Bautista-Teruel *et al.*, 2003; Durazo-Beltran *et al.*, 2004). An understanding of nutritional requirements of abalone is important to optimise delivery of nutrients. Optimum growth is obtained through proper balance of dietary nutrients and fulfilment of requirements of essential nutrients and energy (Tahil & Juinio-Menez, 1999; García-Carreño *et al.*, 2003; Gómez-Montes *et al.*, 2003).

Abalone exhibit the most primitive form of reproduction where fertile eggs and sperm are broadcast and the embryos develop into free-living trochophore larvae (Purchon, 1968; Branch *et al.*, 1994; Leighton, 2000; Tarr, 2000b). The gravid individual is characterised by an enlarged gonad. Gonad tissue surrounds the digestive gland and extends, when ripe, from the posterior visceral curvature, overlying the stomach, fully two thirds of the body length along the right side of the columellar muscle (Leighton, 2000). The position of the digestive gland relative to gonads has been indicated to complicate studies of the digestive gland (Carefoot *et al.*, 2000). Investment in the relatively large gonad mass has energetic implications for the animals, which may have significant implications in an aquacultural environment (Leighton, 2000; Tarr, 2000a).

1.2. South African abalone

Five species of abalone occur only along the South African Coast: *Haliotis midae*, *H. speciosa*, *H. queketti*, *H. spadicea* and *H. parva*. *H. speciosa* and *H. queketti* are extremely rare species of abalone while *H. spadicea* and *H. parva* are small species, which are of no economical importance. The South African species associated with the word abalone, is *Haliotis midae*, which is also called the perlemoen (mother-of-pearl). *H. midae* takes eight to ten years to reach sexual maturity, and up to 13 years to reach the legal size of 114 mm shell breadth (138 mm shell length) at which they may be collected. This abalone species can reach sizes in excess of 190 mm (Branch *et al.*, 1994; Tarr, 2000a; Tarr, 2000b; Troell *et al.*, 2006).

1.3. Economical importance of abalone

The importance of abalone to man extends back to prehistoric time. Both meat and shell were important as protein and the manufacture of ornaments (McBride & Conte, 1996; Leighton, 2000; Tarr, 2000a). The foot of the abalone is highly sought-after seafood, commanding high prices, particularly in the far Eastern countries. It is reputed to have aphrodisiac properties, but is more commonly prepared for special or ceremonial occasions (Tarr, 2000b; Sales, 2004). More than 20 species of abalone are classified as commercially important, most of which are relatively large species (Jarayabhand & Paphavasit, 1996; Sales, 2004; Fishtech, 2006). The growing importance of the abalone industry is demonstrated by the fact that since the early 1990s, six international symposia on abalone have been held in various countries. The last of these symposia was held in Chile (2006), and was attended by delegates from more than 15 countries.

The industry is driven by a demand, far exceeding the supply. In 1999 demand exceeded supply by about 6 000 – 7 000 metric tons, and in 2004 supply was still about 5 000 mt lower than the demand for about 29 000 tons of abalone (Gordon & Cook, 2003; Roberts, 2005). The big demand has lead to increased prices for quality abalone products resulting in an economic environment in which abalone culture became an attractive financial investment (Freeman, 2001).

Canned abalone meat is sold for $900 to $1 200/case and live abalone for $40 to $50/kg, with increases in price resulting from the diminishing supply of abalone products (Du Plessis, 2006).

1.3.1 Abalone fisheries

Abalone fisheries have seemingly been around forever, having value both in terms of the meat they supply, as well as the ornamental value of shells as a whole, or transformed into ornaments. Evidence of abalone fishery activities, dating back 125 000 years has been found on the African continent (Tarr, 2000a). During the 1850s, Chinese Americans started a fishery in California that targeted intertidal green and black abalones (Haaker *et al.*, 2001). Countries with large abalone fisheries include Japan, Australia, New Zealand, South Africa, Korea, Taiwan and China. The abalone world of the 1970s was one with minimal regulations of the fisheries sector, little illegal catch and a cultured "market size" industry measuring production in kilograms, not tons (Gordon & Cook, 2003).

All of the major aquacultural producers of abalone have shown a significant decrease in fisheries catches in the last two decades (Britz, 1991; Gordon & Cook, 2003; Fishtech, 2006). Factors that have contributed to the decrease in fisheries production include:
(1) over-fishing (including poaching), (2) diseases (such as abalone withering syndrome), (3) environmental changes (such as habitat loss and the influence of other species) and (4) lack of effective management of fisheries at sustainable levels (Britz, 1991; Leighton, 2000; Tarr, 2000a; Tarr, 2000b; Gordon & Cook, 2003). In the light of this, two countries (USA and South Africa) have either closed, or considered closing abalone fisheries (Gordon & Cook, 2003).

The abalone industry in South Africa has been reliant on a single commercially exploited species, *Haliotis midae*. Commercial abalone fisheries in South Africa started in 1949, and by the year 2000 there were 47 licensed right holders (Tarr, 2000a; Tarr, 2000b; Du Plessis, 2006). Although the abalone fishery is among the smallest sea-fisheries in South Africa with respect to yield, it is the most lucrative in terms of unit value (Hauck & Sweijd, 1999). South African abalone is exported frozen, canned or live, mainly to the East. Since 1970, the previously unregulated commercial fishery has been limited by the introduction of quota systems. By 2001, commercial catch has been limited to 371 tons in terms of the Total Allowable Catch (TAC). Recreational fisheries have also seen the introduction of quota systems, with regulations becoming increasingly restrictive over the last decade, with recreational fisheries being closed in 2003 (Britz, 1991; Tarr, 2000a; Tarr, 2000b; Du Plessis, 2006). It is now believed that the wild population of South African abalone is verging on the brink of extinction (Steinberg, 2005).

1.3.2 Illicit abalone trade

Poaching is one of the most serious threats to natural abalone populations in many of the major abalone producing countries worldwide. Despite stringent laws, penalties and law enforcement attempts, poaching is continuing at unprecedented rates (Britz, 1991; Tarr, 2000b; Gordon & Cook, 2003; Steinberg, 2005). Although the USA closed its fishery in 1997, illegal catch is reported to be continuing at around 120 metric tons per year. This proliferation in illegal catch is not only threatening to irreparably impair the natural resources, but also applies an important downward pressure on abalone prices (Gordon & Cook, 2003).

Although poaching in South Africa has remained containable for the first two decades after the introduction of quota systems in 1970, there has been an exponential increase in illegal catch from the early 1990s totalling more than an estimated 500 metric tons per year (Tarr, 2000b; Steinberg, 2005). By 2002 more abalone was being confiscated by law enforcement authorities annually, than was harvested by the commercial fishery. Important factors that contributed to the proliferation of the illegal harvesting in South Africa include: (1) ease of capture, (2) high value, (3) weakening of the Rand that began in the early 1990s and continued steadily for the following decade, (4) the pre-existence of a highly efficient Chinese organised-crime network and (5) problems with effective border control (Hauck & Sweijd, 1999; Steinberg, 2005). The estimated poaching tonnage for 2004/2005 was 1185 tons compared to the commercial TAC of 237 tons (Du Plessis, 2006).

1.3.3. Abalone aquaculture

Japanese researchers did the pioneering work for the development of techniques of cultivation of abalones, a decade before the culture of abalone on commercial scale commenced. Although they initially cultivated abalone for the purposes of restocking natural habitats, they paved the way for the rest of the abalone producing community (Britz, 1991; Leighton, 2000). Similar research conducted by the USA and Taiwan led to the development of intensive culture in shore based systems, and by 1989, aquaculture was contributing 5% of the global abalone supply. The development of abalone aquaculture technology internationally is largely driven by private enterprise, although state sponsorship played an important role (Britz, 1991; Leighton, 2000). Cultivating abalone on a commercial scale is a capital intensive venture, with high cost of initial set-up and running costs, and a slow return on investment (Leighton, 2000; Tarr, 2000b; Sales, 2004). Abalone are cultured in man-made shore-based systems at high stocking densities, and are fed natural and/or formulated artificial feeds (Sales, 2004).

The aquaculture industry is becoming a reliable, year-round source of high quality abalone products. The abalone market is known for both high demand and high prices. Japan (the main consumer of live abalone) and China (the main consumer of canned abalone) purchase around 80% of the world abalone supply. In 1997 Hong Kong was regarded as one of the world's largest importers of abalone with total imports reaching over 2.3 million kg worth US$ 135 million (Freeman, 2001; Leighton, 2000). The phenomenal growth of global cultured-abalone production is evident when comparing the 8 696 metric tons for 2002 with just 689 metric tons 15 years earlier. Abalone aquaculture, even at its fast growing pace, will take many years to fill the declining supply of the world's commercial abalone fisheries (Gordon & Cook, 2003). Advancement of abalone aquaculture is being sought with focus on acceleration of growth, improvement of meat quality, controlled nutrition and genetic engineering (Leighton, 2000).

In South Africa, initial reluctance to invest in abalone aquaculture resulted from factors such as the low growth rate, especially to legal size (up to 13 years), and provision of sufficient quantities of kelp (*Ecklonia maxima*) for full-scale production. The potential for culturing abalone in South Africa became of interest after 1981 when researchers demonstrated that the South African abalone, *H. midae,* can be spawned in captivity (Cook, 1991; Sales & Britz, 2001). Aquaculture of the South African abalone (*H. midae*) developed through the 1990s, in parallel with the emergence of the abalone aquaculture industry in Asian countries, the USA, New Zealand, Australia and Chile. South African culture technology was based on a combination of technology transfer and local innovation by industry in partnership with research institutions. By 2001, twelve abalone farms were established, with an estimated capital investment of more than US$ 12 million, and production of 500 - 800 tons (Sales & Britz, 2001). In 2006, 90% of the operational farms have their own hatcheries. The farms rely exclusively on pump-ashore, land-based systems, with two farms employing re-circulation systems, and one farm using a partial re-circulation system. Some of the top farms have produced 100 tons or more of abalone per annum, while smaller farms aim for 50 – 60 tons per annum. The total production for 2005 was about 700 tons, and this is projected to rise to 1 200 tons by 2010 (Du Plessis, 2006).

1.4. Project Rationale

The farming of the South African Abalone (*Haliotis midae*) on commercial scale in pump-ashore on-growing systems is a highly successful and lucrative industry. The success of this industry is based on decreased fisheries capture, dwindling natural stocks and concomitant increased international demand. Between 1989 and 2005, there has been a 33% decline in abalone fisheries worldwide (From 15 000 mt to 10 000 mt). In the same period, there was a 1000% increase in abalone aquaculture (from 900 mt to 9 000 mt). Demand in that period increased by about 141%

(from about 18 000 mt to 24 000 mt), thus establishing an unfulfilled demand of about 5 000 mt in 2005 (Roberts, 2005).

Because technologies in South Africa are still relatively young, and under development, the technologies applied to the culture of *H. midae*, are generally adapted with some success from those developed for other *Haliotis* species globally (Britz, 1991; Carter, 1991). Increasing competition in this market sector is placing pressure on the local industry to become more competitive in terms of lowering production cost and increasing product quality and yield (Mouton *et al.*, 2003).

Thus far, technology innovations in the field of abalone aquaculture have in most part been funded by private enterprise with little help from the government. The Small, Medium Enterprise Development Programme (SMEDP) of grants to aquaculture started in 2003, but ground to a halt due to administrative problems. More recently, the government "Frontier Programme" awarded R5 million for research and development in 2006/2007. The Department of Trade and Industry (DTI) and the Department of Science and Technology (DST) both allocated funds on a 1:1 or 1:2 basis with industry. Other governmental initiatives and support of aquaculture are also on the rise. An animal Health Management Programme is run by Dr. Anna Mouton, and most farms take part in this programme, which has played a role in stock improvement and management of parasites and other health related aspects (Du Plessis, 2006).

The effects of animal health, growth stage and physiological condition on production quality and yield have long been recognised in conventional animal husbandry systems. Abalone is no different. Experience over the past few years has clearly shown that the condition of the abalone affects the final product, both during live transport and canning. Problems have been experienced with mortalities, excessive mass loss during live exports, lowered yields and inferior flesh quality on canning. This impacts on individual producers and is detrimental to the market for South African abalone as a whole. There is a lack in understanding of the environmental and management factors that impact on animal condition, making it difficult to prevent or mitigate losses (Mouton *et al.*, 2003).

During live exports these aquatic marine animals are packed in plastic bags that are filled with 100% oxygen and humidified with sponges containing sea water. These bags of abalone are transported in polystyrene containers with ice packs to maintain low temperatures (Sales & Britz, 2001). During the 36 to 40 hours of aerial exposure animals lose on average 15% of their body mass. As farmers are paid on landed mass, this corresponds to a 15% loss of foreign income, which, based on market values of the product, amounts to millions of Rand per year (Vosloo & Vosloo, 2006).

Until now, much of the research done on abalone has concentrated on providing essential needs for culture such as captive breeding, larval rearing and settlement, system design and an elementary artificial diet (Laas & Vosloo, 2006) (see Paper I). For abalone culture to become a sustainable and economically viable mariculture activity, it is necessary to shift the focus to aspects of intensive animal management such as yields and product quality (Mouton *et al.*, 2003).

Physiology studies the functions of living organisms. It is not only a description of function; it also asks why and how and provides an understanding of how organisms function in their environment. Understanding how animals fulfil these functions requires detailed knowledge of the molecular interactions that set the stage for cellular processes. Examining how an animal copes with its environment often tends to show what is good for the animal. Animal physiology studies have yielded knowledge central to many commercial and agricultural advanced during the last few decades allowing farmers to improve the yield and quality of their products (Randall *et al.*, 1997; Schmidt-Nielsen, 1998).

The physiological condition of the animal could be used to make management decisions in order to identify poor quality animals before they are committed to processing and more importantly to predict quality and yield. This will allow abalone farmers to manage pro-actively. At present, producers only identify problems at the very end of the production cycle in terms of mass loss and mortalities during live export, or when poor yields are obtained on canning (Mouton *et al.*, 2003).

1.5. Research aims and objectives

Key Questions:

1. What literature is generally available to South African researchers?
2. Are there any clear research trends in the available literature, and how do research trends in South Africa compare to global research trends?
3. Can physiological parameters be prioritised in order of their effect on production parameters?
4. Can physiological condition of abalone be used to predict export performance during live export?

Objectives:

1. To study past and present trends in abalone research by analysis of available literature.
2. To identify and prioritise some physiological parameters of abalone that correlate with production parameters
3. To determine the optimal feeding regime for abalone in aquaculture systems.
4. To investigate the effects of animal size, diet, season and water temperature on soft tissue composition.
5. To define abalone condition in a way that is meaningful in the production environment.
6. To predict export performance during live export in abalone aquaculture systems from physiological condition parameters

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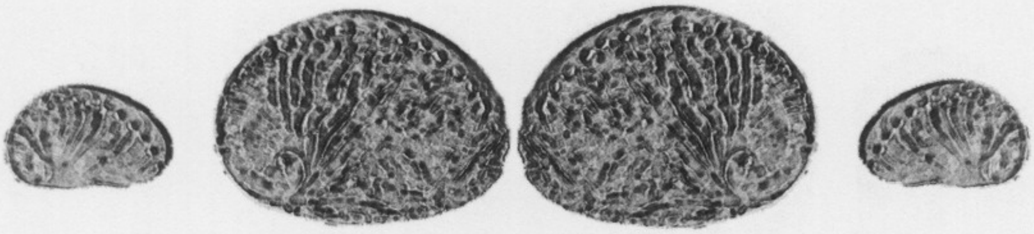
Chapter 2:

Materials and Methods



2.1. Analysis of literature

All literature relevant to abalone research were collected from a variety of sources mainly using Internet-based searches and library resources. Search engines used included: Science Direct, Scopus, Scirus, EBSCO Host, Waterlit, Biblioline, ISI Web of Knowledge, Google Scholar and SA Cat. Keywords used (either alone or in different combinations) in the searches included Abalone, ormer, paua, *Haliotis*, mother-of-pearl, perlemoen, fisheries, aquaculture, and mariculture. Literature were sorted and stored in a database, alphabetically, according to the last name of the first author.

Peer-reviewed, published research publications of which full text copies were readily available to researchers in South Africa were selected for inclusion in an article on internationally published abalone research. The publications used for the study included papers on all subjects relating to abalone research for the period from 1986 to 2005. For the purposes of this article, chapters in books, technical papers and research reports were omitted from the study. Articles were analysed to determine past and current trends in abalone research internationally and locally.

2.2. General sampling methods

The project was conducted in two phases, on six farms between Paternoster on the Cape West Coast and Dangerpoint (Gansbaai) on the Cape South-West Coast (Figure 1).

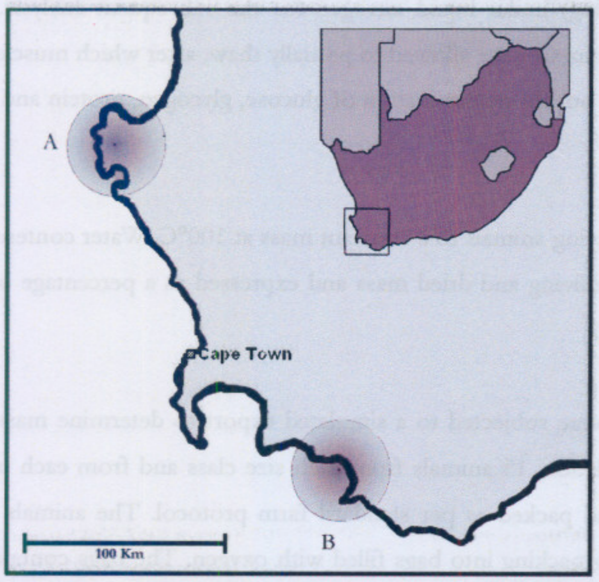


Figure 1: Map of South Africa, with enlargement indicating the approximate localities of the farms studied on (A) the Cape West Coast and (B) the Cape South-West Coast.

Phase one was an exploratory phase conducted at Aquafarm in Hermanus. The selected criteria for this phase of the project were laid down in conjunction with all members at an AFASA research meeting held in Hermanus. Two size classes were selected, 40 – 50 g and 50 – 60 g and a subset of animals from each size class was committed to four different feeding types. The feeding types set out at the research meeting were as follows: (1) kelp only (K), (2) Abfeed™ only (A), (3) kelp and Abfeed™ on a three weekly rotational basis (KAT: two weeks kelp; one week Abfeed™), (4) kelp and Abfeed™ on a weekly rotational basis (KAW: four days kelp; three days Abfeed™).

Animals dedicated to the project were graded in February 2003, and placed into 24 baskets (12 baskets large animals and 12 baskets small animals) at a density of 30 animals per basket. These baskets were then placed in position A (position closest to the inlet) of 24 tanks in the farm system.

Eight samplings were conducted in six-week intervals from April 2003 to the end of January 2004. During each sampling growth was determined in six animals. A further six animals from each size class and from all four feeding groups were collected, weighed and measured. The animals were subsequently snap-frozen whole in liquid nitrogen to preserve physiological condition of the animals for future analysis of physiological parameters. Another sub-set of six animals were collected, weighed and snap frozen for water content analysis.

All samples were transported to the North-West University, Potchefstroom Campus, (Laboratory for Ecophysiology and Biomonitoring) under liquid nitrogen for the subsequent analysis. For physiological purposes, the frozen animals were allowed to partially thaw, after which muscle and digestive gland tissue were dissected out for determination of glucose, glycogen, protein and lipid content.

Water content was determined by drying animals to a constant mass at 100°C. Water content was calculated as the difference between living and dried mass and expressed as a percentage of the living mass (Vosloo & Vosloo, 2006).

At the end of Phase one, animals were subjected to a simulated export to determine mass loss during live transport. For this simulation, 15 animals from each size class and from each of the four feeding types, were purged and packed as per standard farm protocol. The animals were weighed and measured before being packing into bags filled with oxygen. The bags contained a wet sponge to maintain a relatively high humidity, and ice packs were added to the containers to keep a constant low temperature for the transportation process. Animals were then air-freighted in sealed polystyrene transport boxes to Johannesburg International Airport, and were transported from there by car to Potchefstroom. After 36 hours (estimated time of normal live export) the containers were opened, and the animals were re-weighed to determine mass loss experienced during transport.

Phase two was a follow-on phase, based on the results from Phase one. On an AFASA meeting in September 2004, five farms were selected to participate in Phase two. The farms were selected based on (1) the willingness of the farms' managers to participate in the project and (2) geographical positioning of the farms. The size of animals to be used was fixed in two size cases, 40 – 50 g and 70 – 80 g. It was subsequently decided that two feeding regimes will be used, kelp only and Abfeed™ only. As the project progressed, however, some farms were forced to deviate from the original plan due to financial and other external difficulties.

Sampling was conducted seasonally on the five farms during the months of September 2004 (spring), January 2005 (summer), April 2005 (autumn) and July 2005 (winter). During each sampling, eight animals from each size class and each feeding group were collected, weighed and measured. Haemolymph samples were extracted from the pallial sinus of the foot muscle and snap frozen in liquid nitrogen. Part of the foot muscle was subsequently dissected out, and the dissected tissue was snap-frozen in liquid nitrogen to preserve physiological integrity. Physical parameters (pH, dissolved oxygen, conductivity, ammonia concentration and temperature) were measured at the inflow, middle and outflow of each tank that animals were collected from.

Tissue samples were transported back to Potchefstroom under liquid nitrogen where they were stored at -20°C for subsequent analysis. Within a week after each sampling, 15 animals from each size class from the two feeding treatments were committed to a live export trial, as per Phase one. Farmers committed to completing a farm questionnaire (Appendix 1) for each sampling, and these questionnaires were sent electronically

Tissue samples were analysed for free glucose, glycogen, total protein and total lipids. The haemolymph samples were analysed for free glucose concentration.

2.3. Tissue analysis methods

2.3.1. Equipment used

All tissue samples for laboratory analysis were weighed, accurately to three decimals, on a Scaltec balance. Tissues were homogenised using a Diax 900 homogeniser with the 10G tip (Heidolph). Spectrophotometric analyses were conducted on 96 microwell plates using the Powerwave X microwell reader and KC4 software.

2.3.2. Glucose

For tissue glucose determination approximately 0.4 g (weighed accurately to three decimals) of the deep-frozen tissue sample was cut off, and transferred to an 8 mL vial. A 5 x volume by weight of perchloric acid (PCA) was added, and the tissue/PCA was then homogenized on ice to a fine suspension. One aliquot was removed for glycogen determination, and the rest of the homogenate was centrifuged at maximum speed for 15 minutes for free glucose determination.

One aliquot of the supernatant were used for enzymatic glucose determination on microwell plates using a GOD-PAP kit from Roche. Absorbance was read at 546 nm (see Appendix 2). Glucose concentrations were calculated, taking into account tissue water content of 63% (Vosloo, 2003). The rest of the supernatant was stored at -20ºC for future use.

Haemolymph glucose determination was carried out by removing 4 µl of frozen, centrifuged blood, and subjecting it to glucose determination following the same protocol used for tissue glucose determination (Appendix 2).

2.3.3. Glycogen

The homogenates were neutralised with $KHCO_3$ ($1\ mol{\cdot}L^{-1}$) and subsequently digested with amyloglucosidase to liberate glucose from glycogen. Glycogen digestions were carried out by means of shaking in a water bath at 40ºC for two hours. The digestions were terminated by the addition of PCA, and the samples were centrifuged at 9 000 rpm for 15 minutes (Appendix 3). Total glucose content after digestion was measured using the protocol for free glucose (Appendix 2). Final glycogen content was calculated by subtracting the free glucose content of the sample from the total glucose content after digestion, and was expressed as mmol glucose from glycogen per gram wet tissue mass (Keppler & Decker, 1983).

2.3.4. Proteins

For the quantification of total tissue proteins approximately 0.4 g (weighed accurately to three decimals) of deep-frozen tissue was cut of and transferred to an 8 mL vial. The tissue was subsequently homogenised in a 5 x volume by weight of a protein buffer (Buffer C). After homogenisation to a fine suspension, the samples were transferred to a microtube and centrifuged at 9 000 rpm for 15 minutes. The samples were then stored at -80ºC until they were analysed (Van Heerden *et al.*, 2004). Protein quantifications were conducted on microwell plates using a protein kit form Pierce (see Appendix 4 for a detailed protocol). Absorbance was read at 562 nm.

2.3.5. Lipids

For the quantification of total lipids, approximately 0.2 g (weighed accurately to three decimals) of tissue was removed to a glass test-tube. Lipid quantification was carried out using a modification of the method described by Folch (1957). The tissue was homogenised in 4 mL of a 2:1 Chloroform:Methanol mixture, and then allowed to stand, sealed, for 12 hours at room temperature in a fume hood to ensure the complete extraction of lipids. Phase separation was induced by the addition of 400 μL of a 0.73% NaCl solution, and allowing the process to complete for 12 hours. The top phase containing the non-lipid components was then discarded and 1 mL of the bottom phase, containing the lipids, was transferred to a pre-weighed foil pan. The foil pan was then placed in a fume hood, and 12 hours was allowed for the evaporation of the chloroform:methanol solution, leaving the lipids on the pan. The pan was then re-weighed and lipid content calculated, taking the dilution factor into account (see Appendix 5 for detailed protocol).

2.4. Statistical analysis

Statistical data-analysis were conducted in consultation with the Statistical Consultation Services of the North-West University, using the STATISTICA (Statsoft Inc, 2005), and SAS (SAS Institute, 2003) software packages. Factorial ANOVA's were conducted, and data were tested for homogeneity by means of Levene's test. In the case of non-conformance to homogeneity, data were transformed with logarithmic and/or Box-Cox transformations. Subsequently Tukey's HSD tests were performed to test for significant differences ($p<0.05$) between cases. Values are given as mean ± SEM. Effect sizes are reported as partial η^2 (partial-eta squared) values, which is the proportion of the variability in the dependent variables that is explained by the effect. Multiple regression analysis was performed using SAS software using the R-SQUARE procedure. The multiple regression results were reported in terms of the adjusted-R^2 values, as it measures the goodness of fit while taking into account the number of predictors in the model.

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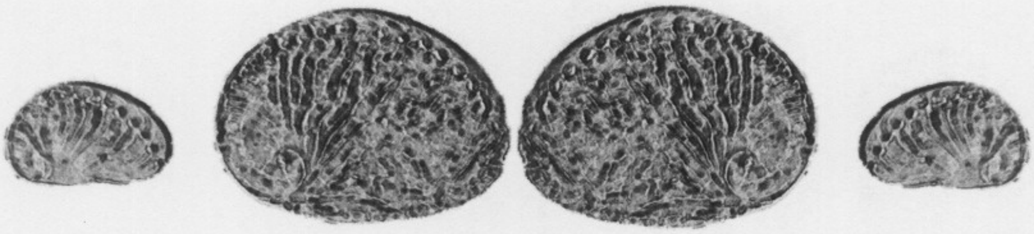
Chapter 3:

Original papers



3.1. Paper I:

Internationally Published Abalone Research: National and International trends.

Submitted to African Journal of Aquatic Science, July 2006.

Faculty requirements:

Letter of consent (Appendix 6)

Instructions to authors (Appendix 7)

A full bibliography is presented in Appendix 8

3.1.1. Original Paper

Internationally Published Abalone Research: National and International trends

André Laas[1] and André Vosloo

[1]Corresponding author:

School for Environmental Sciences and Development; Zoology

North-West University (Potchefstroom campus)

Private Bag x6001

Potchefstroom

2520

E-mail: 11777915@nwu.ac.za

Cell phone: 083 661 9421

Abstract

In this paper we attempted to give an overview of journal articles on abalone research available to South African researchers. A total of 354 full text papers were included. The papers originate from 24 countries and include research on 18 species of *Haliotis* in 11 research fields. Results indicate that the United States, Australia and South Africa published the most papers on abalone. *Haliotis rufescens*, *Haliotis midae* and *Haliotis discus* were the most prominent species on which research was focussed. The best covered research fields were physiology & biochemistry, feeding & nutrition and genetics. In the last decade there has been an increase in the number of papers published, and in the number of contributing countries. There has also been a notable increase in the number of species researched, as well as in the number of research fields. There have been some shifts in the focus of the research, indicating growing importance of aquaculture, and the presentation of optimum foods to animals selected for best traits in the aquaculture environment.

1. Introduction

Abalone are large marine snails and are highly sought after seafood species, commanding high prices internationally (Tarr 2000b; Gallardo & Buen 2003, Huchette *et al.* 2003, O'omolo *et al.* 2003, Sales 2004). There are approximately 90 species of abalone worldwide, of which about 15 are harvested commercially (Sales 2004). There are five species of abalone endemic to South Africa, of which only one, *Haliotis midae*, is collected commercially (Tarr 2000a).

Abalone fisheries worldwide have declined by about 30% between 1989 and 1999. In 1999, the demand for abalone exceeded the supply by approximately 7 000 metric tons, and by 2004, there was still a shortfall of about 5 000 metric tons (Gordon & Cook 2001, Roberts, 2005).

In contrast to the decline in abalone fisheries, abalone culture increased by over 600% in the same period (Gordon & Cook 2001). Due to over-exploitation of natural abalone resources by fisheries in many countries, measures, including legislation and quota systems, have been introduced to enhance this resource and manage it sustainably (Cook 1998, Maliao *et al.* 2004, Moriyama & Kawauchi 2004).

Poaching, pollution, ecological changes and habitat degradation are further important factors putting strain on wild stock of abalone (Cook 1998, Hamm & Burton 2000).

The increased pressure on wild abalone resources and the decline in supply from fisheries have led to a movement towards abalone culture worldwide (Gallardo & Buen 2003, O'omolo *et al.* 2003, Davis & Carrington 2005).

The farming of South African Abalone (*Haliotis midae*) on commercial scale in land based on-growing systems is a highly profitable venture. Abalone farming is a relatively new activity in South Africa and all farm-produced abalone in South Africa are intended for the export market, selling the product at about 80 to 100 mm shell length (Cook 1998). Although *H. midae* has been harvested commercially in South Africa since 1949 (Tarr 2000b), abalone farming only started in the late 1980s when captured specimens were successfully spawned to produce spat (Cook 1998, Sales & Britz 2001). Because South African abalone farming initiatives are still relatively young, technologies have generally been adapted, with some success, on the basis of technology transfer and local innovation by industry in partnership with research institutions (Cook 1998, Sales & Britz 2001). Abalone farming in South Africa has significant economic importance in terms of generation of foreign revenue and job creation, but may also play an important ecological role by relieving the increasing pressures on dwindling wild populations of *H. midae* (Cook 1998, Sales & Britz 2001, Macey & Coyne 2005).

According to Fleming and Hone (1996b) there was a clear shift in the emphasis of research reports from the first International Symposium on Abalone Biology, Fisheries and Culture in 1989, which reflected the importance of establishing techniques for spawning, fertilisation, hatching, larval care and induction to settle, towards survival, growth of post-settlement larvae, environmental and nutritional needs and the development of artificial diets for on-growing at the second symposium in 1994.

Many farmers are constantly refining the design of their culture systems in search of an ideal system and husbandry management during the grow-out phase continues to generate questions or problems (Fleming & Hone 1996) that could be answered by research.

The aim of this paper is primarily to assess the type and amount of scientific, peer-reviewed data that have been published on abalone research in general and to look for shifts in the emphasis of research initiatives over time. The secondary objective was to determine where South Africa stands in relation to other countries with regard to abalone research and publication of abalone-related papers to put into perspective what types of research are currently being undertaken in South Africa, and where future research efforts should be focused.

2. Methods

Publications on abalone research were collected from a variety of sources mainly using Internet-based searches and library resources. Publications that could not be obtained locally were ordered through inter-library loans, or through correspondence with authors and/or institutions. Only peer-reviewed, published research publications of which full text copies were readily available to researchers in South Africa were selected. The publications used for the study included papers on all subjects relating to abalone research for the period from 1986 to 2005.

Chapters in books, technical papers and research reports were omitted from the study. Reasons for the exclusion of these sources included difficulty to acquire and the selective availability of these publications to researchers. The fact that many of these are not peer reviewed, contributed to their omission. Papers of which only the title and/or abstract could be acquired were also excluded. These were usually older papers published in journals that have since gone out of press, and of which copies could not be obtained.

Search engines used included Science Direct, Scopus, Scirus, EBSCO Host, Waterlit, Biblioline, ISI Web of Knowledge, Google Scholar and SA Cat. Keywords used (either alone or in different combinations) in the searches included: abalone, ormer, paua, *Haliotis*, mother-of-pearl, perlemoen, fisheries, aquaculture, and mariculture. Only publications related directly to abalone research were included in the study.

Publications collected from the searches were entered into an Excel database under the following headings: authors(s), year, title, journal, country, haliotid species and research field.

The country field was entered with the country of origin of the first author in the paper. Haliotid species were entered where the article concentrated on a certain species. Where articles incorporated research on several different species, no species name was indicated for that article. Sub-specific names were ignored for the purposes of this paper. To evaluate the direction of research, 11 research fields (Table 1) were identified and each paper was classified into one of these research fields. Articles fitting into more than one of the 11 fields identified were classified based on the main focus of the research conducted.

Table 1: Research fields chosen for describing various aspects of abalone research.

1	Diseases, parasitology & immunology
2	Ecology, biology & behaviour
3	Farm management, economics & markets
4	Feeding & nutrition
5	Fisheries
6	Genetics
7	Husbandry (hatchery & nursery)
8	Microbiology
9	Physiology & biochemistry
10	Shell properties
11	Toxicology

Data were entered into the database, which was sorted alphabetically according to the first authors. Once entered into the main list, data were analysed by sorting into different year groups, haliotid species, research fields, and country of origin. The results were summarised and presented graphically.

3. Results and Discussion

3.1. General Analysis

A total of 354 full-text articles published between 1986 an 2005 were analysed in order to gain some insight on what aspects of which *Haliotis* species were researched and by whom.

Twenty-four countries contributed to the papers, and the main contributors are summarised in Figure 1.

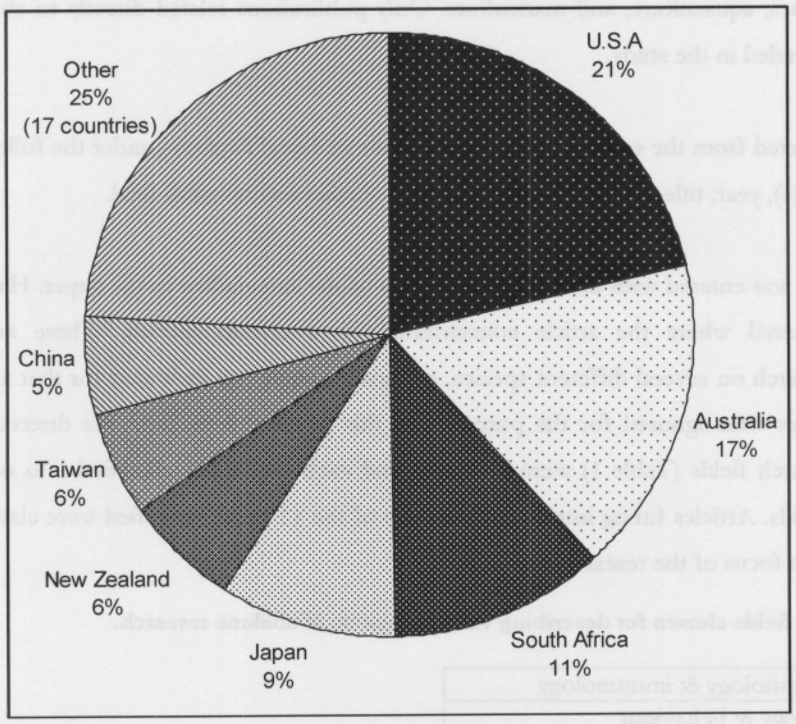


Figure 1: Relative contributions by different countries towards publishing of abalone research, expressed as % of the total number of publications (354) used in the study.

The United States of America played a leading role, but Australia and South Africa also made substantial contributions. In combination, these three countries published as many papers (49%) as the rest of the combined contributing countries.

Figure 2 indicates that the top three species (*Haliotis rufescens, H. midae and H. discus*) were the topic of >50% of the published research. This indicates the level of interest in the South African abalone, *H. midae*.

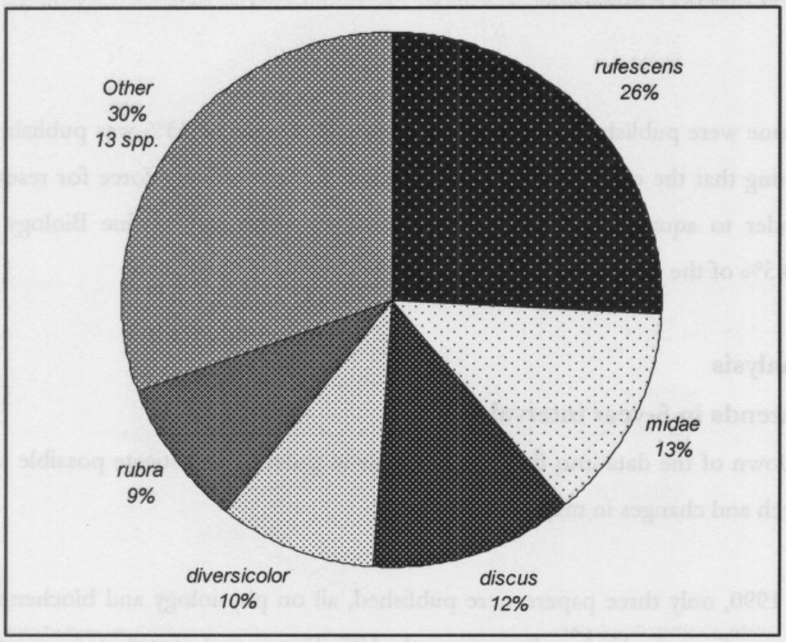


Figure 2: Species of *Haliotis* researched over the last 20 years, expressed as % of papers in which the species appeared relative to the total number of papers (354).

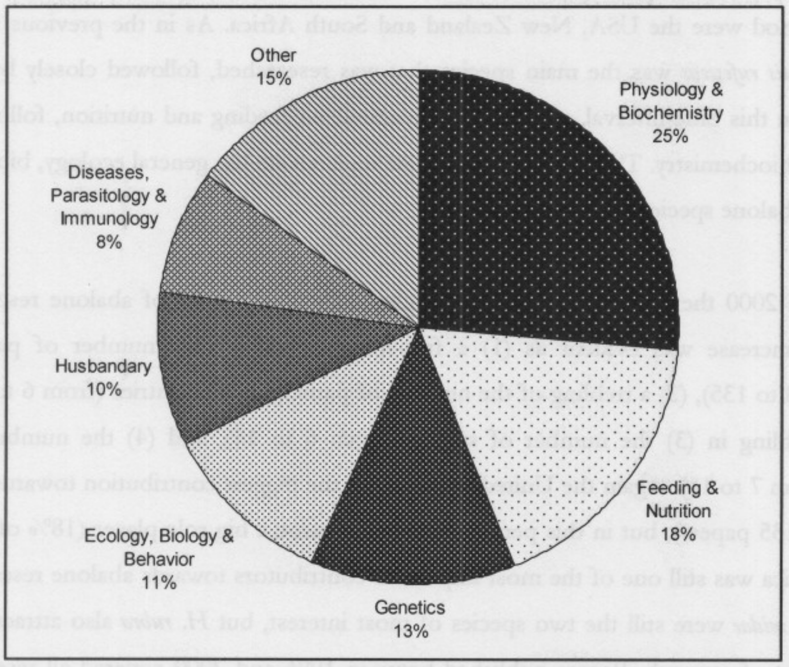


Figure 3: Contribution of research papers to selected research fields, expressed as % of publications relative to the total amount of papers (354).

Coverage of the different research fields is depicted in Figure 3, from which it is apparent that gaining an understanding of the physiology and biochemistry of the animals has attracted considerable attention. The substantial amount of research towards the feeding and nutrition of abalone indicates that there is a great need to find an optimum, balanced diet for the culture of abalone.

The papers on abalone were published in 94 different journals. A total of 33% was published in Aquaculture, indicating that the culturing of these animals is a major driving force for research. The closest contender to aquaculture was the Journal of Experimental Marine Biology and Ecology, in which 4.5% of the papers were published.

3.1. Temporal analysis

3.2.1. Temporal trends in 5-year intervals

Table 2 is a break down of the data into five-year intervals in order to investigate possible shifts in the area of research and changes in major role players.

Between 1986 and 1990, only three papers were published, all on physiology and biochemistry. Two of the papers were from the USA and one from the UK. This period contributed a mere 1% of the total number of papers for the last 20 years.

Between 1991 and 1995, 28 papers were published, by a total of six countries. The major role players for this period were the USA, New Zealand and South Africa. As in the previous five-year interval, *Haliotis rufescens* was the main species that was researched, followed closely by *H. midae, and H. iris.* In this time-interval, research concentrated on feeding and nutrition, followed by physiology and biochemistry. This time-span also saw research on the general ecology, biology and behaviour of abalone species.

Between 1996 and 2000 there was a considerable increase in the quantity of abalone research published. This increase was evident as (1) a five-fold increase in the number of papers published (from 28 to 135), (2) a trebling of the number of participating countries (from 6 to 17) and almost a doubling in (3) the number of species (from 6 to 14), and (4) the number of research fields (from 7 to 11). Again the United States made the biggest contribution towards the research (24% of 135 papers), but in this period Australia became a big role player (18% of 135 papers). South Africa was still one of the most important contributors towards abalone research. *H. rufescens and H. midae* were still the two species of most interest, but *H. rubra* also attracted a noteworthy amount of research. Papers published between 1996 and 2000 covered all research fields. The physiology and biochemistry as well as feeding and nutrition fields of research were still most prominent, but husbandry also becomes an important research field.

Between 2001 and 2005, there was a further increase in the number of papers (188) published. Even more countries made contributions during this time, and all research fields attracted attention. The number of species (14) remained the same as for the previous 5-year interval. This may illustrate that the most important species of abalone for aquaculture have already been identified. Australia and the United States made about even contributions in this period, and Japan now featured as the third most prolific role player. In this period South Africa published 21 of the 188 papers (11%), and thus was still an important contributor. *H. rufescens* remained the best-researched species (19%), and *H. discus* (17%) and *H. diversicolor* (14%) became prominent species reflecting a boom in research by the far eastern countries. Physiology and biochemistry remained at the top of research interest (21%), but genetics (16%) became a more prominent research field. This could be indicative of a movement towards selecting and breeding for the best traits of abalone for culture. The fact that feeding and nutrition (15%) were still major research topics might confirm that the research was becoming more focused towards breeding stronger animals on more specialised feeds.

Table 2: Relative contributions towards abalone research publications in four consecutive five-year intervals between 1986 and 2005.

1986 - 1990	#	**% of 20 yr Total**
# Papers	3	1%
# Countries	2	8%
# Species	2	11%
# Research Fields	1	9%
		% of 5 yr Total
USA	2	67%
United Kingdom	1	33%
Haliotis rufescens	2	67%
Haliotis corrugata	1	33%
Physiology and Biochemistry	3	100%

1991 - 1995	#	**% of 20 yr Total**
# Papers	28	8%
# Countries	6	25%
# Species	6	33%
# Research Fields	7	64%
		% of 5 yr Total
USA	9	32%
New Zealand	7	25%
South Africa	5	18%
Haliotis rufescens	6	30%
Haliotis midae	5	25%
Haliotis iris	5	25%
Feeding & Nutrition	8	29%
Physiology & Biochemistry	6	21%
Ecology, Biology & Behavior	4	14%

1996 - 2000	#	**% of 20 yr Total**
# Papers	135	38%
# Countries	17	71%
# Species	14	78%
# Research Fields	11	100%
		% of 5 yr Total
USA	32	24%
Australia	24	18%
South Africa	14	10%
Haliotis rufescens	40	34%
Haliotis midae	14	12%
Haliotis rubra	9	8%
Physiology & Biochemistry	44	33%
Feeding & Nutrition	25	19%
Husbandry	12	9%

2001 - 2005	#	**% of 20 yr Total**
# Papers	188	53%
# Countries	22	92%
# Species	14	78%
# Research Fields	11	100%
		% of 5 yr Total
Australia	34	18%
USA	33	18%
Japan	22	12%
Haliotis rufescens	31	19%
Haliotis discus	28	17%
Haliotis diversicolor	23	14%
Physiology & Biochemistry	40	21%
Genetics	31	16%
Feeding & Nutrition	29	15%

3.2.2. Temporal analysis of leading countries

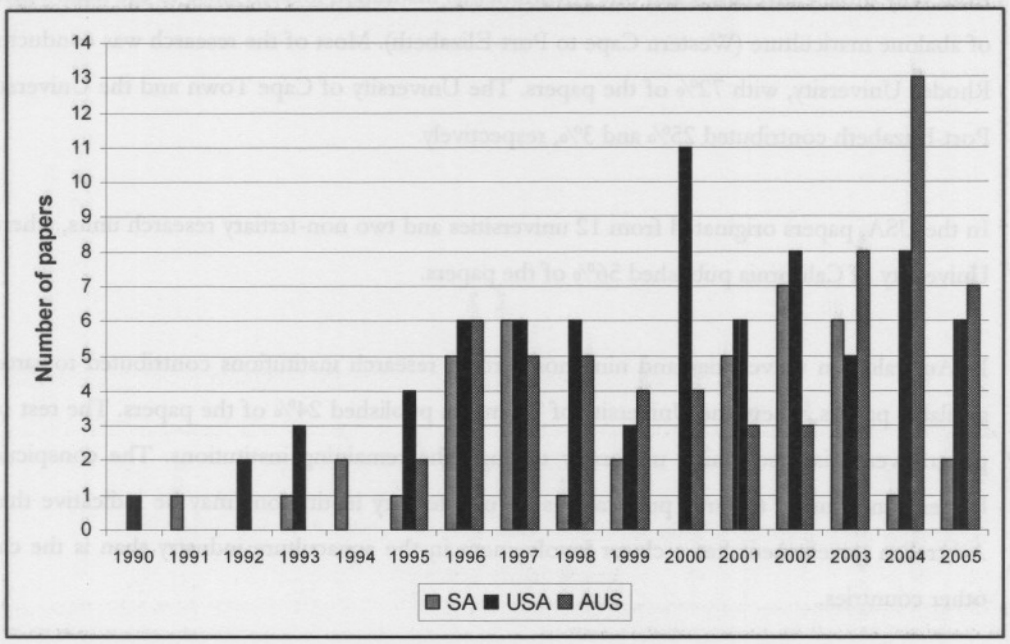


Figure 4: Contributions, in terms of number of papers, by the three leading countries that published abalone research between 1990 and 2005.

The USA was the most frequent publisher of the three leading countries (Fig. 4). South Africa compared well with the USA, but produced fewer papers. Australia only came into play from 1995, but has published constantly, and at high levels, since then. There was a prominent increase in the number of publications by the USA in 2000, and this can be attributed to the fact that articles were published in six research fields that year (Figure 5b). In 2004 there was a similar increase in the number of publications by Australia (Figure 6b) that can also be ascribed to the fact that research from six research fields was published.

It is interesting to note that relatively few papers were published prior to the 1990s. This does not mean that abalone research only started in the 1990s, but may be indicative of the source of funding for the research. If pioneering work was conducted and funded by the enterprise itself, fewer publications scientific journals would be expected. As the industry expanded, increased research by tertiary and governmental institutions would have facilitated increased publication of research in peer-reviewed journals.

When interpreting the data it is important to keep in mind that all of the research published by South African researchers was on the only commercially important species, *H. midae*. Publications by USA researchers covered six species of which *H. rufescens* was the most-researched species. Australian researchers published papers on seven species, of which *H. rubra* was most prominent.

Research in South Africa has been confined to the Cape region, with all publications coming from only three Universities and one governmental institution located close to the main centrums of abalone mariculture (Western Cape to Port-Elizabeth). Most of the research was conducted by Rhodes University, with 72% of the papers. The University of Cape Town and the University of Port-Elizabeth contributed 25% and 3%, respectively.

In the USA, papers originated from 12 universities and two non-tertiary research units, where the University of California published 56% of the papers.

In Australia ten universities and nine non-tertiary research institutions contributed towards the available papers, where the University of Tasmania published 24% of the papers. The rest of the papers were dispersed fairly uniformly through the remaining institutions. The conspicuously higher contribution towards publications by non-tertiary institutions may be indicative that the Australian government has a closer involvement in the aquaculture industry than is the case in other countries.

3.2.3. Analysis of contributions by top three countries

Figures 5 through to 7 depict a breakdown of publications, between 1990 and 2005, from South Africa, the USA and Australia. In this time-frame 176 papers originated from these three countries, contributing to about 50% of the total number of publications (354).

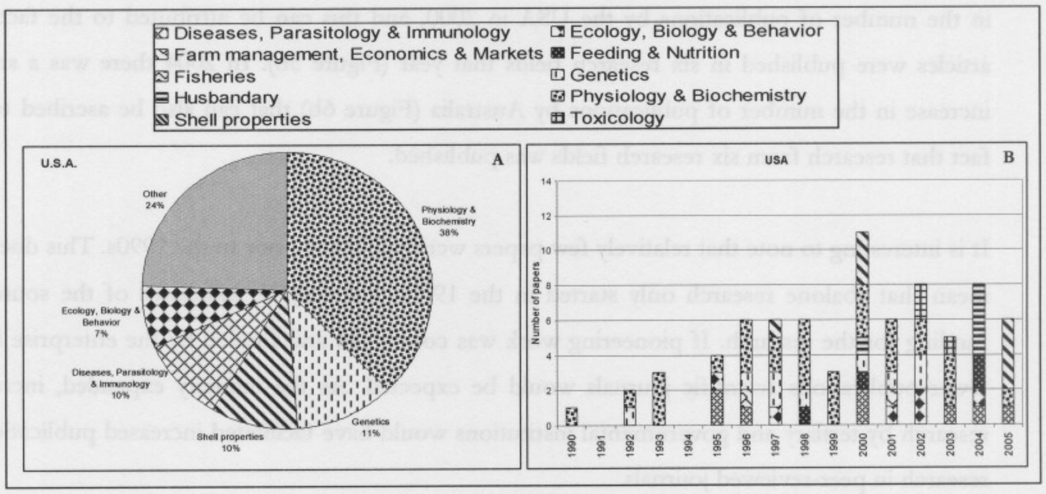


Figure 5: USA: (a) Relative contribution of papers towards the different research fields between 1990 and 2005 and (b) Temporal analysis of these contributions on a year to year basis.

The USA (Figure 5) published regularly and at relatively-high volumes since 1990, and about 43 % of the 176 papers published, came from USA researchers. Physiology and Biochemistry were represented by 40% of the 76 papers. Contributions towards the remaining research fields were distributed fairly even. It seems that the USA has already identified the abalone species best suited for their conditions, and mastered the fundamentals of abalone aquaculture. USA researchers are now focusing on the physiology and biochemistry of the animals, which may be indicative that they are searching for more cryptic possibilities to enhance the aquaculture of abalone. This may be confirmed by the fact that genetics are also playing a bigger role in American research.

The first Australian publications appeared in 1995 (Figure 6), and since then Australian researchers have published 60 papers at regular intervals and at relatively-high volumes. Genetics, at 21%, was the best-represented field in papers from Australia. There was a much more even distribution of contributions towards the different research fields by Australian researchers. Research on physiology and biochemistry has also received attention by Australian researchers, which compares well to what is happening in the USA.

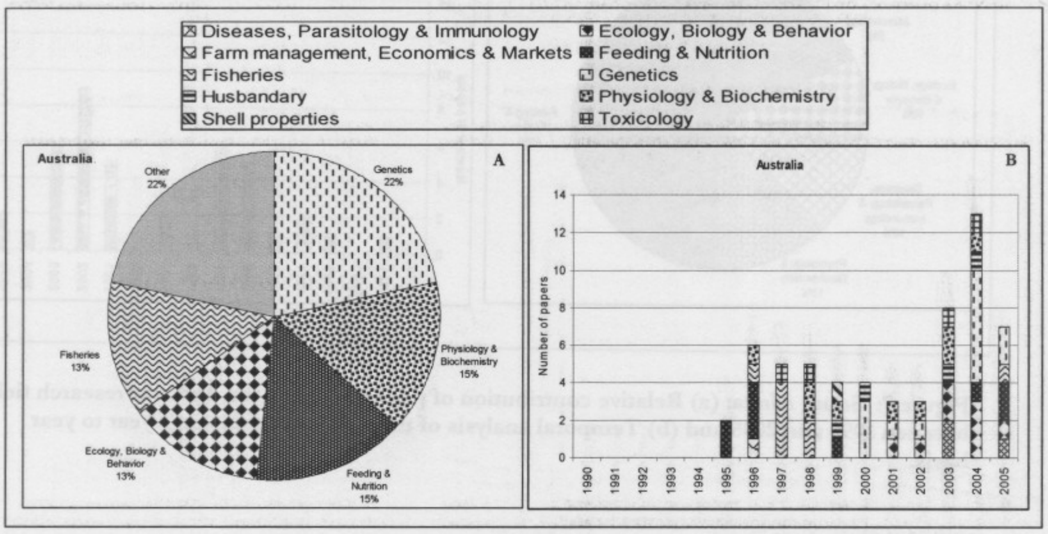


Figure 6: Australia: (a) Relative contribution of papers towards the different research fields between 1990 and 2005 and (b) Temporal analysis of these contributions on a year to year basis

Research outputs by South Africa (Figure 7), in terms of publications, were lower and more irregular than compared to the USA and Australia in the same period. South Africa published 40 papers, of which 50% have been on feeding and nutrition. The rest of the papers were divided between nine other research fields. The 20 (50%) papers on feeding and nutrition from South Africa stands in sharp contrast with the USA and Australia where four (5%) and nine (15%)

papers respectively were published on this field. This may indicate that the USA and Australia have already sorted out the dietary requirements of their animals, or that South African researchers are putting too much emphasis on feeding and nutrition. Research on physiology and biochemistry as well as genetics in South Africa is also apparently lagging behind the other two leading countries.

Although South Africa is the greatest producer of cultured abalone outside of Asia (Roberts 2005), to remain competitive at the highest levels, it would be advisable to conform to the research trends set by countries with high volumes of research outputs. There seems to be a great need for research in South Africa to be diversified. The amount of physiological and genetics research should be dramatically increased. One way of doing this, might be to get more research institutions, with more diverse specialities, involved in abalone research.

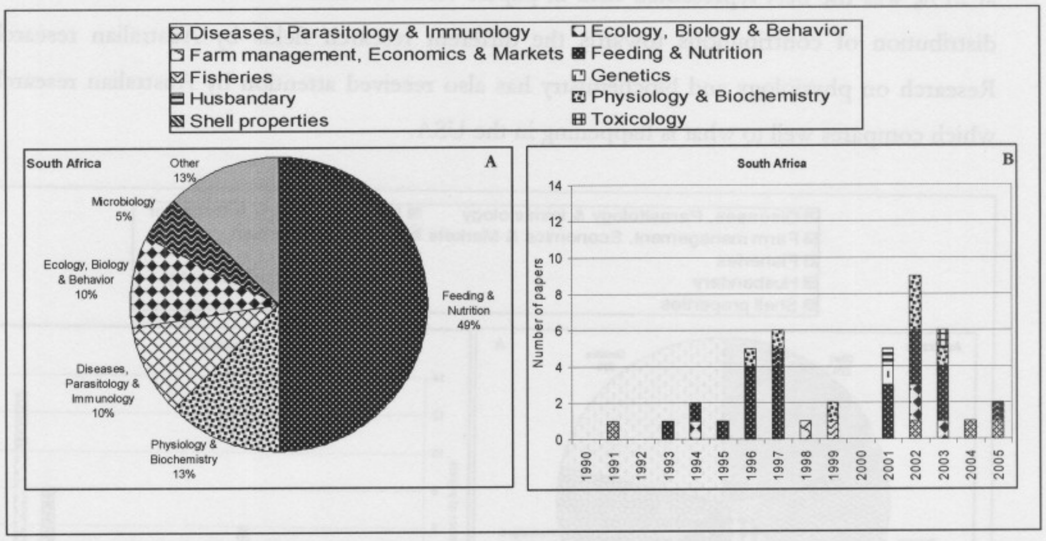


Figure 7: South Africa: (a) Relative contribution of papers towards the different research fields between 1990 and 2005 and (b) Temporal analysis of these contributions on a year to year basis.

From the results presented here, it is evident that there was significant growth in terms of abalone research worldwide, with the USA, Australia and South Africa at the fore-front of abalone research. South Africa compares favourably with the other leaders in the field, and *H. midae* is clearly an important species in terms of world-wide abalone research. For South Africa it will make good sense to diversify both research fields, as well as diversifying the sources of research on abalone.

Acknowledgements

I would like to acknowledge the National Research Foundation for funding during this project.

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3.2. Paper II:

Towards using physiological parameters in the management of South African abalone (*Haliotis midae*) mariculture: Exploration of basic physiology.

Submitted to Aquaculture, August 2006.

Faculty requirements

Letter of consent (Appendix 7)

Instructions to authors (Appendix 9)

3.2.1. Original paper

Towards using physiological parameters in the management of South African abalone (*Haliotis midae*) mariculture: Exploration of basic physiology

André Laas* and André Vosloo

* Corresponding author:

School for Environmental Sciences and Development; Zoology
North-West University (Potchefstroom campus)
Private Bag x6001
Potchefstroom
2520

E-mail: 11777915@nwu.ac.za
Cell phone: 083 661 9421

Abstract

A single-farm physiological study was conducted from April 2003 to January 2004 with the aim of quantifying effects of season, body size and feeding regime on basic constituents of abalone tissues (glucose, glycogen, proteins and lipids). A further aim was to determine whether, based on these variables, water loss during live export can be predicted. Animals of two size classes were subjected to four dietary regimes. Total protein, free glucose, glycogen and total lipid content of the animals were determined in tissue samples from the digestive gland and foot/adductor muscle. One subset of large animals, from each feeding treatment, was subjected to water content analysis by drying. Subsets of large and small animals were subjected to a 36-hour simulated live export trial to determine mass loss.

Digestive gland tissue yielded highly variable results, and proved to be of little value for routine monitoring of physiological constituents. Muscle glucose concentration (average: .38±0.05 $g \cdot kg^{-1}$), muscle lipid concentration (average: 24.37±1.03 $g \cdot kg^{-1}$) and muscle protein concentration (average: 30.27±0.4 $g \cdot kg^{-1}$) were affected by season and body size. Muscle glycogen concentration (average: 43.28±1.90 $g \cdot kg^{-1}$) was affected by body size and feeding type. The average water content of the large animals was 66.21 ± 0.33% M_b. During the live export trials, animals lost on average 15% M_b. Mass loss was significantly higher ($p<0.01$) in small animals relative to large animals. Feeding regime did not influence susceptibility to mass loss during export. Mass loss was best correlated to water content (Adj $R^2=0.79$) and muscle glucose (Adj $R^2 =0.53$). Animal size was a highly important factor, influencing physiological condition as well as export performance. Much of the size effect can be related to the effect of allometric scaling in animals. This study provided reference values for important physiological parameters, and indicated that physiological condition monitoring may be a useful management tool in the abalone production environment.

Introduction

Abalone are marine molluscs that belong to the largest and most diverse class, the Gastropoda (Leighton, 2000; Pechenik, 2000). The South African species associated with the word abalone, is *Haliotis midae*, which is also called the perlemoen (mother-of-pearl) (Tarr, 2000a). Abalone are a highly sought-after seafood species internationally, commanding high prices, particularly in the eastern countries (Tarr, 2000b; Sales, 2004). Cultivating abalone on a commercial scale is a capital intensive venture, with high cost of initial set-up and running costs, but with very lucrative returns in the long-run (Leighton, 2000; Tarr, 2000b; Sales, 2004).

Animals are cultured in man-made shore-based systems at high stocking densities, and are raised on natural and/or formulated artificial feeds (Sales, 2004). The natural diet of *H. midae* consists primarily of the kelp species, *Ecklonia maxima*, that is harvested from the West and South-West coast of South Africa (Tarr, 2000a; Troell *et al.*, 2006). The haliotid digestive system is anatomically and biochemically suited to feed on macro-algae and is well suited for their carbohydrate-based metabolism (Carefoot *et al.*, 2000; Picos-García *et al.*, 2000; García-Carreño *et al.*, 2003). In recent years there has been a rapid increase in the development of artificial feeds to supplement or replace macro-algae in abalone aquaculture (Fleming *et al.*, 1996). Currently Abfeed™ (fishmeal-based) and Midae Meal™ (Seaweed-based) are the only two artificial diets available to the South African abalone farmers (Troell *et al.*, 2006). There is a distinct difference in the composition of the natural kelp and the formulated diet being fed to the abalone. The kelp, *Ecklonia maxima*, contains about 7.8% protein, 0.45% lipids and 43.15% carbohydrates (Algaeron, 2006), while the proximate analysis for Abfeed™ is 34.6% protein, 5.3% lipids and 43.3% carbohydrates (Sea Plant Products, personal communication, 2003).

Abalone farmers in South Africa export about 62% of their products live, and about 33% canned (Du Plessis, 2006). During the live export process abalone lose up to 15% of their body mass, resulting in significant loss of foreign revenue. Studies conducted by Vosloo and Vosloo (2006) indicated that this mass-loss could be attributed to the loss of body water.

The effects of animal health, growth stage and physiological condition on production quality and yield have long been recognised in conventional animal husbandry systems (Mouton *et al.*, 2003). Animal physiology studies have yielded knowledge central to many commercial and agricultural advances during the last few decades. Based on the results of physiological studies, farmers have been able to improve the yield and quality of the animal products they produce (Randall *et al.*, 1997; Schmidt-Nielsen, 1998).

Comparative physiological studies have the power to define the normal range of stressors that animals can cope with. Such studies can be useful in understanding the mechanisms of stress, and to document convenient indicators thereof. Stress responses imply an energetic cost that interferes with the energy budget available for other vital processes, such as growth and reproduction (Baldwin *et al.*, 1992; Taylor *et al.*, 1997; Ansaldo *et al.*, 2006). A specific biochemical method for monitoring metabolic stress would be useful, particularly for stress induced by muscle work and low environmental oxygen during handling and transport of live abalone (Baldwin *et al.*, 1992).

Commissioned by the Abalone Farmers Association of South Africa (AFASA) a research project was launched to study various physiological parameters in *H. midae*. The aim of this study was to evaluate different physiological parameters in abalone muscle tissue and digestive gland, and to investigate (1) seasonal patterns (2) size specific differences and (3) the influence of natural (kelp) and artificial feed (Abfeed™), or combinations thereof, on these parameters. Such information could be invaluable for the purposes of managing the condition of abalone in mariculture systems.

Materials and Methods

The research was conducted in part on-farm, at Aquafarm in Hermanus, and at the Laboratory for Ecophysiology at the North-West University in Potchefstroom. The study was conducted on animals in two size classes of approximately 50 mm (30 – 40 g) and 70 mm (50 – 60 g) starting size respectively.

Four dietary regimes were chosen for the study:

(1) kelp (K): Animals were continually fed on freshly harvested kelp;

(2) Abfeed™* (A): Animals were continually fed on this artificial feed;

(3) kelp and Abfeed™ three weekly (KAT): Animals were rotationally fed on a three-weekly basis, two weeks kelp and one week Abfeed™.

(4) kelp and Abfeed™ weekly (KAW): Animals were rotationally fed on a weekly basis, four days kelp, and three days Abfeed™.

* Abfeed™ is an artificial diet produced locally

Two different rotational feeding regimes were chosen to determine whether differing proportions of a mix between the natural diet and the artificial diet would have an influence on physiological constituents in abalone tissue, and on export performance of the animals.

Sampling was conducted at six-week intervals from April 2003 to January 2004. At each sampling, six animals from each size class, from each of the four feeding groups were collected, weighed and measured. Growth rates were determined from the mass and length data of the sampled animals. The animals were subsequently snap-frozen whole in liquid nitrogen to preserve physiological condition of the animals for future analysis of physiological parameters. Another sub-set of six large animals were collected, weighed and snap-frozen for water content analysis.

To investigate seasonal effects, results of consecutive six-week samplings were pooled for late summer/autumn (January & April), winter (May & July), spring (August & September) and summer (November & December). The pooling of data also served to increase the sample numbers for the purposes of statistical analysis. A temperature profile was constructed from data provided by Aquafarm. Temperatures were measured in the aquaculture systems by a continuous data logger at one-hour intervals. Average daily temperatures were used to calculate weekly average temperatures.

Muscle and digestive gland tissue were dissected out and analysed for free glucose (Enzymatic colorimetric reaction; Roche GOD-PAP), glycogen (Keppler and Decker, 1983), proteins (Van Heerden *et al.*, 2004) and lipid content (Folch *et al.*, 1957). Water content was determined by drying to a constant mass at 100ºC as described by Vosloo & Vosloo (2006). All results are reported per unit wet mass.

After the last sampling in January 2004, 15 animals of each of the two size classes and feeding regimes were committed to a simulated export trial. The animals were purged, weighed and length measured and packed on the farm as per standard farm protocol and subsequently sent to Johannesburg International Airport where they were collected. The polystyrene boxes remained sealed for 36 hours, which corresponds to time of a normal export to the East. After 36 hours the cases were opened, and the animals were weighed and measured again, to determine the loss of body mass during the export.

Statistical analyses were conducted in consultation with the Statistical Consulting Services of the North-West University, using the STATISTICA (Statsoft Inc, 2005), and SAS (SAS Institute, 2003) software packages. Factorial ANOVA's were conducted, and data were tested for homogeneity by means of Levene's test. In the case of non-conformance to homogeneity, data were transformed with logarithmic and/or Box-Cox transformations. Subsequently Tukey's HSD tests were performed to test for significant differences ($p<0.05$) between cases. Values are given as mean ± SEM. Effect sizes are reported as partial η^2 (partial-eta squared) values, which is the proportion of the variability in the dependent variables that is explained by the effect. Multiple regression analysis was performed using SAS software using the R-SQUARE procedure. The multiple regression results were reported in terms of the adjusted-R^2 values, which measure the goodness of fit while taking into account the number of predictors in the model.

Results

Temperature profile

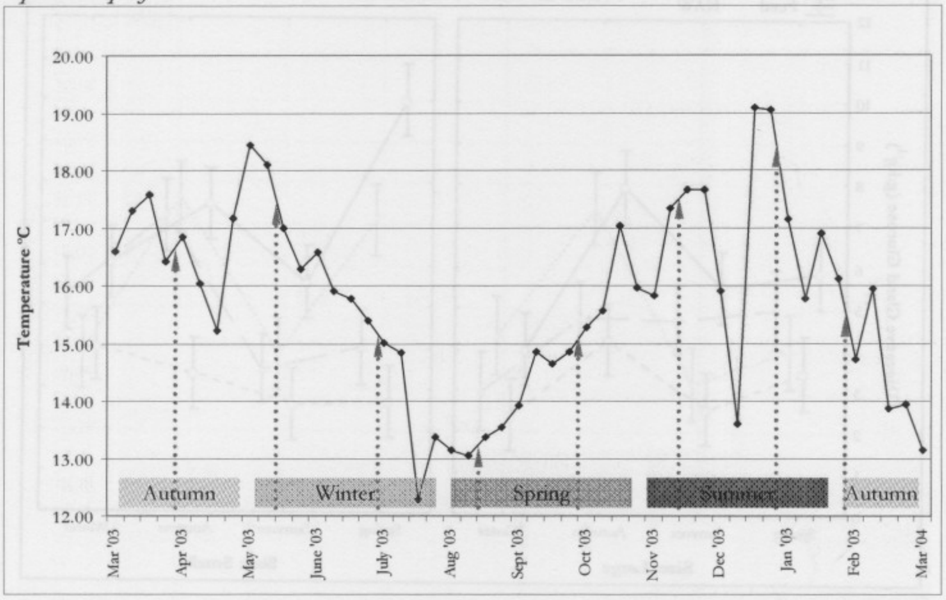

Figure 1: Average weekly aquaculture system water temperatures from March 2003 to March 2004 as provided by Aquafarm. Arrows indicate the dates that sampling was conducted.

The water temperatures in the system showed short-term as well as seasonal variations (Figure 1). The minimum water temperatures (around 12ºC) occurred in July and the maximum temperatures (around 19ºC) occurred in December.

Digestive gland glucose

The average digestive gland glucose concentration for all small and large animals in all seasons on all feed types was 4.91 ± 0.18 $g\cdot kg^{-1}$ (n=383).

The glucose concentrations (Figure 2) of both large and small animals were affected by both season and feed type ($p<0.001$) with feed type having the largest effect size (Partial $\eta^2 = 0.15$). The only significant difference observed in digestive gland glucose concentration of large and small animals occurred in spring, when the glucose concentration in small animals was significantly higher than in large animals ($p<0.05$). Any other differences in digestive gland glucose concentration were obscured by the high variability in the results.

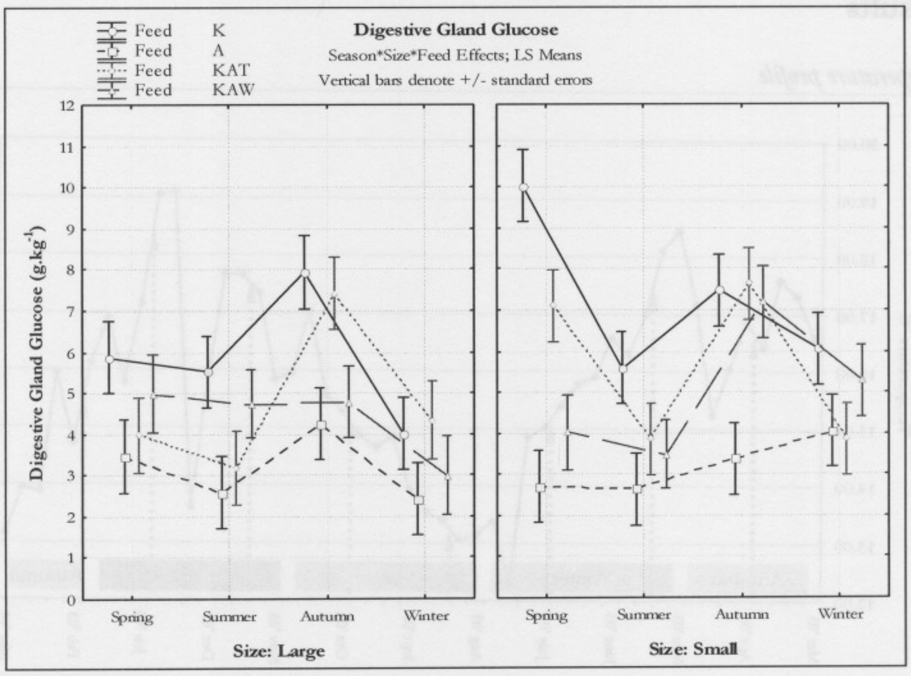

Figure 2: Summary of the combined effects of season and feed on digestive gland glucose concentrations (expressed as g·kg^{-1}) for large and small animals on four different feeding regimes. Each data point within a season represents the average values for two distinct samplings (n=12).

Digestive gland glycogen

Attempts to liberate glucose from glycogen in the digestive gland, using amyloglucosidase, were unsuccessful.

Digestive gland lipids

The average lipid concentration in the digestive glands of all small and large animals in all seasons on all feeding regimes was 56.31 ± 1.67 g·kg^{-1} (n=329). This compares to the 36 – 131 g·kg^{-1} reported for *Haliotis fulgens* by Nelson *et al.* (2002).

As was the case with glucose, the lipid content of the digestive gland (Figure 3) was highly variable. There was no significant difference between the lipid contents of animals fed on different feeds, during different seasons or between large and small animals.

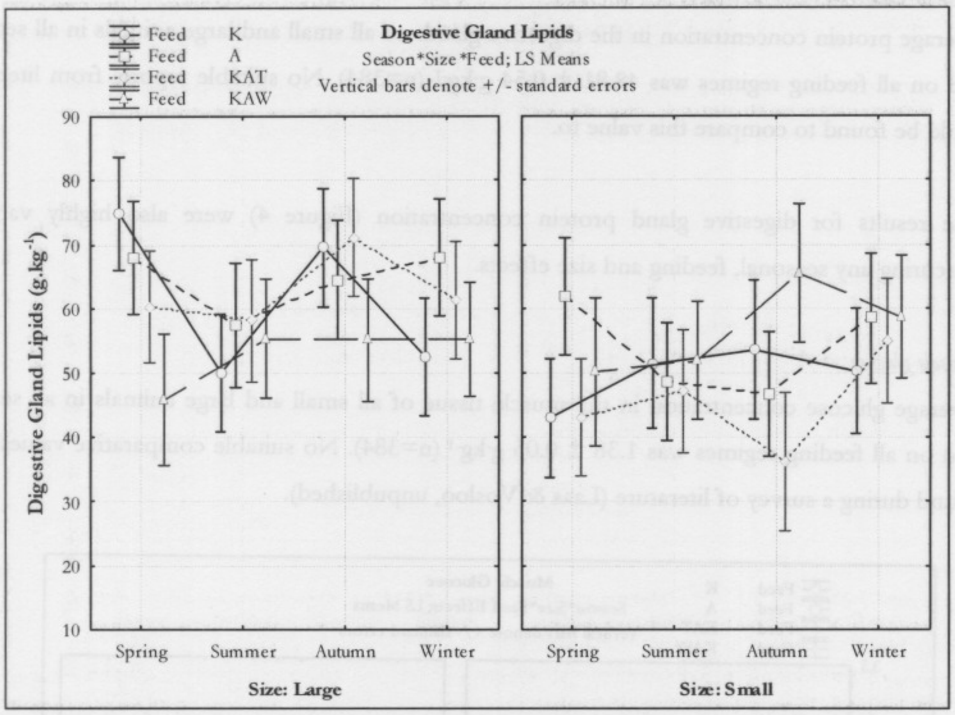

Figure 3: Summary of the combined effects of season and feed on digestive gland lipid concentrations (expressed as g·kg^{-1}) for large and small animals on four different feeding regimes. Each data point within a season represents the average values for two distinct samplings (n=12).

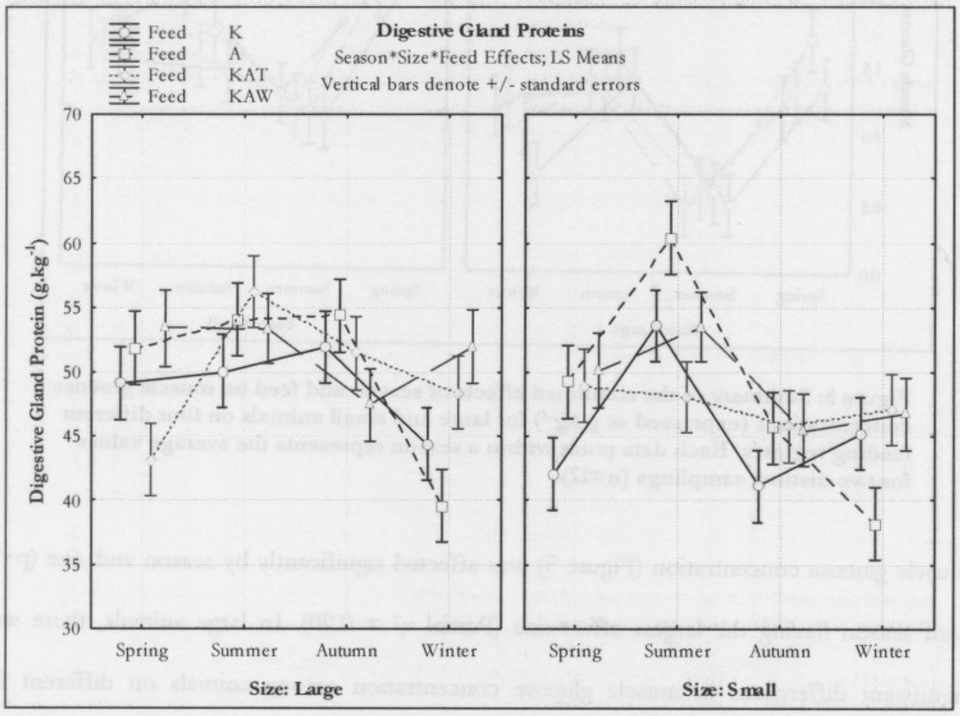

Figure 4: Summary of the combined effects of season and feed on digestive gland protein concentrations (expressed as g·kg^{-1}) for large and small animals on four different feeding regimes. Each data point within a season represents the average values for two distinct samplings (n=12).

Digestive gland proteins

Average protein concentration in the digestive glands of all small and large animals in all seasons and on all feeding regimes was 48.81 ± 0.54 g·kg^{-1} (n=384). No suitable reports from literature could be found to compare this value to.

The results for digestive gland protein concentration (Figure 4) were also highly variable, obscuring any seasonal, feeding and size effects.

Muscle glucose

Average glucose concentration in the muscle tissue of all small and large animals in all seasons and on all feeding regimes was 1.38 ± 0.05 g·kg^{-1} (n=384). No suitable comparative values were found during a survey of literature (Laas & Vosloo, unpublished).

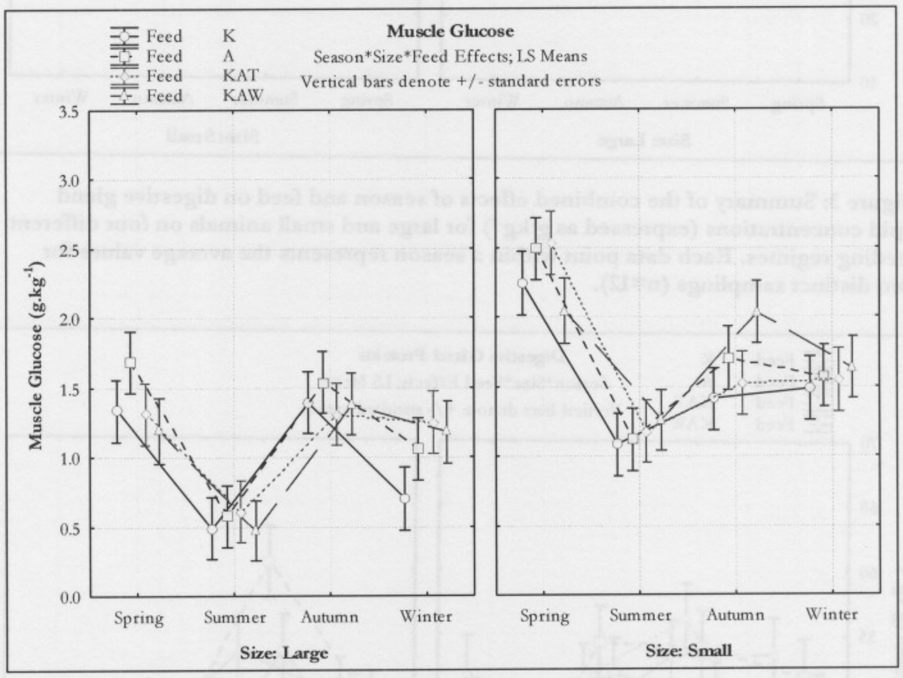

Figure 5: Summary of the combined effects of season and feed on muscle glucose concentrations (expressed as g·kg^{-1}) for large and small animals on four different feeding regimes. Each data point within a season represents the average values for two distinct samplings (n=12).

Muscle glucose concentration (Figure 5) was affected significantly by season and size ($p<0.001$) with season having the largest effect-size (Partial $\eta^2 = 0.20$). In large animals, there were no significant differences in muscle glucose concentration among animals on different feeding regimes within the different seasons. Muscle glycogen concentrations in all large animals during summer, was significantly lower than that of large animals in spring, autumn and winter ($p<0.05$).

Autumn, spring and winter muscle glucose concentration in large animals did not differ.

In small animals, the only significant difference among animals on different feeds within any season, was between K and KAT during autumn ($p<0.05$). Muscle glucose concentration was significantly higher in spring than in summer ($p<0.01$), autumn and winter ($p<0.05$). Summer, autumn and winter muscle glucose concentrations in small animals did not differ. Small animals had significantly higher muscle glucose concentrations compared to large animals in spring and summer ($p<0.05$), but there were no differences between size classes in autumn and winter.

Muscle glycogen

The average glycogen concentration in the muscle tissue of all small and large abalone in all seasons and on all feeding regimes was 43.28 ± 1.90 $g{\cdot}kg^{-1}$ (n=383). This compares to the 60 $g{\cdot}kg^{-1}$ reported for *H. midae*, with shell lengths of between 90 and 100 mm, by O'omolo *et al.* (2003), and the 34 $g{\cdot}kg^{-1}$ for limpets, *Patella rustica*, of between 18 and 40 mm shell length (Santini and Chelazzi, 1995).

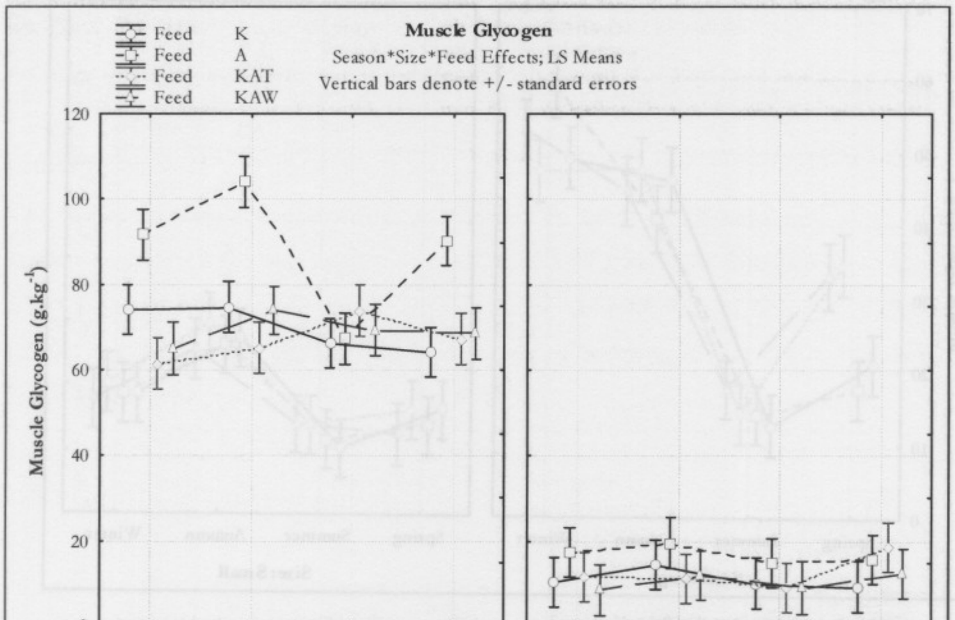

Figure 6: Summary of the combined effects of season and feed on muscle glycogen concentrations (expressed as $g{\cdot}kg^{-1}$) for large and small animals on four different feeding regimes. Each data point within a season represents the average values for two distinct samplings (n=12).

Muscle glycogen concentrations (Figure 6) were significantly affected by size and feeding type ($p<0.001$) with size having the greatest effect size (Partial $\eta^2 = 0.71$). In large animals, the muscle glycogen concentration of animals on Abfeed™ was significantly higher than that of animals on any other feeding regime in all seasons ($p<0.05$), except for autumn. The muscle glycogen concentration in small animals did not vary significantly either seasonally or within different feeding regimes. The difference in muscle glycogen concentrations between large and small animals, were highly significant in all seasons ($p<0.001$). Large animals had glycogen concentrations more than three times higher than that of small animals.

Muscle lipids

Average muscle lipid concentration in all abalone sampled was 24.37 ± 1.03 g·kg^{-1} (n=363). This corresponds well to the 15 – 34 g·kg^{-1} reported for *H. fulgens* specimens, of around 55 mm shell length, by Nelson *et al.* (2002), but differed from the 87.9 - 113.1 g·kg^{-1} reported for *H. asinina,* of 11-12 mm shell length, by Thongrod *et al.* (2003).

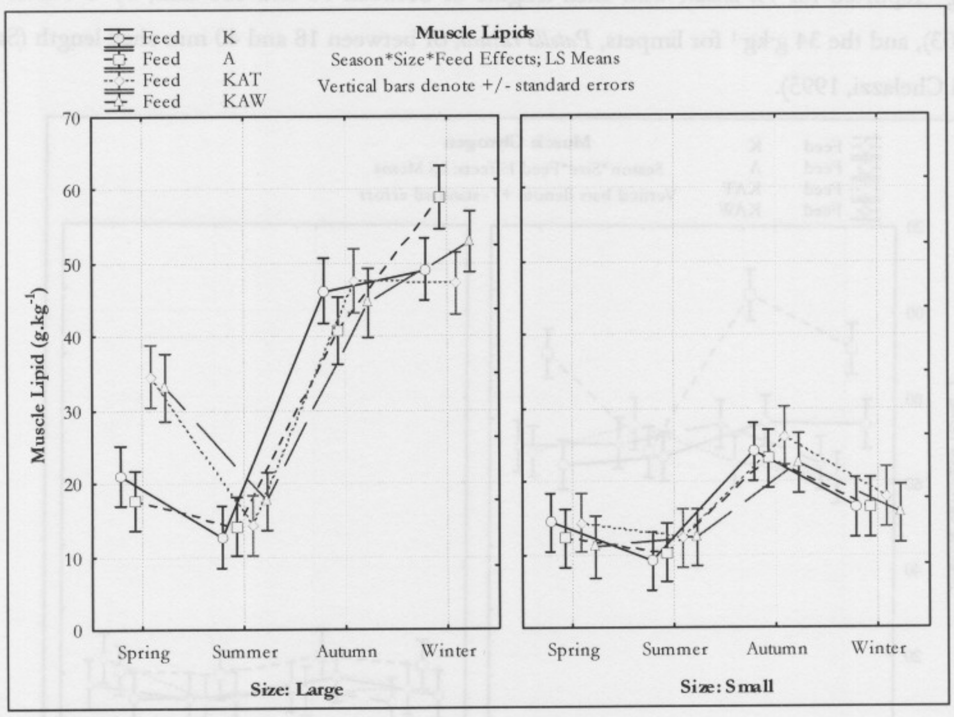

Figure 7: Summary of the combined effects of season and feed on muscle lipid concentrations (expressed as g·kg^{-1}) for large and small animals on four different feeding regimes. Each data point within a season represents the average values for two distinct samplings (n=12).

Muscle lipid concentration (Figure 7) was affected by season and size ($p<0.001$), with season having the greatest effect size (Partial $\eta^2 = 0.33$). In large animals, there were no differences in muscle lipid concentration in animals on different feeding regimes in summer, autumn and winter. In spring, animals on K and KAW, had significantly higher muscle lipid concentrations than animals on A and KAT ($p<0.05$). Muscle lipid concentrations of animals on all feeds were significantly higher in autumn and winter than in spring and summer ($p<0.01$), with the exception of animals on K and KAW in spring, which differed significantly from winter ($p<0.05$), but not from autumn.

In the small animals, there were no significant differences among animals on different feeding types within seasons. Muscle lipid concentration in autumn was significantly higher than in summer ($p<0.05$), but did not differ from spring and winter. In spring and summer there were no significant differences in muscle lipid concentration of large and small animals, with the exception of large animals on K and KAW in spring, which was significantly higher ($p<0.05$). In autumn and winter, muscle lipid concentration in large animals was significantly higher than in small animals ($p<0.05$).

Muscle proteins

The average muscle protein concentration for all small and large animals in all seasons and on all feeding regimes was 30.27 ± 0.40 $g \cdot kg^{-1}$ ($n=384$).

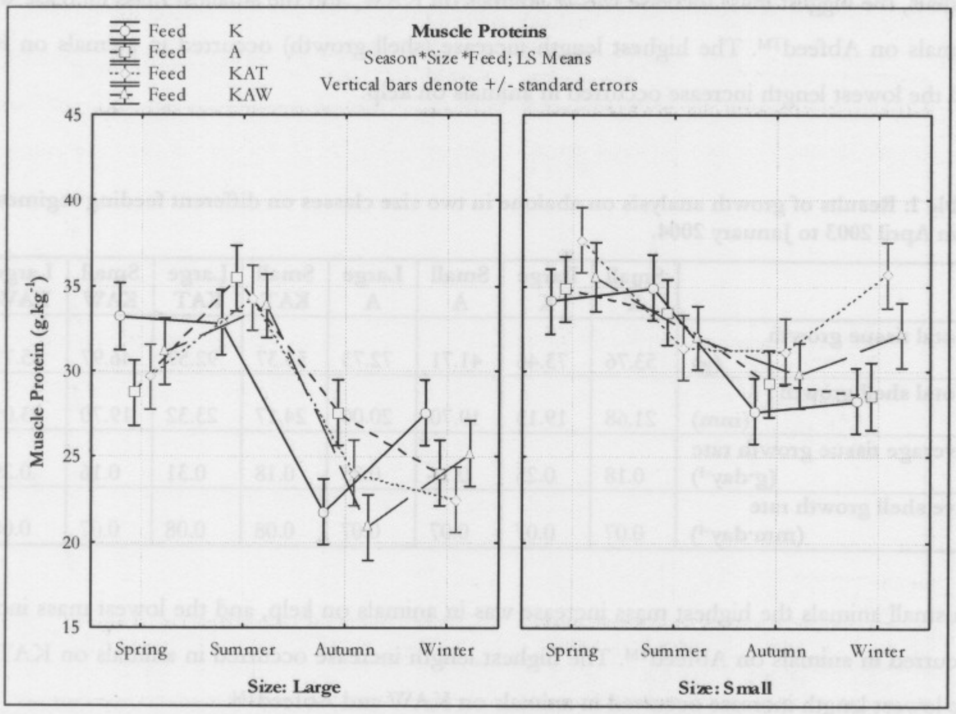

Figure 8: Summary of the combined effects of season and feed on muscle protein concentrations (expressed as $g \cdot kg^{-1}$) for large and small animals on four different feeding regimes. Each data point within a season represents the average values for two distinct samplings ($n=12$).

Muscle protein concentrations (Figure 8) were affected by season and size ($p<0.001$), with season having the greatest effect (Partial $\eta^2 = 0.33$). In large animals, the only significant difference in muscle protein concentration within seasons occurred between animals on A and on K in autumn ($p<0.05$). Muscle protein concentrations in large animals was significantly higher in spring and summer than in autumn and winter ($p<0.05$).

In small animals, muscle protein concentration did not differ among animals on different feeds within any season other than winter. In winter, the muscle protein concentration of animals on K was significantly higher than in animals on A, KAW and KAT ($p<0.05$). In autumn and winter, muscle protein concentration of small animals was significantly higher than that of large animals ($p<0.05$). There were no significant differences between large and small animals during spring and summer.

Growth

The growth results for all animals over the entire study period are summarised in Table 1. Total tissue and shell growth were on average higher in large animals than in small animals. In large animals, the biggest mass increase was in animals on KAW, and the smallest mass increase was in animals on Abfeed™. The highest length increase (shell growth) occurred in animals on KAT, and the lowest length increase occurred in animals on kelp.

Table 1: Results of growth analysis on abalone in two size classes on different feeding regimes from April 2003 to January 2004.

	Small K	Large K	Small A	Large A	Small KAT	Large KAT	Small KAW	Large KAW
Total tissue growth (g)	53.76	73.46	41.71	72.73	53.37	92.59	46.97	85.71
Total shell growth (mm)	21.68	19.13	19.70	20.00	24.17	23.32	19.70	23.05
Average tissue growth rate ($g \cdot day^{-1}$)	0.18	0.25	0.14	0.25	0.18	0.31	0.16	0.29
Ave shell growth rate ($mm \cdot day^{-1}$)	0.07	0.07	0.07	0.07	0.08	0.08	0.07	0.08

In small animals the highest mass increase was in animals on kelp, and the lowest mass increase occurred in animals on Abfeed™. The highest length increase occurred in animals on KAT, and the lowest length increase occurred in animals on KAW and Abfeed™.

Table 2: Average seasonal growth rates for abalone in two size classes and on four different feeding regimes.

	Winter	Spring	Summer	Autumn
Ave tissue growth rate (g·day^{-1})	0.199	0.204	0.214	0.313
Ave shell growth rate (mm·day^{-1})	0.066	0.072	0.063	0.105

Highest average tissue growth rate (Table 2) occurred in late summer and autumn, and lowest tissue growth rates occurred in winter. Highest shell growth rate occurred in autumn, and the lowest shell growth rate occurred in summer.

Small animals reached the starting size of large animals (≈70 mm, April 2004) in January 2005. Statistical analysis revealed that although there were no significant differences in the length or mass of large animals in April and small animals in January ($p>0.05$), there were highly significant differences in physiological constituents between the two groups (all $p<0.001$). Muscle glucose, muscle glycogen and muscle lipid concentrations were higher in large animals than in small animals, while muscle protein concentrations were higher in small animals than in large animals.

Feed type effects

Feed type had differing effects on different tissue types and physiological constituents. Digestive gland glucose concentrations were affected by feed type in both large and small animals. Animals on kelp had higher digestive gland glucose concentrations than animals on Abfeed™, which had the lowest overall digestive gland glucose concentrations. The two rotational diets did not differ from one another or from kelp or Abfeed™. Digestive gland lipid and protein concentrations, however, were not clearly affected by different diets.

In muscle tissue, glucose, lipid and protein concentrations were unaffected by diet. Muscle glycogen concentrations in large animals were, however, raised in animals on Abfeed™. This was not the case in small animals.

The total mass and shell gains in small animals on different feeds did not differ from one another. In large animals, however, the two rotational diets resulted in higher final mass than either kelp or Abfeed™. Final shell lengths in large animals did not differ in different feeding types.

Water content

The average water content for all large animals in all seasons and on all feed types was 66.21 ± 0.33% (n=191).

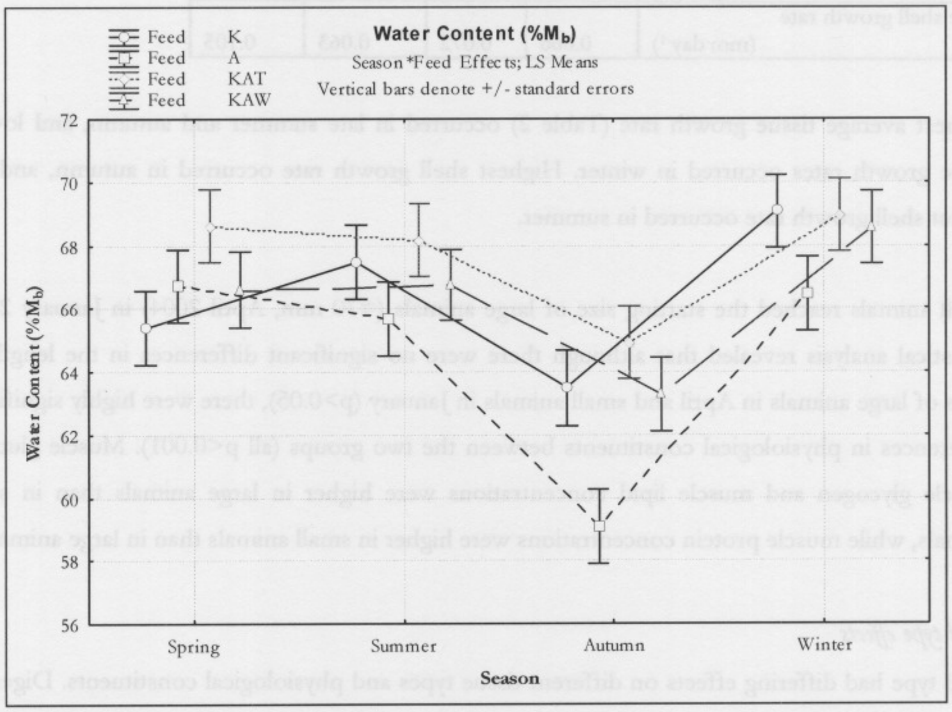

Figure 9: Summary of the combined effects of season and feed on water content (expressed as % body mass) for large animals on four different feeding regimes. Each data point within a season represents the average values for two distinct samplings (n=12).

The only significant difference in the water content (Figure 9) of large animals within any season occurred in autumn, where the water content of animals on A was significantly lower than animals on the other feeding treatments ($p<0.01$). Water content remained fairly constant (66.83 – 68.26% M$_b$) during spring, summer and winter, however, in autumn, water content was significantly lower than in winter ($p<0.05$), with the exception of animals on A. The only physiological parameter that correlated with water content was muscle glucose concentration ($p<0.05$; Adj $R^2=0.29$)

Mass loss

The average mass loss of all large and small animals on all feeding types was 13.211 ± 0.61% (n=105). This compares to the 15.06 ± 0.94% mass loss reported by Vosloo & Vosloo (2006).

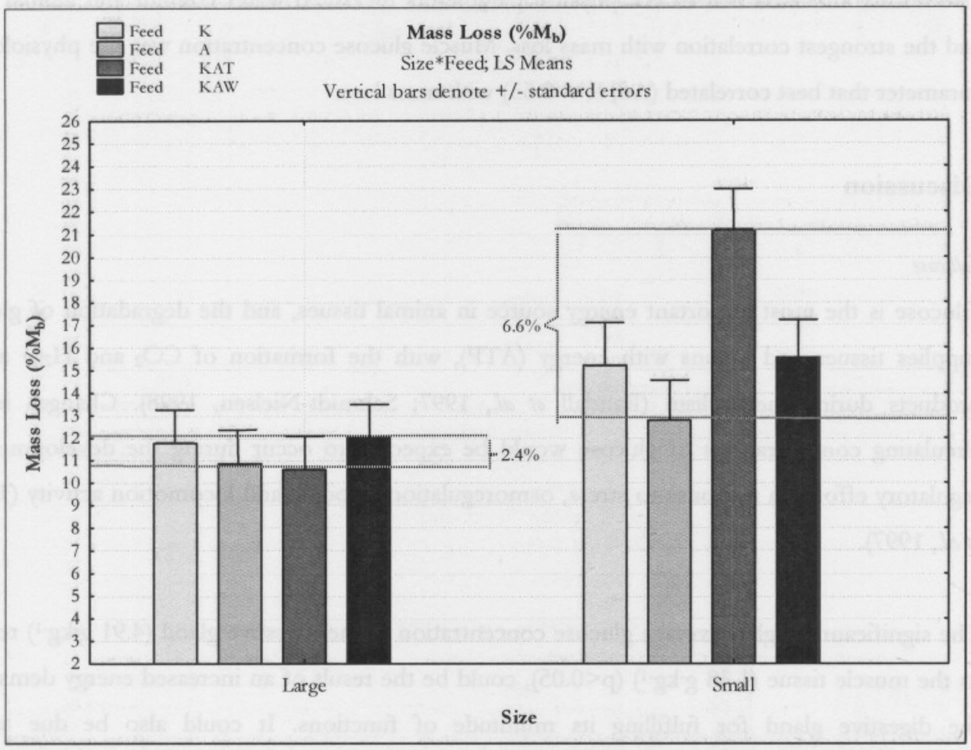

Figure 10: Summary of the mass loss (expressed as %Mb) experienced by large and small animals on four different feeding regimes during a simulated export event.

There were no significant differences in the mass loss (Figure 10) experienced during simulated export by large animals on different feeding regimes. Mass loss in small animals on KAT was significantly higher than mass loss in small animals on A ($p<0.05$), as well as in all large animals ($p<0.01$).

The question arises whether the observed mass loss correlates with any of the measured physiological parameters. The aim of the multiple regression analysis is to identify physiological variables that can accurately predict mass loss prior to live export events.

Table 3: Results from multiple regression analysis performed on physical and physiological parameters measured in *Haliotis midae* in order to find correlations between the measured parameters and observed mass loss.

Parameter Measured	Adjusted R^2 value
Water Content	0.79
Animal Mass	0.60
Muscle Glucose	0.53
Muscle Glycogen	0.28
Muscle Lipids	0.01
Muscle Proteins	-0.04

The results indicated that of the physical parameters measured water content and animal mass had the strongest correlation with mass loss. Muscle glucose concentration was the physiological parameter that best correlated (Adj $R^2 = 0.53$) with mass loss.

Discussion

Glucose

Glucose is the most important energy source in animal tissues, and the degradation of glucose supplies tissues and organs with energy (ATP), with the formation of CO_2 and H_2O as by-products during metabolism (Randall *et al.*, 1997; Schmidt-Nielsen, 1998). Changes in the circulating concentrations of glucose would be expected to occur during the development of regulatory efforts in response to stress, osmoregulation, hypoxia and locomotion activity (Taylor *et al.*, 1997).

The significantly higher average glucose concentration in the digestive gland (4.91 $g \cdot kg^{-1}$) relative to the muscle tissue (1.38 $g \cdot kg^{-1}$) ($p<0.05$), could be the result of an increased energy demand in the digestive gland for fulfilling its multitude of functions. It could also be due to the sequestration of glucose from ingested feed, as feed had a significant effect on the digestive gland glucose concentration. The significant effect of season on digestive gland might be related to the involvement of the digestive gland in gametogenesis (Carefoot, *et al.*, 2000). Season, size and feeding regime effect on digestive gland glucose concentration will, however, have been obscured by the high variability in the data.

In muscle tissue, glucose plays an important role in the normal cellular metabolism, and provision of energy for normal muscle functioning. The effect of season on muscle glucose concentration is illustrated by the low muscle glucose concentration in summer (Figure 5), which corresponds to high average temperatures (Figure 1) and relatively high average growth rates (Table 2). A study by Britz *et al.* (1997) indicated significant increases in weight-gain with increasing temperature. A plausible explanation is thus that active growth is stimulated by higher temperatures in summer, and that glucose concentrations in the muscle tissues will decrease as a result of increased metabolic demand associated with increased growth.

Smaller animals generally had slower growth rates than larger animals (Table 1). Similar results for *H. midae* were illustrated by Shipton and Britz (2001). This would explain the higher glucose concentrations in the muscle tissues of small animals in spring and summer.

Glycogen

Glycogen is a two-branched polymer of D-glucose, and can provide fuel for carbohydrate metabolism very quickly (Schmidt-Nielsen, 1998). Glycogen is the major storage form of energy in abalone tissues owing to their carbohydrate-based metabolism (Baldwin *et al.*, 1992; Picos-García *et al.*, 2000; Gómez-Montes *et al.*, 2003; Braid *et al.*, 2005).

Glycogen could not be liberated from the digestive gland in this study. The reason for this inability to liberate glycogen is unclear as Braid *et al.* (2005) indicated the presence of glycogen, albeit in low concentrations, in the digestive gland of *H. rufescens*.

The high average glycogen concentration in the muscle tissue of large animals (Figure 6) is indicative of the important role of glycogen as energy storage product in abalone. The significant difference ($p<0.001$) in muscle glycogen concentration between large and small animals, is indicative that abalone build up a large glycogen store in muscle tissue with increasing animal size. The fact that the artificial diet yielded higher average glycogen concentrations could be attributed to the higher nutritional value of the diet (Fleming *et al.*, 1996). With the exception of large animals on Abfeed™, the relatively unchanged muscle glycogen concentrations in the muscle tissues of both large and small animals demonstrate tight regulation of muscle glycogen reserves in *H. midae*.

Lipids

Lipids comprise a diverse group of water insoluble biological molecules with rather simple chemical structures and the various lipids have a variety of functions including energy storage and cellular components (Randall *et al.*, 1997).

The low concentration of lipids in abalone tissues corresponds to the low concentration of lipids in their natural diet (Mai *et al.*, 1995; Dunstan *et al.*, 1996; Durazo-Beltrán *et al.*, 2004). Indications are that the lipid requirements of abalone are more dependent on the presence of sufficient amounts of essential polyunsaturated fatty acids than on gross intake of lipid substance (Dunstan *et al.*, 1994; Leighton, 2000; Durazo-Beltrán *et al.*, 2003; 2004). Mai *et al.* (1995) indicated that elevated lipid concentrations in the diet result in decreased deposition of muscle proteins in *H. tuberculata* and *H. discus hannai*. In their study, feed intake, and thus protein intake was reduced in animals fed on a high-lipid diet, which led to the suppression of growth. This is supported by findings that a dietary lipid levels of more than 4 – 5% suppressed growth in abalone (Dunstan *et al.*, 1994; Durazo-Beltrán *et al.*, 2003).

The average lipid content of the digestive gland (Figure 3) was significantly higher ($p<0.05$) than in the muscle tissue (Figure 7). This could be due to the importance of lipids in reproduction (Mai *et al.*, 1995; Santini and Chelazzi, 1995; Nelson *et al.*, 2002). Lipid is a major source of energy for the development from egg to competent larva of *H. fulgens* (Moran & Manahan, 2003). The digestive gland is a nutrient store, and as maturation of gonads proceeds, nutrients are drawn from the digestive gland (Nelson *et al.*, 2002; Litaay & De Silva, 2003). The higher lipid concentrations in both the digestive gland and muscle tissue of larger animals, is indicative of accumulation of lipids in abalone. Similar results were reported by Nelson *et al.* (2002) and Dunstan *et al.* (1996). Thus, although lipids are not primarily used in energy metabolism, lipids are stored in the digestive gland due to its importance in growth and gonadal development.

Different feed types generally did not have any effect on muscle lipid concentrations. In studies by Leighton (2000), lipid absorption was not detected in *H. rufescens*, and faeces were rather enriched with lipid substances. Lipase activities in the gut of many abalone are also low (Mai *et al.*, 1995; Leighton, 2000). This indicate that the artificial feed with its higher lipid content would not contribute towards higher tissue lipid concentrations, due to the inability of abalone to effectively hydrolyse high concentrations of lipids. The higher lipid concentrations in autumn and winter relative to spring and summer correspond to findings by Nelson *et al.* (2002) for *H. fulgens*. This indicates seasonal regulation of lipid concentrations in the muscles.

Proteins

Proteins are the most complex and abundant organic molecules in living cells, and are crucial for soft tissue growth (Randall *et al.*, 1997; Guzmán and Viana, 1998). Abalone do not have a requirement for protein as such, but rather for an appropriate level and balance of individual amino acids (King *et al.*, 1996; Shipton and Britz, 2002).

The average protein concentration in the digestive gland (Figure 4) was significantly ($p<0.05$) higher than that of the muscle tissue (Figure 8). This can relate to the abundance of enzymes present in the digestive gland required for digestion.

It was interesting to note that there were no significant differences in the protein concentrations of animals on different feeds in either the muscle tissue or the digestive gland. This indicates that diets with higher protein concentrations in this study did not result in higher protein concentration in the soft tissues of the abalone. This is in contrast with findings by Britz & Hecht (1997) who found increased soft tissue protein content with increasing protein content in diets with similar lipid content. The artificial diet, used in this study, had a lipid content of approximately 5% (Sea Plant Products, personal communication). Studies by Dunstan *et al.* (1994), Mai *et al.* (1995) and Durazo-Beltrán *et al.* (2003) have indicted that lipid levels of more than 4 - 5% suppressed protein deposition in abalone. Thus, it is likely that the lipid high lipid concentration in the artificial feed would prevent the abalone from fully utilising the high protein levels provided in the diet.

Suitability of digestive gland tissues for routine physiological monitoring

The digestive gland performs multiple functions of energy storage, metabolic transformation, enzyme and anti-oxidant manufacture, and plays a protective role in the sequestration of heavy metals and pollutants. It was also indicated to be intimately involved with gametogenesis (Carefoot *et al.*, 2000). The high degree of variability in the digestive gland may be due to (1) the relative position of the digestive gland to the gonads, (2) the involvement of digestive gland with gametogenesis and (3) the fact that the digestive gland surrounds the stomach and loops of the small intestine (Carefoot *et al.*, 2000; Leighton, 2000; Grubert *et al.*, 2004; Johnston *et al.*, 2005). The variability in metabolites observed in this study may be due to "contamination" from tissue integrated with the digestive gland, but more likely reflects the central role and multiple functions of this organ. Due to this high variability in the concentrations of physiological constituents in the digestive gland tissues and the multitude of functions performed by this organ, it is not advisable for use in routine monitoring of physiological constituents.

Water content

Water content in large animals was only significantly lower in autumn ($p<0.001$), compared to the other seasons. The reason for this lower concentration in autumn is unclear, and a literature survey did not produce comparative results. Water content was correlated to the glucose concentration in the muscle tissues, so that animals with higher muscle glucose concentrations generally had somewhat higher water content. A possible explanation for this would be that 0.6 g water is released per 1 g of glucose during metabolic hydrolysis (Schmidt-Nielsen, 1998).

Mass loss (Simulated export)

The higher degree of mass loss in small animals relative to large animals could implicate that the different size classes react different to culture conditions. Similar observations have been made for *H. australis* and *H. iris* by Wells and Baldwin (1995). It has been indicated by Leighton (2000), that the feeding rate, of *H. rufescens*, decline with an increase in size. This indicates that smaller abalone have higher metabolic needs relative to larger animals, and would have less energy reserves available for surviving the extreme physiological stress of live export. The significantly higher glycogen stores in the muscle tissue of large animals relative to small animals, observed in this study, in combination with the effect of allometric scaling on metabolism (Randall *et al.*, 1997), support this finding. The surface-to-volume ratio is greater for small animals than for large animals (McGrowan, 1994; Randall *et al.*, 1997; Schmidt-Nielsen, 1998). This implicates a larger surface area of the integument, through which water can be lost due to evaporation, in small animals relative to large animals.

Animal size

Within all the results of this study it was evident that animal size has a significant effect on all physiological and physical parameters measured, as well as on the export performance of animals. These differences could be linked to the effect of allometric scaling in animals. Changes in body size introduce changes in the physiology and morphology of animals that are not always directly proportional. Changes in body size influence the metabolic rate of animals so that small animals need to respire at higher rate per unit body mass than larger animals. Thus metabolic rate generally increases with body size, but the mass-specific metabolic rate (metabolic rate of a unit mass of tissue) decrease with increasing body size (McGrowan, 1994; Randall *et al.*, 1997; Schmidt-Nielsen, 1998). It follows that small animals with a proportionally higher metabolic rates will spend more time and energy on searching for appropriate food sources and feeding. This means that nutritional needs of small and large abalone will probably differ in terms of energy value and composition of amino and fatty acids.

At the last sampling in January 2005, small animals have attained the same size of large animals at the first sampling in April 2004. It might thus have been expected that the concentrations of physiological constituents in these two size classes at the different dates would correspond. This was, however, not the case. Although water temperatures were somewhat higher in January than in April, the rest of the study has shown that not all physiological parameters examined were affected by season. Thus temperature differences alone cannot be the only source of variation. Although no water quality parameters were measured during the study, changes in both physical and chemical water quality of the seawater that have been supplied to the holding systems could have an effect on the condition of the animals at the different samplings.

In terms of mass loss, the results of this study indicated a higher susceptibility to mass loss in small animals relative to large animals during live export. Similar observations have been made for *H. australis* and *H. iris* by Wells and Baldwin (1995). The surface-to-volume ratio is greater for small animals than for large animals (McGrowan, 1994; Randall *et al.*, 1997; Schmidt-Nielsen, 1998). This implicates a larger surface area of the integument, through which water can be lost due to evaporation, in small animals relative to large animals.

Can physiological variables be used to predict mass loss during live export?

Multiple regression analysis was conducted in order to find correlations between mass loss and physical measurements, measured physiological parameters and various combinations of these parameters. Due to the large variation and lack of significant differences in the physiological data from digestive gland tissues, regression analysis was only conducted on physiological parameters from the muscle tissues.

Water content had the highest correlation to mass loss during simulated export (Table 3), indicating that high water content in animals will increase their susceptibility to mass loss during export. It was also clear that animal size had a significant effect on the ability of animals to sustain exposure to the rigours of live export. Larger animals were more resistant to mass loss than small animals, indicating that exporting yields may be increased by increasing the size at which animals are exported. This should, however, be viewed in context, as animals would have to be sustained longer in the culture systems, which implicates higher production costs. Theoretically there should be an ideal size for live export, at which the benefits of animal size, and culture time would be at a maximum.

Conclusions

The study indicated that the basic physiology of abalone under culture conditions would be a very useful tool in the management of abalone aquaculture systems. Based on this study, muscle tissue should be utilised for monitoring changes in physiological constituents in abalone, as digestive gland tissue, due to the complexity of the multitude of functions performed by this organ, is unsuitable. The measurement of glucose, glycogen, protein and lipid concentrations in muscle tissue proved to be of value in monitoring the physiological condition of abalone. Animal size played an important role in the levels of physiological constituents in abalone, and small animals were more prone to experiencing mass loss during simulated export than large animals. In this study, different diets and combinations of diets had resulted in very little variation in the physiological make-up of the abalone. Season had a strong influence on the physiological composition of abalone. Water content, animal size and muscle glucose concentration were good predictors of mass loss during export conditions.

Predictive power in modelling and multivariate regression analysis is improved when the variation in factors can be maximised. It is thus recommended that the studies should be continued, and that variation should be maximised by increasing the number of farms participating in the research effort. This will give a better understanding of changes in physiological parameters in abalone based on different farming techniques and also with regard to different geographical locations. It is also advisable that the physical measurement of pH, water temperature, salinity, oxygen content and ammonia levels in the systems should be included in the sampling regime. The combination of all these variables will significantly increase the predictive and modelling power of regression statistics. The end results of this research might be the development of a model by which farmers can pro-actively manage their animals to be in the best possible state for the intended market, be it live export or canning.

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3.3. Paper III:

Advances in the use of physiological parameters in the management of South African abalone (*Haliotis midae*) mariculture: Seasonal physiological changes on three geographically separated farms.

Submitted to Aquaculture, October 2006.

Faculty requirements:

- Letter of consent (Appendix 7)
- Instructions to authors (Appendix 9)

3.3.1. Original paper

Advances in the use of physiological parameters in the management of South African abalone (*Haliotis midae*) mariculture: Seasonal physiological changes on three geographically separated farms.

André Laas* and André Vosloo

* Corresponding author:

School for Environmental Sciences and Development; Zoology
North-West University (Potchefstroom campus)
Private Bag X6001
Potchefstroom
2520

E-mail: 11777915@nwu.ac.za
Cell phone: 083 661 9421

Abstract

This study explores some aspects of the basic physiology of *Haliotis midae* to gain insight into the functioning of this commercially important abalone species under aquaculture conditions. Two size classes of animals on a natural and artificial diet were sampled seasonally on three separate farms. Haemolymph was analysed for glucose content, and foot/abductor muscle was analysed for free glucose, glycogen, lipid and protein content. After each sampling, a subset of animals was committed to a simulated live export, to measure export performance in terms of mass loss.

Average water temperatures in summer was significantly higher than in autumn and winter ($p<0.05$) on all farms. Average muscle glucose concentration was 0.67±0.02 $g{\cdot}kg^{-1}$ ($n=359$), and was affected by season and farm. Average muscle glycogen was 58.95±2.82 $g{\cdot}kg^{-1}$ ($n=351$), and was affected by size, season and farm. Average haemolymph glucose concentration was 0.71±0.01 $g{\cdot}kg^{-1}$ ($n=355$), and was affected only by season. Average muscle lipid concentration was 25.87±0.84 $g{\cdot}kg^{-1}$ ($n=344$), and was affected by season and farm. Average muscle protein concentration was 48.89±1.34 $g{\cdot}kg^{-1}$ ($n=347$), and was affected only by season. The average mass loss for the simulated export was 14.49±0.28% ($n=639$), and was influenced by mainly by season. Summer proved to be the worst season for live export of abalone.

The study resulted in a comprehensive set of reference data on the basic physiology of abalone, including explorations into the effect of season, size, feed type, and farm as well as seasonal export performances. The results proved physiology to be a useful tool for describing and managing the condition of abalone.

Introduction

Abalone are found throughout the majority of the world's temperate oceans (Branch *et al.*, 1994; Leighton, 2000; Stevens, 2003). South Africa has five species of abalone, including *Haliotis midae*, *H. speciosa*, *H. queketti*, *H. spadicea* and *H. parva*. The South African species associated with the word abalone, is *H. midae*, which is also called the "perlemoen" (mother-of-pearl) (Branch *et al.*, 1994; Tarr, 2000a; b).

Abalone stocks in the wild are declining at a rapid rate, and major producers, like China, Japan, Korea, New Zealand, South Africa, Taiwan and the USA, have shown a significant decrease in fisheries catches in the last two decades (Britz, 1991; Fishtech, 2006; Gordon and Cook, 2003). Factors that have contributed to the decrease in fisheries production include (1) over-fishing (including poaching), (2) diseases (such as abalone withering syndrome), (3) environmental changes (such as habitat loss and the influence of other species) and (4) lack of effective management of fisheries at sustainable levels (Britz, 1991; Gordon and Cook, 2003; Leighton, 2000; Tarr, 2000a; Tarr, 2000b).

In the light of the decline in natural stocks, culturing abalone in pump-ashore land-based systems has become a lucrative business. The phenomenal growth in the world cultured abalone industry is evident when comparing the 8 696 metric tons for 2002 with just 689 metric tons 15 years earlier. The industry is driven by a demand, far exceeding the supply. In 1999 demand outbalanced supply by about 6 000 – 7 000 metric tons, and in 2004 supply was still about 5 000 mt lower than the demand for about 29 000 tons of abalone (Gordon and Cook, 2003; Roberts, 2005). The demand has led to increased prices for quality abalone products, resulting in an economic environment in which abalone culture became an attractive financial investment (Freeman, 2001). Advancement of abalone aquaculture is being sought with focus on acceleration of growth, improvement of meat quality, controlled nutrition and genetic engineering (Leighton, 2000).

Animal physiology studies have yielded knowledge central to many commercial and agricultural advances during the last few decades and farmers have been able to improve the yield and quality of their products (Randall *et al.*, 1997). Economic viability in animal production can be enhanced by optimising the culture conditions for a specific species. An important objective would be achieving growth rates that ensure that animals reach a marketable size and condition within an economically viable time frame (Fleming, 1995). Comparative studies on animals in natural and culture environments, which proceed alongside studies of the physiological responses, can provide a logical basis for optimising systems (Taylor *et al.*, 1997). Physiological stress responses start at the sub-cellular level, and usually consist of the disruption of normal metabolic pathways, which imply an energetic cost that interferes with the energetic budget available for other vital processes, such as growth and reproduction (Ansaldo *et al.*, 2006). The physiological condition of an animal can thus be used to make management decisions in order to identify poor quality animals before they are committed to processing and more importantly to predict quality and yield. At present, producers only identify problems at the very end of the production cycle in terms of mass loss and mortalities during live export, or when poor yields are obtained on canning. A better understanding of basic abalone physiology will allow abalone farmers to manage their stocks and product-yields pro-actively.

We hypothesised that the levels of basic physiological constituents of abalone will give some indication of the condition of the animals. Animals in optimal condition will be more resistant to the rigours of live export, resulting in better yields as a result of lower mass loss. By studying some basic physiological constituents in abalone (over different seasons and under different culture conditions and feeding regimes on separate farms), one can assess the extent to which these parameters correlate to export performance. Identifying parameters that constitute optimum condition prior to export can aid in the management of the abalone culture industry.

Materials and Methods

At a meeting of the Abalone Farmers Association of South Africa (AFASA) in September 2004, five farms were selected to participate in the study. The farms were selected based on the willingness of the farms' managers to participate in the project and geographic location (Figure 1) of the farms

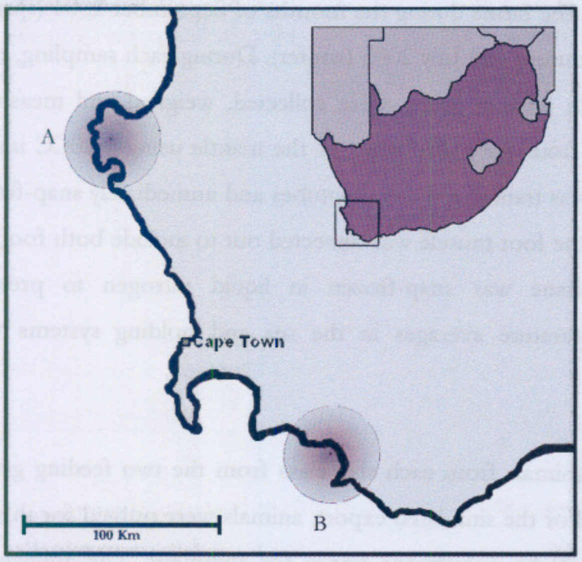

Figure 1: Map indicating the approximate localities of the farms where abalone sampling were conducted. A was situated on the Cape West Coast, and B on the Cape South-West Coast.

Farms were randomly numbered from F1 to F5 to protect the identity of the individual farms. Eventually only farms F2, F3 (Figure 1: B region) and F4 (Figure 1: A region) provided meaningful results, as two of the farms were forced to deviate from the study plan due to unforeseen circumstances. For the purposes of comparisons, results were included from a similar study that was conducted a year prior to the current study (F0, Figure 1: B region). The size of animals to be used was fixed in two size classes, 50 mm (± 40 g) and 70 mm (±60g). For each season new batches of animals were prepared so that animals from the same size class would be sampled throughout the project. Two diets were chosen for the purposes of this study. The diets consisted of kelp (*Ecklonia maxima*), which is a natural diet of *H. midae* and Abfeed™, which is a locally developed and produced artificial diet. The differences in typical composition of the two diets are illustrated in Table 1.

Table 1: Typical analysis (as % dry weight) for kelp* and Abfeed™ fed to experimental animals in the current study.**

	Kelp	Abfeed™
Carbohydrates	43.15%	43.3%
Crude lipids	0.045%	5.3%
Crude protein	7.80%	34.6%
Crude ash	24.30%	5.7%

*Algaeron, 2006.
** Sea Plant Products, 2006. (Electronic communication)

Sampling was conducted seasonally on the farms during the months of September 2004 (spring), January 2005 (summer), April 2005 (autumn) and July 2005 (winter). During each sampling, eight animals from each size class and each feeding group were collected, weighed and measured. Haemolymph samples were extracted from the pallial sinus of the mantle using a 10CC insulin syringe and needle. The haemolymph was transferred to microtubes and immediately snap-frozen in liquid nitrogen. The central part of the foot muscle was dissected out to include both foot and columellar muscle. The dissected tissue was snap-frozen in liquid nitrogen to preserve physiological integrity. Monthly temperature averages in the sea and holding systems were obtained from the farmers.

Within a week of each sampling, 15 animals from each size class from the two feeding groups were committed to a live export trial. For the simulated export, animals were purged for three to four days before being packed into 20 kg polystyrene boxes for shipment. The animals are packed on thin, perforated polystyrene sheets, which serve as substrate for attachment. A sponge layer, slightly wetted with sea water, is added below the animals for sustaining high humidity and for absorbing excess water. The animals are packed into plastic bags filled with 100% oxygen and sealed. Finally gel type ice-packs are packed on a layer of sponge (to prevent direct contact between abalone and ice-packs) on top of the abalone. The polystyrene boxes were kept sealed for a total of 36 hours after which they were re-opened and the animals were re-weighed to determine the mass loss during the 36 hours simulated export.

Tissue samples were analysed for free glucose (Enzymatic colorimetric reaction; Roche GOD-PAP), glycogen (Keppler and Decker, 1983), proteins (Van Heerden *et al.*, 2004) and lipid content (Folch *et al.*, 1957). Haemolymph samples were analysed for free glucose (Enzymatic colorimetric reaction; Roche GOD-PAP).

Statistical analyses were conducted in collaboration with the Statistical Consulting Services of the North-West University, using the STATISTICA 7 (Statsoft Inc, 2005) and SAS 9.1 (SAS Institute, 2003) software packages. Factorial ANOVA's were conducted, and data were tested for homogeneity by means of Levene's test. In the case of non-conformance to homogeneity, data were transformed with logarithmic and/or Box-Cox transformations. Univariate tests for significance were used to test the effects of environmental parameters on the physiological parameters measured, and effect size was expressed as the p-η^2 (partial eta-squared) result. Subsequently Tukey's HSD tests were performed to test for significant differences ($p<0.05$) between cases. Multiple regression analysis was performed using SAS 9.1 software applying the R-SQUARE procedure.

Since the first sampling was conducted within a week after the planning meeting, some of the farms could not supply animals from both size classes and/or in both feeding regimes, and thus some of the data were not available for the spring sampling. The results are displayed as the Mean ± standard error of the mean (SEM) on the graphs, and in the discussion of the results. Differences were considered significant when $p<0.05$.

Results

Water temperature

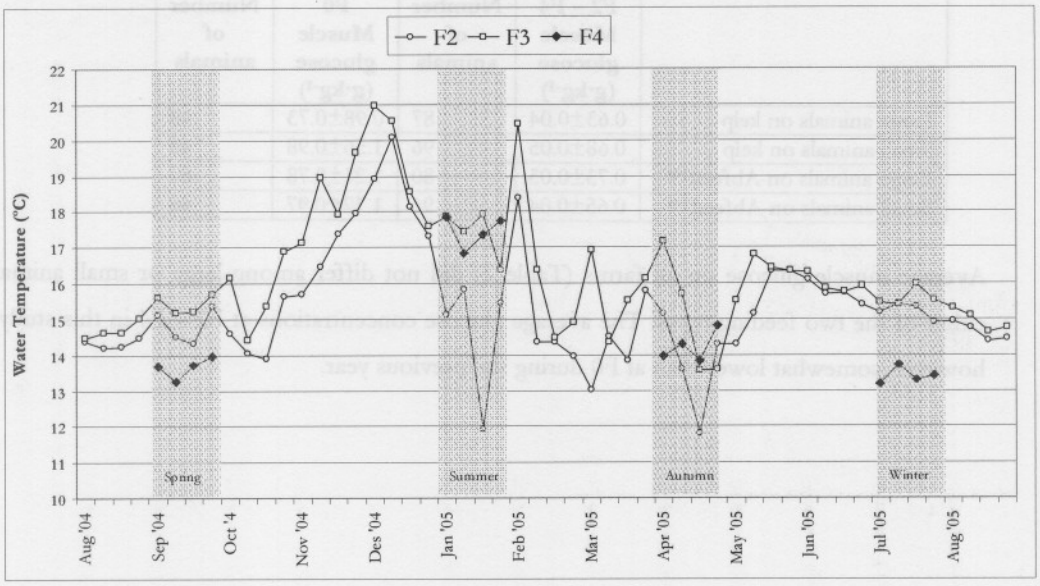

Figure 2: Water temperature profiles from August 2004 to August 2005 for farms F2 - F4. Shaded areas indicate the months that sampling was conducted. Only partial data were available for F4.

The average water temperature in the holding systems were not significantly different from the sea temperatures ($p>0.05$). There were no significant differences in temperature between farms in September 2004 (spring sampling), April 2005 (autumn sampling) and July 2005 (winter sampling). In January 2005 (summer sampling), however, water temperature at F2 was significantly lower than at F3 and F4 ($p<0.05$). Water temperature at F2 and F3 did not differ in summer. At F2, there were no significant differences in water temperatures in any sampling month. At F3, water temperature in January 2005 was significantly higher than in April 2005 ($p<0.05$), but did not differ from September 2004 or July 2005. At F4, water temperature in January 2005 was significantly higher than in September 2004, April 2005, and July 2005. Water temperatures in September 2004, April 2005, and July 2005 did not differ from one another.

Muscle Glucose

Muscle glucose concentration in animals on both kelp and Abfeed™ was affected by season ($p<0.001$) ($p\text{-}\eta^2=0.62$), and to a lesser extent, farm ($p<0.001$) ($p\text{-}\eta^2=0.20$). Animal size had no significant effect on muscle glucose concentration. The average muscle glucose concentration over all seasons, farms and feeds was $0.67 \pm 0.02\ g \cdot kg^{-1}$ ($n=359$), compared to $1.38 \pm 0.05\ g \cdot kg^{-1}$ ($n=384$) measured at F0 the previous season.

Table 2: Average muscle glucose concentration in animals of two size classes on different feed types.

	F2 – F4 Muscle glucose ($g \cdot kg^{-1}$)	Number of animals	F0 Muscle glucose ($g \cdot kg^{-1}$)	Number of animals
Large animals on kelp	0.63±0.04	87	0.98±0.73	48
Small animals on kelp	0.68±0.05	96	1.56±0.98	48
Large animals on Abfeed™	0.73±0.05	80	1.21±0.78	87
Small animals on Abfeed™	0.65±0.04	96	1.72±0.97	48

Average muscle glucose on all farms (Table 2) did not differ among large or small animals on either of the two feeding types. The average glucose concentrations at F2 – F4 in this study was, however, somewhat lower than at F0 during the previous year.

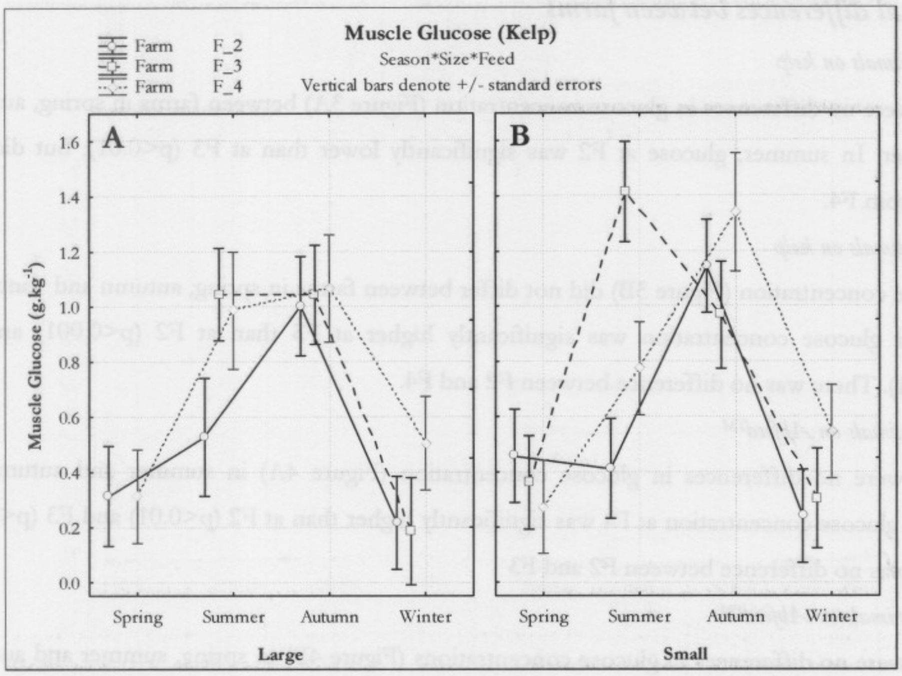

Figure 3: Seasonal changes in muscle glucose concentrations in (A) large and (B) small abalone from three farms, fed on a natural diet (kelp).

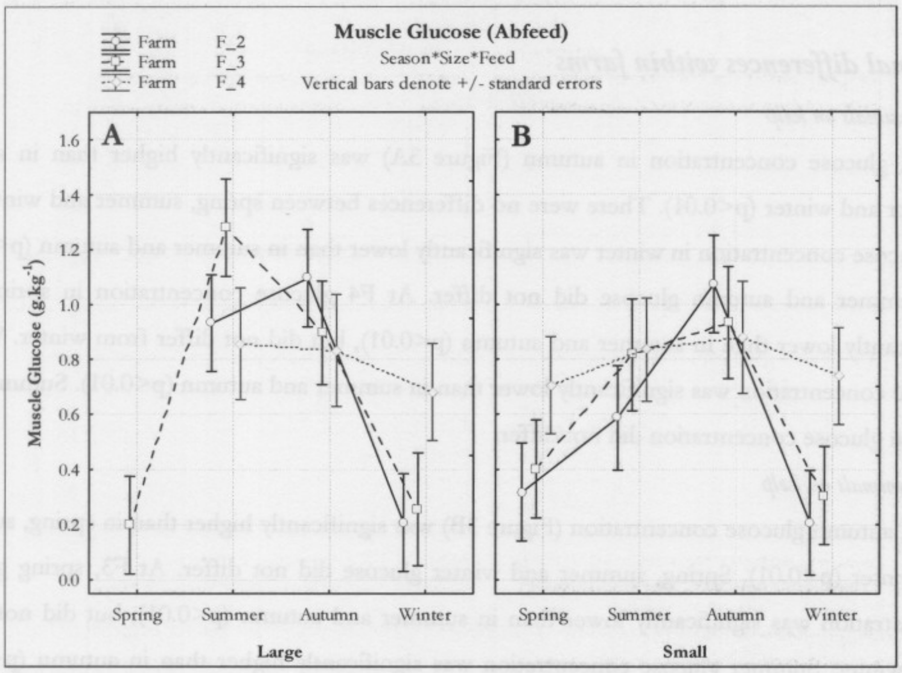

Figure 4: Seasonal changes in muscle glucose concentrations in (A) large and (B) small abalone from three farms, fed on a artificial feed (Abfeed™).

Seasonal differences between farms

Large animals on kelp

There were no differences in glucose concentration (Figure 3A) between farms in spring, autumn or winter. In summer, glucose at F2 was significantly lower than at F3 ($p<0.01$), but did not differ from F4.

Small animals on kelp

Glucose concentration (Figure 3B) did not differ between farms in spring, autumn and winter. In summer glucose concentration was significantly higher at F3 than at F2 ($p<0.001$) and F4 ($p<0.01$). There was no difference between F2 and F4.

Large animals on Abfeed™

There were no differences in glucose concentration (Figure 4A) in summer and autumn. In winter, glucose concentration at F4 was significantly higher than at F2 ($p<0.01$) and F3 ($p<0.05$). There was no difference between F2 and F3

Small animals on Abfeed™

There were no differences in glucose concentrations (Figure 4B) in spring, summer and autumn. In winter, glucose concentration at F4 was significantly higher than at F2 ($p<0.05$) and F3 ($p<0.05$). There was no difference between F2 and F3

Seasonal differences within farms

Large animals on kelp

At F2, glucose concentration in autumn (Figure 3A) was significantly higher than in spring, summer and winter ($p<0.01$). There were no differences between spring, summer and winter. At F3, glucose concentration in winter was significantly lower than in summer and autumn ($p<0.01$), but summer and autumn glucose did not differ. At F4 glucose concentration in spring was significantly lower than in summer and autumn ($p<0.01$), but did not differ from winter. Winter glucose concentration was significantly lower than in summer and autumn ($p<0.01$). Summer and autumn glucose concentration did not differ.

Small animals on kelp

At F2, autumn glucose concentration (Figure 3B) was significantly higher than in spring, summer and winter ($p<0.01$). Spring, summer and winter glucose did not differ. At F3, spring glucose concentration was significantly lower than in summer and autumn ($p<0.01$), but did not differ from winter. Summer glucose concentration was significantly higher than in autumn ($p<0.05$). Winter glucose was significantly lower than in summer and autumn ($p<0.01$). At F4, spring glucose was significantly lower than in summer and autumn ($p<0.01$) but did not differ from winter. Summer glucose was significantly lower than in autumn ($p<0.01$). Winter glucose concentration was significantly lower than in autumn ($p<0.01$), but did not differ from summer.

Large animals on Abfeed™

At F2, winter glucose concentration (Figure 4A) was significantly lower in summer and autumn ($p<0.001$). At F3, spring glucose concentration was significantly lower than in summer and autumn ($p<0.001$), but did not differ from winter. Winter glucose concentration was significantly lower than in summer and autumn ($p<0.001$). There was no difference between summer and autumn.

Small animals on Abfeed™

At F2, autumn glucose concentration (Figure 4B) was significantly higher than in spring, summer and winter ($p<0.01$). Spring, summer and winter concentrations did not differ. At F3, spring glucose concentration was significantly lower than in autumn ($p<0.01$), but did not differ from summer and winter. Winter glucose concentration was significantly lower than in summer ($p<0.05$) and autumn ($p<0.01$). Summer and autumn glucose concentrations did not differ.

Muscle Glycogen

Muscle glycogen concentration in animals on kelp was affected by size ($p<0.001$) ($p\text{-}\eta^2=0.29$) and to a lesser extent, by season ($p<0.001$) ($p\text{-}\eta^2=0.19$). In animals on Abfeed™, muscle glycogen concentration was affected by season ($p<0.001$) ($p\text{-}\eta^2=0.41$) and to a lesser extent, by farm ($p<0.001$) ($p\text{-}\eta^2=0.15$) and size ($p<0.001$) ($p\text{-}\eta^2=0.13$). The average glycogen concentration over all seasons, farms and feeds was 58.98 ± 2.82 g·kg^{-1} (n=351), compared to 43.28 ± 1.90 g·kg^{-1} (n=383) measured at F0 the previous year.

Table 3: Average muscle glycogen concentration in animals of two size classes on different feed types

	F2 – F4 Muscle glycogen (g·kg^{-1})	**Number of animals**	**F0 Muscle glycogen (g·kg^{-1})**	**Number of animals**
Large animals on kelp	38.79 ± 3.21	86	69.78 ± 4.35	48
Small animals on kelp	68.82±5.74	94	11.04±0.74	48
Large animals on Abfeed™	51.64±6.08	78	88.34±5.10	48
Small animals on Abfeed™	73.86±6.15	93	16.93±0.78	48

Average glycogen concentrations on the three farms in this study differed in terms of size and diet (Table 3). Large animals on kelp had the lowest overall glycogen content, which was also much lower than at F0 the previous year. Glycogen in large animals on Abfeed™ was higher than in large animals on kelp, but lower than at F0 the previous year and than small animals on either of the two feeds. Small animals on both feeds had similar glycogen concentrations, which were much higher than at F0 the previous year.

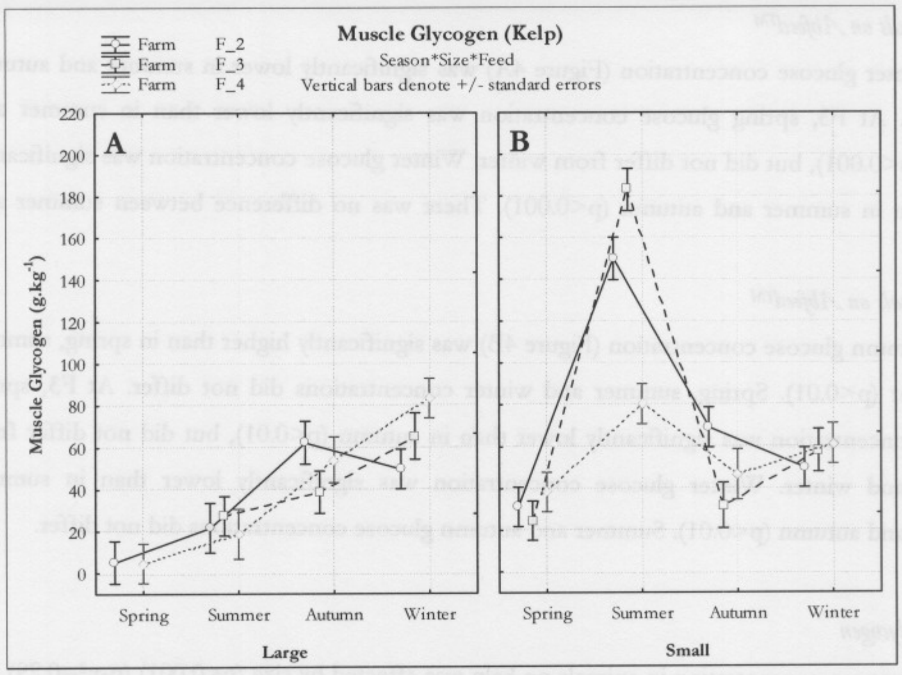

Figure 5: Seasonal changes in muscle glycogen concentration of (A) large and (B) small abalone fed on a natural diet of kelp.

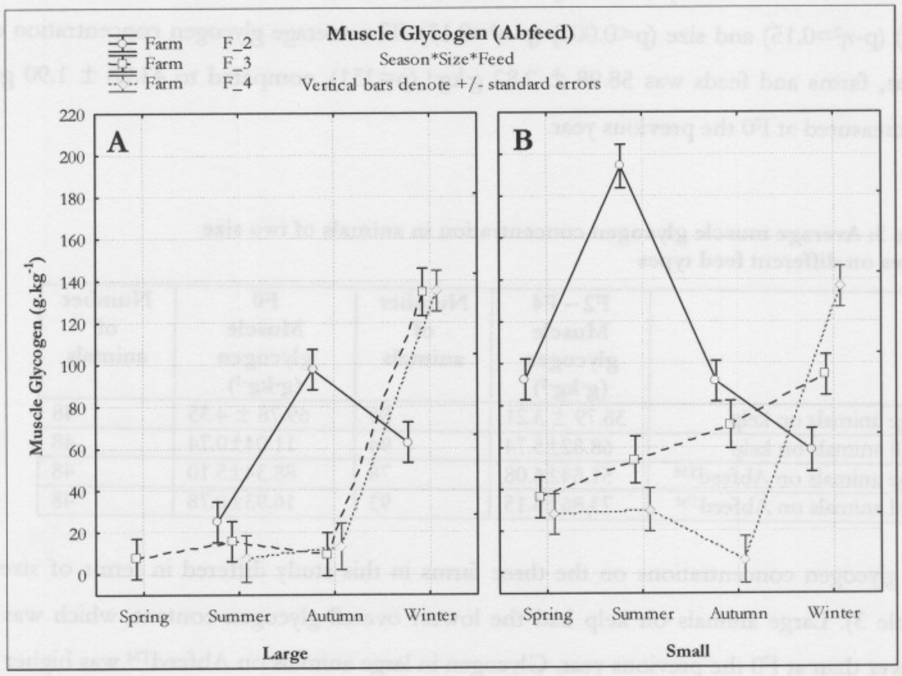

Figure 6: Seasonal changes in muscle glycogen concentration of (A) large and (B) small abalone fed on the artificial diet, Abfeed^{TM}.

Seasonal differences between farms

Large animals on kelp

There were no differences in glycogen concentration (Figure 5A) between farms in any season.

Small animals on kelp

In spring, autumn and winter, there were no differences in glycogen concentration between farms (Figure 5B). In summer, glycogen concentration was significantly lower at F4 than at F2 and F3 ($p<0.001$). Glycogen concentration at F2 and F3 did not differ.

Large animals on Abfeed™

In summer there were no differences in glycogen concentration between farms (Figure 6A). In autumn, glycogen concentration at F2 was significantly higher than at F3 and F4 ($p<0.001$). F3 and F4 did not differ. In winter, glycogen concentration was significantly lower at F2 than at F3 and F4 ($p<0.01$). F3 and F4 did not differ.

Small animals on Abfeed™

In spring, glycogen concentration (Figure 6B) at F2 was significantly higher than at F3 and F4 ($p<0.05$). F3 and F4 did not differ. In summer, glycogen concentration at F2 was significantly higher than at F3 and F4 ($p<0.05$). F3 and F4 did not differ. In autumn, glycogen concentration at F4 was significantly lower than at F2 and F3 ($p<0.01$). F2 and F3 did not differ. In winter, glycogen concentration at F2 was significantly lower than at F4 ($p<0.05$). Glycogen concentration at F3 did not differ from either F2 or F4.

Seasonal differences within farms

Large animals on kelp

At F2, spring glycogen concentration (Figure 5A) was significantly lower than in autumn and winter ($p<0.01$), but did not differ from summer. Autumn glycogen concentration was significantly higher than in summer ($p<0.01$), but did not differ from winter. At F3, summer glycogen concentration differed significantly from that in winter ($p<0.01$). Autumn glycogen concentration did not differ from either summer or winter. At F4, spring glycogen concentration was significantly lower than in autumn and winter ($p<0.01$). Spring and summer glycogen concentration did not differ. Summer glycogen concentration was significantly lower than those in autumn ($p<0.05$) and winter ($p<0.01$). Autumn and winter concentration did not differ.

Small animals on kelp

At F2, spring glycogen concentration (Figure 5B) was significantly lower than that in summer ($p<0.001$). Spring glycogen concentration did not differ from those in autumn and winter. Summer glycogen concentration was significantly higher than in autumn and winter ($p<0.001$). Autumn and winter concentrations did not differ. At F3 spring glycogen concentration was significantly lower than that in summer ($p<0.001$). Spring glycogen concentration did not differ from those in autumn and winter. Summer glycogen concentration was significantly higher than in autumn and winter ($p<0.001$). Autumn and winter concentration did not differ. At F4 there were no seasonal differences in glycogen concentration.

Large animals on Abfeed™

At F2, summer glycogen concentration (Figure 6A) was significantly lower than in autumn ($p<0.001$) and winter ($p<0.05$). Autumn glycogen concentration was significantly higher than in winter ($p<0.05$). At F3, spring glycogen concentration did not differ from those in spring or summer. Winter glycogen concentration was significantly higher than in spring, summer and autumn ($p<0.001$). At F4, summer and autumn glycogen concentrations did not differ. Winter glycogen concentration was significantly higher than in summer and autumn ($p<0.001$).

Small animals on Abfeed™

At F2, spring glycogen concentration (Figure 6B) did not differ from those in autumn and winter. Summer glycogen concentration was significantly higher than in spring, autumn and winter ($p<0.001$). Autumn and winter glycogen concentrations did not differ. At F3 there were no seasonal differences in glycogen concentration. At F4, spring, summer and autumn glycogen concentrations did not differ. Winter glycogen concentration was significantly higher than in spring, summer and autumn ($p<0.001$).

Haemolymph Glucose

Haemolymph glucose concentration in animals on both kelp and Abfeed™ was affected only by season ($p<0.001$) ($p\text{-}\eta^2=0.17$). The average haemolymph glucose concentration over all seasons, farms and feeds was 0.07 ± 0.01 $g \cdot kg^{-1}$ ($n=355$). Haemolymph glucose concentration was not measured at F0 the previous year, thus no comparison is possible.

Table 4: Average haemolymph glucose concentration in animals of two size classes on different feed types.

	F2 – F4 Haemolymph glucose ($g \cdot L^{-1}$)	**Number of animals**
Large animals on kelp	0.08 ± 0.01	87
Small animals on kelp	0.07 ± 0.01	96
Large animals on Abfeed™	0.07 ± 0.01	76
Small animals on Abfeed™	0.08 ± 0.01	96

Based on average haemolymph glucose concentrations (Table 4) there were clearly no differences between different size classes or feeding types.

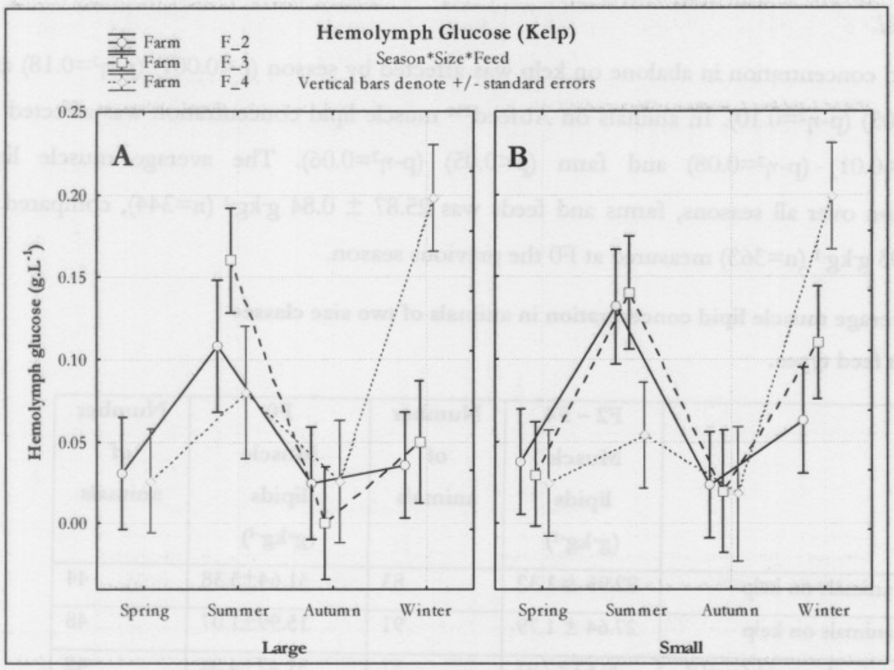

Figure 7: Seasonal change in haemolymph glucose concentration in (A) large and (B) small abalone fed a natural diet (kelp)

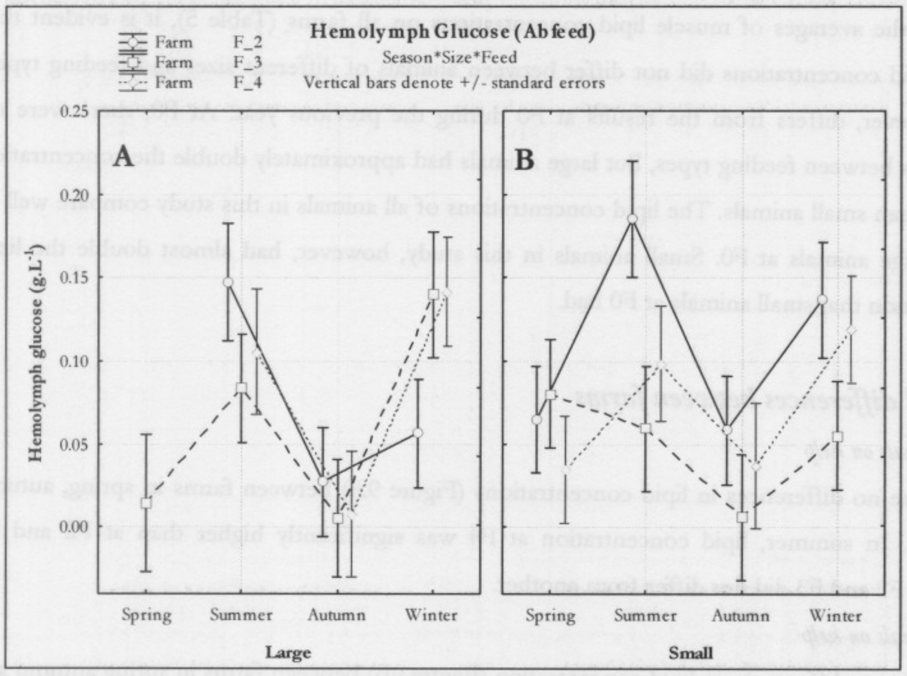

Figure 8: Seasonal changes in haemolymph glucose concentration in (A) large and (B) small abalone fed an artificial diet (Abfeed^{TM}).

Haemolymph glucose concentrations were highly variable. There were no significant differences in haemolymph glucose concentrations between different farms and feed types.

Muscle Lipid

Muscle lipid concentration in abalone on kelp was affected by season ($p<0.001$) ($p\text{-}\eta^2=0.18$) and farm ($p<0.05$) ($p\text{-}\eta^2=0.10$). In animals on Abfeed™ muscle lipid concentration was affected by season ($p<0.01$) ($p\text{-}\eta^2=0.08$) and farm ($p<0.05$) ($p\text{-}\eta^2=0.06$). The average muscle lipid concentration over all seasons, farms and feeds was 25.87 ± 0.84 $g \cdot kg^{-1}$ (n=344), compared to 24.37 ± 1.03 $g \cdot kg^{-1}$ (n=363) measured at F0 the previous season.

Table 5: Average muscle lipid concentration in animals of two size classes on different feed types.

	F2 – F4 Muscle lipids ($g \cdot kg^{-1}$)	**Number of animals**	**F0 Muscle lipids ($g \cdot kg^{-1}$)**	**Number of animals**
Large animals on kelp	22.95 ± 1.32	83	31.64±3.38	44
Small animals on kelp	27.64 ± 1.79	91	15.99±1.07	48
Large animals on Abfeed™	29.63±2.01	77	31.42±4.84	42
Small animals on Abfeed™	27.64±1.79	91	15.51±0.94	48

Based on the averages of muscle lipid concentrations on all farms (Table 5), it is evident that muscle lipid concentrations did not differ between animals of different sizes and feeding types. This, however, differs from the results at F0 during the previous year. At F0, there were no differences between feeding types, but large animals had approximately double the concentration of lipids than small animals. The lipid concentrations of all animals in this study compare well to that of large animals at F0. Small animals in this study, however, had almost double the lipid concentration that small animals at F0 had.

Seasonal differences between farms

Large animals on kelp

There were no differences in lipid concentrations (Figure 9A) between farms in spring, autumn or winter. In summer, lipid concentration at F4 was significantly higher than at F2 and F3 ($p<0.05$). F2 and F3 did not differ from another.

Small animals on kelp

There were no differences in lipid concentration (Figure 9B) between farms in spring autumn and winter. In summer, lipid concentration at F4 was significantly higher than at F2 and F3 ($p<0.05$). F2 and F3 did not differ from another.

Large animals on Abfeed™

There were no differences in lipid concentration (Figure 10A) between farms in any season.

Small animals on Abfeed™

There were no differences in lipid concentration (Figure 10B) between farms in spring autumn and winter. In summer, lipid concentration at F3 was significantly different from that at F2 ($p<0.01$). Lipid concentration at F4 did not differ from that of F2 or F3.

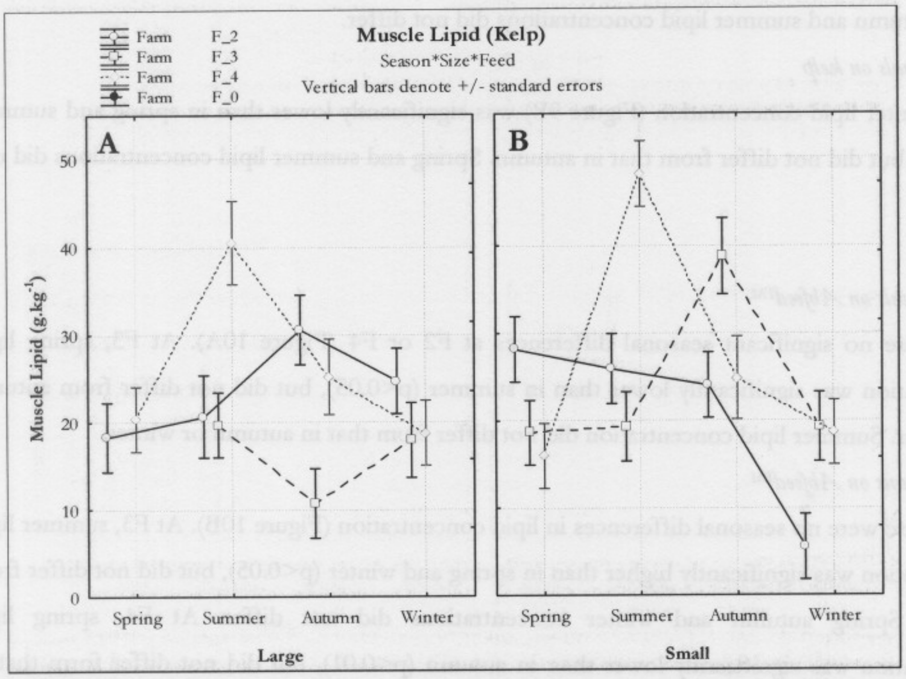

Figure 9: Seasonal change in muscle lipid concentration in (A) large and (B) small abalone fed a natural diet (kelp).

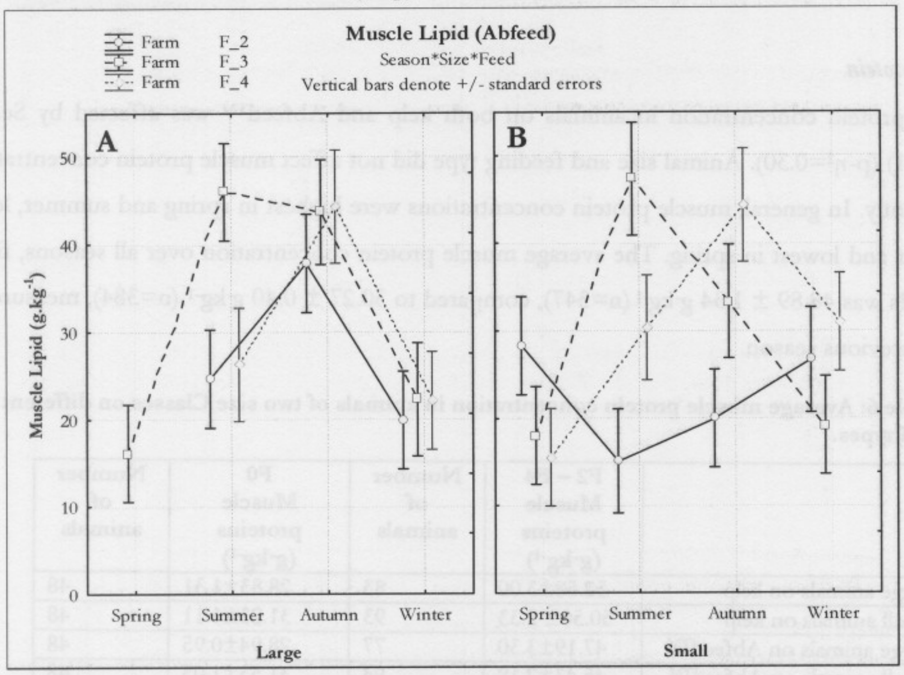

Figure 10: Seasonal changes in muscle lipid concentration in (A) large and (B) small abalone fed an artificial diet (Abfeed™).

Seasonal differences within farms

Large animals on kelp

Neither F2 nor F3 had significant differences in seasonal lipid concentration (Figure 9A). At F4, summer lipid concentration was significantly higher than in spring ($p<0.05$) and winter ($p<0.01$). Spring autumn and summer lipid concentrations did not differ.

Small animals on kelp

At F2, winter lipid concentration (Figure 9B) was significantly lower than in spring and summer ($p<0.05$), but did not differ from that in autumn. Spring and summer lipid concentrations did not differ.

Large animals on Abfeed™

There were no significant seasonal differences at F2 or F4 (Figure 10A). At F3, spring lipid concentration was significantly lower than in summer ($p<0.05$), but did not differ from autumn and winter. Summer lipid concentration did not differ from that in autumn or winter.

Small animals on Abfeed™

At F2 there were no seasonal differences in lipid concentration (Figure 10B). At F3, summer lipid concentration was significantly higher than in spring and winter ($p<0.05$), but did not differ from autumn. Spring autumn and winter concentrations did not differ. At F4, spring lipid concentration was significantly lower than in autumn ($p<0.01$), but did not differ form that in summer and winter. Summer autumn and winter lipid concentrations did not differ.

Muscle protein

Muscle protein concentration in animals on both kelp and Abfeed™ was affected by Season ($p<0.001$) ($p\text{-}\eta^2=0.30$). Animal size and feeding type did not affect muscle protein concentrations significantly. In general, muscle protein concentrations were highest in spring and summer, lower in winter and lowest in spring. The average muscle protein concentration over all seasons, farms and feeds was 48.89 ± 1.34 $g \cdot kg^{-1}$ ($n=347$), compared to 30.27 ± 0.40 $g \cdot kg^{-1}$ ($n=384$), measured at F0 the previous season.

Table 6: Average muscle protein concentration in animals of two size Classes on different feed types.

	F2 – F4 Muscle proteins ($g \cdot kg^{-1}$)	Number of animals	F0 Muscle proteins ($g \cdot kg^{-1}$)	Number of animals
Large animals on kelp	52.58±3.00	83	28.83±1.31	48
Small animals on kelp	50.50± 2.33	93	31.23±1.11	48
Large animals on Abfeed™	47.19±3.30	77	28.94±0.95	48
Small animals on Abfeed™	45.43±2.19	94	31.53±1.05	48

Average protein concentration for all farms in this study (Table 6), did not vary much between different size classes and feeding types. The average protein concentration in this study was, however, somewhat higher than average protein concentrations at F0 the previous year.

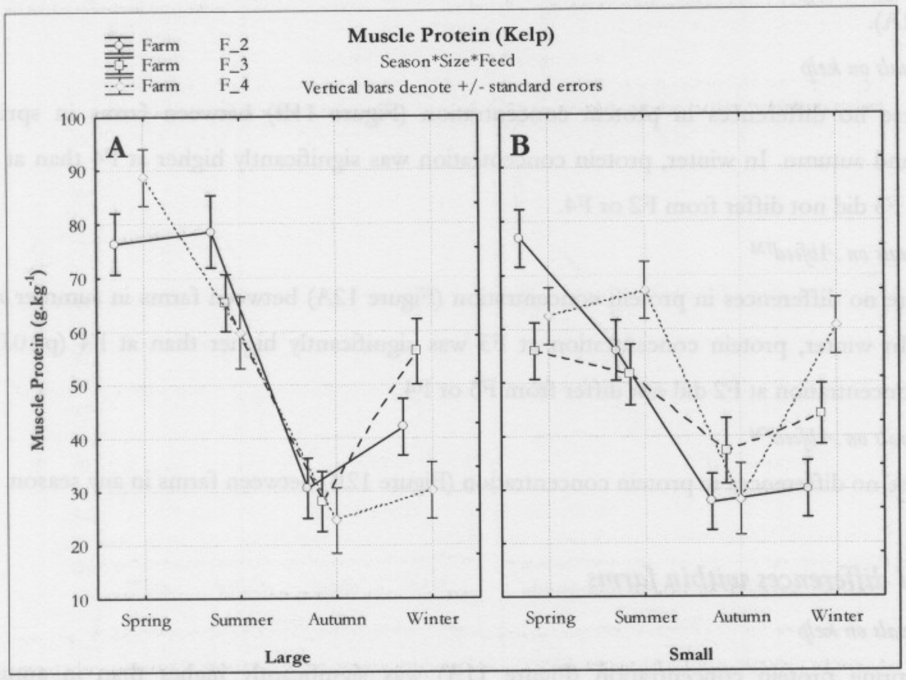

Figure 11: Seasonal change in muscle protein concentration in (A) large and (B) small abalone fed a natural diet (kelp).

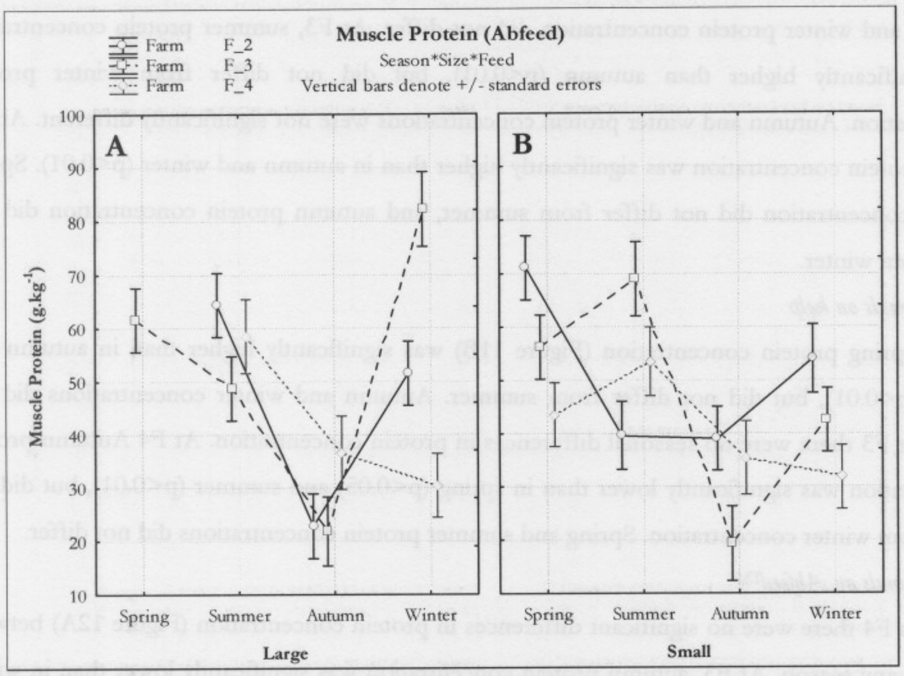

Figure 12: Seasonal change in muscle protein concentration in (A) large and (B) small abalone fed an artificial diet (Abfeed^{TM}).

Seasonal differences between farms

Large animals on kelp

There were no significant differences in protein concentrations between farms in any season (Figure 11A).

Small animals on kelp

There were no differences in protein concentration (Figure 11B) between farms in spring, summer and autumn. In winter, protein concentration was significantly higher at F4 than at F2 ($p<0.05$). F3 did not differ from F2 or F4.

Large animals on Abfeed™

There were no differences in protein concentration (Figure 12A) between farms in summer and autumn. In winter, protein concentration at F3 was significantly higher than at F4 ($p<0.05$). Protein concentration at F2 did not differ from F3 or F4.

Small animals on Abfeed™

There were no differences in protein concentration (Figure 12B) between farms in any season.

Seasonal differences within farms

Large animals on kelp

At F2, spring protein concentration (Figure 11A) was significantly higher than in autumn ($p<0.01$) and winter ($p<0.05$), but did not differ from summer. Summer protein concentration was significantly higher than autumn ($p<0.01$) and winter ($p<0.05$) protein concentrations. Autumn and winter protein concentration did not differ. At F3, summer protein concentration was significantly higher than autumn ($p<0.01$), but did not differ from winter protein concentration. Autumn and winter protein concentrations were not significantly different. At F4, spring protein concentration was significantly higher than in autumn and winter ($p<0.01$). Spring protein concentration did not differ from summer, and autumn protein concentration did not differ from winter.

Small animals on kelp

At F2, spring protein concentration (Figure 11B) was significantly higher than in autumn and winter ($p<0.01$), but did not differ from summer. Autumn and winter concentrations did not differ. At F3 there were no seasonal differences in protein concentration. At F4 Autumn protein concentration was significantly lower than in spring ($p<0.05$) and summer ($p<0.01$), but did not differ from winter concentration. Spring and summer protein concentrations did not differ.

Large animals on Abfeed™

At F2 an F4 there were no significant differences in protein concentration (Figure 12A) between farms in any season. At F3, autumn protein concentration was significantly lower than in winter ($p<0.05$). Spring, summer and winter concentrations did not differ.

Small animals on Abfeed™

At F2, spring protein concentration (Figure 12B) was significantly higher than in autumn and winter ($p<0.01$), but did not differ from summer protein concentration. Summer, autumn and winter protein concentrations did not differ. At F3, autumn protein concentration was significantly lower than in spring ($p<0.05$) and summer ($p<0.01$), but did not differ from winter. Spring and summer concentrations did not differ. At F4, there were no seasonal differences in protein concentration.

Mass Loss

Mass loss in animals on kelp was affected only by season ($p<0.001$) ($p\text{-}\eta^2=0.32$). In animals on Abfeed™, mass loss was affected by season ($p<0.001$) ($p\text{-}\eta^2=0.10$) and size ($p<0.05$) ($p\text{-}\eta^2=0.03$). Overall, animals over all seasons from all farms, sizes and feeding types lost between 1.63% and 57.25% of their body mass during the live export trials. The average mass loss of 14.49±0.28% (n=639) compares to the 13.211 ± 0.61% (n=105) measured at F0 the previous season, and with the 15% average mass loss reported by Vosloo and Vosloo (2006).

Table 7: Average simulated export mass loss in animals of two size classes on different feed types.

	F2 – F4 Mass loss (%M_b)	Number of animals
Large animals on kelp	14.45±0.47	186
Small animals on kelp	14.44±0.60	164
Large animals on Abfeed™	14.89±0.59	127
Small animals on Abfeed™	14.29±0.56	162

Based on average mass losses on all farms (Table 7), it is clear that neither size nor feeding type influenced export performance in this study.

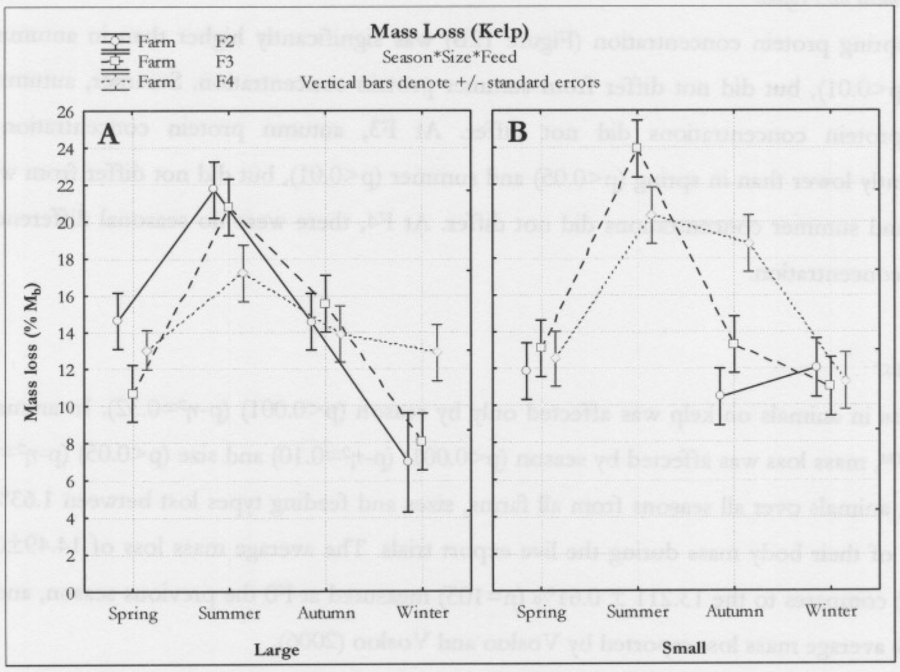

Figure13: Mass loss (%M_b) experienced during 36-hour simulated export by (A) large and (B) small animals from three farms on a natural diet (kelp).

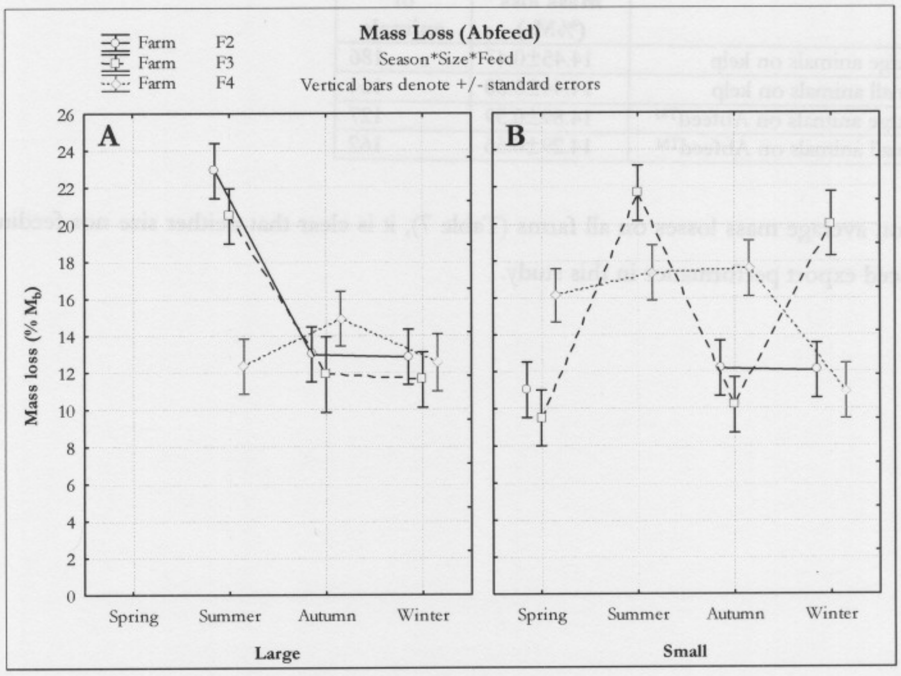

Figure 14: Mass loss (%M_b) experienced during 36-hour simulated export by (A) large and (B) small animals from three farms on an artificial diet (Abfeed^{TM}).

Seasonal differences between farms

Large animals on kelp

There were no differences in mass loss (Figure 13A) experienced between different farms in any season.

Small animals on kelp

There were no differences in mass loss (Figure 13B) experienced between different farms in any season.

Large animals on Abfeed™

In summer, mass loss at F4 (Figure 14A) was significantly lower than at F2 ($p<0.001$) and F3 ($p<0.01$). Mass loss at F2 and F3 did not differ. In autumn and winter there were no differences in mass loss experienced between different farms.

Small animals on Abfeed™

In spring, there were no differences in mass loss experienced between different farms (Figure 14B). In summer, mass loss was significantly higher at F3 than at F4 ($p<0.05$). In autumn, mass loss was significantly higher at F4 than at F2 ($p<0.05$), but did not differ from F3. Mass loss at F2 and F3 did not differ. In winter, there were no differences in mass loss experienced between different farms.

Seasonal differences within farms

Large animals on kelp

At F2, mass loss in summer (Figure 13A) was significantly higher than in spring, autumn and winter ($p<0.001$). Spring, autumn and winter mass loss did not differ. At F3, mass loss in summer was significantly higher than in spring, autumn and winter ($p<0.001$). Spring, autumn and winter mass loss did not differ. At F4, mass loss in summer was significantly higher in summer than in spring and winter ($p<0.01$), but did not differ from autumn. Mass loss did not differ between spring and winter, or between summer and autumn.

Small animals on kelp

At F2, mass loss in summer (Figure 13B) was significantly higher than in spring, autumn and winter ($p<0.001$). Spring, autumn and winter mass loss did not differ. At F3, mass loss in summer was significantly higher than in spring, autumn and winter ($p<0.001$). Spring, autumn and winter mass loss did not differ. At F4, mass loss in summer was significantly higher than in spring and winter ($p<0.01$), but did not differ from autumn. Mass loss did not differ between spring and winter, or between summer and autumn

Large animals on Abfeed™

At F2, mass loss in summer (Figure 14A) was significantly higher than in spring, autumn and winter ($p<0.001$). Spring, autumn and winter mass loss did not differ. At F3, mass loss in summer was significantly higher than in spring, autumn ($p<0.001$) and winter ($p<0.05$). Spring, autumn and winter mass loss did not differ. At F4, mass loss did not differ in any season.

Small animals on Abfeed™

At F2, mass loss in summer (Figure 14B) was significantly higher than in spring, autumn and winter ($p<0.001$). Spring, autumn and winter mass loss did not differ. At F3, mass loss in summer was significantly higher than in spring, autumn ($p<0.001$) and winter ($p<0.05$). Spring, autumn and winter mass loss did not differ. At F4, mass loss did not differ in any season.

Discussion

Several studies have been conducted to assess basic physiological constituents like proteins (Mai *et al.*, 1995b; Shipton and Britz, 2002a; Bautista-Teruel *et al.*, 2003; Gómez-Montes *et al.*, 2003; Sales *et al.*, 2003), lipids (Dunstan *et al.*, 1994, 1996; Mai *et al.*, 1995a; Nelson *et al.*, 2002; Thongrod *et al.*, 2003; Durazo-Beltrán *et al.*, 2004) and carbohydrates (Donovan *et al.*, 1999; Gómez-Montes *et al.*, 2003; O'omolo *et al.*, 2003; Vosloo *et al.*, 2003) in abalone. Most of these studies, however, measured physiological constituents, or changes thereof, in reaction to natural or induced stresses. Studies on seasonal variations in chemical composition in wild abalone have been investigated by Watanabe *et al.* (1992) and by Hatae *et al.* (1995) on *H. discus*. No long-term studies relating the basic physiological composition to the condition of abalone in aquaculture systems have been published.

The nutrition of abalone is strongly carbohydrate based, with carbohydrates making up 12-64% of organic matter ingested. (Leighton, 2000; Picos-García *et al.*, 2000; Gómez-Montes *et al.*, 2003; Braid *et al.*, 2005). It thus follows that glucose and glycogen would be important constituents in abalone muscle tissue, as sources of available and stored energy.

The significantly higher muscle glucose concentrations in summer and autumn may be indicative of increased energy needs in these seasons. Glucose can be liberated from glycogen stores, or from the carbohydrate-rich food preferred by abalone. Results from a study on farm raised *H. midae* by Laas & Vosloo (unpublished) the previous year indicated that growth rate was highest in summer and autumn. This corresponds to findings by Britz *et al.* (1997), which indicated increased growth rates with increased temperatures up to 20°C, but is in contrast with findings by Tarr (2000a) in natural populations of *H. midae*. Higher growth rates would, however, increase the energy demand of the abalone that could result in increased glucose provision to the muscle tissues. As water temperatures generally reached higher temperatures in summer and autumn (Figure 2) than in spring and winter, temperature could be an important factor regulating the higher energy demand in summer and autumn. Metabolic reactions are facilitated by enzymes that are highly energy sensitive, and metabolic rate usually increases with increased temperature (Randall *et al.*, 1997; Schmidt-Nielsen, 1998).

The lack of significant differences in glucose concentrations of large and small animals indicates that both large and small animals are faced with similar elevated energy needs during summer and autumn. Feeding type had no significant effect on the muscle glucose concentration of either large or small animals, indicating that sufficient glucose for energetic needs can be liberated from either natural or artificial feed.

The significantly lower ($p<0.05$) muscle glucose concentrations in this study compared to a study in the preceding year (Laas and Vosloo, unpublished), might be indicative of different prevailing conditions between the two years or it may be indicative of different management strategies applied. This is, however, difficult to confirm, as no other farms were included in the preceding exploratory study.

Haemolymph Glucose

Haemolymph glucose concentration overall was affected by season, but not by farm or feeding type. This is indicative of fairly tight regulation of haemolymph glucose concentrations in *H. midae.* Haemolymph concentrations did not follow the same trends observed in muscle glucose concentrations, indicating that haemolymph and muscle glucose concentrations are regulated independently. Jorgensen *et al.* (1984) observed that although the circulatory system is open, haemolymph flow is directed in a tissue specific manner in *H. cracherodii.* The foot of *H. cracherodii* that makes up 66% of the wet mass (excluding shell) and receives 27% of the cardiac output, while the digestive gland represents only 6% of the body mass and yet receives 13% of the cardiac output. Thus it is likely that haemolymph glucose is preferentially obtained from the glucose liberated from food in the digestive system. The high variability in haemolymph glucose concentration, however, has obscured any clear patterns.

Muscle Glycogen

Glycogen is the main energy storage component (Baldwin *et al.*, 1992; Picos-García *et al.*, 2000; Gómez-Montes *et al.*, 2003; Braid *et al.*, 2005), and thus differences in muscle glycogen concentrations can be linked to differences in the ability to acquire and store energy from their diet. Reduced glycogen levels can, however, also be an indication of stress in the animals, requiring them to mobilise glucose from glycogen stores to deal with elevated energetic needs (Baldwin *et al.*, 1992; Taylor *et al.*, 1997; O'omolo *et al.*, 2003; Nollens *et al.*, 2004; Braid *et al.*, 2005). This decrease in muscle glycogen concentration with increasing energetic demand is, however, normally associated with a similar increase in haemolymph glucose concentrations

(Taylor *et al.*, 1997; Nollens *et al.*, 2004), which was not evident in this study. One plausible explanation for this observation is that the increase in muscle glucose associated with a decrease in muscle glycogen levels will be on a short-term basis, as the glucose liberated from glycogen will be used to fulfil increased energetic needs during times of stress.

Large animals on kelp and Abfeed™ had on average significantly lower ($p<0.05$) muscle glycogen concentrations than was measured at F0 the previous year, and small animals on the other hand, had significantly higher ($p<0.05$) muscle glycogen concentrations than was the case at F0. This could be indicative of changes in the culture environment over the two years of sampling, or of different management practices. The exact reasons for these differences are, however, unclear.

Muscle Lipids

Various studies have indicated that lipid nutrition is more dependent on the presence of certain essential fatty acid, than on gross lipid intake (Mai *et al.*, 1995a; Dunstan *et al.*, 1996; Leighton, 2000; Durazo-Beltrán *et al.*, 2004). The lack of significant difference in lipid concentration of large and small animals compare to findings by Dunstan *et al.* (1996) on juvenile and adult *H. laevigata.* In contrast with findings by Mai *et al.* (1995), Dunstan *et al.* (1996) and Nelson *et al.* (2002), feeding type did not have a significant effect on the muscle lipid concentrations in this study. This indicates that the high lipid concentration in the artificial diet (5.3%) relative to the natural diet (0.45%), does not contribute towards a higher total lipid content of the animals, although it may, however, have an advantage at the level of essential fatty acids. Studies have revealed low lipase activities in the gut of *H. tuberculata, H. discus* (Mai *et al.,* 1995) and *H. rufescens* (Leighton, 2000), indicating that abalone may not be able to utilise large lipid concentrations in their diets. Lipid concentrations of higher than 4 - 5% have also been indicated to have a negative effect on protein uptake and growth (Mai *et al.*, 1995a; Dunstan *et al.*, 1994; Durazo-Beltrán *et al.*, 2003). Although season did affect the muscle lipid content, large and small animals on different farms and different feeding types showed different trends in their seasonal lipid content. This may be indicative that muscle lipid content might be too variable to successfully implement as a predictive management tool.

Muscle Protein

The seasonal variation in muscle protein concentration in this study was similar to seasonal variation in protein concentration of *H. discus.* (Hatae *et al.*, 1995). This indicates seasonal variation in the protein requirements of abalone, which has implications for the formulated feed industry. Abalone do not have a need for protein as such, but rather for an appropriate level and balance of individual amino acids (King *et al.*, 1996; Shipton *et al.*, 2002b). Thus it is possible that the seasonal differences in tissue protein concentration reflect seasonal differences in the amino acid balance of abalone. The lack of differences in protein concentration of abalone on different feeds is contradictory to findings by Britz & Hecht (1997). This result, however, indicates that increased dietary protein concentrations do not increase tissue protein concentration. Results from a previous study by Laas & Vosloo (unpublished) indicated that neither tissue protein concentration, nor average growth rate differed between two diets with different protein concentrations.

Mass Loss

The average mass loss experienced by animals during the simulated export trials compares well to mass loss reported by industry. This makes it possible to study export mass loss under controlled conditions. The significant difference between minimum and maximum mass losses ($p<0.001$) clearly indicates that some animals are better capable to withstand the rigours of live export than others. The significantly higher mass losses observed for all animals in summer indicate that seasonal changes in the condition of abalone will affect their ability to withstand live export conditions. It will be advantageous to limit the live export of animals in summer. The results indicate that summer is the worst season for exporting live animals. In this study, animal size did not have the same pronounced effect on the export performance of the animals in terms of mass loss than was observed in a previous study. Maximum mass losses in small animals, however, were higher than in large animals. Animals from different farms did not differ significantly in terms of mass loss experienced. This indicates that neither management practices nor different environmental conditions had a significant effect on the performance of animals in terms of mass loss. Different diets did not influence the ability of animals to withstand the stresses of live export. This indicates that current diets do not contribute towards better performance in terms of live-export mass loss. Like in a previous study by the authors (Laas & Vosloo, Unpublished), season and animal size were the main factors influencing live-export performance in *H. midae.*

Conclusions

Season is the most important driver of abalone physiology, and it affected muscle glucose, glycogen, lipid and protein concentration, haemolymph glucose concentration and ultimately mass loss during simulated export. There were signs of increased metabolic energy demands in summer, which may be responsible for much of the seasonal effects on abalone physiology. Animal size did not prove to be an important factor, but further studies, expanding the range of size classes analysed are strongly advisable to validate this observation. Feed type also had limited effect on physiological constituents in abalone. Thus, although the feeds currently available may satisfy the basic nutritional needs of abalone, there are leeway for further development and improvement. The reasons for differentiation between different farms were not clear, but the observed differences may have been brought about by different management practices. Mass loss experienced during simulated live export was significantly higher in summer, relative to other seasons, and it would be advantageous to limit live exports during summer to the minimum. It is important to determine to what extent farm and environmental parameters to influence export performance of animals. Multiple regressions and canonical analysis of variances should provide insight as to which physiological, farm and environmental parameters govern the performance and quality of the animals destined for the export market. With this knowledge on hand, abalone farmers can manage pro-actively for best quality and yield of their abalone.

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3.4. Paper IV:

Advances in using physiological parameters in the management of South African abalone (*Haliotis midae*) mariculture: Correlating physiological parameters to mass loss.

To be submitted to Aquaculture.

Faculty Requirements

Letter of consent (Appendix 7)

Instructions to Authors (Appendix 9)

3.4.1. Original paper

Advances in using physiological parameters in the management of South African abalone (*Haliotis midae*) mariculture: Correlating physiological parameters to mass loss

André Laas* and André Vosloo

* Corresponding author:

School for Environmental Sciences and Development, Zoology
North-West University (Potchefstroom campus)
Private Bag x6001
Potchefstroom
2520

E-mail: 11777915@nwu.ac.za
Cell phone: 083 661 9421

Abstract

During live export, abalone are exposed to unnatural stressors, causing animals to lose on average 15% of their body mass. This relates to an equal loss of revenue to aquacultural abalone producers. Physiological condition of abalone has been studied seasonally in two phases on four farms between 2003 and 2005. Physiological parameters have been correlated to mass loss during simulated export events to find ways to mitigate mass loss during live export.

Phase 1 was a single farm experiment where large and small abalone were kept on four feeding regimes and sampled six-weekly, with a single simulated live export event at the end of sampling. Average simulated export mass loss by all animals was 13.20±0.62 (n=104) ranging between 12.78±5.89% M_b and 21.8±6.78% M_b. Larger animals were more resistant to mass loss than small animals. Muscle glycogen (Adj R^2=0.53), animal size (Adj R^2=0.47) and muscle glucose (Adj R^2=0.28) were the best predictors of mass loss during Phase 1. Principle Component Analysis (PCA) indicated that feed constituents did not influence mass loss during simulated export. High mass loss associated with high tissue glucose, while low mass loss associated with high tissue glycogen and lipid concentrations.

Phase 2 was conducted on three farms, where animals were fed on two diets. Samples for analysis of physiological parameters were collected seasonally, followed by simulated export events. Average mass loss by all animals during Phase 2 was 14.53±0.28% M_b ranging between 6.94±1.52% M_b and 23.92±0.70% M_b. Mass loss was significantly influenced ($p<0.0001$) by season, with highest mass losses in summer, and lowest mass losses in winter. Animal size and feeding type did not have significant effects on mass loss ($p>0.005$). Muscle glucose concentration was the only predictor of mass loss (Adj R^2=0.22). PCA results indicated that dietary constituents did not associate with mass loss. High mass loss associated with high tissue glucose, high water temperature and high tissue lipids, and low mass loss associated with high tissue protein and glycogen and with high haemolymph glucose.

The study provides a comprehensive set of reference values and concluded that optimal physiological conditions for simulated export are reached during winter and are characterised by high muscle glycogen and protein and low muscle glucose and lipid concentrations.

Introduction

Driven by an almost insatiable international market, high prices and dwindling natural stock, investment in the abalone aquaculture industry is an expensive, but lucrative venture. In 2005 the aquacultural production by South African farms was estimated to be around 700 tons, and is projected to increase to 1200 ton by 2010. An approximate 62% of the abalone produced by aquaculture in South Africa is exported live (Du Plessis, 2006). During live exports these aquatic marine animals are packed in bags filled with 100% oxygen, humidified by seawater, and transported on ice in sealed polystyrene containers (Sales & Britz, 2001). During the 30 to 42 hours of aerial exposure, abalone on average loose 15% body mass. As farmers are paid on landed mass, this corresponds to a 15% loss of foreign revenue (Vosloo & Vosloo, 2006).

Studies by Vosloo (2003) on the water balance of *Haliotis midae* during simulated live export attributed the total mass loss during export conditions to water loss. Further studies by Vosloo and Vosloo (2006) concluded that routes of water loss in *Haliotis midae* were attributable to evaporation (55% of total water loss) and mucus production (45% of total water loss).

Abalone are faced with several unnatural stressors prior to, and during the live export process. Animals are removed from the rearing containers, and moved to purging systems, where they are starved for up to three days to clean the digestive system of waste products. They are then removed from the purging systems and packed into the shipping containers. Due to transportation costs, the animals cannot be transported in water, so they spend up to 40 hours out of water. Abalone, unlike many other marine gastropods, cannot retract the large foot into the shell and do not possess an operculum, and thus are faced with the stress of desiccation when exposed to air (Vosloo & Vosloo, 2006). The physical properties of air and water are strikingly different, and consequently, biochemical, anatomical and physical features of aquatic and terrestrial animals differ markedly (Dejours, 1987). An important stressor during air exposure of abalone is gas-exchange. When exposed to air, the gills of abalone collapse and become non-functional (Eduardo *et al.*, 1987; Baldwin *et al.*, 1992). Studies on *H. iris* indicated that the gills are responsible for essentially the whole oxygen uptake and that accessory integumental surfaces are unimportant in this respect (Taylor & Ragg, 2005). Abalone transported in air are therefore faced with anaerobic conditions as well as elevation of blood carbon dioxide, and a progressive respiratory acidosis (Eduardo *et al.*, 1987; Truchot, 1987; Wells & Baldwin, 1995). Excretion of nitrogenous end-products of metabolism is also impaired during aerial exposure, as the principle route of ammonia excretion is by diffusion and ion exchange at the gills (Wells & Baldwin, 1995).

The ability of *H. midae* to withstand such adverse conditions may have evolutionary roots, as Tarr (2000) indicated, based on large depositions of abalone shells on beaches, that in the past large abalone were common in the inter-tidal zone. Thus these animals would have been exposed to regular periods of desiccation and anoxia associated with low tide. Anoxic conditions in the marine environment may develop periodically as a result of high concentrations of dinoflagellate algae associated with red tide (Bigelow Laboratory, 2006; HAB, 2006). Behavioural adaptations to these adverse conditions include clamping down onto the substrate by means of the adductor muscle of the shell (Behrens *et al.*, 2002), and taking refuge in temporary pools (Truchot, 1987) during low tide. Abalone have well developed physiological adaptations that allow them to survive adverse conditions. During functional or environmental hypoxia, abalone rely on anaerobic metabolism to supply their energetic needs (Baldwin *et al.*, 1992; Wells & Baldwin, 1995; Behrens *et al.*, 2002; O'omolo *et al.*, 2003; Dahlhoff, 2004). The end-products of anaerobic glycolysis in abalone under environmental anoxia are tauropine and D-lactate, which are specialised pyruvate reductase compounds (Baldwin *et al.*, 1992; Wells & Baldwin, 1995; Behrens *et al.*, 2002; O'omolo *et al.*, 2003). Associated physiological adaptations include reduced metabolic rates during hypoxia, large glycogen reserves, high activities of specific pyruvate reductases and a circulatory system adjusted towards storage rather than rapid delivery of oxygen (Wells & Baldwin, 1995; Donovan *et al.*, 1999; O'omolo *et al.*, 2003).

There has been increasing interest in determining the physiological condition of organisms in a natural context (Dahlhoff, 2004). Stress responses in animals require adjustments in rates of physiological processes, which imply changes in energy demands. Studies that define the normal range of stressors and homeostatic mechanisms may help to explain if some animals are more prone to the rigours of live transport than others (Taylor *et al.*, 1997). The premise of this study was that there should be correlations between environmental, management and physiological parameters and mass loss experienced during export. Animals in optimal condition should be better able to withstand the stressors associated with live export. The main aim of this study was to (1) define which physiological constituents are associated with abalone best able to withstand export mass loss and (2) to determine which managerial practices and environmental conditions will result in the production of abalone are in the optimal physiological condition for export.

Materials and Methods

The data used in this paper emanate from a two-phased project by the authors, where the seasonal changes in basic physiological constituents in abalone muscle tissue and haemolymph were measured. In Phase 1 (Laas & Vosloo, unpublished), free glucose, glycogen, total protein and total lipids were determined in a combination of foot and columellar muscle of abalone. During Phase 1 four feeding regimes were tested: kelp only (K), Abfeed™ only (A), three-weekly rotation of kelp and Abfeed™ (two weeks kelp; one week Abfeed™; KAT) and weekly rotation of kelp and Abfeed™ (four days kelp; three days Abfeed™; KAW). Proximate dietary composition of kelp and Abfeed™ is summarised in Table 1.

Table 1: **Typical analysis (as % dry weight) for kelp* and Abfeed™** fed to experimental animals in the current study.**

	*Kelp	**Abfeed™	***KAT	***KAW
Carbohydrates	43.15%	43.30%	53.20%	43.21%
Crude lipids	0.045%	5.30%	1.80%	2.30%
Crude protein	7.80%	34.60%	16.73%	19.29%
Crude ash	24.30%	5.70%	18.10%	16.33%

*Algaeron, 2006.
** Sea Plant Products, 2006. (Electronic communication).
*** Calculated.

Proximate dietary composition of the two rotational diets was calculated as a function of relative contributions of kelp and Abfeed™ in the two rotational schemes.

Sampling was conducted six-weekly at one farm (F0) in Hermanus (see Figure 1B) between April 2003 and January 2004. One simulated export trial was conducted in January 2004.

In Phase 2 (Laas & Vosloo, unpublished), free glucose, glycogen, total protein and total lipids were measured in a combination of foot and columellar muscle, as well as haemolymph free glucose content of abalone. During Phase 2 only the natural diet (kelp) and artificial diet (Abfeed™) were applied. Sampling was conducted seasonally on five farms (F1-5) situated on the Cape West Coast, and South-West Coast (Figure 1).

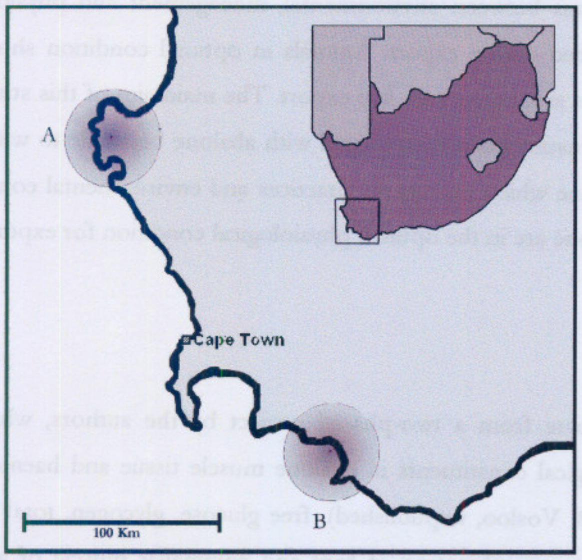

Figure 1: Map indicating the relative positions of farms on the Cape West Coast (A) and Cape South-West Coast (B).

Simulated export trials were conducted seasonally during the months of September (spring), January (summer), April (autumn) and July (winter). Physical parameters, including pH, conductivity and dissolved oxygen were measured in the holding systems during each sampling. Total ammonia concentration in the holding systems was determined by means of a colorimetric phenol-hypochlorite reaction (Bayne *et al.*, 1985). Data provided by the individual farms were used to determine water cycling characteristics at each farm.

Free glucose concentration in muscle tissues was determined by an enzymatic colorimetric reaction (Roche GOD-PAP). Glycogen was extracted from tissue samples by enzymatic digestion with amyloglucosidase (Keppler & Decker 1983), and subsequent determination of total glycogen. Final glycogen concentration was obtained by subtracting the free glucose concentration from total glucose concentration after digestion with amyloglucosidase, and was expressed in terms of total glycocyl units extracted from glycogen. Total protein content was determined by an enzymatic colorimetric reaction (Van Heerden *et al.* 2004). Total lipid content was determined applying a modified Folch technique (Folch *et al.* 1957).

Haemolymph was drawn from the pallial sinus in the mantle of the abalone using a standard 1 mL insulin syringe and needle. Samples were immediately snap-frozen in liquid nitrogen. Free glucose in the haemolymph was determined by an enzymatic colorimetric reaction (Roche GOD-PAP).

Live export conditions were simulated by purging animals on-farm for three days, after which the animals were individually weighed and packed on damp sponge linings in plastic bags that were filled with humidified oxygen. The bags were sealed and packed on thin sponges over gel-type ice-packs in polystyrene transport boxes, which were then sealed and sent by air freight to Johannesburg International Airport. The polystyrene containers were moved by road from the airport to the laboratory in Potchefstroom, where the boxes were opened after exactly 36 h, and the animals were re-weighed.

Statistical data analyses were conducted in consultation with the Statistical Consultation Services of the North-West University, using the Statistica 7 (Statsoft Inc, 2005) and SAS 9.1 (SAS Institute, 2003) software packages. Factorial ANOVAs were conducted to test for significant differences between means. Data were tested for homogeneity by means of Levene's test. In the case of non-conformance to homogeneity, data were transformed with logarithmic and/or Box-Cox transformations. Subsequently Tukey's HSD tests were performed to test for significant differences ($p<0.05$) between cases. Values are given as mean ± standard error of the mean (SEM). Multiple regression analysis was performed with SAS software using the R-SQUARE procedure, to investigate the relationships between variables, and to explain observed similarities and dissimilarities between variables. The multiple regression results were reported in terms of the adjusted-R^2 values, which measures the goodness of fit while taking into account the number of predictors in the model. Principle Component Analysis (PCA) of the data were conducted with Statistica to analyse the structure of the data. The quality of representation by the PCA was included to indicate the percentage of cumulative variance explained by the given number of factors.

T-tests with Welch's correction for unpaired, non-parametric data with unequal variances, was used to evaluate the difference in means between various groupings (based on size, feeding type and season) of results from Phase 2, using GraphPad Prism version 4.00 (GraphPad, 2005).

Results

Phase 1

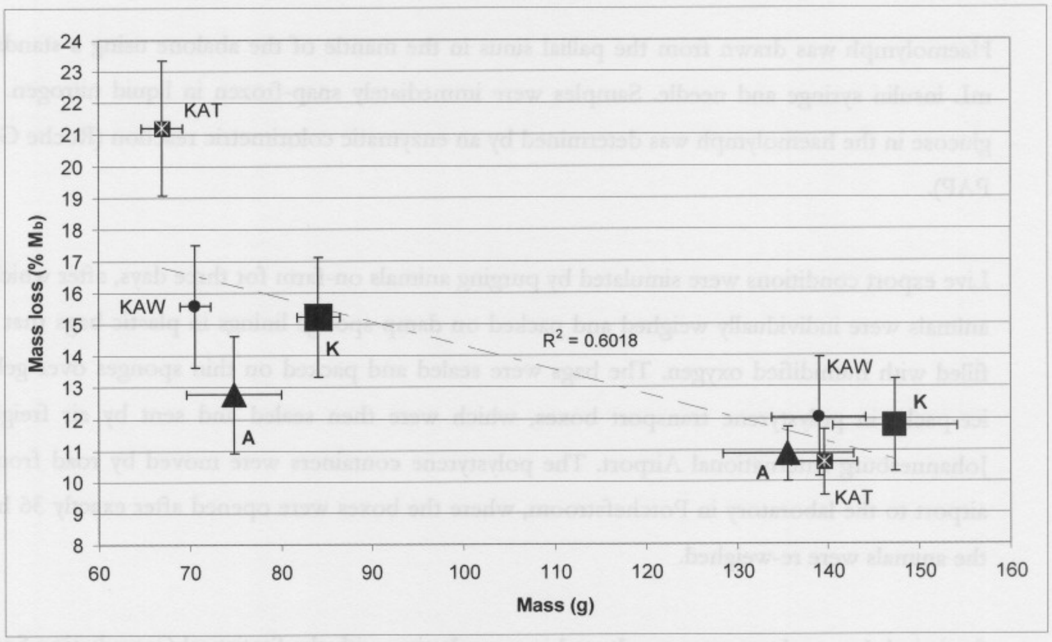

Figure 2: Summarised mass loss results for two size classes of abalone on four feeding regimes during a simulated export trial in January 2004 (K = kelp only. A = Abfeed™ only, KAT = kelp Abfeed™ three-weekly, KAW = Kelp Abfeed™ weekly).

Figure 2 indicates that on F0 in January 2004, mass loss was fairly strongly correlated to body mass (R^2=0.6). Small animals were more prone to mass loss, during simulated export, than large animals. Small animals on different feeding types also showed some degree of differentiation in their susceptibility to mass loss during export. In small animals, the rotational feeding regime, KAT, yielded the highest susceptibility to mass loss during simulated export with an average mass loss of 21.18±6.78% M_b. Abfeed™ yielded the lowest susceptibility to mass loss during simulated export with an average mass loss of 12.78±5.89% M_b. The difference in average mass loss between KAT and A was 8.4%, which was statistically significant ($p<0.05$). The measured mass loss in animals on K and KAW did not differ significantly from one another, or from either K or KAT.

In large animals, the differences between animals on different feeding regimes were less pronounced, and there were no statistically significant difference in the mass loss susceptibility of animals on different feeds. The difference between highest and lowest average mass loss in large animals was 1.44%, which was not statistically significant ($p>0.05$).

Table 2: Multiple regression results for physiological parameters measured during Phase one at F0.

Variable	**Adjusted R^2**
Muscle glycogen	0.53
Size class	0.47
Muscle glucose	0.28
Muscle lipid	0.01
Muscle protein	-0.04

The multiple regression results for Phase one are summarised in Table 2. The results should be interpreted with caution as the power of the regression statistics is impaired by the small sample size (n=8). The results indicate that muscle glycogen was the single physiological parameter that explained the most variation in the live export results. Size explained 47% of the variability in mass loss results. This confirms that, during Phase one, small animals were more prone to mass loss than large animals. Muscle glucose concentration explained 28% of the variability in the results, indicating that glucose concentration has some potential as predictor of mass loss in abalone. Muscle lipid and protein concentrations did not explain any variation in the results of simulated export trials.

The PCA results of the contribution of physiological parameters relative to export performance are summarised in Figure 3. Quality of representation by the two factors analysed was 85.3%. High muscle glucose concentration was associated with high mass loss, whereas high mass, lipid and glycogen concentrations resulted in lowered mass loss during simulated export. There was no direct relationship between tissue protein and mass loss. Dietary carbohydrates, lipids and proteins had no association with the mass loss experienced. High dietary protein concentrations resulted in higher tissue protein concentrations, while high dietary lipid concentrations did not have a direct effect on tissue lipid concentrations. Tissue lipid concentration was, however, associated with muscle glycogen concentration.

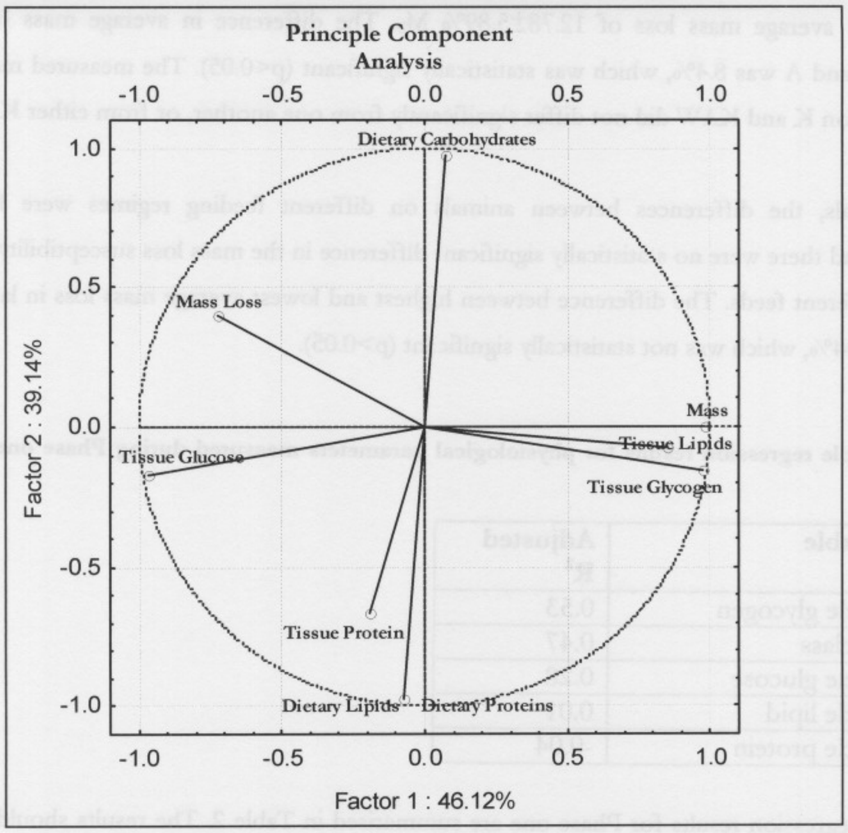

Figure 3: Principle component analysis of the relative contribution of various physiological parameters, in the muscle tissue of large and small abalone on all feeding types, to mass loss experienced during a single simulated export trial.

Phase 2

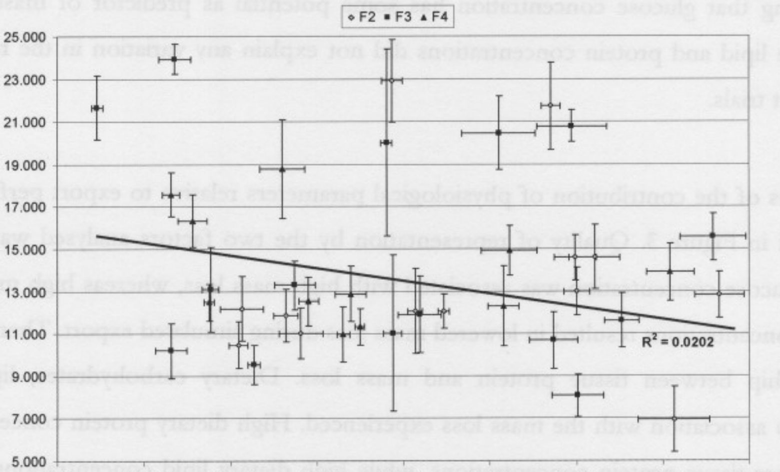

Figure 4: Summarised mass loss results for two size classes of abalone on two feeding treatments from three geographically separate farms during simulated export trials conducted seasonally from September 2004 to July 2005.

Figure 4 summarises simulated export results for all animals, from all farms, feeding types and during all seasons (n=620) for Phase 2 of the project. Each data point represents the average mass loss of 12 - 15 abalone from one of the three farms in one of the size classes, on a specific feed and during a specific season. The highest average mass loss (23.92±0.70% M_b; n=15) was experienced in small animals on kelp in summer on F3, and the lowest average mass loss (6.94±1.52% M_b; n=15) experienced was in large animals on kelp during winter on F2. The difference between highest and lowest average mass loss was highly significant (t-test: $p<0.0001$) at 16.98%. Animal size had no correlation with mass loss, and there were no significant differences in mass loss between animals of different sizes.

Table 3: Averages of mass loss of large and small abalone, and of abalone from different feeding regimes sampled over four seasons on three farms.

Description	N	Mean	T-test (p-value)
All animals	*620*	*14.53±0.28*	
Small animals	326	14.36±0.41	0.54
Large animals	294	14.70±0.38	
All animals on kelp	*331*	*14.50±0.39*	0.93
All animals on Abfeed™	*289*	*14.55±0.40*	
Small animals on kelp	164	14.44±0.60	0.87
Large animals on kelp	167	14.56±0.50	
Small animals on Abfeed™	162	14.29±0.56	0.46
Large animals on Abfeed™	127	14.89±0.59	

Table 3 summarises the simulated export results for different size classes of animals on the two feeding types studied. The average mass loss by all animals in the study (n=620) was 14.53±0.28%, compared to the 13.211±0.61% (n=105) measured at F0 the previous season and with the 15% reported by Vosloo and Vosloo (2006). There were no significant differences ($p>0.05$) in the mass loss experienced by large and small animals on either the natural or artificial feeds.

Table 4: Seasonal mass loss results *Haliotis midae* sampled on three farms.

Description	N	Mean
All animals during spring	*164*	*13.94±0.49*
All animals during summer	*120*	*19.67±0.61*
All animals during autumn	*173*	*13.94±0.43*
All animals during winter	*163*	*11.95±0.57*

When mass loss is compared on a seasonal basis (Table 4), the importance of season on live export performance is clearly visible. Combined results of mass loss in all animals from all three farms, feeding types and size classes showed no significant differences in spring and autumn. Mass loss in spring and autumn was, however, significantly lower than in summer ($p<0.001$) and significantly higher than in winter ($p<0.05$). Summer mass loss in all abalone was also significantly higher than in winter ($p<0.001$). The difference in average mass loss during summer and winter was 7.72%. Seasonal comparisons of mass loss within animals from different farms, in different size classes and on different feeds yielded the same result.

Table 5: Multiple regression results for physiological parameters measured during Phase 2 on farms F2 – F4. (M = muscle; H = Haemolymph).

Variable	**Adjusted R^2**		
	Combined size classes	**Small animals**	**Large animals**
Farm	-0.01	0.09	-0.03
Season	0.08	-0.02	0.17
Feed	-0.03	-0.05	-0.05
Size	-0.02		-
Glucose (M)	0.22	0.12	0.29
Glycogen (M)	-0.02	0.01	0.07
Glucose (H)	0.01	-0.05	0.09
Lipids (M)	0.04	0.05	-0.02
Proteins (M)	0.02	-0.02	0.01

The multiple regression results for Phase 2 are summarised in Table 5. It is evident that none of the physiological parameters had significant predictive value in terms of mass loss. The best predictor in data from all farms was muscle glucose, which could explain 22% of the observed variation. In large animals, season also played a more significant role compared to smaller animals.

The principle component analysis of the contribution of physiological and environmental parameters relative to mass loss for Phase 2 (Figure 5) has a 47.9% representation on two factor axes. High mass loss was associated with high tissue glucose concentration, high water temperature and high tissue lipid concentrations. Lower mass loss was associated with high tissue protein and haemolymph glucose concentrations, and to a lesser extent with higher tissue glycogen concentration. The levels of dietary protein, lipids and carbohydrates did not affect the ability of the animals to withstand mass loss.

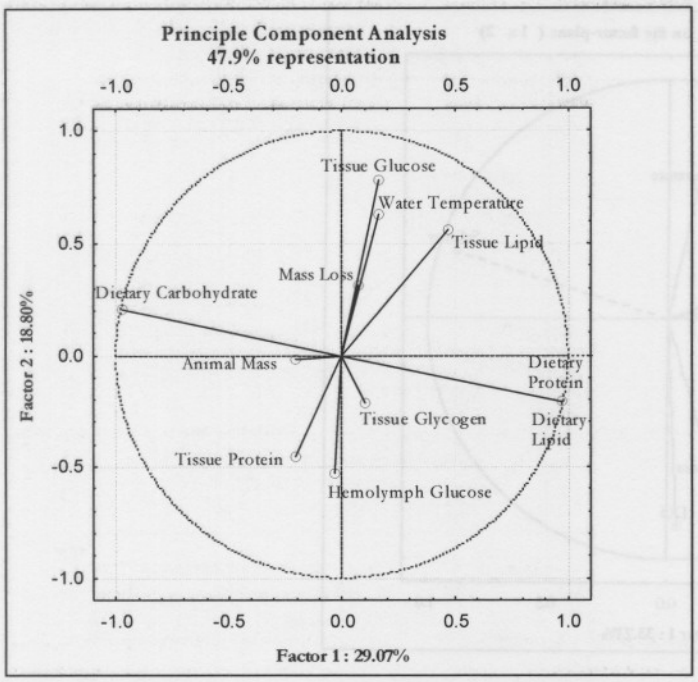

Figure 5: Principle component analysis of the relative contribution of various physiological parameters to mass loss experienced during the simulated export trial.

The physical parameters (Figure 6) that were measured in the holding systems were highly variable between farms as well as seasonally. Conductivity varied between 51.5 and 53.5 (μSi·cm^{-1}) and differed significantly between farms and in different seasons ($p<0.001$). Conductivity had no association with mass loss (Figure 6). The pH in the holding systems varied between 7.4 and 8.4, and differed significantly between farms and all seasons. No association between pH and mass loss occurred. Ammonia concentration in the systems varied between levels below the detection limit and 30 μmol·L^{-1}, and varied significantly between farms and seasons. Ammonia concentration did not associate with mass loss. Dissolved oxygen (DO) varied between 85 and 110% saturation, and varied significantly between farms and in seasons. Dissolved oxygen was the only physical variable that associated with mass loss during simulated export ($R^2=0.14$).

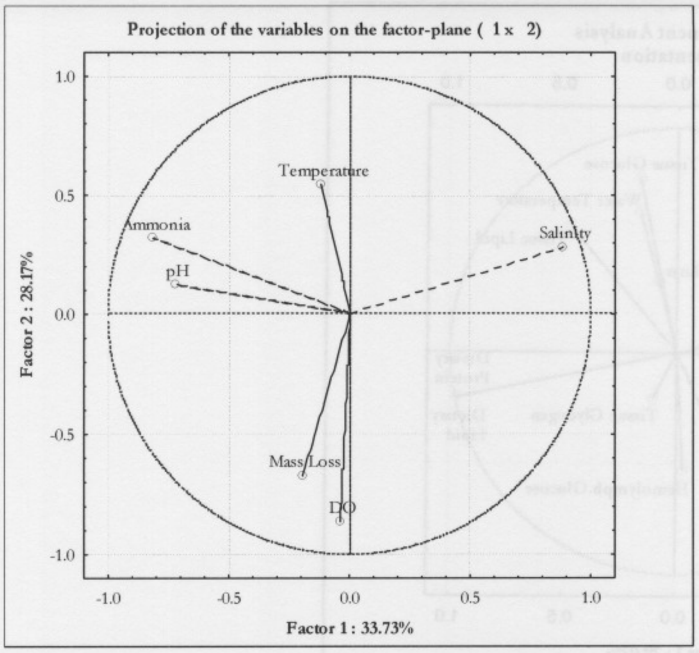

Figure 6: Principle component analysis of the relative contribution of various physical parameters to mass loss experienced during the simulated export trial.

Table 6: Water cycling characteristics and reported growth rates for farm F2 - F3. Data (tank volumes, biomass per tank and flow rate through the system) were provided by the farmers. Stocking density was calculated from tank volumes and biomass per tank. Specific flow rate was calculated from stocking density and flow rate.

	Specific flow rate ($L{\cdot}kg^{-1}{\cdot}h^{-1}$)	**Stocking density ($kg{\cdot}L^{-1}$)**	**Reported average growth rate ($mm{\cdot}month^{-1}$)**
F2	15.63	0.02	1.83
F3	11.11	0.04	1.80
F4	6.65	0.02	1.70

Water cycling characteristics differed to some extent between the three farms (Table 6). The specific flow rate at F2 and F3 was approximately double that of F4. Stocking density at F3 was double that of F2 and F4. Reported growth rates at the different farms did not differ much, but there was some indication that it was correlated to specific flow rate at the different farms.

Discussion

The results have shown that *Haliotis midae* can survive at least 36 h out of water when exposed to the conditions of simulated live export. During this period of emersion, abalone lose on average up to 15% of their body mass, which, in the live export industry, represents a 15% loss of revenue (Vosloo & Vosloo, 2006).

The results for mass loss during simulated export from Phase one should be interpreted with some caution as the results are from a single export trial. The results indicated that small animals were more prone to mass loss problems during simulated export than large animals (Figure 2). Studies by Wells and Baldwin (1995) on *H. australis* and *H. iris* also indicated that large animals were less susceptible to anaerobic stress than small individuals. As the rates of most physiological processes are related to body size (McGrowan, 1994; Randall *et al.*, 1997; Schmidt-Nielsen, 1998; Farias *et al.*, 2003), large animals may be better equipped, in terms of glycogen stores and ability to depress metabolic rate, to survive periods of anoxic stress.

The mass loss in large animals did not differ for animals on different feeding regimes, indicating that neither artificial feeds nor rotational diets improved the susceptibility of large animals to mass loss during export condition. Small animals were more susceptible to mass loss on the rotational diet KAT, than on any other diet. The reason for this increased susceptibility to mass loss on a rotational diet is unclear. In the natural environment, *H. midae* feed on a variety of macro-algae (Tarr, 2000; Sales & Britz, 2001;), and one would expect that variation in diet under culture conditions would be beneficial to abalone. Further investigation into rotational diets is, however, warranted, as these were results for a single export trial, which was insufficient to make final conclusions regarding the effect of dietary variation under aquacultural conditions.

The multiple regression results for Phase one (Table 2) confirmed animal size to be an important driver in explaining observed mass loss. Muscle glycogen concentration had some predictive value in terms of mass loss under export conditions. This can be explained by the fact that glycogen is the main energy source utilised during anaerobic metabolism (Baldwin *et al.*, 1992; Donovan *et al.*, 1999; O'omolo *et al.*, 2003; Braid *et al.*, 2005). High glycogen stores in the muscles of abalone prior to prolonged incidences of anoxia may enhance the ability of the abalone to cope with adverse conditions as experienced during live export events. Muscle glucose also seemed to be a promising mass loss predictor. It has been documented that haemolymph glucose concentration increase during times of stress in abalone (Nollens *et al.*, 2004), which corresponds with higher energetic demands for abalone under stressful conditions (Baldwin *et al.*, 1992; Wells & Baldwin, 1995; Dahlhoff, 2004; Braid *et al.*, 2005). Free muscle glucose levels may be similarly affected by stressful conditions. Thus tissue glucose and glycogen concentrations might give an indication of the condition of animals, in terms of levels of stress, in the culture environment.

Muscle lipid and muscle protein concentrations did not have any predictive powers in terms of observed mass loss. This may be due to the fact that abalone are not dependent on either of these two constituents to maintain anaerobic metabolism. Abalone have a carbohydrate based metabolism (Picos-García *et al.*, 2000; Gómez-Montes *et al.*, 2003; Braid *et al.*, 2005), and both lipids and proteins play an important structural role in abalone. Abalone do not have requirements for proteins or lipids *per se*, but rather for the right combination and amounts of specific amino and fatty acids (Mai *et al.*, 1995; King *et al.*, 1996; Leighton, 2000; Shipton & Britz, 2002; Durazo-Beltran *et al.*, 2004). Thus it would follow that as long as the needs for amino and fatty acids are met by the diet, dietary proteins and lipids should not have an effect on the export performance in terms of mass loss.

The principle component analysis (PCA) (Figure 3) of the data from Phase 1 confirms the observations of the effects of tissue glycogen and glucose concentration on mass loss during export. The PCA results show that dietary carbohydrates, proteins and lipids did not play any role in the ability of animals to deal with export conditions. It would, however, be possible that an increase in the diversity of diets with differing dietary constituents (and thus increased variability) could prove otherwise. The PCA results confirmed that tissue protein concentration did not have any effect on mass loss experienced by the animals, but indicated that muscle lipid concentration was to some extent related to mass loss, although it was not responsible for any of the variation in mass loss (Table 2)

During Phase 2 the variability was increased by increasing the number of farms, as well as the number of animals sampled on each farm, and the number of simulated export events. Thus the results of Phase 2 should give more accurate results on the effects of different environmental and physiological parameters on the export performance of *H. midae*. The trend of lower mass loss with increased animal size (mass), was again visible in Phase 2 (Figure 4), but was much less pronounced than in Phase 1 (Figure 2). One possible explanation for this observation might be the variability observed in the mass of animals in the different size classes. This could be due to unexpected slow or fast growth of animals after grading for the purposes of later sampling. There are, however, still indications that, as in Phase 1, larger animals are better able to cope with the rigours of live export, as the highest mass loss (23.92%) was observed in small animals and the lowest mass loss (6.94%) was observed in large animals. Any significant differences in mass loss susceptibility of large and small animals would have been obscured by the high variability in animal mass in the two size classes. The highly significant ($p<0.0001$) difference (16.98%)

between highest and lowest average mass loss is a clear indication that animal size can play a significant role in the live export performance of abalone. A study by Vosloo & Vosloo (2006) indicted that 54.8% of water loss during export is attributable to evaporation. As evaporation is dependant on surface-area, one would expect small animals to have higher evaporative water loss than larger animals, which would explain the size-related difference in mass loss during live export. There were no significant differences in mass loss during export of animals on either the natural or artificial diet (Table 3). This is in accordance with the results from Phase 1 and indicates that the currently employed diets do not play any role in the ability of abalone to handle the stresses of simulated export. Further proof for this observation is displayed by the PCA results for Phase 2 (Figure 5) where there was no association between mass loss observed and the various dietary constituents.

The results for Phase 2 revealed that season had a highly significant effect on mass loss during simulated export (Table 4). Mass loss was highest in summer and lowest in winter. Temperature has been indicated to have pronounced effects on abalone in terms of feeding rate (Leighton, 2000), susceptibility to disease (Braid *et al.*, 2005), physiological processes (Britz *et al.*, 1997) and growth (Britz & Hecht, 1997; Hickey & Wells, 2003). Thus high temperature in the summer months affects abalone in a variety of ways, leading to increased susceptibility to mass loss. Season affected large and small animals similarly. This is in contrast with what would be expected, as there are usually clear differences between large and small animals due to allometric scaling (McGrowan, 1994; Randall *et al.*, 1997; Schmidt-Nielsen, 1998).

The multiple regression analysis of the data from Phase two (Table 5) indicated that muscle glucose was the only useful predictor of mass loss in abalone. This indicates that muscle glucose concentrations will be elevated in animals that are not in optimal condition for simulated export. In contrast with the results of Phase one (Table 1), muscle glycogen concentration and animal size had no predictive power during Phase two. This could be due to the increased variability in the results of phase two, that would increase the explanatory power of the regression statistics. The multiple regression results for tissue proteins and lipids in Phase two indicated that these two constituents had no powers to predict mass loss in abalone, which corresponded to the observation in Phase one.

In contrast to the results of Phase one, animal size (mass) did not associate well with mass loss observed. This indicates that overall, the effect of animal size may not have the pronounced effect that was expected. High tissue lipid concentration was associated with high mass loss, which was also in contrast with the observation from the results for Phase one. In Phase two, high tissue protein concentration was associated with low mass loss, contrary to Phase one where tissue protein concentration had no association with mass loss. The reasons for this shift is unclear, but may again be the result of the more accurate results brought about by increasing the variability. Haemolymph glucose concentration did not explain any of the variance in export mass loss results. This indicates that haemolymph glucose concentration was not a condition limiting factor prior to export.

The PCA results for physiological parameters in Phase two indicate that high temperatures were associated with high mass loss, which is in agreement with the seasonal mass loss statistics (Table 4) for Phase two. Thus it is highly likely that temperature is the main driver for seasonal differences in terms of the susceptibility to mass loss. High haemolymph glucose concentration was associated with low mass loss, unlike high tissue glucose concentration that was associated with high mass loss. Reasons for this observations is unclear as one would expect high haemolymph glucose concentration to be the result of stress prior to export that should have a negative impact on the ability of the abalone to deal with the stresses associated with export conditions. One possible explanation for this observation may be that haemolymph was drawn from the pallial sinus in the mantle tissue of abalone, and not from the pedal sinus associated with the foot and columellar tissue of the abalone. This explanation, however, warrants further investigation before final conclusions on this observation can be reached.

The PCA results for physiological parameters in Phase two (Figure 5) corresponded well with the PCA results for Phase one (Figure 3) in that high mass loss was associated with high tissue glucose concentration, and low mass loss was associated with high glycogen concentrations. As in the results for Phase one, dietary carbohydrates, proteins and lipids had no correlation with observed mass loss. This provides further proof that the diets applied in this study had no effect on the susceptibility of abalone to mass loss.

Physical parameters in the aquaculture systems were highly variable between different farms, and in different seasons. The only physical parameter that could be linked to mass loss was oxygen saturation (DO) (Figure 6), and high mass loss was associated with high oxygen concentration. It should, however, be noted that high oxygen concentrations associated with high water temperatures. Reasons for the association of high oxygen saturation with high water temperatures is unclear, as oxygen concentration generally decrease with increased temperature. The differences in water cycling characteristics at different farms (Table 6) did not result in differences in export mass loss on different farms. Water cycling characteristics may, however, have indirect effects by increasing oxygenation of the water and removal of waste products.

Conclusions

Preliminary results have indicated that animal size plays an important role in the ability of abalone to deal with the adversities associated with live export, but results of further studies only partially confirm this observation. Further investigations involving more size classes and narrower limits within the selected size classes are necessary to explain this observation. The feed types applied in this study did not have any significant impact on the performance of animals during simulated export events, indicating that neither the natural nor artificial diet had any limiting effect on animal condition prior to export. Different feed types may, however, have implications for growth, and thus, size of the animals at export. Season was the most important factor in terms of the performance of the animals, and it would seem that temperature has a notable influence on the ability of abalone to withstand the rigours of live export. Summer is the worst season for live export and winter the best. The preliminary study indicated animal size, tissue glycogen and tissue glucose concentrations to be good predictors of mass loss. Further investigations, however, could only confirm tissue glucose to be a useful predictor of mass loss. Principle component analysis indicated that high mass loss was associated high tissue glucose and lipid concentrations, as well as with high temperatures. Low mass loss was associated with high tissue glycogen and protein concentrations, and with high haemolymph glucose concentration. Based on the results of this study, animals in optimal physiological condition for the purposes of simulated export should be animals with high tissue glycogen and protein concentrations and low tissue glucose and lipid concentrations. Animals should preferable be exported in winter when average water temperature are lowest.

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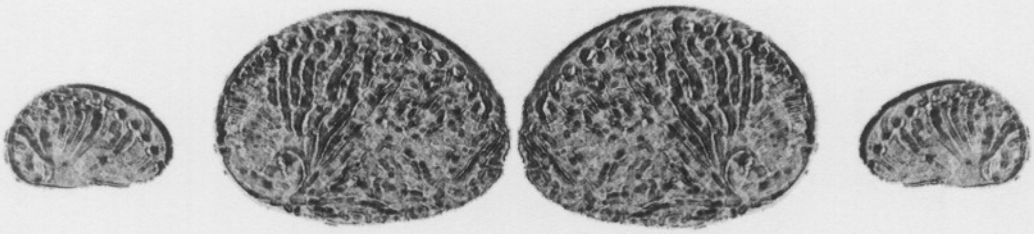
Chapter 4:

Discussion of original papers



4.1. Discussion of original papers

The survey of literature generally accessible to South African researchers (Paper I) showed that publications of research on abalone have increased rapidly in recent years. The USA, South Africa and Australia are the most prolific publishers of research on abalone research. The species that attracted the most research interest were *Haliotis rufescens*, *H. midae* and *H. discus*. Physiology and Biochemistry were the most productive research fields, although the substantial amount of research on the feeding and nutrition of abalone indicate the importance of finding an optimum, balanced diet for abalone under aquacultural conditions. Research on abalone genetics has increased substantially in recent years, signifying a shift in research focus by prominent role-players. Although South Africa is the top aquacultural producer of abalone outside of Asia (Roberts, 2005), the research focus of South African researchers differs somewhat from that of leaders like the USA and Australia. More than 50% of research in South Africa is on feeding and nutrition, and there is a need to diversify the research focus to include more research efforts in important fields, like physiology, biochemistry and genetics.

In the first phase of the project (Paper II) the suitability of different tissues was investigated for monitoring of basic physiological parameters. Results for physiological parameters from the digestive gland were highly variable and proved to be unsuitable for the purposes of this study. This high degree of variability of physiological parameters in the digestive gland were ascribed to (1) the multitude of functions performed by the digestive gland, (2) the position of the digestive gland relative to the gonads, (3) the involvement of digestive gland with gametogenesis and (4) the fact that the digestive gland surrounds the stomach and loops of the small intestine (Carefoot *et al.*, 2000; Leighton, 2000; Grubert *et al.*, 2004; Johnston *et al.*, 2005). It was concluded that investigations into basic physiological parameters should be limited to muscle tissues for the purposes of this study.

Glucose is the most important energy source in animal tissues, and changes in the circulating concentrations of glucose would be expected as a result of regulatory efforts in response to stress experienced by animals in natural or aquacultural conditions (Randall *et al.*, 1997; Taylor *et al.*, 1997; Schmidt-Nielsen, 1998). The average muscle glucose concentration of all animals in Phase one (Paper II) was 1.38 ± 0.05 $g \cdot kg^{-1}$ (n=384) and was affected mainly by season with high glucose concentrations occurring in spring and autumn. In Phase two (Paper III) the average glucose concentration was 0.67 ± 0.02 $g \cdot kg^{-1}$ (n=359) and was also affected mainly by season. This indicates a strong seasonal regulatory effect on muscle glucose concentrations. Average glucose concentration differed between Phase one and Phase two, indicating that environmental and/or culture conditions may influence muscle glucose concentrations at different farms. In both phases, muscle glucose concentrations showed correlations with mass loss (Paper IV). Muscle glucose concentrations explained 22 – 28% of variation in simulated export mass loss results. Thus, muscle glucose concentration may be a useful tool for monitoring the condition of abalone at different stages of the rearing process, and for predicting mass loss prior to committing abalone to live export.

The results indicate that muscle and haemolymph glucose concentrations are regulated separately, and that tissue glucose concentrations are not dependant on haemolymph glucose concentrations. Jorgensen *et al.* (1984) observed that although the circulatory system of abalone is open, that haemolymph flow is directed in a tissue-specific manner in *H. cracherodii.* In this study, haemolymph glucose concentration had no predictive powers in terms of export mass loss.

Glycogen is the major storage form of energy in abalone tissues owing to their carbohydrate-based metabolism (Baldwin *et al.*, 1992; Picos-García *et al.*, 2000; Gómez-Montes *et al.*, 2003; Braid *et al.*, 2005). In Phase one, the average muscle glycogen concentration for all animals was 43.28 ± 1.90 g·kg^{-1} (n=383), and was affected mainly by size. Small animals had significantly lower glycogen concentrations than larger animals. Feed type and season had no significant effect. In Phase 2 the average muscle glycogen concentration for all animals was 58.98 ± 2.82 g·kg^{-1} (n=351), and was affected significantly by season and size. As in the case of muscle glucose, muscle glycogen concentrations between Phase one and Phase two differed to some extent. Thus both muscle glucose and glycogen concentrations show some degree of change with differing environmental and aquacultural differences. Reduction in glycogen levels have been indicated as an indicator of stress, as animals mobilise glucose from glycogen stores to deal with increased energetic demand (Baldwin *et al.*, 1992; Taylor *et al.*, 1997; O'omolo *et al.*, 2003; Nollens *et al.*, 2004; Braid *et al.*, 2005). Jorgensen (1984), however, indicated that haemolymph flow in abalone are directed in a tissue specific direction. Thus glucose liberated from muscle glycogen reserves may not necessarily be deposited in the haemolymph. Another plausible explanation for this phenomenon is that the increase in glucose concentration would be short term in nature, as the glucose would be used to fulfil increased energy demands. Thus the glucose liberated from muscle glycogen stores could already have been used at the time of sampling. Although PCA analysis in Phase one indicated some potential for the use of glycogen as a predictor of mass loss, PCA analysis of results from Phase two indicated that glycogen did not have sufficient predictive power to be of use.

Neither lipid or protein concentrations in abalone had any predictive power in terms of export mass loss in either Phase one of Phase two of this project. A plausible reason for this is that abalone do not have a need for lipids of proteins *per se*, but rather for the right proportions of fatty acids and amino acids (King *et al.*, 1996; Shipton & Britz, 2002; Leighton, 2000; Durazo-Beltran *et al.*, 2004). Thus, as long as sufficient protein and lipids are available in the diet, these two constituents would not influence the mass loss of abalone.

Feed type did not significantly influence export performance of abalone. Feeding abalone on the artificial feed, or rotational regimes instead of the natural feed, kelp, had little effect on constituents in abalone muscle tissues. Both the natural diet and artificial diet have approximately the same carbohydrate content, which would explain the lack of differences in final glucose and glycogen concentrations in animals on either of the two feeds. The high lipid concentration in the artificial diet relative to the natural diet did not contribute to increased lipid deposition in the muscle tissue of abalone. Lipase activities in the digestive system of abalone are low (Mai *et al.*, 1995a; Leighton, 2000) and thus abalone cannot utilise high concentrations of lipids in their diets. There have also been indications that dietary lipid concentrations higher than 4-5% have a negative effect on protein uptake and growth (Dunstan *et al.*, 1994; Mai *et al.*, 1995b; Durazo-Beltrán *et al.*, 2003). Thus high lipid concentrations in the diet are not beneficial to abalone. The artificial diet with its higher protein concentrations in this study did not result in higher protein concentration in the soft tissues of the abalone. The major factors influencing protein utilisation are its digestibility, the balance of amino-acids and their availability, amount of protein supplied and amount of energy supplied (Fleming *et al.*, 1996). It is thus possible that the proteins in the artificial diet are not readily utilisable by *H. midae*.

The observed mass loss during simulated export corresponds well with the situation faced by industry, making it possible to study mass loss under controlled conditions. Mass loss during simulated export was generally lower in large animals than in small animals. It has been indicated by Leighton (2000), that feeding rate, and thus growth rate of *H. rufescens*, decline with increase in size. This effect of size on the export performance of animals can be explained by the principle of allometric scaling. Mass-specific metabolic rate (metabolic rate per unit mass of tissue) decreases with increasing body size (McGrowan, 1994; Randall *et al.*, 1997; Schmidt-Nielsen, 1998). Small animals with a proportionally higher metabolic rate will spend more time and energy on searching for appropriate food sources and feeding. Observations of higher export mass loss in small animals relative to larger animals have been made for *H. australis* and *H. iris* by Wells and Baldwin (1995). The surface-to-volume ratio is greater for small animals than for large animals (McGrowan, 1994; Randall *et al.*, 1997; Schmidt-Nielsen, 1998). This implicates a larger surface area of the integument, through which water can be lost due to evaporation, in small animals relative to large animals.

Export mass loss was unchanged in spring and autumn, but was significantly higher in summer and significantly lower in winter. The difference between average mass loss during live export in summer and winter was 7.72% M_b, which was highly significant ($p<0.0001$). This 7.72% difference relates to a difference in revenue of approximately ZAR 10 885 000 per year. Temperature has been indicated to have pronounced effects on abalone in terms of feeding rate (Leighton, 2000), susceptibility to disease (Braid *et al.*, 2005), physiological processes (Britz *et al.*, 1997) and growth (Britz *et al.*, 1997; Hickey & Wells, 2003). Thus increased temperatures in summer may have significant negative effects on the physiological condition of abalone, making summer the worst season for the live export market This indicates that the physiological condition of abalone in summer is not optimal, and that it is best not to submit the animals to live export in the summer months. Winter is the best time for live exports.

There were no significant differences in the export performance of animals from different farms, indicating that different management practices on different farms did not influence the ability of animals to handle the stresses of live export.

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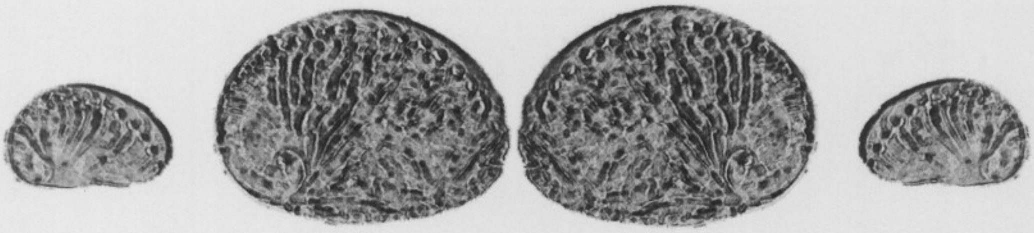
Chapter 5:

Conclusions and Recommendations



5.1. Conclusions

South Africa is a strong contender in the field of abalone research that compares favourably with other world leaders in the field. Research in South Africa is to a large extent focussed on feeding and nutrition, and there is a need for abalone research to be diversified to include other important research fields like physiology, biochemistry and genetics.

Muscle tissue proved to yield the best results for the study of physiological constituents in abalone. The study indicated that the digestive gland is not a suitable organ for the routine study of basic physiological constituents for the purposes of abalone farm management. Muscle glucose was the only physiological constituent that had predictive power on export mass loss. Elevated muscle glucose concentrations seem to be a good indication of stress in the animals. Stress decreases the ability of abalone to cope with live export. Muscle glucose concentration changed independently of changes in the glucose concentration in haemolymph sampled from the pallial sinus in the mantle tissue of abalone. This is indicative of the tissue specific circulation of haemolymph in abalone.

Neither the artificial feed nor rotational diets employed in this study had any effect on the export performance of the abalone. The study also indicated that the artificial diet did not affect the final concentrations of physiological constituents in abalone. This has serious implications for the abalone feed industry. The high lipid and protein content in the artificial diet is not readily utilised by *Haliotis midae*. Abalone have a carbohydrate based metabolism, and increasing carbohydrates and lowering lipids in the diet may be beneficial for protein utilisation by the abalone.

Animal size was also an important factor in terms of live export of abalone. Small animals generally experience higher mass loss during export, and as far as possible, larger animals should be selected for the purposes of live export.

Correlating physiological constituents to mass loss during simulated live export proved to be an effective way of exploring the physiological condition of abalone regarding their ability to handle the rigours of simulated live export.

The study provide comprehensive reference values on the physiological parameters of abalone in aquacultural systems, and their relation to mass loss experienced during live export. It has also opened several new pathways for future research in terms of abalone physiology as well as feeding and nutrition of abalone in aquaculture systems. The study has concluded that animals in prime physiological condition for export purposes should ideally have low muscle glucose. Large abalone, exported in winter, will yield the best performance in terms of live export, relating to greater financial gain for the abalone aquacultural industry in South Africa.

5.2. Recommendations and future directions

5.2.1. Animal size

Due to the effect of allometric scaling, the physiological and energetic requirements and functioning of abalone in different size classes would differ, which could have significant implications for the abalone aquaculture industry. The effect of animal size should be investigated further by increasing the range of size classes studied. Physiological parameters that may prove useful in further studies of the animal size effect include tissue glucose, glycogen, protein and lipid concentrations, as well as glucose concentrations in haemolymph samples obtained from both the pallial and pedal sinuses of the animals. These results should be correlated to mass loss during simulated live export events.

5.2.2. Simulations of live export

In the current study, the simulated live export trials were based on the mass of the animals only, and related to physiological constituents of representative individuals a week prior to export. It is recommended that sampling of physiological constituents be done before purging, after purging and at the end of the simulated export event for comparative analysis. As live export is a highly stressful occurrence for abalone, markers of stress, and end-products of anaerobic metabolism should be measured before, during and after the simulated export event. Conditions (including temperature, humidity and oxygen concentration) inside the polystyrene containers should be monitored closely for the duration of the export trial. These data could prove invaluable for the abalone aquaculture industry.

5.2.3. Diet development

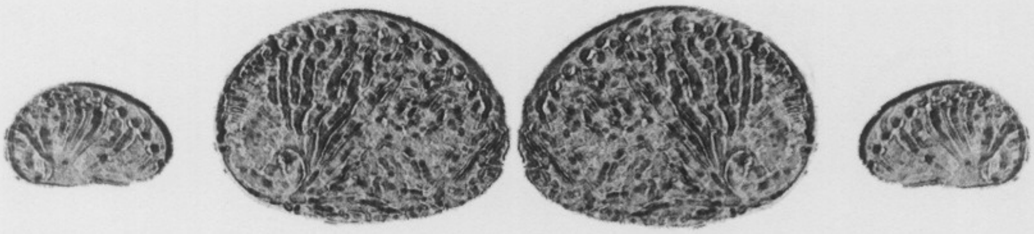
There is some leeway for research to be conducted on the artificial diets for abalone. As the study indicated, abalone cannot necessarily utilise dietary constituents in current dietary formulation, regardless of the high concentrations in which these constituents are available. The correct ratios and forms of dietary constituents in the artificial diets of abalone could improve the physiological condition of abalone. More comprehensive research on the dietary requirement of different size classes of abalone and during different seasons could provide a suite of artificial diets that will maximise the yield of abalone in aquaculture systems, and improve the performance of animals subjected to live export conditions.

5.2.5. Temperature effect

The study indicated that summer is the worst season for abalone in terms of physiological condition and export performance. As there were indications that the higher temperatures during summer caused some degree of distress in abalone, investigations into the effect of temperature should be increased. As both large and small animals were affected similarly by temperature, it is possible that the same physiological processes are involved. Short and long-term temperature studies should be conducted to determine the influence of exposure length, as well as recovery time of abalone exposed to high temperatures. As temperature is a highly important environmental factor in terms of the physiology of abalone, finding the optimum temperature and the exposure time needed at the optimum temperature could have far-reaching implications for the abalone aquaculture industry.

Chapter 6:

Appendices



6.1. Appendix 1: Farm questionnaire

Questionnaire and site visit form

Farm code	*to be inserted by operator*	
Date	*to be inserted by operator*	
Size Class	40g	70g

Section 1: To be completed during site visit									
1	*What is the flow rate into the tanks	$L.h^{-1}$							
2	What is the volume of the holding tanks	L							
3	What are the dimensions of the tanks?	M	*l*	*w*	*h*				
4	*What is the stocking density of the tanks?	$Kg{\cdot}basket^{-1}$							
		$Baskets{\cdot}tank^{-1}$							
5	What are the animals fed?		A	K	A/K combi	other			
6	What is the specific feeding regime on the farm?		S	M	T	W	T	F	S
	Further notes on feeding . . .								
7	What is the size of the animals?	mm, g, months	length	mass	age				
8	Date of last size split		*dd / mm / yyyy*						
9	*Average Growth rate	mm/month							
☐	Reported mass loss (%) from receiving parties								
☐	Reported mortalities								
	☐ Estimated time (h) from packing to receiving								

6.2. Appendix 2: Glucose protocol

SOURCE

Roche GOD-PAP kit.

REAGENTS

	Cat. No	Notes	Supplier
Perchloric Acid	380083	70%	(Sigma)
GOD-PAP Buffer enzyme	1448 668	SYS1	(Roche)
0.9% Saline solution for dilutions			
Glucose standard (22.2 mmol·mL $^{-1}$)	G7021	≥ 99.0%	(Sigma)

RECIPES

Perchloric acid (0.6 mmol·mL $^{-1}$):
Dilute 5.2 mL 70% $HClO_4$ (sp. Gr. 1.67) with water to 100 mL. Store at 4°C.

R1 (GOD-PAP):
Ready for use. Store unopened at 2-8°C. Store opened for 28 days refrigerated.

Saline solution (0.9%):
Dissolve 0.9 g NaCl in 100 mL de-ionised water.

PRINCIPLE

- Enzymatic colorimetric assay
  Addition of sample to buffer enzyme R1 start the reaction:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconolactone} + H_2O_2$$

$$2H_2O_2 + 4\text{-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} 4\text{-(p-benzoquinone-monoimino)phenazone} + 4H_2O$$

- Glucose is oxidised by Glucose Oxidase (GOD) to gluconolactone in the presence of atmospheric oxygen.
- The resultant hydrogen peroxide oxidizes 4 aminophenazone and phenol to 4-(p-benzoquinone-monoimino)-phenazone in the presence of peroxidase (POD).
- The colour intensity of the red dye is directly proportional to the glucose concentration.
- The colour intensity can be measured photometrically.

METHODOLOGY

Cuvette method:

Remove ± 400 mg tissue
Homogenise in 5 parts by weight ice cold Perchloric Acid (±2000 µl).
Centrifuge for 15 min at max speed in pre-chilled centrifuge (4°C).
Remove 20 µl supernatant for analysis.
Pipette into test tubes:

	Blank	Standard	Sample
Standard	---	0.02 mL (20 µl)	---
Sample	---	---	0.02 mL (20 µl)
Reagent	2.000 mL	2.000 mL	2.000 mL

Mix and incubate at room temperature for ± 30 min, but no longer than 90 min.

Use blank to zero the photometer.

Read absorbance of standards and samples at 546 nm. ($A_{standard}$ and A_{sample})

Do three replicates for the standard and for each sample.

Calculation:

$$\text{Concentration}_{\text{sample}} = \text{Standard concentration (mm/l)} \times \left(\frac{A_{\text{sample}}}{A_{\text{standard}}}\right) \times \text{Dil factor}$$

If the concentration exceeds 22.2 mmol·L^{-1}, dilute the sample 1 + 2 with saline and repeat the assay. (Final result x 3!)

Microwell method:

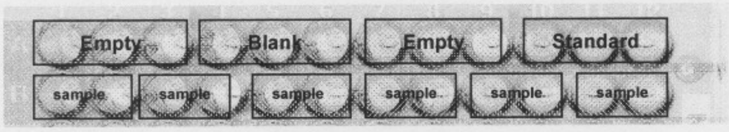
Remove ± 400 mg tissue

Homogenise in 5 parts by weight ice cold Perchloric Acid (±2000 µl).

Centrifuge for 15 min at max speed in pre-chilled centrifuge (4°C).

Remove 4 µl supernatant for analysis

Set up microwell plate as follows:



Into each microwell pipette 4 µl sample + 150 µl reagent.

Incubate for 10 min with shaking every minute.

Read absorbance at 546 nm.

Do two replicates for the standard and each sample.

Calculation

$$\text{Concentration}_{\text{sample}} = \text{Standard concentration (mm/l)} \times \left(\frac{A_{\text{sample}}}{A_{\text{standard}}}\right) \times \text{Dil factor}$$

Calculation of Dilution factor:

Dilution factor = (tissue water content) + 5 parts perchloric acid.

E.g. Abalone tissue contains 63% water
That is 0.63mL of water per 1 gram tissue
Addition of 5 x perchloric acid = 5 x dilution
Thus Dil factor = 5.63

Notes:

Samples should be kept cold on ice until ready for incubation.

SAFETY

Please see the following web sites for explanation of the risk and safety phrases
http://www.sigmaaldrich.com/Help_Pages/Help_Welcome/Product_Search/Risk___Safety_Statements.html#Risk Phrases
http://www.sigmaaldrich.com/Help_Pages/Help_Welcome/Product_Search/Risk___Safety_Statements.html#Safety Phrases

Alcohol
Hazard Codes
F,Xn

Phenol
Hazard Codes
T,C

Sodium nitroprusside
Hazard Codes
T+,N

Tri-sodium citrate
No risk or safety hazard

Sodium hydroxide
Hazard Codes
C

Ammonium chloride
Hazard Codes
Xn

6.3. Appendix 3: Glycogen protocol

SOURCE

KEPPLER, D. & DECKER, K. 1983. Glycogen in Methods of enzymatic analysis, Vol. II, third edition, Metabolites 1: Carbohydrates. Edited by BERGMEYER, H.U. pp 11-18. Weinheim: VCH Verslaggesellschaft.

REAGENTS

	Cat. No.	Notes	Supplier
Perchloric Acid	380083	70.0%	(Sigma)
0.9% Saline solution for dilutions			
Potassium Hydrogen Carbonate	P9144	≥ 99.5%	(Sigma)
Acetate Buffer			
Acetic acid	338826	≥ 99.9%	(Sigma)
Sodium acetate	S8750	≥ 99.5%	(Sigma)
Amyloglucosidase	10115		(Sigma)

RECIPES

Perchloric acid (0.6 mol·L^{-1}):

Dilute 5.2 mL 70% $HClO_4$ (sp. Gr. 1.67) with water to 100 mL. Store at 4°C.

Saline solution (0.9%):

Dissolve 0.9 g NaCl in 100 mL de-ionised water.

Potassium Hydrogen Carbonate (1 mmol·L $^{-1}$):

Dissolve 2 g $KHCO_3$ in de-ionised water and make up to 20 mL. Store at 4°C

Acetate Buffer (0.2 mol·L $^{-1}$; pH 4.8):

Dissolve 4.8 mL 96% acetic acid and 9.75 g sodium acetate in de-ionised water and make up to 1000 mL. Check pH with glass electrode. Store at 4°C

Amyloglucosidase:

dissolve 20 mg lyophilized enzyme preparation with ≥ 10 U/mg protein (at 25°C) in 20 mL acetate buffer (4.4). Store at -20°C

PRINCIPLE

- Glycogen is a storage form of D-glucose in animal tissues.
- Enzymatic method for glycogen degradation.
- Glycogen is hydrolysed to glucose monomers by amyloglucosidase and determined as free glucose concentration.

$$\text{Glucose} + H_2O \xrightarrow[\text{pH4.8}]{\text{Amyloglucosidase}} (\alpha - \text{glycosyl})_{n-1} + \text{Glucose}$$

- Free glucose in the sample is also determined.
- The tissue glycogen concentration will then be the difference between the total glucose concentration (free glucose + liberated glucose) and the free glucose concentration.

METHODOLOGY

Cuvette method:

Remove ± 400 mg tissue
Homogenise in 5 parts by weight ice cold Perchloric Acid (±2000 µl).
Centrifuge for 15 min at max speed in pre-chilled centrifuge (4°C).
Remove 20 µl homogenate for free glucose determination
Remove 200 µl supernatant for glycogen hydrolysis
In an 8mL vial add:
    200 µl Homogenate
    100 µl $KHCO_3$
    2000 µl Amyloglucosidase
Incubate at 40°C for 2 hours with shaking (pH 4.8)
Add 1000 µl Perchloric acid
Centrifuge at maximum velocity for 15 minutes.
Remove 20 µl supernatant for total glucose determination
Determine glucose as per free glucose protocol

Microwell method:

Remove ± 400 mg tissue
Homogenise in 5 parts by weight ice cold Perchloric Acid (±2000 µl).
Centrifuge for 15 min at max speed in pre-chilled centrifuge (4°C).
Remove 4 µl supernatant for free glucose determination
Remove 50 µl supernatant for glycogen hydrolysis
In a microtube add:
    50 µl Homogenate
    25 µl $KHCO_3$
    500 µl Amyloglucosidase
Incubate at 40°C for 2 hours with shaking (pH 4.8)
Add 250 µl Perchloric acid
Centrifuge at maximum velocity for 15 minutes.
Remove 4 µl supernatant for total glucose determination
Determine glucose as per free glucose protocol

Calculation

$$\text{Concentration}_{\text{sample}} = \text{Standard concentration}\,(\text{mm/l}) \times \left(\frac{A_{\text{sample}}}{A_{\text{standard}}}\right) \times \text{Dil.factor}_{\text{glycogen}} \times \text{Dil.factor}_{\text{free glucose}}$$

Final Glycogen = Total glucose (free glucose + liberated glucose) – Free glucose.

Calculation of Dilution factor for glycogen samples:

Dil. factor = 1 x homogenate + 0.5 x $KHCO_3$ + 10 x Amyloglucosidase + 5 x Perchloric acid.
Dil. factor = 16.5

Notes:

Glycogen homogenate is stable in Perchloric acid for several days, stored in the cold.

SAFETY

Please see the following web sites for explanation of the risk and safety phrases
http://www.sigmaaldrich.com/Help_Pages/Help_Welcome/Product_Search/Risk___Safety_Statements.html#Risk Phrases
http://www.sigmaaldrich.com/Help_Pages/Help_Welcome/Product_Search/Risk___Safety_Statements.html#Safety Phrases

Alcohol
Hazard Codes
F,Xn

Phenol
Hazard Codes
T,C

Sodium nitroprusside
Hazard Codes
T+,N

Tri-sodium citrate
No risk or safety hazard

Sodium hydroxide
Hazard Codes
C

Ammonium chloride
Hazard Codes
Xn

6.4. Appendix 4: Protein protocol

SOURCE

Pierce Biotechnology, BCATM Protein Assay Kit.

REAGENTS

	Cat. No.	Notes	Supplier
Glycerol (50%)	G7893	≥99.5%	(Sigma)
NaCl (5M)	S9888	≥99.0%	(Sigma)
$MgCl_2$ (1.5mM)	M8266	≥98.0%	(Sigma)
EDTA (0.5M pH 8)	431788	≥ 99.9%	(Sigma)
HEPES (1M)	H6147	≥ 99.5%	(Sigma)
Sterile Water			
PMSF (0.1M)	P7626	≥ 98.5%	(Sigma)
DTT (0.5M)	07-6130	≥ 98.5%	(Sigma)
BCA™ Reagent A	23225		(Pierce)
BCA™ Reagent B	23225		(Pierce)
Albumin standard (2 $mg \cdot mL^{-1}$)	23209		(Pierce)
0.9% Saline solution			

RECIPES

Reagent stock solutions (for preparation of 100mL buffer):

Glycerol (50%):
Combine 25 mL glycerol (100%) with 25 mL de-ionised water (DW).
Store at 4°C

NaCl (5M):
Dissolve 2.922 g NaCl in DW and make up to 10 mL. Store at 4°C

$MgCl_2$ (1.5mM):
Dissolve 0.203 g $MgCl_2$ in DW and make up to 1 mL. Store at 4°C

EDTA (0.5M pH 8):
Dissolve 0.9306 g EDTA in 3 mL DW. Adjust pH to 8 with
NaOH pellets. Store at 4°C

HEPES (1M):
Dissolve 1.1915 HEPES in DW and make up to 5 mL. Store at 4°C

Sterile Water:
Autoclave de-ionised water, store under sterile conditions. Store at 4°C

PMSF (0.1M):
Dissolve 0.0174 PMFS in DW and make up to 1mL. Store at -20°C

DTT (0.5M):
Dissolve 0.0174 PMFS in DW and make up to 1mL. Store at -20°C

BCA stock solution:

In a 150 mL flask add the following:

Buffer C stock solution	Final concentration	Per 100 mL
50 % Glycerol	25%	50.000 mL
5M NaCl	0.42M	8.400 mL
1M $MgCl_2$	1.5mM	0.150 mL
0.5M EDTA (pH 8)	0.2mM	0.040 mL
1M HEPES	20mM	2.000 mL
Sterile water		39.400 mL

Store at 4ºC

Buffer C final working solution:

In a 150 mL Flask add the following:

Buffer C Final mix	
Buffer C stock solution	99.400 mL
PMSF	0.500 mL
DTT	0.100 mL
Total	100mL

Important: Buffer C final mix only stable for 1.5 hours!

BCA™ working reagent:

Use the following formula to determine the total volume of BCA™ working reagent (WR) required:

$$\boxed{WR_{BCA^{TM}}\ required = (\#\ Standards + \#\ unknowns) \times (\#\,replicates) \times (volume\ of\ WR\ per\ sample)}$$

Prepare working reagent by mixing 50 parts of BCA™ Reagent A with one part of BCA™ Reagent B (50:1, Reagent A: B) (e.g. mix 50 mL Reagent A with 1 mL Reagent B).Mixing reagents yield a clear, green working reagent.

Prepare sufficient working reagent based on the number of samples to be analysed. The working reagent is stable for several days when stored in a closed container at room temperature.

Albumin standard (BSA: 2 mg·mL^{-1}):

Dilute albumin standard with DW to the following standard concentrations: 0 mg·mL^{-1}; 0.1 mg·mL^{-1}; 0.25 mg·mL^{-1}; 0.5 mg·mL^{-1}; 1.0 mg·mL^{-1}; 2 mg·mL^{-1} .

0.9% Saline solution:

Dissolve 0.9 g NaCl in 100 mL de-ionised water.

PRINCIPLE

- The BCA™ Protein assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein.
- This method combines the reduction of $Cu^{+2}$to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid.
- The purple-colored reaction product of this assay is formed by the chelation of two molecules BCA with one cuprous ion.

- This water-soluble complex exhibits a strong absorbance at 562nm.
- Protein concentrations are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA).
- A series of dilutions of known concentrations are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve.

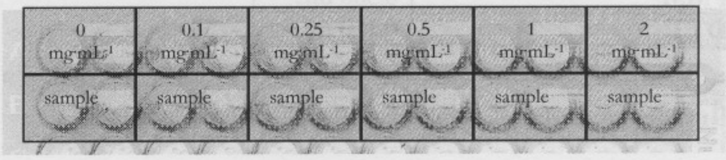
- **METHODOLOGY**

Sample preparation:

Remove ± 400 mg tissue
Homogenise in 5 parts by weight, freshly made, Buffer C working reagent (±2000 µl).
Centrifuge for 15 min at max speed in pre-chilled centrifuge (4°C).
Remove 500 µl supernatant to eppendorff microtube.
Analyse or store at -80°C

Sample analysis:

Set up microwell plate as follows:



0 mg.mL^{-1}	0.1 mg.mL^{-1}	0.25 mg.mL^{-1}	0.5 mg.mL^{-1}	1 mg.mL^{-1}	2 mg.mL^{-1}
sample	sample	sample	sample	sample	sample

Add 10 µl of each of the standards to the designated wells
Add 1 µl of sample to each of the designated wells
Add 9 µl of DW to each sample.
Add 200 µl of BCA™ working reagent to each microwell.
Incubate for 30 min at 37°C
Read absorbance of standards and samples at 562 nm. (A_{standard} and A_{sample})
Do two replicates for the standards and for each sample.

Calculation:

Each microplate analysed should contain a range of standards as well as samples.
A standard graph is constructed from the absorbance values of the known standards.
Concentrations of unknown samples are determined by the regression equation of the trendline:

$$\boxed{y = mx + c}$$

where: y = absorbance value
c = intercept
m = x variable

Rearranged, and with integration of Dilution factors:

$$\boxed{x = (\frac{y-c}{m}) \times Dil\ \ factor_1 \times Dil\ \ factor_2}$$

where:
Dil $factor_1$ = Tissue dilution factor + Buffer C
(5.00 + 0.63 = 5.63)
Dil $factor_2$ = Microwell dilution factor
(1 + 9 = 10)

SAFETY

Please see the following web sites for explanation of the risk and safety phrases
http://www.sigmaaldrich.com/Help_Pages/Help_Welcome/Product_Search/Risk___Safety_Statements.html#Risk Phrases
http://www.sigmaaldrich.com/Help_Pages/Help_Welcome/Product_Search/Risk___Safety_Statements.html#Safety Phrases

EDTA
Hazard Codes
Xi

DTT
Hazard Codes
Xn

PMSF
Hazard Codes
T

6.5. Appendix 5: Lipid protocol

SOURCE

FOLCH, J., LEES, M. & STANLEY, G.H.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226:497-509.
Anon.

REAGENTS

	Cat. No.	Notes	Supplier
Chloroform	C2432	≥99.0%	(Sigma)
Methanol	322415	99.8%	(Sigma)
Sodium Chloride	S9888	≥ 99.0%	(Sigma)

RECIPES

Chloroform/Methanol:

The Chloroform/methanol solution should be made in a properly functional fume hood.
The solutions should be prepared in a glass flask with glass stopper.
Ad a Volumetrically measured volume of Chloroform to the flask.
Carefully add a volumetrically measured amount of methanol equal to half the chloroform volume.

0.73% Sodium Chloride:

Add 730 mg NaCl to a suitable glass flask.
Make up to 100mL with Deionised water.
Store stoppered at room temperature for no longer than one week.

PRINCIPLE

- The aim is to separate cellular or fluid lipids from other constituents.
- Based on a solvent extraction of lipids.
- The efficiency of solvent extraction depends on the polarity of lipids present compared to that of the solvent.
- Polar lipids (such as glycolipids and phospholipids) are more soluble in polar solvents (such as alcohols).
- Non-polar lipids (such as triacylglycerols) are more soluble in non-polar solvents (such as hexane).
- It is impossible to select a single organic solvent that will extract both polar and non-polar lipids.
- The tissue sample is homogenised in a 2:1 chloroform/methanol solution to a dilution 20 times the volume of the tissue sample.
- The extract is shaken and equilibrated with 0.2x its volume with a 0.73% saline solution, and allowed to separate into two phases.
- The lower phase will contain chloroform-methanol-water in the proportions 86:14:1 and contains virtually all the lipids.
- The upper phase will contain the same solvents in the proportions of 3:48:47 and most of the non-lipile components.

METHODOLOGY

Lipid extraction:

Homogenise a known amount of tissue with 2:1 Chloroform/methanol to a final dilution 20 times the tissue volume (1g in 20mL 2:1 chloroform) in a glass test tube.
Allow the extraction process to proceed in the stoppered glass test tube for at least 12 hours.
Ad to the extract 0.2x its volume (4mL/20mL) of 0.73% NaCl and shake well.
Allow phase separation to take place in stoppered glass test tube for at least 12 hours.
Discard the upper phase containing non-lipile components.
Determine total lipid content from bottom phase gravimetrically.

Gravimetric lipid determination:

Pre-weight an foil pan on an analytical balance.
Remove 1mL of bottom phase to the pre-weighed foil pan.
Allow evaporation to take place in a fume hood for at least 12 hours.
Re-weight foil pan.

CALCULATION OF LIPID CONTENT:

The lipid mass is calculated as follows:

$$Lipid\_mass(mg/ml) = \frac{(pan + lipid\_residue) - Pan\_tare}{volume\_dried}$$

Lipid content per g tissue is calculated as follows:

$$Lipid\_content(mg/g) = \frac{lipid\_mass}{volume\_evaporated} \times \frac{Volume\_C:M\_added}{Sample\_volume}$$

Notes:

Care should be taken to avoid contact between plastic/ rubber and the Chloroform solutions!

SAFETY

Please see the following web sites for explanation of the risk and safety phrases
http://www.sigmaaldrich.com/Help_Pages/Help_Welcome/Product_Search/Risk___Safety_Statements.html#Risk Phrases
http://www.sigmaaldrich.com/Help_Pages/Help_Welcome/Product_Search/Risk___Safety_Statements.html#Safety Phrases

Alcohol
Hazard Codes
F,Xn

Phenol
Hazard Codes
T,C

Sodium nitroprusside
Hazard Codes

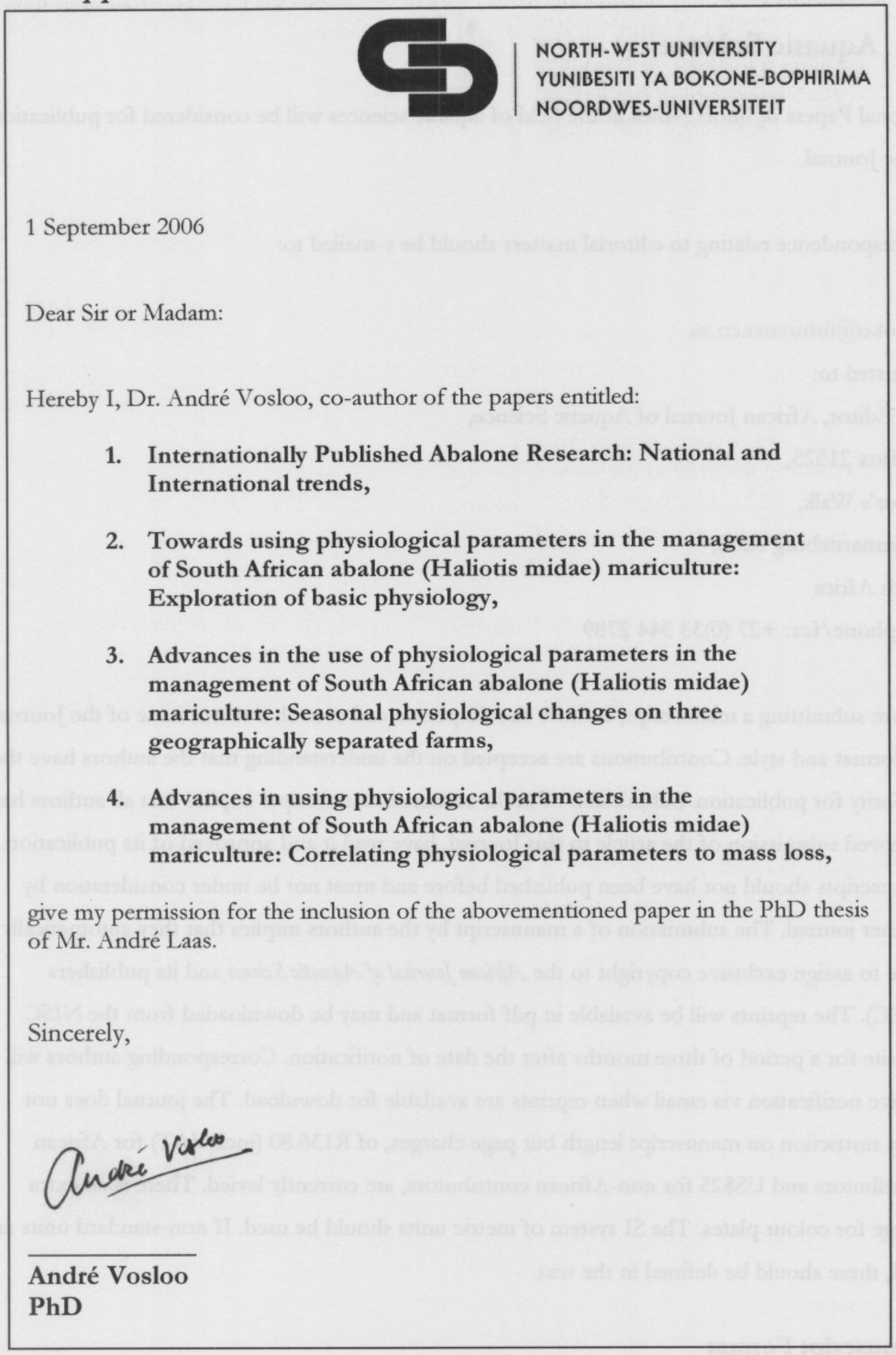
T+,N

Tri-sodium citrate
No risk or safety hazard

Sodium hydroxide
Hazard Codes
C

Ammonium chloride
Hazard Codes
Xn

6.6. Appendix 6: Letter of consent



NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

1 September 2006

Dear Sir or Madam:

Hereby I, Dr. André Vosloo, co-author of the papers entitled:

1. **Internationally Published Abalone Research: National and International trends,**
2. **Towards using physiological parameters in the management of South African abalone (Haliotis midae) mariculture: Exploration of basic physiology,**
3. **Advances in the use of physiological parameters in the management of South African abalone (Haliotis midae) mariculture: Seasonal physiological changes on three geographically separated farms,**
4. **Advances in using physiological parameters in the management of South African abalone (Haliotis midae) mariculture: Correlating physiological parameters to mass loss,**

give my permission for the inclusion of the abovementioned paper in the PhD thesis of Mr. André Laas.

Sincerely,

André Vosloo

André Vosloo
PhD

6.7. Appendix 7: Instructions to authors: African Journal of Aquatic Science

Original Papers or Short Notes in the field of aquatic sciences will be considered for publication in the Journal.

Correspondence relating to editorial matters should be e-mailed to:

mdcoke@futurenet.co.za
or posted to:
The Editor, African Journal of Aquatic Science,
PO Box 21525,
Mayor's Walk,
Pietermaritzburg 3208,
South Africa
Telephone/fax: +27 (0)33 344 2789

Before submitting a manuscript, authors should peruse and consult a recent issue of the Journal for format and style. Contributions are accepted on the understanding that the authors have the authority for publication. Submission of multi-authored manuscripts implies that all authors have approved submission of the article to this Journal, have read it and approved of its publication. Manuscripts should not have been published before and must not be under consideration by another journal. The submission of a manuscript by the authors implies that they automatically agree to assign exclusive copyright to the *African Journal of Aquatic Science* and its publishers (NISC). The reprints will be available in pdf format and may be downloaded from the NISC website for a period of three months after the date of notification. Corresponding authors will receive notification via email when reprints are available for download. The journal does not place restriction on manuscript length but page charges, of R136.80 (incl. VAT) for African contributors and US$25 for non-African contributors, are currently levied. There is an extra charge for colour plates. The SI system of metric units should be used. If non-standard units are used, these should be defined in the text.

Manuscript Format

Manuscripts must be in English in WordPerfect or MS-Word format, double-spaced with all tables and figures attached at the end of the text.

Figures should also be submitted as separate files according to the instructions pertaining to figures that follow.

An electronic copy of the manuscript should be e-mailed to the Editor, followed by one hard copy on white A4 paper together with the original illustrations and photographs.

Summary - A *summary* of not more than 200 words, at the beginning of each paper, is required for articles and notes. Please submit a maximum of eight relevant keywords.

Tables – must be numbered with Arabic numerals and including an explanatory title at the head, should fit an overall printed area of 175 x 235mm (including title). Tables must be planned to fit the page vertically with a printed width of either 80mm or 170mm. Tables may include up to five horizontal lines but no vertical lines.

Figures - high quality originals must be provided. They must be prepared on separate A4 sheets, with the lead author's name, the figure number and the top of the figure indicated on the reverse. Arabic numerals must be used. Figures that are grouped together must be numbered using lowercase chronological letters. Figures should be planned with a maximum final width of either 80mm or 175mm.

Lettering - must be provided by the author(s). Freehand lettering is not acceptable. Letters, numbers and symbols must appear clearly, but not oversized. Lettering must be in a sanserif font, e.g. Arial. It is recommended that one uniform size be used throughout the manuscript. Complicated symbols or patterns must be avoided.

Graphs and histograms - should preferably be two-dimensional and scale marks (turning inwards) provided. All lines (including boxes) should be clear, but not too thick and heavy.

Line artwork (including drawings and maps) **-** must be high-quality laser output (not photocopies). The use of grey tones should be avoided; pattern textures should rather be used.

Photographs - should be excellent quality on glossy paper, with clear details and sufficient contrast.

In addition to the print versions, illustrations, including all graphs and chemical formulae, must be submitted in electronic format, e.g. tif, gif, jpg or eps, with each figure saved as a separate file. The source file of each graphic should also be included. We favour dedicated illustration packages over tools such as PowerPoint and WordPerfect graphics although Excel graphs, correctly sized, are acceptable. It is important to indicate with your submission the software package(s) used for all files supplied.

References - should be quoted in the text as surname and year within brackets, and listed alphabetically at the end of the manuscript. For references with more than two authors please cite the reference in the text as (Allanson *et al.* 1999). Where reference is made to more than one work by the same author, identify each citation in the text as (Allanson 1999, 2000a, b).

References must be submitted in the following style:

Allanson BR, Baird D and Heydorn AE (1999) Perspectives. In: Allanson BR and Baird D (eds) Estuaries of South Africa. Cambridge University Press, Cambridge, pp 321–327.

Paterson AW (1998) Aspects of the Ecology of Fishes Associated with Salt Marshes and Adjacent Habitats in a Temperate South African Estuary. PhD Thesis, Rhodes University, Grahamstown, 104pp.

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Proofs - will be sent to the 'corresponding author' for checking. Corrections to the proofs should be restricted to those that have been introduced during the production process. Other than such corrections, substantial alterations may be charged to the author. Proofs are sent via e-mail as pdf files that can be viewed using Adobe Acrobat Reader, which can be downloaded from the Internet free of charge. Prompt return of corrected manuscripts, preferably within five days of receipt, will minimise the risk of the paper being held over to a later issue

6.8. Appendix 8: Complete bibliography for Paper I

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6.9. Appendix 9: Instructions to authors: Aquaculture

Types of contribution

1. Original Research Papers (Regular Papers)
2. Review Articles
3. Short Communications
4. Technical Papers
5. Letters to the Editor
6. Book Reviews

Original Research Papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form.

Review Articles can cover either narrow disciplinary subjects or broad issues requiring interdisciplinary discussion. They should provide objective critical evaluation of a defined subject. Reviews should not consist solely of a summary of published data. Evaluation of the quality of existing data, the status of knowledge, and the research required to advance knowledge of the subject are essential.

Short Communications are used to communicate results, which represent a major breakthrough or startling new discovery and which should therefore be published quickly. They should *not* be used for preliminary results. Papers must contain sufficient data to establish that the research has achieved reliable and significant results.

Technical Papers should present new methods and procedures for either research methodology or culture related techniques.

The *Letters to the Editor* section is intended to provide a forum for discussion of aquacultural science emanating from material published in the journal.

Book Reviews will be solicited by the Book Review Editor. Unsolicited reviews will not usually be accepted, but suggestions for appropriate books for review may be sent to the Book Review Editor: Mrs. A.A.C. de Groot, Brederoodseweg 49, 2082 BS Santpoort-Zuid, The Netherlands.

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R. P. Wilson, Mississippi State University, Department of Biochemistry and Molecular Biology, Box 9650,
Mississippi State, MS 39762, USA. Tel.: +1 622 325 2640. Fax: +1 622 325 8644. E-mail: rpwl@Ra.MsState.Edu

Husbandry and Management:

B.Costa-Pierce, Rhode Island Sea Grant College Program, Graduate School
of Oceanography, University of
Rhode Island, 129 Coastal Institute, Narragansett, RI 02882-1197, USA. E-mail: aquaculture@gso.uri.edu

Physiology and Endocrinology:

E.M. Donaldson, West Vancouver Laboratory, Department of Fisheries and Oceans, 4160 Marine Drive, West Vancouver, B.C. V7W 1N6, Canada. Tel: +1 604 666 7928. Fax: +1 604 666 3497. E-mail: donaldso@direct.ca

Diseases:

D.J. Alderman, CEFAS, Weymouth Laboratory, The Nothe, Weymouth, Dorset DT4 8UB, UK. Tel.: +44 1305 206 600. Fax: +44 1305 206 601. E-mail: d.j.alderman@cefas.co.uk

Genetics: G. Hulata, Agricultural Research Organisation, Volcani Centre, Department of Aquaculture, P.O. Box 6, Bet Dagan 50250, Israel; Tel.: +972 3 968 3388; Fax: +972 3 9605667; E-mail: vlaqua@volcani.agri.gov.il

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