Toward best management practices for the growth of the abalone *Haliotis midae* Linnaeus on a commercial South African abalone farm

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I declare that

"Toward best management practices for the growth of the abalone Haliotis midae Linnaeus on a commercial South African abalone farm"

is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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Elizabeth van der Merwe Date

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1.1 Aquaculture

Globally, aquaculture practises have been in existence for nearly 4000 years (Ackefors et al. 1994). Aquaculture is currently considered the most vastly expanding food production system in the world (Li 1999; Williams 1999; Semoli 2007). The main factors responsible for this rapid expansion have been cited as including: the increased social demand and open policies; the increase in available aquaculture area; the improvement and development of new culture techniques; the increase in culture species' diversity; genetic enhancement of aquaculture species; development of formulated feeds; and the improved control and understanding of disease related to aquaculture practices (Li 1999).

The United Nations Food and Agriculture Organization (FAO) (1995) defines aquaculture as … "the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants". According to the FAO (1995), farming implies some form of intervention (e.g. regular stocking, feeding, protection from predators, etc.) in the rearing process to enhance production and also implies individual or corporate ownership of the stock being cultivated. Beveridge and Little (2002) emphasized that in this definition, the aspects of husbandry and ownership are considered as being intrinsic. Many authors (see e.g. Reay 1979; Beveridge 1987; Nash 1991a; 1995; Landau 1992; Smoley 1992; Swann 1992; Swift 1993; Ackefors et al. 1994; Barnabé 1994; Parker 2002) have attempted to define aquaculture in various other ways, but none have been universally accepted. Troell et al. (2004) support Costa-Pierce (2002) by emphasizing the industrial perspective of aquaculture, the main aim of which is to maximize production of a preferred or demanded species with the objective of gaining maximum monetary remuneration. However, it should be stressed that the latter perspective has only been developed recently and is not applicable to the numerous traditional[1] systems which primarily concentrate on rural and household subsistence consumption (Troell et al. 2004).

1.1.1. Aquaculture vs Agriculture: Several authors (see e.g. Bardach et al. 1972; Reay 1979; Swann 1992; Parker 2002) realized the similarities between aquaculture and agriculture. However, it should be stressed that even though the principles and the majority of problems are similar (e.g. production activities, management strategies, supplies needed, services required, processing techniques, marketing and distribution activities), the aquatic medium as an environment differs and totally different groups of organisms are cultured. For example, when animals are cultured, homeotherms (agriculture) as opposed to poikilotherms (aquaculture) are the target organisms (Reay 1979; Swann 1992; Parker 2002).

1.1.2 Aquaculture vs Fisheries: Capture fisheries can be considered as the final hunting and gathering activity, while aquaculture includes cultivation, applying methods that enhance the yield to levels far above those naturally present in the environment (Ackefors et al. 1994). While both aquaculture and fisheries have the same fundamental aim (i.e. to maximize productivity - Beveridge 1987), two main factors[2] (i.e. husbandry and ownership of the stock) distinguish fisheries from aquaculture (Naylor et al. 2000). However, as final food product there is not much difference to the consumer where these two activities are concerned. While there are many examples of successfully managed fisheries that are both biologically and economically profitable (see e.g. Muse 1998; Karpov et al. 2000; Leiva and Castilla 2002; Hilborn et al. 2005), it has been argued that the greatest negative impacts of

[1] Traditional aquaculture is an activity based on the utilization of multipurpose water bodies with the cultured organisms therein considered as belonging to everyone and not specifically owned by any individual, or governmental body, or other corporate company (Beveridge and Little 2002).

[2] A number of other fundamental differences are described by Beverton and Holt (1957), Reay (1979), Beveridge (1987), Swift (1993), Ackefors et al. (1994) and Bailey et al. (1996).

fishing have been on marine ecosystems (Watling and Norse 1998). Consequently, aquaculture has been promoted as an alternative to capture fisheries (Reay 1979; Folke et al. 1998; Muir and Young 1998; Naylor et al. 2000; Tidwell and Allan 2001; Hannesson 2003 see also Williams 1999).

Aquaculture is a highly diverse activity and selection of species is significant from an energy perspective due to the differences in characteristics of various species that limit the conditions under which they can be cultured (Troell et al. 2004). As such, aquaculture encompasses two main domains including commercial/industrial and rural/subsistence-based operations (Muir and Young 1998; Naylor et al. 2000; Troell et al. 2004). The diversity of aquaculture can be attributed primarily to three factors: the multiplicity of the cultured species (each with diverse life-histories, behaviour, and environmental requirements); the numerous forms of intervention undertaken by man; and the timing of that intervention in the life-cycle of the cultivated species (Nash 1995).

1.2 Abalone Aquaculture

1.2.1 Distribution and habitat

Except for the western coast of South America and eastern North America, abalone (Mollusca, Haliotidae) are found world-wide in both temperate and tropical waters (Imai 1977; Hahn 1989a; Nash 1991a; Brown and Murray 1992; Lindberg 1992; Lee and Vacquier 1995; Geiger 2000). However, there is no single species of abalone that is globally distributed and the various species appear to have quite restricted distribution ranges (Fallu 1991; Geiger 2000). The geographic range and depth distributions of the different abalone species are largely determined by their temperature requirements and by the distribution of suitable algal food species (Hooker and Morse 1985). The larger species are generally found in temperate regions, while the smaller species are found in tropical and arctic regions (Imai 1977). The greatest number of species is found in the central and south Pacific, and parts of the Indian Ocean, but none of these species are large in size (Cox 1960; Ino 1980; Fallu 1991).

In general, abalone are found in the lower intertidal zone and sublittoral fringe of rocky shores (Newman 1969; Hooker and Morse 1985; Prince and Shepherd 1992; Miller and Lawrenz-Miller 1993; Richards and Davis 1993; Preece and Mladenov 1999; Geiger 2000), the shallow to deep subtidal (Hooker and Morse 1985; Prince and Shepherd 1992; Preece and Mladenov 1999; Geiger 2000) to quite deep (400m) in the open ocean (Hooker and Morse 1985; White 1995). Due to their strict substrate requirements (rocky bottoms), abalone are conspicuously absent from sandy and muddy bottom areas, as well as estuaries (Kato and Schroeter 1985; Fallu 1991; Geiger 2000). McBride et al. (2001) consider abalone to be benthic and motile to semi-sessile animals. However, some authors consider them to be sedentary only (Wells et al. 1998), while others consider them sedentary, but make allowances for local movements in search of food (Momma and Sato 1969, 1970; Poore 1972c; Shepherd 1973). While several species may occur in a given habitat, there appears to be little microhabitat overlap, with similar species aggregating in large groups (Shepherd 1973; Douros 1987; *S*hepherd and Partington 1995; Wells et al. 1998).

1.2.2 Taxonomy

Investigating all 200 taxa known to have been ascribed to the Haliotidae (Mollusca: Archaeogastropoda), Geiger (1998) argued that the genus *Haliotis* was the only valid taxon. Geiger (1998, 2000) did, however, not rule out the possibility of other valid genera, but suggested that there was currently still inadequate evidence to substantiate claims to the contrary. While many authors (see e.g. Hahn 1989a; Fallu 1991; Lyon 1995) suggested that there were more than 90 *Haliotis* species globally, Geiger (1998, 2000) believes that there are only about 56 extant species and 10 extant subspecies. Despite these summations, many modern-day authors (e.g. Jarayabhand and Paphavasit 1996; Elliott 2000; Selvamani et al. 2001; Bester et al. 2004; Sales and Janssens 2004) still report substantially more (up to 90) extant species worldwide and yet others (e.g. Suzuki and Imai 1998; Nakamura and Archdale 2001; Weber and Vinogradov 2001; Baine and Side 2003; Yuasa and Suzuki 2005) report on genera other than *Haliotis*. As with most taxonomic issues, complete consensus is seldom reached.

1.2.3 Factors affecting growth

Growth can be defined as the result of ingested food being converted into body (somatic) tissue (Barnabé 1994). The maximization of growth is fundamental in the management of commercially exploited species to ensure successful commercial production that is economically viable (Keesing and Wells 1989; Capinpin et al. 1999; Lee 2004; Setyono 2005; Naidoo et al. 2006). The suppression of growth reduces productivity, overall yield and profitability (Lyon 1995).

Abalone growth is in general very slow and heterogeneous (Poore 1972b; Leighton 1974; Reay 1979; Shepherd and Hearn 1983; Hooker and Morse 1985; Hahn 1989e; Ebert 1991; Day and Fleming 1992; Viana et al. 1993; Mgaya and Mercer 1995; Preece and Mladenov 1999; Huchette et al. 2003a; Li et al. 2004; Naidoo et al. 2006). Consequently, the commercial exploitation of abalone has prompted numerous studies on their growth (e.g. Leighton and Boolootian 1963; Forster 1967; Newman 1968; Leighton 1972; Poore 1972a) and the effects of the following factors on growth must be known and managed effectively to maintain conditions within acceptable limits (Boyd 1985; Avault 1996; Colt and Tomasso 2001).

1.2.3.1 Water quality

"Water" quality is usually defined as the suitability of water for the survival and growth of aquatic organisms (Boyd 1982). The term "water quality" in the context of aquaculture, generally refers to the physical, chemical and biological factors that influence the well-being of aquatic biota (Ackefors et al. 1994). The term "quality" on the other hand, implies that no one factor should exceed certain upper limits for toxicity or fail to remain within some minimum-maximum range for life sustaining physico-chemical factors (Ackefors et al. 1994). There are a host of water quality parameters that are important to consider for optimal cultivation of any aquatic organism. These include salinity (Alabaster and Lloyd 1980; Brix 1983; Chen 1984; Beveridge 1987; Higashi et al. 1989; Anderson 1990; Fallu 1991; Landau 1992; Jarayabhand and Phapavisit 1996; Boarder and Shpigel 2001; Edwards 2003; Wuenschel et al. 2004), organic waste (faeces, metabolic, feed, fertilizers, etc.) (Rychly 1980; Pillay 1992, 1994; Goldburg et al. 2001), nitrogenous waste (ammonia, ammonium, nitrite, nitrate) (Burrows and Combs 1968; Kinne 1976; Spotte 1979; Colt and Armstrong 1981; Barkai and Griffiths 1987, 1988; Huguenin and Colt 1989; Wajsbrot et al. 1991; Swann 1992; Ackefors et al. 1994; Lyon 1995; Harris et al. 1997; Basuyaux and Mathieu 1999; Reddy-Lopata et al. 2000; Parker 2002; Lopez and Tyler 2006; Camargo et al. 2007), dissolved gasses (oxygen, carbon dioxide, nitrogen, hydrogen sulphide) (Marcello and Strawn 1973; Sylvester 1975; Spotte 1979; Boyd 1982; Elston 1983; Chen 1984, 1989; Sebert et al. 1984; Innes and Houlihan 1985; Beveridge 1987; Boyd and Watten 1989; Fallu 1991; Pillay 1992; Ackefors et al. 1994; Harris et al. 1997; Boyd 1998; Harris et al. 1999a, b;

Hindrum et al. 2001; Parker 2002; Cheng et al. 2004a; Lopez and Tyler 2006; Camargo et al. 2007), pH (McDonald 1983; Beveridge 1987; Randall 1991; Swann 1992; Barnabé 1994; Harris et al. 1999a), alkalinity and hardness (Moyle 1945; Boyd 1990; Landau 1992; Swann 1992; Stickney 1994), and pathogens (Lester and Davis 1981; Bower 1987; Pillay 1992, 1994; Douillett and Langdon 1994; Bachére et al. 1995; Nakatsugawa et al. 1999; Rengpipat et al. 2000; Olafsen 2001; Nicolas et al. 2002; Bower 2003; Macey and Coyne 2005).

1.2.3.2 Temperature

Most aquaculture species are poikilothermic, meaning that their body temperatures conform to the temperature of their environments (Landau 1992; Ackefors et al. 1994; Barnabé 1994; Stickney 1994; Poxton 2003). However, most poikilotherms are able to maintain internal temperatures within certain limits by means of physiological and behavioural mechanisms (Landau 1992). Ambient temperatures directly determine rates of gonadal recrudescence (Uki and Kikuchi 1984; Hahn 1989a, b, e; Ackefors et al. 1994; Parker 2002; Grubert and Ritar 2005), larval development (Leighton 1972, 1974; Ebert and Houk 1984; Owen et al. 1984; Hahn 1989c, e; Sawatpeera et al. 2001), feed consumption (Vermeij 1978; Uki 1981; Hahn 1989e; Peck 1989; Parker 2002) excretion (Barkai and Griffiths 1987; Lyon 1995), oxygen consumption (Uki and Kikuchi 1975; Barkai and Griffiths 1987; Lyon 1995; Poxton 2003), growth rate (Leighton 1974; Hahn 1989e; Peck 1989; Fallu 1991; Landau 1992; Swift 1993; Ackefors et al. 1994; Hinshaw et al. 2004) and survival (Hahn 1989d; Hinshaw et al. 2004).

All species have minimum and maximum lethal temperature limits that should be avoided. Of equal importance, however, is the need to provide the optimal temperature (Reay 1979). All abalone show different behavioural responses toward a thermal habitat and avoidance of lethal temperatures (Giattina and Garton 1982; Ackefors et al. 1994; Barnabé 1994) and the preferred temperature is primarily a function of recent thermal history or a thermal acclimation state (Windsor et al. 2005). Too rapid or too great a thermal change may be deadly because the organisms are unable to accommodate these changes metabolically (Ackefors et al. 1994). The preferred temperature thus represents the thermal range in which the processes that control activity are effective and their performance of efficiency increased and optimized (Brett 1956; Jobling 1981; Prosser and Nelson 1981; Kelsch and Neill 1990; Hecht 1994; Diaz et al. 2000). Examples of the preferred temperatures for abalone are well documented in the literature (see e.g. Hahn 1989d; Hecht 1994; Gilroy and Edwards 1998; Diaz et al. 2000, 2006).

The temperature requirements of abalone change during the development of their various life history stages (Leighton et al. 1981; Chen 1984; Hahn 1989e). Diaz et al. (2006) emphasized that two thermal groups of abalone exist: those adapted to cold climates species (e.g. *H. rubra*, *H. midae*, *H. laevigata* and *H. rufescens*) and those adapted to tropical climates species (e.g. *H. diversicolor supertexta*, *H. fulgens* and *H. corrugata*). It is possible to rear most species over a wide range of temperatures but, away from the optimal, growth is so slow that the time to reach market size is too great for rearing to be profitable (Barnabé 1994). However, animals and plants from temperate climates, can better tolerate exposure to higher temperatures, and often have accelerated growth rates under such conditions (Nash 1995). As a general rule, a coldwater or midrange species may attain a maximum size and weight as large as, or larger than those of a warmwater species, but the growth rate of the warmwater species will often be more rapid because of the higher metabolic rate (Stickney 1994). Finally, the effects of temperature and elevation on water quality parameters must be known and managed to maintain conditions within acceptable limits (Boyd 1985; Avault 1996; Colt and Tomasso 2001).

1.2.3.3 Feed

Under experimental conditions, abalone growth during short-term studies is largely dependent upon the nutritional value of the food and the abalone feeding rate (Stuart and Brown 1994). The growth rate of abalone fed natural algal diets is generally low and variable over time (Viana et al. 1993, 1996; Britz 1996b; Simpson and Cook 1998; Bautista-Teruel and Millamena 1999; Chen and Lee 1999; Coote et al. 2000; Serviere-Zaragoza et al. 2001). Formulated diets on the other hand, provide better growth rates in cultured abalone because they provide a balance of nutrients that may otherwise be lacking in single-feed natural diets (Hahn 1989e; Uki and Watanabe 1992; Viana et al. 1993, 1996; Kruatrachue et al. 2004).

Feed requirements differ during the different life history stages. Lecithotrophic larvae (pelagic or planktonic stage) do not possess any feeding apparatus and use their yolk to supply the energy for development during this early stage until they settle and begin to feed (Vance 1973; Huner and Brown 1985; Landau 1992; Barnabé 1994; Takami et al. 2000). It has been suggested that these non-feeding larvae have attained the ability to absorb nutrients (e.g. dissolved amino acids) directly from the external environment, allowing the larvae to conserve some egg nutrient reserves (Jaeckle and Manahan 1989a, b; 1992; Fallu 1991; Shilling et al. 1996). The absence of the need for food simplifies the operation of the hatchery (see Jaeckle and Manahan 1989b, 1992).

Postlarvae (spat, newly metamorphosed abalone) begin feeding immediately after settlement (Crofts 1937; Tutschulte and Connell 1988; Kawamura et al. 1995), initially feeding on microalgal biofilm, bacteria and mucus trails (Shepherd 1973; Saito 1981; Slattery 1992; Kawamura and Takami 1995; Kawamura 1996; Takami et al. 1997a, b; Stott et al. 2002, 2003, 2004a, b, c). Only when the radula is fully developed at approximately 800 µm shell length (SL), do spat start feeding on diatoms, turf and crustose coralline algae (Tong and Moss 1992; Dunstan et al. 1996; Takami et al. 1997a; Daume et al. 2000; Kawamura et al. 2001). This diet is maintained until the spat are large enough to undergo the final diet transition from diatoms to macroalgae (Jarayabhand and Paphavasit 1996; Kawamura et al. 2001) and the size of the individuals at this final transition varies among species (see Hahn 1989e; Jarayabhand and Paphavasit 1996; Kawamura et al. 2001). Animals are referred to juveniles or grow-out animals following formation of the first respiratory pore of the post larval stage, and prior to the onset of sexual maturity (Hooker and Morse 1985; Hahn 1989c).

Grow-out rearing is the final phase, during which the juveniles are placed into the adult environment and reared until they are harvested (Southgate and Lucas 2003). Juveniles begin to eat macroalgae at about 10 mm shell length and will eat about 10-30 % of their body weight in algae daily, the high feeding rates being attributed to the high water content and relatively low protein content of macroalgae (Hahn 1989e). Abalone food preferences have been studied from both the analysis of gut contents and feeding experiments (see Leighton and Boolootian 1963; Leighton 1966; Uki et al. 1986; Mercer et al. 1993; Corazani and Illanes 1998; Simpson and Cook 1998) and it is well documented that abalone from the northern hemisphere show a general preference for brown algae and kelps (Tomita and Tazawa 1971; Fallu 1991) while those from the southern hemisphere usually have more red algae in their guts (Poore 1972b; Fallu 1991). The higher presence of red algae in the guts of southern hemisphere abalone, may be attributed to the characteristics of these algae, namely that they lack polyphenolics which are often deterrents to feeding (Steinberg 1988), are easier to digest (Shepherd and Steinberg 1992), and are relatively higher in their protein content compared to green and brown algae (Fallu 1991; Stuart and Brown 1994).

1.2.3.4 Hardiness

Hardiness is described as the ability to tolerate crowding, handling (e.g. grading), variable physical and chemical conditions, and changes in the rearing conditions (Reay 1979; Barnabé 1994). To achieve an acceptable level of production, cultured species will no doubt experience conditions that differ considerably from their environment (Appleford et al. 2003). In culture, animals will generally experience social crowding, poorer water quality and handling, all of which induce stress. The cultured species should be able to adapt to these stresses and maintain high survival and optimal growth (Appleford et al. 2003).

1.2.3.5 Culture environment

Besides the above factors, the growth of abalone are also affected by a variety of culture environment factors including the culture system or flow system (Swift 1993; Alcantara and Noro 2006; Dlaza 2006); feeding regime (Hooker and Morse 1985; Hahn 1989e; Viana et al. 1993; Mai et al. 1994, 1995; Britz 1996a; Fleming et al. 1996, 1998; Guzmán and Viana 1998; Tahil and Juinio-Menez 1999; Boarder and Shpigel 2001; Fermin 2002; Bautista-Teruel et al. 2003; Kruatrachue et al. 2004; Alcantara and Noro 2006; Francis et al. 2007); abalone age (Chen 1989; Neori et al. 2000; Steinarsson and Imsland 2003; Alcantara and Noro 2006; Naidoo et al. 2006); abalone size (Barnabé 1994; Mgaya and Mercer 1995; Shipton and Britz 2001; Naidoo et al. 2006); light or photoperiod (Day and Fleming 1992; Clarke and Creese 1998); stocking density (Koike et al. 1979; Chen 1984; Douros 1987; Day and Fleming 1992; Knauer 1994; Mgaya and Mercer 1995; Marsden and Williams 1996; Capinpin et al. 1999; Huchette et al. 2003a, b); water velocity or water flow (Leighton and

Boolootian 1963; Shepherd 1973; Benson et al. 1986; Day and Fleming 1992; Higham et al. 1998; Capinpin et al. 1999; Huchette et al. 2003b; Dlaza 2006); handling techniques (Freeman 2001); water depth (Liu and Chen 1999); reproductive activity (Shepherd and Hearn 1983); species of abalone or genotype (Reay 1979); tank or rearing system design (Moss 1997; Chen and Lee 1999; Friedman et al. 2000; Benson et al. 1986; Preece and Mladenov 1999); basket design (Dlaza 2006); and growth hormones (Morse 1984; Fallu 1991; Kawauchi and Moriyama 1991; Landau 1992; Moriyama and Kawauchi 2004; but see Taylor et al. 1996).

1.2.3.6 Stress

Parker (2002) defines stress as … "a condition in which an animal is unable to maintain a normal physiologic state because of various factors adversely affecting its well-being" and classifies stress factors into four groups. Firstly there are chemical stressors such as poor water quality (low DO, unsuitable pH, etc.), pollution (intentional or accidental chemical treatments or pesticides), diet composition (type of protein or amino acids) and metabolic wastes (accumulation of ammonia or nitrite). Secondly there are physical stressors such as temperature, light, sounds, and dissolved gases. Thirdly there are biological stressors such as stocking density (overcrowding), food competition, diseases, stress, introduced species, genetically modified species, etc. Fourthly there are procedural stressors such as handling, shipping and disease treatments.

While impossible in some instances (e.g. grading), stress should in general be avoided. Properly dealt with, many managerial practices can be effectively managed to minimize the stress responses of animals. These include the choice of species, history of the animals cultured, the water chemistry, water flow rate, water temperature, ambient light conditions and cycles, bottom substrates, noise and other physical stimuli, stocking density, degree of handling and use of anaesthetics (AFS 2004). Stressed animals typically consume much more energy, are therefore less likely to grow, and consequently their chances of mortality increase (Reay 1979; Fallu 1991).

One of the main factors inducing stress in abalone includes aerial exposure during handling (e.g. grading) largely because the abalone are forced to undergo anaerobic respiration (Olley and Thrower 1977; Gäde 1988). Due to the ability of abalone to respire anaerobically for short periods, it is generally accepted that they can survive short intervals of aerial exposure (Gäde 1988; Baldwin et al. 1992; Behrens et al. 2002; Lleonart et al. 2003). It should, however, be emphasized that abalone gills collapse during aerial exposure and so respiration becomes impossible (Fallu 1991; Ragg and Taylor 2006; Song et al. 2007). Under farming conditions most handling techniques require animals to be exposed to air for prolonged periods of time in environments where it is nearly impossible to control the air temperature (Song et al. 2007).

1.2.3.7 Handling

Farming practices require the periodic removal of abalone from their holding structures in order to perform activities such as system maintenance, transfers between culturing units, adjustment of stocking densities, size-grading (sorting), tagging, harvesting (capturing), export preparation and transport, all of which require regular dislodging and removal from the rearing substratum (Hahn 1989d; Tegner and Butler 1989; Shepherd et al. 1992; Tong et al. 1992; White et al. 1996; Reaburn and Edwards 2003). Due to their large muscular foot which functions as an adhesive organ, possessing an exceptional ability to pull their shells quickly and tightly down on the substratum, removal during routine procedures becomes extremely difficult, often impossible without the use of mechanical devices (Fretter and Graham 1962; Barnes 1987; White et al. 1996). The use of equipment such as knifes ("knifing") or spatulas (scrapers) to remove abalone mechanically often causes injury, which may lead to infection with bacteria, and due to their slow healing rates, may eventually lead to mortality (Armstrong et al. 1971; Genade et al. 1988; White et al. 1996; Reaburn and Edwards 2003). Death by injury (even small injuries) has also been caused by the failure of blood to clot (Cox 1962; Armstrong et al. 1971; Genade et al. 1988; Hahn 1989d; Tong et al. 1992). Consequently, handling is considered an important cause of mortality in abalone (Reay 1979; Genade et al. 1988; White et al. 1996; Ragg et al. 2000; Edwards et al. 2000). Forced removal of abalone may also result in excess mucus production, with subsequent energy losses (Peck et al. 1987; Davies and Williams 1995; McBride et al. 2001). In addition, as stated earlier, during routine handling procedures, abalone are regularly exposed to air. This is stressful because the abalone are unable to irrigate their gills during aerial exposure (Fallu 1991; Ryder et al. 1994; Gorfine 2001; Song et al. 2007). Currently, handling practises during activities such as size-grading, harvesting, live storage, and transport, do not allow for recovery time from aerial exposure (Ryder et al. 1994). For good productivity, it is therefore important to avoid injury and stress to abalone to minimize mortalities during farming activities.

1.2.3.8 Grading

Heterogeneous growth rates within a cohort of abalone often result in noticeable size variation among individuals (see Cambell 1985; Beveridge 1996; Chua and Tech 2002; Johnston et al. 2005). Research has shown that larger aquatic animals are competitively superior to smaller ones and this has resulted in the need for size-grading in order to prevent intraspecific grazing competition (see Campbell 1985; Masser 1995, 2004; Mgaya and Mercer 1995; Beveridge 1996; Chua and Tech 2002; Johnston et al. 2005; Setyono 2005).

1.2.3.9 The use of anaesthetics

Abalone farms have tested various methods for removing abalone from the rearing substrate without causing injury (Tong et al. 1992; Stuart and Brown 1994; White et al. 1996; Chacón et al. 2003). Anaesthetics have subsequently proved to be important for the well-being of the cultured animals particularly during handling and transportation (Stoskopf 1992; Alderman 1999). The purpose of an anaesthetic is to cause relaxation of the soft tissue as well as to reduce the degree of awareness in the abalone (Hahn 1989d; Tong et al. 1992; White et al. 1996; Aquilina and Roberts 2000).

Several anaesthetics have been suggested for abalone (see e.g. Prince and Ford 1985; McShane and Smith 1988; Hahn 1989d; Tong et al. 1992). While some anaesthetics are pharmaceutically controlled and thus not easily available, others are costly and unsafe (Hahn 1989d; Marking and Meyer 1985; Schnick et al. 1986; White et al. 1996; Bernstein et al. 1997; Holloway et al. 2004). The restrictions on the use of anaesthetics in farming operations are not determined solely by its effectivity, but moreover by the legality and safety of their use (Marking and Meyer 1985; Schnick et al. 1986; Schnick 2001).

Carbon dioxide has been used as an anaesthetic in almost every animal phylum because it is efficient and cost-effective (Sugiyama and Tanaka 1982; Ross and Ross 1984 see also Stefan 1992; Bernier and Randall 1998; Summerfelt 2000; Coyle et al. 2004). Some advantages of carbon dioxide are that it leaves no residues, requires no depuration period, and requires no registration, since its use in food fish is already allowed under the present GRAS (generally regarded as safe) declaration by the U.S.A. Food and Drug Administration (FDA) (Gilderhus and Marking 1987). However, some disadvantages are listed by Smart (1981) and Summerfeldt et al. (2000) including acidosis, hypercapnia, sedation, reduced growth, decreased food conversion ratios, lower condition factors, reduced hematocrit and plasma chloride factors and even death, emphasizing that no anaesthetic is problem free.

1.3 Mariculture of the South African abalone, *Haliotis midae* Linnaeus

1.3.1 South African abalone

Of six extant species (*H. saldanhae* Kensley is extinct) of abalone occurring along the South African coastline, five are endemic (Kensley 1972; Kensley and Pether 1986; Geiger 2000; Evans et al. 2004). Among these endemics, *H. speciosa* Reeve and *H. queketti* Smith are particularly rare, and *H. spadicea* Donovan and *H. parva* Linnaeus are small and cryptic (Muller 1986; Branch et al. 1994). *Haliotis midae* Linnaeus is the largest and most commonly observed of the five endemic species (Branch et al. 1994). Of all species, *H. midae* is also the only one that occurs in adequate quantitites for commercial exploitation (Newman 1967; Hecht 1994; White 1995); the small size and low biomass (rarity) of the other species make them commercially unattractive (Newman 1969; Lyon 1995). Within the distribution of *H. midae*, "generally smaller-sized geographic races of the species are found in the warmer parts of the species range, while larger-sized geographic races are found in cooler environments" (Newman 1969). For additional comments on the distributions of these species, see Britz et al. (1997) and Evans et al. (2004).

1.3.2 Suitability of *H. midae* as a culture species

Like many other Haliotids, *H. midae* possess many attributes that make it suitable for cultivation. These include: prolific gamete production; minimal numbers of adults as broodstock; a planktonic larval stage that is non-feeding, carrying a vital supply of yolk material; an algal food supply to juveniles that is relatively non-fouling; and high survival under crowded conditions (Tarr 1991). In addition to these, and despite their slow growth rates, Britz (1995) listed some fundamental attributes that contribute to *H. midae*'s suitability, including: having a high stress threshold; is generally good adaptation to captive conditions; their growth and health are not easily compromised by man-made environments and handling; their non-aggressive or non-territorial nature allowing for successful rearing at high stocking densities; disease resistance; a captive breeding cycle that is closed due to hatchery technology that is currently well established; seed that can be produced annually on an industrial scale; and acceptance of dry pelleted, formulated feeds.

1.3.2.1 Reproductive biology

Although the South African abalone fishery has existed since 1949 (Tarr 1992), the first attempt to cultivate *H. midae* was only made in 1981 when captured specimens were successfully spawned (Genade et al. 1985, 1988). In general, water temperature appears to be the overriding factor that determines when *H. midae* will reach sexual maturity. Newman (1969) and Tarr (1993) found that *H. midae* reached sexual maturity at 33-40 mm shell width in the Eastern Cape and in the Western and south-Western Cape, where the growth rate was slower, sexual maturity was reached at 80 mm shell width (age 4-7 years). In addition, 100% sexual maturity may occur as early as 3 years of age on the warmer east coast and under culture conditions (Wood 1993) or as late as 7.2 years of age on the west coast (Tarr 1995).

1.3.2.2 Size and growth rate

A cultured species must preferably display a rapid growth rate in order to reach market-size quickly and historically a growth period of more than two years was considered inadequate (Appleford et al. 2003). Low risk and high economic value as in the case of abalone such as *H. midae*, however, have overcome the traditional *status quo* (Appleford et al. 2003). The commercial interest of an abalone species is not only determined by its fecundity but also by its size as larger species are considered more suitable for culturing (Nash 1991b). The large size of *H. midae* (up to 230 mm) (Newman 1968; Tarr 1987; Hecht 1994; Steinberg 2005) makes it ideal for culturing (Roodt-Wilding and Slabbert 2006).

Generally, *H. midae* is a slow grower (Tarr 1987; Nash 1991b) obtaining a maximum shell growth rate in culture of up to only 2.9 mm/month (Britz 1995; Shipton and Britz 2001). Due to its large size and slow growth rate it is impractical to attempt to culture *H. midae* to its full size in an aquaculture environment (Dixon 1992). Therefore, to reduce production time, cultured *H. midae* are harvested as “cocktail”-sized abalone when they are between 2.5 and 5 years old and have reached 50 to 80 mm shell length (Hooker and Morse 1985).

1.3.2.3 Abundance

Haliotis midae are most abundant where the sea temperatures vary between 15 and 17 ºC (Newman 1969), and variable in densities between St. Helena Bay and Cape Agulhas (Barkai and Griffiths 1986; Hecht 1994). It is the only South African abalone species that occurs in quantities large enough to make commercial exploitation feasible (Genade et al. 1988). The high abundance of *H. midae* along the south west Cape has been attributed to the abundance of kelp (Branch and Branch 1989; Hecht 1994). Their CTM (critical thermal maximum; maximum bearable temperature) can, however, explain the eastern limit of their distributional range as the sea temperatures often exceed 26-27 ºC in the vicinity of East London (Hecht 1994). Hecht (1994) suggested other possible reasons for the higher abundance of *H. midae* in cooler waters, including reduced predation pressures, or greater susceptibility to disease at higher temperatures.

1.3.2.4 Water quality

The South African coastline generally experiences severe swells and wave action and has few protected bays, making on-growing systems using in-water barrels or cages impractical (Cook 1998). The benefit of the South African coastal environment, however, is that much of it is reasonably free of industrial or domestic pollution and locations where abalone farms are situated usually have relatively good water quality (Cook 1998). Water conditions along the coastline are also such that it supports large beds of kelp (*Ecklonia maxima* and *Laminaria pallida*) which serve as the main food item for *H. midae* (Rotmann 1999).

1.3.2.5 Optimal temperature requirements

Temperatures ranging from 12 to 20 ºC are physiologically optimal for juvenile and grow-out *H. midae* (Britz et al. 1997). Above 20 ºC ammonia excretion and oxygen consumption rates increase significantly (Lyon 1995) and consequently growth rates decline, feed conversion and protein-efficiency ratios deteriorate and mortality increases significantly because of a gradual breakdown in physiological processes (Britz et al. 1997; Sales and Britz 2001). From a farming perspective, feeding should be stopped once water temperatures exceed 20 ºC.

1.3.2.6 Metabolism

Poikilotherms such as *H. midae* do not need to use energy for swimming or to maintain a constant body temperature (Fallu 1991) and in general, the metabolic rate is a predictable function of body size and ambient temperatures (Schmidt-Nielson 1979; Britz et al. 1997). In general, there is an inverted relationship between respiration (oxygen consumption) rate and abalone body size and temperature (Uki and Kikuchi 1975; Hahn 1989d; Peck et al. 1987; Lyon 1995). Higher temperatures, however, will only have a positive effect on growth rate if there is an increase in the digestion efficiency of the food (Newell and Branch 1980), or that nutrient ingestion exceeds the metabolic demand of the animal (Brett and Groves 1979; Hughes 1986).

1.3.2.7 Feeding habit

Haliotis midae are generalist, opportunistic herbivores feeding nocturnally (Muller 1984; Knauer et al. 1995; Wood and Buxton 1996) and consuming a wide variety of algal species that are abundant in their vicinity (Barkai and Griffiths 1986; Wood and Buxton 1996; Sales and Britz 2001; Troell et al. 2006). The preferred algal species are, however, not necessarily the species that produce the best growth (Leighton 1966). The kelps *Ecklonia maxima* and *Laminaria pallida* (south coast) and the red alga *Plocamium corallorhiza* (east coast) have been identified as the main food source for wild *H. midae* (Newman 1968, 1969; Barkai and Griffiths 1986; Tarr 1987, 2000; Wood 1993; Britz 1996b; Wood and Buxton 1996; Sales and Britz 2001). *Ecklonia maxima* is the most abundant algal species along the southwest coast of southern Africa and is therefore the most widely used food source for abalone farms developing along this area of coast (along the northern west coast of South Africa and in Namibia it is largely replaced by *L. pallida*) (Stegenga et al. 1997). Wild *H. midae* consume about 7-10 % of their body mass per day in fresh *E. maxima* fronds (Rotmann 1999; Levitt et al. 2002). Stepto and Cook (1996) found, however, that *E. maxima* was the least preferred of three algae fed to *H. midae* and suggested that this might be due to the high phenolic levels. Other, less desirable properties of kelp include its low protein (5 – 15 %) and high water content (68 - 83 %) (Hahn 1989e; Robertson-Andersson 2004).

A number of other algal species are also actively consumed. Wood and Buxton (1996) found that adult *H. midae* readily consumed the encrusting alga *Ralfsia verrucosa* and it was suggested that this alga was actively consumed because it was never found between the drift algae. As abalone in smaller size classes and *Ulva* spp. are both abundant in shallow water, the diets of smaller sized *H. midae* are often comprised largely of *Ulva* spp. (Barkai and Griffiths 1986; Sales and Britz 2001). Wood and Buxton (1996) argued that despite the low levels of *Ralfsia* and *Ulva* spp. in the environment, their high percentage in abalone gut samples suggested a preference for these algal species by *H. midae*. In addition to consuming various fleshy rhodophytes, juvenile *H. midae* have a high percentage of coralline red algae in their guts (Wood and Buxton (1996). While Wood and Buxton (1996) suggested that this feeding behaviour may be related to shell growth requirements during the early years, in all likelihood it may simply be due to the fact that abalone larvae (and thus juveniles) often recruit to the surfaces of coralline red algae and thus start feeding on these algae as a food source (see Morse and Morse 1984; Day and Branch 2000).

1.3.2.8 Natural vs formulated diets

The majority of South African abalone farms are located along the South African south, south west and west coasts (see Rothman et al. 2006; Troell et al. 2006). This is largely so because they initially all took advantage of the main food source (kelp) that occurs so abundantly along these shores (Simons 1990; Simpson and Cook 1998; Gray 2003), making it the most logical and economical feed for commercial abalone culture (Dixon 1992; Simpson and Cook 1998; Troell et al. 2006). There are, however, a number of reasons why kelp and other natural feeds are proving less viable. One of the main drawbacks of feeding kelp is that its harvesting is dependent on sea conditions; this complicates farm management and increases the risk of such ventures (Dixon 1992; Britz 1995). The use of kelp is considered by some more costly and labour intensive when the expenses of harvesting, transport and storage are taken in account (Hahn 1989e; Dixon 1992; Britz 1993, 1995; Dunstan et al. 1996; Reyes and Fermin 2003; Lee et al. 2004). In addition to these factors, kelp has been reported to be approaching limits of sustainable harvesting, particularly in kelp concession areas with high abalone farm concentrations (Anderson et al. 2001, 2006; Rothman et al. 2006). These factors have lead to the development of nutritionally complete formulated feeds.

Some of the advantages of using formulated feeds include: reliability (Fallu 1991; Dixon 1992; Britz 1993, 1995; Lyon 1995; Tahil and Juinio-Menez 1999); convenience and ease of application (Dixon 1992; Britz 1993, 1995; Britz et al. 1994; Sales and Britz 2001); balanced nutritional composition (Lyon 1995; Britz 1996a, b; Fleming et al. 1996; Viana et al. 1996; Mai et al. 2001; Montaño-Vargas et al. 2005); ease of production (Britz 1993, 1995; Lyon 1995); higher growth rates (Britz 1993, 1995, 1996a, b; Britz et al. 1994; Lyon 1995; Viana et al. 1996); lower food conversion ratios (FCR) (Britz 1993; 1995; 1996b; Knauer 1994) and thus higher feed conversion efficiencies; easier digestibility (Lyon 1995); ease of storage and transportation (Britz 1993, 1995; Lyon 1995); easier handling (Britz 1993, 1995; Lyon 1995); year-round availability (Britz et al. 1994; Lyon 1995); and the overriding advantage of being geographical and location independent (Dixon 1992; Britz 1993, 1995; Lyon 1995). Britz (1993) had previously emphasized that the use of formulated, balanced diets was fundamental to the success of any intensive farming industry taking into account that the overall objective of applied nutrition was to rear an animal to market-size in the shortest possible time, for the least possible cost, and by providing the animal with the correct nutrient concentration in a readily available form. Britz (1993) found that *H. midae* readily accepted formulated feeds and when given a choice, it was found to consistantly choose formulated feed over natural feeds (seaweed).

Currently two brands of formulated feeds are available in South Africa: Abfeed® (Marifeed Pty Ltd, South Africa), a fishmeal-based formulated feed; and Midae Meal™ or "Midae Meal MM-1c" (Eric-Piet [Pty] Ltd, Luderitzbucht, Namibia), a seaweed-based formulated feed (Troell et al. 2006). Abfeed®-S34 contains mainly fishmeal, soya bean meal, starch, vitamins and minerals with an approximate compositional analysis of 34.6 % protein, 43.3 % carbohydrates, 5.3 % fat, 1.2 % Crude fibre, 5.7 % ash and ~10 % moisture (Marifeed Pty Ltd, South Africa). For a number of reasons (that will not be covered here), a series of low-protein variants of Abfeed® (e.g. Abfeed®-K26 [26% protein]; Abfeed®-K22 [22% protein]) are now currently under production, all aimed at achieving different outcomes. Midae Meal™ consists entirely of seaweeds, including *Laminaria* and *E. maxima* (stipes and fronds), *Graciliaria* spp., *Gelidium* spp. (including the epiphyte *G. vittatum*), *Porphyra capensis* and "agar-agar" (Troell et al. 2006). While the Abfeed® variants have been extensively tested (see e.g. Chalmers 2002; Gray 2003; Simon et al. 2004; Naidoo et al. 2006; Dlaza et al. 2008; Francis 2008), Midae Meal™ has not, although Dlaza et al. (2008) have provided a comparative analysis of various fishmeal- and one seaweed-based formulated feeds.

While many studies have focused on single-species feeds (Day and Fleming 1992; Day and Cook 1995; Fleming 1995; Simpson and Cook 1998) and formulated feeds (see above), multiple-species diets are proving to be of tremendous benefit to the abalone industry. In particular, a number of such studies involving *H. midae* have been conducted ranging from mixed diets (e.g. Owen et al. 1984; Day and Fleming 1992; Fleming 1995; Simpson and Cook 1998; Naidoo et al. 2006; Daume et al. 2007), to rotational diets (Simpson and Cook 1998; Naidoo et al. 2006), to supplementation with fresh wild seaweeds (Dlaza et al. 2008). All of these studies have confirmed that variation in the diet often results in enhanced growth in abalone that have become accustomed to a monotone diet.

1.3.3 Marketing and economics

The main criterion for the selection of species for culture is market demand and profitability (Nash 1995; Appleford et al. 2003). Abalone are globally considered as one of the most valued seafood species (Setyono 2005; Cai et al. 2006; Reddy-Lopata et al. 2006). Asians (particularly the Chinese and Japanese) are the foremost consumers of abalone because they consider them as part of traditional cuisine and ceremony (Chen 1989; Ikenoue and Kafuku 1992; Britz 1995; Sales 1999; Sales and Britz 2001). But, not all abalone (for a number of reasons) are considered equally attractive and so species differ in their market value and consequently their suitability for cultivation (Hooker and Morse 1985; Fallu 1991; Nash 1995; Oakes and Ponte 1996; Elliot 2000). *Haliotis midae* is an attractive species that has several marketing opportunities. Farm-grown animals of various sizes are sold for restocking, for sea ranching (farming) (Cook 1998), or sold live, frozen, dried, canned, in soups, as food to the Eastern markets (Olley and Thrower 1977; Tarr 1987; Britz 1990; Landau 1992; Vosloo and Vosloo 2006). Due to their slow growth, however, *H. midae* is mostly sold as cocktail- or "medallion"-sized [6-8 cm] abalone (Britz 1990; Gordon and Cook 2001).

In South Africa the development of a viable *H. midae* cultivation industry is currently the foremost success story in mariculture (Vosloo and Vosloo 2006). Investment in this industry has largely been driven by decreasing commercial fishing quotas (Cook 1998), the existence of a seemingly avid foreign market demand (Britz 1991; Oakes and Ponte 1996; O'Omolo et al. 2003), and the positive exchange rates experienced by exporters (Huchette et al. 2003a; Macey and Coyne 2005; Vosloo and Vosloo 2006). Due to the fact that numerous countries apart from South Africa (e.g. Chilli, Australia, China, Taiwan, Japan, United States of America and Mexico) are now also concentrating on the production of market abalone, the local industry is being forced to become more competitive in terms of decreasing production costs and simultaneously improving product quality and yield (Vosloo and Vosloo 2006).

The Asian market, however, generally considers the South African *H. midae* to be superior to many other abalone species (including Australian and New Zealand species) increasing the demand which constantly exceeds supply. Consequently, the meat of the muscular foot of *H. midae* fetches high market prices (Britz 1990; Alvarez-Tinajero et al. 2001). These high market prizes have largely also been the reason for the poaching of wild abalone and exploitation of secondary stocks (marginal size wild stock) formerly considered not viable to harvest (Britz 1991). These negative spin-offs have, however, contributed to improved fisheries management and upgrading of abalone aquaculture (Britz 1991; Brugère and Ridler 2004) with the local abalone cultivation industry growing exponentially (Troell et al. 2006).

With the exception of Asia, South Africa is currently the largest abalone producer in the world (FAO 2004). Commercial farms in South Africa produced 750 tons of abalone in 2005 (Loubser 2005). This increased to 890 tons in 2006 (Wayne Barnes, AFASA, *pers. com*. 2007). While being estimated to exceed the 1000 ton mark (Belemane Semoli, Marine Aquaculture Management Unit, *pers. comm.* 2007), by the end of 2007 production achieved 783 tons (Wayne Barnes, AFASA, *pers. com*. 2008). With an average of roughly 808 tons per annum for the period 2005-2007, abalone aquaculture in South Africa is becoming an increasingly profitable enterprise. Currently there are 18 abalone rights-holders of which 14 are operational and of which 13 are exporting (Wayne Barnes, AFASA, *pers. com*., 2008). With countries such as Chili now becoming a major abalone producer (see e.g. Flores-Aguilar et al. 2007), Roodt-Wilding and Slabbert (2006) advise that if South Africa wants to stay competitive in this growing international market, the focus should be on the application of advanced management strategies and the development of innovative technologies.

2.1 Current emphasis of abalone research in South Africa

In 2007, the South African Department of Environmental Affairs and Tourism (DEAT) established the Marine Aquaculture Management (MAM) unit (Semoli 2007). The Frontier Program, which is the research component of this unit, drives research initiatives and provides technical inputs that will eventually be incorporated into management strategic components (Semoli 2007). The following areas of research are seen as particularly important for the strengthening of the South African aquaculture industry: 1) nutrition (i.e. nutritional requirements of all life-cycle stages of abalone, including spat, juveniles and adults; seasonal, regional and geographical nutritional variability and polyphenolic content of kelp; the use and development of probiotics; and seasonal succession of diatoms and their role as food for spat); 2) genetic and molecular studies; 3) animal husbandry (i.e. improving water quality; nitrogen supersaturation and gas bubble disease; winter clay; inducers of larval settlement; effects of handling/grading; and development of live export protocols); 4) farm design (i.e. tank and basket design); 5) optimal carrying capacities; 6) environmental impacts; 7) integrated mariculture; 8) animal health (i.e. national survey of animal diseases; the use of haematology and blood chemistry to determine abalone health status; and polydorid polychaete infestations); 9) harmful algal blooms (for the prevention of spat mortalities and Paralytic Shellfish Poisoning); and 10) overall food quality, safety and public health (Pitcher 2005).

2.2 Focus of the current research

South African abalone farmers have expressed concern about the effects that grading has on subsequent growth and survivorship of their animals (Wayne Barns, AFASA, *pers. comm.*). Grading has been advocated to induce stress in abalone and their subsequent recovery from grading is often variable (AFS 2004). The debate as to whether juvenile abalone younger than 30-months should be graded or simply split is ongoing and not yet critically evaluated. In addition, it has also been suggested that larger, machine-graded animals tend to be more stressed by the grading process than hand-graded animals, and that there are higher counts of shell damage and subsequent abalone mortalities from the machine-graded process (Jonathan Venter, Jacobsbaai Sea Products, *pers. comm*.). Of concern too is the use or lack thereof of carbon dioxide as an anaesthetic during the grading process (Jonathan Venter, Jacobsbaai Sea Products, *pers. comm*.). Nothing is currently known of the effects of the grading methods on the recovery and survivorship of abalone, and while much speculation exists amongst abalone farmers, there is little scientific data or published material to warrant such speculation.

The primary aim of the current study falls within the main framework of the Frontier Program (Pitcher 2005), namely to address aspects of "Animal Husbandry". Within this research area, the determination of the effects of handling/grading and chemicals (anaesthetics) on the growth and survivorship of abalone are highlighted as key research areas and these are thus one of the focuses of this research. In addition to these research initiatives, two questionnaires were developed to ascertain the variable farm management practices surrounding these research areas. These questionnaires were also used to gauge the level of communication between abalone farmers and the broader scientific community with the explicit goal of making recommendations towards best farm management practices pertaining to handling/grading on South African abalone farms.

Chapter 3

The effects of hand-grading vs. splitting on the growth of < 30-month old *Haliotis midae*

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3.1 Abstract

Size grading is a routine practice in shellfish hatcheries and generally stimulates the growth of smaller individuals in the absence of larger, presumably dominant competitors. Abalone farmers have, however, expressed concern over the effects that the grading process has on subsequent growth and survivorship of animals. Grading has been advocated to induce stress in abalone and their subsequently recovery from grading is often variable. The debate as to whether juvenile abalone < 30 months, that have never undergone a grade before, should be graded or splitted, is ongoing and not yet critically evaluated and it has been suggested that grading so early is unnecessary. The aim of this study was to determine the recovery response and survival rate of juvenile abalone < 30 months old, undergoing a hand-grading versus a splitting exercise, without the application of an anaesthetic. Growth of the abalone was monitored on a commercial abalone farm over a period of 4 months and consisted of 2 treatments with 4 replicates (n = 200 abalone per replicate). The treatments were: hand-grading; and direct splitting. While abalone weight increases showed no significant differences between treatments (p = 0.282), growth in shell length showed significant differences with hand graded abalone (60.39 ± 0.984 µm.day^{-1}) performing worse than split abalone (72.42 ± 1.143 µm.day^{-1}; p < 0.001). This study shows that it is better to perform a splitting exercise for size-sorting abalone at an early age. Not only does this appear to be less stressful to the abalone, but it is also more cost-effective to the abalone farmer because of the savings in costs associated with the grading process

Keywords: abalone, grading process, growth, *Haliotis midae*, hand-grading, splitting

3.2 Introduction

The commercial exploitation of abalone has resulted in many studies on their growth (see e.g. Leighton and Boolootian 1963; Newman 1968; Leighton 1972; Hayashi 1980; Shepherd et al. 1988; Day and Fleming 1992; Britz 1996a; Hindrum et al. 2001; Dlaza 2006; Garcia-Esquivel et al. 2007). Growth of abalone on macroalgae and formulated diets in particular, has been widely studied (see e.g. Koike et al. 1979; Nie et al. 1986; Viana et al. 1993; Britz and Claydon 1996; Chen and Lee 1999; Nelson et al. 2002; Taylor and Tsvetnenko 2004; Naidoo et al. 2006; Dlaza et al. 2008). Despite extensive published accounts on the growth of abalone and the causes for their differential growth, many areas of research into their commercial exploitation still remain largely unexplored. One such area of research is the effects that the grading process has on recovery, subsequent growth and survival.

Size grading is a routine practice in shellfish hatcheries and generally stimulates the growth of smaller individuals in the absence of larger, presumably dominant competitors (Jarayabhand and Newkirk 1989; Kamstra 1993; Mgaya and Mercer 1995; Setyono 2005; Ashley 2007). Abalone farmers have, however, expressed concern about the effects that the grading process has on recovery, subsequent growth and survivorship of the animals. Grading has been advocated to induce stress (Cambell 1985; Flos et al. 1988; Qin et al. 2001) and the subsequent recovery of abalone from the grading process is often variable. The debate as to whether juvenile abalone < 30 months, that have never undergone a grade before, should be graded or splitted, is ongoing and not yet critically evaluated (Gavin Johnston, HIK abalone, *pers comm*.). It has even been suggested that grading so early is unnecessary (Jonathan Venter, Jacobsbaai Sea Products, *pers. comm*.). The aim of this research was therefore to determine the recovery response and survivorship of juvenile abalone < 30 months old, undergoing a hand-grading versus a splitting exercise, without the use of an anaesthetic. This was attempted to make recommendations as to which practice was most appropriate for such young abalone in light of suggestions by some abalone farmers that possible anaesthesia may not be necessary.

3.3 Materials and Methods

3.3.1 Experimental system

The research was conducted on the Jacobsbaai Sea Products (JSP - 17º 53' 12.5" E, 32º 58' 2.5" S, Western Cape, South Africa) commercial abalone farm. Moderately aerated seawater (supplied by means of conduit pipes with holes of 1 mm diameter) with a flow rate of 1000 ±100 $L.h^{-1}$ was supplied at 13.8 ± 1 ˚C in concrete production tanks (5500 x 1300 x 550 mm; length, width, depth) with a total water holding capacity of 4500 litres. Abalone were grown in mesh culture baskets (1200 x 500 x 500 mm; length, width, depth; mesh diameter = 12 mm) ideally suited for culturing abalone fed kelp. Culture baskets were subdivided with vertically orientated plates to increase the surface area for attachment and replicate baskets were arranged according to the Latin square model (Zar 1984) to compensate for end effects[1]. Both the flow-through tanks and the culture baskets were cleaned every two weeks.

3.3.2 Experimental animals

Hatchery-reared juvenile abalone < 30 months were supplied by the Jacobsbaai Sea Products farm. Since abalone have a very heterogeneous growth rate, depending mainly on size, age and feeding rate (Viana et al. 1993; Mgaya and Mercer 1995; Preece and Mladenov 1999; Huchette et al. 2003a; Lee 2004), abalone of similar size and from the same gene pool (cohort) were used. All juvenile abalone used were spawned in October 2005 and were approximately 20 months old at commencement of the experiment. The abalone were subdivided into four replicate baskets of 200 individuals per replicate, per treatment. The initial body weight and shell length of the post-sorted abalone were measured at 6.8 ± 0.10 g and 33.8 ± 0.16 mm respectively.

[1] End effects refer to the potential negative impacts (e.g. reduced water flow-rate, accumulation of faeces and silt, etc) that may arise from a unidirectional flow.

3.3.3 Feed

Prior to commencement of the experiment, the abalone were cultured on a mixture of kelp (*Ecklonia maxima* [Osbeck] Papenfuss) and Abfeed-S34 (Marifeed Pty Ltd, South Africa). For this experiment, however, kelp was chosen as the sole dietary feed because it is relatively cheap. The experimental animals were initially fed kelp *ad libitum* once per week, but in order to determine the food conversion efficiency (FCE), a fixed amount of 1.5 kg of kelp per basket per week was supplied for part of the experimental period. Before each feed, left-over kelp was removed and fresh kelp topped up.

3.3.4 Experimental Procedure

Half the experimental animals were hand-graded with a standard abalone hand-grader as per the commercial abalone farm practice (see below), while the other half underwent a splitting[2] exercise in which animals were simply subdivided in two. It should be emphasized that the grading ring measurements are based on the shell width and so greater variability in shell length is inevitable. No anaesthetic was used during either of the two procedures.

The hand-grading process on the JSP farm consisted of the following sequence of events. First, animals were removed from the holding tanks while still in their on-growing baskets and transferred to the grading station where they were then bulk weighed. This was followed by temporary placement into a smaller flow-through tank with a flow rate of 1500 $L.h^{-1}$. One basket at a time was then removed from the temporary flow-through tank and their contents inverted onto the grading table whose upper surface consisted of a small-size mesh material required to drain the excess water. The abalone were loosened from one another by hand and then size-sorted according to various shell widths using a grading ring with fixed diameters of

[2] Splitting was done by visual size separation.

24, 30, 38, and 46 mm. Size-sorted abalone were then placed into smaller grading baskets (600 x 200 x 300 mm; length, width, depth) that were immersed in another flow-through tank (1000 x 660 x 560 mm). These smaller baskets were marked according to the applicable size-classes of the grading ring size. Size-sorted abalone were then removed from the temporary flow-through system while still in their grading baskets and transferred to a weighing table. Here the abalone were weighed yet again. The original (now empty) grading basket was returned to the grading-table flow-through system and filled up again with newly graded abalone. Graded abalone were then placed in a larger mesh-net bag from where they were returned to their newly marked, color-coded baskets in the larger flow-through system. It took 8-10 minutes to grade a basket of abalone. This same routine was followed for the animals undergoing the splitting exercise, but instead of being graded with the hand-grading device, the abalone were simply separated by visual inspection and placed by hand into their color-coded baskets. It took 3-5 minutes to split a basket of abalone.

3.3.5 Sampling and data collection

The experiment was conducted over 4 months beginning in June 2007 and ending in October 2007 (winter through early spring). Monthly measurements of 30 individuals from each of the four replicates were randomly chosen. Before all weight measurements, abalone were blotted dry to remove excess water. The blotted wet body weight was recorded to the nearest 0.001 g using a top loading digital electronic balance while shell length was measured along the longest axis to the nearest 0.01 mm using a digital electronic vernier callipers.

Daily increment increase in shell length (DISL in $\mu m.day^{-1}$) was calculated using the formula of Mai et al. (2001) and Zhu et al. (2002):

$$DISL = [(SL_t - SL_i)/t] \times 1000$$

Where SL_t = final mean shell length; SL_i = initial mean shell length; t = the feeding trial period in days.

Specific growth rate (SGR in % weight.day^{-1}) was calculated as in Britz (1996b) using the formula:

$$SGR = [(\ln(W_f) - \ln(W_i)) / t] \times 100$$

Where $\ln(W_f)$ = the natural log of the final mean weight; $\ln(W_i)$ = the natural log of the initial mean weight; t = the feeding trial period in days.

The feed conversion efficiency (FCE) was calculated using the formula of Simpson and Cook (1998):

$$FCE = (growth/ration) \times 100$$

Where growth = the blotted wet weight (g) gained per day; ration = the blotted wet feed (g) intake.

The condition factor (CF in g.mm^{-1}), which is an index that was developed to account for the relationship between the weight of abalone gained per unit shell length, was calculated using the formula of Britz (1996b).

$$CF = [BW / SL^{2.99}] \times 5575$$

Where CF = the condition factor, BW = the mean body weight (g), SL = mean shell length (mm); 2.99 and 5575 are constants.

During the regular monthly measurements, baskets were also checked for mortalities. This was recorded and expressed as a percentage of the total stocking density for each treatment.

3.3.6 Statistical analysis

All data are expressed as means ± se. Data for all experimental replicates were pooled as no significant differences were found between them. The student's T-test was used to test for differences between means. To test for correlation, the body weight and shell length of abalone from each sorting treatment were compared by means of a linear regression test (Pearson's product-moment correlation test). All results were considered statistically significant at $p < 0.05$.

3.4 Results

What is immediately evident is that those animals that underwent the splitting exercise, maintained a relatively constant growth rate (SGR = 0.563 ± 0.009 g; DISL = 72.42 ± 0.011 mm) from the start of the experiment (Figs 1 & 2). The data clearly show that the effects of the two sorting processes were only evident within the first month of the experiment. Thereafter (i.e. from month 1), both sets of animals grew at roughly the same rate (weight p = 0.368; length p = 0.164) (Table 1). While there was no significant difference in overall weight gain between the two treatments (p = 0.282), the hand-grading process did have a significant impact on shell length gain (p < 0.001) largely because of the animals' responses to the grading process within the first month (p = 0.004). These trends are supported by the DISL, SGR, FCE and CF values obtained for the treatments (Table 1). In addition, despite being very low, the hand-grading process (1.3 %) produced significantly higher mortalities than the splitting exercise (0.67 %; p = 0.025) (Table 1).

3.5 Discussion

While there were no apparent long-term negative impacts of the hand-grading procedure, splitting juvenile abalone that are < 30 months old resulted in unhampered growth rates. Despite the fact that grading was only of a consequence during the first month following the procedure, this initial impact, particularly on shell length, produced meaningful differences in the growth responses between hand-graded and split juvenile *H. midae*. This was no doubt due to the stress induced by the grading procedure (see Cambell 1985; Flos et al. 1988; Qin et al. 2001; Malham et al. 2003).

Grading has been classified as a mechanical stressor (Ragg et al. 2000; Reaburn and Edwards 2003; Malham et al. 2003). In general, mild stress is considered beneficial to animals as it often tends to have a positive effect on their physiological responses (Anderson 1990). The natural habitat of abalone is generally highly energetic and dynamic, subjecting abalone to a variety of stress factors that are comparable to size-sorting (Malham et al. 2003). The mechanical disturbances (dislodging, handling, aerial exposure, etc.) associated with grading, however, impact abalone to a much greater extent (Ryder et al. 1994; Ragg et al. 2000; Ragg and Taylor 2006; Vosloo and Vosloo 2006; Song et al. 2007). In particular, excessive handling results in higher levels of the chemicals catecholamines, noradrenaline dopamine, tauropine and D-lactate, levels far greater than those found in natural populations (Gäde 1988; Baldwin et al 1992; Wells and Baldwin 1995; Donovan et al. 1999; Malham et al. 2003; Baldwin et al. 2007; see also Lacoste et al. 2002a, b). Some studies (e.g. Reaburn and Edwards 2003) have emphasized that the common procedure of grading is extremely disturbing for abalone because they need to be removed from the rearing substrate mostly with the aid of a knife or ab-iron (spatula), quite often without the use of an anaesthetic. It has long been known that such mechanical removal with the use of knives or ab-irons often cause injuries which may result at a later stage in death due to stress, slow healing rates, bacterial infections (Genade et al. 1988) or due to bleeding to death as abalone lack a blood clotting mechanism (Cox 1962; Armstrong et al. 1971; Genade et al. 1988; Hahn 1989d). In addition, excessive weight loading of abalone on the grading table often leads to shell damage and/or cuts and bruises of the pedal foot muscle (White 1995; White et al. 1996), all of which invariably influences the animal's growth response.

Growth is a fundamental factor in the management of commercially exploited species (Keesing and Wells 1989; Lee 2004) and the maximisation thereof is a necessity for successful commercial production of cultured animals (Capinpin et al. 1999; Setyono 2005). Abalone fed kelp generally invest more into shell length, quite often at the expense of body weight (Troell et al. 2006). This can mainly be attributed to kelp possessing low protein contents (5-15 %), unbalanced amino acid profiles, and more importantly, high ash contents (about 25 %) (Troell et al. 2006). Growth trends in shell length are therefore often considered more reliable when determining farm management procedures for abalone grown on kelp. Optimal shell growth (including shell strengthening) is particularly important at the juvenile stage largely to protect the vulnerable muscular body parts and to improve respiration and excretion (Vermeij 1977, 1993; Bengtsson 1994; Sälgeback 2006). For these reasons gastropods invest heavily in the development and strengthening of their shells as a thicker shell is less prone to breakage and boring (Barnes 1987). During grading, the chances of abalone undergoing shell damage and acquiring cuts and bruises to the pedal foot is increased (White 1995; White et al. 1996). This may be particularly prevalent in juvenile abalone that have not yet developed thick protective shells and that lack strong adhesive powers (Tarr et al. 1996).

Disturbance of any form will ultimately reduce growth and/or induce mortality (Ragg et al. 2000; Edwards et al. 2000). While the effects of the grading process in this study were only evident within the first month following the grading procedure, that initial impact was sufficient to cause a substantial difference overall, particularly in growth in shell length. In addition, mortalities were double that in the graded animals than the split animals. While mortality values were notably low, if we were to extrapolate them to the scale of the farm (JSP has roughly 3 million abalone on the farm at any one time) they may actually amount to very high numbers. Other factors such as tank cleaning, deplating of abalone, etc., may, however, also have an impact, making it impossible to determine a more accurate figure for grading mortalities. Reay (1979) had long ago stated that animals reared in captivity are generally prone to higher mortalities because their artificial rearing environments are different from their natural environments. Bearing this in mind, it should be noted that abalone farms experience natural mortalities of around 1 – 2 % per year (Jonathan Venter, Jacobsbaai Sea Products, *pers. comm.*) placing these figures in context. None-the-less, the benefits of the splitting exercise are clearly evident from this study.

Most of the smaller farms, or those under development, use either the splitting exercise or the hand-grading process, or a combination thereof to size-sort their juvenile and grow-out abalone (Abalone farmers, *pers. comm.*; see Chapter 5). This is largely because it is still too expensive for them to convert to a mechanized or computerized grading system. These two processes (splitting and hand-grading) therefore are part of common farm practice particularly for emerging farmers. Until this study, no formal investigation had been made to investigate these two opposing practices and till now, all abalone, even juvenile abalone < 30 months old had undergone hand-grading. Not only is the splitting exercise a much faster process than the hand-grading procedure (resulting in less handling and aerial exposure of the abalone), clearly results in better growth. While only a few studies have investigated the effects of grading on cultured Haliotids (see e.g. Mgaya and Mercer 1995; Malham et al. 2003), none that we are aware of have investigated the opposing methods of hand-grading vs splitting of juvenile < 30-month old abalone.

In conclusion, despite the obvious negative impacts of stress associated with handling during the grading process, a number of benefits are gained by the procedure. These include: a reduction of the size differences that may decrease possible intraspecific competition for space and feed, and in so doing improve performance and overall yield (Campbell 1985; Jarayabhand and Newkirk 1989; Kamstra 1993; Mgaya and Mercer 1995; Masser 1995, 2004; Beveridge 1996; Lazur 1996; Chua and Tech 2002; Setyono 2005; Ashley 2007); improved feed conversion ratios/efficiencies (Hugenin and Ansuini 1978; Lazur 1996); an opportunity to inspect the general health and condition of the animals (Goggin and Lester 1995; Oakes and Fields 1996; Diggles et al. 2002; McDiarmid et al. 2004); and easier identification of market ready animals (Hugenin and Ansuini 1978; Baardvik and Jobling 1990; Lazur 1996; Huguenin 1997). These benefits, however, are largely of relevance to larger, older abalone that no doubt may have become accustomed to the grading procedure. The results of this study have shown that smaller abalone that have never undergone a grading before would benefit more by an initial splitting exercise because this results in unhampered growth notably because of the comparative reduction in handling and stress associated therewith. In addition, the process is substantially less time consuming and therefore more cost-effective, a factor that clearly should be considered in any commercial enterprise.

3.6 Table

Table 1. Growth parameters of juvenile abalone for the two treatments. Daily increment increase in shell length (DISL -µm.d^{-1}), specific growth rate (SGR - % body weight.d^{-1}), initial, final and difference in condition factor (CF - g.mm^{-1}), feed conversion efficiency (FCE), and mortalities are also provided. Comparative means with the same letter are not statistically different at $p > 0.05$.

~~Treatment~~	~~Final length (mm)~~	~~Final weight (g)~~	~~DISL~~	~~SGR~~	~~FCE~~	~~CF Initi~~
~~Hand-grading~~	~~40.06 ± 0.26~~	~~11.96 ± 0.24~~	~~60.02^{a}~~	~~0.551^{a}~~	~~0.023^{a}~~	~~1.00~~
~~Direct splitting~~	~~41.42 ± 0.29~~	~~12.22 ±0.27~~	~~72.16^{b}~~	~~0.571^{a}~~	~~0.026^{a}~~	~~0.98~~

Treatment	Hand-grading	Direct Splitting
Final length (mm)	40.06 ± 0.258^{a}	41.41 ± 0.292^{b}
Final weight (g)	11.99 ± 0.239^{a}	12.20 ± 0.266^{a}
DISL (months 0-1)	46.78 ± 3.162^{a}	81.21 ± 3.573^{b}
DISL (months 1-4)	64.93 ± 0.258^{a}	69.49 ± 0.333^{a}
DISL (months 0-4)	60.39 ± 0.984^{a}	72.42 ± 1.143^{b}
SGR (months 0-1)	0.701 ± 0.024^{a}	0.739 ± 0.021^{a}
SGR (months 1-4)	0.497 ± 0.003^{a}	0.504 ± 0.005^{a}
SGR (months 0-4)	0.548 ± 0.008^{a}	0.563 ± 0.009^{a}
CF (Initial)	1.012	1.003
CF (Final)	1.035	1.031
CF (Diff.)	0.023^{a}	0.028^{b}

FCE	0.023[a]	0.026[a]
Correlation (r)	0.94	0.94
Correlation (r^2)	0.88	0.87
Mortalities (Total)	8[a]	4[b]
Mortalities (%)	1.3[a]	0.67[b]

Figure Captions

Figure 1. Increase in body weight (mean ± se) of juvenile < 30-month old *H. midae* undergoing a hand-grading and a splitting exercise.

Figure 2. Increase in shell length weight (mean ± se) of juvenile < 30-month old *H. midae* undergoing a hand-grading and a splitting exercise.

Figure 1

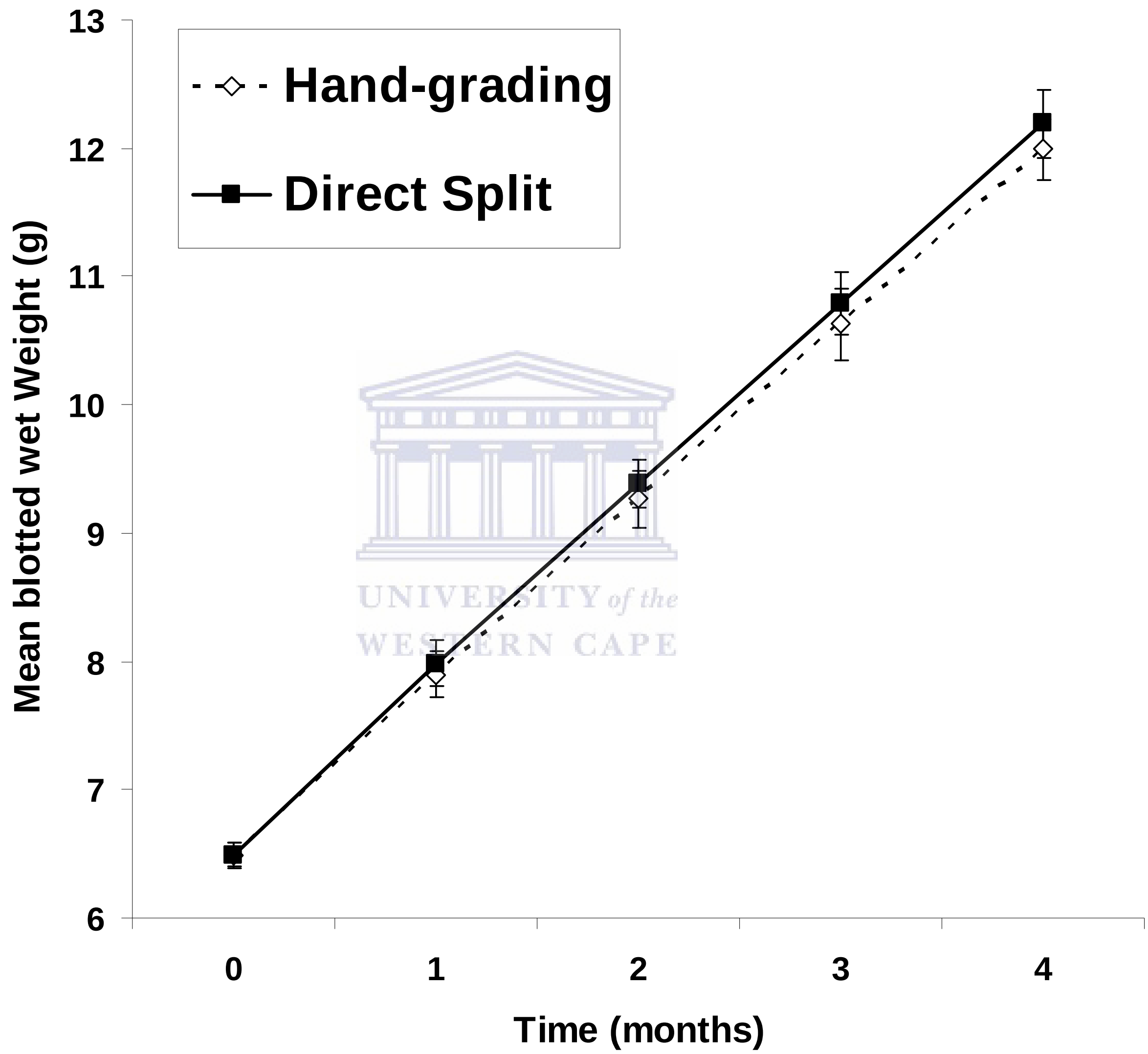
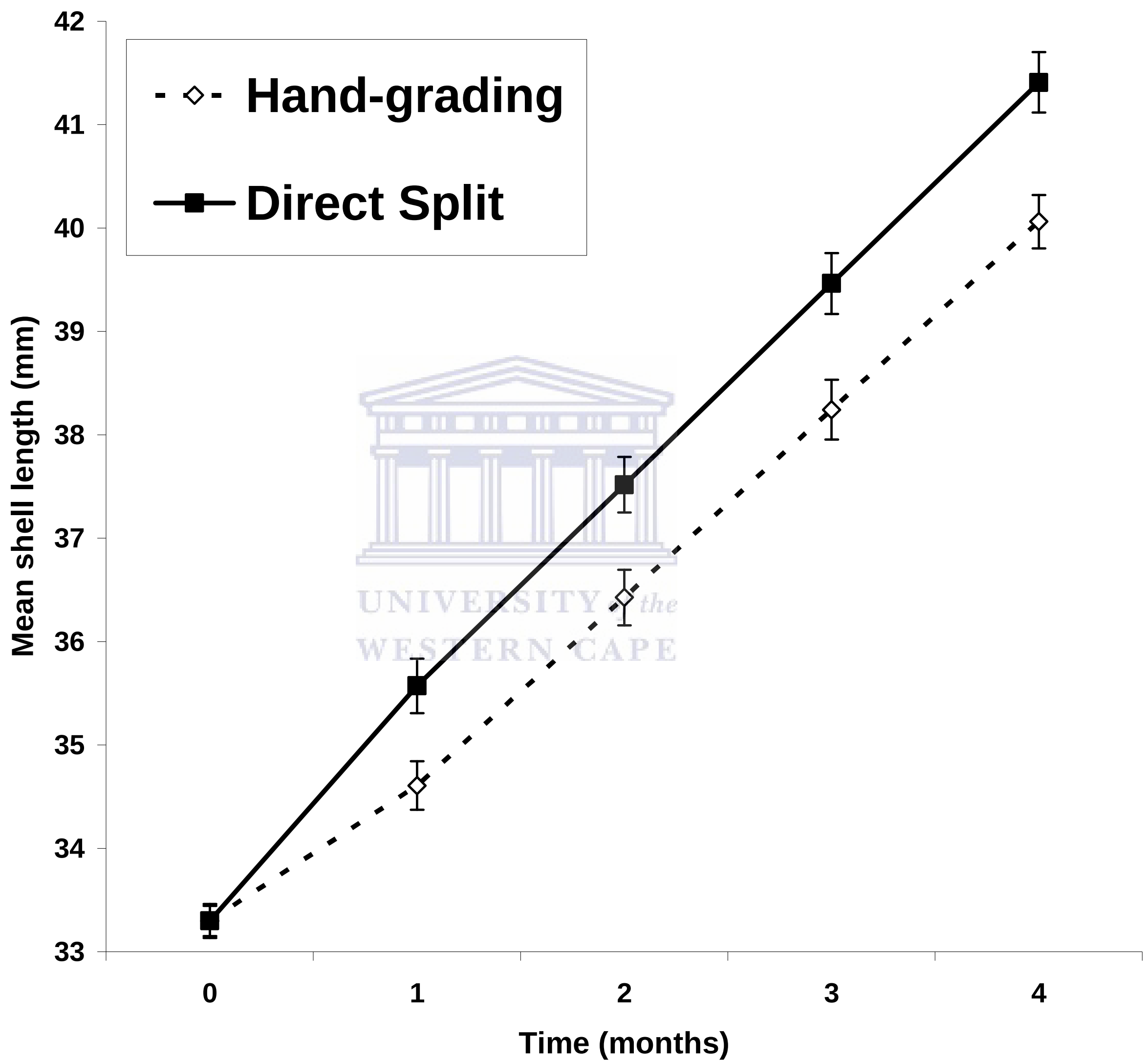

Hand-grading
Direct Split
Mean blotted wet Weight (g)
Time (months)
13
12
11
10
9
8
7
6
0
1
2
3
4

Figure 2



Hand-grading
Direct Split
Mean shell length (mm)
Time (months)
42
41
40
39
38
37
36
35
34
33
0
1
2
3
4

Chapter 4

The effect of hand- vs. machine-grading

on growth and survivorship of grow-out abalone

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4.1 Abstract

Larger abalone are generally competitively superior to smaller ones and this has resulted in a need for size-sorting in order to minimize intraspecific grazing competition. As the grading process is on-going and labour intensive, it is considered a costly farming activity. South African abalone farmers have tested various methods for removing abalone from the rearing environment without intentionally causing injuries, the main objective being to reduce the degree of awareness in the abalone. For this reason, the use of anaesthetics is considered important for reducing stress in animals. It has been suggested that machine-graded animals are more stressed by the grading process than hand-graded animals and that there are higher mortalities from the former process. Of concern also is the use of carbon dioxide (CO_2) as an anaesthetic. Till now, nothing has been known of the effects of grading on the recovery and survivorship of abalone. The aim of this study was to determine the recovery response and survival rate of grow-out abalone (*Haliotis midae* Linnaeus) undergoing three grading scenarios. Abalone growth was monitored on a commercial abalone farm over a period of 4 months and consisted of 3 treatments with 4 replicates (n = 30 abalone per replicate). The treatments were: machine-grading with CO_2 (M+); hand-grading with CO_2 (H+); and hand-grading without CO_2 (H-). The data showed that there were no significant differences between machine- (Daily increment increase in shell length (DISL) = 58.19 ± 0.63; Specific growth rate (SGR) = 4.110 ± 0.02) and hand-graded treatments (DISL = 57.97 ± 0.26; SGR = 4.214 ± 0.02) for growth in both shell length (p = 0.481) and weight (p = 0.343) when an anaesthetic was used. Notably, the effect of grading was evident in the weight gain measurements when an anaesthetic was not used, with hand-graded animals in which an anaesthetic was used (SGR = 4.214 ± 0.02) performing better than those in which no anaesthetic (SGR = 3.703 ± 0.02) (p = 0.020) was used. The effects of the grading processes

was, however, only evident within the first month of the experiment. Similarly, while there were no significant differences between feed conversion efficiency (FCE) values (M+ [0.025] vs. H+ [0.022], p = 0.477; H+ [0.022] vs. H- [0.025]; p = 0.301), those abalone that were hand-graded without the use of an anaesthetic had relatively poorer condition factor (CF) values (M+ = 1.174; H+ = 1.153; H- = 1.127; $p < 0.05$). In addition, while the machine-grading (7.88 g) process resulted in substantially more shell fragments than either of the two hand-grading (H+ = 0.5 g, H- = 0.7 g) processes, it produced no mortalities compared to the hand-grading processes (H+ = 1.33 %, H- = 1 %; p = 0.025). This study has shown that the methods of grading are far less important than the application of an anaesthetic. Based on other factors (e.g. time, labour costs and available man-power), abalone farmers clearly have a number of options available to them as long as they use an effective anaesthetic.

Keywords: abalone, anaesthetics, carbon dioxide, grading, growth, *Haliotis midae*, hand-grading, machine-grading.

4.2 Introduction

Maximisation of growth in the cultured organism is of primary importance in any commercial enterprise to ensure financial health and success (Keesing and Wells 1989; Capinpin et al. 1999). Size-sorting (also known as grading or size-separation) of animals is one of many efforts being made to ensure maximum growth of cultured organisms. However, results from many studies on various aquatic organisms suggest that the benefit of size-sorting appears to be ambiguous and not as straight forward as one might have expected (see e.g. Gunnes 1976; Jobling and Reinsnes 1987; Baardvik and Jobling 1990; Makinen and Rouhonen 1992; Kamstra 1993; Jobling and Baardvik 1994; Mgaya and Mercer 1995; Lazur 1996; Endemann et al. 1997; Strand and Øiestad 1997; Sunde et al. 1998; Barki et al. 2000; Lambert and Dutil 2001; Qin et al. 2001; Tidwell et al. 2004; Ahvenharju et al. 2005). But, just why the need for size-sorting?

Variable growth rates within a cohort often result in noticeable size variation among cultured organisms (Johnston et al. 2005). Research has shown that larger organisms are generally competitively superior to smaller ones and this has stressed the need for size-sorting in order to minimize intraspecific grazing competition (Mgaya and Mercer 1995; Setyono 2005). Ultimately, it is this reduction in intraspecific competition that leads to overall improved growth and productivity.

Even though size-sorting has been advocated as a necessity in aquaculture practices, it inherently contributes toward the stress experienced by the animals due to the handling associated with the grading process (Cambell 1985; Flos et al. 1988). It is impossible to completely avoid stress and the ultimate goal should therefore be to reduce stress and the accompanying harmful effects, in order to ensure optimal growth and welfare of the cultured animals (Ashley 2007). To this end, anaesthetics are widely used in aquaculture practices to minimize stress during handling (Wagner et al. 2002) and carbon dioxide (CO_2) as an anaesthetic has for various reasons been approved by the U.S. Food and Drug Administration (FDA) as the most effective anaesthetic (Wagner et al. 2002).

As the grading process is ongoing and labour intensive, it is considered a costly farming activity (Sunde et al. 1998). Currently South African abalone farmers use machine-grading and/or hand-grading to size-sort their abalone and many farmers have suggested that machine-graded animals appear more stressed by the grading process than hand-graded animals. In addition, some farmers have reported higher mortalities with the former process. Studies involving size-sorting of abalone in particular, are scarce and very little is presently known of the effects of the grading process on the recovery time and survivorship of grow-out abalone (Malham et al. 2003). Of concern too is the use, or lack of use of carbon dioxide as an anaesthetic during the grading process. The primary aim of this research was therefore to determine the effects of handling/grading and of CO_2 as an anaesthetic on the recovery response and survival of grow-out *Haliotis midae* Linnaeus.

4.3 Materials and Methods

4.3.1 Experimental system

The research was conducted on the Jacobsbaai Sea Products (JSP - 17º 53' 12.5" E, 32º 58' 2.5" S, Western Cape, South Africa) commercial abalone farm. Moderately aerated seawater (supplied by means of conduit pipes with holes of 1 mm diameter) with a flow rate of 1000 ± 100 $L.h^{-1}$ was supplied at 13.8 ± 1 ºC in concrete production tanks (5500 x 1300 x 550 mm; length, width, depth) with a total water holding capacity of 4500 litres. Abalone were grown in mesh culture baskets (1200 x 500 x 500 mm; length, width, depth; mesh diameter = 12 mm) ideally suited for culturing abalone fed on kelp. Baskets were subdivided with vertically orientated plates to increase the surface area for attachment and replicate baskets were arranged according to the Latin square model (Zar 1984) to compensate for end effects[1]. Both the flow-through tanks and culture baskets were cleaned every two weeks.

4.3.2 Experimental animals

Hatchery-reared grow-out abalone (shell length > 20 mm) were supplied by the Jacobsbaai Sea Products farm. Since abalone have a very heterogeneous growth rate, depending mainly on size, age and feeding rate (Viana et al. 1993; Mgaya and Mercer 1995; Preece and Mladenov 1999; Huchette et al. 2003a; Lee 2004) abalone of corresponding size and from the same gene pool (cohort) were used. All abalone used were spawned in September 2003 and were approximately 44 months old at commencement of the experiment. The abalone were subdivided into four replicate baskets of 100 individuals per replicate, per treatment. The initial body weight and shell length of the post-sorted abalone were measured at 55.3 ± 0.37 g and 65.4 ± 0.14 mm respectively.

[1] End effects refer to the potential negative impacts (e.g. reduced water flow-rate, accumulation of faeces and silt, etc) that may arise from a unidirectional flow.

4.3.3 Feed

Prior to commencement of the experiment, the abalone were grown on the kelp *Ecklonia maxima* [Osbeck] Papenfuss. For this experiment, kelp was continued as the sole dietary feed because it was relatively cheap. The experimental animals were initially fed kelp *ad libitum* once per week, but in order to determine the food conversion efficiency (FCE), a fixed amount of 4 kg of kelp per basket per week was supplied for part of the experimental period. Before each feed, left-over kelp was removed and fresh kelp topped up.

4.3.4 Experimental Procedure

The following three experimental procedures were followed: 1) machine-grading under CO_2 anaesthesia (M+); 2) hand-grading under CO_2 anaesthesia (H+); and 3) hand-grading without any anaesthesia (H-).

4.3.4.1 Grading

The machine-grading process on the JSP farm consisted of the following sequence of events. First, animals were removed from the holding tanks while still in their on-growing baskets and transferred to the grading station where they were then bulk weighed. This was followed by temporary placement into a smaller flow-through tank with a flow rate of 1500 $L.h^{-1}$. Three baskets at a time were then removed from the small seawater flow-through tank and placed into the anaesthesia tank (2000 x 1200 x 500 mm; length, width, depth) that was saturated with CO_2. Anaesthesia was maintained for 10 minutes. Thereafter, each basket was removed and its contents of abalone inverted onto a grading table whose upper surface consisted of a small-size mesh material required to drain the excess water. Abalone still clinging to one another after sedation were loosened by hand. The abalone were then transferred to the loading tray of an Aquagrader (Global Ocean, Kleinmond, Western Cape, South Africa) set to size-sort abalone at shell widths of 24, 30, 38, and 46 mm diameters. It should be emphasized that since the grading measurements are based on the shell width, greater variability in shell length was inevitable. Once the abalone had been size-sorted into smaller grading baskets, they were placed on a weighing table where each basket yet again had its contents of abalone inverted onto a weighting table for bulk weighing and stocking. The abalone were then placed in a larger mesh-net bag from where they were returned to their newly marked, colour-coded baskets back in the larger flow-through system. Using this method, it took 3 - 4 minutes to grade one basket of abalone.

The hand-grading process consisted of the following sequence of events. First, animals were removed from the holding tanks while still in their grow-out baskets and transferred to the anaesthetic tank and submerged in seawater saturated with CO_2 for 5 minutes. Thereafter, the baskets were transferred to the grading station where they were bulk weighed. This was followed by temporary placement into a smaller flow-through tank with a flow rate of 1500 $L.h^{-1}$. One basket at a time was then removed from the temporary flow-through tank and their contents inverted onto a grading table whose upper surface consists of a small-size mesh material required to drain the excess water. The abalone were then loosened from one another by hand and then size-sorted according to various shell widths using a grading ring with fixed diameters of 24, 30, 38, and 46 mm. Size-sorted abalone were then placed into smaller grading baskets (600 x 200 x 300 mm; length, width, depth) that were immersed in another flow-through tank (1000 x 660 x 560 mm; length, width, depth). These smaller baskets were marked according to the applicable size-classes of the grading ring size. Size-sorted abalone were then removed from the temporary flow-through system while still in their grading baskets and transferred to a weighing table. Here the abalone were weighed yet again. The original (now empty) grading basket was returned to the grading table flow-through system and filled up again with newly graded abalone. Graded abalone were then placed in a larger mesh-net bag from where they were returned to their newly marked, colour-coded baskets in the larger flow-through system. Using this method, it took 5 - 6 minutes to grade one basket of abalone.

For animals undergoing hand-grading without CO_2 anaesthesia, the first step in the above procedure was omitted. Abalone were transferred from the holding tanks while in their grow-out baskets straight to the grading station where they were then bulk weighed. Hereafter, the procedure follows exactly that of the hand-grading procedure above. Using this method, it took 7-10 minutes to grade one basket of abalone. The difference in time between the three procedures was largely due to that time taken to separate responsive animals.

During the grading processes, shell fragments at the machine-grader outlet pipe were captured with a fine meshed bag and weighed. Similarly, the shell fragments remaining on the grading table after each hand-grading were gathered and weighed.

4.3.4.2 Anaesthesia

Within the anaesthesia (sedation) tank, CO_2 was supplied via a pipe system and was bubbled into the water with the aid of a micro-pore tube in order to ensure equal distribution throughout the tank. The pH in the sedation tank was kept constant at 4.8 - 5.4 (the optimal being > 5; see Wurtz and Durborow 1992) and the water exchanged once it became dirty. The timing of anaesthesia differed between treatments for two reasons. Hand-graded animals only needed to be loosened from the basket surfaces while machine-graded animals needed to be prevented from adhering to the Aquagrader as well.

4.3.5 Sampling and data collection

The experiment was conducted over 4 months beginning in May 2007 and ending in September 2007 (winter through early spring). Thirty individuals from each of the four replicates were randomly measured each month. Before all weight measurements, abalone were blotted dry to remove excess water. The blotted wet body weight was recorded to the nearest 0.01 g using a top loading digital electronic balance while shell length was measured along the longest axis to the nearest 0.01 mm using digital electronic vernier callipers.

Daily increment increase in shell length (DISL in µm.day^{-1}) was calculated using the formula of Mai et al. (2001) and Zhu et al. (2002):

$$DISL = [(SL_t – SL_i)/t] \times 1000$$

Where SL_t = final mean shell length; SL_i = initial mean shell length and t = the feeding trial period in days.

Specific growth rate (SGR in % weight.day^{-1}) was calculated as in Britz (1996b) using the formula:

$$SGR = [(\ln(W_f) – \ln(W_i)) / t] \times 100$$

Where $\ln(W_f)$ = the natural log of the final mean weight; $\ln(W_i)$ = the natural log of the initial mean weight, and t = the feeding trial period in days.

The feed conversion efficiency (FCE) was calculated using the formula of Simpson and Cook (1998):

$$FCE = (growth/ration) \times 100$$

Where growth = the blotted wet weight (g) gained per day and ration = the blotted wet feed (g) intake.

The condition factor (CF in $g.mm^{-1}$), which is an index that was developed to account for the relationship between the weight of abalone per unit shell length, was calculated using the formula of Britz (1996b).

$$CF = [BW / SL^{2.99}] \times 5575$$

Where CF = the condition factor, BW = the mean body weight (g) and SL = mean shell length (mm); 2.99 and 5575 are constants.

During the regular monthly measurements, baskets were also checked for mortalities. This was recorded and expressed as a percentage of the total stocking density for each treatment.

4.3.6 Statistical analysis

All data are expressed as means ± se. Data for all experimental replicates were pooled as no significant differences were found between them. Multiple student's T-test were run to test for differences between means. To test for correlation, the body weight and shell length of abalone from each treatment were compared by means of a linear regression test (Pearson's product-moment correlation test). All results were considered statistically significant at $p < 0.05$. Finally, machine-grading with CO_2 and hand-grading without CO_2 were not compared because there was no common variable.

4.3.7 Key

1. Machine + (M+) refers to machine-graded animals in which CO_2 was used as an anaesthetic.
2. Hand + (H+) refers to hand-graded animals in which CO_2 was used as an anaesthetic.
3. Hand – (H-) refers to hand-graded animals in which no anaesthetic was used.

4.4 Results

4.4.1 *Growth*

What is immediately evident is that variability in growth between the three treatments only occurred in weight gain with much of this variability occuring within the first month of the experiment (Table 1, Figures 1 & 2). Overall, the data show that there were no significant differences between the machine- (DISL = 58.19 ± 0.633, SGR = 4.110 ± 0.02) and hand-grading (DISL = 57.97 ± 0.264, SGR = 4.214 ± 0.02) methods for growth in both length (p = 0.481) and weight (p = 0.343) when an anaesthetic was used. However, in terms of weight gain, the use or lack of use of an anaesthetic appeared to be critical in that abalone that were hand-graded without the use of an anaesthetic (SGR = 3.703 ± 0.02) fared significantly poorer than those that were graded with an anaesthetic (SGR = 4.214 ± 0.02; p = 0.020). In addition, the data show that the effects of the grading processes were only evident within the first month of the experiment. Notably, all animals lost weight during the first month. Thereafter, all animals grew at roughly similar rates (length: M+ vs. H+, p = 0.313; H+ vs. H-, p = 0.341; weight: M+ vs. H+, p = 0.188; H+ vs. H-, p = 0.132) with the machine-graded animals growing slightly better than the two hand-graded treatments (Table 1).

4.4.2 *FCE and CF*

While there were no significant differences between FCE values (M+ [0.025] vs. H+ [0.022], p = 0.477; H+ [0.022] vs. H- [0.025], p = 0.301), those abalone that were hand-graded without the use of an anaesthetic had significantly poorer CF values (M+ = 1.174; H+ = 1.153; H- = 1.127; $p < 0.05$). This latter treatment (H-) was the only treatment whose animals put on relatively more shell length than weight (CF difference of -0.027). Despite this decrease, all treatments had positive (>1) CF values.

4.4.3 *Shell fragmentation and Mortalities*

The machine-grading process resulted in substantially more shell fragments than either of the two hand-grading processes (Table 1). Despite this, however, there were no recorded mortalities for the machine-graded animals, with significantly more mortalities recorded for the two hand-grading processes (M+ = 0 %, H+ = 1.33 %, H- = 1 %; $p = 0.025$) (Table 1).

4.5 Discussion

Despite the fact that grading was only of consequence during the first month following the procedure, this initial impact, particularly on body weight, produced meaningful differences in the growth responses of abalone graded with and without an anaesthetic. The use of an anaesthetic during the grading procedure appears to be critical and the type of grading (machine- or hand-grading) seems less important. This was no doubt due to the stress induced by the grading procedure on animals that were not sedated (Iwama et al. 1989; Malham et al. 2003).

Abalone are mainly subtidal organisms and therefore not well adapted to cope and survive desiccation, hypoxia and thermal stress due to lengthened aerial exposure (Branch and Branch 1989; Gorfine 2001). *Haliotis midae*, however, appears to be an exceptionally hardened species as it possesses the ability to survive under extended emersion conditions (O'Omolo et al. 2003). Despite this advantage, abalone that were graded without anaesthesia faired worse overall, no doubt due to the extended emersion time resulting from the grading and separation of unsedated animals. Sedation is thus of immense importance and the use of anaesthetics to reduce stress in aquaculture species is not new (see Fish 1943; Post 1979; Ross and Ross 1984; Marking and Meyer 1985; Summerfelt and Smith 1990; Prince et al. 1995; Anderson et al. 1997).

An anaesthetic such as CO_2 has several side affects including lowering the pH of the culture medium (Izutsu 1972; Saborowski et al. 1973; Thomas 1974; Malan et al. 1976; Spotte 1979; Poxton 2003), lowering the oxygen transfer capability (Eddy et al. 1977; Hugenin and Colt 1989; Wurtz and Durborow 1992; Karwacki et al 2001; Sladky et al. 2001), and lowering the adaptability of the blood pigment hemocyanin (Brix et al. 1979, 1990; Petrovich et al. 1990; Wells et al. 1998). Despite these drawbacks, CO_2 is generally considered an effective sedative (because it reduces the overall effect of a greater stressor – grading) that is also cost-effective (see Gelwicks et al. 1998; Sunde et al. 1998, Qin et al. 2001). Consequently, more South African abalone farmers are resorting to sedation of their graded abalone (Wayne Barnes, Abalone Farmers Association of South Africa, *pers. com.*)

Despite the increasing importance of using an anaesthetic, other aspects of the grading procedure still need to be addressed. These include such aspects as the handling time (Cambell 1985; Flos et al. 1988; Gorfine 2001), the duration of exposure (Olley and Thrower 1977; Gorfine 2001; Vosloo and Vosloo 2006), the accuracy of the grading and measuring apparatus (Ashley 2007), the intensity of the labour involved (Cambell 1985; Sunde et al. 1998; Qin et al. 2001), the degree of injury to the aquaculture organism (Sunde et al. 1998; Qin et al. 2001), the percentage mortality (Gunnes 1976; Cambell 1985; Kamstra 1993; Qin et al. 2001), the stress induced (Flos et al. 1988; Iwama et al. 1989; Malham et al. 2003; Cheng et al. 2004b; Hooper et al. 2007), the growth reduction (Baardvik and Jobling 1990; Sunde et al. 1998; Qin et al. 2001), the costs involved (Strand and Øiestad 1997; Sunde et al. 1998; Qin et al. 2001) and the health of the graded animals (Oakes and Fields 1996; Sunde et al. 1998; Qin et al. 2001; McDiarmid et al. 2004).

The shell damage and potential mortality associated with the grading procedure is of particular concern to abalone farmers (Barnes 1987; Tarr 1992; Sälgeback 2006). Avoiding shell damage during the grading process is impossible, particularly as a great amount of handling (manually or mechanically) is involved. While the machine-grader notably produced higher amounts of shell grit, this did not result in mortality. Nevertheless, any shell damage should be considered in a serious light as the shell fulfills many important physiological functions (e.g. aids in respiration and excretion, protects against injury and desiccation, etc - see Vermeij 1977, 1993; Barnes 1987; Bengtsson 1994; Sälgeback 2006); all efforts should therefore be made to limit or prevent shell damage.

It is difficult to interpret and analyze mortality data as a limited amount of information is available to pinpoint the exact course thereof. While mortalities, notably with hand-graded animals, had occurred, it is not possible to attribute it solely to the grading procedure as various other factors (e.g. kelp quality, pathogens, basket cleaning procedures, etc.) may have contributed (see also Reay 1979). While mortality values were generally low, if we were to extrapolate them to the scale of the farm (JSP has roughly 3 million abalone on the farm at any one time) they may actually amount to very high numbers, and so any amount of mortality should be taken seriously.

In conclusion, this research has shown that the methods of grading (machine- or hand-grading) abalone are far less important than the application of an anaesthetic. This is no doubt so because of the stress that is induced in animals that are not sedated. Based on such important factors as time, material costs, man-power, etc, abalone farmers clearly have a number of options available to them as long as they use an effective anaesthetic.

4.6 Table

Table 1. Growth parameters of grow-out abalone for the three treatments. Daily increment increase in shell length (DISL -µm.d^{-1}), specific growth rate (SGR - % body weight.d^{-1}), initial and final condition factor (CF - g.mm^{-1}) and feed conversion efficiency (FCE) and are also provided. Comparative means with the same letter are not statistically different at $p \geq 0.05$.

~~Treatment~~	~~Final length (mm±se)~~	~~Final weight (g±se)~~	~~DISL~~	~~SGR~~	~~FCE~~	~~CF Initial (g.mm^{-1})~~	~~CF Final (g.mm^{-1})~~	~~CF Diff. (g.mm~~
~~Machine-grading with CO_2~~	~~71.82 ± 0.31~~	~~734.3295 ± 1.00~~	~~59.1297^a~~	~~4.1857^a~~	~~0.025$^{(a)}$~~	~~1.1736~~	~~1.1742~~	~~0.0006~~
~~Hand-grading with CO_2~~	~~71.96 ± 0.25~~	~~74.49 ±0.86~~	~~60.3684^a~~	~~4.1536^a~~	~~0.022$^{a(b)}$~~	~~1.1604~~	~~1.1531~~	~~-0.0073~~
~~Hand-grading without CO_2~~	~~71.79 ± 0.28~~	~~71.875 ± 0.92~~	~~58.8889^a~~	~~3.7044^b~~	~~0.025^b~~	~~1.1541~~	~~1.1267~~	~~-0.0274~~

Treatment	**Machine-grading with CO_2**	**Hand-grading with CO_2**	**Hand-grading without CO_2**
Grading time (mins) per basket	3 – 4	5 – 6	7 – 10
Final length (mm)	71.84 ± 0.357^a	71.82 ± 0.290^a	71.92 ± 0.326^a
Final weight (g)	73.95 ± 0.997^a	74.49 ± 0.861^a	71.87 ± 0.992^b
DISL (months 0-1)	62.66 ± 2.184^a	69.84 ± 0.986^a	67.14 ± 0.359^a
DISL (months 1-4)	56.70 ± 0.115^a	54.02 ± 0.681^a	56.14 ± 0.531^a
DISL (months 0-4)	58.19 ± 0.633^a	57.97 ± 0.264^a	58.89 ± 0.309^a
SGR (months 0-1)	-0.144 ± 0.0063^a	-0.031 ± 0.0463^b	-0.111 ± 0.0167^{ab}
SGR (months 1-4)	0.391 ± 0.0279^b	0.362 ± 0.0466^{ab}	0.345 ± 0.0363^a
SGR (months 0-4)	4.110 ± 0.02^b	4.214 ± 0.02^b	3.703 ± 0.02^a
CF (Initial)	1.176^a	1.140^a	1.154^a
CF (Final)	1.174^a	1.153^a	1.127^b

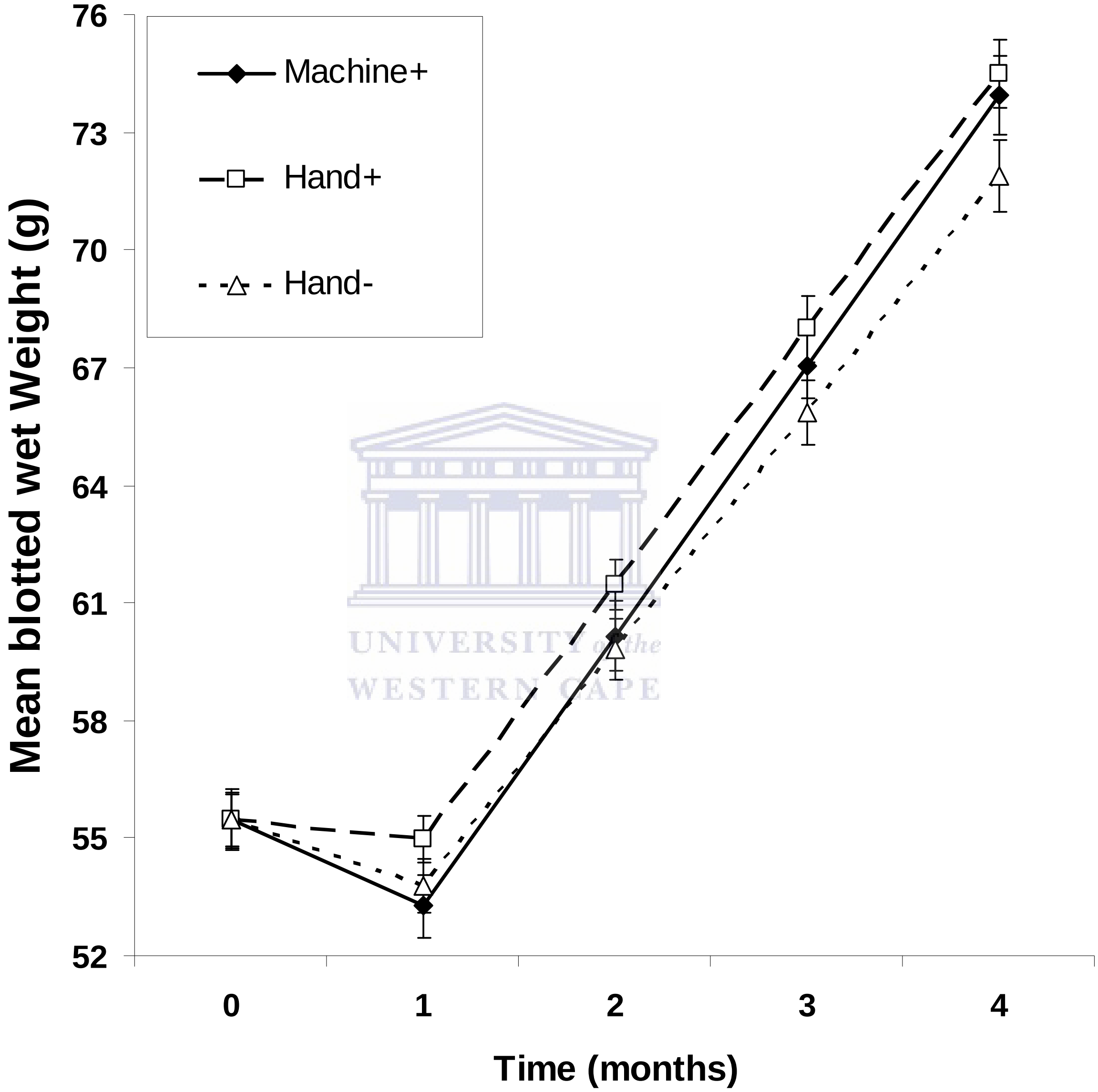
FCE	0.025[a]	0.022[a]	0.025[a]
Correlation (r)	0.88	0.86	0.89
Correlation (r^2)	0.77	0.74	0.79
Mortalities (Total)	0[a]	4[b]	3[b]
Mortalities %	0[a]	1.33[b]	1[b]
Total shell fragments (g)	7.88	0.50	0.70

Figure Captions

Figure 1. Increase in body weight (mean ± se) of grow-out *H. midae* undergoing the various grading procedures.

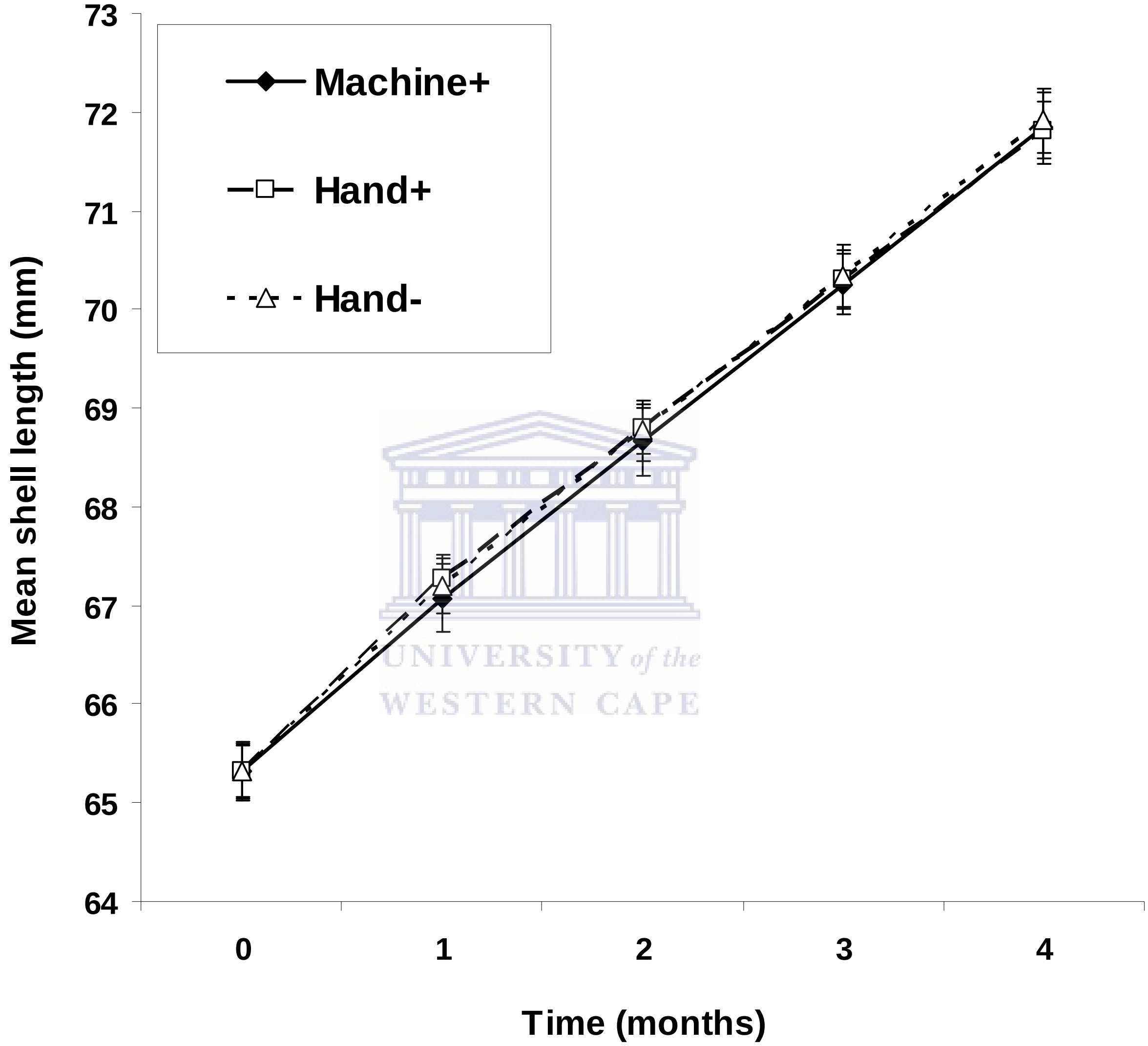
Figure 2. Increase in shell length (mean ± se) of grow-out *H. midae* undergoing the various grading procedures.

Figure 1



76
73
70
67
64
61
58
55
52
Mean blotted wet Weight (g)
Machine+
Hand+
Hand-
0
1
2
3
4
Time (months)

Figure 2


Machine+
Hand+
Hand-
Mean shell length (mm)
73
72
71
70
69
68
67
66
65
64
0
1
2
3
4
Time (months)

Chapter 5

South African abalone farm management practices

Supervisor: Gavin W. Maneveldt

5.1 Introduction

During the course of this study it has been ascertained that while there is much communication between abalone farmers, the dissemination of scientific knowledge to farmers and the general research community is sorely lacking. While the Abalone Farmers Association of South Africa (AFASA[1]) has been most helpful in providing answers to many of the questions posed throughout this study, many questions still remain unanswered, receiving the following response: "… sadly we are not at liberty to provide all the information for which you ask because some of it is treated as proprietary (and therefore confidential) by most members of our Association …" (Wayne Barnes, AFASA, *pers. comm*.). Even though the abalone farmers registered to AFASA apparently do have access to scientific information, it is questionable as to whether all are aware thereof. In addition, whether the accessibility of such research information is reasonably easy, is unclear as some farmers appear to be unaware of the research already done by AFASA, research that may aid their individual farm management practices.

More notably, experimental research (particularly that directed at increased productivity) initiated by farmers who bear the costs involved in such research, generally consider such research as confidential and do not feel obliged to make this information known, or available for publication in society magazines or peer-reviewed journals. Gerber (2004) described a similar scenario stating that as abalone farmers bore the cost of research without any participation of the government, the so-called "technical knowledge" was kept in the industry and are not made public. Gerber (2004) did, however, state that some farms were prepared to share some of their knowledge (within limits) to newcomers joining AFASA.

[1] AFASA is the interactive body with the main objective to promote the interest and image of the abalone aquaculture industry in South Africa through the establishment of communication, interaction opportunities and the general assistance in the provision of funding.

While many abalone farms do not generally disclose certain farming practices (e.g. growth rates, stocking densities, mortalities) there are benefits for the abalone farmers belonging to AFASA. These benefits are incorporated in the following extract from the AFASA constitution (Wayne Barnes, AFASA, *pers. comm*.):

> "The Association's object is to promote the interest and image of the abalone aquaculture industry in South Africa, inter alia as follows:
>
> (a) To establish communication and co-operation amongst members of the Association and to create a unified front in dealings with regulatory bodies.
>
> (b) To serve as a forum for debate on the opinions of members of the Association.
>
> (c) To commission research, collect and disseminate information on abalone, abalone production and research findings to members.
>
> (d) To support or organize seminars, workshops and symposia which have a bearing on abalone production and research.
>
> (e) To assist with the identification of risks to the abalone aquaculture industry in South Africa and their elimination or mitigation.
>
> (f) To procure funds and to apply such funds for the furtherance of the stated objectives."

According to Wayne Barnes (AFASA, *pers. comm*.) "…a system is available within the Constitution for joint funding of research by all members, or selected group of members (if the deliverables are not pertinent to the operations of all members) whereby the amount of 'control' over the research is proportional (on a stepped scale) to the financial contribution of the specific member. As the farms have matured the quantity of generic R&D managed by AFASA has in fact declined but there is still considerable research taking place by individual farms or groups with the same or similar interest." It should be mentioned that abalone farmers value their membership with AFASA (this ascertained by means of a questionnaire - details of which follows) and consider the Association to provide useful assistance to farmers in the various ways mentioned above.

5.2 Management Practices

Currently there are 18 abalone farm rights-holders of which 14 are operational and 13 are exporting; 12 of these latter farms are members of AFASA (Wayne Barnes, AFASA, *pers. comm*.). Two questionnaires were sent to the twelve AFASA member abalone farms, ten of which responded. While all farms were both helpful and generous in their co-operation, some questions were not answered for the following reasons: 1) the question was not applicable to them; 2) they did not know the answer; or 3) they retained the right not to divulge such information because of their confidentiality policy that protected their intellectual property. The following are the responses provided by the abalone farms.

5.2.1 Questionnaire 1

Questionnaire 1 was designed to determine general grading practices on AFASA member farms. Percentages represent either: 1) the number of responses to individual questions received from the ten farms who had returned the questionnaires; or 2) the number of responses to individual questions that were of relevance to a particular subset of respondents. The following 15 questions are cited as they appear in the questionnaire and the answers received are listed accordingly.

1. **Which method(s) of grading (e.g. direct split exercise, hand-grading, machine-grading) do you use?**

100 % (N = 10) of the respondents answered this question. The answers received ranged from: 1) only use splitting exercise (10 %); to 2) hand-grading and direct split exercise (20 %); to 3) hand-grading only (40 %); to 4) computerized weight grading combined with hand-grading (10 %); to 5) machine-grading only (10 %); and 6) both hand- and machine-grading (10 %). One respondent farm was not sure what a direct split was.

2. How does your grading system operate (Just a short description. If it differs for juvenile and grow-out abalone please include how)?

90 % (N = 9) of the respondents answered this question. The answers received ranged from: 1) juveniles are manually graded by size and larger market-ready animals are graded by weight (33 %); to 2) manual sorting by size only (44 %); to 3) computerised separation by weight into various predetermined size classes (11 %); and 4) size-grader rotating drum and weight-grader belt driven (11 %).

3. Do you use an anaesthetic during your hand-grading procedure?

100 % (N = 9) of the applicable respondents answered this question. All indicated that they never size-sort without the use of an anaesthetic.

4. Do you use an anaesthetic during your machine-grading procedure?

100 % (N = 2) of the applicable respondents answered this question, both of which answered yes to the question.

5. What do you think are the main benefits or disadvantages of using anaesthetics?

70 % (N = 7) of the respondents answered this question. The answers received ranged from: 1) ease of handling (7 %); to 2) weight loss (17 %); to 3) reduced growth (9 %); to 4) less damage (31 %); to 5) reduced stress (36 %).

6. What kind of anaesthetic (just the name, e.g. carbon dioxide or magnesium sulphate, etc.) do you use during grading? If it differs for post-larvae, juveniles and grow-out abalone please specify.

90 % (N = 9) of the respondents answered this question. The answers received ranged from: 1) juveniles = magnesium sulphate ($MgSO_4$) and abalone 30 g + = carbon dioxide (CO_2) (44 %); to 2) CO_2 only (44 %); and 3) sodium metabisulphate ($Na_2S_2O_5$) for weaners and CO_2 for larger abalone (11 %).

7. Why do you use this particular anaesthetic compared to others available on the market?

90 % (N = 9) of the respondents answered this question. The answers received ranged from: 1) it is cheap, effective and animals recover faster (44 %); to 2) less weight loss and faster recovery time (11 %); to 3) AFASA study suggests it to be best anaesthetic (11 %); to 4) it is most cost effective, readily available and industrial norm (22 %); and 5) it has proven to work well (11 %).

8. What is the name of the machine-grading device you are currently using? Please also supply the specifications and details of the supplier.

100 % (N = 2) of the applicable respondents answered this question. Only two machine graders are used: both farms use the Gestalt Aquagrader (Global Ocean, 35 Harbour Road, Kleinmond) for size-grading and one farm uses the Scanvaegt Scangrader 7100 (Marel, Batis and Langebergweg, Frazitta Business Complex, Durbanville) for weight-grading.

9. Are there any other comparative grading devices available that you are aware of? If so, have you tried some of these and what then is the reason for your decision to continue using your existing device?

90 % (N = 9) of the respondents answered this question. Of those who already did not use a top-of-the-range machine-grading device, the answers received ranged from: 1) no (37.5 %); to 2) not tried (12.5 %); to 3) not sure (12.5 %); to 4) we have tried the grading machines, but they are not that effective and are extremely expensive and our farm is too small so it is not really necessary (25 %); and 6) yes, Marel (12.5 %).

10. If you use a mechanical grading device, how much did it cost you?

100 % (N = 2) of those farms who possess mechanical grading devices answered this question. The answers received ranged from: 1) R80,000.00; to 2) weight-grader (Scangrader) = R500,000.00 and size-grader (Aquagrader) ± R100,000.00.

11. Are you aware of any other abalone farms that use the same grading system that you are currently using?

90 % (N = 9) of the respondents answered this question. The answers received ranged from: 1) no (22 %); to 2) not sure (11 %); to 3) yes (67 %)

12. Are you aware of the Aquagrader™ that is supplied by Global Ocean? If so, what are the main reasons for not using this device?

80 % (N = 8) of the respondents answered this question. All respondents confirmed that they were aware of the grading device and the reasons for their lack of use of the machine ranged from: 1) too expensive (34 %); to 2) causes shell damage (47 %); to 3) it is less accurate (16 %); and 4) too high maintenance (3 %).

13. If you are using the Aquagrader™, what are the main benefits that have been gained from the use thereof compared to other systems to have used?

Only two farms indicated that they use the Gestalt Aquagrader™ and both responded to the question. The one farm found it cost effective, but not very accurate. The other farm indicated that more abalone could be graded this way than by hand.

14. Are there any side-effects (e.g. shell damage) that you are aware of that impact on your abalone during grading, which you would like to reduce in some way or the other.

70 % (N = 7) of the respondents answered this question. The answers received ranged from: 1) shell damage (50 %); to 2) stress (21 %); to 3) damage to the foot (14 %); and 4) other effects (14 %). Those who stated "other effects" did not specify the nature of these effects.

15. In general, is there communication between you and other abalone farmers pertaining to the use of grading devices and/or anaesthetics?

90 % (N = 9) of the farms answered this question. All respondents answered "yes" to this question although the degree of communication varied between farmers depending on the nature of their associations.

5.2.2 Questionnaire 2

After examining the responses to the first questionnaire, a second one was sent to all farms that employed some kind of splitting exercise to grade their abalone. What follows are the responses to this second questionnaire. Similarly, the percentages represent either: 1) the number of responses to individual questions received from the 9 farms who were approached and who had returned the questionnaires; or 2) the number of responses to individual questions that were of relevance to a particular subset of respondents. Seven of the nine farms approached, responded to the questionnaire. The following 7 questions are cited as they appear in the questionnaire and the answers received are listed accordingly.

1. **Have you always used the splitting exercise, or have you only started using it recently and if so why?**

   100 % (N = 7) of the respondents answered this question. All farms replied that splitting has variably been part of farm practice from the start.

2. **For what size animals are you using the splitting exercise to size-sort?**

   100 % (N = 7) of the respondents answered this question. The answers received encompassed the full size range of abalone from about 12 months to processing at about 5 years. Due to the variable nature of the responses, it was difficult to provide a percentage breakdown of the answers.

3. What would you say are the main benefit(s) of using the splitting method of size-sorting?

57 % (N = 4) of the respondents answered this question. The answers received ranged from: 1) feed competition is minimized (8 %); to 2) stocking densities are reduced (8 %); to 3) less shell damage (8 %); and 4) it reduces handling times and therefore stress (75 %).

4. Do you anaesthetize your animals during the splitting exercise or (if appropriate) only during hand-grading?

100 % (N = 7) of the respondents answered this question. All respondent farms anaesthetise their abalone for splitting and hand-grading.

5. Do you prefer to split or to hand-grade?

71 % (N = 5) of the respondents answered this question. The answers received ranged from: 1) hand-grade (60 %) to; 2) the method used depends entirely on the size of the abalone, smaller abalone are split and larger abalone are hand-graded (40 %).

6. For those of you who prefer to split, what is the main benefit of using the splitting method as opposed to grading?

100 % (N = 2) of the applicable respondents answered this question. The one farm responded by saying that "feed competition is minimized, stocking densities reduced and there is less damage". The second farm responded by saying that "it is quicker and therefore less stressful".

7. **For those of you who prefer the hand-grading method, what was the reason for your choice?**

   33 % (N = 1) of the applicable respondents answered this question. The answer supplied was that it reduced feed competition and due to lower stocking densities, the abalone were less prone to damage.

5.3 Summary

While there is much conformity between abalone farmers as far as the grading methodology in concerned, many abalone farms tend to preserve their own individuality by following differential ways of applying the standards (grading methods and anaesthetics) at different time-scales during the developmental phases of abalone. Farmers appear to be aware of the general management strategies followed by other farmers, but lack detailed knowledge of procedures and protocols. This is understandable because some information "is treated as proprietary (and therefore confidential) by most members" (Wayne Barnes, AFASA, *pers. comm*.). The choice of grading methodology and thus management strategies appear to be determined mainly by factors such as personal preference, management experience (tertiary training and research), farm size, economic output, and financial capabilities. Even though it is clear from the questionnaire that all the farmers currently use an anaesthetic for the sedation of the animals during both splitting and hand-grading, the question had been raised as to whether it was essential to use an anaesthetic during these two processes and whether it actually made a big difference. Some of the inconveniences experienced by farmers using carbon dioxide as an anaesthetic are: 1) the availability thereof; 2) getting the needed stock in time as the distributors are distant 3) regular control whether the supply system is in proper working order 4) regular testing of the anaesthesion bath for pH changes which is time-consuming, etc.. This emphasizes the need for assessment of the use of anesthetics during splitting and hand-grading procedures.

Communication between farmers does exist, with the main aim being to provide support and advice. Furthermore, the Aquaculture Association of Southern Africa (AASA) which represents the aquaculture industry, organizes a biannual, internationally represented conference. As AFASA is a member association of AASA, it acts as a means to promote communication between abalone farmers and associated participants, directly contributing towards the broadening of scientific information and its dissemination to abalone farmers.

6. Summary and Recommendations

In 2007, the South African Department of Environmental Affairs and Tourism (DEAT) established the Marine Aquaculture Management (MAM) unit (Semoli 2007). The Frontier Program, which is the research component of this unit, drives research initiates and provides technical inputs that will eventually be incorporated into management strategic components (Semoli 2007). Various areas of importance were identified (see Chapter 2) for research focus with the aim of strengthening the South African aquaculture industry. The primary aim of the current study falls within the main framework of the Frontier Program (see Pitcher 2005), namely to address aspects of "Animal Husbandry". Within this research area, the determination of the effects of handling/grading and chemicals (anaesthetics) on the growth and survivorship of abalone were explicitly highlighted as key research areas. In addition to these research initiatives, two questionnaires were developed to ascertain the farm management practices pertaining to these areas of research. The questionnaires were also used to gauge the level of communication between the various abalone farmers and the degree of knowledge dissemination to the broader scientific community. The current research has attempted to address these questions, with the aim of promoting knowledge sharing and transfer to the local abalone industry as well as to the global mariculture industry. With reference to the Frontier Programme document (see Chapter 2), the following key areas have been addressed.

Chapter 6 – Summary and Recommendations

6.1 Grading Procedures

6.1.1 Key Research Area – Effects of grading and handling.

6.1.1.1 Subsection Title 1: The effects of hand-grading vs. splitting on the growth of < 30-month old *Haliotis midae*.

Background/Findings: Size grading is a routine practice in shellfish hatcheries and generally stimulates the growth of smaller individuals in the absence of larger, presumably dominant competitors (Jarayabhand and Newkirk 1989; Kamstra 1993; Mgaya and Mercer 1995; Setyono 2005; Ashley 2007). South African abalone farmers have, however, expressed concern about the effects that the grading process has on recovery, subsequent growth and survivorship of the animals. Until now, little research (see e.g. Mgaya and Mercer 1995) has been done evaluating the effects of hand-grading, even though it is currently considered standard practice on all South African abalone farms. In addition, research evaluating the comparative effects of direct splitting as an alternative handling procedure for juvenile abalone does not exist, despite the fact that this procedure too is fairly common practice on many abalone farms.

Our research has found that the main difference between the two sorting processes was the growth response within the first month of the experiment, this is largely evident from shell length gain. Even though there were no apparent long-term negative impacts of the hand-grading procedure, simply splitting juvenile abalone that are < 30 months old resulted in unhampered growth. This initial, yet temporary response was no doubt due to the stress induced by the hand-grading procedure (see also Cambell 1985; Flos et al. 1988; Qin et al. 2001; Malham et al. 2003).

Farm Recommendations:

Based on the conclusions above, the following recommendation can be made:

- While there are no notable long-term effects of hand-grading abalone that are < 30 months old, direct splitting should instead be exercised because it results in unhampered growth. Direct splitting is also relatively faster and thus reduces the stress induced in sorted abalone.

No doubt, despite the obvious initial negative impacts of stress associated with handling during the grading process, a number of benefits are gained by the procedure. These benefits, however, are largely of relevance to larger, older abalone that may have become accustomed to being handled. The results of this study have shown that smaller abalone that have never undergone a grading before would benefit more by an initial splitting exercise because this results in unhampered growth notably because of the comparative reduction in handling and the stress associated therewith. In addition, the process is substantially less time consuming and therefore more cost-effective, a factor that clearly should be considered in any commercial enterprise.

Globally there have been many studies done on the effects of grading (or size-sorting) on the growth of various cultured aquatic organisms such as crayfish (e.g. Ahvenharju et al. 2005), prawns (e.g. Karplus et al. 1986, 1987; Daniels and D'Ambro 1994; Tidwell et al. 2004) and fish (e.g. Gunnes 1976; Campbell 1985; Jobling and Reinsnes 1987; Wallace and Kolbeinshavn 1988; Baardvik and Jobling 1990; Kamstra 1993; Lazur 1996; Endemann et al. 1997; Strand and Øiestad 1997; Sunde et al. 1998; Barki et al. 2000; Lambert and Dutil

2001; Qin et al. 2001; Saillant et al. 2003; Dou et al. 2004; Zakęś et al. 2004;). These studies, like the current research, have emphasized the importance of selecting the appropriate sorting procedure throughout the lifespan of the cultured organism. Ultimately the main reason for choosing a particular grading procedure should be to reduce stress and in so doing increase growth rates and overall biomass gain, and decrease labour costs associated therewith.

6.1.1.2 Subsection Title 2: The effect of hand- vs. machine-grading on growth and survivorship of grow-out abalone.

Background/Findings: Maximisation of growth in the cultured organism is of primary importance in any commercial enterprise to ensure financial health and success (Keesing and Wells 1989; Capinpin et al. 1999). Grading (also known as size-sorting or size-separation) of animals is one of many efforts being made to ensure maximum growth of the cultured organisms. Even though grading has been advocated as a necessity in aquaculture practices, it inherently contributes toward the stress experienced by the animals due to the handling associated with the procedure (Cambell 1985; Flos et al. 1988). Currently South African abalone farmers use machine-grading and/or hand-grading to size-sort their grow-out abalone and many farmers have suggested that machine-graded animals appear more stressed by the grading process than hand-graded animals. Studies involving grading of abalone in particular, are scarce and very little is presently known of the effects of the grading procedure on the recovery time and survivorship of grow-out abalone (Malham et al. 2003). Of concern too is the use, or lack of use of an anaesthetic during the grading procedure.

Similarly as above, our research has found that variability in abalone growth (but this time in weight gain) between the three grading procedures (machine-grading with CO_2, hand-grading with CO_2, hand-grading without CO_2) only occurred within the first month of the experiment. Despite this initial impact, the procedure did produce meaningful differences in the growth responses of abalone graded with and without an anaesthetic. The use of an anaesthetic during the grading procedure appears to be critical and the type of grading (machine- or hand-grading) of secondary importance. Again, this was no doubt due to the stress induced by the grading procedure on animals that were not sedated (Iwama et al. 1989; Malham et al. 2003).

Farm recommendations:

Based on the conclusions above, the following recommendations can be made:

- The use of an anaesthetic during the grading procedure is critical for reducing stress.
- When an anaesthetic is in use, any grading method (hand- or machine-grading) can be employed.
- In the case of a shortage or unavailability of an anaesthetic, it may be possible to continue hand-grading without the anaesthesia because there was no statistical difference in mortalities.

Haliotis midae is an exceptionally hardy species as it possesses the ability to survive under extended emersion conditions (O'Omolo et al. 2003). Despite this advantage, however, unsedated abalone faired poorer overall, no doubt due to the stress associated with the grading procedure. Sedation is thus of immense importance and the use of anaesthetics in aquaculture research to reduce stress in aquaculture species is not new (see Fish 1943; Post 1979; Ross and Ross 1984; Marking and Meyer 1985; Summerfelt and Smith 1990; Prince et al. 1995; Anderson et al. 1997). The results of this study have shown that the methods of grading (machine- or hand-grading) abalone are far less important than the application of an anaesthetic. Based on important factors such as time, material costs, man-power, etc., abalone farmers clearly have a number of options available to them as long as they use an effective anaesthetic.

Grading is one of many efforts being made to reach the financial goals of various aquaculture production systems. However, results from many international studies on various aquatic organisms are ambiguous and not as straight forward as one might have expected; the expected benefits such as an increase in growth rate and overall biomass were not found in all studies (see Gunnes 1976; Jobling and Reinsnes 1987; Wallace and Kolbeinshavn 1988; Baardvik and Jobling 1990; Makinen and Rouhonen 1992; Kamstra 1993; Daniels and D'Ambro 1994; Jobling and Baardvik 1994; Mgaya and Mercer 1995; Lazur 1996; Endemann et al. 1997; Strand and Øiestad 1997; Sunde et al. 1998; Barki et al. 2000; Lambert and Dutil 2001; Qin et al. 2001; Tidwell et al. 2004; Zakęś et al. 2004; Ahvenharju et al. 2005; Wallat et al. 2005). While these studies concentrated largely on the amount of weight gain and increased production from the effects of grading, none has investigated the comparable effects from the various grading procedures (e.g. hand-grading vs. machine-grading), or whether the use or lack of use of an anaesthetic influences the growth response of graded animals. Our research appears to be the first of its kind to investigate the comparative importance of different grading procedures and the influence of sedation on the recovery response in graded animals.

6.2 South African abalone farm management practices

Two questionnaires were sent to the AFASA member abalone farms in order to gain insight into the various handling/grading procedures employed by the various farms. The overall purpose of the first questionnaire was to determine the general grading practices, while that of the second questionnaire was to gain more information regarding the splitting exercise. In general, the abalone farmers were most helpful and cooperative in supplying information, but some questions remained unanswered for various reasons (see Chapter 5).

From questionnaire 1 the following were clear. The majority of farms use either direct splitting or hand-grading; few use machine-grading. All farmers use anaesthetics during their grading procedures, no matter which procedure is applied. The use of anaesthetics was considered by most as beneficial and the majority of farms use magnesium sulphate for juvenile abalone and / or carbon dioxide for both juvenile and adult abalone. Machine-grading is not generally practiced on South African farms because most farmers consider it a costly exercise. Abalone farmers generally speculate about the possible negative effects of machine-grading, but have no scientific evidence to back up their claims. Although the grading procedure (both hand- and machine-grading) has a degree of negative impact on the abalone, farmers have adopted it widely as part of their farming practice because it is beneficial over the long term, easing the harvesting process by generally producing uniformity in size (and weight) of animals. Overall, communication between abalone farmers does exist, but is generally limited and depends entirely on their individual interrelationships.

From questionnaire 2 the following were clear. Farmers tend to use the splitting procedure for no particular age group i.e. splitting is used throughout the lifespan of the abalone. Splitting is generally considered beneficial by those applying it. Sedation by anaesthesia is also a general practice during splitting. The majority of farmers, however, prefer to hand-grade rather than perform splitting exercises; this may be attributed to the lack of available scientific information i.e. research.

6.2.1 Recommendations

Based on the conclusions above, the following recommendation can be made:

- Improved collaboration and communication (scientific knowledge) is needed between the scientific community, the abalone farmers association and the farmers themselves.

6.3 Conclusion

As the Frontier Programme (see Pitcher 2005) has made explicit reference to the need for studies on "Animal Husbandry" in the abalone aquaculture industry, the information obtained from this research will contribute greatly to the knowledge lacking in this sector of aquaculture research. From our research it is clear that: 1) it is better to perform a splitting exercise for size-sorting very young abalone; 2) the methods of grading are less important than the application of an anaesthetic; and 3) greater scientific collaboration and communication would be beneficial to the abalone farmer. No doubt, the information obtained during this study will go a long way in assisting the South African abalone farming industry to undertake more informed decisions when it comes to handling and grading practices.

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